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'Simple' can be good, too: testing three hard bottom sampling methods on macrobenthic and meiobenthic assemblages

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Original Article

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Abstract

Subtidal hard bottoms are of particular scientific and economic value as they are highly productive systems. They are less well studied compared with soft bottoms, as they often require manual sample collection via scuba diving. Although a multitude of sampling devices is available for soft bottoms, only a few are suitable for hard substrates, and their performance is largely unstudied. In the present study, three hard bottom sampling methods were compared, regarding their sampling efficiency and the damage they may cause to macrobenthic and meiobenthic organisms. Two of the sampling methods examined are typically employed for the study of hard bottom substrates (manual collection, airlift device), while the third involves a newly constructed sampler (MANOSS – Manual Operated Suction Sampler). All three sampling methods were tested at 12 m depth on a hard bottom substrate with algal coverage dominated by *Cystoseira* spp. No overall significant differences were observed between the sampling efficiency and the damage caused by the three sampling methods regarding the macrofaunal assemblages, with the exception of the MANOSS method which collected more species than the manual method. In addition, significant differences were observed in the collecting performance for the meiobenthic assemblages, presenting significantly higher densities of meiofauna sampled by the MANOSS compared with the manual collection method, while the airlift device presented an intermediate efficiency. However, taking into account other factors such as cost, ease of use and the scope of each study, none of the methods clearly outperforms the others.

Introduction

The degradation of coastal ecosystem functioning and the loss of important habitats, as a result of human activities and climate change, are widely recognized (e.g. Bianchi & Morri, 2000; Lotze *et al.*, 2006). Coastal rocky habitats are among the most productive systems, characterized by a high biodiversity, primarily due to their structural heterogeneity (Bianchi *et al.*, 2004; Guidetti *et al.*, 2004). They are important fishing grounds, as they host species of great commercial value, and they are highly valued for recreational diving (Bianchi *et al.*, 2004). They are, however, also rapidly degrading as a consequence of human activities (Airoldi *et al.*, 2009), and despite their importance they are much less studied than soft substrates, as the complexity of this environment often requires scuba diving for a manual collection of samples (Hiscock, 1987; Karalis *et al.*, 2003; Bianchi *et al.*, 2004; Antoniadou & Chintiroglou, 2005; Chintiroglou *et al.*, 2005). This is also reflected by the existence of a wide variety of samplers for the study of soft substrates, such as box samplers and corers, grabs, dredges and trawls (Eleftheriou & McIntyre, 2005), while, in contrast, methods for sampling hard substrates are limited and their efficiency is much less known (Gibbons & Griffiths, 1988; Bianchi *et al.*, 2004).

The choice of the sampling method is a challenge as it should be as effective as possible in terms of qualitative and quantitative collection of samples (Kikuchi *et al.*, 2006). Hard bottom sampling methods can be either non-destructive (e.g. visual census) or destructive. The destructive methods are carried out in three successive steps: (a) blocking a surface, usually by means of a rectangle frame with soft material on the side which is attached on the surface and a net on its back, (b) surface scraping with a spatula or a similar tool and (c) collection of the scraped sample, either manually or with a suction device. Surface scraping is a widely known sampling method for collecting benthic organisms but there is evidence of escape ability of the mobile organisms (Abbiati, 1991). The first use of a suction device for the collection of benthic organisms was by Brett (1964). Subsequently, several variations of suction devices have been developed (e.g. Hiscock & Hoare, 1973; Elliott & Tullett, 1983; Rostron, 2001) which included modifications related to the convenience of handling, the targeted organisms and the habitat type. Finally, two types of suction devices have prevailed, differing in how the suction

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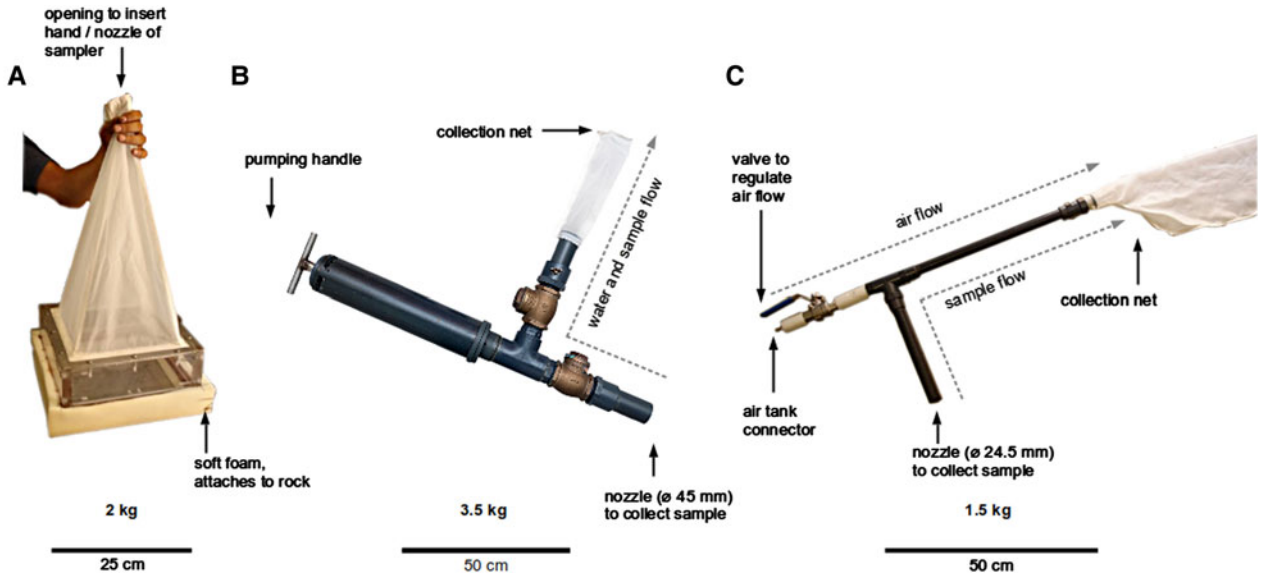


Fig. 1. Illustration of the three different sampling methods: (A) FRAME, (B) MANOSS and (C) SUCTION.

effect is created: through a water pump or through compressed air (Drake and Elliott, 1982; Hiscock, 1987).

