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Important Coldwater Fish Diseases and their Control

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Foreword

The country has significant coldwater or hill fishery resources in terms of gene pool and some of them are suitable for food, sport and ornamental value. Moreover, this sector contributes to the livelihood for a large section of economically underprivileged population of the country. The Indian contribution of coldwater fish production is nearly 3% which is expected to increase by more than 10% till 2030. Although this region can be a promising area for the development of aquaculture, the causes of certain problems associated with the poor production have to be dealt. Scarcity of water, increase in temperature due to global warming, eutrophication and pollution of the water bodies, contribute to tremendous alteration in the environment of the fish. As such, the fish in this ecological zone is susceptible to diseases caused by pathogens like fungi, bacteria, viruses and protozoan and metazoan parasites. Therefore, for better production, coldwater bodies have to be managed well and effective health management practices have to be in place.

The document has been conceived with an idea to disseminate the knowledge on the common diseases of coldwater fish species and has covered almost every aspect of infectious and non-infectious diseases that one may encounter in this sector. I hope this effort of DCFR would be very useful for the researchers, fisheries officers and the farmers.

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Preface

The coldwater sector is progressively making significant contribution in the nation's agenda for food security. The popularity and rate of success in coldwater aquaculture achieved by Jammu & Kashmir as well as Himachal Pradesh have demonstrated that rural areas can become direct beneficiaries by the implementation of intensive aquaculture technologies. Significant progress has been made in mid altitude carp farming in Uttarakhand, trout farming in Sikkim and Arunachal Pradesh during last few years. As a consequence, the fish farmers have achieved significant improvement in their standard of living.

With the expansion of the coldwater aquaculture practices, the Directorate has felt a strong need to combat fish diseases which were being ignored for quite a while. With the establishment of the Coldwater Fish Health Facility, the Directorate took an important step to address fish health issues pertaining to this sector. Fish diseases if not prevented and controlled, can lead to serious economic losses that can hamper the development of coldwater fisheries. Therefore, to have a good production, healthy fish is required and hence the knowledge on fish diseases is quite important to be aware of for their prevention and control.

The document has been designed keeping the field level workers and farmers in mind so that they are aware of the diseases associated with coldwater aquaculture. Thus, this publication will be useful source of information on diseases and their control, preventive measures as well as collection of samples for disease

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Introduction

A disease is an abnormal condition affecting the body of an organism, associated with specific symptoms and signs. It may be defined as a condition responsible for pain, dysfunction, distress, and finally death. However in a broader sense, it sometimes includes injuries, disabilities, disorders, syndromes, infections, isolated symptoms, deviant behaviors, and atypical deviation in structure and function.

Fish being a poikilothermic animal, is very sensitive to its surrounding environment i.e. water. Even minor changes in environment can lead to stress in fish. Fish resides in a continuous state of equilibrium with the environment and pathogen. Pathogens are always present in the aquatic environment, but, they can cause disease in fish only when this equilibrium gets disturbed. This may be due to the deterioration of environment which could lead to stress or immune-compromised state of the animal that in turn enables infection by multiplication of pathogens. In aquatic environment, fish continuously adjust itself to the changing physico-chemical parameters. This imposes a great stress on their limited homeostatic mechanism and severely upsets the physiological control over defense mechanism or immune system resulting in susceptibility to various diseases.

Defense mechanisms of the fish against disease are both non-specific and specific. Non-specific defenses include skin and scales, as well as the mucus layer secreted by the epidermis that traps microorganisms and inhibits their growth. If pathogens breach these defenses, fish can develop inflammatory responses that increase the flow of blood to infected areas and deliver white blood cells that attempt to destroy the pathogens. Specific defenses are specialized responses to particular pathogens recognized by a fish's body and may also be referred to as adaptive immune responses.

Disease is a prime agent affecting fish mortality, especially when fish are young. Fish

Significance

Prevention of fish diseases is essential for the betterment of the fisheries industry as well as for the conservation of fish resources. Because of the complexity of their environment, fish are susceptible to viral, bacterial, fungal, and parasitic infections. These infections will adversely affect "growth and development and," serious infections can be fatal. An outbreak of disease jeopardizes regular aquaculture and threatens fish yields. Therefore, controlling disease is one of the most vital tasks in fish culture.

Signs and diagnosis

In fish, a disease can be characterized by certain signs- for example, abnormal behavior in ponds, scraping along the pond side or bottom, whirling or loss of equilibrium, loss of vitality, weakness, inability to withstand handling during sampling or other farm activities, discoloration of body, eroded areas or sores on head, body fins and other parts of the body, pop-eye, hemorrhages, swelling of body or gills, cyst formation on the body and swollen belly are some signs that one could record. Once such signs are observed, a postmortem is normally conducted to establish the possible cause.

Postmortem findings may include- pale coloration of liver, congestion of kidney or other organs, accumulation of fluid in the abdominal cavity, swollen lesions, cyst containing parasites, intestinal hemorrhages etc. which give a clue for the possible cause. Further, a disease may be caused by external factors, such as pathogenic diseases which are sometimes referred to as extrinsic diseases. However, internal dysfunctions due to physiological or nutritional reasons that lead to intrinsic diseases are also quite important to be dealt with.

Principle of disease control

The principle of fish disease control is an all-round prophylaxis.The proverb, prevention is better than cure, has to be seriously implemented. In the event of an outbreak of disease, fish should be treated in the early stages. However, because of the difficulty in observing the activities of the fish, it is difficult to correctly diagnose and treat an infection in its early stages. In addition, because many diseases severely interfere with the feeding process, orally administered drugs may be ineffective. Parenteral drug administration is an alternative however dip treatments are confined to small containers or spread measures to fish ponds. This type of treatment is impractical for large lakes, rivers, and reservoirs. Therefore, prevention is the key for disease control.

Major types of fish diseases

Table 1: Types of fish diseases

Types	Pathogens /causes	Mode	Source	Control
Infectious Diseases	viruses	acute	Diseased fish or seed, polluted water, contaminated feed & equipments	Prevention, Disease free seed & feed, disinfection, treatment
	bacteria	acute, subacute		
	Fungi	chronic		
Invasive Disease	Parasites	chronic	Diseased fish, polluted water, contaminated feed, gear & silt.	Prevention, disinfection, treatment
Other diseases	Nutritional Environmental Genetic	chronic	Nutritional deficiency, polluted water, genetic abnormality	Balanced feeding, water quality monitoring, quality seed

Infectious Diseases

Infectious diseases are mainly caused by viruses, bacteria, fungi, or unicellular algae "ichthyomicrobial diseases"; e.g. bacterial enteritis, bacterial gill rot, and bacterial erythema. Infectious diseases account for 60 per cent of the loss in production. Thus, investigating infectious disease is quite important for the development of aquaculture. Infectious diseases can be divided into acute, sub-acute, and chronic forms based on the clinical picture. For example, if enteritis of grass carp or black carp occurs in an acute form, it develops rapidly and soon results in a high rate of mortality. Chronic enteritis, however, kills only a few fish per day but lasts for a long time.

Bacterial pathogens of infectious diseases are not strictly parasitic micro-organisms. If the conditions for parasitism are unsuitable, saprophytic relationship may develop. Bacterial pathogens have a high adaptability to environmental changes. The epidemic season is usually observed from late spring to early summer. For example, the pathogen causing enteritis in grass carp and black carp is dormant at water temperatures below 20°C however the same organism becomes virulent between 22-25°C. Likewise, zoospores of *Saprolegnia* frequently come in contact with fish skin but chances of occurrence of saprolegniosis is more if the host has been previously injured as the zoospores get an opportunity grow and multiply thus infecting a fish.

causative agent) and mixed (having two or more pathogens on a single fish). Examples of a mixed infection are grass carp suffering from both saprolegniasis and gill rot and a black carp with enteritis and red skin disease.

Sources of infection can be either primary or secondary. A primary infection originates within the pond. The pathogen may infect the fish directly or through the discharge of pathogenic agents into the water and an asymptomatic fish may act as a disease carrier leading to disease outbreak under certain conditions. A secondary infection originates outside the pond. For example, a disease-free pond could be polluted with water from a diseased pond, diseased or contaminated silt, feed and equipment and these factors help in the spreading a disease.

Infectious diseases normally attack the fish through tissues and organs (skin, gills, intestines or excretory organs). Fish, however, do possess resistance mechanisms against pathogenic microbes. For example, skin texture and mucous membranes of the fish function as barriers to infectious microorganisms. The entry of pathogenic microbes via the digestive tract is prevented by certain enzymes. White blood cells, lymphoid tissue cells, and reticulo-endothelial cells of the spleen, liver, and blood vessels can eliminate foreign bodies, including pathogenic microorganisms. In addition, fish blood contains bactericidin, which is toxic to pathogenic bacteria.

Invasive Disease

Invasive diseases are caused by animal parasites such as trichodinasis, ichthyophthiriasis, lernaesis, argulosis, etc. Fish carrying parasites or the carcasses of diseased fish are a primary source of invasive diseases. Contaminated feeds, gears, pond water, silt, etc., are secondary sources. For example, mature oocytes of *Eimeria* or mature myxosporidia may enter the water with the fish and precipitate to the bottom of the pond. The pond silt is contaminated as a secondary source of invasive disease.

Like infectious diseases, invasive diseases often appear in different seasons. This is because the pathogens and the fish are influenced by external factors (location, climate, physico-chemical properties of the water, farming skills, etc.) and internal factors (growth and physiological status). Invasive pathogens may also be species specific or organotropic.

Other Diseases

Physical and chemical factors or the influence of pond environment may retard growth or even kill the fish. For example, gasping and suffocation may upset the physiological balance of the fish and if serious, cause mass mortality. These diseases can be broadly categorized as

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Viral Diseases

Fish viruses have been found to be similar to mammalian and avian counterparts in most of their characteristics. However, the main difference between the animal and fish viruses is their optimum temperature of replication. The optimum temperature for the viruses infecting the coldwater fish like salmonids, is 10-20°C while the viruses infecting warm water species like the carps, have an optimum temperature of 20-27°C. The mammalian and avian viruses replicate at temperature range of 33-38°C. So far, there is no report on public health issues of the viruses infecting fish unlike their mammalian counterparts. The fish viruses infecting salmonids fail to infect fish of other families like carps etc., however, infectious pancreatic necrosis virus is an exception as it can be transmitted from molluscs to fish and vice versa.

Important viral diseases of coldwater fish

Some of the important viral diseases of fish from the perspective of cold water aquaculture are infectious pancreatic necrosis (IPN), infectious hematopoietic necrosis (IHN) viral hemorrhagic septicaemia (VHS), spring viremia of carp (SVC) and epizootic hematopoietic necrosis (EHN).

