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- --- improve the quality of post-graduate education in mariculture;
- -- make available the modern facilities, equipments and the literature;
- enhance the competence of professional staff;
- develop linkages between the Centre and other Institutions in the country and overseas;
- -- undertake collaboration programmes; and
- organise seminars and workshops.

Under the programmes of the Centre, Post-graduate courses leading to M.Sc. (Mariculture) and Ph.D. are offered in collaboration with the University of Cochin since 1980.

HANDBOOK ON DIAGNOSIS AND CONTROL OF BACTERIAL DISEASES IN FINFISH AND SHELLFISH CULTURE

C. THANKAPPAN PILLAI Central Marine Fisheries Research Institute, Cochin - 682 018



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Edited by : K. Rengarajan Scientist, Central Marine Fisheries Research Institute, Cochin - 682 018.

PRINTED IN INDIA AT PAICO PRINTING DIVISION, ERNAKULAM, COCHIN - 682 031 A national priority has been set for the development of coastal and inland aquaculture in India. Besides seed and feed, one of the major constraints that has been identified towards the development of extensive as well as intensive aquaculture is the problem of diseases. This could affect any stage from hatchery production to the grow-out system. The importance of fish diseases in aquaculture system is also evident from the large volume of literature that is appearing each year in print.

At the Central Marine Fisheries Research Institute, we have identified fish pathology as an important area for research, as specific disease problems are encountered in the culture of some of the species; often resulting in the heavy loss of or mortality of the stocked species. While a beginning has been made in the study of marine fish pathology, a lot has to be investigated. Dr. C. Thankappan Pillai who has been working in this field for the past few years, has prepared this "Handbook on Diagnosis and Control of Bacterial Diseases in Finfish and Shellfish Culture" as a guide to those involved with aquaculture particularly coastal aquaculture. Besides describing the various types of diseases encountered and those to be met with in brackishwatar and marine environment, he has discussed matters relating to defenses against infection, prophylaxis and disease check-up, sampling techniques for disease diagnosis, classification of pathogens, screening of bacteria for identification and methods to be adopted for despatch of diseased specimens for study. It is hoped that this hand book will be a ready guide to our aquaculturists in upgrading the environmental quality and for the well being of the organisms cultivated.

I wish to express my thanks to Dr. C. Thankappan Pillai for the effort he has put in for the preparation of the hand book based on his own experience and from the active guidance and advice he received from Dr. A. L. S. Munro, Principal Scientific Officer, Marine Laboratory, Department of Agriculture and Fisheries, Aberdeen, Scotland, who visited the Centre of Advanced Studies in Mariculture at the Central Marine Fisheries Research Institute as an expert consultant in 1983. I wish to record my sincere thanks to Dr. Munro for the consultancy afforded and the keen interest shown towards the development of research infrastructure in fish pathology at the Central Marine Fisheries Research Institute, Cochin. Dr. Munro also took great pains to critically go through this Manual and suggested changes which has greatly helped for the improvements of the contents. I am thankful to Dr. P. Vedavyasa Rao, Head, Physiology, Nutrition and Pathology Division and Shri C. Mukundan, Head, Demersal Fisheries Division, Central Marine Fisheries Research Institute, Cochin who have read and commented on the Handbook. I am also very thankful to Shri K. Rengarajan for editing this Handbook.

> E. G. SILAS Director, Central Marine Fisheries Research Institute

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CONCEPT OF DISEASE AND PATHOGEN

Just as in the husbandry of land animals or in the production of agricultural crops, disease occurs in the culture of fishes and shellfishes. The problem of diseases has assumed considerable significance with the rapid developments that are now occurring in the culture of aquatic species. New diseases are occurring in intensive culture systems, whether they are under experimental conditions or in grow out systems, and unless we have a sufficient knowledge of the nature of these diseases and their control, aquaculture practices can hardly become successful. An attempt is made here to define disease and pathogen, and different categories of diseases.

The term 'disease' by its popular acceptance denotes 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: defect of heredity, infection by disease producing organisms, insufficiency or excess in diet, or inadequate 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, amoebiasis, a parasitic disease of man. Amoebiasis is characterised by intestinal infection and consequent pathological changes. It is caused by a protozoan parasite *Entamoeba histolytica* which causes different disorders, illness and suffering in the afflicted, of which the most important is an acute dysentery phase. The infection leads to a succession of malfunctions and subsequent disorders of which the manifested ones are called signs. Signs are medically important in as much as they are signposts - indicative of the disease. They must be systematically recorded and evaluated in the process of investigation leading to diagnosis of the cause of a disease condition. In human medicine symptoms are described by the patient to the doctor. What the doctor sees from this study of the sick patient are called signs. It follows that in the study of morbid or sick aquatic animals, the doctor or in this situation the pathologist, will only see the signs of disease as his patients cannot describe their symptoms to him.

In a diseased aquatic animal, the pathologist will look for gross signs on the exterior of the animal as well as in the internal organs. He will also use other means of looking for signs of disease. For example, by histological methods, he will examine with the light microscope chemically fixed and stained sections of tissues for changes in the architecture of organs and cells and with the electron microscope even finer changes in the architecture of individual cells of particular organ. He will also use the methods of clinical chemistry to look for signs of change indicating pathology. For example, changes in the numbers of RBC and WBC in blood, the levels of proteins, lipids, minerals and enzymes of blood.

Now, we may define disease as any state or condition, resulting from an abnormal change in the anatomy and/or physiology of cell(s) or organ(s) which harms the normal life of a living organism. Diseases may be classified as:

- i. Communicable diseases: Involving infection by viruses, bacteria, algae, fungi or attack by parasites.
- ii. Abiotic diseases: Due to malfunctioning of cell(s)/ organ(s) and/or associated changes due to unfavourable ecological or other accidental conditions, *e.g.* pollution or poisoning.
- iii. Nutritional diseases: Owing to lack of suitable balanced diet, e.g. deficiency of vitamin, minerals, amino acid or essential fatty acids.
- iv. Genetic diseases: Due to lack of a gene or failure to express it, resulting in inherent susceptibility to disease, e.g. melanoma in fish.

Of these, communicable diseases are very important and merit considerable attention in research. It has to be emphasized that to prevent a disease, the cause has to be identified and at best removed or at least controlled. Signs of the disease generally help to diagnose the case to a certain extent. In the case of vibriosis of fish,

detection of the aetiological agent Vibrio anguillarum, confirms the disease. Such causative agents are called pathogens. However, it should be borne in mind that every organism which causes disease is not necessarily a pathogen. Sometimes, certain phyto-flagellates, for example, *Prymnesium parvum*, when present in blooms, may cause fish mortality which is known as 'fish kill'. 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 signs which result in a diseased condition. Moreover, in order to be termed the aetiological agent of a disease, the organism must satisfy certain stringent stipulations known as 'Koch's postulates' which are:

- i. The suspected organism should be associated regularly with all cases of the suspected disease, in logical pathological relationship to the disease and its signs.
- ii. The suspected organism should be isolated in pure culture from the suspected case.
- iii. When such pure culture is inoculated into suitable animals, the disease should be reproduced.
- iv. The same organism should be reisolated in pure culture from the inoculated animals.

Only when an organism satisfies these four requisites, it can be calimed as the pathogen causing a particular disease. Viral diseases such as lymphocystis, infectious pancreatic necrosis, and infectious haematopoietic necrosis, and bacterial diseases such as furunculosis, mycobacteriosis are typical communicable diseases. Tests to see whether these postulates are fulfilled become necessary only when dealing with a new disease of unknown case. In cases of regular occurrence, the signs and isolation of the pathogen will be sufficient identification.

Abiotic diseases which are often common in the fish culture systems, are hypoxia, acidosis, alkalosis and intoxication (Hydrogen sulphide, ammonia).

Another aspect of prime importance for successfully managing culture systems is the provision of adequately balanced diets. Imbalances in the diet of cultured animals may result in mass mortality.

Therefore, care should be taken in formulating balanced diets in accordance with available knowledge to avoid nutritional diseases such as avitaminosis, hypervitaminosis, nephrocalcinosis, lipoid liver disease and sekoke disease.

