

A Review of Some Bacteria Diseases in Africa Culture Fisheries

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Abstract: Streptococcosis, Infectious Abdominal Dropsy of Carp, Furunculosis, Motile Aeromonad Disease, Vibrosis, Columnaris disease, Bacteria kidney disease, Peduncle disease (fin rot), Bacteria gill disease, Pasteurellosis, *Aeromonas hydrophila* and other ubiquitous facultative bacteria, Myxobacterial infections - gill and fin rot, Mycobacteriosis of fishes, Epitheliocystis are some bacteria diseases reviewed to educate fish culturist on some likely challenges and solutions in culture fisheries. Bacteria diseases are responsible for heavy mortality in both wild and cultured fish. Understanding the Life history, biology, diagnosis, pathology and epizootiology and control of Streptococcosis, Infectious Abdominal Dropsy of Carp, Furunculosis, Motile Aeromonad Disease, Vibrosis, Columnaris disease, Bacteria kidney disease, Peduncle disease (fin rot), Bacteria gill disease, Pasteurellosis, *Aeromonas hydrophila* and other ubiquitous facultative bacteria, Myxobacterial infections - gill and fin rot, Mycobacteriosis of fishes, Epitheliocystis educates fish culturist on some likely challenges and solutions in culture fisheries.

Key words: Bacteria diseases, diagnosis, epizootiology and control, life history and biology, pathology

INTRODUCTION

Bacteria disease is an illness of fish body caused by bacteria organisms creating infection or internal disorder. It is an expression of a complex interaction between a susceptible host, a pathogen and the environment. In the presence of an infective agent in an effective number, a susceptible host suffers an infection in adverse conditions. Bacteria diseases manifest in various ways for the impairment of the normal physiology in the host (Bassey, 2011).

Bacteria are single cell microscopic organisms occurring in different sizes and forms. These forms include rods, spherical, spiral, comma and filamentous shape. Bacterial organisms have cell wall, which maintains the cell integrity (Bassey, 2011). Mycoplasma species do not have cell wall. Some are capsular, carrying specific antigen on their capsules. Most bacterial pathogens of fish are rod shaped or flagellated, hence motile. They are psychrophilic, having a wide temperature range for growth. The pH range of bacterial growth is 5.5-10.00. Bacteria pathogens are aerobes or facultative anaerobes. Some are chromogenic, forming various pigments. A great number of them grow well on common laboratory media (Bassey, 2011).

Bacterial diseases are responsible for heavy mortality in both wild and cultured fish. The actual role of these micro-organisms may vary from that of a primary pathogen to that of an opportunist invader of a host

rendered moribund by some other disease process" (Richards and Roberts, 1978). The non-specific nature of diseases induced by non-fastidious and opportunistic bacterial organisms makes them unpredictable and complicates their differential diagnosis. Many of these organisms are a usual component of the bacterial flora in aquatic habitats, particularly eutrophic systems. Stressors, often inevitable in most culture systems, predispose fish to bacterial-born diseases (Snieszko, 1974).

The course of events from stress to predisposition to infection, include physiological changes described as a general alarm response syndrome the consequences of which are to enforce barriers, normally preventing entry of bacteria to fish inner systems and at the same time incapacitating fish defense responses and immune reactions (Mazeaud *et al.*, 1977). Diagnosis of bacterial disease of fishes required observation of common clinical signs associated with the disease, isolation and identification of the causative bacterial organisms and confirmation with animal inoculation is important to fish culturist and fisheries managers. The article reviews the species affected geographic range, diagnosis, pathology, epizootiology of some bacterial diseases.

Streptococcosis: Streptococcus-related diseases occur in many cultured marine and freshwater fish. Several streptococcal species are involved, and the relationship between aetiological agents, isolated from the different fish has not been fully determined. In Israel,

Streptococcosis affects *Oreochromis* spp. as well as grey mullets (*Mugil cephalus* and *Liza ramada*) and silver carp, but not common carp (Hubbert, 1989). Streptococcosis also affected coldwater reared rainbow trout (Eldar *et al.*, 1994).

Streptococcus-related diseases were first reported from *Oreochromis* spp. and *Sarotherodon* spp., kept in Japan (Kitao *et al.*, 1981). Outbreaks, caused by Beta and Gamma-haemolytic strains, in Japan (Miyazaki *et al.*, 1984) as well as in Taiwan (Tung *et al.*, 1985) have similar clinical and gross pathological signs to the streptococcal meningoencephalitis found in cultured tilapia hybrids, in Israel (Hubbert, 1989). Isolates from Japan and Taiwan were shown to be conspecific with Israeli isolates (Eldar *et al.*, 1994).

Diagnosis: In acute infection, signs develop within 3-4 days, gross ascites occurs, as well as dermal lesions. Exophthalmus is characteristic of the chronic stage, which may be clinically manifested or subclinical. The chronic condition does not occur in silver carp. Clinical symptoms are characteristically of the neuro-motor type; erratic swimming, swimming in circles and lack of control of the pectoral fins, leaving fish in an erect position. Bacteria are readily isolated from the brain, optic nerve and fatty tissue around the eyes, but only from the viscera in a late chronic condition.

Isolated bacteria were gram-positive, non-sporulating, facultatively anaerobic chains forming cocci and catalase negative. They were able to grow at pH 9.6, but not at 10°C nor at 45°C, nor in the presence of 40% (v/v) bile salts or the presence of 6.5% NaCl (w/v). Deoxyribonucleic acid base composition (G+C% = 37%), deoxyribonucleic acid-deoxyribonucleic acid hybridisations, biochemical and serological studies indicate that these strains constitute two distinct species of streptococci; *S. difficile* (Manitol +ve) and *S. shiloi* (manitol -ve). Both strains were isolated from cultured tilapia (*Oreochromis* hybrids), in Israel. Isolates from Israeli grey mullet and tilapia from Japan were identified as *S. difficile*, while isolates from Taiwanese tilapia and trout corresponded to *S. shiloi* (Eldar *et al.*, 1994).

