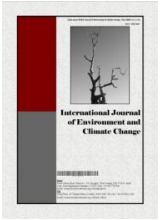

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Infestation of Ostracoda *Vargula tsujii* (*Myodocopa*: Cypridinidae) in *Lethrinus ornatus* and *Carangoides gymnostethus* from Pamban, Southeast Coast India and Its Variation in Prevalence and Abundance with Respect to Seasonality

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Authors' contributions

This work was carried out in collaboration among all authors. Author AG conceived and designed the research, administered and supervised the study, reviewed and edited the final manuscript. Author SJ carried out the research, compiled and analysed the data and wrote the original manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Ostracoda is a diverse group of aquatic crustacean and often infest the fishes and cause huge economic losses. In the present study, the infestation of Ostracoda *Vargula tsuji* in major food fishes *Lethrinus ornatus* and *Carangoides gymnostethus* was studied. A detailed investigation by using biotechnological and molecular tools, it was identified that Ostracoda present in these fishes was *Vargula tsujii* and the sample was deposited with GeneBank (NCBI MN889442). An attempt was also made to study the abundance and degree of infestation for different seasonality *viz* post-monsoon, monsoon, presmonson and summer during 2019. Weekly samples were made from

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Pamban (9.27°N, 79.22°E), Gulf of Mannar, fish landing center and reported the monthly average values. Total 1405 ± 296.5 of *L. ornatus* were examined during Jan-Dec 2019, of which 285.5 ±70.2 (20.31%) were found infested with Ostracoda and in the case of *Carangoides gymnostethus,* out of total 1235.9 ± 205.2 fishes examined, 201.4 ± 47.2 fishes were found with infestation i.e. 16.30% but varying with seasonality. Both *L. ornatus* and *C. gymnostethus*e fishes had *V. tsujii* attacked to their gills at a significant level ($p < 0.05$), was an incidence of occurrence of infestation of *V. tsujii* in their buccal cavity of the intestinal track but not to the significant level. The infestation of *V. tsujii* in fishes from Indian water is reported for the first time and its prevalence and abundance level for seasonality are presented in this study.

Keywords: Ostracoda; Vargula tsujii; infestation; molecular tools; PCR; abundance; seasonality.

1. INTRODUCTION

The infestation of metazoan and protozoan animals play an important role in the ecology of aquatic ecosystems. They can cause harm to the host by tissue damage and can also make the host more susceptible to secondary infection, by weakening host immunity and subsequent economic losses resulting from fish mortality [1]. Endoparasitic diseases affect the normal health conditions and cause reduction of growth, abnormal metabolic activities, and even death of affected fish. According to Kabata [2], factors that directly influence the abundance and prevalence of endoparasitic fauna of fishes include; age, diet, environment of fishes, and season.

The estimation of the economic cost of a parasite event is frequently complicated by the complex interplay of numerous factors associated with a specific incident, which may range from direct production losses to downstream socio-economic impacts on livelihoods and satellite industries associated with the primary producer and landed fished [3]. Cypridinid (myodocopid) ostracods are a diverse group of small aquatic crustaceans usually around 0.3 to 5mm in length. Their most distinctive feature is their calcitic carapace, a hard bivalve, hinger shell that can entirely cover and protect the non-mineralized bodyparts and appendages. The scientific reports available on the Ostracoda are very limited. Ostracoda is an ancient, ecologically diverse, monophyletic group of crustaceans with a dense stratigraphic record. Ostracods are a nexus for interdisciplinary studies in evolution, ecology, limnology, geology, paleontology and luminescent in nature [4-6]. Recently, Goodheart et al have cultured California Sea Firefly *Vargula tsujii* (Ostracoda: Cypridinidae) on a laboratory scale and attempted to develop a model system for the evolution of marine bioluminescence [7].

The feeding habits of the species belonging to the family Cypridinidae have been reported as collectors [8], scavengers [9-11], predators [12,13], and parasites [14]. It is also reported that some cypridinid ostracods bite live fishes. Stepien and Brusca observed that adult nearshore fishes confined in cages on the seafloor were attacked at night by swarms of crustacean zooplankton [15]. Ostracods *Vargulu tsujii*, in hundreds attacked the fishes, however, they caused only minor external damage and were found only inside fishes that had lesions produced by cirolanid isopods. Stepien and Brusca have observed that *V. tsujii* clustered around uncaged fishes but they periodically shook them off or moved to other locations [15]. Some ostracods may therefore be common scavengers:'micropredators on fishes restrained in traps, but the normal behaviors of uncaged fishes (burying, cocooning, or swimming higher in the water column) "protect" them from attack [15].

Except for a few old reports, not much study on the feeding habits particularly parasitic behavior of cyprinidis were done. Wilson described *Vargula parasitica* from the gills and nostrils of *Sphyrna zygaena* (smooth hammerhead shark) and the gills of ~~a~~ sea bass, *Epinephelus adscensionis*, and a jackfish, *Caranx crysos* [16]. The author had concluded that since there were many ostracods regularly arranged on the gills with a sort of pocket-like structure was the evidence for the ostracods had remained in position for some time, providing proof of a parasitic mode of life. Monod had observed the occurrence of *Skogybergia squamosa* on both dead fishes in a trap and also on the live scorpionfish (*Scorpaena scrofa*), hence he considered it as a facultative parasite. Monod observed the ostracods attached firmly to the mucus and was very likely feeding on the blood of their host [17]. Harding had scrapped the Ostracoda *Sheina orri* from *Taeniura lymna* and

Herniscyllium ocellatum and described that they were parasitic [18]. However, Cohen had suggested that no myodocopids are truly parasitic and he observed in his studies that the ostracods only attacked fishes that were injured or unhealthy as a result of trapping [9]. None of the previous studies of possible parasitism in cypridinids have investigated the attachment of the ostracod or its effects on the host tissue. Bennett et al. had re-examined the occurrence and distribution of *Sheina orri* on the gills of the epaulette shark, *Hemiscyllum ocellatum* and observed that ostracoda were often located in the distinct pocket formed by local distortion of shark respiratory lamellae due to considerable time of attachment suggested that ostracoda are parasitic nature [14]. Kornicker [19] reported that a cypridinid feeding adaptation is seen in the articulation of the mandible which allows being taken directly to the mouth region, Kornicker [20] also speculated that Maxillula and mandibular claws of Ostracoda *Sheina orri* are used to cling to the gills of host fish.