Despite the development of various sampling methods, few studies have been carried out to compare their efficiency. These studies focus on the collection ability and not on the damage caused to the organisms by each of these methods (e.g. Emery, 1968; Gale & Thompson, 1975; Tanner *et al.*, 1977; Brooks, 1994; Metaxas & Scheibling, 1994). Most studies on the damaging effect of sampling devices investigate fishing gears and their effects on benthic organisms (e.g. Hall-Spencer *et al.*, 1999; Bergmann *et al.*, 2001; Jennings *et al.*, 2001; Pranovi *et al.*, 2001).

The present study attempts to fill the aforementioned gap by testing three different sampling methods for hard substrates. Two of these are commonly used for the sampling of benthic organisms in hard substrates: simple surface scraping and manual sample collection, and surface scraping and the use of a suction device connected to a compressed air tank. The third method is a manually operated suction device developed recently by the Hellenic Center for Marine Research (HCMR) (Chatzigeorgiou *et al.*, 2012).

We quantify the effectiveness of the three different sampling methods for macrofaunal and meiofaunal assemblages and assess the severity of organismal damage caused by each method, thus contributing to the effectiveness of hard substrate studies. Our null hypothesis, therefore, is that there are no differences in the capacity of the three sampling methods, concerning (a) the collection of macrofaunal and meiofaunal assemblages and (b) the organismal damage caused by each sampling method.

Materials and methods

Collection of samples

Samples were taken in December 2012, on a single sampling site located at the North coast of Crete (Alykes, Eastern Mediterranean, 35°24′52″N 24°59′18″E). The sampling area is characterized by a continuous hard bottom substrate with dense algal coverage (*Cystoseira* spp., *Sargassum* sp., *Jania rubens*), moderate wave exposure and no records of significant anthropogenic impact.

In total, eight replicate units per method were collected, randomly, by scuba divers going in a random direction and for a random distance at 12 m depth and sampled using each of the following sampling methods (Figure 1): (a) scraping and manual collection of the sample (hereafter referred to as 'FRAME'), (b) scraping and use of a Manually Operated Suction Sampler (hereafter referred to as 'MANOSS', described in detail in Chatzigeorgiou *et al.*, 2012) and (c) scraping and use of an airlift sampler (hereafter referred to as 'SUCTION'). For all methods, a plexiglass frame (25 × 25 cm) with a 63 µm mesh size net was attached to the rock and the framed surface was scraped. With the FRAME method, the scraped material was collected into the attached net by the diver by hand and subsequently the net was removed and placed into a plastic bag. The scraped material of the MANOSS and SUCTION methods were collected by placing the nozzle of the respective collecting device into the opening of the net attached to the frame and sucking the sample into a collecting bag (63 µm mesh size). The suction was achieved by means of a manually operated suction sampler with a hand operated plunger (MANOSS) and an airlift sampler connected with an air tank (SUCTION). Samples were subsequently washed through two sieves with mesh sizes of 500 and 63 µm to separate macro- and meiobenthic organisms and fixed in 4% formalin buffered in filtered (63 µm) seawater. More details about the sampling methods can be found in Chatzigeorgiou *et al.* (2012).

Laboratory procedures

The eight replicate units collected for each sampling method were analysed for both meio- and macrofauna. Macrofauna samples were washed to remove the remaining formalin and were stored in 70% ethanol. All specimens were identified to the lowest possible taxonomic level.

Meiofaunal samples were washed through a 63 µm mesh to remove any material with a size below 63 µm and meiofaunal organisms were extracted through centrifugation with Ludox (1.15 specific gravity) as a flotation medium (de Jonge & Bouwman, 1977). Centrifugation was repeated two more times, as this is considered sufficient for the extraction of ∼97% of the organisms (Austen & Warwick, 1989). Finally, the treated samples were stained in rose bengal (1 g l^{-1}) and specimens were sorted and identified to major taxonomic groups under a stereoscopic microscope.

Traits analysis

Three biological traits describing body shape, body design and movement method were selected to potentially identify specific

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Table 1. Biological traits and the relative categories

Trait	Category
Body shape	Complex Conical Elongated compressed Elongated cylindrical Oval Round
Body design	Hard exoskeleton Hard shell Soft Soft-protected (tube/tunic cover)
Movement method	None/semi-motile Crawling Swimming

characteristics of macrofaunal species related to sampling selectivity and to the susceptibility to damage induced by each sampling method. The selected traits were subdivided into 13 categories (Table 1), describing the species' geometric shape, their ability to escape and their fragility which may lead to difficulties during the identification procedure.

All trait categories were scored as presence or absence (1 or 0, respectively) for each species and they were weighted according to their abundances. Missing information for trait categories at the species level was derived from congeners.

Trait analysis was not performed on meiofaunal assemblages since individuals were identified to major taxa.