Pathology: The brain seems to be the primary site of infection. In the acute state the ascitic fluid is fibrinous and contains bacteria-laden macrophages, all vital organs become heavily infected and mortality becomes massive (50-60% of the stock). Epizootic infection, in a variety of estuarine fish, caused clinical signs of erratic swimming, whirling motion and gross pathological signs of haemorrhagic lesions on the body, exophthalmus, corneal opacity, some macroscopic changes in the liver and spleen, and ascites (dropsy) with mucoid inflammation of the gut. Histopathological changes in the heart result from acute pericarditis, with aggregation of

bacteria-laden macrophages and accumulation of fibrinous exudate in the outer layer of the heart muscle. In the brain, meningoencephalitis is evident by accumulation of bacteria-laden macrophages in the meninges. In the liver, multiple necrotic areas develop, holding bacteria-laden macrophages and sometimes with a formation of granulomata. Proliferative changes have also been reported to occur in the spleen, with the kidney containing free bacteria, and granulomatous inflammation also occurring in the ovaries and the testes (Miyazaki *et al.*, 1984; Hubbert, 1989).

Epizootiology: Case reports suggest stress-related epizootiology and pathogenesis. Handling promotes a transition from the chronic state to outbreaks of acute disease and mass mortality.

Variation in the pathological description between case reports from Japan, Taiwan and Israel, may be due to the stage of pathogenesis, which is both environmental and handling stress-mediated, rather than aetiological, although, there were differences in the representation of species in the different countries. Co specificity between isolates from different fish species (Eldar *et al.*, 1994) suggests interspecific cross transmission (Hubbert, 1989).

Experience with disease incidences, in farmed fish, suggests that *S. shiloi* epizootiology is linked with cold water conditions (in reared trout) and therefore is the aetiological origin for streptococcosis in overwintering (cold stressed) tilapia, while other species are the aetiological agents of the other infections not related to cold stress. Circumstantial evidence suggests water, rather than feed, as the aetiological origin (Hubbert, 1989).

In Japan, streptococci strains isolated from fish were widespread in the environment, both in mud samples and in water (Kitao *et al.*, 1981). Food has been implicated as a source of infection in mari cultured fish (Taniguchi, 1982), but it is unlikely to be relevant in cases of tilapia fed on dry and pelleted feeds (Hubbert, 1989).

Infectious abdominal dropsy of carp: This is a dreaded disease in carp culture. *Aeromonas punctuata* is the causative organism. Fish mortality with increase in water temperature during rainy season are signs of the disease. Prevalence of the disease declines during dry season. Two type of infectious abnormal dropsy are intestinal and ulcerative abdominal dropsy. Intestinal dropsy is characterized by swollenness of belly, resulting from accumulation of a yellow or pink colored liquid in the body cavity. The fish jumps when infected. The ulcerative form manifest by bloody spots on the skin. The muscles can be gray scars or partial destruction of external body organs. Avoid stress, long storage, unnecessary handling and inadequate natural food to prevent and cure the disease.

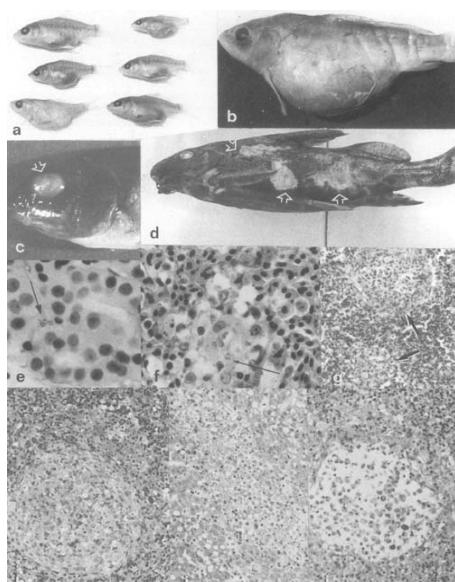


Plate 1: Bacterial infections

In other to control the disease, leave pond to dry for three months and disinfect with quick lime. Carefully remove and destroy dead fishes. Always separate fishes from different source and select disease resistance carp species. Antibiotics are effective curative measures for the bacteria. The antibiotics are injected in a bath or mixed with food. Chloramphenicol is most effective against *Aeromonas punctata*. For resistant strains, oxytetracycline and streptomycin is more effective. Fishes are injected in the body cavity in a ratio of 1 to 1.5 mg of chloramphenicol for each 100 g of fish body weight. Antibiotics can be mixed with food in such a way that fish, weighing 100 mg absorbs 1mg per day.

Plate 1 shows bacterial infections: a,b. Dropsy in carp from Kajansi farm, Uganda ($\times 0.8$). c. Exophthalmus and cornea hyperplasia in eyes of *Oreochromis* sp. from Kajansi farm, Uganda ($\times 2.5$). d. Ulcerating skin lesions in *Synodontis afrofisheri* from north Lake Victoria ($\times 0.8$). e. *Vibrio parahemolyticus* (arrow) in kidney blood of farmed tilapia hybrid in Israel. f. *V. parahemolyticus* (arrow) in macrophage aggregate in spleen of a fish of the same group as e. g. Inflammatory lesions in winter stored tilapia hybrid with *Pseudomonas* sp. as presumptive causative agent. h-g. Histopathological process in tilapia hybrid spleen (h,j) and liver (i) due to pasteurellosis, from granulomatous lesion to liquefactive necrosis.

Furunculosis: This disease is characterized by formation of boil like lesions. A gram negative short to oval (Coccibailary) rod shaped organism, *Aeromonas salmonicida* is the causative organism. The disease kills

fish rapidly and can be transmitted through the digestive tract or small wounds on the skin. *Aeromonas salmonicida* breeds in the blood, liver, spleen and kidney. The symptoms are bloody boils of various sizes from a pea to a nut or small lumps under the skin. All the internal organs can be affected.

Infested ponds should be isolated and dead fish carefully removed and destroyed. Disinfect ponds with quicklime and calcium cyanamide before stocking. Antibiotics can be administered in food; sulfamerazine, oxytetracycline, chloramphenicol and furazolidon are effective drugs. A daily dose of mixture of 10 g sulphamerazine and 3 mg of sulphagnadine can be administered to 100 kg of fish for a period of eight days. Ten grams of chlormphenicol can be administered daily for 100 kg of fry for a period of 10 days. Five g of chlomaphenicol can be administered for 100kg of fingerlings for a period of 6 days. Eggs can be disinfected with one percent of providone iodine. Antibiotics such as chloramphenicol, terramycin or oxytetracycline are applied as additives into the feed. Dosage in feeds range from 5 to 7.5 g/100 kg of fish per day for the period of 5-15 days. Initially treat only a few fish, so that the treatment will not cause more harm than good.