Infectious Pancreatic Necrosis

Infectious pancreatic necrosis (IPN) is globally an economically significant viral disease of salmonid fish. The disease is widespread and the mortality in salmonids is inversely proportional to the age of fish, being higher in fry and fingerling of salmonids and relatively rare in older fish. Highly virulent strains of IPNV can cause greater than 90% mortality in hatchery stocks in less than four-month old fry, survivors of infections are usually persistently infected and serve as reservoirs of infection. IPNV is the prototype virus of the family *Birnaviridae*. The name birna refers to the most important feature of this family i.e. the two segmented viral genome as well as its double-stranded nature and rna refers to the viral

urine, water or other materials and the vertical transmission takes place through reproductive fluids, contaminated eggs being the major source.

Clinical signs

First sign in salmonid fry is frequently a sudden and usually progressive increase in daily mortality. Clinical signs include darkening pigmentation, a pronounced distended abdomen. The behavior of infected fish varies from quiescence with weak respiration to periods of sporadic hyperactivity during which fish swim in a corkscrew manner, rotating about their long axis or whirling violently. Cumulative mortalities may vary from less than 10% to more than 90% depending on a combination of various factors such as virus strain, host and environment.

Post mortem lesions

Internally the fish can display swollen intestine and catarrhal exudates in the lumen. There may also be petechiae on the caecal fat. The liver of infected fish is generally pale yellowish in colour.

Prevention and control

Prevention can be achieved by avoiding the use of fertilized eggs/milt originating from virus carrier broodstock. The use of protected water supply (e.g. spring or borehole) can also help in the production of specific pathogen free seed. In outbreaks, a reduction in the stocking density may help to reduce the overall mortality. Alternatively, a short period of increased water temperature (>18°C) also appears to be beneficial.

Infectious hematopoietic necrosis

Infectious hematopoietic necrosis virus (IHNV) is a negative-sense single-stranded, bullet-shaped RNA virus that is a member of the genus *Novirhabdovirus* within the family *Rhabdoviridae*. It causes a disease known as infectious hematopoietic necrosis in salmonid fish like trout and salmon. The disease may be referred to by a number of other names such as Chinook Salmon Disease, Coleman Disease, Columbia River Sockeye Disease, Cultus Lake Virus Disease, Oregon Sockeye Disease, Sacramento River Chinook Disease and Sockeye Salmon Viral Disease. IHNV is commonly found in the Pacific Coast of Canada and the USA, and has also been found in Europe and Japan. The first reported epidemics of IHNV occurred in the United States at the Washington and the Oregon fish hatcheries during the 1950s.

IHNV is transmitted by clinically sick fish and asymptomatic carriers. This virus is shed in the feces, urine, sexual fluids and external mucus. Horizontal transmission from fish to fish is primarily by direct contact but also through water. IHNV can survive in water for at least a month, particularly if the water contains organic material. The virus can also spread via contaminated feed. The gills or the digestive tract have been suggested as the major sites of virus entry, but recent evidence suggests that IHNV may enter at the base of the fins. Vertical transmission is usually egg-associated but whether IHNV can be present inside the egg or on the surface is controversial.

Clinical signs

Clinical disease usually occurs when the water temperature is between 8°C to 15°C, but outbreaks have occasionally been reported at temperatures warmer than 15°C. Disease outbreaks are generally seen between spring and early summer. Young fish are most susceptible to disease, particularly during the first two months of life. Cumulative mortality in young animals can reach 90-95%. Older fish are comparatively resistant to the disease. Clinical signs include ascities, exophthalmia, darkened skin and pale gills. Long, semi-transparent fecal casts often trail from the anus. Affected fish are typically lethargic, with bouts of hyper-excitability and abnormal activity. Petechial hemorrhages commonly occur at the base of the pectoral fins, the mouth, and the skin posterior to the skull above the lateral line and the yolk sac in sac fry. In sac fry, the yolk sac often swells with fluid. In fry less than two months old, there may be few clinical signs despite a high mortality rate. Surviving fish often have scoliosis.

Postmortem lesions

The abdomen, stomach, and intestines often contain white to yellowish fluid, but food is usually absent from the digestive tract. The kidney, liver, spleen and heart are typically very pale. Necrosis is common in the kidney and spleen, and focal necrosis may be noted in the liver. Petechiae are often found in the internal organs including the pyloric caeca, spleen, peritoneum, intestines, and the membranes surrounding the heart and brain. Hemorrhages may occur in the kidney, peritoneum and swim bladder.

Prevention and Control

Most epizootics have been linked to the importation of infected eggs or fry, but IHNV can also be introduced in asymptomatic carriers. In areas where this disease is not endemic, outbreaks are controlled by destruction of the whole stock, disinfection, quarantines and other measures. Where IHNV is endemic, good bio-security and sanitation may decrease the risk of introducing the virus to a farm. Eggs should be disinfected with an iodophor

Viral hemorrhagic septicemia

Viral hemorrhagic septicemia virus (VHSV) is considered by many nations and international organizations to be one of the most important viral pathogens of finfish. This virus belongs to the genus *Novirhabdovirus*, within the family *Rhabdoviridae*. VHS is also known as "Egtved disease," and VHSV as "Egtved virus." VHS was first reported from a rainbow trout farm in Denmark and has been causing significant losses in rainbow trout farms in many countries in continental Europe. It has also been reported in Japan and Russia. A highly virulent new strain of VHS has been reported in a range of freshwater fish species in the USA.

VHSV has been isolated from at least 50 species of marine and freshwater fish from the Northern Hemisphere, and other species have been infected in the laboratory. Species susceptible to infection include members of the Salmoniformes (salmon and trout), Pleuronectiformes (flounders, soles and other flatfishes), Gadiformes (cod), Esociformes (pike), Clupeiformes (herring and anchovy), Osmeriformes (smelt), Perciformes (perch and drum), Scorpaeniformes (rockfishes and sculpins), Anguilliformes (eels), Cyprinodontiformes (mummichog) and Gasterosteiformes (sticklebacks). Additional species continue to be reported. Many species of marine fish appear to be infected asymptomatically, suggesting that VHSV is probably endemic in marine environments.

VHSV is shed primarily in the urine and reproductive fluids (ovarian fluids, sperm). This virus has also been reported in the feces, but shedding is low. Reservoirs include clinically ill fish and asymptomatic carriers. Virus carriage seems to be life long, but shedding appears to be intermittent in carriers. Transmission can occur through the water or by contact. VHSV is thought to enter the body through the gills or possibly through wounds. Predation on infected fish is also thought to be a route of transmission. Fish-eating birds can introduce VHSV into areas by acting as mechanical vectors.

Clinical Signs

Affected rainbow trout are usually anorexic and may be either lethargic or hyperactive. Swimming behavior can also be abnormal. The colour is usually darker than normal but the gills are pale due to anemia and may have petechial hemorrhages. Hemorrhages can also be seen in the eyes and at the base of the fins, and sometimes on the body surface. Bilateral or unilateral exophthalmia and ascites may be present. A neurologic form characterized only by abnormal swimming behavior, such as constant flashing and/or spiraling, can also occur in this species. Chronic carriers may be asymptomatic. Clinical disease can occur at any age, but younger fish appear to be most susceptible. Stress is a predisposing factor, and outbreaks can occur in subclinical carriers after a stressful event. Water temperature also influences

in the abdomen, swim (air) bladder, intestines, gills and other organs. The spleen is usually enlarged and darker red than normal.

The liver is also dark red in early stage of infection but may later be pale or chalky grey. It can contain petechiae or mottling. The kidneys are dark red in the early stage of disease, but can be severely necrotic in moribund fish. The body cavity may be filled with ascitic fluid, and the gastrointestinal tract is usually lacks food. Fish with the nervous form may have no significant gross lesions. Histopathologic lesions typically include extensive focal necrosis and degeneration in the kidney, liver and spleen. Evidence of hemorrhages may be seen in the muscles.

Prevention and Control

Viral hemorrhagic septicemia is a highly contagious disease; quarantines are necessary to control outbreaks. There is an evidence that VHSV is transferred from wild fish to farmed fish and vice versa. VHSV can survive for long periods in the bottom of farm ponds if the ponds are not dried and disinfected. VHSV is sensitive to many common disinfectants including formalin, iodophor disinfectants, sodium hydroxide and sodium hypochlorite. Methods to decrease the impact of viral hemorrhagic septicemia in endemic areas include hatchery disinfection, the use of specific-pathogen free (SPF) stock, spring or bore water, and management practices that decrease physiological stress. Co-cultivation of wild fish and salmonids (particularly rainbow trout) should be avoided, as VHSV can be transmitted between species and non-virulent isolates could become virulent. There are no effective anti-viral agents for the control of this disease, and no commercial vaccines exist.

Spring Viraemia of carp

Spring viraemia of carp, also known as swim bladder inflammation, is caused by spring viraemia of carp virus. This virus is a member of the family *Rhabdoviridae* and has been tentatively placed in the genus *Vesiculovirus*. It is a contagious infection seen in all species of carp around 1 or 2 years of age in the spring. The disease is usually observed during the spring and the outcome depends on the general condition of the fish, the water temperature, and any secondary bacterial infections. At low water temperatures usually less than 10°C, the disease is fatal as the fish immune response is poor. All ages are susceptible but the most severe losses occur in juveniles and yearlings. The causative virus can be spread by fomites, parasitic invertebrates and is difficult to eradicate once it is established in a pond. Elimination of the virus may require destruction of all aquatic life. Virus enters through gills and infects swim bladder, leading to inflammation and buoyancy problems.

(*Silurusglanis*). Common carp are the most susceptible species and are considered to be the principal host.

SVCV is carried in clinically ill fish and asymptomatic carriers. This virus is shed in the faeces and urine, as well as gill and skin mucus of infected fish. It is also found in the exudate of skin blisters and oedematous scale pockets. Transmission is by direct contact or through the water. The virus enters the host most often through gills. SVCV has been found in ovarian fluids and "egg-associated" (vertical) transmission has not been ruled out, however, this does not appear to be an important route of spread. SVCV can also be spread by fomites and invertebrate vectors. Infectious virus can persist in 10°C water for more than four weeks and in 4°C mud for at least six weeks. Known vectors include the carp louse *Argulus foliaceus* and leech *Piscicola geometra,* but other aquatic arthropods might also transmit the virus. Fish-eating birds are also potential vectors.

Clinical Signs

Fish can carry SVCV with or without symptoms. SVC outbreaks are most common in farmed carp, but can also occur in wild fish. Although fish of any age can become ill, the disease is most common in young fish up to a year of age. Fish up to a year old are most likely to be affected, but illness also occurs in older animals. Water temperature affects the development of disease. Clinical signs are most common at 17°C or below. The infection is characterized by dark body coloration, popeye, pale gills and a swollen abdomen. Sometimes pin-point haemorrhages can be observed in the skin, and there can be a thick mucoid cast trailing from an inflamed and protruding vent. Affected fish breathe weakly and are lethargic although some may display sporadic hyperactivity. The fish can lose equilibrium and swimming can be uncoordinated. It is important to note that not all the signs may be present, particularly during the earliest and very late stages of a disease outbreak.

