

Simultaneous Multiple Parasitic Crustacean Infestation on Banded Needlefish, *Strongylura leiura* (Belonidae) from the Malabar Coast, India

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Abstract- The simultaneous multiple infestation by parasitic crustacean species involving a cymothoid isopod, *Mothocya renardi* (Bleeker 1857) and three copepods such as *Lernanthropus tylosuri* Richiardi 1880, *Caligodes lacinatus* Heller 1868 and *Bomolochus bellones* Burmeister 1833 was frequently observed on Banded needlefish, *Strongylura leiura* (Belonidae) captured from the Malabar Coast. 130 of 132 fish specimens (*S. leiura*) collected from January 2011 to February 2012, were hyperinfested by 698 parasitic crustaceans including 226 *M. renardi*, 262 *L. tylosuri*, 158 *C. lacinatus* and 52 *B. bellones*. Out of 132 host fishes (*S. leiura*) examined, four members showed a quadruple parasitism involving *M. renardi*, *L. tylosuri*, *C. lacinatus* and *B. bellones*. 87 host fishes showed triple parasitism and 38 others a double parasitism, with different parasitic associations. In triple parasitism 4 and in double parasitism 5 possible combinations were noticed. Two fish were parasitized only by *M. renardi*. The sites of infestation by the parasitic crustaceans are highly specific.

Index Terms- Isopod, Cymothoid, Copepod, Infestation, Prevalence, Quadruple parasitism.

I. INTRODUCTION

Parasitic crustaceans infesting fishes invite attention of researchers world over inasmuch as it could adversely affect the health of food fishes (Brusca, 1981; Johnson *et al.*, 1996, 2004; Williams and Williams, 1998; Izawa and Choi, 2000; Ho *et al.*, 2000; Karlsbakk *et al.*, 2001; Ravichandran, 2007; Hadfield *et al.*, 2010, 2011; Trilles *et al.*, 2011, 2012, 2013; Aneesh *et al.*, 2013). Most of the parasitic crustaceans belong to Isopoda, Branchiura and Copepoda (Margolis *et al.*, 1975; Oktener and Sezgin, 2000). Cymothoidae are oligoxenous isopods and often induce deleterious effects on the host (Overstreet, 1978; Kabata, 1985; Trilles and Jacquotte, 1996, 2012; Aneesh *et al.*, 2013). Significant proportion of the parasitic copepods is known parasitizing fishes (Kabata, 1979; Love and Moser, 1983; Hogans and Dadswell, 1985; Pillai 1985; Benz, 1986; Oldewage and Smale, 1993; Benz *et al.*, 2003; Cheng *et al.*, 2009, 2011; Ho *et al.*, 2010; Rashidy and Boxshall, 2010, 2012). Indian fishes have been shown to possess high rate of susceptibility for parasitization by isopods and copepods as well (Pillai, 1985; Aneesh *et al.*, 2010, 2012, 2013; Trilles *et al.*, 2011, 2012, 2013; Helna *et al.*, 2012).

Reports are scanty on the simultaneous occurrence of multiple parasitism involving exclusively parasitic crustaceans. Daniel and Rao (1967) and Daniel and Premkumar (1967) reported the simultaneous infestation of flying fish (*Cypselurus speculiger*) by a copepod *Pennella* sp. and the cirriped *Conchoderma virgatum*. Hewitt (1979) and Benz *et al.*, (2003) observed the multiple infestation of Pacific white shark (*Carcharodon carcharias*) by 5-8 different siphonostomatoid (copepod) species. In India, incidence of double parasitism involving the isopod *Nerocila phaiopleura* and the copepod *Lernaeenicus sprattae* was reported in anchovy fish *Stolephorus commersonii* (Rajkumar *et al.*, 2006). Another Indian fish (*Hemiramphus far*) also showed simultaneously infestation by the isopod *Mothocya plagulophora* and the copepod *Lernaeenicus hemiramphi* (Gopalakrishnan *et al.*, 2010).

The present study indicates the frequent and year round occurrence of double and triple parasitism and also the few incidence of quadruple parasitism exclusively by the species of parasitic crustaceans including isopods and copepods on the host fish, *Strongylura leiura* (Belonidae) distributed along the Malabar Coast (Kerala, India).

II. MATERIALS AND METHODS

The present study was conducted from January 2011 to February 2012. Living or fresh host fishes (*Strongylura leiura*) were collected from the Ayyikkara fish landing center (Lat. 11°51'N, Long. 75°22'E; Malabar Coast, Kerala, India). As soon as they were collected and transferred to the laboratory, various parts of the fish body (the body surface, the lateral line region, the base of the pectoral fin, the branchial cavity and gill filaments, the beak, the inner wall of the operculum, etc) were carefully examined for the presence of parasitic crustaceans using hand lens. Recovered parasitic crustaceans were removed from the host and preserved in 70% ethanol for further detailed examination. The identification was performed, using a dissecting microscope and a stereo microscope Leica-S6D, according to Pillai, (1985) and Bruce (1986). The prevalence (P) was calculated according to Margolis *et al.*, (1982) and Bush *et al.*, (1997). The seasonal fluctuations of the prevalence, if any, were assessed through the *P*-values calculated by Turkey-Kramer multiple comparisons test or through Student's *t*-test, using InStat Software (Graphpad InStat, Version 2.00, 2007). Host nomenclature and fish taxonomy are according to Fish Base (Froese and Pauly, 2013).