Diseases of genetic origin such as certain kinds of tumours and deformities may not be of serious nature as they occur infrequently in culture systems.

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HETEROTROPHIC BACTERIA, INFECTION AND DISEASE

Diseases by bacterial infection, are amongst the more frequent and harmful in fish and shellfish culture systems.

Bacteria, procaryotic microorganisms of the PROCARYOTAE, have always been an object of study by researchers, because of their activities, both harmful and beneficial. Heterotrophic bacterial species (those which obtain their energy by breaking down organic nutrients) are widely distributed in nature and live in many 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 live hosts for their existence.

The flora of live hosts may be divided into commensals and pathogens. The commensal species live in harmony with the healthy host while those organisms that harm the host and provoke disease are called pathogens. Host animals in poor health may be compromised with respect to their defence against microbial attack. In such circumstances, opportunistic pathogens may cause disease and they can be either commensals or saprophytes. The agents which can only live by causing disease as a result of infection in the host are called obligate pathogens *e.g. Aeromonas salmonicida* the aetiological agent of the fish disease called furunculosis.

Infection is the lodging and multiplication of a parasite in or on a host. A material contaminated with a microbe(s) is designated as *infective*. In the case of the bacterial flora of fish, the mucus, skin, gills, stomach or intestine will be infected and the bacterial load commonly ranges between 10^3-10^6 per gram weight of these materials. However, the flesh and body fluids of newly

caught healthy fish are found to be sterile. Disease can be recognised when infection results in significant host responses and damage to the host. Disease is normally dependent on various factors such as the virulence of the attacking agent, the state of the host's defensive system, inherent genetically determined resistance factors and the environmental circumstances in which the host is located.

Other definitions in disease processes can be classified based on the time sequence and place of infection. The initial infection in a host with a parasite is called *Primary infection* and subsequent infection by the same parasite is termed as *reinfection*.

When a host's resistance is lowered by a pre-existing disease or parasite infection, an additional disease is called *secondary infection*. If the infection is only in a localised area in a host (*e.g.* gill rot), it is known as *local infection* or *local sepsis*, but if the microbes multiply internally in the vital organ, then it is described as a systemic infection (*e.g.* furunculosis).

In the host, if signs of infection are not apparent, but the disease gaent can be detected, then it may be denoted as *inapparent* or *latent infection*. Continuous infection with a potential pathogen is known as *persistent infection*, *e.g.* most fish surviving infection with infectious pancreatic necrosis virus (IPNV) carry virus in other tissues for life.

The presence of a known or potential bacterial pathogen in the blood is called *bacteremia*. Previously in the older tests this condition was called as *septicaemia*.

A condition in which the organism remains localised in a host and releases its toxin in the blood stream is called *toxemia*.

An ailment is said to be *contagious* when it is transmitted by direct contact whereas *infectious* implies all (means of sources) including direct contact, *e.g.* via water, food or by other animals.

Epidemiology is the study of the distribution and spread of diseases in man and animals. When animal diseases are discussed, some changes in epidemiological terminology are sometimes made to denote that diseases of animals are being discussed. Thus, *enzootic* replaces endemic and *epiozotic* epidemic. Enzootic means when a disease is widespread in a disease in a population. It may be in tank or pond population or all such population in an aquaculture farm or all the farms in an area or even a country.

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DEFENSES AGAINST INFECTION

In the previous chapter infection and disease have been discussed. Now, let us examine how infection may be overcome by host's defenses. The protective measures of the host against infection(s) are called defense mechanisms. They may be classified as the nonspecific which include structural, humoral and cellular defenses and the specific or adaptive defenses. Higher vertebrates such as mammals including man and birds possess all the defenses and almost certainly most fish do as well. Molluscs and crustaceans probably possess most or all of the nonspecific defenses, but much less is known of their ability to mount adaptive responses.

STRUCTURAL DEFENSES

The main body surfaces of finfish and shellfish are skin, shell, gills and alimentary canal through which invading microbes must pass to get access to the host's tissue. Moreover, many of these surfaces are covered by a layer of mucus which is continually shed making microbial attachment and penetration difficult. If the mucus is breached, the invading microbes may get attached and subsequently gain entry into the epithelium and deeper tissues and in consequence, finally cause disease.

HUMORAL DEFENSES

The various humoral defenses mostly contained in the blood of fish, crustaceans and molluses are as follows.

a. Natural non-specific antibodies

These natural antibodies are considered to have antipathogen properties. They attach to the surface of pathogens and as a consequence increase the efficiency of complement and phagocytosis.

b. Complement

This system is a complex of enzymes which may be activated by the products produced by pathogens or by antibody attaching to them or the pathogen itself. It has effective antipathogen (killing) properties mainly as a result of damage to pathogen cell membranes.

c. C-reactive protein (CRP)

It is considered to be normally present in the serum of fish and functions as a defence factor against pathogens.

d. Interferon

This is an important antiviral agent probably produced by all fish and it is likely to be produced by crustaceans and molluscs, but no proof yet exists.

e. Lysozyme

Lysozyme, an enzyme, is an important weapon in the host's defense and functions against various pathogens. It is present in the mucus, skin and serum and in phagocytic cells.

f. Natural haemolysins

These haemolysins are in fact the complement system recognizing foreign red cells as foreign and lysing them.

CELLULAR DEFENSES

When the pathogen gains entry and begins dividing and causing damage to host cells, defense systems become altered. The cellular defense system has a series of comporents which collectively are called the inflammatory reaction. It is well defined in finfishes, less in crustacean and least in molluscs. A characteristic feature of inflammatory reaction is invasion of the damaged area with leucocytic cells of different functions. Now, there are several alternative fates. As the infection progresses, vasodilation causing swelling and fluid accumulation takes place. Various leucocytic cell types including phagocytes are attracted to the site of infection by the release of specific chemicals from damaged host cells. This is called positive chemotaxis. Moreover, infectious agents are phagocytosed often with the help of humoral factors in the blood, known as complement and lysozyme.

If the invasive microbes are not fully destroyed at the site of attack, they may be carried by the blood stream to the various organs of the body. In fish, the spleen and kidney are the main blood filtering organs and they have a primary role in dealing with microbes in the blood stream, rather like the lymph node in mammals. In fish the work of destroying the pathogens is carried out mainly by wandering phagocytes. There are also fixed phagocytic cells scattered throughout the body of fish and shellfish.

In fish, the blood contains many monocytes which are the precursors of the macrophage. They will differentiate into macrophages, the principal phagocytic cell in fish, when stimulated by injury and invasive microbes. Some notable features are:

- i. The atrium in fish and schllfish has got significant phagocytic populations.
- ii. There are no lymph nodes in fishes, but organs with lymph node activity such as spleen and kidney are present in fish. Shellfish appear not to have any organ with such activity.

In fish the neutrophile is the dominant granulocyte and it is found circulating in the blood. Unlike mammalian granulocytes it is not phagocytic, but it is chemotactic invading areas of inflammation where it bursts discharging cell contents which are lethal for pathogens. The other granulocyte of fish, the cosinophil, does not have phagocytic activity either. Large populations are present in gut and gill in healthy fish, but these may migrate to the sites of infection in any organ where they behave like neutrophiles. There is speculation that they perform the role of tissue mast cells in higher animals.

In shellfish, it is not established that as many populations of leucocytes with specialised function exist, rather it seems a few populations of less specialised cells exist, performing all or most of the functions of the many specialised leucocytes in higher animals.

In fish, the thrombocytic leucocytes promote the clotting mechanism of the blood. Clotting seals areas of injury preventing escape of body fluids. However, in crustaceans only acellular clotting mechanisms are found, nevertheless they act efficiently to seal any gaps in the vascular system. Clotting in molluscs does occur, but by a very different mechanism.

