

Importance of sieve size in deep-sea macrobenthic studies

Sini Pavithran, *Baban Ingole, Mandar Nanajkar, Reshma Goltekar

National Institute of Oceanography,
Dona-Paula, Goa.
India.

Phone no: +91-832-2450242

Fax no: +91-832-2450606

sinipavithran@gmail.com,

baban@nio.org,

mandar_nanajkar@hotmail.com,

reshmag@nio.org

ABSTRACT

The deep-sea is well known for high benthic biodiversity despite being a low-food environment. However, most deep-sea organisms are very small in size as an adaptation to food limitation. Macrofauna are generally considered to be organisms larger than 0.5 mm and smaller than 3 cm. However, the smaller body size of fauna in the deep sea has led to the use of mesh sizes ranging between 0.25 to 0.5 mm to collect macrofauna, 0.3 and 0.5 mm being the most commonly used mesh sizes for deep-sea sampling. In this study, we tested the effectiveness of sieves of two different mesh sizes (0.3 and 0.5 mm) in assessing macrofaunal diversity, density and biomass. A total of 66 species were obtained with the smaller mesh (0.3 mm), while the larger mesh (0.5 mm) retained only 40 macrofaunal species. Thus, use of larger mesh resulted in the loss of 39% species over the smaller mesh ($p=0.0001$). However, both sieves yielded high densities of organisms, high species diversity and steep rarefaction curves for nematodes and polychaetes. Using the larger mesh resulted in a significant loss in biomass of 90% and 78% for polychaetes and nematodes respectively. Vertically in the sediment, faunal density was sampled more effectively with the smaller mesh sieve. Our results show a significant reduction in the

number of species, organism density, and biomass of macrofauna with use of a 0.5 mm mesh rather than a 0.3 mm mesh and that a sieve of lower mesh size is more suitable for evaluation of deep-sea macrofauna.

Key words: Abyssal, biomass, density, diversity, macrofauna, mesh size

INTRODUCTION

Among deep-sea organisms, both gigantism and dwarfism occur, evolutionary trends that can be explained by selection on optimal foraging strategies (Gage and Tyler 1991). Deep-sea dwarfism is common among a variety of taxa (Shirayama and Horikoshi 1989, Gage et al 2002, Kaariainen and Bett 2006, Rex et al. 2006). In contrast a few deep-sea taxa, arthropods in particular, exhibit deep-sea gigantism (Timofeev 2001) including giant isopods, amphipods, and pycnogonid "spiders". However, studies have revealed that the average size of individuals becomes smaller with increasing water depth, suggesting that overall dwarfism is a more common phenomenon than gigantism in the deep sea (Shirayama and Horikoshi 1989). Numerous biological measures (metabolism, faunal abundance, biomass production, nutrient recycling, home range size) have been shown to correlate strongly with individual body size (Peters 1983; Schmidt-Nielsen 1984; Brown et al. 2004; Kaariainen and Bett 2006). Most studies have argued that food limitation may be the major contributing factor in controlling optimal body size of deep-sea benthic organisms, resulting in the predominance of smaller body sizes (Thiel 1975, 1979; Kaariainen and Bett 2006; Rex et al. 2006). In a food limited environment, the advantages of having either large or small body size is explained by Thiel (1975). He suggests that, although the cost of maintaining a given biomass of smaller organisms is

higher than that required to maintain the same biomass of larger organisms, the high individual food demands of larger animals and the requirements to maintain a critical population density for reproduction generally favours small body size. The benthic body size miniaturization hypothesis states that deep-sea communities are dominated by organisms of small body size (Kaariainen and Bett 2006). Several studies have examined the effect of sieve size on deep-sea faunal collection (Shirayama and Horikoshi 1989, Gage et al, 2002) and have recommended the use of increasingly smaller meshed sieves to study the deep-sea macrofauna. The present study aimed to test the influence of two different mesh sizes (0.3 mm and 0.5 mm) on deep-sea macrofaunal collection.

The present study was undertaken in the Central Indian Ocean Basin (CIOB), an area of potential mining activity due to its very high abundance of polymetallic nodules (Prasad 2007), and an area poorly studied in comparison to the Atlantic and Pacific Ocean in terms of deep-sea biology and taxonomy (Ingole and Koslow 2005).

MATERIALS AND METHODS

Sample processing

A total of 23 stations were sampled onboard R.V. *Akademic Boris Petrov* from the CIOB (Fig. 1a & b) between latitude 10⁰ and 16.1⁰ S and longitudes 74.5⁰ and 76.5⁰ E at a water depth ranging between 4252-5693 m (mean: 5221 m). A single box core sediment sample was collected with a 0.25 m² spade box corer (50X50X50 cm size) from each station. To study the general distribution and composition of macrofauna, three subsamples (15X15X10 cm) were collected from each box core, each subsample from a different

quadrant of the core. In addition, the vertical distribution of macrofauna was studied by using a single sediment core sample (PVC coring tube; 12 cm dia. 50 cm length) from the same box core. The cylindrical core samples were sectioned at a sediment depth of 2, 5, 10, 15, 20, 25 and 30 cm. All sub-samples were preserved in neutralized 5% formalin-Rose bengal solution prepared in filtered seawater. These samples were later sieved using two sieves one above the other, the upper sieve being the 0.5 mm and the lower sieve was 0.3 mm. The organisms retained on the respective sieves were collected and sorted group-wise. Subsequently, specimens were identified to the lowest taxonomic level possible under a stereomicroscope.