Motile aeromonad disease: This is a septicemia disease characterized with erythema of the base of the fins, mouth, grooves of the lower jaw and the opercula. The disease can also exist as hemorrhagic septicemia, red mouth disease, and red sore of pike and bacterial hemorrhagic septicemia disease. Organisms belonging to the genus *Aeromonas hydrophila*, *Bacillus hydrophilus*, *Aerobacter liguifaciens*, *Broteus hydrophilus*, *Pseudomonas hydrophilus*, *Aeromonas punctata*, *Bacillus punctata*, *Pseudomonas punctata* and *Bacterium punctum*, *A. hydrophila* and *A. punctata* are the major causative organisms. Both organisms are gram- negative rod shaped organisms, motile with the aid of flagella, non-spore formers and can be found worldwide.

All fresh water fishes are susceptible to the disease. Saltwater fishes may be susceptible to the hydrophilic strains, which occur in brackish or seawater. Epizootics are stress related. Therefore, overcrowding, poor water quality, nutrient and injury are associated with the occurrence of the disease. Fish mortality of between 20-80% can be observed, while morbidity can be up to 100%.

The clinical signs common to this infection include erythema of the mouth, base of fin, opercula, lower jaw and anus. At autopsy, lesions observed include petechial hemorrhages of the peritoneal and most visceral organs. There can be abdominal dropsy, ulcerative necrosis of the skin, presence of bloody mucus in the stomach, and ertheamosus intestinal mucosa. It can be diagnosed through signs of the disease, isolation and identification on the bacterial organisms. The disease shares the same

histopathology with vibriosis and pseudomonas infection, but can be differentiated with the identification of the bacterial organisms, using biochemical tests.

In the treatment and control of the disease, all kinds of stress factors should be avoided. Eggs can be transmitted through bacterial agents. Therefore, egg disinfection is important. Brood stock purchased from wild or other hatcheries should be quarantined before being stocked into the brood stock ponds. Sulfamethazine and oxytetracycline are effective for the control of this disease.

Vibriosis: This can be acute, sub acute or chronic in both marine and fresh water fishes. Large sores on the skin characterize it and muscles from the rupture of boil like lesions. The causative organisms, *Vibrio anguillarum* is more common in marine and brackish water than fresh water. *Vibrio anguillarum* is gram negative, non-spore forming, rod shaped with a polar flagellum. The organism is a primary pathogen of fish. It can be found in the intestinal tract of healthy fish. Hence, seem to be transmitted orally. The disease can also be transmitted through injuries incurred by the fish. The disease condition is septicemia with presence of the organisms in the blood, liver, spleen, kidney and other vital organs of infected fish.

The signs of the disease are erythema of the base fin, mouth, groove of the lower jaw, opercula and the anus. Ulcerative dermatitis and myositis are common features resulting from rupture of boil like lesions observed at the early stages of the disease. At autopsy, petechial hemorrhages can be observed on the peritoneum with attendant hyperemia of other viscera organs. Muscles show petechiation and large abscesses. These signs, with isolation, identification of the causative agent and histopathology are important in the tentative diagnosis of the disease. However, biochemical test for characterization of the organism confirms the diagnosis. Vibriosis is enzootic but the condition can still be controlled. Avoid any form of stress. Regularly monitor and maintain good water quality in the pond. Sulfamerzine and oxy-tetracycline are drugs of choice. Bacteria of *V. anguillarum* are commercially available in developed countries. Hyper osmotic infiltrations of the bacterium can also control the disease.

Halophilic vibrios were isolated from visceral organs of diseased tilapia in Israel (Hubbert, 1989) and Japan (Sakata and Hattori, 1988).

Diagnosis: Affected *Oreochromis niloticus* in Japan and tilapia hybrids in Israel, showed characteristic haemorrhages around the base of the fins, prostration in the swimming movement and stiffness of the muscles. Gills may have purple colouration. The gut appears to be filled with gas and mucus material. Isolates taken from the blood, livers or kidneys and gut contents of clinical

cases often contain pure culture of the aetiological agent. Several halophilic *Vibrio* were isolated from these fish. *V. vulnificus*, were distinguished from other vibrios by their ability to ferment lactose. They were also positive for fermentation of galactose, cellobiose, and salicin, decarboxylation of lysine and ornitine, grew at 42°C and were negative for arginine dihydrolase, Vogues proskauer test and sucrose fermentation. Japanese strains were pathogenic to both tilapia and carp (Sakata and Hattori, 1988). The aetiological agent in Israel was *Vibrio parahemolyticus*, identified with the aid of API (France) 20 E and 20 NE (Hubbert, 1989).

Pathology: Infection with *V. parahemolyticus* in overwintering tilapia, in Israeli farms, gives rise to fulminating septicaemia which is rapidly fatal to most fish, usually within 48-72 hours, following handling. Experimentally, 10⁴ cells and higher caused 100% mortality (Hubbert, 1989). Vibriosis of tilapia farmed in Japan, occurred as a chronic condition and resulted in a gradual death of 10-20% of the pond fish (Sakata and Hattori, 1988). Pathological changes in acute conditions are mainly haemorrhages in visceral organs, sometimes with haemorrhagic ascitic fluid, and distended guts with gases and mucous fluid. In chronic vibriosis, necrotic nodules occur in the spleen, but thus far has not been reported from infections in tilapia. *Vibrio* may also be found in the blood of clinically unaffected overwintering fish.

Epizootiology: Vibriosis is a stress-mediated disease. In Japan, this disease occurs spontaneously (Sakata and Hattori, 1988), while in Israel, it occurs as a direct result of handling overwintering fish (Hubbert, 1989). *Vibrio vulnificus* is ubiquitous in the haline environment. Clinical isolates, from various fish and from the environment, may differ slightly, only strains isolated from eels were reported to be pathogenic to eels (Muroga *et al.*, 1976).

Columnaris disease: The disease can be chronic or sub acute in freshwater fishes. It is characterized by fluffy, grayish opalescent growth on the injured skin and necrosis on the edges of fins. Columnaris disease was named after its causative agent, *Flexibacter columnaris*, that is unbranching rod or filament shaped bacterium. It produces microcyst in its nesting stage. The organism grows on the skin surface of affected fishes, especially the bruised ones, producing a column like structure. Hence, the name.

