# Mixed infections in tropical freshwater fish culture systems: A potential emerging threat for successful aquaculture

**A. Paul, B. Baral, Samikshya Parida, D. Naveen Naik and P.K. Sahoo***

*National Referral Laboratory for Freshwater Fish Diseases, Fish Health Management Division, ICAR-Central Institute of Freshwater Aquaculture (CIFA), Kausalyaganga, Bhubaneswar 751002, India. Tel: +91 6742465446/Fax: +91 6742465407. *Email: pksahoo1@hotmail.com*

The world population is growing steadily and predicted to reach up to 9.7 billion by 2050. To feed the ever-increasing world population, fish is an important source of nutrition. Fish not only serves as a source of protein but also contain other essential vitamins and minerals, and is considered a healthy food.

Aquaculture is an age-old technology initially began with a backyard pond-based extensive mode of culture, and today has reached the level of an industry with high intensification. Advanced technologies such as biofloc culture and recirculation-based super-intensive aquculture systems are becoming more common.

With intensification there is also an increase in the stress load on fishes, which ultimately suppresses the immune system and leads to disease, morbidity and mortality. Diseases are a major issue in aquaculture that commonly account for 10-15% of production losses. The aquatic environment contains many opportunistic pathogens and fish are frequently infected by a range of parasites, bacteria, viruses, and fungi. When the fish body is challenged by physical, chemical or biological stress factors, these opportunistic pathogens gain the upper hand and cause disease. In most incidences of disease, the involvement of two or more pathogens are often seen, which in turn complicates diagnosis and treatment. In our earlier surveillance-based study, we recorded that mixed bacterial-parasitic infections as being 10.50% of total disease incidences, while mixed parasitic infection was 37.80% of all parasitic cases (Sahoo et al., 2020). Further, parasites constituted 74.88% of total disease incidences in the same study, which was conducted from 2014-2018 in eastern India. The parasite infections were not usually directly detrimental, but they cause severe physiological distress to the host animal which invites other pathogens such as bacteria, viruses or other parasite(s) to infect further. However, mixed or co-infections aggravate the stress load on fish and thus lead to cause mass mortalities in different culture environments.

*Above, below: Mass mortality due to stress induced by multiple infections with erratic isolated swimming of fish in the pond.*

Here we present a few case studies on mixed infections in aquaculture systems, which may become more common in future due to changing climatic patterns and associated adverse impacts on water quality. Mixed infections seem to be a big challenge to diagnostic laboratories and health specialists in terms of providing a correct diagnosis and treatment.

## Case 1: Mixed parasitic infections in larval Indian major carps

**Farm location: Giringo, Balipatna, Khordha, Odisha**

A pond size of 0.15 ha with water depth of 2 m was stocked with 0.80 million Indian major carp spawn for fingerling production. A continuous mortality of up to 15,000 for 5 days with clinical signs of surfacing, anorexia and high mucus mount on gills was noticed from day 20 of culture. The farmer incurred a direct loss of INR 10,500/- (US$ 142). Infected moribund fish were bought to the laboratory for investigation. The fish were found to be infected with myxosporideans, *Trichodina* sp. and *Dactylogyrus* sp. under microscopic examination of damaged gills. No other bacterium was isolated from the kidney tissue. The fish were provided with anti-parasitic treatment, and the mortality was stopped.

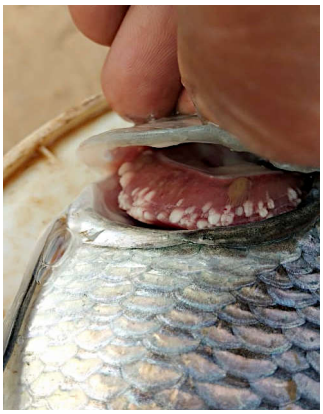
## Case 2: Mixed bacterial and parasitic infections in grow-out of Indian major carps

