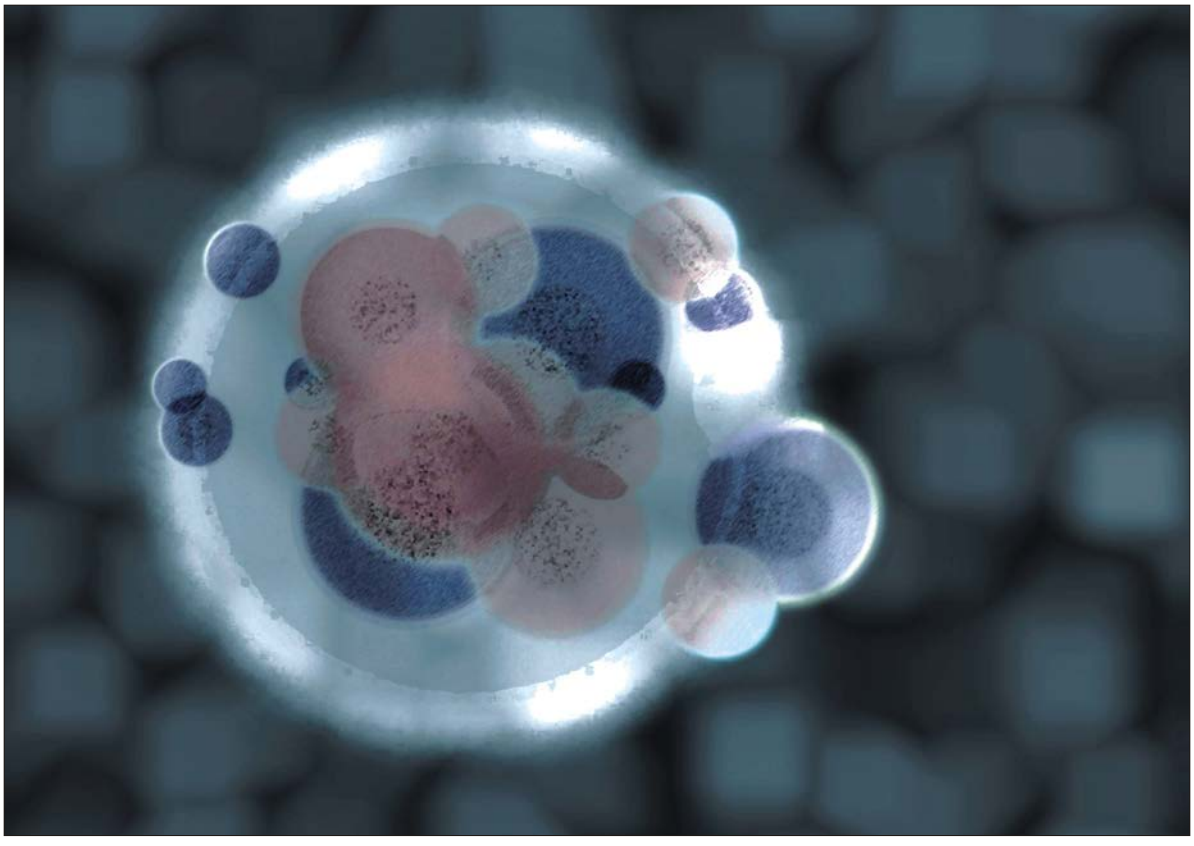

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Presence of an Epibiont *Epistylis niagarae* (Protozoa, Ciliophora) on *Mesocyclops aspericornis* in Velachery Lake Chennai India

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Abstract: Protozoan ciliate epibiont's infestation on the freshwater copepod *Mesocyclops aspericornis* in particular from the Velachery lake Chennai India was studied. The present study investigated species composition, host-epibiont population dynamics, epibiont substrate specificity and attachment preferences of Peritrich epibionts on Cyclops copepods in Velachery lake Chennai. The epibiont *Epistylis niagarae* belongs to the order Peritrichida were measured between 13.3 to 42.6 μm in length and 9.3 to 23.9 μm in width. The cone shaped epibiont with an enlarged disc shaped peristome was lined with conspicuous cilia. The spherical micronucleus was located near the macronucleus. It was observed that the females were more susceptible to epibiont infestation than the males and the copepodites. The percentage frequency of infestation was found to be 53% in females, 31% in males and 16% in copepodites. From our observation it was clearly noticed that the prosome region of *Mesocyclops aspericornis* was highly infested (57%) than the urosome region (43%).