Assessment of damage

Damage may occur to the morphological integrity of benthic organisms through the scraping phase (all samplers) in addition with water flow (in case of MANOSS) and air decompression (in case of SUCTION) as the air that mixes with the water and sampled material is likely to cause damage to the sampled material. To assess the impact of each sampling method on any damage caused to the macrofaunal organisms, a five-point scale of damage was developed for each taxonomic group (Table 2). Damage scores were defined to assess: (a) the impact of each method on the 'identifiability' of the individual to species level and (b) the severity of the damage in terms of mechanical force provoked by each sampling method. A Mean Damage Index (MDI) was calculated for each species, major phyla and trait categories as described by Jenkins *et al.* (2001), using the following formula:

$$\frac{\sum_{i=1}^{i=5} n_i i}{\mathrm{N}}$$

where n_i = number of individuals of damage score i, and N = total number of individuals.

Damage on meiofauna specimens could not be assessed due to their small size.

Statistical analyses

Since the assumptions of parametric ANOVA were violated, non-parametric Kruskal–Wallis tests (Kruskal & Wallis, 1952) were used in order to assess potential differences between the selectivity of the sampling methods based on: (a) macrofaunal diversity, expressed either as species richness, the Margalef index (Margalef, 1958) or the Shannon–Wiener index (Shannon & Weaver, 1963); (b) the abundance (number of individuals) of the most dominant macrofaunal species; (c) the abundance of the trait categories; and (d) the meiofaunal densities (in individuals per 10 cm^2) for each replicate. Regarding the abundance of the most dominant macrofaunal species, the Kruskal–Wallis test was restricted to the most dominant species to avoid potential variation by rare species. For their selection, species' abundance values were ranked and plotted based on the total abundance across all samples; a break-off point was chosen where the curve showed a sudden increase in abundance values. This break-off point was then used as a threshold to exclude rare species.

Non-parametric Kruskal–Wallis tests were also performed in order to assess potential differences between the damage caused by the sampling methods based on the Mean Damage Index (MDI) of: (a) the total macrofaunal species, (b) the major phyla and (c) the trait categories for each replicate.

Mann–Whitney *U* tests were used as post-hoc pairwise comparisons between the sampling methods with a Bonferroni correction lowering the level of significance to 0.017.

To compare multivariate patterns of species distribution between the different sampling methods, abundance values were square-root transformed and the Bray–Curtis coefficient (Bray & Curtis, 1957) was calculated between all possible pairs of samples. The produced dissimilarity matrices were displayed using non-metric Multidimensional Scaling (nMDS) (Clarke & Warwick, 1994). An Analysis of Similarities (ANOSIM; Clarke, 1993) was carried out to test for differences between the sampling methods.

All statistical analyses were performed using the software packages PRIMER (v. 6.1.3, PRIMER-E Ltd) (Clarke & Gorley, 2006) and SPSS (v. 23, IBM SPSS).

Results

Assessment of sampling methods selectivity in macrofaunal assemblages

In total, 6651 individuals were analysed, consisting of 169 species (FRAME: 91 species, MANOSS: 120 species, SUCTION: 117 species). Mollusca, dominated by Gastropoda, were the most abundant group in all sampling methods, followed by Arthropoda, Annelida and Echinodermata (Figure 2).

Table 3 summarizes the mean values of abundances, species number, species richness (Margalef index) and Shannon–Wiener index for each sampling method. No significant differences were observed between the sampling methods in terms of diversity indices or abundances. However, the MANOSS method captured significantly more species than the FRAME method (Mann–Whitney test; $U = 8.5$, $P = 0.013$), while the SUCTION method did not show any significant differences compared with the other two methods.

In general, no significant differences were detected between the different sampling methods regarding the macrofaunal abundances of the most dominant species, as shown Supplementary Table S1. The nMDS plot (not shown here) based on macrofauna species abundances did not reveal any clear pattern and showed a high stress value of >0.2. Accordingly, the ANOSIM test did not detect any significant differences in community structure between the different sampling methods (Supplementary Table S2; $R = -0.002$; $P > 0.05$).

Regarding traits, the most abundant characteristic for body shape, body design and movement method were conical shape, hard shell and crawling movement behaviour, respectively (Figure 3). However, no significant differences were observed between the different sampling methods for each trait category, except for the oval shape (Kruskal–Wallis; $H = 8.162$, $P = 0.017$) where organisms with this characteristic were significantly more abundant in the MANOSS method compared with the FRAME method (Mann–Whitney test; $U = 7.5$, $P = 0.007$).

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Table 2. Damage scores for macrofauna, adapted from Bergmann et al. (2001); Jenkins et al. (2001); Veale et al. (2001); Pranovi et al. (2001); Guyonnet et al. (2008)

Damage score	1	2	3	4	5
Crustaceans	In good condition	Appendages missing	Carapace cracks	Carapace cracks and appendages missing (broken)	Crushed
Ophiuroids	In good condition	Arms broken/missing	Disc damaged	Disc damaged and arms broken/missing	Crushed
Echinoids	In good condition	Minor deformation	<50% spine loss	>50% spine loss	Crushed
Gastropods	In good condition	Edge of shell chipped	Shell slightly cracked	Whole shell cracked	Crushed
Bivalves	In good condition	Edge of shell chipped	One valve cracked	Both valves cracked	Crushed
Polychaetes	In good condition	Appendages broken/missing/ main body damaged	Posterior segments missing	Appendages broken/missing/ main body damaged and posterior segments missing	Crushed

