Efficiency of sampling gears (Quadrate and Core) and Taxonomic Resolution on the Soft Bottom Intertidal Macrobenthic community of Port Blair coast

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Abstract
Ecological studies of a region cannot proceed forward without the evaluation of species diversity. With the ever-increasing demand for studies to understand the change in the macrobenthic communities, the focus has primarily been shifted towards faster results to track down the changes from the prior environments. Therefore, studying the complete benthos faunal diversity of an area leads to an unrealistic approach. Thus, researchers tend to depend on various sampling gears, which ease the work capacity. The present study compared two samplings gears (core and quadrate) in two different habitats to understand the diversity of the macrobenthic communities. In terms of abundance, the core gear showed higher significant differences as compared to quadrate.

However, the gears did not significantly differ among the diversity indices (Margalef's index and Shannon-Winer index) and the cluster analysis (Bray-Curtis similarity index and nMDS). The present study found that the 'information loss' was minimal with the aggregated data at a higher taxonomic level. Spearman rank correlation coefficient revealed that the information loss was low up to family-level and the correlation coefficient decreases as the taxonomic level increases after family-level. Nonetheless, the choice of sampling gears did not influence the diversity of the soft-bottom intertidal macrobenthic communities.

Keywords: Macrobenthos, Sampling gear Comparison andaman Islands.

Introduction
A fundamental step in any ecological investigation is the abundance of organisms[1]. In general, benthic studies are usually expensive and exceedingly time consuming due to strenuous sampling efforts in the field and lengthy laboratory analysis[8]. Though the physical and environmental factors influencing the distribution of intertidal macrobenthos are more complex due to the exposure to both aerial and aquatic environments, the study of intertidal macrobenthos is more accessible than that of subtidal macrobenthos[15].

Studies have been carried in the past few decades to demonstrate the efficiency of various benthic sampling gears[7,12,22,23,35,36]. Different authors have proposed additional sampling gears suitable for sampling benthos. Cores and suction samplers are more efficient than grabs in most soft substrata[9,21,40].

Hand-held core gives the best macrofaunal density and composition than suction sampler[39]. Most of the earlier studies comparing different sampling methods were based only on the numerical count of organisms into major groups to compare the data. Later, non-parametric multivariate measures were applied which demonstrated the inadequacies of univariate diversity measures to detect the variations in macrobenthos community changes[44]. For example, Somerfield et al[36] suggested that the choice of sampling gear does not affect the diversity of the location.

Seagrass meadows have a very high level of primary productivity in the shelf zone; they have a high turnover of seagrass leaves themselves and the epiphytes associated with them[30]. Seagrass ecosystems contribute high productivity to nearby and distant locations by exporting particulate organic matter and living plant and animal biomass[19].

In addition to this, seagrass provides habitat to several benthic, epiphytic and nektonic invertebrates and vertebrates. Larger vertebrates like Turtle and Dugong migrate thousands of nautical miles to feed on seagrass every year in the tropic region[14,20]. The availability of food, contributed by different trophic levels from producers to carnivorous and the habitat stability and complexity, enriches the diversity and abundance of the seagrass ecosystem[6,33]. Bare sediment is less diverse as compared to seagrass habitat[4]. Seagrass bed shows higher species richness whereas it is less in bare sediment[17].

Taxonomic resolution is the most pressing issue in monitoring macrobenthos. In the tropical areas and deep-sea regions, it is very challenging to identify specimens upto species level due to the lack of taxonomic expertise and the presence of a significant number of undescribed species[22,24,29]. Ellis[16] introduced the term "taxonomic sufficiency" (TS); according to this, the information regarding the spatial pattern of biological assemblage or community will be similar using higher taxonomic levels, saving time and taxonomic effort. A group of researchers supported the use of higher taxonomic level in the study[11,27,29,37] whereas some researchers raised the question on this method that how it can reduce the taxonomic expertise[24].

Furthermore, in various studies, the 'information loss' is shallow when using taxonomical data higher than species level such as family level. The results obtained from the family level were similar to that of species-level or genus-level and that family-level is sufficient to detect the changes in studying the impact or monitoring program or detecting an environmental change in strong environmental gradients[22,29,37,38] in the assemblage pattern in different taxonomic levels.

The objective of the present study was to evaluate the efficiency of two frequently used sampling gear (Core and Quadrate). This study theorised that despite the difference in the sizes and volumes of the sampling gear, the community structure of the sampling location remains constant. The present study is conducted in the intertidal area of South Andaman where the taxonomic information is limited. The lack of taxonomic information urges to demonstrate the need for taxonomic sufficiency.

Hence, the taxonomic resolution was implemented on the data to signify the higher taxonomic level adequacy. Therefore, in the present study, an effort has been made to assess the effects of sampling gears and taxonomic resolution on the intertidal softbottom macrobenthos. The study aimed to identify the efficient sampling gear and method for various research purposes to study the intertidal softbottom macrobenthos.

Material and Methods

Study area: Andaman and Nicobar Islands being union territory borders the South-East Asian countries (Thailand, Indonesia and Myanmar). The archipelago is oriented north-south arc between 6° N and 14° N and 92° E to 94° E and covers an area of 8,249 km^2. The archipelago consists of 572 islands and islets, out of which 38 islands are human settlements. The archipelago consists of elongated rocky and softbottom intertidal regions comprising both critical and pristine intertidal ecosystems. Both tropical rainforest and coral reefs occur side by side in these islands.

