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DIVERSITY OF POLYCHAETE FAUNA IN THE GULF OF LIONS (NW MEDITERRANEAN)

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POLYCHAETE
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ABSTRACT. – Ninety two sites (10, 20, 30, 40 and 50 m depth), located on 21 transects along the portion of coast between the Spanish-French border and the mouth of the Rhône River were sampled for sediment and polychaete fauna during September-October 1998. Four assemblages were identified using cluster analysis, namely littoral fine sands (LFS), southern littoral sandy mud (LSMS), northern littoral sandy mud (LSMN) and terrigeneous coastal mud (TCM). Several components of polychaete diversity were assessed: α-diversity (i.e., within sites), β-diversity (i.e., turnover of species over the whole sampled area), estimates of the total number of polychaete species within each assemblage and γ-diversity (i.e., total number of polychaete species over the whole sampled area). Dominance and synthetic indices of α-diversity were strongly influenced by the few dominant species (i.e., mostly *Ditrupa arietina* and *Owenia fusiformis*). Whittaker's β_w was estimated at 9.3 when considering the whole data set, which is high compared to the few other data available for polychaete fauna. This was partly linked to the high diversity of sampled habitats as shown by the drastic decrease in β_w when considering each assemblage separately. In all assemblages but LSMS, β-diversity was more affected by the habitat than by the distance between sites, which seems to reflect more small scale spatial pattern in diversity than true species substitution along a gradient. The number of polychaete species within each assemblage correlated positively with sampling effort. It was nevertheless highest for LFS (67 species) and lower for LSMN (49 species) for a standardized (9 sites) sampling effort. Several non parametric estimators were used to assess the true number of species within each assemblage. All of them resulted in quite similar total number of species per assemblage. Gamma diversity for the whole data set was computed using both the extrapolation of the accumulation curve and the TS method. Total number of observed polychaete species was 173. The extrapolation of the species accumulation curve resulted in an estimation of an overall number of 873 species versus 980 (when splitting the whole data set in 4 geographical areas) and of 1,051 (when splitting the whole data set in 4 assemblages) for the TS method. The variable estimates of total polychaete species richness based on the TS method is discussed in relation with (1) the effect of the interaction between the pattern of spatial heterogeneity and sampling design, and (2) the arbitrary assignment of a sampled surface to a single sample.

INTRODUCTION

During the last decade, biodiversity has received increasing attention due to major threats or even extinction of numerous species (Sisk *et al*. 1994). Most studies dealing with biodiversity relate to terrestrial systems in spite of the fact that oceans cover about 70% of the earth surface. Consequently, most of the biodiversity concepts originate from terrestrial surveys and sometimes prove difficult to apply to marine systems (Gray 2000). Among marine species, macrobenthos inhabiting marine sediments is especially important from an ecological standpoint because: (1) it plays a significant role in a large variety of ecological processes including nutrient and pollutant cycling (Gilek *et al*. 1997, Gunnarsson *et al*. 2000), sediment transport (Aller & Yingst 1985), secondary production and calcification (Medernach *et al*. 2000), and (2) its (specific) composition interferes with ecosystem functions (Snelgrove *et al*. 1997) and can be used to assess the quality of benthic habitats (Pearson & Rosenberg 1978, Borja *et al*. 2000, Simboura & Zenetos 2002, Rosenberg *et al*. 2004). It is thus essential to better assess the biodiversity of macrobenthos both from a functional and a conservational point of view.

Biodiversity concept encompasses a large range of organization levels from genomes to biogeographical provinces. One key point is the scale at which biodiversity is assessed. There is still a large need for more information on variability of biodiversity at different scales (from local and meso-scale, to seascape scale), and to test hypotheses at these scales such as whether the local species pools are random samples from the regional species pools (Duarte *et al*. 2002). This leads to the classic distinction between α– (i.e., the diversity of species occurring at a single site), γ- (i.e., the diversity of species occurring at the regional scale), and β-diversity (i.e., the turnover of species along a gradient or between communities) (Whittaker 1960). A large variety of indices has been proposed to describe these 3 components of biodiversity (Wilson & Shmida 1984, Gray 2000, Foggo *et al.* 2003a, Koleff *et al.* 2003), and at present there is no general agreement on which ones should preferentially be used. For α-diversity, this lack of consensus is linked to the complexity of the concept which refers to: (1) species richness, (2) evenness, and (3) taxonomic relationships between individuals (Warwick & Clarke 1995). Moreover it appears that these different indices of α-diversity are not affected in the same manner by sampling effort (Rumohr *et al.* 2001). The major problem associated with the assessment of γ-diversity is also linked to insufficient sampling, which has led to the development of several mathematical approaches to better infer the true number of species present in a sampled area (Chao 1984, 1987, Grassle & Maciolek 1992, Karakassis 1995, Gray *et al.* 1997). There are 2 ways of assessing β-diversity. First, it can be derived from γ and α-diversity (Whittaker 1972) with the difficulties mentioned above. Second, it can be derived from the relationship linking geographic distance and sample similarity (Whittaker 1972) with the difficulty linked with the existence of potential confounding factors (Ellingsen 2002). These difficulties explain why until recently most studies dealing with marine macrobenthos diversity have been restricted to α-diversity (Gray 2000). As pointed out by Danovaro (2003), there is thus still an urgent need to better assess the full complexity of biodiversity in the marine environment.

Although considered as one of the best-studied seas of the world, there are still major gaps to be covered in the field of biodiversity of the Mediterranean Sea (Duarte *et al.* 2002). Little attention has been devoted to the Mediterranean Sea in this regard despite the fact that it apparently includes 4-18% (depending on considered taxa) of the total world macroscopic marine species (Bianchi & Mori 2000), and that it shows an especially large variety of environmental conditions (Pérès 1967). The mean feature of macrobenthic diversity in the Mediterranean is an eastward decreasing gradient in species richness (Arvanitidis *et al.* 2002). Most of the benthic studies carried out in NW Mediterranean have been either restricted to the description of benthic communities (e.g., Guille 1971) or limited to small areas (e.g., Grémare *et al.* 1998a, Grémare *et al.* 1998b, Sardá *et al.* 1999). Grémare *et al.* (1998a, 1998b) have shown the occurrence of major changes in the composition of soft-bottom benthic macrofauna in the Gulf of Lions mainly, reflected by a dramatic increase in abundance of the serpulid polychaete *Ditrupa arietina*. However, there has been no study focussing on the assessment of the different components of macrobenthos diversity in this particular area.

Polychaeta is one of the richest invertebrate benthic group with regard to species number (Arvanitidis *et al.* 2002). This group often dominates benthic macrofauna (e.g., Grémare *et al.* 1998a). The importance of polychaetes in quality monitoring is also well established (Pocklington & Wells 1992, Hutchings 1998). In the present data set, polychaetes contributed to about 40% of total macrofauna species richness and, to 23% of total macrofauna abundance. The present study is dedicated especially to polychaetes, which probably play an important role in the structure and diversity of total macrofauna, due to their strong dominance. Labrune *et al.* (submitted) studied the structure and diversity of total macrofauna and the relevance of the use of major taxonomic groups as surrogates of total macrofauna.

The aim of the present study was thus to assess the different components of diversity of polychaete fauna in the shallow soft bottoms of the Gulf of Lions. We first consider, independently, each of the assemblages already identified in a previous work (Labrune *et al.* in press) and then the polychaete fauna of the whole studied area.

MATERIAL AND METHODS

Collection and processing of samples: Benthic samples were collected in September-October 1998 on the N.O. Georges Petit between the Spanish-French border and the mouth of the Rhône River (Fig. 1). This section of coast spans about 110 km from South to North and 140 km from West to East.

Data were collected at 92 sites located on 21 inshore-offshore transects. Most (i.e., 16) transects were sampled at 10, 20, 30, 40 and 50 m depth. Transects O, P, Q, S and U were only sampled at 10, 20 and 30 m depth. In addition, transect F was not sampled at 30 m depth. Samples were collected with a 0.1 m^2 van Veen grab. At each site, 3 grabs were taken for the analysis of benthic macrofauna, and 1 for granulometric and organic content analyses. Macrofauna samples were immediately sieved through a 1 mm mesh and the fauna retained was fixed in 5% formalin. At the laboratory, samples were sorted and polychaetes were separated from the remaining fauna, identified to the lowest practical taxonomic level and counted. Unidentified species were only taken into account when they could not be misidentified.