The organism is cosmopolitan in distribution. Presence of injury (abrasion) on the body of fish can result to infection because; the abrasion provides route of entry for the bacteria organisms. The causative agent is easily shed into the water, facilitating the spread of the disease in overcrowded ponds or tanks. The disease is

temperature related because of the occurrence of epizootics at a temperature range of 28-30°C. Morbidity could be 100% while mortality can be as high as 70%.

The fluffy grayish opalescent growth of the organisms on the skin abrasion is pathognomic, hence the name cotton wool disease. Affected fins usually have necrotic lesions on the edges. The gill can be affected, giving a pale appearance initially confined mostly to the tips of the gill filament. This latter gradually progresses to the outer part. Bacteremia can result to hyper-acute infection, if bacterial organisms invade the blood system through the gill or skin abrasion.

Furnace and introfuran is the drug of choice but its use is limited to non food fish (aquarium fishes). The drug can be applied at 1.5 mg/L. It is rapidly absorbed by fish and also acts as a good tropical agent. Oxy- tetracycline at 50 mg/kg of fish per day for 10 days is effective in the control of the epizootics of columnaris disease. However, it does not cure surface infection. Disinfection with 20 mg/L of hyaline for one minute; 500 mg/L of copper sulphate for 1 h and 2 mg/L of potassium permanganate have a good tropical effect. However, proper management practices including maintenance of good water quality, appropriate stocking density in brood stock prior to and after spawning, as well as good handling technique are important.

Bacteria kidney disease: This is a chronic disease characterized by the presence of white to gray abscess in the visceral organs, unilateral or bilateral exophthalmia. Abscesses are present under the skin in serious cases. The causative agent of this disease is *Renibacterium salmoninarum*. It is a gram positive, non-motile rod shaped organism that occurs in pairs (diplobacillus). The optimum growth temperature is 15°C. The possible routes of transmission of the infection are, either, oral or cutaneous. Water quality also has effect on the occurrence of the disease. The disease is more prevalent in soft water than in hard water.

External diagnosis on the disease is difficult. Infected fishes are listless and lethargic. Abscesses are formed on the side of the body. A viscous shows presence of white to gray abscesses containing blood tinged and purulent materials mixed with bacteria. Bilateral and unilateral exophthalmia can be observed among affected fish. The chronic nature of the disease makes it difficult to treat. Response to drug is slow. The possibility of the bacterial organisms being intracellular, explains the slow response. The use of sulfamerazine for 45 mg/kg of fish and erythromycin for 190-220 mg/kg of fish, only control mortality but do not eliminate the disease. Prevention of this disease is the only effective method. This can be achieved through the slaughter method. Regular disinfection of hatchery and culture facilities can also prevent the disease. However, egg disinfection method may not prevent egg transmission of the disease.

Peduncle disease (fin rot): This disease is a chronic infection of fish by several species and sub species of flexi bacteria in cold water. It can also occur in warm water fishes. The disease is characterized by necrosis of the fins. Fin rot and necrosis of caudal peduncle tissues. The causative agent is *Cytophage psychrophilla*. Similar strain of bacteria can be associated with the disease in warm water fishes. They occur singly in pairs or long filaments.

Skin abrasion, presence of irritant, high water pH levels and malnutrition provides a suitable condition for the bacterial invasion. The disease begins with rough appearance of skin and loss of integrity of the tips of the fins. Lesions on the skin appear as gray color along the margins of the fin. If treatment is delayed and the environmental stress factors continue, the lesion on the caudal fin can extend from the base of the fin to the caudal peduncle. Chronic cases can lead to exposure of the vertebrae with very poor prognosis. These signs and detection of causative agent in squash from necrotic fin or caudal peduncle tissues can be useful for tentative diagnosis. Isolation and proper identification of causative agent can aid definitive diagnosis. The disease can be treated with furnaces (1.5 mg/L in water for one hour). Sulfamerazine and oxytetracycline in feed can be combined with tropical use of quaternary ammonium compound. However, the presence of irritants in water, exposure of fish to extreme pH and malnutrition can be avoided to enhance effective treatment.

Bacteria gill disease: This disease can result from exposure of the fish to irritants. These irritants damage the gill membrane, creating an avenue for opportunistic bacteria organisms to invade the tissue. Ammonia, a fish metabolic product is one of such irritant predisposing fish to bacteria gill disease. It implies that environmental irritants are the main causes of gill membrane damage. Bacteria organisms are secondary invaders. Most bacterial organisms associated with this condition are rod shaped gram-negative organisms. Flexibacta, Aeromonads and pseudomonads are typical examples. However, a single species or strains of bacterial organisms are often involved in the disease.

Gasping of infected fishes from the pond are early signs of the disease. Gills appear swollen at autopsy, with hyperemic gill tissue protruding under the operculum. White to gray spots can be seen on the affected gill. Batches of bacteria organisms can be firmly attached to the gill tissue. Infection can be unilateral or bilateral. The clinical signs of this disease, with direct squash of bacteria mass on the infected gill tissues can aid in the diagnosis of the disease. Isolation and identification of the causative organisms and biochemical characterization of

the organism are diagnosis for the disease. Quarantine method and use of ammonium compounds can also control the disease. This can reduce bacterial invasion. Potassium permanganate (KMnO₄) can be used at 5 mg/L for one hour. Treatment for three days is recommended for “flow through” water system. It is important to replace the pond water to improve the water quality and flush out toxic irritants from the pond.

Pasteurellosis: Outbreak of disease, with the aetiological agent identified as *Pasteurella multocida*, occurred in tilapia hybrids from a fish farm on the shore of L. Kinneret, Israel (Nizan and Hammerschlag, 1993). Bipolar, *Pasteurella*-like bacteria were found in histological sections of tilapia hybrids obtained from numerous other farms in the Jordan valley, some with histories of massive mortalities, as well as in moribund commercially reared scalaries (*Pterophyllum scalare*) in the same region.

Diagnosis: Gross pathological signs in affected fish, were white nodules of varied sizes in all visceral organs. Isolates from spleen and kidney nodules yielded bacterial growth on brain and heart infusion +ve yeasts, and Rimmler-Shotts agar. Bacteria were short rods, non-motile, Gram negative, bipolar, Catalase +ve, Oxidase weakly +ve. Colonies were grey/white-yellow, producing acid on triple sugar iron agar. API-20NE tests for reduction of nitrates to nitrites, Indole and acidification of glucose were positive; all other tests were negative.