Sampling was conducted at two locations along the Port Blair coast to monitor the Macrobenthic faunal assemblage. Two sites Chatham (CH; $11^0$41'08″N and $092^0$43'23″E) and Marina Park (MP; $11^0$40'08″N and $092^0$44'58″E) were selected for collection of macrofaunal samples (Fig. 1).

Chatham (CH): It is located in the northeast part of the Port Blair coast (Fig.1). CH is a flat, dissipative beach characterised by heterogeneous sedimentation, sandy silty towards the shoreline and sandy towards the subtidal region. It is influenced by the private and government establishments build around it with a massive amount of sewage discharge coming from 5 different outlets. The station's mid-shore region is covered by dense vegetation of seagrass dominated by *Thalassia hemprichii* and *Halodule uninervis*. This station is also influenced by maritime traffic of commercial as well as fishing ships and boats.

Marina Park (MP): It is located near the central city area (Fig. 1). The seaward coast is comprised of a natural reef system covered with seaweed in some parts. The substratum is mainly sandy with some dead coral fractions at the subtidal areas. 4 freshwater runoffs are coming from the landward side. The freshwater runoffs are relatively less polluted than sewage. Boat activities and fishing activities also influence the station as it is a prominent spot for tourists. The beach is also influenced by the seawall constructed at the landward side. Patches of seaweeds can be seen in the intertidal area when exposed during low tide.

Sampling strategy: The sampling was carried out monthly between September 2016 and March 2017. A quadrate of 25 cm^2 (0.0625 m^2 area) and a core of diameter of 15 cm (0.0047 m^2 area) were used to sample the macrobenthos. A total of 252 samples were collected throughout the study period (2 gears × 3 replicates × 3 zones (high, mid and low tide zone) × 2 locations (CH & MP) × 7 months). The excavated sediment was screened through a test sieve with a mesh size of 0.5 mm in the field after mixing relaxant 73 g $MgCl_2$ per litre of filtered seawater).

The retained matter was transferred to a sample container with 5% buffered formalin mixed with Rose Bengal stain and conditioned for further laboratory analysis. Different infaunal groups were separated from the residue, enumerated, labelled and stored in 70% ethanol (v/v) for further examination in the laboratory. All taxa were first segregated into different groups and then identified up to a species or the possible lowest level in the Linnaean classification.

Data Analysis: Univariate measures (Margalef's species richness, d and Shannon-Wiener diversity, H′ log_2) and multivariate techniques (Cluster analysis, nMDS, PERMANOVA and 2-stage analysis) were performed using PRIMER V6.1[10]. One-way Analysis of variance (ANOVA), Tukey's pairwise test, Kruskal-Wallis test and Mann-Whitney test were performed using Past3 software to test any significant variation in the faunal abundance or diversity among sampling gears (core and quadrate) and locations (CH and MP). Agglomerative hierarchical cluster analysis (AHCA) was performed based on the Bray-Curtis similarity resemblance matrix from square root transformed species abundance data. Non-Metric Multi-dimensional Scaling (nMDS) was obtained from superimposing the assemblage pattern of Bray-Curtis on it. The non-parametric multivariate analysis of variance PERMANOVA was to test the variation in the assemblage pattern both gear-wise within the station and spatially.

The similarity matrices were compared using the non-parametric Spearman's rank correlation and the permutation procedure RELATE[37] determined the significance. The similarity matrix of the *'best'* possible sample was then compared with the other matrices at higher taxonomic levels for sampling gear and location.