Key words: Ciliophora, peritrichs, *Epistylis* sp., *Mesocyclops* sp., peristome, copepodite

INTRODUCTION

Epibiosis is an interspecific association between two organisms: the Epibiont and the Basibiont. The term Epibiont includes organisms that are attached to the surface of a living substratum, while the Basibiont constitutes a support for the Epibiont. The body surface of crustaceans including copepods serve as a convenient living environment for epibionts like protozoa, bacteria, algae and invertebrates (Fernandez-Leborans and Tato-Porto, 2000; Fernandez-Leborans, 2001, 2009; Hanamura, 2000; Gilbert and Schroder, 2003; Song *et al*., 2002). This association has traditionally been regarded as a commensal relationship. However, epibiotic ciliates sometimes affect host crustaceans adversely by increasing their susceptibility to predation, by hindering their movement, growth, reproduction and ability to survive or by reducing food availability through food competition with the epibiont (Utz and Coats, 2005). Over the last decades, the interaction between the parasite and host and its consequences within and/or between ecosystems have been gathering attention (Ohtsuka, 2006; Wahl, 2008). Members of the groups represent protozoan epibionts: apostomatids, chonotrichids, suctorians, peritrichs and heterotrichs (Lynn and Small, 2000).

Botes *et al*. (2001) reported two new species of *Mantoscyphidia*, Ciliophora: Peritrichia in the gills of *Haliotis*, Archaeogastropoda from the south coast of South Africa. Diverse aspects of the colonization patterns of epibiont protozoan communities on *Caridina lanceolata* (Decapoda) from the Malili lakes of Sulawesi (Indonesia) was studied by Fernandez-Leborans *et al*. (2006). Saler and Dorucu (2008) reported the observation of *Epistylis niagarae* on *Cyclops vicinus* in Hazar Lake. Several authors have raised the issue of host specificity when considering epibiosis. In some instances, epibionts appear specific to only one or two host species and sometimes fail to attach to non-living substrates, as reported for *Epistlylis pygmaeum* colonizing *Brachionus calyciflorus* and *Brachionus angularis* (Gilbert and Schroer, 2003). In contrast, other epibionts (e.g., *Lagenophrys denisi*) are able to colonize living host species (e.g., the crayfish *Cambarellus patzcuarensis*), as well as non-living substrates (Mayen-Estrada and Aladro-Lubel, 2000). The assessment of host specificity may help in understanding the seasonal occurrence of epibiosis in aquatic environments, encouraging further studies about the epibiont's life cycle, such as occurrence of encystment when the specific host is not available.

An attempt had been made to focus on epibionts infestation in freshwater habitats which has got local/regional importance towards the quality of water in Velachery Lake. This is the first report of an epibiont *Epistylis niagarae* on *Mesocyclops aspericornis* recorded from the Lake Velachery. None of the copepod nauplii were infested needs further study.

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MATERIALS AND METHODS

Lake Velachery is one of the prime lakes situated in the urbanized area of the metropolitan city of Chennai Tamilnadu. Lake is located at 12' 59' 15" latitude and 80° 30' 45" longitude. It is a shallow water body with a surface area of 265.48 acres and a maximum depth of 3.8 m (Fig. 1). Zooplankton were sampled during Sep to Nov 2009 by duplicate tows of 16 cm diameter and 150 μm mesh net towed vertically from 1 m of the bottom to the surface and preserved in 4% formalin (Edmondson, 1959). Three 1 mL aliquots from each sample were examined using a Nikon stereoscope binocular microscope (Edmondson, 1959; Cheng, 1964). All adult copepods were identified up to species level and immature copepodites up to genus while nauplii were combined under one single group and morphometric measurements were calculated using an ocular micrometer in μm. For convenience sake the whole body was divided into two parts namely prosome and urosome, where prosome consists of head, thorax, antennae and the urosome consists of egg sac, caudal style regions.

Statistical analysis: The analysis was performed using PAST-PAlaeontological Statistics package version 2.07 with data reported in the text as Mean±Standard deviation of the mean (SD) or ranges when appropriate. One-way ANOVA and Welch F test were calculated using PAST to find the significant level.

