

## Recovery of Deep-sea Meiofauna after Artificial Disturbance in the Central Indian Basin

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### Abstract

Colonization of deep-sea (5000-5500-m depths) metazoan meiofaunal assemblage (in terms of abundance and community structure) were investigated in the Central Indian Ocean Basin (CIOB), immediately after and also 44 months after a benthic disturbance experiment. The abundance of nematodes, which are normally dominating the deep-sea meiofauna, was reduced (by 50%;  $p < 0.001$ ) in post-disturbance. The density of meiofauna was generally low during the monitoring phase and varied moderately inside and outside the test area (experimental site). The density values ranged from 8 and 52 no.  $10 \text{ cm}^{-2}$  (mean:  $23.5 \pm 12.2$  SD;  $n=12$ ) in the impacted area. There was steady buildup in nematode population in the test area between the experiment and the monitoring observations 44 months later, as compared to other metazoan density. Meiofauna were vertically recorded down to 30-35-cm sediment layer during the monitoring. Although, bulk of meiofauna (80%) occurred in top 20 cm sediment layer of reference area, only 16% of the fauna was observed in 0-2 cm. On the contrary,  $>30\%$  metazoan population occurred in 0-2 cm section in the test area. Bringing up nutrients from sub-surface and depositing this organically rich material on the sediment surface, could have acted as a stimulating factor for meiofaunal recovery as most of the meiofauna feed on bacteria and dead organic material (DOM). This experimental study suggests that whereas on one hand commercial mining of the deep-sea mineral resources may affect deep-sea benthic communities immediately after the impact, on other hand, availability of food material due to turn over (similar to plowing) of sediments may help recolonisation at temporal scale.

Keywords: Sediment disturbance, meiofauna, restoration, Central Indian Ocean.

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## Introduction

The deep sea floor is known to harbour high biodiversity of benthic fauna (Grassle and Maciolek, 1992; Rex et al., 1993). The temperature, salinity and dissolved oxygen content fluctuate within a very narrow range at abyssal depths, where food availability is limited (Pfannkuche, 1993). The benthic community appears to be adapted to the prevailing conditions at deep sea and any disturbance to their environment could bring changes in the density, diversity and distribution.

As the larger organisms (such as macro- and megafauna) are relatively less on the deep-sea floor, the smaller size class (such as meiofauna) becomes more important (Thurston et al., 1998). The meiofauna constitute an important component of the deep-sea benthos, as they not only act as food material for larger macro- and megabenthic species but also play an important role in making detritus available to macroconsumers (Thiel 1993; Tenore et al., 1977). However, their composition and abundance are limited by availability of food (Thiel, 1983). The labile organic constituents of the deep-sea sediment play a significant role in meiofaunal food (Tietjen 1971; Ingole et al., 1992; Danovaro et al., 1993; Shirayama and Fukushima, 1997; Raghukumar et al., 2001). The growth of bacteria in deep-sea sediments depends upon the availability of dissolved organic carbon in sediment pore water; particulate organic carbon derived from faecal matter of benthos and detritus derived from dead benthos. Thus, bacterial and benthic biomass is tightly coupled in deep-sea sediments (Alongi and Pichon, 1988; Raghukumar et al 2001). Nevertheless, changes in natural conditions due to manmade disturbance in the benthic environment may upset this equilibrium.

To assess the possible effects of future commercial deep-sea mining of manganese nodules on the benthic communities, a benthic disturbance experiment was conducted in the Central Indian Ocean under the Indian Deep-sea Environmental Experiment (INDEX). The seafloor bottom was physically disturbed by moving a benthic disturber in a 3000 m long x 200 m wide area.

Sharma (2001) described the location and characteristics of the test and reference site as well as experimental design and procedures followed. The immediate response of benthic community to sediment disturbance indicated a significant  $\approx 40\%$  reduction in the benthic population at taxon as well as community levels (Ingole et al., 2000; 2001). The present investigation is a part of the monitoring phase of the INDEX programme conducted 44 months after initial disturbance. The purpose of this study is to assess the influence of physical disturbance, on the lateral and vertical distribution of meiofaunal communities in different phases of INDEX experiment. Accordingly, we tested the hypothesis that movements of mining instrument on the seafloor not only diminish the meiofaunal population but also modify the pattern of their spatial and vertical distribution.