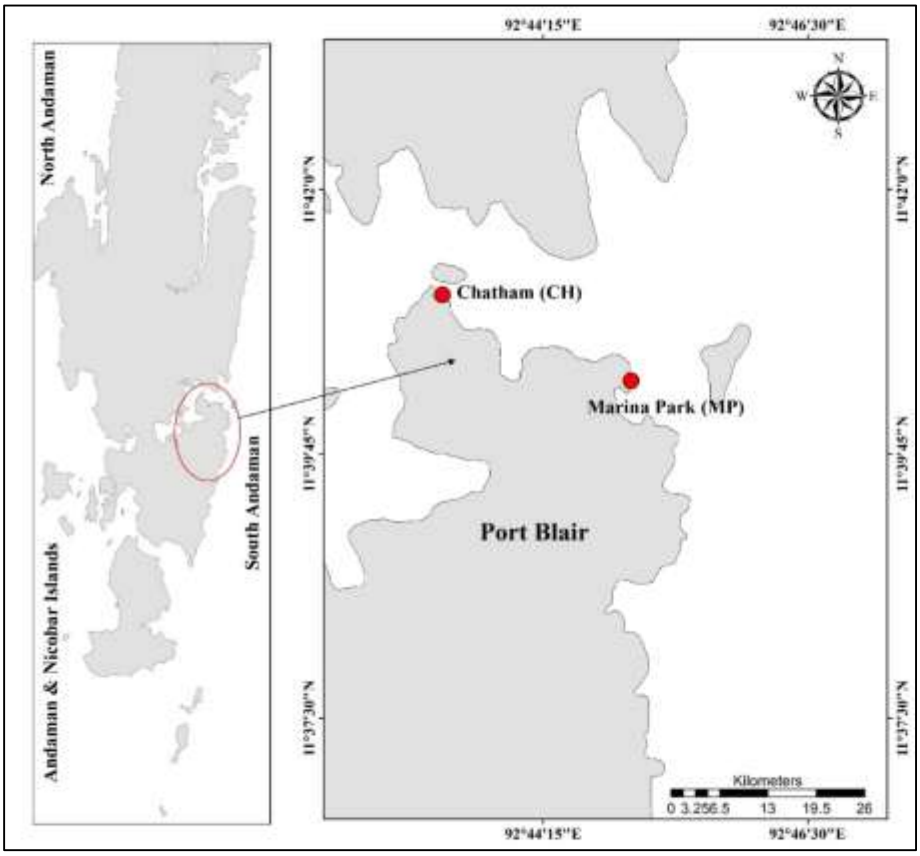


Figure 1: Map showing the study area. Filled red colour circles are representing the sampling stations

A '*2Stage*' orientation was performed using the inter-matrix correlation values of sampling gears and locations at different taxonomic levels[37]. This analysis will provide a clear picture of the interrelationship between different assemblage patterns based on different taxonomic levels (species, genus, family, order, class and phylum) in the Linnean classification.

Results

Macrofauna Community Composition: A total of 78 taxa of macrofauna belong to seven different groups: Polychaeta, Amphipoda, Bivalvia, Gastropoda, Decapoda, Sipuncula and Nemertea which were identified from the collected samples during the study period. The identified taxa belonged to three significant phyla (Annelida, Arthropoda and Mollusca) and two minor phyla (Nemertea and Sipuncula). Polychaetes were the most species-rich group encountered during the study followed by Amphipoda, Bivalvia and Gastropoda and represented by 56 taxa belonging to 22 families and eight orders. Spionidae (11 species), Capitellidae (8 species), Glyceridae (5 species) were species-rich families among polychaetes. Amphipoda and Gastropoda represented five taxa each, belonging to three families each, followed by Bivalvia and Decapoda, representing four species each. Apart from the major phyla, minor phyla such as Nemertea and Sipuncula were also recorded.

There was no remarkable difference found in species numbers between the sampling gears (Core: 78; Quadrate: 78 taxa). Polychaetes were the most abundant and diverse group in both the samplers. Spatially CH recorded the highest number of taxa (77 taxa) than MP (49 taxa). About 29 taxa were recorded exclusively at CH and those species were absent at MP during the study.

Macrofauna Abundance: Altogether 38,327 individuals were recorded during the study period. Polychaetes were the most abundant taxa. *Malacoceros indicus* (2772 ind./m^2), *Microspio* sp. (2458 ind./m^2), *Aricidae* sp.1(1896 ind./m^2) and *Prionospio cirrifera* (1847 ind./m^2) were the most abundant species among Polychaetes. Amphipod was the second most abundant taxa represented by *Hyale* sp.1 (2483 in./m^2) followed by *Urothoe* sp. (1146 ins./m^2). The most abundant Gastropod and Bivalve were represented by *Nassarious globossus* (1026 ind./m^2) and *Tellins* sp. (570 ind./m^2) respectively. A total of 29143 individuals of macrofauna were collected from the core sampler and 9184 from the quadrate sampler. *Malacoceros indicus*, *Microspio* sp. and *Hyale* sp.1 were the most abundant species collected from both the sampler. *Gonida* sp. and *Paraprionospio* sp.2 were absent in core samplers. Spatially, CH (24462 individuals) was more abundant than MP (13865 individuals).

The most dominated group in CH was Polychaetes, represented by *Prionospio cirrifera* (1775 ind./m^2), *Orbiniia* sp.1 (1342 ind./m^2) etc. A spike in the number of sipunculids can be seen in CH (2277 ind./m^2). MP was mostly dominated by *Microspio* sp., *Aricidae* sp.1, *Malacoceros indicus*, *Syllid* sp.1 etc. There was a spike in Amphipod density (*Hyale* sp., 1843 ind./m^2) in MP.

Macrofaunal Diversity: Though the diversity indices values were found high in quadrate samples than core samples, there was no significant difference found in diversity indices between core and quadrate except the number of individuals (N). The number of species (S) was high in quadrate samples (32±17) than core samples (29±15) (Fig.2.a). One-way ANOVA results showed no significant difference in the number of species between quadrate and core (Table.1). The number of individuals (N) was found high in core samples (206.98±93.50) and low in quadrate samples (120.63±55.83) (Fig.2b). There was a significant difference found in the number of individuals from the ANOVA results between quadrat and core (Table 1).

Diversity indices such as Marglef's index (d) and Shannon-Weiner (H'log $_{(2)}$) diversity index, both indices values were found high (d=6.32±3.09; H'log$_{(2)}$= 4.51±0.93) in quadrate samples (Fig. 2c; Fig. 2d). One-way ANOVA results showed no significant difference in both the diversity indices between quadrate and core samples (d: F=1.216, p>0.05; H'log$_{(2)}$: F=0.042, p>0.05) (Table.1). Spatially, the univariate measures were high in CH than MP (Table 1). One-way ANOVA results found a highly significant difference in the diversity indices between CH and MP (Table 1).

