Parasitic Isopod Cymothoa eremita (Brünnich 1783) (Isopoda: Cymothoidae) Affects the Growth of Black Pomfret Parastromateus niger (Bloch 1795) in the Southeast Coast of India

Article *in* Thalassas: An International Journal of Marine Sciences · April 2019

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Parasitic Isopod *Cymothoa eremita* (Brünnich 1783) (Isopoda: Cymothoidae) Affects the Growth of Black Pomfret *Parastromateus niger* (Bloch 1795) in the Southeast Coast of India

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Received: 30 November 2017 /

Abstract
The black pomfret *Parastromateus niger* is often favored host for infestation by the cymothoid, buccal attaching ectoparasite *Cymothoa eremita*. This investigation aimed to evaluate the health status of black pomfret by means of relative condition factor under the existing parasitic cymothoids. Prevalence of *C. eremita* on black pomfret fish was high (42%) but large (>300 mm) and small fish (< 250) showed significantly lesser prevalence than intermediate-sized host fish. The harmful effects of parasite infection on their hosts include basihyal damage, a loss in buccal cavity volume as a result of female cymothoid infestation, and a severe impact on host growth. The diet composition examination was a small indication to suggest a change in either feeding habits or diet of fish where parasitized and non-parasitized fish occupied the similar tropic niche. Due to an infestation of *C. eremita* in *P. niger*, the relative condition factor in infested was found to be lower than that of the uninfested fish. *C. eremita* infestation can impair growth and decrease feeding efficiency in hosts, subsequently affecting health condition and survival.

Keywords Cymothoid · Diet effect · Growth effect · Parasitism · Relative condition factor · Tongue eater

Introduction

Cymothoids isopods inhabit in the different aquatic environment, as ectoparasitic crustaceans infest an array of tropical and temperate fish species (Trilles 1994) and cause incredible critical activity in their hosts (Brusca 1981; Fogelman et al. 2009; Ravichandran et al. 2010; Aneesh 2014). This Cymothoidae are importance group of marine and freshwater isopods, with 384 species in 40 genera were reported globally (Smit et al. 2014) and *Cymothoa* spp. exhibit several host attachment sites including the buccal cavities, body surface and fins, occasionally they burrow into the host musculature (Brusca 1981; Bakenhaster et al. 2006). The large female *Cymothoa* spp. always attached to the host's basihyal, therefore *Cymothoa* spp. are referred to as tongue eating parasitic isopods (Brusca and Gilligan 1983; Hadfield et al. 2011). In general, cymothoid parasites are protandrous hermaphrodites (Brusca 1981; Bakenhaster et al. 2006). The host buccal cavity occupied by free-living manca stage of male parasite and move to the basihyal, then the male converts into efficient female and anticipates the attachments of another male.

The life history of cymothoid (Bakenhaster et al. 2006; Aneesh et al. 2015, 2016) and ecological consequences on *Cymothoa* spp. are restricted to documenting parasite infection rate (Horton and okamura 2001). Limited research has absorbed on the relationship between cymothoids and their host, the succeeding ecological impact and biological consequence on their host (Colorni et al. 1997). Although parasites may have obvious, harmful effect on their hosts, in several cases even acute infection may not strain to host in their wild population (Rohde 2002). Cymothoids continuous feeding on fish tissue and host blood result in the severe localized tissue damage or injuries, decreased growth, behavioral changes and in incredible causes mortality of host noted by several authors (Trilles 1979; Brusca 1981; Colorni et al. 1997; Horton and okamura 2001; Rameshkumar and Ravichandran 2014; Ravichandran et al. 2016). The present study describes the relationship between the isopod *Cymothoa eremita* and the host

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s41208-018-0097-7) contains supplementary material, which is available to authorized users.

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Published online: 01 August 2018

Parastromateus niger, such as the fluctuations of prevalence at different size classes of hosts, effects of the isopods infection on feeding ability and growth of hosts.

