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Rapid assessment of epibenthic communities: A comparison between two visual sampling techniques

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A B S T R A C T

Quantification and comparisons of the structure of subtidal marine communities are essential for answering a suite of basic and applied ecological questions. However, time constraint associated with sampling underwater by scuba diving is a major obstacle to achieve adequate sampling effort. We tested a technique for rapid-assessment of hard substrate epibenthic communities, based on counts of the frequency of different taxa within sampling units (quadrats). This technique was compared to a broadly used technique, based on visual estimation of percent cover of taxa, to determine whether a significant amount of information is lost when using the rapid assessment technique. We applied both techniques in three case studies from the Ligurian Sea, in the NW Mediterranean. Field measurements showed that the same communities can be surveyed twice as fast with the frequency-count compared to the cover-estimation. Structural variables of communities as species richness and diversity yield almost identical values with the two techniques while dominance showed underestimated by frequency-count when compared to the cover-estimation. Multivariate analyses indicated that the two techniques yield similar results, highlighting similar patterns of variation in all three case studies, particularly when data are transformed (square-root or fourth-root transformation). Our findings suggest that applying the frequency-count technique to the assessment of benthic communities is an effective strategy for increasing sampling effort when working underwater at depth.

1. Introduction

The description of pattern is of great importance in ecology (Underwood et al., 2000). Our perception of patterns generates questions and hypotheses, and guides the design and interpretation of experiments (Andrew and Mapstone, 1987). Spatial and temporal patterns of variation provide tests of hypotheses in 'natural' experiments, where natural or anthropogenic variation exists and is utilized in measurative experiments (Underwood and Chapman, 2005). Yet, when attempting to describe patterns in nature, ecologists face the problem of funding and time greatly restricting the number of samples that can be taken (Bianchi et al., 2004; Murray et al., 2006). These problems are particularly serious in monitoring subtidal hard-bottom marine communities, which present the challenge of working underwater. Direct observation by divers is considered as the most effective method to assess the structure of hard substrate benthic communities (Underwood and Jackson, 2009). *In situ* surveys facilitate taxonomic identification of conspicuous species and a comprehensive description of sessile communities but are limited by scuba diving restrictions. These limitations partly explain the scarcity of data on subtidal sessile epibenthic communities below 10–15 m depth.

Rapid visual assessment (RVA), based on time rather than on areas, and photographic sampling may overcome these limitations, but have some disadvantages compared to direct observation. RVAs, which are effective for inventories and hence estimating species richness, do not allow for reliable quantitative estimates (Seytre and Francour, 2008). Photographs, if taken with frames and/or spacers, are adequate to collect quantitative data but: (1) taxonomic identification of species is less reliable, (2) organisms hidden by taller species can not be identified; (3) processing of images in the lab is time consuming; (4) to obtain high-resolution images, the size of sampling units is small (Parravicini et al., 2009). Thus, many scientists think that in situ visual sampling remains the most effective method for assessing hard-substrate subtidal epibenthic communities (Foster et al., 1991). However, their intrinsic high spatial variation and patchiness at all scales (Fraschetti et al., 2005) require a special sampling effort to overcome the ineluctable heterogeneity of the collected data. In hierarchical designs, this sampling effort may be expressed by high replication (Somerfield et al., 2002); in not hierarchical designs, by selection of many sites along gradients and by measuring all the covariates that may represent explanatory factors, thus increasing the

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extent of experiments (Thrush et al., 1997; Hewitt et al., 2007). In any case, increased sampling effort is limited by scuba diving time, particularly at depths greater than 20 m.

With visual sampling techniques, the structure of epibenthic communities is assessed by estimating underwater the 'quantity' of individual species or community descriptors of investigator's choice (Murray et al., 2006). This quantity is commonly expressed by counts of individuals or by percent cover. Abundance counts are used when individual organisms or colonies can be easily told apart, e.g. kelp or gorgonians (Murray et al., 2006). Percent cover is used to quantify organisms that cover the substrate and have modular body organization, including most macroalgae and sponges and colonial animals such as corals, bryozoans and ascidians (Boudouresque, 1971; Benedetti-Cecchi et al., 1996).

The most common sampling units used in direct observation techniques are transects or quadrats (Munro, 2005; Murray et al., 2006). Transects are represented by a line (e.g., 10s of meters in length) laid on the bottom (Loya, 1978), while quadrats are frames enclosing a standard area of the substrate (e.g., 50 × 50 cm or 100 × 100 cm) (Hiscock, 1987; Leujack and Ormond, 2007). Transects are chiefly used in the tropics (English et al., 1997), quadrats in temperate seas (Kingsford and Battershill, 1998).

To measure cover within quadrats, these are normally divided into 25 sub-squares. The percent cover in each sub-square is visually estimated by assigning each species a score ranging from 0 (species absent) to 4 (100% cover), then summing scores across the 25 quadrats (Bianchi et al., 2004); species filling less than 1 are assigned a score of 0.5. Compared with other visual techniques, such as the point intercept (Dodge et al., 1982; Meese and Tomich, 1992), it has the advantage to detect all conspicuous species, including rare ones, within the quadrat. In contrast, the point-intercept technique records only the species found at defined points, thereby possibly missing species with low cover (Dethier et al., 1993).

To address a major limitation of cover estimation, that is the time required to conduct such visual surveys on scuba, we tested a potentially faster technique — the frequency count. To verify the reliability of this technique, we applied both cover estimation and frequency count in three case studies. In each case study, we tested the hypothesis that these two sampling techniques are equally effective in describing benthic community patterns.