There are many reports on the distribution of some Ostracoda in sediments from the Indian coast [21-25], however, no report is available on the occurrence of any Ostracoda in any fish species from Indian waters. The objective of this study is to investigate the infestation of Ostracoda in common and major food fishes *Carangides balabaricus* and *Lethridinae ornatus* off Pamban, the Southeast coast of India, and its degree of infestation for seasonality for the first time.

2. MATERIALS AND METHODS

2.1 Collection of Fish Samples

Samples of food fishes belonging to Carangidae and Lethridinae families were collected from freshly captured (by trawlers) fish stock in Pamban (9.27°N, 79.22°E), Gulf of Mannar, India. All fishes were dead at the time of capture and 15-38 fish samples of each species were made every week and examined the infestation of Ostracoda on the gills. The fishes found with the occurrence of Ostracoda in the gills were preserved in ice-box thermocol and taken to the lab for further examination. The average of 4-5 per month was reported as monthly average readings. The body mass *carangidae gymnostethus* examined were 570 ± 165 g (mean and S.D.), the snout-tail length was 345 ± 67 mm. and in the case of *Lethrinus ornatus*, it was 660±310 g and 348 ± 75 mm respectively. The number of Ostracoda present in the fishes examined was done manually and size was measured by analog vernier caliper (accuracy level up to 0.02 mm). Total 1405 ± 296.5 fish samples of *L. ornatus* and 1235.9 ± 205.2 fish samples *C. gymnostethus,* were examined between January to December 2019 and recorded the prevalence and abundance of Ostracoda *V. tsujii*.

2.2 Examination of Ostracoda

The fishes infested with Ostracoda were dissected and picked up the Ostracoda present in gills, buccal cavity, and intestinal part with help of forceps and needle, and abundance was recorded and reported as monthly average readings by following the procedured described by Gibson [26]. Some of the collected Ostracoda were preserved in IPA for identification and further studies and the remaining were preserved in 4% formaldehyde in seawater. Ostracoda samples preserved in IPA were taken for PCR and SEM studies. Material for scanning electron microscopy was prepared as per the method described by Bennett et al, [14] was fixed in modified Karnovsky's fixative (2% para formaldehyde, 2.5% glutaraldehyde in cacodylate buffer. pH 7.6) at 4 C. Following 3 buffer washes, ostracods were post-fixed at 4 C in 1% OsO_2 for 1 and they were dehydrated with acetone washing. Samples were sputter-coated with gold and were examined using a JEOL JSM-7401F scanning electron microscope at an accelerating voltage of 15 kV GB low. Ostracods collected for light microscopy were fixed in 4% formaldehyde in filtered seawater, dehydrated to acetone (AR grade) infiltrated, and embedded in paraffin wax, secured (7lm). and stained with H & E. All Ostracoda specimens collected were preserved in 4% formalin.

2.3 Examination of Samples and Identification of Parasites

2.3.1 Extraction of DNA

Extraction of DNA from Ostracoda collected from infested fishes was according to the method described by Yamaguchi and Endo [27]. All reagents, consumables, and equipment used throughout the procedure were sterilized, and used Double distilled water was used to prepare all buffers. Carapace-valves of each ostracod was crushed against the side of the tube using a sterile pipette tip, then washed with PBS (1X) for 15 minutes

DNA of the species were isolated with XpressDNA tissue/Cell line DNA isolation Kit (MagGenome, India) as per the manufacturer instruction briefly, microscopically identical samples were grouped to the final weight of ~100 mg into the sterile microcentrifuge tubes and the samples have been minced completely using a homogenizer and Proceed to tissue lysate preparation. A 750 µl of Tissue/Cell Line Lysis Buffer was added to the homogenised sample along with 20 µl of RNase A, the mixture was vortexed for 30 seconds and incubate at room temperature for 15 minutes. To the solution, 20 µl of Proteinase K was added and mix by vortexing the tube for 30 seconds. The solution was incubated at 56°C until the lysate appears clear. The clear lysate mixed completely by pipetting and centrifuge at 14000 rpm for 5 minutes at room temperature. The supernatant was transferred to a fresh 1.5 ml micro centrifuge tube and added 450 µl of Tissue/Cell Line Mg-Na Mix and mixed the solution by inverting the tube 6 - 8 times and kept undisturbed for 5 minutes at room temperature. After incubation, the tube was placed on the MagNa Stand for 5 minutes. Carefully discard the supernatant without removing the tube from MagNa Stand . the pellet 250 µl of Tissue/Cell Line Wash Buffer 1was added and removed the tube from the MagNa Stand to resuspend the pellet by pipette mixing for about 8 - 10 times. Again the tube was placed back on MagNa Stand until the solution becomes clear (30secs – 1min). Carefully discard the supernatant without removing the tube from MagNa Stand. The pellet was washed with 500 µl of Tissue/Cell Line Wash Buffer 2, by gently invert mix the tube without removing it from Mg-Na Stand for about 5 - 6 times. The washed solution was discarded without removing from the MagNa Stand. The pellets were air-dried without removing the tube from MagNa Stand at room temperature for 10 minutes. After drying, remove the tube from MagNa Stand and dissolved the DNA by adding 50 – 100 µl of Tissue/Cell Line Elution Buffer to the tube and resuspend the pellet thoroughly by pipette mixing 10 - 12 times. To enhance the yield the pellets were incubated at 56°C for 5 minutes with intermittent tapping. The DNA was removed from the MagNa by placing the tube on MagNa Stand for 5 minutes or until the solution appears clear, Carefully transfer the supernatant containing the DNA to a fresh sterile 1.5 ml microcentrifuge tube, without removing the tube from Mg-Na Stand and stored at -20°C until it was used.

2.4 PCR and Sequencing

Molecular species identification was studied as reported earlier by Yamaguchi and Endo [27] with minor modification, briefly, 18s rRNA gene was amplified using 18s F1 and R7 primer. The PCR was carried out in a 50 µl reaction solution containing 1X master mix (Xcelris, India) 0.5 µM of each primer with 150 ng of total genomic DNA as a template. PCR has performed over 35 cycles. Each cycle consisted of denaturation at 94 C for 30 s, annealing at 52 C for 30 s, and extension at 72 C for 1 min. The reaction was completed with a final 5 min incubation at 72 C. The PCR products were electrophoresed in 2% agarose gels, excised, and purified for sequencing reactions, using Gel Purification kit (Xcelris, India) and following the guidelines provided with the kit. Further, the amplicon was sequenced with an ABI 3730xl 96 capillary system using Big Dye Terminator v3.1 kit as per the manufacturing kit.