Methods and Materials

General Sampling

Overall, 462 *Parastromateus niger* specimens were collected from February-2016 to January-2017 in the four different landing Centres along the Bay of Bengal, (Chennai-13.0827^{0}N, 80.2707°E; Parangipettai-11.5084^{0}N, 79.7568°E; Nagapattinum-10.7656^{0}N, 79.8424°E; Toothukudi-8.7642^{0}N, 78.1348°E) south-east coast of India. Fishes were measured whole weight (0.1 g) and total length (TL 1.0 mm). Isopod was recovered from the buccal cavity of host and subsequently measured the total length of parasites (0.1 mm), sexed, fixed and preserved using methods commonly applied. The cymothoid parasites were identified according to the morphological descriptions (Rameshkumar et al. 2012; Martin et al. 2016). Voucher specimens were deposited in the CAS in Marine Biology, Annamalai University, India, (collection Ravichandran) (AUCR). Host stomachs were dissected and kept frozen at -20 °C for dietary analyses and the food contents were divided into broad prey classes sorted by large categories and weight of each category was noted.

Changes in Prevalence with Host Length

The linear regression was used to the relationship between female and male parasites length, likewise the relationship between female parasite and host fish length. Also, the non-parametric regression was used to the relationship between prevalence of the cymothoid and black pomfret length based on splines (Marks et al. 1996). Nonparametric regression based on spline was used in theis investigation to evaluate the prevalence relation to host length. The estimated rate of parasitism to host fish length was using randomization procedure was followed by Marks et al. (1996).

Effect of Cymothoiids on Host Body Condition

To determine the effect of cymothoiid on host condition, the relative condition factor (Kn) of infected and uninfected fish were calculated as: $Kn = W/W_e$ where W is weight (g) and W_e is expected weight for a given length was calculated using the assessed weight-length relationship curve calculated as $W = aL^b$ where L is total length, a is a scaling coefficient and b is shaped parameter (Le cren 1951). The logarithmic form of this model was used to adapt this relationship to the straight-line relationship (Fig. S1). A Kruskal-Wallis test was used to test the null hypotheses that mean infected and uninfected fish length, weight and Kn were equal.

Effect of Cymothoids on Host Diet

The consequence of infection on black pomfret diet was assessed by calculating the following dietary indexes; percentage frequency of occurrence *(%F)* of a prey item in fish with food in their stomachs, the percentage of abundance (*%N*) of prey items analyzed percentage of volume *(%V)* of each item out of the total weight of food items. These values were combined to infected and uninfected fish was assessed the index relative importance (IRI) was calculate as $IRI = (\%N + \%V) \times \%F$ and the IRI was the standardised to *%IRI* for each prey items (Pinkas et al. 1971; Hacunda 1981). A visual stomach fullness index was assigned: 0, empty; 1, scarce remains; 2, half full; 3, almost full; 4, completely full (Breiby & Jobling 1985). Obviously, it is not reasonable for stomach fullness index (SFI = mass of stomach content/mass of prey items × 100). The difference in feeding intensity of infected and uninfected fish was determined using indices: percentage of the gastro-somatic index ($\%GaSI$ = mass of stomach / total fish mass × 100). The student t test was used to test the null hypothesis that means infected and uninfected fish *%GaSI* and SFI were equal.

Result

Parasite Characteristics

Out of 462 *Parastromateus niger* were examined, 188 specimens were parasitized by isopods cymothoiid belonging to the species *Cymothoa eremita.* A total of 204 cymothoids were collected. Male parasites were significantly smaller than female parasites (t test = 12.06, df = 202, $p < 0.0001$) ranged in length from 7.9 to 20.9 mm with mean length of 15.66 ± 0.56, n = 38 (Fig. 1). Female parasites ranged in size from 13.4 to 32.9 mm with an average length of 24.93 ± 0.34 mm, n = 166. Statistically significant correlation between the length of male and female parasites ($R^2 = 0.2953$). Likewise, the correlation between the length of female *C. eremita* and *P. niger* was significant ($R^2 = 0.7245$) (Fig. 2). Host weight was increased more rapidly in relation with the length of female parasites. The allometric coefficient b ($W = aL^b$) for the parasite length and host length relationship ($b = 0.0520$, SE = 0.002) differ from that of host length-weight relationship ($b = 6.64$, SE = 0.104).