Granulometric analysis was conducted on fresh sediment using a Malvern® Mastersizer 2000 laser microgranulometer. Organic carbon was measured after acidification (HCl 1N) of freeze-dried sediment using a CHN Perkin Elmer® 2400 analyzer.

Data analysis: Ordination by nonmetric multidimensional scaling (MDS) based on the Bray-Curtis similarity matrix of square root transformed data was used to identify the main polychaete assemblages (see also Labrune *et al.* in press). This analysis was carried out using the Plymouth Routines in Multivariate Ecological Research PRIMER® package.

Values of α-diversity were calculated by using 4 different indices: species richness (S), Shannon-Wiener index (H'(log_2)), Pielou's evenness (J'), and average taxonomic distinctness (Δ^*). S stands for the total number of species present at a given site; H' is the most widely used synthetic diversity index and J' is a measure of evenness. The use of Δ^* has been recently introduced by Warwick and Clarke (1995). This index accounts for both the abundance and the taxonomic distance between all the species present at a given site. Δ^* can be defined as "the expected taxonomic distance apart of any two individuals chosen at random from the sample, provided that those two individuals are not from the same species" (Clarke & Warwick 2001). These 4 indices were computed using the PRIMER® package and compared among assemblages using Kruskal-Wallis ANOVAs and Mann-Whitney U tests.

According to Whittaker (1972), β-diversity can first be regarded as the ratio between the regional (γ) and the average local ($\bar{\alpha}$) diversity. This formulation (β_w) provides a good representation of the species turnover along a gradient (Whittaker 1972) and is one of the simplest and most effective measure of β-diversity (Magurran 2004). It can also be used to measure the proportion by which an overall area is richer than "average sites" even when the samples cannot be arranged along a formal gradient (Wilson & Shmida 1984, Ellingsen 2001, 2002). We looked first at β_w in the whole studied area and then at β_w for each assemblage separately. All these procedures were run using specially designed Matlab® routines. Patterns in β-diversity were also assessed by measuring the relationship between similarity among sites and geographic distance (Whittaker 1972). We computed for all possible pairs of sites within each assemblage: (1) the similarity measure of Bray-Curtis (Bray & Curtis 1957), and (2) taxonomic similarity (Δs; Izsak & Price 2001).

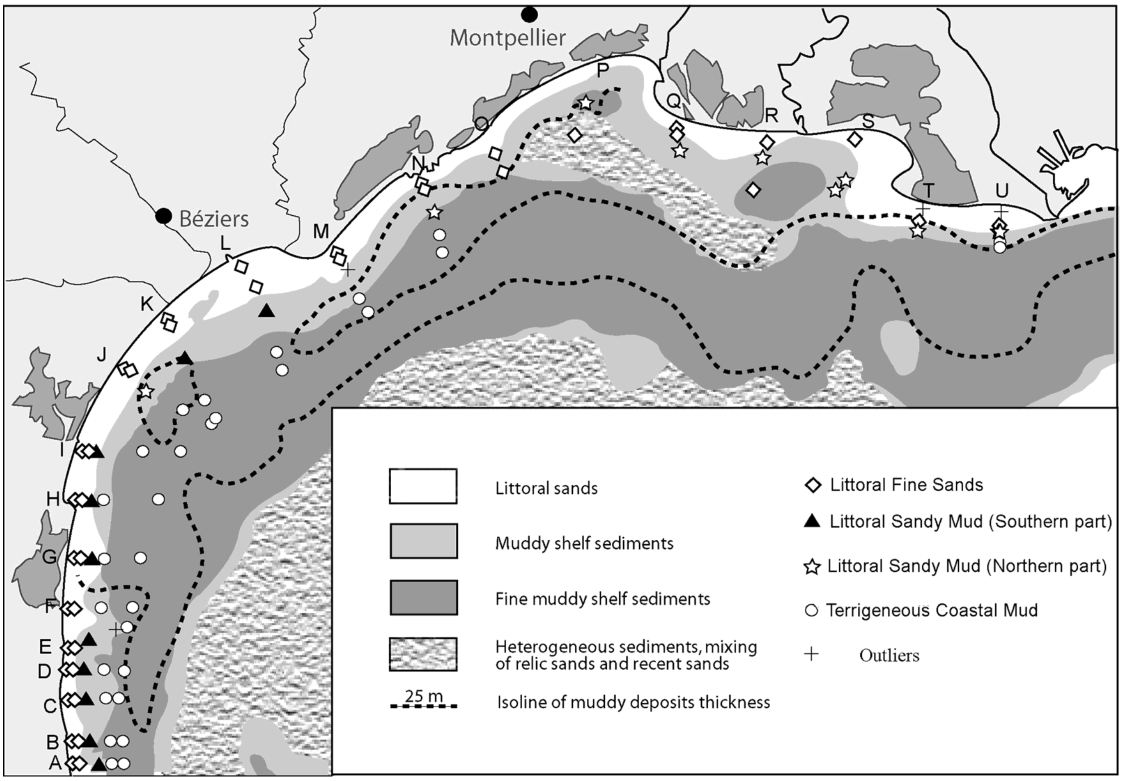

Fig. 1. – Location of the 92 sites sampled during the present study over the sedimentary map drawn by Aloisi *et al.* (1973). Letters from A to U correspond to the 21 transects. Symbols correspond to the polychaete assemblages identified by Labrune *et al.* (in press).

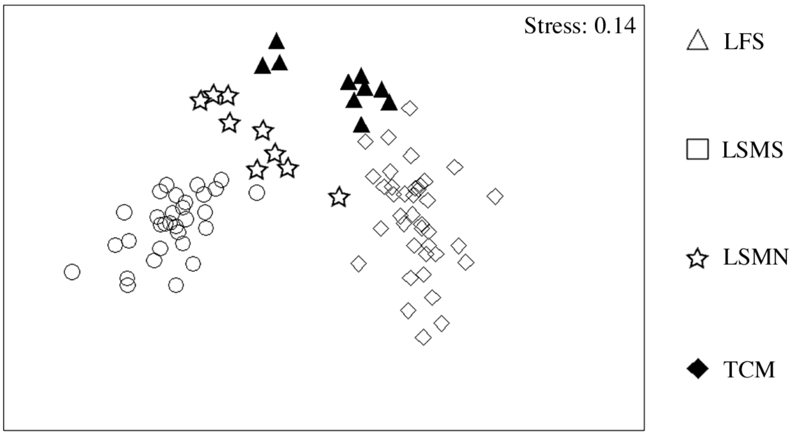

Fig. 2. – Multidimensional scaling ordination based on square-root transformed data of polychaete abundance and on the Bray-Curtis similarity index. Symbols correspond to the polychaete assemblages identified by Labrune *et al.* (in press).

Assemblage	Depth (m)	D50 (μm)	< 63 μm (%)	Corg (%DW)
LFS	10 - 30	47.2 - 278.2	0 - 61.58	0.06 - 0.77
LSMS	30	27.9 - 545.7	14.54 - 81.80	0.29 - 0.63
LSMN	20 - 30	31.4 – 65.7	48.69 - 84.14	0.44 - 0.80
TCM	40 - 50	11.7 - 70.6	49.50 - 99.05	0.50 - 1.13

	LFS (N = 703)	LSMS (N = 45)	LSMN (N = 36)	TCM (N = 465)
Bray-Curtis similarity	**R^2 = 0.04**	**R^2 = 0.20**	R^2 = 0.04	**R^2 = 0.02**
Taxonomic similarity	**R^2 = 0.01**	**R^2 = 0.39**	R^2 = 0.02	R^2 = 0.03

Table I. – Top, Depth ranges and main sediment characteristics ranges of the 4 polychaete assemblages identified by Labrune *et al.* (in press). D50: median grain size; Corg: organic content. Bottom, Determination coefficients of the linear relationships linking Bray-Curtis similarity and taxonomic similarity between sites with the geographic distance between sites. Significant correlation ($p < 0.05$) are in bold.