**Farm location: Kurangsasan, Baranga, Cuttack, Odisha**

A pond size of 0.61 ha with water depth of 2 m was stocked with 5,000 fingerlings (~100 g) and recorded mortality of fish continuously for two days. The fish, weighing on average of 200-500 g, showed sluggishness, anorexia, surfacing and haemorrhages over the body before death (twenty in two days). The moribund fish were bought to the laboratory for further investigation. The fish were found to be infected with myxosporideans, *Trichodina* sp., and *Aeromonas hydrophila* was also isolated from the kidney tissue. Treatment measures using formalin and Sokrena WS (Di-Decyldimethyl Ammonium Chloride of Virbac India) were able to prevent further mortality and the fish were cleared of infection within a week.

*Above, below: Mixed parasitic infection in fry stage of fish.*

*Mixed parasitic (below on gill tissue) and bacterial infections (haemorrhagic ulcer on body) in carps.*

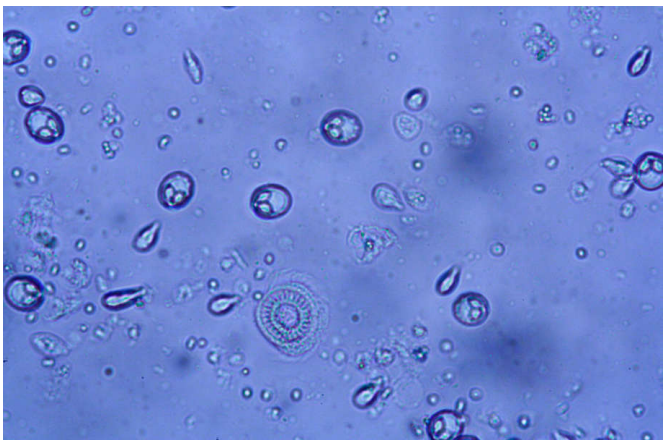


## Case 3: Mixed recurrent parasitic infections in grow-out of Indian major carps

**Farm location: Bhusandpur, Tangi, Khordha, Odisha**

A recurrent infection with mixed parasites was observed in an Indian major carp grow-out pond of 6.47 ha. The fishes showed clinical signs of damaged gills, surfacing and anorexia. During September 2016, moribund catla and rohu samples were diagnosed for mixed parasitic infections with the presence of *Dactylogyrus* sp., *Trichodina* sp. and myxosporideans in gills and lice (*Argulus* sp.) on the body. The farmer was advised to use an antiparasitic drug and subsequently the disease was controlled. Again, during December 2016, 200-250 fishes died in the same pond with clinical signs of haemorrhages, surfacing and gill damage. Diagnosis revealed that the fish harboured the same parasites in gills in addition to the involvement of one bacterium i.e., *Aeromonas hydrophila*, which was isolated and identified using molecular tools from kidney tissue. The farmer was advised to use medication and the infection declined, and mortality was stopped. The pond was completely harvested during December 2016. Again, during early January, 2017, the pond was stocked with fingerlings of Indian major carps and after just 15 days of stocking, a sporadic mortality was noticed. The fish were found to contain a heavy load of myxosporidean cysts in the internal organs such as the spleen, liver and kidney along with presence of *Dactylogyrus* sp. and *Argulus* sp. Anti-parasitic drugs were advocated, and the mortality was controlled. The prolonged winter during 2016-17 might be one of the possible predisposing factors for recurrent parasitic infestations.

*Above, below: Mixed parasitic infection on gills and internal organs.*



## Case 4: Mixed parasitic infections along with fungal infection in juvenile *Anabas testudineus*

**Farm location: Rahandia, Bhadrak, Odisha**

A large-scale mortality was noticed in biofloc culture of Anabas raised in six 100,000 litre tanks stocked at 180,000 fish/tank). Within a span of one week the mortality reached 15,000 fish (size ~100 g) in spite of proper biofloc manage-

ment at the farm level by the farmer. A loss of INR 750,000/- (US$10,140) was incurred because of the mortality. Clinical signs include pale colour of gills, white ulcerative patches on the body, and red spots near the operculum. On examination, the moribund fish were found to be infected with myxosporideans and *Dactylogyrus* sp. Further, fungal elements were also noticed on skin scraping examination. No gross changes in internal organs were found and also the kidney was free of any bacterium upon bacteriological examination. The floc was discontinued and the fish were shifted to isolation tanks for symptomatic treatment to get rid of the infection.