Biomass estimations

The macrofaunal samples were blotted dry with blotting paper. The blotted wet weight of the macrofaunal groups was measured, using a Mettler Toledo balance (0.000001 g precision). This data was used to estimate the total biomass as ash-free dry weight (AFDW) in grams using conventional conversion factors for each of the faunal groups. These were Polychaeta = wet weight X 0.155, Crustacea = wet weight X 0.225, Mollusca = wet weight X 0.085, Echinodermata = wet weight X 0.08, miscellaneous groups including Porifera and Bryozoa = wet weight X 0.155 (Eleftheriou and Basford, 1989).

Data analysis

Macrofaunal group diversity was measured with the software PRIMER (Clark and Warwick 1994) using the Shannon-Wiener diversity (H' ; Shannon and Wiener 1963) function. Evenness (J ; Pielou 1966) and group level richness (d ; Margalef 1968) was also

calculated and rarefaction curves of the expected number of species, $E(S_n)$ were generated using Hurlberts (1971) method. All data were subjected to Shapiro-Wilks test to check for normality (Statistica 5.5 1999). Mann-Whitney U tests and student's t-tests were used to test for significant differences between parameters. Non-parametric Kruskal Wallis ANOVA was used as a global significance test for differences in macrofaunal species counts, density and biomass between stations (Statistica 5.5 1999).

RESULTS

Macrobenthic abundance and diversity in the present study showed a marked difference between the two mesh sizes considered. Density of macrofauna ranged between 0-237 ind.m⁻² with the 0.3 mm mesh, while a density of 0-133 ind.m⁻² was obtained with the 0.5 mm mesh, resulting in more than a 50% loss in macrofaunal abundance, with the larger mesh sieve (Table 1). A significant difference was also obtained for the number of species retained by the two different mesh sizes (Table 1). Significant differences in macrofaunal density, biomass, and species diversity were also observed between stations ($p < 0.001$).

Biomass

The macrobenthic biomass retained on the 0.3 mm mesh, ranged between 0 to 2.98 mg.m⁻² (mean: 0.64 ± 0.86 sd, $n=23$), while biomass values for the 0.5 mm mesh was marginally less, ranging between 0-2.90 mg.m⁻² (mean: 0.51 ± 0.79 sd, $n=23$). Polychaetes had the largest biomass of 0.38 mg.m⁻² among the macrofauna retained on the 0.3 mm mesh, followed next by tanaiids (0.12 mg.m⁻²), then nematodes (0.06 mg.m⁻²), isopods (0.04

mg.m⁻²) and harpacticoid copepods (0.03 mg.m⁻²) (Fig. 2). Bivalves and nemertine worms had a biomass value of 0.001 and 0.005 mg.m⁻², respectively. The biomass of fauna retained by the 0.5 mm mesh was comparatively low. The greatest difference was a 90% reduction in biomass for polychaetes using the 0.5 mm mesh, followed by a 78% loss of biomass for nematodes. Tanaidacea showed a loss of 65%, while, Isopoda and Harpacticoida showed a loss of 45% and 24%, respectively. Bivalves and nemertine worms did not show any change in biomass between the 0.3 and 0.5 mm mesh. The overall difference in biomass of macrofauna between the two mesh sieves was statistically significant (Table 1).

Diversity

A total of 66 species (of 120 individuals) were obtained with the 0.3 mm mesh compared to only 40 species (of 68 individuals) with the 0.5 mm mesh (Table 2). Of the 120 individuals obtained by the 0.3 mm mesh, nematodes were the dominant group which consisted of 47 individuals (23 species) followed by polychaetes with 34 individuals (18 species) (Fig. 3a & b). Similarly, with the larger mesh size (0.5mm), of the 68 individuals obtained, 27 were nematodes belonging to 14 species and 20 were polychaetes belonging to 11 species (Fig. 3a & b).

Thus, the 0.5 mm mesh retained only 57% of the total number of individuals obtained with the 0.3 mm mesh. Furthermore, in terms of number of species, only 61% of the species were retained with the 0.5 mm mesh, illustrating that the use of the larger sieve resulted in the loss of 43% of individuals and 39% of total species. Among the

macrobenthic groups collected, maximum losses with the larger mesh size occurred with harpacticoids both in terms of diversity (67%) as well as density (78%). No differences were observed for bivalves and nemertines which were represented by few specimens (3 bivalves and 1 nemertine). The actual number of species for each group is shown in fig. 3b. Some of the species collected on the 0.3 mm mesh were of very small size and represented only a single individual.

