

The Community Structure of the Deep-Sea Nematode Community Associated with Polymetallic Nodules in the Central Indian Ocean Basin

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Abstract

Technological advances have led to an increase in the number of investigations in the deep sea over the last two decades. However, despite a large number of scientific cruises, few published data on the nematode biodiversity and community structure are available from the abyssal nodule fields of the Central Indian Ocean Basin (CIOB). It is well known that CIOB harbors one of the largest high-grade nodule reservoirs and has therefore gained the most attention from industries and governments. Therefore, to adequately assess the potential environmental impacts of deep-sea polymetallic nodule mining in CIOB, more quantitative samples are needed to better understand trends in nematode diversity, abundance, and community structure in the nodule fields. Recently, on the basis of detailed surveys and analysis, a Test Mine Site (TMS) and Reference Mining Site (TRS) have been identified within the First Generation Mine Site (FGM) for further detailed studies. According to ISA guidelines, the TMS and TRS need to be similar in faunal abundance, composition and diversity, nodule abundances, metal grade and environmental data. As per the deep-sea environment conservation point of view, particularly to plan mining operations, the study has been done to check whether TMS and TRS have a similar type of faunal assemblage so that impacts and the ensuing loss of biota are minimized. To address this, we compared the composition and distribution of nematode communities at genus/species level from TMS and TRS. The average nematode density ranged from 71.68 ± 1.7 inds/10 cm² in the TRS and 91.23 ± 1.3 inds/10 cm² in the TMS. A total of 63 nematode genera belonging to 24 families were identified from TMS. The TRS samples supported 52 genera belonging to 20 families. A homogeneous community was observed at family/genus level from the TMS and TRS, particularly dominated by cosmopolitan nematode genera like *Acantholaimus*, *Halalaimus*, *Thalassomonhystera* and *Leptolaimus*. The TRS and TMS samples did exhibit a similar evenness (J'), and diversity (H') suggesting a homogeneous nematode community throughout the area. Taxon diversity and richness estimators revealed that the current sampling design was able to characterize the majority of the nematode genera present. It can be suggested that the entire area of sampling is inhabited by a single, uniformly distributed, nematode assemblage at the genus level.

This study also addresses whether or not nematode assemblages at genus/ species level inhabiting the bare sediment (soft sediment without nodules) in abyssal nodule fields are unique and differ from those found in the crevices of polymetallic nodules. As expected, there were fewer numbers of total genera recorded from the crevices with the differences being found in the dominant genera and families. *Thalassomonhystera*, *Acantholaimus* and *Desmoscolex* were noted as characteristic of the bare sediment while *Leptolaimus* and *Camacolaimus* showed the highest likelihood in nodule crevices. Hard nodule crevice substratum does favour some species level morphotypes of the genus *Leptolaimus* and *Camacolaimus* since these morphotypes were found exclusively in nodule crevices. However, owing to the still limited sampling effort in the deep sea, it is premature to suggest that these species are endemic to nodules. Implications of the present findings for environmental management and future research needs are provided.

Key words: deepsea; nematodes; nodule crevices; Central Indian Ocean Basin; soft sediment.

1. Introduction

The deep sea, covers more than 65% of the Earth surface, and it represents the world's largest ecosystem (Ramirez-Llodra et al., 2010; Danovaro et al., 2010). It is known that deep-ocean resources involve billions dollars' worth of metals, rare earth elements, and valuable minerals (Cronan, 2001). The most commercially attractive resources include polymetallic nodules covering extensive areas of the deep seafloor (Cronan, 2001; Sharma, 2010). Extremely large deposits of polymetallic nodules have been found to occur in the abyss of three major oceans, the Pacific, the Atlantic and the Indian Ocean. The International Seabed Authority (ISA) is the body that regulates these resources (polymetallic nodules) because they occur in the areas beyond national jurisdiction and are the common heritage of mankind (Wedding et al., 2015). India is among the countries prospecting for polymetallic nodules in the Central Indian basin and the Indian Ridge, far to the south-east of South Africa. Already some of areas in the Central Indian Ocean Basin (CIOB) have been approved by ISA for polymetallic nodules and polymetallic sulfides exploration.

Recently in the 23rd ISA session at Kingston, Jamaica, 2017, ISA extended India's exclusive rights for another 5 years to explore polymetallic nodules from the seabed in CIOB. These rights cover more than 75,000 km² of area in international waters for developmental activities to explore polymetallic nodules. The estimated polymetallic nodule resource potential is 380 million tonnes, containing 4.7 million tonnes of nickel, 4.29 million tonnes of copper, 0.55 million tonnes of cobalt and 92.59 million tonnes of manganese. Many companies are looking to mine these resources and the development towards nodule mining is progressing very fast. At the current stage, several mine sites have been identified, developments of new mining technologies are in progress, and processing technologies are being tested.

An area of about 7,860 km² has been identified in the CIOB for the First Generation Mine Site (FGM) on the basis of detailed surveys and analysis. Furthermore, Test Mining Sites (TMS) and Test Reference Sites (TRS) are identified as per ISA rules. ISA defines TRS as "areas in which no mining will occur to ensure representative and stable biota of the seabed in order to assess any changes in the flora and fauna of the marine environment". The TRS should be carefully located and large enough so as not to be affected by the natural variations of local environmental conditions. The reference sites should have faunal composition comparable to that of the TMS. The reference site should be outside the TMS and the areas influenced by the mining plume (ISA, 1999). Moreover, ISA came out with an environmental policy which recommended that prior to test mining it is important to determine the environmental conditions and baseline biodiversity data in the exploration area (ISA-LTC, 2013).

Since benthic fauna is going to be affected severely, it is important to characterize the benthic community at the micro level.

As per the deep-sea environment conservation point of view and particularly with respect to planning of mining operations, it is important to investigate whether TMS and TRS have similar faunal assemblages so that the impact and the ensuing loss of biota are minimized. To address this, we compared the composition and distribution of nematode communities at genus/species level from the CIOB TMS and TRS. As shown by previous studies (Miljutina et al., 2010; Singh et al., 2016), meiobenthic communities and their dominant component, the free-living nematodes, are suitable candidates for such comparisons.

Furthermore, in order to understand the possible impact of the future mining of deep-sea mineral resources on biodiversity, knowledge of the fauna associated with the nodules is crucial. Mining for polymetallic nodules will not only inevitably impact the sediment fauna by the process of resuspension and redeposition (Jumars, 1981; Ingole et al., 2001; Thiel, 2001), but it will also impact the nodule associated fauna because nodule extraction (mining) will eliminate both the hard surfaces and the specific crevice habitat. It is also of interest to know whether this would irreversibly eliminate some taxa. In other words, to assess the potential deep-sea diversity loss, it is of interest to know if there are in fact unique, nodule-crevice bound taxa. Generally, the nodule field nematofauna is known to differ from the fauna inhabiting nodule-free areas (Singh et al., 2016; Vanreusel et al., 2010), resulting in elevated local diversity in nodule-bearing areas. There is evidence that the nematode assemblages inhabiting the crevices differ in their composition compared to assemblages found in the soft sediment in the immediate vicinity of the nodules (Thiel et al., 1993). Substrate complexity associated with the nodules seems to be responsible for the success of the nodule field community.

However, studies in which a more general comparison of the nematode fauna composition between the bare sediment and nodule-crevices has rarely been attempted (Singh et al. 2016). Moreover, our knowledge of the abyssal benthic fauna, especially the nematode communities from the Indian Ocean is still inadequate. Virtually nothing is known about the faunal communities associated with the polymetallic nodules of this region (Singh et al., 2014). Therefore, along with the TMS and TRS comparison of the nematode community at the genus/species level, we also compared the bare sediment and nodule crevice nematode community at the genus/species level. This was done to find out whether the nematode assemblages in nodule crevices differed from those in the bare sediment and whether or not there are any unique genus/species in the nodule crevices.

2. Materials and Methods

2.1. Study Area

Based on the nodule distribution, abundance, and bathymetry data, a TMS was selected within the FGM and a TRS was selected adjacent to the FGM for assessment of baseline environmental conditions for conducting the nodule collector test during 2019-2020 (Fig.1). The study area lies between 12°00' to 13°00' S and 74°00' to 75°00'E in the depth range of 5000 to 5300 m (Fig.1; Table 1).

In general the CIOB is an abyssal area bounded by ridge systems except at the northern side where it is open towards the Indian peninsula. To the northwest, it is bounded by the Chagos–Laccadive Ridge. The basement age of CIOB is reported to be 50-60 Ma (Mukhopadhyay et al., 1995) and is covered with terrigenous sediments on the northern part (up to 5°S latitude). From 5°S to 14.5°S the major sediment type is siliceous oozes with sporadic calcareous patches found at around 12-14°S latitude and 82.5-83.5°E longitude. The southern part of the basin (south of latitude 14.5°S) is covered with red clay (Nath et al., 1989). The sedimentation rate in CIB is very low (2.7 mm/ka 14°S and 78°E, 3.4 mm/ka 10°S and 82°E) (Udintsev,1975; Banakar et al., 1997; Borole, 1993).

Nodules are found in an area in the Central Indian Ocean that extends from 10°S to 16°30'S and 72°E to 80°E (Mukhopadhyay et al.,2002; Prasad, 2007). This area is identified as the Indian Ocean Nodule field. The nodules are distributed in the Crozet basin, the Wharton basin, the Somali basin and the CIOB is the richest among them (Kamesh Raju, 1993).The nodules in the CIOB vary in size, shape and surface texture. Earlier studies have shown that the environment of deposition of polymetallic nodules is characterized by several parameters such as sea floor morphology, sediment types, water depth and water column conditions, surface productivity and distance from shore (Banerjee and Miura, 2001; Jauhari, 1987; Sudhakar, 1989; Ghosh and Mukhopadhyay, 1999). Nodules with rough surface texture dominate most of the area except the southeastern part of the basin, which is floored more by the smooth nodules. Smaller nodules (<4 cm) are common and are dominant both in density and mass in the southeastern part of the basin, whereas north-western part and the central part show dominance of larger rough nodules with higher density and mass (Vineesh et al., 2009).

The nodules encountered in the study area were of the ellipsoidal, botryoidal and polynucleated shape. As the sampling was concentrated mainly in the polymetallic nodule area, nodules were collected during most of the operations. They have a compact and hard centre surrounded by layers of soft concretionary material. The nodule collection varied from 1.1kg to 3.1 kg. The nodule sizes varied

from very small (<2cm), to small (2-4 cm), medium (4-6 cm) and large (6-8 cm). Nodules recovered from locations in the TMS and TRS areas have a rough surface (Table 1).

2.2. Sample collection and laboratory analysis

A cruise onboard RV SindhuSadhana (SSD 013) was conducted in July-August 2015 during which 10 box corer sediment samples were collected at 10 stations from 2 locations (TMS and TRS) (Table 1; Fig.2). From each box corer, two sub-cores (5.7 diameters) were used for nematode analysis. Following retrieval of the box corer on board, the water overlying the sediment was siphoned off on to 32 μ m mesh size sieve, and the retained material was added to the same sediment samples. The uppermost 5 cm of sediment from each core was then sliced off and fixed with the filtrate in 4% buffered formaldehyde. The sediment sample thus obtained constituted one of the two types of samples provided by each core (the “bare sediment”, i.e., the surrounding sediment, and nodule associated sediment). The nodules were then gently removed from the sediment using forceps, placed in separate containers and fixed in 4% buffered formaldehyde.

In the laboratory, the clean nodules were mechanically broken down to sand-sized grains and fixed in 4% buffered formaldehyde. This produced a sample of the second type, the “nodule crevices”. Nematodes from all the samples were extracted by differential flotation and centrifugation at 4000 rpm using Levasil (McIntyre and Warwick, 1984), and the supernatant was washed onto 32 μ m mesh size sieve. The sieve residue was carefully examined under a stereo-microscope (40 \times magnification) and all nematodes were counted, picked out, transferred to the formalin-ethanol-glycerine mixture and permanently mounted on glass slides for taxonomic identification (Vincx and Hall, 1996). The nematodes were identified to genus/species level using the pictorial keys of Platt and Warwick (1983, 1988) and Warwick et al. (1998), and referring to the NeMys database (Deprez et al., 2005). The dominant genera in the nodule crevices were identified to species level; all the unknown species were denoted as sp.1, sp.2, sp.3....sp.n and drawn using a camera lucida. Individual nematode genus/species were assigned to four feeding categories according to Wieser (1953): selective deposit feeders (1A), non-selective deposit feeders (1B), epigrowth feeders (2A), and predators or omnivores (2B). Gender (female, male or juvenile) was determined for each individual. Total length, excluding tail, and maximum body diameter were measured for each specimen.

