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Occurrence of the isopod, *Norileca indica* (Isopoda: Cymothoidae) on bigeye scad, *Selar crumenophthalmus* (Bloch) off Mumbai coast, India

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ABSTRACT

Parasitological investigations on the occurrence of isopod parasites in carangid fish species collected off Mumbai coast (south-west coast), Arabian Sea, India was carried out for a period of one year from March 2012 to February 2013. Altogether seven fish species were examined *viz., Atropus atropos* (Bloch & Schneider, 1801), *Caranx sexfasciatu*s Quoy & Gaimard, 1825, *Decapterus russelli* (Rüppell, 1830), *Megalaspis cordyla* (Linnaeus, 1758), *Selar crumenophthalmus* (Bloch, 1973), *Scomberoides commersonnianus* Lacepède, 1801 and *Scomberoides tol* (Cuvier, 1832), but only *S. crumenophthalmus* was found to be infested with the cymothoid isopod parasite *Norileca indica* (Milne-Edwards, 1840) Bruce, 1990. During the study period, live specimens of male and female *N. indica* were isolated from the branchial cavities of the bigeye scad, *S. crumenophthalmus*. Out of the 290 specimens of *S. crumenophthalmus* examined, 114 were found to be infested with *N. indica.* The body size of females (N=10) was 24 to 35 (29.70 ± 3.71) mm long and 11 to 16 (13.30 ± 1.57) mm wide at pereonite 4, while the males (N=10) were 15 to 21 (17.20 ± 2.35) mm long and 6 to 8 (6.50 ± 0.71) mm wide at pereonite 4. The isopods were positioned in the branchial cavity of fish with their ventral side facing the inner surface of the operculum. Body of the female parasite twisted to right side in the left branchial cavity, and vice versa. The overall prevalence, mean intensity and mean abundance of infestation with *N. indica* were found to be 37.94%, 1.38 and 0.51 respectively. The prevalence and abundance of infestation was significantly ($p<0.05$) higher during post-monsoon months. Significantly ($p<0.05$) higher prevalence and abundance of infestation was observed in female hosts than in males. However, mean intensity of infestation was non-significant between the months and host sex. Host size does not seem to affect the prevalence, mean intensity and abundance of isopod infestation. The present finding is the first report on the infestation of *N. indica* in *S. crumenophthalmus* from Indian waters.

Keywords: Cymothoidae, India, Isopoda, *Norileca indica, Selar crumenophthalmus*

Introduction

Cymothoid isopods (Crustacea: Isopoda: Cymothoidae) are hematophagous, protandrous hermaphrodites, mostly parasitic on tropical marine fishes (Brusca, 1981; Bruce, 1990; Raibaut and Trilles, 1993; Trilles, 1994; Trilles *et al.,* 2011). They are highly host and site specific (Bunkley-Williams and Williams, 1998). Their life cycle is holoxenic, involves single host (Trilles, 1994; Ramdane *et al.,* 2007). The larval development occurs in the brood pouch of female isopod and the juveniles are released into the water, which in a short period of time will attach to the host, reach the specific site (skin, buccal cavity, branchial cavity or body cavity), loose the swimming setae and metamorphose to males (Bunkley-Williams, 1984). The first male to parasitise the fish converts into a female and the others will remain as males, as the pheromone or neurohormone released by the female will inhibit the sex change of the males (Brusca, 1981; Bunkley-Williams and Williams, 1998; Ravichandran, 2009).

