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First evidence of tumor-like anomaly infestation in Copepods from the Central Indian Ridge

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To investigate the distribution and abundance of mesozooplankton in the Indian Ocean, zooplankton sampling was conducted along the Central Indian Ridge (CIR) from the equatorial waters to 8°S latitude. Of the seven sampling locations, cysts or Tumor Like Anomalies (TLA) of ectoparasites like *Ellobiopsis* sp (Protista, Ellobiopsidae), being known as TLA were observed at the 5 locations. The highest frequency of TLA was observed at Stn. MPN-03 at all the 3 depths sampled (0-500 m, 500-1000 m and 1000-1500 m). Frequency of infected specimens among all the sampled copepods varied from 3.0 – 6.9%. Among the infected genera, *Oncaea* sp (Copepoda: Poecillostomatoida) was the most dominant accounting 21.2% followed by *Clausocalanus* sp (13.6%), *Centropages* sp (12.9%), *Pleuromamma* sp (9.4%) and *Acrocalanus* sp (7.8%). Two types of tumors were observed. Type 'A' was elongated and full of small granular structures in the tumor and type 'B' was spherical with few or no granules in the structure. Type 'B' was the most common that dominated in terms of frequency (96.60%), where as type 'A' which was observed rarely, was in 3.4% of the total parasitized population. Consequent to the physico-chemical anomalous signatures of active hydrothermal mineralisation recorded at station MPN-03, it is hypothesized that high levels of potentially toxic chemicals erupted from the hydrothermal plume caused the weakening of the exoskeleton of *Oncaea* and other copepod species, making them more susceptible to the parasitic attack, especially to ectoparasites like *Ellobiopsi*s sp. These ectoparasites attack the host and feed on their body fluids which may lead to the death of the host. This study emphasis the consequences of parasitic infection in dominant planktonic copepods like *Oncaea* to the deep-sea food chain.

Key words: Deep-sea; mesozooplankton; copepods; ectoparasite; *Ellobiopsis;* sp; infestation; zooplankton; parasites; tumors; ridge; hydrothermal vent; Indian Ocean; Central Indian Ridge]

Introduction

Copepods are the most numerous metazoans in the aquatic community and ubiquitous members of planktonic fauna. Ecologically they are important in the food chain linking microscopic algae to juvenile fish to whales. Their habitats range from lakes on the highest mountains[1] to the deepest ocean trenches, and from the cold polar ice-water interface[2] to the hot active hydrothermal vents[3]. Copepods may be free-living, symbiotic, or internal or external parasites on almost every animal phylum in water. But recently scientists have observed that these crustaceans themselves are host attacked by a mysterious parasite, forming abnormal protuberances on their carapace, being known as Tumor Like Anomalies (TLA)[4].

Most of the studies carried out so far on such TLA are confined either to coastal waters or freshwater[4-8], hence the present study is the first record such abnormalities in copepods from the mid oceanic ridge system of the Indian Ocean.

Materials and Methods

Study area:

The Central Indian Ridge is a part of the 70,000 km long global Mid-Ocean Ridges (MOR) and constitutes an important regime of divergent plate boundaries (Fig. 1). Based on the spreading rates, Raju *et al.*[9] have considered the northern part of CIR as a slow spreading ridge (36 mm yr^{-1}) and southern part as an intermediate spreading ridge (50 mm yr^{-1}). Further, on the basis of a detailed study carried out using multibeam bathymetry, gravity, and magnetics, they suggested the predominance of tectonic extension in this area during the present stage of evolution.

Field sampling and analysis:

Zooplankton was sampled from the equatorial waters of the Indian Ocean (Fig. 1) onboard *ORV Sagar Kanya* (cruise no. SK–201) during March 2003 (Table 1). The sampling area reveals a variable

