STUDIES ON FINFISH AND SHELLFISH DISEASES

THESIS SUBMITTED

IN PARTIAL FULFILMENT OF THE REQUIREMENTS
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DOCTOR OF PHILOSOPHY
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BY

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C E R T I F I C A T E

This is to certify that the Thesis entitled 'Studies on finfish and shellfish diseases' is a bonafide record of the research work carried out by Mr.C.Thankappan Pillai under my supervision and guidance and that no part thereof has been presented for any other Degree.

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S Y N O P S I S

The upsurge of global awareness and interest in aquaculture of commercially important finfishes and shellfishes, to compensate the acute shortage of food, has stimulated the way to the progress of studies in their diseases inorder to frame suitable measures in treatment and prophylaxis for the successful maintenance and management of aquaculture systems. While our knowledge of fish diseases of inland aquaculture system is fairly developed, studies in diseases of cultivable marine and brackish water finfishes and shellfishes are still in their infancy. In fact, little information is available from tropical waters. Hence, the candidate took up the problem 'Studies on finfish and shellfish diseases' for the Thesis leading to the Ph.D. Degree.

In the present study, fifteen types of diseases occurring in twenty one species of finfishes and shellfishes are documented and the causes studied. All these are being reported from India for the first time which adds considerably to our existing knowledge on the subject. In fact, the candidate's studies embodied in

this Thesis has also an applied significance aimed at the proper management of finfish and shellfish aquaculture systems in this country.

The Thesis is presented in seven main chapters as follows:

1. Introduction
2. Isolation and identification
3. Virological studies
4. Pathogenicity studies
5. Testing of therapeutic agents
6. Haematological studies

and 7. Electrocardiography

According to the nature of disease, pathological samples were aseptically dissected out and cultured for isolation of the pathogens, on a variety of media, in two sets, one using aged, filtered sterile sea water base and the other with 0.5 per cent Sodium chloride with distilled water base. The suspected pathogen(s) was isolated from those organisms predominating on the isolation media.

Excluding twenty fungal strains, of the one hundred and fifteen bacterial isolates made, Gram negative

asporogenous rods predominated. The organisms were identified according to three systems of classifications.

Pathogenicity studies were conducted in *Etroplus suratensis*, *Anguilla bicolor bicolor*, *Penaeus indicus*, *Penaeus monodon* and *Panulirus homarus* to evaluate the pathogenicity of the isolates *in vivo* and to satisfy Koch's postulates. Besides intramuscular and intraperitoneal injections, attempts were also made to infect the test species with respective organisms through artificial injuries and site injection.

Only eight organisms, including the fungal strain, were proved to be definitely pathogenic and were reisolated in pure culture.

An instance of disease, proliferative epithelial tumour, in *Arius jella*, was found to be due to viral infection as it satisfied Rivers' postulates.

Haematological study, haemoglobin, made in naturally diseased *Etroplus suratensis* and in normal healthy fish of the same species revealed that the diseased fish was having abnormal haemoglobin value leading to anaemia.

The pathogens were subjected to sensitivity tests against different antibiotics and also to disinfectants,

under *in vitro* and *in vivo*, used for the treatment of finfish and shellfish diseases.

Electrocardiographical studies, conducted in control and diseased *Etroplus suratensis*, the latter as a result of induced infection could prove that cardiological parameters can be considered for stress and disease detection especially the heart rate as an index of microbial disease.

The present studies practically lay the foundation for the systematic study of disease in finfish and shellfish culture systems in the brackish water and marine environments in India.

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PREFACE

From the early seventies, the world fish production has been virtually stagnating causing serious concern all around. One of the major global efforts for augmenting fish production is the accelerated development of aquaculture. For a balanced development of this sector, a considerable amount of scientific inputs are necessary. While great strides have been made in inland aquaculture, particularly in the temperate waters, coastal aquaculture (mariculture) in the tropics has yet to develop on scientific lines. We are greatly deficient in our understanding of areas such as reproductive physiology, nutritional requirements, genetics, pathobiology, farm engineering and so on. Thus, one of the major areas which needs serious attention is fish and shellfish pathology.

We have very little information on the effects of diseases in natural populations of finfish and shellfish occurring in the capture fisheries. The information base is relatively stronger in inland aquaculture and not so in the brackish water and marine aquaculture systems.

Fish and shellfish pathology covers a very wide field of multidisciplinary subjects such as microbiology, virology, histopathology, haematology, parasitology, electron microscopy, tissue culture, nutritional diseases, ecological diseases and genetic disorders. The candidate's main effort thus far has been to develop research on microbial diseases and its control measures. Very few investigations have been carried out in marine and brackish water fishes on microbial diseases in India.

The candidate's work, for the Master of Science Degree in Microbiology of the University of Bombay by research was on the problem 'Studies on microorganisms associated with some fish diseases'. He had taken up in 1976 research in this line realising the great void that exists in fish pathobiology in India. His work for the Masters Degree had considerably helped to strengthen his approach to the studies on fish disease investigation, pertaining to microbial aspects, enabling him presently to set up microbiological laboratory at this Institute and also monitor aquaculture systems for the early detection of microbial diseases.

The candidate's work embodied in this Thesis entitled 'Studies on finfish and shellfish diseases', has

brought to light 15 diseases of finfish and shellfishes from the brackish water and marine environments for the first time in India.

The summary of the candidate's salient findings are as follows:

1. Identification of fifteen microbial diseases (black spot disease, enteritis, eye disease, fin rot, gill rot, hemorrhagic septicemia, muscle necrosis, shell disease, skin lesion, skin spottiness, streptococcosis, tail rot, proliferative epithelial tumour, deep mycosis and dermatomycosis) including a viral case among saline water finfishes and shellfishes.

2. Both in finfishes and shellfishes, bacterial diseases due to Gram negative asporogenous rods predominated.

3. Many diseases could be suitably treated by use of disinfectants and antibiotics.

4. Strict hygiene and suitable prophylaxis seem to be better, than permitting outbreak of diseases, as the applied significance of chemotherapeutics has got only limited importance in the vast aquaculture systems.

5. The basic cause of anaemia is noted to be due to microbial origin as reduced value of haemoglobin is found in diseased, finfishes, with tail rot.

6. Proliferative epithelial tumour is confirmed to be a disease caused by virus satisfying Rivers' postulates.

7. Electrocardiography in normal and diseased finfishes indicated that the heart rate is an useful index of microbial disease.

8. Bacterial infection may lead to cardiac arrest.

The above study has enabled the candidate to have a better perspective of the role of extraneous factors causing microbial diseases, general disease diagnosis of finfishes and shellfishes and their treatment. It has also helped the candidate to understand the significance of strict hygiene and prophylaxis and the possible role of these pathogens in causing cross infection among finfishes and in human beings.

Based on his observations, to check microbial cases, a regular and systematic disease check up is recommended for the successful management of the aquaculture systems.

The salient findings of the present study have considerably enhanced our knowledge in finfish and shellfish diseases and their treatment in this country.

Apart from the present investigation, eight supporting papers of the candidate are appended to the Thesis of which six deal with the diseases of finfishes and shellfishes and two on their microbial populations.

This study was undertaken at the kind suggestion of Dr.E.G.Silas, Director, Central Marine Fisheries Research Institute, Cochin and the candidate has great pleasure in recording his deep sense of gratitude and indebtedness to him for suggesting this problem and guiding his research work. The candidate is also thankful to Dr. Silas for the facilities for research made available to him at the Institute.

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CHAPTER I

INTRODUCTION

INTRODUCTION

India is faced with dire need of augmenting proteinaceous food as a priority programme for increasing the per capita consumption. One of the resources which has considerable potential is fish and ancillary resources of the marine and inland waters. The per capita consumption of fish in India is extremely low being 3.3 kilogram and the production from marine and inland waters stands today around 2.3 million tonnes. Due to various factors, the marine production in the country has been stagnating around 1.2 million tonnes, having reached a peak of 1.4 million tonnes in 1979. A similar trend has been noticed during the last decades in global fish production which for a few years stagnated around 69 million tonnes. This situation has created a greater awareness of increasing fish production through culture means as capture fisheries. Even in this, the industrial sectors which deprive larger fishing vessels are capital intensive. At present, there are major constraints on resources exploitation, the one being the declaration of the exclusive economic zone and the extended jurisdiction in fisheries activities within their own, by

several nations. Some of the major capture fisheries have shown spectacular failure such as the perennial Anchoviella fishery, the herring fishery and the mackerel fisheries of the North Sea. In India, estimates based on exploratory services as well as indirect methods based on productivity show that the marine fisheries production from the present level could be easily doubled or even trebled. However, this would involve heavy capital involvement and the development of diversified fishing activities to tap both conventional and non conventional resources of finfish and shellfish. In view of this, as spectacular increase in production is expected during the decade in this sector. However, the country is blessed with about 1.7 million hectares of brackish waters, saline lagoons and mangrove swamps where part of which could be taken up for productive utilization for the development of coastal aquaculture. Added to this, we have large stretchers of inshore waters free of navigation and fishing activities which could also be utilized for seasonal open sea farming. At present, around 20 thousand metric tonnes of finfish and shellfish (shrimps) are produced from traditional brackish water culture systems primarily in Kerala and West Bengal. Intensive efforts are under way to upgrade

these operations to high productive, selective, stocking and fishing to increase the unit value of the production. Similarly, as a first phase programme, for pilot projects for brackish and saline water finfish and shellfish culture and extensive culture operations through intensive programmes have been planned in the current 6th five year plan of the country. This will be a stepping stone for taking up intensive fish culture which will involve relatively high class technology for use of the large scale hatchery produced seeds, feed input and maintaining the quality of the environment. Since these priorities in our national fisheries development programme has been clearly spelt out, it is imperative that we look at the various constraints that would delay or prevent active development programmes. Today, there is practically no information is available in the country about the role of diseases in natural population of finfish and shellfish (prawns, lobsters, crabs, bivalves such as mussels and oysters etc.) in the country. However, ever since a crash programme on developing suitable technologies for brackish water coastal aquaculture has been taken up from the mid seventies, it has become increasingly evident that almost priority should be bestowed as finfish and shellfish

diseases whether it be in the hatchery rearing stage or in the culture ponds.

The work relating to fish diseases in its broader sense to group finfish or shellfish diseases is practically nil in the country. What little information we have is on the levels of bacterial quality control aspects as contaminants in the fish products from processing and thereafter. Few stray accounts in fish tumours, fin and tail rot and skin lesions of the cultivated organisms are available from the inland waters. Thus, it will be seen that as far as marine species are concerned, practically no work has come out on ichthyopathology.

In view of the great paucity of knowledge about finfish and shellfish diseases in our country and recognising the need for enhancing our knowledge on this, and to safeguard large scale development in coastal aquaculture in our country, the candidate took up for his Ph.D. dissertation, the subject 'Studies on finfish and shellfish diseases'.

This Thesis embodies a brief review of what is known about finfish and shellfish diseases and the candidate's original work on documentation of various

bacterial, fungal and viral diseases, isolation and identification of pathogens, confirmatory test procedures to evaluate the pathogenicity of diseases causing organisms, effect of different disinfectants and antibiotics against the pathogens to treat and control diseases, haematological nature of normal and diseased fishes and the methods of diagnosis of diseases by electrocardiography, apparently entirely a new area which the candidate found will be helpful in diagnosing disease causing factors.

This is the first time that in depth investigations have been carried out on diseases of finfish, prawns, lobsters and some bivalves in India. In this, the candidate has been considerably helped by his research work already he had earlier completed for the Master of Science Degree of the University of Bombay on the topic 'Studies on microorganisms associated with some fish diseases'.

The candidate has produced available literature in fish diseases inorder to obtain back ground knowledge on the subject to enable him to understand and study some of the diseases in finfish and shellfishes whether it be in the capture fisheries or culture fisheries.

Since the subject nature of fish diseases is a vast one, the candidate has limited his Thesis to microbial diseases namely, viral, bacterial and fungal diseases.

RESUME

A resume of the literature on microbial fish diseases indicates that a wide range of pathogenic organisms have been identified as agents responsible for specific diseases. The major - viral, bacterial and fungal diseases hitherto identified in literature are indicated in table 1.1. Particular reference to the works of Conroy and Herman (1970), Ribelin and Migaki (1975), Roberts (1978), Sindermann (1970 and 1977) and van Duijn (1973) help us to have an over view of the problem. Before passing on to the candidate's own contribution, a resume of the specific diseases is given here as back ground information for this dissertation.

In the resume where the various types of diseases and prophylactic measures are discussed, examples of infection on fish and shellfish have been cited chiefly with reference to saline water species. The candidate's work also deals with saline and brackish water species.

TABLE 1.1

Different kinds of microbial diseases

Sl. No.	Diseases	Agent
	A. BACTERIA	
1.	Furunculosis	Aeromonas salmonicida
2.	Columnaris	Flexibacter columnaris
3.	Vibriosis	Vibrio anguillarum
4.	Ulcer	Haemophilus piscium
5.	Tail rot/fin rot	Aeromonas sp./Pseudomonas sp. Vibrio sp.
6.	Hemorrhagic septicemia	Aeromonas sp./Pseudomonas sp.
7.	Cold water/Peduncle disease	Myxobacteria (Cytophaga sp.)
8.	Gill disease	Myxobacteria, Pseudomonas sp.
9.	Exophthalmus	Pseudomonas sp./Aeromonas sp. Flavobacterium sp.
10.	Hagerman red mouth disease	Aeromonas hydrophila
11.	Red mouth disease	Members of Enterobacteriaceae
12.	Kidney disease	Corynebacterium sp.
13.	Tuberculosis	Mycobacterium marinum/ M. fortuitum
14.	Nocardiosis	Nocardia asteroides

TABLE 1.1 (Contd.)

Different kinds of microbial diseases

Sl. No.	Diseases	Agent
15.	Pasteurellosis	*Pasteurella piscida*
16.	Streptococcosis	*Streptococcus* sp.
17.	Micrococcosis	*Micrococcus* sp.
18.	Blue sac disease	No specific organism
19.	Infectious dermatitis	*Pseudomonas* sp.
20.	Scale disease	No specific organism
21.	Enteritis	No specific organism
22.	Erythrodermatitis	Myxobacteria
23.	Emphesematous putrefactive disease	*Edwardsiella tarda*
24.	Spinal disease	*Aeromonas liquefaciens*
25.	Meningitis	*Pseudomonas* sp.
26.	Bankruptcy disease	*Clostridium botulinum*
27.	Red sore disease	*Aeromonas* sp.
28.	Sekoke disease	No specific organism
	B. FUNGI	
1.	Saprolegniasis	*Saprolegnia* sp.
2.	Branchiomycosis	*Branchiomyces* sp.

TABLE 1.1 (Contd.)

Different kinds of microbial diseases

Sl. No.	Diseases	Agent
3.	Ichthyophonus disease	*Ichthyophonus* sp.
4.	Aphanomyces infection	*Aphanomyces* sp.
5.	Achlya infection	*Achlya* sp.
6.	Leptomitus infection	*Leptomitus* sp.
7.	Pithium infection	*Pithium* sp.
8.	Petersenia infection	*Petersenia* sp.
9.	Penicillium infection	*Penicillium* sp.
10.	Pullularia infection	*Pullularium* sp.
11.	Candida infection	*Candida* sp.
12.	Phoma infection	*Phoma* sp.
13.	Exophiala infection	*Exophiala* sp.
14.	Lagnedium infection	*Lagnedium* sp.
15.	Fusarium infection	*Fusarium* sp.
16.	Haliphothoros infection	*Haliphthoros* sp.
17.	Labyrinthomyxa infection	*Labyrinthomyxa* sp.
18.	Sirolpidium infection	*Sirolpidium* sp.
	C. VIRUS	
1.	Channel cat fish virus disease	DNA virus
2.	Herpesvirus disease	DNA virus

TABLE 1.1 (Contd.)

Different kinds of microbial diseases

Sl. No.	Diseases	Agent
3.	Infectious pancreatic necrosis disease	RNA virus
4.	Viral hemorrhagic septicemia	RNA virus
5.	Pike fry virus disease	Rhabdovirus
6.	Lymphocystis	DNA virus
7.	Infectious haematopoietic necrosis disease	RNA virus
8.	Spring viraemia of Carp disease	*Rhabdovirus carpio*
9.	Tumour	Virus
10.	Cancer	Virus (?)
11.	Piscine erythrocytic necrosis virus disease	Virus (?)

Bacterial diseases

(1) *Furunculosis*, results from infection by an obligate pathogenic bacteria, first reported by Emmerich and Weibel (1894), and identified as *Aeromonas salmonicida* (Griffin *et al.*, 1953). Furunculosis is a wide spread disease principally among salmonids and other fishes causing severe mortalities. Signs of this disease appear with variable frequencies (McCraw, 1952). The disease causing bacteria can be detected by culture or serological methods (Rabb *et al.*, 1964; McCarthy, 1975 b).

Outbreaks of this disease among marine fishes have been reported by Scott (1968) and Evelyn (1971). McCarthy (1975 a) has reported an epizootic of furunculosis caused by *Aeromonas salmonicida* var. *achromogenes*. Heavy loss was also noted in minnows, *Phoxinus phoxinus* by Hastein *et al.*, (1978) and in rainbow trout, s *Salmo gairdneri* by Sarig (1979). This disease was also detected in American eel, *Anguilla rostrata* by Hayasaka and Sullivan (1981).

The disease can be treated with drugs (Gutsell, 1948), and cured by oral administration of tetracyclines (McCarthy, 1975 b). Removal of affected fish minimises further outbreaks (O' Donnell, 1947). Selective breeding

for increasing the resistance of the fish is possible (Wolf, 1954; and Ehlinger, 1964). A detailed study of this disease has been made by McCarthy and Roberts (1980).

In India, Almeida (1962) has recorded this disease in *Mugil cunnesius* and isolated the disease causing organism. The candidate (Pillai, 1978) has also documented the disease in *Cichalosoma meeki*, *Barbus pentazona* and *Xiphophorus maculatus* and studied the characteristics of the aetiological agent.

(2) *Columnaris*, affects mainly salmonids and most warm water fishes. The first description of columnaris disease was given by Davis (1922), but the aetiological agent was isolated by Ordal and Rucker (1944) who named the causative agent as *Chondrococcus columnaris*, as it produced fruiting bodies and microcysts. However, Garnjobst (1945) who could not observe the fruiting bodies, named the organism as *Cytophaga columnaris*. The causative organism is now identified as *Flexibacter columnaris* (Buchanan and Gibbons, 1974). As the disease progresses, lesions spread through out the body including the gills (Snieszko and Bullock, 1976). The aetiological agent can also be detected by serological methods in

addition to the normal methods of isolation (McCarthy, 1975 c). An out break of columnaris disease due to *Chondrococcus columnaris* in White suckers (*Catostomus commersoni*) in Canada has been reported by Wobeser and Atton (1973). This disease, affecting rainbow trout, *Salmo gairdneri*, was reported by Conroy and Vasquez (1979). In the case of virulent strains of columnaris pathogen, death of the affected fish occurs within 24-48 hours (Sarig, 1979). Mixed infection of *Flexibacter columnaris* and *Corynebacterium* in Cat fish has been recorded by Marks *et al*., (1980). Heavy mortality of Atlantic salmon, *Salmo salar* due to 'Saddleback' disease caused by *Flexibacter columnaris* was reported by Morrison *et al*., (1981).

Terramycin along with feed at a level of 8 gms per 100 kilogram of fish per day upto 10 days is effective in early stage of the disease (Wood, 1968). Oxytetracycline at a rate of 50 mg per kilogram of fish per day was also found effective (Ashburner, 1973). The outbreak of the disease can be prevented by avoiding the external injuries, overcrowding, unnecessary handling and poor sanitary conditions (Snieszko and Bullock, 1976).

(3) Vibriosis, a disease otherwise known as red pest or red disease is world wide in occurrence. It is caused by the invasion of a Gram negative motile rod shaped bacterium of the genus, Vibrio, which causes severe mortality. Canestrini (1893) first described the aetiological agent of this disease. Bruun and Heiberg (1932) estimated that 30% mortality in eels in Danish waters due to this disease. Rushton (1937) described eel mortalities possibly due to vibriosis in Scotland in 1933. Anderson and Conroy (1970) reported its occurrence in eels (Anguilla sp.) causing catastrophic mortalities. Skin and muscle lesions due to infection by Vibrio anguillarum in Winter flounder (Pseudopleuronectes americanus) was detected by Levin et al.,(1972). The same species caused an epizootic in rainbow trout, Salmo gairdneri during an experimental acclimatisation in sea water (McCarthy et al., 1974). Vibriosis was also seen in rainbow trout cultivated experimentally in cages floating in sea water in Italy (Ghittino et al., 1975). McCarthy (1976) reported mass mortality of Anguilla anguilla in Great Britain. V. anguillarum causing epidemic case of red spot disease in Chanos chanos was recorded by Huang (1977). Vibriosis is considered to be a serious disease among salmonid fishes from fingerlings to adult (Kawatsu

et al., 1979). According to Sarig (1979) Vibriosis causes considerable damage to all species of fish.

Oral use of Terramycin, 3-4 gms for 100 lbs of fish for 10 days is recommended and the diseases can be prevented by avoiding overcrowding, infected feeds and poor sanitary conditions (Ross, 1970). The application of vaccine is also quite useful in preventing the disease.

In India, this disease has been documented by the candidate (Pillai, 1978) in *Arius dussumieri* by studying the isolated disease causing organism.

(4) *Ulcer*, is a disease, mostly prevalent among salmonids causing severe mortality. This disease is due to the attack by a highly fastidious Gram negative rod, *Haemophilus piscium*. Calkins (1899) was the first to describe the ulcer disease and Fish (1934) made a comprehensive study of the disease in trout. Wolf (1938) studied the mechanism of infection and transmission. Snieszko *et al*., (1950) and Snieszko (1952) also attempted to make a detailed study of the disease. As a serious fish disease, in Japan, ulcer has been reported by Kawatsu *et al*., (1979).

Ulcer can be treated by timely application of the broad spectrum antibiotics and prevented by avoiding the infected fishes, feeds and poor sanitary conditions.

In India, Almeida (1962) observed skin infection on various species of fishes. This disease has been detected in Indian major carps, and Gopalakrishnan (1963) has recommended distruction of fish with advanced state of the disease and treatment of the fishes, in an early stage of infection, with 1:2000 Copper sulphate solution for 3-4 days.

(5) Tail rot and fin rot, are world wide in occurrence and result in mass mortalities and severe loss. Finfishes are susceptible to this disease and no specific aetiological agents have been recognised so far. It is noticed that different species of bacteria can cause this disease (Snieszko, 1953; Conroy, 1961, 1964 b; Bullock, 1968; Bullock and Snieszko, 1970). An epidemic case of fin rot in *Chanos chanos* has been recorded by Rabanal *et al.*, (1951). Mass mortality of *Salmo gairdneri* due to fin rot has been reported in Moravian District in Czechoslovakia (Tesarcik and Rehulka, 1972). It has been identified as a major disease, affecting rainbow trout (Conroy and Vasquez, 1979). In, *Solea sole* L. *Pseudomonas* sp.

associated with tail rot disease was reported by Fluechter (1979).

Application of chlorine has been suggested for the treatment of the disease (Connell, 1937). 3 ppm acriflavine has been found to be a good prophylactic treatment (Meyer, 1968). A combined use of iodine and chlorotetracycline (aureomycin) is also advocated (Risely, 1971). In advanced cases of tail rot or fin rot, surgical treatment is advised (van Duijn, 1973). This disease can be prevented by avoiding infected feeds, poor sanitary conditions and overcrowding (Bullock and Snieszko, 1970).

In India, this disease has been found affecting fingerlings of Osphronemus goramy and it was suggested that overcrowding and dirty environments favoured the spread of the disease (Khan, 1939). Almeida (1962) detected the disease in Tilapia mossambica due to Pseudomonas sp., Flavobacterium sp., Bacillus sp.; in Mugil cunnesius due to Achromobacter sp.; in Megalops cyprinoides due to Pseudomonas sp.; in Drepane punctata due to Achromobacter sp. and Brevibacterium sp.; in Trachynotus ovatus due to Flavobacterium sp. and Micrococcus sp. and in Abudefduf saxatilis due to

Aeromonas sp. and Escherichia sp. This disease has also been detected in fry, fingerlings and adults of Carps from different parts of India (Gopalakrishnan, 1963). The candidate (Pillai, 1978) recorded this disease in Etroplus suratensis, Lutianus johni, Plotosus arab, Gerres sp., Therapon jarbua, Apogon quadrifasciatus due to Vibrio parahaemolyticus and in Gerres sp., Holocentrus rubrum, Glyphidodon coelestinus due to Pseudomonas sp. In Sillago sihama, Pomacentrus coeruleus due to Vibrio fischeri and in Arius dussumieri due to Vibrio sp. were the tail rot diseases documented by the candidate (Pillai, 1978). The candidate, Pillai (1978) had also studied fin rot diseases in Gerres sp. due to Pseudomonas mendocina.; in Gerres sp., Cynoglossus puncticeps due to Pseudomonas sp; in Lutianus johni, Drepane punctata due to Vibrio parahaemolyticus and in Drepane punctata as a result of Aeromonas hydrophila attack.

It is difficult to save the fishes in advanced stage of tail rot or fin rot (Gopalakrishnan, 1968). However, Copper sulphate solution, 1:20000 for 10-15 minutes as dip treatment has been suggested (Khan, 1939), while 1:3000 solution of Copper sulphate for treatment has been advocated by Gopalakrishnan (1963). Use of

Copper sulphate on the infected area has been found more effective (Pal and Ghosh, 1975).

(6) *Bacterial haemorrhagic septicemia* This is caused by two species of bacteria, *Aeromonas liquefaciens* and *Pseudomonas fluorescens* singly or collectively. *Aeromonas liquefaciens* is pathogenic for many species of wild and propagated species of finfishes (Bullock and McLaughlin, 1970). There is also a view that eventhough *Aeromonas liquefaciens* is a pathogen, low levels of oxygen initiate the epizootics of hemorrhagic septicemia (Haley, Davis and Hyde, 1967). *Pseudomonas fluorescens* also act similarly as *Aeromonas liquefaciens* in causing disease. Outbreaks of this disease were recorded by Schaperclaus (1954); Bullock, (1965); Bullock and McLaughlin (1970); Bullock *et al.*, (1971); Shotts *et al.*, (1972) and Conroy and Vasquez (1979). Ross (1970) has observed a more or less similar disease in marine and estuarine fishes due to the invasion of *Vibrio anguillarum*. Bullock and McLaughlin (1970) have also described the infection, due to *Vibrio anguillarum* in marine and estuarine fishes, as hemorrhagic septicemia. Rightly, the infection of *Vibrio anguillarum* should be regarded as vibriosis instead of hemorrhagic septicemia.

This disease can be treated by administering broad spectrum antibiotics, orally, 50-75 mg per kilogram of fish for 10 days and prevented by avoiding overcrowding, poor environmental conditions and infected fishes (Snieszko and Bullock, 1974).

In India, Aeromonas and Pseudomonas infections have been reported by Gopalakrishnan (1971 a, b; 1963, 1968) and Almeida (1962). This disease has been documented by the candidate, (Pillai, 1978) in Lutianus johni, Epinephilus pantherinus, Trachynotus ovatus and Platax teira. He has also studied the aetiological agent.

(7) Cold water and Peduncle diseases are identical diseases which mainly affect salmonid fishes causing mass mortalities. These diseases are mostly due to strains of myxobacteria. - Gram negative slender rods showing creeping motility. In advanced cases of both types of diseases, myxobacteria (Cytophaga sp.) are found in the internal organs of the fish. Davis (1929) has recorded peduncle disease in trout fingerlings. Wood and Yasutake (1956) have also made a detailed study of this disease in Pacific salmon. It has been reported by Wolke (1975) that this disease causes acute mortality in salmonid fingerlings.

These diseases can be treated with sulphonamides and prevented by avoiding overcrowding and poor sanitary measures (Bullock and Snieszko, 1970).

(8) Dropsy, swelling of belly, is a serious disease. It is mostly noticed in fishes in temperate (European) countries. A detailed study of this disease in fishes has been made by Schaperclaus (1930 and 1933), who found *Aeromonas punctata* in fishes suffering from dropsy. The disease was later suspected to be of viral origin which was confirmed by the works of Roegner Aust and Schleich (1951); Goncarov (1959) and Gaines and Rogers (1975). Epidemics of this disease resulted in heavy loss in Poland and Germany (van Duijn, 1973).

The disease can be treated with chloramphenicol at a dose of 50 mg per lbs of fish or litre of water body. Surgical treatment, is also advocated in the case of fish in advanced stages of the disease, by removing the accumulated fluid in the body using a syringe before the chloramphenicol treatment (van Duijn, 1973).

In India, this case has been observed in Carps (Gopalakrishnan, 1961 a). A species of *Aeromonas* is considered to be the disease causing agent (Gopalakrishnan,

1961 a; Jhingran, 1975). 5 ppm dip treatment of Potassium permanganate for two minutes has been suggested to limit the spread of this disease (Gopalakrishnan, 1963). Almeida (1962) has also noted this disease in various fish species.

As the aetiological agent of infectious dropsy has already been confirmed as *Rhabdovirus carpio* by Fijan (1972), the disease due to the invasion of the species of *Aeromonas*, *Pseudomonas* is now called as bacterial hemorrhagic septicemia (Snieszko and Bullock, 1974); the term infectious dropsy for bacterial disease is no more in vogue, as it is considered a symptom of viral disease complex.

(9) *Gill disease* is considered to be due to the attack by certain species of myxobacteria. Protozoan parasites, fungi (*Branchiomyces* sp.) trematodes have also been associated with gill disease (Nigrelli, 1954; Wales, 1958; Halver, 1972; Ramamurthy, 1972; and Lightner *et al.*, 1975). Osburn (1910) first described the pathological condition of the gills of salmonid fishes. Fish (1935) could record a case of gill disease, but the isolates could not be proved to be the aetiological agents. Mass mortality of European eels, *Anguilla*

anguilla, due to gill disease as a result of infection with myxobacteria has been reported (Ghittino, 1972). Nutritional gill disease, aneurysmal gill disease and gill necrosis of unknown aetiology are important gill diseases other than bacterial gill diseases (Eller, 1975). For disease outbreak, overcrowding is one of the most important factors which was confirmed by the studies on gill rot in Carps (Wunder, 1947). The gill epithelial proliferation and the presence of large numbers of myxobacteria currently classified as *Flexibacter* sp. on the swollen gill lamellae are the characteristic symptoms of the disease.

The disease can be treated using disinfectants and prevented by avoiding the overcrowding (Snieszko, 1970).

In India, cases of gill disease caused by species of *Pseudomonas* were recorded in *Tilapia mossambica* by Almeida (1962) and in *Glyphidodon coelestinus* by the candidate (Pillai, 1978).

(10) *Exophthalmus*, or *pop eye disease*, is world wide in distribution and causes often mass mortality. No specific agent has been established so far as the causative agent of the disease. Bacteria, mostly

species of *Pseudomonas*., are considered to be the chief causative agent eventhough viruses, fungi and certain worms (*Diplostomum* sp.) and changes in the harmones of hypophysis are also suspected as disease causing factors (Williamson, 1927; Belding and Merrill, 1935; and van Duijn, 1973). Dukes (1975) has stated that species of *Aeromonas*, *Pseudomonas*, *Flavobacterium* and *Vibrio* are mostly associated in the exophthalmic condition.

The disease can be treated by administering antibiotics and prevented by good sanitation and destroying the infected animals. Surgical treatment, by operating out the infected tissues in the case of advanced stage of the disease, is possible followed by antibiotic administration.

In India, a few cases of eye diseases have been reported. This disease has been observed to cause mortality in major carps and the aetiological agent was found to be a species of *Aeromonas* (Gopalakrishnan and Gupta, 1960; and Gopalakrishnan, 1961 b). Almeida (1962) has detected cases of this disease in *Tilapia mossambica* due to *Pseudomonas* sp., *Achromobacter* sp., *Aeromonas* sp., *Micrococcus* sp., and *Bacillus* sp./ in *Scatophagus argus* due to *Pseudomonas* sp.; in

Diagramma griseum due to *Pseudomonas* sp.; and in *Trachynotus ovatus* due to species of *Achromobacter*. The candidate (Pillai, 1978) has documented this disease in *Astronotus ocellatus*, *Gerres* sp., *Therapon jarbua*, *Lutianus johni* and *Drepane punctata* due to *Vibrio parahaemolyticus* and in *Gerres* sp. due to *Bacillus thuringiensis*. He also noticed this disease in *Apogon quadrifasciatus* due to *Vibrio fischeri* and in *Tilapia mossambica* due to *Aeromonas hydrophila*.

Gopalakrishnan (1961 b) has suggested hourly baths in 8-10 mg of chloromycetin per litre of water for three consecutive days as effective in the case of early stages of infection. Application of 1 ppm Potassium permanganate as prophylactic treatment and provision of high dissolved oxygen content in the medium could be helpful in preventing the spread of the disease.

(11) Redmouth disease, a main disease of the salmonid fishes, causes high mortalites. It was first reported in 1948 in California and in Colorado in 1951 by Wagner and Perkins (1952). The disease causing organism is considered to be *Pseudomonas hydrophila* now classified as *Aeromonas hydrophila*, a motile Gram negative rod (Buchanan and Gibbons, 1974). There

is also a similar disease called redmouth disease occurring in Hagerman (Bullock and Snieszko, 1975), in which the causative agent was found to be a member of *Enterobacteriaceae*, and Ross *et al*., (1966) suggested the name RM bacterium for it. Later, McDaniel (1971) suggested the name HRM disease to distinguish redmouth caused by RM bacterium from a similar type of disease caused by *Aeromonas hydrophila*. An epizootic of redmouth disease in hatchery rainbow trout due to infection by *Vibrio anguillarum* has also been reported (Ghittino *et al*., 1972).

The disease can be treated using broad spectrum antibiotics and prevented by avoiding the aetiological agent.

In India, no such disease is found documented.

(12) *Kidney disease* a chronic to acute case of infection which usually results in significant mortality especially among salmonid and other species of fishes (Bullock *et al*., 1974). The aetiological organism of this disease is a Gram positive, non motile and non acid fast diplobacillus, now classified in the genus, *Corynebacterium*. Most of the reports on the outbreaks of the disease have appeared since the 1950's eventhough

this disease was reported in U.S.A. in 1935, and an identical disease reported in 1933 from Scotland. While Rucker *et al.*, (1951) and Wood *et al.*, (1955) believed that the disease could be due to a Gram positive bacillus, Ordal and Earp (1956) established that the aetiological agent is a species of *Corynebacterium*. Bacteria caused kidney disease is reported in the rainbow trout, *Salmo gairdneri*, from Loon lake, British Columbia by Evelyn *et al.*, (1973). This disease, usually results in serious losses in wild or cultured fish (Richards and Roberts, 1978). Proliferative kidney disease posibly due to infection by amoeba in combination with poor quality water resulting in losses upto 75% fingerlings of rainbow trout, *Salmo gairdneri* has been reported by Ferguson and Needham (1978) . At present, a direct fluorescent antibody technique (FAT) has been developed for detecting *Corynebacterium salmonis*, the causative agent of bacterial kidney disease, by Bullock *et al.*, (1980).

This disease is difficult to treat as the organisms are mostly beyond the reach of antimicrobials, but, the disease can be prevented by avoiding the causative agent (Bullock *et al.*, 1975). Warren (1963) has shown that the disease severity increased with softness of water.

(13) *Mycobacteriosis* is an infectious disease causing severe loss especially in cultured species of fishes. Mycobacteriosis was first observed in 1897 in Carps in a contaminated pond by Bataillon, *et al.*, (1897) and Bullock *et al.*, (1971). Later, the causative *Mycobacterium* sp. was isolated from fish grown in aquaria and hatcheries and also from fishes from their natural habitats, including fresh water, brackish and salt water (Parisot, 1970). Acid fast, Gram positive, non motile rods belonging to the genus, *Mycobacterium* are responsible for mycobacteriosis. The three recognised piscine tuberculosis causing organisms are *M. marinum*, *M. fortuitum* and *M. platypoecilia* (Buchanan and Gibbons, 1974). *M. marinum* isolated from marine fish was found to infect warm blodded animals when injected into the lower extremities which had a lower temperature than the main body (Clark and Shephard, 1963). Similarly, *M. leprae* multiplied when injected into perch (Chaussinand and Besse, 1951). A species of *Mycobacterium* isolated from an abscess in a human being and the mycobacterial isolates from neon tetra, *Hyphenobrycon innesi* (Nigrelli, 1953) were found to fit the species description of *M. fortuitum* which represents the first report of *M. fortuitum* in fresh water fishes (Ross and Broncato, 1959). Spontaneous

occurrence of tuberculosis like lesions carrying acid fast bacteria, in three marine fish was recorded in halibut (Sutherland, 1922) in cod (Alexander, 1913; Johnstone, 1913) and in halibut roe (Griffith, 1930). Symptoms of piscine tuberculosis are variable (Wolke, 1975). Lack of acute external symptoms at an early stage of the disease may prevent the easy detection of diseased fish. So, a reliable diagnosis is based on the isolation of the aetiological *Mycobacterium* sp. Mycobacteriosis causing severe loss in Chinook salmon, *Oncorhynchus tshawytscha* has been reported by Ashburner (1977).

The oral administration of broad spectrum antibiotics, with feed especially kanamycin, has been found successful. Improved sanitary conditions and isolation of the infected fishes help to prevent the disease (Parisot, 1970). As the disease is zoonotic (Richards and Roberts, 1978), adequate care has to be taken in handling the fish.

(14) *Nocardiosis* is not a frequently appearing disease. It can be clearly differentiated by observing the Nocardial aerial hyphae and branching vegetative cells which are not seen in mycobacterial species. Heuschmann-

Brunner (1965) stressed the need for careful examinations of fish diseases caused by acid fast bacteria which may appear like both *Mycobacterium* and *Streptomyces*. Nocardial (*N. asteroides*) diseases were reported and the agents isolated from rainbow trout (Snieszko *et al.*, 1964 a; Heuschmann-Brunner, 1966; Ghittino and Penna, 1968), from brook trout, (Campbell and Mackelvie, 1968) and from neon tetra (Conroy, 1964 a). This disease has also been reported from chinook salmon (Wolke and Meade, 1974). Nocardial infection causing severe mortality in yellow tail culture in Japan is mentioned by Richards and Roberts (1978).

Broad spectrum antibiotics are effective and the disease can be prevented by increasing the resistance of the fish and avoiding the disease causing agents, *N. asteroides* and *N. kampachi*.

(15) *Pasteurellosis* is a disease due to the attack by a species of the *Pasteurella* genus, causing mass mortality among finfishes. In the summer of 1963, mass mortalities of white perch, *Roccus americanus* in Chesapeake Bay were recorded and the causative agents were noted as *Pasteurella* sp. (Snieszko *et al.*, 1964 b). The organisms were found pathogenic for healthy white

perch (Allen and Pelczar, 1967). Later, these *Pasteurella* sp. were classified as *P. piscida* in comparison with known *Pasteurella* sp. (Janssen and Surgalla, 1968). Bacteria considered to be species of *Pasteurella* were recorded as causing mass mortalities in brown trout (*Salmo trutta*) and Atlantic salmon (*Salmo salar*) (Hastein and Bullock, 1976). As a serious disease, it has been reported in yellow tail cultures from Japanese waters (Cichards and Roberts, 1978).

The disease can be treated by administering antibiotics and prevented by avoiding infected hosts and maintaining good sanitary measures.

(16) *Streptococcosis*, due to haemolytic and non haemolytic forms of Gram positive cocci, is also an important disease. Hoshina *et al*., (1958) first recorded the disease due to Gram positive cocci in rainbow trout and the bacteria were identified as *Streptococcus faecalis*. Robinson and Meyer (1966) isolated group B haemolytic *Streptococcus* from two epizootics of golden shiner, *Notemigonus crysoleucas*. Streptococcosis has also been reported in marine fishes by Plumb *et al*., (1974). Roode (1977) documented streptococcosis in rainbow trout, *Salmo gairdneri*. Richards and Roberts

(1978) has stated that the aetiological agent could be isolated from peritonitis of salmonid fishes. Kusuda *et al.*, (1978) has observed *Streptococcus* sp. causing epizootic diseases in eels, *Anguilla japonica*. This disease causing mass mortality of rainbow trout, *Salmo gairdneri* was recorded by Boomker *et al.*, (1979).

The disease can be treated with the help of broad spectrum antibiotics and prevented by avoiding infected feeds and poor sanitary conditions.

(17) *Micrococcosis*, as a result of infection due to *Micrococcus* sp., has only been infrequently observed. This disease in rainbow trout, *Salmo gairdneri* was recorded from Argentina by Conroy (1966). Bullock *et al.*, (1971) have described this disease in gudgeon (*Gobio fluviatilis*), possibly due to *Micrococcus pyogenes aureus*. Armas (1979) has reported Micrococci, as potential fish pathogen, isolating them from mullet alevins, *Mugil cephalus L.* from Rio Moche coastal lagoon of Peru.

Broad spectrum antibiotics are effective against the disease and the disease can be prevented by getting rid off the infected fishes.

(18) *Blue sac disease*, also known as dropsy, yolk sac disease and hydrocoele embryonalis, is an

edematous condition commonly observed in sac fry. The infected fry show an abnormal accumulation of fluid usually blue in colour. Various causes such as bacterial, physiological, hereditary and environmental have been postulated for the blue sac disease (Wolf, 1969 a). The early investigators, von Betegh (1912), Guberlet et al., (1931), and Atkinson (1932) believed that bacteria are responsible for this disease. But later, Scherdschewsky (1935) and Dieterich (1939) were of the view that the disease is due to physiological causes. Gottwald (1960) reported that the disease is due to incubation of eggs under continuous deficiency of oxygen. Wolf (1957 a, b) found that the disease was induced in several species of fry by incubating eggs in closed systems where metabolic wastes accumulated. Blue sac disease was also induced in running water in which ammonia was added. This proved that the blue sac disease could be physiological and brought about by unfavourable conditions. This disease could result in heavy mortality (Roberts, 1978).

This disease can be prevented by observing strict standards of water quality in hatcheries and taking adequate care in avoiding handling stress during incubation of eggs.

Besides these, a number of atypical or aberrant types of diseases have been reported to literature. The more interesting among them are as follows:

1. David (1927) has reported scale protrusion as a disease sometimes causing severe mortality.

2. Hamlyn-Harris and Duhig (1930) have described an epizootic of intestinal disease due to attack of bacteria in some fresh water fishes.

3. Wells and ZoBell (1934) have reported the bacterium Pseudomonas ichthyodermis as the causative agent of infectious dermatitis in the marine fish.

4. Peres (1944) reported scale disease in marine teleosts in which rod like bacteria were noted in the advanced stage of the disease.

5. Hodgkiss and Shewan (1950) have observed a case of Pseudomonas infection (infective dermatitis) in Plaice, Pleuronectes platessa.

6. Seaman (1951) reported bacterial intestinal disease in Mugil, Tilapia and Abudefduf saxatilis.

7. Bootsma (1973 a) has documented an outbreak of erythrodermatitis, in Cyprinus carpio, due to certain myxobacteria.

8. A case of Emphysematous putrefactive disease of catfish (EPDC) has been reported in the channel catfish, *Ictalurus punctatus*, as a result of infection due to a new species of bacterium, *Edwardsiella tarda* (Meyer and Bullock, 1973).

9. *Aeromonas liquefasciens* has been observed to cause spinal damage to the sea bream, *Chrysophrus major*, by Munro (1974).

10. Serious infections due to *Flavobacterium* sp. in finfish, *Molliensia sphenops* and due to *Staphylococcus* sp. in *Pollachius virens* were reported by Wolke (1975).

11. *Edwardsiella tarda* causing epizootic disease in mullets, *Mugil cephalus* has been documented by Kusuda *et al.*, (1976).

12. In rainbow trout, *Salmo gairdneri*, *Pseudomonas fluorescens* associated meningitis has been recorded by Roberts and Horne (1977).

13. Trout mortalities due to bankruptcy disease as a result of anaerobic bacterium, *Clostridium botulinum*, is also well recognised (Richards and Roberts, 1978).

14. Sekoke disease of finfishes causing considerable losses, has been reported by Cowey and Roberts (1978).

15. Mass mortality of silver carps, *Hypophthalmichthys molitrix* (Valenciennes) associated with bacterial infection due to *Proteus rettgeri* was reported by Bejerano *et al.*, (1979).

16. Red sore disease due to *Aeromonas hydrophila* in large mouth bass, *Micropterus salmoides* (Lacepede) was noted by Huizinga *et al.*, (1979).

17. Nutritional deficiency disease in Indian major carps, *Cirrhina mrigala* Hamilton due to avitaminosis C resulting in lordosis, scoliosis and hypochromic macrocytic anaemia was reported by Agrawal and Mahajan (1980).

Sporadic cases of diseases in shellfishes are also noteworthy. Significant among them are:

1. Kusuda and Watada's (1969) record of vibrio disease in prawns, *Penaeus japonicus*.

2. Lightner (1977 a) has reported *Vibrio* disease in prawns, *Penaeus* sp. affecting juvenile to adult stages in severe mortality. The disease, can be treated adding antibiotics with food and prevented with minimal handling, maintaining high water quality and reducing the stocking density.

3. Lightner (1977 b) has reported the brown spot disease known as shell disease or burned spot disease or rust disease in shrimps, *Penaeus* sp., and bacteria belonging to *Beneckea* and *Pseudomonas* are considered to be the causative organisms. Broad spectrum antibiotics are useful in treating the disease and it can be prevented by avoiding the aetiological agents and maintaining high water quality.

4. Lightner (1977 c) has observed filamentous bacterial disease in the brown shrimp, *Penaeus aztecus*, white shrimp, *Penaeus setiferus*, Mexican white shrimp, *P. vannamei*, California brown shrimp, *P. californiensis* and blue shrimp, *P. stylirostris*. 5-10 ppm Potassium permanganate for 1 hour static treatment is found to be effective. This disease can be prevented by maintaining good water quality.

5. Black gill disease (Lightner, 1977 d) in shrimps, *Penaeus vannamei*, *P. duorarum*, *P. californiensis* and *P. stylirostris* has also been reported, but the causative factor is unknown.

6. Lightner (1977 d) has also documented black death disease in *P. californiensis* and *P. aztecus* due to ascorbic acid deficiency and cases of blisters in

P. aztecus, P. setiferus, P. californiensis, P. vannamei and P. stylirostris for which again the cause is not known. He could also notice cases like cramped tails in P. aztecus, P. setiferus, P. brasiliensis and P. californiensis due to unidentified causes.

7. Due to unidentified causes, Lightner (1977 f) has recorded a disease called muscle necrosis in shrimps, P. aztecus, and P. californiensis.

8. A case of muscle necrosis in Macrobrachium rosenbergii has been recorded by Sindermann (1977) and the cause is considered to be environmental or physiological stress.

9. Sindermann (1977) has noticed black spot disease also called as shell disease or brown spot disease in shrimps, Macrobrachium vollenhovenii and M. rosenbergii caused by chitinoclastic bacteria, Beneckea sp. The disease can be treated with Furanace and prevented by careful handling, avoiding the infected organisms and maintaining good water supply.

10. Sindermann (1977) has also recorded filamentous bacterial disease in Macrobrachium rosembergii due to Leucothrix sp. This disease, can be treated with Furanace,

and prevented by reducing the population density and removing the infected animals.

11. Bacterial disease in blue crab, *Callinectes sapidus* due to infection with *Vibrio* sp. causing severe mortality was reported by Johnson (1977 a). The disease can be prevented by timely removal of the infected organisms and by careful handling.

12. Sindermann (1977) recorded Gaffkemia in American lobster, *Homarus americanus* due to *Gaffkya homari*, currently renamed as *Pediococcus homari* resulting in severe loss within a few days of infection. Sulfonamides are effective in treating the disease. Removing the infected organisms and by maintaining strict cleanliness will help to prevent the disease.

13. Bacillary necrosis, of the American oyster, *Crassostrea virginica* larvae, due to infection by species of *Vibrio*, resulting in mass mortality has been reported by Sindermann (1977). For treatment, antibiotics such as chloramphenicol and erythromycin are said to be effective. By observing good sanitation and improved water qualities, this disease can be prevented.

14. Sindermann (1977) has also noticed certain unidentified Gram positive bacteria causing focal necrosis in Pacific oyster, *Crassostrea gigas*.

15. Infection in the hard clam, *Mercenaria mercenaria* due to *Vibrio* sp. resulting in mass mortality has been recorded by Sindermann (1977) suggesting erythromycin treatment and improved sanitation and water qualities to prevent the disease.

16. Chitin degrading bacteria, *Pseudomonas*, *Vibrio* and *Beneckea* causing shell disease in lobsters, *Homarus americanus* was reported by Malloy (1978).

17. The bacterial pathogen, *Gaffkya homari* has been reported causing serious disease in the lobsters, *Homarus americanus*, in the U.S.A., Canada and Europe (Stewart and Castell, 1979).

Fungal diseases

Fungal diseases are widely prevalent among finfishes and their eggs in natural and artificial waters. Most of the aquatic animals are affected by fungi (Weston, 1941). Species of *Saprolegnia*, especially *Saprolegnia parasitica* can usually affect fish and fish eggs, although attack by species of *Achlya*,

Aphanomyces, Leptomitus, and Pithium have also been documented (Scott and O'Bier, 1962).