2.3. Statistical analysis

Multivariate community analysis was carried out using PERMANOVA+ module of the PRIMER v6 software (Anderson et al., 2008; Clarke and Gorley, 2006). Community analyses at the genus level data were performed using the Bray-Curtis Dissimilarity (Bray and Curtis, 1957) index and the S7

Jaccard similarity index on presence/absence data. Differences between TMS and TRS sites were visualized using non-metric Multi-Dimensional Scaling (nMDS). The statistical difference between the TMS and TRS was tested with Permutational Multivariate Analysis of Variance (PERMANOVA) (Anderson, 2001; McArdle and Anderson, 2001) designed with two factors: “station” nested in “habitat”. To explore the nematode community structure of TMS and TRS, univariate analyses were run using Margalef’s index (Margalef, 1968) for species richness (d), Pielou’s index (Pielou, 1966) for species evenness (J'), and the Shannon–Wiener index (Shannon and Weaver, 1963) for species diversity (H' , \log_e). The analysis was performed in PERMANOVA+ module of the PRIMER v6 software (Anderson et al., 2008; Clarke and Gorley, 2006). Non-parametric Mann-Whitney tests were used as a global significance test for differences in univariate parameters using a PAST program (Hammer et al., 2001).

To document the accumulation of species and to estimate total species richness in the TMS and TRS during the course of our field study, we used species accumulation curves (SACs), a technique common for estimating diversity in ecology (Gotelli and Colwel, 2001). SACs, plotting the cumulative number of taxa recorded as a function of the number of sites/samples studied, were produced by randomly adding sites/samples and repeating this procedure 9,999 times. Additionally, the Chao1, Jackknife2, and Bootstrap estimators were used to extrapolate and estimate the total taxon richness in the bare sediment (TMS, TRS; Magurran, 2004). The minimum number of additional samples required to detect 95 and 100% of the estimated asymptotic taxon richness was calculated using the non-parametric method proposed by Chao et al. (2009).

However, owing to the few number of individuals in nodule crevices, a species accumulation curve was plotted only for the bare sediment sample. Specimens found in nodule crevices cannot be identified hence they were presented in raw numbers. Comparison between bare sediment and nodule crevices can suffer from large differences in nematode abundances. In our case, the nodule crevice samples were lower in abundance compared to the sediment samples. We used similarity indices to highlight differences in community structure.

Indicator species analysis (Dufrêne and Legendre, 1997) was performed using multi-level pattern analysis (De Cáceres et al., 2010) in R software (R Development Core Team, 2011) with function “indicspecies” to determine the representative indicator genera characteristic of each type of sample (TMS and TRS). The Indicator Value (IndVal) index measures the strength of association between a species and a site group and is based on the product of the mean abundance and relative frequency of occurrence of each species within a given dataset. Statistical significance of the relationship between genera and site is tested using Monte Carlo randomizations with 1000 permutations, as described in

Dufrène and Legendre (1997). All the analyses were performed using R software (R Development Core Team, 2010; Dimitriadou et al., 2011), and the PERMANOVA+ module of the PRIMER v6 software (Anderson et al., 2008; Clarke and Gorley, 2006).

3. Results

3.1. Nematode density and composition in TMS and TRS

The average nematode density ranged from 71.68 ± 1.7 inds/10 cm² in the TRS and 91.23 ± 1.3 ind/10 cm² in the TMS and did not vary significantly among sites (1-factor Permanova, pseudo- $F = 1.5258$, $p_{\text{PERM}} = 0.06$). A total of 63 nematode genera belonging to 24 families were identified from TMS. The TRS samples supported 52 genera belonging to 20 families. The families Monhysteridae, Xyalidae, and Chromadoridae, were dominant in the TMS (17.1, 15.1 and 14.0% respectively) whereas the TRS samples were dominated by the families Chromadoridae (19.3%), Xyalidae (17.6%), and Diplopeltidae (9.0%). Among the genera, *Acantholaimus* (8.8%), *Thalassomonhystera* (7.0%), *Leptolaimus* (6.6%), and *Halalaimus* (6.5%) made the largest contribution in TRS. The TMS assemblages were dominated by the genera *Acantholaimus*, (17.1%), *Halalaimus* (8.3%) followed by *Leptolaimus* (6.1%), (7.0%) and *Thalassomonhystera* (5.0%) (Fig.3). The relative abundances of the dominant taxa did not vary significantly between sites (Permanova, for all tests $p_{\text{PERM}} > 0.05$).

3.2. Differences between nematode assemblages

The Bray-Curtis similarity measure based on non-metric multidimensional scaling (nMDS) ordination of the nematode genus abundance data showed a clear separation of the TMS samples from the TRS samples, although the samples from both the sites did not form a group and were randomly distributed (Fig. 4a). In addition, the result of SIMPER showed that the samples from TMS were more homogenous (64%) than those from TRS (71%). The average dissimilarity between the TMS and TRS samples was 38%. The average dissimilarity between the TMS and TRS was 39.24% and it was primarily due to differences in the relative abundances of *Chromadorita*, *Syringolaimus* and *Marisalbinema* (Table 2). PERMANOVA pair-wise comparisons between the TMS and TRS detected a mild significant difference between the TMS and TRS pair ($P=0.05$). Moreover, the S7 Jaccard similarity index measure based on non-metric multidimensional scaling (nMDS) ordination of the nematode genus on presence/absence also showed that all the samples were randomly distributed and no particular grouping was been observed (Fig.4b). The PERMANOVA did not detect any significant differences ($P>0.05$) between the TMS and TRS.

3.3. Nodule crevice fauna from TMS and TRS

Nodule associated fauna was represented in original raw numbers. Total numbers of nematode specimens found in nodule crevices were 155 in TMS, with varying composition of top dominant nematode genera *Leptolaimus* (maximum 28 specimens), *Camacolaimus* (maximum 27 specimens), *Acantholaimus* (maximum 25 specimens), and *Thalassomonhystera* (maximum 21 specimens). However, in the nodules crevices, 148 nematode specimens were recorded in TRS varying with the composition of nematode genera, *Camacolaimus* (max. 28), *Acantholaimus* (max. 22), *Leptolaimus* (max. 18), *Thalassomonhystera* (max. 17) and *Theristus* (max. 14). However, in total *Camacolaimus* (15.1%), *Acantholaimus* (13.7%), *Leptolaimus* (13.3%), *Thalassomonhystera* (11.9%) and *Theristus* (10.5%) made up maximum contribution in the nodule crevices of TRS and TMS (Fig. 5). Since the genera *Camacolaimus*, *Leptolaimus*, and *Acantholaimus* were dominant they were sorted to species level from the materials collected. The average dissimilarity between the bare sediment and nodule crevices was 51% and it was primarily due to differences in the relative abundances of *Camacolaimus*, *Aegialoalaimus*, *Daptonema* and *Theristus*. At species level, SIMPER identified *Acantholaimus* sp.3, *Leptolaimus* sp.2, *Camacolaimus* sp.3, and *Camacolaimus* sp.4 as the species which contributed most to the dissimilarity between nodule crevices and bare sediment. The average dissimilarity between the bare sediment and nodule crevices was 37 % and it was primarily due to differences in the relative abundances of *Camacolaimus* sp.3, *Camacolaimus* sp.4, *Acantholaimus* sp.2, *Acantholaimus* sp.3, and *Leptolaimus* sp.2 (Table 3).

Among the described species, the most abundant were *Acantholaimus iubilis* (9.3%) *Acantholaimus arthrochaeta* (8.0%), *Acantholaimus akvavitus* (8.0%) in TMS and *Acantholaimus formosus* (13.8 %) followed by *Acantholaimus iubilis* (7.7%), *Acantholaimus angustus* (6.2%), and *Acantholaimus barbatus* (6.2%) in TRS. In nodule crevices *Acantholaimus akvavitus* (17.9%), and *Acantholaimus angustus* (12.8%) were dominant in TRS, while *Acantholaimus arthrochaeta* (21.5%) and *Acantholaimus barbatus* (12.5%) were dominant in TMS. In addition, these species were present in both the types of samples but in different proportions. The majority of the other described species of *Acantholaimus* (*A. quintus*, *A. robustus*, *A. setosus*, *A. sieglere*, and *A. veitkoehlerae*) recorded in this study were restricted to the bare sediment. Similarly, undescribed species were represented by identical morphotypes in each of the two habitats, with the exception of a few species of *Leptolaimus* and *Camacolaimus* that were restricted to the nodule crevices (Table 4).

3.4. Habitat-specific genera

The IndVal index produced a list of indicator genera for each habitat group: Two genera each were significantly associated with the TMS (*Axonolaimus* and *Cyatholaimus*, both at $P < 0.05$) and TRS (*Pselionema* and *Chromadorita*, both at $P < 0.05$). One genus (*Camacolaimus*) was associated with the

nodule crevices ($P = 0.005$) (Table 5). The highest IndVal values ($\text{IndVal} > 1$) were produced for some specific genera (e.g., *Acantholaimus*, *Actinonema*, *Aegiolalaimus*, *Anticoma*, *Arealaimus* and *Axonolaimus*), but their associations could not be statistically tested with the set of all samples because of the lack of an external group for comparison. At the species level the IndVal index produced a list of indicator species for each of the two habitats: Two species of *Camacolaimus* (sp3, sp4) were significantly associated with the nodule crevices ($P = 0.005$) (Table 5). The IndVal index also indicated that *Leptolaimus* sp2 was associated with the nodule crevices, but the association was not significant. Similarly, with bare sediment the IndVal index identified *Acantholaimus akvavitus* as significant ($P = 0.005$). The highest IndVal values were produced for identified species belonging to *Acantholaimus*, but their associations could not, again, be statistically tested with the set of all samples because of the lack of an external group for comparison.

3.5. Diversity Indices

As seen in Table 6, taking into account all genus level data, Shannon-Wiener diversity H' , Pielou's evenness J' , and Hurlbert rarefaction ES_{51} were not significantly affected by the factor Site (Table 6). Mann-Whitney U test showed no significant difference between the TMS and TRS in any of the diversity measures. The values of diversity measures were similar in the TRS and TMS samples. 85% of the taxa were shared among these two sites. SACs suggest that 68-70% of the total number of nematode genera from the TMS and TRS in the sampling area had been identified (Fig.6). Bootstrap estimates of the total number of nematode genera gave 64 with increasing number of sites and samples, whereas Chao1 and Jackknife2 estimated 65 and 68 taxa, respectively. TMS had a higher number of observed taxa (S_{obs}) and a higher estimated total taxon richness (Chao1, Jackknife2, and Bootstrap) than TRS (Fig.6).

Feeding Habit:

In nodule crevices both in TMS and TRS the assemblages were dominated by epigrowth feeders (2A) followed by selective feeders (1A) and non-selective feeders (1B), however predators were completely absent (Fig 7). In contrast, in bare sediment both in TMS and TRS these patterns were not observed (Fig 8). In TMS the selective feeders (1A) were found to be dominant (37 %) followed by epigrowth feeders (33 %) whereas the proportions of predators (2B) were comparatively low (8%). In TRS non-selective feeders were dominant (45 %) followed by epigrowth feeders (25 %) and selective feeders (22%). The area was dominated by non-selective deposit feeders (1B) and predators (2B), whereas the proportions of predators (2B) were comparatively low (8%).

Size spectra and sex ratios

Females were most abundant (TRS 62.5%; TMS 65%) averaged across all samples, whilst the proportions of males (TRS 30.5%; TMS 26%) and juveniles (TRS 7.3%; TMS 9.0%) were lower. Averaged across all specimens, both in TMS and TRS more than 50% had body length in the range 1000–1500 µm which was followed by specimens that had body lengths in the range of 500–1000 µm. 12% of the specimens had body lengths in the range 1500–2000 µm, whereas nematodes of longer body lengths (2000 to 4000 µm) were very rare. In nodule crevices the pattern was similar as more than 70% females were found both in TMS and TRS followed by males (< 25%) and juveniles (5%). The most dominant body length of the specimens in nodule crevices (< 60%) were found in the range of (500–1000 µm) followed by (100-500), whereas the other body lengths were very rare.