RESULTS

Based on the microscopical observation it was observed that out of 422 ind L^{-1} of *M. aspericornis* isolated, only 325 ind L^{-1} were infested with the epibiont *E. niagarae* and the remaining 97 ind L^{-1} were non- infested. The percentage of infestation estimated (Fig. 2) revealed that the females were more susceptible towards epibiont's infestation (53%) than the males and the copepodites (31 and 16%). To our surprise, nauplii encountered during the study were not infested by these epibionts. The reason for this is yet to explore. Analyses of samples collected during the month of Nov-09 showed a maximum percentage (23%) of infestation on

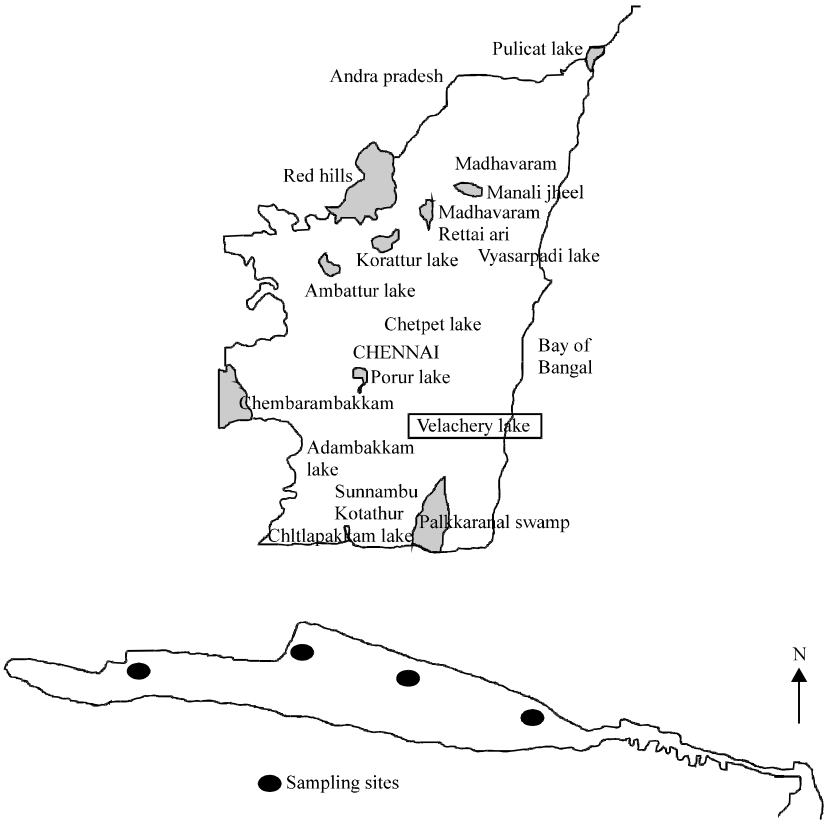

Fig. 1: Map of velachery lake showing the sampling sites

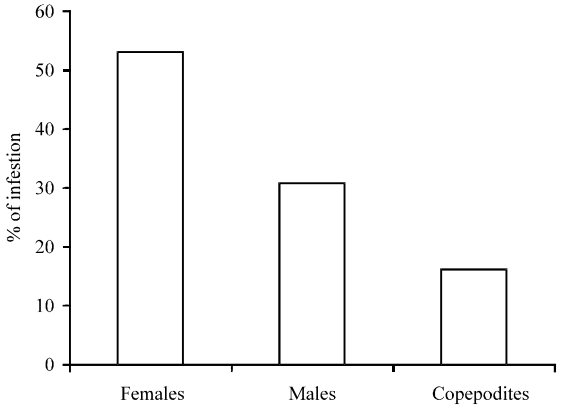

Fig. 2: Infestation of *E. niagarae* on *M. aspericornis*

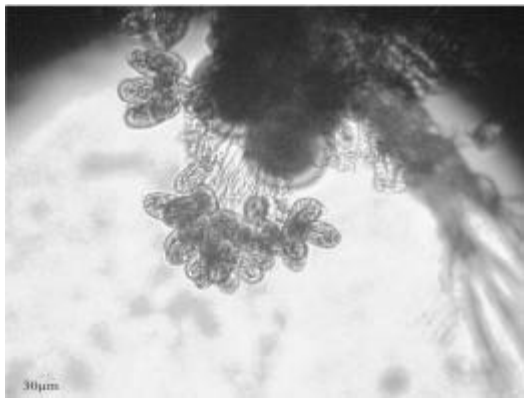

Fig. 3: *Epistylis niagarae* located on the urosome (egg sac) region of *Mesocyclops aspericornis*. Scale bar = 30 µm

the prosome of *M. aspericornis* and minimum percentage (15%) of infestation was noticed during Oct-09 (Table 1).