Shannon's index (H') values across all taxa ranged between 0-2.5 for the 0.3 mm mesh, and were slightly less with the 0.5 mm mesh ranging between 0-2.2. The maximum H' value obtained using a 0.3 mm mesh was for polychaetes (2.8) followed closely by nematodes (2.7). With the 0.5 mm mesh the highest diversity values obtained were with nematodes (2.4), followed by polychaetes (2.2) (Fig. 4b), there were no significant differences in H' between the two mesh sizes (Table 1).

Pielous evenness parameter (J) ranged from 0.91 to 1 for the 0.3 mm mesh samples and for the 0.5 mm mesh between 0.93-1. There were no significant differences in J between the two mesh sizes (Table 1). With the 0.3 mm mesh, polychaetes had the highest J value (0.95), whereas the highest J values with the 0.5 mm mesh was observed for harpacticoids (1.0) (Fig. 4b).

Margalef's richness measure (d) ranged from 0.9 to 4.6 with the 0.3 mm mesh, while d values ranged from 1.4-3.6 for the 0.5 mm mesh. However, differences were not

significant between the two mesh sieves (Table 1). The highest d values obtained were with nematodes and polychaetes with both mesh sizes (Fig. 4a & b).

Hulbert's expected number of species ($E(Sn)$) ranged between 0-13 for the 0.3 mm mesh samples, while values were between 0-9 for the 0.5 mm mesh (Fig. 5). But $E(Sn)$ did not significantly differ between the two meshes (Table 1). The highest $E(Sn)$ value obtained was with nematodes followed by polychaetes in both meshes (Fig. 4a & b).

Vertical distribution

Density and Diversity

The average density of macrofauna was much higher with the 0.3 mm mesh (60 ind.m⁻²) compared to the 0.5 mm mesh (32 ind.m⁻²) in the top 0-5 cm sediment. Below this depth, density was similar for both mesh sizes (Fig. 6a). Faunal diversity was also higher in the top 0-5 cm of sediment using a 0.3 mm mesh compared to the 0.5 mm mesh. Diversity remained constant for both mesh sizes below 5 cm. Although the average value of faunal density was lower with the 0.5 mm mesh in the top 5 cm, values did not differ significantly between the two mesh sizes for the top 5cm sediment ($t=1.37$, $df=6$, $p=0.22$) as well as for the entire core (40 cm, Table 1).

Biomass

Similar to faunal density measures, average biomass values were higher with the 0.3 mm mesh compared to the 0.5 mm mesh. The upper 0-2 cm and 2-5 cm sediment layers averaged, respectively, a 40% and 78% loss of biomass with the 0.5 mm mesh. Below 5

cm, biomass values remained constant for both mesh sizes (Fig. 6b). However, macrofaunal biomass did not significantly differ between the two sieves in the top 5 cm ($t=0.867$, $df=6$, $p=0.42$) as well as when the entire core was considered (Table 1).

DISCUSSION

Sieve size influence on the estimation of macrofaunal density, biomass and species diversity was first documented in coastal waters, wherein use of a 0.5 mm mesh sieve was found to be more effective than a 1 mm mesh sieve (Reish 1959). Thus 0.5 mm mesh has been considered the standard size for macrofaunal sampling in the coastal waters.

Due to the low abundance and smaller body size of deep-sea macrofauna, researchers have been prompted to use smaller mesh sizes in order to retrieve macrofauna in the deep-sea. Literature surveys have shown the use of sieve sizes ranging between 0.25 to 0.5 mm in deep-sea studies (Sanders et al. 1965; Hessler and Jumars 1974; Gage et al. 2002).

Meio- and macrofaunal organisms in the deep-sea tend towards body size miniaturization with increased water depth, primarily due to limited food availability, especially at abyssal depths (Thiel 1979). The Central Indian Ocean has very low surface primary productivity ranging between $9.06 \text{ mg C m}^{-2}\text{d}^{-1}$ to $103.4 \text{ mg C m}^{-2}\text{d}^{-1}$ (Matondkar et al. 2005) leading to low sediment organic carbon (Avg. 0.2%). Hence, the food available to the deep-sea communities in this area is also low, which could explain in part the diminutive size of macrofauna observed in the present study.

Thus, use of larger sieve sizes (eg. 0.5 mm or 1 mm) for extracting deep-sea metazoans could potentially underestimate their densities. A significant difference was obtained for macrofaunal density (>50% loss using 0.5 mm mesh sieve) and biomass between the two mesh sizes studied (Table 1). Vertical distribution of macrofauna in the sediment did not generally show a significant difference between the mesh sizes, except for a marginal increase in density (Fig. 6a) and biomass (Fig. 6b) in the upper 5 cm of sediment with the finer mesh. In coastal ecosystems, high densities of macrobenthic individuals are also retained on fine meshed sieves, but due to the extraction of juvenile stages rather than species with smaller average body sizes (Schlacher and Wooldridge 1996). The present study reveals that higher numbers of individual organisms obtained with finer mesh actually include an increase of small sized adults of species that would have passed through a coarser mesh.

