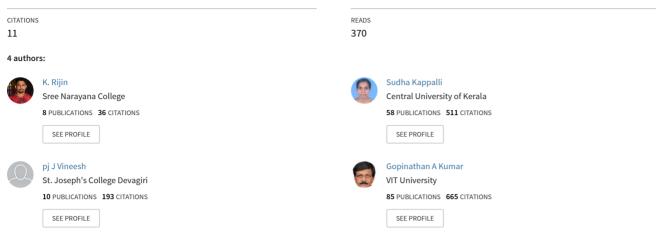
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Seasonal variation in the occurrence of parasitic isopods and copepods (Crustacea) infecting the clupeidaen fishes of Malabar Coast, India

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Seasonal Variation in the Occurrence of Parasitic Isopods and Copepods (Crustacea) Infecting the Clupeidaen Fishes of Malabar Coast, India

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Abstract

Occurrence of parasitic isopods and copepods infecting the clupeidaen fishes of Malabar coast (India) was assessed in terms of prevalence, intensity, host/ site specificity and seasonal variation. 3 isopods (Joryma brachysoma, Anilocra leptosoma and Agarna malayi) and 4 copepods (Clavellisa hilsae, Peniculus fistula fistula, Pseudorbitacolax varunae and Naobranchia cygniformis) were recovered from the fishes, Escualosa thoracata, Tenualosa toli (hosts two parasites), Sardinella fimbriata and Anodontostoma chacunda (hosts three parasites) respectively. The prevalence and/or intensity of each recovered parasitic species showed statistically significant variation (P<0.05) according to seasons; the prevalence of isopod species was high during pre-monsoon and least in monsoon. Except P. varunae, which is more prevalent in pre- monsoon, all copepod species exhibited high prevalence during post- monsoon. The floor of branchial cavity forms the major site of infection for J. brachysoma and A. malayi recovered from E. thoracata and T. toli respectively. A. leptosoma prefers dorsal body surface, behind the head of T. toli for infection. P. fistula fistula, P. varunae and N. cygniformis prefer to infect respectively caudal fin, anterior and posterior mucus layer of inner operculum of A. chacunda indicating their microhabitat preference likely to avoid niche competition during the circumstance of triple parasitism.

Introduction

Crustacean parasitism is one of the important factors affecting the viability of captive and cultured fish populations (Athanassopoulou, Bouboulis, & Martinsen, 2001; Barber & Poulin, 2002; Rijin & Sudha, 2017; Başusta, Mutlu, & Deval, 2017), but the assessment of their possible effects on their fish hosts is quite difficult especially in fish under wild conditions (Pillai, 1985; Williams Jr & Bunkley-Williams, 2000; Yu & LI, 2003; Carr & Whoriskey, 2004). Further, it still remains to be explained why some fish species have a higher parasite species richness than others, and how parasite communities build up on these hosts (Trilles & Oktener, 2004; Smit, Bruce, & Hadfield, 2014; Rameshkumar & When the prevalence of a Ravichandran, 2014). parasitic crustacean is high, they can significantly diminish reproductive output of host populations (Lafferty & Kuris, 1993; Leonardos & Trilles, 2003; Johnson *et al.*, 2004) and also host density (Fogelman, Kuris, & Grutter, 2009).

Even though the incidence of many parasites varies conspicuously from season to season (Altizer *et al.*, 2006) studies were still meager on the pattern of seasonality of parasitic occurrence.

Recent report from our laboratory on the parasitic isopod, *Nerocila sp.*, infecting the marine fishes along the Malabar Coast showed that four species under this genus were more prevalent during post- monsoon/ premonsoon seasons and by the onset of monsoon, the prevalence showed a gradual and significant decrease (Panakkool-Thamban, Kappalli, Keethadath, Gopinathan & Jean-Paul, 2013).

Clupeidaen fishes represent a large part of the fish biomass in Malabar coast of India and our preliminary observation indicates that these fishes are under the heavy infection of parasitic crustaceans including both isopods and copepods (Aneesh, 2014). The present paper reports the pattern of occurrence of parasitic isopods and copepods infecting the Clupeidaen fish species of Malabar coast in terms of prevalence, intensity, host and site specificity and seasonal variation.

