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Isopod Parasite Induced Secondary Microbial Infection in Marine Food Fishes

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Isopods are parasitic crustaceans that pose serious threat to fisheries. Several studies have tried to explore the host-pathogen relationship between marine fishes and isopods. The present study aims to understanding the secondary infections in marine fishes pertaining to isopods. To assess the secondary infection in infected fishes, parasite infested and healthy tissues of fishes were collected. The samples were subjected to standard microbiological procedure to identify the presence of pathogenic bacteria and fungi. Our results showed the branchial region had the higher microbial load of non-sporulating cenocytic fungi in infected fishes. Moreover, fungal strains isolated from the parasitic lesion confirmed that the parasitation and body lesion facilitates the entry of several pathogenic microbes at the damaged host tissue. More over the immune regulation of fish fights back by producing minute cysts, trying to encapsulate the growing fungus. But this may eventually lead to systemic infestation and death of the fish.

Key words: Isopod parasites, Marine fishes, Secondary microbial infection, Cymothoids

Isopods are associated with many species of commercially important fishes around the world and cause significant economic losses to fisheries by killing, stunting, or damaging these fishes. They can also kill or impair immature fishes so that they do not survive. Isopods occur very commonly as parasites in edible fishes and crustaceans. Only a few related works are available on the nature of parasitism of isopod parasites. More information is still warranted to understand host – parasite relationships. 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.

Lesions due to parasitic infection are generally the sites for secondary microbial infection (Ravichandran et al., 2001; Ravichandran et al., 2008). Lernaea sp. and isopod parasites have been blamed for the introduction of virus causing dermal tumors in fishes (Simudu and Tsummoto 1985). *Aeromonas punctata* was reported to have infested the fish secondarily at the site where the parasite *Argulus* sp. was attached (Cusack and Cone 1985). Parasitic infection becomes an entry route for opportunistic bacterial and fungal pathogens. These microbial infections combined with the parasitic attachment plays a very important role on the physiology of the infected animal leading to

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death. Studies of cymothoid parasites in aquaculture systems can thus complement studies of cymothoids in wild fish and provide an opportunity to obtain critical information on the effects of these parasites on their hosts. Such studies are very contemptible in Indian coastal environment. Therefore, the present study was designed to investigate the occurrence and secondary infection by the microbes in both infected fish and healthy fish of marine environment in Tamil Nadu coastal areas.

Materials and methods

Fish samples were examined thoroughly from different localities in various landing centers along the Tamil Nadu coastal environment for the presence of isopod parasites. The infested fish samples were brought immediately to the laboratory and examined for analyses. Isopods were removed from the body surface, buccal cavities and branchial region of the fish hosts and immediately placed into 70% ethanol. Mouthparts and appendages were carefully dissected using dissecting needles and forceps. Host nomenclature and fish taxonomy are given according to Fish Base (Froese and Pauly 2010). The collected samples were subjected to standard microbiological producers and total microbial counts were expressed as colony forming unit/gram (CFU) (Cusack and Cone 1985).

Results

Infestation of parasitic isopod on the host fishes

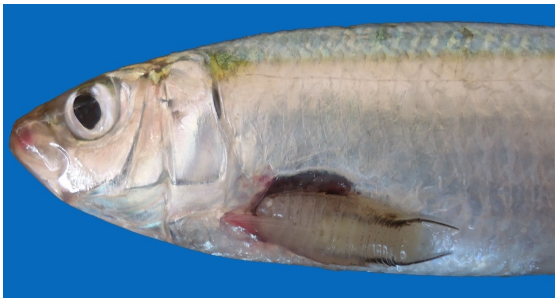

Fig. 1. Infestation of *N. phaeopleura* on *S. longiceps*.

Fig. 2. Skin lesion caused by *N. phaeopleura*.

Fig. 3. Erosion of skin due to *N. phaeopleura*.

Fig. 4. Infestation of *M. plagulophora* in *H. far*.

Fig. 5. Eroded gill racks due to *M. plagulophora*.

causes serious erotic lesions on the skin of the fishes (Fig. 1-5). These lesions paved the way for entry of microbes into the attachment sites as the secondary invaders. A comparative study of microbes present in both infected and uninfected region of fishes was analyzed. In general region of infested by parasites had the more microbial load when compared to the uninfected fishes. The microbial load was also calculated in the branchial and body surface of parasites attached fishes. More bacterial colonies (6.3±0.45 $\times 10^5$ cfu g-1) were observed and recorded in *Hemiramphus far* infested by *Mothocya plagulophora* in the branchial region and less colonies (5.0±0.28$\times 10^5$cfu g-1) were noticed and recorded in *Sardinella gibbosa* infested by *Nerocila phaeopleura* on the body surface. In the uninfected fishes bacterial load was higher (4.5±0.35$\times 10^5$cfu g-1) in *Carangids malabaricus* infested by *Catoessa boscii* in the buccal region and lower (2.8±0.12$\times 10^5$cfu g-1) in *S. gibbosa* infested by *N. phaeopleura* on the body surface (Table 1).

