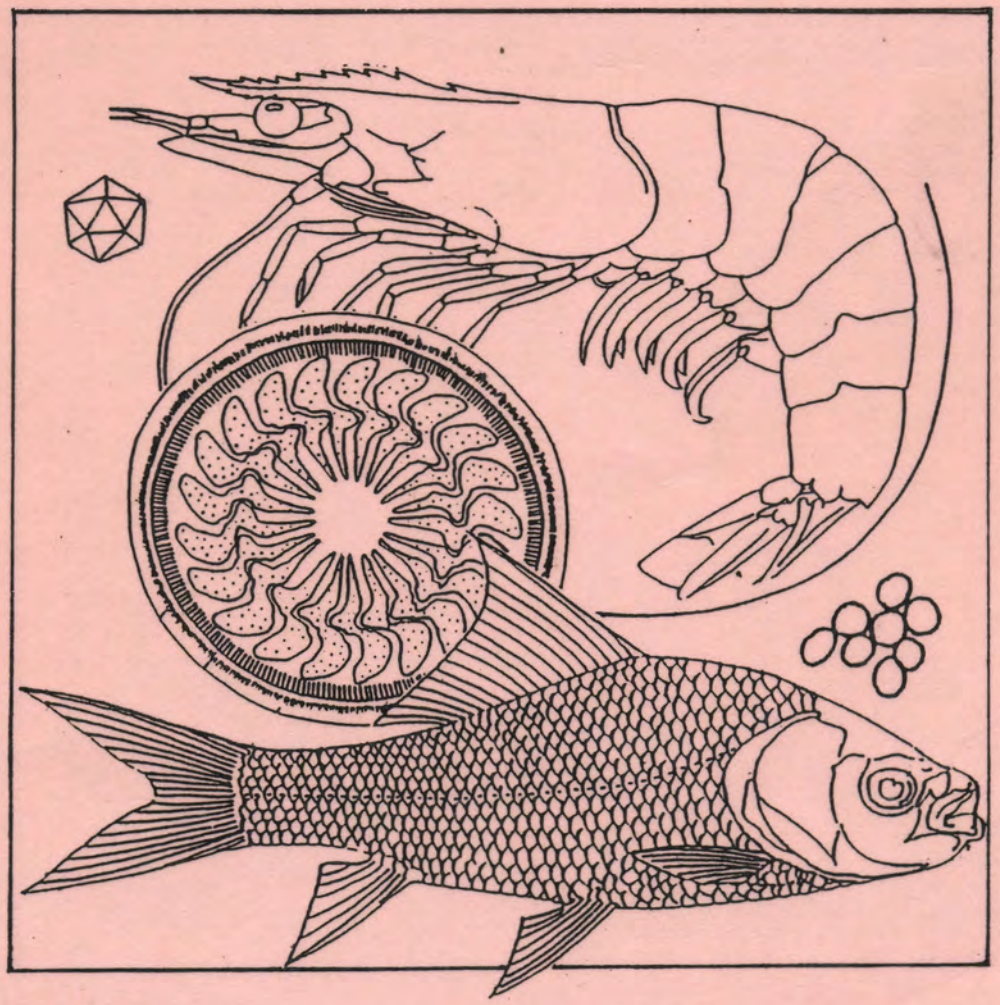
Methods for Diagnosis and Treatment of Fish Disease



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Foreword

Anthropogenic pressure and intensification in aquaculture in the last two decades has created problems of environmental stress, resulting in outbreak of fish diseases. This has given a serious jolt to inland fisheries and aquaculture. In recent past high incidence of diseases in fish has become an important factor limiting fish production and is a matter of nation-wide concern. This situation brought out few significant lacunae in this field. They are in the form of insufficient knowledge on fish diseases, dearth of trained manpower and lack of required infrastructure. The problem is further aggravated by paucity of information available on diagnosis and treatment of fish diseases. Realising the above said deficiencies, ICAR sanctioned a Short Course on *"Methods for Diagnosis and treatment of Fish Diseases"* to be conducted at Central Inland Capture Fisheries Research Institute, Barrackpore from July 15 to 24 1998 for various categories of fishery workers.

This manual is a compilation of lectures delivered at the above said course. The resource persons have made effort to project the information available so far on fish diseases caused by various pathogens, symptoms, their identification, pathology, epidemiology and treatment methods. It is hoped this manual will be of use to the participants of the short course as well as others interested in inland fisheries and aquaculture in general.

M. Sinha
Director

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Fish disease problems in India – an overview

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The last two decades witnessed the transformation of aquaculture and fisheries from its traditional nature to an important economic activity in India. This has been possible because of development and adoption of various scientific technologies in this field. However, anthropogenic pressure and intensification in aquaculture has created some problems in the form of environmental stress resulting in outbreak of disease. Pollutants (industrial, pesticides and intensive fish farming effluents) discharged in various water bodies are creating stress to fishes and endangering their life. The recent outbreak of Epizootic Ulcerative Syndrome in fishes throughout India showed that fish disease can be a major limiting factor for enhancing fish production. It also brought to focus the lack of sufficient knowledge on fish disease and lack of required infrastructure for fish health management in India.

There are many types of fish diseases known so far affecting the fishes externally as well as internally. They are caused by various types of organisms (fungus, bacteria, protozoans, crustaceans, helminths etc.) , causing damage to fish of minor as well major magnitude. Whereas, therapeutic measures for some diseases are still not well known , there are even such diseases known whose causative organism is yet to be ascertained. As such, though considerable know-how in this field is available, a lot more is still desired. Even many of the fish health management measures, known to the Scientists, have not reached the field functionaries. These gaps are due to many reasons, including lack of trained manpower and inadequate infrastructure.

Trained manpower for tackling fish disease problems is a must. In a vast country like India it is grossly inadequate even at the level of scientists, teachers, technicians and fishery extension personnel. There is no specific course on fish disease at graduate or post-graduate level. There is shortage of skilled manpower or infrastructural facilities for the same. Moreover, good books on fish diseases available in India, for study at the graduate or post-graduate level, are meagre. Only recently Inland Fisheries Society of India has published the first comprehensive book on "Fish and Prawn Diseases of India", giving details of fish diseases known in the country till now, their diagnostic characters, therapeutics and the methodology for fish pathology studies.

Lot still deserves to be done to develop the **required infrastructure**, both for research and fish health management in the field. The country has few national laboratories for the purpose. Even in existing laboratories the level of expertise available is often inadequate, especially in terms of histopathological studies. Properly equipped fish virology laboratory is not yet in existence in India. There is an urgent need to develop some distinct areas of research , *viz*. fish virology, fish microbiology, clinical pathology, immunology and environmental toxicology; to tackle the problem of fish health management in totality. Field stations, at least at block level is a must to tackle the fish disease problems, both at prophylactic and therapeutic stages.

Undertaking **prophylactic measures** for fish disease prevention are till now given least importance in our country. Any deterioration in water quality creates stress in fish and suppresses their immune system. Consequently fishes become susceptible to pathogens present in water. Thus, one of the important required strategy for disease prevention is to adopt proper prophylactic measures before disease outbreak. There is every chance of preventing the disease outbreak if proper prophylactic measures are taken in time. If prophylactic measures fail, proper therapeutic measure would have to be taken. Here also, various chemotherapeutic compounds are being used in our country, empirically without proper trials. This endangers the ecosystem. Proper risk assessment of the use of chemicals and drugs for disease prevention and control should be done.

The aspect of **disease surveillance** is of paramount importance in India. With the outbreak of EUS our inadequacies in this regard were exposed. Till now the monitoring of outbreak of fish disease in India by the Fisheries Institutes is limited to the report of outbreak received either from farmers or state/ central government agencies. A network for monitoring has not yet developed. The first attempt of this kind was initiated by Ministry of Agriculture, Govt. of India with the questionnaire developed by CIFRI / NACA for monitoring EUS in India. The attempt has been fairly successful.

There is no system of fish or fish seed **quarantine** in this country. It is true that introduction of certain exotic fishes has significantly enhanced the fish production in this country. But possibility of introduction of exotic pathogens, alongwith non-quarantined exotic fish or fish seed, also exists and have rather been observed. Parasites, previously unknown in India, have been recorded from introduced fishes. Thus, it is essential that import of various culture and ornamental fishes is subjected to quarantine procedure. Transfer of fry and fingerlings within the country should also be checked and fish health certification procedure be introduced. Unfortunately, proper fish quarantine procedure, trained manpower for the same and facilities for implementation is lacking.

Some of the prevalent relevant deficiencies in the area of fish health management and disease control in our country have been enumerated above. To overcome the same **certain suggestions** are as below:

To create opportunities/ facilities and impart training for creating the required expertise at laboratory as well as field level.

To upgrade the research capabilities , particularly in field of fish virology, fish microbiology, immunology, clinical and general pathology, environmental toxicology and histopathology.

To upgrade the facilities for fish disease research and diagnosis in Research Institutes , Universities as well as farm stations and develop a network to coordinate the research efforts.

Development of quarantine and fish health certification procedures and facilities in India

Create a general awareness regarding fish health management and its importance at all levels from the primary fish farmer to policy maker.

Anatomy of fish

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Introduction

Intensive fish culture has tremendously increased probability of occurrence of fish diseases. Even sometimes diseases occur in the form of out-breaks, wiping out all the stocks of fish/fin fish from the ponds which are used for intensive fish farming. In India in recent years, shell fish has been devastated by spreading of the diseases, thus incurring huge losses to the farmers. Therefore, disease is one of the most seriously limiting factors in aquaculture. Unnaturally high population density favours the spread of many diseases and parasites.

Much economic loss is preventable with proper fish health management. Since diseases are heralded by the appearance of structural or behavioural abnormalities, it is important to understand what is normal for any fish species with which we deal. This knowledge constitutes the basis for all fish health activities. Therefore, in the following, the anatomy of fish is discussed in order to provide an idea of various fish organs which are frequently attacked by pathogens and their possible relationships between pathogen. As organs and tissues are viewed here as constituents of a substrate as parts of environment that can be colonized and modified by various pathogens in consequence of complicated interactions.

The ailments of the fish may be placed under one of the following categories: 1) dis orders resulting from abiotic factors, mechanical injuries or pollution 2) dietary deficiencies followed by misfunctions and malformation 3) tumours and atypical cell growth 4) diseases including those caused by infections agents and parasites. Studies of these disorder falls in domain of fish pathology, with etiological, morphological, physiological and therapeutic aspects.

The form of the fish body

Fish are generally supposed to have spindle shaped bodies which are laterally somewhat compressed with blunt head and long thin tails. Each species has optimally adapted itself to its environment and a variety of different shapes have resulted. More extreme examples of such adaptations are many eels which live in crevasses in the rocks.

External morphology (anatomy) of the fish

Integument and fins:- Skin provide a safe protective covering for underlying tissues and organs while fins pectoral, pelvic and dorsal assist in locomotion of the fish. In some fishes skin is protected by scales which may be of various types (ganoid, cycloid, ctenoid etc). The skin as a result of injuries can be breached either due to mechanical or parasites and pathogen activities. Some Myxozoa form cysts under the scales causing loss of scales leaving open lesions free to secondary infection. Even, some crustacean found refuge under the scales, a microorganisms can cause local inflammation. These and other causes often manifest in the form of haemorrhages.

Further, scales provide an excellent substrate for attachment of ectoparasites (monogeneans and copepods). Certain protozoan and monogenia formed a thick mucous coating on scales which can disturb respiration, thus effecting oxygen intake.

Openings

The mouth, the gill apertures and the anus (vent) are the main openings connected with the alimentary tract in the fish body. The anus offers a point of penetration to certain helminths and crustaceans ectoparasites that have been adapted to life in rectum.

Mouth or buccal cavity

The fish mouth is situated anteriorly on the head in terminal position, but adaptively its position may be superior or inferior. In relative size it ranges from small (Phoximus) to huge (gulpers). The mouth is bordered by the lips. The buccal cavity generally is not a good place for the pathogens attachment. But some pathogens found protected place around the fold of skin surrounding the tongue and around the teeth. Ciliates such as trichodinids may settle on the roof of the mouth on the tongue while *Lernaea* can attach at any site in the mouth..

Gill apertures

In many fishes with gill covers, there is a single opening on each side of the head . Normally this opening is infront of the pectoral fin bases but in bat fishes, it is behind them. The openings are a single pair in the Chimaeras (Holocephali) and in some hagfishes (myxine). In the other hag fishes, the openings vary from 5-14 on each side. All lampreys have seven gills while sharks have five to seven besides a spiracle which is located between the anterior most gill slit and eye. Inside the spiracle a small tuft of gill filaments persists. Gills which are main respiratory organs in fishes offer a good substratum for attachment of parasites especially to crustacea (*Ergasilus*) which squeeze the gill filament with their second antennae and stop blood supply to distal portion. Circulation might also be occluded by parasites, such as the trematode *Sangunicola* which are carried in the blood stream and trapped in the capillary network of the gills. Myxosporea and certain bacteria are the other pathogens which are associated with the gills. Fortunately, fish are capable of surviving with much reduced surfaces but pathogens may impair the ability of the fish to cope with stressful challenges and further manifest in the form of slow growth and weight gain.

Operculum

It is the bony covering of the gills and is made of several bones such as preopercle, opercle, inter opercle, and sub opercle, the opercle is typically the largest bone in the series and extend farthest posteriorly. Generally, outer opercular surface is devoid of pathogens but inner surface, however, is frequently colonise (copepods and monogenea). Most of the parasites cause only superficial tissue damage and are not very dangerous unless they facilitate secondary infection.

Sensory organs

Eyes:- Eyes are lidless and that cannot be closed are situated in orbits, one on each side of the mid-line of the fish head. Most often, the eyes are lateral, with partially independent fields of vision and movement. In many bottom dwellers the eyes are dorsal and in flounders and flat fishes both eyes are on one side of the head. The eyes are reduced or absent in cave fishes (Amblyopsidae). Despite the protection the eyes are susceptible to pathogen attack. Some infect the orbit outside the eye proper (myxozoans) forming the macroscopic cysts, other cause eye to protrude. The conjunctival surface can be damaged or covered by protozoan, metazoan or fungal parasites. Pathogen may also infect the eye itself, living in both chambers or even in the lens. Trematodes (*Diplostomulum*) lodging in the lens cause opaqueness and result in loss of eye sight due to parasite cataract.

Lateral line

Numerous microscopically small openings of skin sensory organs are developed on the surface of the fish body. In most fishes, a series of these pores, extending along each side in a single row from the head to the caudal fin, comprises the lateral line. In rare cases they do also provide entry points for parasites whose lodging in the canal results in the form of ovoid swellings.

Nose or Nares

It is an olfactory organ and may be single or paired. Most of the fishes have narial openings at the top of the sides of the snout. To perform its functions, the nose must continually intake water inside the chamber. While at the time of entering water inside the nose may carry microorganisms and helminth parasites. These may settle on a nourishing substrate, the olfactory epithelium. Generally *Trichodina* and *Gyrodactylus* are observed inside the nose. Sites of attachment of various pathogens on the body of fish is shown in Fig. 1.

B. Internal Anatomy

In highly developed animals, muscular body wall surrounds a cavity which is called coelom or secondary body cavity. The coelom is lined by a single layer of coelom-epithelium or coelothal, this together with the connective tissue which lies beneath it forms the peritoneum. However the peritoneum is restricted to posterior portion of the coelom which does not enclose the heart, the abdominal cavity. The heart is enclosed inside the pericardial cavity which is separated by abdominal cavity by a septum. The abdominal cavity and pericardial cavity are inter connected in some less developed groups but in higher fishes (Teleostei) they are not connected with each other. All important organs lie inside these cavities except kidney and gonads. The description of some of the important systems and of the pathogens associated with these is given below. The general plan of the other vital fish organs inside the fish body is depicted in Figs. 2 & 3.

Digestive system

The following form the digestive tract and is valid for most of the fishes.

1. Mouth is bordered by toothed jaw and rimmed interally by oral valves

2. Oral cavity with vomerine and palatine teeth in roof and tongue with tongue teeth in the floor.

3. Pharynx with pharyngeal tooth pads on the throat sides of the gill arches and gill rakers that guards the internal branchial openings.

4. Esophagus or gullet

5. Stomach

6. Pylorus (pyloric valve; followed by openings into pyloric caeca in many fishes)

7. Small intestine passing the openings of the duct (s) that bring in bile and pancreatic secretions, and going through atleast a major s-curve (duodenum) into the next section.

8. Large intestine which open via rectum into anus. Liver with a gall bladder and spleen are also present.

Among the bony fishes there are however, many special adaptations of the digestive tract depending upon type of feeding *ie* predator, omnivorous herbivorous and parasites (Figs. 5a & 5b).

Digestive tract is having altogether different environment than outside world, so the pathogens which attack this system develop special organs for their survival. Most pathogens enters the alimentary canal with food, particularly with dietary components that serves an intermediate host. A few entered by penetrating the rectum via anal opening. The common parasites associated with digestive system are: bacteria, protozoan, nematodes, acanthocephala, and cestodes.

Intestinal parasites are generally well adapted to their specialised habitat and tend to be rather, mild pathogenic. Reactions to them are generally restricted to localised inflammatory response. Some bacteria can become vary pathogenic and seriously affect the intestine. Parasites might also block the food absorption, so that the fish could starve even though sufficient food is ingested. The liver is not only the largest but also the most versatile of the metabolic organs. It is often used by nematode as attachment substrate. Large scale destruction of hepatic tissues by penetrating nematodes has been frequently observed. Myxosporea infect the liver tissue, as well as the bile duct. The gall-bladder is a common site for myxosporean but also harbours other parasites. These parasites sometimes cause jaundice and cirrhosis of the liver.

Circulatory system

The blood in fishes circulates by means of a more or less continuous tubular system of heart and vessels. The heart is a valved pump that forces blood forward toward the gills for oxygenation. Having traversed aortic arches in passing to and from gills, arterial blood is ultimately dispersed in capillaries of the tissues. Venous blood from the tissues returns to the heart although that flowing through the kidneys and the liver is first again dispersed in capillaries respectively in the renal (except cyclostomes) and the hepatic portal systems. Arteries carry away the blood from the heart; the veins, towards it.

Blood

Fish blood like other higher animals, has two parts fluid and solid. The fluid part is plasma in which the solids part the blood cells are transported. The cells are red (erythrocyte) and white (lymphocytes, leucocytes). Haemoglobin in red cells greatly enhances the ability of blood to transport the oxygen. In certain fishes (ice fishes) haemoglobin is absent, thus blood is colourless lacking erythrocytes.

Heart

The fish heart is constructed fundamentally to offer a single circulation, rather than a completely double one as in the mammals. Typically blood returning to the heart enters a sinus venosus, passes through the auricles or atrium and is propelled through the gills for aeration by the thick walled mud ventricle. Such type of arrangement is observed in cyclostomes. The chondrichthyes add a contractile, muscular valved base (the conus arteriosus) to the ventral aorta where it leaves the ventricle. In higher bony fishes conus is replaced by non contractile bulbus arteriosus. The above is the basic plan of the heart, but in certain group some modification such as partial partition of the atrium, ventricle and ventral aorta do occur (Fig. 4). The ventral aorta in a fish is median in position beneath the gills. From it branch the afferent branchial arteries to each gill pouch or arch. Within the gills afferent break down into capillaries and collect again into efferent that form the dorsal aorta, main vessel for distribution of the blood to the body.

Blood vessels

Oxygenated blood leave the gills to supply the head region principally through carotid arteries and to supply the body by branches of the dorsal aorta, including many that are segmentally arranged. In the tail the dorsal aorta is termed the caudal artery and traverses the haemal arches of the caudal vertebrae, blood from the tail is mainly collected by caudal vein. In fish group above the cyclostomes, the caudal vein drains into the renal portal system of the kidneys and upon leaving the kidneys blood from the dorsal musculature in the posterior cardinal veins. Post cardinals receives blood from anterior cardinals. Cardinals join the duct of Curvier on each side of the esophagus. The ducts of Curvier receive additional blood from beneath the head and from the lateral body wall. In addition to receiving the ducts of Curvier, the sinus venosus also collects blood from the liver. The liver is supplied from the viscera through hepatic portal system.

From disease point of view all part of the circulatory system can be affected by pathogens. A frequent occur is cardiac failure, recognized externally by oedema. Its symptoms are distended bally, pop-eye and abnormally soft flesh. Heart failure is often due to atrial muscle damage in toxic myocardial necrosis resulting from acute bacterial infections. Aeromonad bacteria and vibriosis are mainly responsible. Generalized rhabdovirus infection causes haemorrhages of the heart musculature. Myxozoa may live in cardiac tissues, and indeed, some species appear to be adapted exclusively to life in heart. Similarly, larvae may penetrate heart tissue with their attachment organs.

Gas-bladder or swim-bladder

This structure appeared as a respiratory organs derived from the last pair of embryonic gill pouches but with the passage of time this organ has been modified to serve as many functions such as gravity adjustment, hydrostatic equilibrium sound production and assistance in reception. This is totally absent in cyclostomes and chondrichthyans. The embryonic connection between the gut and gas-bladder has been retained as a functional pneumatic duct in most soft-rayed fishes (physostomous), but lost generally in spiny-rayed fishes (physoclistous). Additionally, in several fishes (herring family), the gas-bladder communicates with the exterior by a pore near the anus.

Gas-bladder is often attacked by helminths, specially nematode and protozoa, coccidia can produce masses of cysts, filling and distending it completely. Fungal and bacterial infection have also been recorded. They are mainly manifest in abnormal distensions of the bladder, indicating disruption of its gas regulation mechanism.

Ear

In fishes only inner ear is present and out and middle like man are absent. The function of ear in fishes is hearing and balancing. Very few pathogens have been recorded from the ear, *Myxosoma cerebralis* is one of them. It destroys the cartilage surrounding the ear of salmonoid (juvenile), thus impairing the balance, causing the fish to move in a characteristic whirling fashion that gave rise the name whirling disease.

Brain and spinal cord

The fish brain is an extension of the anterior end of the spinal cord. Its parts progress linearly from forebrain region (the enlarged cerebral hemispheres and the connecting between brain) through the mid-brain with its swelling (the optic lobes), to the hind brain (cerebellum and medulla) and continue towards the caudal fin with the spinal card. Both the brain and spinal cord are whitish and soft. The brain is enclosed inside cranium of the skull. The spinal cord runs length-wise of the fish in the neural canal of the vertebral column. The cerebral hemispheres and the cerebellum are more prominent in sharks and bony fishes than in the lampreys and hag fishes. The mid-brain is prominent both in the cyclostomes and bony fishes. The cavities of the brain are continuous with that of the spinal cord.

Not much information is available of the pathogens which attack the fish brain. Some leisons have been associated with rhabdovirus infections and with fungal penetration, possibly as a secondary pathogen. Infection in the brain through trematode cercariae is known evoking a hyperplastic response but not causing ill effect on brain. Damage to brain vessels by heavy trematode infection might prove fatal.

Excretory system

The kidney is retroperitoneally located in all vertebrates; *ie* it is situated exterior to the dorsal wall of the body cavity or coelom. Kidneys are the main paired excretory organs. Commonly they are reddish brown, pulpy structure .Kidney in fishes are of two types, 1) Pronephric and 2) Mesonephric the former is a simple and the latter is complecated structure. Many nitrogenous wastes pass through the kidneys that also assist in water- salt balance (Homeostasis) by the excretion or retention of certain minerals. Besides, kidneys, gills also take a prominent role in waste excretion, eliminating mainly amonia. A typical fish kidney is made up of many individual units or nephrons, each consisting of a renal corpuscles (Malpighian body) and a kidney tubule. The tubules join in collecting ducts that finally lead to the outside through the mesonephric duct with it various terminal modifications. The Malpighian body is made up of a glomerulus, a blood vessel tightly coiled with efferent and efferent arterioles and encapsulated by thin kidneys with frequent small glomeruli. The glomerulus and the capsule together act as ultrafilters. The excretory fluid undergoes alteration on its way through the tubules where glucose, various minerals, other solutes, and in some cases water are reabsorbed into the blood by an energy-requiring process.

Reproductive system

Fishes may be unisexual, bisexual and a few hermaphrodite. Even parthenogenesis has been reported in a few fish (*Poecila formosa)*. The sex glands are testes in male and ovary in females with their ducts. The testes of males are long, smooth, whitish organs which extend throughout most of the length of the abdominal cavity. Each is continued posteriorly into a duct which opens into the urogenital sinus. They lie just beneath the air bladder while in certain fishes (featherback) testis is a triangular single structure. The ovary also run the length of the abdominal cavity and contain numerous ova. Oviducts are lacking. Mature eggs find their way to exterior through urinogenital sinus. The endocrine system plays an important role in reproduction. The process by which sperm cells are formed is called spermatogenesis while oogenesis is the process of egg development in ovaries parallel to that of sperm manufacture. Fecundity is a general term used to describe the number of ova produce in a spawning season. On set of maturity is early in short life spanned fishes and late in long life fishes. Fertilization may be external but in a few families internal fertilization is also prevalent. Most of the fishes are oviparous but a few are live bearer (guppies etc) *ie* young one is borne. The living coelocanth (latemaria) is ovoviviparous. Similarly in certain fishes phenomenon of parental care is highly developed (*Tilapia* spp.) while most of the fishes leave eggs to nature for their development.

Gonads are choked sometimes by bacteria (tuberculosis, furunculosis and virus (rhabdoviruses) which results in diffuse local damage, haemorrhage and sterility or reduction in fecundity. Even, protozoan too invade the gonads. They form cyst and provoke necrosis and fibrotic reaction the latter resulting in thickening and hardening of the gonads with loss of reproductive function. Certain large parasites *(Ligula intestinalis)* of the visceral cavity indirectly impaired gonads by exerting sustained pressure.

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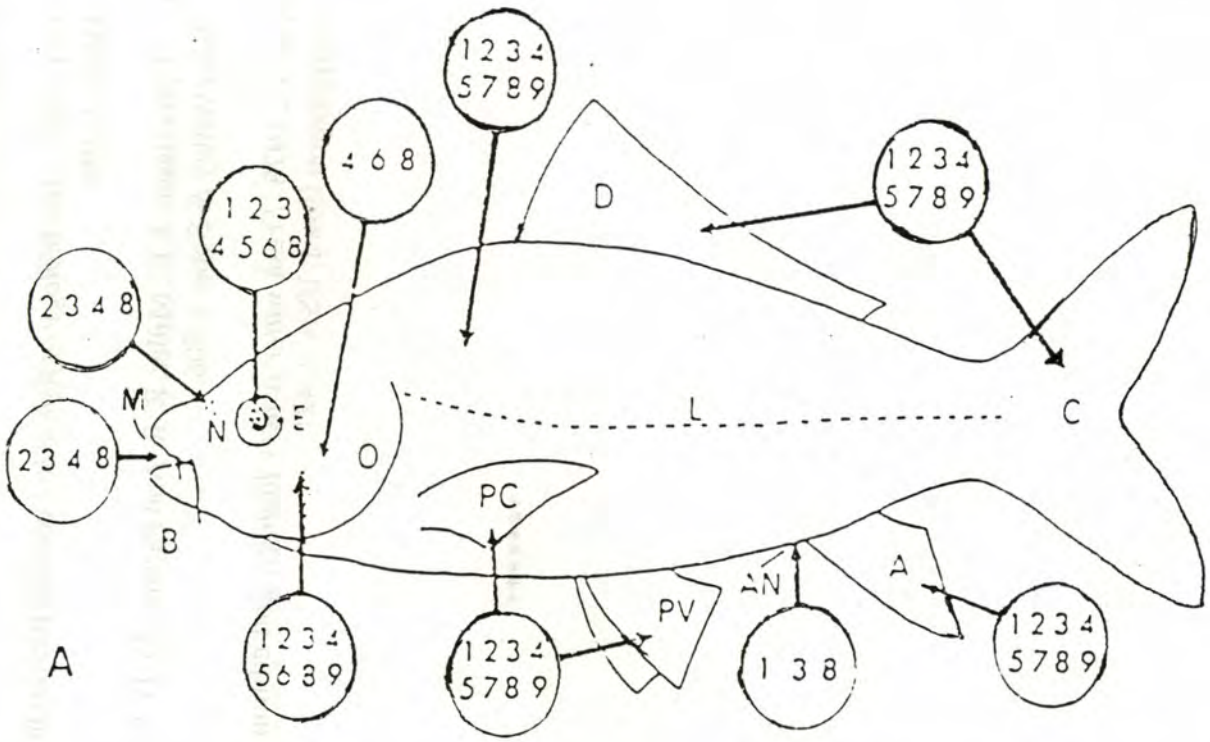

Figure 1. External morphology of ***Cyprinus carpio***, with indication of pathogens attacking various parts of the fish.

Abbreviations: A = anal fin; AN = anus; B = barbels; C = caudal fin; D = dorsal fin; E = eye; L = lateral line; M = mouth; N = nose; O = operculum; PC = pectoral fin; PV = pelvic fin; 1 = micro-organisms; 2 = fungi; 3 = Protozoa; 4 = Monogenea; 5 = Digenea; 6 = Nematoda; 7 = leeches; 8 = Arthropoda; 9 = Mollusca (glochidia).

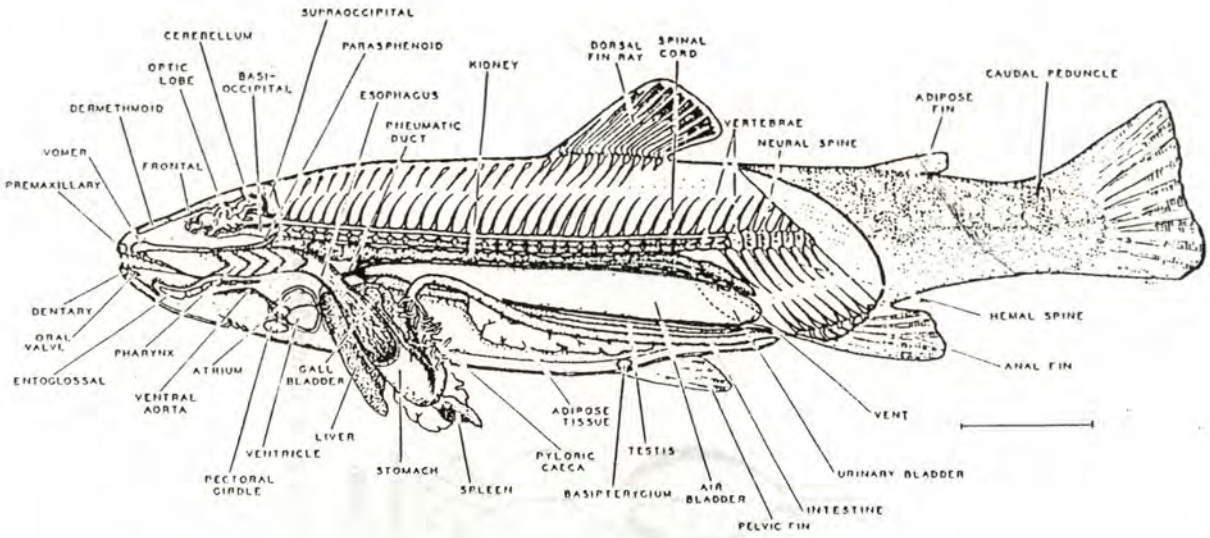

Fig. 2 ANATOMY OF A SOFT-RAYED FISH.