2. Materials and methods

2.1. Study sites and sampling methods

Three distinct locations in the Ligurian Sea (NW Mediterranean Sea) were investigated: Golfo Tigullio (Genoa, Italy), Bergeggi (Savona, Italy) and Gallinara (Savona, Italy) (Fig. 1). At each location, surveys addressed specific questions. At Golfo Tigullio, sampling was aimed at assessing variation in benthic community structure associated with different mineralogical composition of the substrate (conglomerates, limestone and sandstone) and with the presence of two outfalls and one river mouth. At Bergeggi, sampling was aimed at examining possible short-term responses of benthic communities to the establishment of the Marine Protected Area (MPA) "Isola di Bergeggi", established 2 years before this study. At Gallinara, sampling had the objective of assessing variation in benthic communities within the proposed "Isola Gallinara" MPA.

At each location, sampling was designed to compare the efficacy of the two visual techniques in revealing benthic community patterns. We compared the two techniques at different locations addressing different questions in order to assess the generality of our results and thus to rule out their potential context-dependence.

At each location, we surveyed hard bottom communities using both cover estimation and frequency count, on each quadrat sampled. Cover estimation was performed as described above (see Introduction). Frequency count was conducted with the same quadrat frame used for cover estimation (i.e. divided in 25 sub-squares with monofilament line). Frequency was quantified as the number of squares in which each species occurred. As many species, sometime partially overlapping each other, normally occur in the same sub-square, total frequency may surpass 25; similarly, total cover may exceed 100%. Frequency count and cover estimation were conducted separately for each quadrat by the same observer and the time needed for data collection was recorded for each technique. The technique (cover estimation or frequency count) to be conducted first was randomly chosen at the beginning of each dive. To avoid any observer effect, observers were randomly assigned to sampling stations and locations. In all cases, only conspicuous species, easily recognized underwater, were considered (Hiscock, 1987); these were identified at the lowest possible taxonomic level.

Although we did not formally test the potential effect of sampling unit size, this may alter both frequency and cover measurements and influence sampling times (Andrew and Mapstone, 1987; Cattaneo-Vietti et al., 2002). Thus, we chose to employ the two most commonly used quadrat sizes, i.e. 50 × 50 cm (at Bergeggi) and 100 × 100 cm (at Golfo Tigullio and Gallinara).

2.1.1. Golfo Tigullio: sampling design

Sampling at Golfo Tigullio was conducted during summer 2008. Three sampling stations were randomly chosen within each mineralogical type (i.e., conglomerate, limestone and sandstone), totaling 9 sampling stations. At each station, four 100 × 100 cm quadrats were randomly placed on vertical rocky walls at 5 m depth. Each quadrat was visually surveyed once. The exposure of each quadrat to dominant current and wave directions was recorded using a compass, and the distance of each station from the outfalls and the Entella river mouth were noted.

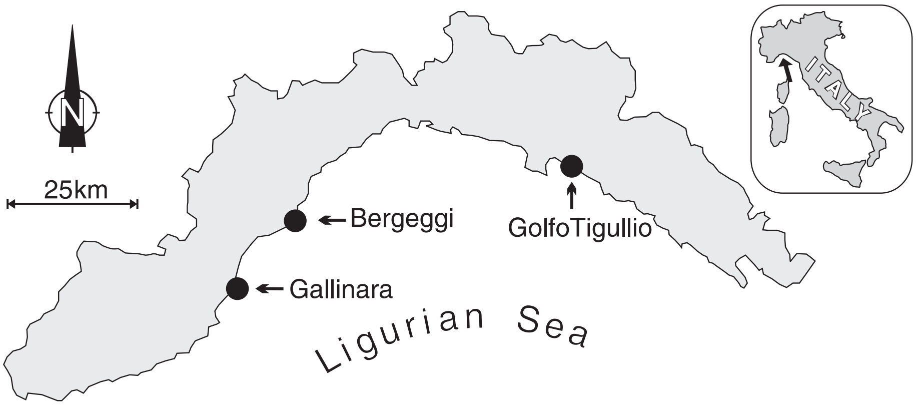

Fig. 1. Geographical position of the three sampling locations.

2.1.2. Gallinara: sampling design

Sampling at Gallinara was conducted during summer 2009. Sampling was designed to compare the two sampling techniques and characterize communities as a baseline for assessing future effects of the planned MPA. Thirteen sampling stations were randomly selected around the Gallinara Island, the proposed location of a new MPA, ranging from 2 m to 35 m depth. At each sampling station two 100 × 100 cm quadrats were randomly placed on vertical rocky cliffs.

2.1.3. Bergeggi: sampling design

Sampling at Bergeggi was conducted during summer 2009. The sampling design was aimed at comparing the two sampling techniques in providing a first snapshot of the variation across protection levels two years after the establishment of an MPA. The MPA comprises three zones with varying levels of protection: A zone (i.e. no-take zone), B zone (i.e. general reserve zone — touristic activities allowed and fishing allowed with restrictions) and C zone (i.e. partial reserve zone — buffer zone). Two sampling stations were randomly positioned within each of these three zones. At each station, six 50 × 50 cm quadrats were randomly placed on vertical rocky walls at 5 m depth.

2.2. Data analysis

2.2.1. Underwater sampling time

Differences in the mean underwater time for data collection were compared between the two sampling techniques (i.e. frequency count and cover estimation), separately for each location, by performing paired *t*-tests.

A general linear model was used to test possible relationships between sampling time and depth for Gallinara, the only dataset exhibiting a depth gradient.