In the present study there was a significant loss of macrofauna using a 0.5 mm mesh sieve. The loss was greatest for polychaetes, nematodes, harpacticoids and isopods both in terms of number of specimens and species obtained. Nematodes and harpacticoid copepods are generally considered to be part of the meiofauna, but their presence in the 0.5 mm and 0.3 mm mesh sieves in the present study has lead to their inclusion with the macrofauna. Nematodes outnumbered the polychaetes in the present study. A similar observation was reported by Ingole et al. (2001).

Gage et al. (2002), concluded that, use of 0.5 mm mesh sieves would miss a number of species that could be collected on 0.425, 0.3 and 0.25 mm mesh sieves. The deep-sea is thought to harbor a large number of rare species of macrofauna, with samples typically being composed of many species occurring as singletons or in low numbers (Grassle and Maciolek 1992). Hence, use of coarse sieves could greatly undersample many small uncommon species. Accordingly, Gage et al. (2002) recommended a sieve with 0.5 mm mesh for the purpose of describing macrobenthic biomass in the deep sea (but not abundance and species richness).

Polychaetes, nematodes and crustaceans (harpacticoids and isopods) considered separately showed notable differences in occurrence between the two sieve sizes examined. There were also varying responses in diversity indices for different macrofaunal groups with the different sieve sizes used, which agrees with the findings by Gage et al. (2002). It may therefore be inaccurate to use results from one taxonomic group as a proxy for the total assemblage. Hence, it is suggested that the total macrofaunal community as a whole be studied and that a mesh of 0.3 mm or finer be used.

In the abyss, many new species have been found with no sign of the number of species reaching an asymptote (Gage 1996) and the loss of even a few species in any deep-sea study could lead to the loss of rare or unknown species. The use of finer mesh will directly aid in documenting the diversity of the deep-sea habitat more precisely, a crucial element in understanding global species distributions and their ecological roles.

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Table 1: Mann-Whitney U and Students t-tests of macrofaunal density, biomass, number of species, and three diversity indices for sieves of two different mesh sizes.

Mann-Whitney U test	U	p	n
Macrofaunal density	1296	0.0001	132
Macrofaunal biomass	1543	0.004	132
Species	1289	0.0001	132
<i>J</i>	107.5	0.78	29
<i>E(Sn)</i>	190.5	0.101	46
<i>H'</i>	190.5	0.09	46
Student's t-test	t	p	df
<i>d</i>	1.141	0.264	27
Sub-samples of PVC pipe			
Density	0.718	0.486	12
Biomass	0.771	0.298	12

Table 2: List of genus/species obtained on 0.5mm and 0.3 mm mesh sieve

Major taxa	Family	Genus/Species	
		0.3 mm sieve	0.5 mm sieve
Nematoda	Oncholaimidae	<i>Viscocia</i> sp. <i>Filoncholaimus</i> sp.. <i>Adoncholaimus</i> sp.	<i>Viscocia</i> sp. <i>Filoncholaimus</i> sp. <i>Adoncholaimus</i> sp.
	Oxystominidae	<i>Halalaimus</i> sp.	<i>Halalaimus</i> sp.
	Ironidae	<i>Dolicholaimus</i> sp. <i>Trissonchulus</i> sp.	<i>Dolicholaimus</i> sp.
	Microlaimidae	<i>Paramicrolaimus</i> sp.	
	Anticomidae	<i>Odantanticoma</i> sp.. <i>Cephalanticoma</i> sp.	<i>Cephalanticoma</i> sp.
	Ethmolaimidae	<i>Comesa</i> sp.	<i>Comesa</i> sp.
	Enchelidiidae	<i>Polygastrophora</i> sp. <i>Belbolla</i> sp. <i>Calyptronema</i> sp.	<i>Polygastrophora</i> sp. <i>Belbolla</i> sp. <i>Calyptronema</i> sp.
	Linhomoeidae	<i>Megadesmolaimus</i> sp..	
	Phanodermatidae	<i>Micoletzkyia</i> sp.	
	Thoracostomopsidae	<i>Enoplolaimus</i> sp.	
	Xyalidae	<i>Linhystera</i> sp.	
	Leptsomatidae	<i>Pseudocella</i> sp. <i>Melercylicolaimus</i> sp.	<i>Melercylicolaimus</i> sp.
	Comesomatidae	<i>Sabatieria</i> sp.	<i>Sabatieria</i> sp.
	Phanodermatidae	<i>Phanoderma</i> sp.	<i>Phanoderma</i> sp.
	Aegialoalaimidae	<i>Diplopelloides</i> sp.	
	Unidentified	sp. 1	sp. 1
	Polychaeta	Maldanidae	<i>Axiothella</i> sp. <i>Maldane</i> sp.
Hesionidae		<i>Hesionone</i> sp. Genus 2	<i>Hesionone</i> sp. Genus 2
Spionidae		<i>Prinospio</i> sp.	<i>Prinospio</i> sp.
Capitellidae		<i>Capitella minima</i>	
Paraonidae		<i>Levisennia</i> sp. <i>Paradoneis</i> sp.	
Orbiniidae		<i>Scoloplos</i> sp.	
Flabelligeridae		<i>Flabelligera</i> sp. <i>Brada</i> sp. Genus 3	<i>Flabelligera</i> sp. <i>Brada</i> sp.
Phyllodoceidae		<i>Phyllodoce</i> sp.	
Terebellidae		species 1	
Goniadidae		<i>Glycinde</i> sp.	<i>Glycinde</i> sp.
Glyceridae		<i>Glycera</i> sp.	<i>Glycera</i> sp.
Eunicidae		<i>Lumbriconeries latreilli</i>	<i>Lumbriconeries latreilli</i>
Unidentified		sp. 1	sp. 1
Tanaidacea		Agathotanaidae	<i>Paranathura</i> sp.
	Leptognathiidae	<i>Paraleptognathia</i> sp. <i>Leptognathia</i> sp.	<i>Paraleptognathia</i> sp. <i>Leptognathia</i> sp.