Voucher specimens of all parasites were collected by Aneesh, Helna and Sudha from Ayyikkara fish landing centre (Lat. 11°51'N, Long. 75°22'E), Malabar Coast, Kerala, India and deposited in the Parasitic Crustacean Museum, Crustacean Biology Research Laboratory, Sree Narayana College, Kannur, Kerala, India. Abbreviations used: PCM-Parasitic Crustacean Museum, Crustacean Biology Research Laboratory, Sree Narayana College, Kannur, Kerala, India; LT - Total length; OgF - Oviparous female; BP- Brood pouch; ES - Egg sac.

Mothocya renardi (Bleeker 1857): All from *Strongylura leiura*, Ayyikkara fish landing centre (Lat. 11°51'N, Long. 75°22'E), Malabar Coast (Kerala, India); collected by Aneesh, Helna and Sudha and deposited in PCM. Transitional stage (LT, 17 mm) (PCM N° MR -10), 13 March 2011; OgF releasing manca larvae (LT, 21 mm) possessed only 2-5 manca larvae in the BP (PCM N° MR-11), 23 March 2011; OgF (LT, 24 mm) possessed pullus larvae in the BP (PCM N° MR-12), 23 March 2011; Transitional stage (LT, 23 mm) (PCM N° MR -17), 21 May 2011; Male (LT, 14 mm) (PCM N° MR- 23), 14 January 2012.

Lernanthropus tylosuri Richiardi, 1880: All from *Strongylura leiura*, Ayyikkara fish landing centre (Lat. 11°51'N, Long. 75°22'E), Malabar Coast (Kerala, India); collected by Aneesh, Helna and Sudha and deposited in PCM. OgF (LT, 7.8 mm) with ES (PCM N° Lt-06), 13 March 2011; OgF (LT, 4.6 mm) without ES (PCM N° Lt-11), 23 March 2011; Male (LT, 1.6 mm) detached from the specimen PCM N° Lt -11 (PCM N° Lt -12), 23 March 2011; OgF (LT, 5.5 mm) without ES and a male (LT, 1.8 mm) clinging on OgF (PCM N° Lt -13), 23 March 2011

Caligodes lacinatus Heller 1868: All from *Strongylura leiura*, Ayyikkara fish landing centre (Lat. 11°51'N, Long. 75°22'E) Malabar Coast (Kerala, India); collected by Aneesh, Helna and Sudha and deposited in PCM. OgF (LT, 9.6 mm) with ES (PCM N° Cl-02), 13 March 2011; OgF (LT, 6 mm) without ES (PCM N° Cl -03), 23 March 2011.

Bomolochus bellones Burmeister 1833: All from *Strongylura leiura*, Ayyikkara fish landing centre (Lat. 11°51'N, Long. 75°22'E) Malabar Coast (Kerala, India); collected by Aneesh, Helna and Sudha and deposited in PCM. OgF (LT, 1.8 mm) with ES (PCM N° Bb-02), 13 March 2011; OgF (LT, 1.4 mm) without ES (PCM N° Bb -03), 23 March 2011.

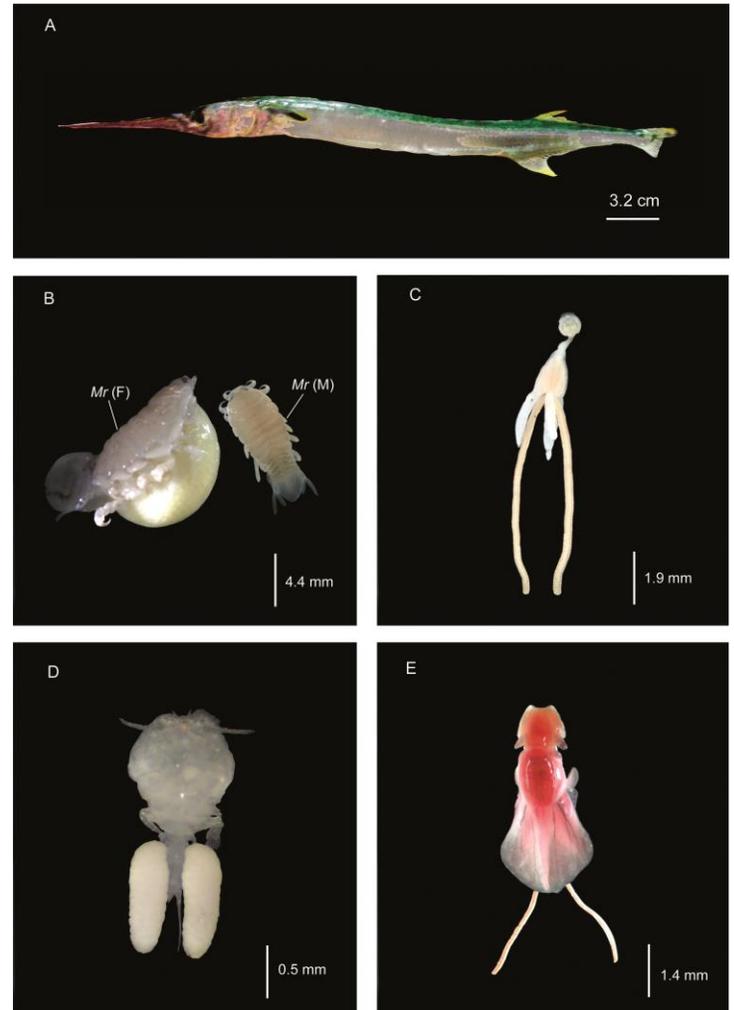
III. RESULTS

132 *Strongylura leiura* (Figure 1 A) collected from January 2011 to February 2012, were found to be simultaneously infested by maximum four different species of parasitic crustaceans (Table I). Recovered parasitic crustaceans include a cymothoid isopod, *Mothocya renardi* (Bleeker 1857) and three copepods, *Caligodes lacinatus* Heller 1868, *Lernanthropus tylosuri* Richard 1880 and *Bomolochus bellones* Burmeister 1835 (Tables I - III; Figures 1 B - E).