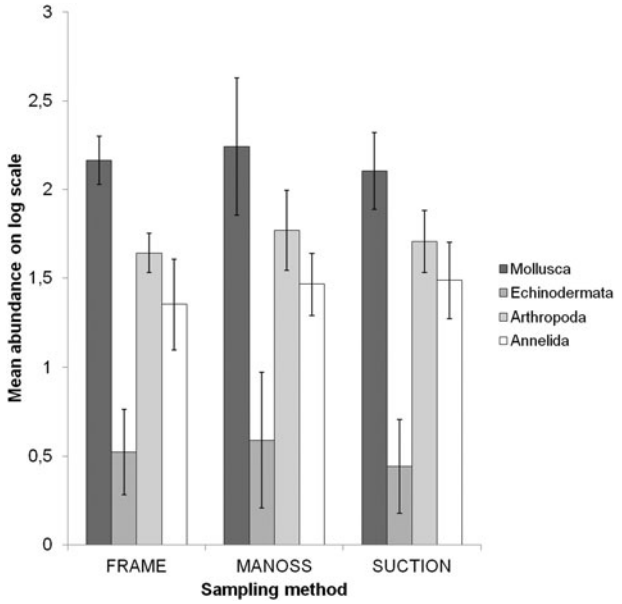


Fig. 2. Mean abundance ± SD of the major macrofaunal phyla on log scale, for each sampling method.

Assessment of damage caused by sampling methods

Concerning the damage caused by the different sampling methods among the most dominant species, most species were preserved intact (damage score 1) or with minor or moderate damage (damage scores 2, 3), with the exception of some very fragile polychaete species (Supplementary Table S3). No significant differences between the sampling methods were observed in the MDI of the total number of species, nor within the major phyla or different trait categories (Table 4).

Percentages of identified and unidentified (due to damage) organisms were estimated in order to assess the damage effect on the identifiability of the organisms. More than 95% of the individuals were identified to species level for all sampling methods (FRAME: 96.88%; MANOSS: 97.09%; SUCTION: 96.54%). No significant differences were observed between the sampling methods regarding the number of identified and unidentified organisms.

Sampling methods efficiency in meiofaunal assemblages

In total, 21 major meiofaunal groups were identified, out of which four presented higher densities for all three different sampling methods (Table 5). More specifically, nematodes, copepods, copepod nauplii and polychaetes represented the 95.82 ± 0.3% of the total meiofauna while the remaining percentage (4.18 ± 0.3%, 'Others') included 17 meiofaunal groups (Table 6) with low densities. Significant differences were detected between the different sampling methods regarding the meiofaunal densities of only copepods, polychaetes and 'others' (Table 5), which revealed significantly higher densities in MANOSS samples than in FRAME samples (Mann–Whitney test; copepods: $U = 5$, $P = 0.005$; polychaetes: $U = 5$, $P = 0.005$; others: $U = 8.5$, $P = 0.014$). However, no significant differences were observed between the sampling methods regarding the total meiofaunal and nematode densities (Table 5).

The nMDS plot (not shown here) of the meiofaunal densities similarity matrix, illustrated no discrimination for the community structure of the different sampling methods. The value of the ANOSIM test supported the hypothesis that there are no significant differences between the community structure of the sampling methodologies (Supplementary Table S2; $R = 0.06$; $P > 0.05$).

Discussion

Sampling efficiency in macrofaunal assemblages

Mollusca, specifically Gastropoda, were the most abundant taxonomic group (>50%) followed by Arthropoda (~20%) and Annelida (10–15%) in each sampling method. This contribution pattern of the major phyla seems to be common in hard bottom areas in the Eastern Mediterranean Sea according to related biodiversity studies (Antoniadou & Chintiroglou, 2005; Antoniadou *et al.*, 2005). In general, no significant differences were detected between the different sampling methods regarding the macrofaunal abundances of the most dominant species and the diversity indices; in addition, the sampling methods presented a similar pattern regarding the community structure indicating similar sampling efficiency. However, the MANOSS method captured significantly more species than the FRAME method, indicating a potential loss of species that can easily escape from the scraping procedure, while the SUCTION method showed an intermediate efficiency. This may be related to the collection of the sample by hand in the FRAME method where loss of the scraped material is more likely to happen than when using a suction device. Furthermore, according to Abbiati (1991), mobile fauna can more easily escape from the sampling net during the scraping method, therefore this procedure is more effective for flora and sessile organisms. However, no significant differences were observed between the different sampling methods for the mobility traits, indicating that the

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Table 3. Mean values of abundance, species number, species richness (Margalef) and Shannon–Wiener with their standard deviation (SD) for each sampling method

	Sampling method				
	FRAME	MANOSS	SUCTION	*H*	*P*
Mean abundance ± SD	235 ± 66	347.13 ± 161.91	249.25 ± 82.01	1.836	0.4
Mean species number ± SD	29.25 ± 3.49	36.75 ± 5.92	34 ± 11.93	5.789	**0.05**
Mean species richness (Margalef) ± SD	5.20 ± 0.53	6.22 ± 0.97	6.03 ± 2.04	2.625	0.27
Mean Shannon–Wiener ± SD	1.54 ± 0.21	1.69 ± 0.62	1.88 ± 0.58	1.461	0.48

Kruskal–Wallis results are shown between the different sampling methods. Significant results are marked in bold ($P < 0.05$).