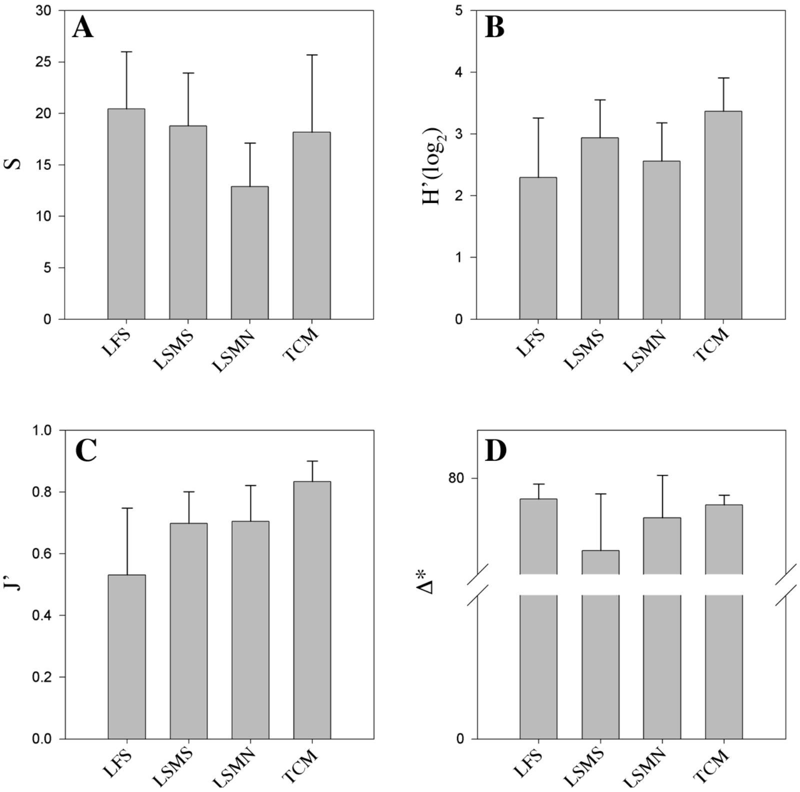

Fig. 3. – α-diversity. Average and standard deviation of Species richness (A), Shannon index (Log_2) (B), Pielou's evenness (C), and Average Taxonomic distinctness (D) recorded in each polychaete assemblage.

Bray-Curtis similarity was computed after a square root transformation to reduce the importance of most dominant species (Clarke & Ainsworth 1993, Olsgard *et al.* 1997). Δs was computed using a Matlab® routine. The relationships linking these 2 indices and geographic distances among sites were assessed for each polychaete assemblage using simple linear regression models.

We used several methods to estimate the true number of polychaete species in each assemblage: S_{obs}, $Chao_1$, $Chao_2$ and ICE. The measure S_{obs} refers to the total number of species recorded in the whole data set. $Chao_1$ (Chao 1984, 1987) is an abundance-based estimator of species richness based on the number of species that are represented by only one or two individuals. $Chao_2$ (Chao 1987) is an incidence-based estimator of species richness, which applies the same approach that $Chao_1$ but based on the numbers of species that occur only in one or two samples. Lee & Chao (1994) introduced ICE which is an incidence-based Coverage Estimator of species richness, determining the number of species in a sample as a function of the numbers of common and rare species. Cumulative curves of these indices were obtained by randomly generating increasing in size subsets of sampling sites. This procedure (50 randomizations) was carried out using the EstimateS freeware (Colwell 1997). Because of the bias induced by the heterogeneity of the data on these indices (Foggo *et al.* 2003a, 2003b), and the fact that both Chao's estimators assume homogeneity among samples (Magurran 2004), these estimates were computed for each assemblage separately.

Gamma diversity is traditionally defined as the diversity observed at the regional scale. By extension, this concept is often ascribed to the diversity of all the sites of a given data set (Ellingsen 2001, 2002). During the present study, we used two different indices to infer γ-diversity of polychaete fauna: the extrapolation of species accumulation curve and the "Total Species-accumulation curve" (TS). This last method was recently introduced by Ugland *et al.* (2003). It consists in constructing a regression of the average number of species in all combinations of, respectively, one, two, three and four combinations of predefined subsets of stations against the logarithm of the cumulative number of samples in each of these combinations. This regression is then extrapolated to the true surface area of the studied zone which corresponds to surface area between the coast, the 50 m depth isoline and transects A and U. According to Ugland *et al.* (2003, 2005) this method gives more realistic estimates of true species richness than the other estimators. We used the TS method based on: (1) the 4 polychaete assemblages and (2) 4 geographical subareas (area 1 = transect A to E, area 2 = transect F to J, area 3 = transect K to P, area 4 = transect Q to U).

RESULTS

Identification and location of assemblages

Three main polychaete assemblages, one of them being separated into two sub-assemblages were identified from the samples (Labrune *et al.* in press). Their location can be observed in Fig. 1. The groups of stations corresponding to these assemblages are shown on the MDS (Fig. 2). These assemblages were tightly associated with depth and sediment granulometry as indicated by the concordance between their spatial distributions and the sedimentary map of the Gulf of Lions (Labrune *et al.* in press). Littoral Fine Sands (LFS) assemblage contained most of the 10 and 20 m deep stations and was characterized by high abundance of the serpulid *Ditrupa arietina*. Littoral sandy mud (LSM) was mostly composed of 30 m deep stations and was composed of two sub-assemblages in relation with latitude and sediment granulometry. For clarity, these 2 sub-assemblages will be considered as full assemblages in the present study, namely: (1) the portion of the LSM assemblage, which was only found in the Southern part of the Gulf of Lions (LSMS) and (2) the portion of the Littoral sandy mud assemblage which was mostly found in the Northern part of the Gulf of Lions (LSMN) (Fig. 1). Terrigeneous Coastal Mud (TCM) assemblage was composed of 40 and 50 m deep stations; it was found all over the Gulf of Lions.

The depth and the main sediment characteristics (median grain size (D50), silt-clay and organic carbon contents) ranges of these 4 assemblages are presented in Table I. LFS and LSMS featured the widest range of sediment median grain size and silt-clay contents whereas LFS and TCM featured the widest range of organic carbon contents.

Alpha diversity

Polychaete species richness was highly variable among sites. It ranged between 4 (site U10) and 33 (sites C40 and E40). There were 7 sites with less than 10 polychaete species and 4 sites with more than 30 polychaete species. Average species richness significantly differed between assemblages (Kruskal-Wallis ANOVA, $p = 0.014$). It was minimal for LSMN (12.9), which differs significantly from LFS and LSMS (Mann-Whitney U test, $p = 0.001$ and $p = 0.022$, respectively). Polychaete species richness was relatively close for the 3 other assemblages (20.4, 18.8 and 18.2 for LFS, LSMS and TCM, respectively, Fig. 3A), and did not present any significant differences (Kruskal-Wallis ANOVA, $p = 0.345$).

Average H' and J' significantly differed between assemblages and both tended to increase between LFS and TCM (Kruskal-Wallis ANOVA, $p < 0.001$ in both cases). Mann-Whitney U test indicated significant differences in H' between TCM and each of the other assemblages ($p < 0.05$, in all cases). Differences in J' values were more marked since only LSMS and LSMN were not significantly different ($p = 0.932$). Average H' was between 2.3 for LFS and 3.4 for TCM and average J' was between 0.5 for LFS and 0.8 for TCM (Fig. 3B, C). This pattern reflected the dominance of *Ditrupa arietina* in LFS, LSMS and LSMN.

