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Invasion of parasitic isopods in marine fishes

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PEER REVIEW

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Comments
In this study, large numbers of parasite infected marine fishes of known ages have provided the first strong evidence that cymothoid infections reduce the growth of fish hosts. The results also clearly illustrate the importance of incorporating the age of fish hosts in any assessment of parasite effects.
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ABSTRACT

Objective: To carry out a detailed three-year observation study on isopod parasites infestation in fish.
Methods: Fish samples were collected from different localities in various landing centers along the Tamil Nadu coastal area. The prevalence and mean intensity were calculated. The proximate composition of infestation and uninfestation were studied in different marine fishes. A comparative analysis of bacteria and fungi in the infected and uninfected regions of fishes were analysed.
Results: Tweenty six species including 12 genera of isopods (Cymothoidae) distributed in 39 species of marine fishes along the Tamil Nadu coast. The isopod parasites were attached in three different microhabitats in host fishes *viz.*, buccal, branchial and body surfaces. They exhibited host and site specific occurrence. Maximum prevalence 17.11% was recorded in March 2010 and minimum 0.27% in Febuary 2010. The intensity ranged from 1 to 1.7 parasites per fish during the different months from Decmber 2008 to November 2011. There was a decrease in the protein, carbohydrate and lipid content in the infested fishes compared to uninfected fishes. A comparative analysis of bacteria and fungi in the infected and uninfected region of fishes were analysed. It revealed that infected portions had dense bacterial load as observed in the lesions of infected fishes than uninfected fishes.
Conclusion: Factors which are able to induce parasitic manifestation are stock quality, stocking density, environmental conditions, biological and physiological characteristics of parasite, zoo technical measures, food quantity, feeding strategies, etc.

KEYWORDS
Isopod parasite, Invasion, Microhabitat, Occurrence, Proximate composition, Microbial load

1. Introduction

Isopods are dorsoventrally flattened crustaceans. Parasitic isopods are among the dominant groups of crustacean ectoparasites of fish; about 450 species are parasites of marine and freshwater fish[1]. The cymothoid isopods inhabit freshwater, brackish water and the sea environment, as ectoparasites of various fish species. They may be observed on the body, buccal cavity or gill cavity of the host[2]. Most cymothoid are highly host and site specific. Isopod parasites are usually large and fierce looking and the damage they cause to the host fishes is considerable[3]. The specificity of isopod parasites, zoogeography and the vertical distribution of isopod parasites in host systems was studied in the north-west African shelf[4]. Host specificity is the tendency of a parasite to occur on one or a few host species and is a product of co-existence between both parasite and host lineages[5]. A few related works are available on the nature of infestation of isopods parasites in fishes[6,7]. The Indian cymothoid fauna is relatively poorly known and until now studies on these parasitic isopods in marine fishes from the Indian coasts were scanty. Most of the studies were from

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the east coast of India[8–14]. But no specific studies on the distribution and infestation isopod parasites in marine fishes were done. Hence an attempt has been made to study their infestation in marine fishes.

2. Materials and methods

The study was based on three-year observations during December 2008 to November 2011. Fish samples were collected from different localities in various landing centers along the Tamil Nadu coastal area covered for isopod infestation from the 8°5′N to 13°35′N latitude and 76°15′E to 80°20′E longitude *viz*., Parangipettai, Mudasalodai, Pazhaiyar, Nagapattinam, Kodiakarai, Vedaranyam, Mandapam, Tuticorin, Kanyakumari, Colachal (Figure 1). Fish samples were examined thoroughly for the presence of isopod parasites. Isopods were removed from the body surface and the buccal cavities 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 FishBase[15]. Specimens are deposited at the Annamalai University Ravichandran Collection.