2.2.2. Species richness, diversity and dominance

While the number of species recorded by the two techniques within each quadrat is exactly the same, common indices to assess community structure — e.g., Margalef species richness (d), Shannon–Wiener diversity (H'), and Simpson dominance (λ) — may lead to different results when assessed through frequency or cover.

Possible relationships between d, H' and λ calculated on frequency data and the same indices calculated on cover data were assessed through general linear mixed models considering location as a fixed factor and sampling station as a random factor.

2.2.3. Benthic community multivariate structure

For each case study, similarity in species composition and relative abundance (expressed as % cover or frequency) was calculated using Bray–Curtis similarity. In order to test whether the multivariate pattern described by frequency count was correlated to the multivariate pattern expressed through visual estimation of percent cover, we used the RELATE routine of Primer/Permanova + (Clarke and Gorley, 2006). This analysis was performed to compute the Spearman correlation coefficient (ρ) between the Bray–Curtis similarity matrices obtained by cover estimation with those obtained through frequency count for all the three datasets.

Since data transformation may alter variability in community structure (Underwood and Chapman, 2005), correlations between frequency-count and cover-estimation matrices were computed on untransformed data and with the most commonly used data transformations (i.e. square-root and fourth-root, which give increasingly more weight to the less abundant species).

Multivariate patterns among samples were visualized using non-metric multidimensional scaling ordination (nMDS) for each dataset. nMDS plots were obtained in all cases from untransformed, square-root transformed and fourth-root transformed data.

Non-parametric multivariate analyses of variance (PERMANOVA; Anderson, 2001) were used on frequency-count and cover-estimation datasets obtained at Golfo Tigullio and Bergeggi. In both cases, analyses were performed on frequency-count and cover-estimation datasets separately using untransformed, square-root transformed and fourth-root transformed data.

At Golfo Tigullio PERMANOVAs were used to determine whether the two sampling techniques produced similar results. All analyses were conducted considering mineralogical type (i.e. conglomerates, limestone and sandstone) as a fixed factor and sampling station as a random factor nested within mineralogical type. Physical exposure of the coastline and distance from outfalls (O1 and O2) and from the river mouth were used as covariates.

PERMANOVA was applied to the datasets collected at Bergeggi to see whether the two sampling techniques provide similar results for a first snapshot of the variability across protection levels of the Bergeggi MPA. We considered the three zones of the MPA (A, B and C) as a fixed factor, and the sampling station as a random factor nested within zone.

At Gallinara we examined whether the two sampling techniques yield similar patterns of community variation using cluster analyses (complete linkage method). Significant clusters ($p < 0.01$) were identified through the similarity profile (SIMPROF) permutation test (Clarke and Gorley, 2006). PERMANOVA was not used on this dataset because no explicit hypothesis was tested and sampling was aimed at producing a baseline characterization of these communities.

In order to identify which taxa drove the differences highlighted by PERMANOVAs at Golfo Tigullio and Bergeggi and cluster analyses at Gallinara, we used different routines, depending on the sampling design of each case study. At Gallinara, we used SIMPER analyses to identify the taxa responsible for significant differences among the clusters identified with the frequency-count and the cover-estimation techniques. SIMPER was also used to determine the percentage contribution of taxa to the significant dissimilarities among stations within zones at Bergeggi (see Results).

At Golfo Tigullio, the relationship between categorical and continuous explanatory variables with multivariate species data was analyzed using non-parametric multiple regression (McArdle and Anderson, 2001) on both frequency-count and estimated cover-datasets. Next, distance-based redundancy analyses (dbRDA; Legendre and Anderson, 1999) were used on the frequency-count and cover-estimation datasets to identify the taxa driving differences among individual stations.

2.2.4. Relationship between cover and frequency

To test whether frequency counts provide a reliable estimate of the spatial variation in the abundance of individual taxa, we examined Pearson correlation (r) between cover and frequency of each taxa present in more than three quadrats, separately for each dataset. We used the Bonferroni correction of significance levels to account for multiple comparisons (Rice, 1999).

3. Results

3.1. Underwater sampling time

Underwater surveys conducted using the frequency-count technique were significantly faster than cover-estimation in all case studies (Golfo Tigullio: $df = 35$, $t = 16.39$, $p < 0.001$; Gallinara: $df = 23$, $t = 8.8$, $p < 0.001$; Bergeggi: $df = 35$, $t = 12.08$, $p < 0.001$; Fig. 2). Cover estimation required, on average, 20.2 ± 0.6 min/quadrat at Golfo Tigullio, 20.96 ± 0.7 min/quadrat at Gallinara (100 × 100 cm quadrat employed), and 16.14 ± 0.4 min/quadrat at Bergeggi (50 × 50 cm quadrat). Performing frequency count required, on average, about half the time needed for cover estimation (Golfo Tigullio: 11.54 ± 0.3 min/quadrat; Gallinara: 12.1 ± 0.4 min/quadrat; Bergeggi: 9.11 ±

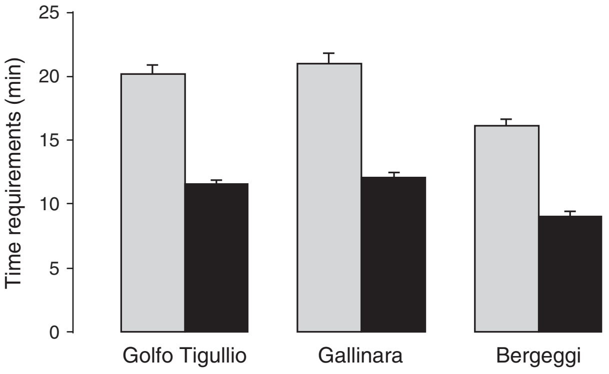

Fig. 2. Underwater time (mean + SE) required for collecting data by cover estimation (grey bars) and frequency count (black bars) in quadrat at Golfo Tigullio ($n = 36$, 100 × 100 cm quadrats), Gallinara ($n = 24$, 100 × 100 cm quadrats) and Bergeggi ($n = 36$, 50 × 50 cm quadrats).