## **Materials and Methods**

This study is based on the benthic samples collected on two Russian research vessels *R.V. Yuzhmorgeologia* and *R.V.A.A. Sidorenko* in the polymetallic nodule field within the Central Indian Ocean Basin. Deep-sea sediment sampling was conducted prior to benthic disturbance (i.e., in June 1997, termed as pre-disturbance sampling) and immediately after the disturbance in August 1997 (termed as post-disturbance). In order to monitor the recolonisation and restoration of the deep-sea environment, another set of observations were made in April 2001 in the test and reference sites. Conductivity Temperature Density (CTD) profile, taken in the vicinity of the area, indicated that temperature decreased from about  $32^{\circ}\text{C}$  at surface to  $-2^{\circ}\text{C}$  below 4500 m depth (Murthy et al., 2001). Salinity profiles showed a superficial thin layer of high salinity at bottom water layer (34.72 PSU at 3000m depth; Murthy et al., 2001). To study the process of meiofaunal recolonisation, benthic samples were taken with the help of a box corer (50x50x50 cm size). Samples from total 14 box corers were analyzed; 12 from the test site and 2 from the reference site. Details of the sampling location and sediment characteristics are given in table 1 and figure 1. Sub-samples (2-3 replicates) for meiofauna were taken by inserting an acrylic

core tube (inner diameter 5.7 cm) in the box core (plate 1). The core tube was capped and the uppermost 2-cm thick sediment layer was carefully sectioned at 0.5-cm intervals, followed by 2-cm intervals sections down to 20 cm core depth and rest of the core was sectioned at 5-cm intervals. Each sub-section was processed separately, which involves fixation in 5% formalin- Rose Bengal solution. In the laboratory, the samples were washed through a series of different mesh size sieves (500, 63 and 45  $\mu\text{m}$ ), sorted and identified under stereoscopic binocular microscope. Metazoan meiofauna in this paper is defined as organisms passing through a 500  $\mu\text{m}$  sieves and retained on 45  $\mu\text{m}$  sieves.

Statistical analysis was performed using the general linear model method of least squares, the T-test and the Mann-Whitney a-parametric test. One-way analysis of variance (ANOVA) was used to test differences in meiofaunal density between experimental (test) and reference sites as well as differences between the pre-and post-disturbance and monitoring phase. All the tests were performed under the assumption of 95% confidence level.

## **Results**

### ***Composition and abundance***

The study area has siliceous ooze sediments and low ferromanganese nodules abundance ( $<2\text{kg/m}^2$ ; Sharma, 1999). The meiobenthic assemblage was made up of 8 metazoan groups. The depth integrated average abundance in the test area varied between 8 and 52 meiofauna per  $10\text{ cm}^2$  (mean:  $23.5 \pm 12.2$  SD;  $n=12$ ; Table 2). Meiofaunal counts in individual cores varied from 28-37 no. $10\text{ cm}^{-2}$  and 8-52 no. $10\text{ cm}^{-2}$  inside (stns. 2,3 and 5) and outside (all other stns. shown in Table 2) of the test area (Fig. 2). Nematodes were numerically the most abundant taxa with a relative abundance of 37% of the total number collected from the test area (Table 3). They were followed by nemertin worms, turbellarians, gastrotrichs, polychaetes and harpacticoid copepods with a relative abundance of 35%, 11%, 9%, 4% and 1%, respectively (Table 3). Mean abundance of nematodes and nemertines, was  $12.0 \pm 9.6$  SD ( $n=7$ ) and 11.3

$\pm 10.2$  SD (n=7), respectively. Nematode density was slightly higher in the test area compared to reference.

### ***Vertical distribution of meiofauna in the sediment column***

The vertical distribution of meiofauna in the sediment column was expressed as total counts and percentage (%) in the respective sediment section. Differences between abundance and vertical distribution at different sites (test and reference) are given separately in Table 2 and figure 3. It shows that, the vertical profile of meiofauna in the sediment column differed considerably, especially in the top 0-2 cm layers. In the test area, metazoans were distributed down to the sediment depth of 35-40 cm, although, >60% of the population was recorded in top 10-cm layer, >30% fauna was found in the uppermost 0-2 cm section (Fig.3A). However, only 16% of the metazoan population was observed in the same section (0-2 cm) of reference area (Fig.3B). The cumulative frequency (%) of specimens down to a sediment depth of 35-40 cm in test and reference area is shown in figure 4. It indicated that a bulk of meiofauna (80%) occurred in the top 20 cm sediment layer of reference area, only 10% of the fauna was observed in top 0-2 cm. In case of the test area, >30% metazoan population occurred in 0-2 cm and accumulative value of 90% occurred at the sediment depth of 30 cm. This indicates that metazoan concentration was much higher in the uppermost sediment layer of test area.

### ***Comparison with pre- and post-disturbance study***

The comparison of the depth integrated meiofauna abundance on the test and reference area during monitoring phase to that of pre- and post-disturbance phase is given in figure 2 and 5. The density of meiofauna was decreased from 45 no.  $10\text{cm}^{-2}$  to 21 no.  $10\text{cm}^{-2}$  in post-disturbance and maximum reduction was in the test area (stn. 2,3, & 5; Fig.2 & 5). The monitoring study conducted after 44 months, showed wider variation in mean meiofaunal density with overall increase not only in the test area (stn.2, 3 and 5; Fig.2) but also in either side of the test area (Fig.5). The nematode: copepod (N:C) ratio which was increased from 2.18 (pre-disturbance) 3.00 in post-disturbance was depressed towards copepods

showing further increase to 30.0. Two-way ANOVA showed significant variation in mean meiofaunal density between and within the test as well as reference area (Table 5).