The clinical signs are nonspecific. In carp, the most common symptoms include abdominal distension, exophthalmia, inflammation or edema of the vent (often with trailing mucoid fecal casts), and petechial hemorrhages of the skin, gills and eyes. The body is often darkened with pale gills. Diseased fish tend to gather at the water inlet or sides of the pond, swim and breathe more slowly than normal, and react sluggishly to stimuli. Loss of equilibrium, with resting and leaning, are seen in the late stages. Concurrent bacterial infections (carp-dropsy complex) or parasitic infections influence the symptoms and mortality rate.

Post-Mortem Lesions

The body is often darkened with pale gills, and petechial hemorrhages may be seen in the

necrotic material. The spleen is frequently swollen, with a coarse surface texture. Other lesions may include degeneration of the gill lamellae, edema of other internal organs, hepatic necrosis, jaundice, cardiac inflammation and pericarditis. In fish that die suddenly, gross lesions may be absent.

Prevention and control

There is no known cure for SVC, but fish held at temperatures above 18°C have a greater chance of survival while 20-22°C is optimum. Disinfection of ponds, sterilization of equipment, decontamination of eggs by iodophor treatment, and stocking fish known to be free from the virus are advisable measures that can be taken. Vaccines are available and should be used in the summer or autumn to prevent acute disease in the spring. Vaccination is more effective above 20°C. Temperature control is not usually practical in large open ponds. Fish have an increased susceptibility to disease when they are stressed, so the risk of disease and mortalities can be significantly reduced by keeping stress to a minimum wherever possible. Newly acquired stock must be under quarantine for at least 2 weeks, and mixing fish from different sites should be avoided. Any unusual mortality should be investigated by an appropriate laboratory. Good sanitation and management techniques also decrease the incidence of disease. Reducing the fish stocking density in winter and early spring can decrease spread of virus. Stress should be minimized. In facilities with controlled environmental conditions, increasing the water temperature to at least 19-20°C may prevent or check outbreaks, or reduce the mortality rate. Antibiotics can be used to control the bacterial component of carp-dropsy complex (co-infection with *Aeromonas* or other systemic bacteria). Vaccines for SVC are in development, but are not yet available.

Epizootic hematopoietic necrosis

Epizootic hematopoietic necrosis (EHN) is a systemic iridoviral disease of fish. It is caused by Epizootic hematopoietic necrosis virus (EHNV), a member of the genus *Ranavirus* in the family *Iridoviridae* with the type species Frog virus. It causes systemic clinical or subclinical infection in rainbow trout and redfin perch. This disease is highly fatal in redfin perch. Affected farms usually have serious economic losses and severe losses can occur in wild populations. Rainbow trout are less likely to be infected and the mortality rate is usually low.

Natural EHNV infections are known from only two teleost species, redfin perch (*Perca fluviatilis*) and rainbow trout (*Oncorhynchus mykiss*), however, a number of other finfish species are susceptible to EHNV experimentally e.g. Macquarie perch

these are true carriers. Vertical (egg-associated) transmission has not yet been observed. The EHN group of viruses can be transmitted on fomites, and birds may act as mechanical vectors. Virus is shed into water from infected tissues and carcasses as they disintegrate. EHNV can survive in the avian digestive tract for a few hours, and might be transmitted in regurgitated food. It can also be carried on the feathers and feet. EHNV is highly resistant to drying. This virus can remain infective for more than 97 days in the water and for at least 113 days in dried fish tissues.

Clinical Signs

The clinical signs are nonspecific. In perch, sudden death is the most common sign. Darkening of the body surface, lethargy and reddening of skin around the nostrils and brain region have also been seen. Haemorrhages may occur in the gills and at the base of the fins. Symptoms reported in experimentally infected rainbow trout include darkening of the body surface, lethargy, abdominal distension and loss of equilibrium. Skin ulcers, flared opercula and reddening at the base of the fins have also been reported in outbreaks; however, these lesions may be due to secondary infections, suboptimal water quality and other husbandry problems.

Postmortem lesions

In redfin perch, there may be swelling of the kidney, spleen and liver, haemorrhages at the base of the fins and focal haemorrhages in the gills. Focal necrosis is the most consistent finding in head kidney and liver of naturally and experimentally infected fish. The spleen is often swollen, but it is occasionally pale and shrunken. Petechiae may be found on the viscera. In rainbow trout, the lesions may include abdominal distension with ascitic fluid, and swelling of the spleen or kidney. Petechial haemorrhages have been seen on the viscera in a few fish. Focal necrosis in the liver is rare. The gross lesions may be minimal in this species.

Control

In areas where epizootic hematopoietic necrosis is not prevalent, it is controlled by culling, disinfection, quarantines and good health management practices (low stocking density, adequate water quality etc.). In regions where this disease is prevalent, good bio-security and sanitation are necessary to prevent the virus from entering a farm. As the virus is resistant to inactivation, EHNV is presumed to persist for months or years on infected farms in the water, pond sediments, plants and equipment. It is highly resistant to drying and disinfection. In dried surface films, this virus can be destroyed by 70% ethanol in two hours but it is resistant to

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Bacterial Diseases

Most of the bacteria associated with fish diseases are naturally saprophytic organisms, widely distributed in the aquatic environment. Comparatively few species are classified as true obligate pathogens. Both groups of organisms may be present on the external body surface or in the tissues of apparently healthy fish and their pathogenic role will only manifest itself as a consequence of stress. Some of the important bacterial diseases that are major threat to rainbow trout culture are being discussed below.

Bacterial Cold Water Disease (CWD)

Bacterial cold water disease (CWD) is a serious septicemic infection of hatchery-reared salmonids, especially young coho salmon. This disease has also been referred to peduncle disease, rainbow trout fry syndrome (RTFS) or rainbow trout fry anemia. The causal agent of both the diseases is *Flavobacterium psychrophilum*. *Flavobacterium psychrophilum* is usually pathogenic at less than 10°C and can cause disease up to 16°C.The diseases usually occurs in spring, when temperatures are 4 -10°C.Mortality decreases at higher temperatures which begins within 5-10days after infection and peaks 20-60 days later. Mortalities typically are 5-10% but may reach 90% in some epidemics. Colonization of fish may be forerunner to disease.

Clinical signs

Clinical signs most common in sub-acute to acute infections in young fish which include hemorrhages at the base of fins, pale gills, hemorrhagic ulceration in muscle, tail rot, lethargy, darkened skin, ascites, and exophthalmos. Skin ulcerations or eroded/dissolving jaw in older fish is pathognomonic of the disease. Fish with brain infections may have soft hemorrhagic swelling on the head, and fish may exhibit spiral swimming.

Diagnosis

Clinical observations, fresh microscopy and histopathology with biochemical or serological characterization of the isolated bacteria are the usual methods of diagnosis.

Treatment and control

Broad-spectrum antibiotics have been partially ineffective in controlling an outbreak, but improving the environment and using 3-4 times recommended doses of antibiotics have shown benefits. Florfenicol, a chloramphenicol related antibiotic with trade name Aquaflor also appears effective at rate of 10mg/kg body weight/day for 10 days. As *Flavobacterium psychrophilum* rapidly develops antibiotic resistance thus the antibiotic sensitivity of the isolate should be determined. Autogenous vaccines are is use in the UK.

Bacterial Kidney Disease (BKD)

Bacterial Kidney Disease (BKD) is a systemic infection of salmonids caused by *Rennibacterium salmonarium* that is slowly progressive and frequently fatal leading to an acute to chronic systemic granulomatous disease. Salmonids are clinically susceptible, especially belonging to the genus *Oncorhynchus*(Pacific salmon and rainbow trout). The disease is transmitted by both vertical and horizontal transmission. The infected salmonids remain as carriers of the infection for life. Horizontal route is by "direct contact" with diseased fish and contaminated water, or indirect, through handling materials or feeding with fisheries residues. Vertical transmission is the most frequent route of contamination. The bacteria are present in the ovarian fluids and are likely to be the source of the *R. salmoninarum* that have been detected in eggs from some infected female salmonids.

Clinical signs

Pale gills, exophthalmia, abdominal distension (due to ascites), skin blisters (filled with clear or turbid fluid), shallow ulcers (the results of broken skin blisters), hemorrhages (particularly around the vent) and more rarely, cavities in the musculature, filled with blood tinged caeseous or necrotic material. Internally, there may be fluid in the abdomen, varying hemorrhage on the abdominal walls and viscera, a membranous layer on one or more of the visceral organs, and most characteristically, creamy-white glaucomatous lesions in the kidney and less frequently in the liver, spleen and heart.

Etiology

*Renibacterium salmoninarum*is a small fastidious, slow growing (0.5x1.0µm), non-acid-

Diagnosis

Clinical signs, Grams smear, ELISA, FAT, IFAT, histopathology, isolation (2-3 weeks at 15°C) in cysteine-enriched media such as kidney disease medium (KDM2), or selective kidney disease medium (SKDM) with agglutination tests and PCR. Histological sections show large area of focal necrosis and chronic interstitial necrosis with hematopoietic tissue replaced with macrophage infiltration with hematoxylin and eosin (H&E) staining. The disease must be differentiated from other kidney diseases of chronic progression including pseudo-kidney disease (*Carnobacterium piscicola*), nephrocalcinosis, and proliferative kidney disease. Confirmation must be made by observations and identification of the bacteria.

Treatment and control

Control of BKD with conventional treatment methods, such as chemotherapeutics remains a challenge due to the intracellular nature of *R. salmoninarum.* The bacteria can survive and multiply within phagocytic cells. Prevention is the first and strongest line of defense.Chemotherapy (erythromycin) provides limited and only temporary relief two techniques are followed to deliver the antibiotics. In first technique, erythromycin phosphate is injected subcutaneously into the median dorsal sinus just anterior to the dorsal fin of adult salmon and steelhead trouts. The adults are given additional injections at 30-days intervals until 30thday prior to spawning. Each fish is given erythromycin phosphate at 1.0 mg/kg of body weight. Eggs taken from infected females have been shown to carry drug levels that are inhibitory to *R. salmoninarum*in laboratory. The second technique involves water-hardening in which freshly fertilized eggs are treated in a 2-ppm bath of erythromycin phosphate for up to one hour.