Table 1
Results of RELATE routine performed on cover-estimation and frequency-count data for each on the three dataset. Matrices of similarities among samples were used.

Dataset	Quadrat size	Data transformation					
		None		Square root		Fourth root	
		ρ	p value	ρ	p value	ρ	p value
Golfo Tigullio	100 × 100 cm	0.89	$p < 0.01$	0.909	$p < 0.01$	0.911	$p < 0.01$
Gallinara	100 × 100 cm	0.9	$p < 0.01$	0.943	$p < 0.01$	0.955	$p < 0.01$
Bergeggi	50 × 50 cm	0.73	$p < 0.01$	0.811	$p < 0.01$	0.901	$p < 0.01$

0.2 min/quadrat). For Gallinara Island, the general linear model showed that the time to survey a single quadrat increased with depth ($F_{1,44} = 36.5$, $p < 0.0001$), but this increase was less relevant using frequency rather than cover ($F_{1,44} = 4.1$, $p < 0.05$).

3.2. Species richness, diversity and dominance

Significant interaction between locations and cover-based indices were recorded for Shannon–Wiener diversity (H': $F_{2,62} = 4.14$; $P = 0.02$) and Simpson dominance (λ: $F_{2,62} = 6.03$; $P = 0.004$) but not for Margalef species richness (d). Frequency-based indices were always significantly correlated with those based on cover (d: $F_{1,62} = 1365.42$; $p < 0.0001$; H': $F_{1,62} = 180.70$; $p < 0.0001$; λ: $F_{1,62} = 58.69$; $p < 0.0001$). Relations among the indices, without considering location effect, explained 94% of the total variation for d, 74% for H' and 42% for λ.

3.3. Benthic community multivariate structure

Bray–Curtis similarity matrices obtained by frequency-count were significantly correlated with those obtained by cover estimation in all cases. Spearman correlation coefficients (ρ) had high values in all comparisons (Table 1). Data transformation increased ρ values, with the highest correlations found with the fourth-root data transformation.

3.3.1. Golfo Tigullio dataset

The pattern described by cover estimation resulted analogous to that expressed by frequency count. With both techniques, and independently of data transformation, there were significant effects of distance from the Entella river mouth, distance from outfall O2, and station nested within mineralogical type, but no significant overall effect of mineralogical type and physical exposure of stations (Table 2). Mean of squares, sum of squares and p values of PERMANOVA performed on cover-estimation data showed similar to those obtained by frequency-count data. Differences among data transformations were more marked than those between sampling techniques. Frequency count and cover estimation provided similar results also when using distance based linear models (DISTLM, see

Table 2
Results of PERMANOVAs performed on cover-estimation and frequency-count data from Golfo Tigullio dataset. Physical exposure of the coastline and distances from the river mouth, outfall 1 (O1) and outfall 2 (O2) were included as covariates. Significant p values are shown in bold.

Source of variation	df	Cover estimation				Frequency count			
		SS	MS	F	p	SS	MS	F	p
Untransformed data									
Distance from river mouth	1	8104.1	8104.1	2.4026	**0.0382**	9563	9563	2.4668	**0.0315**
Distance from O1	1	3777.1	3777.1	1.1198	0.3987	4142.1	4142.1	1.0684	0.4188
Distance from O2	1	7940.1	7940.1	2.554	**0.0252**	9978	9978	2.5738	**0.0293**
Exposure	1	3761.8	3761.8	1.1152	0.3872	4303.7	4303.7	1.1101	0.4041
Mineralogy	2	3905.6	1952.8	0.57894	0.7672	4446.4	2223.2	0.57348	0.7747
Station (Mineralogy)	2	6746.1	3373.1	3.0202	**0.0001**	7753.5	3876.7	2.8618	**0.0002**
Residuals	27	30155	1116.8			36576	1354.7		
Total	35	64390				76762			
Square-root transformed data									
Distance from river mouth	1	7876.2	7876.2	2.8507	**0.015**	6993.9	6993.9	2.7511	**0.0211**
Distance from O1	1	3793.4	3793.4	1.373	0.2438	3353.1	3353.1	1.319	0.2692
Distance from O2	1	7034.5	7034.5	2.5461	**0.0263**	5916.3	5916.3	2.3272	**0.0401**
Exposure	1	3090	3090	1.1184	0.3902	3199.1	3199.1	1.2584	0.3108
Mineralogy	2	3230.8	1615.4	0.58468	0.7844	3050	1525	0.59987	0.7734
Station (Mineralogy)	2	5525.7	2762.9	2.8357	**0.0001**	5084.4	2542.2	2.8566	**0.0001**
Residuals	27	26307	974.33			24029	889.95		
Total	35	56857				51625			
Fourth-root transformed data									
Distance from river mouth	1	6945.5	6945.5	3.0607	**0.0128**	6326.3	6326.3	2.9286	**0.0176**
Distance from O1	1	3622.8	3622.8	1.5965	0.1742	3171	3171	1.4679	0.2112
Distance from O2	1	5663.3	5663.3	2.4957	**0.0303**	4940.8	4940.8	2.3172	**0.0407**
Exposure	1	2470	2470	1.0885	0.415	2851.4	2851.4	1.32	0.2915
Mineralogy	2	2609.1	1304.5	0.57488	0.8025	2659.7	1329.9	0.61562	0.7803
Station (Mineralogy)	2	4538.4	2269.2	2.7226	**0.0002**	4320.4	2160.2	2.7117	**0.0001**
Residuals	27	22504	833.47			21509	796.62		
Total	35	48353				45778			