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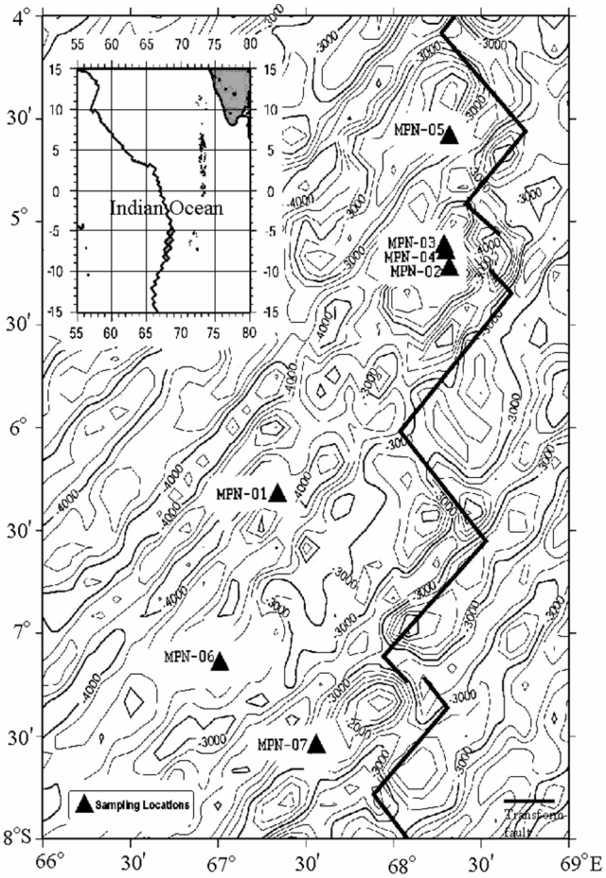

Fig. 1—Geographical position of the sampling stations along the Central Indian Ridge (depth counters in meters).

Table 1—Details of the zooplankton sampling

Station No.	Sampling date	Lat. (S)	Long. (E)	Dominant Species
MPN-01	15-03-2004	06° 33'	67° 34'	Oncaea sp.
MPN-02	16-03-2004	05° 23'	68° 32'	*Oncaea* sp.
MPN-03	16-03-2004	05° 12'	68° 29'	*Oncaea* sp.
MPN-04	17-03-2004	05° 15'	68° 30'	*Oncaea* sp.
MPN-05	18-03-2004	04° 59'	68° 32'	*Oncaea* sp.
MPN-06	21-03-2004	07° 15'	67° 01'	*Oncaea* sp.
MPN-07	22-03-2004	07° 55'	67° 56'	*Oncaea* sp.

Depth range (m): 1500 -1000; 1000-500 and 500-0
No. of nets operated at each sampling station : 03

bottom topography structured by the systems of sea mounts, plains and typical ridges. The objective of the multidiciplinary cruise was to study the tectonic and oceanic processes along the ridge and hence the cruise track strictly followed the ridge segment. Vertical tows were taken at 7 stations from 1500 m water depth up to surface using a Multiple Plankton Net (MPN) of 200μ mesh size lowered from the aft diving platform and then hauled back to the surface. All the nets of the MPN were opened acoustically. The sampling depth was measured by a built in pressure sensor of MPN. A minimum of 3 nets were operated at each location with the sampling depth interval of 500 m. Each tow duration was ~45 min. After arrival of the MPN on the deck of the vessel, the nets were washed with filtered seawater from outer side of the net to collect the zooplankton fauna in net bottles. Samples were preserved in nutralized 5% formaldehyde solution. Biomass of zooplankton was measured onboard the vessel as displacement volume by standardized methods[10]. 'Jelly like' organisms were excluded from the biomass measurements and the individual counts were made to include in density values. Quantitative analysis of the zooplankton samples were subsequently carried out in the shore laboratory. Copepods were identified[11] up to generic level, but only to broader assemblages with some specialist groups; immature and damaged species. All copepods with the protuberances were sorted, counted and studied individually. Based on the identification keys as well as personal communications with some of the experts, we conclude that all TLA recorded here could be due to the parasites.

Results and Discussion

Copepod composition:

Zooplankton community of the study area was composed of 27 taxonomic groups and was dominated by copepods, which comprised of 79.76% of the total zooplankton abundance followed by Chaetognatha (3.63%), Bryozoa (3.43%), Larvacea (2.37%) and Ostracoda (1.22%). Other groups including Radiolaria, Cladocera, Thaliacea, Euphausiidae and others accounted collectively 8.63% of the composition of the planktonic fauna.

The sampled Copepoda comprised of 52 genera belonging to orders Calanoida, Cyclopoida, Poecillostomatoida and Harpacticoida. TLA occurred in orders Poecillostomatoida (2 genera) and Calanoida (18 genera). The highest frequency of TLA was observed at Stn. MPN-03 at all the 3 depths sampled (0-500 m, 500-1000 m, 1000-1500 m). Among the infected species, *Oncaea* sp. (Copepoda: Poecillostomatoida) was the most dominant accounting for 21.24% of the infected population (Fig. 2) followed by *Clausocalanus* sp. (13.60%), *Centropages* sp. (12.90%), *Pleuromamma* sp.