Enteric Red Mouth disease (ERM)

Enteric Red Mouth disease (ERM) is a systemic bacterial infection of fishes, but is principally a problem for young farmed rainbow trout, however, all salmonids are susceptible. *Yersinia ruckeri,* a Gram-negative, motile rod-shaped bacterium is the causal agent. Feral fish, imported baitfish or even ornamentals have been suggested as sources of ERM. *Y. ruckeri* is also isolated from faecal matter ofbirds.

Clinical Signs

Gross external signs first described were lethargy, skin darkening and congestion around the mouth and operculum, and at the base of the fins. Other signs seen include exophthalmos, ulceration and coetaneous petechiae. Internally the fish show signs of hemorrhagic septicemia

isolation during epidemics. FAT and ELISA tests have also been used but isolation is usually necessary for antibiotic sensitivity. Histologically, there is colonization of well vascularized tissues, causing hemorrhage of gills, kidney, liver, spleen and heart as well as muscle.

Prevention, Treatment and control

A bacterin produced using *Y. ruckeri* was the first commercial fish vaccine, and a considerable amount of work in vaccine development and field-testing has involved oral, injection and immersion products against ERM. Broad-spectrum antibiotics are effective in controlling an outbreak, but increasing antibiotic resistance are observed and sensitivity should be tested. Sulfadimethoxine-Ormetoprim at the rate of 50mg/kg weight can be used but it is quite expensive.

Furunculosis

Furunculosis is a fatal epizootic disease, primarily of salmonids, caused by the bacterium *Aeromonas salmonicida*. This organism can also cause clinical disease in other fish species where it is named as ulcer disease or carp erythrodermatitis. *Aeromonas salmonicida*is a Gram-negative, non-motile rod and most strains produce a brown diffusible pigment on agar containing tryptone. It grows best at 22°C or lower tempratures. Atypical furunculosis is caused by a slower growing non-pigmenting isolate *A. salmonicida achromogenes.* Salmonids (wild and farmed) can carry the organism and when these fish are stressed, such as with high water temperatures or low oxygen levels, then clinical disease can break.

Clinical Signs

In a population affected by typical/classical furunculosis there will be examples of both chronic and acute forms of the disease. High mortalities, without external signs of infection are often associated with acute furunculosis, although anorexia may be present. Other fish may appear dark in colour, lethargic with reddening at the fin bases or head. Internally there may be widespread petechiae in the viscera and a swollen spleen. In chronic furunculosis, usually seen in older fish, there may be similar clinical signs to a subacute form. Liquefactive, hemorrhagic lesions may be present in the musculature with bloody discharge from the vent and splenomegaly also present. Atypical furunculosis may cause lower level mortalities and small skin ulcers with a dark, pigmented periphery.

Diagnosis

Gross and histological signs (H & E) are helpful but confirmation requires isolation on general nutrient agar (24 - 48 hours at 22°C) such as TSA or BHI. Isolation of the bacteria is

amoxicillin trihydrate / kg of body weight/day for 10 days is effective against the resistant isolates.

Bacterial Gill Disease

Bacterial gill disease is an important disease in farmed freshwater salmonids caused by bacterium *Flavobacterium branchiophila* that results in proliferative response in gill tissue. *Flavobacterium branchiophila* is a Gram-negative, long, thin, filamentous rod shaped bacteria. Global distribution and most reports are in farmed salmonids, although most teleosts are susceptible. Probably in wild fish and aquatic ecosystems the organism may be present naturally as carrier of infection. The factors favoring the disease are low oxygen, high turbidity, high ammonia and overcrowding.

Clinical Signs

Gill is the only target organ and clinical signs include lethargy, dyspnea, coughing and flared opercula. Strands of mucus may trail from the gills and exhibit pale and/or swollen areas.

Diagnosis

Wet gill smears or histopathology. Isolation on Cytophaga Agar at 18°C. Histological section shows hyperplasia and fusion of adjacent secondary lamellae and hyperplastic lesions surrounded by bacteria and cell debris.

Treatment and control

BGD usually responds well to antiseptic and surfactant baths such as chloramine T and benzalkonium chloride. Providing adequate oxygen is useful supportive therapy.

Mycobacteriosis (Fish tuberculosis)

Mycobacteriosis in fish is a chronic progressive disease caused by certain bacterial species within the genus *Mycobacterium*. The species in fish are non-tuberculous mycobacteria (NTM) and do not cause major disease in healthy humans. But, some species can infect humans usually causing localized, non-healing ulcers that may be difficult to treat because of the resistance some isolates have to most anti-tuberculosis drugs. *Mycobacterium marinum, M. fortuitum, M. salmoniphilum* and *M. chelonae* are all considered pathogenic for fish. All are aerobic, acid-fast, Gram positive and non-spore forming. Many of these bacteria occur naturally in the aquatic environment. Contaminated feed prepared from uncooked trash

Diagnosis

Clinical signs, histopathology, growth of organisms on Lowenstein-Jensen or Middlebrook 7H10 can be used for diagnosis. Histological sections show granuloma with H&E staining and numerous acid-fast mycobacteria in a granuloma of the fish.

Zoonotic Potential

Transmission from infected fish to humans is rare but "fish handler's disease" or "fish tank granuloma" is a condition that can occur in humans as a result of skin infection from these bacteria. Gloves should be worn when handling suspect or infected fish or when cleaning contaminated surfaces.

Treatment and control

There is no effective treatment therefore the best course is to cull and disinfect premises. 10,000ppm chlorine or 60-85% alcohol can be used as disinfectants. Other disinfectants include sodium hypochlorite @ of 200mg/L for 60 minute.

Rainbow trout gastroenteritis (RTGE)

Rainbow trout gastroenteritis (RTGE) is an enteric syndrome of freshwater-farmed rainbow trout reported in several European countries, which results in significant economic loss and daily mortalities of 0.5 to 1.0% are common. Rainbow trout in freshwater are the affected species, although there have been a low number of suspect (unconfirmed) cases in Atlantic salmon in freshwater. No specific cause of the disease is established. However the "*Candidatus* arthromitis" may have some role to play in the disease. This bacterium has not yet been cultured *in vitro*.

Clinical signs

Lethargy, discoloration and slight swelling or oedema of the body gives a hunched up appearance. Internally, congestion and oedema of the intestinal wall with a yellow catarrhal faecal cast can be observed. Mortalities can persist for many weeks and the condition is associated with warmer water temperatures in the summer months (Plate-2). The disease is diagnosed on the basis of clinical signs, pathology and histopathology.

Treatment and Control

Changing the diet type or the adding salt to diet and broad spectrum antibiotics appear to be effective once the disease is present however none appear to be preventative. Bio-security

pleomorphic) organism. Salmonids, particularly Pacific salmon is vulnerable. Wild fish, shellfish, crustaceans all are reported to harbor rickettsia but the true source not established. Vectors (lice, isopods) may also be involved in transmission.

Clinical signs

Skin lesions, dark skin, lethargy, anorexia, nervous signs in some cases, and internal petechiae, peritonitis, ascites, white nodules in liver and kidney.

Diagnosis

Confirmation can be done using immune-histochemistry, isolation of the organism in cell culture (CHSE-214) in the absence of antibiotics. PCR has also been developed.

Treatment and Control

Broad-spectrum antibiotic therapy is used, although some resistance has been reported to be developing. However, oral antibiotics have been mostly ineffective probably because of *P. salmonis*is an intracellular pathogen. Outbreaks usually associated with stressful event, such as algal bloom, sudden change in environment or grading.

Red mark syndrome (RMS) or Cold water strawberry disease

Red mark syndrome (RMS) is an infectious dermatitis of rainbow trout, which does not cause mortality but presents as dramatic hemorrhagic marks on the skin. Not fully established *Flavobacterium psychrophilum* and rickettsia-like organisms have been associated with the condition in Scotland and the USA respectively. An adeno-like virus was observed from two cases in France. Rainbow trout in freshwater are the affected species, although there have been a low number of suspect (unconfirmed) cases in brown trout in freshwater and rainbow trout in seawater.

Clinical signs

Red, hemorrhagic marks on the flanks of trout can appear suddenly and then resolve within a few weeks (or months) without treatment. There are no mortalities or internal abnormalities associated with the disease. Diagnosis is based on clinical signs and histopathology.

Treatment and control

The lesions resolve eventually without treatment, however, broad-spectrum antibiotics do induce a rapid healing of the condition. Livestock from infected farm, nets etc., should not

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Fungal Diseases

Fungal infections are generally restricted to chronic, but steady losses. In fish, fungi are known to attack eggs, fry, fingerlings and adults. Mostly the fungal infection starts when the host gets injured or as a result of infection other than fungal origin. Coldwater aquaculture industry, particularly rainbow trout farming has been rapidly developing in many areas of hill states in India. One of the serious problems in coldwater aquaculture is fungal infections that can be observed in cultured rainbow trout, mahseer and exotic carps (Figure -1).

Fungal infected trout

Fungal infected carp

Saprolegniosis

Saprolegniosis is caused by *Saprolegnia* sp., which affects a wide variety of fish species including coldwater fishes. *Saprolegnia* is generally a secondary pathogen but under some circumstances it can act as primary pathogen as well. Most frequently, it targets fish both in wild and in tank environment through cellular necrosis and other epidermal damage. The disease has been observed most frequently in brood stock ponds during the post spawning period. Initially the disease appears as white mats over the skin which gradually spreads and invades in deeper tissues causing mortality in acute cases. All the life stages of fish including the eggs are prone to such infections. As the infection progresses the fish becomes increasingly lethargic due to restriction of movement because of overgrown mycelium. Infected fish becomes lethargic, listless and less responsive to external stimuli, loss of equilibrium often occurs shortly before death. *Saprolegnia diclina* infections found more common in winter months, whereas *Saprolegnia ferax* occurs predominantly in the spring and autumn.

Branchiomycosis

Branchimycosis also termed as "gill rot" is characterized by a yellow to brownish discolorations of the gills sometimes leading to disorganization and loss of a portion of the gill in the infected fish. The affected fish gasps for air and ultimately dies due to asphyxiation. Gills are affected due to serious obstruction of blood vessels. Affected gills lose their normal bright red colour and show brownish areas due to hemorrhage and thrombosis and lighter whitish or grayish areas as results of ischemia. The disease is common in ponds having abundant decaying organic matter. Branchiomycosis is caused by the fungus *Branchiomyces sanguinis* and *B. demigrans*. The disease appears suddenly and spreads rapidly with losses as high as 30 to 50% during two to four days. The disease occurs in carps, murrels and weed

sporangium opening. In species of the genus *Aphanomyces* the cysts are produced in the same way as in *Achlya* but unlike the latter, the former have much thinner hyphae and the zoospores in their zoosporangium are arranged in a single line. The infected fish shows physical abnormality and abnormal behavior with white to grey cotton like growth on skin, fins and gills.