Appendix A). Both sampling techniques identified distance from the river mouth as the major correlate of the observed variation in community structure. Distance from the outfall O2 showed values close to significance (Appendix A). Finally, nMDS ordination plots did not reveal obvious differences between the two sampling techniques (Fig. 3). nMDS on untransformed, square-root and fourth-root transformed data identified two main groups of samples: the first group is composed of samples from the station closest to outfall O2 (station C) and the second group comprises the other stations.

Cover estimation and frequency count identified the same taxa as major drivers of the observed patterns (Fig. 4) Redundancy analysis (dbRDA) indicated that algal turf, hydroid mat and the coral *Cladocora caespitosa* characterize station C with both sampling techniques and with all data transformation employed. The brown alga *Dictyota*

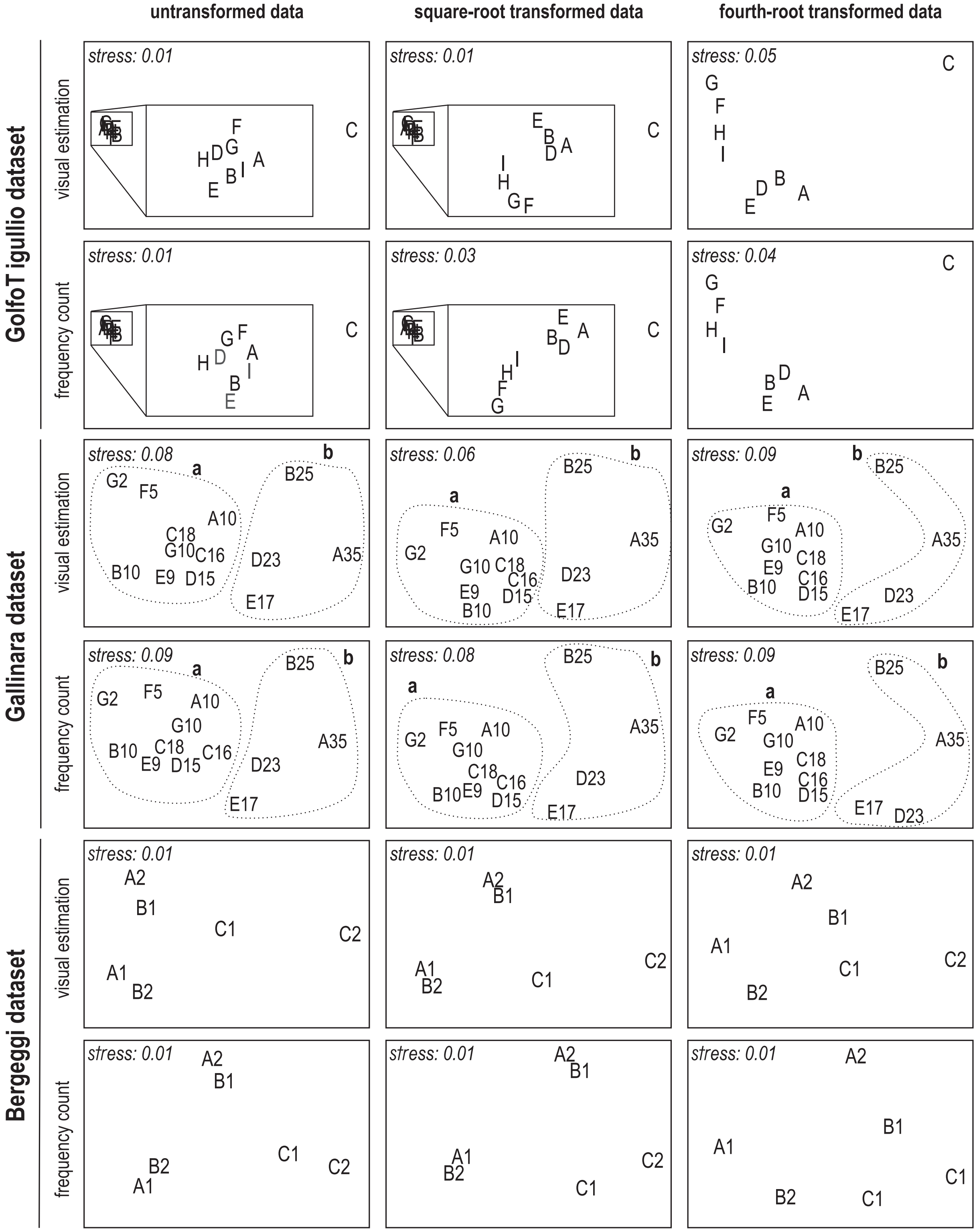

Fig. 3. Non-metric multidimensional scaling ordination plots of sampling stations obtained with cover estimation and frequency count from the three datasets (Golfo Tigullio, Gallinara, and Bergeggi). Plots are derived from Bray–Curtis similarity matrices on untransformed, square-root transformed and fourth-root transformed data. Dotted lines indicate significant clusters ($p < 0.01$) highlighted by similarity profile permutation analysis (SIMPROF).