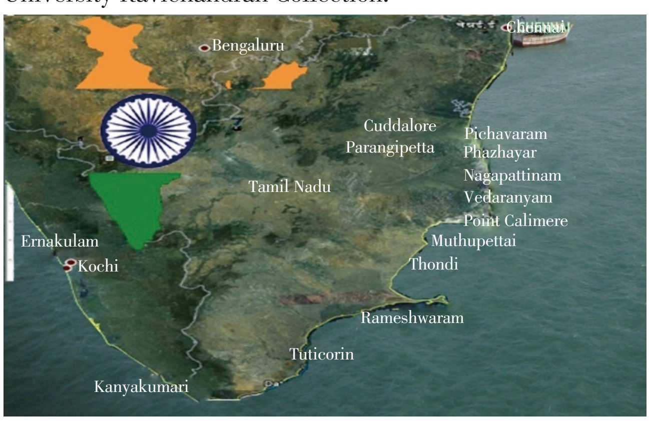

Figure 1. Sample collection area.

The prevalence and mean intensity were calculated. The proximate composition of infestation and uninfestation were studied in different marine fishes. The protein, carbohydrate and lipid contents from the fishes were estimated following standard methods respectively[16–18]. Samples were taken for enumeration of total heterotrophic bacteria (THB) and fungi. For enumeration of THB and fungi the pour plating technique was adopted. THB was enumerated using Zobel marine agar and fungi using 2% malt extract agar. The total count was expressed as CFU/g. With a view to assess the nature of damage, fish tissues were taken from the parasite attachment area of infested and uninfested fishes and were cut out in fresh condition and fixed in 10% buffered neutral formalin for examination.

3. Results

3.1. Distribution of isopod parasites

In the present study twenty six species including twelve genera (*Alitropus, Anilocra, Ceratothoa, Cymothoa, Glossobius, Joryma, Lironeca, Lobothorax, Mothocya, Nerocila, Norileca* and *Ryukyua*) of isopods belonging to the family Cymothoidae were found in thirty nine species of marine fishes along the Tamil Nadu coast. The isopod parasites mainly attached in three different microhabitats of host fishes (Table 1) *viz.*, buccal, branchial and body surfaces. They exhibited host and site specific occurrence. Two of them are new record (*Catoessa boscii* and *Nerocila loveni*) for the Indian fauna. Sixteen species have been recorded previously, but the 10 new records are reported for the first time from India including *Anilocra dimidiata, Ceratothoa angulata, Lobothorax typus, Mothocya renardi, N. longispina, Nerocila trichiura, Nerocila depressa, Nerocila arres, Nerocila loveni* and *Norileca indica*.

Table 1
Microhabitats of isopod parasites in host fishes.

Branchial parasites	Buccal parasites	Bodysurface parasites
Joryma brachysoma	*Alitropus typus*	*Anilocra dimidiata*
Joryma hilsae	*Ceratothoa angulata*	*Nerocila arres*
Joryma tartoor	*Cymothoa eremita*	*Nerocila depressa*
Lironeca puhi	*Cymothoa indica*	*Nerocila exocoeti*
Mothocya plagulophora	*Glossobius* sp.	*Nerocila phaeopleura*
Mothocya renardi	*Lobothorax typus*	*Nerocila poruvae*
Norileca indica		*Nerocila longispina*
Ryukyua circularis		*Nerocila loveni*
		Nerocila serra
		Nerocila sundaica
		Nerocila trichiura
		Nerocila trivitata

3.2. Prevalence and intensity of parasites

The prevalence and intensity of parasites in fishes during the different months from Decmber 2008 to November 2011 were examined. The maximum prevalence 17.11% was recorded in March 2010 and the minimum 0.27% in Febuary 2010 (Figure 2). The intensity ranged from 1 to 1.7 parasites per fish in different months from Decmber 2008 to November 2011 (Figure 3).