Among the recovered parasitic crustacean species, *Mothocya renardi* exhibits the highest prevalence (P = 93.18%) (Table II; Figure 2) by infesting 123 (out of 132) host fishes (*S. leiura*); the intensity being equal to 1.71 (Table II). Of 226 members of *M. renardi*, there were 103 males and 107 females and the remaining 16 were under the transitional phase. 206 members of this cymothoid parasite were appeared in pairs

with two different combinations such as male - female (89 +89 =178) and male - transitional stage (14 +14=28) during their infestation on the fish (*S. leiura*); invariably, member in the pair being settled the floor of either of the host fish branchial cavity. The remaining unpaired 20 (226-206) members of *M. renardi*, including 18 females and 2 transitional stages were also found to be settled the host branchial cavity. The large sized females usually orient their cephalon towards the host mouth.

Figure 1: A – host fish *Strongylura leiura*; B – *M. renardi* - male and female; C - *C. lacinatus* - female; D - *B. bellones* - female; E - *L. tylosuri* - female



(Legends: Mr (M) - *M. renardi*, male; Mr (F) - *M. renardi*, female)

91 of the 132 *S. leiura* examined were also infested by 262 members of the copepod *Lernanthropus tylosuri* (236 females and 26 males). The prevalence and intensity reach 68.9 % and 2.88 respectively (Table II; Figure 2). All females are reproductive active evidenced by the presence growing ovaries and/or egg sacs. The recovered males were not independent but found to be in a clinging/ copulatory position attaching the genital segment of the females with their maxilliped. *L. tylosuri* shows strict site specificity by infesting only the gill filament of the host.

Table I: Showing data on the multiple parasitism on *Strongylura leiura* by parasitic crustaceans

Month	Host fish series	Infestation of <i>S. leiura</i> by Parasitic Crustaceans								Remarks
		Isopod		Copepods						
		<i>Mothocya renardi</i>		<i>Lernanthropus tylosuri</i>		<i>Caligodes lacinatus</i>		<i>Bomolochus bellones</i>		
		Female	Male	Female	Male	Female	Male	Female	Male	
January 2011	1	1	1	2	-	-	-	-	-	Double
	2	1	1	3	-	2	-	-	-	Triple
	3	1	1	2	-	-	-	1	-	Triple
	4	1	1	-	-	1	-	-	-	Double
	5	1	1	-	-	2	-	-	-	Double
	6	1	1	2	-	-	-	2	-	Triple
	7	-	-	4	-	1	-	-	-	Double
	8	1*	1	-	-	-	-	-	-	Single
	9	1	1	2	-	2	-	-	-	Triple
	10	1	1	2	-	1	-	-	-	Triple
	11	1	1	3	-	-	-	-	-	Double
	12	1	1	2	-	-	-	-	-	Double
	13	1	1	2	-	-	-	1	-	Triple
	14	1	1	2	-	2	-	-	-	Triple
February 2011	15	1	1	2	-	2	-	-	-	Triple
	16	1	1	3	-	2	-	-	-	Triple
	17	1*	1	-	-	2	-	-	-	Double
	18	1	1	-	-	1	-	1	-	Triple
	19	1	1	2	-	-	-	2	-	Triple
	20	1	1	-	-	-	-	1	-	Double
	21	1	-	3	-	-	-	1	-	Triple
	22	1	-	2	-	2	-	-	-	Triple
March 2011	23	1	1	3	-	1	-	-	-	Triple
	24	1*	1	2	-	1	-	-	-	Triple
	25	1	-	-	-	1	-	-	-	Double
	26	1*	1	-	-	2	-	1	-	Triple
	27	1	1	-	-	1	-	1	-	Triple
April 2011	28	1	1	-	-	2	-	1	-	Triple
	29	1	1	3	-	1	-	-	-	Triple
	30	1	1	3	-	-	-	-	-	Double
May 2011	31	1	1	2	-	-	-	1	-	Triple
	32	1	1	3	-	2	-	-	-	Triple
	33	1	1	2	-	2	-	-	-	Triple
	34	1	1	-	-	1	-	-	-	Double
	35	1	1	-	-	-	-	1	-	Double
	36	1*	-	2	-	2	-	-	-	Triple
	37	1	1	2	-	-	-	-	-	Double
	38	1	1	3	-	2	-	1	-	Multiple
	39	-	-	3	-	-	-	1	-	Double
	40	1	1	2	-	1	-	-	-	Triple
June 2011	41	1	1	3	-	-	-	1	-	Triple
	42	1	1	-	-	2	-	1	-	Triple
	43	1	1	2	-	1	-	-	-	Triple
	44	1	1	3	-	-	-	-	-	Double
July 2011	45	1	1	3	-	1	-	-	-	Triple
	46	1	-	-	-	2	-	1	-	Triple
	47	1	1	2	-	2	-	-	-	Triple
	48	1	1	2	-	2	-	-	-	Triple
	49	1	1	-	-	2	-	-	-	Double
	50	1	1	3	-	-	-	1	-	Triple
August 2011	51	1	1	3	-	1	-	-	-	Triple
	52	1	1	-	-	1	-	1	-	Triple
	53	1	1	3	-	1	-	-	-	Triple
	54	1*	1	-	-	2	-	1	-	Triple
	55	1*	1	2	-	2	-	-	-	Triple
	56	1	1	1	-	-	-	1	-	Triple
	57	1	1	2	-	1	-	-	-	Triple
	58	1	1	2	-	-	-	-	-	Double
	59	1	1	2	-	-	-	2	-	Triple
	60	1	1	-	-	1	-	-	-	Double
	61	-	-	3	-	2	-	-	-	Double
	62	1	1	2	-	1	-	-	-	Triple
	63	1	1	2	-	-	-	1	-	Triple