Δ^* showed a much narrower range of variation than species richness, H' and J'. It was between 59.1 at site L30 and 79.9 at site N10. However, average Δ^* signifi-

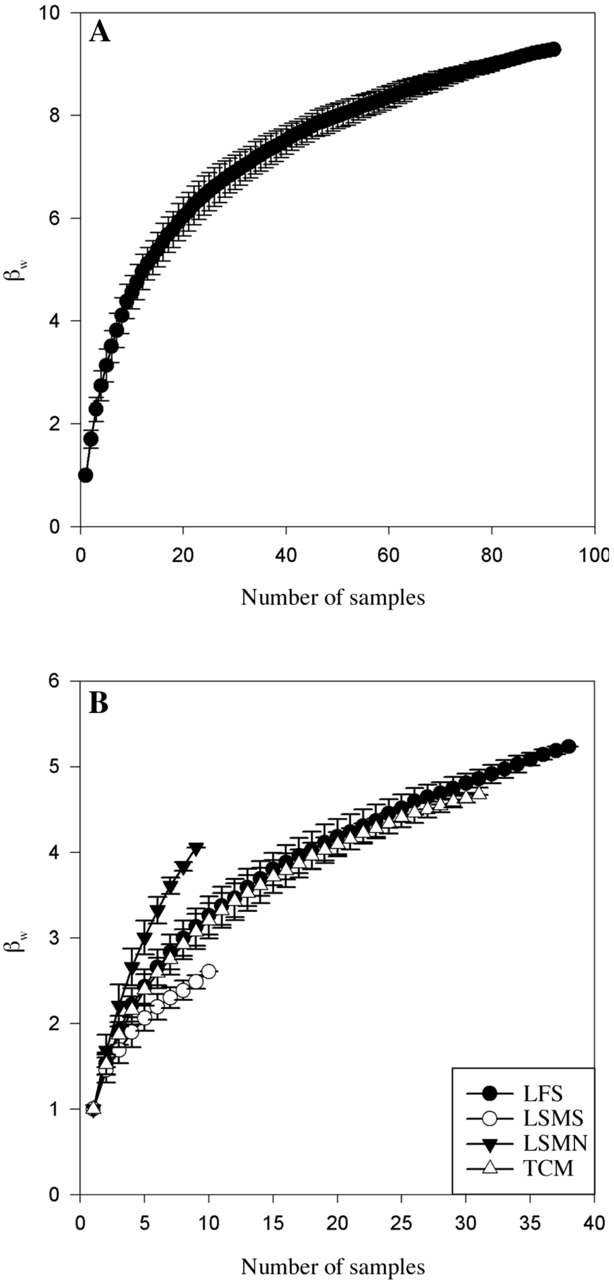

Fig. 4. – β-diversity. Accumulation curves of β_w based on (A) the whole data set and (B) each polychaete assemblage separately. Plotted values are means +/- SD of 50 randomly generated combinations of samples.

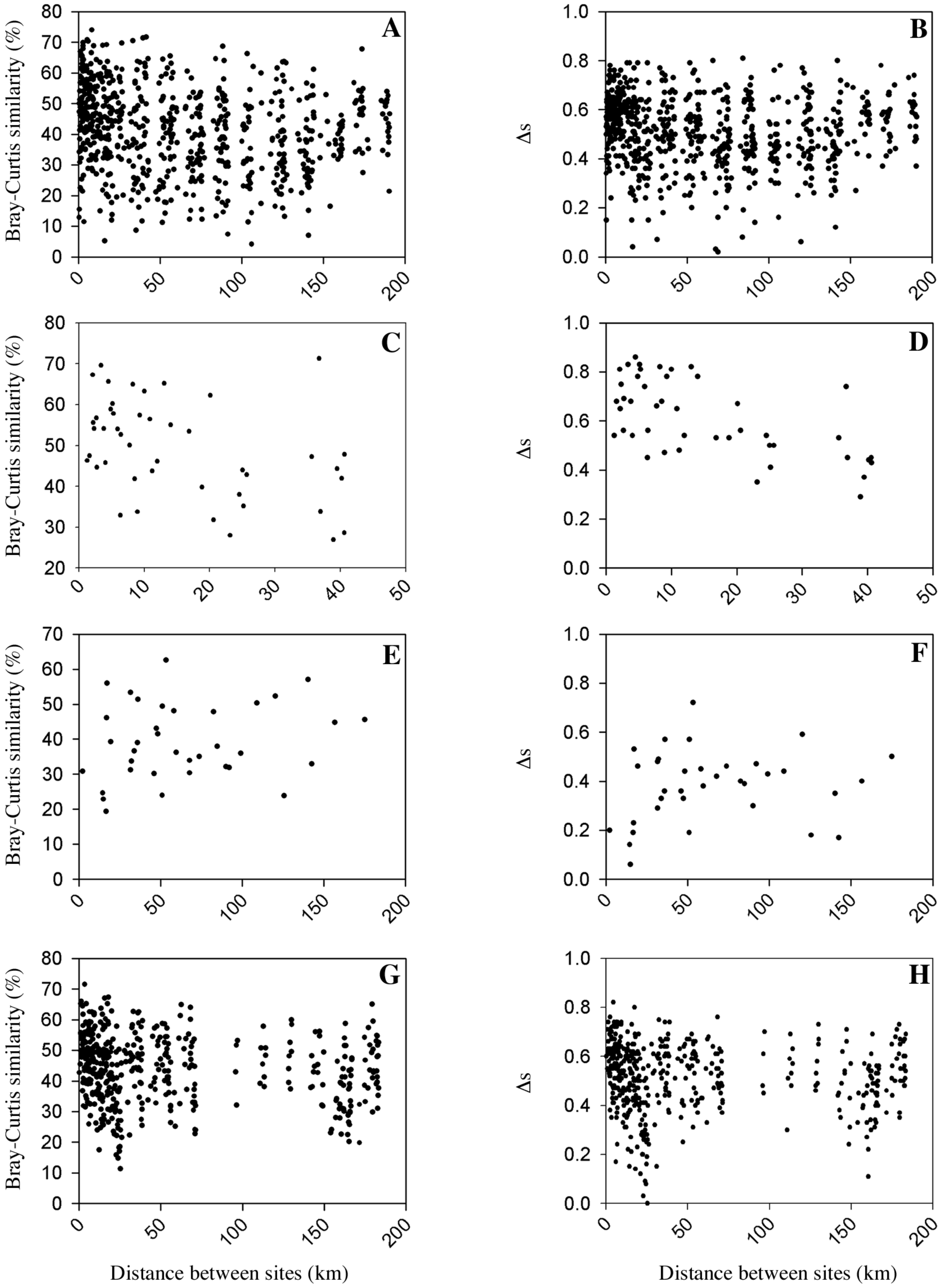

Fig. 5. – β-diversity. Relationships between the geographic distance between two samples and Bray-Curtis similarity and Taxonomic similarity (Δs). Each point corresponds to a possible combination of two different samples of the same assemblage: (A and B) LFS, (C and D) LSMS, (E and F) LSMN and (G and H) TCM.

cantly differed between assemblages (Kruskal-Wallis ANOVA, $p < 0.001$), and particularly between LFS and TCM and between LSMS and TCM (Mann-Whitney U test, $p = 0.008$ in the two cases). Average Δ^* was between 72.5 for LSMS and 77.8 for LFS.

Beta diversity

The overall β_w was 9.3 (Fig. 4A). The cumulative β_w curves for each assemblage are presented Fig. 4B. The value of β_w was higher for LSMN (4.1 for 9 sites), followed by TCM, LFS (3.1 for 9 sites) and by LSMS (2.5 for 9 sites).