Discussion

4.1. Comparison of nematode assemblages between the test mining and reference sites

In general, nematode abundance, diversity, and community composition were similar between the sites (TMS, TRS) in the CIOB license area. The nematode community investigated here in TMS and TRS is dominated by specific families - Monhysteridae, Chromadoridae, Xylidae and Leptolaimidae which were also reported previously in other nodule fields; e.g., the Clarion-Clipperton Fracture Zone in the tropical NE Pacific (Renaud-Mornant and Gourbault, 1990; Miljutina et al., 2010) and the Peru Basin in the tropical SE Pacific (Bussau, 1993). Earlier, Singh et al. (2014) also found that Chromadoridae and Xyalidae are dominant in the CIOB region. Monhysterids were also found to be the dominant family in the central part of the Clairion-Clepperton Fracture Zone (Singh et al., 2016; Lamshead et al., 2003).

The majority of the genera identified in this study were common and recorded from both the sites (TMS, TRS). The dominant nematode genera recorded (*Acantholaimus*, *Thalassomonhystera*, *Leptolaimus* and *Halalaimus*) in our study are cosmopolitan and can inhabit a variety of deep-sea habitats (Vanreusel et al., 2010). *Acantholaimus* and *Leptolaimus* are known as typical abundant nematode genera in the deep sea (Soltwedel et al., 2017; Lins et al., 2015; Miljutina et al., 2010; Singh et al., 2014). Similarly, *Halalaimus*, a widespread deep-sea genus characterized by a long and thin body, also shows a preference for surface sediment (De Mesel et al., 2006; Ingels et al., 2011; Leduc et al., 2010). *Thalassomonhystera* always accounts for the maximum contribution in the deep-sea bottom (Vanreusel et al., 2010). Moreover, in general, the composition of the nematode genera also corresponds well with many other deep-sea studies (see, for example, Dinet and Vivier, 1979; Tietjen, 1984; Soetaert and Heip, 1995; Gambi et al., 2003; Vanreusel et al., 2010; Hasemann and Soltwedel, 2011). This indicates that the nematofauna of the CIOB nodule fields is very similar to other nodules

regions, with nematode families and genera exhibiting a relatively uniform distribution (Singh et al., 2016; Thistle and Sherman, 1985; Vanreusel et al., 2010). Moreover, the nematode density and diversity between the TMS and TRS did not prove to be statistically significant.

Nematode abundances observed in our study fell within the lower to middle range of nematofaunal abundances reported for other nodule-bearing sites (Table 7). The low densities recorded in CIOB by this study and earlier studies (Ingole et al., 2005; Singh et al., 2014) could be related to the lower primary productivity in the surface waters of the CIOB (Matondkar et al. 2005). The abundance of each genera is presented in percentage and given in Table 8.

The TRS and TMS samples did exhibit a similar nematode evenness (J'), and Shannon-Wiener diversity index (H') suggesting a uniform distribution of the nematode community throughout the area under investigation. In both the sites (TMS, TRS), the coexistence of genera was always relatively high, therefore no significant variation in diversity indices was observed. The diversity values revealed in our study were quite similar to those reported for other abyssal environments, probably due to the similar environmental conditions throughout the abyssal plain. Despite the limited sampling in our study (10 box cores), we appear to have captured a large fraction (70%) of the nematode genus diversity belonging to this specific habitat type (i.e., TMS, TRS). However, more extensive sampling is required to get a more realistic picture of nematode abundance and diversity.

Nevertheless, the IndVal Index analysis identified genera which can serve as indicators for TRS and TMS. The indicator genera for TMS were *Axonolaimus* and *Cyatholaimus* and for TRS, they were *Pselionema* and *Chromadorita*. Although these genera were rare in both of the sites, to make any ecological management plan rare species have to be considered particularly for disturbed habitats. There were also genera which characterised both the sampling sites like *Acantholaimus*, *Diplopeltullah*, *Actinonema*, *Desmoscolex*, *Camacolaimus* and *Halallaimus*. In such cases, IndVal index reaches a maximum (100%) when the individuals of target genera are observed at all sites (Dufrêne and Legendre, 1997). The association with the set of all the sites cannot be statistically tested because there is no external group for comparison. Therefore, these genera cannot be treated as indicators of a particular habitat; instead, they can be considered cosmopolitan as they were found in large proportions, in both the sites. Nonetheless, the IndVal index (Dufrêne and Legendre, 1997) expresses a value for each genus that characterizes a particular environment. Therefore it is an important tool to assess and monitor a habitat. The IndVal index could also be used to explore the strength of association of the genera with the particular habitat. Most genera have the potential of colonizing a variety of deep-sea substrates, although some that are dominant in one habitat are not found in others (Vanreusel et al., 2010). The IndVal index has been applied to nematodes in only a

limited number of previous studies. Pinto et al. (2013) successfully used this index on mangrove nematode fauna, whereas Singh et al. (2016) used it to characterize the nodule and nodule-free abyssal nematofauna. In addition, Singh and Ingole (2016) used this index to identify the species that characterize the shelf, slope, and basin (including the OMZ) on the west coast of India.

In general, TMS and TRS are inhabited with the similar topography and nodule distribution. Therefore, most of the genera were reported commonly in both the habitats. This study therefore reveals that these sites satisfy the ISA regulations that prior to testing mining, TRS should be representative of the site to be mined in terms of faunal composition. Ideally, to get a more realistic view, fauna should be analysed to species level with more replicates, but as pointed out by Wilson (2016) and De Smet et al. (2017), capturing all species is unrealistic because of the logistics of processing large numbers of samples to species level.

Size spectra and sex ratios

In bare sediment the majority of specimens (> 50 %) had a body length in the range 1000–1500 μm , which is similar to that found in the earlier study by Singh et al., (2014) from CIOB. Similarly, Gambi et al. (2003) observed that 50–70% of nematodes in Atacama Trench by were < 1.5 mm in length. Bussau (1993) also reported that 51 % of nematodes were in the length range of 501–1500 μm in the Peru Basin.

Previous studies on deep-sea nematodes found higher numbers of individuals in the smaller size fractions with increasing water depth (Pfannkuche, 1985) and decreasing food availability (Vanreusel et al., 1995; Soltwedel et al., 1996). The deep sea is well known to be food limited and this appears to be particularly true of CIOB. The surface water primary production (PP) values in the CIOB is lower than in other oceans (Matondkar et al. 2005; Pavithran et al. 2009). Low primary production contributes to low sediment organic carbon in CIOB (Pavithran et al. 2009). Schewe and Soltwedel, (1998) has hypothesized that increasing nematode dwarfism with depth is due to the increasing “oligotrophy” with depth. However, in nodule crevices even smaller size nematodes were found to be dominant, which could be related to the space and habitat available in the nodule crevices.

Females were three times more abundant than males at all CIOB stations which is in agreement with the earlier study from CIOB (Singh et al., 2014). However, this is in contrast to the Atacama Trench where males were five times more abundant than females (Gambi et al. 2003), whilst in the Clairion-Clepperton Fracture Zone females also dominated (Miljutina et al. 2010). Such a high proportion of females may be a strategy to minimize the energetic investment required for reproduction

Nodule crevices and bare sediment fauna

Knowledge about the benthic communities associated with nodule crevice fauna is greatly in need particularly in view of the potential impacts of nodule mining. To assess potential deep-sea diversity loss, it would be of interest to know if there are in fact unique, nodule-crevice bound taxa. However, technical constraints and uncertainties associated with collection make it difficult to obtain reliable quantitative data in the deep sea.

It has been shown that the nematode assemblage inhabiting the nodule crevices is smaller than those found in the bare sediment. In earlier studies, Thiel et al. (1993) and Bussau et al. (1995) also found low densities of nodule crevice fauna compared to the surrounding sediment. In the abyss, about 70–90% of the nematodes inhabit the upper surface layer of sediment (Snider et al., 1984; Bussau, 1993; Vanreusel et al., 1995). This suggests that the difference is related to the available space – bare sediment provides more space which can be inhabited in a continuous manner, whereas the nodule crevices on the nodule surface provide a limited number of sediment-filled pockets. While the nodule surface can be successfully utilized by sessile organisms which can be quite dense on the nodule surface (as shown by both Mulineaux, (1987) and Veillette et al., 2007), it may be less suitable for sediment-dwelling motile organisms such as nematodes.

Overall, at the family level, this study showed a high degree of similarity with earlier work, except for the Leptolaimidae, a family dominant in the nodule crevice samples. At the genus level, *Camacolaimus*, *Leptolaimus*, and *Acantholaimus* had their maximum abundance in the nodules crevices. *Camacolaimus* was also reported to be dominant by Thiel et al. (1993) in Pacific nodule crevice sample.

In this study, *Camacolaimus* was particularly dominant in nodule crevice samples, although it was also found in bare sediment though with a very low contribution. The high abundance of *Camacolaimus* in the nodule crevices may be related to the accumulation of organic matter at the nodule surface. *Camacolaimus* and *Acantholaimus* are epigrowth feeders which are capable of piercing or scraping hard surfaces (Tietjen and Lee, 1977). This strategy fits well with the nodulized substratum, although the *Acantholaimus* was dominant in both bare sediment and nodule crevice habitats. *Acantholaimus* has been found to be the dominant genera in soft sediments and in the crevices of nodules in previous deep sea studies (Bussau, 1993). Possibly these nematode genera are supported by microbial biomass derived from the labile organic matter in the sediment. Another genus, *Leptolaimus*, has been found to dominate sediments in the Laptev Sea (Vanaverbeke et al., 1997) in the Arctic Ocean (Makarov Basin) (Vanreusel et al., 2000). In our study, it was found to be abundant in the nodule crevices.

Leptolaimus, classified as a selective deposit feeder (feeding type 1A), are the main consumers of deep-sea bacteria (Jensen, 1988; Soeteart and Heip, 1995).

Identification of nematofauna to a lower taxonomical level, preferably to species, is required to fully characterize biogeographical patterns in the CIOB (ISA, 2015). Because of the dominance of *Acantholaimus*, *Leptolaimus* and *Camacolaimus*, they were identified to species level. Our species-level data suggests that there are a few species that are restricted to specific types of habitat. This suggestion is supported by the PERMANOVA results that show significant differences in species between the nodule crevice and bare sediment samples. This also reveals that the species level is more sensitive to habitat changes than the genus level, which stresses the need for species-level taxonomic studies in the deep-sea meiobenthos. Although we have encountered preference within the bare sediment for some species of *Acantholaimus* that were restricted to TMS or TRS, the majority of described species of *Acantholaimus* (*A. quintus*, *A. robustus*, *A. setosus*, *A. sieglere*, and *A. veitkoehlerae*), and the undescribed species of *Leptolaimus* and *Camacolaimus* were represented in both sites (TMS and TRS). With the exception of a few morphotypes of *Leptolaimus* and *Camacolaimus* that were restricted only to the nodule crevices, the majority of the described species of *Acantholaimus* reported here have been reported earlier in the deep sea by Bussau (1993). Interestingly, in earlier reports *Acantholaimus maks* was found to live abundantly in the nodules, but in our samples, it was completely absent, both in the surrounding sediments and the nodule crevices. Thiel et al. (1993) and Bussau (1993) also found that some nematodes species live only in nodule crevices. Thiel et al. (1993) reported *Camacolaimus*, *Syringolaimus*, *Paracyatholaimus*, *Trefusia* and *Cervonema* as the dominant genera in nodules crevices, whereas *Thalassomonhystera*, *Desmoscolex* and *Tricoma* mainly dwelled in surrounding ooze. In this study, only a few species morphotypes of *Camacolaimus* and *Leptolaimus* were found only inside the nodules. This was the reason that these morphotypes contributed most to the dissimilarity between the nodule crevice and bare sediment fauna. The results from this study clearly show that the nodule crevice fauna, when identified to the genus level, is not distinctly different from the bare sediment fauna, but there are some species which are restricted to nodule crevices. In the deep sea, species diversity varies geographically and bathymetrically, and many hypotheses have been proposed concerning the factors that drive the patterns of species diversity in the deep sea, but so far it has not been possible to draw any firm conclusion about which of these hypotheses are correct (Etter, and Grassle, 1992). This study suggests that presence of nodules contributes to increased species diversity.