Prevalence and Cymothoid Infections on Host Fish

The estimated function of cymothoid prevalence in relation to black pomfret fish length was determined. The overall

prevalence of infection was 40.62%. The results suggested that infection of *C. eremita* in the larger fishes was more susceptible than that of the smaller fishes and the highest prevalence rate (42%) was found in black pomfret more than 300 mm TL. The prevalence ratio correlated with the length class of the host was 1:2.6 (infected: uninfected) for the length class of 150 mm to 300 mm, and increased gradually to reach a ratio of 1:1.8 (infected: uninfected) for lengths >300 mm TL (Figs. 3 and 4). A total of 96 (21%) black pomfret were observed to have significant destruction to their basihyal with the presence of parasites. *P. niger* was basihyal damage ranged in size between 150 and 400 mm TL (Fig. 4).

The cymothoid parasites come across in the buccal cavities occupied the surface of the lower jaw, and were clung firmly to the basihyal with their heads arranged towards the inside of the host mouth (Fig. 5). In some fish, two *C. eremita* are found, a substantial ovigerous female in the mouth and a small dynamic male in the buccal cavity. The infected fishes exposed small pin-hole in the basihyal region, through which dactyls of pereopods penetrating claws dig into the host tissues. As the parasite developed, there was an expansion in the amount of mechanical and physical harm done to the buccal cavity of fish.

Effects of Cymothoid on the Body Condition and Growth of Host

The parasite infection acute effect on the growth of *P. niger*. The linear regression analysis produced the logarithmic equivalent of the weight-length relationship of infected and uninfected fish data that estimates were respectively log (*a*) and *b* value of infected fish ($a = -1.8443$; $b = 1.9435$) and uninfected fish ($a = -1.7578$; $b = 1.9153$) (Subsidiary information Fig. S1 and S2). This relationship enabled us to calculate the theoretically expected weight (*We*) and then the relative condition factor as defined before. The analysis the Kruskal-Wallis test of equality of mean length, weight and *Kn* of different length grope of infected and uninfected fish were significantly varied (Table 1). The overall relative condition factor (*Kn*) of infected and uninfected fish showed differ in significantly ($t = 15.84$, $df = 460$, $p < 0.0001$).

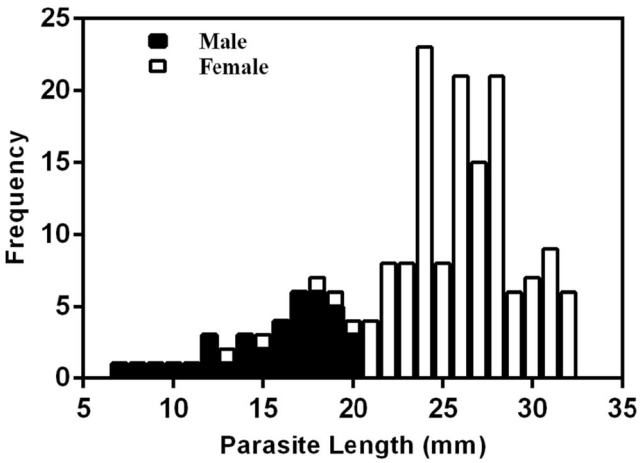

Fig. 1 Length frequency of male and female cymothoid *Cymothoa eremita* occurred in the buccal region of *Parastromateus niger*

Effect of Cymotoids on Diet in Host Fish

The diet composition examination stated that *P. niger* (<400 mm TL) (supplementary material Table SI) were primarily dependents on Polychaeta, Copepoda and Penaeidae as their prey source and there were unequally similar in the diet of infected and uninfected fish (Fig. 6). The teleosts were coequally alike in the diet of infected and uninfected fish. Uninfected fish feed more on polychaeta and penaeidaes, while infected fish feed large copepods. The percentage of index relative importance (*%IRI*) of infected and uninfected fish showed significantly different (t test = 7.494, $df = 14$, $p < 0.0001$). The Gastro-somatic index (*%GaSI*) showed significantly varied over the uninfected, infected and tongue damaged fish (t test = 2.573, $df = 306$, $p < 0.0105$). The examination of stomach