Macrobenthic Community Structure: The dendrogram for hierarchical clustering was constructed on Bray–Curtis similarity matrix obtained from square–root transformed faunal abundance data. The resulting dendrogram was possible to recognise two distinct assemblages representing the two-sampling locations at 46% similarity (Fig. 3). There was no definite assemblage pattern observed within the locations as well as between the sampling gears. The assemblage pattern of both the stations was distinct whereas the assemblage pattern of the samples collected from quadrate and core was mixed (Fig. 4). The assemblage pattern was superimposed to the nMDS plot to understand better the assemblage pattern between the samples collected from quadrate and core. The nMDS plot showed that macrofaunal assemblage pattern is more evident in the spatial scale (Fig. 4a).

Statistically, it is clear from PERMANOVA that the assemblage pattern between the station is highly significant (F=14.361, p<0.001). Visually there was no evidence of a distinct pattern of macrofauna between quadrate and core samples. PERMANOVA results showed no significant difference between sampling gear within MP (F=1.2339, p>0.05). However, a significant difference was found in the assemblage pattern of quadrate and core of CH (PARMANOVA: F= 2.231; p= 0.021).

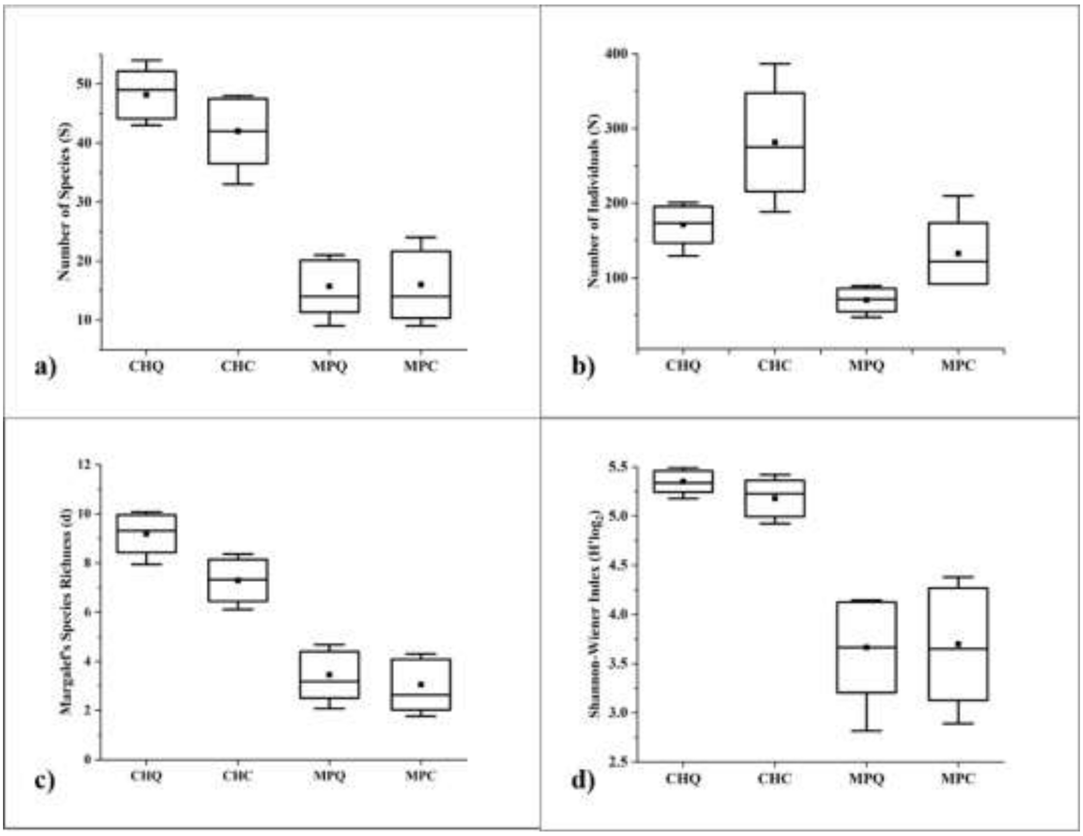


Figure 2: Univariate measures of macrobenthos: a) Number of species; b) Number of individuals; c) Margalef's species richness; (d) Shannon-Wiener index. CHQ: Chatham Quadrate samples; CHC: Chatham Core samples; MPQ: Marina Park Quadrate samples; MPC: Marina Park Core samples.

Table 1
One-way ANOVA results of community attributes of macrobenthos, comparing sampling gears and sampling locations. Values are mean±standard deviation.