September 2011	64	1	1	3	-	1	-	-	-	Triple
	65	1	1	3	-	2	-	-	-	Triple
	66	1	-	-	-	2	-	-	-	Double
	67	1	-	3	-	1	-	1	-	Multiple
	68	1	1	4	-	2	-	-	-	Triple
	69	1	1	-	-	1	-	-	-	Double
	70	1	1	3	-	-	-	-	-	Double
	71	1	1	2	-	1	-	-	-	Triple
	72	-	-	3	-	-	-	2	-	Double
	73	-	-	2	-	2	-	-	-	Double
	74	1	1	-	-	2	-	1	-	Triple
	75	1	1	2	-	2	-	1	-	Multiple
	76	1	-	3	-	1	-	-	-	Triple
	77	1	1	2	-	1	-	-	-	Triple
78	1	1	3	-	1	-	-	-	Triple	
October 2011	79	1	1	1	-	2	-	-	-	Triple
	80	-	-	3	-	1	-	-	-	Double
	81	1	1	-	-	2	-	-	-	Double
	82	1	1	-	-	2	-	1	-	Triple
	83	1	-	4	-	-	-	1	-	Triple
	84	1	1	-	-	1	-	1	-	Triple
	85	1	-	2	-	1	-	-	-	Triple
	86	1	-	3	-	2	-	-	-	Triple
	87	1	1	2	-	2	-	-	-	Triple
	88	1	1	3	-	-	-	-	-	Double
	89	1	1	-	-	1	-	2	-	Triple
November 2011	90	1	1	2	-	-	-	-	-	Double
	91	1	-	2	-	-	-	-	-	Double
	92	1	1	-	-	2	-	1	-	Triple
	93	-	-	2	-	2	-	1	-	Triple
	94	-	-	3	-	1	-	-	-	Double
	95	1	1	-	-	2	-	-	-	Double
	96	1*	1	3	1	2	-	-	-	Triple
	97	1	-	2	1	1	-	-	-	Triple
	98	1	-	3	1	-	-	1	-	Triple
	99	1	1	2	-	-	-	1	-	Triple
	100	1*	1	4	2	2	-	-	-	Triple
	101	1	1	-	-	2	-	1	-	Triple
	102	1	1	-	-	2	-	1	-	Triple
	103	1	1	4	4	-	-	-	-	Double
	104	1	-	-	-	2	-	1	-	Triple
	105	1*	1	-	-	2	-	-	-	Double
	106	1*	1	4	2	1	-	-	-	Triple
107	1	1	4	-	2	-	-	-	Triple	
December 2011	108	1	-	1	-	2	-	-	-	Triple
	109	1	1	2	1	2	-	-	-	Triple
	110	1	1	4	2	2	-	-	-	Triple
	111	1*	1	3	-	1	-	-	-	Triple
	112	1	1	-	-	2	-	2	-	Triple
	113	1	1	-	-	1	-	1	-	Triple
	114	1	1	-	-	2	-	-	-	Double
	115	1*	1	4	3	-	-	-	-	Double
	116	1	1	-	-	-	-	-	-	Single
	117	1	1	-	-	2	-	-	-	Double
January 2012	118	1	1	1	-	-	-	1	-	Triple
	119	1	-	-	-	2	-	1	-	Triple
	120	1	-	3	2	2	-	-	-	Triple
	121	1	1	4	2	2	-	-	-	Triple
	122	1*	1	3	-	1	-	-	-	Triple
February 2012	123	1	-	-	-	2	-	2	-	Triple
	124	1*	-	2	-	2	-	-	-	Triple
	125	1	1	-	-	2	-	-	-	Double
	126	1	1	2	-	2	-	-	-	Triple
	127	1*	1	3	-	1	-	-	-	Triple
	128	1	1	-	-	2	-	1	-	Triple
	129	1	1	4	3	2	-	-	-	Triple
	130	-	-	3	2	2	-	-	-	Double
	131	1	1	-	-	1	-	-	-	Double
	132	1	1	3	-	2	-	1	-	Multiple
		123	103	236	26	158	0	52	0	Single - 2 Double- 39 Triple - 87 Multiple- 4
		123(226) * Transitional stage = 16		91(262)		98(158)		45(52)		

Table II: Parasitological index of the parasitic crustaceans on *Strongylura leiura* under multiple parasitism