2.5 Prevalence of Abundance of Ostracoda

Prevalence and abundance of ostracoda infested in *Carangides gymnostethus* and *Lethrinus ornatus* specimen was calculated using the following formula:

Prevalence = Total no. of infected fish (x100) / Total no. of fish hosts examined.

Abundance was calculated according to Ekanem et al. [3] as follows:

Abundance (%) = Total No. of parasites recovered / Total no. of fish host examined X 100

Calculation of affected parasites was estimated as follows

Affected fishes (%) = (Total No. of fishes with parasitic infestation / Total no. of fish host examined X 100.

2.6 Statistical Analysis

Data are presented as means ± SD of at least twelve independent measurements. A one-way analysis of variance (ANOVA, SYSTAT version 7) was used to determine the prevalence and abundances of Ostracoda infested in fishes. A Tukey's HSD test was applied for post-hoc comparison studies and data were considered statistically significant when $p < 0$

3. RESULTS AND DISCUSSION

3.1 PCR and Molecular Tools for Species Identification

PCR studies (Fig. 1) and molecular tools revealed that the species collected was *Vargula tsujii* and it is in agreement with a previous report (Yamaguchi and Endo, 2003). 18s rRNA gene was amplified using 18s F1 and R7 primer. The nucleotide sequence as shown below confirmed that the Ostracoda species infested in *C. gymnostethus* and *L. ornatus* was *Vargula tsujii* [GeneBank (NCBI MN889442)]

>ST1_R

GCTAAAAGCCACCTCTTGAAAATGCGCCACG
CAATCAAGGGACGCACATCCAGTGGCATC

AAAACCAAAAACCAACCAACAAACAAAAAAAT
AAACCTTACTTTATCTCTTCTTTCTTTA

TCAACATCTACCAACACTGTCTCATACAAGAA
TTAAATCTTAGTACGAAGGAACAGCATG

TGGCAGTAATAGTAAATTTATTACTGTTAATG
ATCCTTCCGCAGGTTCACCTACGGAAAC
CTTGTTACGACTTTTACTTCC

>ST2_R

AGCTTTCTCGCCCAGGGGGTTCTCAGACAAC
TTTGGGTTTTTATAGGAAAAACGGAGCTC
GGGCGCTCCATCCCGCTAAGCCGCCACCCG
GAGGCGGGCGGACAGAGCAGGGTAGGAGG
C
ATTGAGCCCCCCTCCTCTCCCCGGAAGGGA
GGGGAAGGTCCACGGGCACCCGGAGGCGC
G

CGGAGGGCGGTCAGGGCGAGGCCGCCGCG
GCGCGCTAGAGTGTTAAGGTTCCGACTGTCG

GCGCGCGCGTTGGGGCCGGACCCAGAACCA
CGAAGGGGAACCGCTCGAAACCCCCCCCCC

CCCCCTGCTCCCCCCCCAAAAAAAGACCGC
CTTGCCCTCCCGGAAGGGGGGACGGGGGG
G

GGGGGGGGGTAAAAAATGAGAAAAAGGGGG
GGGTTTTTCATCCAGCGGACCCCCCCCGGG
GAAAGAAGGAGGGCCGGCCGAGCCCCCCC
CGCCCCACCAAA

3.2 SEM and SM Image of *V. tsujii*

Scanning Electron Microscope (SEM) image of Ostracoda *V. tsujii* collected from *C. gymnostethus* is given in Fig. 2 and is measured to have 3.5 mm length and 2 mm width with 0.9 mm of an antenna and its furcae and appendages are shown clearly in its Stereo Microscope (SM) image (Fig. 3).

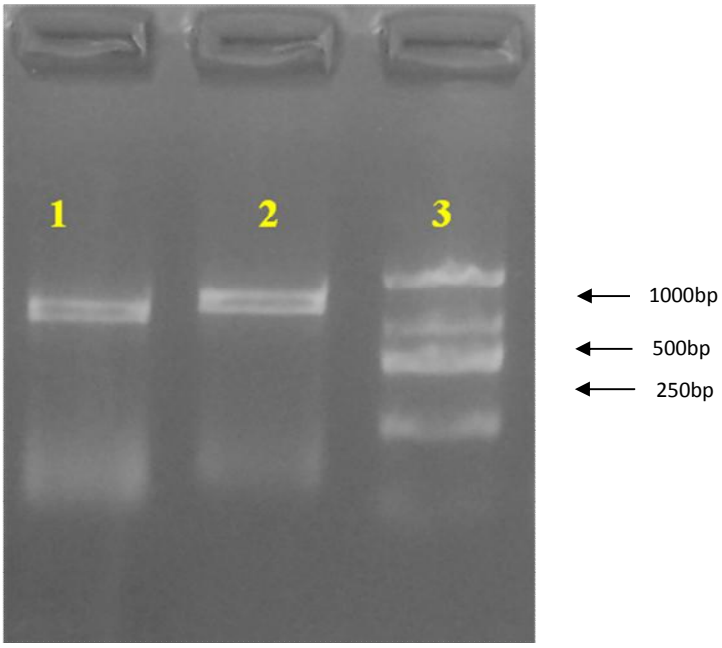

Fig. 1. PCR amplification images of the 18S rRNA gene bands of the ostracoda *V. tsujii* isolated from *C. gymnostethus*, Lane (3): Ladder; Lane 1 & 2: 18S rRNA (ribosomal RNA) of F1 & F2 isolates