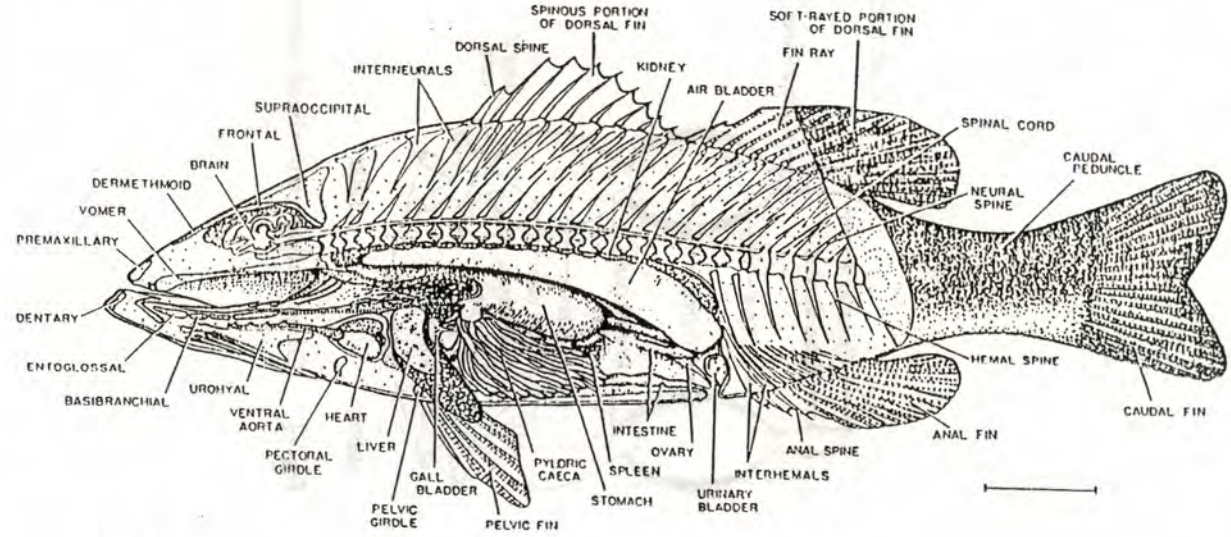

Fig. 3 ANATOMY OF A SPINY-RAYED FISH.

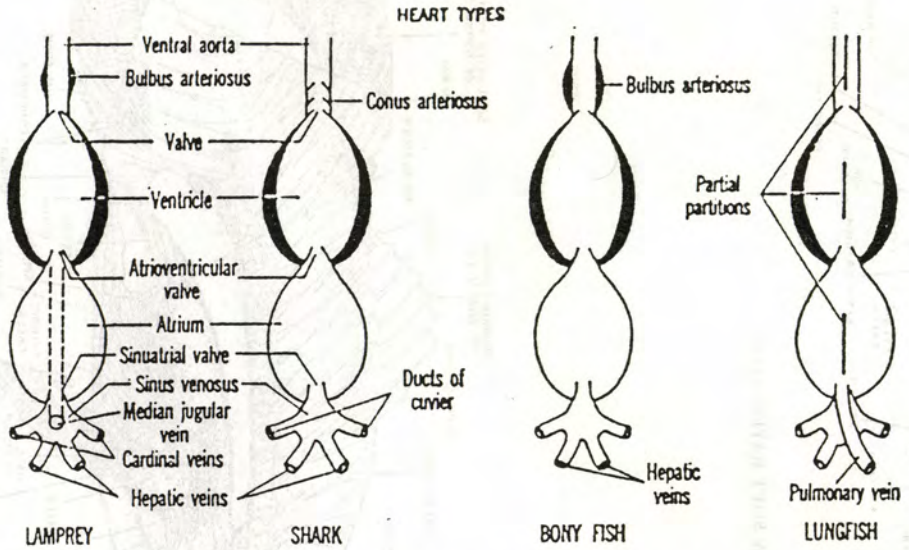

Fig. 4 Comparative generalized schemes of the heart in major groups of living fishes.

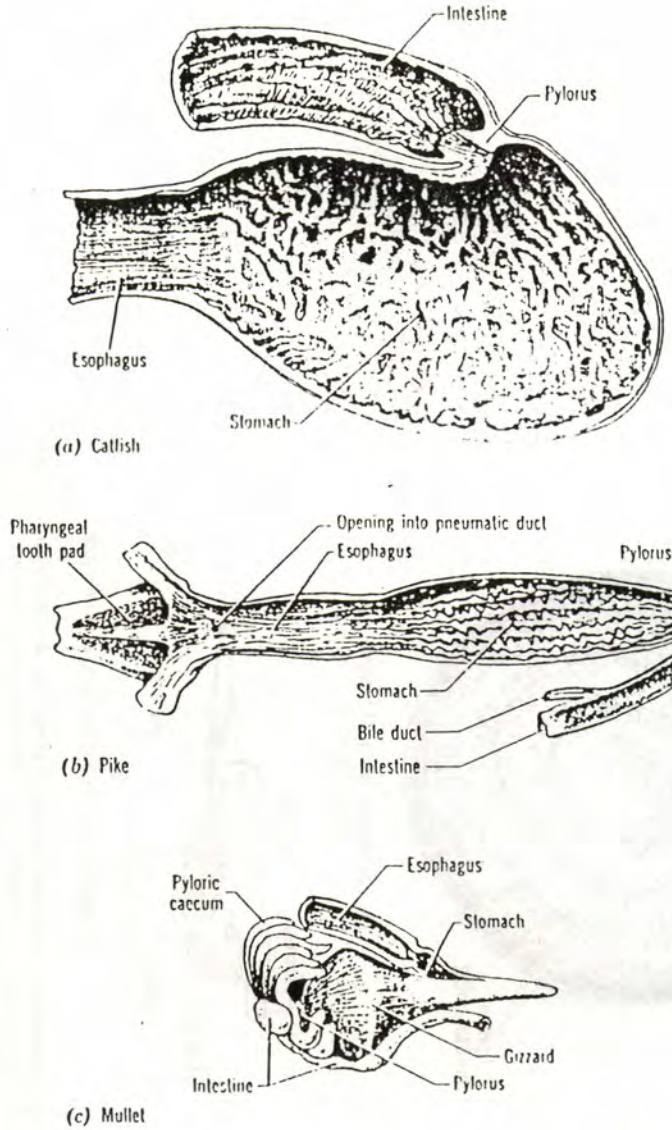

Fig. 5a Variations in shape, appendages, and lining of the anterior portion of the digestive tract in three fishes: (*a*) an omnivore, a catfish, the European Wels (*Silurus glanis*); (*b*) a carnivore, the northern pike (*Esox lucius*); (*c*) a bottom-grubber, the striped mullet (*Mugil cephalus*). (Based on Pernkopf in Bolk, Göppert, Kallius, and Lubosch, 1937).

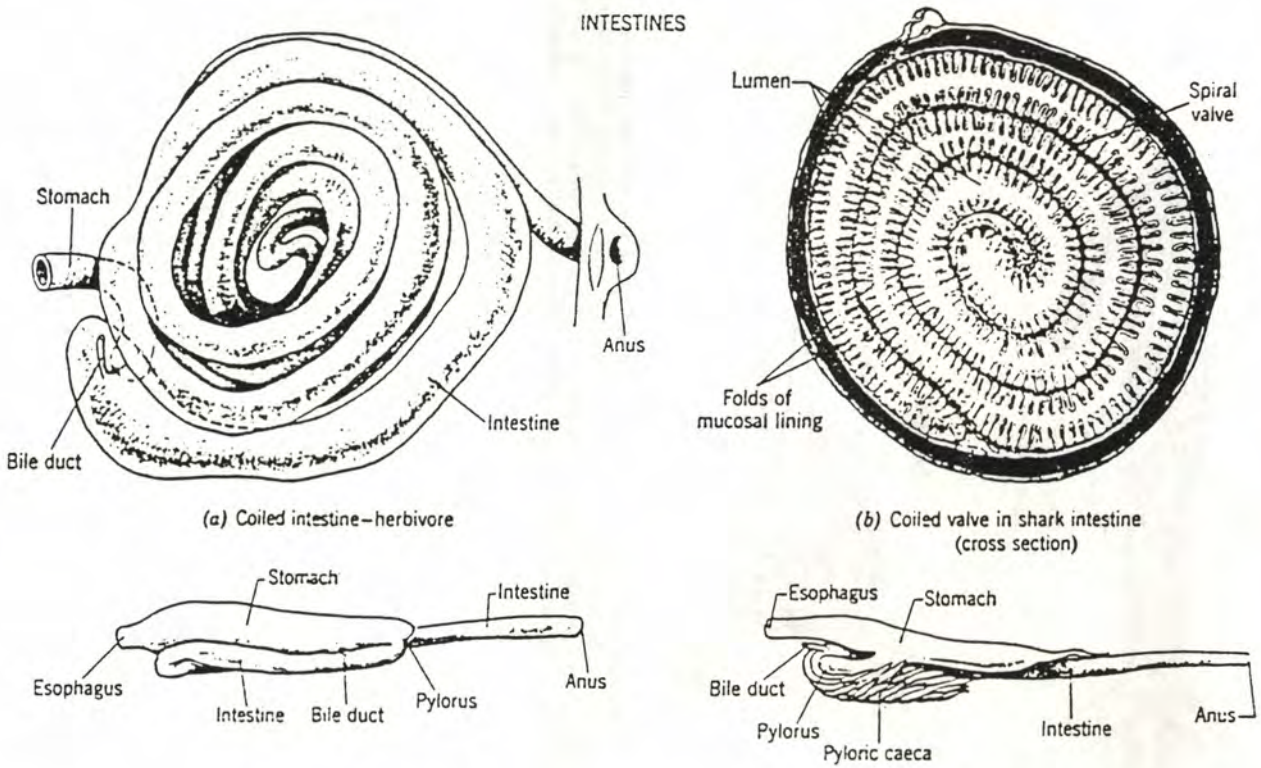

Fig. 5 b Variation in intestinal length and other features among carnivorous and herbivorous fishes: (*a*) an herbivorous catfish (*Loricaria*); (*b*) spiral valve in cross section of intestine of a carnivorous shark (*Scyllium*); (*c*) a carnivore, the northern pike (*Esox lucius*); (*d*) a carnivore, a perch (*Perca*). (Based on Bolk, Göppert, Kallius, and Lubosch, 1937).

Role of abiotic factors in fish disease outbreak

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Any outbreak of fish disease is the result of prolonged overlooking of some stress factors acting upon aquatic environment. For, a disease is the ultimate expression of the deterioration of water quality that provides the opportunity to pathogenicity.

pH

One of the most important abiotic factors behind the outbreak of fish disease is the uncongenial pH of the ambient water. Fish do not grow well below pH 6.5 and this provides favourable environment for fungal diseases. EUS, which is now supposed to be a fungal infection has also been seen to be favoured under acidic pH. Fishes, including tilapia have observed to be affected with severe ulcers at or below pH 4. Similar disorder has also been said to occur above pH 9.5. Fishes grow comfortably in the pH range of 6.5 to 8.5. However, for *P.monodon* the congenial range of pH is 7.5 to 8.5 and best being around 8.2. Any sharp change even within this congenial range may act as a severe stress to the growing *P.monodon.* Acidic waters below pH 6.5 and alkaline waters above pH 9.5 retards reproduction and growth of fish (Swingle, 1961; Mount, 1973) and indulges diseases.

Dissolved oxygen

Concentration of dissolved oxygen is another important abiotic factors which has a direct bearing on fish health and outbreak of fish disease. Dissolved oxygen should not be lower than 5 mg/l over a period of 16 hours of any 24 hours. Less than 5 mg/l over a period of 8 hours may act as a stress on fish health and at no time it should be less than 3 mg/l (Boyd, 1982, McKee and Wolf, 1963).

Prolonged exposure to low dissolved oxygen is known to be a precursor to bacterial infection in fish (Snieszko, 1973). Walters and Plumb, 1980 have shown that high dissolved carbon dioxide (> 15 mg/l) and low DO, together are more effective in causing bacterial infection in fish. Low DO itself (Stewart, *et al.*, 1967) retards intake of food and growth. Again, supersaturation of oxygen has been found to cause gas bubble disease and mortality in fish (Nebeker and Brett, 1976).

Unionised Ammonia

Chronic exposure to water containing high amount of unionised ammonia has been known to produce lesion on fish body. The impact of unionised ammonia increases with rise of pH and temperature and fishes become victims to opportunistic pathogens. Similar observation has also been made by Smith and Piper, 1975. The present author has also seen a number of cases of fish pathogenicity under high concentration of unionised ammonia. Histological

changes take place even at low concentration of unionised ammonia (Boyd, 1982). Fish growth suffers severely even when the concentration of unionised ammonia is below 1 mg/l. Therefore, it may be concluded hat accumulation of unionised ammonia in water affects growth in fish and favours the pathogenicity by opportunistic pathogens.

Hydrogen sulphide

Similarly, unionised hydrogen sulphide has also been found to be highly toxic for fish health. Adelman and Smith, 1970, have shown that the presence of 0.06 mg/l of unionised hydrogen sulphide results in low rate of egg survival and fry development in northern pike. Bioassay experiment conducted by Smith *et al.*, 1976, has indicated that any detectable concentration of unionised hydrogen sulphide is detrimental to fish health and may cause fish mortality directly or through pathogenicity.

Transparency (Secchi Disc)

The value of Secchi disc transparency measured at noon under bright sunlight is a good indicator of fish health. In natural waters a Secchi disc transparency of 30 to 80 cm is good for fish health. In intensive fish culture pond this value may range from 15 to 40 cm. Fish kills are quite common when the Secchi disc transparency goes down below 12 cm. This low transparency causes stress to fish and outbreak of disease has frequently been seen in sewage fed freshwater bheries.

Carbon-di-oxide

Respiration of aquatic plants and animals is responsible for the accumulation of carbon dioxide in water. Its concentration below 15 mg/l, is quite conducive for fish health but when the accumulation of carbondioxide exceeds 15 mg/l, it hinders the uptake of oxygen by fish (Boyd, 1982). At higher concentration water becomes acidic and this favours selective pathogens to invade fish and indulges in the outbreak of fish disease. It also causes the loading of oxygen more difficult for fish.

Alkalinity

Alkalinities actually mean the total concentration of bases in water expressed in mg/l of calcium carbonate. Waters having alkalinities below 20 mg/l have been found to favour the outbreak of EUS and other parasitic diseases more virulently in fish (Das and Das, 1995 and other robservations of Das and Das, at Bethuadahari sanctuary Nadia, 1988). Waters with low alkalinity show sharp rise or fall of pH even with a small change in the concentration of bases because of its poor buffering capacity. Waters having alkalinities above 40 mg/l have been found to be more productive having less possibilities of outbrak of disease (Moyle, 1945).

Hardness

Fishes are seen to be more susceptible to diseases in water having hardness below 20 mg/l. Virulent outbreak of EUS has been noted more frequently in waters with low hardness (20 mg/l<) (Das and Das, 1995). Productive water should have its hardness greater than 20 mg/l, calcium content > 5 mg/l and magnesium content > 2 mg/l. Very hard water (> 300 mg/l) is also

not congenial for fish health because at higher pH nutrient availability is not optimum. Most suitable hardness for fish cultue has been found to be in the range of 75 to 150 mg/l. This hardness also acts as a preventive against the outbreak of common diseases of fish.

Turbidity

Appreciable fish mortality has been noted in highly turbid water (> 175000 mg/l). Productive waters are generally have clay turbidity around 25 mg/l. Turbid waters (> 25 mg/l) are having more bacterial density than those of less turbid waters and consequently have greater chances of outbreak of diseases.

Decomposing organic matter

High accumulation of humic substances through the decomposition of profuse organic matter and macrovegetation, harbours higher number of disease producing organisms than those of clean waters, having transparency above 20 cm. It has been observed by the author that the bheries having large amount of decomposing organic matter favoured the virulent manifestation of incurable diseases in prawn in bheries of 24-Parganas South during 1996-1997 period. These type of diseases were found to be much less in bheries having clean water but located in the same areas.

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Fungal and bacterial diseases of fish - diagnosis and therapy

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Introduction

Fish husbandry has been practised for at least 3000 years in China and less so in other parts of the world. But only over the last few decades aquaculture has grown into a significant industry in many countries through gradual transition from extensive to semi-intensive and intensive farming technologies. However, due to increased farming intensity and high population density in culture system occurrence of infections diseases, caused by many fungi, bacteria, viruses and parasites, are becoming more common and pose a constant and highly costly threat to successful fish and prawn husbandry.

With time extensive works have been done on fish pathogens in developed fish farming countries. But such study in India is scarce and has been started in some accelerated way at recent days. In this text we shall restrict ourselves to only fungal and bacterial fish diseases with special emphasis to those prevalent in India.

Bacterial diseases

Bacteria are small prokaryotes distributed ubiquitously and are common cause of illness to man and animals including fish. Fortunately, number of pathogenic strains of bacteria are only few and fish are very efficient in dealing with bacterial invaders. There are two hypotheses about causation of bacterial diseases: one, the bacterial strain must be pathogenic to the host and be virulent, and the other, total bacterial load should cross a critical concentration in the milieu. Possibly, both the factors play simultaneously in disease production very often. Sometimes, disease producing bacteria are obligate pathogen, but they often are facultative in nature and produce disease in weak victimised fishes.

Table 1 : Important bacterial pathogens of fishes

Bacteria	Disease/Pathological feature
Aeromonas salmonicida,	Furunculosis
A.salmonicida, atypical strain	Carp erythrodermatitis
A. hydrophila	Haemorrhagic septicaemia, dropsy, skin lesions
Vibrio anguillarum, Vibrio spp.	Vibriosis
Yersinia ruckeri	Enteric red mouth disease
Renibacterium salmoninarum	Bacterial kidney disease
Edwardsiella tarda	Edwardsiellosis (Haemorrhage, septicemia)
E.ictaluri	Enteric septicaemia of channel catfish
Flexibacter columnaris	Columnaris disease
Pseudomonas spp.	Septicemia, haemorrhage, eye disease
Nocardia spp.	Granulomatous lesions
Mycobacterium spp.	Granulomatous lesions
Staphylococcus aureus	Eye disease
Clostridium botulinum	Botulism

i) *Aeromonas salmonicida* infection : *A. salmonicida,* gram-negative non-motile bacteria, cause furunculosis in salmonid and non-salmonid fishes. The disease is essentially a rapidly fatal septicemia or furuncles in chronically infected fishes. Furunculosis has not been reported in India. However, Reddy *et al.* (1994) reported mass mortality of common carp and Tilapia in Kalyani reservoir, Andhra Pradesh, from per-acute septicemia and bacteriosis caused by *A.salmonicida.* Fishes died suddenly and a few moribund fishes showed symptoms of petechiae on lateral surface of abdomen, swollen belly, exophthalmus, and characteristically spongy and oily mass in Tilapia in bucco-pharyngeal cavity. Histologically, there were degenerative and inflammatory changes in gills, kidney, intestine and hepatopancreas, simulating the changes typically found in furunculosis among salmonids.

Aeromonas hydrophila infection : *A. hydrophila* is a common inhabitant of aquatic environment and has been isolated from both unpolluted and polluted freshwaters, natural springs, sewage, estuarine waters and intestinal tract of healthy fish. The organism has also been associated with a wide-range of infections and is a facultative pathogen. Virulence of the strain and environmental stress and injury precipitates disease in aquaculture practice.

ii) Infectious dropsy : Acute infections abdominal dropsy, a condition characterised by an abnormal accumulation of fluid in whole body, especially in abdomen or localized in some organs, occur in Indian major carps, particularly *Catla catla* and *Cirrhinus mrigala* and less so in *Labeo rohita.* The condition is caused by virulent strains of *A.hydrophila.* The infected fishes show loose scale, distended abdomen with reddish fluid accumulation, lethargy,

exophthalmus, swirling movement, muscular degeneration and petechial hemorrhages on the epidermis just under the loose scale. Skin ulceration may occur due to secondary bacterial infection. There may be terminal septicaemia.

iii) Haemorrhagic septicaemia : It is an acute, rapidly fatal septicaemia of Indian major carps and common carp with a few gross symptoms and high mortality rate. Fishes may die with apparently no signs other than some dark patches on the body or the fishes may show lethargy, refuse to feed, crowd at water surface, body colour becomes dark following extensive haemorrhagic necrosis. Blood may ooze through the anal region. Some of the fishes jumps over the water surface in distress and then collapse. The disease is caused by a highly virulent strain of *A. hydrophila.*

iv) Ulcer disease : The ulcerative form of *A. hydrophila* infection, which is a milder form, appears to be not frequently encountered in Indian major carps. The predominant signs studied in Catla are white cutaneons lesions on the snout, loose scales at the base of the fins and mild disintegration of fin margins. Perhpas the infection is secondary to injury and invasion of blood stream does not occur explaining the absence of dropsy, septicaemia and other accompanying signs.

v) Eye disease

Epidemic eye disease affecting the eyes of *Catla catla* is cased by a variant of *Aeromonas liquefaciens.* Initially the infected eye becomes vascularized and subsequently turns milky white and later becomes opaque. The eye ball may either withers-off or the contents become lysed and fall out. From eye the bacteria may spread to brain, leading to death.

Bacterial eye diseases of silver carp and airbreathing fish *Channa marulius* have been described by different workers. Causative agent was identified as *Staphylococcus aureus.*

A number of factors have been identified, which contribute to virulence of a strain of *A.hydrophila*, such as haemolysin, protease, haemagglutinin, amylase, gelatinase, cytotoxin, dermonecrotic factor etc. However, role of these virulence factors in the natural disease process has yet to be elucidated. Many workers claim the predisposing factor of dropsy and some ulcerative syndrome of fish as rhabdovirus infection.

vi) *Pseudomonas* infections : *P. aeruginosa* have been reported to cause mortality in *Clarias batrachus.* Fishes show *echymotic* skin haemorrhages, tissue lysis and fin-rot. In the acute stage deep irregular crater-like lessions appear on the skin besides dorsal and caudal fin-rot. Other *Pseudomonas* spp. have been reported to cause ulcerative skin necrosis and epithelial hyperplasia, ascites in cyprinids, and haemorrhagic septicemia.

vii) Columnaris disease : *Flexibacter columnaris* cauge the so-called columnaris disease in Indian major carps and other carps. Initially raised whitish or greyish plaques are seen over head and back giving very often a shaddle back like appearance. The gills are often necrotic. The disease is found to be stress mediated.

viii) Enteric red mouth (ERM) disease : *Yersinia ruckeri* cause enteric red mouth (ERM) in several trout-and-salmon-rearing areas of the USA, Canada, Australia and other countries. Mandeep Sharma *et al.* (1995) first reported such disease outbreak among rainbow trouts in Himachal Pradesh with 10-40% mortality and comparatively higher morbidity. The fishes were moribund, lethargic, exophthalmic with blackish coloration on ventral side. On pressure thick yellow fluid dribbled out through anus. Petechial haemorrhages were seen on liver, kidney, lateral musculature, lower intestine and occasionally on swim bladder. The spleen was enlarged and friable. Histologically, the changes were congestion and haemorrhages with infiltration of mononuclear cells or with heterophils. It is widely accepted that ERM outbreak occur due to poor environmental condition and stress factors, such as low D.O., high water temperature, poor water quality and handling etc.

Fungal diseases

Diseases caused by fungi have long been recognized in fish, but far less is known about these diseases than those of bacterial or viral origin. Fungi associated with fish diseases belong to a wide range of taxa, the most frequent are the so-called water molds - the Oomycetes, the principal members are *Saprolegnia, Achyla* and *Aphanomyces.* Two important pathogens, *Branchiomyces* and *Ichthyophonus* are still of uncertain taxonomic affinity. Majority of these fungi are at best facultative parasites and attack their host when injured or secondary to other infection. Only *Aphanomyces astaci, Ichthyophonus* and *Trichomaris invadens* are obligate parasites and may cause severe mortalities.

Mycotic infections of fish by freshwater Oomycetes can develop at all stages of the fish's life cycle and are of considerable economic significance. Unless treated, such infections are usually lethal to the fish and extensive zoospore production ensures that infections spread rapidly through a population.

Saprolegnia infections : Nearly every important fish species in India have been reported to be affected by this fungi. Infected eggs become white, darken gradually and finally become black in color and fail to hatch. In post-hatch stages and in adults cotton-like growth appears on fish. These wool-like lesions are normally white in color but may be discolored by the accumulation of debris between the fungal hyphae or as a result of simultaneous bacterial infection. Fishes become lethargic, listless and less responsive to external stimuli. There may be sex difference in fishes in distribution of lesions. Generally fungal growth is restricted to epidermis and dermis and occasionally growth extends to or beyond muscles. Histologically, there are focal areas of cellular necrosis, spongiosis or intercellular edema, and ultimately epidermis sloughs-off. Inflammatory responses are normally absent or weakly developed. Fishes dies from massive osmoregulatory problems caused by destruction of the superficial tissues. The most common pathogens is the *S.parasitica.* Other species of interest are *S.diclina* and *S.ferox.*

The genus *Saprolegnia* is identified by filamentous non-septate hyphae without a conspicuous hold fast organs. Primary and secondary zoospores are motile and different in shape.

Fungal gill rot (*Branchiomycosis*) : *Branchiomyces* is known only as a parasite of gill tissues. *B.sanguinis* has been reported to infect gills of carps; the gill lamillae loose their normal color and turn yellowish brown due to degenerative changes. The infected fishes gasp for air on the

water surface for some time and die soon afterwards. The fungal hyphae are branched and coenocytic with varying thickness, and the fungi reproduce by aplanospore. Taxonomic position of this fungi is unknown.

Other fungal infections

Infection of fishes by *Achyla, Pythium* and *Aphanomyces* have been recorded by many workers from time to time. Srivastava *et al.*, (1994) have reported deep mycosis of *Chela laubuca* Ham. with varying degrees of destruction of epidermis, hypodermis and underlying musculature. Eyes were also affected. The causal watermolds were identified as *Achyla orion, Saprolegnia diclina, S.ferax* and *Pythium aphanidermatum*. Occasionally, some non-aquatic fungi such as *Aspergillus niger* and *Helminthosporium nodulosum* have been related to fish mortality.

Epizootic Ulcerative Syndrome

Epizootic ulcerative syndrom (EUS) is the most dreaded fish disease causing severe and chronic skin ulceration and heavy mortality. Nearly all the fish species, both cultured and wild, are affected. In India the disease was first reported in 1988 in North Eastern states which gradually spreaded nearly all over India. The disease gradually diminished in subsequent years and are presently restricted in some pockets.

The disease is most commonly observed during post-monsoon period and at the time of-fall in water tempeature. The incidence is more in acidic low calcium soil areas. Pesticides, fertilizers and heavy metals are suspected to have some role in EUS outbreak.

Clinically disease commences with inflammatory red spots which gradually ulcerate. Histologically, the changes are mycotic granuloma with cellular infiltrations.

Etiology of EUS is still confusing. Though some rhabdovirus like particles could be found from affected fishes, its pathogenicity could not be proved. Many bacteria such as *Aeromonas hydrophila, Pseudomonas* spp., *Staphylococcus* sp., members of the Enterobacteriaceae group, chemoautotrophic nocardiform bacteria etc. have been isolated from diseased fishes but no one is truely accepted as an etiology. Initially *Achyla* and *Saprolegnia* spp. were identified from affected fishes, but these were later dismissed as secondary agents. Presently, a strain of *Aphanomyces,* now named as *Aphanomyces invaderis,* has been claimed to be the cause of this epizootic syndrome.

Diagnosis of infectious diseases

Classically, diagnosis of infectious diseases is done from natural history, clinical findings, gross-and histopathology and, the most important, isolation and identification of the causative organism. In fish disease diagnosis the same principle applies but with careful consideration. Often, particularly for sporadic outbreaks, natural history is not available. Clinical signs exhibited by affected fishes are a few and often overlapping and confusing. Environmental impact on fish health makes the problem more complex.

The best way of diagnosis is to isolate and identify the causative agent through Koch's postulate. Many bacteria are associated with fishes as normal flora which may come in culture and complicate the isolation and identification of the pathogenic one. For surface lesions such as skin ulcers etc. the process of isolation of pathogenic bacteria is very cumbersome. Pathogenicity testing of an isolate also needs special attention. One isolate may be found virulent by its virulent factors but may not induce the disease when tested in fish. Concentration of bacteria used to induce disease may be critical or particular environmental situation may be required for clinical manifestation of the disease. For example pathogenic strain of *Aeromonas hydrophila* may not induce disease unless fishes are exposed to environmental stress of low D.O. and high stocking density and acute injury. *Saprolegnia* affects fishes only when there are surface abrasions.

Bacteria, are traditionally isolated on different bacteriological media and are identified by their morphology, cultural and biochemical characters, genetic relatedness and production of virulent factors such as toxins and enzymes. Fungi are identified by their morphology and reproduction characteristics. Serodiagnosis of bacterial fish diseases are becoming common with availability of diagnostic antiserum.

Epizootiology and control

Epizootiology is the study of occurence of disease, the spread of pathogen, mode of infection, time of incidence, environmental factors, geographical distribution of the disease and control of the disease in animals other than humans. In a culture system fishes are confined to a restricted environment and as such spread of pathogen increases with increase in susceptible population. In open water system the infectd fish travel over a wide area carrying the pathogen, but as the availability of susceptible population at contact is less the disease spreads slowly but over a large area. In temperate areas the incidence of fish disease is generally seasonal. But this does not hold true in tropical climate. Bacterial diseases tend to predominate as water temperature increases.

There are basically two types of organisms involved in infectious diseases : (i) obligate pathogens such as viruses, *Mycobacterium marinum* and *Renibacterium salmoninarum*, and (ii) facultative or non-obligate pathogens which are able to survive in water but under certain conditions, usually environmentally induced stress, they cause infectious diseases in fish. *Aeromonas hydrophila* is one such well known facultative bacteria.

There is a theory that to have an infectious disease in fish, in addition to a host and pathogen, an unfavourable environmental condition must act as a trigger or stressor for the disease to develop. Often, especially in warmwater fish, potential infectious disease organisms are endemic in the environment and only environmental conditions, and/or the host's natural resistance can dictate to begining of the disease process. Some commonly known stressors related to fish health are insufficient oxygen or high concentration of CO2, high concentrations of unionized ammonia, nitrite and H2S, excess of suspended solids, chronic exposure to low concentrations of pesticides or heavy metals, rapid changing or extremes of pH, extreme or rapidly changing water temperature, insufficient or bad quality food, transportation, rough handling, injury and high stocking density. Heavy metals and pesticides suppress the fish immune system making them more susceptible to infectious diseases. Environmental stress on fish increases geometrically when environmental conditions approach the tolerance limit of the

host. And when more than one stressor is involved detrimental effects are magnified. So, for control of fish diseases and protection of fish health maintenance of a good and wholesome environment is essential.

For prevention and control of fish diseases the following points are to be followed -

i) Keeping the environmental parameters within limits.

ii) Maintain good nutritional quality. Avoid over-feeding or over-manuring.

iii) Avoid over-crowding. If there is over crowding reduce the standing crop, increase water volume, increase aeration, exchange freshwater and limit the quantity of food.

iv) Protect through segregation. Infected fishes must be segregated from the whole stock. Fishes may be segregated and cultured species-wise.

v) Youngs of a species are more susceptible to diseases. Older and wild fishes suffer less but may be carriers. So, at least young fishes should be cultured separately.

vi) Stock your farm from a disease-free farm. Brooders are potential place of infection. So, maintain maximum hygiene in the brooder.

vii) If possible freshwater should be exchanged periodically.

viii) There should not be unauthorized entry of adult and wild fishes from other water areas.

ix) Fishing appliances should be regularly disinfected and dried before use.

x) Fishes should be given bath treatment of disinfectants before stocking. Avoid rough handling.

xi) There should be facilities for early disease diagnosis.

xii) Where vaccine is available against particular disease vaccination schedule should be followed.

xiii) In face of outbreak use chemicals, disinfectants or antibiotics as recommended. Generally, waterbody is treated with 1-5 mg/lit potassium permanganate or bleaching powder @ 1 mg/lit. Calcium oxide is used @ 50-100 kg/ha. Fishes may be treated by bath tratment with potassium permangate @ 500 mg/lit, formalin @ 1000-1500 ul/litre, hydrogen peroxide @ 500-1000 ul/lit, or common salt @ 30000 mg/lit for 15 min. every one or two days alternate. Chemotherapeutics and antibiotics such as oxytetracycline, sulpher drugs, tricaine methansulphonate may be used with feed but with legislative care.

xiv) There should be facilities and legislative measures for quarantine and certification atleast for exotic breeds of fishes.