About 330 species of cymothoid isopods were reported worldwide (Trilles, 1994), but only around 56 species are recorded from India (Rameshkumar *et al.,* 2012). Most of the reports on marine cymothoidae in India are from east coast and such studies are very few on west coast (Trilles *et al.,* 2011). Further, despite the commercial importance of carangids in Indian fishery, only two fish species were investigated for isopod infestation so far (Trilles *et al.,* 2011, 2012; Rameshkumar and Ravichandran, 2013). Hence, in view of dearth of knowledge on isopod infestations in the marine fishes, an investigation has been undertaken on isopod parasites infestations in seven species of carangids from south-west coast of India (off Mumbai coast). In this study, a cymothoid isopod *Norileca indica* (Milne-Edwards, 1840) Bruce, 1990 was found infesting the branchial cavities of big-eye scad, *Selar crumenophthalmus* (Bloch, 1793). The present study represents the first report on the occurrence of *N. indica* in carangid fish from Indian waters. The occurrence of *N. indica* on bigeye scad in relation to month, host sex and size were investigated.

Materials and methods

Sampling area and fish samples

Carangid fishes caught by the trawlers along the coast of Mumbai (18° 54' N, 72° 49' E, south-west coast of India), were collected monthly at its major landing centers over a period of one year from March 2012 to February 2013 except during June and July 2012 on the account of monsoon ban on trawl fishing. A total of 794 carangid fishes were examined comprising 60 specimens of *Atropus atropos* (Bloch & Schneider, 1801); 42 *Caranx sexfasciatus* Quoy & Gaimard, 1825; 82 *Decapterus russelli* (Rüppell, 1830); 180 *Megalaspis cordyla* (Linnaeus, 1758); 290 *Selar crumenophthalmus* (Bloch, 1793); 84 *Scomberoides commersonnianus* Lacepède, 1801 and 56 *Scomberoides tol* (Cuvier, 1832). The collected fish samples brought packed in ice to the laboratory were weighed (g), total length (cm) measured and dissected to know the sexuality. Nomenclature and taxonomic identification for hosts were done according to Froese and Pauly (2012).

Parasitological examination

Fishes were examined for the presence of isopod parasites on the body surface, buccal, branchial and body cavities and the number and sexuality of isopods, the site of attachment and their orientation on each host were recorded. The number of isopod infested and non-infested fishes were also recorded. Photographs of isopod infested host fish and the parasites were taken using a Nikon digital camera D300. The live specimens of female and male cymothoid isopods were carefully removed with forceps from the branchial cavities of host, weighed, taken measurements (total length and width at perionite 4) and then fixed in 70% ethanol. Taxonomic identification of the parasites was according to Bruce (1990). Representative specimens of the isopod were deposited at the Aquatic Animal Health Management laboratory of Central Institute of Fisheries Education, Mumbai, India.

Data analyses

The ecological terminology *viz.,* prevalence, mean intensity and abundance were followed from Margolis *et al.* (1982) and Bush *et al.* (1997).

The effect of month, host sex and size on the prevalence, mean intensity and abundance of *N. indica* was estimated by adopting a general linear model. Proc GLM of SAS version 9.3 (SAS Institute, 2011) was employed to carry out the Analysis of Variance (ANOVA). The significant difference between the means within a group was estimated by Duncan's test and comparisons were made at 5% probability level. The graphs were obtained using Microsoft Excel spread sheet version 2007.

Results

Among the seven species of carangid fishes examined during the study period, only the bigeye scad, *Selar crumenophthalmus* (Bloch, 1793) were found infested by cymothoid isopod parasites in their branchial cavities. The cymothoid isopods observed in this study (Fig. 1) were all identified as female and male *Norileca indica* (Milne-Edwards, 1840). The body of female parasites (N=10) were 24 to 35 (mean ± standard deviation 29.70 ± 3.71) mm long and 11 to 16 (13.30 ± 1.57) mm wide at pereonite 4, while the male parasites (N=10) were 15 to 21 (17.20 ± 2.35) mm long and 6 to 8 (6.50 ± 0.71) mm wide at pereonite 4. Male parasites were much