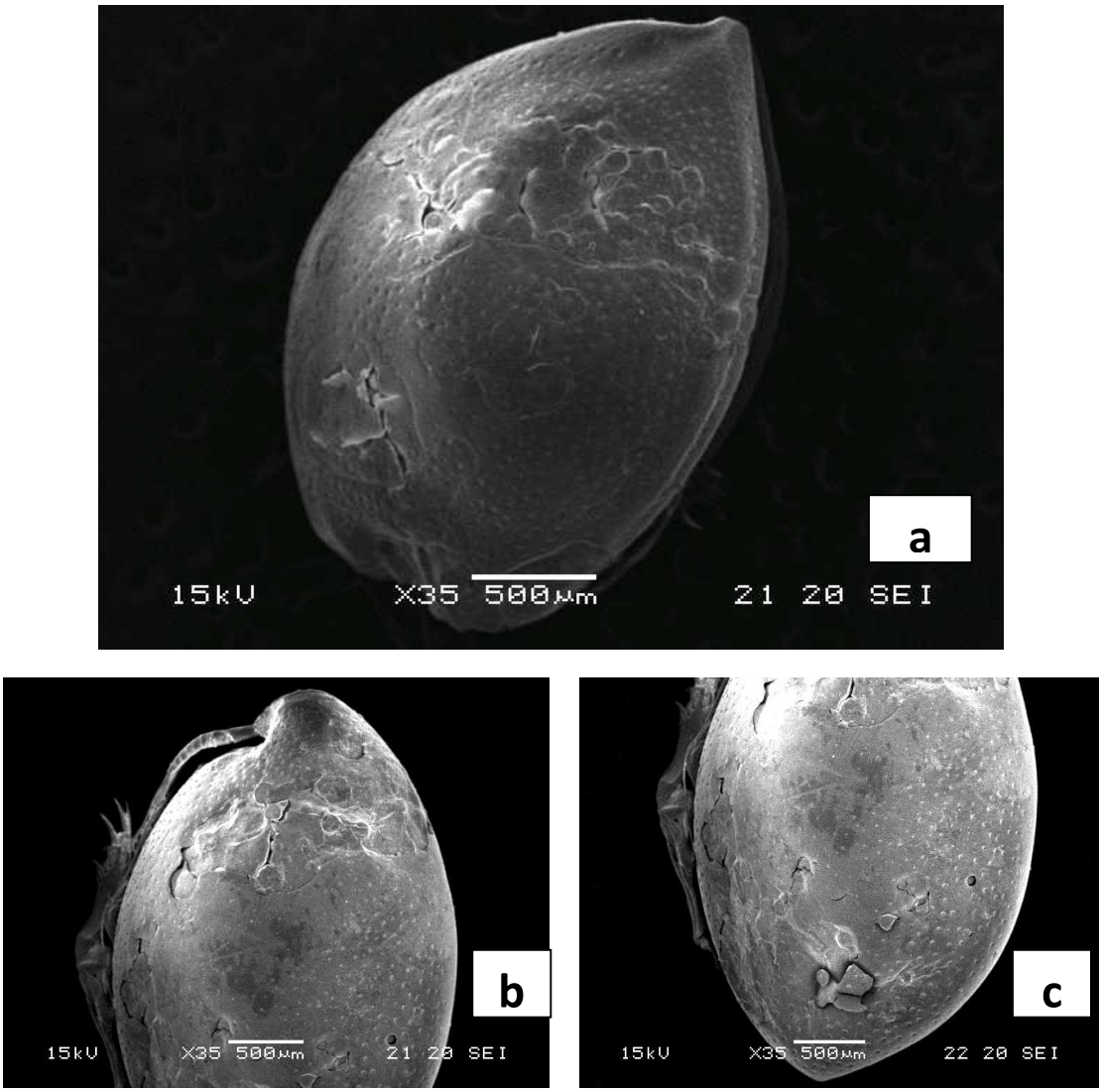

Fig. 2. Scanning Electron Microscope (SEM) image of Ostracoda *V. tsujii* observed from *Carangoides gymnostethus,* a – Full view , b – Anterior view, c – Posterior view of *V. tsujii*

3.3 Prevalence and abundance of *Ostracods* infestation in *C. gymnostethus* and *L. ornatus*

An infestation of *V. tsujii* in *Carangoides gymnostethus* and *ethrinus ornatus* is shown in Figs. 4-6. Total 1405 ± 296.5 of *Lethrinus ornatus* were examined during Jan-Dec 2019, of which 285.5 ±70.2 (20.31%) were found infested with Ostracoda. The body mass *Lethrinus ornatus* examined was 660 ± 310 g (mean and S.D.), the snout-tail length was 348 ± 75 mm. In the case of *Carangoides gymnostethus* 1235.9 ± 205.2 were examined in total and infestation of *V. tsujii* found with it was 201.4 ± 47.2 i.e. 16.30% and body mass of total fishes examined was 570 ± 165 g (mean and S.D.) with length (snout-tail)of 345 ± 67 mm. The highest number found in the gills of both the species studied, i.e. 369 in *L. ornatus* (Fig. 5b) and 377 in *C. gymnostethus* (Fig. 4a). A small number of ostracods were attached to the buccal cavity and intestines of both the fishes tested but it was not statistically significant. Close examination of the surface of the filament margin in the gills showed no discrete puncture marks or grooves in the epithelium cells. The largest number of Ostracoda *V. tusjii* was found between adjacent gill filaments and on the out margin of gills as well. The size of *V. tusjii* examined in this study

varied and it was 3.45 ± 0.38mm by anterior-posterior position and 1.3 ± 0.13 mm of a dorso-ventral region with a width of 2.0 ± 0.15mm (Figs. 2 and 3).

3.4 Seasonality in the Infestation of *V. tsujii* in *C. gymnostethus* and *L. ornatus*

Prevalence of infestation of Ostracoda *V. tsujii* in *C. gymnostethus* and *L. ornatus* in different seasons during 2019 is given in Table 1. The prevalence of infestation of *V. tsujii* in both these species was found higher in pre-monsoon months (Jul – Sep) and it was 24.84 % and 30.91% in *C. gymnostethus* and *L. ornatus* respectively, followed by summer (15.98% and 16.86%) and winter seasons (16.71% and 12.68%). The degree of infestation of *V. tsujii* in *C. gymnostethus* and *L. ornatus* in monsoon was 9.98 and 13.90% respectively but not statistically significant. A similar trend was observed abundance of *V. tsujii* with respect to seasonality in both species studied (Table 2 and Fig. 7). The higher percentage abundance was recorded in the month of August and it was 66.3 ± 13.7 (*C. gymnostethus*) and 77.0 ± 21.1 (*L. ornatus*). The occurrence of ostracoda in the gills of *C. gymnostethus* in different seasons winter, summer, pre-monsoon, and monsoon was 16.67%, 34.30%, 56.33%, and 18.60% respectively and a similar trend was recorded in *L. ornatus* also (20.3%, 26.70%, 65.7% and 10.30%), thus higher degree of infestation was observed in pre-monsoon months. There was sporadic incidence of infestation of *V. tsujiiin* in the mouth of *C. gymnostethus* (2.7%, 9.3%, 9.0%, and 4.33% with different seasons) and in *L. ornatus* (3.0%, 5.0%, 10.7%, and 0.0%), the infestation was found only in the pre-monsoon season in the intestinal tract and not to the significant level.