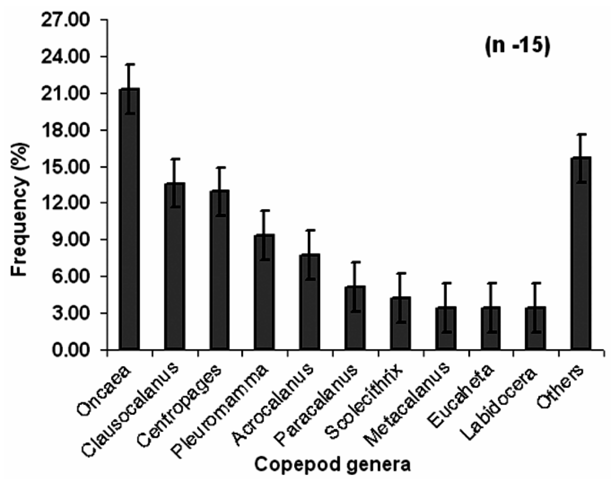

Fig. 2—Frequency of tumour like anomalies in different copepod genera

(9.35%), *Acrocalanus* sp. (7.75%), *Paracalanus* sp. (5.10%) and *Scolecithrix* sp. (4.25%). Other genera with such abnormalities were *Metacalanus* sp., *Euchaeta* sp. and *Labidocera* sp. (3.40% each), *Scolecithricella* sp., *Haloptilus* sp. and *Eucalanus* sp. (2.55% each), *Mesocalanus* sp., *Candacia* sp., *Paracandacia* sp. and *Undeuchaeta* sp. (1.70% each), *Corycaeus* sp. (0.85%), *Phaenna* sp. (0.16%) and *Pareucalanus* sp. (0.16%).

Nature of the TLA:

Transparent pyriform TLA co-occurred in copepods at 5 of 7 stations sampled from the Central Indian Ridge. TLA were most commonly observed at the articulation of the second and third prosomal segment (Figs. 3, 4). They were commonly found on the most abundant and highly predacious *Oncaea* sp (Fig. 4). Two types of TLA were observed. Type 'A' was elongated with full of small granular structures (Fig. 3A) and type 'B' was spherical with few or no granules in the structure (Fig. 3B). Type 'B' was the most common (96.60%) whereas the Type 'A' made up only 3.4% of the total infected population.

Aquatic food web is always subjected to different kinds of threats to its well-being and it is difficult to say what the next threat could be. Skovgaard[12] hypothesized that ectoparasite *Ellobiopsis* sp. (Protista:Ellobiopsidae) pierce weak shelled copepods, usually the anterior part of the body and feed on their body fluids. Some dinoflagellate endoparasites such as *Blastodinium* sp. are also known to infect the marine copepods[13], which may produce protuberances that persist even after the parasite has left the host[12]. Even though, the exact etiology of these abnormalities is not confirmed, it is believed that carcinogenic chemicals, ultraviolet radiation, injury, or viral or bacterial infections are also known to cause tumors or TLA[14]. It is said to be a global phenomenon that has a common cause[7], but the factors responsible for the susceptibility of the copepods to such parasitic attacks may be site specific and the frequency as well as type of infection could vary from area to area.

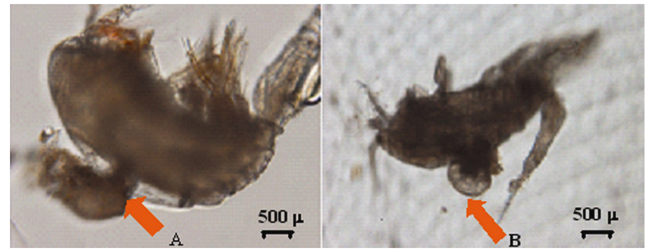

Fig. 3—Different types of abnormalities found on *Oncaea* sp. (A = granular tumor; B = spherical agranular tumor)