	Neotanaidae Unidentified New Family	<i>Neotanais</i> sp. sp. 1 sp. 1	<i>Neotanais</i> sp. sp. 1 sp. 1
Harpacticoida	Canuellidae Zosimidae Pseudotachidiidae Neobradidae Argestidae Huntemanniidae Aegisthidae Ameiridae	<i>Canuella</i> sp. <i>Zosime</i> sp. <i>Pseudotachidius coronatus</i> <i>Pseudotachidius similis</i> <i>Marsteinia</i> sp. <i>Mesocletodes</i> sp. <i>Metahuntemannia</i> sp. <i>Cervinia</i> sp. <i>Cerviniella</i> sp. Genus 3 <i>Parameiropsis</i> sp. Genus 2	<i>Canuella</i> sp. <i>Pseudotachidius coronatus</i> <i>Cerviniella</i> sp. <i>Parameiropsis</i> sp.
Bivalvia	Montacutidae Thyasiridae	<i>Montacuta</i> sp. <i>Mendicula</i> sp.	<i>Montacuta</i> sp. <i>Mendicula</i> sp.
Isopoda	Macrostylidea Desmosomatidae Haploniscidae.	<i>Macrostylis</i> sp. Genus 2 <i>Pseudomesus</i> sp. <i>Haploniscus</i> sp.	<i>Macrostylis</i> sp. Genus 2
Nemertine	Unidentified	sp. 1	sp.1