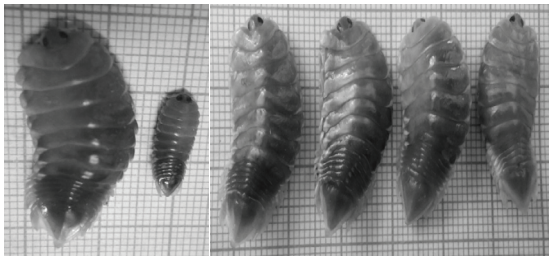

Fig. 1. Fresh specimens of *Norileca indica* (dorsal view) recovered from the branchial cavities of *Selar crumenophthalmus*: a) Adult female (left) and male (right); b) Females showing the two patterns of twisting

Fig. 2. Ovigerous female *Norileca indica* in the branchial cavity of *Selar crumenophthalmus* (scale bar: 10 mm)

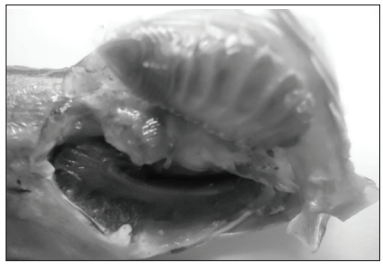

Fig. 3. Atrophy and necrosis (arrows) in the gill filaments of first gill arch of *Selar crumenophthalmus* due to infestation of female *Norileca indica*

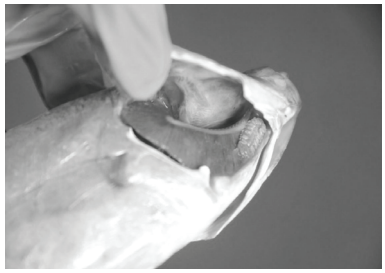

Fig. 4. Adult male *Norileca indica* in the branchial cavity of *Selar crumenophthalmus*

smaller and narrower than females. The female parasites were found positioned on host (Fig. 2) with the ventral side facing the inner surface of the operculum and dorsal side deeply plunged in the first gill arch with the gill filaments showing considerable atrophy and necrosis (Fig. 3), cephalon directed to the anterior in ventral portion of branchial chamber with sternum facing out. The body of the female parasite twisted to one side, to right side in the left branchial chamber, and *vice versa*. The male counterparts however positioned ventrally to the inner operculum, orientation with the head directed towards the upper half of the operculum (Fig. 4). In both female and male parasites the dorsal body surface was coloured pale reddish brown on pereon, medium reddish brown on pleon to blackish brown on pleotelson.

Of the 290 bigeye scad specimens examined, 147 (50.7%) were females (212.60 ± 23.14 mm in total length) and 143 (49.3%) were males (207.44 ± 25.47 mm in total length). *N. indica* infestation was recorded in 114 (73 female and 41 male) fishes, carrying 155 parasites (Table 1) corresponding to an overall prevalence, mean intensity and abundance of 37.94%, 1.38 and 0.51 respectively (Table 2). The effect of month, host sex and size on prevalence, mean intensity and abundance of *N. indica* infestation was analysed using one-way ANOVA model. The monthly prevalence has shown marked variation during the year, with a significantly higher ($p<0.05$) infestation in November (77.99%), October (71.43%) and April (55.9%) 2012 than during the other months (Table 2). Similarly the abundance of infestation was significantly greater ($p<0.05$) during November (1.09) and October (0.97) 2012. However, there was no significant difference ($p>0.05$) between months in the mean intensity of infestation by *N. indica*.

The parasite prevalence was significantly higher ($p<0.05$) in female fishes (47.85%) than in male fishes (28.04%) (Table 2). The highest prevalence in female fishes was observed during November 2012 (93.3%) followed by October (81.9%) and lowest (15.0%) in August 2012. In male fishes also the prevalence was significantly higher in November (60.5%) and October (57.8%), though the lowest prevalence (12.2%) was observed during September, it was not significant from the remaining months (Fig. 5).