During the sampling period the percentage of infestation 57% occurred on the prosome of *M. aspericornis* was found to be more than the urosome 43% (Table 1). It was noticed that the epibionts were found to be attached to the body surface (thorax), egg sac and on the setae of *M. aspericornis* (Fig. 3, 4). Figure 5 clearly showed the presence of *Epistylis niagarae* on the prosome (head) region of *Mesocyclops aspericornis*. The freshwater copepod *M. aspericornis* occurred more frequently in the Velachery Lake during the months of Sep, Oct and Nov 2009. The total length of the female ranged from 720-810 µm. The cephalothorax ranged from 390-480 µm. The antennule of the female is composed of seventeen segments and reached the distal margin of the third thoracic segment (Fig. 7).

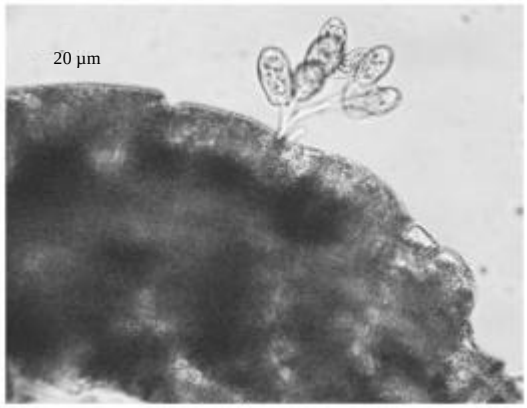

Fig. 4: Epibiont *Epistylis niagarae* observed on the prosome (thorax) region of *Mesocyclops aspericornis*. Scale bar = 20 µm

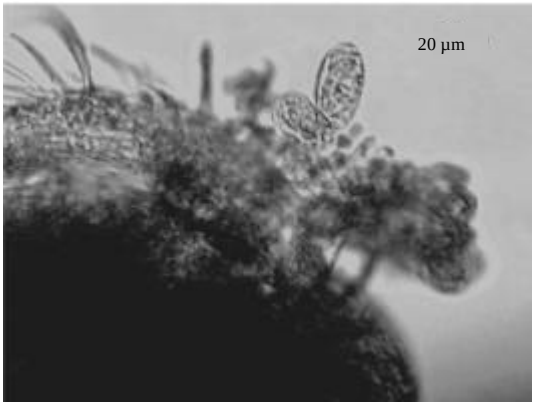

Fig. 5: View of *Epistylis niagarae* on the prosome (head) region of *Mesocyclops aspericornis*. Scale bar = 20 µm

Epistylis niagarae, exhibited a buccal ciliature consisting of a haplokinety and a three- component polykinety, both extending to the middle of the zooid, where P3 was shorter than P1 and P2. The peristome was broad with striated peristomial lip. Body was striated. Pellicular striations were evenly spaced, parallel and uniform. Oral infraciliature of *E. nigarae* was divisible into peristomial part and infundibular part as in other sessiline peritrichs. Adoral zone completed spiral of 360° counter-clockwise around epistomial disc, with haplokinety and polykinety starting almost at same point. Haplokinety and polykinety ran together around peristome and separated before plunging into infundibulum. Symbiotic algae were present throughout

Table 1: Appearance of *E. niagarae* on *Maspericornis* during the sampling period in velachery lake Chennai

	Prosome of *M. aspericornis.*				Urosome of *Maspericornis*		
Months	Head	Thorax	Antenna	(%)	Egg sac	Caudal style	(%)
Sep-09	29	21	13	19	31	6	11
Oct-09	23	17	9	15	43	8	16
Nov-09	36	25	12	23	36	16	16
Total	88	63	34	-	110	30	-
%	-	-	-	57	-	-	43