INDUCED OR ADAPTIVE IMMUNITY

Another defense system possessed by higher animals and fish, recognises foreign material if it penetrates the structural defenses. There are both cellular and humoral responses to foreign material. They are brought about by leucocytic cells called lymphocytes. In fish the lymphocytes carry receptor sites capable of recognizing foreign materials which are called antigens. There are two types of lymphocytes called T and B lymphocytes. T lymphocytes are produced in the thymus and spleen and they are responsible for cell mediated immunity a mechanism whereby pathogens are killed by the T lymphocytes making physical and chemical content. B lymphocytes are responsible for antibody production.

While in mammals five distinct classes of immunoglobulins (Ig) are identified, in teleost fishes only one class of Ig is detected. The antibodies can act in a variety of ways. Based on the nature of the foreign material, they may agglutinate particulate antigens, such as whole bacteria. They also precipitate soluble antigens and thus neutralise toxins. Antigens which are coated with antibody are much more readily phagocytosed.

The structural, humoral and cellular defenses are the first line of defense against invaders. They are non-specific in their activity. A significant difference between the defense system of fishes and shellfishes is that acquired immunity detected in fishes is limited to a primitive antibody-like substance in some crustaceans, but no immune response is found in molluscs.

Pathogenic microbes entering the host's body may escape from phagocytes by producing aggressins which kill the phagocytes, by not eliciting host cells to release biologically active chemicals. *i.e.*, negative chemotaxis thus not attracting leucocytes to their point of entry, by producing capsules which prevent phagocytosis or are motile and escape from the phagocyte. In these situations, the host becomes susceptible and the pathogens enter directly into the blood stream or through the lymphatics.

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BACTERIAL DISEASES

All living beings, in their natural surroundings, are exposed and periodically saccumb to diseases. Fishes and shellfishes are no exception. In their natural conditions, finfishes and shellfishes become diseased, but most of the cases pass unnoticed possibly due to lack of interest and/or closer observation. Occurrence of diseases of fish and shellfish in culture systems is more obvious and will adversely affect production. Therefore, a thorough study of disease and its control is essential, especially in the present context of the objective of enhancing food production through aquaculture.

Considerable work has been carried out in several countries in this field and substantial literature is available (Conroy and Herman, 1970; Sindermann, 1970, 1977; Snieszko, 1970; Bullock, 1971; Bullock *et al.*, 1971; Mawdesley Thomas, 1972; Reichenbach Klinke, 1973; van Duijan, 1973; Roberts and Shepherd, 1974; Ribelin and Migaki, 1975; Dulin, 1976; Hoffman, 1977; Roberts, 1978, 1982; Ahne, 1980). 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, preferably before our emerging aquaculture industry is faced with significant disease problems.

Because fish and shellfishes are mute and live in a dynamic environment, detection of their diseases and also diagnosis presents many problems.

The signs produced by a disease may be of considerable help to provisionally diagnose a case. However, detection of the aetiological agent of the disease will be necessary to confirm diagnosis. Additionally, the latter may be especially important in order to ensure correct treatment, e, g, by antibiotic therapy or by adopting

Disease	Actiological Agent(s)	Host species	Gross clinical signs	Drug(s)	Administration
1	2	3	4	5	6
Hemorrhagic Septicemia (PL. I A)	Pseudomonas aeruginosa	Epinephilus pantherinus, Lutianus johni, Platax teira, Trachinotus ovatus	Body reddening, skin lesions with fluid and or blood, swollen belly, protruding scales, sunken or protruded eyes, inflamed anus, discharge from the vent, pale liver, swollen spleen, inflamed swim bladder and anaemia	Terramycin, Chloromycin	Alongwith feed, at a rate of 75 mg of either of the drug per kilogram body weight of the fish for 7-21 days or mass injection (intraperitoneal of either of the drugs at a level of 20-40 mg per kilogram body weight of the fish.
	Pseudomonas aeruginosa	Etroplus suratensis, Mugil cephalus, Chanos chanos		Gentamycin	As stated above.

TABLE 1. Some important finfish and shellfish diseases (common in India) of bacterial aetiology, host species, gross clinical signs, recommended drugs and treatments. The figure references are given in parentheses.

	2	3	4	5	6
Furunculosis* (PL. I B)	Aeromonas salmonicida	Mugil cunnesius	Boils and/or	Terramycin, Chloromycin	Alongwith feed 75 mg of either of the drug per kilogram body weight of the fish for 7-21 days.
Skin lesion (PL, IC)	Vib r io cholerae	Gerres spp.	Skin lesion clearly visible in water, blood in the lesion, sluggishness and anorexia.	Nif urpi rinol	As bath for one hour in 1-10 mg of the drug per one litre of habitat water for 7-21 days.
	Aeromonas hydrophila sub. sp. hydrophila	Tilapia mossambica	As above	Terramycin, Chloromycin	Alongwith feed at a rate of 75 mg of either of the drug per kilogram body weight of one fish for 7-21 days.

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TABLE 1 (Contd.)

1	2	3	4	5	6
Vibriosis (PL. 1 D)	Vibrio anguillurum	Etrolpus suratensis	Erythemia of the skin and fins, petechiae in the mouth, swimming at water surface, body darkening, abdominal disten- sion, corneal opacity, anorexia, pale gills, enlarge- ment or liquefaction of the spleen and kidney and myocardial lesions.	Terramycin, Chloromycin	As above stated
Skin spottiness (PL. II A)	Vibrio fischeri	Etroplus suratensis	Shallow to deep sores with fluid and/or blood and weak movements.	Terramycin	As above stated
Gill rot (PL. 11 B)	Kiesiella pneumoniae	Etroplus suratensis, Tilapia mossambica	Isolated movements anorexia, restless- ness, floating at surface, orienta- tion against the current, gill tissue decay or prolifera- tion and weakness.	Nifurpirinol	1-10 mg of the drug in a litre of habitat water as one hour bat for 7-21 days.

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TABLE 1 (Contd.)

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1	2	3	4	5	6		
Tail rot (PL. II C)	Proteus vulgaris	Etroplus suratensis, Tilapia mossambica	Loss of natural colour beginning from the outer margin of tail/fin, fraying of the tail/fin, progressive disintegration of the tail/fin tissue, swimming near water surface. Ecchymosis may be noticed.	Acriflavine	Bath in 1-5 mg of the drug in a litre of habitat water for 7-21 day or Nifurpirinol may be used.		
Fin rot (PL. II C)	Pseudomonas stutzeri	Chanos chanos, Etroplus suratensis, Tilapia mossambica	As above		As stated above.		
Eye disease (PL. H D)	Vibrio parahaemolyticus	Gerres spp., Therapon jarbua, Lutianus johni, Drepane punciata	Eye opacity with tissue proliferation weak movements, pale gills and anorexia.	Terramycin, Chloromycin	As already stated.		

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1	2	3	4	5	6
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Enteritis (PL. III A)	Escherichia coli	Etroplus suratensis	Enteritis, sluggish movements, body lesions, body discolouration, reddening of the anus, cyclic movements, swimming near water surface. Kidney may be infected.	Sulphadiazine, Sulphisoxazole	Alongwith feed, 75 mg of either ol the drug per kilogram body weight of the fish for 7–10 days.
Black spot disease (PL, III B)	Alcaligenes eutrophus Bacilius thuringiensis	Mugil cephalus	Depressed black spots in the skin and pale gills.	Nifurpirinol	As already stated.
Scale disease (PL. III C)	Vibrio parahaemolyticu	Chanos chanos Is	Scale protrusion with liquid and/ or blood and weakness.	Nifurpirinol	As already stated.
Skin uker (PL, III D)	<i>Haemophilus</i> sp.	Drepane punctata	Shallow open sores mostly with white rim, presence of liquid and/or blood eroded fins and mouth.	Terramycin, Chloromycin	Alongwith feed, 75 mg of either of the drug per kilogram body weight of the fish for 7-21 days.