(1) Saprolegniasis is an important fungal disease affecting eggs, fry, fingerlings and adults of most of the finfishes. The Saprolegnia sp. grow on different types of organic matter and Davis (1953) reported that there is no evidence that Saprolegnia can develop on normal eggs, unless foreign organic matter is present. Usually injuries caused by spawning activities or lesions due to injuries facilitate fungal infection (Hoffman, 1969; Vishniac and Nigrelli, 1957; Scott and O'Bier, 1962). The presence of these saprophytic opportunists can be detected by the presence of ulcers on the body with or without tufts of minute white cotton like outgrowths, or by microscopic examination of the pathological samples. Saprolegniasis has been detected in the milkfish, Chanos chanos (Forskal) in Philippines by Rabanal et al., (1951). Bootsma (1973 b) has observed Saprolegnia infection, in the Dutch pike hatcheries, resulting in the damage of vital organs such as the liver. Many of the fungi causing saprolegniasis are primary pathogens and damage finfishes being cultured (Wolke, 1975). Outbreak of saprolegniasis has been

reported, in brown trouts, by Richards and Pickering (1978). This fungal disease can be treated by immersing the infected fishes in 1:15000 solution of malachite green for 10-30 seconds (Foster and Woodbury, 1936 and O'Donnell, 1941). Formalin is also effective in 1:500 to 1:1000 dilution for 15 minutes (Burrows, 1949; Reddecliff, 1958 and 1961; and Steffens, 1962). For treatment of diseases due to *Saprolegnia*, *Achlya*, *Aphanomyces*, *Leptomitus* and *Pithium*, Hoffman (1969) has suggested malachite green, 1:15000 for 10-30 seconds. Malachite green has also been reported to be an effective fungicide by Cline and Post (1972) and Bootsma (1973 b). This disease can be prevented by observing strict cleanliness, good sanitation and removing the infected fishes (Hoffman, 1969).

In India, Khan (1922) has recorded *Saprolegnia* disease resulting in mass mortality of fry of the murrel, *Ophiocephalus marulius* (Hamilton-Buchanan). Chidambaram (1942) has observed saprolegniasis in the gourami, *Osphronemus goramy* at Madras and suggested that overcorwding, pollution due to decaying matter and injuries on the fish are the contributing factors for the spread of the disease. The incidence of the disease has been observed in major

carps (Gopalakrishnan, 1963 and 1964). Dip treatment in 3% common salt solution or 1:2000 Potassium permanganate solution for roughly 5-10 minutes has been advocated by Hora and Pillai (1962). One minute dip treatment in 1:3000 Copper sulphate solution once daily for 3-4 days has been recommended by Gopalakrishnan (1963, 1964).

(2) Branchiomycosis is yet another serious disease due to infection by Branchiomyces sp. which often causes gill rot first reported by Plehn (1912). Branchiomycosis has been recorded several times in carps (Rehulka and Tesarcik, 1972). This disease was also noted in a number of finfishes in Italy (Grimaldi et al., 1973). Infections due to Branchiomyces sanguinis and B. demigrans were reported in a number of finfishes by Wolke (1975). Although Reichenbach Klinke (1969) has recommended the use of 1-2 gm of Copper sulphate per m^3 of water body, there is no other effective treatment of the disease (Richards, 1978). By observing strict hygiene, timely removal of infected fishes and avoiding overfeeding, the disease can be prevented.

In India, branchiomycosis has been reported to be common in cultivated fishes in ponds with large amount of

decaying matter (Hora and Pillai, 1962). Treatment, in the case of mild infections, by baths in 3-5% common salt solution for 5 to 10 minutes, has been suggested (Alikunhi, 1957).

(3) *Ichthyophonus* disease, infection due to *Ichthyophonus hoferi*, is a systemic granulomatosis and is detected in both fresh water and marine finfishes. Caullery and Mesnil (1905) named the aetiological agent as *Ichthyosporidium gasterophilum*. Pettit (1911) and Plehn and Mulsow (1911) identified the organism as *Ichthyophonus hoferi*. It often causes mass mortalities in the North Atlantic herring, *Clupea harengus harengus* (Wolke, 1975). Outbreaks of this disease in finfishes were recorded by McVicar and Mackenzie (1972) and Richards (1978). The latter author mentioned that the infection can be easily noticed by the 'sand paper' effect of the skin of the infected animals. This disease is also noticed in deep sea scabbard fish, *Aphanopus carbo* (Lowe) in North East Atlantic (Agius, 1978). In fish reared under controlled conditions, in the early case of infection, phenoxethol treatment is effective(Reichenbach Klinke and Elkan, 1965).

Other miscellaneous fungal diseases have also been reported:

1. Sparrow (1936) studied a case of fungal infection caused by a species of *Petersenia* on marine rotifer eggs.

2. Sproston (1947) documented a case of superficial infection by a fungal parasite in the mackerel.

3. Ermin (1952) reported a disease of fungal granuloma in *Aphanus chantrei* Gaillard.

4. *Penicillium piscium* (Reichenbach Klinke) in the internal organs of Carps; *Pullularia* sp. in the ray, *Trigon pastinacea* were also noticed (van Duijn, 1973).

5. *Candida albicans* was found to cause gill disease in Carps (Goreglyad and Vylegzhanin, 1975).

6. Serious infections due to *Phoma herbarum* among coho and chinook salmon fry; *Exophiala salmonis* in trouts; *Scolecobasidium humicola* in coho salmon were reported (Wolke, 1975).

7. Richards (1978) and Richards *et al.*, (1978) have reported *Dermocystidium* (*Labyrinthomyxa*) infection in chinook salmon and *Exophiala salmonis* infection in Atlantic salmon, *Salmo salar* respectively.

8. Blazer and Wolke (1979) have also reported *Exophiala* like fungus causing systemic mycosis in Cod, *Gadus morhua* L.

In India fungal diseases in finfishes were recorded by Bhargava *et al.*, (1971); Srivastava and Srivastava (1975); the candidate Pillai (1978) and Srivastava (1979).

Fungal diseases, in shellfishes too, were recorded:

1. Lightner (1977 g) has documented cases of fungal infections in *Penaeus* sp. due to *Lagenidium callinectes* and found malachite green effective in arresting the infection, if the drug is used before the infection is well established. To prevent the infection, spawning of gravid females in clean containers with 0.006 ppm malachite green and removal of infected organisms are imperative.

2. Lightner (1977 h) has also noted cases of *Fusarium solani* infections in the shrimps, *Penaeus duorarum*, *P. aztecus*, *P. californiensis*, *P. stylirostris* and *P. occidentalis*. Fungicides are effective in treating the disease and destruction of the infected animals at

the proper time will be of value in preventing the disease.

3. Sindermann (1977) found *Lagenidium callinectes* infection in the blue crab *Callinectes sapidus* and advocated the use of fungicides for treatment and destruction of the infected female crabs with eggs.

4. Armstrong and Fisher (1977) have noticed a fungus disease due to *Lagenidium* sp. infection in eggs and larvae of Dungenes crab, *Cancer magister* Dana and recommended avoidance of infected feeds and maintenance of strict cleanliness.

5. Mortalities due to infection with *Lagenidium* sp. in the larvae of the American lobster, *Homarus americanus* was documented by Nilson and Fisher (1977) who suggested malachite green treatment and observance of perfect cleanliness of culture systems.

6. Fisher and Nilson (1977) reported extensive loss due to mycosis as a result of infection with *Haliphthoros milfordensis* in the American lobster, *Homarus americanus* and the European lobster, *Homarus gammarus* recommending malachite green treatment and practice of hygienic measures to prevent the disease.

7. Sindermann (1977) has noticed a case of *Fusarium* infection in the American lobster, *Homarus americanus*. He has also reported larval mycosis of the oyster, *Crassostrea virginica* due to *Sirolpidium zoophthorum* resulting in mass mortality. Ultraviolet treatment of the water helps to prevent the disease.

8. Quick (1977) documented mycotic disease, due to *Labyrinthomyxa marinum* (earlier known by *Dermocystidium marinum*) infection, in American oyster, *Crassostrea virginica*, for which antifungal antibiotic treatment and planting the seeds at lower densities has been suggested as preventive measures.

9. *Dermocystidium marinum* causing oyster mortalities has also been reported by Ray (1954 a b c). According to McVicar and Wooten (1980) *Dermocystidium* is a protozoan parasite.

10. Sindermann (1977) has detected a case of *Labyrinthomyxa* infection, in American oyster, *Crassostrea virginica* denoted as Malpeque Bay disease. He has also noted a mycotic disease, as a result of an attack of *Sirolpidium zoophthorum* in hard clams *Mercenaria mercenaria*, resulting in severe loss. To prevent the

disease ultraviolet treatment of the water is suggested.

11. *Labyrinthomyxa* infection resulted in mass mortality of the mussel, *Mytilus edulis* (Li and Clyburne, 1979).

12. Gacutan and Baticados (1979) documented a mycotic disease due to *Lagenidium* affecting the nauplii to post larval stages of the prawn, *P. monodon*.

Viral diseases

Fish virology is still a virgin field and many of the pathogenic viruses are yet to be isolated and identified. Weissenberg (1914) who studied lymphocystis disease is the earliest researcher on virus diseases of finfishes. The important virus diseases of finfish are discussed below:

(1) *Channel cat fish virus* (CCV) disease is an important virus disease found mostly in America. It was first described by Fijan, Wellborn and Naftel (1970), as an acute case of infection in the channel cat fish, *Ictalurus punctatus* and its fingerlings. The aetiological agent of this disease is demonstrated to be a DNA virus. So far, only the channel cat fish has been found susceptible to this disease (Wellborn *et al.*, 1969).

Wolf and Darlington (1971) gave a detailed picture of this disease causing virus. Fish surviving the disease are suspected to be having latent infection (Liversidge and Munro, 1978). There is no treatment for the disease except the destruction of infected fish stock.

(2) Herpes virus disease, is a severe disease, noticed in Japan and the U.S.A., which causes significant losses in finfishes. No treatment is suggested, but the disease can be prevented by removing the infected fish (Wolf et al., 1975).

(3) Infectious pancreatic necrosis (IPN), an infection by a stable virus, causes diseases in fry, fingerlings and adult fishes and this disease has been observed mostly in the United States of America, France, Denmark, Sweden, Italy and United Kingdom. This disease is mostly noted in salmonid fishes and a detailed account of the disease causing agent is given by Yasutake (1975) and Liversidge and Munro (1978). There is no treatment for this disease, but it can be prevented by avoiding the aetiological agent and also by selective breeding (Wolf, 1966 a).

(4) Viral hemorrhagic septicemia (VHS) is also a serious disease affecting finfishes and resulting in mass

mortalities. The first report of this disease is that of Schaperclaus (1938). The disease is usually found in the rainbow trout and the disease outbreaks was recorded from European countries (Wolf, 1972 b). It is difficult to treat the diseased fishes. But the disease can be prevented by slaughtering the infected fishes, propagating the pathogen free fish and using the virus free water (Liversidge and Munro, 1978).

(5) Rhabdovirus disease, of Northern pike fry, is an acute case of virus infection causing severe mortality and is mostly noticed in Holland. Incidence of Rhabdovirus in diseased Northern pike fry was reported by Bootsma (1971); Bootsma and Vorstenbosch (1973); deKinkelin and Galimard (1973) and Wolf (1974). This pathogenic infection resulting in a state of disease condition known as hydrocephalus or red disease is also reported by Bootsma et al., (1975). The treatment of the diseased fry or fish is difficult (Liversidge and Munro, 1978).

(6) Lymphocystis, a highly infectious disease is world wide in distribution, causing non lethal infection, among fresh water as well as marine finfishes, resulting neoplastic like changes on various parts of the body.

It is a benign disease due to attack by lymphocystis disease virus which is considered to be the oldest known fish virus. The disease has been reported in plaice and flounders around the British Isles by Lowe (1874); M'Intosh (1885); Perkins *et al.*, (1972) and Shelton and Wilson (1973). This disease in three species of Sciaenids, *Cynoscion nothus*, *Cynoscion regalis* and *Stellifer lanceolatus* was recorded from Georgia coast (Smith, 1972). For the first time from the lakes in Africa, lymphocystis was recorded in Cichlid fishes (Paperna, 1973). A case of spontaneous lymphocystis has been reported in the silver perch, *Bairdiella chrysaura* from the gulf of Mexico (Lawler *et al.*, 1974). Dukes and Lawler (1975) have recorded ocular lesions, as a result of lymphocystis, in finfishes. It is difficult to treat the infected fishes, but the disease can be prevented by timely removal of the infected fish and also by disinfecting the eggs (Liversidge and Munro, 1978).

In India, this disease has been reported in *Anabas testudineus* (Thakur and Nasar, 1977), but no virological studies were carried out.

(7) *Infectious hematopoietic necrosis* (IHN) disease is due to infection by an IHN virus classified as

Rhabdovirus. Chinook and sock eye salmon and rainbow trout are found susceptible to the disease (Amend, 1974). An epizootic case in rainbow trout, *Salmo gairdneri*, due to infectious hematopoietic necrosis (IHN) virus at two hatcheries in Minnesota was detected by Plumb (1972). This case has also been noted occurring in the rainbow trout fingerlings, *Salmo gairdneri* during an epizootic case at a hatchery in Minnesota (Holway and Smith, 1973). Outbreaks of this disease were also reported from United States and Japan in salmonid fishes (Liversidge and Munro, 1978). There is no treatment for the infected fish. However, the disease can be prevented by avoiding the pathogen and maintaining good water supply.

(8) *Spring viraemia of carp* (SVC) The aetiology of the disease was a subject of dispute quite some time. However, Fijan *et al.*, (1971) isolated the aetiological viral agent as *Rhabdovirus carpio* by fulfilling Rivers' postulates. The disease is mostly noticed in Carps in European countries and the infection can occur at any age of the fish (Liversidge and Munro, 1978). There is no method of treatment, but the disease can be prevented by maintaining disease free stocks and observing strict hygienic measures.

Tumours both benign and malignant are another unresolved problem in the case of finfishes. The real importance of oncogenic viruses in neoplastic diseases in aquatic animals has now been realized and the subject was recently reviewed (Wolf, 1972 a; Mawdesley Thomas, 1972 a b). The role of viruses in the aetiology of such diseases as Burkitt's lymphoma, Marek's disease and lymphosarcoma in fish has already been established. There is absolutely no doubt that the viral aetiology of many other types of tumours may definitely be established in due course. The mode of infection of most of fish viruses is similar to that in other vertebrates (Wolf, 1972 a), but effective treatment for cure of the disease is still lacking.

1. Mortality due to *Herpesvirus scophthalmi* in turbot fish, *Scophthalmus maximus* was reported by Buchanan and Madeley (1978).

2. Papillomatosis (Cauliflower disease) in eels and Atlantic salmon, Pox disease in carps, Piscine erythrocytic necrosis in wide range of finfishes are documented by Liversidge and Munro (1978).

3. Lymphosarcoma in an Atlantic salmon, *Salmo salar* was recently reported by Roald and Hastein (1979).

4. McCain *et al.*, (1979) noted pseudobranchial tumours in Pacific Cod, *Gadus macrocephalus*, lymphocystis in the yellowfin sole, *Limanda aspera* and in the rock sole, *Lepidopsetta bilinecta* with epidermal papillomas.

5. Hepathomas in Atlantic tomcod, *Microgadus tomcod* was recorded by Smith *et al.*, (1979).

6. In the rainbow trout, *Salmo gairdneri*, a case of papillomatosis was reported by Roberts and Bullock (1979).

7. Two kinds of tumours - lymphocytic lymphoma and tubular eosinophilic adeno carcinoma were recorded by Haller and Roberts (1980) in *Tilapia spilurus*.

A few cases of tumours have also been reported from India. Incidence of hyperostosis has been recorded among sciaenids by Chabanaud (1926); in carangids by Gopinath (1951); in ribbon fishes by Bhatt and Murti (1960) and James (1960) and in bat fishes by Murty (1967). Different types of tumours in various parts of the body of the cat fish, *Tachysurus jella* (Day) were discussed by Selvaraj *et al.*, (1973) and incidence of osteoma in the marine cat fish, *Arius maculatus*, from the Porto Novo waters was reported by Lakshmanaperumalsamy *et al.*, (1976). Desai and Srivastava (1979) have reported gonadal tumour in *Catla catla* (Hamilton).

It will be interesting to find out in future whether such cases could be the result of viral infestation. Viral diseases in shellfishes are also documented:

1. Virus disease due to infection with *Baculovirus penaei* Couch in the Pink shrimp, *Penaeus duorarum* and the Brown shrimp, *P. aztecus* has been described by Lightner (1977 i).

2. HLV disease in the Blue crab, *Callinectes sapidus* due to Herpesvirus like agent has been reported by Johnson (1977 b). No treatment is possible. However, to prevent the disease disposal of infected crabs and use of lysol are said to be helpful.

3. Johnson (1977 c) has also detected RVL disease in the Blue crab due to infection with virus related to Reo virus. No effective treatment is known except removal of infected crabs and use of lysol.

4. Sindermann (1977) has documented a case of HLV disease as a result of infection with a herpes type virus resulting in mass mortality of the American oyster *Crassostrea virginica*. Diseased oysters are difficult to treat in natural waters, but the disease can be

prevented by selecting the stocks free of the pathogen.

5. A case of rickettsial infection in the marine shellfish, Carcinus mediterraneus was reported by Bonami and Pappalardo (1980).

Algae and Protozoa are other groups of organisms causing finfish and shellfish diseases, probably by serving as disease vectors. Conroy and Herman (1970) have proved that leeches serve as disease vectors by carrying pathogenic bacteria and spreading various diseases.

CHAPTER II

ISOLATION AND IDENTIFICATION

ISOLATION AND IDENTIFICATION

Isolation and identification of aetiological agent(s) of any disease is essential for diagnosing and treating disease(s). For every disease there must be a cause or a combination of causative factors (mixed aetiology) and detection of these factors is imperative for a rapid diagnosis and treatment of the disease(s) and also to take suitable timely prophylactic measures.

Microbial diseases are the predominant group of diseases among finfish and shellfishes. Obviously, identity of the disease causing microbe is absolutely essential to provide means for treating diseases and for preventing disease outbreak. Occurrence of infection and diseases are noticed not only in the culture system, but also in the natural environment. However, severity of the disease is left to the virulence of the pathogen. Mild cases of infections often escape attention and is detected when large scale mortality occurs among the animals and it is too late for any remedial measures to be effectively taken. Whether finfish or shellfish, early detection of infection may be difficult partly due to

variation in the refractive index between the water and air which results in changes in the visual properties of the objects. For example, disease spots in the body of these animals become unnoticed when they are removed from their habitat. Moreover, diseased fishes become incapaciated and easily fall prey to the predators. This may minimise or completely wipe out the infected stock even before the fish farmers realise it.

So, in large scale culture systems, it is essential that continuous monitoring be carried out to detect finfish and shellfish diseases at the very outset.

In the present investigation, diseased finfishes and shellfishes were mainly obtained from the culture systems at the Central Marine Fisheries Research Institute (CMFRI) Prawn Research Centre, Narakkal (Cochin); the CMFRI Mussel farm, Vizhinjam; the CMFRI Oyster farm, Tuticorin and the CMFRI Kovalam Research Centre, Madras. A few cases were also detected from among the natural populations of finfish from the inshore waters off Cochin and from the Vellayani Lake, near Trivandrum.

The diseased finfish and shellfishes under moribund conditions (except the case of tumour in *Arius jella*) were aseptically collected in suitable

containers which were clean and sterile. When needed, long sterile forceps were used for handling the materials to minimise damage and contamination. The collected samples were taken to the laboratory immediately.

To clearly pinpoint and confirm the microbial causes of the diseases, the Koch's postulates to be satisfied are:

1. The suspected organism should be associated regularly with all cases of the suspected disease and in logical relationship to the disease, its symptoms and lesions.

2. The suspected organism should be isolated in pure culture from the suspected case.

3. When such pure culture is inoculated into suitable animals, the disease should remanifest itself.

4. The same organism should be reisolated in pure culture from the inoculated animals.

The different species of diseased finfishes and shellfishes, analysed for investigating the causes of diseases, are listed in table 2.1.

TABLE 2.1

List of diseased finfishes and shellfishes studied by the candidate from India

Sl. No.	Scientific name	Popular name	Habitat
1.	*Anguilla bicolor bicolor*	Level finned eel	F
2.	*Arius jella*	Small eye cat fish	M
3.	*Clarias batrachus*	Teysmann's spotted cat fish	F/E
4.	*Chanos chanos*	Milk fish	F/E/M
5.	*Etroplus suratensis*	Pearl spot	F/E
6.	*Tilapia mossambica*	African mouth breeder	F/E
7.	*Sillago sihama*	Lady fish	E/M
8.	*Chirocentrus dorab*	Wolf herring	M
9.	*Pampus argenteus*	Silver pomfret	M
10.	*Mugil cephalus*	Grey mullet	F/E/M
11.	*Lates calcarifer*	Bekti	E/M
12.	*Lactarius lactarius*	White fish	M
13.	*Megalops cyprinoides*	Indian tarpon	E/M
14.	*Wallago attu*	Wallago	F/E
15.	*Penaeus indicus*	Indian white prawn	M
16.	*Penaeus monodon*	Giant tiger prawn	M
17.	*Metapenaeus monoceros*	Speckled shrimp	M
18.	*Panulirus homarus*	Spiny lobster	M

F = Fresh water E = Estuarine M = Marine

TABLE 2.1 (Contd.)

List of diseased finfishes and shellfishes studied by the candidate from India

Sl. No.	Scientific name	Popular name	Habitat
19.	*Perna indica*	Brown mussel	M
20.	*Crassostrea madrasensis*	Indian oyster	M
21.	*Scylla serrata*	Green crab	E

F = Fresh water E = Estuarine M = Marine

PRE STERILIZATION OF SURGERY CELL

During the present investigation, a large surgery cell in which the candidate could enter and carry out the work was used.

The cell was first rendered dust free and clean with a neat towel. The surgery cell was then disinfected with dettol followed by absolute alcohol by the swab method. The cell was then sealed and sterilized by switching on ultraviolet tube lights for a period of 60 minutes.

SURGERY

The diseased specimens were placed, in a clean and decontaminated dissection tray, from the container. The specimens found alive were killed by a hard blow on the head or by decapitation. The pathological samples from the external parts such as skin, fins, tail, eyes, gills and tumours were removed as follows:

External parts

(i) Skin scrapings:

Skin scrapings in the case of finfish and

shell scrapings from the shellfish (prawns) from the infected area were taken aseptically using sterile surgical blades with necessary help of the sterile scalpel, forceps and scissors.

(ii) Fins/tail/uropods/telson:

Infected fins/tail/uropods/telson were clipped off aseptically with the help of sterile surgical instruments.

(iii) Eyes:

The infected eye tissue was removed aseptically from the affected area.

(iv) Gills:

The operculum was lifted first by using sterile forceps and the infected gill filaments and tissues were cut off and transferred aseptically.

(v) Tumours:

The tumours were excised, with the help of sterile scissors, surgical blades and forceps and transferred aseptically.

Internal parts

Using sterile surgical instruments, the internal parts were removed as follows

(i) Kidney:

The ventral area of the specimen was sterilized first by carefully applying absolute alcohol and burning off the excess, after descaling the region. The body was then slit open mid ventrally from operculum to vent exposing the visceral organs. The kidney was aseptically removed without any contamination from other internal parts, with the help of surgical instruments. During the operation, the colour of the various internal organs and other changes if any were also noted.

(ii) Blood:

Blood was removed directly from the open heart by using a sterile one ml tuberculin syringe with a one inch needle of gauge size no. 22 or by cardiac puncture from the anaesthetised fish using a similar syringe. Sterile platinum wire was also used to transfer the blood.

(iii) Hepatopancreas:

By dissecting open the carapace, along the post rostral carina, the hepatopancreas was removed aseptically.

(iv) Mussel and oyster tissue:

By aseptically dissecting open the bivalves, the infected tissue was transferred.

All the pathological samples transferred were immediately placed in sterile Petri dishes. In the case of blood, the sample was removed into sterile glass tubes having sterile anticoagulant (EDTA) to avoid clotting.

For the sterility check, inside the surgery cell, open nutrient agar plates were also kept exposed inside the cell during surgery. The sterile nature of the surgery cell was confirmed by absence of microbial growth in the plates.

All the surgical instruments were either autoclaved or dipped in absolute alcohol and the excess burned off.

DILUTION OF THE PATHOLOGICAL SAMPLES

The majority of marine microorganisms require

sea water or its equivalent for their optimal activity (ZoBell, 1959, 1963). So, aged and filtered sterile sea water was used to dilute the marine pathological samples.

In the present investigation, procedures for the dilution of the samples as given by Bullock (1972, 1976) were adopted with slight modifications.

The pathological samples were ground in a clean and sterile 25 ml mortar using a sterile pestle inside the surgery cell, in 1-3 ml of the suitable suspension fluid. The properly ground and diluted samples were aseptically collected in convenient sterile test tubes for timely inoculation on to a suitable media.

MEDIA

The wet mount of the pathological sample was prepared and an examination of it under the microscope was made to select the suitable media for culturing bacteria or fungi (Bullock, 1971, 1976; van Duijn, 1973; Roberts and Shepherd, 1974). The pathological samples were cultured on appropriate media based on sea water or habitat water as the base. Fresh water with 0.5% Sodium chloride was also tried as the base.

In addition to culturing in solid media, samples were also cultured in the corresponding liquid media, sea water peptone or peptone water or in both according to the nature and source of the samples. The following media were used for culture:

1. Nutrient broth with sea water base (ZoBell and Morita, 1959)
2. Nutrient broth
3. Nutrient agar
4. Nutrient sea water agar
5. ZoBell's medium, 2216 (ZoBell, 1946)
6. Fish infusion agar
7. Tripticase soy agar
8. Blood agar
9. MacConkey's agar (Collins and Lyne, 1970)
10. Sabouraud's glucose agar
11. Leetown medium (Bullock, 1971)

Nutrient sea water agar

Peptone (Bacto)	1.00 gm
Ferric phosphate (BDH, AR)	0.01 ''
Beef extract (Oxoid)	0.30 ''

Nutrient sea water agar (Contd.)

Agar (Bacto)	1.50 gm
Aged and filtered sea water	100 ml

Fish infusion agar

Peptone (Bacto)	1.00 gm
Agar (Bacto)	1.50 ''
Fish infusion*	100 ml

*Preparation of fish infusion

Fresh fish muscle weighing 454 gm was mixed into one litre of water (aged and filtered sea water or habitat water). The minced fish muscle overnight stored in a refrigerator at about 4 °C and later boiling was done in a water bath for 30 minutes. The precipitated and coagulable proteins were removed by filtering through lint and finally using Whatman 1 filter paper.

Fish muscle consists of a high amount of protein, sugar and amino acids (Shewan, 1971). So, fish infusion medium is nutrionally rich and favours the growth of most forms of bacteria (Snieszko and Friddle, 1948; and Fiakas, 1950). This medium was found to be one of the best media giving luxurient growth.

All the media were in regular routine use except ZoBell's agar as the purpose of this medium was satisfied by the rest of the media. MacConkey's media did not give satisfactory growth. Tripticase soy agar, Leetown medium and Sabouraud's agar were used only when the need arose.

pH of the media and autoclaving

The available information regarding the pH of fresh fish muscle shows that it is approximately neutral in reaction. Usually the pH of the sea water is 7-8 (Wood, 1967). The maximum growth of marine microbes is in the pH range between 7-8 (ZoBell, 1946; ZoBell and Morita, 1959; Scholes and Shewan, 1964). Normally, high alkaline and acid ranges are not favourable for the growth of marine and terrestrial microbes respectively. Inorder to obtain the maximum counts and number of species, the pH of the media was adjusted to 7.2 to 7.5 unless and otherwise mentioned.

All the media were sterilized at 121 °C for 10 - 15 minutes, if not otherwise mentioned.

CULTURING OF THE SAMPLES

A 0.1 ml aliquot of the pathological sample, was taken with a sterile pipette and aseptically spread

over the surface of the media according to the modified methods of Bullock (1972). The time lag between capture of the fish and the commencement of culture of the samples was generally within one to two hours.

Incubation temperature

The most suitable temperature for the culture and cultivation of the microbes from the fish and shellfish depends on the ambient water temperature as the microbes associated with various types of fish whether pelagic or deep sea, will have corresponding varying optimum growth temperatures. However, the optimum growth temperature for marine microbes varies from 18 - 22 °C, for the development of the maximum plate counts (ZoBell and Conn, 1940). The growth of the marine forms at 37 °C is very poor because most of the marine bacteria are thermosensitive (ZoBell, 1963).

The surface temperature of the tropical waters in the open sea ranges generally between 28 - 30 °C and a little higher in certain areas of the littoral zone (ZoBell, 1959; Wood, 1967) and the same is the case with fresh water systems. Eventhough the temperature of the marine environment is relatively low, quite surprisingly

enough, many of the marine bacteria grow at 20 - 25 °C than at 12 - 15 °C (ZoBell, 1959, 1963). Inorder to isolate the pathogenic forms from the diseased specimen, from both marine and fresh water, the media were incubated at aerobic temperature, 28 ± 2 °C, for 24 to 72 hours.

The predominating bacteria represented by most numerous colonies similar in colonial characteristics were selected and considered as the causative agents (Bullock, 1971). Representatives of the isolated colonies, after studying the colonial morphology - size, shape, margin, elevation, consistency, opacity and pigment were transferred into sea water nutrient or nutrient broth and each isolate was restreaked to check for its purity. After ascertaining the purity of the isolates, they were streaked on to the corresponding fish infusion agar slants and stocked at 4 °C for future studies, since ZoBell and Upham (1944) observed that refrigerator temperatures favour the constancy of the species characteristics.

The organisms were sub cultured in fish infusion agar slants once for every 30 days.

Identification of microorganisms is useful and essential in many ways as they form the indicator organisms

in several respects (Kriss, 1963; Scholes and Shewan, 1964). In nature, many of the bacteria autothonus to the sea form the indigenous flora of the finfish and shellfishes. It is also important to take into consideration the fact that a few microbes of terrestrial origin also find a place and survive in the sea. Eventhough there are no imfallible criteria to differentiate marine bacteria from the terrestrial forms, it is an established fact that marine bacteria indigenous to the sea do exist, and it has been calculated that they form atleast 12% of the existing forms of the total bacteria (ZoBell, 1946, 1959 and 1963; Kriss, 1963; Scholes and Shewan, 1964).

It is a well established fact that most of the bacteria of marine fish are Gram negative rods (Shewan, 1962 and 1971; Scholes and Shewan, 1964) but, there are considerable difficulties in identifying the Gram negative rods other than the species belonging to the family Enterobacteriaceae (Hendrie et al., 1964; Scholes and Shewan, 1964; Buchanan and Gibbons, 1974).

To tentatively identify the isolates obtained in the present study, two systems of identification, a modified scheme of Simidu and Aiso (1962) and Buchanan

and Gibbons (1974), were used to identify the bacterial isolates, while Bessey (1961) was followed to identify the fungal strains.

CHARACTERS EXAMINED

Unless specially mentioned, all the tests were carried out in freshly prepared media, inculated at room temperature 28 ± 2 °C for 24 hours to 14 days.

<u>The following characters were studied</u>

1. Colonial morphology on fish infusion agar.
2. Cell morphology and Gram's staining of the isolates from fish infusion agar according to Hucker's modifications (Bullock, 1971).
3. Motility in hanging drop preparations from cultures following incubation for 6 - 18 hours in peptone water or sea water peptone (Bacto peptone, 1% in aged and filtered sea water pH 7.2 - 7.5).
4. Indole production in tryptone water.
5. Methyl Red and Voges Proskauer reactions (Mackie and McCartney, 1962; Cruickshank et al., 1975).

6. Utilization of citrate in Koser's citrate medium.

7. Nitrate reduction in peptone water containing 0.2% Potassium nitrate, and testing at 24 - 72 hour culture with Dimethyl alpha naphthyl amine sulphanilic acid reagent. Zinc dust was added to the cultures showing negative reactions, to detect if nitrate remained and decomposition had not proceeded beyond the nitrite stage.

8. Ammonia from peptone in peptone water cultures aged 18 - 22 hr with Nessler's reagent.

9. Hydrogen sulphide production in nutrient broth with lead acetate papers suspended over the medium.

10. Gelatin liquefaction (Collins and Lyne, 1970).

11. Starch hydrolysis in starch media using Lugol's iodine as an indicator.

12. Catalase by emulsifying an 18 - 24 hr culture, from fish infusion agar, with a few drops of 20 vol Hydrogen peroxide.

13. Oxidase according to Kovacs (1956).

14. Urease by inoculating the organism in Christensen's agar (Mackie and McCartney, 1962).

15. Metabolism of glucose (Hugh and Leifson, 1953).

16. Reaction in litmus milk (Mackie and McCartney, 1962).

17. Haemolysis in blood agar (cooled sterile nutrient agar plus 5% sterile defibrinated blood).

18. Casein hydrolysis and pigmentation observed in milk agar.

19. Lypolytic activity in plates of tributyrin agar.

20. Sensitivity to antibiotics according to Shewan _et al._, (1954). The vibriostatic compound, 'O/129' (2,4 Diamino 6,7 diisopropyl pteridine) was also tested by using filter paper discs.

21. Luminescence in Yeast peptone medium according to Hendrie _et al._, (1970).

22. Peptone water including 1% of the respective

sugars with Andrade's indicator to observe the production of acid and gas.

Each organism was studied individually for its characteristics and results are presented in tables 2.2 - 2.9.

The cases are recorded according to the clinical symptoms noted at the time of documentation of diseases.

RESULTS AND DISCUSSION

In total, 135 strains were isolated and identified to the species level and they belong to the genera, *Alcaligenes*, *Bacillus*, *Escherichia*, *Flavobacterium*, *Klebsiella*, *Micrococcus*, *Photobacterium*, *Proteus*, *Pseudomonas*, *Staphylococcus*, *Streptococcus* and *Vibrio*. The fungal isolates were classified as *Penicillium* sp.

Table 2.2

The strains Sl 1,2,3,4,5,6 and 7 isolated from skin lesion were identified as *Pseudomonas fluorescens* as they were catalase and oxidase positive, produced alkalinity in Hugh and Leifson's medium, insensitive to penicillin and the compound 'O/129', unable to ferment most of the sugars tested and produced fluorescent

TABLE 2.2 (a)

Gross Morphological Characters

Species	Disease	Isolate(s)	Shape, size, Gram reaction	Motility
Chanos chanos (Pl.1: fig. 1); *Etroplus suratensis* (Pl.2: fig. 2); *Mugil cephalus*; *Pampus argenteus*	Skin lesion	SL 1,2,3; SL 4; SL 5,6; SL 7	Rods: 0.6-1.2 x 1.2 - 3.7 μ, with rounded ends, occurring singly. Gm$^-$	Motile
Etroplus suratensis (Pl.3: fig. 3)	Skin lesion	SL 8	Curved rods: 0.6-1.0 x 1.2 - 3.7 μ, with rounded ends, occurring singly. Gm$^-$	Motile
Etroplus suratensis (Pl.4: fig. 4)	Skin spottiness	SP 1	Rods: 0.7-1.2 x 1.2 - 2.5 μ, with rounded ends, occurring singly. Gm$^-$	Motile

TABLE 2.2 (b)

Cultural Characters

Isolate no(s)	Fish infusion agar	Peptone water	Sea water peptone	Pigmentation on milk agar
SL 1,2,3; SL 4; SL 5,6; SL 7	Circular, 2-3 mm diameter, entire margin, convex, opaque, butyrous, light orange with bluish fluorescence.	No pellicle, good growth with uniform turbidity. Soluble pigment, powdery deposit observed.	Pellicle present, good growth with uniform turbidity. Soluble pigment, powdery deposit noted.	Light orange
SL 8	Circular, 3 mm diameter, entire margin, convex, translucent, butyrous, light yellow.	No pellicle, moderate growth with uniform turbidity. Soluble pigment.	No pellicle, good growth with uniform turbidity. Soluble pigment.	Light yellow
SP 1	Circular, 2 mm diameter, entire margin, convex, opaque, butyrous, white.	No pellicle, good growth with uniform turbidity.	No pellicle, good growth with uniform turbidity.	White

TABLE 2.2 (c)

Gross Physiological Characters

Isolate no(s)	Indole	Methyl red	Voges Proskauer	Citrate utilization	Nitrate to nitrate	NH_3 from peptone	H_2S production	Gelatin liquefaction	Starch hydrolysis	Catalase	Oxidase (Kovacs)	Urease	Reaction in Hugh and Leifson's medium	Litmus milk	Haemolysis in blood agar	Casein hydrolysis	Lipolytic activity	Penicillin (2.5 i.u.)	O/129	Luminescence
SL 1,2,3;	-	-	-	+	+	+	-	+	+	+	+	-	ALK	A+P	A	+	-	-	-	-
SL 4;																				
SL 5,6;																				
SL 7																				
SL 8	+	-	-	-	+	-	-	+	+	+	+	-	F	A+P	A	+	-	-	+	-
SP 1	+	+	-	-	+	+	-	+	+	+	+	-	F	-	-	+	-	-	+	+

+ = Positive reaction   - = No reaction   ALK = Alkaline

A+P = Acid and peptonisation   F = Fermentative   A = Alpha

O/129 = A vibriostatic compound: 2,4 Diamino 6,7 diisopropyl pteridine

TABLE 2.2 (d)

Fermentative Characters

Isolate no(s)	Arabinose	Galactose	Glucose	Glycerol	Lactose	Maltose	Mannitol	Sucrose	Xylose	Identity
SL 1,2,3; SL 4; SL 5,6; SL 7	+	-	-	-	-	-	-	-	-	*Pseudomonas fluorescens*
SL 8	+	+	+	+	-	+	+	+	-	*Vibrio anguillarum*
SP 1	-	-	+	-	-	-	-	+	-	*Vibrio fischeri*

+ = Acid

- = No acid

TABLE 2.3 (a)

Gross Morphological Characters

Species	Disease	Isolate(s)	Shape, size, Gram reaction	Motility
Metapenaeus monoceros; *Penaeus indicus* (Pl.5: fig. 5);	Shell disease	SD 1,2; SD 3,4,5, 6,7,8;	Cocci: 0.6–1.2 μ, occurring singly, in pairs and groups. Gm^+	Non motile
Penaeus monodon		SD 9,10,11, 12,13,14;		
Scylla serrata		SD 15		
Chanos chanos;	Hemorrhagic septicemia	HS 1,2,3, 4,5;	Rods: 0.6–1.2 x 1.2 – 5.0μ, with rounded ends, occurring singly and in pairs. Gm^-	Motile
Etroplus suratensis (Pl.6: fig.6);		HS 6,7,8, 9,10;		
Mugil cephalus		HS 11,12,13, 14,15		
Chanos chanos; *Etroplus suratensis* (Pl.7: fig.7); *Tilapia mossambica*	Eye disease	ED 1,2; ED 3,4,5,6,7; ED 8,9,10,11	Cocci: 0.6–1.2 μ, occurring singly and in groups. Gm^+	Non motile

TABLE 2.3 (b)

Cultural Characters

Isolate no(s)	Fish infusion agar	Peptone water	Sea water peptone	Pigmentation on milk agar
SD 1,2; SD 3,4,5, 6,7,8; SD 9,10,11, 12,13,14; SD 15	Circular, 2 mm diameter, entire margin, convex, opaque, butyrous, golden yellow.	No pellicle, good growth with uniform turbidity.	No pellicle, good growth with uniform turbidity.	Golden yellow
HS 1,2,3,4,5; HS 6,7,8,9,10; HS 11,12,13, 14,15	Circular, 2-3 mm diameter, entire margin, convex, opaque, butyrous, blue green.	Thin pellicle present, good growth with uniform turbidity. Slight deposit with bluish green fluorescence.	Thin pellicle present, good growth with uniform turbidity. Powdery deposit with bluish green fluorescence.	Light blue
ED 1,2; ED 3,4,5,6,7; ED 8,9,10,11	Punctiform, 1 mm diameter, entire margin, convex, opaque, butyrous, light yellow.	No pellicle, good growth with uniform turbidity. Soluble pigment.	No pellicle, moderate growth with uniform turbidity. Soluble pigment.	Yellow

TABLE 2.3 (c)

Gross Physiological Characters

Isolate no(s)	Indole	Methyl red	Voges Proskauer	Citrate utilization	Nitrate to nitrate	NH_3 from peptone	H_2S production	Gelatin liquefaction	Starch hydrolysis	Catalase	Oxidase (Kovacs)	Urease	Reaction in Hugh and Leifson's medium	Litmus milk	Haemolysis in blood agar	Casein hydrolysis	Lipolytic activity	Penicillin (2.5 i.u.)	O/129	Luminescence
SD 1,2;	+	-	-	-	+	+	+	-	+	+	+	-	F	D	B	+	+	-	-	-
SD 3,4,5,6,7,8;																				
SD 9,10,11,12,13,14;																				
SD 15																				
HS 1,2,3,4,5;	+	-	-	+	+	+	-	+	-	+	+	-	O	D	B	+	-	-	-	-
HS 6,7,8,9,10;																				
HS 11,12,13,14,15																				
ED 1,2;	-	-	-	-	+	-	-	+	-	+	+	-	O	D	-	+	-	-	-	-
ED 3,4,5,6,7;																				
ED 8,9,10,11																				

+ = Positive reaction   - = No reaction   F = Fermentative

D = Decolourised   B = Beta   O = Oxidative

O/129 = A vibriostatic compound: 2,4 Diamino 6,7 diisopropyl pteridine

TABLE 2.3 (d)

Fermentative Characters

Isolate no(s)	Arabinose	Galactose	Glucose	Glycerol	Lactose	Maltose	Mannitol	Sucrose	Xylose	Identity
SD 1,2; SD 3,4,5, 6,7,8; SD 9,10,11; 12,13,14; SD 15	+	+	+	−	−	+	+	+	−	*Staphylococcus aureus*
HS 1,2,3, 4,5; HS 6,7,8, 9,10; HS 11,12,13, 14,15	+	+	+	+	−	+	+	−	+	*Pseudomonas aeruginosa*
ED 1,2; ED 3,4,5, 6,7; ED 8,9,10,11	−	−	+	−	−	+	−	+	+	*Micrococcus varians*

\+ = Acid

− = No acid

TABLE 2.4 (a)

Gross Morphological Characters

Species	Disease	Isolate(s)	Shape, size, Gram reaction	Motility
Etroplus suratensis (Pl.8: fig. 8); *Tilapia mossambica* (Pl.9: fig. 9)	Gill rot	GR 1,2,3; GR 4,5	Rods: 0.6–1.2 x 1.2 – 3.7μ, with rounded ends, occurring singly. Gm^-	Non motile
Mugil cephalus (Pl.10: fig. 10)	Black spot disease	BS 1,2,3	Rods: 0.6–1.2 x 1.2 – 3.7μ, with rounded ends, occurring singly. Gm^-	Non motile
Penaeus indicus (Pl.11: fig. 11); *Penaeus monodon*	Black spot disease	BS 4,5,6,7,8; BS 9,10,11,12,13	Rods: 0.6–1.2 x 1.0 – 4.0μ, with rounded ends, occurring singly. Gm^-	Motile

TABLE 2.4 (b)

Cultural Characters

Isolate no(s)	Fish infusion agar	Peptone water	Sea water peptone	Pigmentation on milk agar
GR 1,2,3; GR 4,5	Circular, 3 mm diameter, entire margin, convex, opaque, butyrous, off white.	No pellicle, good growth with uniform turbidity.	No pellicle, good growth with uniform turbidity.	Off white
BS 1,2,3	Circular, 3 mm diameter, entire margin, concave, opaque, butyrous, off white.	No pellicle, good growth with uniform turbidity. Powdery deposit noted.	No pellicle, good growth with uniform turbidity. Powdery deposit observed.	Off white
BS 4,5,6,7,8; BS 9,10,11,12,13	Circular, 2-3 mm diameter, convex, opaque, butyrous, yellow.	No pellicle, good growth with uniform turbidity. Soluble pigment.	No pellicle, good growth with uniform turbidity. Soluble pigment.	Yellow

TABLE 2.4 (c)

Gross Physiological Characters

Isolate no(s)	Indole	Methyl red	Voges Proskauer	Citrate utilization	Nitrate to nitrite	NH_3 from peptone	H_2S production	Gelatin liquefaction	Starch hydrolysis	Catalase	Oxidase (Kovacs)	Urease	Reaction in Hugh and Leifson's medium	Litmus milk	Haemolysis in blood agar	Casein hydrolysis	Lipolytic activity	Penicillin (2.5 i.u.)	O/129	Luminescence
GR 1,2,3; GR 4,5	+	-	+	+	+	-	-	-	-	-	-	-	F	D	B	-	-	-	-	-
BS 1,2,3	+	-	-	-	+	-	-	+	-	+	+	-	O	-	-	+	-	+	-	-
BS 4,5,6,7,8; BS 9,10,11,12,13	+	+	-	-	+	-	-	+	+	+	+	-	F	-	-	+	-	-	+	-

+ = Positive reaction   - = No reaction   F = Fermentative

D = Decolourised   B = Beta   O = Oxidative

O/129 = A vibriostatic compound: 2,4 Diamino 6,7 diisopropyl pteridine

TABLE 2.4 (d)

Fermentative Characters

Isolate no(s)	Arabinose	Galactose	Glucose	Glycerol	Lactose	Maltose	Mannitol	Sucrose	Xylose	Identity
GR 1,2,3; GR 4,5	++	++	++	++	++	++	++	++	++	*Klebsiella pneumoniae*
BS 1,2,3	+	-	+	+	+	+	+	+	-	*Alcaligenes eutrophus*
BS 4,5,6, 7,8; BS 9,10,11, 12,13	+	+	+	+	-	+	-	-	-	*Vibrio fischeri*

++ = Acid and gas

\+ = Acid

\- = No acid

TABLE 2.5 (a)

Gross Morphological Characters

Species	Disease	Isolate(s)	Shape, size, Gram reaction	Motility
Mugil cephalus	Black spot disease	BS 14	Rods: 0.6–1.2 x 1.2 – 5.0μ, with rounded ends, occurring singly and in groups. Gm^{+} Spores 0.6–0.8μ, oval	Motile
Crassostrea madrasensis	Muscle necrosis	MN 1;	Rods: 0.6–1.0 x 1.2 – 3.7μ, with rounded ends, occurring singly and in groups. Gm^{-}	Motile
Penaeus indicus (Pl.12: fig. 12);		MN 2,3,4, 5,6;		
Perna indica		MN 7		

TABLE 2.5 (b)

Cultural Characters

Isolate no(s)	Fish infusion agar	Peptone water	Sea water peptone	Pigmentation on milk agar
BS 14	Circular, 3–4 mm diameter, wavy margin, flat, opaque, butyrous, off white.	No pellicle, good growth with uniform turbidity.	No pellicle, good growth with uniform turbidity.	Off white
MN 1; MN 2,3,4,5,6; MN 7	Circular, 3 mm diameter, entire margin, convex, opaque, butyrous, yellow.	No pellicle, good growth with uniform turbidity. Soluble pigment.	No pellicle, good growth with uniform turbidity. Soluble pigment.	Yellow

TABLE 2.5 (c)

Gross Physiological Characters

Isolate no(s)	Indole	Methyl red	Voges Proskauer	Citrate utilization	Nitrate to nitrite	NH_3 from peptone	H_2S production	Gelatin liquefaction	Starch hydrolysis	Catalase	Oxidase (Kovacs)	Urease	Reaction in Hugh and Leifson's medium	Litmus milk	Haemolysis in blood agar	Casein hydrolysis	Lipolytic activity	Penicillin (2.5 i.u.)	O/129	Luminescence
BS 14	+	−	−	−	+	−	−	+	+	+	+	−	O	D	−	−	+	−	−	−
MN 1, MN 2,3,4,5,6; MN 7	+	−	−	+	+	+	−	+	+	+	+	−	F	D	−	+	−	−	+	−

+ = Positive reaction   − = No reaction

O = Oxidative   D = Decolourised

F = Fermentative

O/129 = A vibriostatic compound: 2,4 Diamino 6,7 diisopropyl pteridine

TABLE 2.5 (d)

Fermentative Characters

Isolate no(s)	Arabinose	Galactose	Glucose	Glycerol	Lactose	Maltose	Mannitol	Sucrose	Xylose	Identity
BS 14	-	-	+	-	-	+	-	+	-	Bacillus thuringiensis
MN 1; MN 2,3,4, 5,6; MN 7	-	+	+	+	-	+	+	-	-	Vibrio fischeri

+ = Acid

- = No acid

TABLE 2.6 (a)

Gross Morphological Characters

Species	Disease	Isolate(s)	Shape, size, Gram reaction	Motility
Etroplus suratensis (Pl.13: fig.13);	Enteritis	E 1,2,3	Rods: 0.6–1.2 x 1.2 – 3.5μ, with rounded ends, occurring singly. Gm^-	Motile
Arius jella (Pl.14: fig.14)	Proliferative epithelial tumour	PET 1,2	Rods: 0.6–1.2 x 1.2 – 3.8μ, with rounded ends, occurring singly and in pairs. Gm^-	Motile
Panulirus homarus (Pl.15: fig.15)	Streptococcosis	S 1,2	Cocci: 0.6–1.0μ, occurring singly and in pairs. Gm^+	Non motile

TABLE 2.6 (b)

Cultural Characters

Isolate no(s)	Fish infusion agar	Peptone water	Sea water peptone	Pigmentation on milk agar
E 1,2,3	Circular, 2-3 mm diameter, convex, entire margin, opaque, butyrous, off white.	No pellicle, good growth with uniform turbidity. Powdery deposit observed.	No pellicle, good growth with uniform turbidity. Powdery deposit observed.	Off white
PET 1,2	Circular, 3 mm diameter, convex, opaque, butyrous, light yellow.	Pellicle present, good growth with uniform turbidity.	Pellicle present, good growth with uniform turbidity.	Light yellow
S 1,2	Punctiform, 0.2-0.5 mm diameter, convex, entire margin, opaque, butyrous, off white.	No pellicle, moderate growth with uniform turbidity.	No pellicle, moderate growth with uniform turbidity.	Off white

TABLE 2.6 (c)

Gross Physiological Characters

Isolate no(s)	Indole	Methyl red	Voges Proskauer	Citrate utilization	Nitrate to nitrite	NH_3 from peptone	H_2S production	Gelatin liquefaction	Starch hydrolysis	Catalase	Oxidase (Kovacs)	Urease	Reaction in Hugh and Leifson's medium	Litmus milk	Haemolysis in blood agar	Casein hydrolysis	Lipolytic activity	Penicillin (2.5 i.u.)	O/129	Luminescence
E 1,2,3	+	+	−	−	+	−	−	+	−	+	+	−	F	A+P	B	−	−	−	−	−
PET 1,2	−	−	−	+	+	+	+	−	−	+	+	−	ALK	A+P	A	+	−	−	−	−
S 1,2	−	−	−	−	+	−	+	−	−	+	+	−	F	D	B	−	−	−	−	−