Parasites	NFO	NFI	P	NPR	I	Site of infestation
<i>Mothocya renardi</i> Bleeker 1857	132	123	93.18	226	1.71	floor of the branchial cavity, the cephalon of the parasite usually facing towards the host mouth
<i>Lernanthropus tylosuri</i> Richard 1880	132	91	68.9	262	2.88	on the gill filament
<i>Caligodes lacinatus</i> Heller 1868	132	98	74.2	158	1.61	penetrating the fleshy part of the lower beak
<i>Bomolochus bellones</i> Burmeister 1835	132	45	34.1	52	1.16	attached on the inner side of the operculum

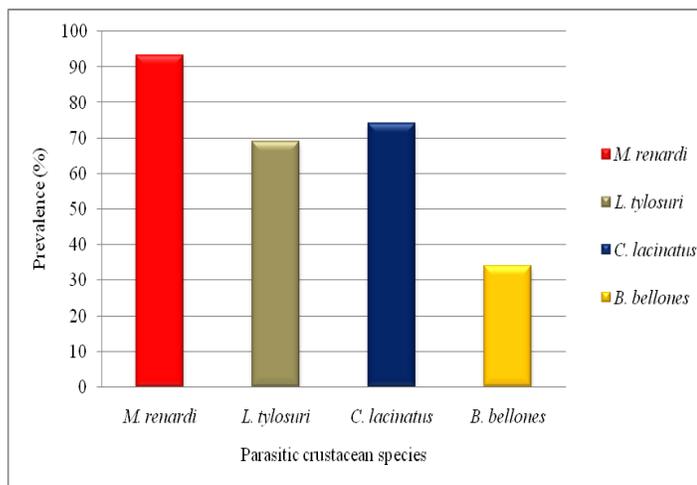
(P – Prevalence; NFO- Number of fishes examined; NFI- Number of fishes infested; NPR: Number of parasite recovered; I: Intensity)

Table III: Showing parasitic combinations in different levels of multiple parasitism

NFO	Single Parasitism	Double Parasitism					Triple Parasitism				Quadruple Parasitism
	M	ML	MC	LC	MB	LB	MLC	MLB	MCB	LCB	MLCB
132	Total instances of single parasitism - 2	13	16	6	2	2	50	15	21	1	Total instances of quadruple parasitism - 4
		Total instances of double parasitism - 39					Total instances of triple parasitism - 87				
	Total instances of multiple parasitism (double, triple and quadruple) - 130 Total : 132										

(NFO- Number of examined fishes; MLC - *M. renardi*, *L. tylosuri* and *C. lacinatus*; MLB - *M. renardi*, *L. tylosuri* and *B. bellones*; MCB - *M. renardi*, *C. lacinatus* and *B. bellones*; LCB - *L. tylosuri*, *C. lacinatus* and *B. bellones*; ML - *M. renardi* and *L. tylosuri*; MC - *M. renardi* and *C. lacinatus*; LC - *L. tylosuri* and *C. lacinatus*; MB - *M. renardi* and *B. bellones* and LB - *L. tylosuri* and *B. bellones*)

Figure 2: Occurrence of parasitic crustaceans (*Mothocya renardi*, *Lernanthropus tylosuri*, *Caligodes lacinatus* and *Bomolochus bellones*) parasitizing the fish *Strongylura leiura* during the period from January 2011 to February 2012



The copepod species *Caligodes lacinatus* was collected from 98 out of the 132 *S. leiura* examined; the prevalence reached 74.2%. A total of 158 parasites were recovered from the infested host fishes, the intensity being equal

to 1.61 (Table II; Figure 2). All the recovered members of this parasite were exclusively females carrying growing ovaries and egg sacs as well. *C. lacinatus* was found to penetrate the fleshy part of the lower beak of the host fish; swelling and tissue damages were frequently observed at the penetration site.

Bomolochus bellones showed the lowest prevalence (34.1%), only 45 of the 132 *S. leiura* examined being infested; 52 parasites were collected from the infested host fishes, the intensity being equal to 1.16 (Table II; Figure 2). All specimens were females and reproductively active possessing egg sacs or maturing ovaries. The inner side of the operculum forms the specific site for the attachment of this species.

Interestingly, the host fish (*S. leiura*) was under frequent and simultaneous multi infestation (quadruple/triple/double) by these four parasitic crustaceans (*M. renardi*, *C. lacinatus*, *L. tylosuri* and *B. bellones*) throughout the study period (January 2011 to February 2012) (Figure 3). In order to check the seasonal fluctuation, if any, in the prevalence of parasitisation by these four parasitic crustaceans on the host fish, the data (on Prevalence) was grouped according to Summer/ Pre-monsoon (January-May), Monsoon (June-August), Post-monsoon (September-December) seasons and a statistical comparison was made. None of the recovered parasitic crustaceans showed any statistically significant seasonal-wise fluctuation in their prevalence (Table IV).