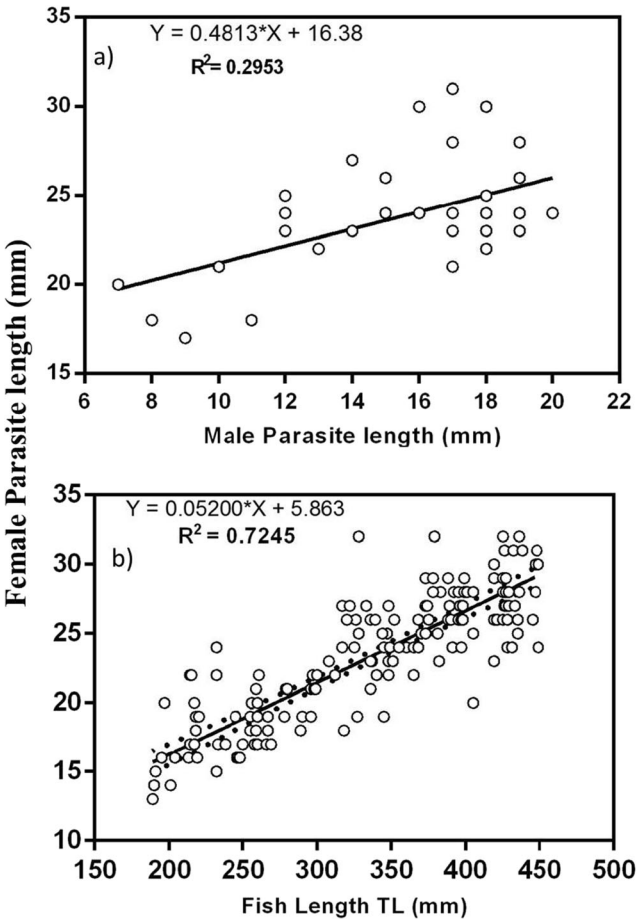

Fig. 2 The linear correlation between (**a**), the length of male and female *Cymothoa eremita*; (**b**), the length of female cymothoid and *Parastromateus niger*. Samples were collected between February-2016 to January-2017 from Bay of Bengal, India

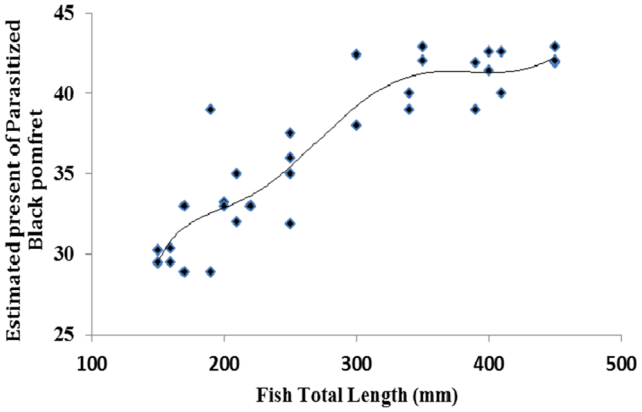

Fig. 3 The relationship between prevalence of the cymithoid and blach pomfret length was evaluated using nonparametric regression based on splines

fullness index of infected and uninfected fish showed did not significantly different (t test = 0.7397, df = 306, p < 0.4601).

Discussion

According to our data, it appears that *Parastromateus niger* almost always infected with female parasitic isopod *Cymothoa eremita*. Male-female pairs were observed with a few exceptions of their host. In general, cymothoiid parasites are protandrous hermaphrodites or male was found at presampling but was dislocated during handling or capture. The correlation between the lengths of male parasites was smaller than the female parasites. The characteristic of the Cymothoidae family is that the males are smaller than females, this characters being most strongly expressed in the branchial and buccal attaching genera reported by Smit et al. (2014). The analysis of the relationship between the length of the cymothoid and its host indicated that parasites occur once, and the parasite grows together with its host. The present