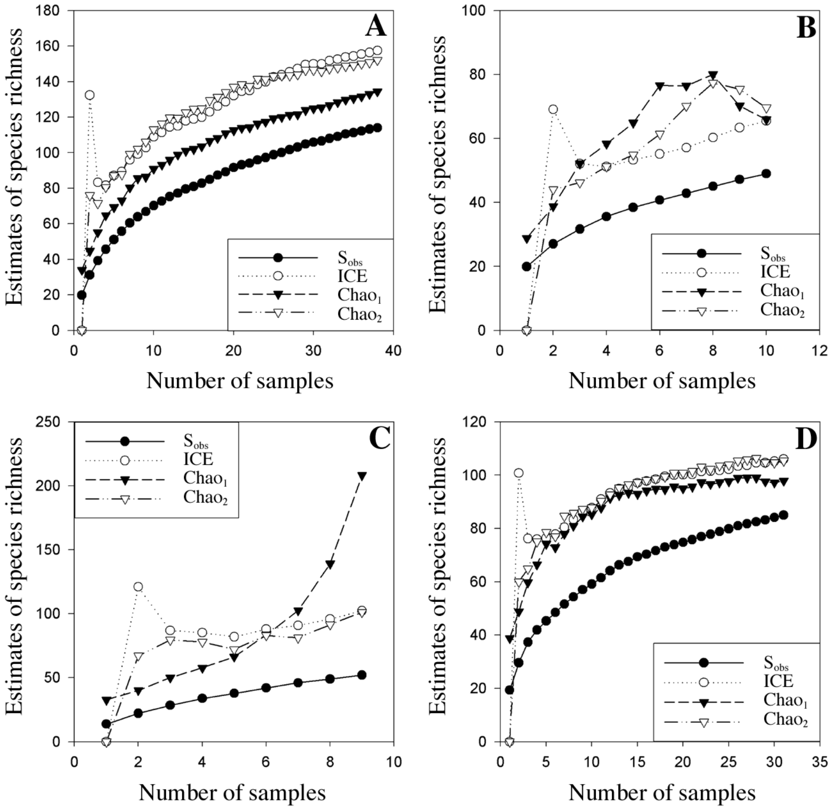

Fig. 6. – Total number of polychaete species in each assemblage. Species accumulation curves. Indicators of species richness are the total number of species observed (S_{obs}) and estimator of true species richness ($Chao_1$, $Chao_2$ and ICE). Plotted values are means of 50 estimates based on 50 randomisations of sample accumulation order (without replacement). (A) LFS, (B) LSMS, (C) LSMN and (D) TCM. For clarity reasons, standard deviations are not shown.

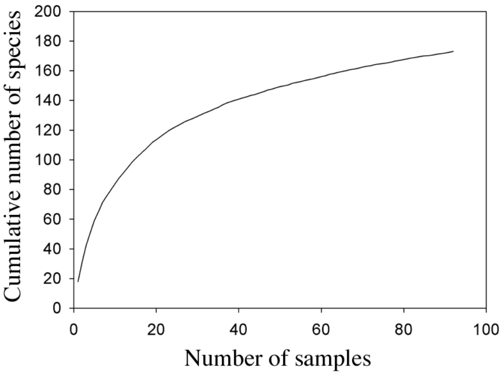

Fig. 7. – γ diversity. Species accumulation curve based on the whole data set (S_{obs})

In both LFS and LSMS, there were significant negative relationships between distance among sites and similarity indices (Fig. 5, Table I Bottom). However, corresponding determination coefficients were very low, which indicates very weak relations between variables, particularly for LSMS. Conversely, there was no significant relationship between geographic distance between sites and taxonomic similarity neither in LSMN nor in TCM. For TCM, relationship between Bray-Curtis similarity and geographic distance between sites was significant but determination coefficient was very low. Overall, our results showed that there was no clear trend between geographic distance and similarity indices. This suggested that the 4 assemblages were homogeneous enough to allow for the computation of non parametric estimators of species richness within each of them.

Total number of species per assemblage

Estimates of total number of species in each assemblage are presented in Fig. 6. For all 4 assemblages, S_{obs} was smaller than the 3 non parametric estimators. $Chao_1$, $Chao_2$ and ICE gave almost identical results taken into account the variability associated with each estimator for all assemblages.

LSMS showed the smallest estimator of total number of species (S_{obs} = 49 and $Chao_2$ (highest estimate) = 70+/-13 (mean+/-SD)). LSMN presented a lower S_{obs} than TCM (52 and 85 species, respectively). Despite the fact that TCM contained more stations than LSMN, $Chao_2$ and ICE estimated almost similar total number of species for both assemblages ($Chao_2$ = 106+/-11 *vs* 101+/-26 and ICE = 105 and 102 respectively). Conversely, $Chao_1$ estimated a higher number of polychaete species for LSMN than for TCM ($Chao_1$ = 208+/-127 and 98+/-8, respectively). Moreover the total numbers of polychaete species for a given sampling effort (9 samples) were very close ($Chao_1$ = 84+/-18 and ICE = 86+/-12 for TCM).

LFS featured the highest estimates of the total number of species (S_{obs} = 114; $Chao_1$ = 134+/-11 $Chao_2$ = 152 +/-16; ICE = 157). However, for a sampling effort of 9 samples, these estimates were rather similar to those obtained for TCM (S_{obs} = 67+/-8, $Chao_1$ = 86+/-13, $Chao_2$ = 106+/-20 and ICE = 103+/-22).

Gamma diversity

The species accumulation curve based on the randomization of samples collected over the whole studied area is shown Fig. 7. Total number of polychaete species observed (S_{obs}) was 173 and showed little sign of approaching an asymptote. By making the correspondence between the number of samples and the sampled

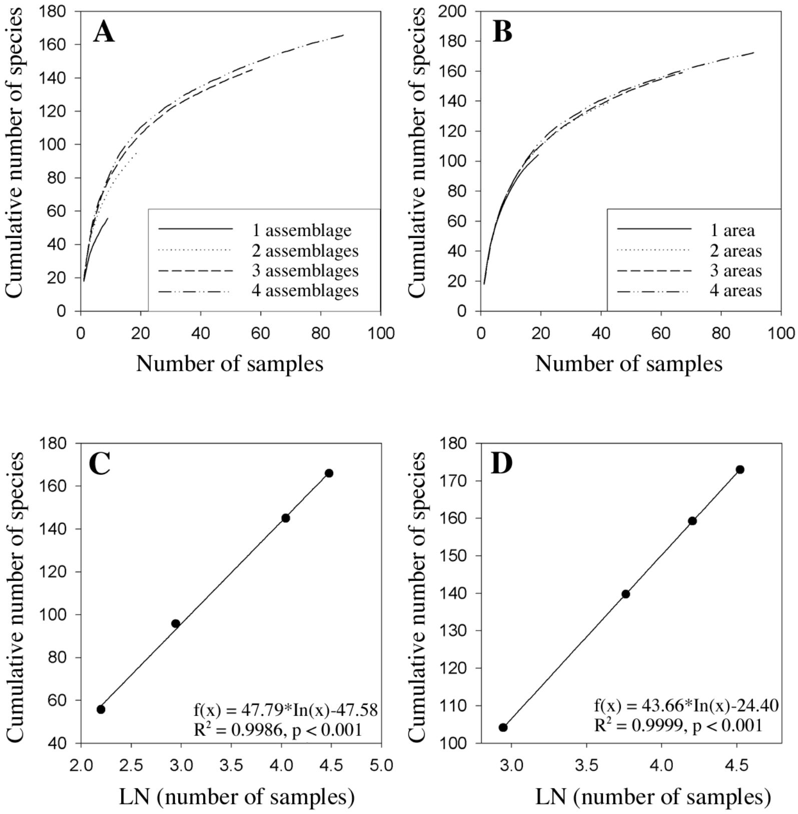

Fig. 8. – Gamma diversity. Species accumulation curve for all combinations and corresponding semi-logarithmic regressions used for the estimation of true species richness (TS) for 1 to 4 assemblages (A, C) and 1 to 4 geographical sub-areas (B, D).

area, this curve was extrapolated to the whole surface area of the studied zone which was estimated to be 2921 km^2 and led to an estimation of a true species richness of 873 species. The average number of species in all combinations of, respectively, one, two, three and four combinations of predefined subsets of stations against the cumulative number of samples in each of these combinations is presented (Fig. 8A, 8B). The regression between these average numbers of species and the logarithm of the cumulative number of samples (Fig. 8C, 8D) is then extrapolated to the true surface area (2921 km^2) of the studied zone considering that each sample corresponds to 0.3 m^2. TS estimate of the total number of polychaete species was 980 when based on the 4 assemblages (Fig. 8A, C) and 1,051 when based on the 4 geographic areas (Fig. 8B, D).