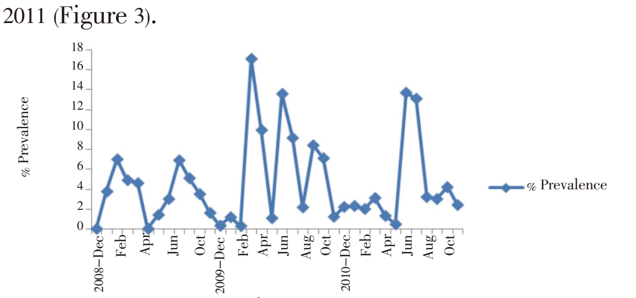

Figure 2. Prevalence of parasites on fishes in relation to different months (December 2008–November 2011).

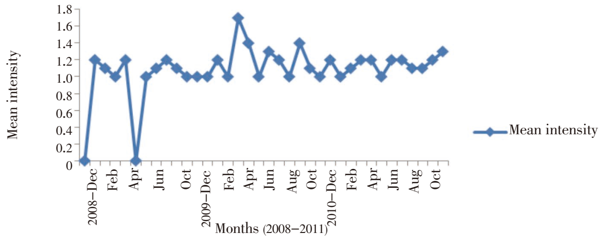

Figure 3. Mean intensity of parasites on fishes in relation to different months (December 2008-November 2011).

3.3. Proximate composition in relation to infestation

The proximate composition of infestation and uninfestation were studied in 14 species of fishes (selected on the basis of isopods infestation at body regions). There was a decrease in the protein, carbohydrate and lipid content in the infested fishes compared to uninfected fishes (Figures 4-6). In infested fishes, the protein content was maximum in *Thryssa mystax* (71.77%) and minimum in *Otolithes ruber* (58.49%). It was also clear that higher protein content was recorded in the uninfested fish *Thryssa mystax* (76.49%) and lower in *Otolithes ruber* (62.39%).

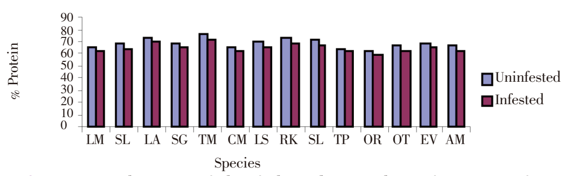

Figure 4. Protein changes of the fishes due to the infestation of isopod parasites.
LM-*Llisha melastoma*, SL-*Sardinella longiceps*, LA-*Lelognathus* sp, SG-*Sardinella gibbosa*, TM-*Thryssa mystax*, CM-*Carangids malabaricus*, LS-*Lelognathus splendens*, RK-*Rastrelliger kanakurta*, SL-*Selaroides leptolepis*, TP-*Terapon puta*, OR-*Otolithes ruber*, OT-*Opisthopterus tardoore*, EV-*Exocoetus volitans*, AM-*Arius maculatus*.

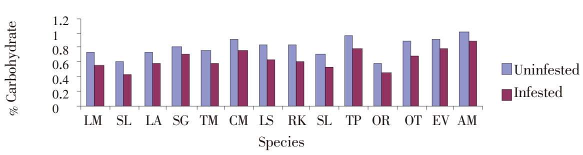

Figure 5. Carbohydrate changes of the fishes due to the infestation of isopod parasites.
LM-*Llisha melastoma*, SL-*Sardinella longiceps*, LA-*Lelognathus* sp, SG-*Sardinella gibbosa*, TM-*Thryssa mystax*, CM-*Carangids malabaricus*, LS-*Lelognathus splendens*, RK-*Rastrelliger kanakurta*, SL-*Selaroides leptolepis*, TP-*Terapon puta*, OR-*Otolithes ruber*, OT-*Opisthopterus tardoore*, EV-*Exocoetus volitans*, AM-*Arius maculatus*.

Figure 6. Lipid changes of the fishes due to the infestation of isopod parasites.
LM-*Llisha melastoma*, SL-*Sardinella longiceps*, LA-*Lelognathus* sp, SG-*Sardinella gibbosa*, TM-*Thryssa mystax*, CM-*Carangids malabaricus*, LS-*Lelognathus splendens*, RK-*Rastrelliger kanakurta*, SL-*Selaroides leptolepis*, TP-*Terapon puta*, OR-*Otolithes ruber*, OT-*Opisthopterus tardoore*, EV-*Exocoetus volitans*, AM-*Arius maculatus*.