Although, it is generally useful to compare studies, it is difficult to compare nematode species assemblages from different deep-sea regions owing to the limited number of studies and the fact that

the species identification tends to rely mostly on putative species/morphotype level. Generally, owing to the limited number of nodule samples, it is difficult to unequivocally conclude that the species/morphotype presence or absence is specific for particular habitats. In both of the habitats (bare sediment and nodule crevice), the coexistence of genera/species was always relatively high, therefore no significant variation in diversity indices was observed. The addition of nodule crevice fauna, therefore, appears to complement the existing knowledge of deep-sea nematode assemblage patterns.

4.2.Recommendations for Management

Polymetallic nodule mining at the abyssal depths is likely to impact one of the most remote and least known environments on earth. This study is particularly pertinent in CIOB as contractors are considering to initiate test mining in 2019 at the end of the exploration phase of mining.

In this context, ISA has developed the regulatory framework for preserving habitat and benthic species in the areas where commercial mining of the deep-sea polymetallic nodules is being done, where habitat and benthic species in TRS adjacent to TMS are not impacted during a mining operation, and so can be used as the areas at which the unaffected biota is preserved; this biota could then perhaps serve as donors for re-colonization of sites impacted by mining.

In this study, we have found a homogeneous nematode community at the family/genus level from the TMS and TRS which are separated by ~60 nautical miles. However, this is likely not the case for each and every benthic taxa because there is always a uncertainty in the spatial and temporal variation of the fauna (Wedding et al., 2015). The ISA has recommended that test reference mining site should have species composition comparable to that of the TMS but there are also uncertainties in producing species-level data. The following describes a few shortcomings to the ISA recommendations and uncertainties that will likely continue to plague efforts to obtain reliable datasets from the deep sea.

First, in studies that penetrate to the species level there is a high chance of getting distinct species-specific assemblages in both the sites because there are millions of species which are not even discovered yet. Moreover, marine species diversity often shows inhomogeneous patterns as different oceanographic processes tend to influence community structure. Earlier studies of Miljutina et al. (2010) found a significant difference in species composition of the nematode community between nodule-bearing and nodule-free sites in the French licence area in the Clairion-Clepperton Fracture Zone.

In addition, the abyssal deep sea has typically low densities of meiofauna, as compared to larger size classes (Zeppilli et al., 2015). However, species diversity in general, is high (Glover et al., 2002; Grassle et al., 1989) and many species are represented as singletons in small sediment samples

(Jones et al., 2017). Therefore, large samples along with numerous replicates are required to properly quantify the faunal density and the diversity particularly of meiofauna which most of the time is not possible in the deep sea. Therefore, prior to investigating the impact of test mining a better understanding of reference areas and nodule associated fauna should be achieved. Moreover, the biotic and abiotic environmental condition of the areas along with habitat mapping is also very much needed (Cordes et al., 2016). More studies of the fauna associated with ferromanganese nodules are critically needed to ensure nodule mining is environmentally sustainable. This is challenging and will require careful planning and a multi-disciplinary approach.

5. Conclusions

This study provides one of the first insights into the abundance, diversity, and community composition of nematofauna from the test mining and reference sites in the first generation site of CIOB. The objective of this study was to improve our knowledge of sediment fauna from test and reference sites in order to facilitate environmentally sustainable management of future mining activities. A homogeneous community was observed at family/genus level from the TMS and TRS, particularly dominated by cosmopolitan nematode genera like *Acantholaimus* and *Halalaimus*, *Thalassomonhystera* and *Leptolaimus*. Indeed, the data and analysis presented in this paper indicate that the entire area of our sampling is inhabited by a single, uniformly distributed, nematode assemblage at the genus level. Despite the limited sampling in this study (10 box cores), we characterized a large fraction (70%) of the nematode genus diversity belonging to this specific habitat type (i.e., TMS, TRS).

This study aimed to determine whether nematode assemblages inhabiting nodule crevices would differ in terms of nematode community structure and diversity from those in adjacent soft sediments. As expected, there were fewer numbers of total genera recorded from nodule crevices compared to the soft sediments. These differences were found in the dominant genera and families. The nematode assemblages inhabiting the nodule crevices were dominated by *Camacolaimus* and *Leptolaimus* and unique colonization of the nodule-crevice micro-habitat by specific genera or families was not observed. However, it was also shown that *Camacolaimus* and *Leptolaimus* were most likely to represent the nodule crevice microhabitat, whilst typical deep-sea genera such as *Acantholaimus* and *Thalassomonhystera* were more representative of the bare sediment. Furthermore, we can postulate that the hard nodule crevice substratum does favor some nematode species since three undescribed species were found exclusively in nodule crevices. However, owing to the still limited sampling effort in the deep sea, it is premature to suggest that these species are endemic to nodules.

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Table 1: Details of the Central Indian Ocean Basin sampling stations, sub sampling, depth, nodule abundance, latitude, and longitude detail.

| Location | Station | Box core | Sub-sampling | Lat (S) | Long (E) | Depth | Nodule abundance (Kg/m ²) | Description |
|----------|---------|----------|--------------|-----------|-----------|-------|---------------------------------------|--|
| TRS | TRS-1 | 1 | 2 | 12 52.537 | 74 37.444 | 4908 | 8.4 | Small to medium size, flat, smooth surface |
| | TRS-2 | 1 | 2 | 12 52.509 | 74 45.009 | 5094 | 5.8 | Rough surface nodules of 2-5cm diameter |
| | TRS-3 | 1 | 2 | 12 56.216 | 74 41.217 | 5152 | 7.2 | Smooth surface big nodules and rough surface small nodules |
| | TRS-4 | 1 | 2 | 13 00.003 | 74 37.494 | 5055 | NA | 6-7 Small (~1cm), flat, rough surface nodules |
| | TRS-5 | 1 | 2 | 13 00.014 | 74 44.998 | 4900 | NA | Small Pieces |
| TMS | TMS-1 | 1 | 2 | 13 29.999 | 75 29.982 | 5227 | 12.44 | 3 large botryoidal, others medium, rough surface |
| | TMS-2 | 1 | 2 | 13 37.498 | 75 29.995 | 5280 | 6.52 | Small to medium, rough surface nodules. |
| | TMS-3 | 1 | 2 | 13 37.496 | 75 37.491 | 5234 | 4.4 | Rough surface 2-5cm diameter nodules |
| | TMS-4 | 1 | 2 | 13 29.995 | 75 37.493 | 5180 | 5.6 | Rough surface 2-5cm diameter nodules. |
| | TMS-5 | 1 | 2 | 13 33.743 | 75 33.743 | 5187 | 6 | Rough surface small (3-4) and big (5-6) nodules |

Table 2: Pair-wise analysis of genus-level taxonomic composition. Average relative abundances (%) of the ten genera who contributed most to pair-wise dissimilarity (SIMPER)

| Groups Mining site & Reference site | | | | | | |
|-------------------------------------|-------------------------------|----------------------------------|---------|---------|----------|-------|
| Average dissimilarity = 38.51 | | | | | | |
| Genus | Group Mining site Av.Abund | Group Reference site Av.Abund | Av.Diss | Diss/SD | Contrib% | Cum.% |
| <i>Chromadorita</i> | 0.42 | 1.77 | 1.5 | 1.9 | 3.9 | 3.9 |
| <i>Syngolaimus</i> | 0.31 | 1.28 | 1.19 | 1.54 | 3.08 | 6.98 |
| <i>Marisalbinema</i> | 1.13 | 0 | 1.18 | 2.85 | 3.07 | 10.05 |
| <i>Araeolaimus</i> | 0.6 | 1.54 | 1.1 | 1.56 | 2.87 | 12.92 |
| <i>Thalassomonhystera</i> | 2.98 | 2.07 | 1.05 | 1.43 | 2.73 | 15.65 |
| <i>Axonolaimus</i> | 1.05 | 0.73 | 1.02 | 1.08 | 2.65 | 18.29 |
| <i>Cobbia</i> | 0.7 | 1.46 | 0.98 | 1.52 | 2.54 | 20.83 |
| <i>Desmodora</i> | 0.34 | 0.89 | 0.94 | 3.3 | 2.44 | 23.27 |
| <i>Camacolaimus</i> | 1.44 | 0.77 | 0.92 | 1.37 | 2.39 | 25.67 |
| <i>Actinonema</i> | 1.77 | 0.96 | 0.91 | 1.81 | 2.36 | 28.02 |

Table 3: Pair-wise analysis of species-level taxonomic composition. Average relative abundances (%) of the top species who contributed most to pair-wise dissimilarity (SIMPER)

| Groups Bare sediment & Nodule crevice | | | | | | |
|---|---------------------------------|----------------------------------|---------|---------|----------|-------|
| Average dissimilarity = 51.49 | | | | | | |
| Genera | Group Bare sediment Av.Abund | Group Nodule crevice Av.Abund | Av.Diss | Diss/SD | Contrib% | Cum.% |
| <i>Camacolaimus</i> | 1.11 | 3.28 | 3.27 | 2.57 | 6.34 | 6.34 |
| <i>Aegialoalaimus</i> | 1.57 | 0 | 2.38 | 3.1 | 4.63 | 10.98 |
| <i>Daptonema</i> | 1.44 | 0 | 2.16 | 3.23 | 4.2 | 15.18 |
| <i>Theristus</i> | 1.68 | 2.33 | 2.03 | 2.27 | 3.95 | 19.13 |
| <i>Chromadorita</i> | 1.01 | 2.04 | 1.7 | 1.4 | 3.3 | 22.43 |
| <i>Araeolaimus</i> | 1.04 | 0 | 1.63 | 1.49 | 3.17 | 25.6 |
| <i>Cobbia</i> | 1.09 | 0 | 1.62 | 1.6 | 3.15 | 28.75 |
| <i>Leptolaimus</i> | 2 | 3.02 | 1.55 | 2.27 | 3.01 | 31.75 |
| <i>Ceramonema</i> | 0.98 | 0 | 1.47 | 1.95 | 2.85 | 34.61 |
| <i>Halalaimus</i> | 2.33 | 1.54 | 1.39 | 0.99 | 2.71 | 37.31 |
| Groups Bare sediment & Nodule crevice Species | | | | | | |
| Average dissimilarity = 37.60 | | | | | | |
| Species | Group Bare sediment Av.Abund | Group Nodule crevice Av.Abund | Av.Diss | Diss/SD | Contrib% | Cum.% |
| <i>Camacolaimus</i> sp.3 | 0 | 17 | 5.54 | 5.1 | 14.73 | 14.73 |
| <i>Camacolaimus</i> sp.4 | 0 | 12 | 3.93 | 6.99 | 10.46 | 25.19 |
| <i>Acantholaimus</i> sp.2 | 13.5 | 5 | 2.98 | 1.23 | 7.93 | 33.12 |
| <i>Acantholaimus</i> sp.3 | 8 | 0 | 2.72 | 4.22 | 7.24 | 40.37 |
| <i>Leptolaimus</i> sp.2 | 17 | 14.5 | 2.45 | 1.17 | 6.51 | 46.87 |

Table 4: List of the valid species and species morphotypes identified in the dominant genera, with their relative abundances (% from overall) (-, Absent).