Plate 2 Illustrates Pasteurellosis in cichlid fishes: a-g. a. Hepatocyte degeneration in tilapia-hybrid liver. b. degeneration and death of cells invaded by the bacteria; c, d. the resulting disintegration of the tissue due to infection (same fish). e-g. Pasteurellosis in angel fish (*Pterophyllum scalare*), showing nodular, bacteria loaded (arrows) lesions (abscesses) with gradually disintegrating macrophage-like cells. h, i. Epithelioid granulomatous lesions with acid fast positive bacteria (*Mycobacteria*) (arrows) in estuarine grey mullets from Israel (E, epithelioid cells; F, loose outer and, Fi, dense inner layers of fibroblasts; L, surrounding spleen tissue).

Pathology: Histopathology is very characteristic; variable sized granulomata with a necrotic core and peripheral layers of macrophages and also, in the later stage, fibroblasts assemble into a fibrous capsule. Free bacteria aggregate in the periphery and inside the nodule. Many macrophages contain ingested bacteria. Necrotic nodules persist for a long time after the systemic bacterial infection subsides.

Epizootiology: Pasteurellosis, occurred in stored overwintering tilapia, at 15-17°C ambient water temperatures and the abundance of nodules in fish

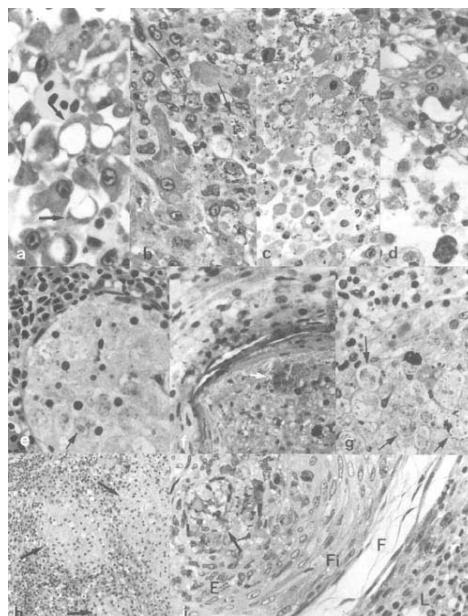


Plate 2: Pasteurellosis in cichlid fishes

examined from many different farms in Israel suggests the pathogen is widely distributed. Several species of *Pasteurella* were incriminated as the causative agent of morbid diseases in striped bass (*Morone saxatilis*), white perch (*M. americanus*) and grey mullet in marine and estuarine habitats, in the USA (Lewis *et al.*, 1970; Paperna and Zwerner, 1976), also in cultured yellowtail and red sea bream in Japan (Kusuda *et al.*, 1978; Yasunaga *et al.*, 1983) and lately in sea cultured European sea bass (*Dicentrarchus labrax*) and bass (*Morone*) hybrids in Israel.

A relationship between aetiological agents from tilapia and those from the European sea bass and bass hybrids has yet to be established. *P. multocida* is a known pathogen of poultry (Bredy and Botzler, 1989), which suggests, if both piscine and avian agents are the same, that chicken manure used to fertilize ponds may be the aetiological source (Nizan and Hammerschlag, 1993).

Aeromonas hydrophila and other ubiquitous facultative bacteria: Haemorrhagic septicaemia, due to *Aeromonas hydrophila* and sometimes other gram negatives (*Edwardsiella tarda*), has been reported from farmed tilapia in the Mombasa region of Kenya (Roberts and Sommerville, 1982). Septicaemia, caused by *A. hydrophila*, was reported from farmed *O. niloticus* in Egypt, while the bacterium was also isolated from the same fish species in the Nile (Amin *et al.*, 1985; Faisal *et al.*, 1984). A variety of gram negative bacteria, predominantly *Aeromonas hydrophila* (in 36% of cases) but also other facultative pathogenic genera or species (*Edwardsiella* sp. and *Yershinia enterocolitica*), were

isolated from moribund cultivated tilapia (*Oreochromis mossambicus-hornorum* hybrids and *O. niloticus*) in the Ivory coast. *A. hydrophila* and *Pseudomonas putida* were isolated from moribund, septicemic *Anguilla mossambica* reared in Grahamstown, South Africa (Jackson, 1978).

Aeromonas and *Pseudomonas* spp. and unidentified gram-negatives were reported to be the cause of high mortalities in reared *O. mossambicus*, *O. niloticus* and *Tilapia zillii*, in the Philippines (Lio-Po *et al.*, 1983) Haemorrhagic septicemia has been reported from pond cultured tilapia (*O. niloticus*) in Japan, involving *Pseudomonas fluorescens* (Miyazaki *et al.*, 1984) and *Edwardsiella tarda* (Miyashita, 1984). The latter, as well as causing acute infections in overwintering farmed tilapia, in Israel, cause a characteristic chronic clinical condition (Paperna, 1984).

Mortality of carp at Kajansi farm, Uganda, caused by extreme dropsy and furunculosis-like lesions in *Synodontis afrofisheri* in the Volta lake, Ghana, provide further documented evidence of clinical conditions with presumptive bacterial aetiology.

Diagnosis: External and internal gross pathological signs exhibit a similar pattern in all acute systemic, gram negative, bacterial infections and are therefore not very different from that observed in acute vibriosis. Routine standard methodologies are available (Bullock *et al.*, 1971; Lewis, 1973) for isolation, culture and differential diagnosis of *Aeromonas* and other gram-negative facultative bacteria. For biochemical profiles for the diagnosis of *E. tarda* see Darunee *et al.* (1984) and Wakabayashi and Eugusa (1973) and for *Pseudomonas fluorescens* see Miyashita (1984).

Pathology: Acute clinical conditions, associated with systemic infections, resulted in mortalities within 24-48 hours. Tank-reared, two-week-old fry died at a rate of 15% daily. Affected fish were heavily pigmented, with petechial haemorrhages. In more chronic-type clinical conditions, eroded fins occur as well as skin lesions and sluggish swimming (Roberts and Sommerville, 1982; Lio-Po *et al.*, 1983). Internally, the liver is usually pale and there may be focal haemorrhages over the visceral and peritoneal surfaces. Some reports (Faisal *et al.*, 1989) also note swelling and haemorrhaging of the spleen and the kidneys and accumulation of ascitic fluid. Histological changes become distinct only if clinical conditions are prolonged, being mainly focal cellular necrosis in the liver and haemopoietic cells, heart and skeletal muscles. Strands of fibrin and bacteria aggregate at the periphery of the necrotic areas. Cellular inflammatory infiltrate, when present, consists of macrophages with ingested bacteria or melanosomes (Roberts and Sommerville, 1982).