The carbohydrate content in the infested fish was higher in *Arius maculatus* (0.88%) and lower in *Sardinella longiceps* (0.43%). In uninfested fishes the carbohydrate were calculatedto be maximum in *Arius maculatus* (1.02%) and minimum in *Otolithes ruber* (0.57%). Among the infested fishes lipid content was maximum in *Rastrelliger kanakurta* (9.47%) and minimum in *Opisthopterus tardoore* (5.87%). The result of that uninfested fish lipid content was high in *Thryssa mystax* (11.44%) and low in *Opisthopterus tardoore* (6.64%).

3.4. Secondary microbial infection

A comparative analysis of bacteria and fungi in the infected and uninfected region of fishes were analysed and it revealed that infected portions had dense bacterial load as observed in the lesions of infected fishes than uninfected fishes.

The bacterial and fungal load was calculated in the branchial and body surface of parasites attached fishes. The THB load was maximum in *Rastrelliger kanakurta* (5.9×10^5 CFU/g) in the infected fishes and the minimum was noticed in *Ilisha melastoma* (3.5×10^5 CFU/g). In the uninfected fishes THB load was higher in *Rastrelliger kanakurta* (3.3×10^5 CFU/g) and lower in *Exocoetus volitans* (1.7×10^5 CFU/g) (Table 2).

The presence of fungal load in the infected fishes, maximum (1.9×10^3 CFU/g) in *Amblygaster sirm* and minimum (0.9×10^3 CFU/g) in *Leiognathus splendens* were noticed. Also, total fungal load in uninfested fishes were higher (1.1×10^3 CFU/g) in *Arius maculatus* and lower (0.2×10^3 CFU/g) in *Leiognathus splendens* (Table 2).

Table 2
Total heterotrophic bacteria and fungal counts on infected and uninfected fishes (CFU/g).

Fish Name	Parasites	Region	Bacteria count		Fungi count	
			Infected fishes	Uninfected fishes	Infected fishes	Uninfected fishes
Rastrelliger kanakurta	*Norileca indica*	branchial	5.9×10^5	3.3×10^5	1.3×10^3	0.5×10^3
Amblygaster sirm	*Ryukyua circularis*	branchial	5.6×10^5	2.9×10^5	1.9×10^3	0.4×10^3
Arius maculatus	*Nerocila trivittata*	Body surface	3.9×10^5	1.8×10^5	1.7×10^3	1.1×10^3
Sardinella gibbosa	*Nerocila phaeopleura*	Body surface	4.8×10^5	1.9×10^5	0.8×10^3	0.3×10^3
Leiognathus splendens	*Nerocila loveni*	Body surface	3.8×10^5	2.1×10^5	0.8×10^3	0.2×10^3
Ilisha melastoma	*Nerocila sundaica*	Body surface	3.5×10^5	2.5×10^5	1.2×10^3	0.7×10^3
Exocoetus volitans	*Nerocila trichiura*	Body surface	3.8×10^5	1.7×10^5	1.2×10^3	0.8×10^3

CFU/g: Colony forming units/g.

From the biochemical identification the identified isolates were *Flavobacterium*, *Aeromonas hydrophila*, *Pseudomonas fluorescens*, *Pseudomonas putida*, *Citrobacter*, *Photobacterium*, *Bacillus*, *Mycobacterium marinum*, *Flexibacter*, *Vibrio salmonicida*, *Aeromonas salmonicida*, *Flexibacter* and *Bacillus* sp. in the parasite attached region of host fishes (Table 3).

Fungal strains were identified as *Exophiala salmonis*, *Ichthyosporidiosis*, *Aspergillus niger*, *Aspergillus* sp, *Candida* sp. and *Mucuor* sp. based on their microscopic, morphological characters.