| | species | Bare sediment | | Nodule crevice | |
|---|---------|---------------|------|----------------|------|
| | | TRS% | TMS% | TRS% | TMS% |
| <i>Acantholaimus</i> | sp1 | 14.7 | 10.8 | 25.6 | 10.9 |
| <i>Acantholaimus</i> | sp2 | 25.3 | 12.3 | 10.3 | 9.4 |
| <i>Acantholaimus</i> | sp3 | 12.0 | 10.8 | | |
| <i>Acantholaimus</i> | sp4 | | 9.2 | 12.8 | 6.3 |
| <i>Acantholaimus akvavitus</i> Gerlach, 1979 | | 8.0 | | 17.9 | 9.4 |
| <i>Acantholaimus angustus</i> Bussau, 1993 | | 2.7 | 6.2 | 12.8 | 6.3 |
| <i>Acantholaimus arthrochaeta</i> Miljutina and Miljutin, 2012 | | 8.0 | 3.1 | 5.1 | 21.9 |
| <i>Acantholaimus barbatus</i> Miljutina and Miljutin, 2012 | | 4.0 | 6.2 | 10.3 | 12.5 |
| <i>Acantholaimus formosus</i> Miljutina et al. 2012 | | 6.7 | 13.8 | 2.6 | 7.8 |
| <i>Acantholaimus iubilus</i> Gerlach, Schrage, Riemann, 1979 | | 9.3 | 7.7 | 2.6 | 9.4 |
| <i>Acantholaimus microdontus</i> Gourbault and Vincx, 1985 | | 1.3 | 3.1 | | 6.3 |
| <i>Acantholaimus quintus</i> Schrage and Riemann, 1979 | | 2.7 | | | |
| <i>Acantholaimus robustus</i> | | 1.3 | 3.1 | | |
| <i>Acantholaimus setosus</i> Vitiello, 1970 | | 1.3 | 3.1 | | |
| <i>Acantholaimus sieglere</i> | | 1.3 | 6.2 | | |
| <i>Acantholaimus veitkoehlerae</i> Miljutina and Miljutin, 2012 | | 1.3 | 3.1 | | |
| <i>Camacolaimus</i> | sp1 | 46.2 | 53.8 | 30.2 | 26.1 |
| <i>Camacolaimus</i> | sp2 | 53.8 | 46.2 | 20.9 | 20.3 |
| <i>Camacolaimus</i> | sp3 | | | 27.9 | 31.9 |
| <i>Camacolaimus</i> | sp4 | | | 20.9 | 21.7 |
| <i>Leptolaimus</i> | sp1 | 38.6 | 28.8 | 28.9 | 23.9 |
| <i>Leptolaimus</i> | sp2 | 27.3 | 37.3 | 21.1 | 31.3 |
| <i>Leptolaimus</i> | sp3 | 15.9 | 22.0 | 18.4 | 17.9 |
| <i>Leptolaimus</i> | sp4 | 18.2 | 11.9 | 18.4 | 20.9 |

Table 5: Genera associated with individual group and the significance level of the associations

| Genera | Group | Indval | p-value | freq |
|--------------------------|----------------|----------|---------|------|
| <i>Axonolaimus</i> | TMS | 1.000 | 0.01 | 2 |
| <i>Cyatholaimus</i> | TMS | 1.000 | 0.011 | 10 |
| <i>Marisalbinema</i> | TMS | 1.000 | 0.012 | 3 |
| <i>Viscosia</i> | TMS | 1.000 | 0.008 | 9 |
| <i>Pselionema</i> | TRS | 1.000 | 0.008 | 1 |
| <i>Chromadorita</i> | TRS | 0.877551 | 0.023 | 8 |
| <i>Southerniella</i> | TRS | 0.833333 | 0.029 | 5 |
| <i>Aegialolaimus</i> | TRS | 0.698413 | 0.026 | 10 |
| Species | Group | Indval | pvalue | freq |
| <i>Camacolaimus sp3</i> | Nodule crevice | 0.877551 | 0.023 | 8 |
| <i>Camacolaimus sp4</i> | Nodule crevice | 0.833333 | 0.029 | 5 |
| <i>Acantholaimus sp3</i> | Bare sediment | 0.698413 | 0.026 | 10 |

Table 6: Diversity indices (mean and standard deviation) for nematode assemblages at the genus level: Margelf's index (d), Pielou's evenness (J'), expected no. of species ES (51) and Shannon-Wiener diversity (H')

| Site | S | d | J' | ES(51) | H'(loge) |
|-----------------|-----------|-----------|-----------|---------------|-----------------|
| Nodule crevices | 10.4±0.6 | 24±1.9 | 2.9±0.12 | 0.97±0.01 | 2.27±0.06 |
| Bare sediment | 34.4±2.7 | 45.6±3.68 | 8.72±0.52 | 0.97±0.01 | 3.42±0.07 |
| TMS | 37.4±2.18 | 7.9±0.5 | 1.0±0.02 | 28.4±1.2 | 3.5±0.1 |
| TRS | 39.2±1.59 | 8.3±0.35 | 1.0±0.01 | 29.5±0.60 | 3.5±0.04 |

Table 7: Comparison of nematode abundance previously recorded from different sites of Oceans

| Location | Depth (m) | Latitude | Longitude | Abundance inds/10cm ² | Types of sediment | Mesh Size | Reference | Sampling Gear |
|---|-------------------------------|-----------------------|------------------------|-------------------------------------|-------------------------------|--------------|---|----------------------------------|
| Atlantic Ocean | | | | | | | | |
| Porcupine Seabight, NE Atlantic | 4167 - 4850 | 49.3°- 52.2°N | 13.0°- 14.2°W | 345±103 | - | 42µm | Pfannkuche (1985) | Multiple corer |
| Porcupine Abyssal Plain | 4843 - 4850 | 48°50.4'- 48°51.9N | 16°28.8'- 16°29.9'W | 118±35 | - | - | Lamshead et al. (1995) | Multiple corer |
| NE Atlantic BIOTRANS NE Atlantic | 4330 - 4561 | 47-48°N | 19°30'- 19°34.7'W | 117±63 | - | 30µm | Soltwedel et al. (1996) | Box corer and Multiple corer |
| Pacific Ocean | | | | | | | | |
| Clarion Clipperton fracture zone, eastern, central Pacific | 4905 - 5140 | 14°N | 130°W | 35±15 | radiolarian siliceous ooze | 40µm | Renaud- Mornant andGourbault -1990 | Box corer |
| Western Pacific Eastern south Pacific | 5580 - 5820 | 10-29°N | 144-153°E | 145±108 | sand,silt,red clay | | Shirayama (1984) | Box corer |
| (Peru Basin) | 4100 - 4200 | 07°S | 88°W | 133 | - | | Bussau et al. (1993) | Multiple corer |
| (Peru Basin) HOT Station central Pacific | 4100 - 4200 4871 - 4884 | 07°04'40"S 22°N | 88°27'60"W 157°W | 121 40±9 | nodule,nodule crevice - | 45µm | Bussau et al. (1995) Brown et al. (2001) | Multiple corer Multiple corer |

| | | | | | | | | |
|---|---------------|--------------------|--------------------|----------------------|--|------|-------------------------------|--|
| JGOFS Eq.Pac. transect 9°N, Equatorial Pacific NE Pacific Basin, CCNP, 14°N, NN site | 4986 - 4994 | | | 45±38 | - | 45µm | Brown et al. (2001) | Box corer |
| NE Pacific Basin, CCNP, 14°N, NB site | 4,983 - 5,042 | ≈14°N | 130°W | 137±28 | fine grained,radiolarian oozes,deep sea red clays | 40µm | Miljutina et al. (2010) | Multiple corer |
| Kermadec Trench Southwest Pacific Ocean | 4,947 - 5,000 | ≈14°N | 130°W | 69±19 | fine grained,radiolarian oozes,deep sea red clays | 40µm | Miljutina et al. (2010) | Multiple corer |
| | 6013-9177 | 32-35°S | 177-178°W | 42-312 | | 20µm | Leduc and Bowden (2018) | hybrid re-motely operated vehicle (HROV) Nereus manned submersible SHINKAI 6500 and core samplers |
| Tonga Trench Indian Ocean | 6250-10817 | 16-26° S | 172-178°W | 43.2-489.2 | Silty | 20µm | Leduc et al. (2016b) | |
| Central Indian Ocean Basin | 5200 - 5300 | 10°S | 75-76°W | 23.5±12.2 | Siliceous ooze sedimet,ferromanganese nodules | 45µm | Ingole et al. (2005) | Box corer |
| Central Indian Ocean Basin | 5300 - 5330 | 10°S | 75-76°W | 52±27 | Siliceous ooze sedimet,ferromanganese nodules | 45µm | Ingole et al. (2000) | Box corer |
| South West Indian Ocean | 2007 - 2053 | 03-04°S | 41°W | 251-406 | medium sand,fine sand,very fine sand, silt | 32µm | Muthumbi et al. (2004) | Box corer |
| Central Indian Ocean Basin | 5000 - 5200 | 12°20'- 13°00'S | 74°18'- 75°30'E | 27.17 ±2.7 71.68- | silt,clay,sand | 32µm | Singh et al. (2014) | Box corer |
| Central Indian Ocean Basin | 4900-5280 | 12°-13°S | 74°-75°E | 91.23 | silt,clay,sand | 32µm | Present Study | Box corer |

Table 8: The abundance of nematode genera recorded from TMS and TRS is presented in percentage (%)

| Genus | TMS1 % | TMS2% | TMS3% | TMS4% | TMS5% | TRS1% | TRS2% | TRS3% | TRS4% | TRS5% |
|-------------------------|-----------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| <i>Acantholaimus</i> | 1.9 | 2.8 | 1.0 | 3.7 | 7.4 | 1.3 | 2.0 | 2.5 | 2.0 | 1.1 |
| <i>Actinonema</i> | 1.2 | 0.7 | 1.4 | 0.6 | 0.4 | 0.1 | 0.1 | 0.4 | 0.8 | 0.1 |
| <i>Aegialoalaimus</i> | 0.5 | 0.5 | 0.4 | 0.3 | 0.2 | 0.6 | 0.7 | 1.1 | 1.3 | 1.5 |
| <i>Anticoma</i> | 0.4 | 0.2 | 0.1 | 0.1 | 0.1 | 0.1 | 0.5 | 0.1 | 0.7 | 0.1 |
| <i>Araeolaimus</i> | 0.1 | 0.4 | 0.0 | 0.0 | 0.3 | 1.5 | 0.2 | 0.9 | 0.1 | 1.1 |
| <i>Axonolaimus</i> | 0.6 | 0.8 | 1.0 | 0.6 | 1.2 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| <i>Bathyeurystomina</i> | 0.0 | 0.0 | 0.0 | 0.3 | 0.3 | 0.2 | 0.2 | 0.0 | 0.0 | 0.0 |
| <i>Bolbolaimus</i> | 0.2 | 0.0 | 0.1 | 0.2 | 0.6 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| <i>Calligyus</i> | 0.2 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| <i>Camacolaimus</i> | 0.4 | 0.8 | 0.2 | 0.5 | 0.6 | 0.9 | 0.0 | 0.2 | 0.4 | 0.0 |
| <i>Campylaimus</i> | 0.0 | 0.0 | 0.0 | 0.0 | 0.2 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| <i>Ceramonema</i> | 0.5 | 0.0 | 0.1 | 0.6 | 0.0 | 0.1 | 0.9 | 0.2 | 1.8 | 0.2 |
| <i>Cervonema</i> | 0.4 | 0.5 | 0.1 | 0.0 | 0.0 | 0.1 | 0.5 | 0.8 | 1.1 | 0.0 |
| <i>Chromadora</i> | 0.5 | 0.0 | 0.0 | 0.0 | 0.1 | 0.1 | 0.0 | 0.1 | 0.6 | 0.4 |
| <i>Chromadorella</i> | 0.2 | 0.1 | 0.0 | 0.1 | 0.1 | 0.0 | 0.0 | 0.1 | 0.1 | 0.0 |
| Chromadoridae_Genus_1 | 0.1 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| <i>Chromadorina</i> | 0.6 | 0.1 | 0.0 | 0.0 | 0.0 | 0.5 | 0.1 | 0.6 | 0.1 | 0.2 |
| <i>Chromadorita</i> | 0.3 | 0.1 | 0.0 | 0.1 | 0.1 | 0.5 | 1.3 | 1.6 | 1.2 | 0.2 |
| <i>Cobbia</i> | 0.6 | 0.3 | 0.1 | 0.0 | 0.0 | 0.9 | 0.5 | 1.1 | 0.2 | 0.5 |
| Cyatholaimidae_Genus_1 | 0.0 | 0.2 | 0.5 | 0.3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| <i>Cyatholaimus</i> | 0.7 | 0.2 | 0.3 | 0.4 | 0.1 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 |
| <i>Daptonema</i> | 0.5 | 1.0 | 0.5 | 1.3 | 1.2 | 0.6 | 0.7 | 0.8 | 0.4 | 0.1 |
| <i>Desmodora</i> | 0.5 | 0.2 | 0.0 | 0.0 | 0.0 | 0.1 | 0.4 | 0.2 | 0.1 | 0.4 |