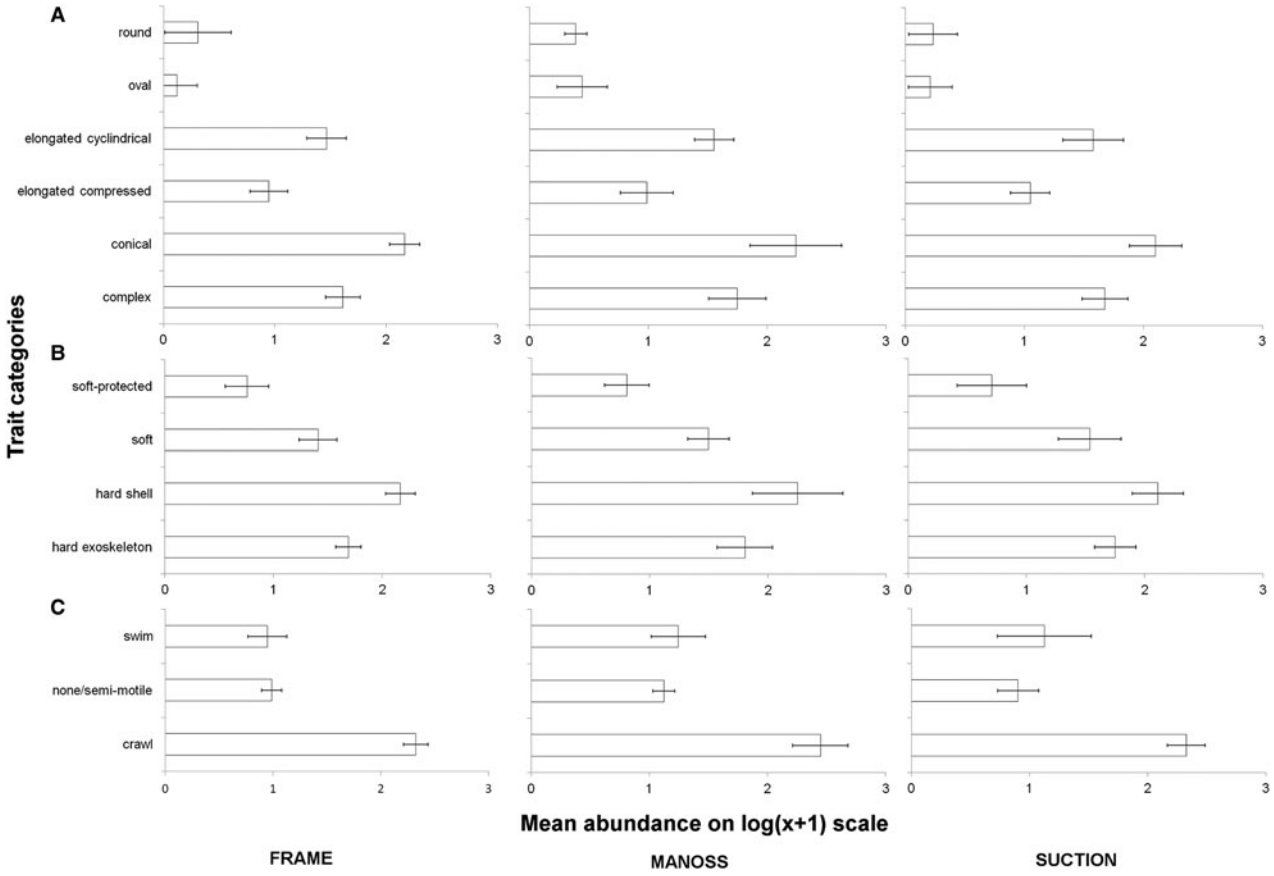


Fig. 3. Mean abundance ± SD on log(x + 1) scale of the each trait category of (A) body shape, (B) body design and (C) movement method, for each sampling method.

loss of species is a mechanical characteristic of the FRAME method.

The sampling selectivity and the fragility of the organisms may be influenced by specific biological traits. For example, the existence of protective shells could reduce the severity of damage by sampling methods (Bergmann *et al.*, 2001) and the movement method could influence the sampling selectivity, as fast swimmers can more easily escape from the sampling methods in comparison to species with a low mobility (Sutherland, 2006). All methods exhibited a similar distribution of biological traits, with the most representative trait categories being the conical shape, hard shell and crawling behaviour. These categories are mostly found in gastropods which were the most abundant taxonomic group. Furthermore, no significant differences were observed between the different sampling methods for each trait category, except for the oval shape where organisms with this characteristic were significantly more abundant in the MANOSS method compared with the FRAME method. However, these differences may be related to random effects as the oval shape is represented by organisms with low abundances.

In a study by Pranovi *et al.* (2001), the percentages of damage induced by the studied gears were related to the morphology (with or without appendages), the body structure (hard or soft tissues) and the size of the organisms. Several indices have been used for the assessment of damage of macrofaunal organisms, mostly for assessing the impact of different fishing gears (e.g. Mensink *et al.*, 2000; Bergmann *et al.*, 2001; Jenkins *et al.*, 2001; Moschino *et al.*, 2003). In the present study, no significant differences were observed in terms of MDI between the sampling methods for the most dominant species, the major phyla and the different trait categories as the observed damage may not be related to the sampling methods but to procedures common to all methods: scraping, washing, sieving and sorting (Bianchi *et al.*, 2004; Eleftheriou & McIntyre, 2005).

