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Secondary Microbial Infection in *Ilisha melastoma* Due to Isopod Fish Parasites

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Abstract: Parasitation of isopod, *Joryma brachysoma* was observed in *Ilisha melastoma* collected from fish catches off Parangipettai coastal environment, southeast coast of India. In parasitized fish skin lesions were observed in the gill and body surface due to the attachment of parasite. In the lesioned spots a heavy load of Total Heterotrophic Bacteria was observed. *Vibrio parahaemolyticus* and *V. anguillarum* were abundant at the site of parasitic lesion. The microbial load in the parasitized fish was significantly higher. Cenocytic fungal pathogens were also isolated from the lesioned sites.

Key words: Isopod parasites, secondary microbial infection, lesion, *Vibrio parahaemolyticus*, *V. anguillarum*

INTRODUCTION

Crustacea and fishes were thrown into ecological propinquity by the very fact of their aquatic existence. Both in marine and freshwater environments fishes are, undoubtedly, among the best potential hosts for any prospective parasite. Several facultative and obligatory parasitic members of the order Isopoda are deleterious parasites on fishes. Pathogenic microorganisms in the aquatic habitat pose problems to the economic important fishes due to their secondary invasion on the body of the fish, which got parasitic infestation primarily. *Aeromonas punctata* was reported to have infected the fish secondarily at the site where the parasite *Argulus* sp. was attached (Cusack and Cone, 1985). *Lernaea* sp. and isopod parasite have been blamed for the introduction of virus causing dermal tumors in fishes (Simudu and Tsummoto, 1985). The copepod parasite *Ergasilus* sp. was reported be the etiological agent for a bacterial disease in fishes (Nigrelli, 1950). The trematode parasite *Gyrodactylus avalonia* was identified to carry pathogenic bacteria into the fish (Cusack and Cone, 1985). In the present study incidence of bacterial and fungal infection in the fish *Ilisha melastoma* were studied and the causative agent that facilitate the secondary infection by bacteria was identified.

MATERIALS AND METHODS

In the routine observation of *Ilisha melastoma* fishery in the Parangipettai landing center (Southeast coast of India) an intensity incidence of isopod parasitization was observed in several fishes. The isopod parasite was identified to be *Joryma brachysoma* (Ravichandran *et al*., 1999). The incidence of parasite attachment was maximum in the branchial and shoulder region of the host fish (Fig. 1). At the site of parasite attachment skin lesion was observed in all the parasitized fish.

The tissues exercised out from the parasitized (host) fish were separately homogenized with sterilized sea water and subjected to further microbiological testing (Totally five samples for each region).

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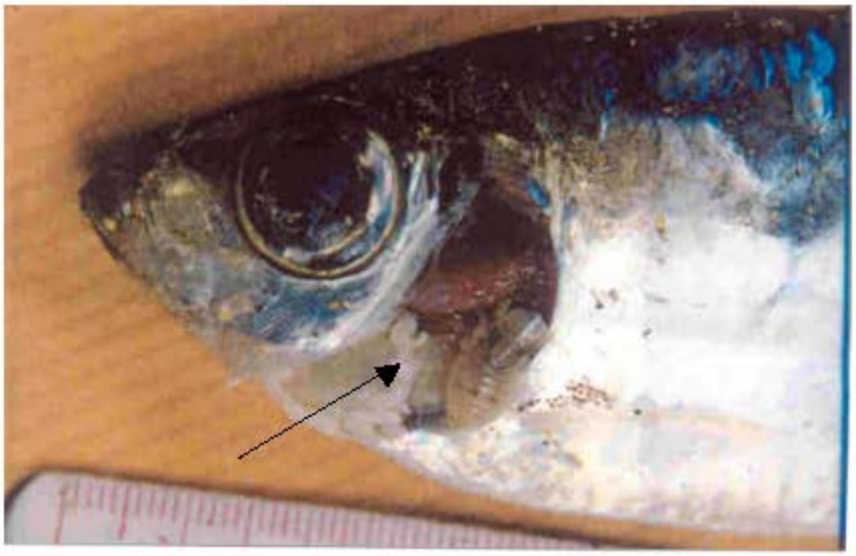

Fig. 1: *Joryma brachysoma* in *Ilisha melastoma*

One milliliter of the serially diluted samples was taken for enumeration of Total Heterotrophic Bacteria, vibrio and fungi using the plating technique. THB was enumerated using Zobell Marine Agar, Vibrios using Thiosulphate Cirarate Bilesalts Sucrose Agar (TCBS) and fungi using 2% Malt Extract Agar (MEA). The total counts were expressed as colony forming unit per gram (cfu g^{-1}). Different colonies of vibrios isolated were repeatedly streaked for purity and stored in nutrient agar slants under refrigeration. Identification of vibrios was carried out by performing Parker and Smith method (Parker and Smith, 1984). Fungus was grown in Rosebengal medium and was identified. The fungal growth in different salinity concentration was also studied.