| | | | | | | | | | | |
|------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| <i>Desmoscolex</i> | 0.8 | 0.7 | 1.8 | 0.9 | 0.8 | 1.2 | 1.2 | 1.5 | 1.3 | 1.1 |
| <i>Dichromadora</i> | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.2 |
| Diplopeltidae_Genus_1 | 0.0 | 1.0 | 0.0 | 0.4 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| <i>Diplopeltula</i> | 1.4 | 1.0 | 0.8 | 1.2 | 0.9 | 1.1 | 0.4 | 1.4 | 1.3 | 1.1 |
| <i>Doliolaimus</i> | 0.0 | 0.1 | 0.0 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| <i>Greeffiella</i> | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| <i>Halalaimus</i> | 1.7 | 1.0 | 1.2 | 1.9 | 2.4 | 1.3 | 1.6 | 2.2 | 0.4 | 0.9 |
| <i>Hopperia</i> | 0.0 | 0.5 | 0.2 | 0.1 | 0.0 | 0.0 | 0.2 | 0.4 | 0.0 | 0.2 |
| <i>Kraspedonema</i> | 0.0 | 0.0 | 0.0 | 0.2 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| <i>Ledovitia</i> | 0.8 | 0.9 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 |
| <i>Leptolaimus</i> | 0.9 | 1.7 | 0.8 | 1.5 | 1.0 | 1.5 | 1.3 | 0.9 | 1.4 | 1.4 |
| Linhomoeidae_Genus_1 | 0.0 | 0.0 | 0.3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| <i>Linhomoeus</i> | 0.2 | 0.0 | 0.0 | 0.0 | 0.1 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 |
| <i>Linhystra</i> | 0.2 | 0.0 | 0.0 | 0.2 | 0.0 | 0.2 | 0.1 | 0.0 | 0.2 | 0.1 |
| <i>Manganonema</i> | 0.5 | 0.6 | 0.2 | 0.6 | 0.4 | 0.1 | 0.4 | 0.2 | 0.1 | 0.1 |
| <i>Marisalbinema</i> | 0.6 | 0.1 | 0.5 | 0.1 | 0.4 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| <i>Marylynnia</i> | 0.1 | 0.0 | 0.3 | 0.2 | 0.0 | 0.1 | 0.1 | 0.0 | 0.0 | 0.0 |
| <i>Mesacanthion</i> | 0.0 | 0.0 | 0.2 | 0.0 | 0.1 | 0.1 | 0.0 | 0.2 | 0.1 | 0.1 |
| <i>Metachromadora</i> | 0.1 | 0.0 | 0.0 | 0.1 | 0.2 | 0.2 | 0.0 | 0.0 | 0.1 | 0.0 |
| <i>Metadesmolaimus</i> | 0.0 | 0.0 | 0.0 | 0.0 | 0.2 | 0.1 | 0.0 | 0.1 | 0.1 | 0.0 |
| <i>Metalinhomoeus</i> | 0.0 | 0.1 | 0.0 | 0.0 | 0.3 | 0.1 | 0.0 | 0.1 | 0.0 | 0.1 |
| <i>Microlaimus</i> | 0.6 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.4 | 0.0 | 0.0 |
| <i>Molgolaimus</i> | 0.0 | 0.0 | 0.3 | 0.1 | 0.0 | 0.5 | 0.1 | 0.1 | 0.0 | 0.0 |
| <i>Nannolaimus</i> | 0.0 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| <i>Nemanema</i> | 0.0 | 0.2 | 0.0 | 0.0 | 0.1 | 0.2 | 0.0 | 0.1 | 0.1 | 0.1 |
| <i>Neochromadora</i> | 0.1 | 0.0 | 0.1 | 0.0 | 0.0 | 0.1 | 0.0 | 0.2 | 0.2 | 0.1 |
| <i>Oncholaimus</i> | 0.0 | 0.1 | 0.1 | 0.0 | 0.0 | 0.1 | 0.0 | 0.2 | 0.1 | 0.0 |
| <i>Paralinhomoeus</i> | 0.1 | 0.0 | 0.2 | 0.0 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 | 0.1 |
| <i>Paramonohystra</i> | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.2 | 0.1 | 0.2 | 0.0 |
| <i>Pselionema</i> | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.6 | 0.4 | 0.1 | 0.1 | 0.1 |

| | | | | | | | | | | |
|---------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| <i>Rhips</i> | 0.6 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.1 |
| <i>Siphonolaimus</i> | 0.6 | 0.1 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| <i>Southerniella</i> | 0.0 | 0.0 | 0.2 | 0.0 | 0.0 | 0.4 | 0.5 | 0.1 | 0.1 | 0.1 |
| <i>Sphaerolaimus</i> | 0.2 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.1 | 0.1 | 0.1 | 0.0 |
| <i>Syringolaimus</i> | 0.3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.8 | 0.2 | 0.0 | 0.9 | 1.1 |
| <i>Thalassomonhystera</i> | 1.3 | 1.5 | 0.7 | 0.8 | 1.1 | 1.2 | 1.3 | 1.8 | 0.7 | 2.1 |
| <i>Theristus</i> | 0.6 | 0.7 | 1.0 | 1.3 | 0.6 | 0.7 | 1.1 | 1.5 | 1.6 | 1.1 |
| <i>Tricoma</i> | 0.1 | 0.0 | 0.0 | 0.0 | 1.1 | 0.0 | 0.0 | 0.2 | 0.1 | 0.0 |
| <i>Tubolaimoides</i> | 0.0 | 0.0 | 0.0 | 0.1 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| <i>Valvaelaimus</i> | 0.1 | 0.0 | 0.0 | 0.1 | 0.0 | 0.0 | 0.1 | 0.0 | 0.0 | 0.2 |
| <i>Viscosia</i> | 0.4 | 0.4 | 0.2 | 0.3 | 0.5 | 0.0 | 0.2 | 0.4 | 0.1 | 0.0 |
| Xyalidae_Genus_1 | 0.1 | 0.3 | 0.6 | 0.1 | 0.0 | 0.2 | 0.1 | 0.1 | 0.2 | 0.2 |
| Xyalidae_Genus_2 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.1 | 0.1 | 0.8 | 0.0 |
| Xyalidae_Genus_3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.1 | 0.0 | 0.2 | 0.1 |