N = No. of individuals

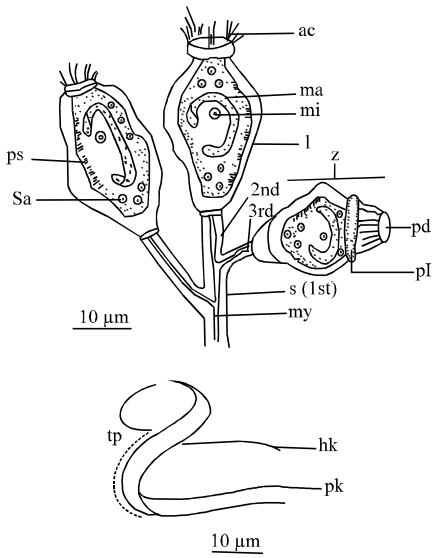

Fig. 6: Epibiont, *Epistylis niagarae* with infraciliature. my-myoneme, ad-adoral cilia, ma-macronucleus, mi-micronucleus, l-lorica, s-stalk, ps-pellicle striations, pd-peristomial disc, pl-peristomial lip, 1st, 2nd, 3rd order stalks, z-zooid, hk-haplokinety, pk-polykinety, tp-third polykinetid

cytoplasm varying in number and size which obscuring the position and shape of nuclear apparatus (micro and macronuclei) in *Epistylis niagarae* (Fig. 6).

Taxonomic position of *E. niagarae* (Sprague and Couch, 1971):

Phylum	:	Protozoa (Honigberg *et al.*, 1964)
Sub phylum	:	Ciliophora (Doflein, 1897)
Class	:	Oligohymenophorea (De Puytorac *et al.*, 1974)
Sub class	:	Peritrichia (Calkins, 1933)
Order	:	Peritrichida (Stein, 1852)
Sub order	:	Sessilina (Kahl, 1933)
Family	:	Epistylididae (Kahl, 1933)
Genus	:	*Epistylis* (Ehrenberg, 1832)
Species	:	*niagarae* (Kellicott, 1883)

Fig. 7: The freshwater copepod *Mesocyclops aspericornis*

Morphological characteristics of *E. niagarae*: Among members of the epizoic genus Epistylis the *E.niagarae* is a colonial peritrich consists of 40 to 50 telotrochs/colony. The cone shaped body was measured between 13.3-42.56 µm long and 9.31-23.94 µm wide with an enlarged disc shaped peristome lined with conspicuous cilia on the anterior side (Fig. 6). The diameter of peristomial lip measured from 10.64 to 13.30 µm. The macronucleus which resembled the English alphabet C was located longitudinally (10.64-15.96 µm long, 5.32-9.31 µm wide). The spherical micronucleus was located near the macronucleus. The stalk of the epibiont

Table 2: ANOVA test

SoV	Sum of Sqrs	df	Mean square	F	P
Between groups	5163.43	6	860.571	1.701	0.1702
Within groups	10625	21	505.952		
Total	15788.4	27			

p>0.05 not significant

Table 3: Morphometric features of *Epistylis niagarae* in μm, n = 30

Features	Mean	SD	Minimum	Maximum
Body length	23.49	8.96	13.30	42.56
Body width	15.51	5.02	9.31	23.94
Stalk length	38.26	7.54	26.60	49.21
Stalk width	3.35	1.54	2.66	6.65
Macro nucleus length	14.98	3.49	10.64	15.96
Macro nucleus width	8.72	1.43	5.32	9.31
Peristomial lip diameter	10.62	1.16	10.64	13.30

SD: Standard deviation, n = 30, values in μm

was elongated 26.6- 49.2 μm long, 2.66- 6.65 μm wide. The stalk contained a contractile myoneme along the whole of its length (Table 3). Welch F test in the case of unequal variances, f = 1.102 df = 9.199 p = 0.4284 which was (p>0.05) not significant. The results got from One-way ANOVA where p = 0.1702 (p>0.05) which denoted that there were no significant differences between the infestation found on the prosome and urosome (Table 2).

DISCUSSION

The first observation of *E. niagarae* infests on freshwater *Cyclops vicinus* living in Hazar Lake was reported by Saler and Dorucu (2008). We reported here the same fortuitous observations of freshwater copepod *Mesocyclops aspericornis* which was heavily infested by peritrichous ciliates namely *E. niagarae*. In natural environments ciliate epibionts rarely produce mortality in their crustacean basibionts. These epibionts can cause gill and surface fouling disease in shrimps at high densities, typically under stress conditions (Lightner, 1996). In some cases, they become opportunistic pathogens in culture ponds during periods of low oxygen concentration (Johnson, 1978). Present findings could represent that the ciliate protozoans act as epibionts without causing any harmful effect on the host by giving contradictory views of the above. Saler and Dorucu (2008) also noticed that there was no incidents of infestation occurred on *Cyclops vicinus* by the ciliate protozoan *E. niagarae* from Hazar Lake in Turkey. Present findings revealed that the females were more susceptible towards epibiont's infestation in Velachery Lake. Similar results have been reported in ciliate-crustacean associations (Xu, 1992; Hanamura, 2000; Fernandez-Leborans, 2009).