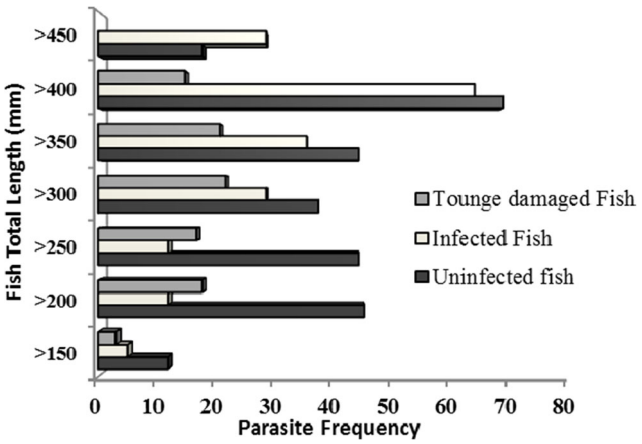

Fig. 4 Parasite frequency (Prevalence) against the mean length of black pomfret, infected by *C. eremita*, uninfected fish, tongue damage fish as a consequence of possible previous infection

investigation is similar correlation was noted by Parker and Booth (2013) for the tongue-eating parasitic cymothoid *Cymothoa borbonica* and body surface parasitic isopod *Anilocra apogonae* were noted by Fogelman et al. (2009). The strong relationship suggested that the growth of fish and parasite was synchronized and infections by small parasites only occurred when hosts were young. The related findings were documented by Adlard and Lester (1994) where the small size of *Anilocra pamacentri* infested on a young host of *Chromic nitida* and *Anilocra haemuli* infested on *Haemulon flavolineatum* were noted by Welicky and Sikkel (2014).

The estimated functions of cymothoid prevalence in relation to black pomfret fish length were highly vulnerable to infection while the success of attachment of parasite length was increased as the length of host increased. This, result also observed by Adlard and Lester (1994), who proved that cymothoid *Anilocra pamacentri* were more likely to attach to the smaller than larger *Chromic nitida* in laboratory studies and Trilles et al. (2012), who have reported that the parasitic prevalence of *Catoessa boscii* was increased to the smaller than larger *Carangoides malabaricus*. According to our length data for *P. niger* intensely propose that the lower prevalence of cymothoid at smaller host size is the result of the short life span of *C. eremita*, but this may be confounded if infested fish have a higher mortality rate than uninfected fish. However, these findings do not support the result of Bakenhaster et al. (2006) who reported a parasitic prevalence of *Glossobius hemiramphi* in *Hamiramphus brasilinsis* was high and decreased with increased host size.

C. eremita was to date collected from the buccal cavity of *P. niger*. The parasites occur in the buccal cavity of the host fish and their position is highly specific. In some fish, two *C. eremita* is found, a large ovigerous female in the mouth and a small dynamic male in the buccal cavity. Most of the females were attached to the fish tongue, infrequently on the roof of the buccal cavity. Other authors found the same site of attachment for other genus *Cymothoa* and their host (Rameshkumar et al. 2012; Parker and Booth 2013; Aneesh et al. 2015; Martin et al. 2016). The active males were constantly attached to the second and third gill filaments in one branchial chamber of the infected fish. The ends of the *C. eremita* pereopods have sharply curved hooks which allow attaching to the buccal cavity of host and Smit et al. (2014) who have reviewed that genera and species of what was *Cymothoa* by morphological characteristics also occurred on the buccal cavity of the host.

The parasitic cymothoids are recounted to cause severe damage to their host fishes. Our findings stated that the gill arches and branchial cavity of the infected fish shown a substantial effect on their host including acute basihyal damage which was more distinct in the gill chamber where the female was protected. The parasitic infected basihyal showed a serious effect when compared to non-infected ones. However,