Table 1. Infestation of *Norileca indica* on *Selar crumenophthalmus* in relation to month and host size

Sub-group	Female fish				Male fish			
	No. examined	No. infested	Parasites collected		No. examined	No. infested	Parasites collected	
			Female	Male			Female	Male
Month-wise								
Mar' 12	16	4	4	1	14	2	2	1
Apr' 12	16	11	11	2	12	5	5	1
May' 12	12	5	5	2	13	3	3	1
Aug' 12	11	2	2	1	15	2	2	1
Sep' 12	17	6	6	3	15	2	2	1
Oct' 12	17	14	14	6	13	8	8	2
Nov' 12	17	16	16	7	18	11	11	4
Dec' 12	12	7	7	2	16	3	3	1
Jan' 13	14	4	4	1	10	2	2	1
Feb'13	15	4	4	2	17	3	3	1
Total	147	73	73	27	143	41	41	14
Length class (mm)								
< 175	22	11	11	3	22	8	8	2
175 - 190	20	6	6	2	21	9	9	2
190 - 205	40	22	22	10	40	12	12	3
205 - 220	35	21	21	7	28	5	5	3
220 - 232	15	7	7	3	18	3	3	2
> 232	15	6	6	2	14	4	4	2

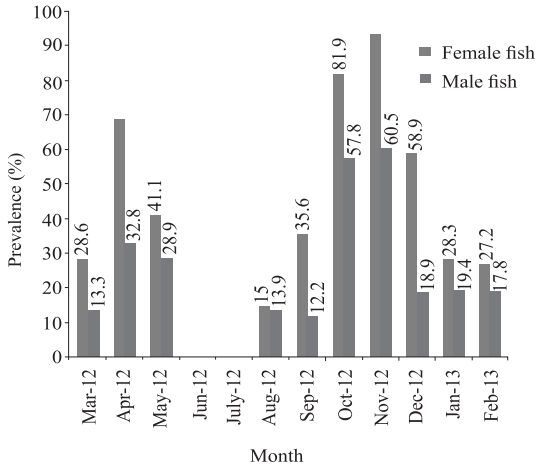

Fig. 5. Prevalence of *Norileca indica* in relation to the sex of *Selar crumenophthalmus* during different months

Table 2. The occurrence of *Norileca indica* on *Selar crumenophthalmus* in relation to month, host sex and size

Sub-group	*Prevalence (%) ± S.E	*Mean Intensity ± S.E	*Abundance ± S.E
Overall	37.94 ± 2.74	1.38 ± 0.06	0.51 ± 0.04
Month-wise			
Mar' 12	18.97^{c} ± 7.95	1.34^{a} ± 0.21	0.25^{d} ± 0.12
Apr' 12	55.90^{ab} ± 8.24	1.19^{a} ± 0.13	0.66^{cb} ± 0.12
May' 12	31.75^{c} ± 8.75	1.38^{a} ± 0.18	0.44^{cd} ±0.13
Aug' 12	17.15^{c} ± 8.67	1.52^{a} ±0.25	0.25^{d} ± 0.13
Sep' 12	24.30^{c} ± 7.77	1.46^{a} ± 0.18	0.36^{cd} ± 0.12
Oct' 12	71.43^{a} ± 8.20	1.38^{a} ± 0.12	0.97^{ab} ± 0.12
Nov' 12	77.99^{a} ± 7.60	1.42^{a} ± 0.17	1.09^{a} ± 0.11
Dec' 12	36.71^{bc} ± 8.26	1.27^{a} ± 0.16	0.47^{cd} ± 0.12
Jan' 13	23.27^{c} ± 8.99	1.33^{a} ± 0.21	0.31^{cd} ± 0.13
Feb'13	21.90^{c} ± 7.79	1.49^{a} ± 0.19	0.31^{cd} ± 0.12
Host sex			
Female	47.85^{a} ± 3.74	1.38^{a} ± 0.07	0.65^{a} ± 0.05
Male	28.04^{b} ± 3.76	1.37^{a} ± 0.09	0.37^{b} ± 0.05
Length class (mm)			
< 175	45.50^{a} ± 6.59	1.28^{a} ± 0.11	0.58^{a} ± 0.10
175 - 190	35.34^{a} ± 6.81	1.26^{a} ± 0.13	0.45^{a} ± 0.10
190 - 205	37.15^{a} ± 4.99	1.37^{a} ± 0.10	0.51^{a} ± 0.08
205 - 220	38.30^{a} ± 5.51	1.43^{a} ± 0.11	0.54^{a} ± 0.08
220 - 232	36.23^{a} ± 7.71	1.54^{a} ± 0.16	0.53^{a} ± 0.11
> 232	35.10^{a} ± 8.17	1.39^{a} ±0.17	0.46^{a} ± 0.12