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Immunodiagnostic methods for microbial fish diseases

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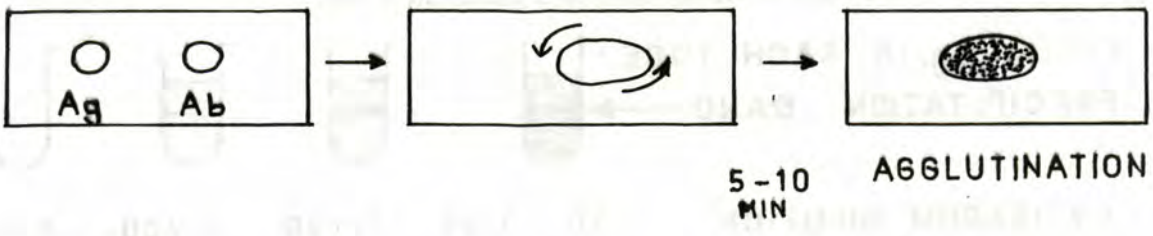
The term immunodiagnosis refers to any diagnostic technique based on detection of either antigen or antibody inside or outside the host system (eg. Fish). The antigen which is defined as a macromolecule (usually a protein of viral or bacterial origin) which can evoke specific immune response inside the body of the host and the antibody which is a globular protein which can neutalize a specific antigen, are the two most important variables of an immune process. Nearly all the immunodiagnostic techniques are based on either the detection of an antigen with antibody of known specificity or detection of an antibody with a known antigen. The antigen and antibody molecules are complementary antibody with a known antigen. The antigen and antibody molecules are complementary to each other when mixed, the reaction being manifested by either precipitation or agglutination etc.

On the basis of the type of reaction which results due to interaction of antigen and antibody immunodiagnostic methods can be of following types-

Agglutination test: The test is based on the visible clumping (agglutination) of a particulate antigen with antibody when the two test reagent are mixed together on a glass slide.



This test is mostly used for diagnosis of bacterial diseases *eg.Edwarsiella tarda*

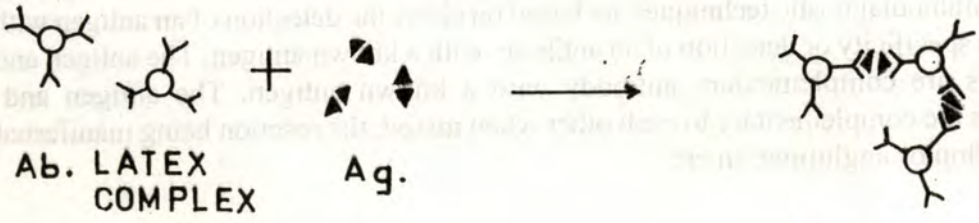
MODIFICATIONS

1. *Quantitative Agglutination Test:* In this method different dilutions of antiserum are allowed to react with a fixed quantity of antigen or *vice versa.* The lowest dilution which shows positive reaction is taken as the titre. For example-

Dilutions of Ab	-	1:10	1:100	1:200	1:300	1:400
Agglutination		+	+	-	-	-

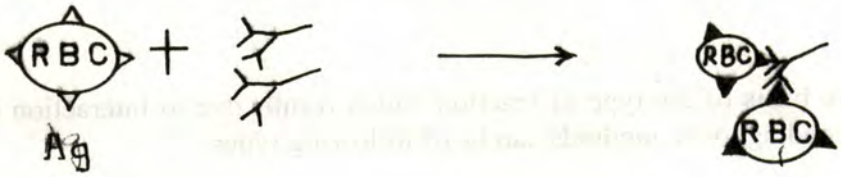
The titre will be 1:200

2. *Latex Agglutination Test:* In this method antigen is adsorbed on latex beads to increase the size of clumps. Such method increases the sensitivity of the test.



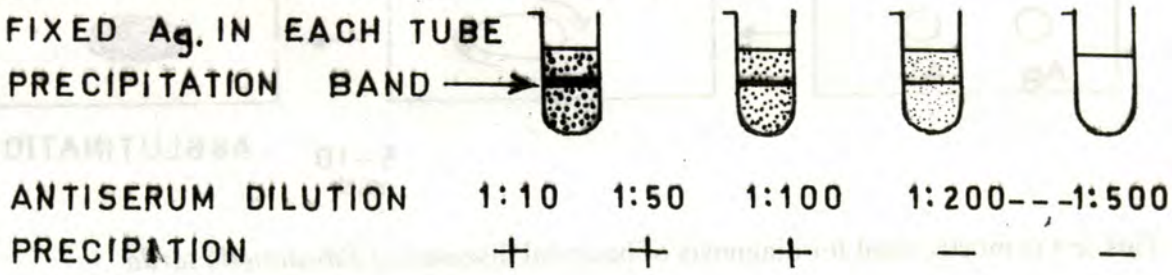
Latex Agglutination

3. *Haemagglutination Test:* In this method either antigen or antibody is adsorbed on RBC surface. There are certain viruses which directly cause agglutination of RBC.



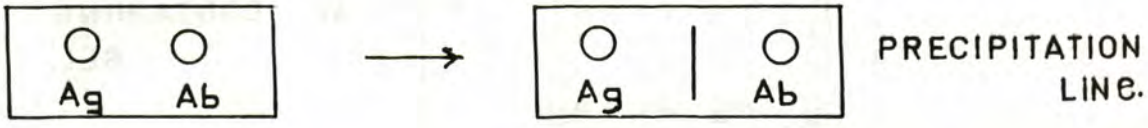
Hacmagglutination

Precipitation Test: The basic principle of this test is as the agglutination tests except that here the antigen is in soluble form and the reaction with antibody results in precipitation. This is generally done in tubes and is mostly used as a quantitative test.



MODIFICATIONS

1. *Agar Gel Precipitation Test (AGPT):* In this method antigen and antibody are allowed to diffuse through agar until they meet to form a precipitation line.



Procedure:

1. Slowly pour 5 ml of 1-2% molten agar (45^{0}C) on a clean glass slide and allow it to solidify.

2. Make two small holes in the agar equidistant from the centre of the slide.

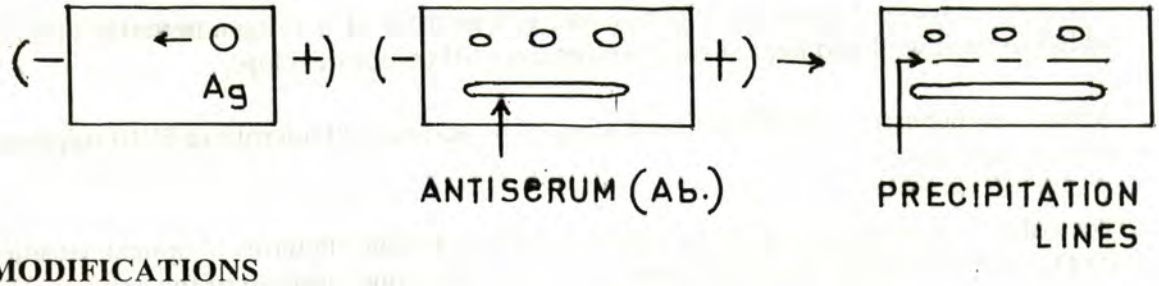
3. Put 50 microlit of the antigen in one well and 50 microlit of antibody in one well

4. Incubate the slide between 25-35^{0}C temperature for 24 hours.

5. A precipitation line between the centre of the two wells indicates positive reaction.

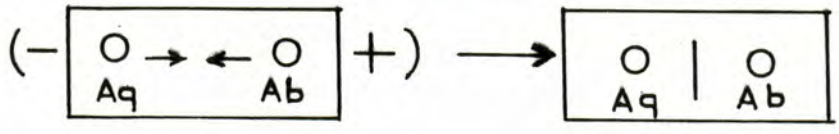
This method can be modified suitably for a quantitative AGPT. For this antigen is kept in the central well and different dilution of the antibody are kept in surrounding wells.

Immunoelectrophoresis: In this method the diffusion of antigen and antibody is enhanced by applying an electric field across the slide.

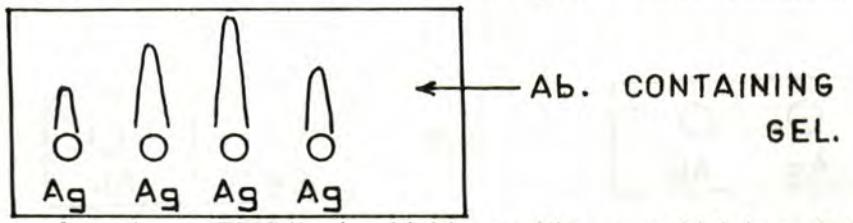


MODIFICATIONS

a) *Counter immunoelectrophoresis test:* is performed in agar gels where pH is chosen so that the antibody is positively charged and the antigen is negatively charged. By applying a voltage across the gel the antigen and antibody move towards each other and precipitate. The principle is the same as the immunodiffusion but the sensitivity is increased 10-20 fold.



b) *Rocket immunoelectrophoresis:* In this method the antigen is quantitated by electrophoresing them into an antibody containing gel. The pH is chosen so that the antibodies are immobile and the antigen is negatively charged. Precipitin rockets form the height of the rocket is proportional to antigen concentration, and unknowns are determined by interpolation from standards.



Enzyme Linked Immunosorbent Assay (ELISA): is a highly sensitive test which is probably the most widely used of all immunological assays since a large number of test can be performed in a relatively short time. The test is based on the recognition of antigen by antibody-enzyme conjugate.

Procedure

a) Different dilution of antigen (50 microdit) are added to the 96 well ELISA plates. The plate is incubated at 37 ^{0}C for one hour.

b) The antigen solution is discarded and the plate is blocked by 1% BSA solution

c) The plate is washed by phosphate buffered solution with Tween-20 (PBS-T) and antibody-enzyme conjugate is added to each well and incubated for 30 minutes.

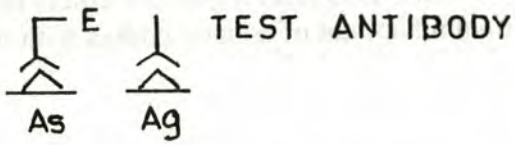
d) The plate is washed again with PBS-T and 100 microlit of hydrogen peroxide (1%) is added to each well and kept at rook temperature till colour develops.

e) After 30 minutes the colour reaction is stopped by adding 100 microlit of N/10 sulphuric acid in each well.

f) The colour intensities are read by ELISA reader to get values in terms of optical densities (O.D.). The OD values are directly proportional to the concentration of the antigen. The usual practice is to plot a standard curve with a set of known concentrations of a specific antigen and then extrapolate the unknown samples.

MODIFICATIONS

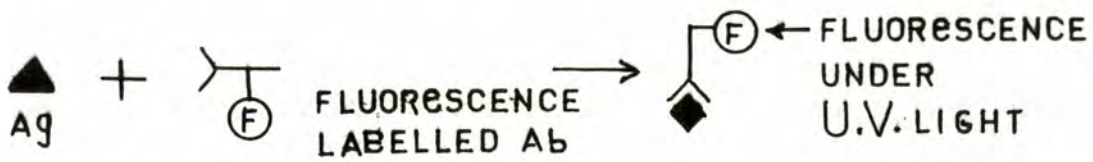
1) *Competitive ELISA:* Here, the unknown samples compete with a known sample for combining with the antigen.



2) *Sandwich ELISA:* The antigen binds with antibody (non conjugated) which is pre coated on the plate.

3) *Dot ELISA:* Here, nitrocellulose membrane is used as a base for reaction instead of plastic plates. The colour reaction appears in the form of a dot on the membrane.

Fluorescent Antibody Technique: This technique uses fluorescin dye tagged antibody to detect specific pathogen inside the tissue itself. The fish tissue suspected to contain a particular pathogen is processed to make sections for microscope. This is then treated with antibody-fluorescin conjugate and the slide is examined under the ultra violet light microscope. The part of the tissue which contain the pathogen are seen as greenish yellow coloured spots. This can be used to detect a particular microbial pathogen as well as to know the part of tissue being attacked or damaged by the pathogen.



Ag Fluoresin Fluorscence under U.V. light

CONCLUSION

The immunodiagnostics have great prospects in fish disease diagnosis since they can specifically detect different microbial diseases with great sensitivity. The advantage of these methods over other conventional tests is that they can differentiate between closely related strains of same species of bacteria or virus. This is an important aspect in case of fish pathogen since many of the bacteria are normal inhabitant of aquatic bodies with only some strains being pathogenic to the fish.

Protozoan diseases of Fish - Diagnosis and control

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Protozoans constitute one of the most important group of animal parasites affecting fish. Most of the organ systems are infected by these parasites and majority of the groups of protozoan parasites can cause mortality in fish. The three main phyla responsible for various protozoan diseases of fish and prawn are the Ciliophora, Sarcomastigophora and Myxozoa

Taxonomy of protozoan parasites afflicting fish (Fig. 1)

Common protozoan diseases

Ichthyophthiriasis

Species affected: Fry and fingerlings of *C. catla*, *L. rohita* and *C. mrigala* in nursery and rearing ponds.

External symptoms : Minute white spots nodular in form and in size are visible on the skin, fins and gills. Affected fishes show irritation, erratic movement and restlessness with tendency to rub on the sides.

Effect on host: Epidermal tissue changes occur in the area where the parasite is lodged and its dislodging brings about formation of epithelial ulcers.

Causative agent: Ichthyopthirius multifilis. Fingerlings of *L. bata* and C. *mrigala* have been experimentally infected with this parasite.

Morphology and life history : The mature trophonts body is circular to ovoid, measuring 60-800 mm in size. It is covered with 36 to 48 meridonial rows of cilia. The mouth or cystosoma is present anteriorly at the bottom of a vestibular depression. Macronucleus present is large and horseshoe shaped.

Life cycle : The infective stage of the host is the migratory thereont, which infests fish skin or gills. Once inside, it starts feeding and growing and this stage is called trophont. The trophont on reaching the size of 1 mm escapes from the host and encyst on a convenient substrate outside as tomont. Within the cyst the tomont divides by a series of 10 to 11 divisions to produce small tomites, which break through the cyst wall to become thereonts again. The thereont is a bit elongated 25-70 x 15-22 mm in size covered by 36 to 48 meridonial cilia. They remain infective upto four days. Once the thereont become lodged in the skin, the life cycle is completed .

Treatment : The control of this infestation is to be concentrated on the life stages outside dermal tissue of the host. The stage of *I. multifilis* within the dermal tissue of the host fishes are almost impossible to remove using chemotherapy without injuring the host.

Therapeutic : (i) Hourly bath in 1 : 5,000 formalin solution for 7 days.

(ii) 2% Sodium chloride solution for 7 days or more.

Trichodiniasis

Species affected : Fry, fingerlings and adults of *C. catla, L. rohita, C. mrigala, C. idella, C. carpio, H. molitrix, T. mossambica* and other cultured carps.

External symptoms : Colour of the gills turn pale and there is a creamish coating due to excessive mucus secretion. Heavily infested fishes gradually become sluggish and loose weight, and very often, there is asphyxia.

Effect on host: Hyperplasia and hypertrophy of the gill filament occur with proliferation of mucous cells. The respiratory function of the gill lamellae is hampered, often resulting in asphyxia.

Causative agent: Urceolariid ciliates, *Trichodina reticulata, Tripartiella bulbosa, T. obtusa* and *T. copiosa.*

Key to genera of trichodinids occurring in fish

1. a) The adoral spiral makes one turn or slightly less or more (300° to 540°)..... 2.
   b) The adoral spiral makes one half to three quarters of turn (150° to 290°).....3

2. The denticles have well developed thorns and blades .....*Trichodina.*

3. a) The denticles have well developed thorns 4
   b) The thorns stunned to form short crooks or platelets .....*Trichodinella.*

4. a) The blades are attached to the central part almost perpendicularly and the denticles are interlocked only by their central conical parts..... *Paratrichodina.*

   b) Blades extend from the central part obliquely backwards, denticles are interlocked by central parts and by anterior projections of blades fitting into corresponding notches in the blades of the preceding denticles .....*Tripartiella.*

Morphology: The shape of the body is grossly hemispherical varying from a flat disc to a bell shaped one. It is concave on its aboral surface. It attaches to the host surface by means of the adhesive disc constituted by skeletal elements. The disc consist of a ring of denticles which has a central part and centrifugal and centripetal projections called blades and thorns respectively. The denticles are held to one another by inserted conical parts and subtended by a ring of fine skeletal rods, called radial pins. The disc is encircled by a movable border membrane reinforced by fine skeletal rays. The border membrane seals off the attachment disc, helping to maintain

the suction that temporarily attaches the ciliate to the host surface. The ring of denticles and radial pins located above the aboral, pellicular surface provide rigidity to the cells allowing elevation of the centre of the attachment disc via a complex system of myofibrils. Apically, above the border membrane there is a ciliary sheath of a single row of cilia. These are covered apically by the velum. The non-contractile oral surface lacks a separate epistomial disc.

Life cycle: The urceolariid ciliates reproduce by binary fission. The adhesive disc separates in two semi circles which then close to form two smaller discs in daughter individuals.

Treatment

- Prophylactic: The presence of these ciliates indicate deteriorating water quality thus the measures to be adopted are:
  (i) Water quality should be improved.
  (ii) Stocking density should not be high

- *Therapeutic*:

  (i) Sodium chloride bath treatment @ to 2-3% till the fishes are stressed.
  (ii) Potassium permanganate treatment @ 4 mgl^{-1} in pond.
  (iii) Formalin treatment @ 25 mgl^{-1} in pond
  iv) Formalin bath treatment @ 100 mgl^{-1} with aeration.

External fouling in prawns

Species affected: *Macrobrachium rosenbergii* and *Penaeus monodon.*

External symptom: The affected prawns are often seen moving on the sides of the pond in a lethargic condition. The appearance of the infested prawn depends not only on the causative agents, mostly sessile protozoans but also on the additional debris which become entangled in it.

Effect on host: The infestation hampers movement and respiration. Prawns have an increased oxygen demand just prior to moulting and heavy fouling can be associated with mortality due to anoxia.

Causative agent: Epistylis sp. *Vorticella* sp. and *Zoothamnium* sp.

Key to genera of Sessilina

1. Ciliates attached by means of the secreted stalk.

2. a) A non contractile stalk is branched, bearing a small colony of several zooids ..... *Epistylis.*

   b) The stalk is contractile, unbranched, bearing a single zooid or branched, bearing colonies of many zoodis .....*Vorticella, Zoothamnium*

Treatment

Prophylactic: The presence of these ciliates indicate poor water quality. As such the measures taken are to improve water quality and to encourage prawn more active and moult regularly.

Therapeutic :

(i) Formalin treatment @ 20-30 mgl^{-1} in the pond preferably with aeration.
(ii) Formalin bath treatment for larval infestation @ 100 mgl^{-1} with aeration.

White gill spot disease

Species affected: *Catla catla, C. mrigala* and *L. rohita.*

External symptoms: Gills of infested fishes are covered with whitish cysts of different sizes ranging between 1 mm to 4 mm or more. In acute cases some of the cysts assume a cauliflower shape, blocking the entire respiratory surface. Excessive mucus secretion occur and the fishes surface for gulping air.

Effect on host: The absorptive surface on gill is reduced hindering normal respiration. In heavy infestation hyperplasia of gill lamellae occur and under oxygen depleted condition these fishes die first.

Causative agent: Thelohanellus catlae Chakravarty & Basu, 1948, *Myxobolus bengalensis, M. catlae Seenappa & Manohar, 1981, M. hosadurgensis.*

Key to myxosporean genera afflicting fish

1. a) Spore pyriform, ovoid or spherical without any posterior process 2

   b) Spore with posterior process.....3

2. a) Only one polar capsule present at the anterior end parallel to longitudinal axis of spore.....*Thelohanellus*.

   b) One polar capsule present at the enterior end perpendicular to longitudinal axis of spore .....*Neothelohanellus.*

   c) Two polar capsules present .....*Myxobolus.*

3. Two polar capsules present .....*Henneguya.*

4. Fourpolarcapsules present.....*Kudoa.*

General morphology of a myxozoan: The infective stage of the myxozoan diseases is the mature myxozoan spore. The spore structure form the basis for identification of different species of myxozoan parasites. The myxozoan spore is of various size and structure but its basic structure is consistent. The spore wall is formed of two valves. The wall encloses in the anterior end polar capsules either one or more. The polar capsules open outside by an aperture separated by inter capsular structure. Spirally coiled polar filament is present which gets ejected out under specific stimuli. The posterior part of the spore contains the sporoplasma with two nuclei.

Life history: The mature spore is ingested by the fish from the water body. On entering the fish the polar filament is ejected and it serves as an anchor. The infective sporoplasma of the spore comes out as a small amoebula and penetrate the gut wall. The amoebula somehow, possibly through the blood stream reaches the infective place or tissue of the host fish. Here it becomes a trophozoite and increases in size with repeated nuclear division and cytoplasmic growth, to form a large cyst. Growth of the trophozoite is accompanied with the sporogenic differentiation. Certain cells (sporonts) become differentiated from the syncytial mass. The nucleus of each sporont divides several times to form the sporoblast. From the sporoblast the mature spores develop.

Treatment :

- Prophylactic : Decrease the density of fishes in ponds.
- Therapeutic :

(i) Treat the pond with mahua oil cake and lime by which the infective spores are destroyed to a great extent.
(ii) Sodium chloride bath treatment @ 3.5% destroy the spores and other developing stages, if present, but not the cysts.

White scale spot disease

Species affected: *C. mrigala* and *L. rohita.*

External symptoms: The scales and the body surface are covered with whitish cysts. In *C. mrigala* the cysts are superficially located on the body surface and scale whereas in *L. rohita* the cysts are situated superficially as well as inside the scales and are released on cutting open the scales. Affected fishes are lethargic.

Effect on host: As the disease advances the scales become loose and perforated in many cases and fall off. There is development of ulcers in such areas.

Causative agent: *Myxobolus mrigale* and *M. sphericum, C. mrigala* and *Myxobolus rohitae, L. rohit.*

Treatment: Same as in white gill spot disease

Suggested reading :

Das, M.K. and Das, R.K., 1997. Fish and Prawn diseases in India - diagnosis and control. *Inland Society of India, India.*

Helminth diseases of fish diagnosis and therapy

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The success of the implementation of various fishery development programmes depend to a certain extent on the intestification of the fish parasitological research, as the improvement of fish yield can mainly be achieved from healthy fish stock. Members of many phyla are parasitic on fishes and impair their health often resulting in mass mortality. Among helminth worms of the groups Monogenea, Digenea and cestoda parasitize various fish species causing their growth retardation and mortality.

Taxonomy of helminth parasites affecting fishes

Key to the classes of Helminth

1. Body dorsoventrally flattened. Attachment organ, the haptor located at the posterior end, with varying number of median and mariginal hooks.
   Ectoparasitees..... Monogenea.....1

2. Body slender ribbon like, segmented. Attachment organ situated anteriorly. Digestive canal absent..... Cestoda.

3. Body unsegmented, dorsoventrally flattened. Two attachment organs. One situted near tip of the anterior end and the other between the middle and posterior tip. Digestive canal present.....1 Trematoda.

Common Helminth diseases

Monogenetic trematode diseases

Typical monogenea is bilaterally symmetrical and body is dorsoventrally flattened (Fig. 1). The most important structure is the haptor which is shallow concave organ at the body's posterior extremity. It is armed with chitinoid structures important for attachment. The anterior end can be rounded or it can be sub-divided into two more more lobes. In some monogenea instead of marginal hooks the opisthaptor is equipped with clamps, and some have auxillary attachment organs known as squamodisc.

Gyrodactylosis (skin fluke, Gyrodactylus sp.)

Morphology: Body enlongated, small with bifid anterior, opisthaptor with 16 marginal hooks and pair of anchor connected by an dorsal and one ventral bar usually with membranous posterior outgrowth. Eye absent, very short oesophagus, intestinal caeca end blindly. Genital pore sub-median, posterior to pharynx. Vagina absent. Uterus containing single embryo which in turn contains embryo of the following generations :

Dactylogyrosis (Gill fluke, *Dactylogyrus* sp.)

Morphology: Opisthaptor with 14 marginal hooks, two of them away from the rim and close to the anchor. Later has barbs pointing in dorsal direction, with one or two connecting bars. Two pairs of eyes, sometimes consisting of loosely grouped pigment grannules. Usually has four head lobes. Intestinal caeca fused posteriorly.

Life cycle: Monogeneans have direct life cycle involving only one host (fig. 2). Most are oviparus, depositing eggs, which on hatching release a ciliated free swimming larva. On locating the host it becomes attached to it and metamorphosis into a mature worn. *Gyrodactylus* sp. is viviparus and give birth to live worm with already well developed reproductive system. These young ones tend to become attach immediately on birth thus building up large population.

Diagnostic characters

Gyrodactylosis

- Colour of the infected fish begins to fade.
- In heavy infection dull bluish film develop on the body of the fish
- Excessive mucus secretion and dropping of scales.

Dactylogyrosis

- Colour of gills of the infected fish fades.
- Excessive mucus
- In heavy infection gill covering stretched wide open, while the gills are expanded and very pale.

In general there is a growth reduction in afflicted fishes for both the disease.

Species affected: Mostly fry and fingerlings of *C. catla, L. rohita* and *C. mrigala* in nursery and rearing ponds.

Effect on host

- Dactylogyrosis destroy the gill tissue and cause the fish to suffocate
- Hyperplasia of the gill epithelium, gill tissue erosion
- Skin of Gyrodactylosis become more slimy and shows small blood spot.

Therapy

- Bath in 5% Nacl for 5 minutes kill the worm on fish/or
- formalin bath treatment @ 100 mgl^{-1} kills the worm
- Formalin treatment in pond @ 25 mgl^{-1} is effective in controlling the worms/or
- Potassium permanganate in pond @ 4 mgl^{-1} effective in controlling the worms.

Digenetic trematode disease

Key to the families of Trematoda with larval stage in fish

1. Larval forms, metacercariae encysted in different organs.

2. Cuticle armed with slender spines, metacercaria usually less than 1 mm long ..... Clinostomidae.

Digenetic trematoda have complex life cycle with several successive larval stages alternating sexual and asexual generations involving two or more hosts.

Morphology: A typical adult diagenean has an unsegmented dorsoventrally flattened body (fig. 3). The shape varies from oval to lanceolate. At the anterior end there is a oral sucker. A ventral sucker or acetabulum is located more towards the posterior end. This adult form may be present in fish. In black spot disease the form that is present in fish is the larval metacercaria.

Black Spot Diseae (Metacercaria larva of *Diplostomum* sp.): A typical metecercaria is bilobed more or less bilaterally symmetrical. The oral sucker is anteriorly located. The digestive tract consists of an elongated pharynx, a slender oesophagus and intestinal caeca extending towards the posterior end. Accessory suckers are located laterally. Acetabulum is situated at the area of bifurcation of oesophagus and a holdfast organ is placed behind the acetabulum. The rudiment of genital organs, ovary and testicles are placed on the portion of the body. The anterior and posterior portion of the body is demarcated by a contradiction (fig. 4).

Life Cycle: The digenean (*Diplostomum* sp.) are oviparous and release eggs into the water and hatches out in a free swimming miracidium. Miracidium penetrates the skin of the molluscs, the first intermediate host. The miracidium penetrates the host digestive tract and migrates in blood system and enters hepatopancreas. In some species the eggs are swallowed by the host and miracidium hatches out in the alimentary that and migrate into the hepatopanereas. Miracidium becomes the next stage larva sporocyst, then into redia and finally into tail bearing cercaria by asexual reproduction which escapes from the snail host and becomes free swimming. When cercaria comes in contact with second host, usually an arthopod, they penetrate the host body and encyst. The life cycle is completed when the infected invertebrate is eaten by a suitable fish which act as a final host (fig. 5).

Diagnostic Characters

- Presence of black cysts containing metacercaric stages on the body of the fish.

Effect on host

- Growth reduction in the affected fish without mortality
- High mortality in the highly affected fishes may be due to the movement of the parasite within the tissue of the host/toxins liberated by the parasite.

Species affected

Fry and fingerlings of *C. catla, L. rohita, C. mrigala* and *H. Molitrix* in nursery and rearing ponds and *C. catla* in reservoir and lakes.

Therapy

Removal of resident molluscan population (first ntermediate host) in the affected water areas and the aquatic birds (final host) around it.

Cestode disease

Ligulosis: Ligula intestinalis (Plerocercoid larva)

Cestodes or tapeworms are one of the most common parasite of fish. Although a large number of cestode species parasitize fish, the most common still remains the *Ligula intestinalis.* The life cycle of the parasite is complicated involving three different hosts.

Key to orders of avian cestode

1. Scolex with two superficial bothria, vitelaria follicular and diffuse..... pseudophyllidea

2. Scolex with 4 suckers, vitellaria compact and unpaired .....Cyclophyllidea

3. Scolex with or without suckers, testes and ovary without ducts, no isolated vitelline gland.....Aporidea

Key to families of the order Pseudophyllidea

1. Genital and uterine apertures ventral, eggs operculated ..... Diphyllobothriidae
   Genital apertures dorsal, uterine aperture ventral.....2.

2. Scolex with deep slit like bothria the margins of which are inrolled, eggs not operculate..... Ptychobothriidae.

Key to genera of Diphyllobothriidae

1. Each proglottis with two sets of reproductive organs..... *Diagramma.*
   Each proglottis with one set of reproductive organs.....2

2. Scolex small, Triangular, with bothria weakly developed.....3
   Scolex broadly oval, bothria well developed.....

3. Segmentation confined to anterior portion of strobilla, proglottids not craspedote.....*Ligula.*

Morphology: A typical adult worm (*Ligula* sp.) lives in the intestine of aquatic bird has a elongated dorsoventrally flattened body. The scolex is triangular, small and pointed. The bothria or attachment organ is represented by dorsoventral groove. The neck is absent and the following body portion is the strobila with external segmentation confined to the anterior portion. The rest of the body portion is unsegmented and transversely wrinkled.

Life Cycle

A typical avian cestode life cycle represented by *Ligula* sp. commences with eggs passed out into the water with the excrement of the bird. They hatch out in free swimming corcacidia larvae which are swallowed by cyclopoid copepods. Here they are transformed into procercoid larvae. These copepods when eaten by fish the larvae transfroms into the infective larval form the plerocercoids. The life cycle is completed when eaten by bird (fig. 6).

Diagnostic Characters

- Dark colouraton, swollen abdomen and erratic swimming.
- Emaciation, anaemia and reduced growth.

Effect on host

- Abdomen of affected fishes gets distended because while growing fill up almost totally the body cavity of fish.
- In acute cases because of increased physical pressure, the abdomen gets burst open.

Species affected

L. rohita, C. catla and *L. calbasu* in the lakes and reservoirs.

Therapy

Control icthyophagus birds which are the definitive host.