+ = Positive reaction   − = No reaction   ALK = Alkaline

A+P = Acid and peptonisation   F = Fermentative   A = Alpha

B = Beta   D = Decolourised

O/129 = A vibriostatic compound: 2,4 Diamino 6,7 diisopropyl pteridine

TABLE 2.6 (d)

Fermentative Characters

Isolate no(s)	Arabinose	Galactose	Glucose	Glycerol	Lactose	Maltose	Mannitol	Sucrose	Xylose	Identity
E 1,2,3	++	++	++	-	++	++	++	+	++	*Escherichia coli*
PET 1,2	+	+	-	+	-	-	-	-	+	*Pseudomonas mendocina*
S 1,2	-	-	+	-	-	-	-	-	-	*Streptococcus pyogenes*

++ = Acid and gas　　+ = Acid

\- = No acid

TABLE 2.7 (a)

Gross Morphological Characters

Species	Disease	Isolate(s)	Shape, size, Gram reaction	Motility
Chanos chanos; *Etroplus suratensis* (Pl.16: fig. 16) *Lates calcerifer*; *Megalops cyprinoides*	Tail rot	TR 1,2; TR 3,4,5,6,7; TR 8,9; TR 10,11	Rods: 0.6-1.0 x 1.2 - 3.7μ, with rounded ends, occurring singly and in groups. Gm^-	Motile
Clarias batrachus; *Sillago sihama*; *Wallago attu*	Tail rot	TR 12; TR 13,14; TR 15	Rods: 0.6-1.2 x 1.2 - 4.2μ, with rounded ends, occurring singly, Gm^-	Motile
Etroplus suratensis; *Tilapia mossambica*	Tail rot	TR 16,17,18; TR 19,20	Rods: 0.6-1.2 x 1.2 - 3.7μ, with rounded ends, occurring singly and in pairs. Gm^-	Motile

TABLE 2.7 (b)

Cultural Characters

Isolate no(s)	Fish infusion agar	Peptone water	Sea water peptone	Pigmentation on milk agar
TR 1,2; TR 3,4,5,6,7; TR 8,9; TR 10,11	Circular, 3 mm diameter, entire margin, convex, opaque, butyrous, shiny white.	No pellicle, good growth with uniform turbidity.	No pellicle, good growth with uniform turbidity.	White
TR 12; TR 13,14; TR 15	Circular, 2 mm diameter, entire margin, convex, opaque, butyrous, yellow.	No pellicle good growth with uniform turbidity. Soluble pigment.	No pellicle, good growth with uniform turbidity. Soluble pigment.	Yellow
TR 16,17,18; TR 19,20	Irregular, 3-4 mm diameter, wavy margin, flat, opaque, butyrous, off white.	No pellicle, good growth with uniform turbidity.	No pellicle, good growth with uniform turbidity.	Off white

TABLE 2.7 (c)

Gross physiological Characters

Isolate no(s)	Indole	Methyl red	Voges Proskauer	Citrate utilization	Nitrate to nitrite	NH_3 from peptone	H_2S production	Gelatin liquefaction	Starch hydrolysis	Catalase	Oxidase (Kovacs)	Urease	Reaction in Hugh and Leifson's medium	Litmus milk	Haemolysis in blood agar	Casein hydrolysis	Lipolytic activity	Penicillin (2.5 i.u.)	O/129	Luminescence
TR 1,2; TR 3,4,5,6,7; TR 8,9; TR 10,11	+	+	-	+	+	-	-	+	+	+	-	-	F	-	-	+	-	-	+	+
TR 12; TR 13,14; TR 15	-	+	-	+	+	-	-	+	+	+	+	-	F	D	-	+	-	-	+	-
TR 16,17,18; TR 19,20	+	-	-	+	+	-	-	+	+	+	+	+	F	D	A	+	-	-	-	-

+ = Positive reaction   - = No reaction

F = Fermentative   D = Decolourised

A = Alpha

O/129 = A vibriostatic compound: 2,4 Diamino 6,7 diisopropyl pteridine

TABLE 2.7 (d)

Fermentative Characters

Isolate no(s)	Arabinose	Galactose	Glucose	Glycerol	Lactose	Maltose	Mannitol	Sucrose	Xylose	Identity
TR 1,2; TR 3,4,5, 6,7; TR 8,9; TR 10,11	+	+	++	+	–	+	–	–	–	<u>Photobacterium phosphoreum</u>
TR 12; TR 13,14; TR 15	–	+	+	+	–	+	+	–	–	<u>Vibrio fischeri</u>
TR 16,17, 18; TR 19,20	+	++	++	+	+	+	+	+	+	<u>Proteus vulgaris</u>

++ = Acid and gas + = Acid – = No acid

TABLE 2.8 (a)

Gross Morphological Characters

Species	Disease	Isolate(s)	Shape, size, Gram reaction	Motility
Chirocentrus dorab; *Lactarius lactarius*; *Penaeus monodon* (Pl.17: fig.17)	Tail rot	TR 21, 22; TR 23; TR 24,25,26,27,28	Rods: 0.6–1.0 x 1.2 – 2.5μ, with rounded ends, occurring singly. Gm⁻	Motile
Chanos chanos; *Etroplus suratensis*; *Tilapia mossambica*	Fin rot	FR 1; FR 2; FR 3,4	Rods: 0.6–1.0 x 1.0 – 3.7μ, with rounded ends, occurring singly. Gm⁻	Motile

TABLE 2.8 (b)

Cultural Characters

Isolate no(s)	Fish infusion agar	Peptone water	Sea water peptone	Pigmentation on milk agar
TR 21,22; TR 23; TR,24,25,26,27,28	Circular, 3 mm diameter, wavy margin, flat, opaque, butyrous, light yellow	No pellicle, moderate growth with uniform turbidity.	Thin pellicle present, good growth with uniform turbidity.	Light yellow
FR 1; FR 2; FR 3,4	Circular, 2 mm diameter, entire margin, convex, opaque, butyrous, off white.	Pellicle present, good growth with uniform turbidity.	Pellicle present, good growth with uniform turbidity.	Off white

TABLE 2.8 (c)

Gross Physiological Characters

Isolate no (s)	Indole	Methyl red	Voges Proskauer	Citrate utilization	Nitrate to nitrite	NH_3 from peptone	H_2S production	Gelatin liquefaction	Starch hydrolysis	Catalase	Oxidase (Kovacs)	Urease	Reaction in Hugh and Leifson(s medium	Litmus milk	Haemolysis in blood agar	Casein hydrolysis	Lipolytic activity	Penicillin (2.5 i.u.)	O/129	Luminescence
TR 21,22; TR 23; TR 24,25,26,27,28	+	-	-	-	+	+	-	+	-	+	+	-	O	D	-	+	-	-	-	-
FR 1; FR 2; FR 3,4	-	-	-	+	+	+	+	-	+	+	+	-	ALK	A+P	A	-	-	-	-	-

+ = Positive reaction - = No reaction O = Oxidative

D = Decolourised ALK = Alkaline A+P = Acid and peptonisation

A = Alpha

O/129 = A vibriostatic compound: 2,4 Diamino 6,7 diisopropyl pteridine

TABLE 2.8 (d)

Fermentative Characters

Isolate no(s)	Arabinose	Galactose	Glucose	Glycerol	Lactose	Maltose	Mannitol	Sucrose	Xylose	Identity
TR 21,22; TR 23; TR 24,25, 26,27, 28	-	-	+	-	-	+	-	+	-	*Flavobacterium uliginosum*
FR 1; FR 2; FR 3,4	+	-	-	-	-	-	-	-	+	*Pseudomonas stutzeri*

+ = Acid   - = No acid

TABLE 2.9

Gross details of the fungal strains

Species	Disease	Isolate no(s)	Cultural characters in Sabouraud's agar	Microscopic characters of wet mount observed under high power and stained under Lactophenol cotton blue	Identity
Anguilla bicolor bicolor (Pl.18: fig.18)	Dermatomycosis	DM 1,2,3, 4,5,6, 7,8,9, 10	Rapid growth producing cottony colonies. Colour white at first which turns to pink later.	Produced abundant globose conidial spores in chains at the tip of sterigmata. Characteristic appearance of *Penicillium* (Brush like) noted. Found conidial heads mostly at the tip of hyphae.	*Penicillium* sp.
Penaeus indicus (larvae)	Deep mycosis	DEM 1,2, 3,4, 5,6, 7,8, 9,10	Rapid growth producing cottony colonies. Colour white at first which turns to dark green later.	Produced abundant globose conidial spores in chains at the tip of sterigmata. Characteristic appearance of *Penicillium* (Brush like) was noted. Observed conidial heads at various centers in the hyphae.	*Penicillium* sp.

pigment. Although *Pseudomonas fluorescens* is found to be pathogenic (Bullock, 1971) in the present study, it could not induce the disease.

The isolate, SL 8 was identified as *Vibrio anguillarum* for the reasons it was oxidase positive but citrate negative, fermentative in Hugh and Leifson's medium, insensitive to penicillin but sensitive to '0/129' and haemolytic in blood agar. Produced acid from all the sugars under test except lactose and xylose. This organism has been recorded as a pathogen both in marine and fresh water teleosts (Richards and Roberts, 1978). In the present study, the organism was noticed to be pathogenic provoking the disease in the Pearl spot, *Etroplus suratensis*.

The organism SP 1 was identified as *Vibrio fischeri* as it did not haemolyse the blood, but hydrolysed starch. Sensitive to the compound '0/129' while it was insensitive to penicillin. It also satisfied the basic characteristics of the genus, *Vibrio*. This species also could not induce the disease.

Table 2.3

The isolates SD 1 to SD 15 were screened as *Staphylococcus aureas* for the reasons for their cellular

nature, golden yellow pigment, fermentative in Hugh and Leifson's medium, haemolytic in blood agar, coagulase positive (not referred in the table) and produced acid from arabinase, galactose, maltose, mannitol and sucrose. Staphylococcal diseases have been reported in plaice and haddock by Bullock *et al.*, (1971). In the present study the organism has been found pathogenic in prawns, *Peneeus indicus* and *Penaeus monodon*.

The strains HS 1 to HS 15 were identified as *Pseudomonas aeruginosa* for the reasons they were oxidative in Hugh and Leifson's medium, catalase and oxidase positive, insensitive to penicillin and 'O/129', liquefied gelatin but not hydrolysed starch and produced the characteristic fluorescent diffusible blue green pigment. That species of *Pseudomonas* are responsible for hemorrhagic septicemia has been well documented (Bullock *et al.*, 1971; van Duijn, 1973; Snieszko and Bullock, 1974; and the candidate Pillai, 1978). In the present study, the organism was found to induce the disease in the Cichlid fish, *Etroplus suratensis*.

The isolates ED 1 to ED 11 were screened as *Micrococcus varians* as they were positive for catalase and oxidase, oxidative in Hugh and Leifson's medium,

insensitive to penicillin and 'O/129' and being non motile and yellow pigmented. It could not cause to any disease.

Table 2.4

The organisms GR 1 to GR 5 were classified as *Klebsiella pneumoniae* as they were catalase positive but oxidase negative, MR negative but VP positive, fermentative in Hugh and Leifson's medium, insensitive to penicillin and 'O/129', haemolytic in blood agar and produced acid and gas in all the sugars under test. The organism is an associate of various pathological conditions (Buchanan and Gibbons, 1974). In the present study, they were found to be pathogenic in *Etroplus suratensis*.

The strains BS 1 to BS 3 were identified as *Alcaligenes eutrophus* as they were oxidative in Hugh and Leifson's medium, sensitive to penicillin but insensitive to 'O/129', catalase and oxidase positive, non pigmented and produced acid in most of the sugars tested. The organism could not provoke the disease.

The isolates BS 4 to BS 13 were identified as *Vibrio fischeri* for the reasons they were nonhaemolytic

and hydrolysed starch satisfying the basic characteristics of the genes, *Vibrio*. The agents could not cause any disease.

Table 2.5

The strain BS 14 was identified as *Bacillus thuringiensis* as it was motile, Gram positive, oxidative in Hugh and Leifson's medium, insensitive to penicillin and 'O/129'. The isolative could not initiate the disease.

The agents MN 1 to MN 7 were classified as *Vibrio fischeri* as they were nonhaemolytic and hydrolysed starch satisfying the basic characteristics of the genus, *Vibrio*. These agents differed in the fermentative characteristics when compared to that of BS 4 to BS 13. *Vibrio fischeri* proved to be non pathogenic.

Table 2.6

The strains E 1,2 and 3 were classified as *Escherichia coli* as they were fermentative in Hugh and Leifson's medium, catalase and oxidase positive, insensitive to penicillin and 'O/129', positive for indole and methyl red but negative for Voges Proskauer and citrate utilization tests and produced acid and gas

in most of the sugars under test. Enteric bacteria causing fish diseases have been reported by Bullock *et al.*, (1971). The isolates produced the disease in *Etroplus suratensis*.

The isolates PET 1 and 2 were identified as *Pseudomonas mendocina* as they did not hydrolyse starch and liquefy gelatin, positive for catalase and oxidase, alkaline in Hugh and Leifson's medium and insensitive to penicillin and 'O/129'. It did not cause any disease.

The organisms S 1 and 2 were screened as *Streptococcus pyogenes* based on their cellular morphology, cultural characteristics and as they were haemolytic in blood agar, fermentative in Hugh and Leifson's medium, did not hydrolyse starch and casein, insensitive to penicillin and 'O/129', decolourised litmus milk and inactive in most of the sugars under test. Streptococcal fish diseases are important (Bullock *et al.*, 1971).

In the present study, the organism was found to be pathogenic, causing the disease in *Panulirus homarus*.

Table 2.7

The agents TR 1 to TR 11 were registered as *Photobacterium phosphoreum* as they were fermentative

in Hugh and Leifson's medium, insensitive to penicillin but sensitive to 'O/129', positive for catalase but oxidase negative, being luminescent and produced acid and gas in glucose. It could not cause any disease.

The organisms TR 12 to TR 15 were identified as *Vibrio fischeri* as they were indole negative and nonhaemolytic, satisfying the basic characteristics of the genus, *Vibrio*. It was found non pathogenic.

The strains TR 16 to TR 20 have been classified as *Proteus vulgaris*, considering their cultural characteristics and also they were fermentative in Hugh and Leifson's medium, haemolytic in blood agar, insensitive to penicillin and 'O/129', positive for indole and acid and gas production in galactose and glucose. *Proteus vulgaris* has been recorded as the causative agent for blotch disease in white fish, *Coregonus marsena* (Bullock *et al.*, 1971; and van Duijn, 1973). Mass mortality of silver carps has also been reported due to *Proteus rettgeri* by Bejerano *et al.*, (1979). In the present study, the organism could induce the disease in *Etroplus suratensis*.

Table 2.8

The isolates TR 21 to TR 28 were screened as

Flavobacterium ulginosum as they were insensitive to penicillin and '0/129', catalase and oxidase positive, oxidative in Hugh and Leifson's medium, liquefied gelatin, hydrolysed casein but not starch, produced yellow pigment and acid in glucose, maltose and sucrose. These organisms did not initiate the disease and is considered non pathogenic.

The organisms FR 1 to FR 4 were classified as *Pseudomonas stutzeri* as they were alkaline in Hugh and Leifson's medium, hydrolysed starch but did not liquefy gelatin, fermented arabinose and xylose with acid satisfying the basic characteristics of the genus, *Pseudomonas*. These strains also could not cause to any disease.

Table 2.9

The fungal isolates DM 1 to DM 10 and DEM 11 to DEM 20 were identified as species of *Penicillium* mainly based on the appearance of the conidial spores at the tip of sterigmata similar to that of the characteristic appearance of a brush. The difference between the isolates of DM and DEM was while DM isolates appeared white to pink in colour DEM isolates were noticed to be white to dark green. Although the

species of *Penicillium* are usually saprophytes, they have been recorded as pathogens for carps (van Duijn, 1973). DM isolates were found to cause the disease in *Anguilla bicolor bicolor*.

Although 135 isolates, belonging to 18 species binding to 13 genera, were made from 15 kinds of diseases, only eight diseases could be confirmed. They are:

1. *Vibrio anguillarum* for skin lesion
2. *Staphylococcus aureus* for shell disease
3. *Pseudomonas aeruginosa* for hemorrhagic septicemia
4. *Klebsiella pneumoniae* for gill rot
5. *Escherichia coli* for enteritis
6. *Streptococcus pyogenes* for streptococcosis
7. *Proteus vulgaris* for tail rot

and 8. *Penicillium* sp. for dermatomycosis

The remaining isolates failed to reproduce the disease. This could be due to various reasons. The pathogens cause disease symptoms mainly when the hosts

are weak and the ecological environment is so favourable for their proliferation and associated epizootics. It is also an established fact that the diseases do not occur as a single base cause (Snieszko, 1973 and Wedemeyer, 1974). Moreover, pathogens were also detected in normal and healthy fishes without disease manifestation under conditions when physiological or environmental imbalances have not developed. This is indicative that they could remain latent and proliferate under certain types of imbalances (Bullock and Snieszko, 1969). So, it needs no emphasis that while studying the fish diseases and their pathological aspects, proper prophylactic and hygienic measures in the field of aquaculture do deserve utmost importance.

VIROLOGICAL STUDIES

Viruses are intracellular parasites. They have long been known to cause fish and shellfish mortality, but detailed studies on viral diseases have been taken up only in the recent past. This may be partly due to the difficulty in maintaining an expensive laboratory cum test animal house facility, as compaired to the one required for the study of other microbial diseases.

Weissenberg (1914) who investigated lymphocystis disease was one of the first to report on virus diseases of fishes. Subsequently a number of workers have contributed to this field and in 1965 to celebrate the achievements of the past 50 years, a symposium on virus diseases in poikilothermic vertebrates was held by the New York Academy of Sciences (Snieszko et al., 1965).

Many of the recent advances in fish virology are due to Wolf (1966 b, 1972 a), who reviewed the situation. Yet, there are many unresolved problems confronting the fish virologists.

The candidate has discussed different types of finfish and shellfish diseases investigated in and outside

India due to viruses in the introductory part of this Thesis. In India, certain tumours and virus induced diseases have been documented without establishing their viral aetiology (Selvaraj *et al.*, 1973; Lakshmanaperumalsamy *et al.*, 1976; and Thakur and Nasar, 1977). Hence it can be said that upto no work has been done in the field of fish virology in this country (Pillai, 1978). This was a challenging task for the candidate to start from work in this area of vitally important research in pathology.

In the present study, only one case could be documented from the inshore waters off Cochin. The case is proliferative epithelial tumour in the marine catfish, *Arius jella*.

The specimen secured was studied immediately for its viral aetiology. The specimen weighed 360 gm (wet weight). The total and standard length were 38 cm and 30 cm respectively. The tumour size ranged 0.5 cm to 2.5 cm. The tumour growth occurred in the dorsal aspect of the body near the origin of the dorsal fin; in the caudal fin towards its base, and on the dorsal fin. The consistency of the tumour was more or less semi-hard and the colour, ash to rose.

Chances of any parasitic attack and its associated inducement of the tumour were checked by observing wet preparations from the pathological samples.

The specimen was dissected for observing the nature of the internal organs.

To satisfy Rivers' postulates (Frobisher, 1968), virological investigations were carried out using two methods:

TRANSMISSION

Operation of the tumour cells

The specimen was placed on a clean and sterile tray. The fresh tumour was selected for operation. The outer surface of the tumour growth was well cleaned with absolute alcohol diluted to 70%. Aseptically, using sterile scissors, surgical blades and forceps, the tumour tissue was excised and transferred into suitable clean and sterile glass containers kept in an ice packed tray.

Method of decontamination of excised tumour tissue:

The excised tumour tissue was transferred aseptically, with the help of sterile forceps, into another clean and sterile Petri dish containing Dulbecco's phosphate buffered saline (PBS) of the following composition incorporated with antibiotics at concentrations of Penicillin G sodium, 100 i.u., Streptomycin, 100 microgram and Mycostatin, 25 - 50 microgram per ml of saline according to the formulae given by Wolf and Quimby (1969).

Composition of PBS (Dulbecco and Vogt, 1954)

NaCl	8.0000 gm
KCl	0.2000 "
Na_2HPO_4	1.1500 "
KH_2PO_4	0.2000 "
$CaCl_2.2H_2O$	0.0125 "
$MgCl_2.6H_2O$	0.0125 "
Distilled water	1000 ml

pH was adjusted to 7.2. The sample was further filtered for sterilization.

A. jella being a marine species, 0.07 M. Sodium chloride was added in the medium (Wolf and Quimby, 1969).

Method of homogenisation of tumour tissue:

Aseptically, using sterile forceps, the decontaminated tumour tissue, avoiding the blood clots, was transferred into a clean and sterile Ten Broek's glass homogeniser into which fresh PBS, with antibiotics, was added at a concentration of 1:10 w/v. The tumour cells were then ground well so as to homogenise the tumour tissue. The grinding was carried out by keeping the homogeniser on a tray of ice, so as to have a low temperature, as some of the fish viruses are heat labile (Wolf, 1970).

The entire cell free portion of the ground suspension was poured off aseptically from the homogeniser into a suitable clean and sterile glass tube. The cell free suspension was conveniently dispensed into suitable sterile tubes and centrifuged at 4 °C at 2000 r.p.m. for 10 - 20 minutes. The supernatant of the cell free suspension was aseptically collected into sterile vials and kept in a refrigerator (4 °C) for further study.

The supernatant was finally filtered through a millipore filter, keeping the filter flasks in an ice

jar. The filtrate was collected aseptically into suitable clean and sterile vials and stored in an ice jar, placed in the refrigerator.

Pathogenicity test

The pathogenicity test was carried out in *Etroplus suratensis* after its quarantine period with controlled food as described in Chapter IV.

The injection of the filtrate was at a dose of 0.2 to 0.5 ml using one sterile tuberculine syringe with half an inch needle of gauge no 26.

The injection was carried out through two routes - intramuscular and at the site of the original tumour, after anaesthetising the fish with chloretone.

TRANSPLANTATION

The excised tumour tissue, without blood clots, was placed in a clean sterile Petri dish containing PBS with the usual antibiotics as incorporated in the transmission method. The tumour tissue was chopped into small fine pieces with the help of sterile scissors until the saline with the tumour cells turned into a

milky suspension. The work was carried out, keeping the dish on an ice jar so as to have a lower temperature. The milky suspension was transferred into a clean sterile glass vial and kept in an ice jar. The suspension was injected into Etroplus suratensis as indicated for the transmission method, the only difference being the use of one inch needle of gauge no 18.

RESULTS AND DISCUSSION

The results of transmission and transplantation methods are presented in table 3.1.

In the case of tumour cells by transmission via intramuscular route, the fish survived for seven days and the one with site injection died on 11th day.

In the case of transplantation via intramuscular and site injections, the survival period was only 6 - 7 days. Here, both routes of injections could provoke skin granulations. But, the continued growth of the granular tissue could not be observed as the fish did not survive for many days, although the experiment was repeated by injecting the suspected skin granulations.

TABLE 3.1

Pathogenicity test for proliferative epithelial tumour in *Arius jella*

Host	Route of injection		Days 1	2	3	4	5	6	7	8	9	10	11
Etroplus suratensis	*Transmission	IM	Isolation	Isolation, sluggishness and anorexia		Isolation, sluggishness, anorexia and lingering near bottom			Death				
		Site injection	Isolation	Isolation, sluggishness and anorexia		Isolation, sluggishness, anorexia, lingering near bottom, fin rot and imbalance in swimming							Death
	*Transplantation	IM	Isolation	Isolation, sluggishness and anorexia		Isolation, sluggishness, anorexia, lingering near bottom and skin granulations of 0.1 - 0.2 mm diameter		Death					
		Site injection	Isolation	Isolation, sluggishness and anorexia		Isolation, sluggishness, lingering near bottom and skin granulations of 0.1 - 0.2 mm diameter			Death				

* = All controls were normal   IM = Intramuscular injection

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In the present study, all the injected fish died at various intervals except the controls which continued to be normal. Relatively, the effect of the transplantation method was rapid and could provoke skin out growth. This reveals the fact that the disease could be possibly due to viral origin (Bullock, 1976 Personal communication). The results of the present study are agreeable to that of Wellings and Chuinard (1964); Wellings (1969) and Wellings *et al.*, (1976).

The occurrence of tumours in marine bony fishes is relatively higher than that in cartilaginous fishes (Mawdesley Thomas, 1975). Even here, the tumours are documented comparatively more in catfishes (Sarkar *et al.*, 1955; Sarkar and Chaudhuri, 1958; Sathyanesan, 1962 and 1966; Selvaraj *et al.*, 1973; Lakshmanaperumalsamy, 1976 and the candidate Pillai, 1978). The reason, why the marine catfishes are found more affected with tumours, is yet to be investigated. It could be due to the increased susceptibility of the species to the oncogenic virus.

Diseases and stress in natural fish population are practically unknown in India. The candidate's present study suggests the need for an intensive monitoring

of natural fish population for detecting viral diseases which may cause mortality significant enough on stock depletion. This is an area which needs urgent attention.

CHAPTER IV

PATHOGENICITY STUDIES

PATHOGENICITY STUDIES

Pathogenicity is considered to be the ability of the parasite to cause either natural disease or induced experimentally in suitable animals (Watson and Brandly, 1949). However, disease can also occur as a result of attack by an oppertunistic pathogen. The biological as well as physiological well being of the host is important in determining the severity of the infection with a microbe (Smith, 1934). It is also noteworthy here to understand that fish disease does not generally occur as a singular event, but is the net result of the interactions between the aetiological agent and the environment (Snieszko, 1973).

To study the pathogenicity of the selected representative strains in the present investigation, the pure cultures were sub-cultured prior to introduction in the experimental host, on fish infusion agar slants, for 18 - 24 hours.

Broth cultures were not used, inorder to avoid unfavourable reactions that might occur as a result of introduction of foreign substances into the body of the

host. The slant culture was washed with sterile normal saline and the cell suspension density was adjusted to a turbidity of 0.3 OD at 450 millimicrons in a photocolorimeter.

Viable cell counts, using the pour plate method, ranged 1.0 - 1.9 x 10^9 per ml of the cell suspension at 0.3 OD.

The dosage of cell suspension introduced into the body of the fish varied from 0.2 - 0.5 ml and was through the intraperitoneal and intramuscular routes in the case of fishes. But, only intramuscular injection was carried out in the case of prawns and lobsters. The culture was also introduced into the host by site injection superficial scratches in the skin, eyes, gills, fins and tail depending upon the diseases for which the isolate was originally intended for.

Since great difficulty was experienced in obtaining the original host fishes, the tropical finfish, Etroplus suratensis which can live in fresh as well as saline water (upto 34.5‰ salinity) was selected as candidate species for the pathogenicity

tests. In the case of prawns and lobster, original host species (Prawns: *Penaeus indicus* and *P. monodon*; Lobster: *Panulirus homarus*) were used. Such induced pathogenicity tests for other diseases have been carried out by Hodgkiss and Shewan (1950) in laboratory animals, in the absence of suitable host fishes.

Since experimental fishes should be healthy, this was ascertained by quarantining the test fishes for two to three weeks in the control tanks under normal conditions. Selection was made only if during the period these fishes were found to be healthy.

Eighty litre capacity aluminium framed glass tanks were used for the experiments. The metallic parts within the tank were coated with bitumen and no plants or other ornamental materials used in the tanks except the aeration.

The ecological factors such as dissolved oxygen, salinity, temperature, pH, turbidity, metabolites and fish food must be ideal and well regulated eventhough the experimental fish selected, *Etroplus suratensis*, is quite adaptable to variations in the environmental parameters (Pillai, 1979).

Oxygen:

Oxygen was supplied by aeration. The dissolved oxygen needs of the fish differs from species to species. However, an oxygen content of 5 - 8 mg per liter is enough for the majority of fishes (Conroy and Herman, 1970). According to van Duijn (1973) sufficient oxygen could be given by aeration.

Salinity:

Changes in salinity lead to variation in osmotic pressures which may be lethal to the fishes and may affect osmoregulations and the metabolic activities of the fish (Almeida, 1962). The candidate species *Etroplus suratensis* though primarily a fresh water fish, can tolerate saline water content upto 34.5‰. (Pillai and Pillai, 1973 and the candidate Pillai, 1979). Stored and filtered clean habitat water was used and was found quite satisfactory for the experiments.

Temperature:

Changes in temperatures of the water body can play a significant role in determining the severity

of a disease. The importance of this parameter appears so in temperate regions. Garnjobst (1945) observed that temperature has an influence on the production of disease by Cytophaga sp. Ross (1970) noted that severity of vibriosis increased with raise in water temperature. Bullock, Conroy and Snieszko (1971) have observed that outbreaks of corynebacterial kidney disease begin when the water temperature decreases to 10 °C or less. In short, normal temperature fluctuations upto ±12 °C may not be harmful (Conroy and Herman, 1970), although there can be slight variations in the temperature requirements for fish from species to species. Tropical fish are known to live and acclimatize to normal variations in water temperature.

In the present study, water temperature was maintained at room temperature, 28 °C (±2 °C).

pH:

Drastic changes in pH can cause stress and strain on fish and it is important that pH should not fluctuate but remain within the appropriate range. Depending upon the season, the sea water varies in

pH from 7 - 9, to which pH the fish also become adapted.

In the present study as the fish was not kept in a flow through system, but in tanks where frequent water change was made. In the present study the pH of water was maintained at 7.0 - 8.0 and care was taken to keep the tank clean.

Turbidity and toxic substances:

It is obvious that fish continuously excrete metabolic waste products. Accumulation of fecal matter facilitates the ammonia production and also causes the increase in the turbidity of the water body. The suspended particles can harm the fish by mechanical irritation, by clogging the gills and also by removing the oxygen from the water by decomposition (Roberts and Shepherd, 1974). So sufficient care was taken to siphon off the excess food, waste materials and also to observe hygienic conditions.

Fish food:

A proper and balanced diet is absolutely essential for the steady maintenance of the fishes

in the tanks. Generally the specimens, collected from their natural habitats, do not acclimatize immediately to formulated feed in captivity. In the present study, a mix of sterile prawn meat and green peas was used to avoid introduction of any pathogenic microbial flora. The fish easily got acclimatized to this feed and thrived well. Besides controls, two fishes were maintained per tank, for each test organism, one for the intramuscular and the other for the intraperitoneal injection. In addition to this, pathogenicity tests were also made by site injection, superficial injuries in the body, tail, fin, eyes and gills of the fishes.

Before injecting or superficially injuring the fish, they were anaesthetised by applying chloretone at a doze of 1:1000 (w/v) with which they became inactive within 2 - 5 minutes. They were then tagged with sterile coloured threads and injected with the test organism after carefully swabbing the site of injection with 70% alcohol.

The intramuscular injection was done through the dorsolateral region just below the posterior end of the dorsal fin. The intraperitoneal injection was

made through the lateral aspect of the abdominal wall, inserting the needle obliquely so that no internal organs were damaged (Conroy and Herman, 1970). In the case of prawns and lobsters, only intramuscular injection, was carried out, by administering the pathogen in the fifth abdominal segment.

The injections were performed with the help of sterile one ml tuberculine syringe with needles of no 26 gauge.

Immediately after injection, the site of injection was again swabbed gently and carefully with absorbent cotton wetted in 70% alcohol. Then, the fish was replaced in the tank. The usual recovery time was 2 - 5 minutes. A set of fish injected similarly, with sterile normal saline, served as controls for the experiments. Controls were also maintained for the experiments with superficial injuries.

Observations were made daily upto a period of four weeks. As soon as the fish started showing disease symptoms and moribund conditions, they were collected in clean and sterile glass containers for the recovery of the test organism, by culturing the

pathological samples removed from the diseased specimen.

The pathogenicity tests were conducted with representative culture (Table 4.1) selected from among the isolates.

RESULTS AND DISCUSSION

Based on the pathogenicity studies, the following strains were observed to be obligate pathogens.

Bacteria:

1. *Vibrio anguillarum* (SL 8)
2. *Staphylococcus aureus* (SD 1)
3. *Pseudomonas aeruginosa* (HS 1)
4. *Klebsiella pneumoniae* (GR 1)
5. *Escherichia coli* (E 1)
6. *Streptococcus pyogenes* (S 1)
7. *Proteus vulgaris* (TR 16)

Fungi:

8. *Penicillium* sp. (DM 1)

TABLE 4.1

Selected isolates tested for pathogenicity studies

Isolate No	Species
SL 1	*Pseudomonas fluorescens*
SL 8	*Vibrio anguillarum*
SP 1	*Vibrio fischeri*
SD 1	*Staphylococcus aureus*
HS 1	*Pseudomonas aeruginosa*
ED 1	*Micrococcus varians*
GR 1	*Klebsiella pneumoniae*
BS 1	*Alcaligenes eutrophus*
BS 14	*Bacillus thuringiensis*
E 1	*Escherichia coli*
PET 1*	*Pseudomonas mendocina*
S 1	*Streptococcus pyogenes*
TR 1	*Photobacterium phosphoreum*
TR 16	*Proteus vulgaris*
TR 21	*Flavobacterium uliginosum*
FR 1	*Pseudomonas stutzeri*
DM 1	*Penicillium* sp.
DEM 1	*Penicillium* sp.

*Tumour isolate

Vibrio anguillarum (SL 8)

Vibrio anguillarum is pathogenic for fishes from estuarine and marine environments (Bullock, Conroy and Snieszko, 1971). This pathogen can also cause disease in fresh water fishes (Ross, *et al.*, 1968; Ross, 1970; and McCarthy, 1976).

Species of *Vibrio* as casual organisms for disease conditions in fish were first recognised by Bergman (19)9). Later, a proposal to bring together the species of *Vibrio* - *Vibrio anguillarum*, *Vibrio piscium* var. *japonicus* and *Vibrio ichthyodermis* into a single species, *Vibrio anguillarum* was made by Hundrie, Hodgkiss and Shewan (1971), in agreement with the recommendations of Ross *et al.*, (1968).

In the present study, the organism, *V. anguillarum* was injected through the intraperitoneal and intramuscular routes. The fishes were quite restless for the first two to four hours. By six hours, signs and symptoms of the disease appeared - erythema at the base of the pectoral and pelvic fins and redness in the skin. By 12 - 15 hr of injection, the fishes were observed to be very sick, loosing equilibrium in swimming and

often falling down to the bottom of the water body and anorexic. At this time, the fishes were removed for further diagnosis. The gills were swollen and bled on slight pressure. The intestines were filled with a yellowish red fluid.

The organisms were reisolated in pure culture from the skin, fins and kidney.

The signs and symptoms observed in this case are similar to those described by Drouin de Bouville (1907); Rucker (1959); Wolter (1960); Rose (1970); van Duijn (1973) and Richards and Roberts (1978).

Staphylococcus aureus (SD 1)

This organism, pathogenic for human beings, is also found causing diseases in plaice and haddock (Bullock, Conroy and Snieszko, 1971).

The pathogen was injected only through the intramuscular route. After injection of the pathogen, they were quite restless and anorexic. By six to eight hours, shell discoloration radiating from the site of injection was noticed. Within 10 - 12 hr, shell decay appeared. By 12 - 14 hr, they appeared to be

quite moribund and they were removed for further examination.

The pathogen was reisolated from the shell with tissue, hepathopancreas and blood.

The species of *Staphylococcus* have been reported from the coastal waters and also found on the brown mussel, *Perna indica* (Pillai, 1980). But diseases caused by Gram positive cocci are rare and staphylococcal diseases are seldom reported (Bullock *et al.*, 1971; Wolke, 1975; Richards and Roberts, 1978).

Pseudomonas aeruginosa (HS 1)

Pseudomonas sp. is one of the important aetiological agents of fish diseases, causing hemorrhagic septicemia (Bullock *et al.*, 1971; van Duijn, 1973; Snieszko and Bullock, 1974 and Richards and Roberts, 1978).

In the case of both intramuscular and intraperitoneal injections, for the first two to four hours, the fishes were quite restless and dashing against the glass walls occasionally. They were anorexic. Within 6 - 10 hr, they developed

erythema at the base of the fins and on the lateral sides of the body, folding of pectoral fins and at times sudden darting movements after which they become inactive, sinking to bottom of the tank. Within 12 - 18 hr, the eyes became 'sunken', sloughing away of scales and loss of equilibrium. At this time, they became sluggish and moribund with reddish discharge from the anus. So, they were removed for examination and the bacteria were reisolated in pure culture from the skin, fins and kidney and the signs and symptoms of the disease are found to agree with the descriptions given by Bullock _et al._, (1971); van Duijn (1973); Snieszko and Bullock (1974) and Richards and Roberts (1978).

Klebsiella pneumoniae (GR 1)

This organism is an associate of various pathological conditions (Buchanan and Gibbons, 1974).

The organism, was isolated from cases of gill rot, was injected both intramuscularly and intraperitoneally. The injections could only result in skin lesions and death within 24 hr of infection. However, more positive results were obtained when the pathogen was introduced in the gills through artificial injuries.

The infection through artificial injuries could result in gill rot with signs and symptoms such as restlessness, imbalance in swimming, isolation, skin lesion, anorexia, weak movements and surface floating within the first fifteen hours of infection. Within eighteen hours, they were found fully inactive with body colour bordening to black. Examination at this stage, showed gill lesions with tissue decay. The pathogen was reisolated in pure culture from the gill filaments and the kidney.

Klebsiella infections are rarely reported (Ahne, 1980). But the signs and symptoms are more or less similar to that of other enterobacterial infection reported by Bullock, Conroy and Snieszko (1971) and Richards and Roberts (1978).

Escherichia coli (E 1)

Enteric bacteria can cause disease in fishes (Bullock *et al.*, 1971; Wolke, 1975; and Richards and Roberts, 1978).

The pathogen, isolated from cases of enteritis, was introduced in the host by intramuscular and intraperitoneal routes.

For the first two hours, the fishes were restless. By six hours of infection, they developed sluggishness and anorexia. Within 12 hr, skin lesion and body discoloration into dark colour were observed. By 15 hr time, they became completely inactive with fecal discharge of reddish colour. At this stage, they were removed for further studies. The pathogen was reisolated in pure culture from the skin, intestine and kidney.

Escherichia coli has been reported occurring in the coastal waters and also associating with the brown mussel, *Perna indica* (Pillai, 1980). But diseases caused by *E. coli* are seldom reported. In the present study, the signs and symptoms are almost similar to that of other enterobacterial infections (Bullock *et al.*, 1971; van Duijn, 1973 and Richards and Roberts, 1978).

Streptococcus pyogenes (S 1)

Streptococcal infections are well known among fishes and in general infections due to Gram positive cocci in lobsters are serious diseases (Bullock *et al.*, 1971; Stewart, 1975; Wolke, 1975 and Richards and Roberts, 1978).

In the present study, the pathogen, *Streptococcus pyogenes* isolated from the spiny lobster, *Panulirus homarus* was introduced through intramuscular route and through artificial injuries.

The infection by injection resulted in shell 'decay' with adjacent tissue necrosis, anorexia and sluggishness. The infection through artificial injuries could also result in typical blister formation within twenty two hours of infection. At this stage, the hosts were found completely inactive and so, they were removed for further examination. The pathogen was reisolated in pure culture from the parts where shell 'decay' and muscle necrosis had setin and from the heart.

The signs and symptoms of the disease are almost similar to that described by Bullock *et al.*, (1971); Sindermann (1970 and 1977); Stewart (1975); Wolke (1975) and Richards and Roberts (1978).

Proteus vulgaris (TR 16)

Instances of diseases as a result of infection due to *Proteus* sp. have been recorded by Bullock *et al.*, (1971); van Duijn (1973) and Bejerano *et al.*, (1979).

In the present study, the pathogen, *Proteus vulgaris*, was introduced into the host both by intramuscular and intraperitoneal routes. The fishes were found restless for the first three hours. By 6 - 10 hr, the signs and symptoms such as loss of body colour and anorexia could be noticed. Within 12 - 22 hr, skin lesion and tail tissue decay were noted. By this time the fishes were quite weak and found swimming, occasionally, near water surface. Their reflex activities were mostly lost. At this time, they were removed for further check up.

The pathogen was also introduced into the host by superficial injuries and this method was also successful in provoking the disease within 20 - 24 hr.

The organism was reisolated in pure culture from the skin, tail and blood.

The signs and symptoms of the disease are almost agreeable to that given by Bullock *et al.*, (1971); van Duijn (1973); Wolke (1975) and Richards and Roberts (1978).

Penicillium sp. (DM 1)

Relatively, fungal infections in fishes are

rare as compared to bacterial infections (van Duijn, 1973; and Richards, 1978).

The fungal suspension (0.2 to 0.5 ml) was injected intramuscularly and intraperitoneally. But, the injections were not very successful in provoking the disease, although the intraperitoneally infected fish died on the third day. The infection through surface injuries was quite successful.

Visible growth, like cotton buds, appeared within 36 - 48 hr and death on 20th day.

The rate of infection was very slow. In the first week, the fish was active and no other visible abnormalities noted. During the 2nd week, the spread of infection over the body was rapid. The eyes were also found infected. At this stage, they were noticed to be anorexic and isolated.

The water had to be changed once a day due to excess turbidity as a result of fungal growth shed from the body.

In the third week, they were very sluggish and found fully infected with ulcers on the body. At this time, they were removed for further examination and

reisolation of the aetiological agent.

Species of Penicillium are usually observed as saprophytes. But, cases of their pathogenicity are also reported by Reichenbach Klinke and Elkan (1965); van Duijn (1973) and Bendele and Klontz (1975).

In the present investigation, of 18 selected representative isolates tested, only eight organisms proved to be pathogenic.

Comparatively, intraperitoneal injection was found to be more effective. Similarly, the pathogenicity tests made by superficial injuries were also noticed to be effective.

All the controls were found to be normal and healthy.

CHAPTER V

TESTING OF THERAPEUTIC AGENTS

TESTING OF THERAPEUTIC AGENTS

As aquaculture of finfish and shellfish has provoked a world wide interest, measures on prophylactic methods and treatment to prevent and treat the diseases among these cultivable animals have also gained significant importance.

The outbreak of disease in a confined culture system could be due to conditions such as, poor sanitary measures and lack of adequate care in observing suitable prophylactic measures such as the proper quarantine system for new arrivals; the removal of unhealthy animals at the right time; supply of good quality water and balanced feed; avoiding overcrowding and unnecessary handling; poor filtration and aeration systems; and lack of disinfection of culture ponds, tanks and allied apparatus used for the culture practices.

The causative factors for the disease are situations created for the proliferation of facultative and obligate pathogenic organisms. Hence, the need for identifying the malady and the prompt treatment of

infected population. The treatment of fish varies according to the clinical signs and symptoms of the diseased fishes and drugs must be administered accordingly. Inorder to device suitable treatment for fish and shellfish diseases, therapeutic investigations on the same, caused by the pathogens isolated in the present study, were carried out. For the treatment of the diseases, both antiseptics and antibiotics were tested for their efficacy.

ANTISEPTICS

The use of antiseptics for the control and treatment of fish and shellfish diseases (van Duijn, 1973; Roberts, 1978 and Brown and Gratzek, 1980) are well known. To cite a few examples, Atkinson (1932) treated hydrocoele embryonalis in trout eggs and fry with acriflavine. Feustel (1935) suggested potassium permanganate for the treatment of bacterial gill disease. Gutsell (1947) and McCraw (1952) used acriflavine to disinfect trout eggs. Axelrod and Schultz (1955) recommended the use of potassium dichromate for the treatment of fin rot. Methylene

blue was used for the improvement of anaemic cases (Tack, 1960) and for the use against fungal diseases (Axelrod, 1962).

Acriflavine is advocated for the treatment of various microbial diseases by Conroy and Herman (1970); Bullock *et al.*, (1971); Herman (1972); van Duijn (1973); Hoffman and Meyer (1974); and the candidate Pillai (1978).

Copper sulphate is an another effective chemotherapeutic agent used for the treatment of bacterial and fungal diseases (Schneberger, 1941; Davis, 1953; Conroy and Herman, 1970; Bullock *et al.*, 1971; van Duijn, 1973; Hoffman and Meyer, 1974; Roberts and Shepherd, 1974; and Roberts, 1978).

Potassium permanganate is also widely used for the treatment of various microbial diseases in fish and shellfishes (Conroy and Herman, 1970; Herman, 1972; van Duijn, 1973; Hoffman and Meyer, 1974; Lightner, 1977 c and Roberts, 1978).

Silver nitrate has also been used for treating different kinds of microbial diseases (van Duijn, 1973; and the candidate Pillai, 1978).

In the light of this, it is clear that antiseptics can be successfully used as chemotherapeutic agents against varieties of diseases.

Four antiseptics were selected for experiments in the present investigation *viz*., acriflavine, copper sulphate, potassium permanganate and silver nitrate as they are used for the treatment of fish diseases, reasonably cheap and locally available.

The pathogens, used in the study for testing the effect of the antiseptics, were:

1. *Vibrio anguillarum* (SL 8)
2. *Staphylococcus aureus* (SD 1)
3. *Pseudomonas aeruginosa* (HS 1)
4. *Klebsiella pneumoniae* (GR 1)
5. *Escherichia coli* (E 1)
6. *Streptococcus pyogenes* (S 1)
7. *Proteus vulgaris* (TR 16)

and 8. *Penicillium* sp. (DM 1)

The evaluation of the effect of the selected antiseptics on the pathogens involved the determination of the inhibition coefficient and the inferior lethal coefficient.

Determination of the inhibition coefficient

Ten fold serial dilutions upto 10^{-6} of all the compounds were prepared in sterile distilled water and sterilized. Sterile fish infusion agar tubes, containing 13.5 ml each, were cooled and 1.5 ml of each dilution of each compound was mixed well with each tube of agar and poured into sterile Petri plates. The dilutions were thus increased ten fold. The test pathogenic organisms which had been cultured for 18 - 24 hr in fish infusion agar were streaked on the plates. Sabouraud's agar was used for the fungi. Observations were made every day upto 3 days. The results are presented in table 5.1 and discussed later under this section.

Determination of the inferior lethal coefficient

Observing the results obtained in the previous experiment, the dilutions selected ranged from the highest dilution showing no growth to a minimum dilution of 1:10. Sterile fish infusion broth, in 4.5 ml amounts, was inoculated and incubated, for 18 - 24 hr. Then, 0.5 ml amounts of the respective dilutions of the compounds were added to each tube of

TABLE 5.1

Inhibition coefficient of the antiseptics

Isolate(s)	Acriflavine						Copper sulphate						Potassium permanganate						Silver nitrate					
	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
Vibrio anguillarum (SL 8)	-	-	-	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	-	-	-	+	+	+
Staphylococcus aureus (SD 1)	-	-	-	+	+	+	-	-	+	+	+	+	-	-	+	+	+	+	-	-	-	-	+	+
Pseudomonas aeruginosa (HS 1)	-	+	+	+	+	+	-	-	+	+	+	+	-	+	+	+	+	+	-	-	-	-	+	+
Klebsiella pneumoniae (GR 1)	-	+	+	+	+	+	-	-	+	+	+	+	-	+	+	+	+	+	-	-	-	-	+	+
Escherichia coli (E 1)	-	+	+	+	+	+	-	-	-	+	+	+	-	+	+	+	+	+	-	-	-	-	+	+

+ = Growth

- = No growth

1 = 10^{-1} 4 = 10^{-4}

2 = 10^{-2} 5 = 10^{-5}

3 = 10^{-3} 6 = 10^{-6}

TABLE 5.1 (Contd.)

Inhibition coefficient of the antiseptics

Isolate(s)	Acriflavine						Copper sulphate						Potassium permanganate						Silver nitrate					
	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
Streptococcus pyogenes (S 1)	−	−	−	−	+	+	−	−	+	+	+	+	−	+	+	+	+	+	−	−	−	−	+	+
Proteus vulgaris (TR16)	−	+	+	+	+	+	−	−	+	+	+	+	−	−	−	+	+	+	−	−	−	−	+	+
Penicillium sp. (DM 1)	−	+	+	+	+	+	−	−	+	+	+	+	−	+	+	+	+	+	−	−	−	−	+	+

+ = Growth
− = No growth

1 = 10^{-1} 4 = 10^{-4}
2 = 10^{-2} 5 = 10^{-5}
3 = 10^{-3} 6 = 10^{-6}

culture. The final dilutions were then increased ten fold. After the sharp exposure periods of 2,4,6,8 and 10 minutes, sub-cultures were made on plates of fish infusion agar. Sabouraud's agar was used for fungi. Observations were made for every 24 hr upto a period of 3 days. The results are presented in table 5.2 and discussed later in this section.

After the *in vitro* studies, of the effect of antiseptics on the test organisms were completed, the effect of antiseptics *in vivo* was conducted. Based on the *in vitro* studies, the effective concentrations of antiseptics used on the test organisms ranged from 10^{-1} to 10^{-3}. Though a dilution of 10^{-4} was sometimes effective after 10 minutes, such an exposure was not selected as it may be lethal to the host.

Since *Pseudomonas aeruginosa* and *Escherichia coli* are found causing septicemic diseases, they were not selected for *in vivo* studies as mostly the use of antiseptics in such cases may not be helpful (Davis, 1946; the candidate Pillai, 1978).

Vibrio anguillarum, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Streptococcus pyogenes*, *Proteus*

TABLE 5.2

Inferior lethal coefficient of the antiseptics

Isolate(s)	Antiseptic dilution	Acriflavine					Copper sulphate					Potassium permanganate					Silver nitrate				
		(exposing time in minutes)																			
		2	4	6	8	10	2	4	6	8	10	2	4	6	8	10	2	4	6	8	10
Vibrio	10^{-1}	-	-	-	-	-			Not tested*					Not tested*			-	-	-	-	-
anguillarum (SL 8)	10^{-2}	+	-	-	-	-			''					''			+	+	-	-	-
	10^{-3}	+	+	+	+	+			''					''			+	+	+	+	+
Staphylococcus	10^{-1}	-	-	-	-	-			Not tested*					Not tested*			-	-	-	-	-
aureus (SD 1)	10^{-2}	+	+	-	-	-			''					''			+	+	+	-	-
	10^{-3}	+	+	+	+	+			''					''			+	+	+	+	-
	10^{-4}			Not tested*					''					''			+	+	+	+	+
Pseudomonas	10^{-1}			Not tested*					Not tested*					Not tested*			-	-	-	-	-
aeruginosa (HS 1)	10^{-2}			''					''					''			+	+	+	-	-
	10^{-3}			''					''					''			+	+	+	+	+
	10^{-4}			''					''					''			+	+	+	+	+

+ = Growth   * = Not tested since the isolate is resistant

- = No growth

TABLE 5.2 (Contd.)