## **Discussion**

The distribution of meiofauna in the sediment column did not show consistence pattern and the values differed considerably between the sampling locations. During the pre-disturbance study, majority (>75%) of the meiofauna was concentrated in the upper most sediment layer and a similar pattern of vertical disturbance was observed during the post-disturbance (Ingole 2000). However, there was considerable (40%) reduction in the total meiofaunal density in the top 2-3 cm sediment layer in post disturbance. Deep-sea meiofauna generally concentrated to a relatively thin surface layer of the sediment (Ingole et al., 1992; Shirayama and Fukushima, 1997; Radziejewska and Modlitba, 1999). Although meiofaunal organisms were vertically distributed down to 30-35 cm layers at both the sites, only 10% population lived in the 0-2 cm layer of the reference area. On the other hand almost >30% population was recorded in top 0-2 cm sediment layer of test area. Considerable difference in the vertical distribution of meiofauna observed in the top 0-2 cm layer of test and reference area (Fig. 3 A & B) was due to the physical disturbance. In earlier studies, Pfankuche and Thiel (1987) and Ingole et al (1992) reported 90% and 70% of metazoan population from 0-2 cm sediment layer, respectively from Arctic Ocean (Nansen Basin) and southwestern Indian Ocean (Rodrigues and Saya de Malha Bank). Thus, the physical disturbance conducted in the CIB 44 months prior to the present sampling, may have created entirely new conditions in the deep-sea environment, such as bringing up dissolved nutrients from the subsurface layers and depositing the organically rich sediment layer on sediment surface (Ingole et al., 1999; (Ragukumar et al., 2001; Nath et al., 2005). It is believed that the sediment disturbance at deep-sea level is deleterious to the buried in-fauna; however it increases the food resources for larger epibenthic animals by exposing the organically rich sediment to the surface (Bluhm, 1994; Ingole et al.,

2002). The increased meiofaunal population on the test as well as in the adjacent area (Fig.5) therefore, was mainly due to the augmented food supply (Raghukumar et al., 2002; Nath et al., 2005). Nevertheless, occurrence of some portion of meiofaunal population in deeper layer of some of the sediment cores collected during the monitoring phase (Table 2), strongly suggested a possible influence of higher sediment reworking by larger macro-and megabenthic organisms (Soetaert et al., 1997; Nath et al., 2005). Presence of live polychaete burrows as deep as 25 cm (Ingole et al., 2005); further support the fresh bioturbation activity in the sediment layer, both in the reference as well as test area. During the pre- and post-disturbance study, sediment samples were collected down to 20 cm sediment depth; however meiofauna was not present below 12 cm. In the monitoring phase, cores were collected down to sediment depth of 40 cm and meiofauna was observed till 30-35 cm (Table 2; fig.3). The occurrence of fauna to the deeper sediment depths (>30cm) could be as a consequence of increased sediment porosity and permeability (Rardziejewska and Modlitba, 1999).

The vertical distribution of meiofauna at reference area (Fig. 3 B) showed totally different pattern, particularly for the top few centimeters, which is very critical for deep-sea meiofauna (Shirayama and Fukushima, 1997) and did not agree with the general pattern reported for the Indian Ocean (Ingole et al., 1992). It probably suggests a wider impact of physical disturbance on sedimentation in CIB and agrees with the observations of Valsangkar (2004). Discussing the results of German deep-sea disturbance experiment, DISCOL Schriever et al, (1997) have also reported the enhanced meiofaunal population at reference area. This indicates that certain processes take place in the abyssal communities even without human interference, supporting the notion of no absolute inherent stability on the abyssal seafloor (Gage and Tyler, 1991). Sporadic flux of phytodetrital material, which sinks from the euphotic zone to the deep-sea floor, is perhaps liable for instability of deep-sea fauna (Galeron et al., 2001 and references there in).

The meiofaunal abundance observed in the Central Indian Basin fall within the reported range from the deep-sea areas of Indian Ocean (Parulekar et al., 1992; Ingole et al., 1992) and from similar depths in Pacific Ocean (Shirayama and Fukushima, 1997; Rardziejewska and Rokicka-Praxmayer, 2001). Nematodes and copepods showed differences between samples collected before and immediately after the disturbance (Ingole et al., 2000). The nematode: copepod (N:C) ratio frequently used as environmental indicator (Warwick, 1981; Danovaro et al., 1995; Ingole et al., 2000; Kim et al., 2000; Ansari and Ingole, 2002) was used to assess the restoration process.