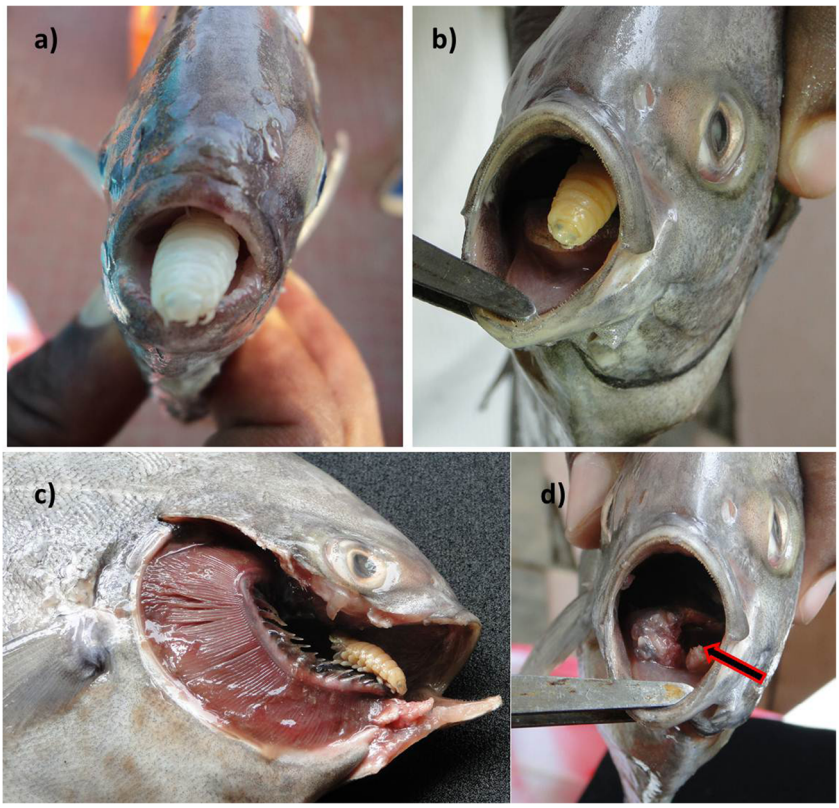

Fig. 5 (**a**) and (**b**), Adult female *Cymothoa eremita* attached in the basihyal of *P. niger*; (**c**), position within the host buccal cavity where the cymothoid attaches itself to the basihyal; (**d**), damaged and deformed basihyal (arrow) infected by *Cymothoa eremita*

these results support the findings of Trilles (1994) reported that the deleterious impacts of branchial cymothoids were also reflected in the heart, pericardium, and respiratory metabolism. Erosion of tongue was the serious gross lesions perceived as a significance of isopod infestation. Similar results were documented by Parker and Booth (2013) where infection by *C. borbonica* had a severe effect on the basihyal of *Trachinotus botla*. Mancas of *C. eremita* were attached to young host at the buccal cavity and then crawled over the tongue of the fish.

Cymothoid had a significant harmful effect on growth and health of fish. Parasitized fish were significantly loss the weight than were uninfected fish of the same length. The evaluation of the slope of the length-weight relationship equation between infected host and non-infected host exhibited those statically significant variances. It was observed that the health condition of non-infected fish was even higher to that of parasitic infected fish. In the present investigation on *P. niger* indicated that some certain combinations of parasitic isopod occur on the host with weaker condition (Table 1)

Table 1 Kruskal-Wallis test for infected and uninfected fish of equality of mean length, mean weight and relative condition factor (*kn*) (± Standard Error) with respect to the fish length group

	Length (mm)		Weight (g)		*Kn*	
	Mean ± SE		Mean ± SE		Mean ± SE	
Length group (mm)	Host fish	Non host fish	Host fish	Non host fish	Host fish	Non host fish
150–200	194.0 ± 1.4	192.3 ± 0.98	384.4 ± 8.94	392.09 ± 7.69	0.96 ± 0.00	0.95 ± 0.02
200–250	220.2 ± 5.15	240.4 ± 1.85	493.56 ± 21.54	632.6 ± 7.80	0.96 ± 0.04	1.10 ± 0.01
250–300	270.9 ± 4.40	279.4 ± 4.43	740.98 ± 10.04	849.88 ± 7.71	0.97 ± 0.01	1.09 ± 0.01
300–350	325.6 ± 2.49	338.8 ± 1.74	1051.65 ± 26.47	1236.18 ± 16.06	0.95 ± 0.01	1.10 ± 0.01
350–400	367.6 ± 2.10	387.8 ± 2.16	1258.08 ± 20.35	1583.84 ± 6.146	0.91 ± 0.01	1.04 ± 0.01
400–450	426.4 ± 1.54	432.9 ± 1.31	1682.46 ± 8.061	1947.37 ± 4.692	0.90 ± 0.01	1.03 ± 0.00
>450	455.0 ± 0.91	459.7 ± 0.60	1890.67 ± 10.33	2189.55 ± 12.17	0.93 ± 0.00	1.01 ± 0.01
Significance for K-W test	0.7493		0.5652		0.0088*	