Inferior lethal coefficient of the antiseptics

Isolate(s)	Antiseptic dilution	Acriflavine					Copper sulphate					Potassium permanganate					Silver nitrate				
		(exposing time in minutes)																			
		2	4	6	8	10	2	4	6	8	10	2	4	6	8	10	2	4	6	8	10
<u>Klebsiella</u>	10^{-1}			Not tested*					Not tested*					Not tested*			-	-	-	-	-
<u>pneumoniae</u> (GR 1)	10^{-2}			"					"					"			+	+	-	-	-
	10^{-3}			"					"					"			+	+	+	+	-
	10^{-4}			"					"					"			+	+	+	+	+
<u>Escherichia</u>																					
<u>coli</u> (E 1)	10^{-1}			Not tested*			-	-	-	-	-			Not tested*			-	-	-	-	-
	10^{-2}			"			+	-	-	-	-			"			+	+	-	-	-
	10^{-3}			"			+	+	+	+	+			"			+	+	+	+	+
	10^{-4}			"					Not tested*					"			+	+	+	+	+
<u>Streptococcus</u>	10^{-1}	-	-	-	-	-			Not tested*					Not tested*			-	-	-	-	-
<u>pyogenes</u> (S 1)	10^{-2}	-	-	-	-	-			"					"			+	+	-	-	-
	10^{-3}	+	+	+	+	-			"					"			+	+	+	+	-
	10^{-4}	+	+	+	+	+			"					"			+	+	+	+	+

\+ = Growth   * = Not tested since the isolate is resistant

\- = No growth

TABLE 5.2 (Contd.)

Inferior lethal coefficient of the antiseptics

Isolate(s)	Antiseptic dilution	Acriflavine					Copper sulphate					Potassium permanganate					Silver nitrate				
		(exposing time in minutes)																			
		2	4	6	8	10	2	4	6	8	10	2	4	6	8	10	2	4	6	8	10
Proteus vulgaris (TR16)	10^{-1}	Not tested*					Not tested*					−	−	−	−	−	−	−	−	−	−
	10^{-2}			''					''			+	+	+	−	−	+	+	−	−	−
	10^{-3}			''					''			+	+	+	+	+	+	+	+	+	+
	10^{-4}			''					''			Not tested*					+	+	+	+	+
Penicillium sp. (DM 1)	10^{-1}	Not tested*					Not tested*					Not tested*					−	−	−	−	−
	10^{-2}			''					''					''			±	±	±	−	−
	10^{-3}			''					''					''			+	+	+	+	+
	10^{-4}			''					''					''			+	+	+	+	+

+ = Growth

± = Poor growth

− = No growth

* = Not tested since the isolate is resistant

vulgaris and *Penicillium* sp. were selected for the *in vivo* studies as they were also found causing mild infections.

Based on the *in vitro* studies, *Vibrio anguillarum*, *Staphylococcus aureas* and *Streptococcus pyogenes* were tested with the disinfectants, acriflavine and silver nitrate; *Klebsiella pneumoniae* with silver nitrate; *Proteus vulgaris* with potassium permanganate and silver nitrate and *Penicillium* sp. with silver nitrate.

In vivo, the experimental animals tended to die in the disinfectant solutions when they were kept for more than thirty seconds time, although in the *in vitro* test, the pathogens were killed in higher dilutions at higher exposure time. To minimise this difficulty, minimum exposure time of the effective lowest concentration was chosen according to Almeida (1962) and the candidate Pillai (1978).

The test animals, selected were these kept in quarantine period under suitable ecological conditions as discussed in Chapter IV, were only chosen for the experiments. Two animals were used for testing each disinfectant. Relevant principles for administering

therapeutic agents were duly followed as suggested by Poupard (1978). Both positive and negative controls were maintained in this study.

DISCUSSION

Vibrio anguillarum (SL 8, tables 5.1 - 5.2)

As soon as the experimental fishes started showing visible signs and symptoms of the disease, the fishes were bathed by dip method, in the disinfectant solution. The dip method was found to be unsuccessful, leading to death of the animals. Gentle swabbing with sterile absorbent cotton wetted in the related disinfectant solution did not affect the fish. Both, acriflavine and silver nitrate were found unsuitable to recover the diseased fish from the effects of the pathogen introduced through the intraperitoneal route. But, intramuscularly infected animals recovered well from the ill effects of the disease with the help of the antiseptics, acriflavine and silver nitrate. The antiseptic was applied once daily for three days.

Staphylococcus aureus (SD 1, tables 5.1 - 5.2)

When the shell of the infected prawns showed signs of decay, they were bathed by dip method, in the disinfectant solution. Here again, the dip method was found to be lethal to the experimental animals. So, swabbing the affected area with absorbent cotton wetted in the disinfectant solution was followed once daily for five days. Both the disinfectants, acriflavine and silver nitrate were effective in treating the disease.

Klebsiella pneumoniae (GR 1, tables 5.1 - 5.2)

Dip method, was not followed as it was not useful in treating the disease, in the selected doze. When the symptoms of the disease appeared, the experimental fishes were swabbed, using the disinfectant, silver nitrate, once daily. The disinfectant was not effective in treating the disease, as the diseased animals died on the second day before the death of the positive control. The negative control was normal.

Streptococcus pyogenes (S 1, tables 5.1 - 5.2)

When the signs and symptoms appeared as a result of infection, the disinfectants, acriflavine and silver

nitrate were tested, by swabbing the affected area, once daily. The disinfectants were effective and complete recovery was noticed by five days treatment.

Proteus vulgaris (TR 16, tables 5.1 - 5.2)

The disinfectants were applied by swab method as soon as the signs and symptoms of the disease were noticed. Potassium permanganate was not potent enough to cure the disease, while silver nitrate was noticed to be quite effective in treating the disease when the drug, was applied by swabbing the affected area, once daily for five days.

Penicillium sp. (DM 1, tables 5.1 - 5.2)

Visible infection was noticed within 36 - 48 hr of the attack with the pathogen. At this stage, the antiseptic, silver nitrate, was applied by the swab method, once daily for seven days. The disinfectant was very effective in treating the disease.

In general, the disinfectants were effective, except potassium permanganate, when administered by swab method. While all the positive controls died, the negative controls were fully normal.

Based on the above experiments with the antiseptics, the salient observations that were made are that the effectiveness of the antiseptics depend upon:

a) The nature and sensitivity of the pathogen

b) Method and time of application of the suitable disinfectant

and c) Toxicity of the antiseptics to the hosts.

ANTIBIOTICS

Broad spectrum antibiotics have gained considerable importance in the field of clinical medicine (Herman, 1969) and these antibiotics have also been found useful for the treatment of fish and shellfish diseases (Snieszko, 1959; Meyer, 1964; Meyer and Collar, 1964; Robinson *et al.*, 1969; Conroy and Herman, 1970; van Duijn, 1973; Sindermann, 1970, 1977 and Roberts, 1978).

Antibiotics have been used in sea water to control bacterial growth in phytoplankton cultures (Fish, 1950; Spencer, 1952) and also for the preservation

of fish (Tarr *et al.*, 1950). Oppenheimer (1955) observed that the use of the proper antibiotics prevents bacterial growth in fish eggs. Hoshina (1957) noted the *in vitro* effect of some antibiotics on the aetiological agent of furunculosis like disease in rainbow trout. Volf and Havelka (1958) reported the activity of the antibiotics, streptomycin, chloromycetin and aureomycin against infectious dropsy in carps. Irwin (1959) successfully treated fin rot with terramycin. For tail and fin rot, kanamycin has been found effective (Conroy, 1962 and 1963) and also for infections due to species of *Aeromonas* and *Pseudomonas* (Meyer and Collar, 1964). While Stewart and Cornick (1967) studied the *in vitro* susceptibility of *Gaffkya homari* (lobster pathogen) to different antibiotics, Rabin and Hughes (1968) treated the disease, gaffkemia, with streptomycin. The antibiotics, chloromycetin, erythromycin, kanamycin, streptomycin, terramycin, gentamycin and griseofulvin have been used for different kinds of bacterial diseases (Conroy and Herman, 1970; Bullock *et al.*, 1971 and van Duijn, 1973). The antibiotic, terramycin has been used for the treatment of bacterial diseases in shrimps, *Penaeus* sp. (Lightner, 1977 a, b). Sindermann (1977) had treated

fresh water shrimps, *Macrobrachium vollenhovenii* and *M. rosenbergii* with furanace against bacterial disease, black spot disease and the antibiotics, penicillin, streptomycin, vancomycin were found very helpful in treating the diseased lobsters *Homarus americanus* with the attack due to *Gaffkya homari*. Sindermann had also suggested to use the antibiotics, chloromycetin, polymyxin B, erythromycin and neomycin against bacterial diseases in oysters, *Crassostrea virginica* and also for the treatment of bacterial disease in clam, *Mercenaria mercenaria*. Le Bitoux (1977) had also advocated the use of antibiotics such as penicillin, streptomycin, erythromycin and furanace against bacterial disease in shrimps, *Macrobrachium rosenbergii*. So, it is clear that different kinds of antibiotics are in active use for the treatment of fish and shellfish diseases (van Duijn, 1973; Sindermann, 1977; Roberts, 1978 and Brown and Gratzek, 1980).

In the present study, four antibiotics, chloromycetin, kanamycin, terramycin and griseofulvin were selected for testing, the eight test pathogens.

Method of testing *in vitro* sensitivity

Under aseptic conditions, the antibiotics were

weighed and diluted in sterile distilled water to get a concentration of one mg per ml.

Fish infusion agar plates for the bacteria and Sabouraud's agar for the fungi, were surface seeded with 18 - 24 hr cultures grown on the respective media. Sterile filter paper discs, 7 mm in diameter, were used for the test. They were soaked in the respective antibiotic solutions and placed on the seeded Petri plates and incubated at room temperature. After 24 - 48 hr of incubation, the results were read and recorded. The results are presented in table 5.3. The antibiotics, showing the maximum sensitivity *in vitro*, were then tested to detect the least concentration of antibiotic effective against the respective cultures. For this, the cylinder plate method was followed. The diluted antibiotics, one mg per ml were further diluted from one mg per ml to $1:10^5$ mg per ml.

Twenty ml amounts of sterile fish infusion agar (Sabouraud's agar for the fungi) were melted and cooled and bulk seeded with the respective 18 - 24 hr cultures and plated out. With a suitable cork borer (10 mm diameter) holes were punched in the agar and the sterile

TABLE 5.3

In vitro sensitivity to antibiotics

(zone size in mm)

Isolate(s)		Chloromycetin	Kanamycin	Terramycin	Griseofulvin
Vibrio anguillarum	(SL 8)	++ 30 mm	++ 30 mm	++ 25 mm	-
Staphylococcus aureus	(SD 1)	++ 30 mm	+ 15 mm	+ 15 mm	-
*_Pseudomonas aeruginosa_	(HS 1)	-	+ 15 mm	-	-
Klebsiella pneumoniae	(GR 1)	++ 35 mm	++ 30 mm	++ 30 mm	-
Escherichia coli	(E 1)	+ 15 mm	++ 25 mm	-	-
Streptococcus pyogenes	(S 1)	++ 25 mm	++ 23 mm	++ 25 mm	-
Proteus vulgaris	(TR16)	-	++ 25 mm	-	-
Penicillium sp.	(DM 1)	-	-	-	-

*_Ps. aeruginosa_, being insensitive to the antibiotics tested, Gentamycin was substituted. (Gentamycin = ++ 30 mm)

+ = Bacteriostatic zone ++ = Bactericidal zone - = No zone

cups were positioned. The antibiotics in each dilution were properly pipetted into the cups and incubated at room temperature.

The results recorded after 24 - 48 hr, are presented in table 5.4.

A drug which is effective against a pathogen in vitro is further tested for its ability to inhibit the pathogen in vivo (Bullock and Collis, 1969). The antibiotics were therefore tested in host species as mentioned in Chapter IV.

The administration of the antibiotics can be done in different ways. The mixing of the antibiotics in the habitat water was not followed, as it was not desirable for at least to reasons, (a) it is not economical and (b) it may destroy the aquatic flora, of the habitat water, which are helpful in breaking down the metabolic waste products accumulating in the tank water. The antibiotics were also not supplied through the feed as this causes leaching and sometimes even the natural food is not eaten by the animals. As such proper evaluation of results may not be possible. Hence, to test the efficacy the best method of administration is by injection and this was preferred.

TABLE 5.4

Effect of antibiotics (*in vitro*)

Isolate(s)	Antibiotic concentration	Chloromycetin	Kanamycin	Terramycin
Vibrio anguillarum (SL 8)	10^{-3}	++	++	++
	10^{-4}	++	++	++
	10^{-5}	+	+	+
	10^{-6}	-	+	-
	10^{-7}	-	-	-
Staphylococcus aureus (SD 1)	10^{-3}	++	Not tested*	Not tested*
	10^{-4}	++	"	"
	10^{-5}	+	"	"
	10^{-6}	-	"	"
	10^{-7}	-	"	"

* = Not tested since the isolate is resistant

\+ = Bacteriostatic zone ++ = Bactericidal zone - = No zone

TABLE 5.4 (Contd.)

Effect of antibiotics (*in vitro*)

Isolate(s)	Antibiotic concentration	Gentamycin	Chloromycetin	Kanamycin	Terramycin
Pseudomonas aeruginosa (HS 1)	10^{-3}	++	Not tested*	Not tested*	Not tested*
	10^{-4}	++	"	"	"
	10^{-5}	++	"	"	"
	10^{-6}	–	"	"	"
	10^{-7}	–	"	"	"
Klebsiella pneumoniae (GR 1)	10^{-3}	Not applicable	++	++	++
	10^{-4}	"	++	++	++
	10^{-5}	"	++	++	++
	10^{-6}	"	+	+	+
	10^{-7}	"	–	–	–

* = Not tested since the isolate is resistant

+ = Bacteriostatic zone ++ = Bactericidal zone – = No zone

TABLE 5.4 (Contd.)

Effect of antibiotics (*in vitro*)

Isolate(s)	Antibiotic concentration	Chloromycetin	Kanamycin	Terramycin
Escherichia coli (S 1)	10^{-3}	Not tested*	++	Not tested*
	10^{-4}	"	++	"
	10^{-5}	"	+	"
	10^{-6}	"	-	"
	10^{-7}	"	-	"
Streptococcus pyogenes (S 1)	10^{-3}	++	++	++
	10^{-4}	++	++	++
	10^{-5}	+	+	+
	10^{-6}	-	-	-
	10^{-7}	-	-	-

* = Not tested since the isolate is resistant

+ = Bacteriostatic zone ++ = Bactericidal zone - = No zone

TABLE 5.4 (Contd.)

Effect of antibiotics (*in vitro*)

Isolate(s)	Antibiotic concentration	Chloromycetin	Kanamycin	Terramycin
Proteus vulgaris (TR 16)	10^{-3}	Not tested*	++	Not tested*
	10^{-4}	"	++	"
	10^{-5}	"	+	"
	10^{-6}	"	+	"
	10^{-7}	"	-	"

* = Not tested since the isolate is resistant

+ = Bacteriostatic zone ++ = Bactericidal zone - = No zone

The selected antibiotics were separately dissolved in sterile distilled water and diluted to concentrations of 4 mg per ml and five mg per ml so as to inject at the level of 20 µg (0.02 mg) per gram of fish according to the methods of Conroy (1963); van Duijn (1973); Snieszko and Bullock (1974) and the candidate Pillai (1978).

The antibiotics were injected through the intramuscular and intraperitoneal routes (Meyer and Collar, 1964; Fribourgh, Robinson and Meyer, 1969; Conroy and Herman, 1970; and van Duijn, 1973), to a maximum of 0.5 ml at a time.

Healthy animals under quarantine were maintained in 3 tanks with suitable ecological conditions as discussed previously. One set was used for the experiment while the other two sets were run as positive and negative controls. In each tank, 4 fishes were kept properly tagged; two for intramuscular injection and the other two for intraperitoneal injection. Intraperitoneal injection was not carried out in the case of shrimps and lobsters.

Maintaining the controls, each test pathogen was injected as in the pathogenicity studies. For

chemotherapy to be more effective, it should be started at the earliest and not delayed until the disease is well established (McCarthy, 1975 b). So, each antibiotic selected was injected after 4 hr of the injection of the pathogenic organism in the host (Pillai, 1978). If the hosts were found to develop any symptoms of the disease in spite of the antibiotic injection, they were then bathed by dip method in the suitable antibiotic solution, for one to five minutes, at a concentration of 50 mg per litre of water, twice daily for 7 days, as suggested by Conroy and Herman (1970). This proved quite successful. After an antibiotic injection, the dip method was followed to avoid the added stress as a result of repeated injections over the pathogenic effect. Zubrod (1947); Gibson (1948) and White et al., (1948) who studied various schedules in mice infected with haemolytic bacteria observed that with intervals upto 12 or even 14 hr, the dose was the deciding factor but not the frequency of the injection. In the present investigation also this was found to be true.

DISCUSSION

Vibrio anguillarum (SL 8, tables 5.3 - 5.4)

The pathogen was introduced, through the normal two routes, in the hosts. After four hours of interval from the time of injection of the pathogen, the fish was given separately the antibiotics, chloromycetin, kanamycin and terramycin by injection. They were slightly sluggish for the first one hour after injection of the antibiotics. No symptoms of the disease developed. The fishes became normal within 24 hr from the administration of the antibiotics. The positive controls developed the disease and died within 18 - 20 hr.

The results of the effect of antibiotics are in concurrence with that of Kubota and Hagita (1963); Bullock *et al.*, (1971) and Richards and Roberts (1978).

Staphylococcus aureus (SD 1, tables 5.3 - 5.4)

After 4 hr of infection of the host with the pathogen, *Staphylococcus aureus*, the hosts were given

the antibiotic, chloromycetin by way of injection. As shell discoloration was noticed, by eight hours time, from the site of infection, the hosts were further bathed by dip method twice daily in chloromycetin solution made of 50 mg per liter of water according to Conroy and Herman (1970). On the 5th day they became normal.

The positive controls died within 16 hr of infection.

Here, the results of the antibiotic therapy are agreeable to that of Bullock *et al.*, (1971) and Richards and Roberts (1978).

Pseudomonas aeruginosa (HS 1, tables 5.3 - 5.4)

The pathogen was injected intramuscularly and intraperitoneally. After 4 hr of infection, the antibiotic, gentamycin was administered intramuscularly and intraperitoneally. The fishes were weak for the first two hours. Although one fish which received the intraperitoneal injection of the antibiotic died after three hours, the remaining fishes did not develop any disease signs or symptoms. They became normal

within 20 hr of antibiotic injection. But, by 18 hr time, the positive controls developed the disease and died.

The results of the therapeutic study are similar to that mentioned by Meyer and Collar (1964); Conroy and Herman (1970); Bullock _et al._, (1971) and Richards and Roberts (1978).

Klebsiella pneumoniae (GR 1, tables 5.3 - 5.4)

Through both the routes, the pathogen was introduced in experimental fishes. After 4 hr, the antibiotics, chloromycetin, kanamycin and terramycin were administered intramuscularly and intraperitoneally.

By eight hours time of infection, the fishes started showing disease symptoms and so they were given bath treatment by dip method.

While the positive controls died by 22 - 24 hr time, the fishes that received treatment although had a long survival time, died by 48 - 50 hr. Here, the antibiotics were effective _in vitro_ but ineffective _in vivo_.

The treatment results are almost similar to that of Conroy and Herman (1970) and Richards and Roberts (1978).

Escherichia coli (E 1, tables 5.3 - 5.4)

The organism was introduced in the experimental fishes through the usual two routes. Four hours later, the antibiotic, kanamycin, was administered intramuscularly and intraperitoneally. The fishes did not develop any disease symptoms, although one fish which received intramuscular injection of the antibiotic died six hours after the infection. All the rest of the fishes recovered back to normal by 20 - 24 hr time. The positive controls died within 18 hr.

These findings are almost in agreement with that of Conroy and Herman (1970); Bullock *et al.*, (1971) and Richards and Roberts (1978).

Streptococcus pyogenes (S 1, tables 5.3 - 5.4)

After 4 hr of infection of the experimental lobsters with the pathogen, they were given intramuscular injection of the antibiotics, chloromycetin, kanamycin, and terramycin. As they started showing disease symptoms

even after the antibiotic therapy, they were given bath treatment by dip method, twice daily. After 6 days, the lobsters returned to normal.

The positive controls died in 22 - 24 hr.

The results of the present study are almost similar to that of Stewart and Cornick (1967); Rabin and Hughes (1968) and Sindermann (1977).

Proteus vulgaris (TR 16, tables 5.3 - 5.4)

The experimental fishes were infected with the pathogen, *Proteus vulgaris* and after 4 hr, the fishes were injected with the antibiotic, kanamycin, intramuscularly and intraperitoneally. As the fishes started showing disease signs and symptoms, by 6 - 8 hr of infection, the fishes were further treated, by dip method in the kanamycin solution. The fishes were bathed twice daily and by the seventh day, they were found to be quite normal.

The positive controls developed the disease and died in 20 - 22 hr.

Here, the results are similar to those reported by Conroy and Herman (1970); Bullock *et al.*, (1971); van Duijn (1973) and Richards and Roberts (1978).

Although the antibiotics, chloromycetin, kanamycin and terramycin were ineffective *in vivo* to cure the disease, gill rot, in general the antibiotics were found useful to treat the diseases and the general effects of the antibiotics are presented in table 5.5.

Microbial diseases are comparatively more prevalent among finfishes and shellfishes than their diseases due to macroparasites. Over and above, microbial diseases are serious and problematic as transmission of pathogenic microbes is zoonotic and invisible to the naked eye, and thus difficult to detect early. Added to this, they have short generation time and proliferate rapidly. So, proper check against disease is through treatment and antibiotics are quite helpful.

Antibiotics, a definite key for the treatment and cure of various diseases of finfishes and shellfishes, are surely a milestone in our achievements. But, a bottleneck is, in the wide culture system, application of such costly drugs involves high expenditure which exceeds the anticipated profit from the culture practices.

TABLE 5.5

In vivo effect of antiseptics and antibiotics

Isolate(s)		Antiseptics	Effect	Antibiotics	Effect
Vibrio anguillarum	(SL 8)	Acriflavine	+	Chloromycetin	+
		Silver nitrate	+	Kanamycin	+
				Terramycin	+
Staphylococcus aureus	(SD 1)	Acriflavine	+	Chloromycetin	+
		Silver nitrate	+		
Pseudomonas aeruginosa	(HS 1)	NT		Gentamycin	+
Klebsiella pneumoniae	(GR 1)	Silver nitrate	-	Chloromycetin	-
				Kanamycin	-
				Terramycin	-
Escherichia coli	(E 1)	NT		Kanamycin	+
Streptococcus pyogenes	(S 1)	Acriflavine	+	Chloromycetin	+
		Silver nitrate	+	Kanamycin	+
				Terramycin	+
Proteus vulgaris	(TR16)	Potassium permanganate	-	Kanamycin	+
		Silver nitrate	+		
Penicillium sp.	(DM 1)	Silver nitrate	+	NT	

+ = Effective   - = Ineffective   NT = Not tested

So, it is always better to maintain strict prophylactic and hygienic conditions as the outbreaks of various diseases are often as a result of poor prophylactic and hygienic measures. Although cure by therapy is possible for the fish and shellfish diseases, it is only second in importance to better prophylaxis and hygienic measures since prevention is better than cure.

CHAPTER VI

HAEMATOLOGICAL STUDIES

HAEMATOLOGICAL STUDIES

Fish haematology is a relatively new field as compared to human haematology. Haematological parameters are useful aids in diagnosing diseases among terrestrial vertebrates and can be used as a tool for investigating fish diseases as well.

With emphasis in aquaculture, haematological studies of fish and shellfishes are quite essential and will be rewarding, as most of these parameters are vital in disease diagnosis.

Hardly any work has been carried out in fish haematology in India (Pradhan, 1961; Rao and Behera, 1973; Radhakrishnan *et al.*, 1976; and Rao *et al.*, 1980), while considerable amount of investigations have been undertaken elsewhere (Bolton, 1933; Catton, 1951; Mawdesley Thomas and Jolly, 1968; Conroy and Herman, 1970; Riggs, 1970; Bullock *et al.*, 1971; Hoffman, 1971; Satchell, 1971; Conroy, 1972; Hawkins and Mawdesley Thomas, 1972; van Duijn, 1973; Ribelin and Migaki, 1975 and Roberts, 1978).

Haematological parameters such as, erythrocyte counts, leucocyte counts, differential counts, haematocrit, haemoglobin, erythrocyte sedimentation rate are of equal significance in disease diagnosis. In the present study, however, only a single factor, haemoglobin, has been carried out due to certain limitations. The blood volume in the investigated fishes thereby giving a very low volume of blood for investigation. Besides, the size of the diseased fish to be investigated can not be foreseen.

Haemoglobin, was studied for only one kind of disease, viz., tail rot, in a single species, Etroplus suratensis, to understand whether any difference occurs comparing to that of normal healthy fish. As such, haemoglobin study was also made from the normal healthy fish kept as control for comparative verification of results and repeat trials.

The diseased and normal healthy fish were secured and brought alive to the laboratory from the Narakkal fish culture farm of the C.M.F.R.Institute.

Haemoglobin, the colouring matter of the erythrocytes, form about 95 per cent of the dry weight

of the erythrocytes. The salient function of the red cell is to store this pigment and transports it for circulation. But, haemoglobin inturn works primarily as a carrier of oxygen and also to a certain extent for the removal of carbon dioxide and to maintain the acid base balanse of the body. Haemoglobin, combines with oxygen by virtue of the iron, it contains. The classical feature of erythrocyte of fish is its nucleated nature, while human erythrocytes are non nucleated.

Since fishes are poikilotherms, blood temperature is low enabling greater amount of oxygen to be carried. Exceptions are certain polar fishes which have no haemoglobin but the blood plasma meets the oxygen demand. In general, the capacity for carrying carbon dioxide for disposal in water is significantly higher in deoxygenated than oxygenated blood.

During respiration, water is entered through the mouth and passed over the gills and out through the operculae. The ventilatory flow is driven by alternate expansion and contraction of the buccal and opercular chambers, acting in a suitable way that a

continuous water flow is maintained over the gills. Here, the blood in the secondary lamellae flows counter to the direction of water flow. So, the oxygen extraction, from the water, can be as high as 80 per cent (Roberts, 1978). Carbon dioxide is highly water soluble and therefore, can be easily released in the water from the gills.

In the light of these facts, the importance of haemoglobin is quite understandable and any sharp declining trend in the haemoglobin value can be suggestive of ill-health probably leading to mass mortality.

PROCEDURE

The haemoglobin was estimated according to the methods suggested by Hoffman (1971) with some modifications.

To estimate the haemoglobin, fish blood was drawn from the inactivated fish. It was done by a stunning blow on the dorsal side of the head or by

anaesthetizing the fish, using the anaesthetizing agent, chlorobutanol at a concentration of 1:1000 (wt/vol).

Extraction of blood

Fish blood sampling, is slightly difficult, due to the absence of superficial blood vessels. Although blood can be drawn out by cardiac puncture or through the renal portal vein, in the present investigation, it was obtained by making an oblique cut to sever the part of the caudal peduncle and fin from the rest of the body and the fish blood therefrom.

Prior to this, the dorsal and anal fins are cut off carefully from the anaesthetized fish. Then, the body is wiped well with a clean soft cloth before severing the caudal peduncle with a sterile surgical knife and bone cutting forceps. The first one to three drops of blood was allowed to flow out and the remaining blood was collected into convenient size test tubes with Ethylene diamine tetra acetic acid (EDTA).

To prepare test tubes, 0.5 ml of 2 percent EDTA solution was removed into a convenient size glass

test tubes with stopper and then the tube with EDTA solution was allowed to be evaporated at 140 °C and allowed to cool to room temperature before use.

Estimation of haemoglobin:

N/10 hydrochloric acid was taken in a clean and dry Sahli's tube upto the mark, 10. The blood to be estimated was taken in the Sahli's pipette upto the mark, 20. Filter paper was carefully used to wipe off the excess blood in the tip of the pipette. Carefully the pipette with the blood was dipped below the surface of the hydrochloric acid in the Sahli's tube and the blood expelled. The blood was mixed well with the acid by sucking back the mixture in the pipette and releasing the same back in the Sahli's tube. The process was repeated for 2 - 3 times. Then, the blood was retained undisturbed in the Sahli's tube for three minutes. The Sahli's tube with the blood and acid (acid haematin) was inserted in the central slot in the Sahli's haemoglobinometer. Distilled water was added slowly drop by drop until achieving the same hue to that of the reference (coloured glasses) in the haemoglobinometer. After five minutes from the time

of mixing of blood with the acid, the reading of the haemoglobin in grams per 100 ml of blood was made from the graduated measure in the Sahli's tube.

In fish blood, to avoid the possibility of high readings owing to the presence of nucleated erythrocytes which increase the turbidity of the fluid for haemoglobin reading and eliminate the possible errors, the readings obtained using the Sahli's haemoglobinometer were corrected according to the methods given by Larsen and Snieszko (1961) using the following formula

$$0.534 \times \text{AH Hb value} + 2.44$$

(AH Hb = acid haematin haemoglobin)

The haemoglobin was estimated within three hours from the time of collection of fish.

RESULTS AND DISCUSSION

The results, obtained in the present study of twenty diseased and a similar number of normal fish, are presented in table 6.1. For the normal healthy

TABLE 6.1

Haematological parameters of healthy and diseased fish, *Etroplus suratensis*

Sl. No.	Wet weight (in gm)		Total length (in cm)		Standard length (in cm)		Haemoglobin* (in gm/100ml)		Haemoglobin** (in gm/100 ml)	
	H	D	H	D	H	D	H	D	H	D
1	150	140	12.5	12.0	10.5	10.0	9.0	7.0	7.2	6.2
2	160	140	13.5	11.5	11.5	9.5	8.6	4.2	7.0	4.7
3	165	145	12.5	12.0	10.5	10.0	10.0	7.0	7.8	6.2
4	170	140	13.0	11.5	11.0	9.5	8.0	5.0	6.7	5.1
5	160	150	12.0	12.0	10.0	10.0	8.0	5.6	6.7	5.4
6	165	140	12.5	12.0	10.0	10.0	9.6	7.0	7.6	6.2
7	150	150	12.5	12.5	10.0	10.0	9.2	7.0	7.4	6.2
8	150	140	12.5	12.0	10.5	10.0	8.0	6.4	6.7	5.9
9	160	150	13.0	12.0	11.0	10.0	10.0	6.0	7.8	5.6
10	160	150	13.0	12.5	11.0	10.5	8.0	6.0	6.7	5.6

H = Healthy
D = Diseased
* = Sahli's readings
** = Readings according to Larsen and Snieszko

TABLE 6.1 (Contd.)

Haematological parameters of healthy and diseased fish, *Etropius suratensis*

Sl. No.	Wet weight (in gm)		Total length (in cm)		Standard length (in cm)		Haemoglobin* (in gm/100 ml)		Haemoglobin** (in gm/100 ml)	
	H	D	H	D	H	D	H	D	H	D
11	165	170	12.5	13.0	10.0	11.0	8.2	7.0	6.8	6.2
12	150	150	12.0	12.0	10.0	10.0	9.0	6.2	7.2	5.8
13	150	145	12.0	12.0	10.0	10.0	9.0	6.4	7.2	5.9
14	165	150	13.0	12.0	11.0	10.0	9.2	7.0	7.4	6.2
15	170	150	13.0	12.0	11.0	10.0	9.6	5.6	7.6	5.4
16	150	140	13.0	12.0	11.0	10.0	8.6	5.0	7.0	5.1
17	155	165	12.5	13.0	10.0	11.0	8.0	4.6	6.7	4.9
18	160	150	12.5	12.0	10.0	10.0	9.0	6.0	7.2	5.6
19	170	160	13.5	12.5	11.0	10.0	10.0	6.4	7.8	5.9
20	140	155	12.0	12.5	10.0	10.0	8.8	4.6	7.1	4.9

H = Healthy

D = Diseased

* = Sahli's readings

** = Readings according to Larsen and Snieszko

fish, the haemoglobin value ranged 6.7 to 7.8 gm per 100 ml and in the case of diseased fish, it declined from 6.2 to 4.7 gm per 100 ml (values according to Larsen and Snieszko, 1961).

Pradhan (1961) reported that the normal haemoglobin value ranged 9 - 12 gm per 100 ml for fresh water fish, *Tilapia* sp. and 8 - 20 gm per 100 ml for brackish water fish, *Macrones* sp., *Ophiocephalus* sp., and *Periopthalmus* sp. stating that she found a comparatively higher haemoglobin rate in brackish water fishes than that of fresh water fishes. 6.4 gm per 100 ml of haemoglobin in normal hake, *Merluccius merluccius* was reported by Conroy and Rodriguez (1965). Conroy and Herman (1970) reported a reduction of haemoglobin to below 5.3 gm per 100 ml in diseased carp with dropsy, while in normal fish, the value was found to be 9.6 gm per 100 ml. Bullock *et al.*, (1971) noticed that haemoglobin value decreased to 5.3 to 2.0 gm per 100 ml in the case of diseased carp with hemorrhagic septicemia while the value for the normal carp was 9.6 gm per 100 ml. Conroy (1972) observed the haemoglobin value for the spawning adults (Atlantic salmon, *Salmo salar*) ranging 6.4 to 11.5 gm

per 100 ml. The haemoglobin value in gm per 100 ml for normal fish, *Labeo umbratus*, 6.8; *Labeo capensis*, 7.3; *Clarias gariepinus*, 5.8; *Cyprinus carpio*, 5.8; *Tilapia mossambica*, 5.6 was recorded by Hattingh (1972). Rao and Behera investigated an average haemoglobin content, 9.1 gm per 100 ml in normal *Catla catla* (Gangetic carp). But, haemoglobin rate in normal and diseased Atlantic salmon, *Salmo salar* was noticed by Foda (1973) as 8.6 - 11.0 and 5.0 down to 3.7 respectively. Mulcahy (1975) observed that the mean haemoglobin rate in normal pike as 8.8 gm per 100 ml and in diseased pike with spontaneous lymphoma, 6.4 gm per 100 ml. Higher content of haemoglobin, in normal air breathing mud eel, *Amphipnous cuchia*, 13.8 to 26.0 gm per 100 ml was recorded by Mishra *et al.*, (1971). The mean haemoglobin concentration in normal rohu, *Labeo rohita* was found to be 9.0 gm per 100 ml by Siddiqui and Naseem (1979). Based on the investigations in normal and diseased rainbow trout, it has been stated by Barham *et al.*, (1980) that they noticed a significant decrease in the haemoglobin value, in diseased fish, comparing to that of normal fish.

While it is understood that haemoglobin concentration can also be changed due to ecological, interspecies and even intraspecies variations, the above investigations establish the fact that microbial infection or associated diseases can definitely decrease the haemoglobin concentration.

Comparing to the haemoglobin value for the normal fish, a sharp decrease in haemoglobin, 6.2 - 4.7 gm per 100 ml (values according to Larsen and Snieszko, 1961) for the diseased fish in the present investigation could be due to anorexia as a result of bacterial infection. Here, for the tail rot disease in Etroplus suratensis not much blood loss was noticed. Moreover, anorexia due to bacterial infection causes a decrease in haemoglobin concentration. This incapaciates proper oxygen carrying capacity which probably leads to anoxia and mortality.

The low concentration of haemoglobin, in the case of diseased fish, is indicative of anaemia.

Although anaemia can be treated (van Duijn, 1973), if left uncared this would lead to mass mortality.

The result, obtained in the present investigation on a cultivable species of finfish, *Etroplus suratensis*, is yet another instance to corroborate the significance of haemotological studies in fish pathology.

CHAPTER VII

ELECTROCARDIOGRAPHY

ELECTROCARDIOGRAPHY

Electrocardiography is a study of the graphic record (electrocardiogram or E C G) of the electrical activity produced in the myocardium due to the passage of an active depolarising current. The first electrocardiogram was obtained in 1887 by Waller by employing a capilliary electrometer. Wilhem Einthoven became the father of electrocardiography by inventing the string galvanometer, in 1903, which became the standard instrument for recording electrocardiogram.

Although lot of work has been carried out regarding the application of E C G in human beings, very little work has been done in aquatic animals (Roberts, 1978 and Richards, 1979 Personal communication). So, a teleost fish, Etroplus suratensis was chosen for its E C G study inorder to find out its application in stress and disease diagnosis.

In finfish, the heart enclosed by pericardium is situated anterior to the main body cavity at the base of the pharynx. It is a muscular organ for pumping blood to the different parts of the body. The heart consists of four chambers, viz., sinus venosus,

atrium, ventricle and bulbus arteriosus. All chambers except bulbus arteriosus are contractile and an unidirectional flow of blood is maintained by valves at the sino-atrial, atrio-ventricular and bulbo-ventricular orifices.

Deoxygenated venous blood enters the sinus venosus from the ductus cuvieri and main veins through the sino-atrial valves and the blood passes into the atrium which is placed dorsal to the ventricle. By the contraction of the atrium, the blood enters the ventricle. From the ventricle, blood passes to the bulbus arteriosus through a pair of valves.

The blood from the heart passes initially through ventral aorta into the gill circulation and thereafter through the dorsal aorta to the systemic circulation.

The important characteristics of the heart are rhythmicity, contractility, distensibility, excitability and conductivity.

The cells of the heart, unlike those of organs such as the liver, do not regenerate when damaged. Hence diseases affecting the heart would need immediate

attention. Damage to the myocardium or conduction system can show variations in the point of origin, spread and recovery of the electrical activity and these could be recorded in the electrocardiogram.

Cardiac disorders like arrhythmia, myocardial infarction, atrial and ventricular hypertrophy could be easily diagnosed from the E C G tracings. Myocardial necrosis which results in cardiac failure is associated with bacterial infections. Gram negative rods have been noted causing myocardial necrosis (Roberts, 1978). The electrocardiographic changes in such infected fishes are only rarely known and only scanty literature is available showing normal E C G descriptions of finfishes (Kisch, 1948; Oets, 1950; Satchell, 1971 and Roberts, 1978).

E C G can be a quick primary investigation for diagnosing microbial diseases if an E C G criteria for heart rate/rhythm/P/PR is available. Moreover if the E C G could be a tool for early detection of imbalances leading to diseases, it will be yet another aid to take prompt decisions about the actions to be taken to protect the cultivated stock. This will help

in the better management of the culture system and help to also decide whether the standing stock is to be immediately treated or fished out.

For the first time in India, the candidate made an attempt to study the E C G patterns both in healthy and diseased *Etroplus suratensis*. To artificially induce diseases, six types of fish pathogens were used, *viz*.,

1) *Klebsiella pneumoniae*
2) *Escherichia coli*
3) *Pseudomonas aeruginosa*
4) *Proteus vulgaris*
5) *Staphylococcus aureus*

and 6) *Streptococcus pyogenes*

MATERIALS AND METHODS

Suitably quarantined fishes, (*Etroplus suratensis*) were maintained in eighty litre capacity aluminium framed glass tanks. Only two fishes were kept per tank to avoid overcrowding and to minimise any stress during the quarantine period. All the ecological parameters such as suitable salinity,

dissolved oxygen, pH, water temperature, food etc. were maintained optimum to keep the fish in good health. These were kept constant during the control study as well as during the study of the effect of the pathogens on fish E C G.

18 - 24 hr aged slant culture was washed in sterile normal saline and the cell suspension was used for injection into fishes. Viable cell counts of the cell suspension was made using pour plate method and the counts ranged 2.1×10^7 to 4.1×10^9 per millilitre. And, the dose of cell suspension introduced into the body of the fish was 0.3 ml. Sterile one ml tuberculine syringe with needle no 26 gauge was used for injection. For the present experiments, intramuscular or intraperitoneal injection was given. The intramuscular injection was given through the dorso lateral region just below the posterior end of the dorsal fin. The intraperitoneal injection was given through the lateral aspect of the abdominal wall, inserting the needle obliquely, so that no internal organs are damaged (Conroy and Herman, 1970). The intraperitoneally injected fish was differentiated by proper tagging, with sterile coloured threads, in their candal fin.

Immediately before and after injections in the anaesthetized fishes, the site of injection was gently swabbed with absorbent cotton wetted in 70% alcohol. Once the fish was anaesthetized for recording E C G, the fish was maintained in a conditioning tank, for about five minutes, from where the fish was finally replaced in their respective tanks.

A set of fish injected similarly with sterile normal saline was served as controls. Always adequate care was taken for gentle handling to minimise stress.

Chloretone at a concentration of 1 : 1000 (wt/vol) was used as the fish anaesthetic.

E C G was taken from the anaesthetized fishes immediately before injections and at fixed intervals following injections. The anaesthetized fish was gently placed, on a comfortable surgical tray, on its lateral side. Proper gill irrigation was maintained with the help of a rate adjustable polythene drip tube held through the mouth of the fish (Pl. 19 : fig. 19). The gill irrigation was maintained at a rate of 250 - 300 drops of aerated water per minute. The electrocardiogram was recorded using surface disc electrodes and they were found

to be adequate and convenient. It was better than the needle electrodes as they minimised stress. The disc electrodes were connected to the equipment, Cardio-aid (DMS 600, Simonsen and Weel, Type CB 600 M, Denmark). Yellow disc (left arm) was placed on the top dorsal aspect near to the anterior part of the dorsal fin, the red (right arm) on the ventral side near the pectoral fin and the black one (right leg) was placed on the mid caudal region (Pl. 19 : fig. 20). The electrodes were well positioned on the body of the fish with the help of an electrode gel (Aquasonic 100, Parker laboratories Inc., Orange, N.3. 07050 USA). The E C G waves were observed on an Oscilloscope (Pl. 20 : fig. 21). The electrocardiogram was recorded on conventional wax coated, heat sensitive E C G paper (Pl. 20 : fig. 22).

The paper speed was 25 mm per second. The cardiograph papers used for the experiments were Tokushu Menon (London), Electrodyne (USA) and Myeica (London).

All the experiments were carried out at room temperature.

To minimise the diurnal rhythm in the heart rate, the experiments were started in the morning at 06.00 hr.

ANALYSIS OF RESULTS AND DISCUSSION

The data obtained from the present study is presented in table 7.1 (Pl. 21, 22 : fig. 23, 24).

The different wave patterns noticed in the E C G recordings are P, QRS, T and U.

P = Signals the variable deflection as a result of depolarisation of the atrium.

QRS complex :

Q = Initial negative deflection of ventricular depolarisation.

R = Positive deflection of ventricular depolarisation.

S = Negative deflection following R wave.

T = Variable deflection revealing the repolarisation of the ventricular muscle.

U = A deflection (usually positive) seen following T wave. The exact cause is not known.

TR = It is the time taken for the passage of the impulse from atrium to ventricle.

QT = Interval from ventricular depolarisation to repolarisation.

QRS width = Duration taken for ventricular depolarisation.

QRS axis = It shows the direction of electrical depolarisation of the ventricle.

QRS height = It represents the electrical activity generated by the ventricular myocardium.

The wave patterns, P, QRS, T and U (Pl. 23 : fig. 25) are the rhythemic electrical depolarisation or repolarisation of the myocardium which preceeds the contractions (mechanical activity).

Based on the study, the heart rate, rhythm, P, PR, QRS width, QRS axis, QRS height, T, QT and U were studied in detail. To find out the statistical significance, heart rate, QRS height and QT were also studied.

Heart rate :

Clinical experiments, in general, do not have scope for many replications as required for any valid statistical inference due to their inherent variations.

Added to that, there are wide variations among the observations even when they are taken under controlled conditions. The data under analysis are no exceptions.

Six pathogens are employed in this experiment to study their effects upon the heart of the fish, Etroplus suratensis. Under each pathogen, there are only 5 or 6 observations and that too, over different intervals, sometimes not showing any trend over time. Inorder to have a base for comparison of the effects of different pathogens, the results of all controls in this category, were taken together to form the base. In that, the range was 50 - 140 (Table 7.1, Pl. 21, 22 : fig. 23, 24) which is so wide encompassing almost all other observations obtained under the six pathogens.

Inorder to have a better base, interval wise averages were taken together and the range for these average values was considered for the analysis here, under the assumption that controls, over time, should have more or less same effect on the animals considering them as a homogeneous group as test fish did not differ much in size and weight. So far as length and weight of each animal is concerned, the corresponding standard

deviation 'σ' was obtained using the tables for σ/R for different 'n' (Snedecor and Cochran, 1967). For the controls, the result for the 9th hr in the table was not considered as the value was recorded only once.

On the basis of the above method, control charts $\bar{x}$, $\bar{x} \pm 2\sigma$ and $\bar{x} \pm 3\sigma$ have been drawn finding out the values as follows.

Total of five averages = 97.0 + 95.3 + 95.7 + 71.5 + 90.0

= 449.5

n = 5

$\bar{x} = \frac{449.5}{5}$

= 89.9

R, Range (71.5 – 97.0) = 25.5

σ/R = 0.43 (Snedecor and Cochran, 1967)

σ = 0.43 x R

= 0.43 x 25.5

= 10.9

2σ = 10.9 x 2

= 21.8

$3\sigma = 10.9 \times 3$
$= 32.7$

$\bar{x} - 2\sigma = 89.9 - 21.8$
$= 68.1$

$\bar{x} - 3\sigma = 89.9 - 32.7$
$= 57.2$

$\bar{x} + 2\sigma = 89.9 + 21.8$
$= 111.7$

$\bar{x} + 3\sigma = 89.9 + 32.7$
$= 122.6$

The values for heart rate, for the infected, were then plotted in the graph (Pl. 33 : fig. 45) as in table 7.1.

Although heart regulation in fish is not very precise (Randall, 1970) usually heart rate along with the clinical symptoms can be taken as an index of the health status of an organism. Any altered heart rate signifies mostly the pathophysiological condition of the heart.

For Salmo gairdneri, the active heart rate in the normoxic environment was found to vary between 45 - 58 beats per minute (Weintraub, 1975). In relation

to swimming speed, the standard heart rate is 36 - 38 beats per minute and active heart rate is 90 - 94 beats per minute (Priede and Tytler, 1977 and Priede and Young, 1977).

In the present investigation on *Etroplus suratensis*, the mean values for the heart rate for the controls ranged to 72 - 97 per minute (Table 7.2). In comparison with the works of Priede and Tytler (1977) and Priede and Young (1977) a slightly higher rate is noticed here which could be possibly due to the reason that *E. suratensis* is a warm water species.

Regarding the heart rate for the infected, generally a declining trend, over time, is noticed.

Klebsiella pneumoniae

A clear declining trend of heart rate, 100 to 84 beats per minute is noticed within 11 hr of infection (Pl. 24 : fig. 27, 28).

Escherichia coli

Here also, heart rate slowed from 64 to 45 beats per minute, but before death, a slightly increased heart rate, 60 beats per minute is

TABLE 7.2

Heart rate per minute for controls

(*Etroplus suratensis*)

Time in hr \ Heart beat	*Klebsiella pneumoniae*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Proteus vulgaris*	*Staphylococcus aureus*	*Streptococcus pyogenes*	Total	Average
0	140	96	50	100	100	96	582	97.0
6	140	96	60	84	100	92	572	95.3
9	120						120	120
11	140	96	46	100	100	92	574	95.6
15			60		62.5	92	2145	71.5
18			90					90

R, Range (71.5 – 97.0) = 25.5

noticed (Pl. 25 : fig. 29, 30). This should be on account of the sudden decline and cardiac arrest as seen in the case of *Pseudomonas aeruginosa*.

Pseudomonas aeruginosa

In this case, a sharp declining trend of heart rate is observed, starting from 50 beats per minute at the 0 hr with an increased heart beat at the 6th hr after infection. At the 18th hr, the rate slowed down to 20 beats per minute (Pl. 26, 27 : fig. 31 - 34). At the 20th hr, typical bradycardia was registered (Pl. 27 : fig. 33, 34) from the infected fish which was found almost dead. This indicated the ectopic beats and cardiac arrest.

Proteus vulgaris

Within eleven hr of infection, the heart rate declined to a stage of cardiac standstill (Pl. 28 : fig. 35, 36).

Staphylococcus aureus

Here again, a declining trend of heart rate, 100 - 60 beats per minute, was noticed within 11 hr of infection. At the 13th hr, cardiac standstill could be registered. In the case of host where the pathogen was introduced through the intraperitoneal route, the rate slowed down, 92 - 60, giving a rapid effect over time (Pl. 29, 30 : fig. 37 - 40).

Streptococcus pyogenes

The pathogen, introduced in the body through intramuscular and intraperitoneal injections, could exercise an effect of decreased heart rate, 92 to 45 beats per minute and 100 to cardiac standstill, by 15 hr time, respectively. But the effect was rapid in the case of intraperitoneal injection (Pl. 31, 32 : fig. 41 - 44).

Comparing the controls, in the case of infected animals, a general declining trend of heart rate is detected which leads to the conclusion that the pathogens have an effect on retarding heart rate in

some cases resulting in cardiac arrest. Statistical examination of the data also points to this. Among the pathogens tested, Pseudomonas aeruginosa is found to be most potent (Pl. 26, 27 : fig. 31 - 34). The present study thus shows that the heart rate could also be considered as an index of cardiac disease induced by pathogenic organisms as often causative factors.

Partial and complete heart block are characterised by changes in the ventricular rate. When beats are missed occasionally, the pulse is irregular either at regular or irregular intervals. In the more fully developed condition, marked slowing (bradycardia) of the pulse occurs. This was noticed typically in the case of the pathogen, Pseudomonas aeruginosa (Pl. 27 : fig. 33, 34).

It is known that even touching a fish could induce bradycardia via the reflex mechanism which has a gradient sensitivity, beginning at the head and terminating at the tail (Kisch, 1950). But, in the present case, the bradycardia evoked is only because of the pathogen while all the other parameters

were the same for both controls and experimental fishes where the injected fish showed a steep declining trend of heart rate. The significance of infection inducing marked bradycardia is evident from these experiments.