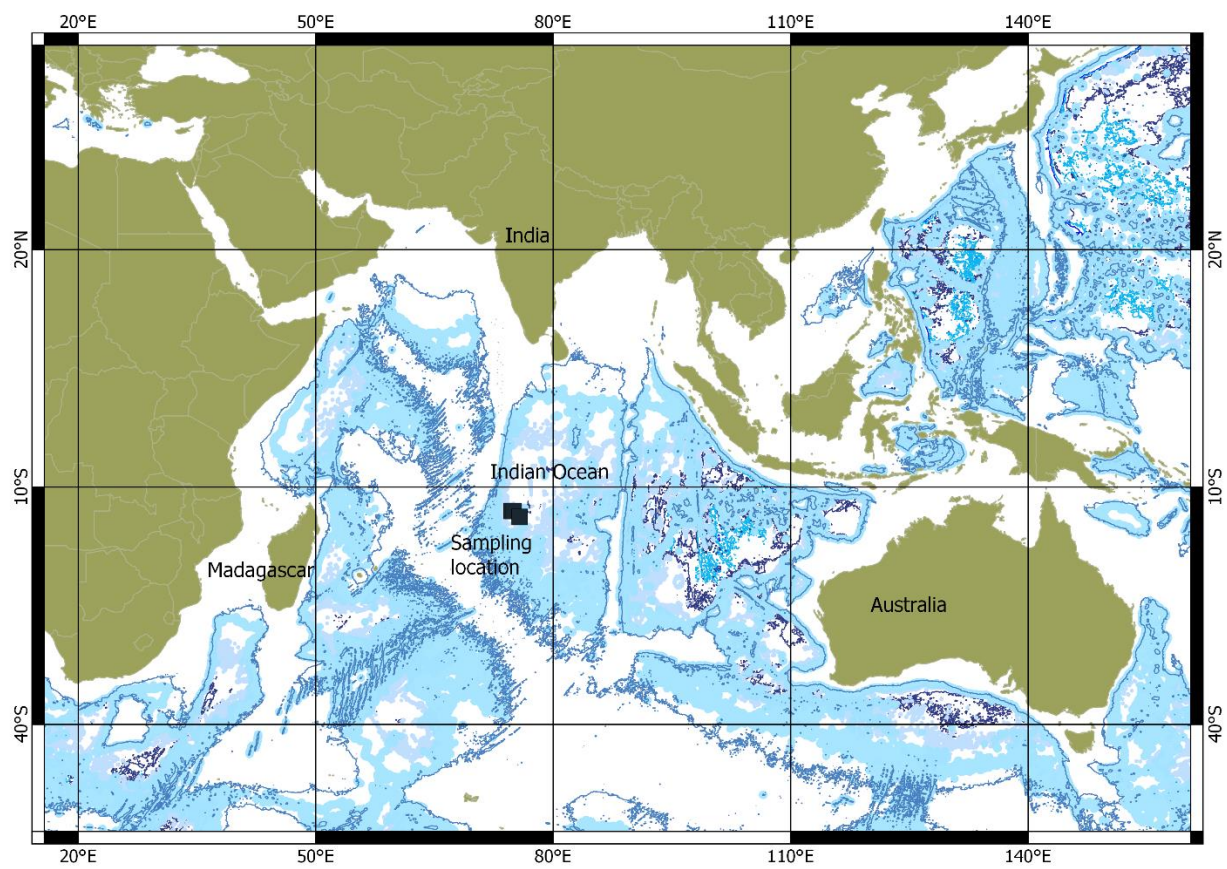


Fig.1

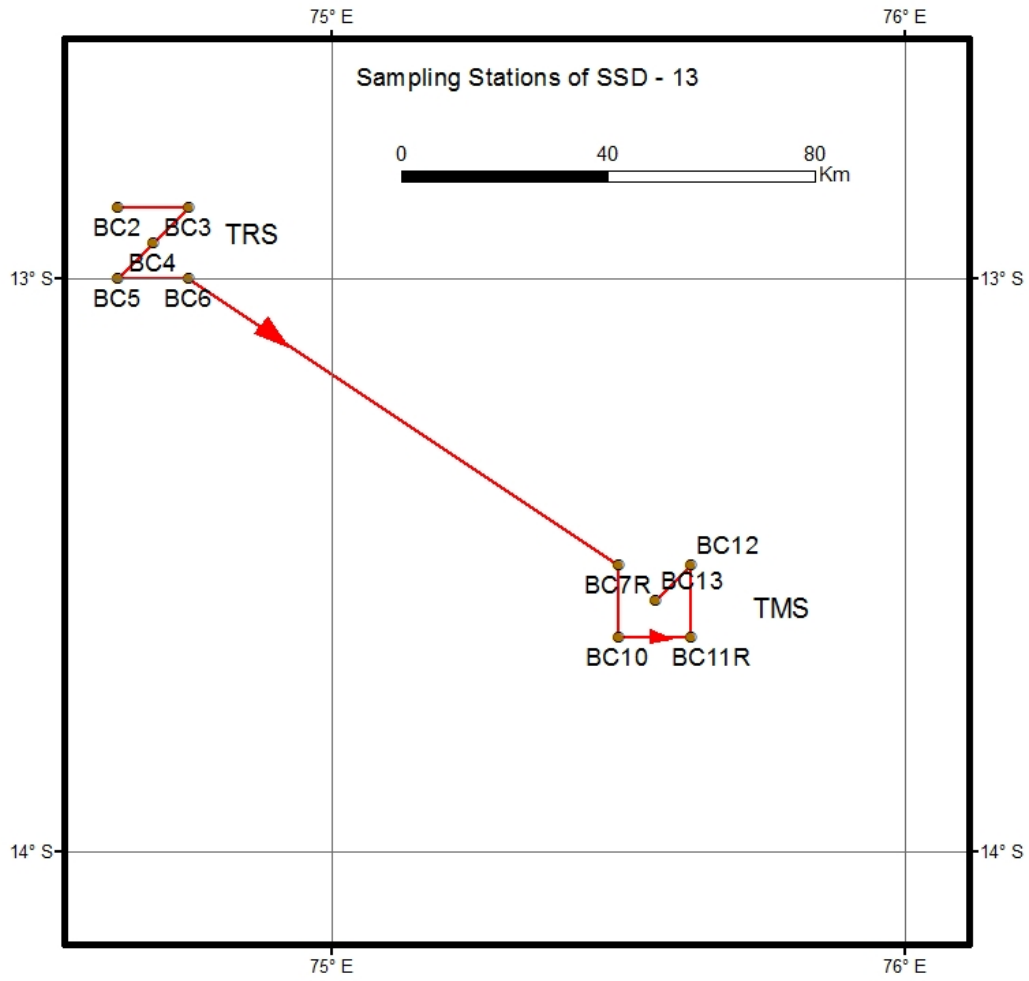


Fig.2

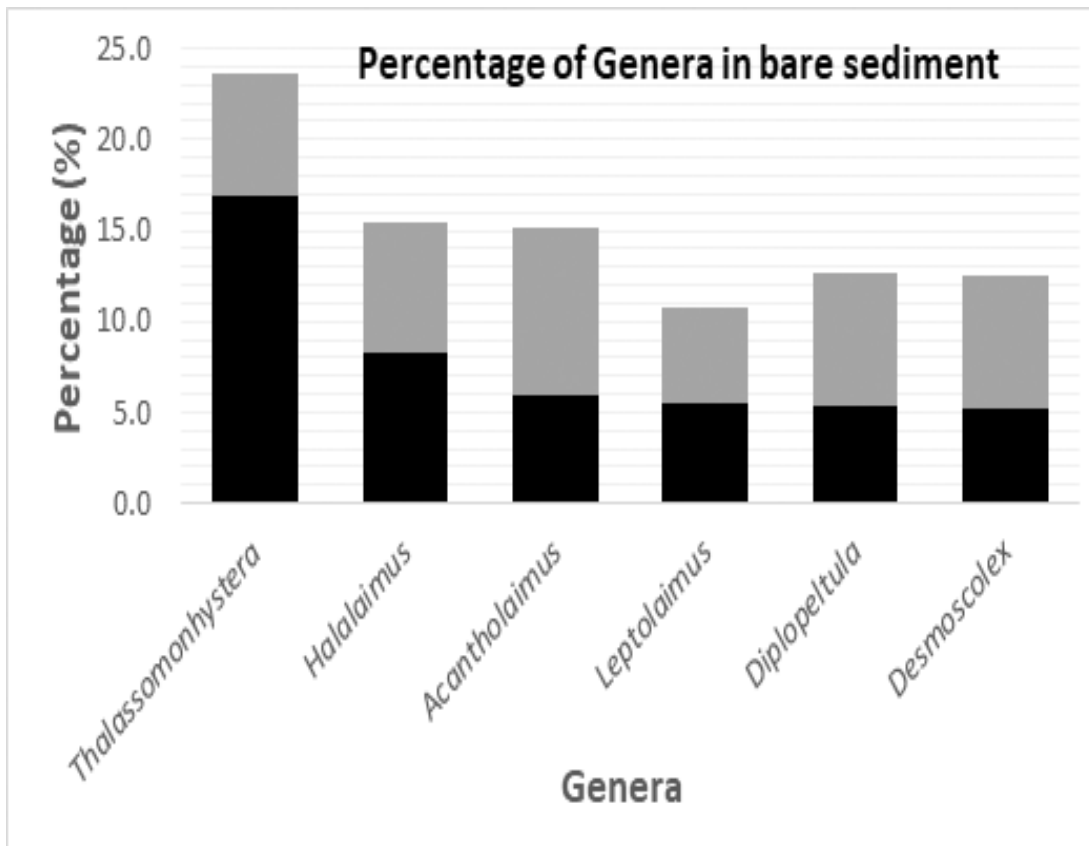


Fig.3

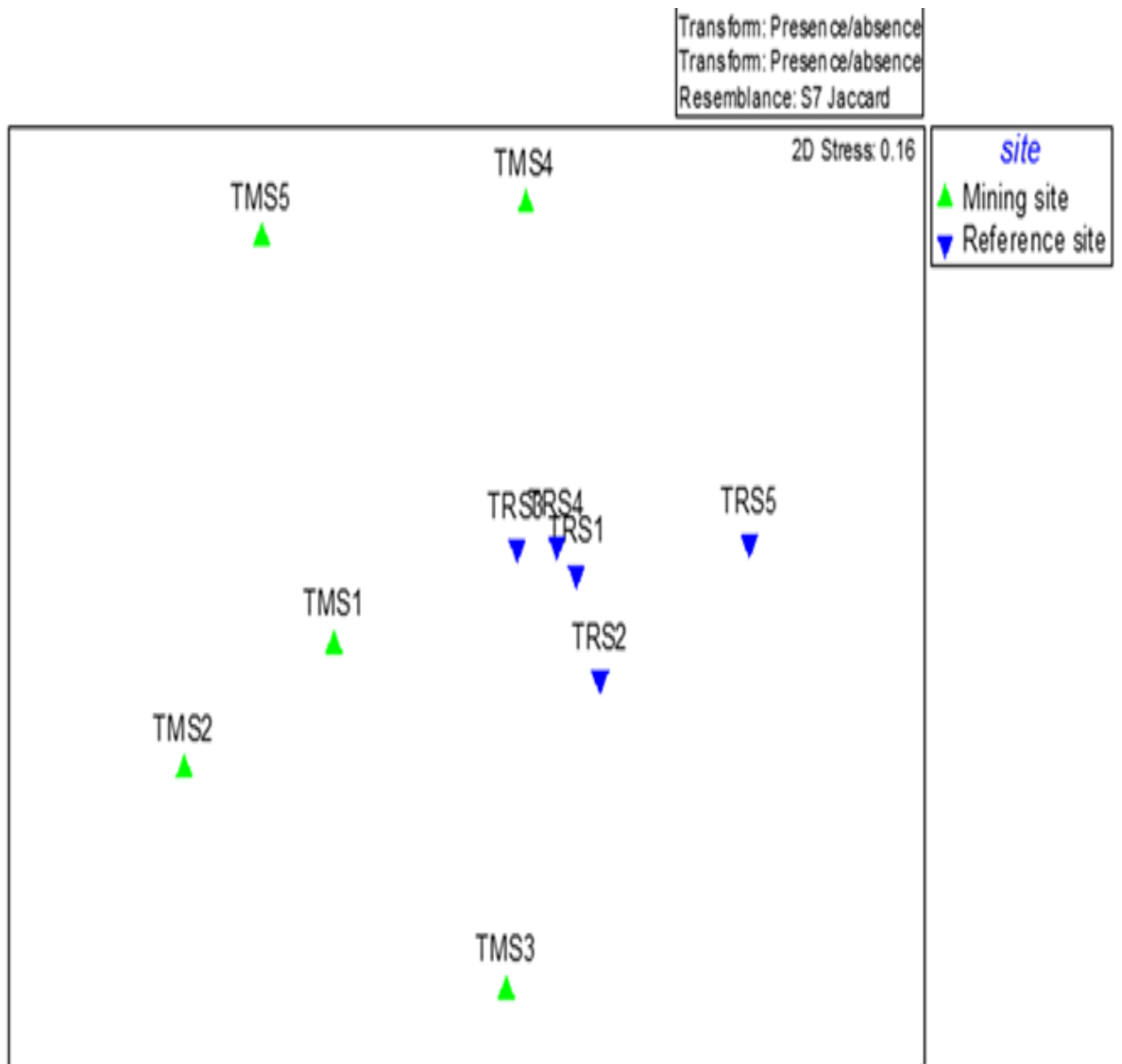


Fig.4a

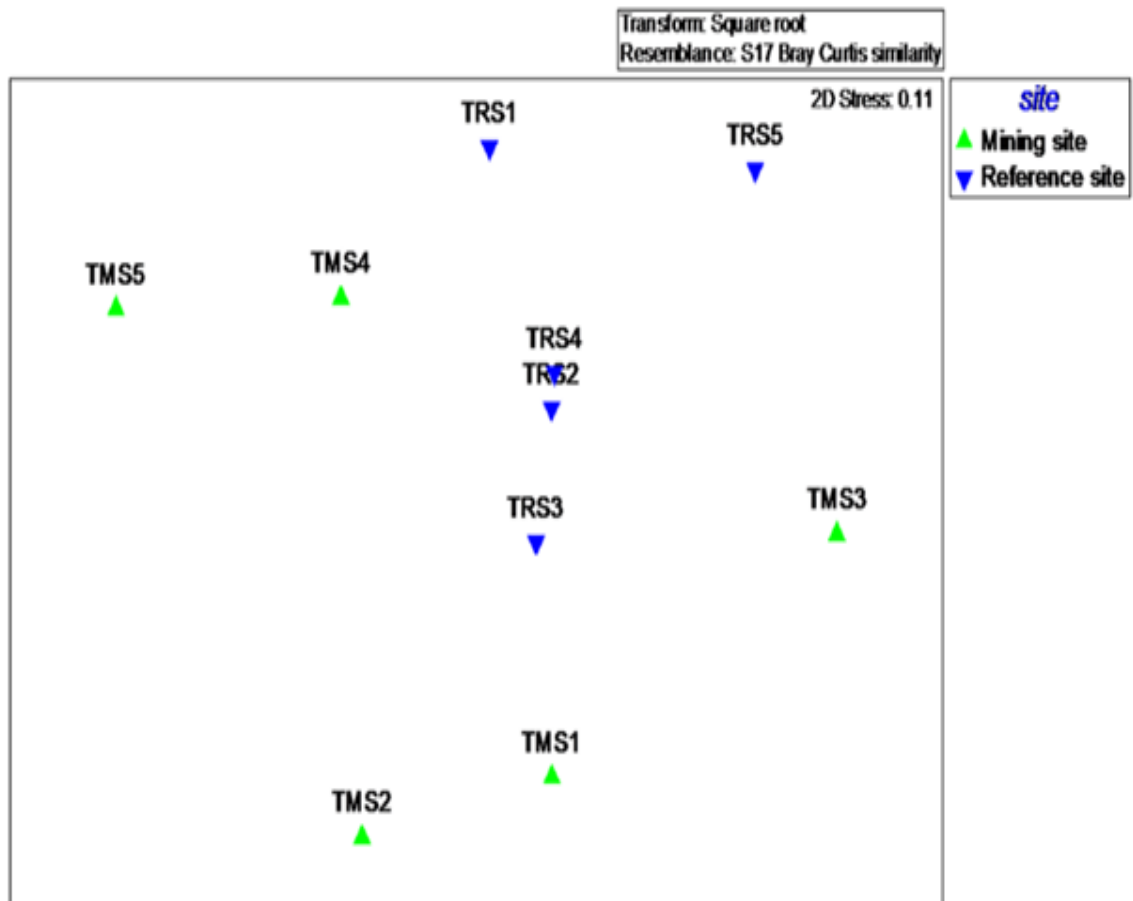