*Significant at 0.05 level

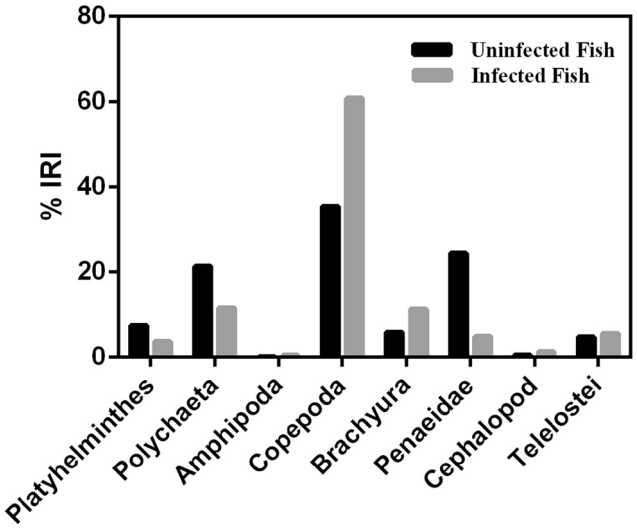

Fig. 6 Percentage index of relative importance of various prey items, consumed by *Parastromateus niger* that was infected by *Cymothoa eremita*. The dietary assess fell within the infection stage (< 400 mm)

and Ozer et al. (2016) who have reported similar results for *Mugil cephalus*. However, Sindermann (1987) found that harmful impact of parasite on host growth and survival has been demonstrating for some parasites-fish host systems. The statistical variances were found between parasitic and non-parasitic black pomfret in relation to stomach fullness index and gastro-somatic index. The decreased fullness index and gastro-somatic index of infected fish indicate that the condition of *P. niger* was compromised by the occurrence of the parasitic cymothoid. Östlund-Nilsson et al. (2005) stated that the decreased reserves may be the source of the metabolic reduction. Feeding intensity and the relative percentage of the different prey consumed was unaffected by the buccal obstruction. Likewise, Parker and Booth (2013) noted that the *C. borbonica* had a slight effect on the stomach fullness index of *Trachinotus botla* and infestation did not affect the host feeding capability and Fogelman et al. (2009) reported that the decreased growth condition and castration of the *Cheilodipterus quinquelineatus* by *Anilocra apogonae*. In their reports stated, a single parasite was required to adequately stress the parasitized fish actively to result in together impaired growth distress. Being parasitized compromise host energy levels and parasitized fish may have to feed more often and therefore taken more risk to reimburse for this energy source. Infected fish may be less able to escape predators compared to non-infected fish due to altered hydrodynamic and minimized energetic source. Östlund-Nilsson et al. (2005) have proved a reduced in sustained swimming speed of parasitized fish.

In conclusion, this study could be explained by the parasitism are the factors influencing the vulnerability of *P. niger* by the parasites and interspecific differences in infection. The infestation of *C. eremita* was negatively correlated with the relative condition factor, caused an alteration in the health status of host fish *P. niger*, This may be related to the growth and decrease feeding efficiency in hosts, subsequently affecting health condition and survival.

Acknowledgements This study was financially supported by Department of Science and Technology, Govt. of India (SB/SO/AS/063/2014). Authors are thankful to Ahmet Ozer and A. J. Booth for the statistical advice.

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