Causes and Clinical signs of the fungal infected fish

The infected fish shows physical abnormality and abnormal behavior. Clinically, affected fish is found with white to grey cotton like growths on skin, fins and gills. Initially the disease appears as white mat over the skin which gradually spreads and invades in deeper tissues causing mortality in acute cases. All the life stages of fish including the eggs are prone to such infections. As the infection progresses the fish become increasingly lethargic due to restriction of movement because of overgrown mycelium. Depending on the degree of infestation, the affected fish stops taking food and lose their escaping reflex. Loss of equilibrium often occurs shortly before death. They contain white-grey wool-like fungal growth of different sizes, prominently on their body surface. The transparent mycelium with a large amount of sporangia can be seen under the microscope. In the fish, fungus remains as an ulcerative mycosis that converts into a deep necrotic lesion involving the muscle. In early infections, skin lesions appear gray or white in colour, with a characteristics circular or crescent shape. Due to this, rapidly, causes destruction of the epidermis. Unfertilized trout eggs are susceptible to fungal infection. During egg incubation, these fungi produce mycelia that grow and spread from the non-viable to the healthy eggs which cause suffocation leading to mortality in eggs.

The causes and pathogenesis of this disease are largely unknown. However, sudden decrease in temperature and a significant number of pathogenic fungal zoospores in water have been identified as risk factors for the disease. The primary risk factor is thought to be the inability of the fish to adapt to rapidly fluctuating water temperature during the winter months. The rapid decrease in water temperature from 22°C down to 12°C in 24 hours have been shown to impair the fish immune system. It causes a loss of mucus from the skin and temporary suppression in mucus production by goblet cell in the dermal layer. Mucous provides a physical barrier that prevents fungal spores from contacting and infecting the skin of fish. Mucous also contains antimicrobial components (including immunoglobulin or antibodies, complement, C-reactive protein, lysozyme, and photolytic enzymes) that can destroy invading zoospores. Without mucus, skin is unprotected and fungal spores develop as masses of fungal hyphae that extend into the muscle tissue. If fungal spores are not present in sufficient numbers to establish infection, fish can adapt to a change in temperature and regain normal function of goblet cells and mucous production within a few days. Once the infection is established, fish

due to osmotic or respiratory problems when large area of skin or gills with are affected. Improper handling and physical injury is also a major cause of the disease. Over stocking, uncleaned pond with clay sediments in bottom, high ammonia level in the pond water and under feeding creates stress on the growing stock and fish becomes more prone to fungal infection.

Prophylactic measures and cure

Fungal infections are difficult to prevent and treat. Malachite green was considered the most effective chemical for controlling *Saprolegnia*. However, because of concerns about potential carcinogenicity, i.e., its teratogenicity and/or mutagenic properties, malachite green is banned in the United States and some other countries. Currently, the most effective strategy for controlling and preventing *Saprolegnia* infections is a combination of good fish management and husbandry techniques, combined with salt treatment. Water flush treatment with 2-3% of common salt, weekly, for 3 weeks can be given to the infected stock. Preventive measures that could be for reducing the risk of fungal infection are -

- Proper handling of stock to avoid the physical injury.
- Ensuring hygienic environment for fish with regularly cleaning of the tanks. Feeding with appropriate rate, frequency and with quality feed.
- Stocking of diseased free seed in appropriate density.
- Maintaining water flow in the trout raceways.

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Parasitic Diseases

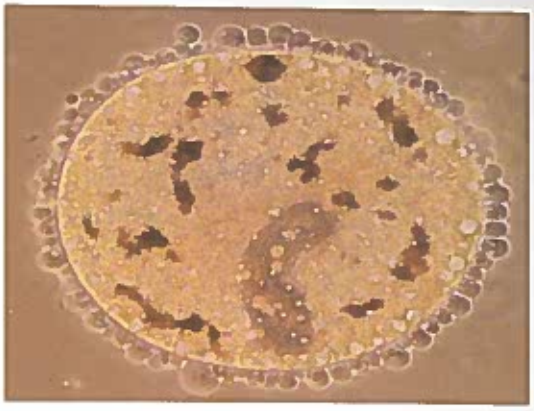
Broadly speaking, a parasite is an organism that requires a host for its survival as it lives on or in the body of its host to meet the demand of its nutrition or food. Parasitism is a sort of relationship in which the parasite benefits from the host. Based on their dependence, these can be classified as obligate and facultative. Obligate parasites are those that cannot survive without a host while facultative can survive without a host but, can opt to live on host as per their needs. Depending upon their habitats also, parasites are grouped either as ecto-parasites or endo-parasites. Ecto-parasites live outside the body of a host for example leech, body louse etc while endo-parasites live inside the body of its host for example myxosporidian, microsporidian, trematodes and nematodes.

Like plants and other animals, fish too are affected by parasites. These organisms can cause direct or indirect serious loss in aquaculture by reducing the production and profit of fish farmers. Protozoans, trematodes and crustaceans are common parasites of the coldwater fish species like trout, mahseer and carps. Generally, these parasites attack fins, skin, and gills besides internal parts of the body for example, liver, alimentary canal. Finally, alteration of normal swimming behaviour, respiration, feed intake, digestion and excretion is occurred. Affected fishes become sluggish, weak and their growth suffers. In severe infection, large scale mortality occurs. Some of the important parasitic diseases are being discussed below.

White spot (Ich) Disease

Holotrichus ciliate protozoan *Ichthyophthirus multifilis* is the etiological agent of white spot disease. It grows within the skin of fish and multiplies both in its host (fish) and in tank/ pond water. The adult parasite is 1mm long round hairy often brown coloured parasite. The parasite is oval with "C" or horse shoe shaped nucleus, moves very slowly. Tomites are pear shaped, infectious and invade fish body. After invading the skin, they take fluid from the blood cells of the fish and increase in size. At infected site

of indigenous and exotic coldwater and warm freshwater fish species. The parasite burrows into the skin, gills and preys on red blood cells of the fish.



Ichthyophthirus

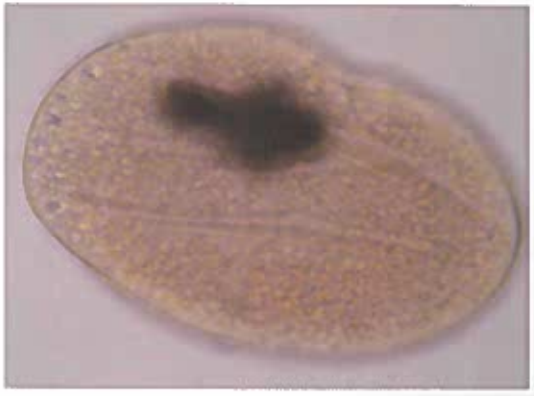
In majority of cases, fry and fingerlings are affected by this parasite. Erratic swimming behavior, infected fishes try to rub their body against hard surfaces of the pond and marginal areas. As the parasite burrows into the skin and gills, white pin head size spots are produced in these organs. Different degree of inflammation and epithelial erosion could be other microscopic changes expressed at tissue level. Irritation and itching is caused, therefore the disease is also named as itching disease.

Some of the control measures are –

- Dip treatment in 2-3% salt solution for 1-2 minute
- Applying lime @30-50mg/l
- Dip treatment with $KMnO_4$ @ 1ppm

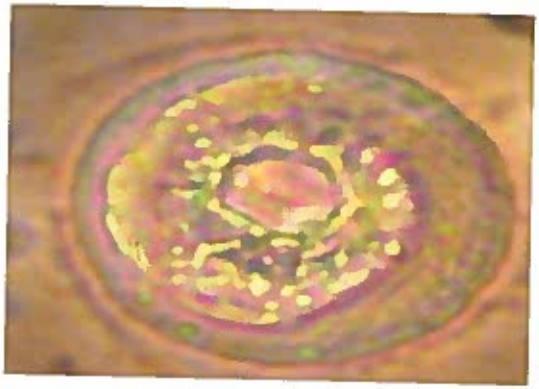
Costiasis

Costiasis is a common disease of salmonid fry. The disease appears in spring with the first warming of the water of the hatchery. Within a few days, the infection can become intense. The causative flagellate agent is *Costianecatrix*, which lives in large in number on the skin, fins and the gills. The major symptoms of the affected fish are: the skin gets covered with a light gray-blue film; the more seriously affected body parts show red patches; the gills becomes brown in colour; and a few a cases, are even partially destroyed. The infected trout weaken, lose appetite and ultimately die. A 30 minute formalin bath in a concentration of 1:4,000 or a 15



Costia

belonging to the genus, *Trichodina, Trichodinella and Tripartiella*. These parasites are 30-40µm in diameter and harbor gills besides the external surface and causes serious irritation at the site of attachment. Presence of one or more predisposing factor such as high stocking density, malnutrition, higher organic load with poor water quality, rise in water temperature etc., increase the risk of disease outbreak.



Trichodina

Occurrence of this disease is very common during monsoon when fry rearing activities are maximal. The parasites get attached to skin, fins and gill. The incidence of trichodinasis is mostly in small fry and fingerlings with large mortality of fry. High organic load along with rise in temperature helps the multiplication of this parasite in intensive fry rearing facilities.

The signs of the disease include reddening of gills, minute red spots over gills, skin, fins and especially anal fins are the most common site for the attachment of the parasite. As these parasites graze the body mucus, fish shows erratic behavior and try to rub their body to hard substances to reduce the itching caused. The body coloration also changes and becomes blackish with excessive mucus secretion from the skin and gills. Disease can be controlled by the following ways:

- Dip treatment in 2-3% salt solution for 2-3 minutes every alternate day. The treatment should be given for a week.
- Dip treatment in 1:1,000 Acetic acid for 2-3 minutes
- Dip treatment with $KMnO_4$ @ 1ppm

Myxosporadian parasites

Thelohanellus, Henneguya and Myxobolus are three major genera that infect coldwater fishes. The parasites attach to gills, skin, muscle, liver and kidney. Spores with polar capsule and eye spots are a characteristic feature of these parasites. Depending upon the size of

schizogamy, gametogamy and sporogamy. Several species of myxosporidians infect coldwater trouts and carp species, produce mucoid cysts over the body surface, fins and gills. In microsporidian infection, internal organs like ovary, kidney, liver, spleen are affected.

The affected fish show erratic swimming behavior, petechial hemorrhages at infected site, decreased feeding, lethargic behavior of fishes could be observed. Fishes with excessive cream coloured cysts over the fish skin fetch low prize in market. Damage of kidney tubules, hepatic cells, poor egg production and breeding response due to gonadal regression are other harmful effects observed during the infection. Thousand of spores encysted in a cream coloured cyst get lodged to fish skin or gills. Histopatholgically disruption of skin and gill epithelium, fusion of lamelle, cellular necrosis, hyperplasia necrosis, dystrophic changes are also observed.