Materials and Methods

Collection and Identification

The present study was conducted during the period from June 2014 to May 2017. Fresh Clupeidaen fishes were collected from the Ayyikkara fish landing centre, one of the major fish landing center in Malabar region (Lat. 11°51'N, Long. 75°22'E; Malabar Coast, Kerala, India). Soon after collection they were brought to the laboratory and closely examined the body surface, lateral line region, base of the pectoral fin, branchial cavity and gill filaments, inner wall of the operculum, buccal cavity etc for the presence of parasitic isopod and/or copepod using hand lens (Rijin & Sudha, 2017). Recovered parasitic crustaceans were preserved in 70% ethanol for further detailed examination under dissection microscope and a stereo microscope Leica-S6D. The taxonomic identification was performed according to Pillai (1985), Aneesh, (2014), Panakkool-Thamban, Kottarathil & Kappalli (2016). Prevalence (P) and intensity (I) of infection were calculated according to Bush, Lafferty, Lotz & Shostak (1997). Host nomenclature and fish taxonomy are done according to Fish Base (Froese & Pauly, 2016). The period February - May, June- September and October - January were considered respectively as Pre-monsoon, Monsoon and Post- monsoon periods with view to assess the seasonal wise variation, if any, in the prevalence and/or intensity of parasitisation.

Voucher specimens of all recovered isopods and copepods were deposited in the Parasitic Crustacean Museum, Crustacean Biology Research Laboratory, Sree Narayana College, Kannur, Kerala, India.

Data Analysis

Seasonal variation, if any, in prevalence and intensity of recovered parasitic crustacean was analyzed using one-way ANOVA followed by Tukey's pairwise comparisons. Significance level was set at P<0.05. Shannon diversity (H') was estimated for all three seasons. Normality of data was analyzed by Jarque-Bera test and opted parametric tests for seasonality studies. All aforesaid statistical analysis were done using PAST software (version 2.17c) (Hammer, Harper & Paul, 2001).

Results

A total of 4272 members of Clupeidaen fishes belonging to eight species were collected during the study period (Table 1). Among these, two fish species, thoracata (Valenciennes, Escualosa 1847) and Tenualosa toli (Valenciennes, 1847), showed infection with three isopods and two clupeids (Sardinella and Anadontostoma chacunda) with fimbriata copepods. E. thoracata hosts Joryma brachysoma (Pillai, 1964) and T. toli hosts two isopods, Anilocra leptosoma (Bleeker, 1857) and Agarna malayi (Tiwari, 1952). The fish, A. chacunda (Hamilton, 1822) is highly potential to host three copepods including Peniculus fistula fistula (Von Nordman, 1832), Pseudorbitacolax varunae (Bennet, 1968) and Naobranchia cygniformis (Hesse, Clavellisa 1863). Copepod hilsae (Pillai, 1962) was recovered from Sardinella fimbriata (Valenciennes, 1847) (Table 2; Figure 1). The clupeids Amblygaster sirm (Walbaum, 1792), Sardinella longiceps (Valenciennes, 1847), Hilsa kelee (Cuvier, 1829) and Tenualosa ilisha (Hamilton, 1822) were found to be completely free from both isopod and copepod infection during the study period (Table 1).

Out of 1039 white sardine, (*E. thoracata*) collected, 271 members were infected with *J. brachysoma*, and prevalence (P) and intensity (I) being 26.11 ± 0.73 and 1.59 ± 0.08 respectively; the floor of the branchial cavity appears to be the site of infection (Table 2; Figure 1, 2 & 3).