Table 3
Identified microbes from the fishes.

Fish name	Bacteria	Fungi
Rastrelliger kanakurta	*Flavobacterium*	*Exophiala salmonis*
	Aeromonas hydrophila	
	Pseudomonas fluorescens	
	Pseudomonas putida	
	Citrobacter	*Ichthyosporidiosis*
	Photobacterium	
	Bacillus sp.	
Amblygaster sirm	*Flavobacterium*	*Exophiala salmonis*
	Aeromonas hydrophila	
	Pseudomonas fluorescens,	
	Pseudomonas putida	
	Mycobacterium marinum	*Ichthyosporidiosis*
	Flexibacter	
Arius maculates	*Vibrio salmonicida*	*Exophiala salmonis*
	Aeromonas salmonicida	
	Bacillus sp.	
	Pseudomonas fluorescens	*Ichthyosporidiosis*
	Aeromonas hydrophila	
Sardinella gibbosa	*Aeromonas hydrophila*	*Aspergillus niger*
	Pseudomonas fluorescens	
	Citrobacter	
	Bacillus sp.	
	Vibrio sp.	*Aspergillus* sp.
	Aeromonas salmonicida	
Leiognathus splendens	*Flavobacterium*	*Aspergillus* niger
	Citrobacter	
	Bacillus sp.	
	Mycobacterium marinum	*Candida* sp.
	Vibrio salmonicida	
	Aeromonas salmonicida	
Ilisha melastoma	*Aeromonas hydrophila*	*Mucuor* sp.
	Flexibacter	
	Vibrio salmonicida	
	Vibrio sp	
	Bacillus sp.	*Aspergillus* sp.
	Pseudomonas sp	
	Aeromonas sp	
Exocoetus volitans	*Mycobacterium marinum*	*Ichthyosporidiosis*
	Flexibacter	
	Aeromonas salmonicida	*Candida* sp.
	Bacillus sp.	
	Pseudomonas sp	*Aspergillus* sp.
	Aeromonas hydrophila	

3.5. Specimens database

This data base is designed to collate distribution records of isopod parasites across the Tamil Nadu coastal area. It includes 26 species of isopods and their taxonomical key for identification, microhabitat and host species etc (Table 4).

Table 4
Species profile database

S. No.	Species name	Identification code
1	*Ceratothoa angulata*	AUCR1
2	*Cymothoa indica*	AUCR2-14
3	*Alitropus typus*	AUCR15,16
4	*Nerocila poruvae*	AUCR17,18
5	*Joryma hilsae*	AUCR19,20
6	*Nerocila longispina*	AUCR21,22
7	*Anilocra dimidiata*	AUCR23,24
8	*Joryma brachysoma*	AUCR25-241
9	*Nerocila phaeopleura*	AUCR242-306
10	*Cymothoa eremita*	AUCR307-313
11	*Glossobius* sp.	AUCR314
12	*Joryma tartoor*	AUCR315-332
13	*Mothocya plagulophora*	AUCR333-405
14	*Mothocya renardi*	AUCR406-459
15	*Nerocila depressa*	AUCR460-463
16	*Nerocila sundaica*	AUCR464-471
17	*Nerocila serra*	AUCR472
18	*Nerocila arres*	AUCR473-489
19	*Nerocila trivitata*	AUCR490-496
20	*Nerocila trichiura*	AUCR497-499
21	*Nerocila loveni*	AUCR500-525
22	*Norilica indica*	AUCR486-499
23	*Nerocila exocoeti*	AUCR500-524
24	*Ryukyua circularis*	AUCR525-562
25	*Lobothorax typus*	AUCR563-612
26	*Lironeca puhi*	AUCR613

AUCR-Annamalai University collection Ravichandran.