Thelohanellus, Henneguya and Myxobolus are three common genera infecting fish species farmed in foot hills and mid Himalayan region. The spores are covered by thick wall and thus making them resistant to many therapeutic agents due to non penetration of drugs used for treatment. The parasite obstruct normal gill functioning on attachment and after detachment of the cyst, the site provides route of entry for secondary pathogens.

Some preventive and control measures include –

- Good husbandry with proper management helps in controlling the disease to some extent
- At the time of pond preparation, fresh bleaching powder applied @ 40-60ppm
- Few parasitic species show specificity to fish, hence, changing the number and ratio frequently species may reduce the chances of outbreak
- Use of anti-coccidial drugs toltrazuril stops developmental stages of *Myxobolus sp.*

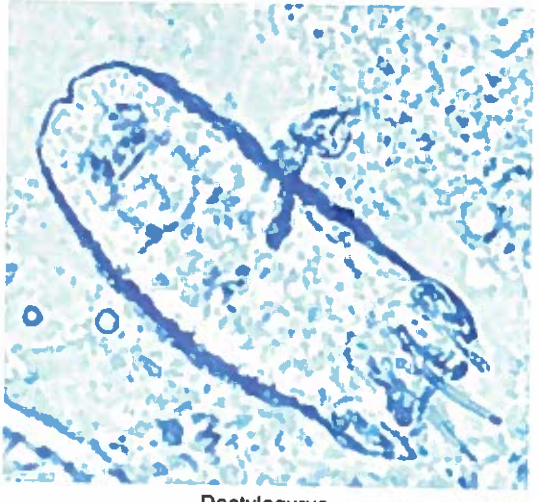
Whirling diseases

Causative agent of the disease is *Myxobolous cerebralis* while *Tubifex tubifex* an oligochaete that dwells in fish the ponds acts as carrier. In this disease, infected fishes show circular or whirling movement due to imbalance and loss of equilibrium, erratic swimming, darkening of tail region, deformity of skeleton and mortality. The disease is very common in small fry and fingerlings. The parasite infests a wide range of fish including touts, exotic carp and indigenous hill stream fishes. Preventive and control measures are the same as for other myxosprideans.

Trematode Infections

In dactylogyrosis, the parasite causes localized infection and invades gills which are a vital organ for exchange of oxygen and survival of fish. Excessive secretion of light bluish mucus, changes in body coloration, hemorrhages, light yellow lining in the gill filaments, fading of gill color are the major signs. In severe cases, cotton wool like growth embedded with soil particles can be seen between the gill filaments. Infected fishes become weak and emaciated resulting in death. Large scale fry kills due to this gill parasite has been observed in both coldwater and warm water fishes. It is considered as one of the serious coldwater parasitic disease encountered during seed production and in grow-out ponds.

Monogenic trematode *Dactylogyrus* sp., is the causative agent of this infestation which infects the gills. The parasite is identified by presence of two eye spots, seven pairs of marginal hooks and two big hooks which help in attachment of parasite to the host. High organic load, intensive stocking, lower dissolved oxygen/ pH, increased free ammonia value are some of the important favorable factors for multiplication of parasite in the environment. Lower pH due to accumulation of residues is also a common phenomenon found in infested facilities. Development of filamentous algal growth in cemented nursery tanks during larval rearing along with rise in temperature also increases the risk of disease outbreak. The intensity of infection can be measured by presence of parasite in gills. Some of the preventive and control measures are-



Dactylogyrus

- Dip treatment in 2-3% salt solution for 2-3 minutes every alternate day followed by dip treatment in 1-4ppm of $KMnO_4$ solution.
- Improving water quality parameters and transfer of small fishes also help in bringing down the infestation.

Gyrodactylosis is another trematode infection in which small red spots over the body, fins and gills, excessive secretion of mucus, emaciation, restlessness in infected fishes, breaking of gills filaments and development of hemorrhagic wounds in gills are observed. Since the parasite infects both skin and gills however, the intensity of infection is greater in fins and skin. Impairment of osmoregulation and secondary infection are the

portion. The parasites increase their number under adverse physicochemical conditions. Preventive and control measures are similar to dactylogyrosis infection.

Black Spot Disease

Disease can be easily identified based on presence of round black nodules or cyst like outgrowths over the body of infected fishes. Mostly smaller fry and fingerlings are affected. Location and number of spots varies from1-2 to 50-60-in each fish and varies with the fish species. Retardation of growth and weight loss is also observed. Although rate of morbidity and mortality is low but fishes look ugly and chances of other secondary infection increases.

Digenictrematode worm *Diplostomum sp.* is responsible for this infestation. The parasite completes its life cycle in three organisms i.e., fish eating bird, snail and fish. Metacerceria find fish as a host and get attached to the skin. Consequently fish body reacts with secretion of melanin pigment and covers the parasite. Some of the preventive and control measures are –

- Aquatic weeds removal reduces the number of snails in the pond
- To break the life cycle of parasite, trees surrounding fish ponds should be cut so that fish eating birds will not get space to sit and release parasite in the pond water.
- Picric acid treatment to control the infection
- While preparing the nurseries, application of bleaching powder @60ppm kills snails of the rearing facility.

Eye Disease of Rainbow Trout

The disease is observed in fishes and initially a white opacity in lens is seen. The disease is more apparent and pronounced in adult trout leading to cataract, dislocation and degeneration of lens, liquefaction of cortical fibers and finally blindness. Feed intake in blind fishes is reduced substantially and fish becomes weak. Other healthy rainbow trout also

Preventive and control measures include –

- Use of Praziquantel @300 mg/kg body weight of fish
- Bath treatment in Praziquantel @ 1mg/l for 9 hours
- Bath treatment in Praziquantel @ 10mg/l for 1 hour
- Breaking the life cycle of the parasite by removing secondary host present in the fish environment.

Argulosis

Argulosis is an important disease of coldwater fishes specially snow trout and carp. The causative agent *Argulus* is known to have more than 100 species. At initial stages of infestation, localized reddish patches are seen at the base of ventral and pectoral fins, which in advance stages spreads to different places of the body. Infected fishes are subjected to severe stress. Excessive mucus secretion, erratic behavior, scratching against hard substrate, surfacing, low feed intake and emaciation are the general signs that can be recorded. With the rise in water temperature beyond 16-17°C, susceptibility of rainbow and brown trout increases during summer months. Therefore, abundance of argulosis is mostly recorded during March-April and June-July, when biomass of ponds increases with rise in temperature.

Argulus

Preventive and control measures include –

- Incoming water should be filtered by fixing nets
- Removal of aquatic weeds and floating hard substrate from the pond reduces the intensity of infestation as area for egg laying reduces. Eggs laid over bottom biota had chances of damage.
- Fixing wooden/ bamboo poles/ FRP plates in the tanks/ponds and their removal

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Non-infectious Diseases

Non-infectious diseases can be broadly categorized as environmental, nutritional, or genetic.

NUTRITIONAL DEFECIENCY DISEASES

Nutritional deficiency diseases of fish may develop as a result of deficiency (under nutrition), excess (over nutrition), or imbalance (malnutrition) of nutrients present in food. The development of nutritional deficiencies is gradual because animals have body reserves that make up for nutritional deficiency up to a certain extent. Signs of disease develop only when supply of any diet component falls below critical level. When there is too much food, the excess is converted to fat and deposited in fish tissues and organs. This may severely affect physiological functions of the fish.

Nutritional management of fish is highly variable, depending on species and system. For established food fish industries, high-quality diets are readily available. For coldwater aquaculture, nutrition is one of the greatest constraints in development of commercial culture. Fish generally require a high-protein diet, with a significant percentage of protein provided by fish meal.

Lipodosis

Lipodosis is a common nutritional disease among cultured food fish. Various degrees of lipodosis have been observed in the liver of fish. Feeding with rancid formulated feeds or with fatty or poorly stored trash fish can cause lipodosis. Fish in the grow-out stage are susceptible to lipodosis. Affected fish grow poorly, are lethargic, with opaque eyes, and shows slight distention of the abdomen. The liver has a pale appearance and histological sections show presence of large fat droplets. The disease may be diagnosed by histopathology of liver and analysis of proximate analysis of artificial feeds.

EFA Deficiency

Trout larvae require essential fatty acids (EFA) for normal growth and development. Fatty acids are essential components of bio-membranes and precursors of some physiological modulators such as the eicosanoids. Essential fatty acids such as docosa-hexaenoic acid [DHA, 22:5(n-3)] and eicosapentaenoic acid [EPA, 20:5(n-3)] are commonly found in live food. Deficiency in these fatty acids is associated with larval mortality known as "shock syndrome" in which the larvae display unusual sensitivity to stress. Visual observation of larval behavior (weak movement) indicates this disease.

Preventive and control methods includes supplementation with DHA and EPA is reported to slow down larval mortality.

EAA Deficiency

Fish shows reduced growth when fed essential amino acid (EAA) deficient diets. Poor feed formulation due to the use of disproportionate amounts of feed proteins leads this disease. Besides the fish meal, which has a well-balanced EAA profile, the majority of protein source presented have amino acid imbalances which render them unsuitable as a sole source of dietary protein for fish. For example, the deficiency of methionine in plant proteins, yeast, meat and bone meal, blood meal, and hydrolysed feather meal; the deficiency of lysine in oilseeds, hydrolyzed feather meal and algae; the deficiency of threonine in some oilseeds and pulses; and the deficiency of tryptophan in fish silage. It is clear from the above that during feed formulation special care must be given to the choice of feedstuffs used, so that the desired overall dietary EAA profile is obtained. Dietary EAA deficiencies may also arise from excessive heat treatment of feed proteins during feed manufacture. Rainbow trout shows dorsal and caudal fin erosion due to lysine deficiency, cataract due to methionine deficiency, scoliosis and lordosis due to tryptophan deficiency.

Preventive and control methods include supplementation of diet with adequate amount of fish meal.

Vitaminosis and mineral deficiency

Most fish do not synthesize ascorbic acid therefore, adequate supplementation is necessary. Classic ascorbic acid deficiency has been referred to as "broken back disease" because of the collapse of the vertebral column that occurs due to inadequate collagen synthesis. Less dramatic signs of deficiency include impaired wound healing and immune function.

Of the B vitamins, folic acid deficiency has been associated with poor growth. Pantothenic

Choline and inositol deficiency have been linked to poor growth. Vitamin A deficiency has been associated with poor growth and retinal atrophy. Vitamin E deficiency has been related to myopathy, including muscular deformities. Skeletal muscle abnormalities have been associated with selenium deficiency and rancid feeds. Thyroid tumor or goiter is primarily caused by the deficiency of iodine in trout. A table spoonful of Lugol's solusion can be mixed in 25 kg fish feed to control the disease.