Similarly the presence of fungal population in the examined infected fish, maximum colonies (1.8±0.25 $\times 10^3$ cfu g-1) were observed in *H. far* and minimum colonies (0.8±0.09$\times 10^3$ cfu g^{-1}) in *S. gibbosa,* where as in uninfested fishes higher (0.9±0.1$\times 10^3$ cfu g^{-1}) population was noticed in *H. far* and lower (0.2±0.1 $\times 10^3$ cfu g^{-1}) in *Exocoetus volitans* (Table 1). Single purified colonies of the isolated bacteria were identified upto genus level by following routine biochemical tests. The identified bacterial strains were characterized under nine genus which includes *Aeromonas hydrophila, Aeromonas salmonicida, Bacillus* sp., *Citrobacter* sp., *Flavobacterium* sp., *Flexibacter, Mycobacterium marinum, Photobacterium* sp., *Pseudomonas fluorescens* and *Vibrio salmonicida,* The isolated fungal strains were also purified into single colony and identified based on their micro morphological characters with the help of microscope. The identified fungal genus were *Aspergillus* sp., *Candida* and *Mucor* (Table 2). The results of the present study also shows that microbial load in the parasitized fish was significantly higher than uninfested fish.

Discussion

Parasitic infestations on marine fishes incur damage to skin and fins that is likely to increase the likelihood of secondary microbial parasite infections (Clayton *et al*., 1998; Ravichandran *et al*., 2009). The pathogenic microbes and parasite presence could damage the physiological and reproductive activities of the host fish (Ranjit Singh and Padmalatha 1997). In the present study both total heterotrophic bacterial (THB) and total fungi counts were found to be in greater numbers on the infested host's than in the uninfested host's. There by a regional difference for the proliferation of microbes was observed. The greater degree of *Vibrio* 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

Table 1. Microbial load on parasite infected and uninfected fishes

Fish Name	Parasites	Region	Colony forming Units/gram			
			Bacteria ($\times 10^5$)		Fungi ($\times 10^3$)	
			Infected	Uninfected	Infected	Uninfected
Hemiramphus far	*Mothocya plagulophora*	Branchial	6.3±0.45	4.1±0.23	1.8±0.25	0.90±0.1
Strongylura leiura	*Mothocya renardi*	Branchial	5.5±0.27	3.3±0.19	1.6±0.13	0.8±0.2
Carangids malabaricus	*Catoessa boscii*	Buccal	6.1±0.94	4.5±0.35	1.3±0.12	0.7±0.2
Trichiurus lepturus	*Lepidopygopsis typus*	Buccal	5.9±0.45	3.9±0.26	0.9±0.06	-
Sardinella gibbosa	*Nerocila phaeopleura*	Body	5.0±0.28	2.8±0.12	0.8±0.09	-
Exocoetus volitans	*Nerocila exocoeti*	Body	5.9±0.35	3.6±0.16	1.2±0.10	0.2±0.10

Table 2. Microbes isolated from the parasite infected and uninfected fishes

Host Name	Bacteria	Fungi
Hemiramphus far	*Flavobacterium* sp. *Aeromonas hydrophila* *P.fluorescens* *Bacillus* sp.	*Candida* *Aspergillus* sp.
Strongylura leiura	*Flavobacterium* sp. *Mycobacterium marinum* *Flexibacter*	*Candida* *Aspergillus* sp.
Carangids malabaricus	*Vibrio salmonicida* *Aeromonas salmonicida* *Bacillus* sp. *P. fluorescens* *Aeromonas hydrophila*	*Penicillium* sp. *Candida*
Trichiurus lepturus	*Aeromonas hydrophila* *Bacillus* sp. *Vibrio* sp. *Aeromonas salmonicida*	*Aspergillus* sp.
Sardinella gibbosa	*Flavobacterium* sp *Bacillus* sp. *Vibrio salmonicida* *Aeromonas salmonicida*	*Aspergillus niger* *Candida*
Exocoetus volitans	*Flexibacter* *Bacillus* sp. *Pseudomonas* sp. *Aeromonas hydrophila*	*Candida* *Mucor* *Aspergillus* sp.

1997). The THB count in the branchial region was significantly higher than the shoulder region (Ravichandran *et al*., 2001).

In the present study infections by microbes are frequently reported in association with wounds caused by parasitic isopods. The isolated fungi from the infected area were found to be non-sporulating cenocytic fungi. Its failure in sporulation indicated that they are strictly parasitic. (Mohan and Shankar 1994) isolated the fungal species *Aphanomyces* which has been isolated from the epizootic ulcerative syndrome of infected fish. In the present study two fungal strains were isolated from the parasitic lesion which further confirms that the parasitation and body lesion facilitates the entry of several pathogenic microbes secondarily at the damaged host tissue. The fish tissues fight back by producing minute cysts, trying to encapsulate the growing fungus. Eventually the whole body may be infected and the fish die.

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