Electrocardiogram of teleost fishes is almost similar to that of other vertebrates, peaking at about 0.7 mV on the QRS level (Roberts, 1978). But in the present study, it ranged between 0.2 - 0.3 mV. This could be possibly due to the reason that *Etroplus suratensis* is an active tropical species. Oets (1950) has also indicated that tropical species may show a different QRS deflection. The wave pattern of the E C G will be variable in different species of fishes (Richards, 1979 Personal communication).

QRS height :

On the basis of the above said method, control charts $\bar{\bar{x}}$, $\bar{\bar{x}} \pm 2\sigma$ and $\bar{\bar{x}} \pm 3\sigma$ have been drawn based on the results in the table 7.3.

$$\text{Total of five averages} = 0.75 + 1.08 + 1.08 + 1.33 + 1.00 = 5.24$$

$$n = 5$$

TABLE 7.3

QRS Height in millimeter for controls

(*Etroplus suratensis*)

QRS Height / Time in hr	*Klebsiella pneumoniae*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Proteus vulgaris*	*Staphylococcus aureus*	*Streptococcus pyogenes*	Total	Average
0	1.0	0.5	1.0	0.5	1.0	0.5	4.5	0.75
6	1.5	1.0	0.5	1.5	1.5	0.5	6.5	1.08
9	2.0						2.0	2.00
11	1.5	1.0	0.5	1.5	1.5	0.5	6.5	1.08
15			0.5		3.0	0.5	4.0	1.33
18			1.0				1.0	1.00

R, Range (0.75 - 1.33) = 0.58

$$\bar{x} = \frac{5.24}{5}$$

$$= 1.04$$

R, Range (0.75 - 1.33)	= 0.58
σ /R	= 0.43
σ	= 0.43 x R
	= 0.43 x 0.58
	= 0.25
2 σ	= 0.25 x 2
	= 0.50
3 σ	= 0.25 x 3
	= 0.75
$\bar{x}$ - 2 σ	= 1.04 - 0.50
	= 0.54
$\bar{x}$ - 3 σ	= 1.04 - 0.75
	= 0.29
$\bar{x}$ + 2 σ	= 1.04 + 0.50
	= 1.54
$\bar{x}$ + 3 σ	= 1.04 + 0.75
	= 1.79

The values, for QRS height for the infected (Table 7.1), were then plotted in the graph (Pl. 34 : fig. 46).

The results revealed an overall declining trend of QRS height for the infected fishes. This sounds that QRS height is also a valid factor in fish disease diagnosis.

QT interval :

Based on the method already discussed, the control charts $\bar{x}$, $\bar{x} \pm 2\sigma$ and $\bar{x} \pm 3\sigma$ have been drawn in consultation with the values in table 7.4.

$$
\begin{aligned}
\text{Total of five averages} &= 0.36 + 0.34 + 0.35 + 0.35 \\
&\quad + 0.34 \\
&= 1.74 \\
n &= 5 \\
\bar{x} &= \frac{1.74}{5} \\
&= 0.35 \\
R,\ \text{Range}\ (0.34 - 0.36) &= 0.02 \\
\sigma / R &= 0.43 \\
\sigma &= 0.43 \times R \\
&= 0.43 \times 0.02 \\
&= 0.0086 \\
&= 0.009 \\
2\sigma &= 0.009 \times 2 \\
&= 0.018
\end{aligned}
$$

TABLE 7.4

QT In seconds for controls

(*Etroplus suratensis*)

Time in hr \ QT Interval	*Klebsiella pneumoniae*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Proteus vulgaris*	*Staphylococcus aureus*	*Streptococcus pyogenes*	Total	Average
0	0.32	0.32	0.40	0.36	0.40	0.36	2.16	0.36
6	0.28	0.36	0.36	0.32	0.34	0.36	2.02	0.34
9	0.36						0.36	0.36
11	0.32	0.36	0.44	0.32	0.32	0.36	2.12	0.35
15			0.36		0.32	0.36	1.04	0.35
18			0.34				0.34	0.34

R, Range (0.34 - 0.36) = 0.02

$3\sigma = 0.009 \times 3$

$= 0.027$

$\bar{x} - 2\sigma = 0.35 - 0.018$

$= 0.33$

$\bar{x} - 3\sigma = 0.35 - 0.027$

$= 0.32$

$\bar{x} + 2\sigma = 0.35 + 0.018$

$= 0.368$

$= 0.37$

$\bar{x} + 3\sigma = 0.35 + 0.027$

$= 0.377$

$= 0.38$

The values for QT interval for the infected (Table 7.1) were then plotted in the graph (Pl. 35 : fig. 47).

QT interval varies from 0.20 - 0.46 seconds (Satchell, 1971). In the present study, the QT interval for the controls ranged 0.34 to 0.36 seconds almost agreeing to that of Satchell (1971). But, the QT interval in the case of infected fishes was relatively high comparing to that of controls, in the present investigation.

The QT interval recorded in the present study

is larger than that of mammals and conforms to the views of Satchell (1971). But, a declining trend of QT interval, in the case of infected fishes, over a period of time, is noticed. In the present QT interval analysis, *Escherichia coli*, *Pseudomonas aeruginosa* and *Streptococcus pyogenes* are found to be statistically significant pathogens.

Rhythm :

While sinus rhythm is noticed for all the controls, in the case of infected fishes irregular rhythm was detected (Table 7.1). Irregular rhythm along with clinical symptoms is useful for disease diagnosis.

The rhythm is affected which possibly could be due to SA, AV node infection which leads to heart block. The ischemic lesions developed in the heart may finally lead to impaired activity. As a chain action, again it leads to heart block leading to bradycardia and cardiac standstill.

P :

In finfishes, P varies from 0.04 to 0.08

seconds (Satchell, 1971). Absence of P reveals the non functioning of atrium. It could be due to necrosis of the tissue and non functioning of the atrium. It could also occur in few other conditions such as mechanical damage to the organ.

In the present study while controls revealed the presence of P and the absence of P in the case of infected fish registers the fact that P become absent due to necrosis of atrium as a result of infection. Hence for convenience, the 'P' can be classified as infective P and non infective P.

If the P is infective, it is potentially dangerous. Because if this is not found out in time, it leads to ventricular necrosis leading finally to cardiac standstill. Moreover, here the vertical and horizontal transmission of pathogens also cannot be ruled out. This is proved further by recording the E C G of naturally infected fishes with the tail rot disease. In two cases (Pl. 23 : fig. 26) studied, the absence of P and occasional ectopia, where the P is infective, were found. Thus, mass mortalities, due to tail rot, reported should be definitely due to

heart necrosis and not merely due to tissue decay, in the caudal aspects, associated with bacterial infection.

PR :

Satchell (1971) has stated that the PR interval varies from 0.08 - 0.24 seconds in finfishes. But, Kisch (1948) recorded a time frequency of 0.12 - 0.26 seconds in bony fishes. PR conduction time is generally shorter in teleost fishes than in selechians. Usually increasing heart rate is accompanied by an increase in PR interval and if the heart rate is slowed down, the PR interval also becomes shorter. This is because of the well known effect of the heart rate on a strained a - v conduction.

In the present study, the PR for the controls ranged to 0.1 to 0.36 seconds for the controls. In the case of infected fishes, the PR was not registered wherever P was absent. In the case of fish infected with the pathogen, Klebsiella pneumoniae, PR ranged 0.12 to 0.20 seconds. Here again, while the control was having, at the 11th hr of the experiment, 0.14 seconds, the infected fish had only 0.12 seconds.

This fact is further confirmed in the case of the pathogen, *Proteus vulgaris* where the infected at the 6th hr of infection was having only 0.20 seconds, the corresponding control registered a time frequency of 0.28 seconds.

QRS width :

QRS width for the controls in the present study was 0.04 seconds which is also the same for the infected fishes. As no change could be noticed, the significance of QRS width in diagnosing diseases is doubtful.

QRS axis :

For both the control and infected fishes, QRS axis was normal. As no change was noticed in the case of infected fishes, QRS axis as a tool for disease diagnosis is doubtful.

T :

In all the controls, the T wave was noted as upright, biphasic and flat. But, once an inverted T was noticed in the case of *Streptococcus pyogenes* infected host. Here, although no significant results

could be achieved and as instances such as inverted T is registered, the T wave may serve as an useful tool for disease diagnosis. Kisch (1948) also has detected this wave system.

U :

The U wave, was noticed in normal fishes, but never recorded in diseased fishes. The exact genesis of this wave is not known. Hence the significance of its absence in diseased fishes is not clear. U wave may also be helpful in detecting diseases. This wave also has been detected by Kisch (1948). The presence or absence of U wave helps in detecting some imbalances and a considerable amount of work is needed to establish the use of U wave in disease diagnosis. At present, the absence of U wave indicates a definite imbalance and in the present study the absence was with the infected fish and not in the controls.

Salient Features

1. Decreasing heart rate is an useful index for diagnosing infection in fishes. The E C G data has been analysed and found to be statistically significant.

2. Ectopic beats (missing beats) occur in diseased fishes.

3. Absence of P (atrial necrosis) and associated heart block are found in diseased fishes.

4. Bradycardia (very slow heart rate) and cardiac arrest in advanced stage of the disease.

5. Changes in QT interval (decreased QT interval) is a sign for disease diagnosis.

6. Absence of U wave is found in diseased fishes.

CHAPTER VIII

SUMMARY

SUMMARY

Sea food can substantially contribute to meet the presently felt need of protein gap. The prospects of increasing fish production from the sea appears to have limitations. One way of supplementing capture fisheries production is by aquaculture of cultivable species of finfishes and shellfishes. However, these farmed species are often plagued by problems of diseases. The fundamental and applied aspect of diseases in hatchery and culture system spelling the success or failure of aquaculture practices needs no emphasis.

In the present investigation by the candidate, fifteen types of diseases due to virus, bacteria and fungi such as black spot disease, enteritis, eye disease, fin rot, gill rot, hemorrhagic septicemia, muscle necrosis, shell disease, skin lesion, skin spottiness, streptococcosis, tail rot, proliferative epithelial tumour, deep mycosis and dermatomycosis are documented in twenty one species of finfishes and shellfishes and the causative factors studied. Among these, the diseases of bacterial origin predominated,

occurring in nineteen species of finfishes and shellfishes. The fungal diseases, dermatomycosis occurred in the finfish, _Anguilla bicolor bicolor_ and deep mycosis in the larvae of _Penaeus indicus_. Proliferative epithelial tumour due to viral infection was detected in a species of finfish, _Arius jella_.

The diseased finfishes and shellfishes were obtained from the fish farms of different Research Centers of the Central Marine Fisheries Research Institute; from Vellayani Lake (Trivandrum); and from the inshore waters off Cochin.

The pathological samples removed from the diseased finfishes and shellfishes were cultured in different types of media, in two sets one with aged and filtered sea water base and the other with 0.5% sodium chloride with distilled water base.

The suspected causative agent(s) was/were isolated from among those organisms predominating in the media.

In total, excluding twenty fungal strains of _Penicillium_ sp. (two different species) as causative agents for dermatomycosis and deep mycosis, 115 bacterial

strains were isolated among which Gram negative asporogenous rods predominated. The isolates belonged to the species of the genera, *Alcaligenes*, *Bacillus*, *Escherichia*, *Flavobacterium*, *Klebsiella*, *Micrococcus*, *Photobacterium*, *Proteus*, *Pseudomonas*, *Staphylococcus*, *Streptococcus* and *Vibrio*. A case of virus infection in *Arius jella* was studied and satisfied Rivers' postulates.

Representative isolates were selected and subjected to pathogenicity studies suitably *in vivo* in the finfishes *Etroplus suratensis*, *Anguilla bicolor bicolor*; prawns *Penaeus indicus*, *Penaeus monodon*; and the lobster *Panulirus homarus*.

The strains were introduced in the test species by intramuscular, intraperitoneal routes and also via superficial injuries and site injections. Only seven bacterial strains, *viz*., *Vibrio anguillarum*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli*, *Streptococcus pyogenes* and *Proteus vulgaris* and one fungal strain, *Penicillium* sp. could be categorised as definitely pathogenic.

The obligate pathogens were further studied for their sensitivity to various therapeutic agents which

are used for the treatment of finfish and shellfish diseases.

The antiseptics tested were acriflavine, copper sulphate, potassium permanganate and silver nitrate. The antibiotics studied were gentamycin, chloromycetin, kanamycin, terramycin and griseofulvin.

After in vitro studies, the effect of the therapeutic agents was tested in the test species, *Etroplus suratensis*, *Penaeus indicus*, *Penaeus monodon* and *Panulirus homarus*. The antiseptics were applied by swab method and the antibiotics were administered by intramuscular, intraperitoneal routes and by dip method.

The effect of the antiseptics and the antibiotics varied from pathogen to pathogen. However, the antiseptics, acriflavine and silver nitrate were found quite effective. The antibiotics gentamycin, chloromycetin, kanamycin and terramycin were powerful enough to cure the microbial diseases. The antibiotic, gentamycin was tested against only one pathogen, *Pseudomonas aeruginosa* as this organism was found insensitive to the other antibiotics under study. This reveals the fact that the cross infection due to this pathogen is potentially dangerous as antibiotic

treatment against this pathogen may be difficult and so, efforts should be made to check this pathogen, at least by strict hygiene and proper prophylaxis. Griseofulvin was completely ineffective against the fungal strain, Penicillium sp.

Studies, on haematological parameter - haemoglobin, carried out in a species of finfish, Etroplus suratensis diseased naturally with tail rot, and in the normal healthy finfish of the same species, revealed the fact that in the case of diseased fishes there existed a sharp decline of value of haemoglobin resulting in anaemia as compared to the normal healthy fish.

Electrocardiographical studies were conducted in normal healthy finfish, Etroplus suratensis, and also in the same species by provoking diseases by injecting the pathogens, Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris, Staphylococcus aureus and Streptococcus pyogenes. The cardiological parameters, P, PR, QRS width, QRS axis, QRS height, T, QT and U were studied. Statistical analysis of these data could extend new waves of light that the heart rate could be considered as an index of microbial diseases and its diagnosis. Studies, on

electrocardiography in _Etroplus suratensis_ diseased with tail rot as a result of natural infection, registered the atrial necrosis and associated cardiac involvement. Disease diagnosis through electrocardiography deserves greater attention.

The present study emphasizes the need that for the successful farming of finfish and shellfish, strict hygiene and suitable prophylaxis are essential. Moreover, inorder to make the wealth of the sea food fully available, the eradication of diseases affecting finfishes and shellfishes is absolutely necessary for which studies such as the present investigation are inevitable.

The present study, though answering only a fraction of the problems, highlights the need for an intensive programme for pathobiological investigations in our country.

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Not referred in original

PLATES I – XXXV

Plate 1: fig. 1 (top) Skin lesion caused by *Pseudomonas fluorescens* in *Chanos chanos*

(bottom) Close up view showing the skin lesion

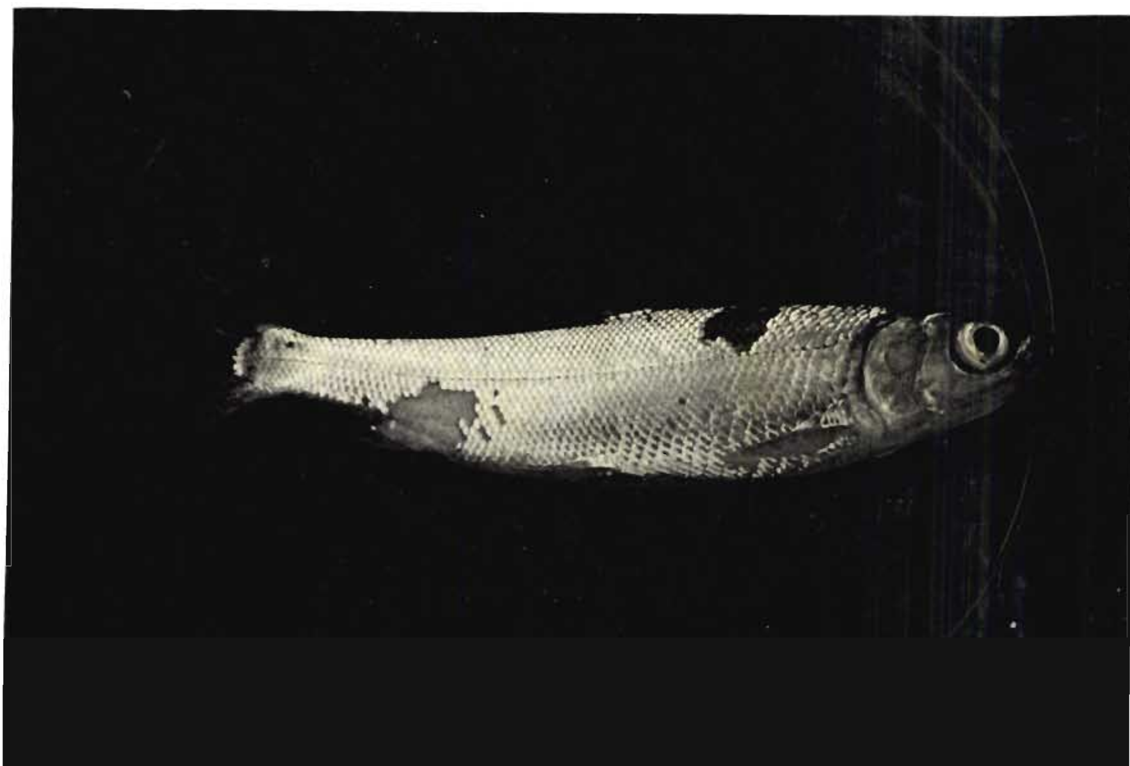
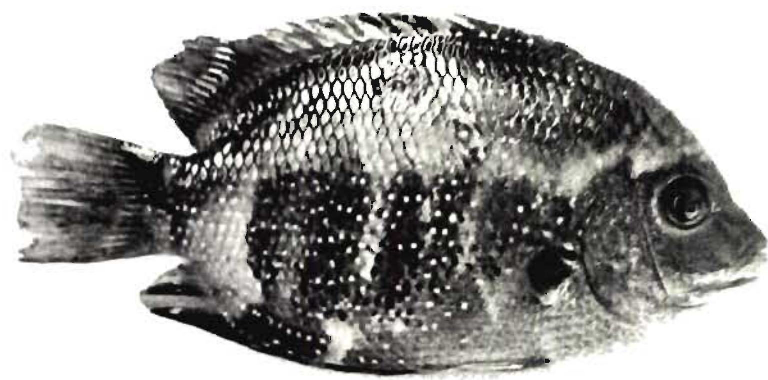


Plate 2: fig. 2 (top) Skin lesion caused by *Pseudomonas fluorescens* in *Etroplus suratensis*

(bottom) Close up view showing the skin lesion



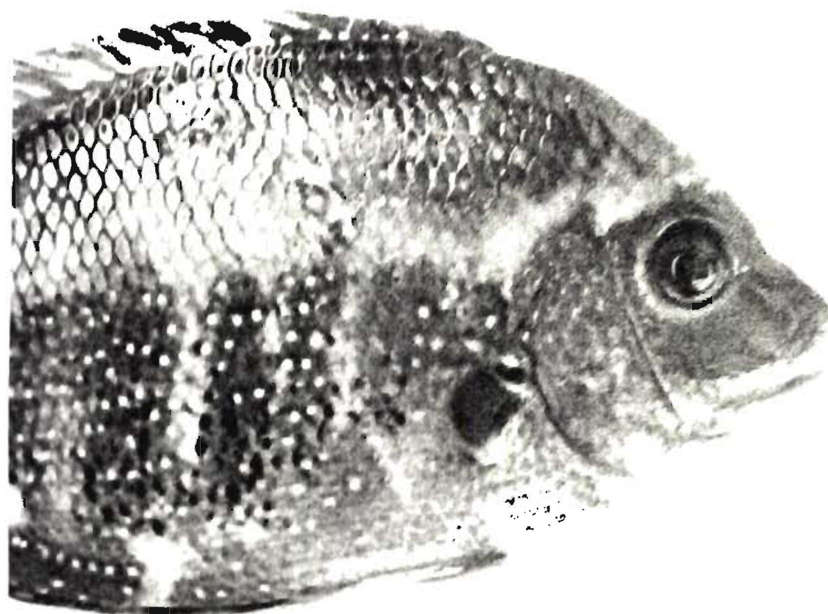


Plate 3: fig. 3

(top) Skin lesion (vibriosis) caused by *Vibrio anguillarum* in *Etroplus suratensis*

(bottom) Close up view showing the skin lesion

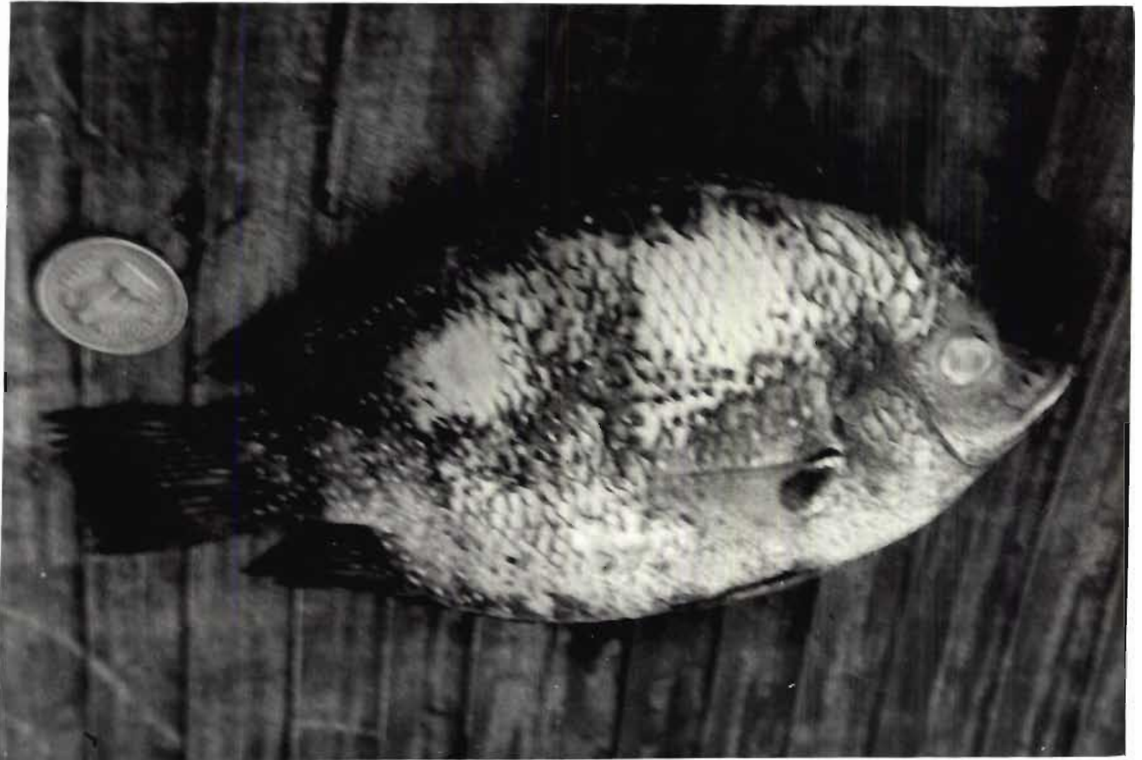
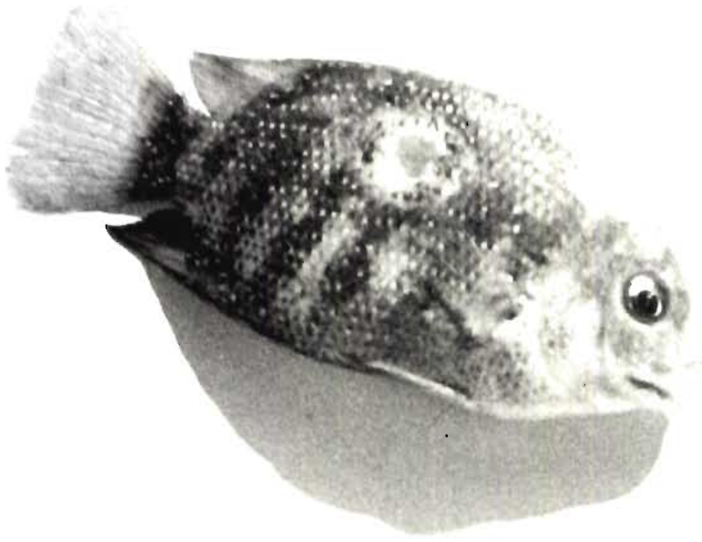


Plate 4: fig. 4 (top) Skin spottiness caused by *Vibrio fischeri* in *Etroplus suratensis*

(bottom) Close up view showing the skin spottiness



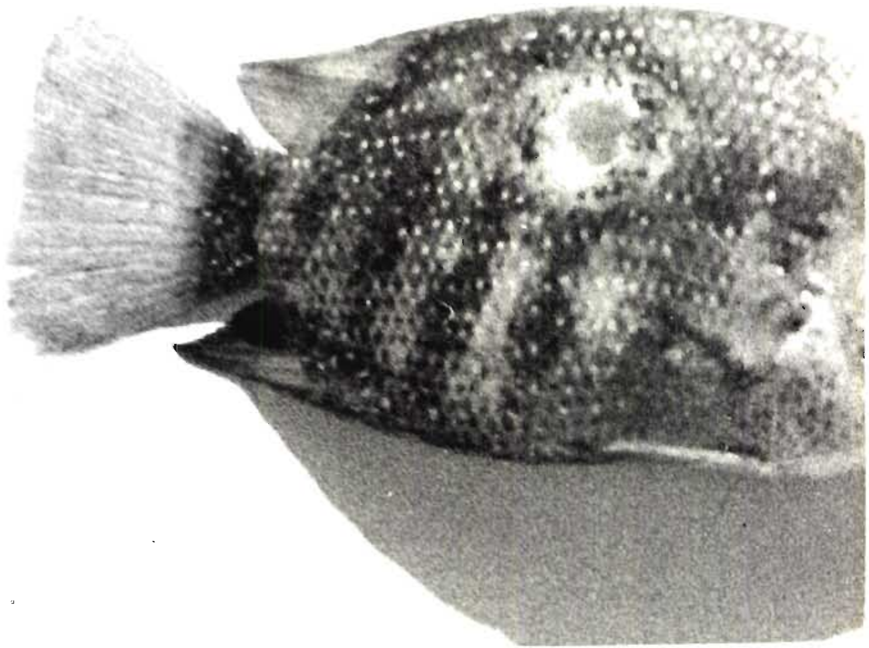


Plate 5: fig. 5 (top) Shell disease due to *Staphylococcus aureus* in *Penaeus indicus*

(bottom) Close up view of the infected exoskeleton

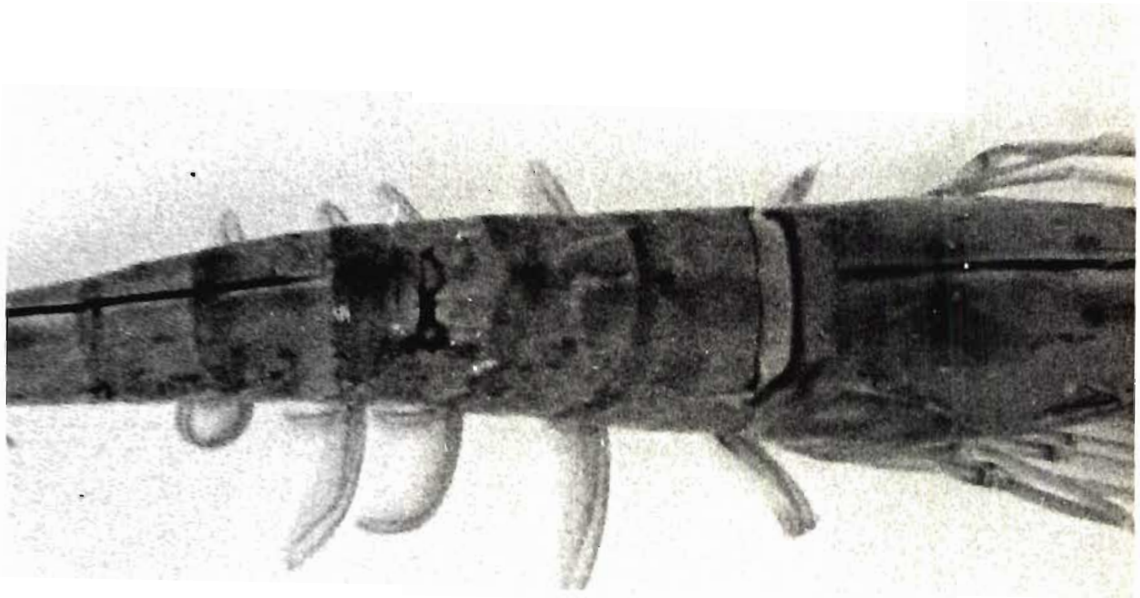


Plate 6: fig. 6 Hemorrhagic septicemia in *Etroplus suratensis* caused by *Pseudomonas aeruginosa*

Plate 7: fig. 7 (top) Eye disease in *Etroplus suratensis* due to *Micrococcus varians*

(bottom) Close up view showing the infected eye

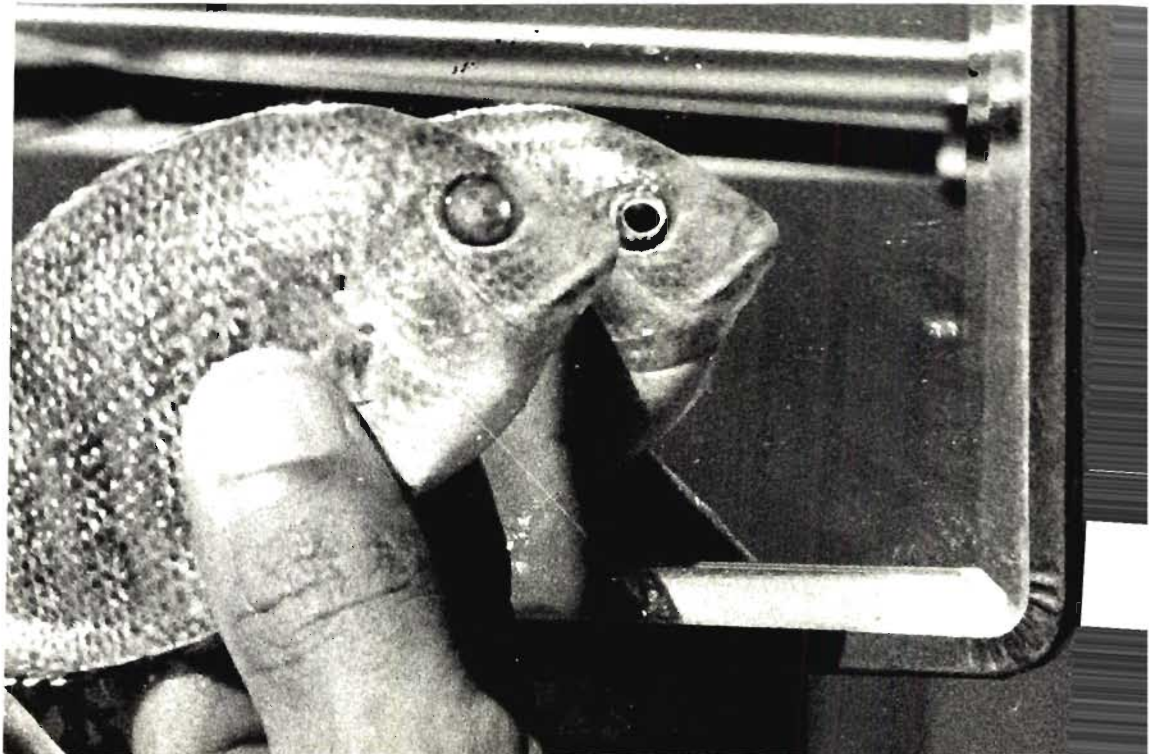
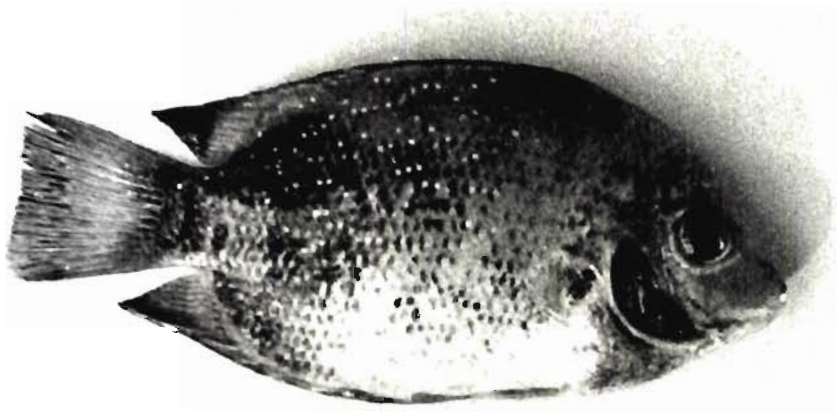


Plate 8: fig. 8 (top) Gill rot in *Etroplus suratensis* caused by *Klebsiella pneumoniae*

(bottom) Close up view showing the infected gills



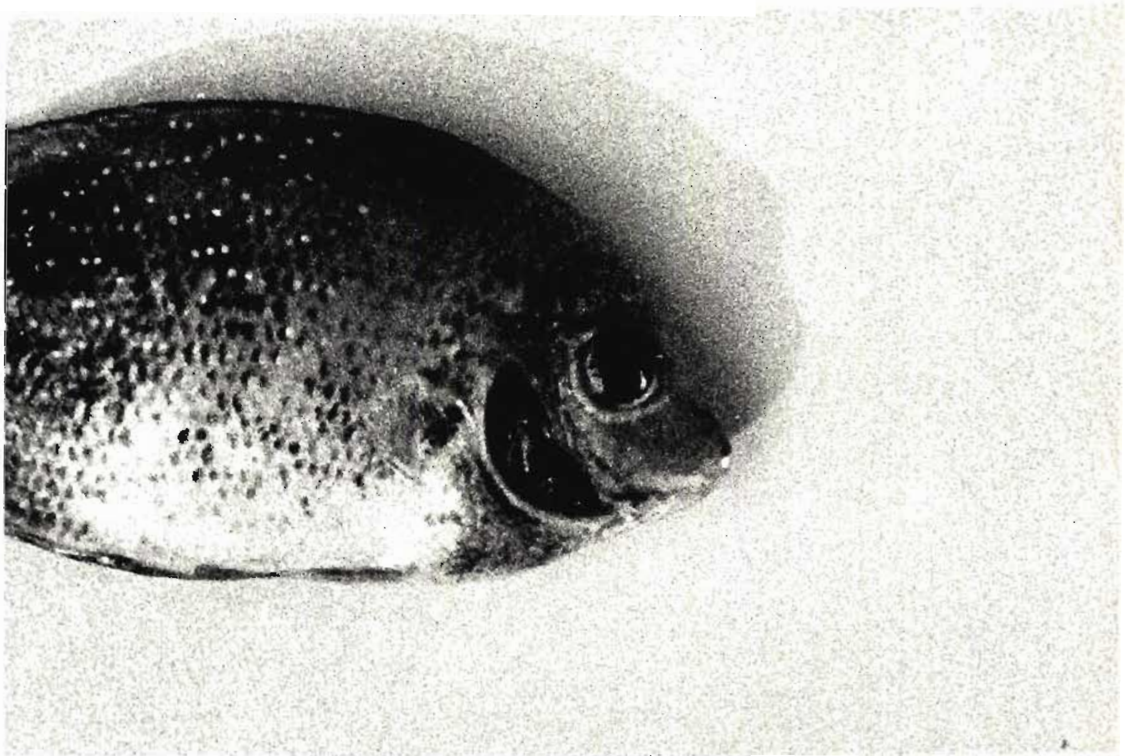


Plate 9: fig. 9 (top) Gill rot in *Tilapia mossambica* due to *Klebsiella pneumoniae*

(bottom) Close up view showing the infected gills

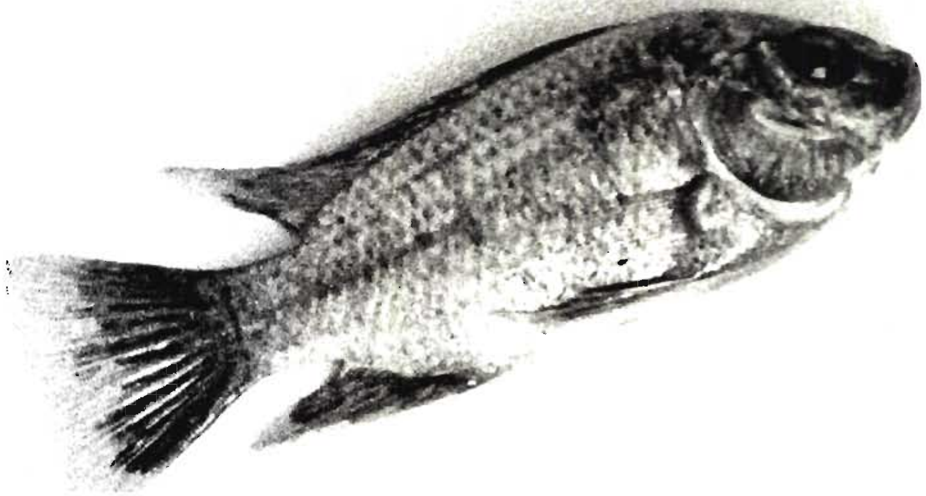


Plate 10: fig. 10 (top) Black spot disease in *Mugil cephalus* caused by *Alcaligenes eutrophus*

(bottom) Close up view showing the infected area

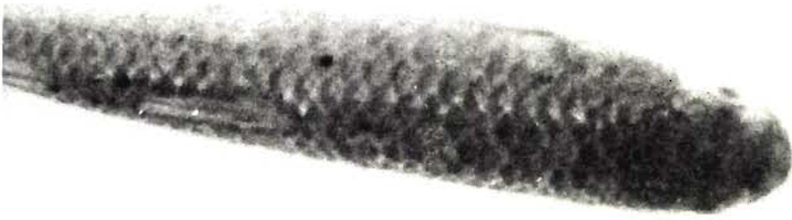
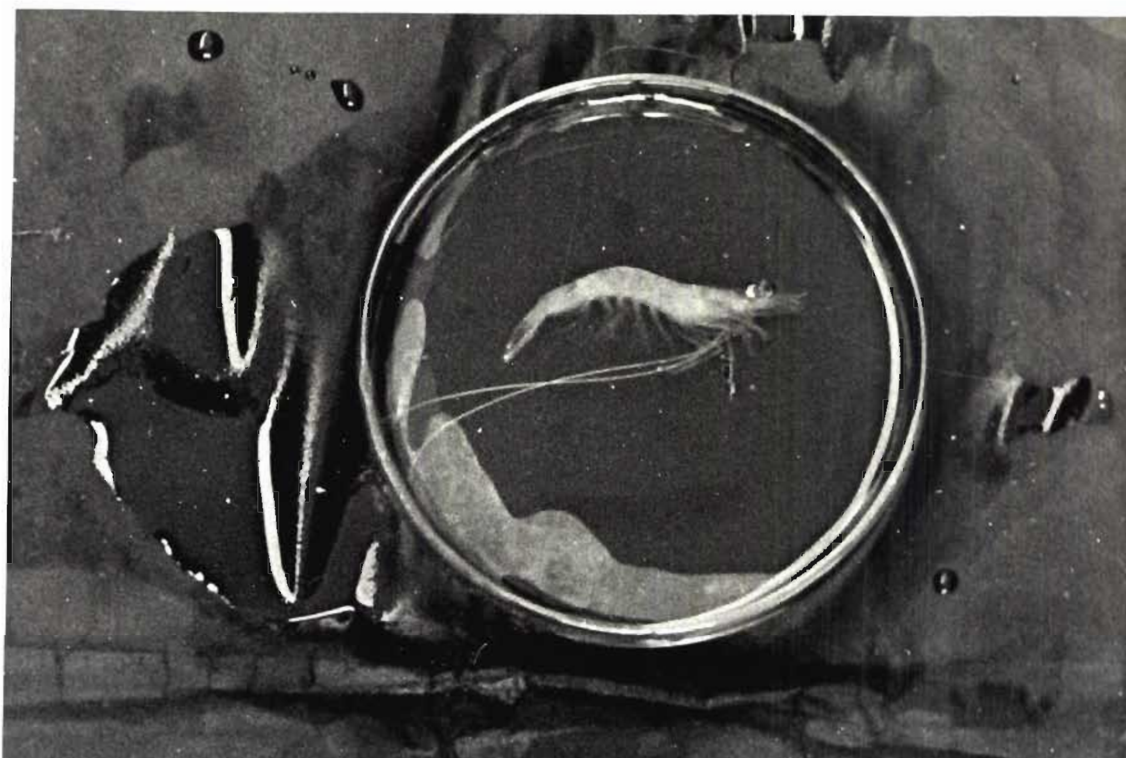


Plate 11: fig. 11 (top) Black spot disease in *Penaeus indicus* due to *Vibrio fischeri*

(bottom) The infected area in close up view

Plate 12: fig. 12 (top) Muscle necrosis in *Penaeus indicus* larvae due to *Vibrio fischeri*

(bottom) Close up view of the infected area in the abdominal segments



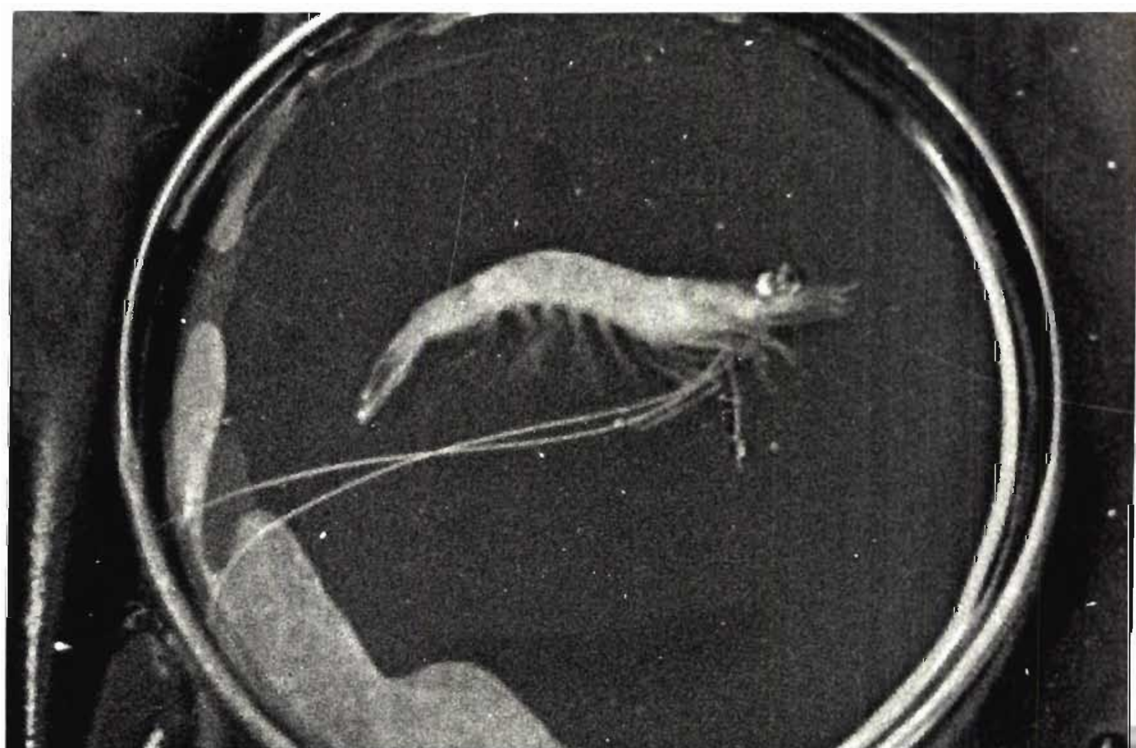
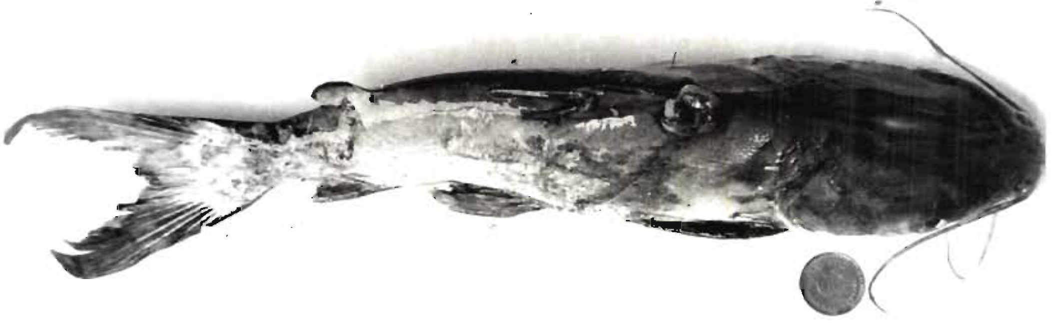


Plate 13: fig. 13 (top) Enteritis in *Etroplus suratensis* due to *Escherichia coli*

(bottom) Close up view showing the characteristic skin lesion on the dorsal and ventral side of the fish

Plate 14: fig. 14 (top) Proliferative epithelial tumour in *Arius jella* (Associate: *Pseudomonas mendocina*)

(bottom) Close up view of the tumour



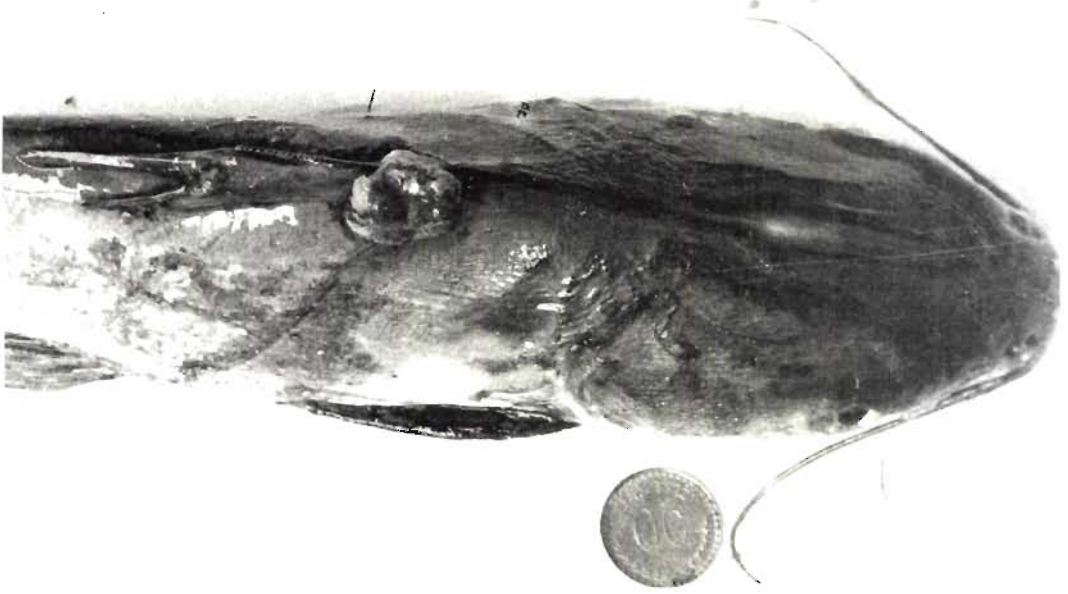


Plate 15: fig. 15 (top) Streptococcosis in *Panulirus homarus* due to *Streptococcus pyogenes*