Preventive and control methods include proper storage of feeds in a cool and dry place to minimize degradation of vitamins. Adequate supplementation of the vitamins and minerals is required according to the requirement of the species.

Nutritional Gill Disease

Gills of fish are affected with a swelling (hyperplasia) in the proximal portions of the gill filaments. In typical gill disease bacteria or parasites are absent. Fish with this condition exhibit a loss of appetite and become lethargic. Fish tend to congregate near the water inlet. The primary cause of the disease is pantothenic acid deficiencies in the diet. The dry pelleted feeds which have been stored for extended periods and exposed to high temperatures are responsible for this condition. The disorder is reversible, although recovery may be gradual.

Sun Burn

Sun burning found only in salmonids, characterized by a peeling or sloughing of outer layers of the skin. Lesions usually occur on the dorsal skin, between head and dorsal fin. Outbreak occurs when fish in clear water are subjected to high levels of UV light. Dietary deficiencies of niacin contribute to the disease. Place sunshades over ponds to eliminate the problem. Sun burn can be a "port of entry" for invasive bacteria or fungal spores.

Aflatoxicosis

Aflatoxicosis is caused by the contamination of feedstuffs by the fungus, *Aspergillus flavus*. The toxins produced by the fungus are collectively known as aflatoxins. Moist conditions and warm temperatures favour the growth of this fungus in feed. Aflatoxins have been known to produce liver hematomas in trout. There are no early external signs of the disease. Tumors can be recognized as small gray-white or yellow nodular lesions within the liver. There is no known treatment for affected fish. Preventive measure is removal of damaged feed and keeping feed sacks in cool and dry place.

Fatty Infiltration of the Liver

Lordosis and Scoliosis

This is a curvature of the spinal column in trout and salmon. Condition is caused by a lack of vitamin C in trout diet. Fish can survive with this condition in a hatchery environment. Once stocked the chances of survival are low due to the erratic behavior of the fish. Increase vitamin C in the diet. Curvature of the spine is not reversible. It is best to destroy these fish than stock them in a stream or lake.

Cataracts

This is a cloudiness or opacity in the crystalline lens of the eye, often leading to blindness. This may be due to a deficiency of vitamin B12, histidine, riboflavin and zinc. These fish lose the capacity to evaluate distance. As a result the fish lose their ability to attack prey or find food in the natural environment. Such fish may also lose the ability to avoid predators besides losing weight and may die due to difficult circemstances.

Whirling disease

This disease is due to the deficiency of the thiamine chloride. Trout shows whirling movement and are not able to swim in its normal upright position. The fish shows a tendency to swim at an angle to the vertical plane. As the intensity of the disease progresses, the angle to the vertical plane becomes acute until the fish starts swimming practically on its sides. Finally, the trout falls in the bottom and remains motionless for some time. One of the most pronounced signs is melanotic appearance of fish. The infected fish picks up a mouthful of food and rejects it. Trout of the all age groups are prone to this disease. Vitamin B1 can be given in the feed @ 0.15-0.186 mg/day/kg.

Nutritional diseases can be prevented to a great extent by following certain practices which are as follows:

- Store feed bags (dry pellet) in dry, well ventilated and cool place.
- Humidity in store room should be less than 75%.
- Avoid excess heat during feed manufacturing, which causes oxidation and loss of vitamins, especially vitamin C.
- Storage time should be strictly adhered to. Storage time is 90 days for dry and moist feeds and 60 days for semi-moist feeds.
- Moist feeds must be kept frozen until ready to use.

- Don't feed fish immediately before a stressful situation.
- Collect information on diet, size and type of feed, storage and feeding procedures, age of diet, abnormalities in the diet, if fish is nutritionally sick.
- Observe the clinical signs, symptoms and behavior of sick fish.
- Send affected fish to fish health laboratory for diagnostic work.

COMMON ENVIRONMENTAL FISH DISEASES

Gas Bubble Disease

Gas bubble disease results when water becomes supersaturated with oxygen or nitrogen gas. Total gas saturation between 105 and 140% produces chronic gas bubble disease. At 140% and above the disease is acute. Gas supersaturated water results when strong sunlight (rapid photosynthesis) produces high oxygen. The disease is characterized by formation of gas bubbles in the eyes, gills, fins, skin, and mouth. The most common lesions occur in the gill tissue where gas bubbles block respiration, ammonia excretion, and osmoregulation. A degassing column with coke rings or an oxygen injection system can control the disease

Nephrocalcinosis

Nephrocalcinosis is a chronic, degenerative, inflammatory disease affecting the kidneys of trout. It is common in rainbow and brook trout and has been reported throughout the United States. Mortality rates are low, but stressing affected fish can result in massive mortalities. Sign of the disease is white chalky material in the kidneys with tubular dilation and degeneration of the nephrons. Cause of the disease is unknown, however high carbon dioxide, magnesium deficiency, selenium toxicity and a diet low in minerals are the major cause of the disease.

Blue-Sac Disease

Commonly observed in salmonid sac fry. Affected fry show an abnormal accumulation of fluid over part of, or the entire yolk sac. Accumulated fluid takes on abluish hue. Exophthalmia is commonly observed in affected fry. Growth is retarded and fry are paler than normal sac fry. Blue sac fry are lethargic and gather in quiet areas such as centers of trays and lower ends of rearing troughs. Cause of disease is not known, however infectious agents, physiological factors, hereditary, physical and chemical parameters have been suggested.

White-Spot or Coagulated Yolk

disease include poor water quality, nitrogen supersaturation, temperature extremes, egg loading and rough egg handling, chemical treatments, bacterial, fungal, and protozoan infections. To reduce or eliminate the disease, nitrogen gas levels must be kept below 100%, maintain proper water temperatures, proper water flows, minimize rough handling, avoid heavy loading of eggs and monitor for soft water or chemical contaminants.

Soft-Egg or Soft-Shell Disease

Soft-egg disease is an abnormal condition found in incubating fish eggs. Affected eggs become soft and flaccid during incubation. This disease is thought to be caused by an amoeba. Strict sanitary measures and frequent disinfection of hatchery equipment are the best methods for avoiding the disease. The disease occurs infrequently, but some hatcheries have had periodic losses over several years.

Brown Blood Disease

Brown blood disease is caused by the buildup of nitrites in the water through winter feeding. Ammonia produced by the catfish is generally broken down by nitrifying bacteria. During winter the ammonia accumulates in ponds, because the water is too cold, and bacteria are dormant. In spring, bacteria which convert ammonia to nitrites are more active than bacteria which convert nitrites to nitrates. As a result nitrites become dangerously high in the water and brown blood disease develops in the catfish. A nitrite ion enters the bloodstream and binds with a red blood cell and prevents it from carrying oxygen to the body. Use salt treatment to prevent or reduce the disease.

Genetic abnormalities include conformational oddities such as lack of a tail or presence of an extra tail. Most of these are of minimal significance; however, it is important to bring in unrelated fish for use as bloodstock every few years to minimize inbreeding.

What to do if Your Fish are Sick

If you suspect that fish are getting sick, the first thing to do is check the water quality. Low oxygen is a frequent cause of fish mortality in ponds, especially in the summer. High levels of ammonia are also commonly associated with disease outbreaks when fish are crowded in vats or tanks. In general, check dissolved oxygen, ammonia, nitrite, and pH, during a minimum water quality screen associated with a fish disease outbreak.

7

Diseases Control

Prevention of Disease

The concept that "prevention is better than treatment" is fundamental to the maintenance of a healthy stock of fish. Since fish are schooling animals, they are hard to observe individually, making the diagnosis and treatment of disease difficult. In addition, some fish diseases are still essentially incurable. Therefore, preventive measures are essential to the control of disease.

Pond regulation

Pond regulation is effective in improving environmental conditions, preventing disease and raising fish yields. There are two main aspects to pond regulation: pond trimming and pond disinfection.

Rearing management

A reliable person should be responsible for the daily management of the pond (stocking, feeding, disease prevention, etc.). The scheduled feeding procedure, which benefits fish yield and disease prevention, should be used. Variations in water quality must be observed carefully. Pond inspection is essential in the morning, particularly in dismal weather or after a torrential rainfall during the epidemic season (May to September). Besides, it is necessary to remove the weeds along the pond sides and clear the feeding platforms to prevent the occurrence of disease. Netting, transferring, and transporting should be performed with great care.

Disinfection of fish seed

Fish seed can be disinfected during their transfer or prior to stocking. The procedure can be done in a bucket or in a jar depending on local conditions.

Disinfection of feed, feeding platform and equipments

disinfected after each use. Large nets can be exposed to sunlight for 1 or 2 days and wooden pails can be sterilized by immersing them in a quicklime solution or in a 1 ppm potassium permanganate solution for 15 min.

Pond treatment

Spreading chemicals over the entire pond is a common method of disease prevention. Before seed stocking ponds should be cleaned properly and must be disinfected with1ppm potassium permanganate.

To improve deteriorated pond water, quicklime should be added. This will improve water quality and thus prevent disease. The quicklime chunks should be dissolved in a little water and the diluted solution be stirred and sprayed evenly over the pond.

Establishing a quarantine system

Geographic and climatic conditions can produce epidemic diseases in certain regions. However, as the freshwater farming industry develops rapidly and the transportation of fry and fingerlings among provinces becomes more frequent, local diseases tend to spread. Under such conditions quarantine should be strictly followed and transportation of diseased fish be prohibited.

Diagnosis

Disease diagnosis is the first step toward effective treatment, therefore due care must be taken. The fish must be alive or recently dead and the body must be kept damp. The dissected organs must be kept as complete as possible. Instruments used for post-mortem must be kept clean and disinfected to avoid contamination of pathogens among organs. Distilled water for microscopic observation of the skin and use 0.85 per cent normal saline for microscopic observation of the internal organs. Samples should be preserved for further identification if there is any doubt about the pathogens or the clinical signs. If complications are observed during the diagnosis, diagnose the primary and secondary disease and implement the appropriate treatments separately or simultaneously.

Methods of diagnosis include surveying the pond and examining the fish with the naked eye and microscopic examination of suspected material.

Survey the diseased pond

Determine if the water source is seriously polluted. If it is, find the source of the pollution.

Saprolegnia. It is also possible to see the pathogens of bacterial erythrodermatitis, albinoderm, stigmatosis, and furunculosis with the naked eye.

Gills- Inspect the gills, with an emphasis on the gill filaments. Observe the colour of the gill lamella, the quantity of mucus, and the congestion and putridity of filament tips after an opercular incision is performed.