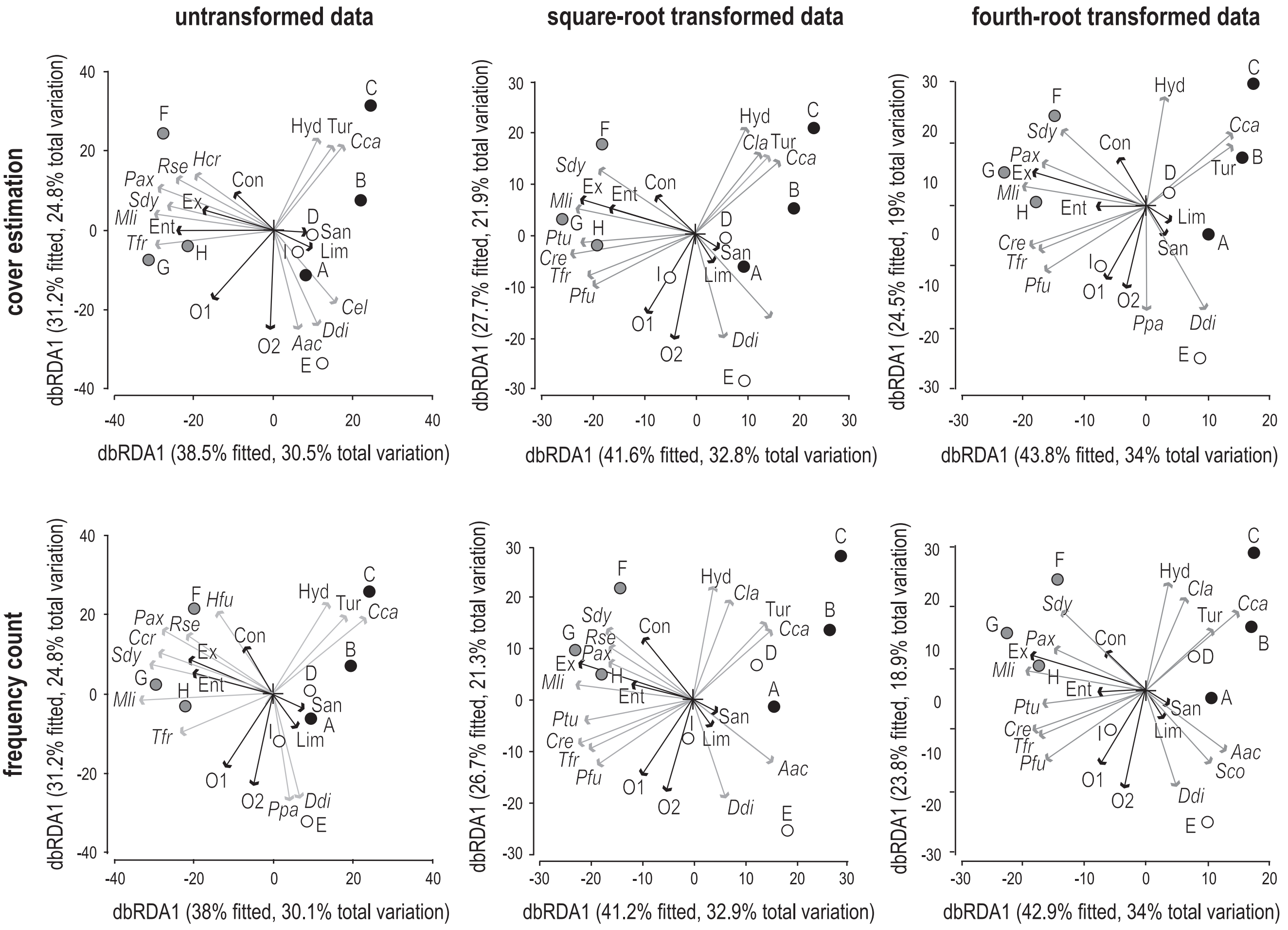

Fig. 4. Distance base Redundancy Analyses performed on the Golfo Tigullio data obtained through cover-estimation and frequency-count techniques. Analyses were based on untransformed, square-root transformed and fourth-root transformed data. Black arrows indicate the correlation of explanatory variables with the first two redundancy axes (dbRDA1 and dbRDA2). Grey arrows indicate the correlation of individual taxa with the same axes. Only species with a correlation higher than 0.75 were included. Black circles: stations on sandstone (A, B, C); white circles: stations on limestone (D, E, I); grey circles: stations on conglomerates (F, G, H). Con: conglomerates; Ent: distance from Entella; Ex: exposure; Lim: limestone; San: sandstone; O1: distance from outfall 1; O2: distance from outfall 2. *Aac*: *Acetabularia acetabulum*; *Cca*: *Cladocora caespitosa*; *Ccr*: *Crambe crambe*; *Cel*: *Corallina elongata*; *Cla*: *Cladophora* sp.; *Ddi*: *Dictyota dichotoma*; *Hcr*: *Halyclona cratera*; *Hfu*: *Halyclona fulva*; *Hyd*: hydroids; *Mli*: *Mesophyllum lichenoides*; *Pax*: *Parazoanthus axinellae*; *Pfu*: *Pseudochlorodesmis furcellata*; *Ppa*: *Padina pavonica*; *Ptu*: *Protula tubularia*; *Rse*: *Reteporella septentrionalis*; *Sdy*: *Salmacina dysteri*; *Tfr*: *Tricleocarpa fragilis*; *Tur*: turf.

dichotoma characterizes station e, placed on limestone and far from the outfalls, but close to the river mouth. Depending on data transformation, the calcareous algae *Acetabularia acetabulum* and *Corallina elongata* were also found to characterize this station. Stations placed on conglomerate rocks, the most distant from the river mouth, showed the algae *Mesophyllum lichenoides* and *Tricleocarpa fragilis* and the serpulid polychaete *Salmacina dysteri* as the most characteristic species with both techniques and regardless of data transformation used (Fig. 4).