(bottom) Close up view of the infected tail

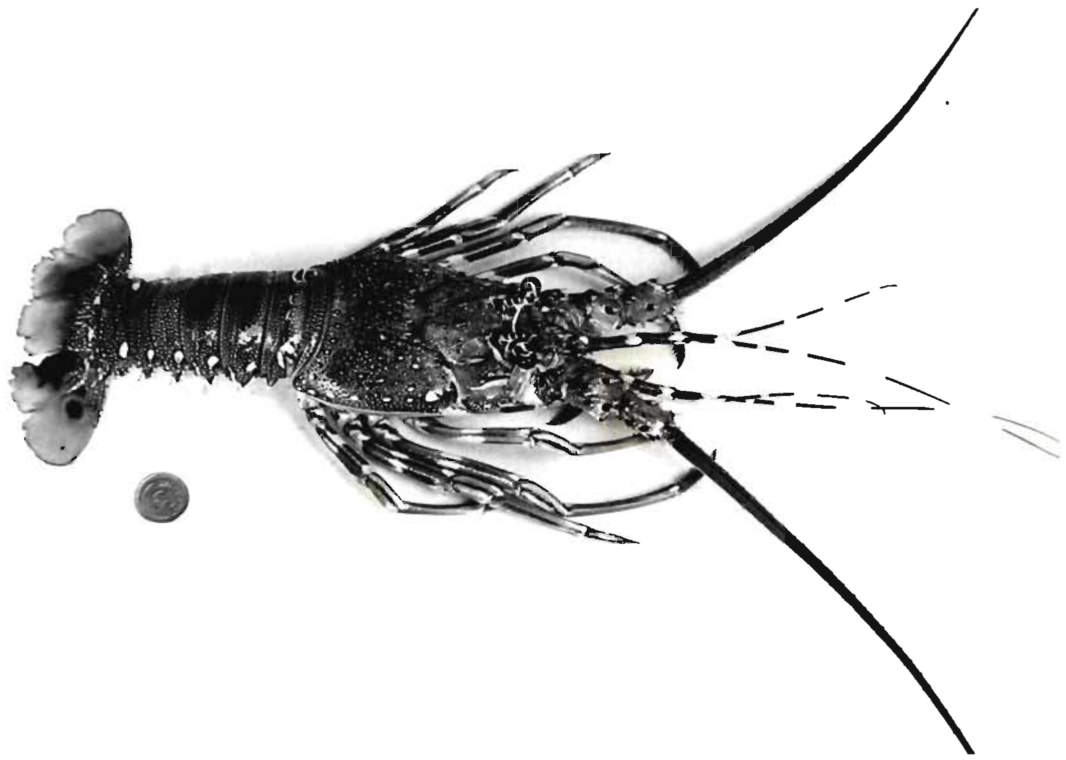
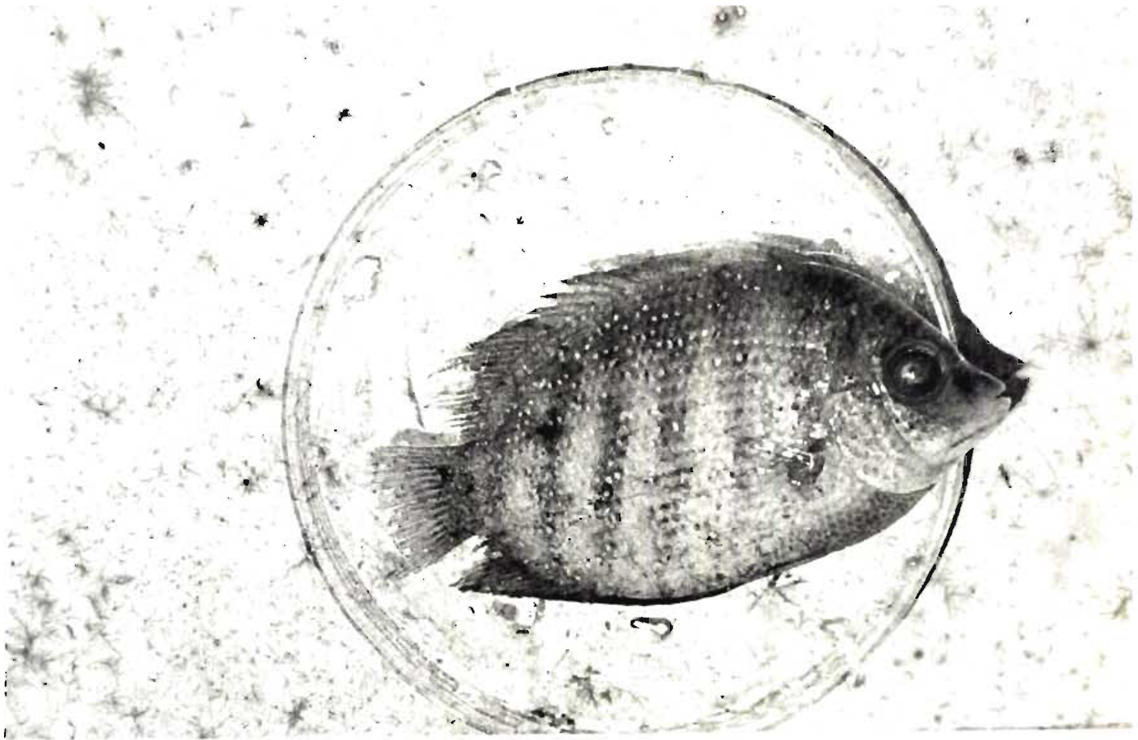


Plate 16: fig. 16 (top) Tail rot in *Etroplus suratensis* due to *Photobacterium phosphoreum*

(bottom) Tail rot in close up view



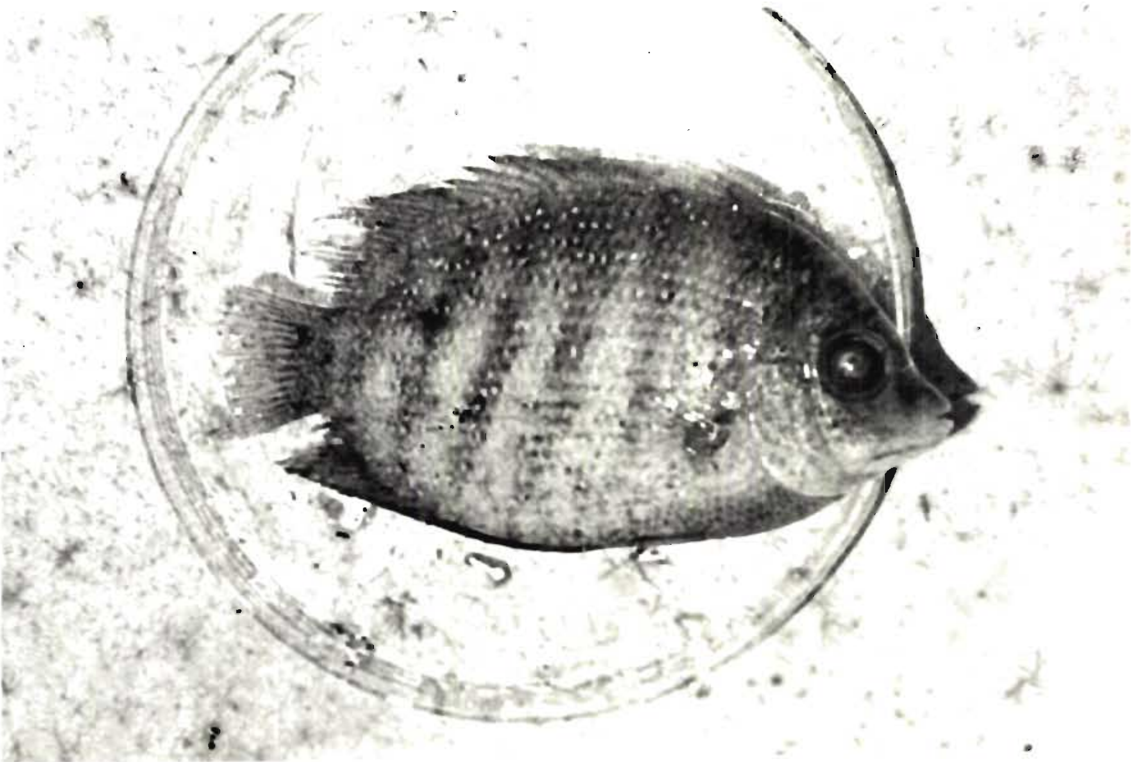
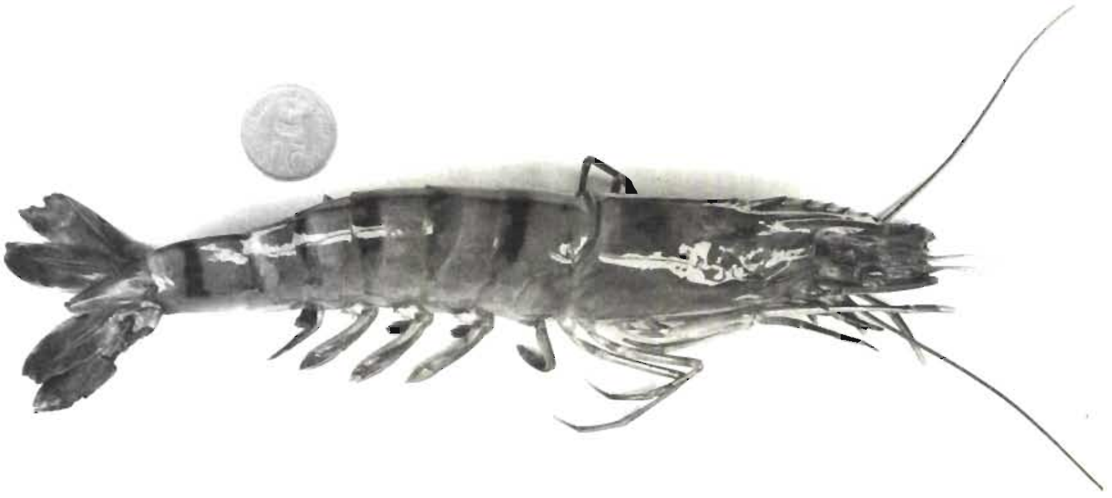


Plate 17: fig. 17	(top)	Tail rot in *Penaeus monodon* due to *Flavobacterium uliginosum*
	(bottom)	Close up view of the infected tail



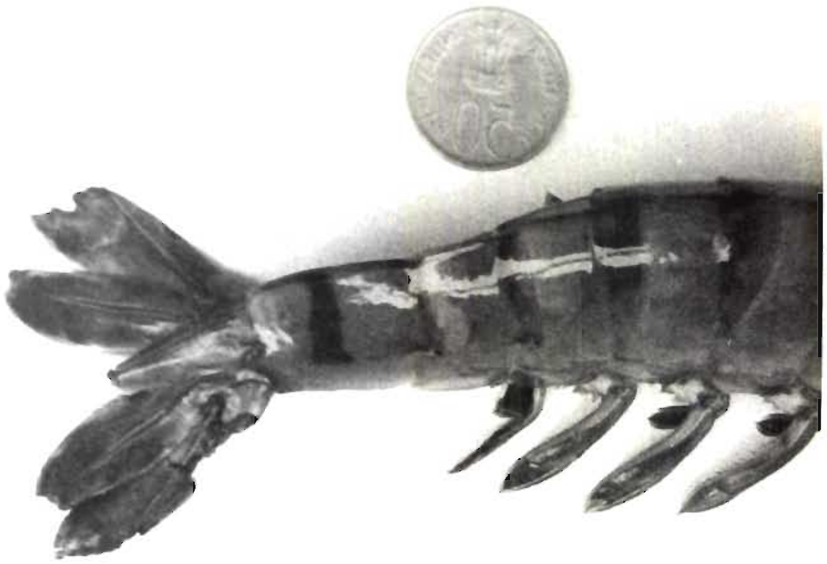


Plate 18: fig. 18 (top) Dermatomycosis in *Anguilla bicolor bicolor* due to *Penicillium* sp.

(bottom) The infected area in close up view

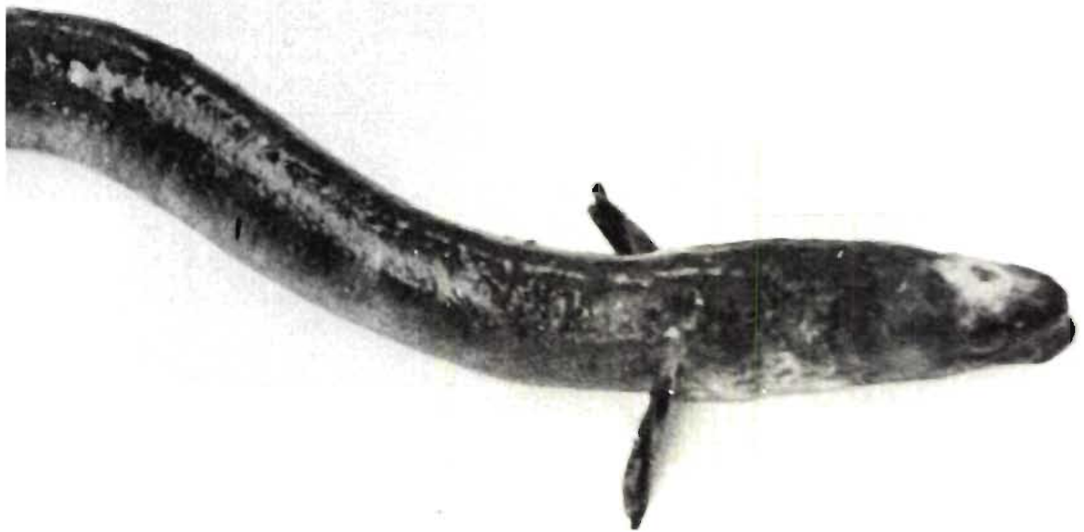
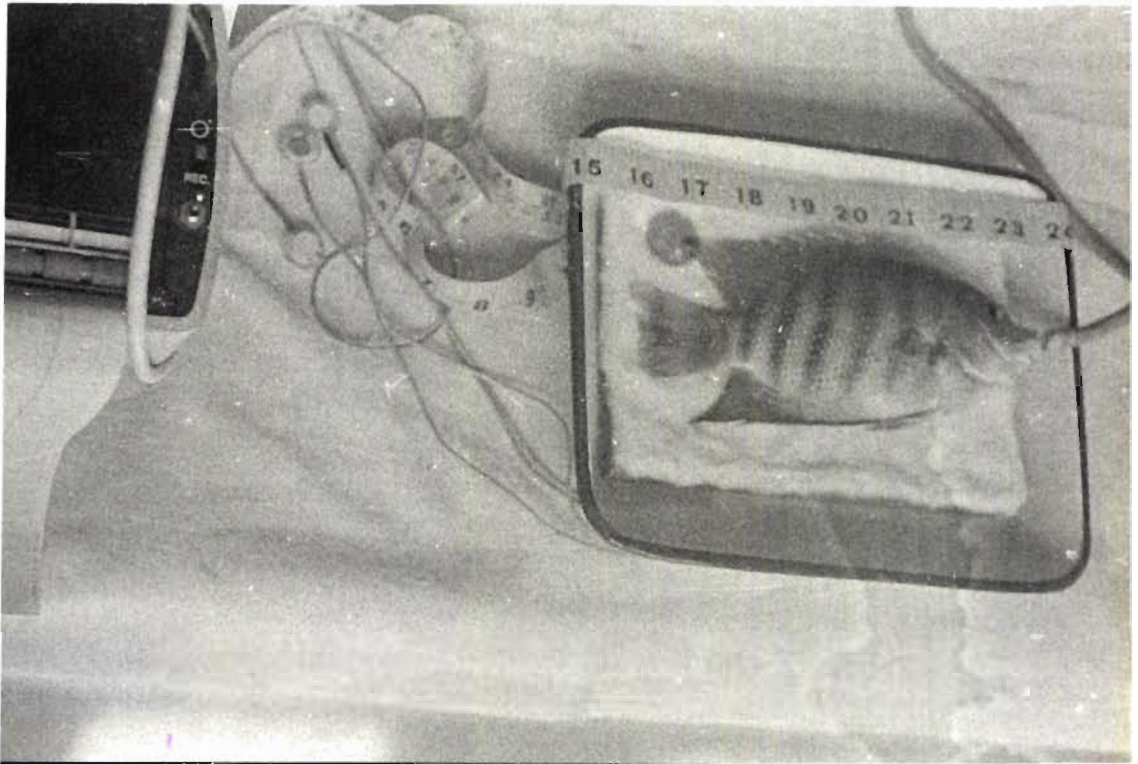


Plate 19: fig. 19 (top) *Etroplus suratensis* with rate adjustable polythene drip tube held through the mouth for gill irrigation

Plate 19: fig. 20 (bottom) *Etroplus suratensis* getting ready with surface disc electrodes


15 16 17 18 19 20 21 22 23

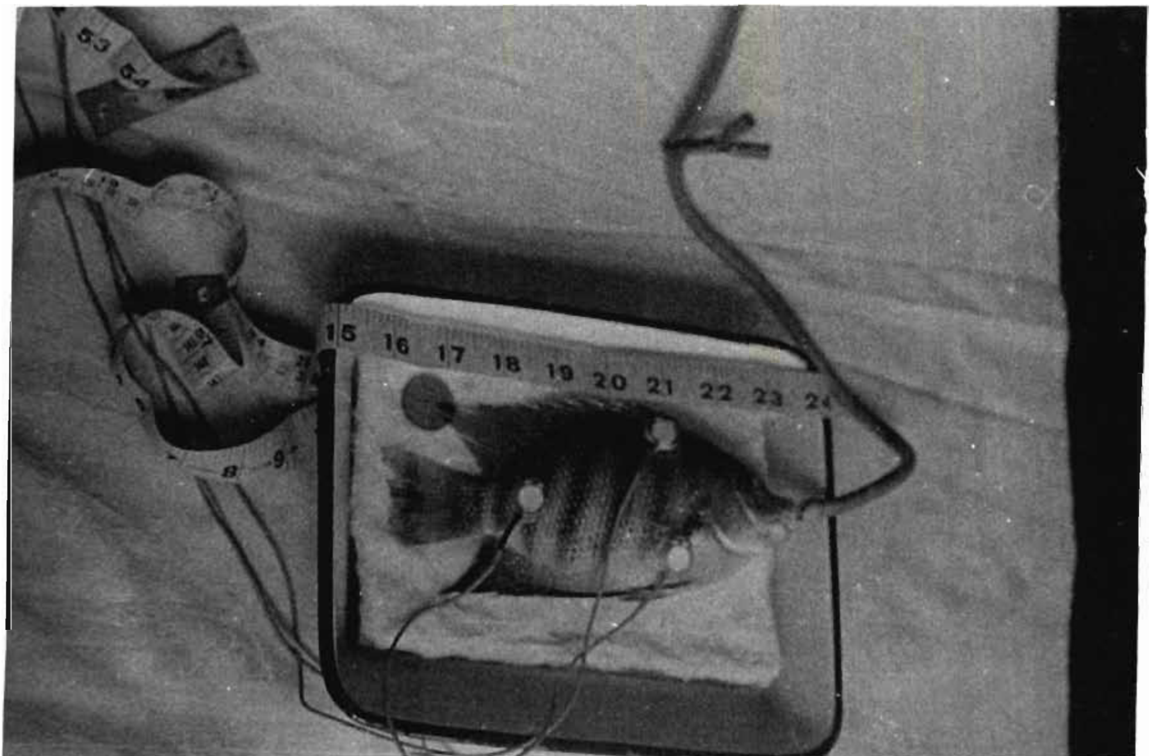
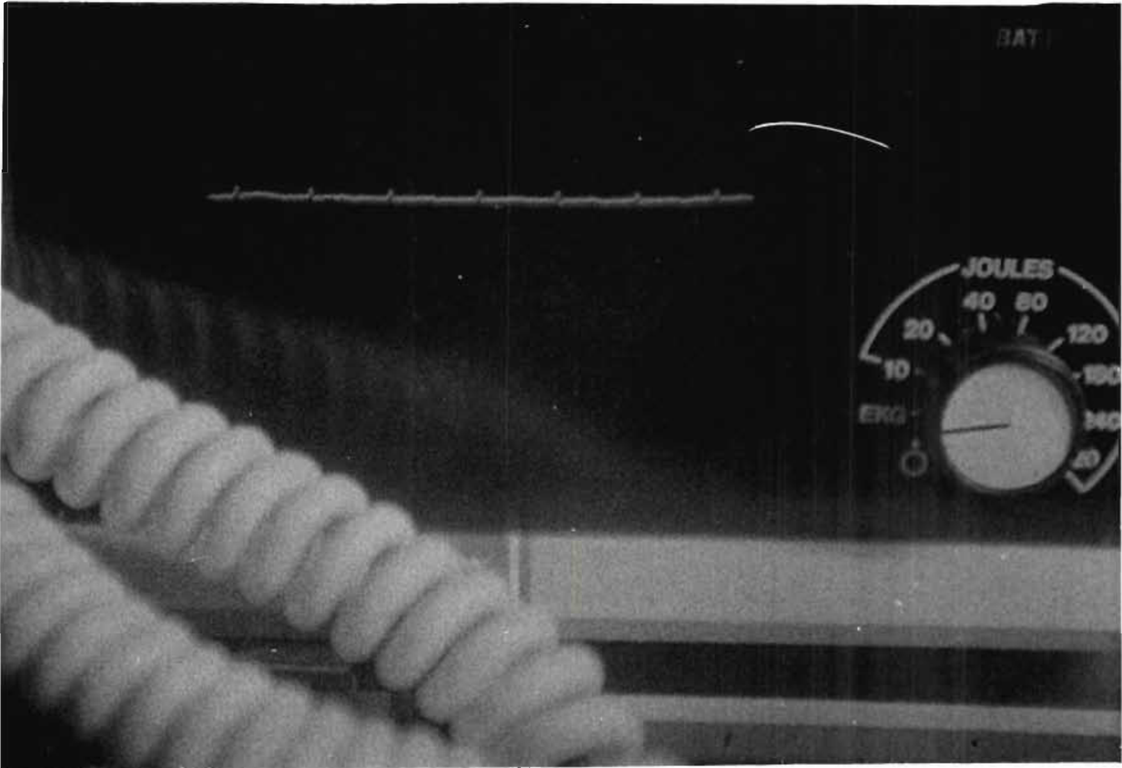

15 16 17 18 19 20 21 22 23

Plate 20: fig. 21 (top) DMS 600 (Oscilloscope) showing the fish E C G

Plate 20: fig. 22 (bottom) The Cardio aid - DMS 600 recording E C G of the Pearl spot *Etroplus suratensis*


JOULES
40
80
20
120
10
EKG

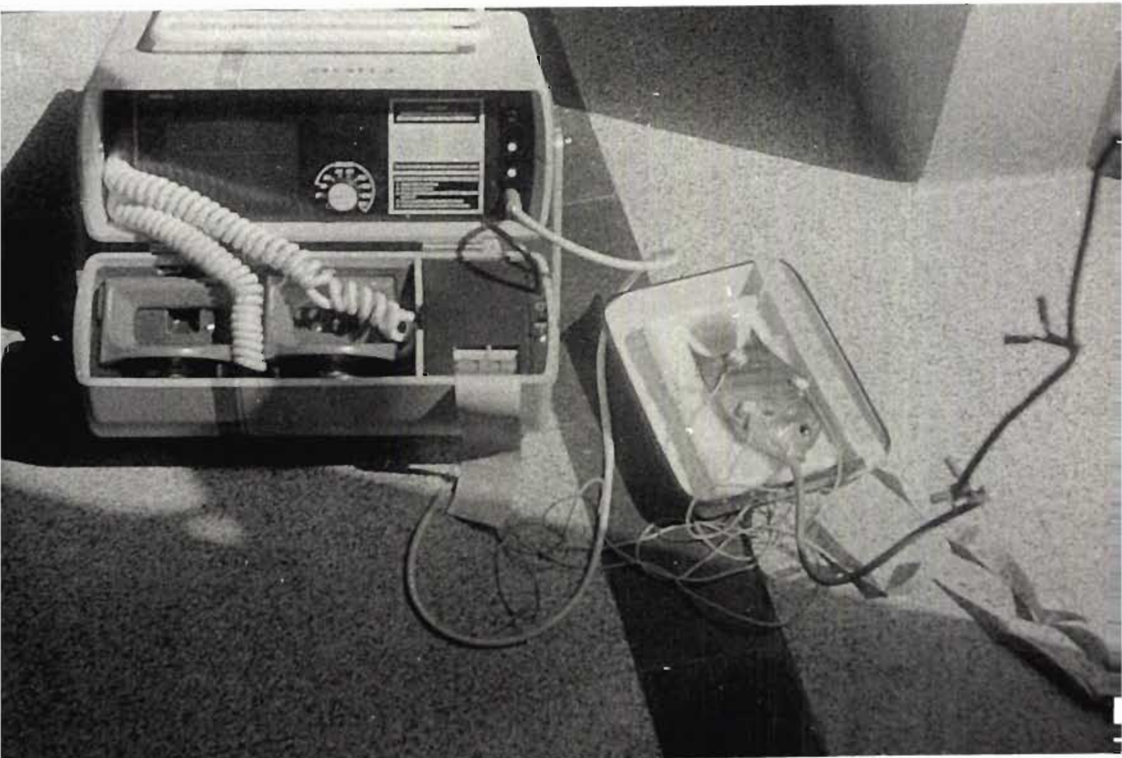


Plate 21: fig. 23 (Table 7.1) E C G data showing the effects of different pathogens in *Etroplus suratensis*

ECG paper number	SPECIES	WET WEIGHT (in gms)	TOTAL LENGTH (in cm)	PATHOGEN (S)	DOSE (in ml)	BACTERIAL COUNTS (per ml)	TIME FREQUENCY (in hours)	CLINICAL SYMPTOMS	RATE PER MINUTE		RHYTHM		P		PR		QRS WIDTH		QRS AXIS		QRS HEIGHT		T		QT		U		REMARKS
									Control	Infected	Control	Infected	Control	Infected	Control	Infected	Control	Infected	Control	Infected	Control	Infected	Control	Infected	Control	Infected	Control	Infected	
1	*ETROPLUS SURATENSIS*	195	16						106		SR		0·08		0·14		0·04		N		1·5		↑		0·28		Present		
2		260	17·5						100		SR		0·04		0·12		0·04		N		1·5		↑		0·32		Present		
3	"	95	10					Progressive tail tissue decay resulting in tail rot, surface floating, imbalance in swimming and anorexia		150		IRR		AB				0·04		N		0·5		Flat		0·24		AB	Frequent ectopics
4	"	120	14					"		100		IRR		AB				0·04		N		1·0		↕		0·28		Present	
5,6	*ETROPLUS SURATENSIS*	120-135	13-14	*KLEBSIELLA PNEUMONIAE*	0·3	$2{\cdot}1 \times 10^7$	0	Control: Normal Infected: Normal	140	100	SR	SR	0·04	0·04	0·2	0·2	0·04	0·04	N	N	1·0	1·0	↑	Flat	0·32	0·36	AB	AB	
7,8		"	"	"	"	"	8	Control: Normal Infected: Restlessness, dashing against the wall and anorexia	140	100	SR	IRR	0·08	0·04	0·12	0·12	0·04	0·04	N	N	1·5	1·5	↑	Flat	0·28	0·32			
9,10	"		"	"	"	"	9	Control: Normal Infected: Weak movements, imbalance in swimming and white patches on the body	120	94	SR		0·04	0·08	0·1	0·12	0·04	0·04	N	N	2·0	1·5	↑	Flat	0·36	0·28	"		
11,12					"	"	11	Control: Normal Infected: Loss of balance and surface floating	140	84	SR	"	0·08	0·06	0·14	0·12	0·04	0·04	N	N	1·5	0·5	↕	Flat	0·32	0·28		-	
13,14	*ETROPLUS SURATENSIS*	140-150	14-14·5	*ESCHERICHIA COLI*	0·3	$1{\cdot}6 \times 10^8$	0	Control: Normal Infected: Normal	96	64	SR	SR	0·08	AB	0·18	AB	0·04	0·04	N	N	0·5	1·5	↑	↕	0·32	0·52	Present	Present	
15,16	"	"	"	"	"	"	8	Control: Normal Infected: Sluggish movements and anorexia	96	40–80	SR	IRR	0·08		0·14	"	0·04	0·04	N	N	1·0	1·0	↕	↕	0·36	·56	AB		
17,18	"					"	11	Control: Normal Infected: Body discolouration, skin lesions, swimming near water surface, belly swelling and faecal discharge of reddish colour	94	60	SR		0·08		0·14	"	0·04	0·04	N	N	1·0	2·0	↑	↕	·36	·44			
19,20	*ETROPLUS SURATENSIS*	140-150	14-14·5	*PSEUDOMONAS AERUGINOSA*	0·3	$3{\cdot}4 \times 10^7$	0	Control: Normal Infected: Normal	80	80	SR	SR	0·08	0·08	0·16	0·14	0·04	0·04	N	N	1·0	1·0	↑	↑	0·4	·4	AB	AB	
21,22					"	"	8	Control: Normal Infected: Restlessness and anorexia	60	150	SR	IRR	0·04	AB	0·12	AB	0·04	0·04	N	N	0·5	1·0	↕	Flat	0·36	0·24		"	
23;24				"	"		11	Control: Normal Infected: Lingering near bottom of the the water body, folded pectoral fins, erythema of the base of fins and on lateral sides of the body and occasional tumbling movements	48	44	SR	"	0·08		0·1		0·04	0·04	N	N	0·5	1·5	↑	Flat	0·44	0·44	"		
25,26	"	"	"	"	"	"	16	Control: Normal Infected: Sloughing away of scales and sunken eyes	60	46	SR	"	0·08	"	0·16	"	0·04	0·04	N	N	0·5	1·5	↑	Flat	0·36	0·4			
27,28	"	"	"	"	"	"	18	Control: Normal Infected: Petechiae on operculum and mouth region and loss of balance	90	20	SR	"	AB	"	AB	"	0·04	0·04	N	N	1·0	2·0	↑	Flat	0·34	0·48			
29,30 31,32		"	"	"	"	"	20	Infected: COMPLETELY INACTIVE	TERMINAL ECG SHOWING ECTOPIC BEATS AND CARDIAC ARREST																				

SR = SINUS RHYTHM, AB = ABSENT, IRR = IRREGULAR

Plate 22: fig. 24 (Table 7.1 contd)

E C G data showing the effects of different pathogens in *Etroplus suratensis*

Specimen Nos.	Species	Wt	Lt	Pathogen (s)	Dose (in ml)	Bacterial counts (per ml)	Time frequency (in hours)	Clinical symptoms
33, 34	E... [illegible]	[illegible]	[illegible]	PROTEUS VULGARIS	0.3	8.0X10^7	0	Control: Normal; Infected: Normal
36							6	Control: Normal; Infected: Dashing against the wall, imbalance in swimming and anorexia
37, 38							11	Control: Normal; Infected: Skin lesions, tail tissue decay and loss of balance
39, 40, 41, 42	ETROPLUS SURATENSIS	125-135	13-14	STAPHYLOCOCCUS AUREUS	0.3	4.1X10^9	0	Control: Normal; Infected: Normal
43, 44, 45, 46							6	Control: Normal; Infected: Skin lesions, imbalance in swimming and anorexia
47, 48							11	Control: Normal; Infected: The fish with IP injection died after erratic swimming
49, 50, 51, 52							15	Control: Normal; Infected: Scale protrusion and loss of balance
53, 54, 55, 56	ETROPLUS SURATENSIS	120-130	12-13	STREPTOCOCCUS PYOGENES	0.3	3.0X10^7	0	Control: Normal; Infected: Normal
57, 58, 59, 60	"						6	Control: Normal; Infected: Sluggishness, anorexia and [illegible] skin lesion
61, 62, 63, 64	"						11	Control: Normal; Infected: Loss of pectoral fin activity and faded body colour
65, 66, 67, 68	"						15	Control: Normal; Infected: Loss of balance. The fish with IP injection died after tumbling movements

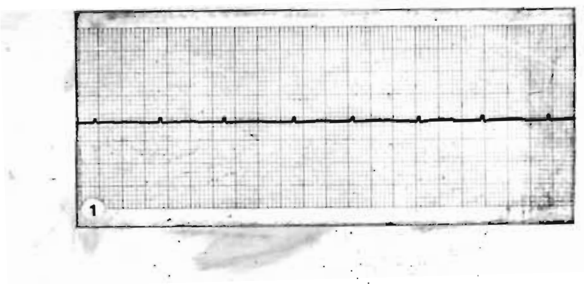
Specimen Nos.	Rate per minute Control IM	Control IP	Infected IM	Infected IP	Rhythm Control IM	Control IP	Infected IM	Infected IP	P Control IM	Control IP	Infected IM	Infected IP	PR Control IM	Control IP	Infected IM	Infected IP
33, 34	100	-	84	-	SR	-	SR	-	0·04	-	0·04	-	0·14	-	0·12	-
36	84	-	80	-	SR		IRR	-	0·08	-	0·08	-	0·28	-	0·2	-
37, 38	100	-	●	-	SR	-			0·08	-		-	0·28	-		-
39, 40, 41, 42	100	96	100	92	SR	SR	SR	SR	0·04	0·04	0·06	0·06	0·12	0·12	0·14	0·14
43, 44, 45, 46	100	100	100	60	SR	SR	IRR	IRR	0·04	0·08	AB	AB	0·12	0·38	AB	AB
47, 48	100	100	60	●	SR	SR			0·04	0·04	"		0·2	0·12	"	
49, 50, 51, 52	75-80		●		IRR				0·08				0·16			
53, 54, 55, 56	96	100	92	100	SR	SR	SR	SR	AB	AB	AB	AB	AB	AB	AB	AB
57, 58, 59, 60	92	96	92	110	SR	SR	IRR	IRR	"	"	"	"	"	"	"	"
61, 62, 63, 64	92	96	80	90	SR	SR	IRR	IRR	"		"	"	"	"	"	"
65, 66, 67, 68	92	96	46	●	SR	SR	IRR		"	"	"	"	"	"	"	

Specimen Nos.	QRS width Control IM	Control IP	Infected IM	Infected IP	QRS axis Control IM	Control IP	Infected IM	Infected IP	QRS height Control IM	Control IP	Infected IM	Infected IP
33, 34	0·04	-	0·04	-	N	-	N	-	0·5	-	1·0	-
36	0·04	-	0·04	-	N	-	N	-	1·5	-	1·0	-
37, 38	0·04	-		-	N	-		-	1·5	-		
39, 40, 41, 42	0·04	0·04	0·04	0·04	N	N	N	N	1	1·5	1·5	2
43, 44, 45, 46	0·04	0·04	0·04	0·04	N	N	N	N	[illegible]	[illegible]	1·5	1·0
47, 48	0·04	0·04	0·04		N	N	N		1·8	0·8	1·0	
49, 50, 51, 52	0·04				N				3·0			
53, 54, 55, 56	0·04	0·04	0·04	0·04	N	N	N	N	0·8	1·8	1·8	1·8
57, 58, 59, 60	[illegible]	[illegible]	[illegible]	[illegible]	N	N	N	N	0·8	1·6	1·8	2·0
61, 62, 63, 64	0·04	0·04	0·04	0·04	N	N	N	N	0·8	2·8	1·0	2·8
65, 66, 67, 68	0·04	0·04	0·04		N	N	N		0·6	2·0	1·6	

Specimen Nos.	[illegible] Control IM	Control IP	Infected IM	Infected IP	[illegible] Control IM	Control IP	Infected IM	Infected IP	U Control IM	Control IP	Infected IM	Infected IP	Remarks
33, 34	↑	-	↑	-	0·36	-	·34	-	AB	-	AB	-	
36	FLAT	-	↑	-	0·32	-	0·32	-	—	—	—	—	
37, 38	FLAT	-		-	0·32	-		-	"	-			
39, 40, 41, 42	↑	↑	↑	↑	0·4	0·4	0·38	0·38	AB	AB	AB	AB	
43, 44, 45, 46	↑	↑	↑	FLAT	0·34	0·28	0·38	0·32	—	—	—	"	
47, 48	↑	↑	↑		0·32	0·28	[illegible]		—	—	—		
49, 50, 51, 52	↑				0·32				"				
53, 54, 55, 56	↑	↑	↑	↑	[illegible]	[illegible]	[illegible]	[illegible]	AB	AB	AB	AB	
57, 58, 59, 60	FLAT	↑	↑	↑	[illegible]	[illegible]	[illegible]	[illegible]	—	—	—	—	
61, 62, 63, 64	FLAT	↑	↓	↑	[illegible]	[illegible]	[illegible]	[illegible]	—	—	"	—	
65, 66, 67, 68	FLAT	↑	FLAT		[illegible]	[illegible]	[illegible]		—	—	—		

IM = INTRAMUSCULAR, IP = INTRA PERITONEAL. ● = ECG Showing basically a cardiac stand still, — = NOT APPLICABLE. SR = SINUS RHYTHM, AB = ABSENT, IRR = IRREGULAR

Plate 23: fig. 25 (left)	Different E C G wave patterns of the normal fish, *Etroplus suratensis*
Plate 23: fig. 26 (right)	E C G of diseased fish, *Etroplus suratensis*, with tail rot (natural infection) showing absence of P and occasional ectopic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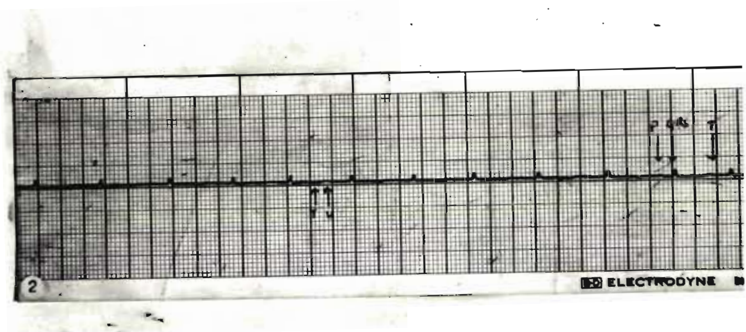
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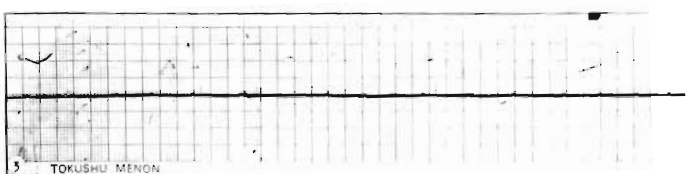
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TOKUSHU MENON

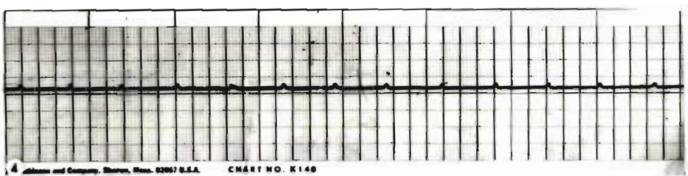
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CHART NO. K140

Plate 24: figs. 27,28 (left and right) E C G showing the effects of the pathogen, *Klebsiella pneumoniae* in *Etroplus suratensis*

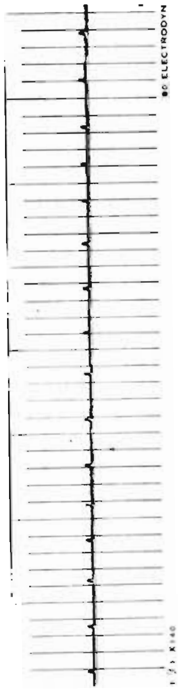
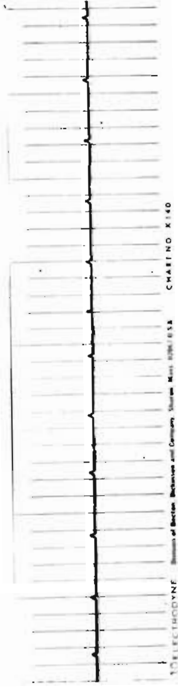
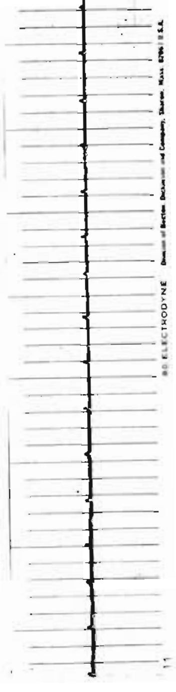
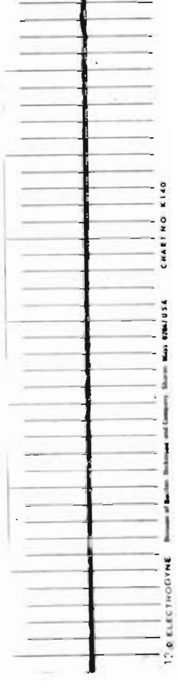
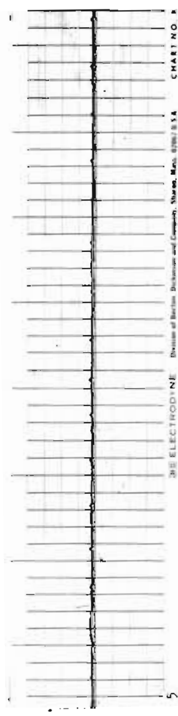
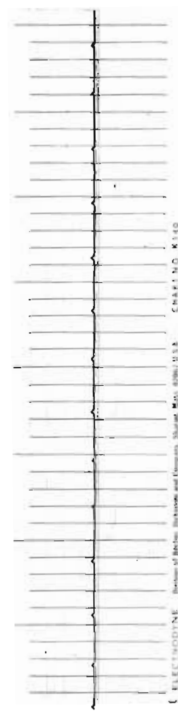
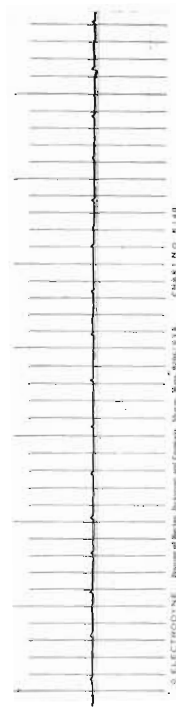
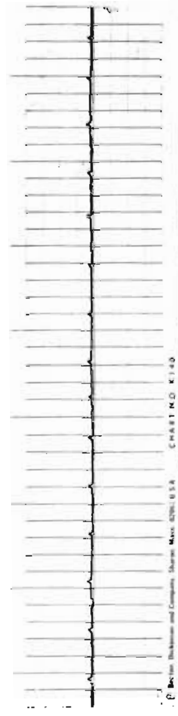
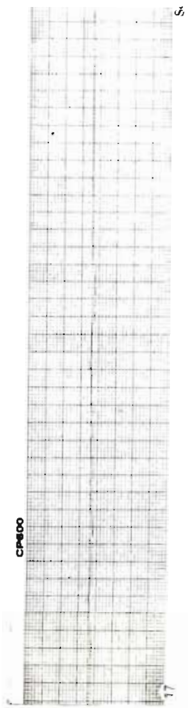
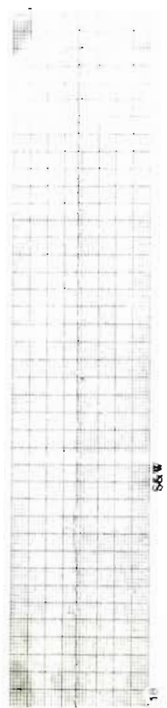
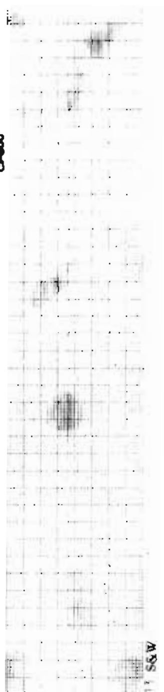
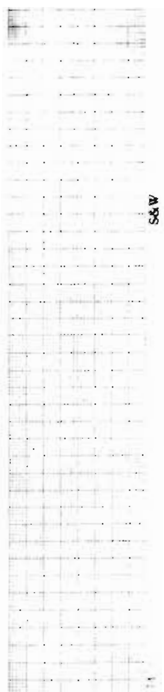









Plate 25: figs. 29,30 (left and right) E C G showing the effects of the pathogen, *Escherichia coli* in *Etroplus suratensis*

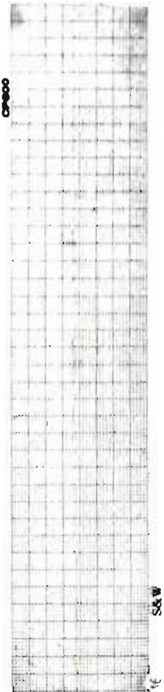
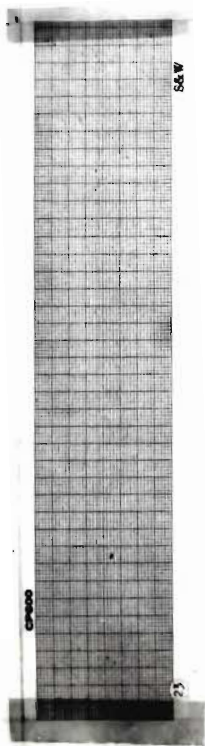
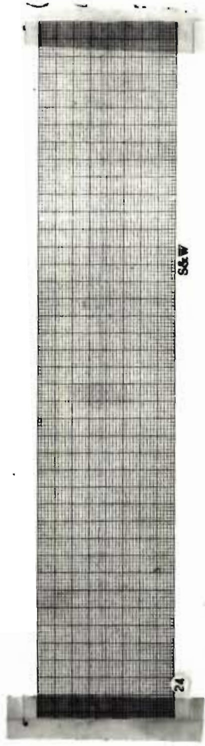
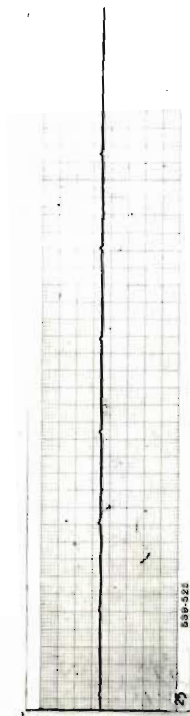
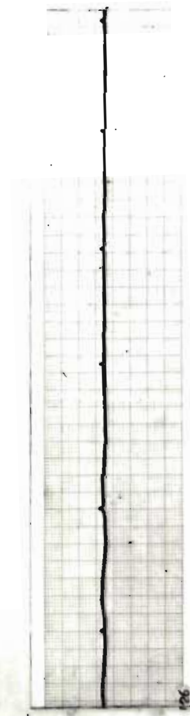
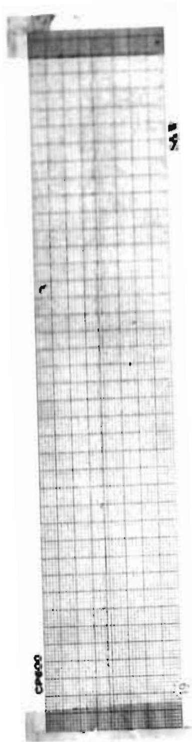


Plate 26: figs. 31,32 (left and right)

E C G showing the primary effects of the pathogen, *Pseudomonas aeruginosa* in *Etroplus suratensis*

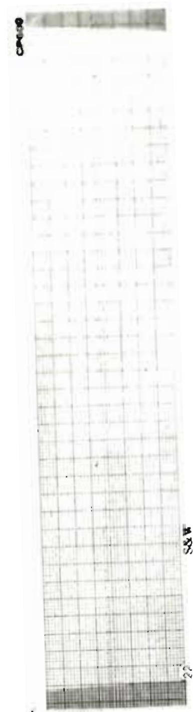
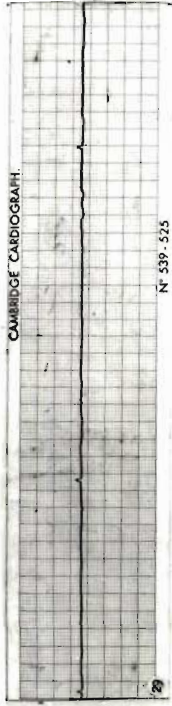


Plate 27: figs. 33,34 (left and right)

E C G showing the terminal effects of the pathogen, *Pseudomonas aeruginosa* in *Etroplus suratensis*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Nº 539·525
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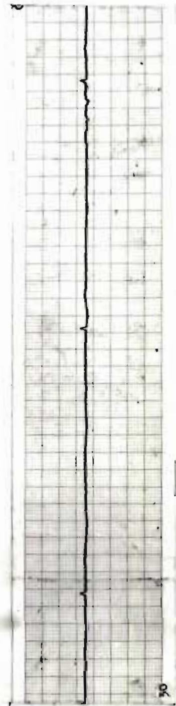
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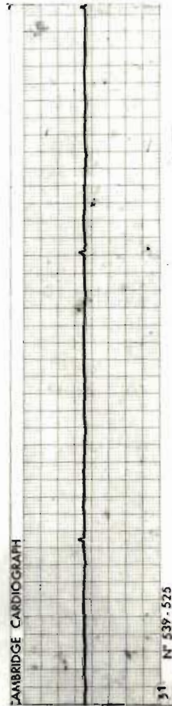
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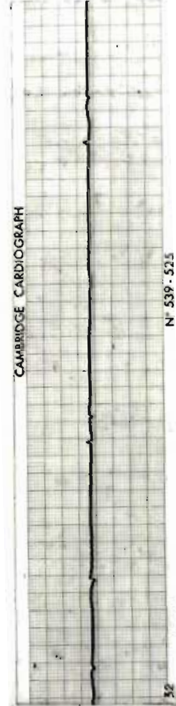
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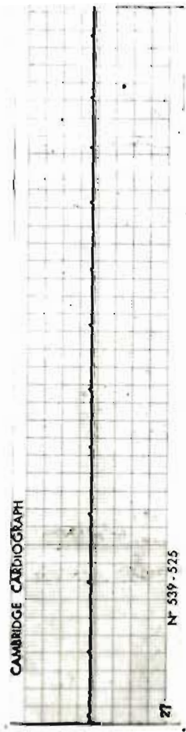
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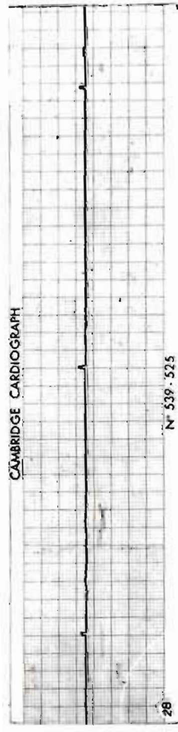
CAMBRIDGE CARDIOGRAPH
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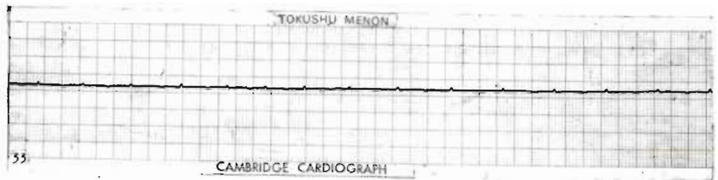
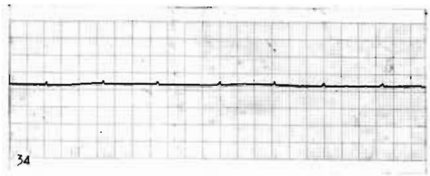
Plate 28: figs. 35,36 (left and right)

E C G showing the effects of the pathogen, *Proteus vulgaris* in *Etroplus suratensis*


TOKUSHU MENON
33
CAMBRIDGE CARDIOGRAPH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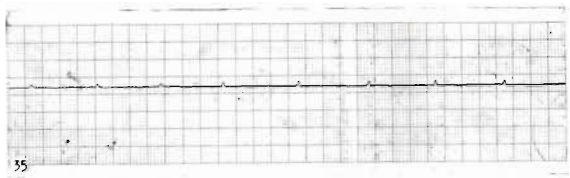
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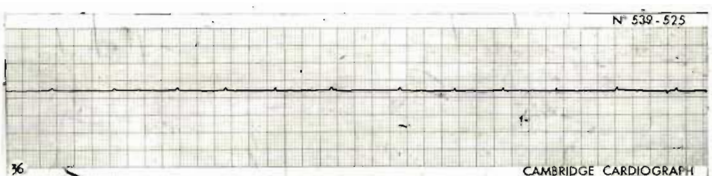
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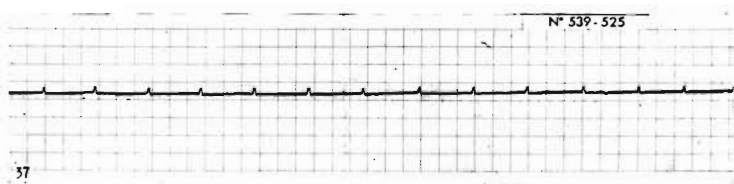
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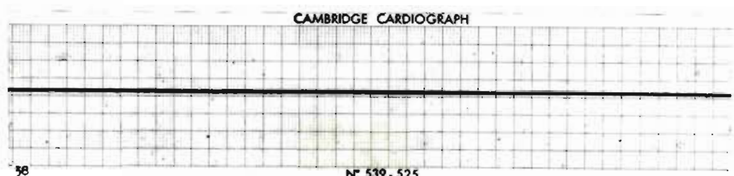
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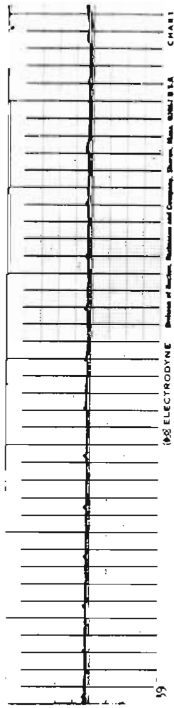
N° 539 - 525