RESULTS AND DISCUSSION

A comparative analysis of bacterial abundance among infected regions and uninfested regions of a fish revealed that infected portion are dense in bacterial load as observed in the lesions of infected fish (Fig. 1). In the parasitized fish Total Heterotrophic Bacterial count was higher of the two sites. The THB count in the branchial region was significantly higher than the shoulder region. Further study reveled the presence of two species of vibrio stain *Vibrio parahaemolyticus* and *V. angullarium*. The total vibrio count in the branchial region of parasite attached was 15±0.5 cfu g^{-1}. In the body surface where the parasite attached had 11.2±0.35 cfu g^{-1} vibrio count (Table 1). Besides vibrio, non-sporulating fungal growth was also observed in the culture of the tissue homogenate taken from parasite attached site. As the fungi did not sporulate in the culture media, its identification could not be carried out. This indicates the salinity tolerance potential of the cenocytic fungi isolated from the parasitic lesion (Fig. 2).

The attachment of the parasitic isopod *Joryma brachysoma* on *Ilisha melastoma* paved way for the entry of pathogenic microbes in to the attachment sites. The pathogenic microbes and parasite presence could damage the physiological and reproductive activities of the host fish Ranjith Singh and Padmalatha (1997). In the present study both Total Heterotrophic Bacterial and total *Vibrio* counts were found to be in greater numbers in the host's branchial region than in the shoulder region. There by a regional difference for the proliferation of microbes was observed. The greater degree of vibrio

Table 1: Mean Total Heterotrophic Bacterial count and Vibrio count in the different regions of parasitised fish *Ilisha melastoma*

Source	Branchial	Body surface
THB count (10^3 cfu g^{-1})	34±0.5	26±1.0
SE	0.03	0.01
P	p<0.05	p<0.05
Vibrio count (10^3 cfu g^{-1})	15±0.5	11.2±0.35
SE	0.01	0.03
P	p<0.05	p<0.05

SE: Standard Error; P: Level of significance

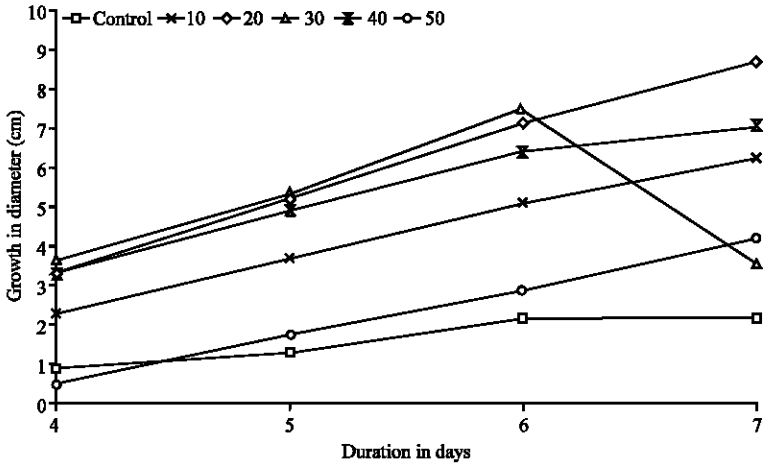

Fig. 2: Growth of pathogenic fungi in different salinity concentrations

count and THB in the branchial region could be due to the severity of lesion at this site as it was reported in the fish Creole parasitized by *Nerocila acumunata* (Vismanis and Kondratovics, 1997). Also the contamination of this area more frequently by the respiratory water current that carries bacteria along with food particles could have enhanced bacterial invasion. The bacterial invasion in the branchial region reduces the respiratory area by clubbing and fusion of gill lamellae and affects respiration as well as nitrogenous wastes excretion (Vismanis and Kondratovics, 1997; Ravichandran *et al*., 1999, 2000, 2004, 2007a,b). Two species of *Vibrio* isolated from the parasitic lesions, *Vibrio anguillarum* and *V. parahaemolyticus* were reported to be potential fish pathogen by previous workers (Parker and Smith, 1984; Kusuda, 1966; Hacking and Budd, 1971; Lohanathan, 1985). Hence the richness of *Vibrio* count at the parasitic lesion may affect the fish population as vibriosis is communicable. So far *V. parahaemolyticus* had been isolated mainly from the crustacean prawn and from cultivable marine fishes (Lighter, 1977; Sindermann, 1970; Raghukumar, 1971).

The fungi isolated from the infected area were found to be non-sporulating cenocytic fungi. Its failure in sporulation indicated that they are strictly parasitic. Survival of fungal species in marine environment is mainly depending upon their growth rate in the salinity of the sea. Raghukumar (1971) stated that higher frequency occurrence of fungi in marine environment and growth in higher saline concentration indicates their survival in these environments. The study on growth of isolated fungus in different saline conditions indicated maximum growth at 20-30 ppt of salinity; this indicates that fungus is well adapted to the optimum salinity concentrations. The fungus isolated from the parasitic lesion further confirms that the parasitation and skin lesion facilitates the entry of several pathogenic microbes secondarily at the damaged host tissue.

The bacterial load involved in the infection depends on the site of attachment. Higher bacterial load was observed in the branchial regions may be attributed to the ingestion of food materials might have facilitated increased bacterial load in the lesions. Rand (1986) while studying the histopathological changes in the creole fish, by *Nerocila acuminata* stated that the bacteria observed in lesions also contribute to more destruction of the host tissue. The gills of fish consist of a large network of capillaries in which the blood is separated from water by one or two layers of cells. These changes have a determental effect on health of fish and threatened their survival.

Hence it is quite evident from the present study that the parasitation of *Ilisha melastoma* by the isopod *Joryma brachysoma* leads to secondary infection by pathogenic microbes which may affect the fish population.

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