		S	N	d	H'($\log_2$)
Q (Mean±SD)		32±17	120.16±55.83	6.32±3.09	4.51±0.93
C (Mean±SD)		29±15	206.98±93.50	5.17±2.38	4.44±0.87
ANOVA for Sampling Gear	F	0.23	8.85	1.21	0.04
	p	>0.05	**<0.05**	>0.05	>0.05
CH (Mean±SD)		45±6	226.18±74.57	8.24±1.25	5.26±0.17
MP (Mean±SD)		16±5	101.41±43.94	3.26±0.97	3.68±0.50
ANOVA for Sampling station	F	216.1	29.09	138.03	126.7
	P	**<0.001**	**<0.001**	**<0.001**	**<0.001**

[For abbreviation of station and sampling gears, refer figure 2]

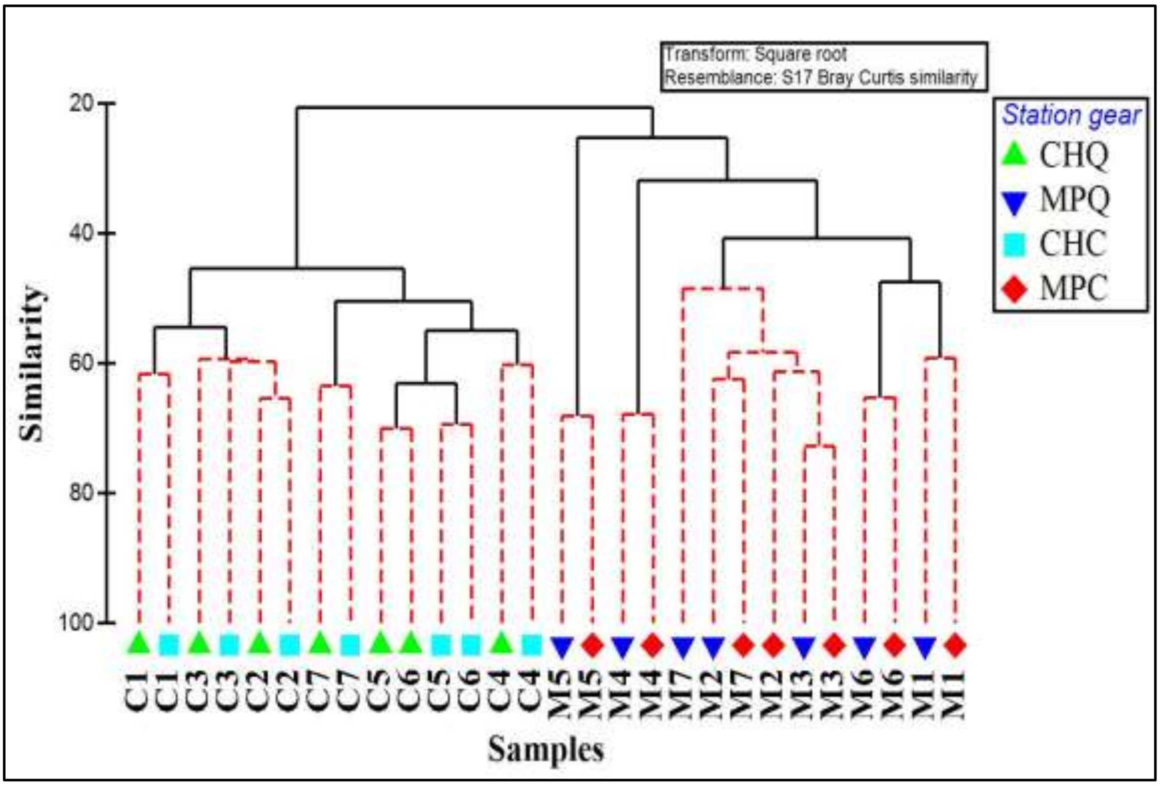
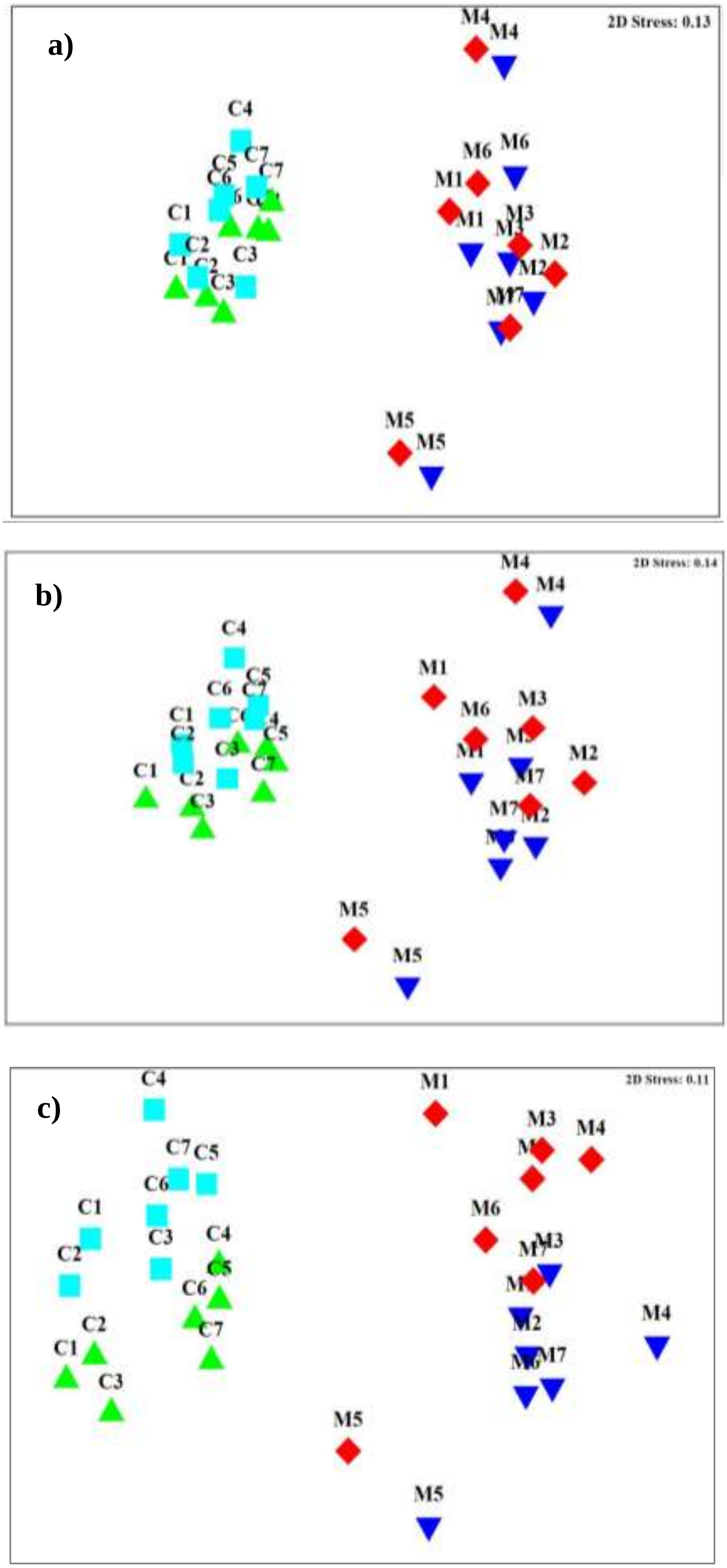