Plate 29: figs. 37,38 (left and right)

E C G showing the primary effects of the pathogen, *Staphylococcus aureus* in *Etroplus suratensis*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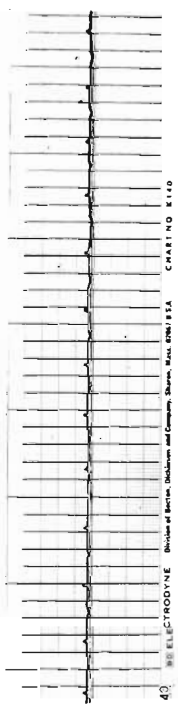
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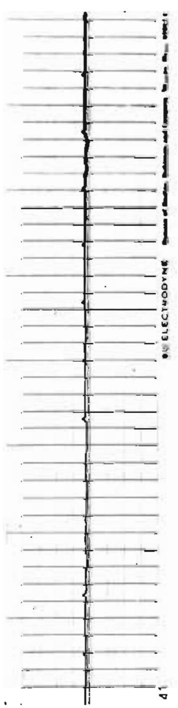
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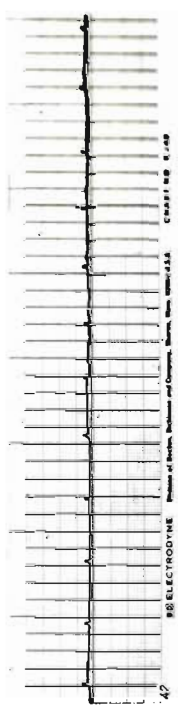
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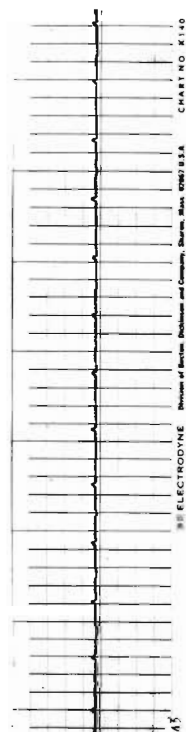
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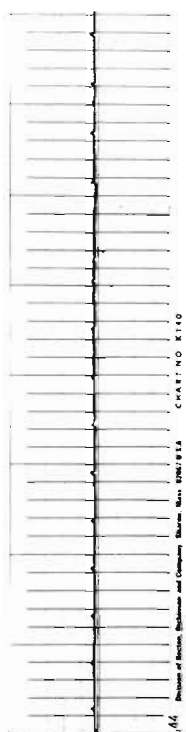
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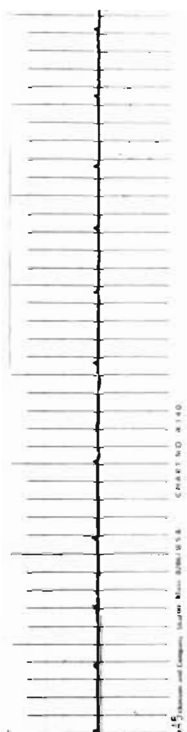
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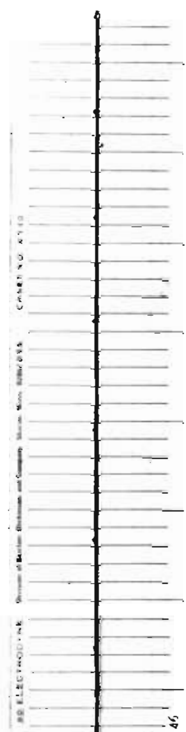
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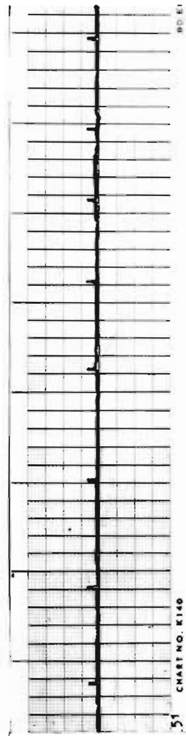
Plate 30: figs. 39,40 (left and right)

E C G showing the terminal effects of the pathogen, *Staphylococcus aureus* in *Etroplus suratensis*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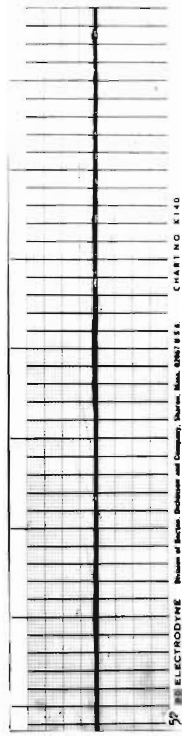
ELECTRODYNE
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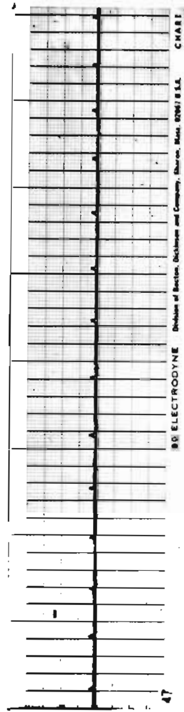
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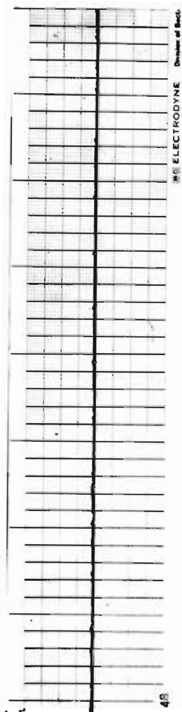
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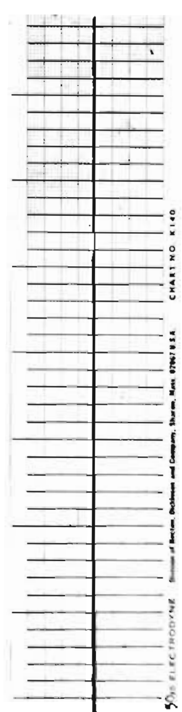
ELECTRODYNE
CHART NO. K140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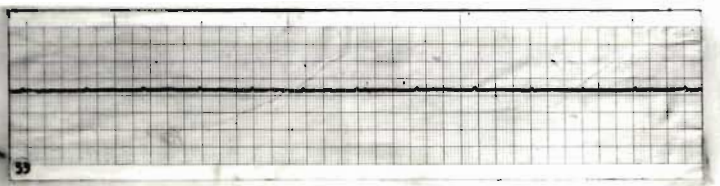
ELECTRODYNE

Plate 31: figs. 41,42 (left and right) E C G showing the primary effects of the pathogen, *Streptococcus pyogenes* in *Etroplus suratensis*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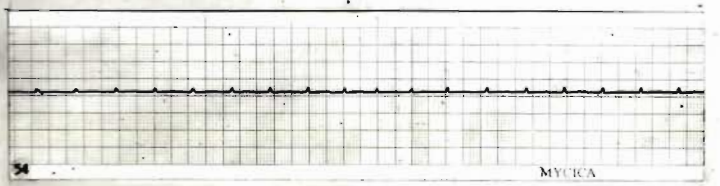
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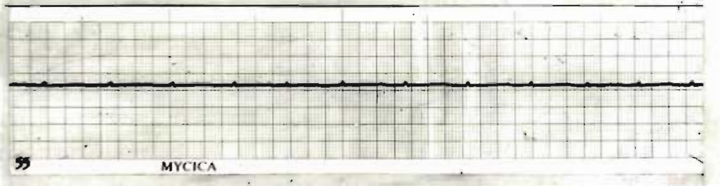
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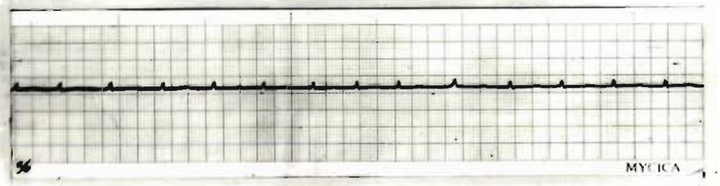
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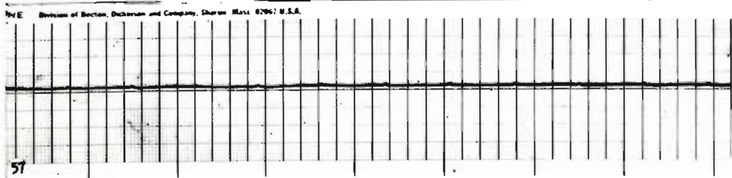
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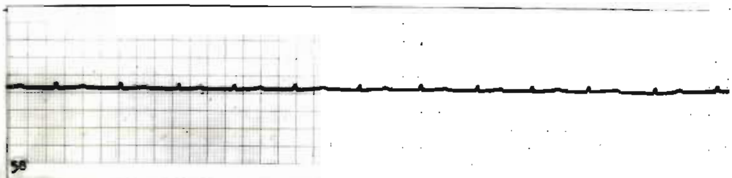
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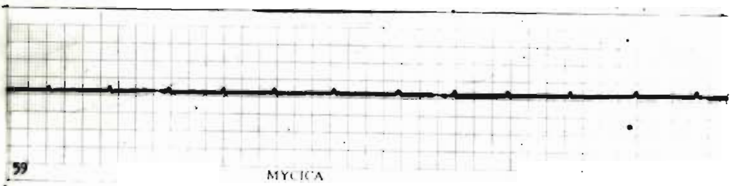
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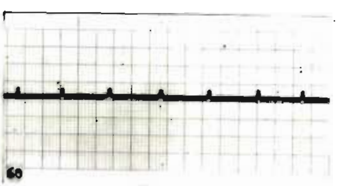

60

Plate 32: figs. 43,44 (left and right) E C G showing the terminal effects of the pathogen, *Streptococcus pyogenes* in *Etroplus suratensis*

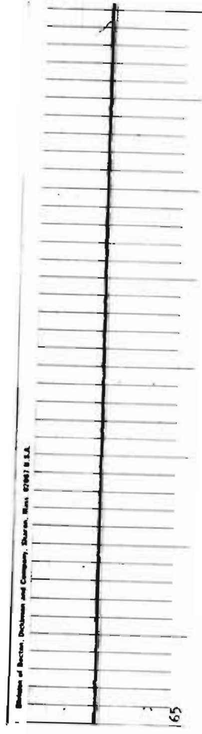
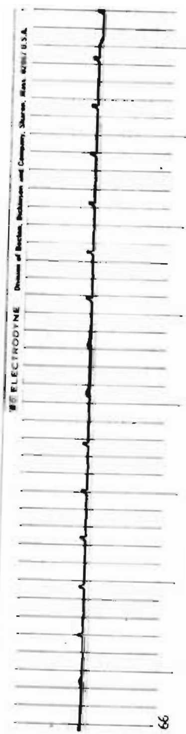
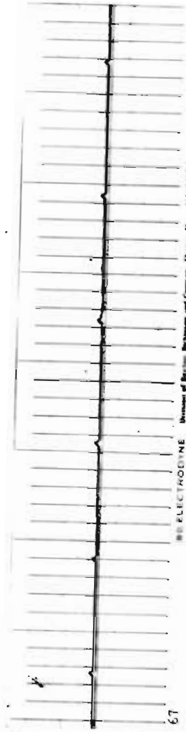
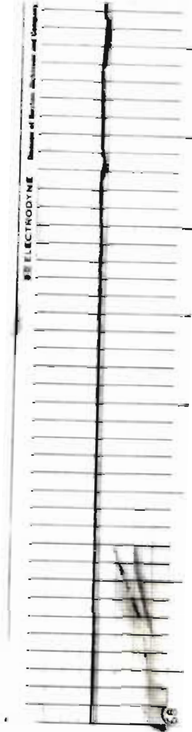
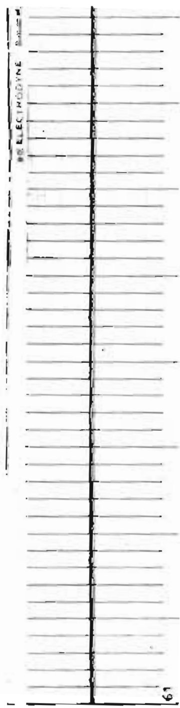
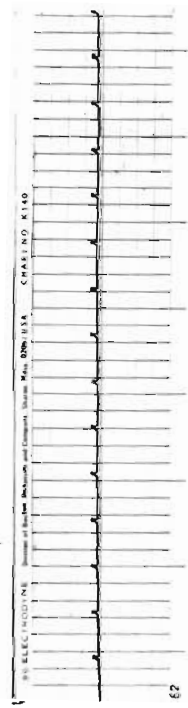
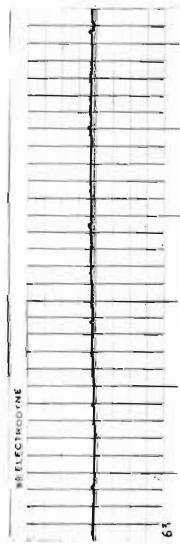
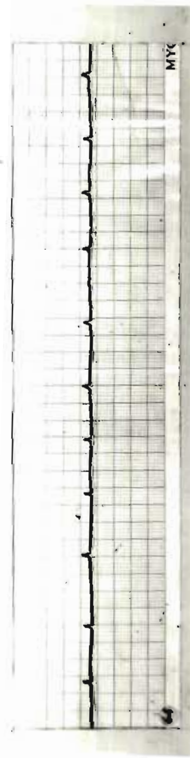









Plate 33: fig. 45 Effects of the pathogens on heart rate in *Etroplus suratensis* (heart rate per minute)

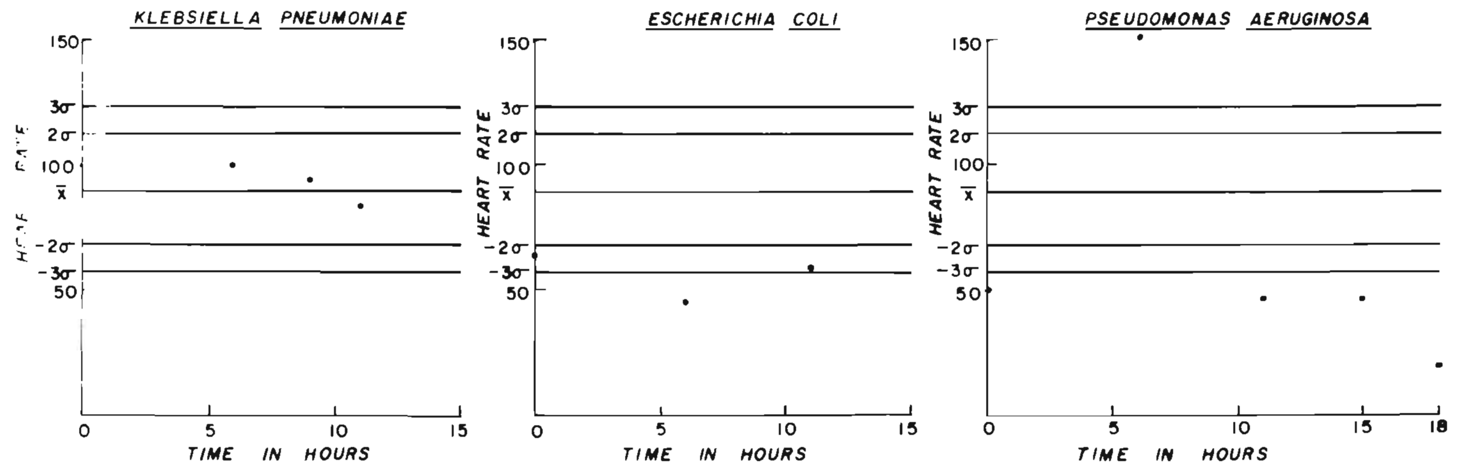
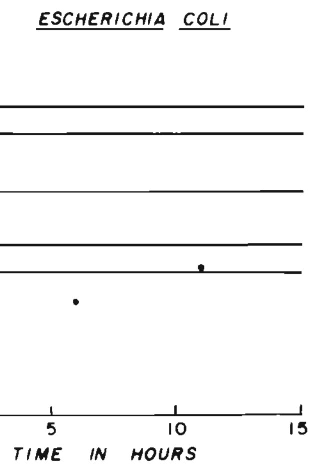
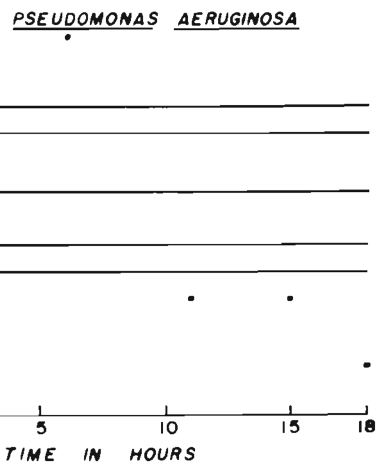
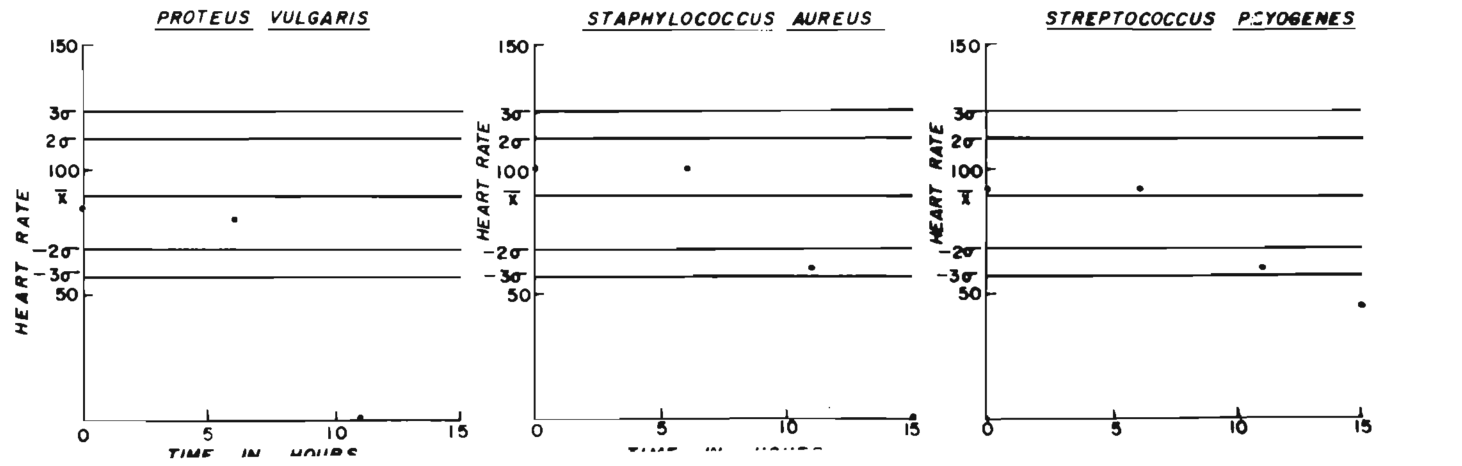
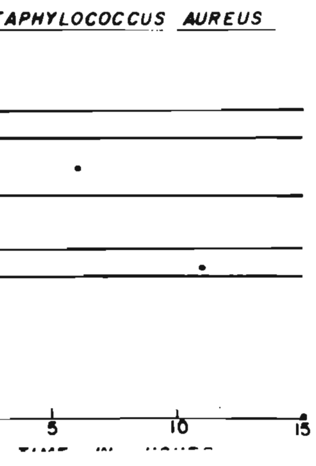
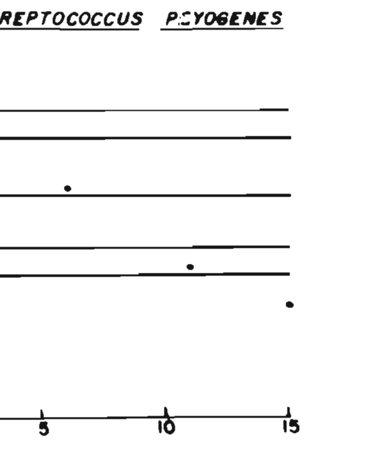
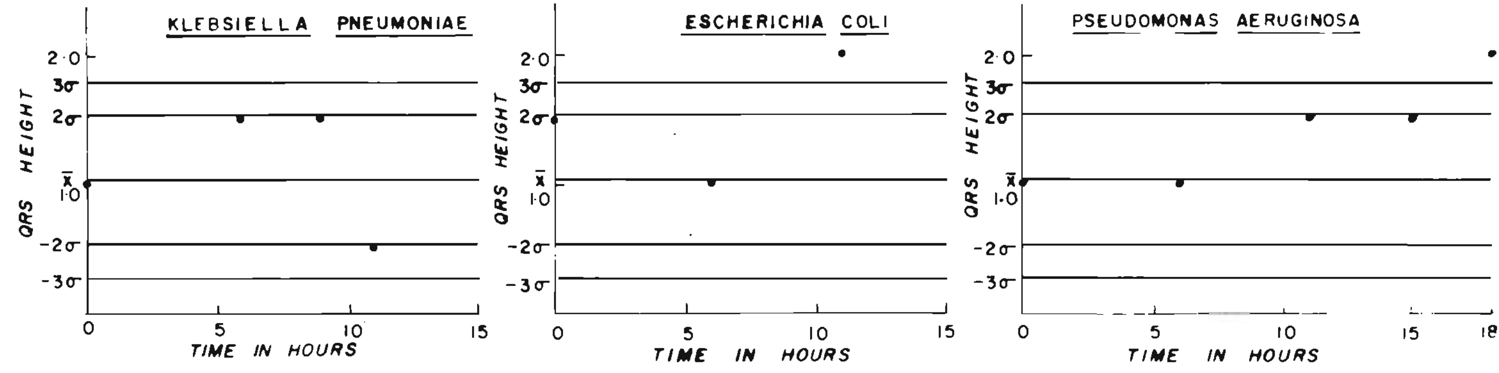
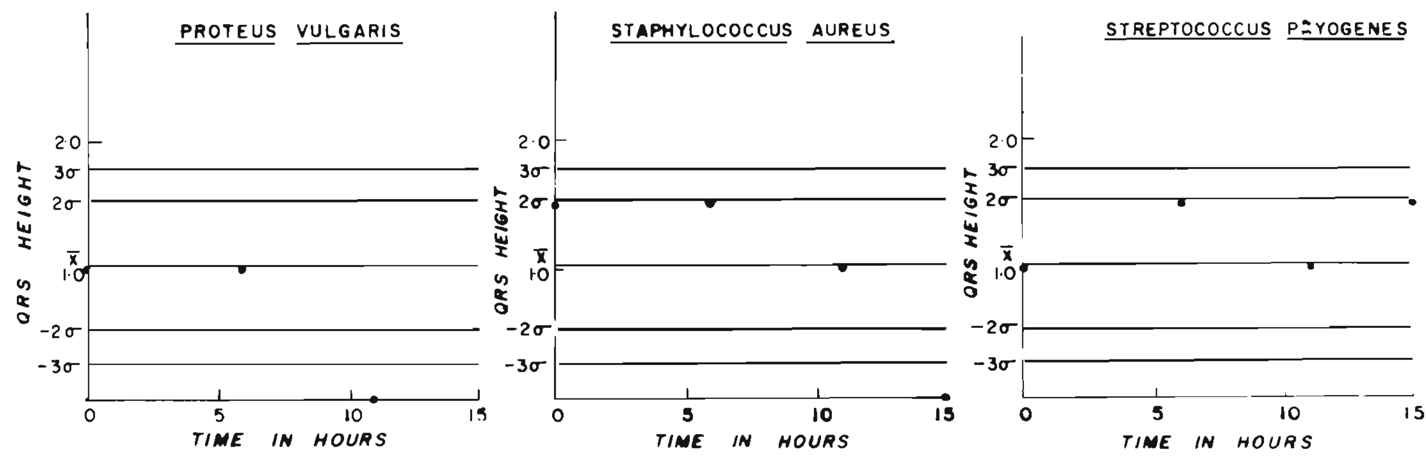

KLEBSIELLA PNEUMONIAE
ESCHERICHIA COLI
PSEUDOMONAS AERUGINOSA
HEART RATE
150
3σ
2σ
100
x̄
-2σ
-3σ
50
0
5
10
15
18
TIME IN HOURS



PROTEUS VULGARIS
STAPHYLOCOCCUS AUREUS
STREPTOCOCCUS PYOGENES
HEART RATE
150
3σ
2σ
100
x̄
-2σ
-3σ
50
0
5
10
15



Plate 34: fig. 46 Effects of pathogens on QRS height in *Etroplus suratensis* (QRS height in mm)

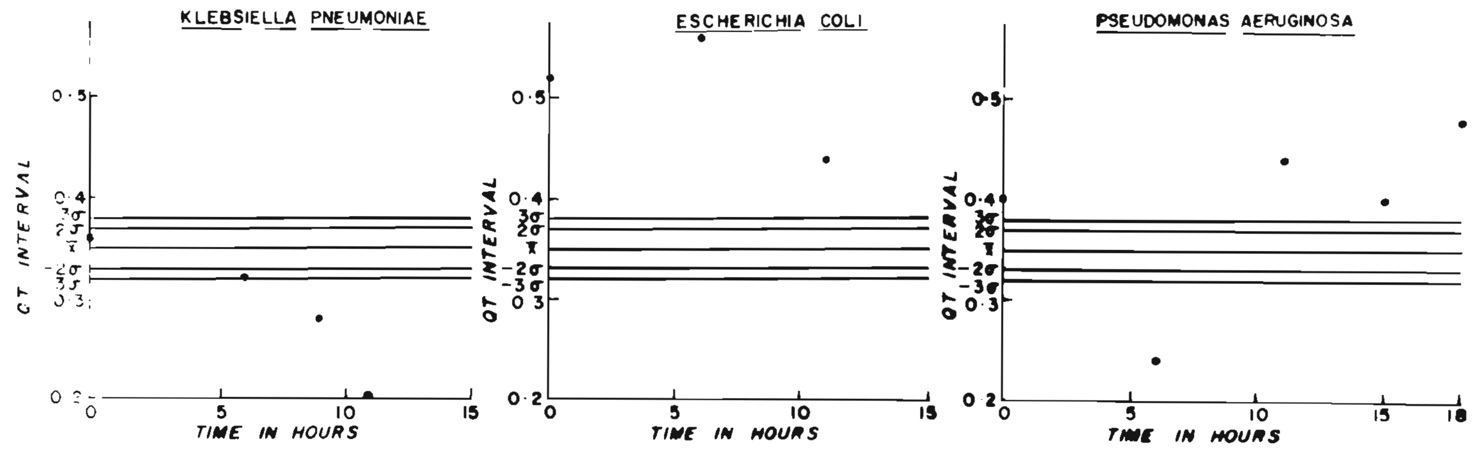
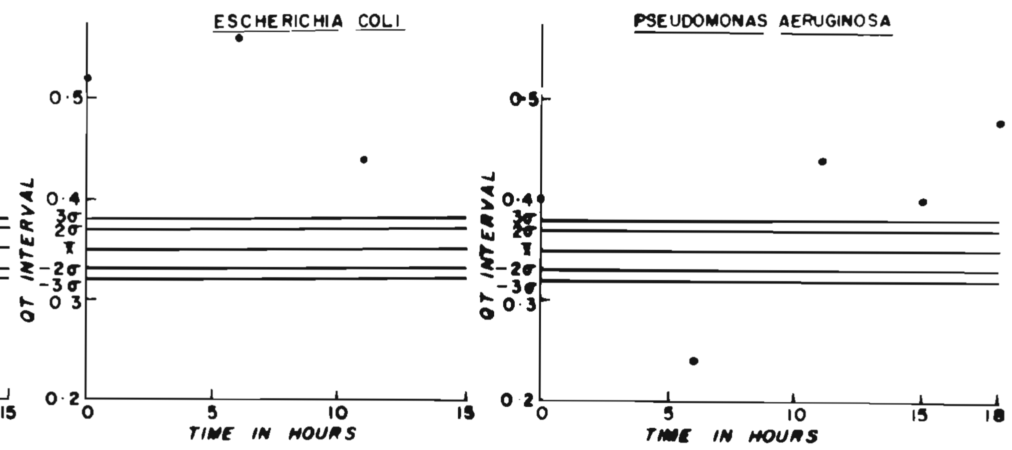
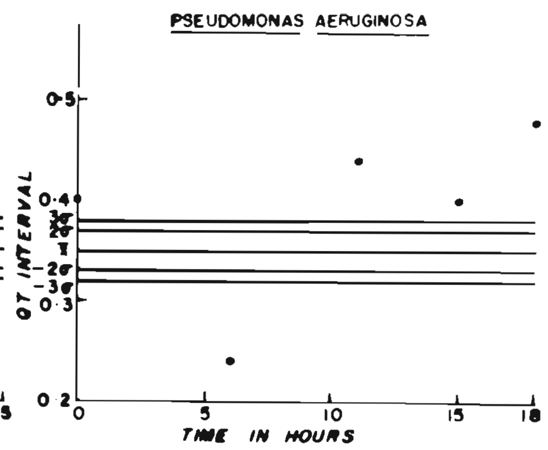


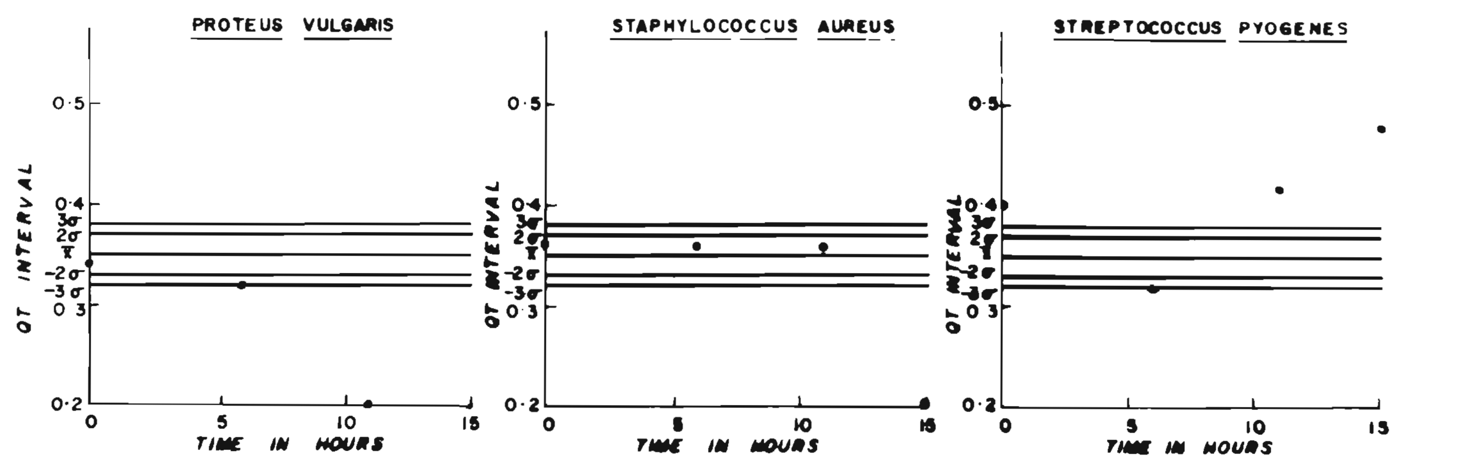
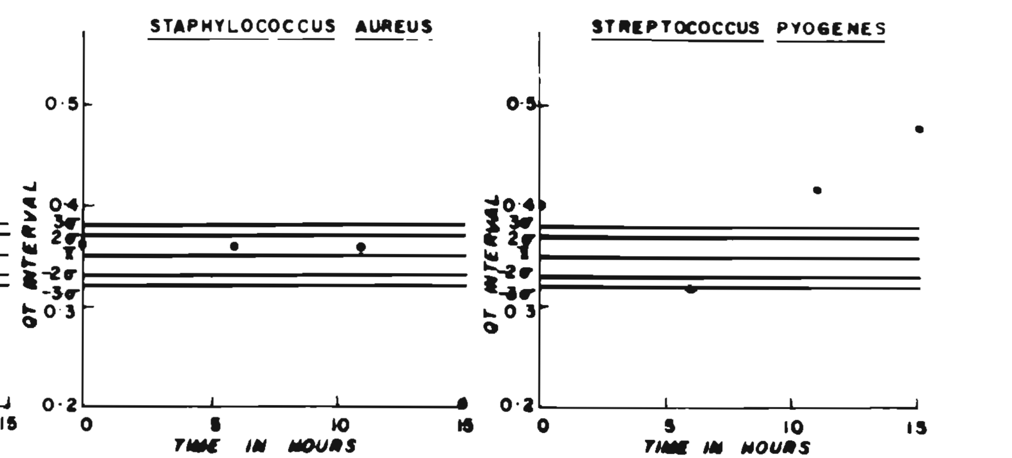
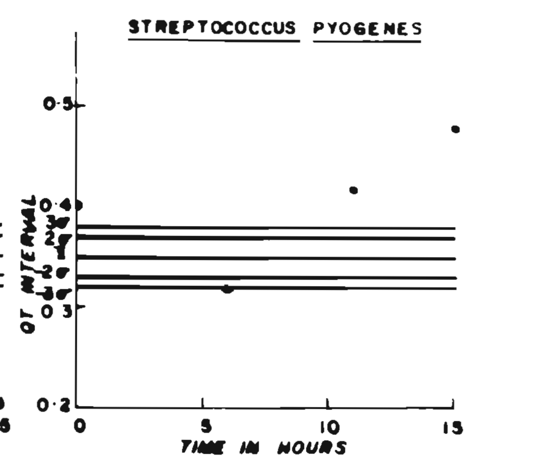
KLEBSIELLA PNEUMONIAE
ESCHERICHIA COLI
PSEUDOMONAS AERUGINOSA
QRS HEIGHT
2·0
3σ
2σ
x̄
1·0
-2σ
-3σ
0
5
10
15
18
TIME IN HOURS



PROTEUS VULGARIS
STAPHYLOCOCCUS AUREUS
STREPTOCOCCUS PYOGENES
QRS HEIGHT
2·0
3σ
2σ
x̄
1·0
-2σ
-3σ
0
5
10
15
TIME IN HOURS

Plate 35: fig. 47 Effects of pathogens on QT interval in *Etroplus suratensis* (QT interval per second)




KLEBSIELLA PNEUMONIAE
ESCHERICHIA COLI
PSEUDOMONAS AERUGINOSA
QT INTERVAL
TIME IN HOURS




PROTEUS VULGARIS
STAPHYLOCOCCUS AUREUS
STREPTOCOCCUS PYOGENES
QT INTERVAL
TIME IN HOURS

A N N E X U R E

1. LIST OF SUPPORTING PAPERS 1-8

LIST OF SUPPORTING PAPERS

1. Fish and shellfish diseases in culture systems

    Part I Concept of disease and pathogen. Seafood Export Journal., 12(2): 13-14(1980)

2. Part II Heterotrophic bacteria and kinds of infections. Seafood Export Journal., 12(9): 21-23(1980)

3. Part III Defenses against infection. Seafood Export Journal., 12(10): 19-21(1980)

4. Part IV Bacterial diseases. Seafood Export Journal., 13(8): 19-26(1981)

5. Part V Prophylaxis and disease check up. Seafood Export Journal., 13(9): 23-26(1981)

6. Studies on the microbes of estuarine and marine fishes of India

    Part I Bacterial flora of the Pearl spot *Etroplus suratensis* Bloch and the White fish *Lactarius lactarius* Schneider. (in press)

7. Microbial flora of mussels in the natural beds and farms. Coastal aquaculture: Mussel farming, *CMFRI Bulletin.*, *29*: 41-43 (1980)

8. Diseases of finfishes and shellfishes cultivated in the coastal waters of India. [Paper presented at the workshop in *Tropical Fish Diseases* held in Puncak, Java, from November 28 to December 1 1978, jointly sponsored by International Development Research Centre (I.D.R.C.) and Indonesia. (in press)]

SUPPORTING PAPER 1

FISH AND SHELLFISH DISEASES IN CULTURE SYSTEMS

Part 1 Concept of disease and pathogen. *Seafood Export Journal.*, **12**(2): 13-14(1980)

Reprint from "Seafood Export Journal"
Vol. XII No. 2. February 1980

FISH AND SHELLFISH DISEASES IN CULTURE SYSTEMS

I. Concept of disease and pathogen

C. Thankappan Pillai
Central Marine Fisheries Research Institute, Cochin-18

"Causes of diseases are reasonable and discoverable causes are removable"

Just as in land animals or in agricultural crops, fishes and shellfishes living in the aquatic environment are susceptible to diseases whenever certain abnormal biological, physological or environmental changes occur. The problem of diseases has assumed greater significance in the context of aquaculture of these organisms in different culture systems. New and new diseases are found coming up in all high productive systems, whether they are on land or in water, and unless we have a sufficient knowledge of the nature of these diseases and their control, the management of the natural resources as well as the culture practices can hardly become successful. This article in the series attempts to define disease and pathogen, and different categories of diseases.

The term 'disease' (absence of ease) by its popular acceptance sets to denote a condition or a state of the body in which there is departure from normal function as a result of one or more of the following: the effect of heredity, the affecting or contamination with disease oroducing organisms, variation in diet or environment. Every disease, generally speaking, has a cause or causes and likewise an effect or a set of effects. Let us take as an example, amebiasis, a parasitic disaase of man. The amebiasis, which is characterised by pathological intestinal manifestations, is caused by an animalcule scientifically named, *Entamoeba histolytica*. It produces different signs and disorders which cause ailment or suffering of the afflicted, of which the most important is an acute dysentary phase. The cause, or causes, lead to a chain of effects (incorrect functions, disorders etc) in succession, of which the manifested ones (apparent ones) are called symptoms. Symptoms are medically of much importance inasmuch as they are signposts — indicative of the disease. However, search for the prime cause of a disease is nearer to the way to put an end to the ailment.

Now, we may define disease as any state or condition, as a result of abnormal change in the anatomy and/ or physiology of cell (s) or organ (s), which harms the normal life of a living thing and disease may be:

1. Biotic disease:
   Involving attack by viruses, bacteria, algae, fungi and other animals eg. tuberculosis.

2. Abiotic disease:
   Due to malfunctioning of cell (s)/organ(s) and/or associated changes due to unfavourable ecological or other accidental conditions eg. gas embolism.
3. Nutritional disease:
   Owing to lack of suitable balanced diet eg. gastroenteritis.
4. Genetic disease:
   Due to inherent susceptibility to a disease eg. melanoma in *Xiphophorus* spp.

Of all these diseases, biotic diseases are most important and merit greater attention in research. It has to be emphasized that to prevent a disease the cause has to be pin-pointed and removed. Clinical symptoms of the disease generally help to diagnose the case to a certain extent. Take the case of vibriosis. It is the detection of the aetiological agent, *Vibrio anguillarum,* which definitely confirms the disease. Such causative agents are called pathogens. However, it should be borne in mind that *anything* which causes disease is not a pathogen. Sometimes, certain phytoflagellates, for example, *Prymnesium parvum,* when present in blooms, may cause fish mortality. Here, the causative factor, the algal toxin, is lethal and is an abiotic factor but not a pathogen. A pathogen is an organism, which survives and flourishes in a host, causing clinical symptoms which result in a diseased condition. Moreover, in order to be termed an aetiological agent of a disease, the organism must statisfy certain stringent stipulations known as "Koch's postulates" which are:

1. The suspected organism should be associated regularly with all cases of the suspected disease, and in logical pathological relationship to the disease, and its symptoms and lesions.
2. The suspected organism should be isolated in pure culture from the suspected case.
3. When such pure culture is inoculated into suitable animals, the disease should be reproduced.
4. The same organism should be reisolated in pure culture from the inoculated animals.

Only when an organism satisfies these four requisites, can it be incriminated as a pathogen causing a particular disease of biotic nature. Viral diseases such as lymphocystis, infectious pancreatic necrosis, and infectious haematopoietic necrosis, and bacterial diseases like furunculosis tuberculosis and ulcer are typical biotic diseases.

Abiotic diseases, which can also harm and upset the fish culture systems, are anoxia, hypoxia, acidosis, alkalosis and intoxication etc. These should also be timely checked and their causes rooted out for safety.

Another aspect of prime importance for successfully managing the culture systems is to provide adequately balanced diet as any imbalance in the diet may also result in mass mortality. So, care should be taken in formulating a suitable balanced diet to avoid nutritional diseases such as avitaminosis, hypervitaminosis and dietary gill disease.

Diseases of genetic origin like certain kinds of tumours and deformities are not of serious problems as they occur only infrequently. ●

SUPPORTING PAPER 2

FISH AND SHELLFISH DISEASES IN CULTURE SYSTEMS

Part II Heterotrophic bacteria and kinds of infections. Seafood Export Journal., 12(9): 21-23(1980)

Reprint from "Seafood Export Journal"
Vol. XII. No. 9. September 1980

Fish and Shellfish Diseases in Culture Systems

II. Heterotrophic bacteria and kinds of infections

C. Thankappan Pillai
(Central Marine Fisheries Research Institute, Cochin-682018

"While infection leads to indisposition
hygiene curtails infection"

The world is facing an acute shortage of food as terrestrial resources are not adequate to meet the increasing needs of the human populations. So, to augment the food production, a suitable alternative is sea food, fish being the best and probably a cheaper source of animal protein than egg, milk and meat. But, here again, the capture fisheries are not sufficient to satisfy the growing demands of the starving population. Hence, aquaculture of fish and shellfish is being made simple and popular, as one of the ideal and attractive sources of supplementing the food production, even among the people in the rural areas through fisheries development and extension programme.

But, for successful management of these aquaculture practices, a thorough knowledge of at least the common diseases that can occur in these animals is absolutely essential in order to control by treatment and take suitable prophylactic measures.

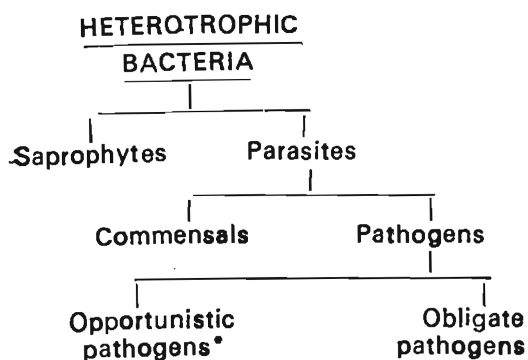
Among diseases, bacterial diseases are the most frequent and harmful in the cultivable organisms. So, this article is intended for providing a better understanding of infections caused by bacteria as investigations in ichthyopathology has been very sketchy in India.

Bacteria, procaryotic microorganisms of the kingdom, PROCARYOTAE, have always been an object of attraction for researchers because of their activities, both harmful and beneficial. Of several groups of bacteria, heterotrophic bacteria (those which obtain their energy by breaking down suitable organic nutrients) are quite important from the point of view of fish diseases. These organisms are widely distributed in nature and live in any diversified situations.

This dynamic group of organisms can be tentatively classified into saprophytes and parasites based on their mode of living. *Saprophytes* subsist on dead and decomposing organic matter, whereas, *parasites* depend on other live hosts for their existence.

Parasites are again divided into commensals and pathogens. The commensals form the normal flora of the healthy host and usually associate in harmony (eg. luminescent *vibrios*) while those organisms that provoke disease and harm the host are called *Pathogens.* Those bacteria, which may cause disease to the host, according to the suitability of the situation in and around them, are termed as *opportunistic* pathogens (*eg. Escherichia coli).* An opportunistic pathogen can be either a commensal or a saprophyte. The agents which mostly cause disease as a result of infection in the host are called *obligate pathogens* (*eg. Aeromonas salmonicida)* (Table 1).

TABLE 1



(* At times, saproyhytes and commensals become opportunistic pathogens)

Sometimes, even saprophytic organisms (eg. *Bacillus subtilis*) may also cause disease when host's vitality is reduced and such organisms are called *Opportunistic pathogens.*

As infection leads to disease, it would be useful and interesting to note the various types of infections.

Infection is the lodgement and multiplication of a parasite in or on a host. A material contaminated with a microbe(s) is designated as *infectiv*
Take the case of bacterial flora of fisl
Normally, the slime, skin, gills, stomac
or intestines in a fish will be infecte
and the bacterial load ranges betwee
10^3 - 10^6 per gram weight although th
flesh and body fluids of newly caugł
healthy fish are considered to be sterile
However, it is not necessary that a
infection, if noticed, should alway
result in disease, as outcome of
disease is normally dependent on variou
factors such as the virulence of th
attacking agent, host's genetic factor
and host's environmental conditions.

Based on the source of infectior
it can be mainly divided into twc
endogenous infection if the host i
infected with an agent from the host'
body itself, and *exogenous infection* i
the infection is due to an agent purel
from outside.

Further, an infection can be classified in various ways based on the tim
and type of infection.

The initial infection in a host witl
a parasite is *primary infection* an
subsequent infection by the sam
parasite is termed as *re-infection.*

At a time when a host's resistanc
is lowered by a pre-existing diseas
and other prasite initiates infection
this condition is called *secondary infection.* But, *cross infection* occur
when a host is already suffering fron
a disease and again a new infectior
takes place from any external source
If the infection is only in a localise
area in a host, (eg. gill rot), it is knowr
as *focal infection* or *focal sepsis,* bu
if the attack exists internally or includin
external, then the situation is *systemi*
infection (eg. furunculosis)

In a host, if the clinical symptoms as a result of infection, are not apparent in helping to diagnose a case then it is denoted as *inapparent infection* and *atypical infection* when the typical clinical symptoms are not manifested. If a parasite remains quite for some time in a host and initiates the clinical symptoms only when the host's resistance is lowered, the situation is known as *latent infection.*

A stage of mere existence of bacteria in the blood is said to be *bacteremia,* while in *septicaemia* the bacteria exist and continue to multiply in the blood. *Pyaemia* is a situation in which pyogenic bacteria cause septicaemia and abscesses in the body.

A condition in which the organism remains localised in a host and releases its toxin in the blood stream is called *toxemia.* Any human-induced infection resulting from diagnostic and other therapeutic measures, is expressed as *iatrogenic infection.*

An ailment is said to be *contagious* when it is transmitted by direct contact and *infectious disease* if the pathogen is acquired by sources other than direct contact.

Based on the spread of infection, diseases may be classified as *enzootic,* when the disease is present only in a localised area; *epizootic,* when the disease is present almost throughout an area; and *panzootic,* when the disease spreads all through the world. ●

SUPPORTING PAPER 3

FISH AND SHELLFISH DISEASES IN CULTURE SYSTEMS

Part III Defenses against infection.
Seafood Export Journal., **12**(10): 19-21(1980)

Reprint from "Seafood Export Journal"
Vol. XII. No. 10. October 1980

Fish and Shellfish Diseases in Culture Systems

III. Defenses against infection

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"If infection leads to indisposition the system offers immunity"

In the previous article of this series, infection and kinds of infection have been discussed. Now, let us examine how a case of infection is overcome by the host before the infection leads to a serious disease succeeding a variety of host's defenses.

In both poikilothermic and homeothermic animals quite an efficient defense mechanism is present and one may be surprised on the incidence of diseases inspite of all such protective measure.

When a pathogen infects a host, there may be some initial abnormalities in the host because of infection, multiplication of the pathogen, release of microbial toxin and due to the efforts made by the host to defend itself from the pathogen. This protective response against tissue damage is called inflammatory reaction. Certain characteristic features of inflammatory reaction are temperature variation, pain, uneasiness, redness, swelling and loss of function. If the cause(s) of inflammation are removed, the tissues come to normal and on account of tissue proliferation, any minimum damaged tissue may also get repaired in due course. When an infection at it's mild stage is overcome by the host, no further symptoms may appear. If not, even a mild attack may change into systemic infection and may finally lead to general death which is the cessation of the complete vital functioning of all the organs as a result of accumulated disorders in the body system. To restrict premature local * / general death due to infection in all the living beings their defense mechanism will systematically be functioning to defend themselves from infections. The protective measures in a host against infection(s) are called defense mechanism and for more convenience they may be classified as structural, cellular and humoral although the present trend is to classify the defense system as cellular and humoral.

STRUCTURAL DEFENSE

Skin and slime or mucous form the structural defense system. The internal tissues and organs of the body

are protected from the external environment by the skin and slime. These structures function as physical barriers against infections. Moreover, slime is considered to have antipathogen activity. Because of such special mechanisms, this system of defense inhibits / removes pathogens that infect the host. The presence of fatty acids in the skin is also believed to possess antipathogen property.

CELLULAR DEFENSE

If the skin and slime are damaged by any means, the pathogen find entry into the body. As soon as the internal cells are attacked, this system of defense become alert and start functioning opening with inflammatory reaction. In a few sceonds time, the blood vessels dilate, become more permeable and plasma from the blood is poured over the attacked area. The pathogens arriving at this area are killed in the plasma because of its antipathogen property and / or they may be made susceptible to further attack of leucocytes. Leucocytes are present in the blood. They are attracted to the site of infection and the pathogens are invaded. In the attack, if the pathogens are not fully destroyed, the leucocytes may be removed from the site by the blood stream and lymph node like organ (spleen) takes over charge of attack against the pathogens. As there is no lymph nodes in fishes (lymph nodes trap and kill pathogens in higher animals) the work for destorying the pathogens is carried out by the spleen which serve as lymph node like organ.