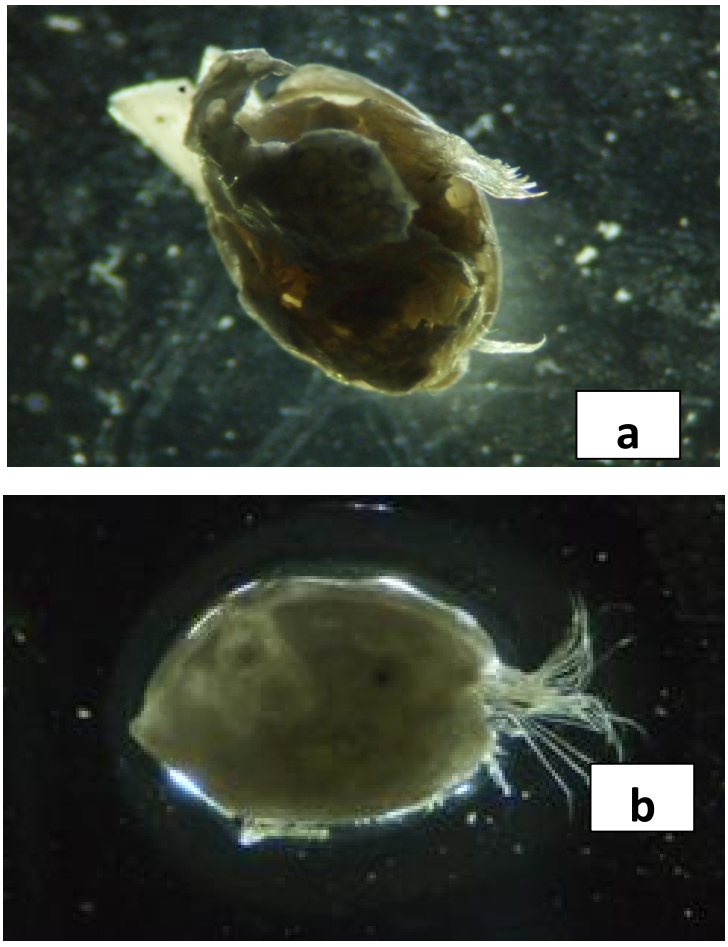

Fig. 3. Stereo microscopic image of Ostracoda *Vargula tsujii* infested in *Carangoides gymnostethus*; a-Lateral view with carapace open; b-Dorsal view with appendages

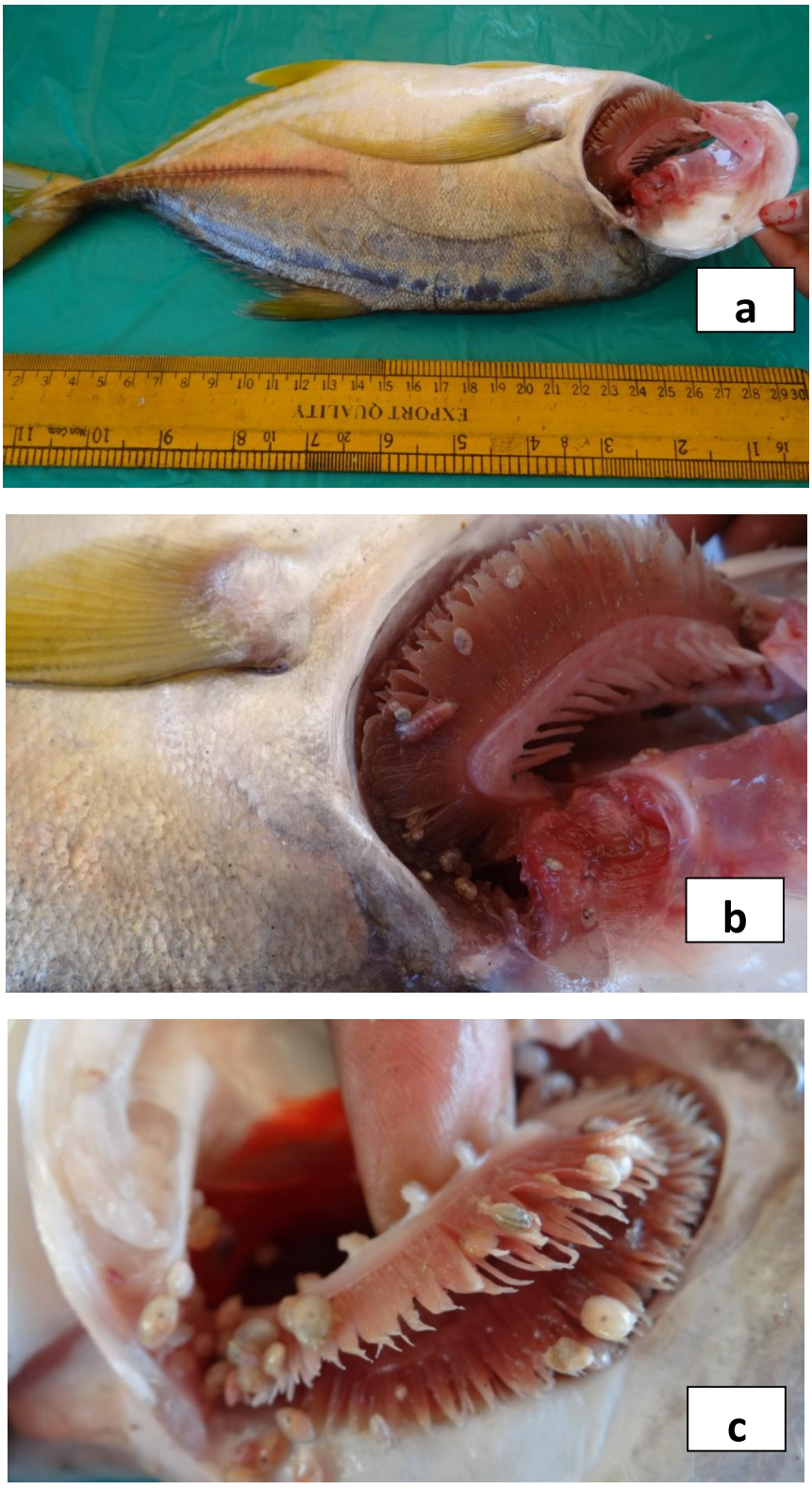

Fig. 4. Infestation of Ostracoda *Vargula tsujii* in gills of *Carangoides gymnostethus*: a – Full view of fish with infestation of *V. tsujii*, b & c close view of gill affected with *V. tsujii*