	1	2	3	4	5	6
	Mycobacteriosis (PL. IV A)	Mycobacterium sp.	Etroplus suratensis	Progressive body weakening, damaged or folded fins, swelling of abdomen, anorexia colour fading, deformities in the skeletal system, sluggish movemen opacity in the cornea, scale defects, listlessness and presence of tubercles.	∕ ts, ∵	Along with feed 75 mg of any one of the drug per kilogram body weight of the fish for 7–21 days.
	Myxobacteriosis (PL, IV B)	Flexibacter succinicans	Penaeus indicus, P. monodon, P. affinis, M. dobsoni, M. monoceros	Patches of lesions due to exoskeleton decay, preference to isolation and weak movements.	Nifurpirinol	As already stated.
	Pseudomonasis (PL, IV C)	Pseudomonas fluorescenes	Penaeus indicus	Reddish marks over the rostrum.	Terramycin Chloromycin	As already stated.
17	Staphylococcosis (PL. IV D)	Staphylococcus aureus	Metapenaeus monoceros, P. indicus, P. monodon, Scylla serrata	Decay of dorsal body surface, weak movements.	Terramycin Chloromycin	As already stated.

TABLE 1 (Contd.)

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	1	2	3	4	5	6	
	Shell disease (PL. V A)	Vibrio fischeri	P. indicus, P. monodon	Depressed black spots with margins in the exoskeleton with or without underlying tissue necrosis.		As already stat e d.	
	Flavobacteriosis (PL. V B)	Flavobacterium ulginosum	Penaeus monodon	Irregular decay of tail region, weak movements.	Terramycin, Chloromycin	As already stated.	
	Streptococcosis (PL. V C)	Streptococcus pyogenes	Panulirus homarus	Shell decay with adjacent tissue neucrosis, sluggishness, presence of blisters and anorexia.	Terratnycin, Chloromycin	Alongwith feed at a rate of 75 mg of either of the drug per kilogram body weight of the shellfish for 7-21 days or intramuscular injec- tion of a drug at a dose of 20-40 mg/kg body weight of the host.	
	Muscle necrosis (PL. VI A)	Vibrio fischeri	P. indicus, Crassostrea madrassensis, P. indicus	Focal necrosis of tissue with varied colour.	Terramycin, Chloromycin	As already stated.	

TABLE 1 (Contd.)

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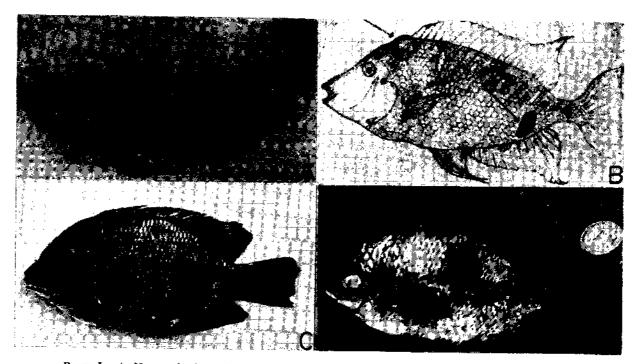


PLATE I: A. Haemorrhagic septicemia caused by Pseudomonas alcaligenes in Epinephelus pantherinus; B. Furunculosis caused by Aeromonas salmonicida in Cichalosoma meeki (diagrammatic); C. Skin lesion caused by Pseudomonas fluorescens in Etroplus suratansis and D. Vibriosis caused by Vibrio anguillarum in Etroplus suratensis.

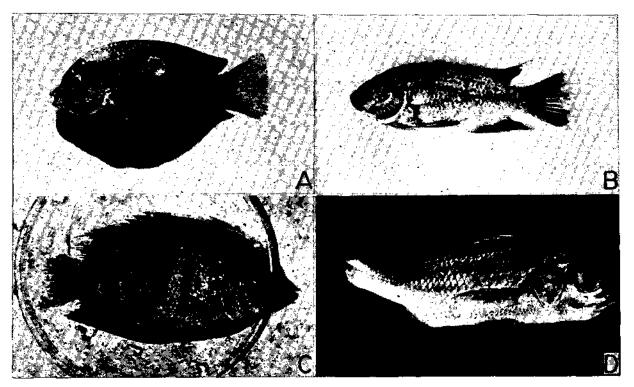


PLATE II: A. Skin spottiness caused by Vibrio fischeri in E. suratensis; B. Gill rot caused by Klebsiella pneumoniae in Tilapia mossambica; C. Tail rot caused by Photobacterium phosphoreum in E. suratensis and D. Eye disease caused by Vibrio parahaemolyticus in Gerres sp.

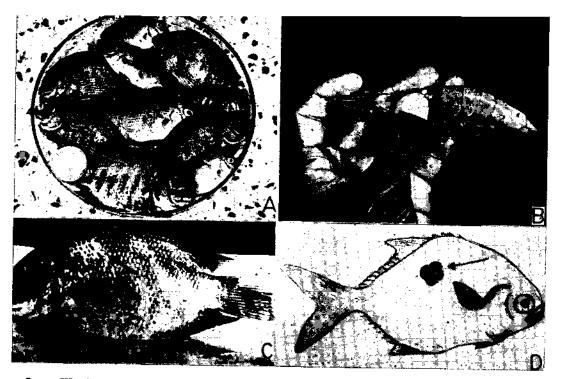


PLATE III: A. Enteritis caused by Escherichia coli in E. suratensis; B. Black spot disease caused by Vibrio fisheri in Penaeus indicus; C. Scale disease caused by Vibrio parahaemolyticus in E. suratensis and D. ulcer caused by Haemophilus sp. in Trachinotus ovatus.

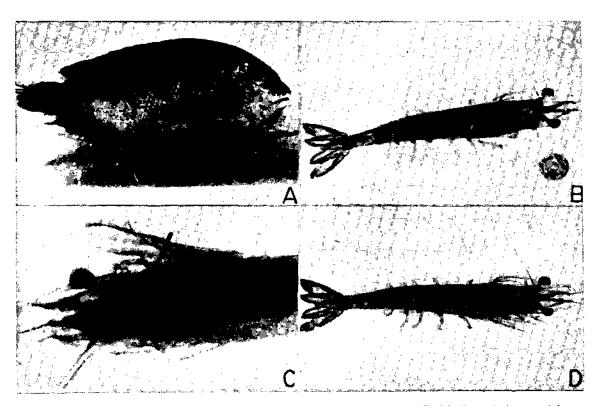


PLATE IV: A. Mycobacteriosis caused by Mycobacterium sp. in E. suratensis; B. Myobacteriosis caused by Flexibacter succinicans in P. indicus; C. Pseudomonasis caused by Pseudomonas fluorescens in P. indicus and D. Staphylococcosis caused by Staphylococcus aureus in P. indicus.

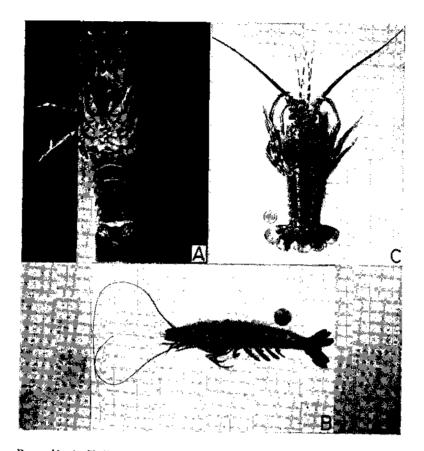


PLATE V: A. Shell disease caused by Vibrio fisheri in Panulirus sp; B. Flavobacteriosis caused by Flavobacterium ulginosum in P. monodon and C. Streptococcosis caused by Streptococcus pyogenes in Panulirus homarus,

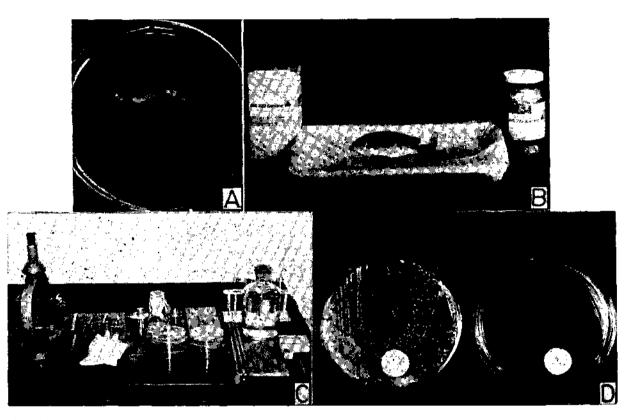


PLATE VI: A. Muscle necrosis caused by Vibrio fisheri in P. indicus; B. The anaesthetised fish E. suratensis and the anaesthetic MSS 222 (Tricaine Methane Sulphonate); C. Instruments for post-mortem examination and D. Inoculated (left) and uninoculated (right) culture plates.