As shown in table 4, the values were depressed towards harpacticoid copepods from 2.18 in pre-disturbance to 3.00 in post-disturbance suggesting that copepods are less resistant to sediment disturbance. This ratio was further depressed towards copepods in monitoring phase (30); indicate the steady recovery of nematodes. Hence, harpacticoid copepods being the second dominant metazoan group during pre-disturbance, were not recovering at the same rates to that of nematodes. These micro-crustaceans are considered very sensitive to environmental changes (Raffaelli and Mason, 1981), whereas nematodes are resistant to the rapidly changed environment (Heip, 1980; Moore and Bett, 1989; Ansari and Ingole, 2002). On the other hand, nemertine worms emerged as second dominant group next to nematodes, during the monitoring phase (Table 3). These proboscis worms were not observed during pre- and post-disturbance phase and their dominance over predictable deep-sea meiofaunal taxon during the monitoring phase was conspicuous. However, as majority of nemertine worms are carnivorous (McDermott and Roe, 1985), their dominance after 44 months could be due to the availability of abundant food material. Hence, the disturber activity provided a suitable habitat for colonization of certain exceptional forms such as nemertine worms, by stimulated production of their food material and thus increasing their population. Nevertheless, this aspect needs further detailed analysis and therefore, the results on emergence of nemertines should be interpreted cautiously.



It is anticipated that the physical disturbance at deep-sea level may have mixed impact on the benthic biota (Dayton and Hessler, 1972). The experimental studies conducted in the CIB suggest that whereas on one hand deep-sea disturbance as in case of mining of the deep-sea mineral resources will affect deep-sea benthic communities immediately after the impact (Foell et al., 1990; Shirayama and Fukushima, 1997; Rardziejewska, 1997; Shriever et al., 1997; Ingole et al., 1999; 2000; Radziejewska and Stoyanova, 2000; Radziejewska and Rokicka-Praxmajer, 2001); on the other hand, their recovery is expected to be slow. However, availability of food due to mining of sediments may help recovery and fast recolonisation of benthic fauna.

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Station No.	Geographical position		Depth (m)	Sediment texture
	Latitude ( <sup>0</sup> S)	Longitude ( <sup>0</sup> E)		
M-2	10 <sup>0</sup> 02.311	76 <sup>0</sup> 01.061	5330	Test area
M-3	10.03443	76.00958	5294	Test area
M-4	10 <sup>0</sup> 01.497	75 <sup>0</sup> 59.673	5249	Adjacent to test area
M-5	10 <sup>0</sup> 01.786	76 <sup>0</sup> 06.120	5306	Test area
M-6	10 <sup>0</sup> 02.651	76 <sup>0</sup> 01.625	5311	Adjacent to test area
M-7	10 <sup>0</sup> 02.320	76 <sup>0</sup> 00.470	5350	-----do-----
M-8	10 <sup>0</sup> 01.788	76 <sup>0</sup> 00.762	5330	-----do-----
M-12	10 <sup>0</sup> 01.328	76 <sup>0</sup> 00.564	5254	-----do-----
M-13	10 <sup>0</sup> 01.510	76 <sup>0</sup> 00.350	5286	-----do-----
M-14	10 <sup>0</sup> 02.200	76 <sup>0</sup> 00.148	5273	-----do-----
M-15	10 <sup>0</sup> 02.418	75 <sup>0</sup> 59.939	5240	-----do-----
M-18	10 <sup>0</sup> 02.729	76 <sup>0</sup> 00.620	5315	-----do-----
M-A1/A	10 <sup>0</sup> 10.836	75 <sup>0</sup> 22.111	5240	Reference area
M-A1/B	10 <sup>0</sup> 06.873	75 <sup>0</sup> 10.638	5292	Reference area

Sediment depth (cm)	Station numbers													
	Test area												Reference area	
	2M	3M	4M	5M	6M	7M	8M	12 M	13 M	14 M	15 M	18 M	A1/1	A1/B
0-0.5	3	3	3	5	4	0	0	3	12	7	2	11	2	1
0.5-1.0	2	2	1	4	4	1	3	1	2	0	1	2	0	2
1.0-1.5	1	2	1	3	3	2	2	0	1	12	0	3	0	2
1.5-2.0	3	2	1	2	2	1	1	0	2	3	0	1	0	1
2.0-4.0	2	1	4	3	4	2	1	0	2	0	4	0	0	2
4.0-6.0	2	2	4	3	3	0	2	0	0	0	2	0	4	1
6.0-8.0	1	1	3	0	3	1	3	0	0	0	0	0	2	1
8.0-10	2	2	1	1	14	2	1	1	0	0	0	0	1	1
10.0-12	1	2	1	0	2	2	3	0	0	0	0	0	2	3
12.0-14	1	1	2	2	3	1	2	2	0	0	0	0	3	1
14-16	2	3	1	3	1	0	1	0	0	0	0	0	1	2
16-18	0	1	2	5	3	0	1	0	0	1	0	0	1	5
18-20	1	1	1	1	1	1	2	1	0	0	0	1	2	1
20-25	2	2	0	2	1	1	1	0	0	0	0	0	2	3
25-30	1	1	2	2	1	1	3	0	0	0	0	0	2	1
30-35	1	1	1	1	1	0	1	0	0	0	0	0	0	0
35-40	3	1	1	0	2	0	3	0	0	0	0	0	0	0
Total Density	28	28	29	37	52	15	30	8	19	23	9	18	22	27

Table,3: Composition (%) and abundance (no. 10 cm<sup>2</sup>) of meiofauna at test and reference site during monitoring phase.