No significant difference ($p>0.05$) was observed in the prevalence, mean intensity and abundance of parasite infestation in relation to the host size (Table 2). The higher prevalence and abundance was recorded in below 175 mm length class and mean intensity of infestation was higher in 220-232 mm. However, these values were not significantly higher from that of other length classes.

Among the infested fishes (Table 1), 27 female and 14 male fishes were infested by two parasites (female and male, one per branchial chamber, Fig. 6) while 46 female and 27 male fishes with one female parasite in their branchial cavities. The sex ratio (female : male) of parasites according to the host's sex was determined at different months. In female hosts it was highest (5.5) in April 2012 and lowest (2.0) during August, September 2012 and February 2013. Similarly maximum sex ratio (5.0) was observed in male hosts in April 2012 and minimum (2.0) during March, August, September 2012 and January 2013. The results indicate higher sex ratio of parasites in female host than in male host (Fig. 7).

The parasite sex ratio was also calculated in relation to the size (length class) of hosts (Table 1). In female hosts, higher (3.7) and lower (2.2) parasite sex ratio values were observed in < 175 mm and 190-205 mm length classes respectively, whereas in male hosts it was higher (4.5) for the length class 175-190 mm and lower (1.5) in the length class 220-232 mm (Fig. 8).

Fig. 6. Male (lower arrow) and female (upper arrow) *Norileca indica* in the branchial cavities *Selar crumenophthalmus*

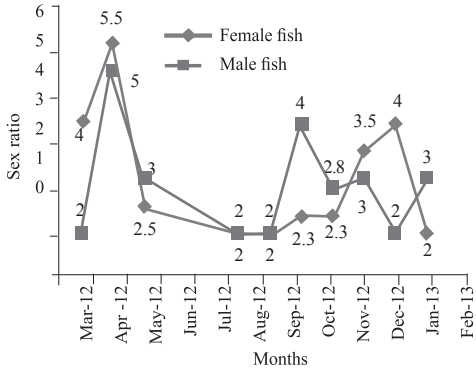

Fig. 7. Sex ratio (female:male) of *Norileca indica* during different months

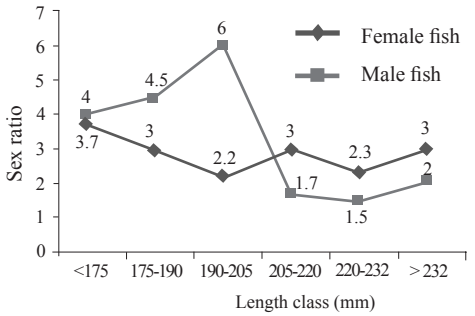

Fig. 8. Sex ratio (female:male) of *Norileca indica* in relation to the size of *Selar crumenophthalmus*

Discussion

Norileca indica (Milne-Edwards, 1840) Bruce, 1990 is a protandrous, hermaphroditic, cymothoid isopod mainly infesting the gill chambers of the pelagic marine teleosts (Bruce, 1990). This species is known to be widely distributed in Sumatra, Indonesia, Philippines and New Guinea (Trilles, 1976), north-western Australia (Avdeev, 1978), off Mozambique (Rokicki, 1982), eastern Australia (Bruce, 1990), Pakistan (Ghani, 2003), China (Yu and Li, 2003), the Philippines (Yamauchi *et al.,* 2005), Thailand (Nagasawa and Petchsupa, 2009) and south-eastern India (Rameshkumar *et al.,* 2012).