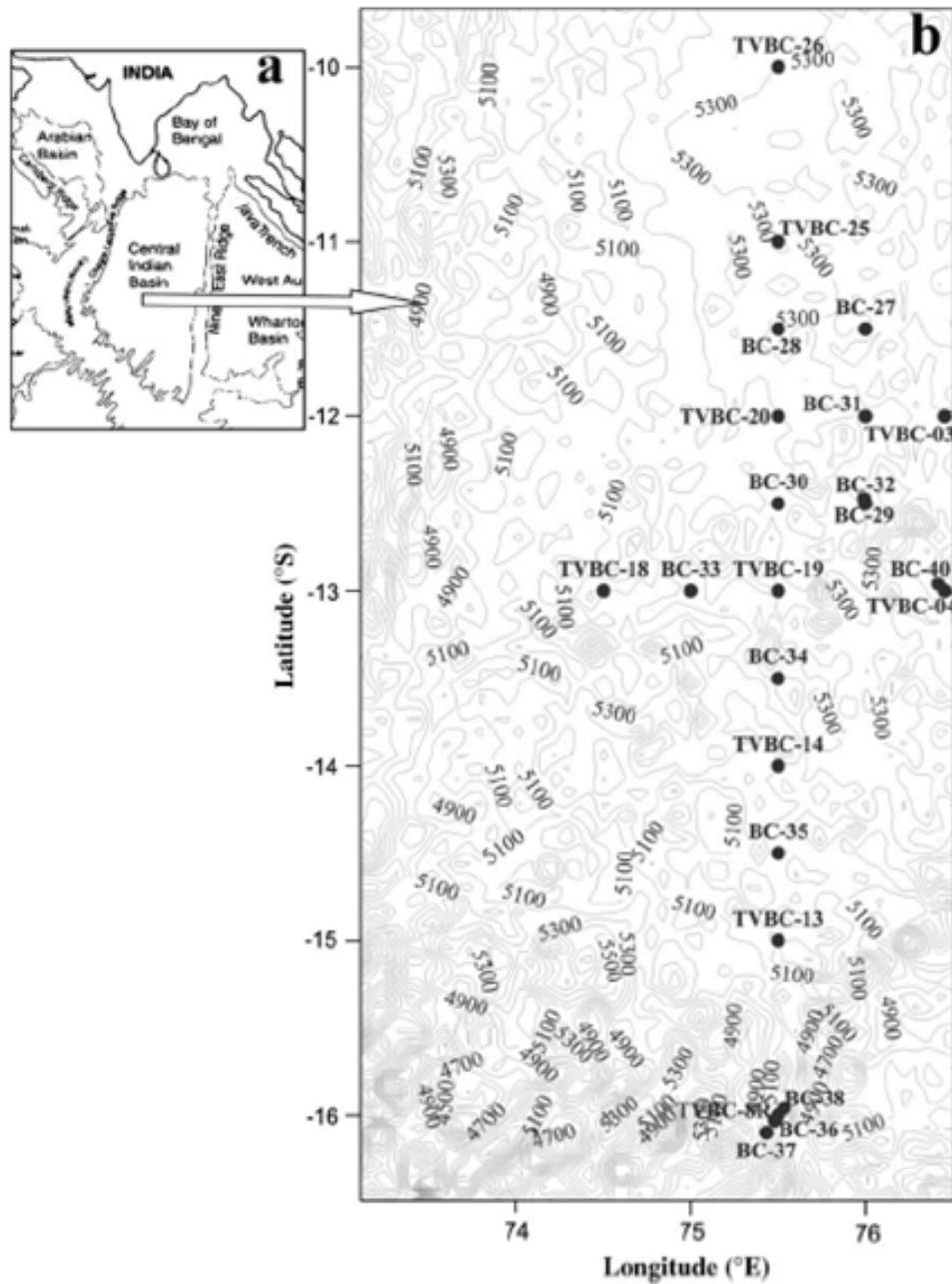


Figure 1. (a) Study area (b) sampling stations and contour map of the site

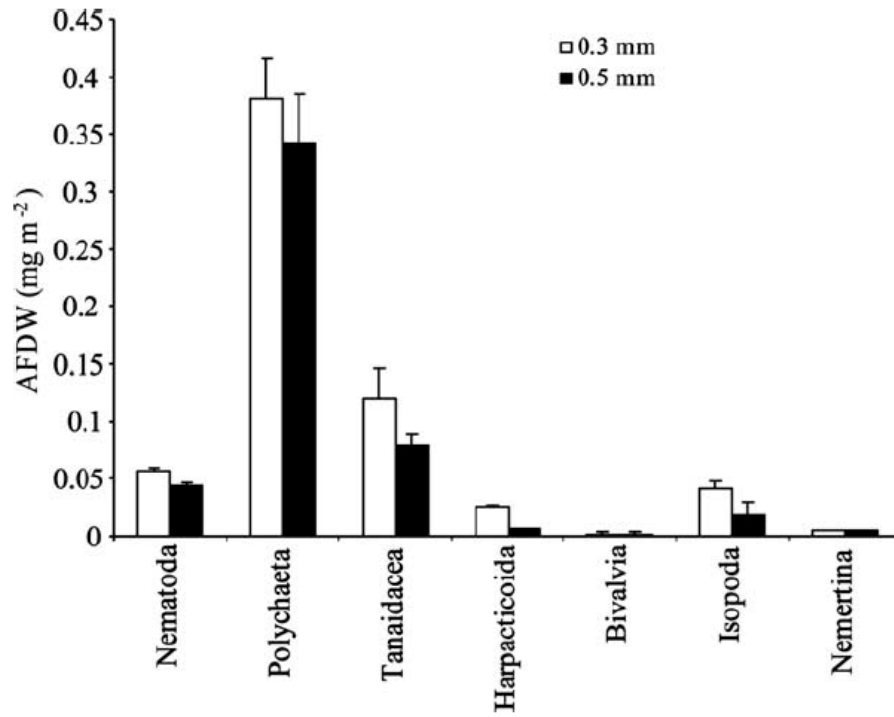


Figure 2. Variation in macrobenthic biomass due to different mesh sizes

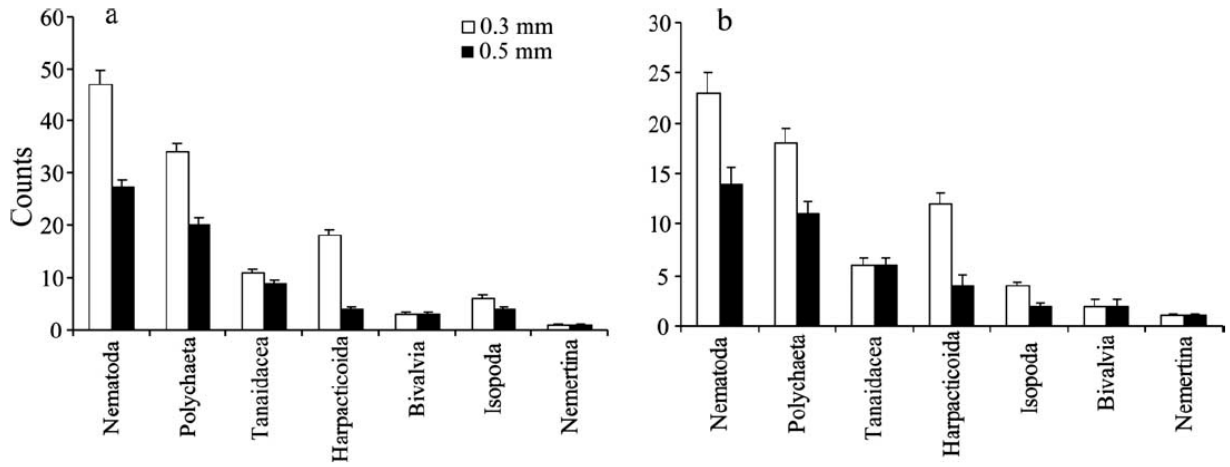