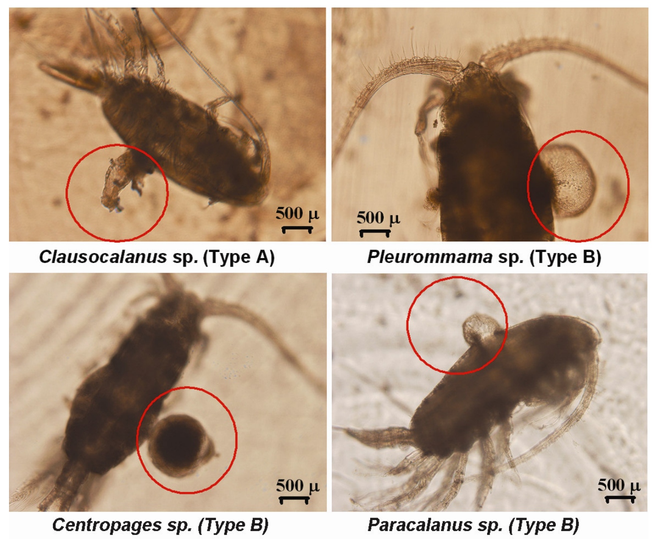

Fig. 4—Tumors observed in different copepod genera.

Frequency of the abnormalities:

The TLA were found at different depths at 5 of 7 stations sampled from CIR. The highest (average) frequency was observed at station MPN-03 (390 no/100m^3). The percentage of infected specimens among all the sampled copepods varied from 3.0-6.9% and maximum infected population was in 500-1000 m water depth (Table 2). The frequency of the parasitized specimen at this station was maximum in 0-500 m water depth followed by 1000-1500 m; comparatively fewer animals with portusion were recorded from the water depth of 500-1000 m (Fig. 5). TLA was observed in copepods collected from four other stations; however, their frequency was significantly low. The highest frequency of TLA (average frequency of 3 depths, 390 no/100m^3) was observed at station MPN-03 at all the 3 depths sampled. As shown in Fig 5, the frequency of TLA infected specimen was highest in 0-500 m (512 no/100m^3) water depth followed by at 1000-1500 m and mid depth of 500-1000 m, recorded low frequency. TLA was also observed in copepods collected from other four stations; however, their frequency was significantly low.

Table 2—Mean abundance (no 100m^{-3}) of planktonic copepods with percent occurrence of TLA infected copepod specimens (n=15)

	Sampling water depth (m)		
Copepod order	0-500	500-1000	1000-1500
Calanoida	14640	7179	4719
Cyclopoida	912	377	379
Harpacticoida	48	0	11
Policilostomatatoida	10032	3638	3383
Total (copepods)	25632	11194	8492
No. of TLA infected copepods	768	752	427
TLA infected copepods (%)	3.0	6.7	5.0

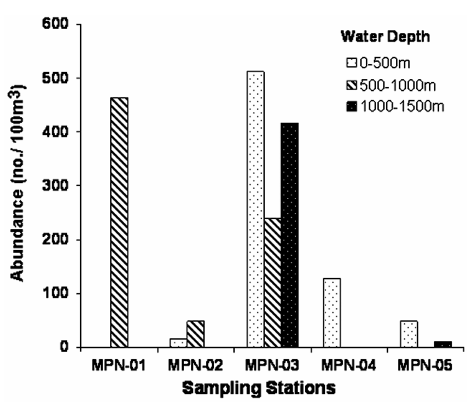

Fig. 5—Abundance of infected copepods at different locations and water depths.

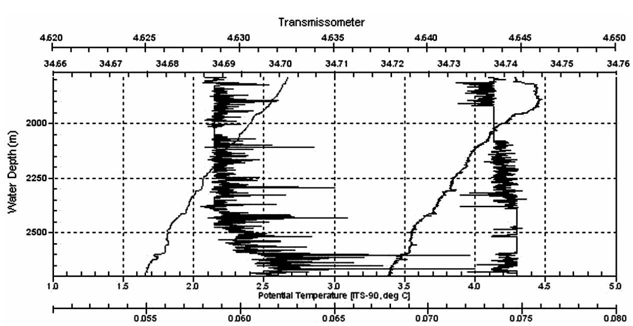

Fig. 6—Physical anomalous signatures of hydrothermal vent at MPN-03. (A=Significant increase in Light Scattering; B = Significant decrease in Light Transmission coincident to 'A')

Could it be related with hydrothermal vent?