So far *N. indica* has been reported to infest six species of marine pelagic fishes, of which four species belongs to the family Carangidae whcih include: the bigeye scad *Selar crumenophthalmus* (Rokicki, 1982; Bruce, 1990; Nagasawa and Petchsupa, 2009), blackfin scad *Atule malam* (Avdeev, 1978), smallmouth scad *Alepes apercna* (Trilles, 1976) and Indian scad *Decapterus russelli* (Ghani, 2003). Non-carangid host fishes include: *Rastrelliger kanagurta* (Rokicki, 1982; Ghani, 2003; Rameshkumar *et al.,* 2012), *Herklotichthy*es sp. (Bruce, 1990; Yu and Li, 2003). In the present investigation, *N. indica* was found in the branchial cavities of *Selar crumenophthalmus* from off Mumbai coast (south-west coast) of India, which further confirms its wide geographic distribution.

Host specificity is the tendency of a parasite to occur on one or a few taxonomically related host species and is a product of co-existence between both parasite and host lineages (Timms and Read, 1999; Poulin, 2007). Isopod parasites, cymothoids in particular are mostly host specific and the development of specificity is promoted by combination of two factors: the uniformity of diet (blood or epithelium) and morphological adaptation to the particular kind of epithelium or scales (Rameshkumar and Ravichandran, 2013). Among the various species of carangids observed in the present study, *N. indica* was found only on bigeye scad indicating high degree of host specificity. Ravichandran and Rameshkumar (2011) also reported the host specificity of most Indian cymothoid fauna, which are limited to one host species in a given environment.

The occurrence of cymothoids in natural populations is patchy, and levels of prevalence are extremely variable (Brusca, 1981). Rokicki (1982) found *N. indica* in gill cavities of all bigeye scad specimens (N=70) examined from off Mozambique. Reports of Nagasawa and Petchsupa (2009) indicate high rate of prevalence (85%) of *N. indica* on the same host species from Pattani Bay of southern Thailand. The present study observed moderate prevalence of 37.94% (maximum of 77.99% in November 2012) of *N. indica* on *S. crumenophthalmus*. Ghani (2003) reported D. russelli as one of the hosts for *N. indica*. However, during our study period, no such parasite infestation was found in *D. russelli.*

The parasite-host interactions among isopods and marine fishes, have shown high parasite prevalence in association with a high abundance of the host fish (de Carvalho-Souza *et al.,* 2009). Along the Maharastra coast of Arabian Sea, carangids are abundant during post-monsoon months with peak during October and November (Radhakrishnan Nair, 2000). Our results are in agreement with de Carvalho-Souza *et al.* (2009), wherein the isopod prevalence was maximum during post-monsoon (October and November, 2012) months in both host sexes (Fig. 5).

Studies on the nature of crustacean parasite infestation in fishes of south-west coast of India (Radhakrishnan and Nair, 1983; Ravichandran *et al.,* 2009), revealed higher prevalence and intensity of parasite infestation in female hosts than that in male hosts. The present investigation (Table 2) recorded similar observations, wherein *N. indica* prevalence was much higher in female hosts (47.85%) in comparison with male hosts (28.04%). Thomas (1964) stated that the lowering of estrogen levels in female fishes during breeding season makes them more susceptible to parasitic infestations.

Romestand and Trilles (1979) stated that the size of fishes that can be infested will be within certain maximum-minimum variables depending upon the species of the host. The preference of parasite for fish at intermediate body size was presented by some species of cymothoids (Marks *et al.*, 1996; Bello *et al.,* 1997). However, in the present study no significant difference was observed in the prevalence, mean intensity and abundance of *N. indica* infestation on different length classes of *S. crumenophthalmus.* The reason might be attributed to more similarity in physiological (matured) condition of the hosts observed, which were more or less equally preferred by *N. indica.* However, this is to be verified further by examining all the length groups of host including juvenile, sub-adult, adult and older-adults to find out the actual nature of *N. indica* infestation in relation to size of *S. crumenophthalmus.*