Figure 3. Variation in number of (a) individuals and (b) species for the two mesh sizes

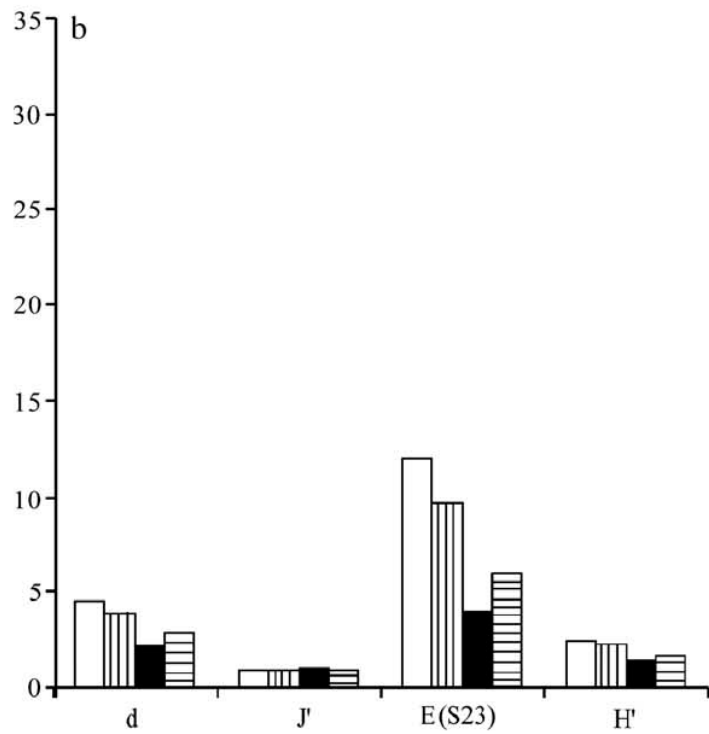
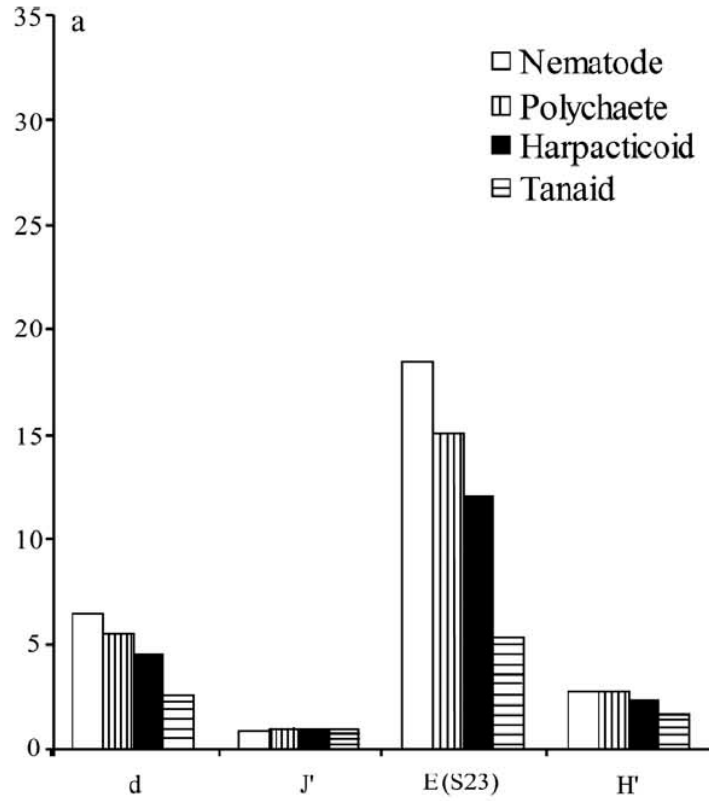


Figure 4. Variation in diversity indices, (a) 0.3 mm mesh sieve, (b) 0.5 mm mesh sieve. *d*, species richness; J' , evenness; $E(S23)$, Hurlbert's rarefaction; H' , species diversity.