4. Discussion

Too little is known about isopods associated with fishes in the southern Caribbean to adequately discuss their zoogeography[19]. A study in Kuwait reported 9 species of Cymothoidae[20]. Latertwenty-nine species from the genera *Anilocra*, *Creniola*, *Nerocila*, *Pleopodias* and *Renocila* are recorded or reported from the Indo-West Pacific[21]. Fourteen species of cymothoids were reported from the eastern Pacific[22]. Fourteen Cymothoidae have been reported from Algeria, the majority being widely distributed in the Mediterranean[23]. Nine new species of *Anilocra* were reported from the West Indian coral reef fishes[24]. In India seven species of cymothoid isopods parasitic on the marine fishes of the Kerala coast were reported[25]. Seven species of isopod parasites were from marine food fishes of Parangipettai. However, twenty six species of parasites were collected from thirty nine species of fishes[26].

A number of cymothoids including *Nerocila orbignyi* and *Nerocila bivittata* are specific in their choice of hosts, whereas other genera are less specific. The results of this investigation indicate that *Nerocila phaeopleura*, although is comparatively primitive in being an external parasite and being highly host specific, it is also highly specialized to a mode of life upon a pelagic, fast swimming host. It lives on a highly specific region of the body. This position is determined by the needs of the parasite and the limitations exerted by the morphology and habits of the host. In the present study specificity was observed in the host of 11 species of *Nerocila* species collected, than other genera of cymothoids.

The main factors determining the fish parasite fauna as well as intensity and prevalence of infestation in marine environments were studied[27]. *Nerocila phaeopleura*, is being host specific, would thus seem to fit in with an apparently generic characteristic and it seems reasonable to assume that *Sardinella gibbosa* is the major host of this species in the South China sea. There was an increase in the prevalence of the parasitosis from 33.4% to 98.2%; concurrently, the total number of parasites on salmon rose from 309 to 3 987 with an increase of infestation intensity from 1.4 to 6.1 parasites per fish[28]. In Parangipettai coast the highest percentage of infestation occurred in January and the lowest in July[29]. In the present study maximum 17.11% was recorded in March 2010 and minimum 0.27% in Febuary 2010. The intensity ranged from 1 to 1.7 parasites per fish during the different months from Decmber 2008 to November 2011.

Parasites have been responsible for delay in fish growth and gain of weight by affecting the food ingestion[30-33]. The results of the biochemical analyses revealed that the fishes were seriously affected by the parasite. The tissue of the infested fish showed changes with respect to its biochemical composition. The major factor for the increase is due to the decrease of organic constituents such as protein, carbohydrate and lipid. The first proposed the protein-water line for muscles of non-fatty fishes[34]. The proximate composition of 5 different species of fishes, *Channa orientalis, Anabas testudineus, Lebistes reticulatus, Tilabia mossambica* and *Macropodus cupanus* was investigated, suggesting depletion due to parasitic infestation[35]. In the present study, proximate composition of infestation and uninfestation were studied in 14 different species of marine fishes.

The results of the biochemical analysis on the marine fishes revealed that the muscle tissue of the infested fishes shows some changes with respect to its biochemical composition. The depletion of glycogen in the infested fishes is due to the feeding of blood by these parasites which utilize the blood sugar as a source of energy reserve. Glycogen may be utilized by arthropod parasite for the synthesis of chitin and also for moulting purposes. The biochemical constituents of fishes in different stages as non infested and infested with parasites. But in the present study the equal sizes of uninfested and infested fishes with parasites were analysed[35]. The conspicuous change in the proximate composition of uninfested fish is the increase in protein, carbohydrate and lipid content compared to infested fish[27]. Similarly, in this study, it was reported that the proximate composition increased when compared to infested fishes. It is quite possible that the parasite utilizes the lipid content for the development of musculature. On the other hand, from the present study percentage reduction of protein in the infested fishes were not as high when compared with carbohydrate and lipid. It is generally recognized that parasites living in oxygen rich surroundings such as blood theoretically derive most of their energy from the oxidation of lipids and proteins.