The prevalence of *A. leptosoma* and *A. malayi* infecting *T. toli* is relatively less comparing to that of *J. brachysoma*. Of 542 members of fish host, only 78 were found parasitized by *A. leptosoma* ($P = 11.93\pm0.95$; I = 1.00) and 99 by *A. malayi* ($P = 14.38\pm1.34$; I = 1.31±0.15) (Table 2; Figure 2 & 3); the dorsal body surface just behind the head of the host fish forms the

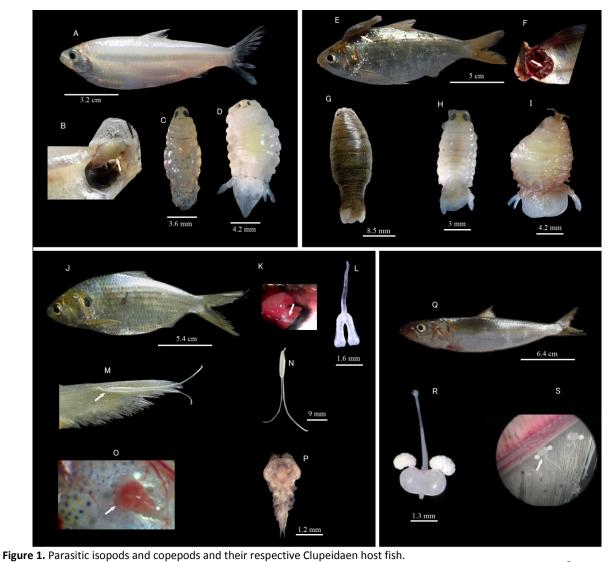
Table:1. List of Clupeidaen fishes observed for the presence of parasitic isopods and copepods

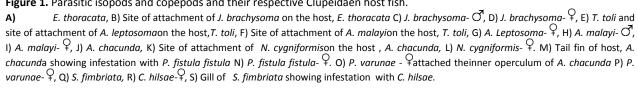
SI No	Clupeid Fish	NFO	Inl	InC
1	Tenualosatoli (Valenciennes, 1847)	670	**	-
2	Escualosathoracata (Valenciennes, 1847)	1039	*	-
3	Amblygastersirm(Walbaum 1792)	368	-	-
4	Sardinellalongiceps (Valenciennes, 1847).	612	-	-
5	Sardinellafimbriata (Valenciennes, 1847)	576	-	*
6	Anodontostomachacunda (Hamilton, 1822)	754	-	***
7	Tenualosailisha (Hamilton, 1822)	190	-	-
8	Hilsakelee (Cuvier, 1829)	63	-	-

(NFO_Number of fishes observed, Inl_Infected with isopod ,InC_ Infected with copepod, * _ Single parasitism, ** _ Double parasitism, *** _ Triple parasitism, - _ Absence of parasite).

Table 2. Prevalence (P), Intensity (I) of recovered parasitic isopods and copepods and the site of attachment on the Clupeidaen host fish

SI	Parasitic			Host Fish	Р	I	Site of attchn	nent
No	Crustace ans	Species/ Family			(Mean±SD)	(Mean±SD)		
1		Joryma brachysoma/ Cymot	hoidae	E. thoracata	26.11±0.73	1.59±0.08	Branchial c floor	avity-
2	Isopod	Anilocra leptosoma/ Cymothoidae		T. toli	11.93±0.95	1.00±0.00		Body ehind
3	Agarna malayi/ Cymothoidae		e	T. toli	14.38±1.34	1.31±0.15	Branchial c Floor	avity-
4		<i>Clavellisa hilsae</i> / Lernaeopo	odidae	S. fimbriata	7.13±1.22	4.03±0.21	Gill racker/ filaments	' gill
5		Peniculus fistula fistula/ Pennellidae		A. chacunda	2.88±0.47	1.21±0.02	Caudal fir upper/lower	
6	Copepod	<i>Pseudorbitacolax</i> Bomolochidae	varunae/	A. chacunda	20.29±3.03	1.02±0.01	inner opercu Upper region	
7		Naobranchia cygniformis / Lernaeopodidae		A. chacunda	0.40±0.56	1.00±0.00	inner opercu lower region	





site of infection for *A. leptosoma*, on the other hand, *A. malayi is* found attached the floor of the branchial cavity of the host fish (Figure 1). Both body surface and branchial cavity of host fish (*T. toli*) where parasite found attached were heavily damaged (Figure 1).