Acanthocephalan Disease *(Pallisentis* sp., *Acanthogyrus acanthogyrus* and *Acanthogyrus antispinus)*

These are mostly cylindrical worms armed with an anterior retractile proboscis carrying hooks. There is no gut and sexes are separate. At least one intermediate host is required in the life cycle.

Key to genera of Acanthocephala commonly afflicting fishes in India

1. Proboscis short, cylindrical to subglobular with Three transverse rows of 6 hooks each ..... *Acanthosentis*
2. Proboscis short subglobular to cylindrical with 8-10 spiral of 4-6 hooks each ..... *Pallisentis*
3. Proboscis subglobular with 6 spiral rows of 3 hooks each ..... *Acanthogyrus*

Morphology

The body of the adult worm shows three externally recognizable regions: the proboscis, the neck and the trunk. The proboscis is a hollow, subglobular or cylindrical structure always armed with a set of posteriorly pointing hooks whose number, size and arrangement are of taxonomic importance. It is retractable being provided with the muscles that invert into a sheath or receptacle. Sheath walls are muscular, their contraction everting the proboscis. The proboscis functions to anchor the worm in place more or less permanantly, by penetrating the host's intestinal wall.

The neck, a short section of the body directly behind the proboscis, is also retractable. It's length varies and it's girth often expands posteriorly, it is never armed. It is deliniated from the trunk by a circular ring.

The trunk is a sac like structure, straight or curving, subcylindrical or bilaterally flattened, usually with many transverse wrinkles or pseudosegmentation. It's wall has a syncytial structure and contain scattered nuclei. In some acanthocephan these nuclei are whole and their number constant, in others they break up into numerous fragments during early development. Trunk wall is transverse by a system of ducts, the lacunar canals, whose arrangement is of taxonomic importance. The trunk is exterior can be unarmed or can carry various hooks (fig. 7).

Life Cycle

The acanthocephala requires an invertebrate host for the completion of their life cycle. Eggs passed in the faeces of the final host contain an acanthor larva. The eggs are eaten by a suitable host normally an arthopod where the acanthor larva hatch out and penetrate into the host body cavity. Here it develops to form a cystacanth which if eaten by a suitable final host, develops into a mature parasite (fig. 8).

Diagnostic Characters

- There is no visible external symptom of diagnostic nature.

Effct on fish

- These parasite inhabit the alimentary tract of the fishes attaching themselves by their proboscis. Tissue at the site of attachment gets swollen and reddish in colour.

Species affected

C. catla, L. rohita, C. mrigala and *M. gulio*

Therapy: None.

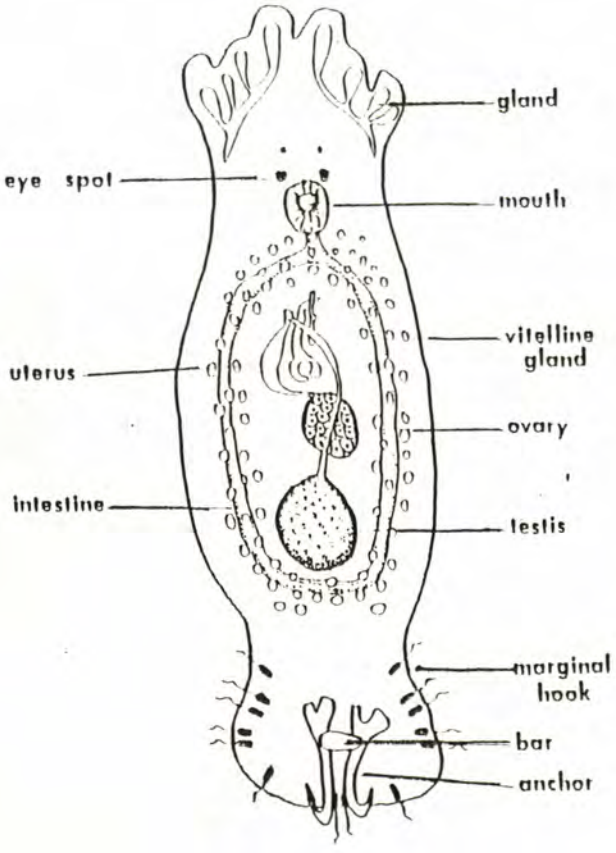

Fig. 1

GENERAL MORPHOLOGY OF MONOGENEA

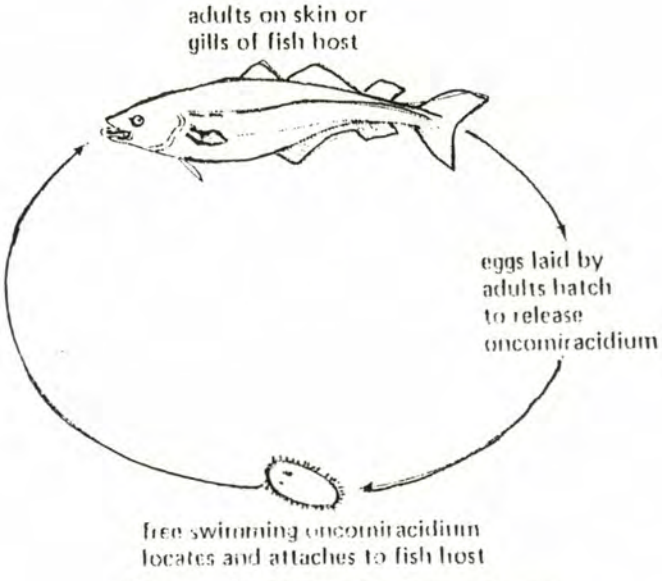

Fig. 2. The life-cycle of monogeneans.

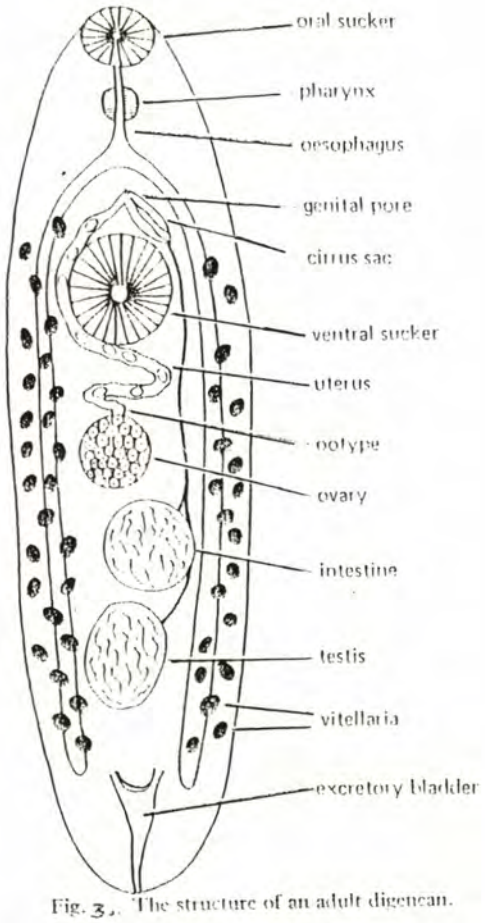

Fig. 3. The structure of an adult digenean.

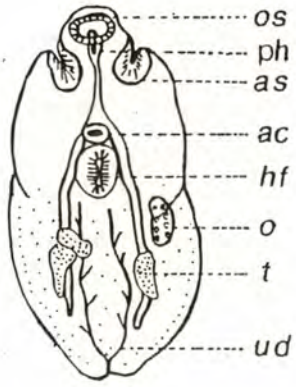

Fig. 4. Morphology of a metacercaria of *Diplostomum* sp.
Abbreviations: ac= acetabulum; as = accessory sucker;
hf = hold fast organ; o = ovary; os = oral sucker; ph = pharynx
t = testes; ud = uterine duct.

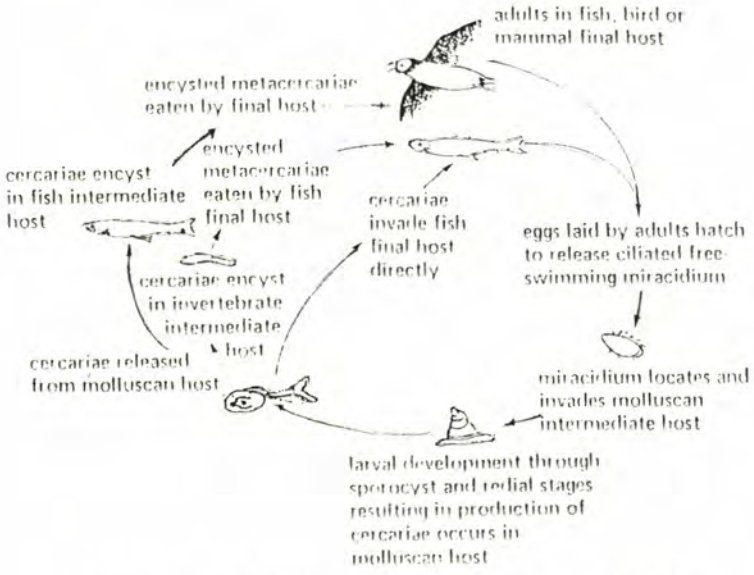

Fig. 5. Possible life-cycle patterns of digeneans parasitizing fish.

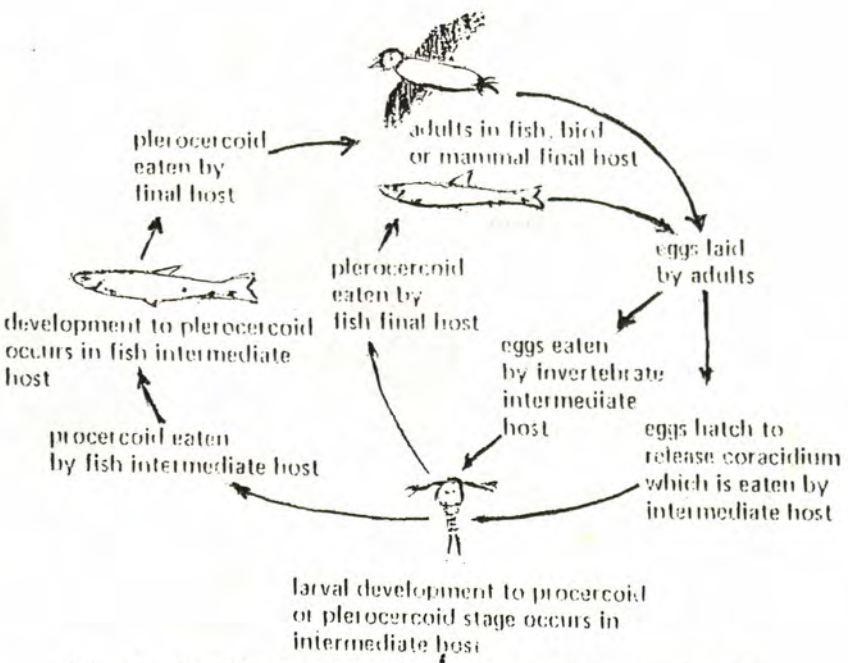

Fig. 6. Possible life-cycle patterns of cestodes parasitizing fish.

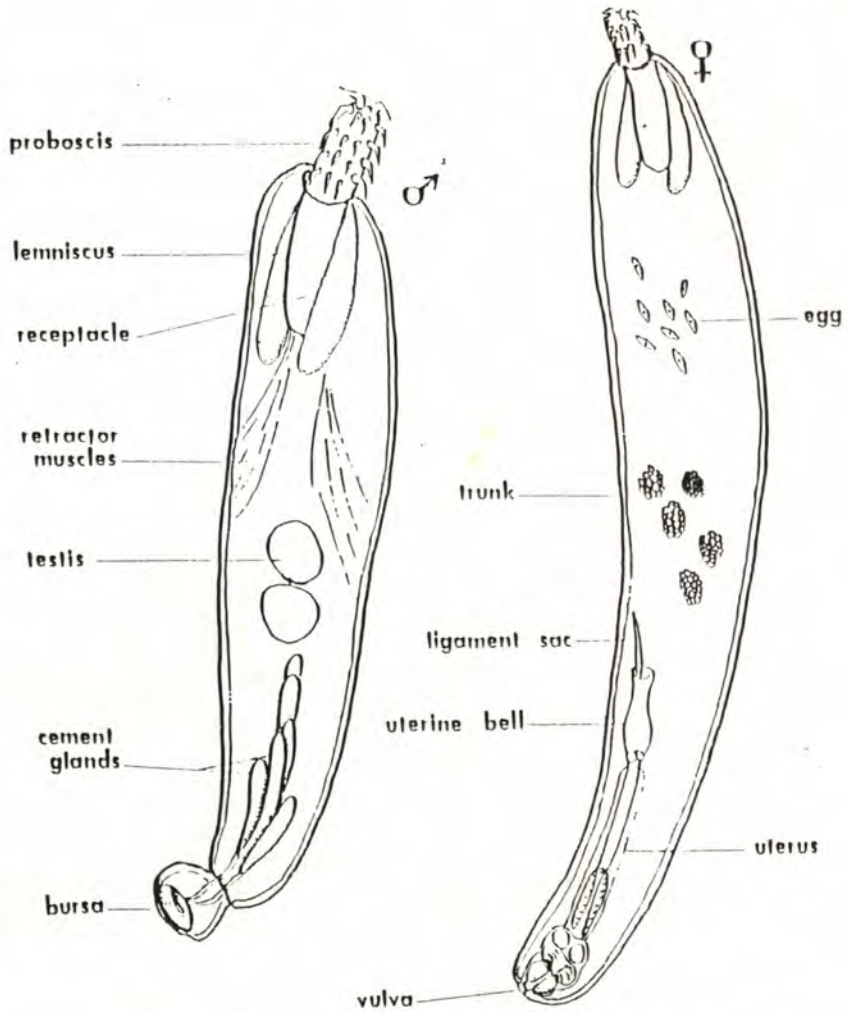

Fig. 7.
GENERAL MORPHOLOGY OF ACANTHOCEPHALA

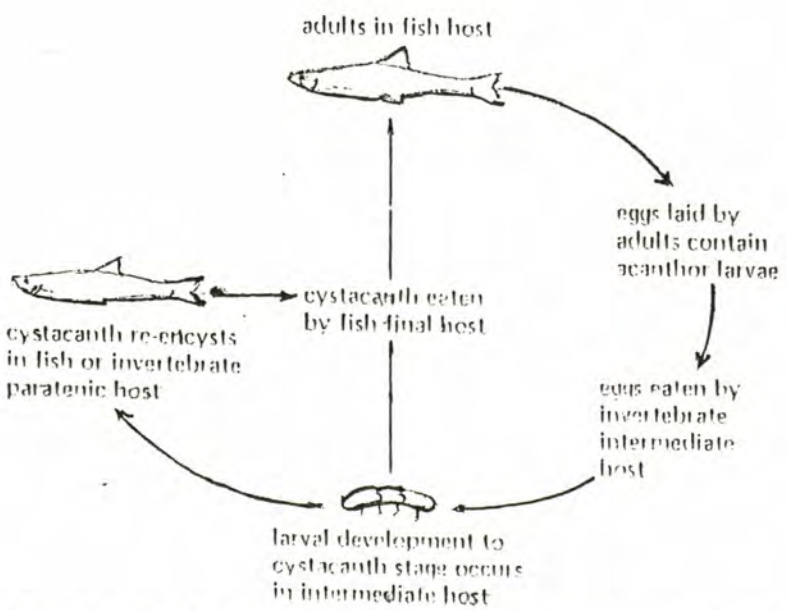

Fig. 8. Possible life-cycle patterns of acanthocephalans parasitizing fish.

Crustacean diseases of fish - diagnosis and therapy

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In recent years, importance has been diverted towards fish capture and culture in many States of India and West Bengal in particular. In some areas it is developing as an industry and thus playing a significant role in integrated rural development. It is further believed that still there exist immense possibility for expanding culture fisheries in the world as a whole. In India fish production in initial phase was restricted wholly to 'hunting' while 'culture of fish' in the true sense has evolved during recent times.

With the intensification of fish farming, where usually very high stocking density under artificial conditions is applied to increase the productivity, the prevalence of parasites and diseases increases accordingly.

Further, the transportation of brood fish as well as fish seed from one place to another is getting more frequent, which then increases the possibility of possibility of the production or spreading of fish parasites and diseases as well. Environmental conditions especially the water plays an important role. As the water now a days is getting more and more heavily polluted with organic matter, it may cause stress to the fish and favours the growth and spreading of parasites. Fish are naturally gifted to acclimate themselves with the changing environment but that too has a limit. Because the respiratory oxygen level in water compared to air, is limited and can become lethal to fish ultimately leading the fish to stress condition. A stressed fish exhibits its uneasiness by its actions such as restlessness, rubbing its body against the pond dykes, splashing water, surfacing and gulping air, whirling on the surface, loss of appetite, non acceptance of food, sluggish movement, vertical hanging etc.

Like all living beings, fishes may always enjoy good health, but with vigilant observation, proper knowledge and adequate care, the danger to fish can be reduced to a minimum. Half of the problem is solved by starting treatment. Often the fish producer observes the results of an infection rather than the organism that causes it. The visible characteristics are signs like a sick fins dropping, and stays at the same place while making wriggling movement as if it is shivering. In many cases, the skin shows cloudiness, patchiness or spots, and the fish may look frayed or bloody which aid in the identification of the infective pathogen. Although they are not positive proof that a certain pathogen is present.

Both fish and pathogens maintain an equilibrium during their existence in a common environment. But sudden change in environment can break this condition when either fish or pathogen gets an upperhand. If the environment is more congenial for fish then it grows better, when there is reverse then the pathogen cause harm to the fish making it ill. This may be subclinical or in clinical level. Most of the chronic cases remain in subclinical stages for a considerable period whereas acute cases are easy to detect by clinical signs. Generally, the fish is having defensive mechanism against its pathogen. The body of the fish is covered with scales

and its skin secretes lot of mucus to drive away its pathogens when it is being attacked by them. The immune system including phage cells of host also acts against its pathogens. Even then fishes are affected with several parasitic diseases particularly by very common Crustacean paracites described below.

Ecological conditions, such as long periods of temperate climate and high organic matter levels in the pond water due to intensive fertilization and feeding, form an excellent basis for increased repetition of life cycles and intensive spread of many external crustacean parasites. Because of heavy infestations many gill, fin or skin parasites, universally known to be less harmful to fish, may become pathogenic in such conditions. The increased density of the fish population in the commercial ponds increases the likelihood of epidemic outbreaks. The external parasites present a specially severe problem because of consumer sensitivity to fish infested with external parasites on gills, fins or skin. Due to stringent specifications, fish infested with larger parasites of crustacean group which can be recognised without magnification are practically unsalaeble. Because of these factors, great efforts and many investigations have been undertaken involving control of external parasites.

Among the many crustaceans which parasitize fish, only certain copepods and isopods cause sufficient damage to the host to be considered as seriously pathogenic, and hence to cause Disease in the sense that is used in this text. Principally because of their relative conspicuosness, the parasitic crustaceans have been recorded frequently; an extensive literature has accumulated. In contrast to almost all other arthropods, the crustaceans (except for terrestrial isopods) are aquatic animals with breathing organs such as gills.

Crustacean parasitic diseases

Morphologically, the structural plan of various groups of crustaceans varies considerably. However, the modifications fundamentally are based on the general structural type, especially among several parasitic forms.

The body typically consists of three parts: the head, the thorax and the posterior region. Only the head and thorax bear the typical biramous appendages characteristics of crustaceans, but variously modified into slender legs or foliaoceous limbs. The first two pairs of cephalic appendages constitute the antennae and the next three pairs constitute oral appendages, the mandibles and the two pairs of maxillae. The respiratory organs comprise processes of the appendages, the inner surface of the carapace and rarely the abdominal appendages. The alimentary canal is characterized by the formation of extensive pouch- like outgrowths of mesenteron, especially in the case of parasitic species. Excretion takes place by specially differentiated cell types and specific glands. Sensory hairs, filaments or setae serve as chemoreceptors and as feeler organs to sense waves of flow and pressure. Two kinds of eyes are present, the unpaired median or frontal eye or paired compound eyes. In most crustaceans sexes are separate. However, some parasitic and sedentary cirripedes and parasitic isopods are hermaphrodites. Parthenogenetic reproduction is also common. While mostly there are only slight diffrences between sexes, cases of extreme sexual dimorphism exist among many parasitic forms. The females often grow to an enormous size during their parasitic life or change their form in such a manner that they can hardly be recognised as crustaceans. On the other hand, the male members mostly retain the typical characteristics of crustaceans and often remain small like larva and can, in some forms, become puny dwarf males.

Embryonal development can take place in the following two forms:

(I) Epimorphosis:- In the case of epimorphic development, the complete development takes place in the egg. On hatching the young animals have all the segments and are essentially no different from the adults; only increase in size and the development of the sexual organs take place in the post-embryonal development. Marked post-embryonal transformations are observed only in those species which either change to parasitism after a long free-living period or those which first live as parasites and, after attaining maturity, are free-living, consuming their reserves. The metamorphosis in adaptation to the respective mode of living is often associated with reduction of the various organs.

(ii) Anamorphosis:- In anamorphosis development, the egg hatches into a larva with a smaller number of segments. Only in the post -embryonal phases, the segments increase to the number which is typical for the species in the course of a viable number of molts. In many of the orders, in which the development is anamorphic, the larvae called nauplius hatches from the egg with three pairs of appendages, for example in Copepoda, Cerripedia etc. In the case of certain forms, a few additional pairs of appendages are found in the egg, for example Branchiura. There are several forms known, according to the mode of development and the respective stage of the ontogenesis *i.e.*,

Nauplius >Metanauplius>Copepodite >Cypris >Zoea> Megalopa and so on.

The development of additional pairs of appendages takes place either regularly or irregularly. The crustaceans with a highly diversified mode of life are found in the sea, in fresh water and on land. The interest lies mainly in predators and sucking parasites, which cause damage in aquaculture practices and among ornamental fish in the hobby of aquarium keeping.

Among the many crustaceans which are common as well as parasitize fish and considered harmful are briefly narrated with their systematic positions.

Systematic position of Crustacean parasites of importance

1) *Ergasilus* spp.:- Subclass: COPEPODA
Order: PODOPLEA
Suborder: CYCLOPOIDEA

Caligus spp.:- Order: CALIGOIDEA

2) *Salmincola* spp.:- Order: LERNEOPODIDEA

3) *Achtheres* spp.:- Order: LERNEOPODIDEA

Tracheliastes spp.:- Order: LERNEOPODIDEA

4) *Lernaea* spp.:- Order: LERNAEOIDEA

5) *Argulus* spp.:- Subclass: BRANCHIURA
Family: ARGULIDAE

6) *Aega* spp.:- Subclass: MALACOSTRACA
Order: ISOPODA
Suborder: FLABELLIFERA
Family: AEGIDAE

Cymothoa spp.:- Family: CYMOTHOIDAE

7) *Palaegyge* spp.:- Family: BOPYRIDAE

Characteristic features of the causative agent

1) *Ergasilus* spp.:- The morphology of ergasilids largely resembles that of the free-living cyclopoid copepods. They are distinctly segmented, slightly flattened in the cross-section and possess four pairs of natatorial limbs. The females lack maxillipeds, the thoracic limbs help in the intake of food. Another morphological difference from the free-living nonparasitic cyclopoid copepods pertains only to the second pair of antennae and are modified into large grasping hooks with sickle-shaped terminal claws. When these are pierced into the tissue of the host, they serve as clasping organs. There is no significant sexual dimorphism. In contrast to the females, the males lead a free swimming nonparasitic life which also explains their retention of the maxillipeds. The parasite grows to about 1.7 mm long. The egg formation in the ovary begins in late summer or winter approximately 200 hundred eggs are extruded from the ovary with a secretion which hardens in water and forms long egg pouches attached to the body of the mother. The duration of the embryonal development up to the hatching of nauplii depends on the temperature. The oogenesis progresses during the embryonal development in the egg pouches. Before hatching, the nauplii have a conspicuous blue colour. The nauplii take 8-10 weeks to grow to a sexually mature stage and copulate. During this period both sexes live freely in the water. It is only after this period that the females change over to a parasitic mode of life.

Therapeutic measures

The initial appearance of ergasilids in water body is attributed either to the entry of adult parasites with the stocking material or to the ingress of larvae with the water supply. The entry of parasites by introducing fish, or by letting in water containing larvae must be absolutely avoided. Once the parasite population is established, it is very difficult to eliminate. The strictest control of all fish introductions is therefore a very important prophylactic measure. The infestation in an open water body zone is more severe than in a zone with vegetation; it is therefore expedient to grow vegetation in the water body. Infested fish must be freed from ergasilids before they are transplanted. This is done by a dip bath with Trichlorphon in a concentration of 5 mg/m^3 for about 10 minutes. (Bromex-50, DDFT, Dylox, Malathion, were tried as therapeutic measures).

2) *Salmincola* sp.:- The parasite has been reported from the gill filaments, gill arches and fins of the fish. The parasite can cause disease and losses in all stages of cultured fish. Severe infestation brings about loss and proliferation of branchial mucous membrane, whereby the respiration obstructed. The fish become sluggish and discoloured, the distal end of gills is destroyed. The record reveals that as many as 12,000 of 14,000 spawn of *Salvelinus* sp. in a

hatchery died from an infestation with this group of parasites. The reproduction of the parasites takes place in the hot summer season. Older fish suffer the most severe attack. The intensity of infestation has been reported to be 500 per fish.

Therapeutic measures

The measures recommended for the preventation of parasitosis are:

i) Seperating fish of different age groups;

ii) arranging powerfull sources of electric light on the water body and capturing the larval parasites attracted by positive phototaxis; biological control by introducing 'cleaning-fish' which consume the larvae of the copepods. (Acetic acid, Bromex-50, Calcium chloride, Dylox, Formalin, Hydrochloric acid, Malathion, nitric acid, Oxalic acid, potassium chlorate, sodium chloride, Tartaric acid etc. were tried with success.

3) *Achtheres* sp.:- The species of *Achtheres* (syn. *Salmincola)* and *Tracheliastes* are closely related to lernaeids and like these, belong to the copepods of the order Lernaeoidea. The parasites reported to occur in both fresh and brackish waters and live in the gills of the host. The adult female of *Achtheres* which are up to 5 mm long, differ from the males, which are about 2 mm long, by the considerable transformation in their shape. The anterior part of the body, which is dorsoventrally flattened in the juvenile stages is round in the adult female and is seperated from the rest of the trunk by a deep constriction in the region of the anterior thorax. In contrast to the male, the segmentation can hardly be seen in the contrast to the male, the segmentation can hardly be seen in the female. The maxillae of the second pair of the antennae are very long, strongly bent into the shape of a clamp and are connected with each other at the tip by an unpaired chitinous adhesive disk, which is firmly anchored to the gill arch, operculum or in the buccal cavity of the host. As such, a host once attacked, is not abandoned, though the oscillatory motion of the clasping arms may continue. In the course of this oscillatory movement, the mouth of the parasite gets at the host tissue, where a cut is made with the sawlike serrated mandibles enclosed in a short suction tube in the upper and lower lips. The parasite obtains its nourishment by the upper and lower lips by sucking in blood. The development of *Achthere* occurs largely inside the egg. In most of the species, the hatching takes place at the first copodite stage and this, after a short planktonic life, attaches itself to a fish.

Therapeutic measures

Specific measures to control *Achtheres* and *Tracheliastes* have not tried so far. Trials can be made to gradually reduce the density of parasite population by constantly capturing and eliminating the infested fish. Control, as in the case lernaeid infestation, is conceivable if the parasites appear in the small water body and other production establishment.

4) *Lernae* sp.:- This cyclopoid parasite is distributed all over the world and appears in small ponds, outdoor tanks and even in aquaria on gold fish. The ripe females grow to a length of 5-22 mm, whereas the males, very much smaller in size, grow and females look alike; they are segmented, resembling the copepodite stage. Thereafter, the females change their shape completely by a rapid, markedly allometric growth of the trunk in favour of the cephalothorax and the appendages, as well as by loss of segmentation. The body then assumes the shape of a

worm, and is characterized by two pairs of horn-shaped cephalic appendages, situated at right angles to the body. The bifurcated abdomen ends in two short protuberances. The life cycle of the lernaeids or anchor worms can be represented by the 0.25 mm long nauplii, with two pairs of antennae and a pair of mandibles, hatch from 0.07-0.1 mm long, oblong, greensish eggs within 24 hours at 27-28 C. The first copepodite stage is reached after about three molts and the formation of metanauplius stage. The cyclopoid stage is attained after five molts. In the copepodite stages, they are temporarily transition to parasitism. The blood and mucin of the host serve as their nourishment. However, the migration of the larvae from host to host does not signify a change of host in the true sense. In this period, the parasites are found mostly on the gills of the host. Mating generally takes place in fourth copepodite stage. Soon after this males perish, while the females penetrate the skin of the host and are, from then on, parasites with definite, firmly fixed localization. The anterior part of the body with the conical mouth and the developing horn-like cephalic appendages are anchored deep under the skin in the musculature. The sac-like, long body then protrudes between the scales, directed to the rear. After attachment, the females are transformed largely to the body shape. Eventhough the lernaeids are warm water parasites, they can also attack trout. These parasites do not seem to occur in bodies of water with pH value below 7; they are infrequent in waters with salinities above 1.8%. This parasite is capable of massive attack with high pathogenicity and mortality, mainly during the hot summer season. Considerable mortalities occur among carps and goldfish and ell in fish farms. Incidents of fish mortality have also been reported from natural lakes and even from streams and rivers.

Therapeutic measures

Baths of common salt 8-11 parts per thousand may be practiced to destory the larval stages. Even formalin (250 ppm) duration 30-60 minutes for a period of three weeks can kill the larval forms. Potassium permanganate baths which may be preferable for females, when employed in flowing waters. Various insecticides have been tried. Due to differences in the active Y-isomer component of BHC in the commercial preparation, trials must be carried out before large scale application. Besides being highly toxic to fish, high rates of accumulation in the body of the fish can be expected.

(Antimycin A, Baygon, Baytex, Benzone hexachloride, Bromex-50, Calcium chloride, Dylox, formalin, Korlan, Menanzon, Methyl parathion, Mitox, Neguvon, potassium permanganate, Sodium chloride, Zectran etc. were used as therapeutic measures).