The attachment of the parasitic isopod on marine fishes paved way for the entry of pathogenic microbes in to the attachment sites. But such behaviours also incur damage to skin and fins that is likely to increase the likelihood of secondary microbial parasite infections[36]. In the present study both 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. Hemorrhagic lesions in the spotted gore parasitized by the cymothoid *Anilocra acuta* were subjected to secondary infection[37]. 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[38]. Higher bacterial load was observed in the branchial regions and may be attributed to the ingestion of food materials which might have facilitated increased bacterial load in the lesions[39]. In the present study, 13 species of bacteria isolated from the parasitic lesions of the body surface and branchial regions, were reported to be potential fish pathogens. Hence the richness of bacterial count at the parasitic lesion may affect the fish population. The bacterial load involved in the infection depends on the site of attachment.

Parameters prevalence and mean intensity indicate that cymothoid is a successful parasite on marine fish population within Indian waters. Generally speaking, fish and fish products are one of the main protein sources worldwide. In this study, large numbers of parasite infected marine fishes of known ages have provided the first strong evidence that cymothoid infections reduce the growth of fish hosts. Our results also clearly illustrate the importance of incorporating the age of fish hosts in any assessment of parasite effects. All of the parasitic species determined both hinders the growth of their host, and may cause death due to blood suction. Factors which are able to induce parasitic manifestation are stock quality, stocking density, environmental conditions, biological and physiological characteristics of parasite, zoo technical measures, food quantity, feeding strategies, etc.

There can be no doubt that economic effect of parasitism should increase the interest concerning the biology and life cycle of this parasite.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

The isopod parasites were attached in three different microhabitats of host fishes *viz*, buccal, branchial and body surfaces. The depletion of glycogen in the infested fishes is due to the feeding of blood by these parasites which utilize the blood sugar as a source of energy reserve. The attachment of the parasitic isopod on marine fishes paved way for the entry of pathogenic microbes in to the attachment sites. Parameters prevalence and mean intensity indicate that cymothoid is a successful parasite on marine fish population within Indian waters. Factors which are able to induce parasitic manifestation are stock quality, stocking density, environmental conditions, biological and physiological characteristics of parasite, zoo technical measures, food quantity, feeding strategies, etc. There can be no doubt that economic effect of parasitism should increase the interest concerning the biology and life cycle of this parasite.

Research frontiers

A detailed study of three-year observations on infestation isopod fish parasites was carried out. There was a decrease in the protein, carbohydrate and lipid content in the infested fishes compared to uninfected fishes. A comparative analysis of bacteria and fungi in the infected and uninfected region of fishes were analysed and it revealed that infected portions had dense bacterial load as observed in the lesions of infected fishes than uninfected fishes.

Related reports

The proximate composition of infestation and uninfestation were studied in different marine fishes. A comparative analysis of bacteria and fungi in the infected and uninfected regions of fishes were analysed. Tweenty six species including 12 genera of isopods (Cymothoidae) distributed 39 species of marine fishes along the Tamil Nadu coast. The isopod parasites were attached in three different microhabitats of host fishes *viz*, buccal, branchial and body surfaces. They exhibited host and site specific occurrence. Maximum prevalence 17.11% was recorded in March 2010 and minimum 0.27% in Febuary 2010. The intensity ranged from 1 to 1.7 parasites per fish during the different months from Decmber 2008 to November 2011.

Innovations and breakthroughs

The innovative outcome of this paper in this successive year leads to new report of three isopods which are entirely new to the Indian coast. This in turn is preceded after the new discovery of two isopods. The host specificity of isopod infection is also a new record. This discovery ultimately paves for the creation of database collection of isopods.

Applications

Cymothoid infestation has a potential to be a useful marine ecosystem health indicator in a changing environment.

Peer review

In this study, large numbers of parasite infected marine fishes of known ages have provided the first strong evidence that cymothoid infections reduce the growth of fish hosts. The results also clearly illustrate the importance of incorporating the age of fish hosts in any assessment of parasite effects.

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