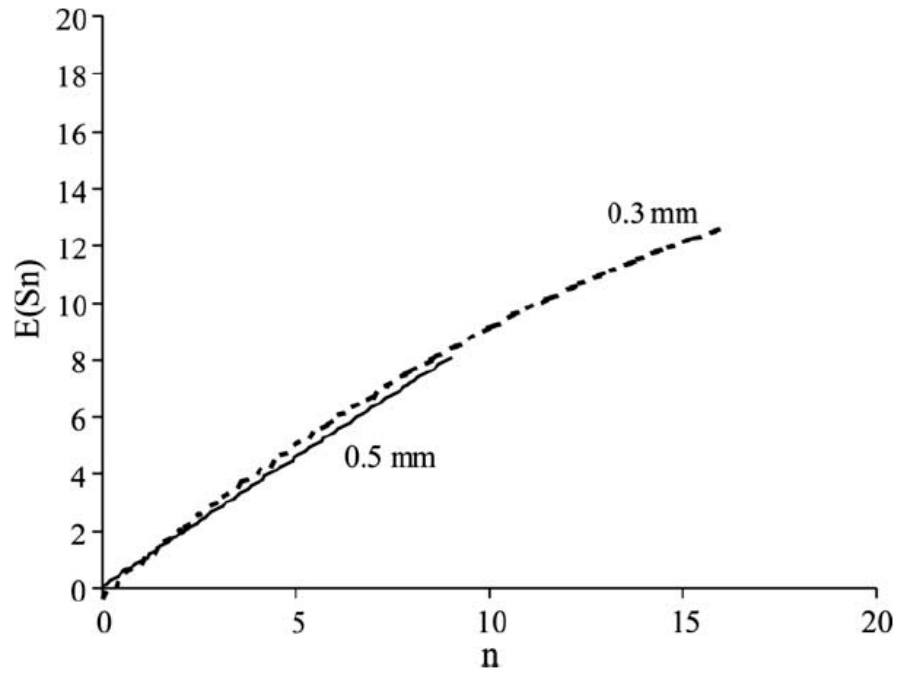


Figure 5. Hurlbert's rarefaction curve for two mesh sizes. $E(S_n)$ = expected number of species; n = observed number of species

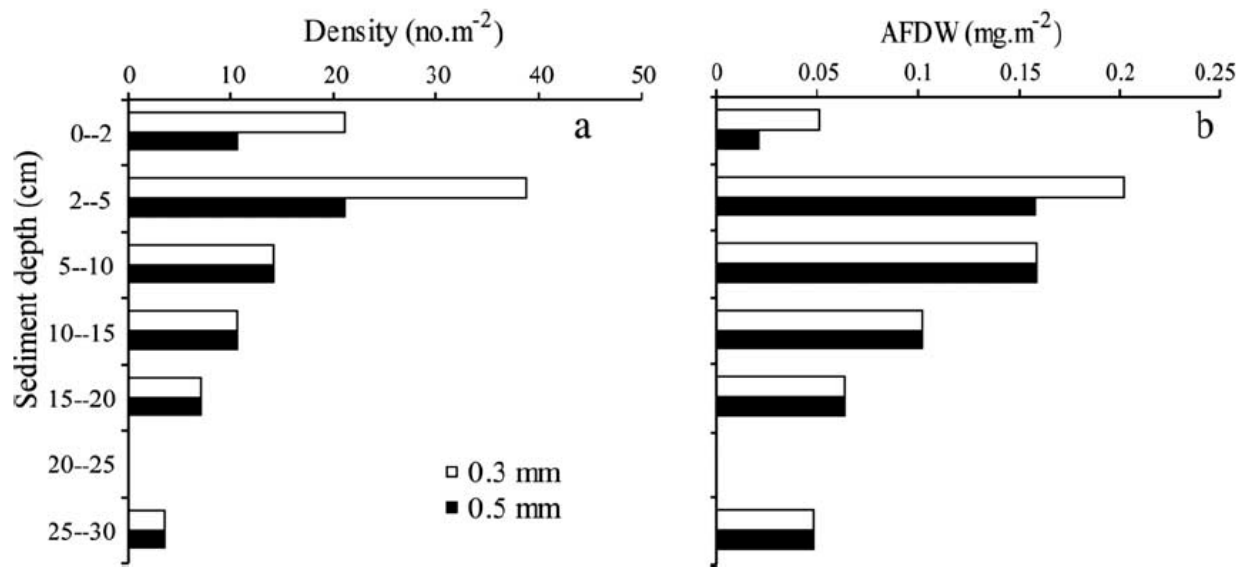


Figure 6. Vertical variation of macrofaunal (a) density and (b) biomass