A. chacunda, a small genus of gizzard shads usually distributed in the Indo-Pacific region showed infection with three parasitic copepod species belong to three different genera such as Bomolochidae, Lernaeopodidae, and Pennellidae (Table 2; figure 1). The bomolochid, P. varunae exhibited greater prevalence (P= 20.29±3.03) and intensity (I= 1.02±0.01) (Table 2; Figure 3 & 4) compared to other copepod species. It preferred upper region of inner operculum of host fish (A. chacunda), for site of infection (Figure 1). Most of the recovered members were females possessing egg sacs containing eggs undergoing embryogenesis. All members of pennellid species, P. fistula fistula recovered from A. chacunda were also females attached to either lobe of caudal fin lying parallel to the host body (Figure 1); prevalence and intensity being 2.88±0.47 and 1.21±0.02 respectively

(Table 2; Figure 2 & 3). A. chacunda also showed infection with lernaeopodid, Ν. cygniformis (Hesse, 1863), but with relatively less prevalence (0.04±0.56) and intensity (1.00) (Table 2, Figure 2 & 3) and site of infection being the lower region of inner operculum of host fish. Selection of different niche by the different copepod parasites on the same host body likely avoids competition among them for space and food. Fringe scale sardinella (S. fimbriata) showed infection by the copepod C. hilsae (Figure 1) with prevalence and intensity respectively 7.13±1.22 and 4.03±0.21 and the site of attachment being both gill filaments and gill rakers (Table 2; Figure 2 & 3).

Seasonal Variation in Prevalence

Season dependent variation was noticed in the intensity and prevalence of parasitic isopods and copepods recovered from clupeids (F=2.788; P=0.022) during the present study period. Irrespective of the individual parasite species,

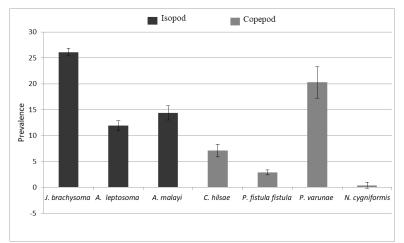


Figure 2. Prevalence (Mean± SD) of parasitic isopods and copepods recovered from the Clupeidaen fishes.

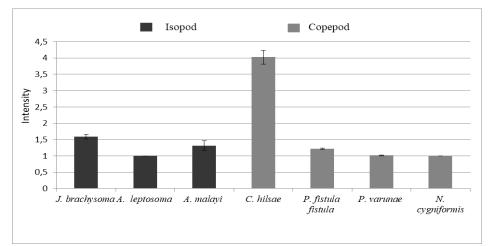


Figure 3. Intensity (Mean± SD) of parasitic isopods and copepods recovered from their Clupeidaen fishes.

isopods shows greater prevalence during the premonsoon (Feb-May) and least in monsoon (June- Sept). Among isopods higher prevalence was found in J. brachysoma (Figure 4) with a sequence of pre-monsoon (P= 32.68±2.90) > post- monsoon (P=26.29±0.99) > monsoon (P=17.02±0.81) (Figure 4). The prevalence shown by A. leptosoma during pre-monsoon, monsoon and post- monsoon was 14.76±2.27, 8.97±0.56 and 13.86±4.25 respectively (Figure 4). Contrary to this pattern, parasitic copepods such as C. hilsae, P. fistula fistula and N. cygniformis showed greater prevalence during post- monsoon season (Figure 4) while the seasonal pattern of prevalence shown by *P. varunae* was resembled to that of isopods. During monsoon, prevalence shown by all isopod and copepod species recovered during the present study was least (Figure 4).

Tukey's pairwise comparison between the prevalence of the recovered isopods and copepods revealed no pairwise variation in the prevalence with respect to seasons (P>0.05) (Table 3) except between *A. malayi* and *N. cygniformis* (P<0.05). Season wise analysis reveals that prevalence shown by the isopods J. *brachysoma* and *A. malayi* between monsoon & postmonsoon, monsoon & pre-monsoon and post-monsoon & pre-monsoon and post-monsoon & pre-monsoon is statistically significant (P<0.05) (Table

4). Statistically significant seasonal variation in prevalence shown by the copepods, *C. hilsae* and *P. fistula fistula*, between post- monsoon & pre- monsoon and monsoon & post- monsoon was also recorded (P<0.05) (Table 4). Prevalence of *P. varunae* also showed notable variation between monsoon & pre- monsoon and post- monsoon & pre- monsoon (P<0.05) (Table 4).