In chronic septicemia caused by *Pseudomonas* spp. in Japan (Miyazaki *et al.*, 1984) and unidentified gram-negatives (presumably pseudomonads) in overwintering tilapia in Israel and southern Africa (Paperna, 1984), white nodules occurred in the spleen, liver, and kidneys. The Japanese fish had exophthalmia with corneal opacity and an inflamed swim bladder which contained milky fluid. Histology revealed granulomata of epithelioid and encapsulated necrotic lesions. Fibrin strands, bacterial aggregates and infiltrating phagocytic cells, with ingested bacteria, precipitated in the periphery of the granulomata. Large abscesses contained cellular infiltrate of necrotic phagocytes with ingested bacteria. Inflammatory foci also occurred on the gills in the Japanese fish and the swimbladder contents consisted of neutrophil infiltrate and bacteria. Follow up of the overwintering fish showed perpetuation of a granulomatous process even after complete disappearance of the bacteria. With the rise in water temperature, lesions regressed into fibroblast encapsulated necrotic foci.

Epizootiology: Generally, fish are predisposed to systemic gram-negative facultative bacterial infections by handling trauma or adverse growth conditions such as inadequate feeding, poor water conditions, overstocking and, outside the tropical region, low temperatures (Roberts and Sommerville, 1982; Paperna, 1984). Stress-mediated infections, due to handling, usually lead to acute haemorrhagic septicemia. In overwintering tilapia farmed outside the tropical regions a chronic condition with visceral granuloma develops, resulting at times in losses of up to 98% of the overwintering stock (Paperna, 1984).

Wounds, caused by tags, were the cause of bacterial septicemia in tilapia brood stocks. *A. hydrophila* infected lesions, caused by attached epistiliid sessilian ciliates, are known as "red sore" disease (Esch *et al.*, 1976; Overstreet and Howse, 1977). Some strains of *A. hydrophila*, *E. tarda* or species of *Pseudomonas* are aetiological causes for systemic infection in diverse species of fish (carp, eels and tilapia) (Miyazaki *et al.*, 1984; Darunee *et al.*, 1984; Faisal *et al.*, 1989). Interspecific differences in vulnerability to these facultative bacterial infections reflects the degree of compatibility between fish and their environment (e.g. carp vs. tilapia in overwintering storage) rather than an innate tolerance or susceptibility to a specific bacterial pathogen.

Myxobacterial infections-gill and fin rot: Myxobacterial skin lesions and gill rot in Africa have been reported from eels reared in South Africa (Jackson, 1978) and cultured tilapia (*Oreochromis niloticus*) in Kenya (Roberts and Sommerville, 1982) and in Egypt (Amin *et al.*, 1988) and outside Africa in cultured tilapia and their hybrids in Israel. In Southeast Asia catfish of the

genus *Clarias* develop myxobacterial skin lesions on the back (“saddle back”) and around the mouth (Lio-Po *et al.*, 1983).

Diagnosis: Myxobacteria of the genus *Flexibacter* (*Cytophaga*), including *F. columnaris*, the commonest aetiological agent of fin rot and skin lesions (“saddle back”) of freshwater fish, are readily recognised in direct microscopic examination of a squash of an affected tissue by their long thin (filamentous) structure. Myxobacteria are isolated on *Cytophaga* agar medium (Anacker and Ordal, 1959; Fijan, 1969), re-cultured on Nutrient, 5% sheep blood, MacConkey and SS agar (Difco) (Amin *et al.*, 1988). Methods of characterization of pure isolates were described by Cruickshank *et al.* (1975).

Pathology: Myxobacterial gill infections, reported from *O. niloticus* tilapia in Egypt (Amin *et al.*, 1988), included both acute and chronic clinical conditions. These data were similar to pathological data on myxobacterial infections in other fish (carp, trout) elsewhere (Richards and Roberts, 1978). Snieszko (1981) differentiated between the acute necrotic disease caused by *Flexibacter columnaris* and the chronic proliferative condition caused by other *Flexibacter* or *Cytophaga* spp. There is also evidence for systemic myxobacterial infections in fish (Richards and Roberts, 1978).

Gill lesions in the acute clinical condition (usually caused by *F. columnaris*) are necrotic and often rapidly expanding, and death is more rapid. On the skin, acute lesions are often confined to the head and back (“saddle back”) (Roberts and Sommerville, 1982). Such lesions are white or yellow with a reddish zone of hyperaemia around the periphery and comprise of bacterial cells and necrotic tissue covering haemorrhagic ulcers. Histology reveals epidermal spongiosis and a subsequent necrosis which extends into the dermis. Chronic myxobacterial infections cause extensive hyperplasia in the gills, with resulting fusion of the lamellae and sometimes also with proliferation of mucus glands and chloride cells. Proliferation in the skin occurs at the tips of fins and at skin folds.

Other pathological manifestations of myxobacterial infection reported elsewhere, which might also be relevant to African fish are as follows (Richards and Roberts, 1978): Fin rot condition has been reported only from fish outside Africa. It induces a severe epidermal and dermal oedema, a fibrinous exudate overlaid with cytophaga bacteria, and cellular exudates, with subsequent sloughing and progressive erosion of the fins. Myxobacteria may be displaced by secondary opportunistic saprophytic bacteria. Cotton wool disease, affecting tropical aquarium fish, is suggested to result from irritation of gill lamellae, producing catarrhal exudate over the gills, which serves as an attractive

substance for cytophaga proliferation (Richards and Roberts, 1978).

Control of infections by facultative pathogenic bacteria: Antibiotic therapy by use of medicated feeds appears to be a feasible undertaking, as long as the infected fish are still willing to eat. Drug sensitivity tests of the pathogen targeted for treatment must be repeatedly performed to ensure efficacy of antibiotics added to feeds. Application of medicated feeds, as a non-specific prophylactic measure, is economically wasteful, harmful to the environment (damaging nitrification processes and primary production) and promotes drug resistance amongst pathogens in the habitat.