5) *Argulus* sp.:- Among the pathogenic curstaceans, the members of the family Argulidae constitute the most widerspread and most dangerous ectoparasites of freshwater and marine fish. Like many other parasites, argulids damage the fish directly by extracting the blood (hymolymph) and tissue fluids and indirectly as pathmakers for even true carriers of secondary infections. The systematics of Argulidae presented difficulties for a long time. Formerly the members were sometimes grouped with Cirripedia and sometimes with Branchiopoda. These are now included in a special subclass, Branchiura, grouped together in a single family. The genus *Argulus* includes the largest number of species and their first mention in the literature as early as 1666 and has distribution all over the world. The biology of argulids and their pathological effects have attracted the attention of biologists and fishermen. With the development of intensive culture in many countries, the problem of controlling this group of dangerous parasites has again been brought to the forefront. The general characteristic of *Argulidae* is the dorsoventrally flattened body, consisting of two large sections of these, the anterior portion is

adapted to the parasitic function and posterior portion for locomotion. The anterior portion constitutes the cephalothorax, comprising head and first thoracic segment; it extends as a shallow-shield or carapace to the front, both sides, and in the various species, more or less far to the posterior. The lateral flaps of the carapace can move up and down a little, like wings. Between the cephalothorax and the abdomen, the three additional segments can be recognised, over which the carapace projects somewhat. The abdomen is insignificant, fin-shaped and medially notched, with the notch varying in depth; in its inner corner, the indentation carries a tiny furca, in front of which lies the anus. The anterior pair of maxilly are transformed into two suctorial disks which are fairly deep, tubular adhesive contrivances. There is a movable spine in the median line of the body which is situated in a sheath, the hollow tube. This spine is directed to the front and is a secondary acquisition. The secretory duct of a large gland situated in the cepalothorax opens at its tip. The poison produced in the gland is sprayed into the wound caused in the host; it prevents coagulation of blood and has a marked paralyzing effect. The sting can be deadly for fry for food fish and aquarium fish. The two black faceted eyes, which have given the Argulids their names (Argulus is the diminutive form of Argus in latin = hundred eyed guard of IO the daughter of King Inachus of Argos). For purposes of reproduction, the argulids leave the host. The mating takes place in open water. The female has a sac-like, somewhat asymmetrical situated ovary. Two circvular, dark receptacula spermathecastore spermatozoa in the impregnated individuals. When eggs are laid, they pass the papillae which are situated below the genital orifice, and are connected with the spermatheca through a duct. It is at the papillae the eggs are inseminated after being pierced by sharp points of papillae. In contrast to the parasitic copepods, the eggs are not carried on the body but laid on the aquatic plants, stones, roots, aquarium glass and the like. The embryonic and postembryonic development by way of naupliar stages to metanauplius takes place inside the egg. The juveniles on hatching have the typical shield-shaped carapace and after a short planktonic mode of life soon attack fish. The formation of the suctorial disks is initiated in the fifth juvenile phase but these these become capable of functioning only after the seventh molt. The argulids are temporary facultative parasites which leave their hosts after sucking blood and then freely swim about. When the parasite is firmly adhered to the host the anterior end is directed against the current. Argulids hold on to the host tightly, primarily with their large suckers. At the same time, it presses itself with the large flaps of the carapace, so that the adhesion to the fish skin is perfect. When the host swims fast, the several caudally directed spinules on the ventral side of the parasite press like barbed hooks into the fish skin, the faster fish swims, the more firmly the parasites adheres. Argulid pierces the skin of the host, with its jaws; it then pricks its poisonous sting into the wound and with its proboscis sucks the blood form the punctured blood vessels. It fills its mesogastral diverticula full, in a short time. When a parasite remains on a fish, a superficial, ring-shaped wall forms around the parasite, due to mucous membrane proliferation. This is followed by modifications of the epithelial cells and intensified formation of mucous cells to more than 2.5 times the normal number. The changes in surface of the skin under the parasites are characterized by the disintegration of the mucous cells, nuclear pycnocis in the epithelial cells and infiltrations of lymphocytes. The argulids are probably true carriers of *Aeromonas* sp., the bacterial pathogen of infectious dropsy. As such, ulcers can form at the places of incision.

Therapeutic measures

Prophylactic measures to prevent argulosis are undertaken as per the life cycle of the parasite. Since the period of development of a new generation lasts for eight weeks, argulids can become dangerous for carp young in fish farms only when impregnated parasites, ready to

spawn, find entry in large numbers in the water body. Care should therefore be taken that the ponds are stocked with parasite-free fish and the water supply is similarly free from parasites. If necessary, parasitized fish must be rid of argulids by short bath treatments with $KMnO_4$ in a concentration of 10 mg/litre for 30 minutes. Since effects depends on the temperature, the mortality of the parasites mostly occurs a long time after the bath in fresh water.

(Acetic acid, Alcohol, Ammonium chloride, Balsam or Peru oil, Baytex, benzene hexachloride, Bromex, Calcium oxide, DDFT, Ddt, DDVP, Dylox, Ethyl parathion, Gix Kerosene, Lysol, Malathion, Methyl parathion, Neguvon, Potassium permanganate, Pyrethrum, Sodium chloride, Teaseed meal, Turpentine etc. were used as therapeutic agents through out globe).

6) *Aega* sp.:- The species of the family Aegidae are moderately large, common ectoparasites on body of fish, sharks and skates. They generally are found clinging to the outside skin of the host, but occasionally they have been found just inside the gill slit. Apparently the species are able to live when not attached to the hosts because many times they have been collected in bottom samples. Fish on which the isopods have been clinging are usually scarred or wounded where the claws of the specimens were attached to the hosts because many times they have been collectged in bottom samples. Fish on which the isopods have been clinging are usually scarred or wounded where the claws of the specimens were attached. They are probably of ecological importance since many economical valuable fish are parasitized by the species of the family. The species with very exceptions have eyes which are generally quite distinct and sometimes extremely large. All peraeonal segments are well developed, and the well developed, and the pleon is usually composed of five segments plus pleostelson; but in some species there are only four free segments plus the pleotelson. The pleotelson and the uropods usually form a broad caudal fan. The first three pairs of peraeopods are modified into distinct prehensile hooks and the next four are usually ambulatory. The distinction between the two types of peraeopods is usually quiate marked. On the tip of the maxillipedal palp there are usually large teeth or spines, but are sometimes absent in gravid females. Eyes large, sometimes converging at center of cephalon; body compact; maxillipedal palp of five articles; front of cephalon with medial point separating basal articles; front of cephalon with medial point separating basal articles of antenna one; flagellum of antenna one with many articles.

7) *Palaegyge* sp.:- There are more described species of Bopyridae than in the other epicardean families. The species are parasitic on decapods (crabs and shrimp). The body of both the male and female is segmented, and praeopods are present and differ little in size and shape. Females have large asymmetrical bodies and males are small and symmetrical, frequently being found attached to or among the pleopods, or in the brood pouch of the female. The cephalon is generally distinct and eyes, if present, are tiny. The antenna and mouth parts are rudimentary. All seven segments of the peraeon are always laterally distinct, but in some instances some of the anterior setgments are fused with the cephalon. Five pairs of ootegites are present, and knowledge of their form is useful since the most commonly encountered is the gravid female. Coxal seperations are present on the edges of the peraeonal segments, and large bosses or swellings are frequently present just medially to the coxal swellings are frequently present just medially to the coxal sutures. Seven pairs of prehensile peraeopods are almost always present. The pleaon is always distinct and generally segmented or with indications of segmentation on the lateral margins. The lateral margins are frequently elongate, the pleopods are sometimes absent and when present they can be uniramus, biramus or triramus. The uropods, if present, are generally simple and composed of lobes which like the ends of the pleonal segments. A distinct

telson is sometimes present, but many times it is fused to a pleonal segment to form a pleotelson. A small male is frequently found clining to the pleopods of the female. The male is small, symmetrical and distinctly segmented. It has very small antennae, mouth parts and prehensile peraeopods that mostly have pointed dactils. The pleon is always distinct, usually lacks appendages, but a well defined pleotelson with long uropods are present in some species. The bopyrids are generally found in the branchial cavity of their decapod hosts.

Nothing is known so far regarding te control of parasitic isopods

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Epizootic Ulcerative Syndrome in fishes - Diagnosis and treatment

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History of the disease

This dreaded fish disease has been a major concern in several countries of Asia-Pacific region. In Queeensland, Australia, an epizootic of marine and estuarine fishes characterised by shallow haemorrhagic ulcers occurred in 1972 with recurrence in subsequent years. The disease was named 'red spot disease'. Papua New Guinea reported a similar type of disease characterized by dermal ulcer from the rivers of the south during 1975-76 and north during 1982-88. Indonesia also reported similar type of disease in Bogor in 1980 which subsequently spread to West Central and Eastern Java. This disease was named infectious dropsy or 'haemorrhagic septicaemia'.

Malaysia reported the disease during 1981-83. The affected fishes had red or necrotic areas of ulceration all over their bodies and was called Webak Kudes. In early 1984, the disease was reported from fishing areas of Kampuchea along with a significant decrease in the natural fish stock. In 1984, a similar disease was reported from the southern and central parts of Laos. Burma experienced the outbreak of the disease during 1984-85 affecting both wild and cultured fish stock. In Thailand, the disease epizootic was first reported in 1980 in the natural water system and thedisease recurred every year during 1980 to 1985 in different water bodies. In Sri Lanka the disease was first reported in 1988 in the Kelani river, Dandugan Oya, and in streams nearby causing severe fish mortality. In Bangladesh, the first outbreak of the disease occurred during February/March 1988 in the rivers Meghna, Padma and jamuna and adjoining water areas with enormous loss of the commercial fish stock. In India, the outbreak of the disease was first noticed in May 1988 among fishs of the rivers, canals, beels, paddy fields, and ponds of the North Eastern states. In 1989 Nepal was affected by the disease.

Areas affected by EUS

Fishes were afflicted with EUS in all types of water areas in India, namely rivers, floodplain wetlands (beels), lakes, irrigation canals, reservoirs and culture ponds (Table 1).

Time of occurrence

The disease is mostly observed during the post monsoon period which is different states, vary from May to February. In some states, for example Kerala which has two monsoon, *i.e.*, south west (June-August) and north east (October-November), the disease is prolonged and is observed throughout the year. Investigations carried out at disease prone sites in West Bengal showed that EUS outbreak occurs at the time of waning of rainfall and onset of gradual stagnation from September and fall in water temperature. The details are given in Table 1.

Spread of the disease

Since May 1988, when the disease first appeared in the north-eastern states, it gradually spread to the eastern, central western, southern and northern states. The disease in any particular area was severe during the first outbreak and gradually diminished in subsequent years, lasting up to three years. In the fourth year also in some areas it remained within pockets of minor incidence. In the north-eastern, eastern, central and some southern states the disease outbreak could be correlated to water-borne transmission. However, in many areas the transmission of the disease could be due to transplanting fish, fry and fingerlings from disease-prone areas.

Major outbreaks

India witnessed the first major outbreak of EUS in May 1988 in the states of Tripura, Assam, Meghalaya and West Bengal. It gradually spread and affected major outbreaks till 1992 in the states of Orissa, Bihar, Uttar Pradesh, Maharashtra, Andhra Pradesh, Tamil Nadu, Kerala, Karnataka, haryana and Rajasthan (Table 1).

Fish species affected

Thirty species of freshwater and brackishwater fishes have been recorded to be afflicted by EUS out of which four are exotic and the rest indigenous (Table 2). The range of incidence of the disease recorded from the various species of fishes and from different types of water bodies (Table 3) reveals that certain genera of fishes, such as *Channa, Puntius, Mastocembelus, Mystus, Glossogobius, Anabas, Clarias* and *Heteropneustes* are highly susceptible to EUS.

Semiotics of the disease

The symptoms and other characters of Epizootic Ulcerative Syndrome are conspicuously different from the other low level ulcerative conditions reported earlier. It has some distinct manifestations; fishes in the rivers as well as in confined waters exhibit abnormal swimming behaviour with head projected out of water. In the rivers, abnormal swimming behaviour was witnessed with several fishes floating listlessly near the bank.

In the initial stages of the disease, the infection usually commences in the form of multiple inflammatory red spot on the body causing localized haemorrhage. In carps these appear within the scale pockets. In advanced stages of infection, the ulceration covers larger areas with sloughing of scales and degeneration of epidermal tissue. With further advancement of the disease, the ulcers become deep haemorrhagic and necrotic often with black melanistic rim. In advanced stages of the disease, large and deep ulcers are very commonly seen in all parts of the fish, especially the head, abdomen and peduncle. Histopathological studies conducted on ulcerated fishes show identical histopathological manifestations. In heavily ulcerated fishes there is degeneration of epidermis of skin at the ulcerated arteas and granulamatous formations. Basically fungal granulomata occur in the dermis and hypodermis. A high degree of inflammatory reactions involving infiltrations by macrophage cells and lymphocytes around some of these granuloma formations were found. Livers affected fishes did not show any significant change except vacuolization in certain cases. However, frequently most of the sinusoidal space and blood vessels were congested (hyperaemia) and wandering lymphocytes wer

plenty in liver parenchyma. No changes were observed in kidney of affected fishes. Hematological parameters of affected fishes showed higher counts of phagocytic cells and reflected initiation of defense phagocytosis in blood circulation.

Investigation on the causative factors of EUS in India

The causative agent of this dreaded disease has been baffling the scientist. It is widely suspected that a biological infectious agent is the primary cause of EUS and certain abiotic factors are responsible for creating stress to fish. The suspected biological agents are viral, bacterial, fungal and other animal parasites. The investigations carried out in India uptil now on the different probable causative agents reveal the following points.

Environmental factors

It is suspected that the physico-chemical parameters of water and anthropogenic factors such as pesticides, fertilizers and heavy metals play an impolrtant role in the outbreak of EUS. As such data was recorded at specific water bodies in EUS disease prone area of West Bengal throughout the year and in various affected water areas in all the affected states on selected physico-chemical prameters having relevance to the EUS outbreak (Table 4). It reveals that the affected wate areas in different states where the intensity of disase was severe had low alkalinity and hardness - a characteristic of acidic low calcium soils (Tables 3,4). The observation is in agreement with earlier reports from other countries affected by EUS that low alkalinity, hardness, chloride concentration and fluctuating pH showed a link with EUS outbreak. Also occurred in water areas with high alkalinity and hardness but with lesser intensity. Investigation carried out at disease prone site in West Bengal shows that EUS outbreak does not commence during the monsoon period. The disease outbreak occurs at the time of waning of rainfall and onset of gradual stagnation from September and fall in water temperature and minimum air temperatures. Sharp fall in the hardness of water from the higher summer values due to dilution during rainy season seems to be another predisposing factor for triggering the disease outbreak.

Heavy metal concentration in water

Though in some affected water areas significantly high values of Zn, Cu and mercury were obtained, the data collected so far (Table 5) do not suggest any perceptible role of the heavy metal content in creating stress to fishes and subsequently predisposing it to EUS outbreak.

Pesticide and other agrochemicals

Since the incidence of EUS is high in rice field environments in India as in case of other countries where EUS occurred pesticides were suspected to be associated with outbreak. Most of the outbreaks of EUS in India occurred after rain fall. This observation is in agreement with reports from other countries leading to suspicion that drainages of agricultural chemicals may have an important role as predisposing factor for EUS outbreak.

Analyses of pesticide residue in water, fish and plankton of some specific EUS affected water areas in India were carried out to assess the relation between pesticide use and EUS outbreak (Table 6). The studies indicate that although occasionally higher concentrations of organochlorine and organophosphorus pesticides have been found in water and fish samples, no correlation can be made with the presence of pesticide residue and disease outbreak.

The study indicated that the extent of pollution may create a stressed condition for aquatic life and may be the predisposing factor for EUS outbreak.

Virus

Virological studies conducted on the EUS affected fishes in India from samples of EUS affected fishes, namely, *C.idella, Colisa sp., P.javanicus, H.molitrix* and *P.sophore* from Assam, *C.catla* and *C.carpio* from Tripura, *C.punctatus, M.armatus, N.nandus, P.sophore* from West Bengal, showed no cytopathic effects on snakehead cell line upto 14 days when tissue extracts (spleen, liver, gills, and ulcereted parts) were inoculated. The monolayer of snakehead cells in the conrol and inoculated flasks were the same. The electron microscopy studies for occurrence of viral agents in the kidney and liver showed negative results.

Though a primary viral aetiology has been consider a likely possibility given the rapid and uncontrollable spread of EUS and its distinct clinical sign. However, from the extensive study conducted on viral aetiology of EUS in different countries, it is opined that although seemingly frequent isolation of rhabdovirus might at first sight present an attractive proposal for causal agent, it should be realized that the virus has never been isolated from more than 5% of diseased fish examined. It is still not known what role any of the viruses so far isolated or visualized play in the pathogenesis or spread of the disease. Seemingly successful transmission of the disease has been achieved on a few occasions in snakehead fish by different investigators by co-habiting infected and clinically healthy fish but attempts of the experimental induction of disease with an isolated and identified virus have failed so far.

Bacteria

Investigations on the bacterial pathogens from EUS affected fishes have been conducted by several workers. These workers isolated a wide variety of pathogenic bacterial forms from lesions and other internal organs such as gills, kidney and liver. Table 7 depicts the variety of bacterial fauna isolated from EUS affected fishes in different states of India. Further, there is no significant relationship between the forms of bacteria isolated and a particular species of diseased fish or a location of disease outbreak. In India as in other countries the predominant bactgerial form isolated is *Aeromonas hydrophila.* However, it is not considered to be the primary causative agent.

Investigators in India have tried to reproduce the disease symptoms inoculating pure bacterial isolates from EUS affected fishes with mixed success.

Though reinoculation of pathogenic bacterial forms isolated from EUS affected fishes could produce ulcers in apparently healthy fishes, further experiments under different environmental conditions are required to produce the disease of similar clinical symptoms.

Fungus

Fungal species is consistently isolated from the lesions of EUS affected fishes especially in an advanced stage of ulceration. The species most frequently isolated in India is *Saprolegnia* sp. *Aspergilus* sp. was also recorded in the liver parenchymatous tissue from severely affected fishes. It is inferred that these fungal species secondarily infect the fish.

Animal parasites

Ureceolariied ciliates of the genus *Tripartiella* and *Trichodina,* myxozoans of the genus *Thelohanellus* and *Myxobolus,* monogenetic trematodes of the genus *Dactylogyrus* and less frequently parasitic copepods *Ergasilus* sp., were encountered predominantly from the gills of EUS affected fishes. These parasites could not be attributed to be the primary cause of ulceration. Most of the parasitic infestations recorded on the sampled fish were at a low intensity.

Present state of knowledge on EUS

Till now, investigators throughout the world have put in a great deal of effort for ascertaining the aetiology of epizootic ulcerative syndrome in fishes, but to date no firm conclusions have been reached regarding the cause of the disease. During January 1994 in the Regional seminar on Epizootic ulcerative syndrome organised by ODA and AAHRI, Bangkok, scientists from affected countries presented upto date findings on EUS and its relationship to Red spot disease in Australia, Menhaden disease in USA and Piscida disease in Japan.

The conclusions and recommendations that emanated from the deliberations reveal the latest state of knowledge on Epizootic Ulcerative Syndrome (EUS).

Definition of EUS

A seasonal epizootic condition of freshwater and estuarine warm water fish of complex infectious aetiology characterised by the presence of invasive *Aphanomyces* infection and necrotizing ulcerative lesions typically leading to a granulomatous response.

Similar diseases

Recent pathological and epizootiological evidence has indicated that the condition known as Red Spot.Disease in Australia is indistinguishable from EUS.

Similarly all available evidence suggests that the condition known in Japan as mycotic granolomatosis is indistinguishable from EUS.

Extension of range

EUS is endemic in many countries and is still extending its geographical range even into sub tropical, sub temperate and temperate climates. Experimental evidence indicates that the Aphanomyces involved is capable of causing disease in temperate species.

Human significance

All available evidence suggests that consumption of EUS infected fish poses no proven specific health problems to humans provided that they are properly prepared in sanitary conditions.

Recommendations for future work

In view of the importance and continuing extension of this serious disease, the meeting recommended the following research areas as being of high priority in terms of future work.

Investigation of early stage

It was clear from the information presented to the seminar that there is a distinct and critical early pre-mycotic stage of the pathogenesis of the disease and it is essential that detailed multi-disciplinary research is carried out on this stage.

Virology

As studies have shown the presence of a wide range of viral agents in fish affected with EUS, it is recommended that further extended work be undertaken to determine more accurately the incidence and distribution of tropical food fish viruses throughout the region, in addition to specific EUS related investigations.

Epidemiology

As proposed studies are likely to produce large bodies of complex data relating to EUS outbreaks in fish populations it is recommended that epidemiological expertise be developed within the region to enable these data to be effectively utilised.

Environment

The evidence presented at the seminar strongly points to a relation between the initiation of Eus and environmental factors. It was recommended that further studies on environmental conditions, including physical, chemical and biological factors, be carried out to better understand their role in outbraks of EUS. The seminar expressed concern over the limited understanding of the relationship between fish health and environmental conditions, in general, and recommended expansion of research effort in this important subject area.

Speciation of fungus

It is essential given the fundamental importance of fungal organisms of *Aphanomyces* sp. to this disease that the detailed mycology andmolecular genetics of these strains be compared in detail. The isolates should be fully characterised and their relationship defined.

Diagnosis

Currently diagnosis is of necessity based upon a number of clinical and pathological features of the disease. It is important that a rapid, specific, accurate, low cost diagnostic test capable of being used under field conditions is developed.

Recommendations for treatment

It would appear from information presented at the seminar that although some measure of control may be achieved for instance, liming, options for te treatment of the disease are currently limited to empirical management of pond situations. There is a need to understand the current inadequate control methods in order to improve them.

Development of resistance

Evidence from some countries suggests that after the initial outbreak, an element of resistance to the disease may develop in the fish. This resistance may be ecological, genetic or associate dwith some acquired immunity. It is important that the mechanism for this is now investigated.

Mode of transmission

The evidence suggests that although the extension of the disase in Asian countries was largely via river systems and natural waterways, there was, nevertheless, evidence of distinct transfer over marine barriers. It is important that an understanding be gained as to the mechanism of transfer between water bodies and it is essential that attention be given to the development of quarantine measutres to prevent the transfer of these via live fish transportation or infected material.

Socio-economic impact of EUS

As in other countries, the outbreak of EUS in India created panic in the affected areas with sizeable loss of valuable edible fish. This unprecendented appearance of the disease caused grave bioecological and socio-economic consequences. The rivers and large water bodies were affected most in the initial stages, with heavy mortality of valuable stock of fish. As a result, depletion of fisheries is evident, with the consequent impact on fisherman development. This is obvious from a case study conducted during 1987-91 at Jorhat Fish Assembly centre in Assam, to evaluate the damage caused by the disease in fisheries of Brahmaptura river system (Table 8).

Social effect

Investigations carried out in five districts of West Bengal reveal that 73% aquaculture operation units were adversely affected by EUS. The outbreak of the disease depressed the fish consumption rate by 28.7%, 23.3% and 20.5% in urban, sub-ruban and rural sectors respectively. Consequently the fish trade was also affected seriously. Owing to consumer resistance, the traders did not accept such fish for selling. In rural markets diseased fishes were sold at a very low price.

About 42.19% of the aquaculturists suffered 31 to 40% loss of fish in their culture ponds followed by 21 to 30% by 25.05%. The pecuniary loss faced by 50% aquaculturists was in the range of Rs. 1,001 to Rs. 5,000/- while 19.73% culturists suffered a greater loss ranging from Rs. 5,001 to 10,000. A section of the farmers had to search for alternate jobs. 88.9% fish traders also suffered losses to some extent during the affected period.

Another study undertaken in 5 Districts of Kerala revealed that the spread of EUS completely paralysed the inland fish market and threw the fishermen out of their occupation and women fish vendors wer particularly subject to severe hardship. They had to seek alternative employment as agricultural labourers, head-load and quarry workers, etc. without much success.

Remedial measures

The remedial measures both prophylactic and therapeutic so far tried in India for either controlling or containing EUS are applicable only in manageable water areas. In large open waters such as rivers, reservoirs, lakes and big beels above 30 hectares and backwaters where EUS outbreak occurred remedial measures developed so far are not applicable.

The difficulty encountered in counytering the disease outbreak at present is primarily lack of knowledge on the primary causative agent, occurrence of the disease in large water bodies affecting wild population.

The chemicals used for therapeutic and prophylactic treatments in manageable water areas are lime, $KMnO_4$, NaCl, bleaching powder, and antibiotics. The chemical treatment is primarily aimed at controlling the external pathogens observed such as bactgeria and fungus.

Liming

Depending upon the pH quick lime at 100-600 kg per hectare has been found effective in manageable water areas. In areas having alkalinity below 40 ppm (Table 4) the higher doses of lime is applied and in areas with higher alkalinity the lower dose of lime is applied at an interval of 1 month during the outbreak period. It is observed that CaO applied at 50 kg/ha in the disease prone water area in the post monsoon period just prior to the outbreak od disease have either arrested the occurrence of the disease or if outbreak occurred the intensity is mild. The information collected from the different states of India through questionaire developed by CIFRI, Barrackpore and distributed to all the states it is gathered that lime treatment has given encouraging results in checking the intensity and spread of the disease. A study conducted in West Bengal revealed that as remedial measure the clientele adopted different remedial measures. The study revealed maximum respondents (358) applied lime to control the disease followed by application of $KMnO_4$ (227). Only limited number of farmers applied antiobiotics. About 68% of the respondents obtained positive result from the treatments.

Potassium permanganate

Application of this chemical as a deterent for EUS is quite widespread in India. An application rate ranging from 1 ppm to 10 ppm has given fairly encouraging result in the different states. While the application rate for bath treatment of fish is 1-6 ppm the pond treatment rate is 5-10 ppm. This rate has been found effective in containing EUS and healing up of initial stage of ulceration of fish.

Bleaching powder

Bleaching powder at 1 mg/litre or 5-10 kg/ha was reported to be useful in healing up of initial lesion of EUS affected fishes and @ 3-5 ppm for desinfecting all fishery equipments used for fishery activities in EUS affected areas. Investigations at CIFRI showed that EUS can be contained in manageable water areas, by applying a prophyolactic dose of 50 kg/ha CaO and after one week bleaching powder @ 0.5 ppm in disease prone water areas.

Therapeutic dose of 100 kg/ha CaO and after one week belaching powder @ 1 ppm when initial symptoms of Eus is seen.

Salt (NaCl)

Application at a concentration of 3-4% dip treatment of affected fishes have given fairly effective result in healing up of ulcers at the initial stage of the disease.

Antibiotics

Pending knowledge of the definitive primary causative agent of the disase what is apparent is that the EUS affected fishes are afflicted by a wide variety of bacteria and in acute cases fungus. A microencapsualted feed containing 30% protein, nalidixic acid, erythromycin along with vitamin A and C has been formulated by CIFRI. Trialwith the pelleted feed to diseased fishes showed the fishes recovering. In general it was found that antibiotics either erythromycin or oxytetracycline or terramycin at 60-100 mg/kg of feed for 7 days cured the ulcers of EUS affected fishes.

CIFAX

A drug formulated by CIFA for application in Eus affected captive waters is reported to show encouraging result in controlling EUS. The drug applied at 1 litre/ha metere of water area with the notice of the symptoms of EUS in the pond is reported to cure affected fishes within 7 days.

Suggested Readings :

Tonguthai, K., 1985. A preliminary account of ulcerative fish diseases in Indo Pacific region - A comprehensive study based on Thai experiences. *FAO TCP/RAS/4508 :* 1-39.

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Table 1. Details of EUS outbreak in different states

State	Period of outbreak	Duration	District affected	Water areas
Tripura	1988 - 1991	May to Sept.	North, South & West Tripura	Rivers, lakes, reservoirs, paddy fields, ponds
Assam	1988-1991	May to Dec.	All districts	-do-
Meghalaya	1988 - 1990	May to Dec.	East Khasi & Garo Hills	Rivers, streams, paddy fields
Mizoram	1989,1990	June to Sept.	Large ponds	Large ponds
Arunachal Pradesh	1989	Sept. to Dec.	Itanagar	Rivers, ponds
Manipur	1989	December		Ponds
West Bengal	1988 - 1991	Sept. to Dec.	All districts	Beels, reservoirs, paddy fields, ponds
Orissa	1989 to 1992	Oct. to Jan.	Cuttack, Puri Balasore, Mayurbhanj, Bhadrok	Beels, ponds, paddy fields
Bihar	1989 to 1992	April to Oct.	29 districts	-do-
U.P.	1989 to 1991	Sept. to Nov.	Gorakhpur, Lucknow, Allahabad, Faizabad, Sultanpur, Gonda, Barabanki, Rai Bareilly, Bahraich, Fatehpur, Varanasi, Unnao, Gazipur, Budaon, Rajgarh, Bilaspur	-do-
M.P.	1990,1991	Nov. to Dec.	Raipur, Durg, Rajnandgaon, Gwalior, Shivpuri, Jabalpur	Irrigation tank, culture ponds
Maharashtra	1990,1991	Sept. to Oct.	Gondia, Bhandra	Beels,culture ponds
Andhra Pradesh	1990,1991	Nov. to Jan.	Eluru	Lakes, canals, drains
Tamil Nadu	1990,1991	Oct. to Feb.	Kancheepuram, Chingleput, MGR Trichy	Lakes, reservoirs
Kerala	1991, 1992	July to Feb.	Wayanad, Kozhikode, Malappuram, Thrissur, Ernakulam, Idukki, Kottayam, Alappuzha, Pathanamthitta and Kollam	Backwaters, lakes, culture ponds
Rajasthan	1991	November	Tonk	Reservoir
Haryana	1991	October	Sonepat	Culture ponds
Karnataka	1990,1991	November	-	Rivers, lakes, ponds

Table 2. Fish species affected by EUS in India

Cultured	Wild
Freshwater	
Catla catla, Cirrhinus mrigala, Labeo rohita, Puntius javanicus, Ctenopharyngodon idella, Hypophthalmichthys molitrix	*Channa striatus, C. punctatus, C. gachua, Clarias batrachus, Heteropneustes fossilis, Puntius sophore, Ambassis ranga, Amblypharyngodon mola, Mystus vittatus, Nandus nandus, Glossogobius giuris, Gadusia chapra, Mastocembelus pancalus, M. armatus, Callichrous pabda, Rhinomugil corsula, Trichogaster* sp., *Acrossocheilus hexagonolepis, Notopterus* sp.
Brackishwater	
Mugil parsia	*Mugil cephalus, Mugil subviridis, Mugil parsia, Etroplus* sp.