The pathogen hes to face another defense system which is called reticulo-endothelial system (RES). This is a system of phagocytic cells scattered throughout the body. This system is considered to be responsible for segregating phagocytosed material from the body. There are two population of macrophages (reticulo - endothelial cell having the capacity for phagocytosis). One is fixed and the other is circulating. The promonocytes of the haemopoitic organs, monocytes of the blood and lymph, macrophages of loose connective tissue, free and fixed macrophages of the spleen, kidney and the fixed macrophages of the atrial lining of the heart form the reticulo-endothelial system.

THE CHARACTERISTIC FEATURES OF THE RES ARE:

1. The atrium has got significant phagocytic activity in fish whereas this activity is not present in other higher animals.
2. There is no lymph nodes in fishes but lymph node like organ, spleen is present.
3. The liver whose Kupffer cells, almost inert in fishes, are alert in phagocytic activity in higher animals.

HUMORAL DEFENSE

In addition to the above defenses, one more set of defense functioning is called humoral (fluid) defense. This is found working at various intervals independently and along with other defenses like structural and cellular. The various humoral defenses are:

NATURAL NON-SPECIFIC ANTIBODIES:

These natural antibodies are considered to have antipathogen properties.

COMPLEMENT:

This system, is a complex of ensymes, and has got an effective antipathogen property.

CREACTIVE PROTEIN (CRP);

It is considered to be normally present in the fish serum and functions as a defense factor against pathogens.

INTEROFERON :

This is an important antiviral agent.

LYSOZYME :

Lysozyme, an important weapon of host's defense factor, functions well against various varieties of pathogens. It is present in the slime, skin, serum and phagocytic cells.

NATURAL HAEMOLYSINS :

Certain substances called natural haemolysins detected in the serum are considered to have antipathogen qualities and the capacity to lyse foreign red cells.

SPECIFIC ANTIBODIES :

Another significant defense is specific antibodies belonging to the class of serum proteins called immunoglobulins (Ig). While in mammals five distinct classes of Ig are identified, in teleosts only one class of immunoglobulin (IgM) is clearly detected. The antibodies can act in a variety of ways. Based on their biological activity, they may be categorised as agglutinating antibodies which work against particulate antigens; precipitating antibodies which function as antitoxins (antitoxic antibodies) by precipitating soluble antigens and neutralising their toxins; and virus-neutralising antibodies which act against viruses and make them inactive.

In general the structural and cellular defenses will be acting against any invader as they are non specific in their activity and the significant difference in the defense system of fishes and shellfishes is that acquired antibodies detected in fishes are not noticed in shellfishes except antibody like substances. ●

SUPPORTING PAPER 4

FISH AND SHELLFISH DISEASES IN CULTURE SYSTEMS

Part IV Bacterial diseases.
Seafood Export Journal., **13**(8): 19-26(1981)

Reprint from "Seafood Export Journal"
Vol. XIII. No. 8. August, 1981

Fish and Shellfish Diseases in Culture Systems

IV. Bacterial diseases

C. Thankappan Pillai
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"Disease may ensure immunity but not life"

All living beings, in their natural surroundings, become susceptible to diseases one time or the other in their life. Fishes and shellfishes are also no exception. Even in the natural conditions, a few of fishes and shellfishes become diseased and most of the cases pass unnoticed possibility due to lack of interest and and/or odservation. Ocuurrecce of diseases in these organisms either in their natural environments or in culture system adversely affect the population. So, a thorough study of the diseases is absolutely essential, especially in the present context of a global awarecess to enhance the food production through aquaculture of fishes and shellfishes in order to meet the requirements of animal protein for the increasing population.

Considerable work has been carried out in severol countries in this field and substantial literature is available (Conroy and Herman, 1970; Sindermann, 1970 and 1977, Snieszko, 1970; Bullock, 1971; Bullock; Conroy and Snieszko 1971; Mawdesley – Thomas, 1972; Reichenback - Klinke, 1973; van Duijn, 1973, Roberts and Shepherd, 1974; Ribelin and Mijaki, 1975; Dulin, 1976; Hoffman, 1977; and Roberts, 1978). However, few investigations have been undertaken in this discipline in our country and it is imperative that concerted efforts should be made to study and understand the various important fish and shellfish diseases affecting our resources.

Some important bacterial diseases, which cause heavy loss to cultivable species of fish and shellfishes are:

1. Hemorhagic septicaemia
2. Vibriosis
3. Furunculosis
4. Eye disease
5. Fin / tail rot
6. Ulcer
7. Skin lesion
8. Myxobacteriosis
9. Kidney disease
10. Gill rot
11. Tuberculosis
12. Enteric bacteriosis

Fish and shellfihhes being mute and living in a dynamic environment,

detection and disease diagnosis in these cultivable species are rather difficult as compared to that of human diseases.

The symptoms of a disease are of considerable help to privisionally diagnose the case. But, detection of the aetiological agent of the disease will be of definite hope to confirm the case and makes it easier to treat the disease as well as to sdopt proper prophylactic measures against its outbreak.

Nevertheless, symptomatic treatment shall be followed as any delay in treatment results in heavy mortality of the afflicted population. Considering the needs, a provisional key, for the diagnosis of cases and treatment, is formulated and presented in table 1.

Bacterial diseases predominate among fishes and shell-fisees. But, it may be surprising to note that these organisms are not necessarily essential to be the cent percenr cause(s) always for an outbreak of disease. Because, in any community, it has been detected that normal individuals / hosts also sometimes harbour or carry potentially pathogenic microbes without showing any apparent disease symptoms or falling sick. For example, species of *Aeromonas* and *Pseudomonas* which are the normal bacteria of the aquatic environments cause diseases to the fishes and shelifishes when they are under stress. Any sort of stress (eg. dense population) explicates the chances of invading organisms to make the host more susceptible and provokes diseases. And, this happens due to failure of the host's defense mechanism in the struggle against the invading agent. Here, it is better to realise that stress always lowers the resistance of animais and enhances susceptibilty to disease. 'Stress' is a state of condition, caused by intrinsic or extrinsic factors, which upsets the adaptive responses of the animals and reduse the chances of their survival. Stress can be short term or chronic. In the case of short term stress (eg. cold shock) the animals return to normalsy within a few (4 – 8) hours. But chronic stress cause severe damage as a result of increased susceptibility to infections probably because of reasons such as decreasing inflammatory responses (basic protective responses to tissue damage), decreasing amount of circulating lymphocytes (white blood cells responsible for immune responses), impairing of gamma - globuling (plasma protein concerned with antibody production against infections) production and depressing interoferon (important defensive factor against virus infection) production etc.

Thus it is essential to maintain the natural equilibrium to a resonably possiole level among the host, parasite and envoronment to minimise diseasas Outbreak of adisease is usually the result of abnormal alternation in the adjustment of the interrelationship of the host: parasite and environment and the severity of the disease depends on the intensity of the alteration. Hence, enough care has to be taken in selecting disease-free and immunised seed, observing hygienic measures, maintaining congenial water and other sanitary conditions, and supplying suitable balanced diet for having healthy and quality fishes and shellfishes.

TABLE-1

Sl. No.	Disease	Clinical symptoms	Drug(s)	Administration
1.	Hemorrhagic septicaemia	Body reddening, skin lesion with pus and/or blood, swollen belley, protruding scales, sunken or ptotruded eyes inflamed anus, bloody discharge from the vent, discolourstion and degeneration of the liver, swollen spleen, inflamed swim bladder, anaemia.	Chloromycetin Terramycin	Short bath in suitable water* having any one of the drugs at a rate of 25 - 75 mg. per liter of watar OR intraperitoneal injection of any one of the drugs at a level 0.02 mg. per gram body weigyt of the fish or any one of the drugs may be given along with feed, at a rate of 1 mg. per 10 gram body weight of the fish, once daily for 7 - 21 days.
2.	Vibriosis	Erythemia or hyperemia of the skin and fins, petechiae in the mouth, swimming with uneasiness near water surface, body darkening, abdominal distension, corneal opacity, anorexia, pale gills, enlargement or liquefaction of the spleen and kidney myocardial infection.	– do –	– do –
3.	Furunculosis	Boils may be present, skin lesion with pus/or blood, pale gills, sluggish movements, fraying of the fins, inflamed intestine, myocardial necrosis, iiquefaction of the kidney and spleen, bleeding from the gills.	Terramycin	Along with feed, the drug may be administered at a rate of 5 – 10 mg. per 100 gram body weight of the fish once daily for 7 – 21 days OR intraperitoneal injection of the drug at a dose of 0.02 mg. per gram body weight of thefish.

TABLE - 1 (Continued)

Sl. No.	Disease	Clinical Symptoms	Drug(s)	Administration
4.	Eye disease	Eye opacity with tissue proliferation, weak movements, pale gills, anorexia.	Brilliant green Chloromycetin	One mg. of chloromycetin may be given in feed per 10 gram body weight of the fish, once daily for 21 days. Swabbimg with brilliant green solution (0.1%) at the area with or without surgical removal of the infected tissue.
5.	Fin / tail rot	Loss of natural colour beginning from the out margin of fin / tail,, fraying of the fin / tail, progressive disintegration of the fin / tail tissue, weak movements, swimming near water surface. Ecchymosis may be noticed.	Acriflavine	Dip in 1 – 5 ppm acriflavine suitable water or surgical removal of the infected tissue and application of the drug (0.1%) in solution in the operated area.
6.	Ulceer	Shallow open sores mostly with white rim, presence of pus and / or blood, erdoded fins and mouth·	Acriflavine Chloromycetin	One mg. of chloromycetin may be given in feed per 10 gram body weight of the fish once daily for 7 – 21 days. Swabbing with acriflavine (0.1%) solution at the site of infection.
7.	Skin lesion	Skin lesion clearly visible in water, pus and / or blood in the lesion, sluggishness, anorexia,	Acriflavine Brilliant green Chloromycetin	Swabbing either with acriflavine or brilliant green (0.1%) solution at the site of infection OR short bath thrice daily in suitable water having the

TABLE-1 (Continued)

Sl. No.	Disease	Clinical Symptoms	Drug(s)	Administration
				antibiotic at a rate of 25-75 mg, per litre of water for 3 - 10 days.
8.	Myxo-bacterious	Grayish white spots or patches in the body and fins with pus and / or blood, swollen lips, anorexia, sluggishness, damaged gill tissue.	Terramycin	One mg. of the drug in feed, per 10 gram body weight of the fish, once daily for 7-21 days OR short bath having the drug in suitable water at a dose of 25 - 75 mg. per liter of waterfor 7 - 21 days.
9.	Kidney disease	Body lesion, bilateral expothalmia, swollen abdomen, balanceless swimming, body darkenining, swollen kidney, swollen spleen, infected liver, pericarditis, false membrane over kidney, liver and spleen may be noticed.	Erythromycin	- do -
10.	Gill rot	Isolated movements, anorexia restlessness, surface floating. orientation against the current, weakness, gill tissue decay or proliferation.	Copper Sulphate Erythromycin	One mg. of the drug (erythromycin) in feed, per 10 gram body weight of the fish, once daily for 7 – 21 days OR dip treatment (for few seconds) in copper sulphate in suitable water at a rate of 1 - 2 mg. per liter of water.

TABLE - 1 (Continued)

Sl No.	Disease	Clinical symptoms	Drug(s)	Administration
11.	Tuberculosis	Progressive body weakening, damaged or folded fins, swelling of abdomen, anorexia, colour fading, deformities in the skeletal system, sluggish movements, opacity in the cornea, scale defects listlessness, presence of tubercles.	Kanamycin	One mg. of the drug in feed, per 10 gram body weight of the fish, once daily for 7-21 days OR intraperitoneal injection of the drug at a rate of 0.02 mg. per gram body weight of the fish.
12.	Enteric bacteriosis	Enteritis, sluggish movements, body lesions, body discolouration, reddening of the anus, cyclic movements, swimming near water surface. Kidney may be ihfected.	Sulphadizine Sulphisoxazole	One mg. of sulphisoxazole in feed, per 10 gram body weight of the fish, once daily for 7 - 10 days OR short bath, having sulphadiazine in suitable water at a rate of 25–75 mg. per liter of water, for 3 - 14 days.

* Habitat water or water similar in quality to that of habitat water should be used.

SUPPORTING PAPER 5

FISH AND SHELLFISH DISEASES IN CULTURE SYSTEMS

Part V Prophylaxis and disease check up.
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Fish and Shellfish Diseases in Culture Systems

V. Prophylaxis and Disease check up

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The importance of aquaculture of cultivable finfishes and shellfishes has already been mentioned in tjhe preceeding series of this article. In all living beings diseases can occur at unpredictable intervale, but by adopting proper management and timely prophylactic measures, to a large extent diseases can be prevented.

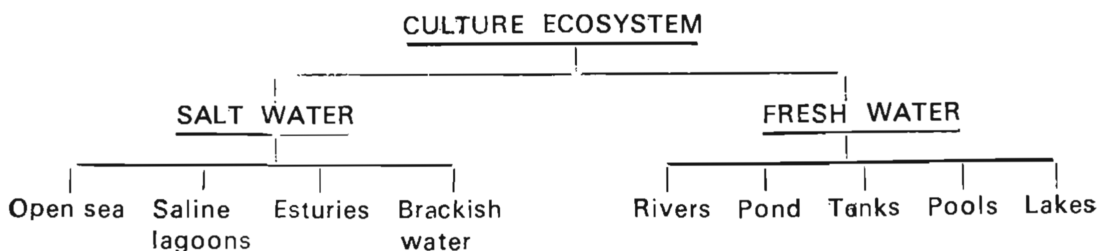
It is essential that finfishes and shellfishes are safeguarded against diseases as they damage the resources in the natural and culture systems, which result in loss of weight anu bodily damages resulting in non-marketability. Above all, the possibility of certain types of infections being transmitted through aquaculure p;oducts to man cannot be ruled out. Further, the occurence of diseases in cultivable organisms pose serious problems to the management of the resources.

It is often believed that incidence of diseases in the vast culture system is unavoidable. This need not be the rule, for many of the diseases can be prevented by following certain measures of prophylaxis.

For convenience, let us classify culture ecosystems as in table 1.

Each system has its own ecological and biological characteristice.

TABLE—1



As the nature of the culture ecosystem differs, the applieability of the undermentioned guidelines may also vary. In the enclosed water culture ecosystem, disease prevention is relatively easier than that of the open sea culture ecosystems in cages and pens wherein control over the ecosystem can be only minimal or precticallyni l.

Methods of prophylaxis are:

1. SELECTION OF CERTIFIED DISEASE FREE SEEDS FOR CULTURE

As in agricultural crops, a practice of certification and issue of disease free finfish and shellfish seeds would be highly useful. This is in practice in some foreign countries such as U. S. A. and Canada and could be adopted in India.

2. DEVELOPMENT OF DISEASE RESISTANT SEEDS

This can be carried out by selective breeding of hardy species. This method has been found quite successful by investigators in Germany in developing disease resistant species (carps) against hemorrhagic septicaemia. Genetic selection with desirable traits is thus very important.

3. SUITABLE SITE SELECTION

Site for culture should have all the required congenial conditions. For example, the culture site should not be contaminated by pollutants and there should be adequate supply of good quality water. The culture system should be monitored for pathogenic orgenisms.

4. DISINFECTION OF CULTURE SITE

It will be desirable to drain off the culture ponds and treat the ponds with any one of the disinfectants (say, quick lime / calcium cyanamide etc.) using permissible levels of contamination. When chemical treatment is made, stocking should be done only after a period of time to allow for biodegradation and flushing. The pH and other parameters will havo to be carefully checked for stocking.

5. DISINFECTION OF SEEDS

If the seeds are not certified ones, and if contamination is suspected on examination, every consignment of seeds before releasing them in the culture system, may be suitably treated with an effective antimicrobial agent (eg. Terramycin at a rate of 15-50 mg. per litre of water.

6. INSTALLATION OF PROPER BUND AND SLUICE

This is applicable in the case of enclosed water culture system. Suitable bunds which are seepage free are essential. Sluices should be installed in the bunds to control the inflow and outflow of water and permit good circulation. The mesh size of the screens of the sluices should be suitable to retain the stocked animals and at the same time prevent the entry of the free swimming larval parasites and predators from outside- Care should be taken for the proper maintainance of the screens and sluices.

7. STOCK DENSITY

The field should be stocked only with optimum number of seeds in consideration of the characteristics of the species selected for culture and the nature of the site as overcrowding can at times lead to disease outbreaks. So, a preferable stock density limit may be fixed according to the size of the site and nature of seeds.

8. CARE ON ENVIRONMENTAL STRESS

Any sudden change in the environmental factors can impose severe stress to the stocked animals. For example, presence of excess of decaying organic matter may result in drastic decrease

in the dissolved oxygen content of the water body. Here, the oxygen deficiency acts as a stress factor to the standing stock. Every effort should be taken to minimise or avoid any kind of stress to the animals.

9. KNOWLEDGE OF LIFE HISTORY OF THE PATHOGENIC PARASITES

A knowledge of the life history of the pathogens in relation to different ecological parameters will help to save the population at the right time. This will also be helpful to adopt suitable prophylactic measures and treatment where necessary.

10. STANDARD FOOD FOR FEEDING

Supply of well balanced diet should be maintained. Artificial food may be made sterile and supplied. In the case of natural food such as phytoplankton and zooplankton, they may be treated with ultraviolet rediation and or deep frozen over 24 hours in clean water for stock.

11. QUALITY OF FOOD

Periodical chek up to ensure that the food items are free from pathogens and microbial toxin(s), is essential to avoid their entry into the culture system. For example, aflatoxin produced by a mould, *Aspergillus flavus* can cause hepatoma disease.

12. MAINTAINANCE OF GENERAL CLEANLINESS

Hygiene is very important in culture practices. Utensils, nets and other instruments should be properly and suitably cleaned and sterilised by UV radiation or using chemicals. In the absence of sufficient facilities' the hatchery instruments may be properly cleaned in clean water and sun dried well before using or reusing. This will help to eliminate contaimination and cross infection.

13. AVOIDANCE OF PATHOGENS IN CIRCULATING WATER

Water may contain pathogens harmful to the stocked animals. Hence in recycling systems, it will be desirable thet the water be suitably filtered and treated with ultraviolet light to kill the pathogens and reduce the high bacterial load.

14. LIMITATION OF FREQUENT HANDLING

Avoid frequent handilng of the animals, especially by inexperienced hands. Moreover, handling should be as gentle as possible as any stress or any injury can cause epizootics and other problems.

15. SEGREGATION OF ANIMALS AND BIRDS

As far as possible, no chances should be given for animals and birds to get into the culture systems as quite often they serve as diseas vectors by transmitting various viral, bacterial and fungal pathogens. Bird and animal dropping could also contaminate the water;

16. SEED IMMUNISATION

As vaccination is effective and possible to prevent certain types of diseases, suitable and timely vaccination may be given promptly. Mass immunisation is successful against vibrosis by immersion method in the coneerned vaccine. Oral vaccine introduced through feed is also effective.

Adequate care may be taken right from the egg stage in order to remove the infected from among the non-

infected as the infected ones will rapidly spread the infection and lead to mass mortality.

18. SCREEINNG OF FREQUENT SEED INTRODUCTION

In a culture system, frequent introduction of seeds, at different intervals without proper quarrantine period and conditioning in the ecosystem in which the seeds are to be introduced, is not desirable, because, as a result of frequent introduction, pathogens may be introduced in the culture system along with the seeds. This will save the standing stock and the introduced animals. Moreover, the microbial flora of the newly introduced seed may serve as pathogens to the standing stock and also the microbial flora of the culture system may in turn become pathogenic to the newly introduced seed due to lack of the conditioning of the animals to the flora and the flora to the animals.

19. DISEASE CHECK UP

Disease check up of the standing stock at frequent intervals is quite essential to estimate the general health of the animals to forecast the occurrence of diseases and to take necessary measures for safeguarding. Along with the disease check up, water analysis is also useful to determine the hydrological and biological conditions.

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SUPPORTING PAPER 6

STUDIES ON THE MICROBES OF ESTUARINE AND MARINE FISHES OF INDIA

Part I Bacterial flora of the Pearl spot *Etroplus suratensis* Bloch and the White fish *Lactarius lactarius* Schneider. (in press)

STUDIES ON THE MICROBES OF ESTUARINE AND MARINE FISHES OF INDIA.

1. BACTERIAL FLORA OF THE PEARLSPOT *ETROPLUS SURATENSIS* BLOCH AND THE WHITE FISH *LACTARIUS LACTARIUS* SCHNEIDER

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ABSTRACT

Bacteriological studies of *Etroplus suratensis* Bloch and *Lactarius lactarius* Schneider from the estuarine and marine environments respectively showed that the average bacterial load was high on the skin (with muscle), 7.6×10^5 per gram for *E. suratensis*, and 1.9×10^6 per gram for *L. lactarius*. Qualitatively, the bacterial species isolated were of the genera, *Pseudomonas*, *Vibrio*, *Achromobacter*, *Flavobacterium/Cytophaga*, *Coryneforms*, *Bacillus* and *Micrococcus*. *Pseudomonas* including fluorescent types and *Vibrio* predominated in both the species.

INTRODUCTION

Fish, as a high protein food, deserves much attention for consumption without any changes in the quality. After death, the quality of fish changes hourly if not properly preserved, due to the bacterial and tissue enzyme action. Therefore, it should be of some interest and importance to study the average bacterial load on fresh and spoiling fishes together with the types of bacteria associated with them at various stages of spoilage inorder to be able to device measures to prevent spoilage.

Published data on the bacteriological aspects on fishes especially from the Indian waters are scanty (Bhat and Albuquerque, 1953; Karthiayani and Iyer, 1967; Venkataraman and Sreenivasan, 1952, 1954, 1955) although a considerable amount of work has been carried out on fishes from other oceans. The present study carried out from March to May, 1974, on the estuarine fish, *Etroplus suratensis* Bloch and the marine fish, *Lactarius lactarius* Schneider, was aimed at investigating the average bacterial loads and the types of bacteria present on the external and internal organs of these two species and thereby determine any quantitative or qualitative differences that

may exist in the bacterial flora of our estuarine and marine fishes.

The author is much obliged and expresses his sincere thanks to Dr.E.G.Silas, Director, C.M.F.R.Institute, Cochin-18, upon whose active interest and sincere encouragement this work is made possible and also for critically going through the manuscript several times and improved the paper. My sincere thanks are also due to Dr.J.M.Shewan, Torry Research Station, Scotland for helping with literature and the efforts shown to modify the manuscript; to Shri K.Mahadeva Iyer, Central Institute of Fisheries Technology, Cochin-3, for going through the manuscript and offering helpful suggestions.

MATERIALS AND METHODS

Fresh specimens of *E. suratensis*, from the Cochin Backwater, a tropical estuary, and *L. lactarius*, from the inshore waters off Alleppey were taken under aspetic conditions, placed in sterilized glass containers and transported to the laboratory immediately. The bacteriological sampling from *E. suratensis* were completed within an hour after the capture of the fish, whereas

with _L. lactarius_ 4-5 hours had elapsed. During the period of storage, the specimens were kept below 10 °C. Samples from gills, skin (with muscle), and intestines were taken from twelve specimens each of _E. auratensis_ and _L. lactarius_ spread out over a three month period.

The samples were pour plated using the modified ZoBell's agar (ZoBell, 1946) of the following composition:

Bacto peptone	= 10.0 gram
Ferric phosphate (BDH,AR)	= 0.1 ''
Bacto agar	= 15.0 ''
Aged and filtered sea water	= 1000.0 ml

(adjusted to pH 7.5 and sterilized at 15 pounds pressure per sq. inch for 30 minutes)

The poured plates were incubated at room temperature (about 30 ± 2 °C) for 24 to 72 hours after which time the colonies were counted. Selected colonies were then transferred to Sea water peptone of the following composition:

Bacto peptone	= 10.0 gram
Potassium nitrate (BDH,AR)	= 2.0 ''
Aged and filtered sea water	= 1000.0 ml

(adjusted to pH 7.5 and sterilized at 15 pounds pressure per sq. inch for 30 minutes)

From sea water peptone, after establishing the purity of the isolates, the organisms were streaked on to Sea water agar slants and kept at room temperature for further investigation.

The bacterial isolates were then classified using Shewan's System (1960a,b).

Modified Casares-Gil flagella staining (Gemmell and Hodgkiss, 1964) was followed for detecting flagella.

The anaerobic floras of these fishes were not investigated.

RESULTS

A total of 27 and 20 strains from the gills, 36 and 24 strains from the skin (with muscle) and 30 and 23 strains from the intestines of E. auratensis and L. lactarius respectively were isolated.

The quantitative and qualitative aspects of bacteriological data obtained from both species of fish examined are given in tables, I to III.

DISCUSSION

One hundred and sixty cultures were isolated from the gills, skin (with muscle) and intestines of the two species of fishes. The majority were motile, Gram negative rods, although Coccoid rods were encountered, a fact concurring with the results of Georgala (1958).

Various pigmented strains were also encountered whose colours ranged from cream white to yellow, orange and rose ro red.

The average aerobic counts in the gills were of the order, 1.1×10^4 per gram in _E. auratensis_ and 8.1×10^4 per gram in _L. lactarius_; on the skin (with muscle) 7.6×10^5 per gram and 1.9×10^6 per gram; and in the intestines 8.8×10^4 per gram and 1.2×10^6 per gram respectively.

The higher counts obtained from the skin as compared with the gills and intestines in both fishes are somewhat similar to the results obtained by Liston (1956).

The higher counts which generally occurred with _L. lactarius_ may be due to the differing habitats as

well as the time factor (4-5 hours) between capture and sampling in the laboratory.

Qualitatively, bacteria belonging to 7 genera including species of *Pseudomonas*, *Vibrio*, *Achromobacter*, *Flavobacterium*/*Cytophaga*, *Coryneforms*, *Bacillus* and *Micrococcus* were identified from the gills, skin (with muscle) and intestines of both fishes.

The gills:

In the gills of both fishes, five genera were encountered in *E. auratensis* and four in *L. lactarius* (Table I).

Table I. Aerobic bacterial flora of the Gills of *E. auratensis* and *L. lactarius* (expressed in percentage of the total isolate made)

Organisms	*E. auratensis*	*L. lactarius*
Species of *Pseudomonas*	66.7	75.0
" " *Vibrio*	14.8	5.0
" " *Flavobacterium*/*Cytophaga*	3.7	15.0
" " *Achromobacter*	7.4	-
" " *Corynebacterium*	7.4	5.0

The species of *Pseudomonas*, *Vibrio*, *Flavobacterium*/ and *Corynebacterium* are common to both the species of fishes, but the genus *Achromobacter* occurred only in *E. auratensis*. From table I, it will be seen that *Pseudomonas* and *Vibrio* predominated in *E. auratensis* and *Pseudomonas* and *Flavobacterium*/*Cytophaga* in *L. lactarius*.

In the present survey, the results are similar to that of Aschehoug and Vesterhus (1940) and Liston (1957).

The skin (with muscle):

On the skin (with muscle) on both fishes, seven genera, *Pseudomonas*, *Vibrio*, *Flavobacterium*/*Cytophaga*, *Achromobacter*, *Corynebacterium*, *Bacillus* and *Micrococcus* were observed (Table II).

Table II. Aerobic bacterial flora of the Skin (with muscle) of *E. auratensis* and *L. lactarius* (expressed in percentage of the total isolates made)

Organisms	*E. auratensis*	*L. lactarius*
Species of *Pseudomonas*	52.8	37.5
" " *Vibrio*	16.7	8.3
" " *Flavobacterium*/*Cytophaga*	-	12.5
" " *Bacillus*	13.8	8.3

Organisms		E. auratensis	L. lactarius
'	' Micrococcus	11.1	4.2
'	' Achromobacter	2.8	29.2
'	' Corynebacterium	2.8	-

But, of these, species of Flavobacterium/Cytophaga were found only in L. lactarius. Likewise, species of Coryneforms were found only in E. auratensis. Again, Pseudomonas sp. predominated on the skin (with muscle) of both the fish species, but comparatively more species of Pseudomonas were found in E. auratensis, which is quite contrary to what was observed in the gills.

Aschehoug and Vesterhus (1940) in Norwegian winter herring, Georgala (1958) in north sea cod and Liston (1957) in sea skate and sea lemon sole recorded species of Pseudomonas as predominating forms. Karthiayani and Iyer (1967) in Sardines, Pivnick (1949) in Canadian Atlantic Cod, Stewart (1932) and Shewan (1938) in North Sea Haddock found species of Achromobacter as dominating organisms. Dyer (1947) in Candadian Atlantic Cod found species of Micrococci including species of Sarcina as dominant. Reed and Spence (1929) in Canadian Haddock and Venkataraman and Sreenivasan (1952) in mackerels recorded species of

Bacillus as dominant forms and the latter authors in (1955) in Shark noticed species of *Coryneforms* and *Micrococcus* and *Sarcina* were equally found dominant. Wood (1940) recorded species of *Micrococcus* and *Sarcina* and in (1953) *Micrococcus*, *Sarcina* in teleosts and Coryneforms in elasmobranchs as predominating forms. Bhat and Albuquerque (1953) in Bombay Duck found *Bacterium phosphoreum* and *Sarcina littoralis* as predominant forms.

The dominance of *Pseudomonas* sp. noticed on the skin (with muscle) of *E. auratensis* and *L. lactarius* is a finding similar to that of Aschehoug and Vesterhus (1943); Georgala (1958) and Liston (1957).

The intestines:

In the intestines of *E. auratensis* and *L. lactarius*, strains belonging to six genera (Table III) occurred, viz., *Pseudomonas*, *Vibrio*, *Flavobacterium*/*Cytophaga*, *Achromobacter*, *Coryneforms*, and *Bacillus*.

Table III. Aerobic bacterial flora of the Intestines of *E. auratensis* and *L. lactarius* (expressed in percentage of the total isolates made)

Organisms	*E. auratensis*	*L. lactarius*
Species of *Pseudomonas*	33.3	47.8

Organisms	E. suratensis	L. lactarius
Species of *Vibrio*	60.0	21.7
" " *Flavobacterium/Cytophaga*	6.7	4.4
" " *Achromobacter*	-	13.0
" " *Coryneforms*	-	8.7
" " *Bacillus*	-	4.4

Of these, in *E. suratensis* only species belonging to the genera, *Pseudomonas*, *Vibrio*, and *Flavobacterium/Cytophaga* were present. An interesting finding was the predominance of *Vibrio* species in *E. suratensis* and *Pseudomonas* sp. in *L. lactarius*.

Aschehoug and Vesterhus (1940), Stewart (1932) found species of *Achromobacter* as predominating forms. But Karthiayani and Iyer (1967) observed both *Achromobacter* and *Pseudomonas* as equally dominating forms. Liston (1957) recorded species of *Vibrio* in the sea skate and *Pseudomonas* and *Vibrio* as equally dominating in the sea lemon sole. Reed and Spence (1929) also detected species of *Pseudomonas* as dominant forms. Dyer (1947) noted species of *Micrococci* including *Sarcina* as dominant. Venkataraman and Sreenivasan (1952) detected species of *Achromobacter* and *Bacillus* and Wood (1940) species of *Bacillus* as predominating forms.

The dominance of the species of *Vibrio* and *Pseudomonas* in the intestines of the two species of fishes is similar to the results of Liston (1957); Reed and Spence (1929) and Karthiayani and Iyer (1967).

In general, *Pseudomonas* sp. predominated in both species of fishes. A major difference is the importance of *Achromobacter* sp. in the results of Aschehoug and Vesterhus (1940); Georgala (1958); Liston (1957); Venkataraman and Sreenivasan (1955) and Wood (1940) which is next in importance to *Pseudomonas* in majority of the cases, whereas in the present investigation, *Vibrio* species are next in order of importance to *Pseudomonas* sp.

The occurrence of *Bacillus* and *Micrococcus* sp. which are supposed to be of land origin. In this investigation, parallel the results recorded by Dyer (1947); Venkataraman and Sreenivasan (1952, 1955) and Wood (1940,1950,1952,1953).

Among the *Pseudomonas* species isolated from *E. suratensis* and *L. lactarius*, fluorescent pigmented types occurred in the gills, on the skin (with muscle) and the intestines of both the species of fishes, but they occurred more frequently on the skin (with muscle) of *L. lactarius*.

In general it may be concluded that there were no great differences in the flora of *E. suratensis* and

L. lactarius despite their coming from two different ecological environments.

The average monthly salinity values of the Cochin backwater varied from 28.4‰ to 21.0‰ during the period when the samples of *E. suratensis* were collected.

Whether this parameter has got any influence on the bacterial floral composition of *E. suratensis* to be similar to that of *L. lactarius* is not known.

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SUPPORTING PAPER 7

MICROBIAL FLORA OF MUSSELS IN THE NATURAL BEDS AND FARMS

Coastal aquaculture: Mussel farming, CMFRI Bulletin., 29: 41-43(1980)

X

MICROBIAL FLORA OF MUSSELS IN THE NATURAL BEDS AND FARMS

C. THANKAPPAN PILLAI

Quantitative estimation of the bacterial load of the brown mussels cultured at Vizhinjam has been shown as 10^6. The occurrence of Coliforms, *Escherichia coli*, faecal streptococci and coagulase positive staphylococci is reported. *Pseudomonas*, *Vibrio* and *Micrococcus* are seen as normal flora.

INTRODUCTION

In India quite encouraging results have been achieved, in culture of the economically important species of brown mussel (*Perna indica*) and green mussel (*P. viridis*). However, adequate care has to be taken against diseases, that can occur to these animals, as they can deplete the stock as a result of mass mortality. Moreover, infestation can result in poor growth, thin meat, change of normal colour and failure of byssal development (Sindermann, 1970 in *Principle diseases of Marine Fish and Shell-fish*, Acad., Press, 369 pp[1].). Paralytic shelfish poisoning is another problem (Mason, 1971, *Underwater Journal*, 3 : 52-59[2]). Hence, studies on the life history of the aetiological agents of various diseases, treatment of diseases and necessary prophylactic measures against diseases are significant areas for

investigations for the success and development of mussel culture.

Material and Methods

In view of the above, a preliminary study of microbial flora of suspended cultured mussels (*P. indica*) in Vizhinjam, Trivandrum and surface sea water in that environment was made. Similar investigations were carried out in the mussels of the natural beds also at Kovalam, Trivandrum for comparison.

The mussels were aseptically collected in sterile containers. Surface sea water samples were collected aseptically in sterile glass bottles both from the culture site and from the environment of the natural mussel beds. The collected samples were immediately transported to the laboratory for further investigation. In the laboratory, the mussels were aseptically opened and the mussel meat along with fluid was separated and suitably diluted in sterile sea water. Similarly, water samples were also diluted. The diluted samples were inoculated into sea water agar. The samples were further plated in selective media for coliforms, *Escherichia coli*, faecal streptococci and coagulase positive staphylococci. The inoculated petri dishes were incubated at room temperature for 2-3 days and readings taken. The selected colonies, from the sea water agar, were transferred into sea water agar slants for further observation.

The isolates, were tested for their purity, and identified using the system of classification of Buchanan and Gibbons (1974, *Bergey's Mannual of Determinative Bacteriology,* 8th ed. Williams & Wilkins Co., Baltimore ; 1246 pp.)

Results

The results of the quantitative and qualitative studies are presented in Table 1 and 2 respectively.

Table 1. *Quantitative Aspects of the Bacterial Flora in Mussels and in Sea-water*

Organisms		Meat emulsion of the cultivated mussels	Meat emulsion of the mussels from the natural bed	Sea water from the culture site	Sea water from the natural mussel bed area
		Average counts per millilitre			
Total bacterial counts :	Oct. 1977	2.5×10^6	2.2×10^5	1.7×10^6	2.0×10^5
	May 1979	3.4×10^5	TNC	1.2×10^6	TNC
Coliforms :	Oct. 1977	1.0×10^4	1.6×10^3	3.5×10^4	2.8×10^4
	May 1979	2.2×10^4	TNC	6.3×10^4	TNC
Escherichia coli :	Oct. 1977	3.0×10^2	1.0×10^3	1.4×10^3	7.0×10^2
	May 1979	7.0×10^2	TNC	1.9×10^2	TNC
Faecal streptococci :	Oct. 1977	Nil	Nil	Nil	Nil
	May 1979	1.0×10^2	TNC	2.7×10^2	TNC
Coagulase positive staphylococci :	Oct. 1977	6.1×10^2	1.5×10^3	2.8×10^3	1.2×10^2
	May 1979	Nil	TNC	Nil	TNC

Nil — No growth. TNC — Test not made

Table 2. *Qualitative Aspects of the Bacterial Flora in Mussels and in Sea water* (In percentage)

Material		*Pseudomonas* Spp.	*Vibrio* Spp.	*Micrococcus* Spp.
Meat emulsion of the cultivated mussels :	Oct. 1977	50	34	16
	May 1979	60	40	—
Meat emulsion of the mussels from the natural bed :	Oct. 1977	50	25	25
Sea water from the culture site :	Oct. 1977	50	25	25
	May 1979	50	33	17
Sea water from the natural bed :	Oct. 1977	37.5	37.5	25

Quantitatively, the bacterial load of the suspended culture of mussels was relatively higher than that of the mussels in the natural bed. It was 10^6 and 10^5 respectively. Similar situation was also noticed in the case of sea water. The occurrence of coliforms, *Escherichia coli*, faecal streptococci and coagulase positive staphylococci were almost steady both in mussels and sea water. Faecal streptococci was not noticed in October 1977 but noted in May 1979. Coagulase positive staphylococci which was present in October 1977 was absent in May 1979.

As the normal flora in mussels and in sea water, species of *Pseudomonas*, *Vibrio* and *Micrococcus* were present. Species of *Pseudomonas* predominated both in the mussel and sea water. All the isolates of *Vibrio* were luminescent. The results of this investigation are almost in agreement with those of Colwell and Liston (1962, *J. Insect. Pathol.*, **4** : 23-33[4]) and Karthiayani and Iyer (1975, *J. mar. biol. Ass. India*, 17(1) : 96-100[5]).

Discussion

In the present investigation, species of *Pseudomonas*, *Vibrio* and *Micrococcus* are found as the normal flora of the mussels. Aquatic bacteria such as *Pseudomonas*, *Aeromonas* and *Vibrio* are potential pathogens and can cause diseases to the aquatic animals especially when the animals are under stress (Bullock, 1964, *Dev. Indust. Microbiol.*, **5** : 101-108[6]).

In the present study, *Escherichia coli*, an indicator organism of faecal pollution, was present in the mussels and sea water both at Kovalam and Vizhinjam. This reveals the possibilities of outbreaks of epidemics like gastro-enteritis if the polluted mussels are consumed without proper washing and cooking. If these mussels are to be marketed alive, they may first be depurated as discussed by Wood (1969, *Lab. leaflet*, **20** (N.S.), Lowestaft Fisheries Lab., 15 pp[7].) and Mason (1971 and 1976, in *Marine mussels : their ecology and physiology*, Camb. Univ. Press : 585-410[8]) by storing the mussels in sterile sea water for 2 days. The depurated mussels should be well washed and cooked for human consumption.

Disease causing bacteria, fungi, viruses, protistans and other parasites have been better studied in the case of oysters and clams than in mussels. Apart from the large-scale mortality of mussels caused by the parasitic attack of *Mytiltcola intestinalis* in American and European waters, pathogens like *Labyrinthomyxa marina*, *Monilia* sp., *Ostracobiabe implexa* and *Sirolpidium zoophthorum* are known to cause mortality among shellfishes. It is quite possible that under certain conditions mussels in the farm might also be affected by the above organisms. A haplosporidian, *Chytridiopsis mytilorum* is known to destroy mussel eggs in North Atlantic waters. Similarly *Haplosporidium tumefacientis* invades the digestive glands of mussels causing mild mortality. The gregarine *Nematopsis schneideri* causes mortality of mussels destroying the gill region. In Baltic waters, *Hypocomides mytili* and *Kidderia mytili* have been identified to cause mortality of mussels. *Ancistrocoma pelseneeri*, a ciliate, also causes considerable damage to the digestive system of mussels resulting in mortality. Species of *Aeromonas* and *Vibrio* are particularly dangerous to the hatchery produced molluscan larvae.

The above instances only go to show the potential dangers to be foreseen from different sources. Fortunately cases of diseases and mortality among mussels in India have not been so far reported. It is possible that this is not because of the absence of the diseases but due to inadequacy of attention to this aspect. Future investigations might throw light on this aspect.

Taking steps to prevent the outbreak of diseases is very important. In this context, the following points appear to warrant our attention.

(1) Selection of farm site free of biological and chemical contamination after studying the extent of contamination.

(2) Selection of disease resistant seed for culture.

(3) Avoiding overcrowded stocking in order to minimise the ill effects of epizootics.

(4) Care in handling the cultured stock to avoid contamination.

(5) Periodical investigation of the level of pathogenic organisms in the culture system to assess the status of the stock population.

(6) Eliminating other source of disease transmission by selective removal of reservoir host of pathogens.

(7) Routine disinfection of materials used in culture (materials like buckets, rope etc.) and

(8) Timely harvesting of stock reducing the vulnerability of older stock to diseases.

SUPPORTING PAPER 8

DISEASES OF FINFISHES AND SHELLFISHES CULTIVATED IN THE COASTAL WATERS OF INDIA

[Paper presented at the workshop in *Tropical Fish Diseases* held in Puncak, Java, from November 28 to December 1, 1978, jointly sponsored by International Development Research Centre (I.D.R.C.) and Indonesia. (in press)]

DISEASES OF FINFISHES AND SHELLFISHES CULTIVATED IN THE COASTAL WATERS OF INDIA

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Paper presented at the Workshop on 'Tropical Fish Diseases' held in Puncak, Java from November 28 to December 1, 1978, jointly sponsored by International Development Research Centre (I.D.R.C.) and Indonesia.

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ABSTRACT

Investigations initiated in 1976 by the Central Marine Fisheries Research Institute to accumulate and document the knowledge relating to Marine fish and shellfish diseases amongst the cultured stock from five project centres have brought to light certain common diseases. 'Hemorrhagic septicemia' due to *Pseudomonas* sp., 'Vibriosis' on account of *Vibrio anguillarum* and 'Myxobacterial infection' caused by *Chondrococcus* sp., were often noticed among *Penaeus indicus*, *P. monodon*, *Parapenaeopsis stylifera*, *Metapenaeus affinis* and *M. monoceros*. In the case of mass mortality of hatched prawn larvae, the etiological agents were identified as enteric forms like *Escherichia coli* and faecal streptococci. In the case of *Crassostrea madrasensis*, the edible oyster cultured in tidal creek, digenetic trematode infection

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of the gonads and viscera by Bucephalopsis haimeanus was observed. Predatory attacks by the gastropd cymatium pileare killing oysterlings was also common during certain months. Scylla serrata, the green crab was susceptible to fungal attack in the gonadial and muscular regions.

INTRODUCTION

Fish and shellfish culturists all over the world are quite often confronted with problems of large scale mortality of the tended stock due to diseases, predation and ecological variations. Successful management of farming culturable species of marine fish and shellfish depends amongst other aspects, prevention and elimination of such causes. Considerable work had been done in advanced countries in this realm and there is a vast literature available to the present day culturists (Borg, 1960; Bullock, 1964 and Bullock et al., 1971; Robinson and Meyer, 1966; Conroy and Herman, 1970; Sindermann, 1970; van Duijn, 1973; Shigueno, 1975). In India, raising of many species of marine fish and shellfish in brackish, estuarine and bay-water environments has been successfully

developed and demonstrated. Intensive, efficient culture of any animal requires crowding them more densely than in nature. Under these conditions they fall prey to many types of diseases, reaching catastrophic proportions which imposes the demand for knowledge about the nature and control of fish diseases. Investigations were initiated in 1976 by the Central Marine Fisheries Research Institute envisaging the following programme of work in five centres where intensive culture of different species of fish and shell fish are in progress.

a. Collecting diseased specimens from different species cultured.

b. Investigating the etiological factors responsible for the disease.

c. Classification of different types of diseases and determination of probable effects on the tended stock.

d. Development of treatment and preventive measures for the control of documented diseases.

Cochin and Vizhinjam in the west coast, Tuticorin, Mandapam and Madras along the east coast were the centres

chosen for implementation of this project work. Details of species cultured, project centres and method of culture are given in Table 1.

Table 1. Places, names of species cultured and method of farming.

Places	Species	Method
Cochin	1. *Penaeus indicus*	Brackish water pond culture
	2. *Metapenaeus monoceros*	
	3. *M. affinis*	
	4. *Parapenaeopsis stylifera**	
	5. *Chanos chanos* (Milk fish)	
	6. *Etroplus suratensis* (Pearl spot)	
Vizhinjam	7. *Perna indica* (Brown mussel)	Rope culture in Harbour Basin
Tuticorin	8. *Crassostrea madrasensis* (edible oyster)	Rack culture in tidal creek
	9. *Chanos chanos*	Salt pan fish culture
	10. *Mugil* sp. (Mullet)	"
	11. *Scylla serrata* (Green crab)	Cage culture in tidal creek
Mandapam camp	12. *Anguilla bicolor* (Eel)	Fresh water tank culture
Madras	13. *Perna viridis* (Green mussel)	Rope culture in open sea

*not cultured, but from experimental tanks

Periodical examination of the specimens in the farm was made. The moribund specimens whenever noticed were collected properly in suitable sterile containers and brought to the laboratory immediately. Pathological samples were aseptically removed and wet mounts examined microscopically before culturing the samples. Samples were cultured in appropriate media to isolate etiological agent(s). The etiological agent(s) were identified using the standard keys (Buchanan and Gibbons, 1974 and Shotts and Bullock, 1975). Pathogenicity of the isolates was studied in appropriate hosts to satisfy Koch's postulates. One of the junior authors (CTP) carried out the entire work relating to the pathogens.

RESULTS

A synopsis of the cases of diseases observed is presented in Table II.

REMARKS

The foregoing account, an outcome of investigations conducted by the Central Marine Fisheries Research Institute from 1976, cites instances of fish and shellfish diseases encountered so far in the animals raised in farms

Table II

SYNOPSIS OF CASES OF DISEASES
(after Pillai, 1978)

Case No.	Clinical symptoms	Causative agent(s)	Host	Disease	Treatment	Remarks
1.	Patches of infection with pus information; preference to isolation; weak movements.	*Chondrococcus* sp. (*Flexibacter succinicans*)	*Penaeus indicus*, *P. monodon*, *P. affinis*, *M. dobsoni*.	Myxobacteriosis	Application of crystal violet at the affected area; administration of chloromycetin (10–15 mg/lit.)	90% cure
2.	Reddish marks over the entire rostrum.	*Pseudomonas fluorescens*	*P. indicus*	Hemorrhagic septicemia	-	-
3.	Erythema on the body with slight pus.	*Pseudomonas fluorescens*	*P. indicus*, *M. monoceros*	Hemorrhagic septicemia	-	-
4.	White patches on abdomen with pus.	*Vibrio anguillarum*	*P. indicus*	Vibriosis	-	-
5.	Disintegration of appendages, uropod and telson.	*Escherichia coli*, faecal streptococci	*P. indicus* larvae	Enteric bacterial infection	-	-
6.	Tail tissue decay.	*Pseudomonas alcaligenes*	*Etroplus suratensis*	Tail rot	-	-

Table II (contd.)

Case No.	Clinical symptoms	Causative agent(s)	Host	Disease	Treatment	Remarks
7.	Scale protrusion with pus.	*Vibrio parahaemolyticus* (Biotype I)	*Chanos chanos*	Scale disease	-	-
8.	Unusual softening of the exoskeleton and muscle; wavy intestine; white patches with or without pus; loss of weight; seasonal	?	*P. indicus*, *P. monodon*, *M. monoceros*	Under investigation	-	-
9.	Parasitic castration; disintegration of fully developed gonad.	*Bucephalopsis haimeanus*	*Crassostrea madrasensis*	Trematode infection	-	-
10.	Mycelial growth; muscle and gonad disintegration.	?	*Scylla* [illegible]	Mycosis	-	-

Case of predation: In the case of the edible oyster grown at Tuticorin it was noticed during the months of July-August, 1978 the cages in which transplanted oysters of size 45-55 mm were kept, large scale predation of the stock by a gastropod tentatively identified as *Cymatium pileare* was noticed. Thorough examination of all trays and removal of the predator was resorted to.

in India. Although the study is but a beginning made, it underlines the importance of bestowing particular attention in tackling the problems in the context of future plans for intensive 'aquaculture' under varied marine environmental habitats. In the cases studied (Table II), bacterial, fungal and protozoan diseases have been recorded. But, in many of them, the percentage of afflicted animals in the farm stock was insignificant, although the bacterial isolates were found pathogens satisfying Koch's postulates. In the project areas at Madras and Vizhinjam, no visible indications of disease were noticed at present in the cultured mussels.
Though not included in the body of the paper it may be mentioned here that in the case of eel grown in fibre glass tanks at Mandapam, sporadic cases of gill rot disease were come across. In one interesting instance, (Case No. 10; Table II) at Cochin, prawns in the culture ponds seemed to have been affected during particular seasons (i.e.) post-monsoon and pre-monsoon time when salinity and temperature variations occur, causing soft shell disease. In the case of edible oyster cultured on racks at Tuticorin occasional invasive disease on account of parasitic trematode infection interfering with metabolic activities and growth, has been observed (Samuel,

1976). Predation of the young oysters by a gastropod species during some months also contributes to the destruction of the growing stock.

It has been established by earlier workers all over the world that unfavourable conditions in the captive populations are often conducive to spread of pathogenic microorganisms proliferating quickly leading directly or indirectly to mass mortality. Therefore the stray cases of afflictions noticed now should not give room to complacency. The culturist in India would be greatly benefited by information on various troublesome diseases, periods of occurrence during each year and the causes of the environmental stress which act as promoters of various disease symptoms among the captive stock. To cope up with this need, the establishment of 'Fish Pathology' division in C.M.F.R.I. is on the cards whose function will be to catalogue the various types of pathogenic organisms responsible for different kinds of diseases in finfish and shellfishes in natural and culture systems, to study the characteristics of the pathogens, histopathology of the host, host specificity, immunology and prophylaxis.

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