In the case of streptococcal infection, use of medicated feeds in poly culture is not cost-effective as carp, which always comprise the greater portion of the pond biomass, are refractory to infection. Vaccination has been repeatedly considered as a potential solution and some research is in progress. There are, however, serious doubts as to how effective this vaccination may be and whether it will be economically worthwhile. The feasibility of vaccination use will largely depend on the degree and durability of protection afforded by immersion vaccination or via feeds. Specific immune response has been induced in tilapia to various antigens (Sailendri and Muthukkaruppan, 1975) and to some bacterial infections. Elevation of antibody titers to a level of 100% protection to challenges during two to five weeks was obtained in Nile tilapia (*O. niloticus*) following injection of formalin-killed *A. hydrophila* and Freund's complement adjuvant vaccines (Ruangpan *et al.*, 1986). Attempts to vaccinate reared tilapia (*O. niloticus*) fingerlings with lyophilized *Aeromonas hydrophila* in the Ivory Coast were, however, unsatisfactory.

Serum agglutinating antibody titer of tilapia hybrids rose following injection with a *V. parahemolyticus* bacterin both with and without Freund's complement adjuvant, while titers for naturally occurring antibodies in the overwintering fish were usually low. Immunization trials by immersion showed significant efficacy in protecting tilapia from a challenge for about 60 days (Hubbert, 1989). Neither significant elevation in agglutinin titers nor protection against challenge was obtained in Nile tilapia immunized with formalin-killed *Edwardsiella tarda*, using the hyperosmotic infiltration method (Lio-Po and Wakabayashi, 1986). Iida *et al.* (1982) and Kusuda and Tagaki (1983) found that vaccination using toxoid, rather than bacterin, elicited a greater antibody response and they recommended the use of detoxified endotoxin in streptococcal infections of yellowtail.

There is a serious risk of introducing bacterial infections into Africa (notably *Streptococcus*, *Pasteurella*

and mycobacteria); which are prevalent in countries outside Africa specializing in tilapia culture, with genetically improved culture seed - breeders, fry and apparently also eggs. Only an adequately enforced ban on such imports will secure African habitats from these infections.

Mycobacteriosis of fishes: "Piscine tuberculosis" is another name for the disease. It is a chronic disease of brackish, fresh and saltwater fishes. *Mycobacterium marinum* can cause the disease in salt-water fishes, as *Mycobacterium fortuitum* in fresh and brackish water fishes. Both organisms are long, rod shaped, gram positive and acid fast. The optimum growth temperature range from 25 to 35°C. They are cosmopolitan. All fish species and most amphibians are susceptible to these bacteria. Transmission is through oral and cutaneous route. Man and other homoiothermic animals are not exempted from "piscine tuberculosis". It causes granuloma on the elbow or knee and unhealed sores.

Clinical signs for "Piscine tuberculosis" include emaciation, loss of appetite, stunted growth, body pigment alteration and curvature of the spine. The affected fishes may not develop. At autopsy most viscera organs develop, while gray white nodular lesion of varying sizes, kidney and liver are mostly affected. Kidney and spleen are mostly affected. Spleen, stomach, heart, gill and intestine are occasionally infected. Diagnosis depends on the clinical signs, isolation, identification of the causative organisms and histopathology. Squash preparation of infected organs shows the gram positive acid fast long rods. Histopathology reveals lesions with necrotic center, surrounded by fibrous connective tissues. The most effective control measure for the disease is regular sanitation and disinfection of hatchery facilities and destruction of infected fishes. Quarantine measure is important.

Reported from many marine and freshwater fish species and also fish families occurring in Africa (Cichlidae, Characidae) (Nigrelli and Vogel, 1963; Noga *et al.*, 1990). There are several case reports from African cichlid species; from *Sarotherodon andersonii* and *T. sparmanii* in the Okavango swamps in Botswana (Roberts and Sommerville, 1982), farmed *O. niloticus* in Kenya (Roberts and Sommerville, 1982) and also from aquarium reared *Haplochromis multicolor*, *Hemichromis bimaculatus* (Nigrelli and Vogel, 1963) and *Oreochromis mossambicus* (Noga *et al.*, 1990).

Mycobacterial infections seen in grey mullet (*Mugilidae*) entering inland waters in Israel are likely to be of marine origin. Mycobacterial infections have also been reported from ornamental cyprinids, and exotic tropical fish reared in Malaysia (Shamsudin *et al.*, 1990). *Mycobacterium* spp. isolated from fish are also infectious

to humans (Nigrelli and Vogel, 1963). Nocardiasis has not yet been reported from African fish, although, it has been reported in tropical fish from South America (*Hyphessobrycon innesi* - Valdez and Conroy, 1963) and Southeast Asia (Gourami, *Trichogaster trichopterus*).

Diagnosis: In piscine tuberculosis white nodules occur in all visceral organs, but mainly in the spleen, which also usually becomes enlarged. Similar granulomatous nodules occur in the viscera of fish with nocardial infection. Skin lesions occur only in some cases of tuberculosis, but are characteristic to nocardial infections. Positive identification of mycobacteria is by acid fast positive staining with Ziehl-Nielson (or other modifications for acid fast staining). Some *Nocardia* are acid fast positive. Both *Mycobacterium* and *Nocardia* are gram-positive but the latter stain more distinctly than the former. *Mycobacterium* are short rods, but may be filamentous or branching and therefore confused with acid fast positive *Nocardia*, which is a pleomorphic actinomycete forming both hyphae, bacilli and cocci. Identification to species requires culture and biochemical characterisation; mycobacteria are fastidious and need special media, whereas *Nocardia* grow well on nutrient agar. Molecular biology methodology has recently been introduced for differential diagnosis of piscine mycobacteria (Knibb *et al.*, 1993).

Pathology: Spleen and kidneys usually contained the highest concentration of mycobacterial tuberculi. Visceral granulomatous lesions were comprised of either epithelioid, with a core of degenerating cells, or necrotic nodules enclosed in epithelioid gradually replaced by encapsulating fibroblasts; the cores contained acid fast staining bacteria. Systemic granulomata of *Nocardia* demonstrate similar configurations.

The mycobacterial infection reported from *O. mossambicus*, by Noga *et al.* (1990), was atypical. The multiple skin lesions were comprised of single nodules or aggregates of small nodules of vacuolated inflammatory cells. Dermal aggregates extended into the epithelial layer. Lymphocytes as well as pigment cells were aggregating around the lesions, resulting in heavy melanization of the skin. Visceral lesions were small and contained mostly pigmented and non pigmented chronic inflammatory cells. In nocardial infections, deep, ulcerating, sometimes haemorrhagic lesions develop in the skin, from which nocardial bacteria are readily isolated.