DISCUSSION

Alpha diversity

We used 4 different indices of α-diversity, namely species richness, H', J' and Δ^*. Species richness, H' and J' values ranged between 4 and 33 species per site, between 0.39 and 4.42, and between 0.1 and 1.0, respectively. Provided that slight differences in sampling gears and strategies, these results appear to be close to those of previous studies carried out at similar depth in the Mediterranean Sea. In NW Mediterranean, Salen-Picard *et al.* (2003) reported species richness between 22 and 27 near the mouth of the Rhône River, whereas Nicolaidou & Papadopoulou (1989) reported between 2 and 64 species per site with H' values between 0.59 and 3.67 and J' between 0.37 and 0.90 in the Amvrakikos Bay.

The values of the Δ^* recorded during the present study were between 59.1 and 79.9. We are not aware of any existing comparable data for the Mediterranean polychaete fauna. Using a similar approach, but based on presence/absence data, Arvanitidis *et al.* (2002) computed a similar index (Δ^+) for the overall polychaete fauna of the Black Sea and the Mediterranean using the same (European Register of Marine Species) taxonomic reference as in this study. These authors reported Δ^+ values of 75.0 and 74.9 for the Black Sea and the Mediterranean respectively, which is very close to the results provided by the present study (i.e., a Δ^+ value of 76.2 for our whole data set). This suggests that the data set originating from the present study is large enough to be considered as a random sample of the Mediterranean Sea as far as taxonomic diversity is concerned. The latter is not really at odds with results deriving from species richness, since taxonomic distinctness is known to be largely sampling effort free (Rumohr *et al.* 2001). Shin & Ellingsen (2004) reported lower Δ^+ values (i.e., between 58 and 65 versus 72 and 78 during the present study) for polychaetes inhabiting the subtropical Hong Kong waters. Comparison between these figures is a difficult task because of the discrepancies in the taxonomic classification used to construct the aggregation tables in these two studies. Nevertheless, Shin & Ellingsen (2004) also mentioned the important impact of heavy pollution, trawling and dredging in Hong Kong waters as a possible cause for the rather low diversity they recorded. Disturbance is known to negatively affect average taxonomic distinctness (Warwick & Clarke 1995, 1998) and may thus contribute as well to lower Δ^+ values in Hong Kong waters than in the Gulf of Lions, which is not yet heavily affected by human activities (Rosenberg *et al.* 2003). Overall, the values of the 4 indices of α-diversity measured during the present study were thus within the range of those reported from the relevant literature.

Conversely, the pattern of changes in both H' and J' indices between LFS, LSM and TCM was not similar to the ones already described for polychaete assemblages in the Tyrrhenian Sea (Fresi *et al.* 1983, Gambi & Giangrande 1986). These authors identified polychaete assemblages in relation with sediment characteristics, which allow for a direct comparison to our own results. They reported maximum H' in LSM (heterogeneous substrates) and maximum J' in LFS, whereas in the present study, both H' and J' were maximum in TCM. This difference in the values of the afore-mentioned indices largely resulted from the strong dominance of *Ditrupa arietina* and *Owenia fusiformis*, which both were primarily associated with shallow depths (i.e., both LFS and LSMS) (see also Grémare *et al.* 1998a, b). During the present study, TCM was the only assemblage which was not largely dominated by one or few species, thus resulting in high H' and J' values. Values of Δ^* significantly differed among assemblages as well. It was maximum in LFS and minimum in LSMS. As mentioned above, Δ^* is not affected by dominance since it represents the average taxonomic distance between two individuals belonging to two different species. This distance tended to be higher in LFS than in TCM, LSMN and LSMS, which is consistent with the high diversity usually reported for sandy bottoms (Karakassis & Eleftheriou 1997, Simboura *et al.* 2000).

Summing up, the differences between the values of the 4 indices used during the present study confirms that the use of a single index is not appropriate to assess entirely α-diversity (Ellingsen 2002).

Faunal assemblages and β-diversity

The calculated value of β_w was 9.3. Shin & Ellingsen (2004) found a β_w of 8.6 for the polychaete fauna identified from 101 sites, sampled between 5 and 47 m in subtropical Hong Kong waters. These authors found a β_w of 11.0 for benthic macrofauna as a whole and considered these two figures as indicative of high β-diversity.

The accordance between the spatial distribution of polychaete assemblages and the bathymetric and sedimentary maps of the Gulf of Lions suggest that depth and granulometry gradients are the main factors structuring the composition of polychaete fauna in this area (Labrune *et al.* in press). The effect of granulometry on the composition of benthic fauna is well known and has already been documented for Mediterranean polychaetes (e.g., Simboura *et al.* 2000). This relationship has important consequences on the assessment of β-diversity. Ellingsen (2002) reported that β_w of North Sea macrobenthos correlated positively with the diversity of sampled habitats. She also noticed that the relationship between geographic distance and similarity indices may be confounded by other factors such as depth. Along the same line, Harrison *et al.* (1992) argued that the strength of the relationship between geographic distance and similarity index may be directly associated with differences in environmental variables. During the present study, we tried to unravel the effects of depth by computing β_w and the relationship between the Bray-Curtis similarity index and geographic distance for each assemblage individually. Corresponding β_w values were: 5.2, 2.6, 4.0 and 4.7 for LFS, LSMS, LSMN and TCM respectively, which was higher (except for LSMS) than the ones (i.e., 1.4 and 3.2) reported for North Sea polychaetes by Ellingsen (2001 and 2002, respectively). When comparing the β_w for equal sampling effort (i.e. 9 samples), LSMS featured the lowest value, LSMN the highest one whereas LFS and TCM both featured the same intermediate value. Conversely, the relationship between geographic distance between sites and similarity indices was strongest, but still weak for LSMS ($R^2 = 0.20$ and $R^2 = 0.39$, $p < 0.001$ for Bray-Curtis and Δs respectively) and almost null for the other assemblages. As underlined by Harrison *et al.* (1992) for β-diversity to be structured by distance, species must tend to occur over continuous (rather than discontinuous) ranges. When species ranges are discontinuous β-diversity is not determined by how well species are able to reach available habitat but becomes more a function of niche breadth combined with the spatial structure of environment. The discrepancies between the 2 ways of measuring β-diversity in the present study may thus be related to spatial patchiness in all assemblages but LSMS, which would induce high β_w values but no significant relationship between geographic distance and similarity indices between sites. Therefore, most of the β_w computed per assemblage seems to reflect more small scale spatial pattern in diversity than true species substitution along a gradient as already reported by Goettsch & Hernandez (2006).

As pointed out by Ellingsen (2002), polychaetes appear to be one of the benthic taxa with the highest proportion of widespread species. Beta diversity is thus likely to be even higher for other benthic taxa. This now stresses the need for a comparison of patterns of β-diversity in polychaetes and in other taxa as already achieved by Ellingsen (2002) in the North Sea and by Shin & Ellingsen (2004) in Hong Kong waters.

Total number of species in each assemblage

The total number of species in a given sampled area often cannot be directly computed as the total number of identified species because of sampling limitations. During the present study, none of the 4 assemblages presented a species accumulation curve that reached an asymptote. Several indices have been proposed to infer true species richness (e.g., Chao 1984, 1987, Lee & Chao 1994). Foggo *et al.* (2003b) showed that these indices are sensitive to heterogeneity. We therefore used them to infer total species richness in each assemblage separately. We used Chao_1, Chao_2 and ICE indices, which represent significant improvements over previous non parametric estimators according to Foggo *et al.* (2003b) and which were recommended by (Colwell & Coddington 1994). Based on repeated sub-samplings of a large data base, Gray (2000) concluded that $Chao_2$ tends to underestimate total species richness. Conversely, Foggo *et al.* (2003a) argued that for intermediate sampling efforts $Chao_2$ and ICE tend to overestimate species richness and show poor precision and accuracy while $Chao_1$ represents the best compromise over a wide range of sampling efforts. Colwell & Coddington (1994) concluded that for small data sets, $Chao_2$ provides an accurate estimate of total species richness. There is thus clearly still no agreement on a single estimator of true species richness. As underlined by Mackie *et al.* (2005) and Magurran (2004), there are considerable differences in the significance of the relative merits of each index. Interestingly, all estimators resulted in quite similar total number of polychaete species per assemblage during the present study.