Shannon diversity index of each parasite (both isopod and copepod) with respect to seasons was also recorded; highest Shannon diversity for all three isopods was during pre- monsoon followed by post- monsoon and monsoon (Figure 5). Maximum Shannon diversity was recorded in the case of J. brachysoma when compared to other two isopod species throughout the study period (Figure 5). Among the copepods, P. varunae showed high Shannon diversity when compared to other copepod species irrespective of the seasons. C. hilsae, and P. fistula fistula, on the other hand, showed greater diversity index during post- monsoon and then it gradually declined towards the pre- monsoon and the lowest value was observed in the monsoon (Figure 6).Interestingly, in the case of N. cygnyformis, no diversity index was observed with respect to seasons as it remained at the base line throughout the study period

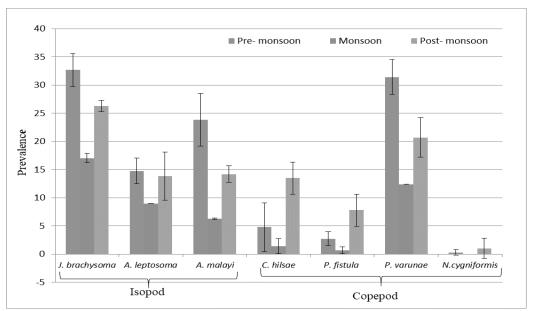


Figure.4. Seasonal variation in Prevalence (Mean± SD) of parasitic isopods and copepods recovered from Clupeidaen fishes.

Table 3. Tukey's pairwise comparisons between prevalence of parasitic isopods and copepods . (Significant P value is given in bold)

	J. brachysoma	A. leptosoma	A. malayi	C. hilsae	P. fistula fistula	P. varunae	N. cygniformis
J. brachysoma		1	1	0.9088	0.6796	0.5579	0.05274
A. leptosoma	0.174		1	0.9451	0.7533	0.6369	0.07058
A. malayi	0.2112	0.3852		0.8489	0.5846	0.4631	0.0365
C. hilsae	1.625	1.451	1.836		0.9993	0.9947	0.4758
P. fistula fistula	2.271	2.097	2.482	0.6457		1	0.7612
P. varunae	2.54	2.366	2.752	0.9156	0.2699		0.8581
N. cygniformis	4.348	4.174	4.559	2.723	2.077	1.807	

(Figure 6).

Seasonal Variation in Intensity

Intensity of infestation signifies the degree of survival of parasite on the host species. Intensity in isopods (except *A. leptosoma* in which the intensity was 1.00 irrespective of the season) is at its peak during

pre- monsoon season followed by post- monsoon and monsoon (Figure 7). In the case of copepods, high rate of intensity was during post- monsoon and least in monsoon (Figure 7). However, results of one-way ANOVA test showed no significant variation in intensity when we consider overall data (F= 1.022; P= 0.387) on both isopods and copepods together; but, there exists significant variation in the intensity when the parasite

Table 4. Significance of variation in prevalence of individual parasitic isopods and copepods with different seasons- *P* value, *F* value and Tukey value. (Significant *P* value is given in bold).

SI No	Name of the parasite	F value	P value	Seasons	Pre-monsoon	Monsoon	Post-monsoon
				Pre-monsoon		14.8	6.052
1	J. brachysoma	55.49	0.000135	Monsoon	0.000312		0.002149
				Post-monsoon	0.01247	8.764	
				Pre-monsoon		10.69	5.853
2	A. malayi	28.66	0.000851	Monsoon	0.000861		0.03272
				Post-monsoon	0.01449	4.839	
				Pre-monsoon		1.886	4.871
3	C. hilsae	12.16	0.007751	Monsoon	0.4293		0.00747
				Post-monsoon	0.03841	6.758	
				Pre-monsoon		1.953	4.829
4	P. fistula fistula	12.19	0.007707	Monsoon	0.4073		0.007345
				Post-monsoon	0.033	6.782	
				Pre-monsoon		9.58	5.393
5	P. varunae	23.06	0.001525	Monsoon	0.001412		0.0573
				Post-monsoon	0.02077	4.187	