3.3.2. Gallinara dataset

Cluster analyses identified two main sample groups with both sampling techniques and all data transformations used (Fig. 3). Stations were grouped according to sampling depth, shallower ones belonging to cluster a and deeper ones belonging to cluster b. Although data transformation had no effect on the identification of significant clusters, nMDS changed its configuration according to the data transformation, but not according to the sampling technique. Both cover estimation and frequency count identified the same taxa as responsible for the differences between the clusters recognized through SIMPER analysis (see Appendix B). However, a greater number of taxa contributing to differences between clusters was found by frequency count compared to cover estimation. With both techniques, differences between the two clusters were due to the algae *Corallina elongata*, *Jania rubens*, *Dictyota dichotoma*, *Padina pavonica*, and *Stypocaulon scoparium*, algal turf, and the encrusting sponge *Crambe crambe*, all more abundant at stations within cluster a, and to *Parazoanthus axinellae*, *Peyssonnelia rubra*, *Peyssonnelia squamaria*, abundant at stations in cluster b.

3.3.3. Bergeggi dataset

Frequency count and cover estimation revealed similar patterns of community variation across the three MPA zones (A, B, C) (Table 3). With both sampling techniques and regardless of data transformation, there is significant variation in community structure among stations, nested within zones, but there are no significant differences among zones (Table 4). Results of PERMANOVAs conducted on the two datasets are virtually identical when analyses are performed on fourth-root transformed data, and very similar for untransformed and square-root transformed data. These results are consistent with the patterns shown by nMDS ordination plots (Fig. 3). SIMPER analyses highlighted that the two techniques identified similar groups of taxa as important for characterizing differences between station and within zone, with similar contributions of taxa to among-station dissimilarities (see Appendix C). Similar to the Gallinara case study, SIMPER applied to frequency-count data tended to identify a greater number of taxa contributing to significant differences among stations than the same analysis applied to cover-estimation data.

Table 3
Results of PERMANOVAs performed on cover-estimation and frequency-count data from Bergeggi dataset. Significant *p* values are shown in bold.

		Cover estimation				Frequency count			
Source of variation	*df*	SS	MS	*F*	*p*	SS	MS	*F*	*p*
Untransformed data									
Zone	2	8747.3	4373.7	1.595	0.1312	6520.9	3260.5	1.3168	0.2629
Station (Zone)	3	8226.1	2742	2.2888	**0.0007**	7428.1	2476	2.1849	**0.0028**
Residuals	30	35941	1198			33997	1133.2		
Total	35	52914				6520.9	3260.5		
Square-root transformed data									
Zone	2	6255.8	3127.9	1.2262	0.1991	5453.8	2726.9	1.1611	0.3541
Station (Zone)	3	7652.8	2550.9	2.5877	**0.0001**	7045.8	2348.6	2.4209	**0.0007**
Residuals	30	29574	985.81			29104	970.15		
Total	35	43483				41604			
Fourth-root transformed data									
Zone	2	5456.2	2728.1	1.1394	0.3334	5216.3	2608.2	1.1469	0.376
Station (Zone)	3	7182.8	2394.3	2.6331	**0.0001**	6822.5	2274.2	2.5028	**0.0008**
Residuals	30	27279	909.3			27260	908.66		
Total	35	39918				39298			

3.4. Relationship between cover and frequency

Significant correlations between estimated percent cover and frequency were found for most of taxa (see Appendix D). 103 of the 130 correlations performed have *p* values smaller than 0.05. The angular coefficients recorded for significant correlations between percent cover and frequency data had similar distributions for the Golfo Tigullio and Gallinara datasets, with interquartile ranges comprised between 0.5 and 0.75 and median values close to 0.5 (Fig. 5). A different picture emerges at Bergeggi, with interquartile range of angular coefficients comprised between 0.5 and 1.2 and median value close to 1.

4. Discussion

Our results indicate that the assessment of benthic community structure based on the frequency of taxa within gridded quadrats reveals the same patterns of community variation as the more time consuming estimation of cover commonly used in ecological studies (Dethier et al., 1993; Fraschetti et al., 2001; Guidetti et al., 2004). These conclusions are robust across three different case studies, suggesting that the frequency-count technique may be effectively applied across different studies and locations as a more time-efficient alternative to cover evaluation. Thus, the frequency-count technique can provide a faster yet thorough technique for quantifying the structure of hard-substrate epibenthic communities that can replace cover estimation at little or no loss of information and statistical power of tests. In particular, if species richness or even Shannon's diversity are the structural variables of interest, the two techniques yield near identical results. If the variable of major concern is dominance, as for example when functional attributes are used instead of species, frequency may underestimate it when compared with cover, although being significantly correlated.