Sampling efficiency in meiofaunal assemblages

Hard bottom areas are dominated by nematodes and copepods (Danovaro & Fraschetti, 2002; Fraschetti *et al.*, 2006), a pattern which was also observed in the hard substrate meiofauna assemblages captured by the three different sampling methods. Different

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Table 4. The Mean Damage Index (MDI) of the total number of species, the major phyla and the trait categories for each sampling method (ranging from 1 to 5; for definition of the score values see Table 2)

	Mean Damage Index (MDI)				
	FRAME	MANOSS	SUCTION	*H*	*P*
Total number of species	2.16	2.12	2.14	0.015	0.99
Major phyla					
Annelida	2.28	2.49	2.33	0.815	0.67
Arthropoda	1.84	1.94	1.86	0.785	0.67
Echinodermata	2.19	2.21	2.64	0.415	0.81
Mollusca	1.82	1.52	1.84	1.635	0.44
Trait categories					
Body shape					
Complex	2.42	2.95	2.75	1.78	0.41
Conical	1.81	1.74	2.03	1.145	0.56
Elongated compressed	2.20	1.82	1.79	2.343	0.31
Elongated cyclindrical	2.24	2.29	2.13	1.535	0.46
Oval	1.83	2.16	1.83	1.532	0.46
Round	3.08	2.73	2.73	0.302	0.86
Body design					
Hard exoskeleton	2.16	2.41	2.23	0.593	0.74
Hard shell	2.06	2.04	2.12	0.105	0.95
Soft	2.29	2.31	2.10	1.505	0.47
Soft-protected (tube/tunic cover)	2.16	2.02	1.96	1.455	0.48
Movement method					
Crawling	2.22	2.17	2.12	0.585	0.75
None/semi-motile	2.05	2.16	2.13	0.740	0.69
Swimming	2.53	2.30	2.31	0.196	0.91

Kruskal–Wallis results are shown between the different sampling methods.

Table 5. Mean values of meiofaunal densities (ind. 10 cm^{-2}) with their standard deviation (SD) for each sampling method and taxonomic group are presented

	Mean Density ± SD				
	FRAME	MANOSS	SUCTION	*H*	*P*
Total	110.5 ± 42.87	150.67 ± 51.37	128.09 ± 48.23	3.875	0.14
Copepod nauplii	14.44 ± 8.83	13.39 ± 5.87	17.65 ± 8.98	0.594	0.74
Copepods	24.13 ± 10.24	54.63 ± 21.01	39.15 ± 18.43	8.512	**0.01**
Nematodes	56.13 ± 23.15	70.85 ± 25.03	61.35 ± 19.78	1.040	0.59
Polychaetes	6.79 ± 1.46	14.81 ± 7.75	10.76 ± 5.39	9.08	**0.01**
Others	0.24 ± 0.55	0.52 ± 1.36	0.40 ± 1.18	7.143	**0.03**

Kruskal–Wallis results are shown between the different sampling methods. Significant results are marked in bold ($P < 0.05$).

sampling efficiency of the major meiofaunal groups was observed between the three sampling methods. Specifically, significant differences were detected regarding the meiofaunal densities of copepods, polychaetes and the rarer ('Others') groups which revealed significantly higher densities in the MANOSS method compared with the FRAME method, with the SUCTION method again presenting an intermediate efficiency. The low densities of copepods and polychaetes in the meiofaunal samples collected with the FRAME method could be attributed to their relatively high mobility. High mobility can facilitate an easier escape from the collection net (Carleton & Hamner, 1987; Abbiati, 1991). Several epibenthic taxa are known to show an emergence behaviour, i.e. they are able to temporarily move into the hyperbenthos for a variety of reasons (e.g. predation, escape behaviour, foraging) (Alldredge & King, 1985; Decho, 1986; Armonies, 1988; Mees & Jones, 1997; Giere, 2009). Sample collection potentially acts as a threat for these organisms, activating this emergence behaviour and resulting in lower densities of these taxonomic groups in the samples.

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Table 6. Contribution of the 4.18 ± 0.3% of meiofaunal taxa to the total density of each sampling method

	Sampling method		
	FRAME (%)	MANOSS (%)	SUCTION (%)
Amphipoda	–	0.07	0.06
Anisopoda	–	0.01	0.01
Aplacophora	0.01	–	–
Ciliophora	0.07	0.06	0.04
Cumacea	–	–	0.01
Gastrotricha	0.01	0.02	0.01
Halacaroidea (Mites)	0.35	0.42	0.38
Isopoda	0.05	0.02	0.01
Kinorhyncha	0.27	0.46	0.41
Loricifera	–	0.01	–
Mollusca (Bivalvia)	0.27	0.01	0.10
Mollusca (Gastropoda)	–	0.02	–
Oligochaeta	0.01	0.01	0.02
Ostracoda	0.71	1.31	1.14
Rotifera	0.67	0.60	0.69
Tardigrada	1.43	0.61	0.87
Turbellaria	0.66	0.30	0.36

Table 7. Summary of the basic advantages and disadvantages of the three different sampling methods

	Advantages	Disadvantages
FRAME	Easy performance Effective method for macrofauna collection	Performance only in calm seas, otherwise there is potential loss of material (Bianchi *et al.*, 2004) Lower densities of major groups in meiofaunal assemblages (copepods and polychaetes) than MANOSS
MANOSS	Effective method for the collection of macrofaunal and meiofaunal assemblages	Slower execution than FRAME. Extra equipment is needed Physical strength is required. Cannot be used out of the water column
SUCTION	Effective method for the collection of macrofaunal and meiofaunal assemblages	Slower execution than FRAME. Except for the sampler, an air tank is needed. Equipment very bulky. Cannot be used out of the water column

Conclusions

In Table 7, the advantages and disadvantages of the three different sampling methods are summarized. In general, the sampling methods do not differ in their efficiency of collecting macrofaunal assemblages with the exception of the MANOSS method which collects more species than the FRAME method. In addition, the MANOSS method showed a higher sampling efficiency in meiofaunal assemblages compared with the FRAME method. However, the selection of a sampling method in order to carry out a biodiversity study in hard substrates should also take into account the scope of the study, the efficiency of the sampling method required, as well as the technical characteristics of the method during its execution. For example, if the focus is to capture the entire diversity of the community, then the MANOSS method shows a clear advantage as it captures more species. Similarly, if the strength of the operating person is an issue, then the SUCTION method is equally effective as it performs similarly to MANOSS with the small price of losing some of the macrofaunal species. We conclude that 'simple' methods such as the FRAME method could be an effective and easy way for macrofauna collection, but a meiofaunal study requires a more advanced method such as MANOSS. Further studies, focusing on the comparison of the sampling methods on several hard substrates, in terms of region and type of substrate (e.g. artificial, 'trottoir') are necessary to establish a standardized sampling protocol for the macro- and meiobenthic assemblages in hard substrates.