Indian Ridge system, although less studied compared to the Pacific and Atlantic Mid Oceanic Ridge systems, is said to be having many active hydrothermal vents[15,16]. During the present cruise (SK-201), Sarma *et al.*[17] recorded some strong physicochemical signatures (Fig. 6) with very high concentration of CH_4 (6.02 nM) and SiO_2 (125.57 µM) at station MPN-03. Further, Bhandare & Ingole[18] also reported very high zooplankton biomass in the deeper waters (1500 m) at the same location (MPN-03). The above studies strongly suggest the possibility of an active hydrothermal vent in the vicinity of station MPN-03. Surprisingly, this was the same station where the incidance of TLA was also highest and the percentage of the infected copepods were highest in the samples collected at water depth of 500-1000 m (Fig. 1; Table 2).

While discussing the effect of hydrothermal effluents on the marine organisms Berg & Van Dover[19] and Burd & Thomson[20] showed that the effect of hydrothermal effluents at mid-ocean ridges is not confined just to the sea-floor environment but the flux from the hydrothermal vent gets mix with the water column and may reach to surface as well as few km from the actual vent site. Thus, the hydrothermal effluents affect the entire water column. Vent effluents contain potentially toxic chemicals with high metal concentration[21, 22] that can be detrimental to the fauna above the vent field. Zooplankton, especially copepods perform periodic cyclic migration from the depth of the plume to the mesopelagic zone, probably in search of the additional food materials[20]. *Oncaea* sp., the most abundant copepod group in this study shows a strong vertical migration with the ability to migrate hundreds of meters[23]. We suspect that the higher abundance of these pelagic copepods species in deeper waters (1000-1500 m) is due to their opportunistic nature[20] to feed on the excessive food material near the hydrothermal vent in the form of vent bacteria[24] and organic carbon[25]. Besides the higher bacterial biomass, the hydrothermally influenced zone could have high concentration of

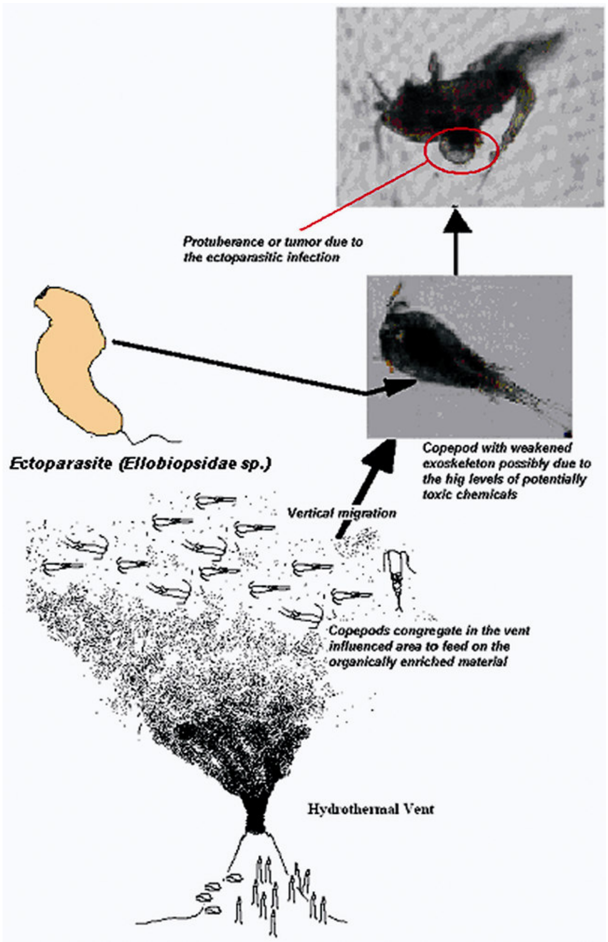

Fig. 7—Schematic representation of the possible relationship between hydrothermal plume and Tumor like anomalies in planktonic copepods

potentially toxic chemicals[21,22] and metails[26]. Thus, as depicted in Fig. 7, pelagic copepods (e.g. *Oncaea* sp.) not adapted to toxic vent environments may get exposed to toxic levels of metal concentration. The toxic chemicals, and excessive metal concentration weakened the exoskeleton of the copepods[7,20] making them more susceptible to the attack of the ectoparasites such as *Ellobiopsis* sp., by means of the tumor formation on their carapace.

Scope for future research:

Although the factors responsible for abnormal protuberances in marine copepods are still unknown[12], high frequency of TLA in dominant copepod groups (e.g. *Oncaea* had >21% TLA among the infected copepods) from the CIR could be an emerging threat to planktonic communities. Therefore, further investigations are urgently needed to examine etiology as well as spatial and temporal distribution of TLA's among various host species. To this approach, some investigations are proposed using the light and electron microscopy of the parasitized specimen.

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