Few studies reported on sex ratio of cymothoid isopods parasitising marine teleosts. Trilles *et al.* (2012) recorded higher sex ratio (female : male) of the cymothoid isopod, *Catoessa boscii* in males than females of *Carangoides malabaricus*, in relation to different months from south-east coast of India. On the other hand, while comparing the host size, sex ratio of *C. boscii* was maximum in male fish (6.0) of the length class 140-150 mm and 150-160 mm in female fish (7.0). However, our findings on the sex ratio of *N. indica* on bigeye scad are contradictory to the above study. We observed higher sex ratio of *N. indica* in female hosts (5.5) than in male hosts (5.0) during April 2012. Further, the sex ratio of *N. indica* in relation to the length class of bigeye scad was found to be higher for 175-190 mm size males (4.5) and

<175 mm size females (3.7). The results indicate dissimilarity in the sex ratio values of *N. indica* among the same length classes of the male and female *S. crumenophthalmus.*

Several studies have indicated harmful effects on host fish by cymothoid infestation citing tissue damage; host behavioral changes; reduction in mean weight, size and growth; and in some instances, death (Kroger and Guthrie, 1972; Sadzikowski and Wallace, 1974; Lindsay and Moran, 1976; Brusca, 1978; Romestand and Trilles, 1979; Brusca, 1981; Kabata, 1984; Moser and Sakanari, 1985; Segal, 1987; Adlard and Lester, 1994; Ravichandran *et al.,* 2007; Fogelman *et al.,* 2009; Rameshkumar and Ravichandran, 2013). In contrary, other researchers (Brusca, 1981; Ruiz and Madrid, 1992; Landau *et al.,* 1995; Marks *et al.,* 1996) stated that individual fish infected with a single parasite were not significantly "less healthy" than non-parasitised fish, and only in cases of multiple infection or stress, would an effect on condition and (or) behaviour of hosts be realised (Keys, 1928; Lanzing and O'Connor, 1975; Brusca, 1981). In the present study, individual fish was infested either by a single female (larger) parasite or a pair (larger female and smaller male), one per branchial cavity and thus pathological effect on host was minimal. Several aspects of isopod life history support the concept that cymothoids may parasitise in a manner that does not constitute a serious threat to host wellbeing.

According to Provenzano (1983) cymothoids feed on blood or other tissues, or both. Bunkley-Williams and Williams (1998) emphasised that the tissue damage to hosts by cymothoids is often impressive, but this damage is caused by crypting (a necrotic eroding reaction of host tissues pressed against the parasite) or deformation (host tissues growing around the parasite). In the present study, it was observed that dorsal body of female *N. indica* was deeply pressed against the gill filaments of first gill arch and occupied two third surface of the first gill, causing pressure atrophy, necrosis and stunted growth of corresponding gill filaments. This has lead to reduction in respiratory surface area of the first gill. Similar observations were reported in *Carangoides malabaricus* infested by branchial isopod, *Joryma tartoor* (Ravichandran *et al.,* 2010).

The present study reports for the first time *N. indica* as a parasite on the carangid fish, *S. crumenophthalmus* from Indian waters. The high prevalence of *N. indica* during post-monsoon months observed in this study was coincided with the peak abundance of *S. crumenophthalmus* along the Mumbai coast. Host sex had a significant effect on prevalence of the parasite. High prevalence of *N. indica* on the female fish observed during the present study could be the result of more number of mature females in the collected samples, which are prone to be more infested due to their higher reproductive stress condition. The nature of *N. indica* infestation in relation to size of the host has to be studied further. In the present study, we observed that adult *N. indica* did not cause serious harm to the host and exhibited self-regulating behaviour by limiting the number (one or two) of individual parasites on any one host, so that parasite will be benefited from continued survival of the host. Further investigations on the occurrence of *N. indica* from the different coasts of Indian waters would be useful for better understanding of environmental and host effects on distribution of this cymothoid isopod.

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