PLATE VII: A. Fish infusion nutrient agar in conical flasks and B. Culture petridishes — Monoculture (left) and Polyculture (right).

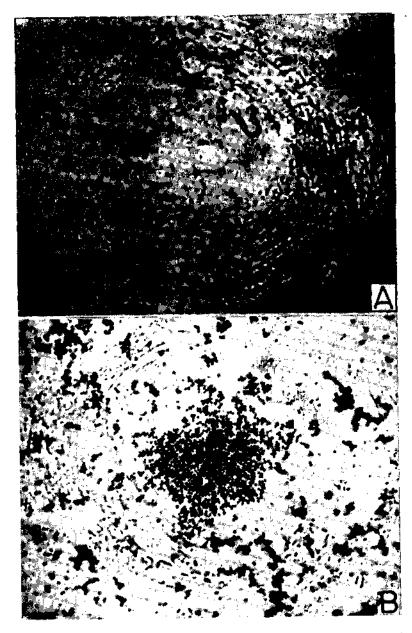


PLATE VIII: A. Bacterial rods (*Pseudomonas auruginosa*) and B. Bacterial cocci (Staphylococcus aureus).

prophylactic measures against further outbreaks. Nevertheless, appropriate treatment on the basis of initial diagnosis must often be followed as delay may result in heavy mortality of the afflicted population. Considering these needs, a provisional key for the diagnosis of some important bacterial diseases which cause heavy loss in culture of fish and shellfishes, and their treatment has been formulated and is presented in Table 1.

Bacterial disease agents are common among fishes and shellfishes However, it should be noted that the presence of these organisms does not necessarily always produce an outbreak of disease. This is because, in any community, it has been found that normal hosts harbour or carry potentially pathogenic microbes without showing any apparent disease (sometimes called latent infection). For example, species of Aeromonas and Pseudomonas which are amongst the normal flora of healthy fish and shellfish, often cause diseases when they are under stress. Any sort of stress (e.g. high population densities, enhances the chances of a potential pathogen to invade the host, thus provoking disease). It is also thought that stress induces the failure of some host defence mechanisms. Genera lising, stress lowers the resistance of animals and enhances susceptibility to disease. 'Stress' is a state or condition, caused by intrinsic or extrinsic factors which upsets the adaptive responses of the animals and reduces the chances of their survival. Stress can be short-term of chronic. In the case of short term stress (e. g. cold shock) the animals return to normal within a few (4-8) hours. Chronic stress causes severe damage as a result of long-term exposure and thus increases susceptibility to infections probably because of reasons such as decreasing inflamatory responses, decreasing amount of circulating lymphocytes (white blood cells responsible for immune responses), impairing production of antibodies and depressing interferon (an important defensive factor against virus infection).

Thus, it is essential to maintain the equilibrium in favour of the host by ensuring that environmental conditions prevent stress. Outbreaks of a disease are often the result of an altered situation in the interrelationship between host, parasite and environment. The severity of the disease may depend on the intensity of the alteration. Hence, great care has to be taken in observing hygienic measures, *i.e.* cleanliness, maintaining appropriate water conditions, supplying suitably balanced diets, choosing suitable stocking densities and ensuring that the husbandry regime for the care and welfare of the animal collectively result in good growth rate and survival, the principal objectives of the mariculture operation.

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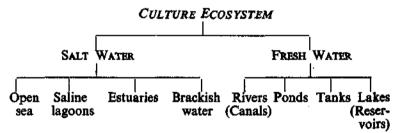
PROPHYLAXIS AND DISEASE CHECK UP

In all animals the occurrence of infectious disease is unpredictable. It is often considered that the occurrence of disease in the culture system is unavoidable. However, this need not be so, for many diseases can be prevented by adopting prophylactic measures or certain management techniques.

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 death, reduced growth and bodily damages resulting in non-marketability. Above all, the possibility of certain types of infections being transmitted through aquaculture products to man cannot be ruled out. Further, the occurrence of diseases in cultivable organisms may pose serious problems to the management of wild resources.

Culture systems use a variety of water sources which are classified in Table 2.

TABLE 2. Classification of culture ecosystem



Each water source has its own ecological and biological characteristics. The applicability of the following guidelines has certain limitations. However, the following prophylactic measures would be helpful.

METHODS OF PROPHYLAXIS

1. Selection of certified disease-free seeds for culture

As for agricultural seed crops, producing finfish and shellfish seeds free of specified disease agents would be highly useful. This is in practice in some foreign countries such as U.S.A., Canada, U.K. and Denmark and might be adopted in India for some species where brood animals can be maintained in a specified pathogen-free status.

2. Development of disease-resistant seeds

This may be carried out by selective breeding from strain resistant to specific pathogens. Although genetic selection using desirable traits could be important, finding resistant strains may be lengthy process and there is no guarantee of success.

3. Suitable site selection

Sites for culture should be chosen according to the criteria known to be required for the cultivar. There should be adequate supplies of good quality water for the volume of production planned. Seasonal variation in quality may occur and therefore it will be important to gain knowledge of the physical properties and biological populations present. Possible sources of pollution (*e.g.* industrial wastes, agricultural sewage and pesticide residues) in the vicinity may necessitate rejection of potential sites.

4. Disinfection of culture site

It may be desirable to drain off culture ponds and treat them with a disinfectant (e.g. Quick lime/Calcium cyanamide/Sodium hydroxide). When such chemical treatment is carried out, stocking should be done only after a period of time to allow for dilution by flushing. The pH and other parameters such as temperature and salinity will have to be checked before stocking.

5. Installation of proper bund and sluice

This is applicable in the case of culture systems with tidal water exchange. Suitable bunds which are seepage proof 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

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stocked animals and the same time prevent the entry of the free swimming larval parasites and predators from outside. Care should be taken for the proper maintenance of the screens and sluices.

6. Stock density

The field should be stocked with the recommended number of seeds for the species being cultured and the nature of the site. Because of the considerable variation in the productive capacity of sites, trial evaluations to determine optimum stocking densities may be necessary.

7. Prevention of environmental stress

Sudden change (e.g. stress in animals caused by occurrence of poor environmental condition) can impose severe imbalance in homeostasis 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 an acute stress factor to the standing stock. Every effort should be taken to minimise or avoid any kind of stress of this kind to the animals.

8. Knowledge of life history of the pathogenic parasites

A knowledge of the life history of opportunistic pathogens may help us in preventing disease. This can be achieved by killing other host organisms necessary to complete the life cycle or preventing their entry into the culture system.

9. Food

Where food is provided, supply of a well-balanced diet free of pathogens should be maintained. Compounded feeds are usally pasteurised by the pelleting process. In the case of natural food collected for feeding, such as phytoplankton and zooplankton, they may be treated with ultraviolet radiation and/or deep-frozen over 24 hours (kills many parasites) for stock, both process reducing the numbers of any potential pathogens which may be present.

10. Maintenance of general cleanliness

Hygiene is very important in culture practices. Ultensils, nets and other instruments should be clean and regularly disinfected using materials such as iodophores or hypoclorite. In the absence

of sufficient facilities, the hatchery instruments may be scrubbed clean with detergent and water and well sun-dried before re-using. The actions will help to eliminate cross infection.

11. Pathogens and recirculating water systems

When an infectious disease occurs in animals in a recirculation system the biofilter will always have to be suspected as a source of infection. Therefore, it is desirable to operate a single filter until the disease has been cured/or the stock killed and then to replace or clean the filter bed.