Taxon	Test area			Reference area		
	Mean	SD	%	Mean	SD	%
Nematoda	12	9.6	36.8	5.2	3.9	17.1
Nermertina	11.3	10.2	34.6	21.0	25.4	70.0
Turbellaria	3.7	6.1	11.4	1.5	3.5	5.0
Gastrotricha	2.9	4.9	8.8	1.0	1.4	3.3
Polychaeta	1.4	3.8	4.4	0	0	0
Unidentified	0.7	1.4	2.2	0	0	0
Harpacticoida	0.4	1.1	1.3	1.5	2.1	5.0
Kinorhyncha	0.1	0.4	0.4	0	0	0
Total	32.5	37.5	100.0	30.0	17.5	100

Table 4: Mean abundance of Nematodes and Copepods and N:C ratio in the test area studied during different INDEX phase

Taxon/group	Baseline study (1996)	Pre-disturbance (June 1997)	Post-disturbance (July 1997)	Monitoring (April 2001)
Nematodes	46	24	12	12
Copepods	22	11	4	0.4
N:C	2.09	2.18	3	30

Table 5: Analysis of TWO-WAY variance of meiofaunal density between the test and reference area

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	139.9412	16	8.746324	2.880378	0.000247	1.685795
Columns	113.9853	15	7.59902	2.502543	0.001886	1.70823
Error	728.7647	240	3.03652			
Total	982.6912	271				

## Legends to text figures

Fig. 1: Position of the sampling stations in INDEX area (counters is depths in m.).

Fig. 2: Meiobenthic abundance (no.10cm<sup>-2</sup>) in individual core during three INDEX phases.

Fig. 3: Vertical distribution of meiofauna in test and reference area

Fig. 4: Vertical distribution and cumulative percentage of in test and reference area.

Fig. 5: Meiofaunal abundance (no.10 cm<sup>-2</sup>) in CIB during different INDEX phases