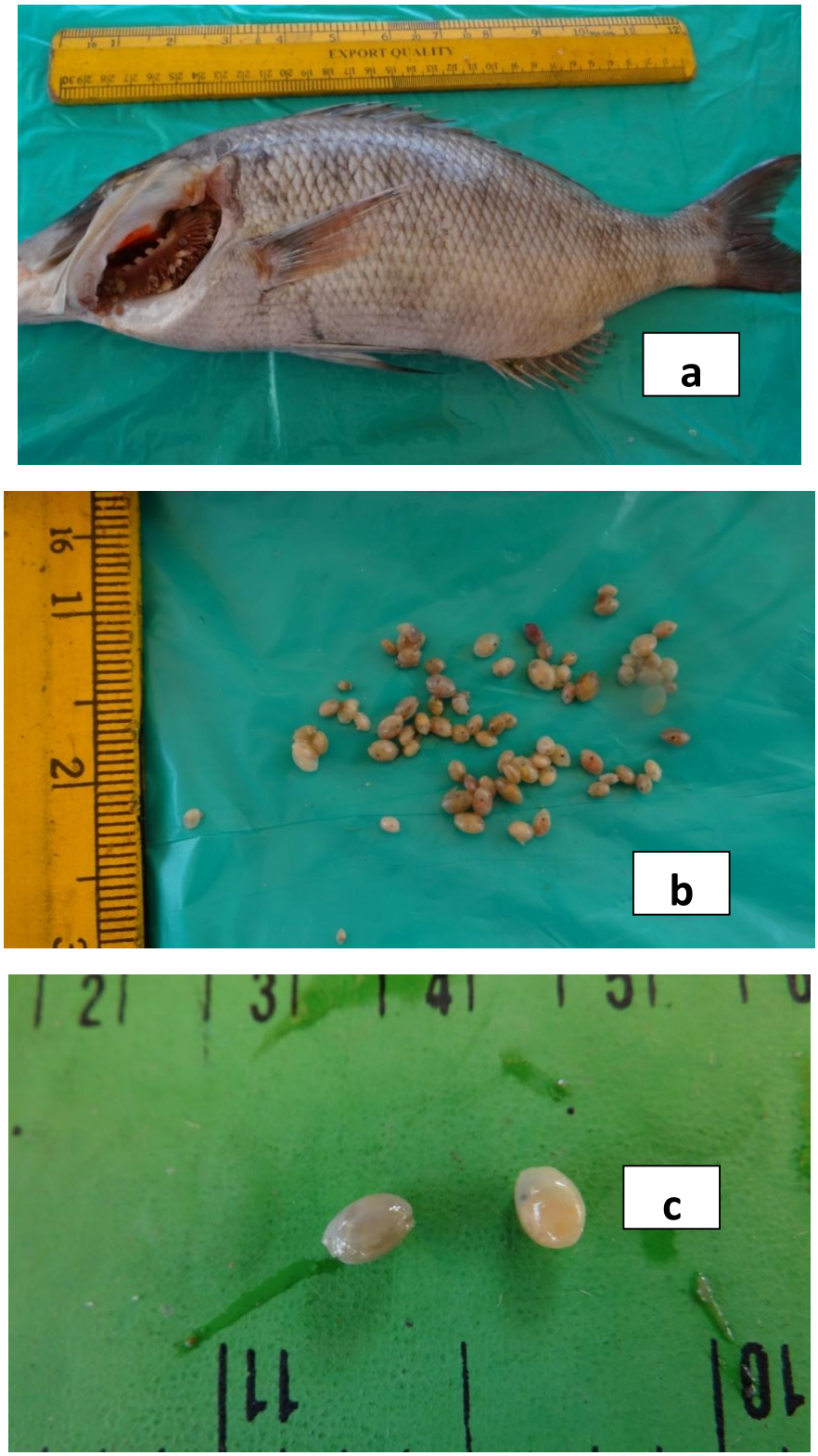

Fig. 5. Infestation of Ostracoda *Vargula tsujii* in gills of *Lethrinus ornatus*: a - Full view of fish with infestation of *V. tsujii;* b - *V. tsujii* collected from gills; c - Close view *V. tsujii*