Gamma diversity

During the present study 173 polychaete species were recorded. The extrapolation of the species accumulation curve to the whole sampled area resulted in an estimated overall number of 873 polychaete species. The TS method (Ugland *et al.* 2003) resulted in a total polychaete species richness number of 980 when the whole data set in 4 geographical areas splitting, and of 1,051 when splitting the whole data set in 4 assemblages. These last two estimates were higher because they account for the increase in habitat heterogeneity with the augmentation of sampling area. As stated by Ugland *et al.* (2003), the extrapolation of the accumulation curve obtained by randomization over all the sampled area ignores such a relationship. Conversely, the TS method takes heterogeneity into account by adding a larger proportion of new species when adding new sub-areas or new assemblages. In the present study, the 4 geographical areas contained sites from the 4 polychaete assemblages. Geographical areas were thus much more homogeneous than the 4 polychaete assemblages, which explains that the total number of species estimated using the TS method was lower when based on geographical areas than on polychaete assemblages. Increase in sampling effort over the whole studied area will most probably not result in new polychaete assemblages since similar benthic communities have been described by Picard (1965) and Guille (1971) along the Provençal and Catalan coasts. Therefore the TS method based on assemblages is likely to lead to an overestimation of the true number of species in the whole sampled area. In this sense it can be considered as the opposite extreme relative to the classical species accumulation curve. Conversely, TS method based on geographical areas is mostly dealing with spatial heterogeneity associated with β-diversity, which is likely to increase with spatial scale and it may well result in an appropriate estimation of total species number as already suggested for the macrobenthos of the Norwegian shelf (Ugland *et al.* 2003). Overall, the difference between the total number of species estimated based on the extrapolation of the accumulation curve and on the TS method based on geographical areas was rather low (i.e., less than 11% of the TS value) when compared to the Norwegian continental shelf (about 78% of the TS value, Ugland *et al.* 2003). This probably reflects the higher homogeneity of the areas sampled during the present study.

The typical way of assessing the pertinence of the estimates of total species richness is to compare them with the total number of historically reported species (Ugland *et al.* 2003). Arvanitidis *et al.* (2002) calculated a total of 884 polychaete species to be reported from the literature relevant to the whole Western Mediterranean Sea. Their data set referred to a large variety of polychaete in the whole Western Mediterranean and it seems rather unlikely that our studied area contained more species than this currently compiled value. A delicate point in all extrapolation methods in general and in the TS method in particular is the assignment of a representative area to one single sample. Ugland *et al.* (2003) assumed that one sample was representative of 100 m^2 by roughly estimating the spatial dispersion of sampling units at the same station. However, generally speaking it seems clear that the true number of benthic species over a 100 m^2 area is much higher than the one measured in a few sampling units as for example shown by intensive sampling replication carried out at a single station (Rumohr *et al.* 2001). During the present study, we therefore considered that a single sample was representative of the true sampled surface area (i.e., 0.3 m^2). As an indication of the effect of the assignment of an area to a single sampling unit, we computed that the TS estimate based on geographical areas would have been 726 polychaete species by considering that a single sample is representative of 100 m^2. This value is lower than the value compiled by Arvanitidis *et al.* (2002) for the NW Mediterranean Sea. There is thus clearly a large uncertainty on the estimates of total polychaete species richness based on the TS method and further works are clearly needed to assess: (1) the effect of the interaction between the pattern of spatial heterogeneity and sampling design, and (2) the area represented by one single sample on TS estimates.

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LITERATURE CITED

Aller RC, Yingst JY 1985. Effects of the marine deposit-feeders *Heteromastus filiformis* (Polychaeta), *Macoma balthica* (Bivalvia), and *Tellina texana* (Bivalvia) on averaged sedimentary solute transport, reaction rates, and microbial distributions. *J Mar Res* 43(3): 615-645.

Aloisi, JC, Got, H, Monaco A 1973. Carte géologique du précontinent languedocien au 1/250000^e. Netherlands: Enschede: Intern Instit Aerial Survey and Earth Sciences.

Arvanitidis C, Bellan G, Drakopoulos P, Valavanis V, Dounas C, Koukouras A, Eleftheriou A 2002. Seascape biodiversity patterns along the Mediterranean and the Black Sea: lessons from the biogeography of benthic polychaetes. *Mar Ecol Prog Ser* 244: 139-152.

Bianchi CN, Mori C 2000. Marine biodiversity of the Mediterranean Sea: situation, problems and prospects for future research. *Mar Poll Bull* 40: 367-376.

Borja A, Franco J, Pérez V 2000. A Marine Biotic Index to Establish the Ecological Quality of Soft-Bottom Benthos Within European Estuarine and Coastal Environments. *Mar Poll Bull* 40(12): 1100-1114.

Bray JR, Curtis JT 1957. An ordination of the upland forest communities of southern Wisconsin. *Ecol Monogr* 27: 325-349.

Chao A 1984. Nonparametric estimation of the number of classes in a population. *Scand J Statistics* 11:265-270.

Chao A 1987. Estimating the Population Size for Capture-Recapture Data with Unequal Catchability. *Biometrics* 43: 783-791.

Clarke KR, Ainsworth M 1993. A method of linking multivariate community structure to environmental variables. *Mar Ecol Prog Ser* 92: 205-219.

Clarke KR, Warwick RM 2001. Change in marine communities: an approach to statistical analysis and interpretation. PRIMER-E: Plymouth.

Colwell RK 1997. EstimateS: statistical estimation of species richness and shared species from samples. Version 5. User's guide and application. Available at: http://viceroy.eeb.uconn.edu/estimates.

Colwell RK, Coddington JA 1994. Estimating terrestrial biodiversity through extrapolation. *Phil Trans Roy Soc London B* 345: 101-118.

Danovaro R 2003. The unknown: identification of the critical information gaps: information gaps for important fish and invertebrates resources and impact of introduced and/or immigrant species; information gaps on species/area relationships in the Mediterranean and the Black Sea; information gaps concerning important habitats in different geographical regions. *In* Arvanitidis C *et al.* Electronic conference on ‘Marine Biodiversity in the Mediterranean and the Black Sea’: summary of discussions, 7 to 20 April, 2003: 37-45.

Duarte C, Jaume D, Vanden Berghe E, van Avesaath PH, Heip CHR, Mees J 2002. Electronic conference on ‘European Heritage under Threat: Marine Biodiversity in Mediterranean Ecosystems’ - Summary of discussions. Flanders Marine Institute (VLIZ): Oostende, Belgium.

Ellingsen KE 2001. Biodiversity of a continental shelf soft-sediment macrobenthos community. *Mar Ecol Prog* Ser 218: 1-15.

Ellingsen KE 2002. Soft-sediment benthic biodiversity on the continental shelf to environmental variability. *Mar Ecol Prog Ser* 232: 15-27.

Foggo A, Attrill MJ, Frost MT, Rowden AA 2003a. Estimating marine species richness: an evaluation of six extrapolative techniques. *Mar Ecol Prog Ser* 248: 15-26.

Foggo A, Rundle SD, Bilton DT 2003b. The net result: evaluating species richness extrapolation techniques for littoral pond invertebrates. *Freshw Biol* 48: 1756-1764.

Fresi E, Gambi MC, Focardi S, Bargagli R, Baldi F, Falciai L 1983. Benthic Community and Sediment Types: A Structural Analysis. *Mar Ecol* 4(2): 101-121.

Gambi MC, Giangrande A 1986. Distribution of soft-bottom polychaetes in two coastal areas of the Tyrrhenian Sea (Italy): Structural analysis. *Est Coast Shelf Sci* 23(6): 847-862.

Gilek M, Björk M, Broman D, Kautsky N, Kautsky U, Näf C 1997. The role of the blue mussel, *Mytilus edulis*, in the cycling of hydrophobic organic contaminants in the Baltic Proper. *Ambio* 26(4): 202-209.