12. Limitation of handling

Frequent handling of the animals is to be avoided, especially by inexperienced staff. Where appropriate, anaesthetise the animal before handling. Moreover, handling should be as gentle as possible, as injuries may cause disease often of epizootic proportions.

13. Exclusion of animals and birds

As far as possible, no opportunity should be given for animals and birds to get into the culture systems. They may act as disease vectors through their faeces. They may also be predators of the culture.

14. Seed immunisation

Mass immunisation against vibriosis is often successful by an immersion in the liquid vaccine. Oral vaccination is not so effective on the commercial scale. However, use of vaccines in aquaculture is as yet in the early stages of development.

15. Disease monitoring

Where certain infectious diseases occur regularly, *e.g.* in a hatchery, one method of control is to monitor their presence and destroy the infected population(s). This can only be contemplated when many non-infected groups are reared (*e.g.* in oyster and prawn hatcheries).

16. Prevention of lateral transmission of disease

Hatcheries and similar establishments which produce young or seed stock for grow-out elsewhere have a crucial role to play in the continuous supply of young stock. Widespread disease occurr-

ence in such a unit would disrupt production in many grow out farms. It is therefore important to design hatcheries so that if disease does occur, it does not spread to the surrounding hatchery populations. Adequate physical separation of stocks, separate utensils for their maintenance and even separate staff for their husbandry may be necessary to achieve this.

17. Growth and survival

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Fish and shellfish farmers are strongly advised to measure growth rates and survival in grow-out populations. If poor survival is indicated, growth is poor or the sampled animals look unhealthy they should seek expert advice.

THE NEED FOR ANAESTHETICS

In general, anaesthetics are central nervous system (CNS) depressants. Because certain manipulative actions are necessary in culture activities, they are used to render the animal unconscious. Their use is important to minimise stress from fright reactions and possible self injury or from handling. Excess exposure to the drug causes paralysis of the respiratory and vasomotor centres which finally leads to death.

The use of anaesthetics in fish culture is often necessary to perform injections, branding, tagging and weight and length measurements.

The methods of inducing anaesthesia in fishes are by inhalation, hypothermia and injection.

Inhalation can be successfully done by adding the anaesthetic in the gill irrigating water. Here, the anaesthetic is absorbed into the blood stream through the gills. Inhalation by bathing is considered the easiest and most effective method of inducing anaesthesia. This is the commonest method in use in aquaculture.

Hypothermia is a physical method of inducing anaesthesia (cooling of the body for 10-15 minutes, inorder to reduce oxygen requirements of the cells). This method has its limitations.

Injection can be intramuscular, intraperitoneal, intravenous and it is the least preferred route for the operation.

Chloretone and benzocaine are good anaesthetics for shallow (short-term) anaesthesia. However, MS 222 (Tricaine methane

TABLE 3. Anaesthetics and their use

Common name	Chemical name	Dose	Time (in minutes) for		Effect	Effect on	Remarks
			Immobi- lisation	Recovery	on fish	human beings	
Chloretone/ Chlorobutanol	βββ Trichloro- tert-butyl alcohol	0.1%	2-5	3-7	Short term anaesthesia	Irritant	When the fish is immers ed in a solution of anaes thetic, an initial phase or excitement takes place followed by erratic swim- ming. Then, the fist become inactive and sink to the bottom of the water body. Anaesthe- tised fish should be re- moved immediately for the 'purpose.' Time for immobilisation and re- covery vary with species. So behaviour of the fish should be observed care- fully while they are being anaesthetised to avoid cardiac arrest due to excess anaesthesia. It is not desirable to use the anaesthetised fish for food within the following 21 days of anaesthetics cheap and locally avail- able.

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	MS 222	Tricaine Methane Sulphonate	0.01%	2-5	2-5	Short and deep anaesthesia	Negli- gible toxic effect	When the fish is immers- ed in a solution of anaes- thetic, an initial phase of excitement takes place followed by erratic swim- ming. Then, the fish become inactive and sink to the bottom of the water body. Anaesthe- tised fish should be re- moved immediately for the 'purpose.' Time for immobilisation and re- covery vary with species. So behaviour of the fish should be observed care- fully while they are being anaesthetised to avoid cardiac arrest due to excess anaesthesia. It is not desirable to use the anaesthetised fish for food within the following 21 days of anaesthetisa- tion. The anaesthetic is expensive and not easily available locally.
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sulphonate) is a better anaesthetic for deep (long-term) anaesthesia, for 10-30 minutes (PL. VI. B). Adequate care has to be taken in administering the correct dosages. Appropriate concentrations are listed in Table 3.

The other fish anaesthetics are Carbon dioxide, Chloral hydrate, Ether, Methylpentynol, Novacaine, 2-Phenoxyethanol, Quinaldine, Sodium amytal, Tribromoethanol and Tertiary-amyl alcohol.

Fish should be held in the anaesthetic for the minimum time possible and returned to fresh anaesthetic-free water as soon as the manipulation to be performed, has been done. Before an anaesthetic is used, a trial on a small number of animals is advised.

The recommended general anaesthetics for use in aquaculture are:

- i. Benzocaine or chlorobutanol for fishes. However, great care must be taken to ensure benzocaine is not used in direct sunlight. In sunlight it decomposes to produce chlorine which is acutely toxic.
- ii. For Crustacea (shrimps) quinaldine may be used as an anaesthetic at a level of 25 ppm.
- iii. To anaesthetise mollusos, menthol is used.

SAMPLING TECHNIQUES FOR DISEASE DIAGNOSIS

Collection and examination of moribund specimens before death is very important for diagnostic purposes because post-mortem (PM) changes may obscure or confuse diagnosis.

Moribund specimens should be collected and sent alive as soon as possible to the Pathology Laboratory furnished with the necessary PM equipment.

The essential items required for the post-mortom examination (PL. VI C) are Surgical knife/scalpel, Scissors, Tissue forceps, Dissection needles and pins, Hand lens, Dissection board, Bacteriological wire loop, Slides and cover slips, Pipettes, Test tubes, Petri dishes, Tissue paper, Gloves, Microscope, Bacteriological medium *e.g.* Nutrient agar/Fish infusion agar plates and Histological fixative *e.g.* 10% buffered formol saline.

Once the moribund specimen collection is over, the minimum procedure for further study is as follows:

Procedure for Post mortem examination

a. Species	: Record species, age, sex, length and weight.
b. Disease signs	: Compare at least 6-20 moribund animals to confirm the signs of disease and study the commonest signs both external and internal.
с.	: Record external and internal patho- logies.

d.	: Extract blood for study of haema- tological parameters e.g., haema- tocrit red and white cell counts, differential counts, etc.
е.	: Make smears/scrapingsfrom lesions, body fluids (blood) and tissue imprints for subsequent staining.
f.	: Take bacteriological samples on loop and plate out.
g.	: Cut out pieces of each of the major organs for histology e.g. liver, kidney, pancreas, spleen, gills, etc. in fish; affected tissue and haemolymph in crustaceans and affected tissue and haemolymph in molluscs.

A case study sheet is appended below to serve as a guide for data aquisition on disease and the concerned biological and environmental factors.

The quality of water is an index for disease investigation. Water samples may be collected in clean, dry and sterile glass containers taking precautions to minimise external contamination. Every effort should be made to avoid delay and post-collection contamination of the samples.

CASE STUDY SHEET

GENERAL

 Farm name Owner Address 	::
Cultivar History	
Species diseased Age Sex Length Weight Kind of feed	

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Α.