Table 3. Intensity of the EUS outbreak in India

EUS affected fish	Av. % of incidence range	State	Av. % of incidence range	affected water area	Av. % of incidence range
Channa sp.	20-100	Assam	30-60	Rivers	4-15
Puntius sp.	5-100	Tripura	35-70	Confined waters	10-55
Glossogobius sp.	10-60	Meghalaya	10-35		
Mystus sp.	5-75	West Bengal	15-65		
Notopterus sp.	3-25	Bihar	20-30		
Wallago attu	7-20	Orissa	20-45		
Mastacembelus sp.	10-35	Uttar Pradesh	15-20		
Anabas testudineus	10-55	Tamil Nadu	5-25		
Amblypharyngodon mola	5-10	Rajasthan			
Rhinomugil sp.	1-5	Maharashtra	5-10		
Clarias batrachus	10-30	Kerala	30-65		
Heteropneustes fossilis	10-20				
Catla catla	5-15				
Labeo rohita	5-10				
Cirrhinus mrigala	5-20				
Cyprinus carpio	10-25				
Ctenopharyngodon idella	2-5				

Table 4. Environmental monitoring in affected water areas in India

State	pH	Alkalinity	Hardness	Chloride	Free CO_2	Ammonia	Salinity
Assam	7.1-7.5	13-74	11-38	4-23	4-10	0-0.4	
Tripura	6.7-7.6	7-49	9-45	3.5-18	2-8	0-0.6	
Meghalaya	6.5-7.5	7-14	10-15	2-12	4-6		
West Bengal	6.7-7.5	10-170	6-180	2.9-13	2-7	0-0.6	
Bihar	6.1-6.8	25-30	13-20	4.7-7	4.0	1.8-2	
Orissa	6.8-7.4	44-138	55-180				1-5
Uttar Pradesh	7.5-8.0	40-217	42-234	0-5.8			
Tamil Nadu	7.8-8.3	103-139	105-158				
Rajasthan	8.0-8.2	140-150	80-90				
Maharashtra	7.5-9.5	30-115	48-140		2.5-3.0		
Kerala	6.3-7.0	0-11	8-17	0-3.4			1.0

Table 5. Heavy metal analysis in affected water areas (µg/b) (1) Levels were not detected (nd)

Site	Fe	Zn	Cu	Chr	Cd	Pb	Hg
Mayapur	280	107	80	8.0	9.0	16.5	0.12
Cooch Behar	200	21	7.0	nd1	nd	nd	nd
Maldah	130	32	3.0	nd	nd	3.8	nd
Jorhat	7,800	62.8	3.9	nd	nd	5.75	nd
Jhalukbari		22.8	1.2	nd	nd	nd	nd
Meghalaya	4,810	53.2	2.12	nd	nd	3.68	0.03

Table 6. Pesticide residues in two EUS affected water bodies near paddy field areas

Pesticide	Balda pond (Antpur) Water (µg/g)	Fish flesh (µg/g)	Gamrapota beel (Bongaon) Water	Fish flesh
		1988		
α - endosulfan	0.00035	1.25		
β - endosulfan	0.008	1.14		
Total endosulfan	0.0088	2.39		
Methyl parathion	0.085	10.85		
Monocrotophos	0.538	523.5		
		1989		
BHC	0.032	1.9	0.108	27.6
BHC	0.011	0.39		3.3
DDT				
OP'DDE		0.97		0.065
PP'DDE		1.82	0.009	2.73
OP'DDD	0.103	12.73	0.019	7.2
OP'DDD	0.25	46.28	0.063	193.1
OP'DDT	0.011	2.07		0.5
PP'DDT	0.023		0.005	11.2

Table7. Bacteria isolated from different organs of EUS affected fish specimens in India

State	Host fish	Predominant bacterial form
Tripura	Channa sp.	*Salmonella sp., Klebsiella sp.*
	Mastacembelus sp.	*A. hydrophila, Shigella sp.*
	Puntius sp.	*Staphylococcus sp., Bacillus sp.*
	C. catla	*Micrococcus sp.*
Assam	Channa sp.	*Pseudomonas mallophila, Shigella sp.*
	Mastacembelus sp.	*Klebsstella ozaenae, Staphylococcus sp.*
	Puntius sp.	*A. hydrophila, E. coli*
	C. catla	*Vibrio sp.*
Meghalaya	Channa sp.	*P. mallophila, Bacillus sp., Micrococcus sp.*
	Puntius sp.	*A. hydrophila*
	Clarias sp.	
West Bengal	Clarias sp.	*Corynbacterium hoffmani, Klebsiella aerugtnusa*
	Puntius sp.	*A. hydrophila, Acid fast Nocardioform CAN)*
	Cyprinus sp.	
	Anabas testudtneus	
	Mugil parsia	
Orissa	Channa sp.	*Shigella sp., A hydrophila, Staphylococcus sp.,*
	Puntius sp.	*Enterobacter agglomerans, Arthobacter sp., Micrococus sp., E. coli, Pseudomonas mallophila*
	Clarias sp.	
Tamil Nadu	Channa sp.	*Citrobacter intermedica*
Kerala	Channa sp.	*Micrococcus lutens, Staphylococcus sp., Citrobacter freundi, NAG Vibrio A. hydrophila, Actnelobacter sp., Streptococcus sp.*

Table 8 •Species-wise landing (kg) EUS affected fish during 1987-91 and percentage increase/decrease* through years with 1987-88 as base

Species group	1987-88	1988-89	1989-90	1990-91
Puntius spp.	34804	12696 (-63.5)	3623 (-89.6)	10401 (-70.1)
Amblypharyngodon mola	22616	7712 (-65.9)	14153 (-37.4)	6601 (-70.8)
Labeo rohita	17316	6350 (-63.3)	8170 (-52.8)	8823 (-49.0)
Catla catla	13046	8434 (-35.4)	8029 (-38.5)	8151 (-37.5)
Puntius sarana	5100	9359 (+83.5)	4072 (-20.2)	5097 (-0.1)
Cirrhinus mrigala	2089	702 (-66.4)	1499 (-28.2)	908 (-56.5)
Labeo bata	1701	783 (-54.0)	219 (-87.1)	651 (-61.7)
Heteropneustes fossilis	13848	13816 (-0.2)	22333 (+61.3)	18291 (+32.1)
Mystus sp.	7006	5219 (-25.4)	3228 (-53.1)	5067 (-27.7)
Ompok spp.	5009	3683 (-26.5)	1926 (-61.5)	2065 (-58.8)
Channa punctatus	30091	4649 (-84.6)	1829 (-93.9)	2622 (-91.3)
Channa striatus	22332	2777 (-87.6)	3941 (-82.4)	3406 (-84.7)
Channa marulius	8079	4309 (-46.7)	7992 (-1.1)	7419 (-8.2)
Anabas testudineus	10189	5963 (-41.5)	15555 (+52.7)	13142 (+29.4)
Colisa spp.	1888	805 (-57.4)	871 (-53.9)	2095 (+11.0)
Nandus nandus	2816	500 (-82.2)	62 (-97.8)	150 (-94.7)
Gadusia chapra	100	883 (+783.0)	339 (+239.0)	376 (+276.0)

Environmental parameters of significance to fish health

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Introduction

Maintenance of a healthy aquatic environment and production of sufficient fish food organisms in a water body are two very important factors for fish production. To keep the water body conducive for fish growth, physical and chemical parameters like temperature, transparency, colour, odour, pH, dissolved oxygen, carbondioxide, toxic gases like ammonia, hydrogen sulphide and nutrient elements like nitrogen, phosphorus, and organic matter may be monitored regularly. When the physicochemical factors are in normal or conducive range, the water body is usually productive; but when they are present in quantities above or below the normal range, the fishes and other aquatic organisms may be under stress which may lead to fish disease or fish mortality in due course. Stress is the sum of the physiological response by which a animal tries to maintain or reestablish a normal metabolism in the face of a physical or chemical force. Brett (1958) correlated stress with the fish disease situation *i.e.,* when the normal functioning is reduced significantly and finally may result in death.

In an aquatic environment, there is a profound and inverse relationship between environmental quality and fish disease. As environmental conditions deteriorate, severity of infectious diseases increases. Thus proper health maintenance practices can play a major role in maintaining a suitable environment where healthy fish can be produced.

Environmental stress and fish disease

Fish prefers an optimal environmental condition for its growth and reporduction. Any change in environmental condition causes stress on the fish. If such a change increases arithmatically, the stress on fish may increase geometrically. Productivity of the ecosystem, which supply food to the fish is also important for the growth and maturation of the fish. The water quality parameters of significance to fish health are as discussed below :

Temperature

Every fish has an optimal temperature for its growth and maturation. Immune response of a fish is also dependent on temperature. Thus warm water mirror carps do not produce antibodies when ambient temperature is less than 12°C but cold water trouts may produce antibodies even at 5°C. Roberts (1975) and Anderson and Roberts (1975) have noted that both defence mechanism and susceptibility to disease in a fish are dependent on temperature. With slightly higher than optimal temperature the wound healing of fish is quicker. Solubility of oxygen and other gases also depend on temperature. At higher temperature the fish metabolism is more but the solubility of oxygen is less.

Light

The growth and maturation rates of fishes are in general dependent on light energy or photoperiod. The growth of fish food organisms are dependent on solar energy for photosynthesis. However, excess of solar radiation may inhibit photosynthesis and may cause sun-burns of the fishes (Roberts, 1978).

Oxygen

Dissolved oxygen is a very important factor influencing the growth and survival of fish in a waterbody. Though oxygen is plenty in air (20.95%) the same is searce in water (0-14 ppm). Solubility of oxygen in water is inversely proportional to both temperature and salinity. A water body may get oxygen by four process :-

a) Diffusion, (b) Wave action, (c) Mechanical agitation and (d) Photosynthesis.

On the other hand the oxygen is consumed from the water body by fishes and other macro and micro organisms present in water and soil.

The oxygen consumption of fish is dependent on fish species, size, activity, temperature. feeding rate, and stress. Oxygen content in a water body ranging between 5.0 and 10.0 ppm during morning may be optimum for fish health. Low level of D.O. (Trace-1 ppm) may be lethal to many species if sustained for a long period. Oxygen content ranging between 1.0 and 5.0 ppm may have some adverse effects on growth, feed conversion and tolerance to disease. (Snieszko, 1973, Plumb *et al.,* 1976). Under culture conditions, CO_2 and NH_3 contents are often high, when D.O. content is low. Walters and Plumb (1980) showed the triad of environmental stresses to be more acute than low D.O. alone in causing bacterial infections in fish. A fish might survive 0.5 ppm dissolved oxygen for a few hours but not for several days.

According to McKee and Wolf (1962), the D.O. content of warmwater fish habitats should not be less than 5.0 ppm during atleast 16 hours of any 24 hrs period, but at no time the D.O. should be less than 3.0 ppm.

Supersaturation with atmospheric gases of waters falling over high dams can cause gas bubble disease and mortality in fish living in streams below. Fish died in a pond when D.O. content reached 300% of saturation, the lethal effect was due to oxygen bubbles surrounding the gills (McKee and Wolf, 1962). In fish ponds, the fish kill due to gas bubble disease is not so common. But D.O. supersaturation may adversely affect the fry and eggs of fish, restricted to surface by lack of mobility.

The dissolved oxygen in water may be estimated by Winkler method as described in A.P.H.A. Polarographic D.O. meter provide an easier and more rapid means of analysis. When using D.O. meters, it is important to calibrate the apparatus by the Winkler technique.

Carbondioxide

Carbondioxide is highly soluble in water but it is present in atmosphere in very small quantity. Less than 1% of CO_2 in water forms carbonic acid :

$$CO_2 + H_2O \rightleftarrows H^+ + CO_3^-$$

At higher CO_2 concentration, pH will be less. 30 ppm CO_2 would give in a pH of 4.8. Carbondioxide does not occur above pH 8.3 and carbonates only occur above this pH.

Carbondioxide is not very toxic to fish; most species will survive for several days in water containing upto 60 ppm CO_2, provided D.O. is plentiful (Hart, 1944). However, CO_2 content is generally quite high when D.O. content is low (Boyd, 1982). Under low D.O. content, high content of CO_2 hinders oxygen uptake by fish, causing respiratory problem and stress.

For healthy growth of fish 3 mg/l or less of free CO2 is permissible in pond or hatchery water. CO_2 content ranging between 12-50 ppm may have sublethal effects which may include respiratory stress and the development of kidney stones. High quantity (50-60 ppm) of free CO_2 is lethal to many fish species with prolonged exposure (Das and Das, 1997).

The free CO_2 content may be estimated by titrating the water sample with $N/_{44}$ NaOH using phenolphthalein as indicator. The end point is slight pink (A.P.H.A., 1980).

Ammonia

Ammonia may enter into a water body as fertilizer. However, in ponds where high densities of fish are fed with supplemental feeds, the ammonia content may increase to undesirably high levels.

In water unionised ammonia exists in a pH and temperatre dependent equilibrium with ammonium ions.

$$NH_3 + H_2O \rightleftarrows NH_4^+ + OH^-$$

Unionised ammonia is highly toxic to fish but ammonium ion is relatively non toxic. The higher the pH and temperature the higher is the percentage of total unionised ammonia (Boyd, 1982).

Ammonia stress : As ammonia level in water increases, ammonia excretion by fish decreases and levels of ammonia in blood and tissue increases. The result is an elevation of blood pH, and adverse effects on enzyme catalysed reactions and membrane stability. In fishes ammonia causes gill hyperplasia, reduced activity and growth. Liver, kidney and brain damage also occur. Sublethal concentration of ammonia causes pathological changes in fish organs and tissues (Smith and Piper, 1975).

Ammonia content in the range of 0.02-0.05 is safe for tropical fishes and prawn. Sublethal effect was noted depending on the species in the range of 0.05-0.4 ppm. But ammonia content ranging between 0.4 - 2.5 ppm may be lethal to many fishes and prawns.

Ammonia content is generally low in carp culture ponds. But ammonia level may be significantly high in intensive culture ponds of airbreathing catfishes (magur or singi) and there may be fish mortality if the ammonia content is not monitored regularly. In CIFRI/IDRC rural aquaculture project, the carp culture ponds had ammonia content in safe limit. But the magur ponds, where large quantity of trash fish was used as fish feed (5-10% of body wt of magur), we frequently noted significantly higher levels of free ammonia. Water replenishment was done to avert fish mortality.

Estimation : Ammonia content in water sample may be estimated colorimetrically by Nesslerization. The yellow to brown colour produced by Nessler ammonia reaction is measured by a Spectrophotometer at 400-425 mm.

Nitrite

In most natural water bodies and fish ponds, nitrite content is generally low. But, if the water body is contaminated with high organic pollution and has low oxygen content, the nitrite content may increase to toxic level.

Nitrite content between 1.0-10.0 ppm is lethal for many warm water fishes. In the range of 0.02 - 1.0 ppm, it is sublethal to many fish species.

Nitrite is highly toxic to fish. When nitrite is absorbed by fish it reacts with haemoglobin to form methemoglobin. Since, methemoglobin is not effective as an oxygen carrier, continuted absorption of nitrite can lead to hypoxia and cyanosis.

Addition of calcium and chloride may reduce the toxicity of nitrite to fish (Boyd, 1982).

Estimation of nitrite: Nitrite is estimated by measuring the colour of the reddish purple azo dye produced at pH 2.0 to 2.5 by coupling diazotized sulphanilic acid with N (1 napthyl) ethelenediamine dihydrochloride. The colour is measured by a spectrophotometer at 543 mm. (APHA, 1980).

Water reaction (pH)

The pH is defined as the negative logarithm of the hydrogen ion activity. $pH = -\log (H+)$.

Most natural waters have pH ranging between 6.5 and 9.0. However, if waters are more acidic than pH 6.5 or more alkaline than pH 9-9.5 for long periods, reproduction and growth of fish will diminish. (Swingle, 1961).

Effect of pH on fish health

pH 4.0	-	direct fish mortality
pH 4-5	-	sublethal effect on fish
pH 5-6.0	-	poor pond productivity, reduced fish growth.
pH 7.4-8.2	-	optimum for fish growth
pH 9.0-10.0	-	sublethal effect on fish
pH 10.0-11.0	-	lethal to many fish if exposed over a prolonged period

Estimation : The pH of water samples may be determined accurately by usuing a pH meter which has been standardised against two buffer solutions of known pH.

Hydrogen sulphide

Under anaerobic conditions, certain heterotropic bacteria use sulphate and other oxidised sulpher compounds as terminal electron acceptors in metabolism and excrete sulphide as follows.

$$SO4^{-2} + 8H^{+} \rightarrow S^{-2} + 4H_2O$$

The sulphide remains in equilibrium with hydrogen sulphide as follows.

$$H_2S = HS^{-} + H^{+}$$
$$HS^{-} = S^{-2} + H^{+}$$

The pH regulates the distribution of total reduced sulpher amongst its species. Unionised H_2S is toxic to fish but the ions resulting from its dissociation are not so toxic. The proportion of unionised hydrogen sulphide decreases with increasing pH.

Effect of hydrogen sulphide on fish health

3 ppm and above	-	Fish and prawn die instantly
0.01 - 0.5 ppm	–	lethal to fish. Any detectable concentration of H_2S should be considered detrimental to fish production (Boyd, 1982).

The toxicity of hydrogen sulphide in a water body may be reduced by frequent exchange of water and by increasing the water pH by liming. Unionised hydrogen sulphide content at different pH.

pH	***unionised H_2S*** (%)
5.0	99.0
6.0	91.1
7.0	50.6
8.0	9.3
9.0	1.0

Estimation

Iodometric method : Iodine reacts with sulphide in acid solution, oxidising it to sulpher. A titration based on this reaction is an accurate method for estimation of sulphide. The method is useful for wastewater and partly oxidised water (APHA, 1980).

Alkalinity

Alkalinity refers to the concentration of bases in water expressed in milligram per litre of equivalent calcium carbonate. In most waters bicarbonate, carbonate or both are the predominant bases.

Effect of alkalinity on fish health

0-20 ppm alkalinity	-	poor fish growth, creates stress in fish (Boyd, 1982).
20-50 ppm alkalinity	-	low to medium fish growth.
80-200 ppm alkalinity-		optimum for fish production.

Liming of fish ponds enhance its total alkalinity content.

Estimation : Total alkalinity is estimated by titration to the methyl orange end point (from yellow to pink) with standard acid (H_2SO_4 or Hcl).

Total hardness

Total hardness refers to the concentration of divalent metal ions in water expressed as ppm on equivalent calcium carbonate. The total hardness in majority of the freshwater ponds should be similar to the total alkalinity. However in quite higher than the total alkalinity. *Liming of fish ponds enhance their hardness contents.*

Effect of hardness of fish health

0 - 20 ppm hardness	-	Create stress on fish leading to poor fish growth
20 ppm and above	-	Congenial for fish production.

Liming of ponds enhance their hardness contents.

Estimation: Total hardness is determined by titration with standard ethelene diamine tetra acetic acid (EDTA) disodium salt usuing Eriochrome black-T as indicator. The end point is from reddish brown to Blue. (APHA, 1980).

Turbidity

Turbidity due to plankton is in general beneficial for fish growth. Turbidity due to high content of humic matter is not directly harmful to fishes but such waters are generally distrophic due to low nutrient levels, acidity and poor light penetration for photosynthesis. Turbidity due to suspended clay particles is most harmful for the fishes. Clay turbidity will reduce light penetration, adversely affecting productivity and some of the particles will settle to the bottom and smoother fish eggs and destroy benthic communities.

Effect of turbidity on fish health

Upto 10000 ppm	-	carps, tilapia and catfishes are generally tolerant to this level.
More than 20,000 ppm	-	create stress in fish.
More than 1,75,000 ppm	-	fish mortality, leading to poor fish production.

Estimation : Turbidity of water may be estimated by Jackson candle turbidimeter (APHA, 1980).

High clay turbidity in a water body may be controlled by alum treatment @ 150-250 kg/ha or application of organic matter (barnyard manure @ 2.5 ton/ha) .

Stress due to metal toxicity

In natural water bodies, the concentration of metals are generally low. But if the water reaction is acidic, the concentration of Iron, manganese and aluminium increases significantly causing stress to aquatic animals. In Jalpaiguri and Assam, where the soil and water reactions were acidic, growth and survival of fish in ponds were poor. Industrial effluents containing significantly higher quantity (chromium, lead, mercury, zinc etc.) when discharged to river, cause considerable harm to fish and fish food organisms.

Aluminium is toxic at pH 5.0 - 5.5
Safe level : 0.1 ppm of Al.
Cadmium : Safe level 0.004 ppm in hard waters and 0.0004 ppm in soft water.

Copper : Safe level 0.005 - 0.04 ppm

Iron : Safe level 1 ppm, 1.2-19.5 ppm - lethal to fishes

Lead : Safe level 0.005 ppm in soft water, 0.05 ppm - toxic to nervous system in fishes.

Mercury : Safe level - 0.0002 ppm in water

Zinc : Safe level 0.01 ppm - 0.05 ppm depending on water hardness.

Chromium : Permissible criteria 0.05 ppm, Desirable - should be absent

Some metals may be either beneficial or toxic, depending on their concentrations. At slightly alkaline pH (8.0-8.5), the metal toxicity is usually low.

Estimation : The metal content in water may be estimated accurately by an Atomic Absorption Spectrophotometer. The pretreated and concentrated samples are estimated for a metal by direct aspiration into an air acetylene flame employing particular lamp for that element.

Pesticides

Pesticides are complex organic compounds, which enter into the water body from adjacent catchment areas such as agricultural fields, forests etc. There are three types of pesticides.

1. Organochlorine (DDT, BHC etc.)
2. Organophosphate (parathion, malathion etc.)
3. Carbamates (carbofuran).

The indiscriminate use of pesticides has threatened the environment of fish and aquatic animals in various parts of the country.

Pesticides in natural water are present in very low concentration. However, organochlorine pesticides are very stable and they may be bioaccumulated in living organisms. The damage due to biological accumulation may be considerable. Even in sublethal concentrations, the pesticides DDT and ethyl parathion significantly affected the standard (basal) metabolism and activity of fishes (Srivastava *et al.*, 1977; Peer Mohamed *et al.*, 1978).

Safe level of common pesticides in water

DDT -	Safe level 0.002 ppm 96 h LC_{50} = 0.03 ppm
Dieldrin	Safe level = 0.005 ppm 96 h LC_{50} = 0.012 ppm
Endrin	Safe level = 0.002 ppm 96 h LC_{50} = 0.46 ppm
Malathion	Safe level = 0.008 ppm 96 h LC_{50} = 0.35 ppm
Carbaryl	96 h LC_{50} = 4.6 - 6.97 ppm

The joint action of a mixture of pesticides may be lethal to fish than effect of a single pesticides. The toxicity of pesticides varies with species and size of fish.

Estimation : Since the pesticide content in water is generally very low, they are extracted from water sample by a mixed solvent (diethyl ethar and hexane). The extract is concentrated by evaporation and if required cleaned up by column absorption chromatography. The individual pesticides are then determined by gas chromatography.

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Stress diagnosis in fish

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Introduction

Mortality of fish or decline in a fish population in a water body is at present the sole indicator that the effects of environmental stress factors are exceeding the acclimation tolerance limit of fish. However several physiological and whole animal changes occur that can be used to provide prior information that the effect of stress will exceed acclimation tolerance limit of fish and lead to dysfunction such as impaired fish health, growth or survival. These changes are a direct or indirect result of the physiological response to environmental stress and can be quantified and used as predictive indices.

Definition of stress

The term stress or stressor or stress factor is defined as the force or challenge in response to which there is a compensatory physiological change in fish. Thus, an environmental or biological stress is of significance if it requires a compensating response by a fish, population or ecosystem.

General Adaptation Syndrome (GAS)

The various physiological changes that occur as a fish respond to stressful stimulus are compensatory or in other words it is adaptive in nature and are required for acclimation. Collectively these phenomenon has been termed General Adaption Syndrome.

Conceptual Frame work of Stress response

The conceptual frame work is to consider the stress response in terms of primary secondary and tertiary changes.

i) *Primary response :* Following perception of a stressful stimulus by the central nervous system the stress hormones *viz.*, cortisol and epinephrine are synthesized and released into the blood stream.

ii) *Secondary response :* Changes in the blood and tissue chemistry and in the haematology occur, such as elevated blood sugar levels and reduced clotting time. Diuresis begins followed by blood electrolyte losses and osmoregulatory dysfunction. Tissue changes, include depletion of liver glycogen and interrenal Vit. C, hypertrophy of interrenal body.

iii) *Tertiary response :* Manifest in reduction of growth, resistance to diseases, reproductive success and survival. These may decrease recruitment to succeeding life stages as a result population decline occur.

Use of the physiological response as indicators

Several of the many changes that occur in response to stress can be used as measurable indices of the severity of stress on fish. These changes are a direct or indirect result of the physiological response to environmental changes and can be quantified and used as predictive indices.

Methods for stress diagnosis

Several biochemical and physiological procedures have been developed to assess the severity of the physiological effects resulting from stress. The physiological parameters of importance for assessing stress in fish at the primary, secondary and tertiary levels are discussed below.

Primary stress response

Plasma cortisol : A relatively direct assessment of the severity and duration of the primary stress response can be obtained by monitoring the rise and fall of plasma cortisol or catecholamines (epinephrine and nor epinephrine) concentrations.

Secondary stress response

The secondary changes that occur mainly in the blood chemistry also characterize the severity of stress in fishes *viz.*, blood glucose, chloride, lactic acid. They are frequently used for assessing stress response. Hyperglycemia for blood glucose and hypochloremia for blood chloride is the physiological effect of concern during stress response. Accumulation of lactic acid in muscle or blood hyperlacticemia is also an indicator of stress due to bright or severe exertion.

The haematological parameters also provide useful information about an animals tolerance to stress.

Haemoglobin/Haematocrit : It increase or decrease following acute stress can indicate whether haemodilution or haemoconcentration has occurred.

Leucocyte decrease (leucopenia) commonly occur during the physiological response to acute stressors. The blood clotting time and changes in the leucocyte count are among the most sensitive parameters indicating stress response.

Histopathology : Since many of the biochemical changes that occur in response to stress are the end result of cellular pathology histological examinations can frequently provide information on the effect of stress factors on fish. For example interrenal hypertrophy, atrophy of the gastric mucosa and cellular changes in gills are indicative of stress response.

The physiological tests of importance and their interpretations are given in Table 1.

Tertiary stress response

Experience have shown that several tertiary stress responses including changes in the metabolic rate, health, behaviour, growth, survival and reproductive success can indicate that unfavourable environmental conditions have exceeded acclimation tolerance limits of fish.

Metabolic rate : It is a fundamental aspect of animals performance and is affected by stress.

Reproduction : Detrimental effects on reproduction as manifested by oocyte atresia, spawning inhibition and decreased fecundity and hatching success are taken into consideration for assessing stress response.

Disease : Incidence of fish disease is an important indicator of environmental stress. Fish disease is actually the outcome of the interaction between the fish, their pathogens and the environment. If the environment deteriorates stressed fish is unable to resist the pathogens that they normally can resist. Certain diseases are proving to be useful indicators that tolerances of adverse environmental conditions have been exceeded.

Conclusion

Thus it is apparent that knowledge of the tolerance limits for acclimation to the single or cumulative effects of various biotic and abiotic stress factors is an important part of the data base for species habitat relationship needed for effective fishery management. Such information will solve many problems ranging from prediction of the tolerance fish will have for proposed habitat alterations to evaluation of the effects on fish health exerted by modern intensive fish culture.

Suggested reading

Stress and Fish. *Ed.* A.D. Pickering, 1981. Academic Press, London

Table 1 : Recommended physiological tests to assess the tolerance limits of fish for abiotic and biotic stress factors (compiled from Passino 1984; Buckley *et al.*, 1985). The interpretations of responses listed are general but not necessarily universal; investigators should be aware that there may be some stressful situations that do not evoke a change in one or more of these physiological conditions.

Physiological test	Interpretation if results are	
	Low	High
Blood cell counts	**Blood variables**	
Erythrocytes	Anemias, hemodilution due to impaired osmoregulation	Stress polycythemia, dehydration, hemoconcentration due to gill damage
Leucocytes	Leucopenia due to acute stress	Leucocytosis due to bacterial infection
Thrombocytes	Abnormal blood-clotting time	Thromobcytosis due to acute or chronic stress
Chloride, plasma	Gill chloride cell damage, compromised osmoregulation	Hemoconcentration, compromised osmoregulation
Cholesterol, plasma	Impaired lipid metabolism	Chronic stress, dietary lipid imbalance
Clotting time, blood	Acute stress, thrombocytopenia	Sulfonamides or antibiotic disease treatments affecting the intestinal microflora
Cortisol, plasma	Normal conditions	Chronic or acute stress
Glucose, plasma	Inanition	Acute or chronic stress
Hematocrit, blood	Anemias , hemodilution	Hemoconcentration due to gill damage, dehydration, stress polycythemia
Hemoglobin, blood	Anemias, hemodilution, nutritional disease	Hemoconcentration due to gill damage, dehydration, stress polycythemia
Hemoglobin, mucus	Normal conditions	Acute stress
Lactic acid, blood	Normal conditions	Acute or chronic stress, swimming fatigue
Leucocrit	Acute stress	Leucocytosis, subclinical infections
Blood osmolality, plasma	External parasite infestation, contaminant exposure, hemodilution	Dehydration, salinity increases in excess of osmoregulatory capacity, diuresis, acidosis
Blood total protein, plasma	Infectious disease, kidney damage, nutritional imbalance, inanition	Hemoconcentration, impaired water balance
	Tissue variables	
Adenylate energy charge, muscle and liver	Bioenergetic demands of chronic stress	No recognized significance
Gastric atrophy	Normal conditions	Chronic stress
Glycogen, liver and muscle	Chronic stress, inanition	Liver damage due to excessive vacuolation, diet dtoo high in carbohydrates
Interrenal hypertrophy, cell size and nuclear diameter	No recognized significance	Chronic stress
RNA : DNA ratios, muscle	Impaired growth, chronic stress	Good growth

Stress effect evaluation in fish through growth performance

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An ecosystem is considered to be ideal if environmentally favourable for recycle and harmonious coexistence of community population in good health and hygiene. Every stress in environment is problematic, either it causes lethality in extrimity or produce sublethal and chronic effect of prolonged and cumulative nature in low profile. Mostly chronic effects inactivate physiological functions ultimately leading to growth retardation and also immunosuppression in affected animals. In this process, the situation further deteriorates as the unhealthy animals become hosts for the pathogens. However, may the reasons be either environmental distress or disease infestation, the effect of physiological inactivation is reflected in poor growth performance of the animals. Thus, growth performance can act as an ideal stress index for the fish in aquatic ecosystems.

1. Growth

The term 'growth' in living beings defines length/height/body area proportionate weight/biomass gain over a limited period of time, Biologically 'growth' is comprehended as compounded effect of physiological activites mainly feeding, digestion, assimilation and conversion with support of environmental necessities.

1.1 Fish growth

These aquatic poikilotherms are unique in respect of growth pattern. In minnows (*A.mola, G.chapra, Puntius* spp.) the growth is at very slow pace, while the same is at faster rate in carps (*L.rohita, C.catla, C.idellus*). Fishes respond very quickly to the natural and artificial feeds and attain faster growth. While on the other hand these creatures withstand food scarcity and starvation for a longer period utilising stored energy. In fish, growth performance depends on various environmental and biological factors.

2. Growth factors

2.1 Environmental factors

All living organisms exhibit a preference for environmental conditions in which their life process takes place most efficiently. In aquatic ecosystems these conditions are mainly physico-chemical qualities of water and sediments. Beside the phenomenal role of water and sediments, factors like accidental or unaware introduction of foreign substances also influences the environment.

2.1.1 Physical factors

Aquatic environment is very complex as regards the role of inter and intra factorial reactions in influencing ecological activities. Some physical factors like temperature and space are directly linked with the growth performance of the biocommunities inclusive of fishes at the top of the trophic levels.

2.1.1.1 Temperature

It is a basic need in maintenance of normal metabolism. Variations either to hypothermic or hyperthermic condition interfere normal metabolism and also other physiological function in thermal affected animals. Apart from playing antagonism to physiological activites, hyperthermism changes viscocity of water and inacts its oxygen retaining capacity. Besides, abnormally high temperature causes luxuriance in macrophytic population. The undesirable over growth of macrophytes creats oxygen imbalance for the other to survive and also eutrophicates environment and makes the ecosystem unhealthy for the inhabitants. In totality thermal extremities counteract many ecological activities including normal life processes also in fish.

2.1.1.2 Space

Like other animals fish also needs optimum space for stress free life. The knowledge about optimum space requirement for fishes, specially of major carps is inadequate. Experimental trials with stocking densities revealed better production possibilities of IMC in 1.0 to 2.5 m^3/fish of water space in culture ponds. Space scarcity beside obstructing the animal movements, results enviromental contamination due to excessive and unmanageable increase in metabolic and other refusal in the system.

2.1.2 Chemical factors

In aquatic systems the chemical and physical factors are closely interrelated. As such segregation of any such factor's role is difficult to ascertain. However, some chemical factors like dissolved oxygen, pH, hardness and CO_2 are known for their important role in maintaining the ecological stability.

The most fluctuating environmental component in aquatic system is oxygen, mainly a *in situ* product of phytosynthetic reactions. Inevitable for every living beings, with diurnal fluctuation, oxygen becomes crucial factor for sustainance of community members at the time of acute depletion in the environment. The other factors like pH, alkalinity, CO_2 etc, though not as essential as oxygen, influence the ecological environment to a great extent. Fishes, at the highest of trophic levels, depend maximum on environmental clarity *i.e.*, the physico-chemical balance for their normal life processes.