Epizootiology: Infection, in cultured marine fish, may reach epizootic proportions (Colomi, 1992). In culture installations, infection spreads apparently via necrophagy of diseased fish. Feeding on trash fish is a potential source for introduction of mycobacterial infection into farmed

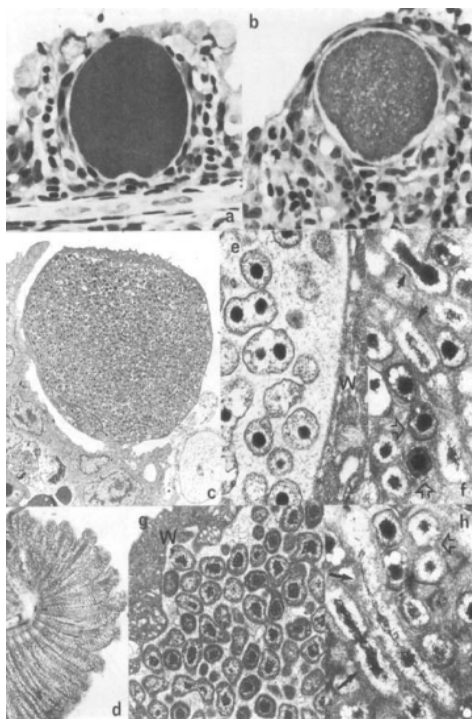


Plate 3: Epitheliocystis

stocks. Transovarian transmission was demonstrated only in viviparous fish (Nigrelli and Vogel, 1963). There is no supporting evidence for transmission via processed feeds. The data are, however, too sporadic for a comprehensive epizootiological evaluation.

Control: Both the relatively high tolerance to antibiotics, demonstrated from *in vitro* drug sensitivity tests, and the granulomatous nature of the pathological process, contribute to the experienced low efficacy of applied drugs for cure of both piscine tuberculosis (Colorni, 1992) and nocardial infections. Avoiding feeding of cultured fish with untreated marine products seems to be an important precautionary measure.

Epitheliocystis: Occurs in both freshwater and marine fish and is widespread in all cichlid species in southern Africa, Kenya and Israel, in the common carp (“Mucophilus” agent) and in the Mediterranean and Red Sea grey mullets (Mugilidae) (Paperna and Sabnai, 1980; Paperna *et al.*, 1981; Paperna and Alves de Matos, 1984).

Diagnosis: Epitheliocystis is chlamydial organisms invading and causing gross hypertrophy of integumental epithelial cells, mostly of the gills; lining and respiratory cells as well as mucus and chloride cells. Infected cells grow into transparent (up to $100 \times 55 \mu\text{m}$ in size) bodies with fine granular contents. Transmission electron

microscopy reveals pleomorphic (round, rods and cocci) prokaryotic organisms bound with a trilaminated membrane and containing a nucleoid. Epitheliocystis organisms thus far have not been cultured *in vitro*.

Plate 3 Illustrates Epitheliocystis: a, b. Hypertrophic epitheliocystis infected cells on gills of *Oreochromis aureus*, Israel. c. Low mag. transmission electron microscopic (TEM) view of hypertrophic epitheliocystis infected cells (approx. $27 \mu\text{m}$ in diam) in gills of carp, Israel. d. Hyperinfected gill of *Mugil cephalus*, Israel. e-h. TEM view of epitheliocystis organisms: e. dividing round bodies ($0.7 \mu\text{m}$ in diam.) and f, h. rods or elongate bodies ($1-2 \mu\text{m}$ long) (same arrows), and cocci (elementary bodies) ($0.5 \times 0.3 \mu\text{m}$) (open arrows) of *Oreochromis Epitheliocystis*; g, round bodies of carp epitheliocystis ($0.4-0.8 \mu\text{m}$ in diam) (W- host cell wall).

Life history and biology: Invaded epithelial cells grow gradually into a grossly hypertrophic body. Organisms are secluded within an inclusion which expands with the increase in the bacterial mass. The border of the inclusion is lined by deformed host cell organelles: a network of microtubules or microfibrils or residue of mucus droplets. Through successive binary divisions, or budding, round chlamydia-like organisms (averaging $0.7 \mu\text{m}$ in diameter) form branching chains which further transform into rickettsia-like rods ($1-2 \mu\text{m}$ long), single or attached into chains, which finally split into cocci ($0.5 \times 0.3 \mu\text{m}$ in size) with condensed cytoplasm, which are the presumed dispersing infective stages. The latter are released into the water with the collapse of the host cell. This stage is structurally, as well as functionally, reminiscent of the chlamydial elementary bodies (Paperna and Sabnai, 1980; Paperna *et al.*, 1981). Epitheliocystis from different fish host species may vary in the morphology of individual stages and demonstrate additional stages with distinct morphology and division patterns (in carp - Paperna and Alves de Matos, 1984).

Pathology: In benign infections, tissue changes are limited to the formation of a thin capsular structure around the hypertrophic cell, the respiratory capillaries may send extensions to the surface of the infected cell. The “proliferative” condition results in massive epithelial hyperplasia which may embed the hypertrophic infected cell as well as part or all of the gill lamellae of the filament. In mullets and carp, the mere overload of the gills with hypertrophic cells caused severe erosion of the gill architecture, which interferes with the respiratory function (Paperna, 1977; Paperna and Sabnai, 1980).

Epizootiology: Infection spreads and accelerates into severe hyper infection very quickly in fry and fingerlings of grey mullet and carp held in overcrowded tanks, but thus far not in young tilapia, which maintain benign infection even in adverse growth conditions. After the

climax, in surviving carp and grey mullets (and marine sparriids) infection spontaneously subsides to become benign and low level (Paperna and Sabnai, 1980).

Control: Applications of antibiotics have, thus far, proved to be ineffective. The proliferative condition may be mediated through immediate reduction of stocking densities.

CONCLUSION

The Life history, biology, diagnosis, pathology and epizootiology and control of Streptococcus, Infectious Abdominal Dropsy of Carp, Furunculosis, Motile Aeromonad Disease, Vibrosis, Columnaris disease, Bacteria kidney disease, Peduncle disease (fin rot), Bacteria gill disease, Pasteurellosis, *Aeromonas hydrophila* and other ubiquitous facultative bacteria, Myxobacterial infections-gill and fin rot, Mycobacteriosis of fishes, Epitheliocystis are some bacteria diseases fish culturist will likely face in culture fisheries and be prepared to manage them.

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