Method of feeding	:
Stocking density	:
Standing stock	:
Recent history of treatments	:
Any recent handling	
of population	:
Estimate of loss (Number)	:

Water chemistry (if relevant)

Habitat water - colour smell turbidity oxygen salinity temperature pH : phytoplankton : zooplankton :

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B. PRESENT COMPLAINTS

C. PHYSICAL EXAMINATION

C-1. Reflex activities

Defensive reflex
Escape reflex
Ocular reflex
Tail reflex

C-2. System history (external)

Visual nature of - slime scale skin/body cephalothorax abdominal segments mouth antenna antennules rostrum pharynx сатарасе oral cavity

nostril	:
eyes ·	:
operculum	:
gills	:
tail/fins	:
postrostral carina	:
dorsal carina	:
belly	
nostril	:
pereiopods	:
pleopods	:
urogenital carina	
vent	:
thelycum/petasma	:

C-3. System history (internal)

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Visual nature of -	body tissue	:
	brain	:
	heart	:
	liver	:
	pancreas	:
	spleen	:
	gall bladder	
	swim bladder	:
	kidney	:
	stomach	:
	intestines	:
	hepatopancreas	:
	pyloric caecae	:
	urinary bladder	:
	gonads	:
	body cavity	
	spine	:
	-	

D. PREVIOUS CASE HISTORY (if any)

E. WET MOUNT MICROSCOPICAL EXAMINATION LABORATORY DATA

F. HAEMATOLOGY

Total counts of RBC WBC	:
Haematocrit	:

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Differential white cell counts

Neutrophils Eosinophils Basophils Lymphocytes Monocytes Haemoglobin ESR

G. VIROLOGY

Tissue culture (fish only) Transmission Transplantation Electron microscopy

H. HISTOPATHOLOGY

I. RADIOGRAPHY

J. CULTURE AND ANTIBIOTIC SENSITIVITY

Locally affected cells	:
Blood (cardiac)	:
Kidney	:
Hepatopancreas	:

K. SATISFACTION OF KOCH'S/RIVERS' POSTULATES

(Optional, only required if a new disease is being studied and the results to be written up for scientific publication).

L. CASE DIAGNOSIS

M. CONCLUDING REPORT

N. DRUG(S) PRESCRIPTION

Laboratory	:	Signature	:
Place	:	Name of the Scientist	:
Date	:	Designation	:

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CLASSIFICATION OF PATHOGEN(S)

In investigations of mortality and morbidity, consideration of an infectious cause is an essential aspect. The signs of dead and dying animals aid in the provisional diagnosis. To prove such a hypothesis microscopical examination of blood (stained smears) and tissues (wet mounts of scrapings, stained histological sections and tissue imprints)must be taken for the cultivation and isolation of probable microbial pathogens, *e.g.* bacteria or fungi. Animal parasites can generally be identified from scrapings and/or histological sections. If a bacterial cause is suspected from the examination (microscopic) aseptically removed sample(s), by bacteriological loop, should be directly inoculated on to the culture medium (PL. VI D) There is no single medium in use for culture and isolation of all the pathogens which may be present in a sample. However, a suitable all-purpose culture medium for bacterial pathogens is fish infusion nutrient agar (Fina).

FISH INFUSION NUTRIENT AGAR (PL. VII A)

Peptone	:	1.0 gm
Agar agar	:	1.5 "
Beef extract	:	0.1 "
Fish infusion*	:	100 ml.

(pH adjusted to 7.2 and sterilized at 121°C for 15 minutes).

454 gm of fresh fish meat is minced and mixed into one litre of water^{**}. The mixture is kept overnight in a refrigerator at 4° C. Then, the mixture is boiled using a water bath for about 30

^{*} Preparation of fish infusion.

^{**} In the case of water, aged and filtered sea water is preferred for isolation of bacterial pathogens from marine fish and distilled water with 0.5% sodium chloride for fresh water fish.

minutes. The precipitated and coagulable protein in the mixture can be removed by filtering through a lint and filter paper.

Shrimp infusion nutrient agar, crab infusion nutrient agar and mussel infusion nutrient agar are prepared in similar manner, replacing the fish muscle by shrimp, crab or mussel meat for isolating bacterial pathogens from the respective species. Fresh media may always be used as the cost of these media is low and the best results are obtained.

The inoculated culture media may be incubated aerobically at room temperature, e.g. $28 \pm 2^{\circ}$ C for 24-72 hours.

The predominant bacterial species in the sample will usually be the most numerous of the colonies growing on the plate (PL. VII B). Select a representative of the dominant or, if more than one all the dominating colonial types. Study their colonial characteristics such as sizes, shape, margin, elevation, consistency, opacity and colour. Transfer by loop the organisms into a fish infusion nutrient broth (a broth tube for each colony type). The isolate has to be checked for its purity (*i.e.* consistent colony morphology) by streaking over a fish infusion nutrient agar. The purified isolate is then subjected to identification procedures.

Generally based on the experience of the pathology team investigating the disease, the signs presented by the morbid animals and the isolation of known pathogens will be sufficient to allow case diagnosis. Only where a new disease involving a pathogen not previously characterised or not characterised as causing that sign of that disease, there is need to resort to Koch's postulates.

SCREENING OF BACTERIA FOR IDENTIFICATION

Identification of bacterial isolates is the next step in the process of disease diagnosis. The problems here are greater for the aquatic microbiologist compared to those of a medical microbiologist, because of the larger number of studies which have been made in medical microbiology.

For identifying bacteria, various systems of classification exist (Scholes and Shewan, 1964). A very detailed and useful key is provided by Buchanan and Gibbons (1974). However, problems still exist in system of classification. The most detailed system of classification described in Bergey's Determinative Bacteriology (Buchanan and Gibbons, 1974) can be handled only by specialists.

In order to aid beginners in microbial taxonomy, a simple and rapid system is called for and this has led to the formation of the keys given in Tables 4 and 5 for screening bacteria: rods and cocci (PL. VIII A, B)

These keys require the following tests for categorising an organism.

1. Cell morphology and Gram's staining according to Hucker's modification (Bullock, 1971). The staining method is given below.

2. Motility in hanging drop preparations from cultures following inoculation for 6-18 hours in peptone water or sea water peptone (1%) peptone in aged and filtered sea water, pH 7.2-7.5).

3. Sensitivity to 2.5 I.U. of pencillin and '0/129'* (Shewan et al., 1954). (1 mg of pencillin is 1667 I.U.)

* '0/129' = 2,4 - Diamino - 6, 7 diisopropyl pteridine

- 4. Breakdown of glucose (Hugh and Leifson, 1953).
- 5. Oxidase according to Kovacs (1956).
- 6. Haemolysis in blood agar (cooled sterile nutrient agar plus 5% sterile defibrinated blood).
- 7. Oxygen relationship (Mackie and McCartney, 1962).
- 8. Coagulase (Mackie and McCartney, 1962).
- 9. Cellulose digestion (Skerman, 1959).
- Acid and gas production in peptone water including respective sugar with Andrade's indicator (Cruickshank et al., 1975).
- 11. Pigmentation in milk agar.

If the disease is suspected to be caused by a mycobacterial species, inoculate the sample in glycerol agar (Nutrient agar plus 1%glycerol) and incubate at room temperature for a few days upto 2 weeks. Colony characters should be noted if acid-fast and Ziehl Neelson-positive organism is observed. The organism may be tentatively classified, based on the pigment, as *Mycobacterium marinum* if the pigment is photochromogenic and *M. fortuitum* if there is no pigment.