*Infection and mass mortality of Anabas in biofloc tank.*

## Case 5: Co-infection with bacteria and parasite in koi carp

**Farm location: Ornamental unit, Kausalyaganga, Odisha**

Mortality was noticed in 90 koi carp stocked in a cement tank. The infected animals showed clinical signs of anorexia, haemorrhages on body, high mucus built up on gills, and sluggishness in movement before death. In total seven fish had died when they were bought to the laboratory for examination. The fish were found to be infected with a heavy load of *Dactylogyrus* sp. on gill squash examination and Staphylococcus epidermidis was isolated from kidney and spleen tissues of the infected fish. Further, symptomatic treatment as able to control the mortality in the farm. Fish were free from carp edema virus on PCR. Gill flukes might have played primary role in causing stress and mortality, besides allowing secondary bacterial infection to further aggravate the condition.

*Mortality of koi carp due to co-infection of bacteria with gill flukes.*

## Case 6: Co-infection with bacteria and parasites in catfish

**Farm location: Ekchahalia, Pipili, Puri, Odisha**

A pond of 0.80 ha with water depth of 2 m stocked with 8,000 numbers of African catfish (*Clarias gariepinus)* along with Indian major carps was recorded mortality in catfish, only. The moribund fish showed marked sluggishness, surfacing and haemorrhages over body before death. Within five days, 300 fish had died of the infection. The fish were diagnosed to be infected with *Staphylococcus aureus* along with a heavy load of myxosporideans on the gills through PCR and microscopic examinations. Symptomatic treatment with parasiticide and disinfectant was able to control the mortality successfully in the farm.

These are few examples of mixed infections which are cited here. Similar types of cases are increasing day by day, not only in freshwater aquaculture systems but also in brackish water aquaculture involving multiple etiological agents. For example, infections with multiple viral etiological agents such as white spot virus, monodon baculovirus and hepatopancreatic parvovirus (Manivannan et al., 2002) and/or with EHP (*Enterocytozoon hepatopenaei*), an intracellular microsporidian (Thamizhvanan et al., 2019) in shrimp are becoming common problems in most of the shrimp producing countries in south east Asia.

*Mortality in catfish due to co-infections with bacteria and parasites.*

## Conclusions

The cases of co- or mixed infections in freshwater fish farming systems are increasing day by day. It is quite often difficult to pinpoint a single etiological agent as a root cause of infection in a mortality event. Similar mortality events are not uncommon in any culture system, particularly in ornamental fish farming. Validating Koch's postulate with single organism most of the times failed to create similar infection upon experimental challenge to establish the primary etiological agent in those mortality events. Earlier we also noticed large scale mortality in goldfish farming with Cyprinid herpes virus-2 along with *Aeromonas hydrophil*a (Sahoo et al., 2016). Further, Swaminathan et al. (2016) noticed carp edema virus infection in koi carp along with co-infection of *Dactylogyrus* sp. and opportunistic bacterial presence in internal organs. Similarly, our laboratory also recorded co-infection of carp edema virus with *A. hydrophila* in a mortality event in koi farming (Sahoo et al., 2020).

Climate change and transboundary movement along with system diversification and intensification are the major factors in the emergence of co- or mixed infections in fish farms. Researchers or health management officials need to be more cautious in disease diagnosis as ultimately this may decide the success of control of infection and effective health management. The mere presence of a parasite or any other opportunistic bacteria or virus may aggravate stressful conditions in the host in a culture environment leading to large scale mortality. Hence, proper care must be given for practising better health management at the farm site along with quality seed stocking. Intermittent use of probiotics and immunostimulants may be practiced in these situations to have a hand on effective management.

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