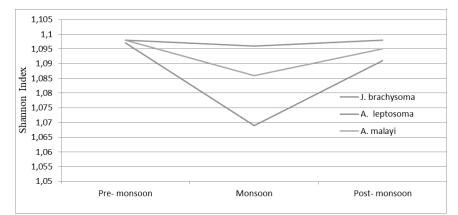


Figure 5. Shannon diversity index for parasitic isopods infesting the clupeid fishes with respect to seasons.

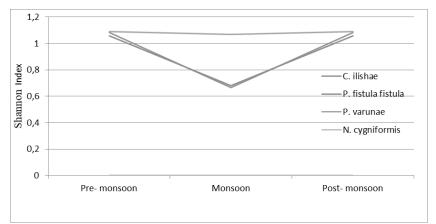


Figure 6. Shannon diversity index for parasitic copepods infesting the clupeid fishes with respect to seasons.

species is considered individually with respect to seasons. For instance, isopods, J. brachysoma and A. malayi exhibit significant variation in intensity (J. brachysoma, F= 7.327, P= 0.02452; A. malayi F= 18.74, P= 0.002627) which is more predominant between premonsoon & monsoon and monsoon & post-monsoon (Table 5).

Discussion

Clupeid fishes from the Malabar coast appear to be potential host for both isopods and copepods. The white sardine, Escualosa thoracata hosts Joryma brachysoma and Tenualosa toli hosts Anilocra leptosoma and Agarna malayi. Among the recovered isopods, J. brachysoma shows highest prevalence (P= 26.11+ 0.073) and intensity (I=1.59 + 0.07). Infection by J. brachysoma has also been reported from the Indian fishes such as Rastrelliger kanagurta, Ilisha melastoma, Pellona brachysoma (Ravichandran & Ajith Kumar, 2008; Ravichandran, Rameshkumar & Kumaravel, 2009; Rameshkumar, Ravichandran & Trilles, 2011). According to the recent report (Panakkool-Thamban et al., 2013), E. thoracata from the Malabar coast is also under the heavy infection with another isopod Nerocila loveni. Panakkool-Thamban et al., (2016) previously reported the presence of parasitic isopod A. malayi on the same host, T. toli with a prevalence of 16.19 and an intensity of 1.89 which is akin to the data (P= 14.38±1.34

and I= 1.31±0.15) obtained from the present study. Among the recovered isopods, *Anilocra leptosoma* shows least prevalence and intensity throughout the study period.

The gizzard shad, A. chacunda is potential to host three different species of copepods (P. fistula fistula, P. varunae and N. cygniformis), but not simultaneously; at a time fish hosts maximum of two species and most instances the combination is with P. varunae and P. fistula fistula. P. varunae shows maximum prevalence (P= 20.29 + 3.03), but their intensity is relatively less comparing to P. fistula fistula. Reports are available on P. fistula fistula infecting the Corvphaenid (Order Perciformes) fish, Coryphaena hippurus from the Aegean Sea coastal waters of Turkey (Öktener, (2008). Vidjak, Zorica, and Sinovčić (2008) reported that garfish, Belone belone (in the eastern Adriatic Sea) infected with Р fistula fistula. Bunkley-Williams and Williams Jr. (2009) listed 34 host fishes in 2 super orders, 6 orders, and 19 families of fishes for this parasite (P. fistula fistula) signifying their non host specific parasitisation. Among the presently recovered parasitic copepods N. cygniformis shows least prevalence. Presence of this parasite has also been reported from the cow-eyed sparid fish, Boops boops in the southern and northern mediterranean (Ramdane, Trilles, Mahe, & Amara, 2013).

During the present study, *S. fimbriata* is shown to have the parasitisation with only the copepod *C. hilsae*.