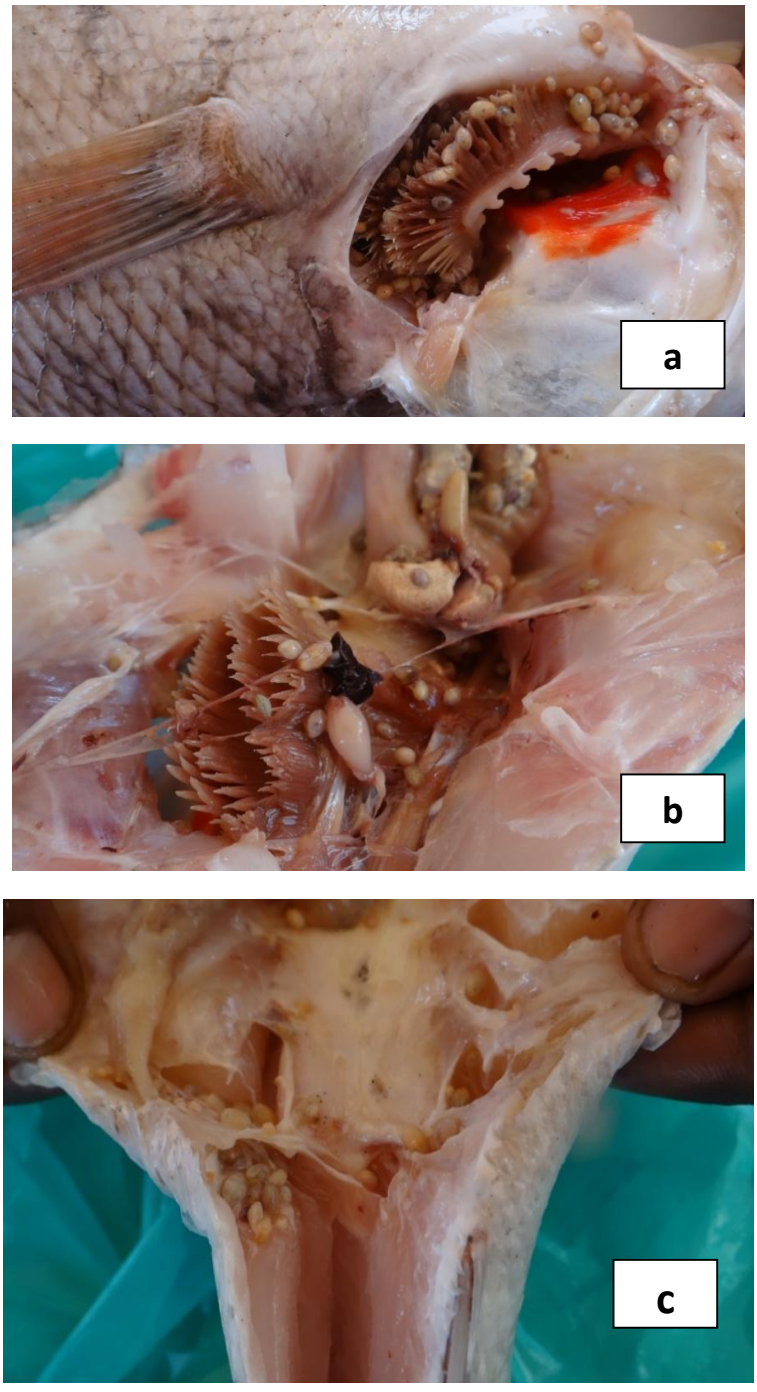

Fig. 6. Infestation of Ostracoda *Vargula tsujii* in different parts of *Lethrinus ornatus* ; a - infesstion in gill region; b – infestation in in smouth region; c – infestation in interstinal region

The infestation of metazoans animals has not an only major impact on global finfish and shellfish aquaculture, having significant effects on-farm production, sustainability and economic viability and it also causes heavy losses to the capture fishes also. PCR studies and molecular tools results confirmed that the ostracoda species infested in fishes studied was *Vargula tsujii* and its nucleotide sequence has been deposited with GeneBank (NCBI MN889442).

Table 1. Prevalence of infestation of Ostracoda *V. tsujii* in *Lethrinus ornatus* and *Carangoides gymnostethus* during Jan-Dec 2019

Seasons	Months	*Lethrinus ornatus**			*Carangoides gymnostethus**		
		Total no. of fishes examined	Total infested	Prevalence (%)	Total no. of fishes examined	Total infested	Prevalence (%)
Post Monsoon	Jan	99.0 ± 15.5	15.1 ± 5.2^{aa}	15.15 ±5.0	83.6 ± 15.8	12.2 ± 3.3 ^{ab}	14.46 ± 5.0
	Feb	125.5 ± 10.2	23.0 ± 4.6^{ab}	18.40 ± 4.4	109.5 ± 12.0	10.9 ± 4.0^{aa}	9.17 ± 6.0
	Mar	162.2 ± 22.5	26.5 ± 7.0^{ab}	16.05 ± 2.0	127.1 ± 21.9	17.5 ± 5.0^{ab}	13.39 ± 5.3
Summer	Apr	123.2 ± 18.1	30.7 ± 5.6^{ab}	24.39 ± 4.7	108.3 ± 25.5	25.6 ± 2.9	4.63 ± 3.2
	May	165.2 ± 20.0	17.6 ± 2.8	10.30 ± 3.6	139.8 ± 24.0	12.6 ± 3.5^{ab}	8.63 ± 2.2
	Jun	128.0 ± 10.5	21.9 ± 5.0	16.41 ± 3.8	113.0 ± 10.2	19.5 ± 4.2^{ab}	16.81 ± 4.2
Pre-monsoon	Jul	120.5 ± 137	35.2 ± 10.2^{ab}	29.17 ± 6.4	91.2 ± 14.3	29.2 ± 7.1^{ab}	31.87 ± 6.2
	Aug	144.4 ± 12.0	33.9 ± 6.0^{ab}	22.92 ±4.8	109.0 ± 18.2	21.1 ± 6.0^{ab}	19.27 ± 5.0
	Sep	128.8 ± 15.0	52.6 ± 8.2^{ab}	32.81 ± 7.8	121.0 ± 16.0	29.5 ± 3.0^{ab}	15.70 ± 5.1
Monsoon	Oct	80.3 ± 12.1	11.5 ± 5.6^{aa}	13.75 ± 6.0	110.2 ± 22.4	11.9 ± 1.8^{aa}	10.00 ± 4.4
	Nov	61.9 ± 11.6	5.0 ± 4.2^{aa}	8.20 ± 5.5	58.2 ± 11.7	5.2 ± 2.2^{aa}	8.62 ± 1.5
	Dec	66.5 ± 12.0	12.5 ± 5.8^{aa}	18.46 ± 7.7	65.0 ± 13.2	6.2 ± 4.2^{aa}	18.05 ± 1.1

**Means followed by same letter (or no letter) are not significant at the 0.05 probability level*

Table 2. Abundance of infestation of Ostracoda *V. tsujii* in different parts *Lethrinus ornatus* and *Carangoides gymnostethus* during Jan-Dec 2019 (Average of 93 - 135 samples per month)

		*Lethrinus ornatus**			*Carangoides gymnostethus**		
Seasons	Months	Gills	Mouth region	Intestinal tract	Gills	Mouth region	Intestinal tract
Post Monsoon	Jan	28.0 ± 6.8^{ab}	6.0 ± 0.0	0.0	22.4 ± 10.1^{ab}	0.0	0.0
	Feb	10.5 ± 4.0^{aa}	0.0	0.0	12.8 ± 5.5^{ab}	0.0	0.0
	Mar	23.1 ± 6.0^{ab}	15.0 ± 0.0	0.0	15.0 ± 8.2^{ab}	8.0 ± 0.0	0.0
Summer	Apr	62.6 ± 10.5^{ab}	12.0 ± 0.0	0.0	44.2 ± 22.5^{ab}	9.0 ± 0.0	7.0 ± 0.0
	May	6.6 ± 5.0^{aa}	3.0 ± 0.0	0.0	40.5 ± 18.2^{ab}	6.0 ± 0.0	0.0
	Jun	12.3 ± 8.2^{ab}	0.0	8.0 ± 0.0	19.6 ± 10.0^{ab}	13.0 ± 0.0	5.0 ± 0.0
Pre-monsoon	Jul	50.9 ± 13.2^{ab}	12.7 ± 5.2	15.0 ± 0.0	53.5 ± 23.0^{ab}	0.0	0.0
	Aug	77.0 ± 21.1^{ab}	16.8 ± 10.0	33.0 ± 0.0	66.3 ± 13.7^{ab}	20.0 ± 0.0	0.0
	Sep	70.4 ± 16.2^{ab}	4.0 ± 0.0	25.0 ± 0.0	50.5 ± 20.4^{ab}	7.0 ± 0.0	0.0
Monsoon	Oct	5.4 ± 3.0^{aa}	0.0	0.0	33.3 ± 12.5^{ab}	11.0 ± 0.0	15.0 ± 0.0
	Nov	19.5 ± 5.5^{ab}	0.0	0.0	13.0 ± 5.8^{aa}	0.0	0.0
	Dec	7.1 ± 3.8^{aa}	0.0	0.0	10.0 ± 8.2^{aa}	2.0 ± 0.0	0.0