Figure 3: Monthly occurrence of multiple parasitism on the fish *Strongylura leiura*

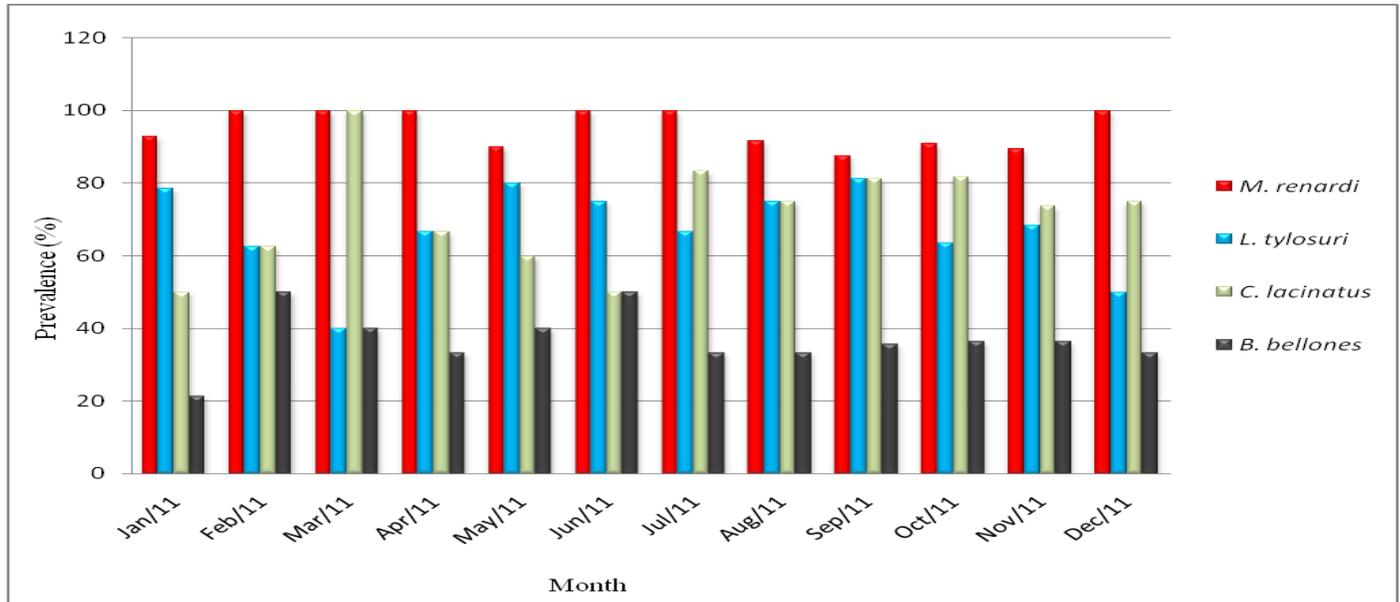


Table IV: Statistical analysis of seasonal occurrence of parasitic crustaceans (*Mothocya renardi*, *Lernanthropus tylosuri*, *Caligodes lacinatus* and *Bomolochus bellones*) if any parasitizing the fish *Strongylura leiura* during the period from January 2011 to February 2012

Parasites	Summer/ Pre-monsoon (January -May) (A)	Monsoon (June -August) (B)	Post-monsoon (September-December) (C)
<i>Mothocya renardi</i>	97.5 % ±2.5 (12)	97.22 % ± 2.78 (8)	92.154 % ±2.151 (12)
<i>Lernanthropus tylosuri</i>	62.29 % ±8.315 (12)	72.22 % ± 2.780 (8)	68.38 % ±5.615 (12)
<i>Caligodes lacinatus</i>	72.29 % ±9.338 (12)	69.443 % ±10.015 (8)	72.348 % ±2.5 (12)
<i>Bomolochus bellones</i>	97.5 % ±2.5 (12)	97.5 % ±2.5 (8)	97.5 % ±2.5 (12)

(*M. renardi* - (A=B (NS) ($t = 0.07441$, $P = 0.471$); A=C (NS) ($t = 1.631$, $P = 0.735$); B=C (NS) ($t = 1.442$, $P = 0.0997$); *L. tylosuri* -(A=B (NS) ($t = 0.982$, $P = 0.1855$); A=C (NS) ($t = 0.6286$, $P = 0.2748$); B=C (NS) ($t = 0.495$, $p = 0.319$); *C. lacinatus* -(A=B (NS) ($t = 0.205$, $P = 0.423$); A=C (NS) ($t = 0.006$, $P = 0.498$); B=C (NS) ($t = 0.272$, $P = 0.397$); *B. bellones* -(A=B (NS) ($t = 0.315$, $P = 0.383$); A=C (NS) ($t = 1.851$, $P = 0.053$); B=C(NS) ($t = 1.123$, $P = 0.152$); NS – Not significant)

A. Quadruple parasitism

Interestingly, 4 of the 132 examined host fish showed a quadruple parasitism, being infested by the four species of parasitic crustaceans identified in this study, but only during February, May and September (Tables I and III; Figure 4).

B. Triple parasitism

87 (out of 132; 65.9%) *S. leiura* were simultaneously infested by three parasitic crustacean species, with four possible combinations (Tables 1 and 3): 1) *M. renardi*, *L. tylosuri* and *C. lacinatus* (MLC), 2) *M. renardi*, *L. tylosuri* and *B. bellones* (MLB), 3) *M. renardi*, *C. lacinatus* and *B. bellones* (MCB) and 4) *L. tylosuri*, *C. lacinatus* and *B. bellones*(LCB). The rates of these combinations were 57.6% (MLC), 24.1% (MCB), 17.2% (MLB) and 1.1 % (LCB) respectively (Table III; Figure 4 and 5). Interestingly, the triple parasitism was observed throughout the study period (Table I).