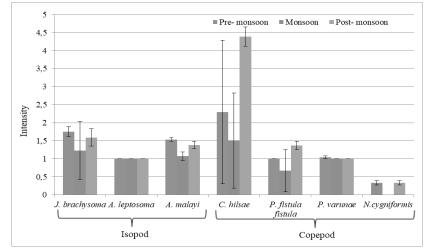


Figure 7. Seasonal variation in intensity (Mean± SD) in the infestation of parasitic isopods and copepods with respect to seasons.

Table 5. Significance of variation in intensity of individual parasitic crustacean with different seasons- *P* value, F value and Tukey value. (Significant *P* value is given in bold)

SI No	Name of the parasite	F value	P value	Seasons	Pre-monsoon	Monsoon	Post-monsoon
				Pre-monsoon		5.28	1.637
1	J. brachysoma	7.327	0.02452	Monsoon	0.023		0.09
				Post-monsoon	0.517	3.65	
				Pre-monsoon		8.45	2.57
2	A. malayi	18.74	0.002627	Monsoon	0.0025		0.0142
				Post-monsoon	0.24	5.87	

But according to the previous reports, this clupeid fish (*S. fimbriata*) distributed at the Malabar coast and Karachi (Pakistan) also harbours another copepod, *Pumiliopes squamosus* (Aneesh, 2014) and an isopod, *Joryma sawayah* (Ghani, 2003).

Parasitic crustaceans usually exhibit strict site specificity apparently for avoiding the inter-parasitic competition for space and food (Rijin & Sudha, 2017). In present study, all the members of J. brachysoma and A. malayi were found specifically attached the branchial chamber of their respective host fish, E. thoracata and T.toli. On the other hand, A. leptosoma prefers the body surface of its host fish, T. toli. All presently recovered copepod parasites also occupy their own niche on their host body; C. hilsae prefers gill for infection, but their microhabitats are restricted to anterior gill rakers and gill filaments. Other microhabitats for parasitic copepods inculde anterio-posterior axis of the gill, gill arches and external/ internal gill filament. (Rijin & Sudha, 2017). The direction and speed of ventilation, water-currents and certain intrinsic factors of the parasite themselves may determine their microhabitat restriction on the gills (Ramasamy, Ramalingam, Hanna & Halton, 1985).

Present study reveals that prevalence and intensity of recovered parasitic isopods and copepods vary with seasons. In the case of isopods, invariably, pre-monsoon (Feb- May) is the period of maximum prevalence and by the onset of the monsoon season, (June- Sept) the prevalence generally showed a downward trend, however, during the post- monsoon season (Oct- Jan) the prevalence gradually increased. This observation supports the previous reports in four species of parasitic isopod representing the genus Nerocila infecting commercially exploited marine fishes of the families Engraulidae, Clupeidae and Ambassidae, from the Malabar coast of India (Panakkool-Thamban et al., 2013). The prevalence and intensity shown by J. brachysoma on R. kanagurta showed considerable monthly variation with maximum in January and minimum in July (Ravichandran et al., 2009). The prevalence could be dependent on environmental parameters like rainfall, salinity and temperature. Low prevalence during the monsoon period is apparently due to the weak salinity (27-29 ppt), resulting from the heavy rain fall, inducing an unfavorable environment for the parasitic infection while the gradual increase of salinity (30-35 ppt) during the post- monsoon season seems to facilitate the parasitic infestation (Panakkool-Thamban et al., 2013). Present study also supports this information.

Conclusion

Present study could demonstrate the diverse pattern of seasonal variation in the prevalence and intensity of parasitic isopods and copepods infection on the Clupeidaen fishes along the Malabar coast using

statistical tools; all three recovered isopod and one copepod species are with their highest prevalence during pre- monsoon period and least in monsoon period. But, post- monsoon season is the favorable period for infection by the remaining three recovered copepod species. This signifies the role apparently played by environmental factors like salinity, temperature and rainfall in the pattern of parasitisation by the isopod and copepods on clupeidaen fishes. So the future research may be focused in such way to demonstrate the role of extrinsic factors bv considering primarily the parameters like salinity, rainfall and temperature, the gathered information would be useful to adopt the strategies for the management of crustacean parasitism in culture fisheries.

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