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References

Abbiati M (ed.) (1991) Metodi di campionamento biologico subacqueo. In *Lezioni del Corso Formativo per Ricercatore Scientifico Subacqueo*. Pisa: International School of Scientific Diving, pp. 3–12.

Airoldi L, Connell SD and Beck MW (2009) The loss of natural habitats and the addition of artificial substrata. In Wahl M (ed.), *Marine Hard Bottom Communities*. Berlin: Springer, pp. 269–280.

Alldredge AL and King JM (1985) The distance demersal zooplankton migrate above the benthos: implications for predation. *Marine Biology* **84**, 253–260.

Antoniadou C and Chintiroglou C (2005) Biodiversity of zoobenthic hard-substrate sublittoral communities in the Eastern Mediterranean (North Aegean Sea). *Estuarine, Coastal and Shelf Science* **62**, 637–653.

Antoniadou C, Koutsoubas D and Chintiroglou CC (2005) Mollusca fauna from infralittoral hard substrate assemblages in the North Aegean Sea. *Belgian Journal of Zoology* **135**, 119–126.

Armonies W (1988) Active emergence of meiofauna from intertidal sediment. *Marine Ecology Progress Series* **43**, 151–159.

Austen MC and Warwick RM (1989) Comparison of univariate and multivariate aspects of estuarine meiobenthic community structure. *Estuarine, Coastal and Shelf Science* **29**, 23–42.

Bergmann M, Beare DJ and Moore PG (2001) Damage sustained by epibenthic invertebrates discarded in the Nephrops fishery of the Clyde Sea area, Scotland. *Journal of Sea Research* **45**, 105–118.

Bianchi CN and Morri C (2000) Marine biodiversity of the Mediterranean Sea: situation, problems and prospects for future research. *Marine Pollution Bulletin* **40**, 367–376.

Bianchi CN, Pronzato R, Cattaneo-Vietti R, Benedetti-Cecchi L, Morri C, Pansini M, Chemello R, Milazzo M, Fraschetti S, Terlizzi A, Peirano A, Salvati E, Benzoni F, Calcinai B, Cerrano C and Bavestrello G (2004) Hard bottoms. In Gambi M and Dappiano M (eds), Mediterranean marine benthos: a manual of methods for its sampling and study. *Biologia Marina Mediterranea* **11** (Suppl. 1), 185–215.

Bray RJ and Curtis JT (1957) An ordination of the upland forest communities of southern Wisconsin. *Ecological Monographs* **27**, 325–349.

Brett C (1964) A portable hydraulic diver-operated dredge-sieve for sampling subtidal macrofauna. *Journal of Marine Research* **22**, 205–209.