Figure 4: Instances of different levels of single, double, triple and quadruple crustacean parasitism on the fish *Strongylura leiura* during the period from January 2011 to February 2012

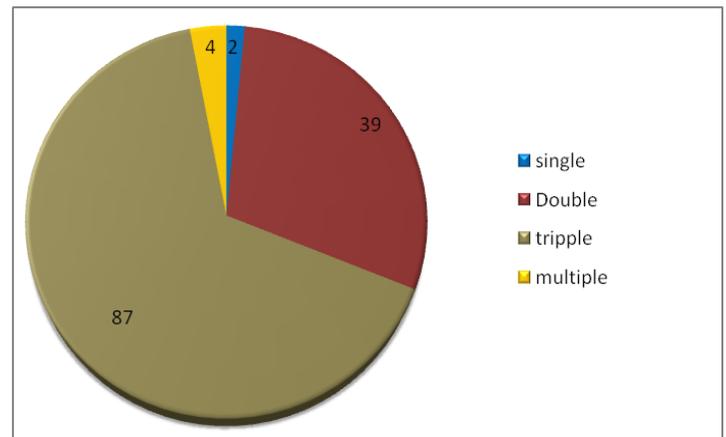
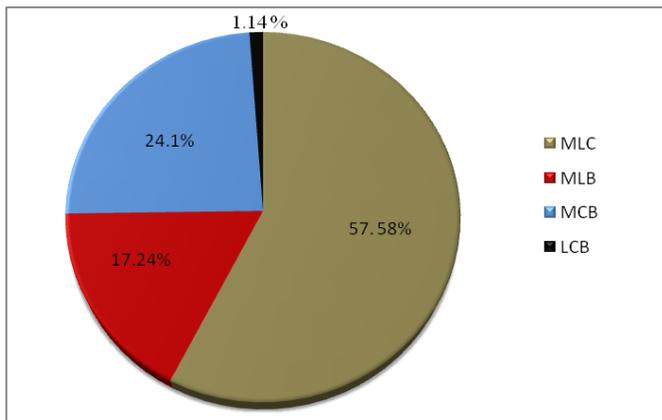


Figure : 5 The percentage of the different combinations in triple parasitism on the fish *Strongylura leiura* during the period from January 2011 to February 2012

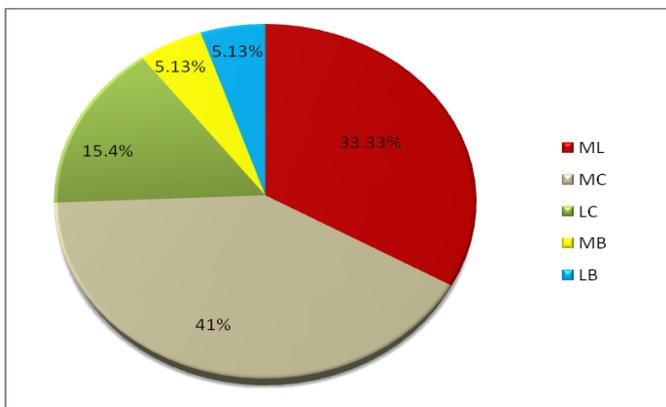


(Legends: MLC - *M. renardi*, *L. tylosuri* and *C. lacinatus*; MLB - *M. renardi*, *L. tylosuri* and *B. bellones*; MCB - *M. renardi*, *C. lacinatus* and *B. bellones* and LCB - *L. tylosuri*, *C. lacinatus* and *B. bellones*)

C. Double parasitism

The occurrence of double parasitism by simultaneous infestation with two parasitic crustacean species was found in 39 (out of 132; 29.5%) members of host fishes (*S. leiura*). Five possible combinations of double parasitism were observed during the present study period 1) *M. renardi* and *L. tylosuri* (ML); 2) *M. renardi* and *C. lacinatus* (MC); 3) *L. tylosuri* and *C. lacinatus* (LC); 4) *M. renardi* and *B. bellones* (MB) and 5) *L. tylosuri* and *B. bellones* (LB). MC and ML combinations were found to be significantly high amounting 41% and 33.3% respectively. LC reached 15.4%; MB and LB combinations were lowest, only 5.13% each (Tables I and III; Figures 4 and 6). The incidence of double parasitism was noticed throughout the year (Table I).

Figure 6: The percentage of different combinations in double parasitism on the fish *Strongylura leiura* during the period from January 2011 to February 2012



(Legends: ML - *M. renardi* and *L. tylosuri*; MC - *M. renardi* and *C. lacinatus*; LC - *L. tylosuri* and *C. lacinatus*; MB - *M. renardi* and *B. bellones* and LB - *L. tylosuri* and *B. bellones*)

D. Single parasitism

Unlike triple and double parasitism noticed in the present host fish (*S. leiura*), infestation with only one species of parasitic crustaceans (single parasitism) was seldom during the present study period; only two fish members (out of 132; 1.5%) showed single parasitism with *M. renardi*, one in January other in December (Table 1; Fig. 4).