Fig.4b

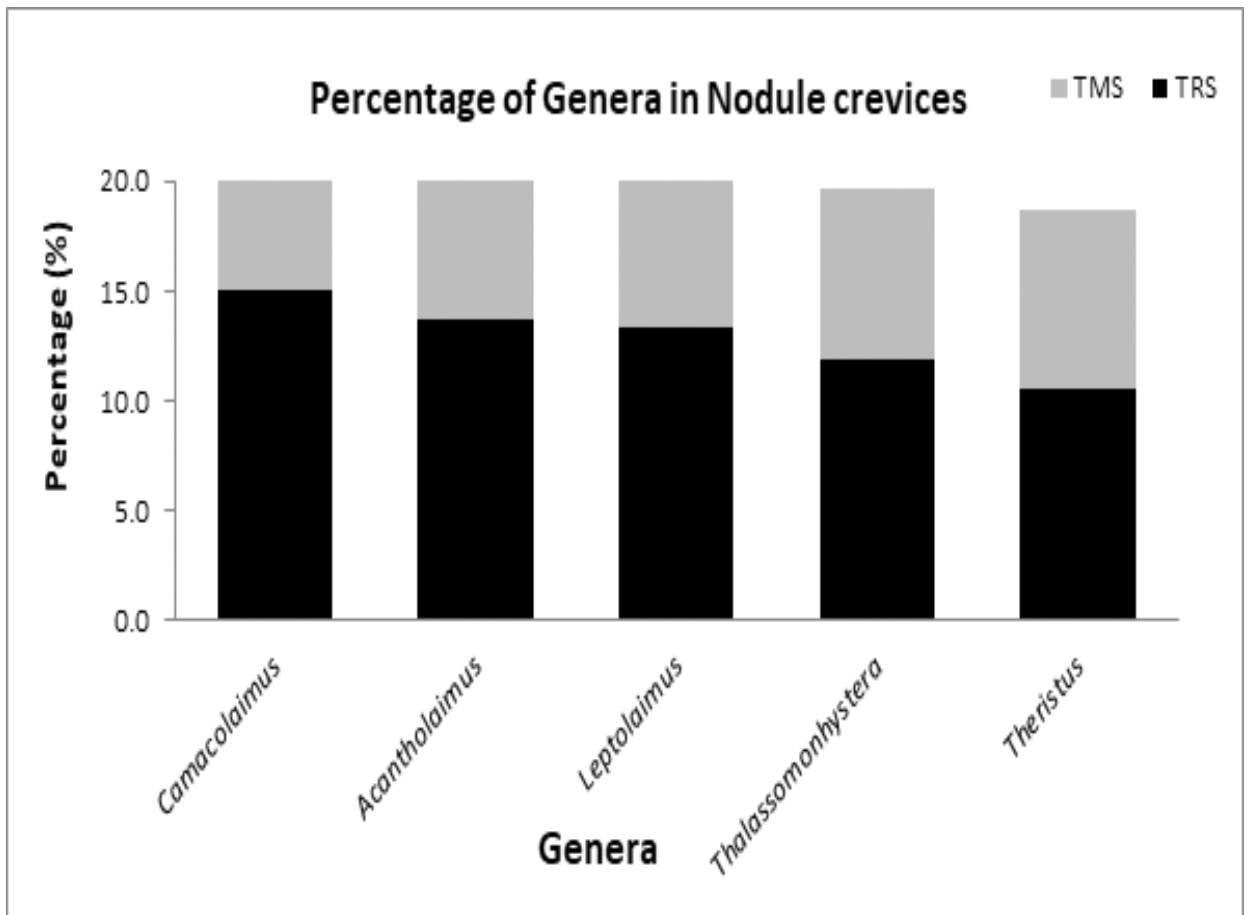


Fig.5

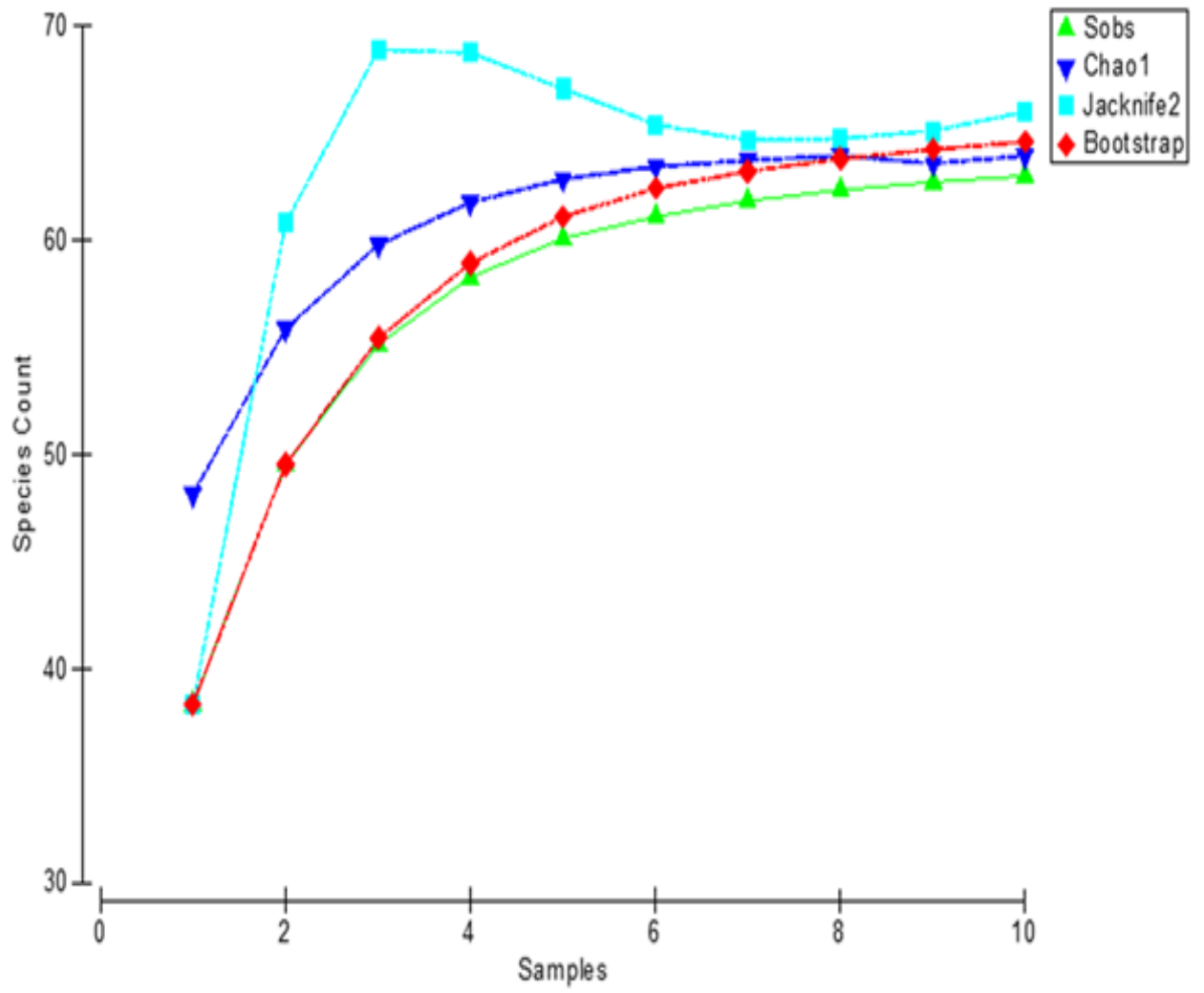


Fig.6

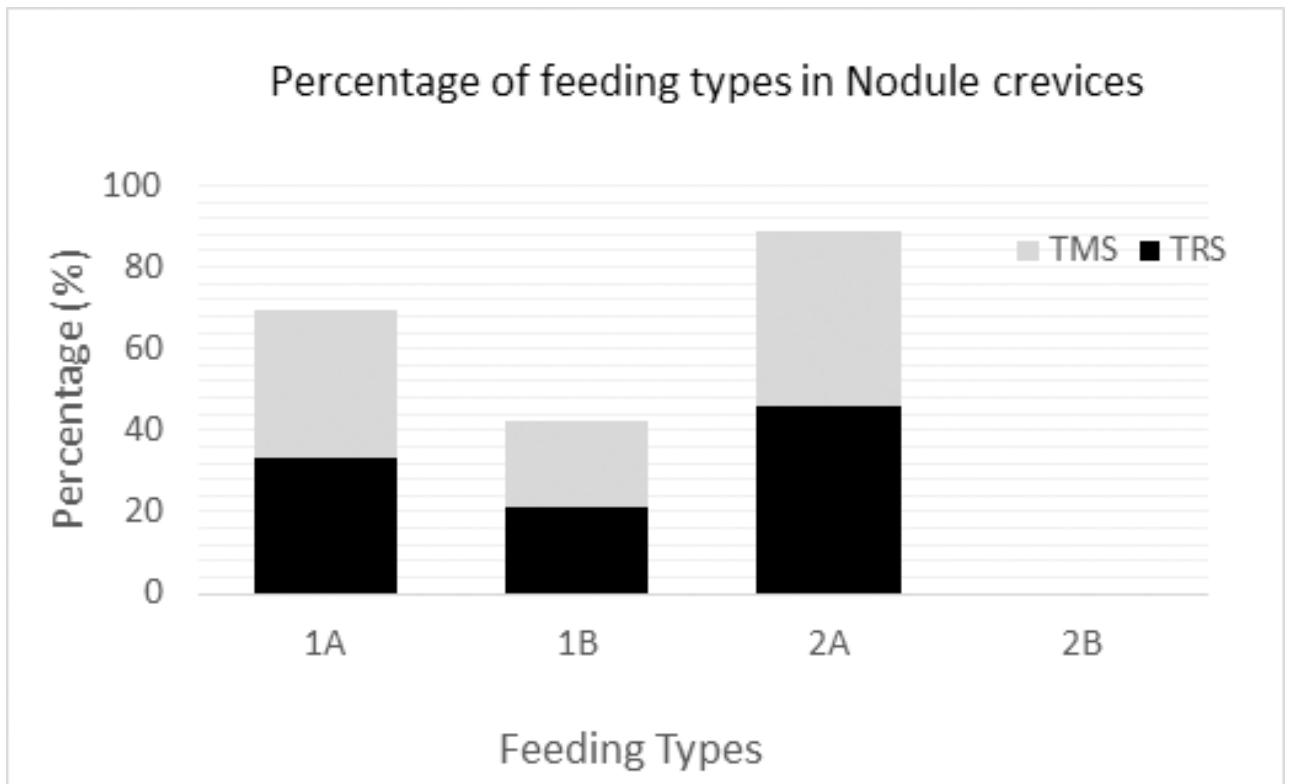


Fig.7

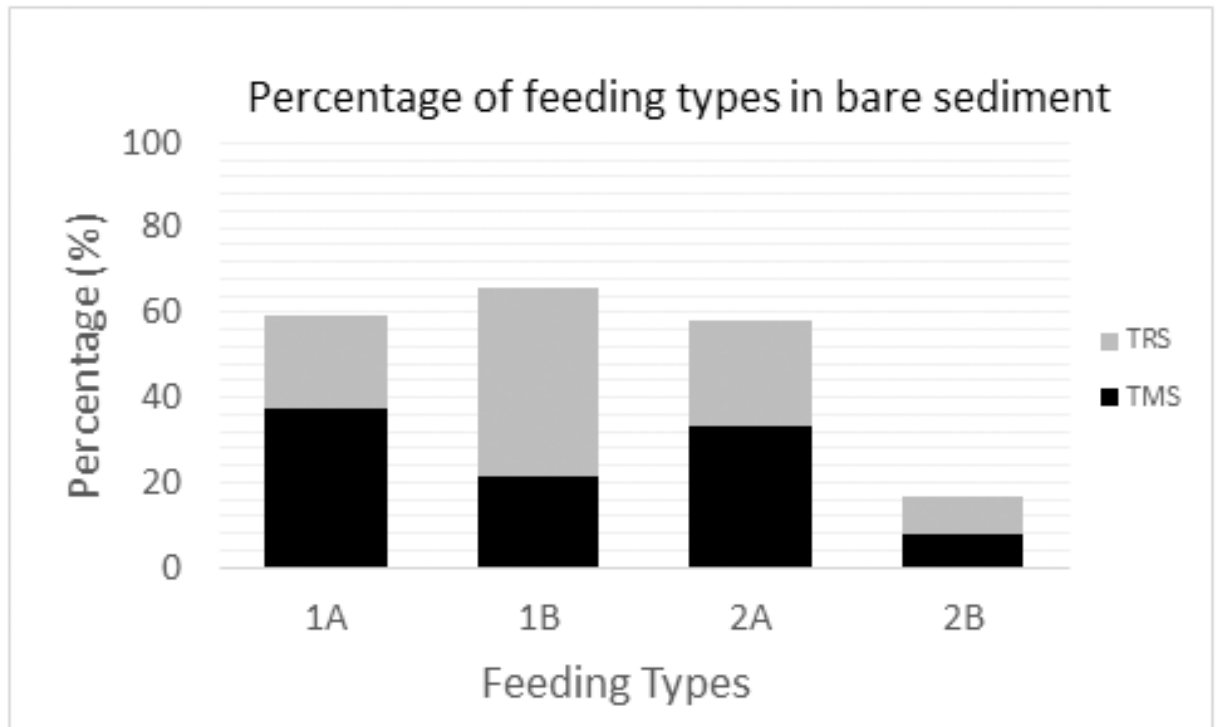


Fig.8