Plate 1: Sub-sampling of meiofauna from box corer



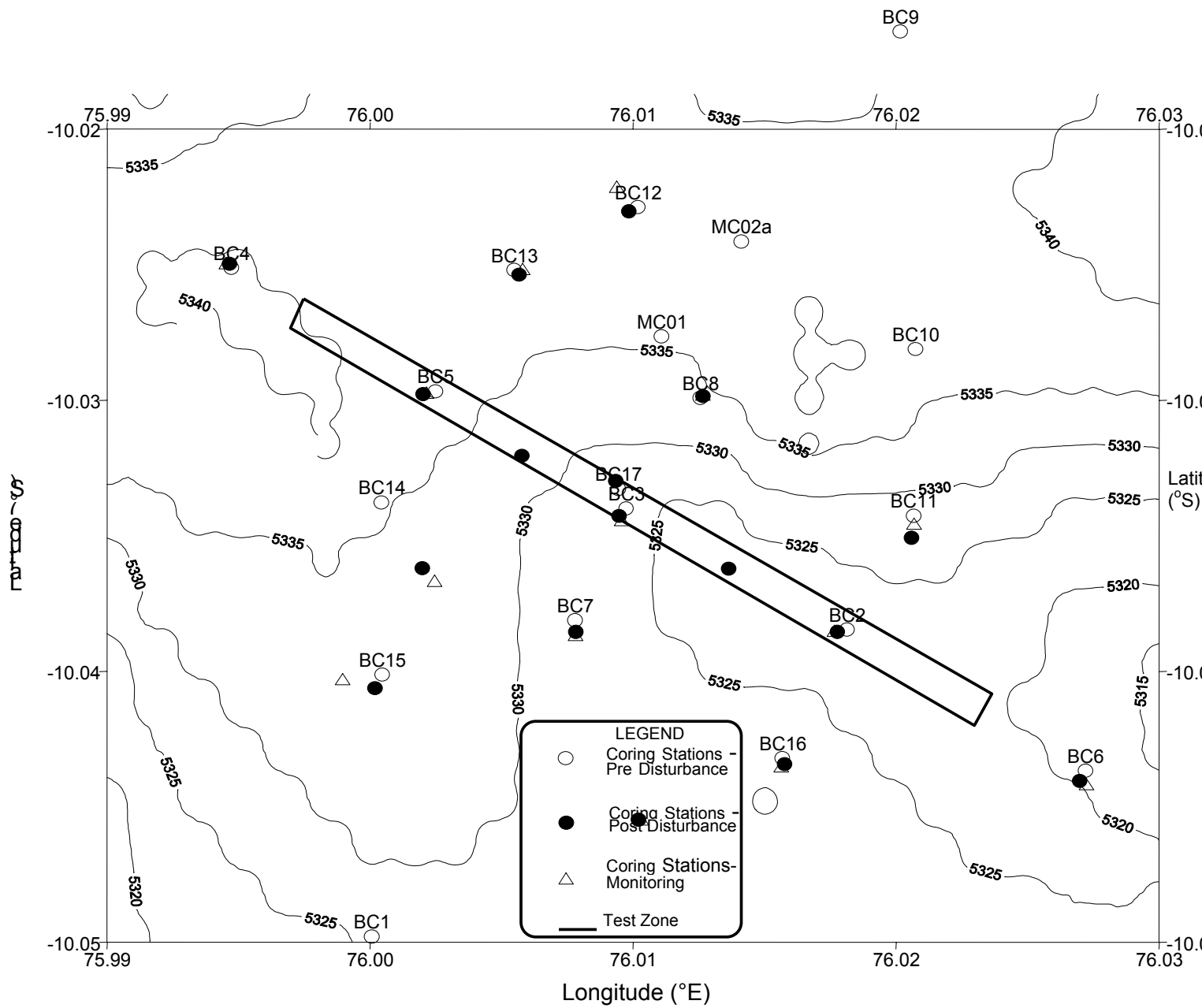


Fig. 1: Position of the sampling stations in INDEX area (counters: depths in m).

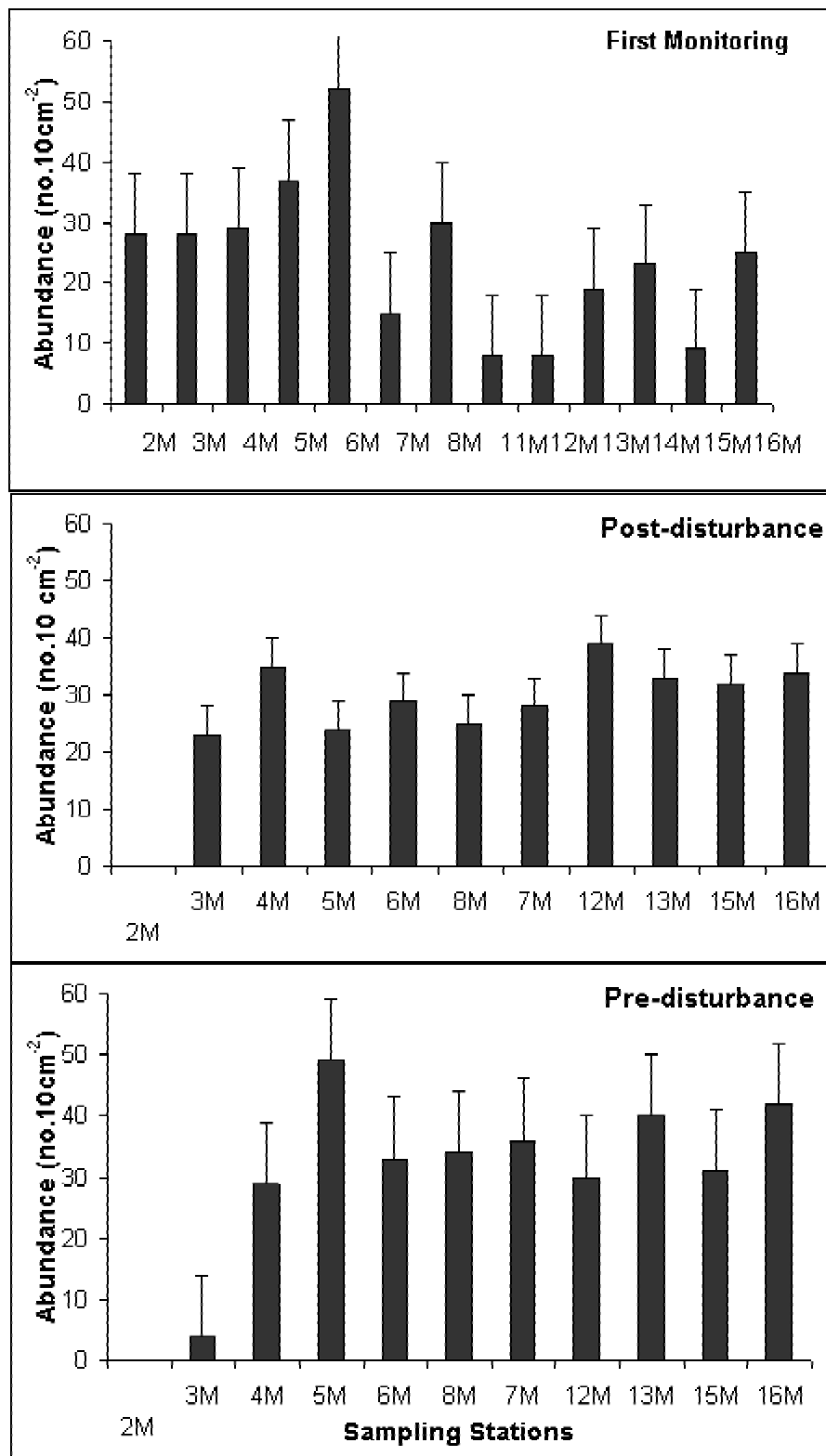


Fig. 2: Meiobenthic density (no.10cm<sup>-2</sup>) in individual core during three INDEX phases.