Goettsch B, Hernandez HM 2006. Beta diversity and similarity among cactus assemblages in the Chihuahuan Desert. *J Arid Env* 65(4): 513-528.

Grassle JF, Maciolek NJ 1992. Deep-sea richness: regional and local diversity estimates from quantitative bottom samples. *Am Nat* 139(2): 313-341.

Gray JS 2000. The measurement of marine species diversity, with an application to the benthic fauna of the Norwegian continental shelf. *J Exp Mar Biol Ecol* 250: 23-49.

Gray JS, Poore GCB, Ugland KI, Wilson RS, Olsgard F, Johannessen Ø 1997. Coastal and deep-sea benthic diversities compared. *Mar Ecol Prog Ser* 159: 97-103.

Grémare A, Amouroux JM, Vétion G 1998a. Long-term comparison of macrobenthos within the soft bottoms of the Bay of Banyuls-sur-Mer (Northwestern Mediterranean Sea). *J Sea Res* 40(3-4): 281-302.

Grémare A, Sardá R, Medernach L, Jordana E, Pinedo S, J.M. A, Martin D, Nozais C, Charles F 1998b. On the dramatic increase of *Ditrupa arietina* O.F. Müller (Annelida: Polychaeta) along the French and Spanish Catalan coasts. *Est Coast Shelf Sci* 47: 447-457.

Guille A 1971. Bionomie benthique du plateau continental de la côte catalane française IV-Densités, biomasses et variations saisonnières de la macrofaune. *Vie Milieu* 22(1B): 93-158.

Gunnarsson J, Björk M, Gilek M, Granberg M, Rosenberg R 2000. Effects of eutrophication on contaminant cycling in marine benthic systems. *Ambio* 29(4): 252-259.

Harrison S, Ross SJ, Lawton JH 1992. Beta diversity on geographic gradients in Britain. *J Anim Ecol* 61: 151-158.

Hutchings P 1998. Biodiversity and functioning of polychaetes in benthic sediments. *Biodiv Cons* 7: 1133-1145.

Izsak C, Price ARG 2001. Measuring ß-diversity using a taxonomic similarity index, and its relation to spatial scale. *Mar Ecol Prog Ser* 215: 69-77.

Karakassis I 1995. S ∞: A new method for calculating macrobenthic species richness. *Mar Ecol Prog Ser* 120: 299-303.

Karakassis I, Eleftheriou A 1997. The continental shelf of Crete: structure of macrobenthic communities. *Mar Ecol Prog Ser* 160: 185-196.

Koleff P, Gaston KJ, Lennon JJ 2003. Measuring beta diversity for presence-absence data. *J Anim Ecol* 72: 367-382.

Labrune C, Grémare A, Amouroux JM, Sardá R, Gil J, Taboada S. Assessment of soft-bottom polychaete assemblages in the Gulf of Lions (NW Mediterranean) based on a mesoscale survey. *Est Coast Shelf Sci* (in press).

Labrune C, Grémare A, Amouroux JM, Sardá R, Gil J, Taboada S. Structure and diversity of shallow soft-bottom macrofauna in the Gulf of Lions (NW Mediterranean). Submitted to *Helgol. Mar Res.*

Lee SM, Chao A 1994. Estimating population size via sample coverage for closed capture-recapture models. *Biometrics* 50: 88-97.

Mackie ASY, Oliver PG, Darbyshire T, Mortimer K 2005. Shallow marine benthic invertebrates of the Seychelles Plateau: high diversity in a tropical oligotrophic environment. *Phil Trans Roy Soc London* A 363: 203-228.

Magurran AE 2004. Measuring Biological Diversity. Blackwell Science. 256p.

Medernach L, Jordana E, Grémare A, Nozais C, Charles F, Amouroux JM 2000. Population dynamics, secondary production and calcification in a Mediterranean population of *Ditrupa arietina* (Annelida: Polychaeta). *Mar Ecol Prog Ser* 199: 171-184.

Nicolaidou A, Papadopoulou KN 1989. Factors affecting the distribution and diversity of Polychaetes in Amvrakikos Bay, Greece. *Mar Ecol* 10(3): 193-204.

Olsgard F, Somerfield PJ, Carr R 1997. Relationship between taxonomic resolution and data transformations in analyses of a macrobenthic community along an established pollution gradient. *Mar Ecol Prog Ser* 149: 173-181.

Pearson TH, Rosenberg R 1978. Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. *Oceanogr Mar Biol Annu Rev* 16: 229-311.

Pérès JM 1967. The Mediterranean benthos. *Oceanogr Mar Biol Annu Rev* 5: 449-533.

Picard J 1965. Recherches qualitatives sur les biocénoses marines des substrats meubles dragables de la région marseillaise. *Rec Trav St Mar Endoume* 52(36): 1-160.

Pocklington P, Wells PG 1992. Polychaete - Key taxa for marine environmental quality monitoring. *Mar Poll Bull* 24(12): 593-598.

Rosenberg R, Blomqvist M, Nilsson HC, Cederwall H, Dimming A 2004. Marine quality assessment by use of benthic species-abundance distributions: a proposed new protocol within the European Union Water Framework Directive. *Mar Poll Bull* 49(9-10): 728-739.

Rosenberg R, Nilsson H, Grémare A, Amouroux JM 2003. Effects of demersal trawling on marine sedimentary habitats analysed by sediment profile imagery. *J Exp Mar Biol Ecol* 285-286: 465-477.

Rumohr H, Karakassis I, Jensen JN 2001. Estimating species richness, abundance and diversity with 70 macrobenthic replicates in the Western Baltic Sea. *Mar Ecol Prog Ser* 214: 103-110.

Salen-Picard C, Arlhac D, Alliot E 2003. Responses of a Mediterranean soft bottom community to short-term (1993-1996) hydrological changes in the Rhône River. *Mar Env Res* 55: 409-427.

Sardá R, Pinedo S, Martin D 1999. Seasonal dynamics of macroinfaunal key species inhabiting shallow soft-bottom in the Bay of Blanes (NW Mediterranean). *Acta Oecol* 20(4): 315-326.

Shin PKS, Ellingsen KE 2004. Spatial patterns of soft-sediment benthic diversity in subtropical Hong Kong waters. *Mar Ecol Prog Ser* 276: 25-35.

Simboura N, Nicolaidou A, Thessalou-Legaki M 2000. Polychaete Communities of Greece: An Ecological Overview. *Mar Ecol* 21: 129-144.

Simboura N, Zenetos A 2002. Benthic indicators to use in Ecological Quality classification of Mediterranean soft bottom marine ecosystems, including a new Biotic Index. *Med Mar Sci* 3(2): 77-111.

Sisk TD, Launer AE, Switky KR, Ehrlich PR 1994. Identifying extinction threats: global analyses of the distribution of biodiversity and the expansion of the human enterprise. *Bioscience* 44: 592-602.

Snelgrove P, Blackburn T, Hutchings P, Alongi D, Grassle J, Hummel H, King G, Koike I, Lambshead P, Ramsing N, Solis-Weiss V 1997. The importance of marine sediment biodiversity in ecosystem processes. *Ambio* 26(8): 578-583.

Ugland KI, Gray JS, Ellingsen KE 2003. The species-accumulation curve and estimation of species richness. *J Anim Ecol* 72: 888-897.

Ugland KI, Gray JS, Lambshead PJD 2005. Species accumulation curves analysed by a class of null models discovered by Arrhenius. *Oikos* 108(2): 263-274.

Warwick RM, Clarke KR 1995. New 'biodiversity' measures reveal a decrease in taxonomic distinctness with increasing stress. *Mar Ecol Prog Ser* 129: 301-305.

Warwick RM, Clarke KR 1998. Taxonomic distinctness and environmental assessment. *J Appl Ecol* 35: 532-543.

Whittaker RH 1960. Vegetation of the Siskiyou Mountains, Oregon and California. *Ecol Monogr* 30: 279-338.

Whittaker RH 1972. Evolution and measurement of species diversity. *Taxon* 21(2/3):213-251.

Wilson MV, Shmida A 1984. Measuring beta diversity with presence-absence data. *J Ecol* 72: 1055-1064.

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