Figure 3: Bray-Curtis similarity-based dendrogram showing the macrobenthos assemblage pattern in the Chatham and Marine Park locations along the Port Blair coast using core and quadrate samplers.

Taxonomic Resolution: The Spearman's correlation coefficients for all the matrices of different taxonomic level, species, genus, family, order, class and phylum were highly significant ($p<0.001$). The Spearman's correlation coefficients, in the low taxonomic levels i.e. species, genus and family level were strongly correlated (Table 2).

The correlation values decrease as the taxonomic group increases above the family level. The lowest correlation value among the 'best' similarity matrix (sample collected by quadrate and core sampler and identified to species level) and similarity matrix at higher taxonomic level was observed in samples taken by core and quadrate. The multivariate analysis of macrofaunal assemblages (nMDS ordinations) aggregated at different taxonomic levels resulted in very similar patterns (Fig. 4a-f). The dissimilarity among some sampling stations decreases as the taxonomic level increases. Nevertheless, the assemblage pattern of quadrate and core remains the same with no specific assemblage pattern.

From the '2Stage' nMDS orientation, the assemblage patterns obtained from resemblance matrices of species, genus and family were very similar to each other (Fig. 5). The assemblage pattern at order, class and phylum level showed a comparatively larger difference. However, the assemblage pattern of sampling gear remains constant, showing no evidence of a distinct pattern.


a)
2D Stress: 0.13
M4
M4
C4
M6
M6
M1
M1
C1
C2
C3
M3
M2
M2
M5
M5
b)
2D Stress: 0.14
M4
M4
C4
M1
C6
M6
M3
C1
C2
M2
M5
M5
c)
2D Stress: 0.11
C4
M1
M3
M4
C7
C5
C6
C1
M6
C3
C4
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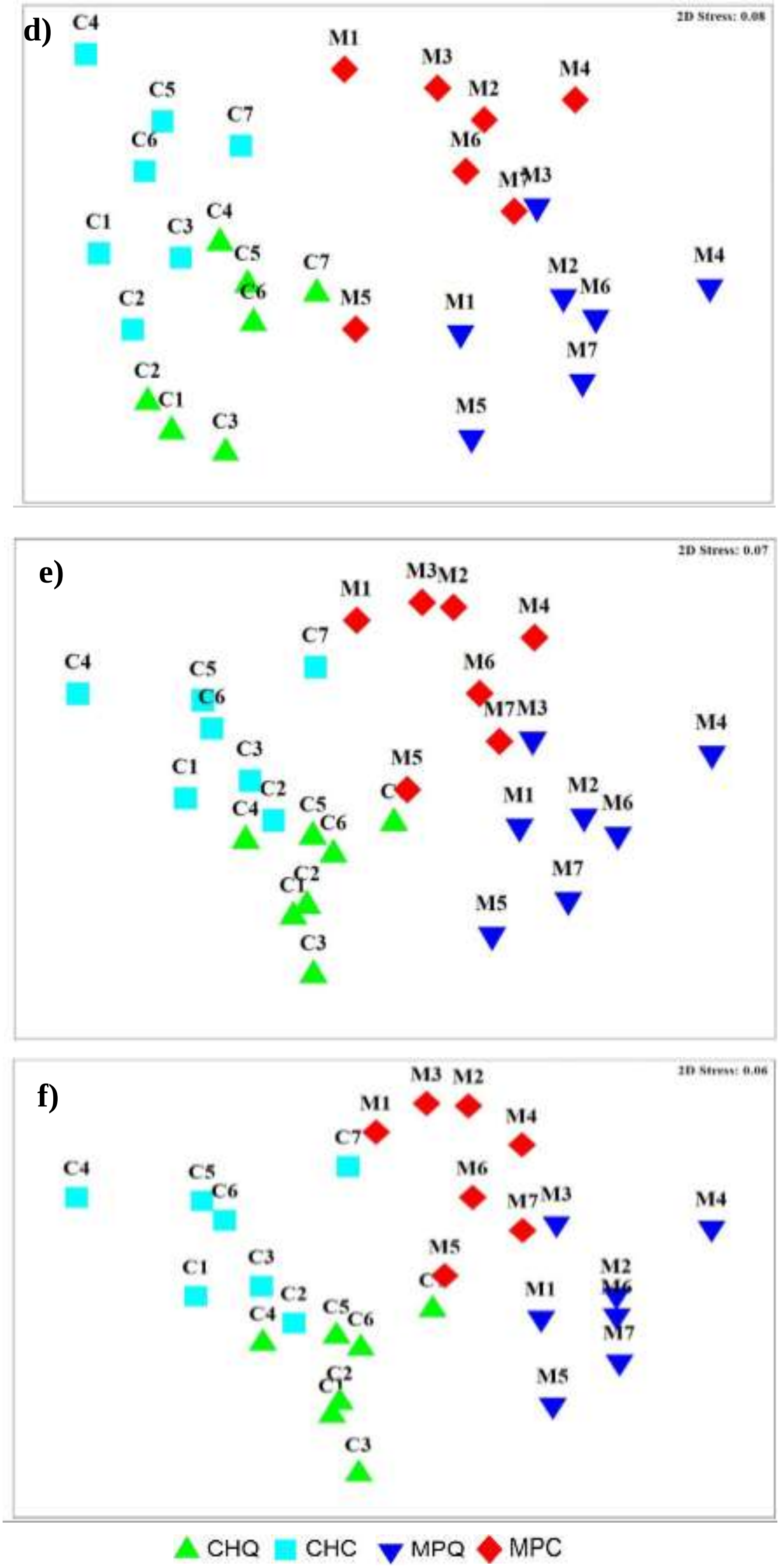