IV. DISCUSSION

The present study reveals that the fish *S. leiura* is a potential host for four parasitic crustacean species. 698 parasitic crustaceans including 226 cymothoid isopod (*Mothocya renardi*) and 472 copepods (262 *Lernanthropus tylosuri*, 158 *Caligodes lacinatus*, and 52 *Bomolochus bellones*) were collected from 132 examined fish, *S. leiura*. The prevalence of *M. renardi* was highest (P = 93.18%) throughout the study period. The parasitic copepod *C. lacinatus* was collected from 98 host fishes, its prevalence being 74.2%. *L. tylosuri* parasitized 91 *S. leiura* (prevalence = 68.9%). The prevalence of *B. bellones*, infesting only 45 of 132 examined fishes, was lowest (34.1%). The mean intensity vary according to the parasitic species. The highest intensity was observed in *L. tylosuri* (I=2.6), *M. renardi* and *C. lacinatus* have an intensity reaching 1.71 and 1.61 respectively. The lowest intensity was observed in *B. bellones* (I=1.16).

These four parasitic crustaceans showed specific attachment sites apparently for avoiding the inter-parasitic competition for space and food. The isopod *M. renardi* and the copepod *L. tylosuri*, the blood feeding parasites prefer buccal cavity and gill filament respectively for their infestation site. On the otherhand, *C. lacinatus* penetrates the tissue lining of the lower beak and *B. bellones* clings the operculum to facilitate tissue feeding.

In the present study, all the collected parasitic copepods belonging to the species *C. lacinatus* (158) and *B. bellones* (52) were invariably mature females carrying egg sacs. No single instance of parasitization was noticed by male members of these copepod species (both *C. lacinatus* and *B. bellones*) apparently due to the existence of sex specific parasitization. Further, among the 262 members of recovered copepod species of *L. tylosuri*, 26 were males, but they were not found attaching the gill filaments of the host fish, but clinging the genital segments of the parasitic females, apparently in copulation process. Reports show that only reproductively mature female copepods are parasitic and the males dying after the copulation (Jithendran *et al.*, 2008).

Notwithstanding few reports, our knowledge on the occurrence of multiple parasitism involving parasitic crustaceans is very poor. The frequent occurrence of double and triple parasitism and few instance of quadruple parasitism by parasitic crustaceans noticed (in the present study) on the fish *S. leiura* invites more observation on the subject. The multiple (double and triple) parasitic infestations found in *S. leiura* throughout the year revealed that it is not an accidental incident. Further, the prevalence was more or less uniform without showing any statistically significant fluctuation throughout the study period suggesting that it (prevalence) is not apparently dependent on seasonal environmental parameters such as the rainfall, salinity and temperature.

Few reports are available on the double parasitism involving crustaceans. The occurrence of double parasitism with a copepod *Pennella* sp. and a cirriped *C. virgatum* was reported from the flying fish *C. speculiger* (Daniel and Premkumar, 1967). The Commerson's anchovy fish *S. commersonii* from the Parangipettai (India) coastal waters was found simultaneously infested by the cymothoid isopod *N. phaiopleura* and the copepod *L. sprattae* (Rajkumar *et al.*, 2006). Double parasitism with the isopod *M. plagulophora* and the copepod *L. hemiramphi* was noticed in *Hemiramphus far* (Gopalakrishnan *et al.*, 2010). Daniel and Rao, (1967) reported the parasitization of the flying fish by an isopod, copepod and cirripede. In the present study, 29.5% (39 out of 132) members of fish, *S. leiura* showed the occurrence of double parasitism involving one isopod and three copepods in five different combinations. The degree of the occurrence of the combinations *M. renardi* and *C. lacinatus* (MC) and *M. renardi* and *L. tylosuri* (ML) was significantly high reaching 41% and 33.3%, respectively. In three combinations (MC, ML and MB), the isopod *M. renardi* was found to be common, signifying its high rate of infestation on the host. The other two double parasitic combinations (LC and LB), all members are copepods; LC amounts 15.4% and LB 5.13% (Tables I and II; Figs. 4 and 6). Apart from this, no further information are available on triple parasitism by crustaceans till date. The present study revealed that 87 members (65.9%) of *S. leiura* have been under triple parasitism with crustacean species and the rate of occurrence is more than two fold of double parasitism. Out of four combinations (MLC, MLB, MCB and LCB) noticed in the triple parasitism, MLC (*M. renardi*, *L. tylosuri* and *C. lacinatus*) scored the highest percentage (57.6)(Tables I and III). Interestingly, the existence of quadruple parasitism (one isopod and three copepods) on the fish *S. leiura* was also exposed first through the present study. However, its frequency was relatively less (3.0%) occurring only during the months of February, May, and September (Tables I and III; Fig. 4). Though seldom, the occurrence of single parasitism with *M. renardi* on the host fish cannot be negated as two fish members (1.5%; 2 out of 132) were under single parasitization with this cymothoid species. Interestingly, *M. renardi* appears as a common parasitic crustacean species infesting *S. leiura* irrespective of the type of parasitism (single, double, triple and quadruple parasitism) involved.

The parasitic isopods and copepods feed host's blood and other soft tissues (John and Nair, 1973; Trilles, 1994). In the present study, we could observe severe damages induced to the branchial cavity, the gill filament, the fleshy part of the lower beak, and the inner side of the operculum of the host. Reports showed that, the infestation by parasitic copepods and isopods induce bacterial and viral diseases in parasitized fishes (Nigrelli, 1950; Cusack and Cone, 1985; Simudu and Tsummoto, 1985; Ravichandran *et al.*, 2001; Ravichandran and Ajithkumar, 2008). The multifestation observed in the present study probably leads the high levels of secondary infections and more studies on this aspect is highly warranted.

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