STAINING METHODS IN BACTERIAL IDENTIFICATION

Gram's staining

Step I	a. Crystal violet (BDH, England) Absolute alcohol		20.0 gm 200 mi
	b. Ammonium oxalate (BDH, AR) Distilled water	::	8.0 gm 800 ml
	Mix solutions a and b		
Step II	Iodine (BDH, AR) Potassium iodide (BDH, AR) Distilled water	:	0.5 gm 1.0 ,, 2.5 ml

(Dissolve the solutes well and then add

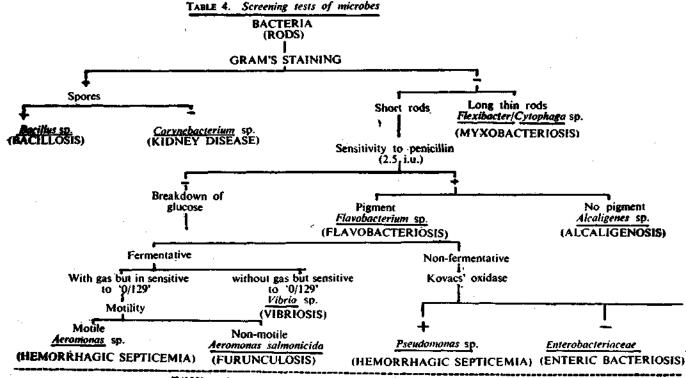
147.5 ml of distilled water)

Step III	Safranin O(BDH, AR)	:	2.5 gm
-	Absolute alcohol	:	10 ml
	Distilled water	:	100 ml

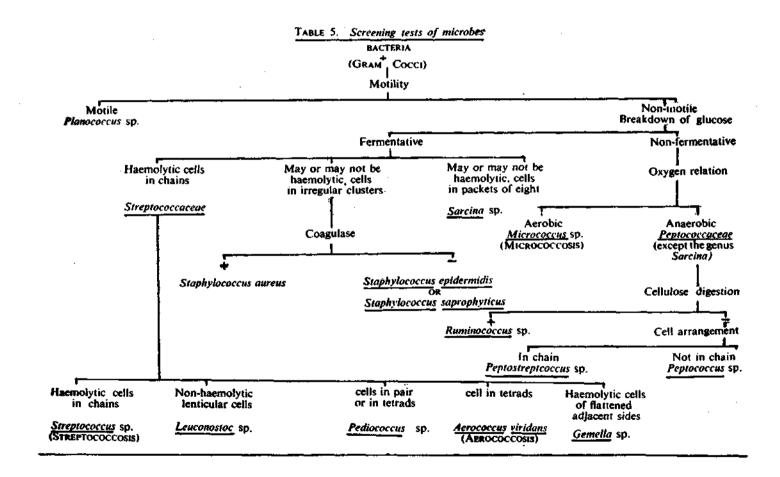
Procedure

- 1. Prepare the slide (Dry the smear well) (Using a sterile inoculating loop, bacteria are picked off the media and mixed with a few drops of sterile physiological saline on a slide. A thin smear is made and allowed to air dry and fixed by passing the slide through a Bunsen flame).
- 2. Flood the smear well enough with ammonium oxalate-crystal violet solution for one minute.
- 3. Wash the smear in tap water for a few seconds.
- 4. Cover the smear with iodine solution and allow to stay for one minute.
- 5. Wash the smear in tap water for a few seconds.
- 6. Decolourise the smear using absolute alcohol for one minute.
- 7. Wash the smear again, in tap water, for a few seconds.
- 8. Counter-stain with Safranin for 30 seconds.
- 9. Wash the smear in tap water for a few seconds.
- 10. Dry the smear for examination.

(Bacteria appearing as violet-blue in colour are Gram-positive and pink-red are Gram negative).



[&]quot;0/129" = 2, 4 Diamino 6, 7 diisopropyl pteridine



THE DESPATCH OF DISEASED SPECIMENS

In some countries where aquaculture is established, fish disease laboratories exist to help the fish and shellfish farmers. In India there is at present little expertise on this subject. However, research organisations such as Central Marine Fisheries Research Institute, Cochin; Central Inland Fisheries Research Institute, Barackpore and the College of Fisheries of Bangalore Agricultural University are centres where the expertise is being gained.

If diseases among the cultivated fishes are detected, live specimens or preserved samples should be sent to the nearest fish pathology laboratory for disease diagnosis. However, there are certain observations to be made before concluding that the fish stock is suffering from an infectious disease. If possible it should be verified whether the stock is getting poisoned (due to consequence of) poor water quality. Poisoning may be suspected if there is lack of macro- or microscopical signs of disease and/or a rapid depletion of the standing stock (often the sudden onset of death of the entire population is suggestive of poisoning). In such cases, it will be important to consult scientists with experience of the ecology of aquatic ecosystems and the chemistry of the waters.

If the case is suspected to be due to infection, the specimens may be despatched in accordance with the following suggestions.

- 1. Forward specimens of the diseased species alive, say, 6 (representative specimens) in a clean container (polythene bags) with 1/3 of the volume filled with water. Protect the bag by placing it in a cardboard box or container. Fill with oxygen, if possible.
- 2. Dead animals should not be sent. If live animals cannot be sent, send preserved tissues in 10% buffered formalin.

- 3. Never deep-freeze the specimens for bacteriological analysis.
- 4. All the available information, regarding the case, may be furnished in the case study sheet (*vide* specimen copy in the earlier chapter).
- 5. Regarding water samples deep freeze upto 3 litres immediately and store until expert advice is available. This will be suitable for certain chemical analysis. Fix some 100-200 ml of water with Lugol's iodine to preserve phytoplankton.

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PATHOLOGY - AN OUTLOOK

In the field of pathology, there has been considerable progress in the human and veterinary clinical medicine. In aquatic animal pathology, finfish and shellfish disease investigation and associated research remains a virgin field. This is because of a lack of awareness and the absence of trained personnel. Such conditions are now changing in India and elsewhere because of the global awareness of the importance of the culture of finfishes and shellfishes. Large scale developmental programmes now being contemplated will require a better knowledge of the diseases and their control in the cultivable species.

Aquatic animal pathology (aquatic veterinary pathology) is concerned with six main areas of study.

- 1. Aetiology: This means the causes of disease and is a subject for study by itself. Moreover, it includes the study of infections and other causes of disease.
- 2. *Pathogenesis*: This is the progressive development of the course of a disease in the affected host from the time of infection to the final stage of recovery or death.
- 3. Pathophysiology: It comprises the changes occurring in the biochemical components of the body tissue and fluids of the diseased host (e.g. changes in red and white cells, etc.).
- 4. Histopathology: Study of the changes in tissue architecture as a consequence of disease e.g. inflammatory responses and necrosis are two of the many possible changes which may occur.

- 5. *Epizootiology:* Systematic study of the origin, distribution and spread of diseases. It involves the recognition of species, sex and age-group affected, percentage of mortality, including the disease distribution.
- 6. Control measures: Control measures mean prevention of disease and treatment of diseased animals. Prevention means to avoid the occurrence of the disease. This can be achieved by several means. In the case of vibriosis, there are several measures, such as immunization, prophylactic chemotherapy, genetic control (breeding resistant species). Several sanitary measures are significant. Physical control by timely removal of the infected population is also useful.
- 7. Treatment: This is to treat and cure or control the disease. There are several methods of chemotherapy. Antibiotics may be administered by way of mass injection, feeding or through mixing in water. For treatment of vibriosis, oxytetracycline is found an effective control by oral administration usually with the animal food.

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GLOSSARY

Aetiology	- Study of the cause(s) of a	disease.
Anaemia	- Lack of red cells in blood.	
Anorexia	 Not feeding. 	
Anoxia	- Absence of oxygen.	
Asphyxiation	Suffocation.	
Biopsy	 Study, usually be histologic tissues removed from a live 	
Diagnosis	Examination of an infected	host.
Ecchymosis	An effusion of blood beneatl	1 the skin.
Enopthalmia	- A condition of retracted en	y e s.
Erythemia	- Superficial redness in the	skin/body.
Exopthalmia	— Protruding eyes.	
Health	- A state of complete well bei	ng of the host.
Haemorrhage	 Loss of blood from any par system. 	t of the vascular
Hyperemia	 Excessive amount of blood is body. 	n any part of the
Hypoxia	- Reduced amount of oxygen.	i
Ischemia	 Deficiency of arterial blood i of an organ. 	n any part or all
Lesion	- Microscopic or macroscopic tissues as a result of infection	
Macrophage	 Group of cells (both free a body having the phagocytic 	

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Necroj	osy —	An examination of the diseased host after death. This is also known as autopsy or post-mortem examination.

Necrosis — Death of cells of a living host.

Pathogenesis - Course and progression of a disease.

- Pathognomonic Typical sign of a disease; may be used to diagnose the cause of a disease.
- Phagocyte A defensive cell in the body having the power to engulf any foreign cell or foreign material.

Prognosis — An opinion of the course, progression and outcome of a disease or treatment of a disease.

Sign of disease — It is a clinical expression, manifested by a living host, as a result of tissue changes of a diseased condition.

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