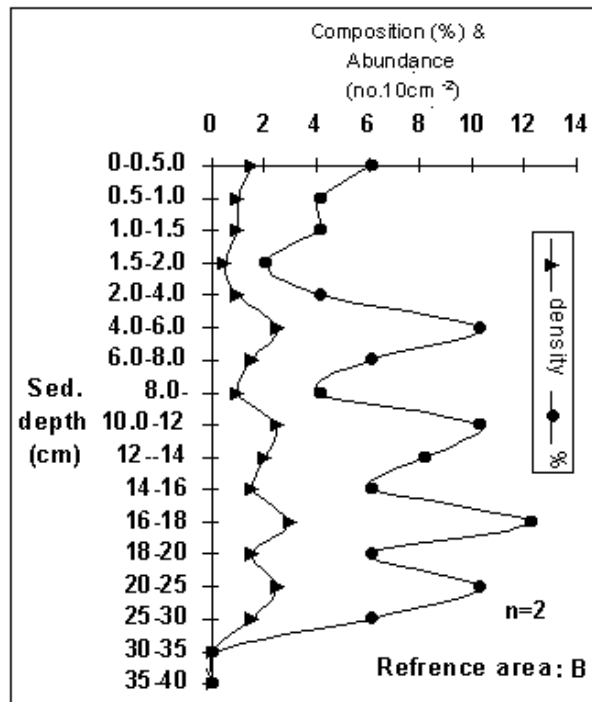
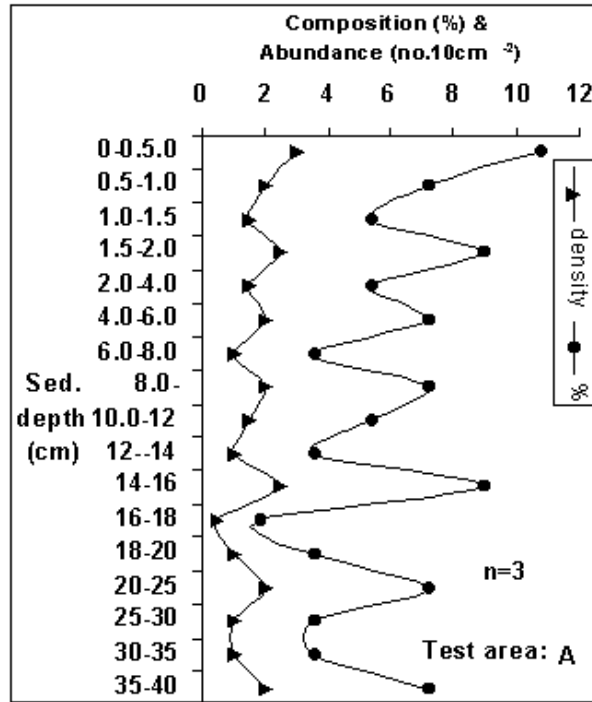


Fig. 3: Vertical distribution of meiofauna in test (A) and reference area (B)