Figure 4: nMDS plots of macrobenthos assemblage at their different taxonomic hierarchy levels; a) Species; b) Genus; c) Family; d) Order; e) Class; f) Phylum.

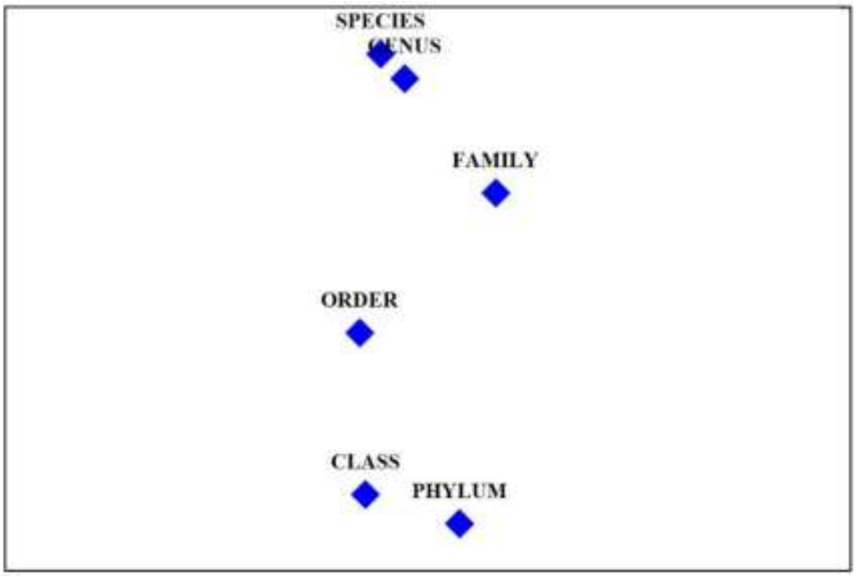

Figure 5: '2stage' nMDS ordination plot of inter-matrix rank correlation of macrobenthos for each sampling gear at different levels of taxonomic resolution: Species, Genus, Family, Order, Class and Phylum.

Table 2
non-parametric Spearman's correlation coefficients from the '2stage' analysis for different levels of taxonomic resolution of macrofaunal assemblages.

	Species	Genus	Family	Order	Class	Phylum
Species						
Genus	0.982298					
Family	0.921558	0.938681				
Order	0.822541	0.842086	0.909613			
Class	0.725162	0.751752	0.806577	0.934962		
Phylum	0.675925	0.699706	0.764925	0.907268	0.975597	

Discussion

Worldwide, numerous studies have been conducted to estimate the efficiency of different sampling gears[22,35,36,38,39] with a higher degree of subtidal sampling and deep-sea sampling. Sampling gears (Core[2], Quadrate[31,32]) in the intertidal area of the Andaman Islands have been robustly used; their efficiency is yet to be evaluated. The present study aimed to determine gear efficiency between two widely used sampling gears for sampling in the intertidal soft-bottom benthos from the Andaman coast The present study recorded 78 macrofaunal taxa from two locations concurring with the other studies from tropical regions[13,17,31,34]. In general, species number is high in tropic than temperate regions.