2.2 Biological factors

Growth, a cumulative progression and reflection of bodily gain in biomass depends on various biological and nonbiological factors. Involvement of physiological processes like feeding, assimilation and conversion are very significant. Through these processes, the building materials like carbohydrate, protein, fat, salts and vitamins enter the system. These energy

materials get stored up within the body tissues after meeting maintenance requirements. Precisely, synchronised functions of ingestion, digestion, circulation and excretion systems are responsive of normal growth attainment. However, food and feeding contribute to the main energy supply from external sources. Inspite of sufficiency in food supply and physiological soundness growth performance may suffer if the animal concerned becomes victim of pathogenic intrusion.

3. Growth estimation

The relationship between length and weight putforth the conversion value for calculating length proportionate weight and *vice-versa* which is most effective method for estimation of growth performance in fish. The general equation for length-weight relationship is expressed as:

$$(W = aL^n)$$

where W = weight, L = length, a is a constant, and n, an exponent. Values for a and n are to be determined numerically. The longarithmic formula for expressing length-weight relationship stands as :

$$\text{Log } W = \log a + n \log L$$

3.1 Data collection

For determining the actual value of exponent (n) in equation $W = aL^n$, length and weight of individual fish should be recorded in millimeter and gram respectively. The sample size should be large to the best of availability. The fishes need be grouped in different size ranges of suitable length. Mean values for each size group worked out and recorded for further calculation.

3.2 Calculation

Record Log values of the individual length and weight. Put the values in the following table and fill-up the columns as required.

L	LogL	W	LogW	Log L x Log W	$(\text{Log L})^2$

Summarise, the column and substitute the following equation, where N = the number of individuals (or number of groups in grouped data).

$$\text{Log } a = \frac{\sum \text{Log } W \cdot \sum (\text{LogL})^2 - \sum \text{LogL} \cdot \sum (\text{Log L} \cdot \text{logW})}{N \cdot \sum (\text{logL})^2 - (\sum \text{logL})^2}$$

Utilising the value for log a find 'n' in the equation.

$$n = \frac{\sum \log W - (N.\text{Log } a)}{\log L}$$

The ascertained Log a and n values now may be substituted in formula.

Log W = Log a + n log l

Determine calculated weight by obtaining antilogs of Log a + n Log L values for each individual or group. Select a weight and calculate corresponding length or vice-versa.

Add up the values obtained by dividing the actual weight by calculated weight of all the individual or group. Thus, the mean value (k) will be 1.0. If this value is less than one it indicates poor growth of the studied population.

A case study

Fish population from two different ecosystems have been considered for the present study. Environmentally one ecosystem (A) is different from the other (B). The K value for fish population of 'A' is calculated and the details are as below :

L	LogL	W	LogW	LogL x LogW	$(Log L)^2$
95	1.9777	19.00	1.2788	2.5290	3.9113
105	2.0218	22.60	1.3541	2.7377	4.0864
115	2.0607	30.33	1.4819	3.0537	4.2465
125	2.0969	37.00	1.5682	3.2854	4.3970
155	2.1903	50.00	1.6990	3.7213	4.7975
165	2.2175	71.40	1.8537	4.1106	4.9173
175	2.2430	80.00	1.9049	4.2726	5.0311
185	2.2672	106.00	2.0253	4.5918	5.1401
195	2.2900	98.50	1.9934	4.5650	5.2442
205	2.3118	110.67	2.0440	4.7254	5.3443
215	2.3324	153.00	2.1847	5.0956	5.4402
225	2.3522	170.00	2.2304	5.2465	5.5328
265	2.4232	281.00	2.4487	5.9337	5.8720
285	2.4548	341.00	2.5328	6.2174	6.0262
	31.2395		26.5999	60.0887	69.9869

$$\text{Log a} = \frac{26.5999 \times 69.9869 - 31.2395 \times 60.0887}{14 \times 69.9869 - 975.9064}$$

$$= \frac{-15.4964}{3.9102}$$

$$= -3.9631$$

$$n = \frac{26.5999 - (14 \times -3.6931)}{31.2395}$$

$$= 2.6275$$

Substituted log a and n values in the formula

$$\text{Log } W = \log a + n \log L$$

L	LogL	n log L	log a + n log L	Calculated W1	W	$W/_{W1}$
95	1.9777	5.1964	1.3112	20.4737	19.00	0.9280
105	2.0218	5.3123	1.3492	22.3450	22.60	1.0114
115	2.0607	5.4145	1.4514	28.2741	30.33	1.0610
125	2.0969	5.5096	1.5465	38.1969	37.00	1.0512
155	2.1903	5.7550	1.7919	61.9317	50.00	0.8073
165	2.2175	5.8265	1.8634	73.0098	71.90	0.9780
175	2.2430	5.8935	1.9304	85.1888	80.00	0.9391
185	2.2672	5.9571	1.9940	98.6207	106.00	1.0748
195	2.2900	6.0170	2.0537	113.2075	98.50	0.8701
205	2.3118	6.0743	2.1112	129.1679	110.67	0.8568
215	2.3324	6.1284	2.1653	146.3124	153.00	1.0457
225	2.3522	6.1804	2.2173	164.9322	170.00	1.0307
265	2.4232	6.3670	2.4039	253.4300	281.00	1.1088
285	2.4548	6.4500	2.4869	306.8224	341.00	1.1114
						K = 0.9910

The log a and n values estimated for the fish population in ecosystem "A" are substituted for calculating "K" for the fish population in ecosystem "B".

L	Log[1]	n log L	log a + n log L	Calculated W	W	W/W1
105	2.6212	5.3107	1.3476	22.2638	20.00	0.8983
115	2.0607	5.4145	1.4514	28.2746	20.00	0.7073
125	2.0969	5.5096	1.5465	35.1965	27.50	1.2799
145	2.1614	5.6791	1.7160	51.9996	40.00	0.7692
155	2.1903	5.7550	1.7919	61.9298	46.50	0.7509
175	2.2430	5.8935	1.9304	85.1922	60.00	0.7043
						K = 0.8517

The study revealed comparatively low "K" value of the fish population in ecosystem B, thus indicated suppressed growth performance due to environmental or any other reason.

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Role of bioassay for estimation of doses of chemotherapeutic compounds

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Introduction

In the last two decades, man has modified his environment beyond limits, as a result of enormous technological advancement. The problem of environmental pollution is more severe in developing countries like India, because of limited economic and technological resources, than the developed countries. The disposal of industrial wastes poses a serious problem due to their diverse composition. Interaction of these components further increase their complexity Environmental contaminants have toxic effects on different types of organisms and affect biological process at cellular, populations, community and ecosystem levels of organisms. The problem of contaminants affecting aquatic system is complex and there is a continuing need for monitoring and mitigating the effect. Since pollution affects living organism, it is logical that it would be measured biologically. A study of the sturucture and function of a biological community in the waste receiving water and laboratory bioassays for determining toxicants are some of the basic approaches that biologists choose for pollution monitoring.

Wedemeyer and MC Leay (1981) while defining the relationship of environment *vis-a-vis* pathogen *vis-a-vis* explained that the presence of fish pathogens will result in epizootics only if unfavourable environmental conditions also exist and the host defence mechanism has been compromised. If the relationship is balanced, good health and growth will occur. If it is marginally changed, chronic disease problems and reduced growth will occur. If it is unsatisfactory, poor growth and disease will occur. Thus environmental parameters of fish and prawn rearing facilities are of utmost importance for raising a healthy crop.

Bioassays are conducted to evaluate the nature of effluent and its components, so that a safe limit can be predicted by laboratory test of animals. Such bioassays are also useful for evaluating the efficiency of a treatment system by comparing the toxicity of the effluent before and after treatment. The bioassay is a test by which aquatic organism are used to detect or measure the presence of effect of one or more substances, waste or environmental factors alone, or in combinations on aquatic organisms. Most pollution problem involve discharges of unknown and variable composition where more than one toxicant or stress is present. In evaluating the criteria for specific toxicants consideration must be given to other environmental influence such as dissolved oxygen, temperature and pH.

In recent years toxicity testing of industrial and domestic wastes has been carried out on a battery of biological organisms .The toxicity measurement through a test species gives correct picture whose dose response relationship could be obtained through time, either experimentally or by continuous field measuring . In aquatic toxicology, fish have been widely and popularly acclaimed as a test species for evaluating the potency of toxicant to cause lethality (acute toxicity) or other sub-lethal responses, using selected behavioural, bio-chemical, or physiological and haematological responses.

Toxicity testing through fish bioassays is a simple basic tool for detection , evaluation and abatement of water pollution. Entry of toxicants into the receiving water triggers a series of events which directly or indirectly affect aquatic life. The possible effects may range from death and mortality of sensitive species, to impairment of growth, reproduction and metabolic functions in organisms or changes in the physical and chemical properties of ambient medium that indirectly affects the resident biota in the water. Assesment of toxicity in aquatic ecosystem is conventionally done through bioassays or testing procedures using fish as a test organisms. Fish is a useful test organisms in aquatic toxicological studies with the logic , that if fish life is protected, the rest of the aquatic food chain is protected as well. data generated from fish bioassays, whether by testing pure chemicals or industrial effluents are used for :

- Prediction of environmental effects.
- Comparison off toxicants
- Comparison of sensivitivity of test animals and conditions
- Regulation of discharge.

Bioassay- Methods

Toxicity bioassays can be categorised as

1. Acute toxicity
2. Chronic toxicity

1. Acute or Lethal toxicity

It is used to determine the level of the toxic agent that produces an adverse effect on a specified percentage of the test organisms in a short period of time .Acute toxicity is measured experimentally for 50% mortality of test animals during 24, 48, and 96 hours exposure period. Results are expressed as 96 hour median lethal concentration (LC_{50}) or 96 hour TLM. For regulatory agencies, the acute data is useful in drafting toxicologically based, water quality and effluent discharge permits.Acute toxicity tests are conducted using four techniques.

i) ***Static Tests*** : Solutions and organisms are kept in the test chambers for a specific duration of the experiment.

ii) ***Recirculation : techniques*** Solutions are continously circulated through an apparatus to maintain water quality by such means as aeration, filteration, and stabilisation and then recyled to the test chambers.

iii) ***Renewal : techniques*** This is improved static test in the sense that an attempt is made to maintain the water quality. The test solutions are periodically (usually once in 24 hrs) or replaced with fresh test solutions of the same composition.

iv) ***Flow Through : technique*** In this method there is a continuous flow of test solutions through the chamber at a fixed rate during the entire test period. Large volumes of test solutions are prepared before the begining of test and these are allowed to flow through the chambers. Fresh test solutions are prepared continously in a special toxicant delivery system and allowed to flow through the chambers.

Flow through test is recommended over static test, since concentration of the compound and DO level is uniformly maintained throughout the test period. Flow through should allow a reasonable rate of flow of test solution per gram of fish, approximately 2 or 3 g/day or higher, for small fish and fairly rapid replacement time of waters, approximately 90% replacement in 6 - 12 hours

2. Chronic or sublethal testing

This type of bioassays demonstrates the effects of long term exposure of test species to toxicant concentrations much lower than the lethal levels. The effects may produce conditions that might interfere with some of normal life functions of the organisms rather than killing directly. Chronic tests data are used for estabilishing water quality standards and prediction but are of little use for control of effluent disposal in receiving water. Chronic tests usually cover the life cycle span of the tests species and are expensive and time consuming. They are however useful for risk evaluation and development of a water quality criteria for protection and conservation of aquatic life.

The procedure includes, exposure of animals to five or six sublethal toxicant concentrations along with a control. Fish tests often start with 40 - 50 individuals per tank. The numbers are reduced at intervals, since a known numbers is removed periodically to observe chronic effects, *viz.*, growth, development, tissue damage, haemtological, biochemical, morphological, and behavioral changes. Changes are considered to be real only when they can positively related to toxicant exposure and compared to control tests. Four procedures are used in chronic testing.

Short term toxicity bioassay has been developed into a useful tool in water quality management. In acute toxicity test or bioassay the harmful properties of a substance which are demonstrated within a short period of exposure, usually in 96 hrs are determined. These tests give information on comparative toxicity of several compounds. Further, data derived from these toxicity studies will help various industrial mangements to take necessary precautions of treating the effluents before discharging them into aquatic systems thereby reducing many ecological problems. Similarly for estimation of doses of chemicals used for therapy in fish diseases control require bioassays to ascertain the doses which are harmful to pathogens but not to fish/ prawn in ponds and lakes.

In the case of aquatic animals, the concentration of the substance in water (mg/l) is taken into account. the mortality data is evaluated statistically by the method of Litchfield and Wilcoxon (1971), Probit analysis (Finney, 1971) or by any other suitable method. The result is LC_{50} or TLM, the concentration necessary to produce death in 50% of the test population tested, 95% confidence limits are derived , which define the accuracy of the test. Interpretation of the acute toxicity is important than just the LC_{50} value and the basis is for comparison with other chemicals and it is the first oppurtunity to observe the biological effects of the compound. Further, an accurate description of the signs of intoxification and behaviour are equally important as the numerical data.

CHEMOTHERAPY OF FISH

Chemotherapeutics or drugs which are capable of affecting or killing micro-organisms especially bacteria , in the lymphatics of organisms or of selectively damaging tumor cells. Antiparasite, disinfectants and anesthetics have been used for a long time for the prophylaxis and treatment of fish diseases. Together with the antibiotics, sulfonamides, nitrofurans, fungistats, coccidiostats, vitamins, and hormones and drugs give good results.

It is quite important that there should be a clear insight on the efficacy and tolerance of drugs in fish, their secondary effects as well as their metabolism and formation of residues. The internal and external dosages for fish mostly depend on the water temperature. The concentration for bath treatments are ascertained generally in aquaria with water of drinking quality. If using pond water, sea water, or the water at fish farms, it must be considered that different concentrations will be necessary on account of chemical oxidative and reducing processes, decomposition and physical absorption. For instance , the variable chlorine binding capacity of surface water is well known , as also the varying consumption of $KMnO_4$ (chemical oxygen consumption, COC) and so on. It is therefore possible to effectively control fish diseases only when diagnosis, prophylaxis and therapy are regarded as an integeral unit, taking at the same time the water temperature and other factors of water analysis to, consideration. Basically dosage distinction comprise four types; ineffective, effective, toxic, and lethal.

The scope between toxic and effective dosage = Therapeutive range, therapeutic index, therapeutic quotient should be as large as possible.

The therapeutic Quotient (Q^{th}) is obtained from

$$Qth = \frac{\text{Toxic dose 50}}{\text{Crative dose 50}}$$

and comes to, for instance about 2-3 for narcotics and 100 for pencillin.

Doses should be differenciated as follows:

SD = Single dose , therapeutically effective single dose.
SMD = Single maximum dose.
DD = Daily dose for 24 hrs
DMD = Daily maximum dose

A dosage measure in mg per kg body weight keeps in conformity to modern therapy. Thus for instance Chloramphenicol ID = 1x 50 mg/kg weight of fish

MD = 3 x 2 daily each 30 mg/kg of fish weight.

The expression measures such as per kg weight of feed per 100 kg weight of feed is also not advisable , since the quantityconsumed is highly dependent on temperature.

Safe Concentrations of Effluents

The statistical methods which estimates the LC_{50} values of the aquatic organisms include graphical interpolation, Litchfield- Wilcoxon, Probit, Logit, Spearman - Karbes, Reed- Muench and moving average methods. Various attempts have been carried out to utilise the data obtained by short term acute toxicity tests to determine the dilution required to protect aquatic populations. Alabaster and Abran (1965) made a detailed study of several methods to find the best method of determining the threshold value of toxicity *(i.e.,* the concentration at which the fish are minimally affected) of several compounds from the data obtained in toxicity bioassays. Their calculations were based on the equations :

$$t = \frac{K}{\log_c = \log_1}$$

Where t = the harmonic mean survival time.
K = a constant.
C = Concentration of toxin.
I = mean threshold concentration.

According to APHA/ AWWA /WPCF "Maximum allowable threshold concentration (MATC) *i.e.*, the concentration of toxic waste that may be present in the receiving waters without causing significant harm to its productivity and all its various uses) is determined by a long term bioassays of a partial life cycle with the sensitive life stages or a full life cycle of the test organisms in which a range of concentrations of the toxicant under test that do not demonstrate significant harm to the test organisms is determined . Various indirect method for estimation of MATC or SC for toxicants including the use of application factor have been proposed.

$$AF = \frac{\text{MATC or SC}}{\text{In cipient } LC_{50}}$$

The mean AF value for one toxicant for different fish species varies by a factor of 2 to 5

The use of this fractional application factors by which LC values are multiplied to arrive at safe concentrations of toxic substances has been favoured widely. Basak and Konar (1977) derived the application factor by dividing 168 hr - LC50 value by 168 hr -LC_{100} value (the concentrations respectively none and all died in 168 hr) under static bioassay condition. The safe concentration is derived by multiplying this factor and LC 50 values (168hr LC_{50} value) . According to them this application rate (SAR) will be safe not only to the species tested (*Cyprinus carpio, Heteropneustes fossilis* and *Tilapia mossambica*) but also to other species having similar tolerance. Further , they claim that SAR will be safe to fish under varried water quality conditions and even at wide temperatures fluctuations (from 21° C to 38° C).

Thus, we can calculate the "Safe concentrations" as follows (from the data given under Litchfield and Wilcoxon method .

LC_0 = Effluent concentration 10%

LC_{100} = Effluent concentration 90%

$$\text{Safe concentration rate} \quad \frac{LC_0}{LC_{100}} = \frac{10}{90} = 0.11$$

Suppose LC_{50} = 54

As such Safe Concentration of effluent Concentration = LC_{50} x SAR

= 54 x 0.11 = 5.94%

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Table 1. Water quality criteria significant to fish/prawn health

Sl.No.	Water quality	Range values	Effects	References
1	**Dissolved oxygen**	5.0 mgl^{-1} 1.0-5.0 mgl^{-1} 0.3-0.8	Optimum Sub lethal effect Lethal to many species	
2	**Ammonia** 1) NH_3 (Unionised form) 2) NH_4^+ ionised Form. (Depends on pH & temperature)	More toxicto fish 0.02-0.05 mgl^{-1}-safe 0.05-0.4 mgl^{-1} sub lethal 0.4-2.5 mgl^{-1} lethal	Maintain equlibrium $NH_3 + H_2O - NH_4 + OH$ cause gill hyperplasia Reduced activity, growth Liver Kidney & brain damage	Robinette (1976)
3	**Nitrite** An intermediate product of biological oxidation of ammonia to nitrate (Nitrification Process)	0.02-1.0 mgl^{-1} 1.0-10 mgl^{-1}	Sub lethal Lethal level, Reacts with haemoglobin of blood to farm methaemoglobin.	
4	**pH** (Conc. of hydrogen in water)	Less than 7.0 (acidic) greater than 7.0 (alkaline) optimum 6-9.0	9-Sub lethal 10-11 lethal 10-11 lethal to all fish/prawn 5.0-6.0 poor 4.0-5.0 Sub lethal 4.0-Direct mortality	Swingle, (1961), Boyd, (1982)
5	**Alkalinity** Concentration of bases (in water) bicarbonate & Ca)	20-3000 mgl^{-1} less than 20-mgl^{-1}	Ideal fish creates stress in fish	(Boyd, 1982)
6	**Total Hardness** (Carbonates of Ca & Mg)	> 20 mgl^{-1} < 20 mgl^{-1} (Treatment with line)	Satisfactory Creates stress in fish	Boyd, 1982
7	**Cabondioxide** free CO_2, bicarbonate ion (HCO_3) & Carbonate (CO_3)	12-50 mgl^{-1} 50-60 mgl^{-1}	Sublethal effect Lethal to manyfish	
8	**Hydrogen sulfide**	3 mgl^{-1} 0.01-0.5 mgl^{-1} 0.1-0.2 mgl^{-1}	Prawn die instantly Lethal fish, creates stress to fish Prawn loose eqlibrium Sub lethal effect	Boyd, 1982
9	Suspended Solid	-	-	-

Table 1 contd.....

10	**Metals** (Most metals dissolve in acid water *ie* < pH 7.0)			
A.	Aluminium (Al) Occur in pH 5.0-5.5	0.1 mgl^{-1}	Safe level	EPA
B.	Cadmium (Cd)	0.004 mgl^{-1} 0.000 4 mgl^{-1} 96ha LC_{50} (*O. mossambicus)*	In hard water In soft water 73.09 mgl-1	EPA Kaviraj & Konar (1983)
C.	Copper (Cu) 96 h LC50 (*O. mossambicus)*	0.00 mgl^{-1} 0.02 mgl^{-1} 0.04 mgl^{-1} 34.7 Mgl^{-1} at pH 7.0	In soft water-safe ,, ,, ,, ,,	10 mgl^{-1} $CaCO_3$ 50 mgl^{-1} $CaCO_3$ 100 mgl^{-1} $CaCO_3$ Mukhopadhyaya *et al* (1984)
D.	Iron (Fe) Due to ferric hydro-oxide ppt. 96 h LC50 (*O. mossambicus)*	1.0 mgl^{-1} 1.2-10.5 mgl^{-1}	In water safe lethal to *C. carpio* 83.20 mgl^{-1} at pH 7.0 118.0 mgl^{-1} at pH 8.5	EPA (1971) ,,
E.	Lead (Pb)	0.005 mgl^{-1} 0.03 mgl^{-1} 0.05 mgl^{-1} 0.0002 mgl^{-1} 0.0005mgl^{-1}	Safe in soft water *ie* 10 mgl^{-1} $CaCO_3$ In other waters Sublethal effects Safe in water Safe aquatic organism	 EPA (1971)
F.	Mercury (Hg) 96 hr LC_{50} for *O. mossambicus)*	0.0722 mgl^{-1}	-	EPA (1971) Kaviraj *et al* (1983)
G.	Niekel (Ni)	0.01 mgl. at 20 mgl^{-1} 0.04 mgl. at 320 mgl^{-1}	Hardness-Safe Safe	EPA (1971)
H.	Zinc (Zn) 96 h LC_{50} for *O. mossambicus*	0.01 0.05 19.09 mg l^{-1} at pH 7.0 62.7 mg l^{-1} at pH 8.5	In safe water 10 mgl-1 $CaCO_3$-safe In safe water 50 mgl^{-1} $CaCO_3$-safe	EPA Mukhopadhyay (1984
I.	Chromium 96 h LC_{50} for *O. mossambicus*	30.03 mgl^{-1}	-	Kaviraj *et al.* (1983)

Table 2. Chemotherapeutic compounds commonly used for controlling diseases

No.	Disease	Chemotherapeutic components used
1	Disease caused by Bacteria	Antibiotic therapy oxytetracycline or erythromycin for 5 days @ 50-90 mgl^{-1} Bath & 500-100 $mgkg^{-1}$ feed treatment
2	Disease caused by virus	i) Liming the pond @ 50 $kgha^{-1}$ ii) bath Treatment of affected fish with Sodium Chloride @ 3-5%
3	Disease caused by Protozoa	i) Treatment of pond with mahua oil cake and lime ii) Sodium Chloride Bath treatment @ 3-5% destroy the spores
A	Ichthyophthiriasis Mainly *C. Carpio, L. rohita* and rearing ponds affected	i) Bath in 1:5,000 form alin solution for 7 days ii) Bath is 2% Sodium Chloride Solution for 7 days or more.
B	Trichodiniasis disease	i) Sodium chloride @ 2-3% fill fishes are stressed. ii) $KMnO_4$ treatment @ 4 mgl^{-1} in pond iii) Formalin treatment @ 25 mgl^{-1} in pond iv) Formalin bath treatment @ 100 mgl^{-1} with aeration
C	External fouling in prawns (*Macrobrachium rosenbergii* and *Penaeus monodon*	i) Formalin treatment @ 20-30 mgl^{-1} in the pond preferably with aeration ii) Formalin bath treatment for larval intestation @ 100 kgl^{-1} with aration.
D	White gill spot (major carps affected mainly)	i) Treat the pond with Mahua oil cake and lime by which the ineffective spores destroyed to a great extent. ii) Sodium Chloride treatment @ 3-5% destroy the spores and other developing stages.
E	White Scale spot (*C. mrigala* and *L. rohita)* mainly affected	-do-
4	Disease caused by Helminth	i) Sodium chloride treatment @ 3-5% for 10-15 minutes. Kills the worms on fish ii) Formalin bath treatment @ 100 mgl^{-1} kills the worms iii) formalin treatment in pond @ 25 mgl^{-1} is effective in controlling the worms. ix) Potassium pamagnate treatment in pond @ 4 mgl^{-1} effective is condtrolling worms.
A	Black spot disease (*C. catla, L. rohita* and *H. molitrix*	Removal of the resident of molluscan population
B	Ligulosis disease (*C. catla, L. rohita, L. calbasu*)	Examination of the definite host

Table 2 ..... Contd /-

5	Disease caused by crustacea	i) Sodium Chloride @ 3-5% till fishes are stressed ii) Gammexane treatment in pond @ 1 mg/l iii) Potasium permangnate treatment in pond @ 4 mg/l
A.	*Argulosis Disease* (Major carps)	
B.	Ergasilosis disease (Major carp *H. molitrix, C. idella* and *L. Parsia*	i) Potsium Permangnate @ 4 mgl^{-1} in pond. ii) Sodium chloride bath @ 2-3% for 15 minutes
C.	Lernaeosis disease	i) Potasium permagnate @ 4 mgl^{-1} in pond ii) Sodium Chloride bath treatment @ 3-5%
6	EUS (Epizootic Ulcerative Syndrome Sodium Chloride bath 3-4% (VU CIFAX-1 litre/../.....	i) Lime-100-600 kg ha^{-1} ii) $KMnO_4$--1-10 mgl^{-1} pond treatment 4 mgl^{-1} iii) Application of CaO- 50 kg ha^{-1} and after..... bleaching powder @ 0.5 mgl^{-1}

Table 3. Freshwater fishes used in bioassay studies

Common name	Scientific name
Major carps	*Laboe rohita* (Ham.) *Ctenopharyngodon idella* (Valenciennes)
Common carps	*Cyprinus carpio* (Var. Communis)
Minor carp	*Puntius sophore* (Ham.) Rosborea daviconies (Ham.)
Air breathers	*Heteropneustes fossilis* (Bloch) C. punctatus (Bloch)
Catfishes	Tilapia mossambica (Peters) *Lelistes reticulatus* (Peters)

Table 4. Classification of water based on hardness as indicated below

Hardness expressed ppm $CaCO_3$	Quality of water
0-50	Soft
50-100	Slightly hard
100-150	Slightly hard
150-200	Moderately hard
200-300	Hard
300 above	Very hard

Table 5. Some commonly used chemotheraputic components used for fish disease control

Sl. No.	Chemotherapeutic	Doses commonly used
1.	Copper sulphate	1 g dissolved in 10 litres of water (1: 10 000 or 100 ppm) Applied for 10-30 minutes.
2.	Ammonia	1 ml/l water
3.	Ammonia Chloride	10-25 g/l (1.5-2.5%) or (15 000-25 000 ppm)
4.	Hydrogen peroxide	5 000 ppm
5.	Salyeylic acid	0.5%- for 30 minutes
6.	Sodium Chloride	2.5% or (25 000 ppm) Solution or 25 g NaCl/l water. Young ones 1.0-1.5% (10 000-15 000 ppm)
7.	Formalin (40% solution)	A strong soln. (1:1000 or 1000 ppm)-15 min. A week soln. (1:5000) or 200 ppm- 30-45 minutes.
8.	Malachite gran	1:5000 000 (0.2 ppm)-60 minutes
9.	Nitorfuran derivatine	1:1000 000 (1 ppm)
10.	Hyaminie-3500	**Hardness** — **Bath conc.** <100 — 1:500 000 (2 ppm) 100-200 — 1:330 000 (3 ppm) > 200 — 1:250 000 (4 ppm)
11.	Quinine sulphate	1g. of Quimine salt 50.75 & 100 l. of water
12.	Synthetic dye bath (Trypaflavin)	1 g. Per 100 l. of water
13.	DDT-Bath	1:50000 00-...% 10.000 000 (0.01-0.0[illegible] ppm)

Application of chemotherapeutic compounds in the aquatic environment for controlling fish diseases

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For preventing fish and prawn diseases, the essential features should form a good husbandry and proper monitoring of the stock. Optimum water quality should be maintained and monitored periodically. Possible prophylactic measures should be taken towards disinfection of water bodies as well as the fish and prawn stock. The fishing appliances should be disinfected. Availability of quality or nutritious food should be ensured. Live food should preferably be checked for the presence of pathogens, if any. Proper quarantine measures should be adopted, if possible, so that exotic pathogens do not get easy access to the local areas. After taking all these cares, if a disease outbreak occurs, one should then embark upon chemotherapeutic measures for controlling the outbreaks of diseases. Since, most of the measures employ chemical compounds for treatment, great care, in general, is needed for their use.

i) Chemotherapy for bacterial diseases

For bacterial fish and prawn diseases like dropsy, the therapeutic application of $KMnO_4$ @ 5 mg lit. appears suitable for containing the diseases.

For columnaris disease, the therapy may be the dip treatment of the affected fishes with 500 mg lit. of $KMnO_4$. The pond water may be treated with 3-5 mg/lit. of the same.

For bacterial ulcerative diseases, the therapy may include treatment of pond water with $KMnO_4$ @ 5 mg lit. Additionally,drug or antibiotic treatment may be taken up. Sulphadiazine incorporation in feed @ 100 mg/kg or terramycin treatment @ 75-80 mg/kg body wt. with feed for about 10 days may control the disease. Also, chloramphenicol injection @ 20-30 mg/kg body wt. intra-muscularly, in two successive treatments, may be carried out.

For controlling vibriosis, the therapeutic measures with antibiotics may be taken up. Oxytetracycline or erythromycin should be used @ 50-90 mg/lit. in both treatment for 5 days and 500-1000 mg kg in feed treatment.

ii) Chemotherapy for fungal diseases

Fish diseases caused by fungus, observed in all stages from eggs to adults, are predominantly of secondary nature as the invador invades the fish when it is either injured or already infected by other organisms.

For fungal diseases like saprolegniasis, the therapeutic measure may include various chemicals that are found to be effective. Bath treatment of the affected adult fishes with 160 mg/lit of $KMnO_4$ for 5 days can be used. Delicate fishes may be treated with $K_2 Cr_2 O_7$ @ 100 mg/lit. for one week using cotton swabs on the affected body part. NaCl bath treatment @ 3-4% to the affected fishes can also be tried. Malachite green bath treatment @ 1-2 mg/lit. for 30 minutes can prove to be useful. If the eggs are seen to be affected, they can be flushed with 2 mg/lit. of malachite green dye for 5 days. Pond water, requiring treatment, can be given formalin @ 20 mg/lit.

Therapeutic measure against branchiomycosis (gill rot) requires pond water to be treated with lime @ 50 kg/ha and bath treatment of the affected stock with NaCl @ 3-5%.

iii) Chemotherapy for protozoan parasitic diseases

Protozoan diseases like ichthyophthiriasis can be controlled therapeutically by giving hourly bath of the affected stock with 1 : 5,000 formalin solution for 7 days. Bath in NaCl @ 2% for more than 7 days can prove effective.

Treatment against trichodiniasis may include NaCl bath treatment @ 2-3%, formalin bath treatment @ 100 mg/lit. with aeration of water, $KMnO_4$ application to pond water @ 4 mg/lit. or formalin application to pond water @ 25 mg/lit.

External fouling in prawn can be controlled by formalin treatment in pond water @ 20-30 mg/lit., preferably with aeration. For larval infection, bath treatment with the same may be carried out @ 100 mg/lit. with aeration.

Therapeutic measures against white gill spot disease may include treatment of pond water with mohua oilcake as well as with lime which can destroy the spores to a large extent. NaCl bath treatment @ 3-5% also destroys the spores and other developing stages, but not the cysts.