Brooks S (1994) An efficient and quantitative aquatic benthos sampler for use in diverse habitats with variable flow regimes. *Hydrobiologia* **281**, 123–128.
Carleton JH and Hamner WM (1987) A diver-operated device for the capture of mobile epibenthic organisms. *Limnology and Oceanography* **32**, 503–510.
Chatzigeorgiou G, Dailianis T, Faulwetter S and Pettas M (2012) MANOSS – a manually operated suction sampler for hard bottom benthos. *Transitional Waters Bulletin* **6**, 42–49.
Chintiroglou C, Antoniadou C, Vafidis D and Koutsoubas D (2005) A review on the biodiversity of hard substrate invertebrate communities in the Aegean Sea. *Mediterranean Marine Science* **6**, 51–62.
Clarke K (1993) Non-parametric multivariate analyses of changes in community structure. *Australian Journal of Ecology* **18**, 117–143.
Clarke K and Gorley R (2006) *PRIMER v6.1.5: User Manual/Tutorial*. Plymouth: PRIMER-E.
Clarke K and Warwick R (1994) Similarity-based testing for community pattern: the two-way layout with no replication. *Marine Biology* **118**, 167–176.
Danovaro R and Fraschetti S (2002) Meiofaunal vertical zonation on hard-bottoms: comparison with soft-bottom meiofauna. *Marine Ecology Progress Series* **230**, 159–169.
Decho AW (1986) Water-cover influences on diatom ingestion rates by meiobenthic copepods. *Marine Ecology Progress Series* **33**, 139–146.
de Jonge VN and Bouwman LA (1977) A simple density separation technique for quantitative isolation of meiobenthos using the Colloidal silica Ludox-TM. *Marine Biology* **42**, 143–148.
Drake CM and Elliott JM (1982) A comparative study of three air-lift samplers used for sampling benthic macro-invertebrates in rivers. *Freshwater Biology* **12**, 511–533.
Eleftheriou A and McIntyre A (2005) *Methods for the Study of Marine Benthos,* 3rd Edn. Oxford: Blackwell Science.
Elliott JM and Tullett PA (1983) A supplement to a bibliography of samplers for benthic invertebrates. *Freshwater Biological Association, Occasional Publication* 20, 27 pp.
Emery AR (1968) Preliminary observations on coral reef plankton. *Limnology and Oceanography* **13**, 293–303.
Fraschetti S, Gambi C, Giangrande A, Musco L, Terlizzi A and Danovaro R (2006) Structural and functional response of meiofauna rocky assemblages to sewage pollution. *Marine Pollution Bulletin* **52**, 540–548.
Gale WF and Thompson JD (1975) A suction sampler for quantitatively sampling benthos on rocky substrates in rivers. *Transactions of the American Fisheries Society* **104**, 398–405.
Gibbons MJ and Griffiths CL (1988) An improved quantitative method for estimating intertidal meiofaunal standing stock on an exposed rocky shore. *South African Journal of Marine Science* **6**, 55–58.
Giere O (2009) *Meiobenthology: The Microscopic Motile Fauna of Aquatic Sediments*, 2nd Edn. Berlin: Springer.
Guidetti P, Bianchi CN, Chiantore M, Schiaparelli S, Morri C and Cattaneo-Vietti R (2004) Living on the rocks: substrate mineralogy and the structure of subtidal rocky substrate communities in the Mediterranean Sea. *Marine Ecology Progress Series* **274**, 57–68.
Guyonnet B, Grall J and Vincent B (2008) Modified otter trawl legs to reduce damage and mortality of benthic organisms in North East Atlantic fisheries (Bay of Biscay). *Journal of Marine Systems* **72**, 2–16.
Hall-Spencer JM, Froglia C, Atkinson RJA and Moore PG (1999) The impact of Rapido trawling for scallops, *Pecten jacobaeus* (L.), on the benthos of the Gulf of Venice. *ICES Journal of Marine Science* **56**, 111–124.
Hiscock K (1987) Subtidal rock and shallow sediments using diving. In Baker JM and Wolff W (eds), *Biological Surveys of Estuaries and Coasts*. New York, NY: Cambridge University Press, pp. 198–237.
Hiscock K and Hoare R (1973) A portable suction sampler for rock epibiota. *Helgolander Wiss. Meeresunters* **25**, 35–38.
Jenkins SR, Beukers-Stewart BD and Brand AR (2001) Impact of scallop dredging on benthic megafauna: a comparison of damage levels in captured and non-captured organisms. *Marine Ecology Progress Series* **215**, 297–301.
Jennings S, Pinnegar J, Polunin N and Warr K (2001) Impacts of trawling disturbance on the trophic structure of benthic invertebrate communities. *Marine Ecology Progress Series* **213**, 127–142.
Karalis P, Antoniadou C and Chintiroglou C (2003) Structure of the artificial hard substrate assemblages in ports in Thermaikos Gulf (North Aegean Sea). *Oceanologica Acta* **26**, 215–224.
Kikuchi RM, Fonseca-Gessner AA and Shimizu GY (2006) Suction sampler for collection of benthic macroinvertebrates in several continental aquatic environments: a comparative study with the Hess and Surber samplers. *Acta Limnologica Brasiliensia* **18**, 29–37.
Kruskal WH and Wallis WA (1952) Use of ranks in one-criterion variance analysis. *Journal of the American Statistical Association* **47**, 583–621.
Lotze HK, Lenihan HS, Bourque BJ, Bradbury RH, Cooke RG, Kay MC, Kidwell SM, Kirby MX, Peterson CH and Jackson JBC (2006) Depletion degradation, and recovery potential of estuaries and coastal seas. *Science* **312**, 1806–1809.
Margalef R (1958) Information theory in ecology. *General Systems* **3**, 36–71.
Mees J and Jones MB (1997) The hyperbenthos. *Oceanography and Marine Biology: An Annual Review* **35**, 221–255.
Mensink BP, Fischer CV, Cadee GC, Ten Hallers-Tjabbes CC and Boon JP (2000) Shell damage and mortality in the common whelk *Buccinum undatum* caused by beam trawl fishery. *Journal of Sea Research* **43**, 53–64.
Metaxas A and Scheibling RE (1994) Spatial and temporal variability of tide-pool hyperbenthos on a rocky shore in Nova Scotia, Canada. *Marine Ecology Progress Series* **108**, 175–184.
Moschino V, Deppieri M and Marin MG (2003) Evaluation of shell damage to the clam *Chamelea gallina* captured by hydraulic dredging in the Northern Adriatic Sea. *Journal of Marine Science* **60**, 393–401.
Pranovi F, Raicevich S, Franceschini G, Torricelli P and Giovanardi O (2001) Discard analysis and damage to non-target species in the 'rapido' trawl fishery. *Marine Biology* **139**, 863–875.
Rostron DM (2001) Procedural guideline No. 3-10. sampling marine benthos using suction samplers. In Davies J, Baxter J, Bradley M, Connor D, Khan J, Murray E, Sanderson W, Turnbull C and Vincent M (eds), *Marine Monitoring Handbook*. Peterborough: Joint Nature Conservation Committee, pp. 293–305.
Shannon C and Weaver W (1963) *The Mathematical Theory of Communication*. Urbana, IL: University of Illinois Press.
Sutherland WJ (2006) *Ecological Census Techniques: A Handbook*, 2nd edn. Cambridge: Cambridge University Press.
Tanner C, Hawkes MW and Lebednic PA (1977) A hand-operated suction sampler for the collection of subtidal organisms. *Journal of the Fisheries Board of Canada* **34**, 1031–1034.
Veale LO, Hill AS, Hawkins SJ and Brand AR (2001) Distribution and damage to the by-catch assemblages of the northern Irish sea scallop dredge fisheries. *Journal of the Marine Biological Association of the United Kingdom* **81**, 85–96.

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