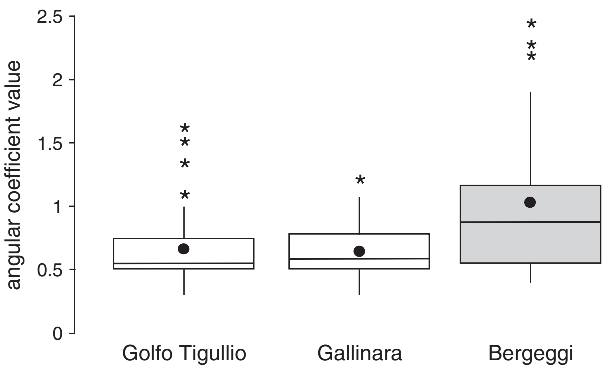

Fig. 5. Box plots of the angular coefficients obtained by the significant linear correlations between frequencies and cover of species identified in the field for Golfo Tigullio, Gallinara and Bergeggi datasets. Line: median; points: mean; stars: outlier; white boxes: 100×100 cm quadrat; grey box: 50×50 cm quadrat.

Frequency counts required approximately half the time needed for cover estimation. This is a key aspect for underwater surveys because bottom time constrains sampling effort. Sampling efficiency has been shown to decrease with depth with both techniques, as the deeper is the quadrat the slower is the diver's work, but at any depth frequency count is faster than cover estimation. On average, with the time needed to obtain data from one quadrat by cover estimation, 1.7 quadrats can be completed by frequency count. Thus, during the time required for sampling 3 quadrats with the cover-estimation technique, 5 quadrats can be sampled with the frequency-count technique. In non-hierarchical sampling designs, if depth is the gradient of interest, frequency-count will allow to increase the extent of the gradient explored compared to the more time consuming cover-estimation (Fig. 6). The greater efficiency of the frequency-count technique is particularly important in surveys of deep communities, where underwater time is greatly reduced by scuba restrictions. In particular, 3 replicate 50×50 cm or 100×100 cm quadrats can be surveyed in one dive at 21 m and 19 m depth, respectively when using the cover-estimation technique, but the same sampling size can be achieved at 30 m and 24 m depth using the frequency-count technique (Fig. 6). Although the data used for this study were all taken within a maximum depth of 35 m where the two techniques used can be safely compared, previous experience with non hierarchical sampling design showed that frequency quadrats can be efficiently surveyed to a depth of 54 m (Bianchi, unpublished data).

As shown by Spearman rank correlations, virtually no information is lost when employing frequency count instead of cover estimation for both similarities among samples and among species. The high heterogeneity of cover data often imposes the need of data transformation to reduce the skewness of the data distribution (Legendre and Gallagher, 2001): due to their metrics, frequency data are intrinsically less skewed.

Importantly, the same patterns of community variation were highlighted using frequency count and cover estimation in all case studies. Results were almost identical for all dataset considered, independent of the location of the study, sampling depth, or the size of sampling unit employed. Both techniques, moreover, identified the same taxa as major drivers for the observed community patters and

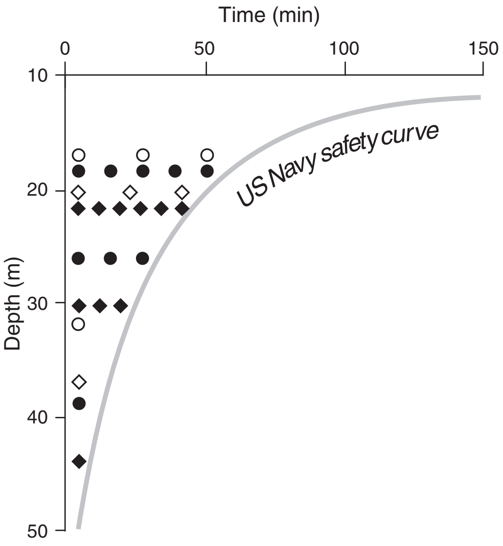

Fig. 6. Diver's safety implications of adopting frequency count (black symbols) instead of cover estimation (white symbols), with either 100 × 100 cm quadrats (circles) or 50 × 50 cm quadrats (diamonds). Grey line indicates the safety curve for no-decompression dives. In the case of hierarchical designs, at around 20 m depth cover estimation allows for no more than three replicates, frequency counts for up to six; if a minimum number of replicates of three is accepted, frequency counts can be performed to a maximum depth of 25 m to 30 m down without decompression, while cover estimations cannot be done below ca 20 m. In non hierarchical designs (with no replication), frequency counts permit the analysis of depth gradients about 10 m deeper than cover estimation.

results were more sensitive to the data transformation than to the visual sampling technique itself.

Frequency count offers the advantage, over the point-intercept technique, to provide the same species list obtained by cover estimation. The frequency count is thus an effective sampling technique when a complete biodiversity inventory is needed and when species richness is an important variable to be studied.

The main drawbacks of the frequency-count technique are that it is not additive and cannot be equate to the most familiar measures of cover or abundance of taxa. Chiarucci et al. (1999) found that cover and biomass estimates describe similar patterns of variation in benthic assemblages and are significantly correlated. Biomass can often be considered ecologically more informative than cover but has the major disadvantage of requiring the collection of physical samples. Similarly, frequency counts entail a loss of ecological information compared to cover estimation, but this technique allows saving time underwater, which is a major advantage when sampling benthic communities by direct observation. Moreover, in this study we found that frequency and cover were almost always significantly correlated, especially when the degrees of freedom of correlations were high. This result indicates that frequency counts can provide a reliable measure of taxa ‘quantity’.

Despite recent major technological advances in marine monitoring, accurate descriptions of population and community patterns are still relying largely on direct underwater observation. Developing reliable rapid-assessment methods remains an important research need. The frequency-count technique we tested provides a robust technique for obtaining information on benthic communities where scuba diving time is limited.

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