**Means followed by same letter (or no letter) are not significant at the 0.05 probability level*

In the present study, the infestation of *V. tsujii* in *C. gymnostethus* and *L. ornatus*, the common food fishes of Pamban landing center varied with different body parts like gills, buccal cavity, and intestines but varying with seasonality. The range of hosts for *V. tsujii* is yet to be ascertained in major fish landing centers of Tamil Nadu, India but this present study could be a crucial one for all future studies. Due to systematic sampling on weekly basis for Jan – Dec 2019, it was possible to determine temporal variations in Ostracoda infestation. The variation in size of ostracods found (3.07 to 3.83mm by anterior-posterior position, 1.17 to 1.43mm dorso-ventral region, and width of 1.85 to 2.15mm) suggests that adults, and perhaps late instars attacked the host. The degree of infestation of *V. tsujii* in *C. gymnostethus* and *L. ornatus* varied with different seasons. The incidence of infestation was highest in pre-monsoon months followed by summer and winter seasons. However, infestation level was found less in monsoon season and this could be due to rough weather prevailed, thereby the wave action was higher than other seasons. It was also noticed that no difference in the size of Ostracoda was found with respect to seasonality.

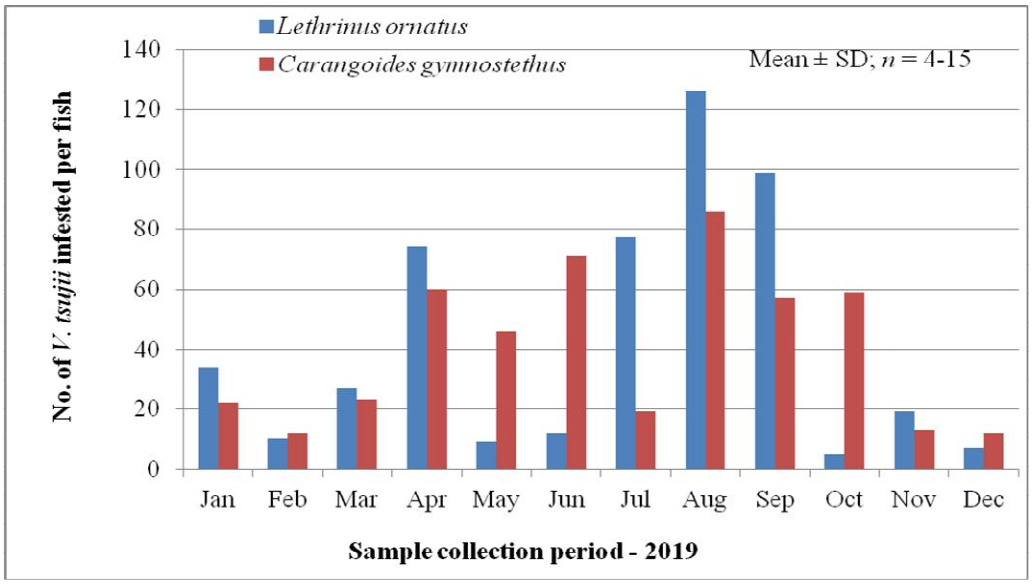

Fig. 7. No. of *V. tsujii* infested in food fishes *L. ornatus* and *C. gymnostethus* from different seasons during 2019

The scientific reports available on the feeding habit of Ostracoda are very limited and within the family Cypridinidae, the crustacean has different feeding behavior like collectors [8], predators [12,13], opportunist scavengers [9], and parasites [14]. Stepien and Brusca [15] reported that Ostracoda attacked the fishes captured in cages during night time. The Ostracoda *V. tsujii* also clustered around uncaged fishes as well but caused no much damages to the host. Bennet et al. [14] observed that individual *V. tsujii* remain attached to the gills for extended periods of time, staying in position long enough to cause the formation of a distinct pocket between the filaments of the gills, that it could be a parasitic kind of feeding habit. In the present investigation, a close examination of the surface of the filament margin in the gills or other body parts where the infestation was noticed showed no discrete puncture marks or grooves in the epithelium and doesn't support that this could be a case of a parasite. The tissue damage in both species examined was more gills and in some fish samples, the damage extended up to intestinal track and in some cases, it was fully eaten away by *V. tsujii.* Since ostracodas are nocturnal in nature, further studies will be undertaken to ascertain it by conducting field studies.

Ostracods have only recently been recognized as a important group of crustaceans for paleolimnology and as an essential instrument for investigating the climatic changes. Ostracoda are considered to indicative of parameters such as salinity, alkalinity, acidity, anhaline environment, structure of sediment, eutrophication, oxygen conditions and prevailing temperature due to their large biodiversity in littoral, sublittoral and profundal zones [28,29]. Ostracod crustaceans are excellent quaternary palaeoclimateproxies, as microfossils they supply evidence of past climatic conditions via indicator species, transfer function and mutual climatic range methods, trace-element and stable-isotope geochemistry of their shells [30]. With the increasing knowledge of temperature and oxygen demands of species, use ostracoda in the study of shallow lakes with respect to climate and lake level changes and the onset of meromictic conditions has become increasingly important [31]. Only since about fifty years ago, ostracods have played an increasingly important role in the evaluation of lake conditions during the quaternary and have become an essential instrument for investigations concerning climatic change [32].

4. CONCLUSION

Based on the results and observation made in the present study, no socket kind of impact was observed in the gills or any other body tissue as reported in the literature as to claim it is a parasite, therefore, the infestation of *V. tisujii* and damages which caused in *C. gymnostethus* and

L. ornatus in the present study support the theory that Ostracoda could have predator kind of feeding behavior. Based on the literature reports, ostracod crustaceans could be good candidates of ecological and climate change indicators.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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