Internal organs- Mainly check the intestines. Begin to observe abdominal hydrops and visible parasites, (e.g., *Ichthyoxenus, Nematodes*, cysts of *Myxosporidia, Ligulos*, then observe other internal organs. Extract the internal organs with a knife and scissors and separate the liver, gall bladder, air bladder, etc. Finally, open the intestine to search for any signs of pathological change.

Microscopic examination

Normally, only the skin, gills, intestines, eyes, and brain need be observed microscopically.

Skin — Scrape a little tissue and mucus from the skin, put them on a slide with a drop of distilled water, and observe the combination under a microscope after pressing with a cover-slip. One should always start with the low power objectives. Samples from at least three different points on the skin should be inspected. Common parasites on the skin are *Trichodina, Ichthyophthirius, Chilodonella, Costia, Glochidium*, and *Myxosporidia*.

Gill — Place some gill filaments and mucus onto a slide. The following parasites may be identified through microscopic observation: *Dactylogyrus, Gyrodactylus, Cryptobia*, and *Myxosporidia*.

Intestines — Transfer a little mucus from the anterior intestinal wall to the slide. Nematodes, *Eimeria*, and *Myxosporidia* may be seen.

Eyes- Press the entire ocular bulb or crystalline body on the slides if cysts of*Diplostomum*are seen, it is an indicator of diplostomiasis.

Brain- Open the central cavity of a fish with whirling disease. White cysts of myxosporidia in the lymphatic fluid beside the brain will be observed. Remove the cysts and place them on a slide. After crushing with a cover slip, the spores can be seen.

How to cure a diseased fish?

Selection of Chemotherapeutic agents: While choosing a treatment following important criteria should be taken into consideration:

Methods of treatment

In order to effectively control the health problem, desired dose of therapeutics could be given in three ways e.g. (i) adding medicine in the water (ii) through feed (iii) through injection. First method is again divided into flush, dip, bath and pond treatment.

Flush Treatment

Higher concentration of chemical is added at the inlet or incoming water site and allowed to pass through the tank. Uniform distribution of chemical depends on the flow of water.

Dip Treatment

In this method fishes are collected by net from the tanks or nurseries and dipped for 1-3 minutes in high dose of medicine and released back in tanks. In this process, attached parasites detach from the body and get killed. Dip treatment is extremely useful in small fry and fingerlings facilities.

Bath Treatment

Employed in small experimental tanks under controlled conditions and after adding chemical/medicine left for specified time. The fishes are kept under observation and as soon as fish shows stress symptoms, either fresh water is added or fishes are taken out from the tanks and released in freshwater.

Pond Treatment

In bigger grow out fish ponds smaller dose of chemical/medicine is used. This method although widely used however before application of medicine economic return of the fish pond should also be taken care of.

7

Collection of samples for Disease investigation

Clinical signs in fish with diseases are rarely pathognomonic, and laboratory examination and tests for identification of pathogens are necessary for diagnosis of infectious diseases. Submissions to a laboratory for diagnosis should include suitable specimens of fish, water and any other material that is suspected to be involved, and a complete history. Fish show few diagnostic clinical and gross signs, so a range of tests may be required and samples and history must be of high quality to ensure a diagnosis. Live affected fish should therefore be submitted where possible. If this is not practical, then fish should be euthanized, and either cooled and dispatched on ice or samples fixed before dispatch. Unlike mammals, which tend to cool after death, limiting some enzyme activity, the body temperature of dead fish in water is unaltered, and those out of water often warm up. Fish enzymes may also operate over a broader temperature range. Fish therefore tend to undergo rapid autolysis and putrefaction after they die. Fish found dead are generally unsuitable for laboratory examination; so dead fish should be submitted only when there are no other options for diagnosis. Poor quality specimens will probably prevent a diagnosis from being established.

Many disease outbreaks have an underlying pre-disposing factor that has caused stress on the aquatic animals. Stress results in a series of events including increased cortisol production and a decrease in lymphocyte production. Common causes of stress include: suboptimal water quality such as low dissolved oxygen, high suspended solids or high amounts of metabolic by-products; overcrowding; intra or interspecies aggression; sudden increases or decreases in water temperature or salinity; moulting or spawning; inadequate nutrition; handling and transport. In addition, very young and very old animals often are less immunocompetent than other animals in the population. Obtaining a full history of the affected animals and any recent events that may have stressed the animals or adversely affected environmental conditions is imperative in an investigation of aquatic animal disease.

that provide information to allow trace-backs on the sample movement from the sample site to the storage facility or laboratory and within those facilities. Storage facilities should record information on the preservation method, storage location, and date and time of storage at each storage freezer along with information on the storage temperature (continuously monitored is preferable). This information should be tracked with a unique sample code for all samples. For laboratories, the date of receipt, storage location information, date of analysis, analysis notes, and report date should be maintained for all uniquely coded samples. These data will greatly facilitate the tracking of sample problems and provide assurance that the samples were properly handled.

History

History to accompany submissions for disease diagnosis from cultured fish should include the following points:

- Characteristics of the problem such as onset, duration and progression of the disease outbreak
- Species, size and age of affected stock
- Number of at risk and affected animals and their value
- Records of water quality parameters such as oxygen saturation, pH, temperature, salinity, hardness.
- Diet, feed intake and recent changes
- Recent stressors such as handling, recent climatic events, malfunction of pumps, equipment or oxygen supply
- Recent introductions
- Type of water and water flow and treatment systems

Collection of tissues for virus isolation

Tissues or fluids from 5 or 10 fish (depending on the specific situation) may be pooled in one container containing transport medium at a ratio of 1 part tissue (weighing a minimum of 0.5 g) to 5 parts medium, representing one pooled sample. Pooled tissues in transport medium may be stored on ice but not frozen during transportation. A microbial transport medium for the collection, transport and storage of samples suspected of having viral pathogens comprises a balanced salt solution, a proteinaceous stabilizer, and carbohydrate and amino acid nutrient sources. The medium is buffered to maintain physiological pH and includes a pH indicator

until virus extraction is performed in the laboratory. Virus extraction should optimally be carried out within 24 hours after fish sampling, but is still acceptable for up to 48 hours if the temperature is maintained at 0°C–4°C, or for longer periods for clinical disease samples held frozen at –20°C to –80°C. However, freezing of samples for testing for subclinical carriers should be avoided. Organ samples may also be transported to the laboratory by placing them in vials containing cell culture medium or Hanks' balanced salt solution (HBSS) with added antibiotics to suppress the growth of bacterial contaminants (one volume of organ in at least five volumes of transportation fluid). Suitable antibiotic concentrations are: gentamycin (1000 ìg ml–1) or penicillin (800 International Units [IU] ml–1) and streptomycin (800 ìg ml–1). Antifungal compounds, such as Mycostatin® or Fungizone®, may also be incorporated into the transport medium at a final concentration of 400 IU ml–1. Serum or albumen (5–10%) may be added to stabilize the virus if the transport time will exceed 12 hours.

Collection of tissues for isolation of bacteria and fungi

Infected tissues can be collected in transport medium that consists of sterile PBS containing 25% glycerol. The medium may be supplemented with a proteinaceous stabilizer like serum. The infected tissue may be directly used for the isolation of bacterial or fungal pathogens.

Viral transport medium:

FCS	:	20ml
Penicillin	:	10 lakhs unit
Streptomycin	:	1g
PBS	:	1000ml (up to)
Glycerol	:	50%

Adjust the pH –7.0, Sterilize by filtration

Storage of samples

Samples should not be frozen before processing but should be maintained between 4 and 10 °C. To maximize sensitivity, samples should be processed and assayed within 24h of sampling but when this is not possible they must be processed within 72h of sampling, during which storage must be at 2-5°C. While it is not recommended, samples to be assayed after 72 h after collection, they may be frozen in the temperature range of -70 °C to -80 °C.

Tissues to be examined

Table 2: Collection of samples from different size of the fish

Fish size (length)	Tissue sample
< 4 cm	entire fish (remove yolk sac if present)
4-6 cm	entire viscera including kidney
> 6 cm	kidney, liver, spleen, encephalon, heart and gill filaments
	Sexually mature ovarian fluids, kidney, liver, spleen, encephalon, heart and gill filaments

Successful diagnosis of microbiological infections by culture is enhanced when the specimen contains as much pathogen as possible upon collection, is protected from thermal inactivation, and is contained in an effective transport system. An ideal transport medium would possess many of the following characteristics. It would preserve the activity of the pathogen, even at room temperatures; it would be nontoxic to cell cultures and not obscure the appearance of viral cytopathic effects; it would have a long shelf life (either in a frozen or non-frozen state); and be applicable for both culture isolations and direct tests such as enzyme immunoassays or immune-fluorescence.

Blood sampling

Blood is sampled for ichthyo-haematological examination as soon as the fish are taken out of the environment in which they have lived. The main criteria for selection a particular method of sampling include the size of the fish, the amount of blood needed and the further fate of the fish caught for different examinations.

Blood sampling in fish fry

Blood from fry at an individual weight of at least 8 gm can be sampled by the methods of cardio-puncture, using a glass capillary long about 200 mm with an inner surface lined with a fine film of heparin before use.

To collect blood from a fish, lift it fixed head down, to the level of the eyes and apply the tip of the capillary at an angle of about 60° (in relation to the longitudinal axis of the fish body) about 1-2 mm cranially from the midpoint, which is the point of intersection of the longitudinal axis of the body with the line connecting cranial edges at the base of both pectoral fins. At this point the carp fry have the so-called stigma: a shallow, usually pigmented depression in the skin up to 1 mm wide. Now drive the tip of the heparinized blood-collecting capillary quickly through the body wall to the pericardium and further to the heart. Blood appearing in

the heart in the same way as the glass capillary. The cardio-puncture technique can also be used with older fish: this is so in those cases when the fish can be killed.

Collecting blood from fish weighing above 200 g, including brood fish

In these fish, the best method is that of collecting blood by puncture of the caudal blood vessels. On the caudal peduncle ventral side the unpaired dermal scale is removed in caudal direction from the anal fin base. Within the central plane, about 1 cm caudally from the anal fin, a sufficiently long needle is introduced, firmly held on the cone of a heparinized disposable syringe, into the fish body in a cranio-dorsal direction at an angle of 45°. The described method is fully recommendable, mainly in larger series of blood collection; collection of 2 ml of blood from cyprinids weighing above 1000 g involves no risk of loss of the treated specimens.

Stabilization of blood

Aqueous solution of heparin sodium salt is the only product used for stabilization of the fish blood. One ml of this aqueous solution contains 5000 I.U. of heparin sodium salt. 0.01 ml (about one drop) of the aqueous solution of heparin suffices to stabilize 1 ml of fish blood: the substance is left to dry on the inner surface of the test tube or flask (bublet) and the blood is collected in the test tube or bublet afterwards. A slight over-dosage of heparin does not produce changes in the blood cells of the fish.

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