The white scale spot disease, an other disease of protozoan origin, requires treatment at par with white gill spot disease.

No method of control however, is available presently for the cotton shrimp disease; only the diseased shrimps should be removed to check spread of it.

iv) chemotherapy for helminth parasitic diseases

Various worms of the groups *Monogenea, Digenea* and *Cestoda* cause parasitic infections that may affect the fish, cause retardation of growth as well as mortality. These parasitic diseases are magnified when they work in association with other parasitic groups.

The common helminth diseases of dactylogyrosis and gyrodactylosis can be checked with NaCl bath treatment of the affected stock @ 3-5% for 10-15 minutes. This kills the worms on fish. Formalin bath treatment @ 100 mg/lit. also destroys the worms. Formalin treatment of the pond water @ 25 mg/lit. is found to be effective in controlling the worms. Alternately, $KMnO_4$ treatment to the pond water @ 4 mg/lit. proves effective.

The black spot disease lacks chemotherapy as yet. However, removal of resident molluscan population from the affected water areas and keeping the aquatic birds at bay may improve the situation to a great extent, as both constitute the first intermediate host and final host respectively for the disease.

The ligulosis also lacks therapeutic treatment. The control measure, however, remains limited to examination of the particular host, the ichthyophagous birds. These birds must be driven away immediately from the surrounding to break the chain of the life cycle. Lakes and reservoirs have shown satisfactory results when this guideline is followed.

Desired treatment procedure has not yet been developed for another disease, named *Acanthocephalan* disease.

v) Chemotherapy for crustacean parasitic diseases

The crustacean parasites infect the fishes and prawns very often, the magnitude of infection is, however, seen to be less in natural open water but more in confined water. Barring local irritations and ulcerations to the host, the disease does not usually pose serious problems.

The common crustacean disease argulosis can be controlled by NaCl bath treatment @ 3-5%. Gamaxene treatment in pond water @ 1 mg/lit or $KMnO_4$ treatment in ponds @ 4 mg/lit. can effectively check such diseases.

Ergasilosis can be controlled both by bath as well as water treatment procedures. NaCl bath treatment @ 2-3% for 15 minutes or $KMnO_4$ treatment in pond water @ 4 mg/lit can be used for this disease.

The lernaeosis requires a control measure by $KMnO_4$ treatment in pond @ 4 mg/lit. NaCl bath treatment of the stock @ 3-5% can also be carried out.

There is no specific therapeutic measure for the control of isopod infestations. However, chemicals employed to check other parasitic copepods and branchiurans can be used.

vi) Chemotherapy for viral diseases

The viral diseases are difficult to treat. Viruses are nucleoproteins chemically and they require a living cell to multiply and grow. By the process, they infect the cells. Hence agents to kill viruses affect the host considerably. Cellular cytotoxicity is also seen often. Viruses can withstand very low temperature of about -75°C. They are, however, destroyed at higher temperature, between 53 and 56°C.

Treatment measures against viral fish diseases like, infectious pancreatic necrosis, viral haemorrhagic septicemia, infectious haemopoietic necrosis (IHNV), spring viremia of carp, channel catfish virus disase etc. may include treatment with synthetic polynucleotides, raising the ambient water temperature to control IHVN infections, immunization of stock with sonicated antigens or live antigens, vaccination by immersing the stock in hyperosmotic solutions containing the antigen etc.

The viral prawn diseases like yellow head disease, white spot disease and Monodon type baculo virus disease etc. can be controlled when suitable drugs developed for the purpose of killing the viruses are made available (therapy). Pending such availability, the viral prawn diseases for the time being, be checked by disease prevention strategies including prophylactic disinfection measures as well as elimination of potential virus carriers like slurry, post larvae, small aquatic animals, other crustaceans etc.

vii) Chemotherapy for epizootic diseases

The remedial measures against epizootic ulcerative diseases can be applied only for small water bodies, preferably below 25-30 ha areas. Remedial measures for natural open water systems like rivers, backwaters etc. as well as for large reservoirs, lakes, beels etc. are, however, not avialble presently.

The therapeutic measures may include NaCl bath treatment of the affected lot @ 3-4% which is effective in healing the ulcers. Small water bodies may be given applications of CaO @ 100 kg/ha, taking into consideration the pH of water. After a week, bleaching powder may be given @ 1 mg/litre when initial symptoms of ulcers are noticed. The two treatments together can be seen to check the spread of the disease.

Additionally, a formulated preparation, named CIFAX, can be applied @ 1 lit. /ha of water area containing the stock with ulcerative syndromes. Effective cure is reported within 7 days.

A micro-encapsulated pelleted feed, containing 30% protein fortified with vitamins A and C and containing nalidixic acid and erythromycin can exhibit healing symptoms of wounds in the affected ulcerative parts of fishes. The stock, on receiving simultaneous chloramphenicol bath @ 15 ppm, can demonstrate complete recovery.

Precautions

There are regulations regarding indiscriminate release of chemicals in the water environment, particularly when drugs and antibiotics are used. Ordinary chemicals like common salt and general disinfection agents like $KMnO_4$, bleaching powder, $CuSO_4$, lime etc. are used for household purposes. These substance, although find their way to water through land drain-offs and rainwaters, do not pose serious problems. But release of durgs and antibiotics in the environment remains restricted. Therefore, it will be desirable to use these chemicals within the framework of law. Drug-resistance and antibiotic resistance phenomena are common and resistant organisms may develop. Antimicrobial sensititivy tests thus should be conducted, before the treatment starts, to ensure the effectiveness of drugs and the dosages required for them. Moreover, the scope of antimicrobial resistance, acquired by animal bacterial pathogens being transmitted in the form of human pathogens, remains. It is for these reasons that regulatory authorities generally impose strict limitations for use of chemotherapeutic agents. Water bodies that are used by human subjects as well as for bathing of domestic animals, washing of clothes, utensils etc. should not be treated with drugs and antibiotics. Feeding and bath-or dip-treatment of the fish and prawn should be carried out in isolation. Care should be taken so that the spent water, after treatment, does not reach the cleaner water bodies. This should preferably be soaked in soil taking care that seepages do not occur to the pond water. Soil microbes, although

destroyed by the spent water, may be regenerated slowly with time. Alternately, the spent water, after treatment, may be boiled with strong oxidizing agents which destroy biopotency of the therapeutic agents by breaking or modifying the molecules. Since most of the drugs and antibiotics are thermo-labile, they may be inactivated by such heat treatments. The treated water may then be soaked in soil.

Chemotherapy has made immense progress nationally and internationally for the treatment of most diversified infectious diseases of fish. For the past quarter of a century, the therapy of fish and prawn diseases has received firm recognition in treating the invasive and infectious diseases. Nowadays, perhaps no efficient fish production on an intensive or semi-intensive scale is possible without a significant combination of both the therapy and prophylaxis.

The chemotherapeutic measures, stated above, may control the disease outbreaks to a great extent. However, there could not be any guarantee that full-proof checks against all outbreaks may always be ensured. Thus it calls for prophylactic measures of developing suitable vaccines (immunoprophylaxis) that can offer long lasting immunities to fish and prawn so that these aquatic living resources do not become susceptible to attack by disease outbreaks for a prolonged time. Hence, efforts should be directed towards the development of suitable vaccines that can be effective against endemic diseases.

Immunoprophylactic measures

Since a discussion on prophylactic measure may be beyond the scope of the present topic on chemotherapy, it is, therefore, stated in brief.

As is known, not all the diseases are caused by pathogens. For example, nutritional deficiency diseases are caused by inadequacies of specific nutrients as well as vitamins and minerals. Heriditary diseases are caused by genetic disorders transmitted through previous generations etc. Majority of the diseases, however, are caused by pathogens. Pathogens like bacteria, virus, parasites etc. or the toxic products elaborated by them cause diseased conditions and to obtain protection, immunoprophylaxis may be employed. This can be of two types : the active immunization (inoculation, vaccination) and the passive immunization (passive inoculation, antisera injection, immunotherapy).

In the former, the pathogen or their toxins are administered in a harmless form offering immunity to the host. This itself develops antibodies, needs some time and immunity is observed as a prophylactic measure. In the latter, antibodies are formed in a foreign homologous or heterologous animal and the preformed antibodies, after extraction, are administerd alongwith the serum to the host to be protected. This passive immunization, however, offers immediate protection. Sometimes, active and passive immunizations are carried out simultaneously.

In active immunization, fish can acquire immunity associated with production of humoral antibodies through oral administration of live or dead pathogens. For example, in the infectious abdominal dropsy (IAD) of carps cultured in pond, the immunoprophylaxis is seen by using a polyvalent inoculum of dead bacteria of *Aeromonas*. This is reported to reduce mortality rates. This type of immunity has been observed in case of another disease, the furunculosis also.

In passive immunization of fish, serum of immunized animal or fish may be used for the purpose. Thus, *antisera* obtained from salmon against *Aeromonas* can be injected to protect fingerlings of *Oncorhynchus* against artificial infection by *Aeromonas*. Also, an effective passive immunity can be seen in salmon for over 60 days by injections of a hyper-immune serum from salmon immunized against *Vibrio*. The passive immunization, obtained for relatively shorter periods, is cost and labour oriented for the reasons of preparation and administration of larger quantities of fish serum. Thus, it finds comparatively lesser application for practical purposes. Active immunization is more practical for protection of fish stock by inoculation and this is gaining more importance. In recent years, consolidated research efforts have been put on immune systems in fish. This has led to understanding of basic principles for obtaining immunoprophylaxis of hazardous infectious fish diseases by way of systematic active immunization. Initial focus was on immunoprophylaxis of diseases like furunculosis, IAD and vibriosis. Afterwards, basic possibilities have been tested for active immunization of fish against pathogens like *C.columnaris,* red mouth enterobacteriaceae, *Corynebacterium,* VHS virus, IPN virus etc. In principle, immunoprophylaxis against parasitic diseases is also possible.

The main aspect of developing an immunity against specific disease producing pathogens in active immunization is to stimulate the specific defense systems in fish. For immunization of fish, since the antigens present in the organisms develop antibodies in host, obtaining immunogenic antigens of the pathogens seems to be the prime requirement. For this, different forms of pathogens (like bacteria, parasite, virus etc.) are to be cultured in suitable nutrient media. Live cell culture techniques can also be adopted.

For the preparation of inoculum, the culture of bacteria, virus etc. can be done on nutrient-agar plates or in cell culture tubes. For the growth of fish pathogens, bacterial cultures can also be taken up in high capacity fermentors employing automatic control for pH, oxygen, nutrients including vitamins etc. The elaborated exotoxins are enriched in the culture media. Either the pathogens or their toxic products of metabolism can be employed as inoculum, the condition being that it should not be harmful to the host but bring about an immunity actively. The inocula from living microorganisms can be used that are weakened variants of pathogens (attenuated). Also, the inocula from living microorganisms can be used which are related to pathogen and domonstrate a lower virulence and pathogenicity. Additionally inocula can be used from killed microorganisms or products elaborated by these organisms that undergo some treatment.

On an experimental basis in fish, live inoculum from attenuated or slightly virulant variants can be used. Live inoculum sometimes can be used for immunoprophylaxis of viral fish diseases. Administration of live bacteria, although exhibiting satisfactory immunization in fish, can sometimes lead to ailments and can become a problem to the stock. Reports for methods on the use of attenuated fish pathogenic bacteria are somewhat lacking.

In active immunization of fish, most of the experiments have been carried out with fish pathogen that were killed by a variety of agents like phenol, formalin, thiomersal, chloroform, alcohol etc. or by simply heating, boiling or deep freezing. Killing of organisms by formalin (final concentration 0.2%) seems to be the most convenient method of preparation for inactivation and preservation of the antigens, while retaining their immunogenicity.

The toxic products elaborted by fish pathogenic bacteria are inactivated and for this a formal maturation or some sort of modification of exotoxins are done from *Aeromonas* and *Vibrio*. The compounds obtained are called toxoids. This is reported to exhibit good results. Mixing with formalin produces formol-toxoids and this is non-toxic to but immunogenic in fish. Another toxin of *Aeromonas* can be used as salt solution extracts which can be injected for active immunization.

For diseases like UAD, *furunculosis*, *vibriosis* etc., the polyvalent vaccines seem to be the most successful. In these, the antigens are not only of endogenic (from bacterial cells) but also of exogenic (exotoxins) origin. These are contained in inactivated form which can stimulate broad immune protection against pathogens. Thus, the refinement of antigen components of pathogens is carried out for exhibiting their optimum immunogenicity.

The composition of antigens is also of some importance. Based on the number of serotypes of a pathogen from which the vaccine is prepared, one can have monovalent, bivalent, trivalent or polyvalent vaccines. This can stimulate an immunity against one, two, three or several types of pathogens. If a vaccine can bring forth an immunity in fish against many types of pathogens, its success of immunoprophylaxis will be much more. Only when serological investigations are done on a large number of strains of pathogens of different origins, it will be possible to select serotypes with a broad antigenic spectrum for a polyvalent vaccine. For the development of effective polyvalent vaccines, studies on antigen structure of various types of fish pathogens are thus of great importance. Most effective vaccines, like the vibrio vaccine, are prepared on such basis. Also, the preparation of polyvalent vaccines can be facilitated when groups of pathogens are having a relatively homologous antigen structure (*e.g. Vibrio anguillarum*, *Aeromonas salmonicidea*, *Chondrococcus columnaris*). Conversely, the preparation may be difficult with heterogenous groups. An example of a polyvalent *Vibrio* vaccine is *Vibrichthyn* which is manufactured in Germany.

The success of immunoprophylaxis against fish diseases also depends on nature, frequency and dosages of antigen administration.

Investigations on different routes of injection of corpuscular and dissolved antigens have revealed that intraperitoneal injection is the most suitable mode of administration for several fishes. This is preferably employed for parenteral mass inoculation of fish. For virus antigens, a subcutaneous or intramuscular route of administration seems suitable. Effective immune protection can be achieved against homologous pathogens by injection of a large amount of antigen, probably of the order of 8-15 x 10^9 bacteria/100 g wt of fish.

The antigenic and immunogenic actions of an injection is considerably strengthened by mixing an adjuvant (helper) to the antigen Aluminium hydroxide, potassium aluminium sulphate etc. act as adjuvants to antigen.

Sometimes, an improvement in active immunization is achieved by repeated injections to obtain boostering effects. The second injection is given usually after 10-15 days of the first injection. This however, implies high cost for the practice. This can be reduced by employing suitable oral vaccines.

The oral vaccines for fish have been experimentally tried and found to exhibit mixed responses. Effective immunoprophylaxis in fish against natural infections by *Aeromonas* and *Vibrio* can be achieved by oral vaccines. The oral immunization depends on the nature of antigen, frequency of administration as well as the species of fish. The same is usually carried out by addition of a vaccine concentrate or lyophilisate to the feed.

Another method of administering antigen is by hyperosmotic infiltration. Here, fish is immersed in hypertonic solutions and then transposed to a 2% antigen solution for about 2 minutes. By this way, the antigen penetrates the host.

Immunogenesis also depends on temperature in case of poikilotherms like fish. At lower temperatures, immunization slows down or becomes ineffective. The optimum temperature for any fish should thus be recorded.

Since antibody synthesis in fish is its protein synthesis, particularly the γ– fractions of globulins, starvation or receiving imbalanced diet may result in reduced effectiveness against diseases. The antibody titer value in fish serum should be monitored. Manifestation of ailment can only provide clue towards the genuinity of protective action in an active immunization.

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Impact assessment of fish disease epizootics - a case study on EUS

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India is endowed with a vast expanse of inland waters. Maximum sustained yield of fish from natural waters and assurance of recurring bountiful harvest of fish without depleting the resources and wastage of fishing effort are necessary for the nation.

Although aquaculture is an age old practice but has acquired the shape of an industry durisng last 2 decades due its rapid development and high profit turn over. During this period there has been rapid expansion of fish and prawn farming in the country and the productions increased many fold. But the growth and development have been hampering due to outbreak of various fish diseases specially prawn diseases of bacterial and viral origin, poor management and improper planning which have been leading to drastic fall in production and heavy economic losses.

Generally during the outbreak of diseases the society is affected in 3-tier level *viz.*, producers, fish traders and consumers. Ofcourse, the producers become worst victim especially when it takes the shape of epidemic form. Thus, it becomes imperative to know the impact of the diseases in the society. Keeping this in view, it is highly essential to know the following facts;

a) the socio-economic conditions of the fish farmers and extent of suffering caused due to disease.

b) the impact of the disease on the fish traders and consumers.

c) the role of communication media towards creation of mass awareness.

It is also imperative to harmonise the aquaculture system for sustainable development, so that fish farmers do not suffer much. It is important to involve all groups of people including scientists, extension functionaries and fish farmers in the process of formulating and implementing management of package of practices towards development process. Effective communication is warranted to motivate fish producers towards adoption of proper mnagement measures for sustainable production for larger benefit.

A case study on the impact on EUS

'Epizootic Ulcerative syndrome' a dreaded fish disease reported in the counry during May, 1988 became a matter of grave concern for the fishery scientists, administrators and policy makers. The disease had a striding gait to several States and caused a havoc in the States of Assam, Tripura, Meghalaya, West Bengal, Bihar, Uttar Pradesh resulting the poor fish farmers still poorer. While fish plays an important role in the diet of 70 percent population of the

country, the consumers were baffled with various rumours relating to the diseased fish and finally, the fish markets were affected. People stopped consuming even the unaffected and healthy fishes. Moreover, some State Governments banned consumption of fish during the outbreak period (Das, 1988). In this sequel, the fish farming community and fish traders started suffering adversely. Thus, the outbreak of the disease established a setback in biological and socioeconomic conditions of the society.

Methodology

An investigation was carried out in 5 progressive districts of West Bengal *viz.*, 24-Parganas (North), 24-Parganas (South), Midnapore, Hooghly and Howrah in 3-tier level *i.e.*, producer, trader and consumer. At producer level, depending on the intensity of the disease, 2 blocks from each district were selected finally. A list of fish farmers were prepared and 500 fish farmers from the list selected at random, constituted the sample for the study. The selected fish farmers were personally inteviewed with a schedule developed to study their socio personal status, extent of suffering due to the disease, remedial measures and role of communication media etc.

At traders and consumer levels the study was categorised into 3 sectors viz., urban, sub-urban and rural areas. The Calcutta was included particularly here, besides above districts. A total of 3 markets of each sector were selected and drawing a sample of 22 from each, a total of 198 fish traders and consumers were personally interviewed with separate schedules developed for studying the impact of the disease in the fish trade and in fish consumption. For assessisng consumption behaviour scores 3,2 and 1 were assigned to most often, often and sometime respectively.

To study the role of communication media on creation of mass awareness towards the disease, 3,2,1 and 0 were assigned to most often, often sometime and never respectively. On the basis of frequency of the respondents reporting, these were finally ranked.

The data thus obtained, were analysed and interpreted as below;

Results and discussion

Table 1 : Socio-personal status of the fish farmers of the sample

Sl.No.	Variables		Frequency (N-500)	Percentage
1	Age	a) 15-20	6	1.2
		b) 21-30	87	17.4
		c) 31-40	249	49.8
		d) 41-50	105	21.0
		e) 51-60	39	7.8
		f) 61-70	14	2.8
2	Caste	a) Scheduled caste	360	72.0
		b) Scheduled tribe	0	0
		c) Other caste	140	28.0
3	Educational level	a) Illiterate	28	5.6
		b) Literate	129	25.8
		c) Primary	205	41.0
		d) High School	117	23.4
		e) Graduate & above	21	4.2
4	Aquaculture as occupation	a) Primary	353	70.6
		b) Secondary	147	29.4
5	Experience in Aquaculture	a) Traditional	224	44.4
		b) Scientific	82	16.5
		c) Semi-scientific	194	39.1
6	Category	a) Marginal	247	49.4
		b) Small	237	47.4
		c) Big	16	3.2

** Figures in parentheses of the text indicate frequency*

Socio-personal status

The socio-personal study (Table 1) of the respondents reveals that the age group of the sample varied between 15 and 70 years but majority of them belonged to the age group 31-40 (249). Most of them belonged to scheduled caste community (360) and they were actively participating in fish farming for their livelihood. The educational level of the sample varied between illiterate and graduate. Majority of the respondents was found with educational qualification upto primary level (205). The primary occupation of most of the respondents was aquaculture (353). Their experimence of fish farming showed that they mostly undertook fish farming adopting traditional method (224), semi-scientific method (194) and scientific (82) method. They were mostly marginal fish farmers (247) followed by small fish farmers (237). Big fish farmers were very limited in number (16).

Table 2 : Distribution of the sample

Sl. No.	Variables	Frequency (N-500)	Break-up of operational trend of fish farming		
			Traditional	*Semi-scientific*	*Scientific*
1	Number of respondents affected with EUS	365 (73.0)	176 (48.2)	129 (35.4)	60 (16.4)
2	Number of respondents not affected with EUS	135 (27.0)	48 (35.6)	65 (48.1)	22 (16.3)

** Figures in parentheses indicate percentage*

Distribution of the sample

The study reveals (Table 2) that in the sample of 500, ponds of 365 fish farmers were affected with the Epizootic Ulcerative syndrome. The breakup of the operational trend of the affected farmers indicates majority of the respondents practice fish farming in traditional way (176), followed by semi-scientific method (129) and scientific method (60).

Table 3 : Extent of the effect of Epizootic Ulcerative Syndrome

Sl. No.	Characteristics		Frequency (N-365)	Percentage
1	Species affected with the disease**	a) Murrels	186	50.9
		b) Catfishes	199	51.8
		c) Misc. fishes	300	82.19
		d) Carps	311	85.20
2	Extent of loss of fish in waterbody (percent of the total crop)	a) 1-10	14	3.83
		b) 11-20	47	12.87
		c) 21-30	106	25.05
		d) 31-40	154	42.19
		e) 41- 50	27	7.39
		f) above 50	17	4.65
3	Pecuniary loss (in Rs.)	a) 100-1000	47	12.87
		b) 1001-5000	213	58.35
		c) 5001 - 10,000	72	19.73
		d) Above 10,000	33	9.04

*** More than one group of fish mentioned*

Extent of the effect of Epizootic Ulcerative syndrome

Fish species most severely affected by EUS were predominantly the bottom dwelling fishes like Murrels, Airbreathing catfishes and other miscellaneous fishes like Puntius, Nandus etc. In culture ponds carps were also affected by the disease (Jhingran & Das, 1990). It is very interesting to note from the study (Table 3) that in all the ponds under traditional system of culture, Murrels were affected at the first stage of outbreak followed by miscellaneous fishes and lastly the Carps. In other scientifically managed ponds where fish toxicants were used to control the predatory fishes the Carps were found to be affected at the outset. The respondents also reported the outbreak of the disease in more than one species in their ponds.

Thus, the pooled data (traditional, semi-scientific and scientific) indicates that extent of the effect of the disease were maximum on Carps (311) followed by miscellaneous fishes (300), Catfishes (191) and Murrels (186). The effect on carps was more pronounced here due to the fact that number of ponds were mostly covered under semi-scientific and scientific management system.

Maximum number of respondents (154) expressed that the percentage of loss of fish from the total crop varied between 31 and 40 while cent percent mortality was reported in 17 cases.

The pecuniary loss faced by the affected fish farmers were found to be maximum in the range of Rs. 1001 to 5000 (213), followed by Rs. 5001 to 10,000 (72).

A section of respondents (17) expressed that during rest of the season they had to search for alternative jobs for their livelihood since they completely lost their fish crop. All of the respondents univocally expressed that they need financial assistance to restore aquaculture and to get relief from the loss incurred by them due to the occurrence of the disease.

Table 4 : Effect of Epizootic Ulcerative Syndrome on fish consumption

Variables	Dimensions	Urban (U)	Sub-urban (SU)	Rural (R)	Total percentage (N-198)
Fish consumption habit before the outbreak of the disease (U,N-66, SU, N-66, R, N-66)	Most often	28 (42.4)	33 (50.0)	17 (29.8)	39.4
	Often	35 (53.1)	31 (47.0)	22 (33.3)	44.4
	Sometime	3 (4.5)	2 (3.0)	27 (40.9)	16.2
Fish consumption habit after the outbreak of the disease (U,N-66,SU,N-66,R,N-66)	Most often	12 (18.2)	16 (24.3)	12 (18.2)	20.3
	Often	22 (33.3)	27 (40.9)	19 (28.8)	34.3
	Sometime	32 (48.5)	23 (34.8)	35 (53.0)	45.5
Comparison of consumption behaviour	Consumption score before outbreak	157	163	128	-
	Consumption score after outbreak	112	125	97	-
	Percentage of decrease	28.7	23.3	20.5	24.4
Fish preferred for consumption (U,N-66,SU,N-66,R,N-66)	Carp	49 (74.2)	43 (65.2)	23 (34.8)	58.1
	Murrel	2 (3.0)	6 (9.1)	16 (24.3)	12.1
	Marine/brackish-water	4 (6.1)	9 (13.6)	2 (3.1)	7.6
	Catfish	1 (1.5)	2 (3.0)	6 (9.1)	4.5
	Misc.	10 (15.2)	6 (9.1)	19 (28.8)	17.7
Consumption of diseased fish	Most often	0	0	0	0
	Often	0	0	6 (9.1)	3.0
	Sometime	0	7 (10.6)	17 (25.8)	12.1
	Never	66 (100)	59 (89.4)	43 (65.1)	84.9
Reason for not consumption (U,N-66,SU,N-66,R,N-66)	Unknown fright	6 (9.1)	4 (6.8)	0	**6.0
	Fright of disease transmission	23 (34.8)	15 (25.4)	0	**22.6
	Hatred	37 (56.1)	40 (67.8)	43 (100)	**71.4

Figures in parentheses indicate percentage ** *N - 168*

Effect of Epizootic Ulcerative syndrome on fish consumption

It appears from the Table-4 that maximum respondents consumed fish 'often' (88) in the sampled areas. The consumption behaviour before the outbreak, as per the score were found to be maximum in the sub-urban sector followed by urban sector and rural sector and the scores were 163, 157 and 122 respectively. Most of the consumers liked carp (115) followed by miscellaneous small fishes (35). The consumers of rural areas had somewhat more affinity towards murrels and catfishes in compared to the consumers of urban and sub-ruban areas.

The study revealed that owing to the outbreak of epizootic Ulcerative syndrome, the rate of consumption of fish declined. Only 15.1 percent of the sample consumed the diseased fish and majority of them belonged to rural sector. The consumption rate, was found to be decreased by 28.7 percent and 23.3 percent and 20.5 percent in urban, sub-urban and rural sector respectively. The respondents who used to consume fish 'most often' (78) and 'often (88) maximum number of them changed their habit of fish consumption to 'sometime' (90) and their preference were found to be restricted mostly with good and healthy carps. The most of the respondents declined to purchase even good and healthy fishes when kept alongwith the diseased fishes in the markets. Again, excepting rural markets, respondents expressed their apathy towards purchase of diseased fish even if it is sold at cheaper rate.

Maximum number of respondents expressed that they did not like to consume diseased fish due to 'hatred' (120), followed by 'fright of transmission of disease' (38) and 'unknown fright' (10) like even death. A negligible percentage of consumers showed interest to change their habit of consumption of marine fish during the affected period. 37 percent of the respondents expressed their anxiousness about the disease and they tried to keep regular information from various sources. No incidence of occurrence of any disease was reported in the sample who consumed or handled the diseased fish.

Table 5 : Effect of Epizootic Ulcerative Syndrome on fish trade

Variables	Dimensions	Urban (U)	Sub-urban (SU)	Rural (R)	Total percentage (N-198)
During outbreak of EUS fish sale (U,N-66,SU,N-66,R,N-66)	Increased	0	0	2 (3.0)	1.0
	No. difference	15 (22.7)	6 (9.1)	22 (33.4)	21.7
	Decreased	51 (77.3)	60 (90.9)	42 (63.6)	77.3
EUS affected fish sale (U,N-66,SU,N-66,R,N-66)	Undertaken	3 (4.5)	7 (10.6)	11 (16.7)	10.6
	Not undertaken	63 (95.5)	59 (89.4)	55 (83.3)	89.4
Reasons for not selling (U,N-66,SU,N-66,R,N-55)	Resistance from public	0	1 (1.7)	2 (3.6)	**1.7
	Lack of customer	11 (17.5)	4 (6.8)	8 (14.6)	**13.0
	Scared	52 (82.5)	54 (91.5)	45 (81.8)	**85.3
Pecuniary loss (U,N-66,SU,N-66,R,N-66)	Suffered	47 (71.2)	63 (95.4)	66 (100)	889
	Not suffered	19 (28.8)	3 (4.6)	0	11.1

** Fisgures in parentheses indicate percentage, ** N-177*

Effect of Epizootic Ulcerative Syndrome of fish trade

Table 5 depicts that majority of the respondents (153) expressed considerable decrease of fish sale in urban, sub-urban and rural markets owing to the outbreak of the disease. This supports the view of the consumers, as explained in Table 4. Moreover, a large number of the respondents (177) did not undertake the sale of diseased fish. Owing to consumers reactions, the traders who once sold diseased fish did never accept such fish for selling thereafter. In rural markets, diseased fish were sold at a considerable lower price. The respondents also expressed that a few customers diverted their choice to marine and brackishwater fishes. The traders did not undertake the sale of diseased fish as they were 'scared' about their business reputation (151), 'lack of customers' (23) and 'resistance from the public' towards sale of affected fishes (3). Even the sale of good and healthy fishes were suffered in the fish stalls where diseased fishes were found and this corresponds the views of the consumers also. Most of the respondents (176) suffered pecuniary loss to some extent during the affected period.

Table 6 : Remedial measure adopted

Sl.No.	Variable **	Frequency (N-365)	Percentage
1	Lime treatment	358	98.1
2	Salt treatment	151	41.3
3	Potassium permanganate treatment	227	62.2
4	Antibiotic treatment	15	4.1

*** More than one treatments mentioned*

Adoption of remedial measure

As per the recommendation of the CIFRI the clientele adopted various remedial measures. The study reveals (Table 6) that maximum respondents (358) applied lime to control the disease followed by application of potassium permanganate (227). Only limited number of farmers used antibiotic (15) mixing with supplementary feed. Some respondents also resorted to more than one treatments with success. 68 percent of the respondents accured positive result from the treatments.

Table 7 : Role of communication media

Sl.No.	Sources	Total score	Rank order
1	Extension functionary of State Fisheries Department	357	II
2	Extension functionary of CIFRI	222	IV
3	Extension functionary of CADC/Voluntary organisations	150	V
4	Publication	48	VII
5	Newspaper	316	III
6	Radio	566	I
7	Television	62	VI

Role of communication media

The fish farmers had been receiving information regarding EUS from various sources of communication media. As per the study (Table 7) communication media ranked according to scores are Radio, Extension functionaries of State Govt., Newspaper, Extension functionaries of CIFRI, TV, Extension functionaries of CADC/Voluntary organisation and publication in descending order. It was observed that Radio played maximum role in transmission of information to the clientele probably maximum number of fish farmers possess a transistor radio which confirmed the study of Bhaumik et al., 1989.

Conclusion

Due to the fish disease epizootic time to time worst affected clientele are the fish farmers. They become victim of considerable quantity of fish loss which cause frustration among the farming community. For restoration of the aquaculture, the services of Financi. Institutions and Insurance Organisations may ease the burden of poverty of the poor clientele. The Mass Media has to play a great role to educate the fish farmers and consumers regarding the disease. The village leaders need be dispensed with concerned literature in regional languages in sufficient numbers for distribution among the needy fish farmers.

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