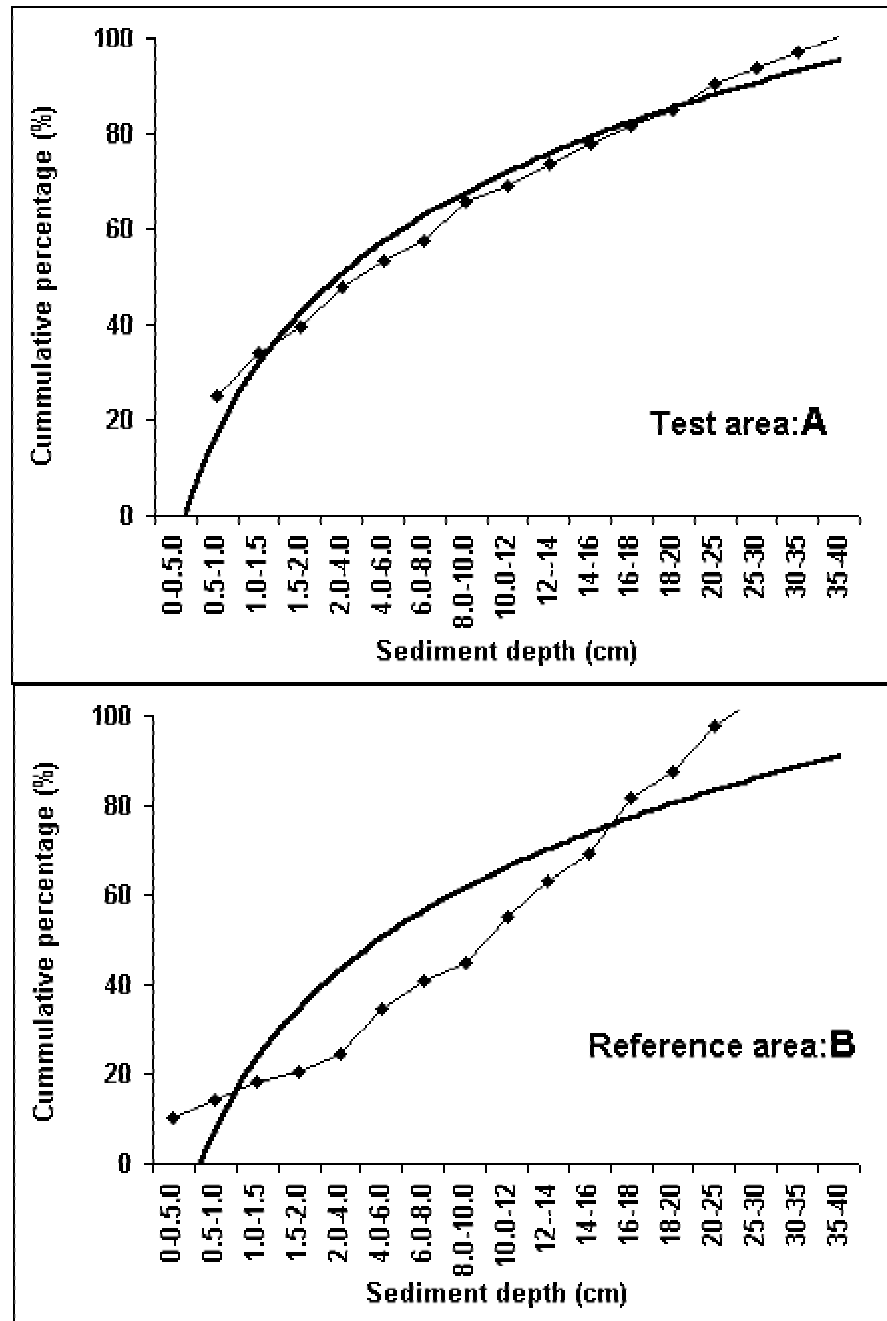


Fig.4: Cumulative percentage of meiofauna in test and reference area

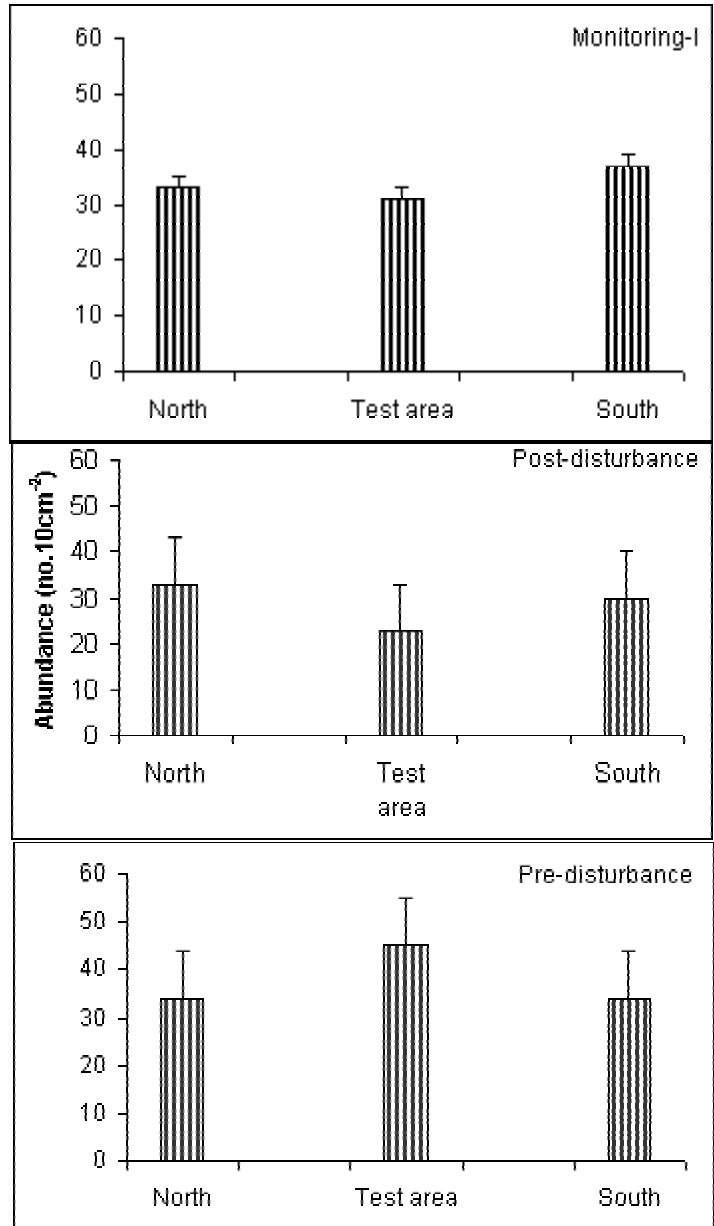


Fig. 5: Abundance (no.10 cm<sup>-2</sup>) of meiofauna in CIB during three INDEX phases (North: north of the test area; South: south of the test area)



**Plate 1: Box core sample being taken for sub-sampling**