The epibionts may leave the basibiont due to moulting. For this reason, the protozoan epibionts like suctorian and peritrich ciliates may adapt their life-cycles in such ways as moulting-induced reproduction and formation of mobile stages which are able to reinfest the basibionts (Overstreet, 1987). The same genera of protozoans are located on different anatomical units of different genera of crustaceans. Location of the protozoan on the host is specific and not random. The reasons for the specificity of protozoans for characteristics sites on their hosts are diverse and related to host habits. Water currents formed by the hosts that carry food particles are exploited by their epibionts (Morado and Small, 1995). Females with broader body surface were highly infected with *E. niagarae* while some other studies have indicated host-size independence. The larger colonies will occupy a larger surface area, thus preventing the attachment of other colonies. In present study, the absence of *E. niagarae* on nauplii and less percentage of infestation on younger copepodites may be due to the process of moulting where shedding of carapace removed epibionts during their developmental stages. The nauplii of Velachery Lake were completely free from epibiont infestation. One of the reasons pointed out by Overstreet (1983) towards infestation on larger animals was the size of the carapace which supports the epibiont to live on and by supporting our results from Velachery Lake.

Epistylis niagarae attached to the entire body of their copepod hosts but overall infestation density and load of *Epistylis* sp. were significantly higher on adults than copepodites and nauplii of *M. aspericornis*.. This result supported from what was found for other systems where high infestation was observed on the adult stages of planktonic crustacea (Xu, 1992; Willey and Threlkeld, 1993). Higher density of colonies and zooids on copepodite stages was not encountered in the result because colonies were shed with the cast skin every time copepodites molt, forcing epibionts to find another substrate. Accordingly, adult copepods should constitute a more stable substrate for epibionts because they do not molt and can accumulate a higher density of colonies.

One possible explanation for the difference in infestation density observed between adults and copepodites in the Velachery Lake, was that heavily infested adults could have a higher predation risk than juvenile stages, since epibionts would increase the apparent size of adults and make them more visible to fish that prey visually. Willey *et al.* (1990) demonstrated that prevalence of *Colacium vesiculosum* on *Daphnia* spp. was significantly lower in experimental tanks containing

fish than in control tanks, suggesting that epibionts made the host more susceptible to predation by increasing size and contrast. Moreover, epibionts may affect swimming behavior and escape reaction by increasing drag forces and making hosts also more susceptible to fishes that are pump filter feeders (Willey *et al*., 1990; Chiavelli *et al*., 1993).

When presence of epibiont *Epistylis niagarae* was analyzed across host body parts, infestation density and load were higher on the cephalothorax and abdomen of *Mesocyclops aspericornis* when compared to antennae or swimming legs. Epibionts have been reported to attach to sites of the host where chances of gathering food are high. This result supported Evans *et al*. (1979) who found that the suctorian *Tokophrya quadripartita* attached especially to the head of *Limnocalanus macrurus* and attributed this preference to the carnivorous habits of this ciliate. Also, *Colacium vesiculosum* colonized the post abdomen of *Daphnia* spp. suggesting that the epibiont was able to take up dissolved nutrients eliminated by its host. The preferential attachment observed for *Epistylis* sp., however, was not apparently related to availability of food but might be related to the mobility of body parts, since copepods use antennae and legs for swimming and food gathering. The presence of epibiont colonies on antennae and swimming legs demonstrated that once epibionts attached to these body parts they can grow but the significantly lower colony density suggested that these mobile parts were harder to colonize than cephalothorax and abdomen.

CONCLUSION

Based on this we concluded that the adult females and males with lengthy body surface area were more susceptible to epibiont infestation than the copepodites and nauplii. The exact reason for epibionts' infestation and its preference needs further study. In the present study, we documented that the epibiont colonies were denser on the cephalothorax and abdomen of copepod hosts when compared to antenna and swimming legs, demonstrating that more mobile parts are harder to colonize.

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