Univariate measures such as diversity indices did not differ significantly between the two gears. However, there was a significant difference in the number of individuals between the gears. The findings are analogous to a study conducted by Somerfield and Clarke[36] who suggested that the diversity of the station remains constant regardless of the gear sampling and methods. However, the abundance, biomass and size can vary when sampled by different gears. For example, an earlier study conducted by Smith et al[35] observed that as the organisms burrow deeper into the sediment and their abundance, biomass and size are high in the core gear than that of those collected through Smith-McIntyre grab.

The study found that the core penetrates deeper into the sediment than the grab. The animals burrow deeper into the sediment; hence, the abundance, biomass and size of the animal are high in the core than grab. The present study found that the abundance of core samples was high than the quadrate samples. The core maneuvers deeper in the soft bottom leaving the sediment undisturbed, unlike the quadrate samples, where a scoop is employed to excavate the sediment, unsettling the sediment and possibly reducing the number of individuals in the sample. Though the abundance may vary, the location's diversity remains unchanged when sampling with a different sampler.

Seagrass habitat plays a significant role in shaping the community structure of intertidal macrobenthos. They are known to reduce the physical disturbance (waves), enhance sediment entrapment[14] and increase food availability (Detritus and sediments),[43] thus supporting the high density and diversity of the macrobenthic community[3,5]. Seagrass bed shows higher species richness whereas it is less in bare sediment[3,18,30]. In the present, sampling location CH (harbouring seagrass patches in the intertidal area), diversity measures were higher. In contrast, the low diversity of MP (non-vegetative) can be determined due to its gentle sloping and moderate wave action[31] which could be why the CH scored higher diversity than the MP.

Multivariate measures suggest that the assemblage pattern of the two stations is distinctly separate from each other. Polychaetes were predominant at both the stations. Spionidae (e.g. *Malacoceros indicus*, *Microspio* sp.) and Orbiniidae (e.g. *Orbinia* spp.*)* were the most dominant species among Polychaetes. Previous studies conducted at this study area also recorded polychaetes as the dominant taxa[31]. However, some studies suggested that Amphipods are the dominant taxa in sand flats[4,26]. In the present study, Amphipods were numerically abundant in MP represented by *Hyale sp.2* with no evidence of distinct assemblage pattern observed between the sampling gears. Somerfield and Clarke[36] observed in their study that although there is a significant difference in the assemblage pattern of sampling gears, the difference is insignificant and does not provide a meaningful ecological explanation.

The present study observed similar research findings. The PERMANOVA results showed a significant difference in the faunal assemblage pattern between sampling gears at CH but could not demonstrate a distinct pattern of assemblage through nMDS plot. Upon utilising different sized sampling gears, the abundance in other sized samplers may change; however, with multivariate method, it is clear that the faunal composition remains the same[7]. The difference could be due to the complexity of the seagrass habitat. When sampled through quadrate during excavation (scoop), the organisms are often disturbed, allowing them to go under hiding to nearby seagrass patches.

Identifying all taxa collected during the sampling is difficult due to the lack of taxonomic expertise, especially in tropics[22,28]. Ellis[16] introduced the term taxonomic sufficiency which suggested that the information will be similar to the assemblage community pattern when using a higher taxonomic level. The present study observed that information loss' was lower when using the family level taxonomy. The assemblage pattern was very similar to species level and genus level. Studies conducted by various researchers worldwide found similar results and concluded that the family level is adequate for analysing spatial pattern. Thompson[41] estimated the cost-efficient method for monitoring marine pollution and concluded that the information about impact is detected correctly even at the family level.

Lampadariou et al[22] studied fish culture areas and found the same results. The macrofauna pattern remains similar while using family-level taxonomic data, but a modification in the spatial pattern has been observed at a taxonomic level higher than the family level. The results are an exact match to the results found by Sauza and Barros[38]. The inter matrices correlation between different taxonomic level starting from species level to phylum level was always significant.

The degree of correlation varied as the taxonomic level increases in all the studies mentioned earlier and the present study. Some taxa such as Nemertea, Sipuncula, Tanaidacea etc., were identified to a high taxonomic level. From the present study, it can be concluded that a higher taxonomic level is suitable for defining the assemblage pattern.

Conclusion

The surface area and volume of the quadrate sampler were more significant than that of the core. Different authors have used both core and quadrate samplers to monitor the intertidal benthic fauna around Andaman and Nicobar Islands. The present study aimed to assess the efficacy of sampling gears core and quadrate. The macrobenthic fauna collected from the two selected sampling gears (core and quadrate) showed a significant difference and this may be attributable to the size difference of the sampling gears. However, the individuals captured in the gears have a different number; the diversity and the assemblage patterns of macrobenthos have no significant difference statistically.

The current study suggests that either core or quadrate can be used to sample macrobenthos regardless of the study purpose. As the island is situated in the tropical region, it has the most species-rich locations. The taxonomical studies conducted in this species-rich area and the taxonomic information available for these islands are limited. In the present study, it was clear that family-level data is sufficient to monitor the macrobenthic community structure. The information loss was observed only after the order level. Though the dissimilarity in the assemblage pattern of macrobenthos reduced as the taxonomic level increases, the dissimilarity patterns of core and quadrate samples remain constant. In cessation, sampling gears did not influence the diversity of intertidal soft-bottom macrobenthos the family-level information is sufficient for monitoring of benthos or rapid impact assessment studies.

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