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Variance estimate and taxonomic resolution: an analysis of macrobenthic spatial patterns at different scales in a Western Mediterranean coastal lagoon

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Abstract

The effects of taxonomic resolution on the variance estimates of macrobenthic assemblages were studied at four spatial scales in a Mediterranean coastal lagoon. The assemblages exhibited significant differences at all the investigated scales; however, spatial variability was mainly associated with the smallest and the largest scales. The decrease of taxonomic resolution (from species to family) was not related to a decrease of the overall variability and similar estimates of variance components were obtained using species and family resolution levels. The ordination models derived from species and family abundances were very similar both in terms of location and dispersion effect, while further aggregation to the class level began to alter the observed spatial patterns. In future studies aimed at assessing changes in the lagoon, resources derived from the cost reductions achieved using family level could be employed to plan more frequent surveys and/or to adopt complex spatial sampling designs with a high number of replicates.

KEY WORDS: spatial scales - taxonomic resolution - multivariate analysis - macrobenthos - coastal lagoon - monitoring - Western Mediterranean

28

29 **1. Introduction**

30 Macrobenthic invertebrates are an essential component in soft-sediment environments playing
 31 important roles in ecosystem processes, such as dispersion, burial, nutrient cycling and energy
 32 flow (Snelgrove, 1998). A deep knowledge of spatial variability patterns in macrobenthic
 33 assemblages is relevant to properly characterise one of the major sources of biotic diversity in
 34 natural environments; moreover, such information represents a requirement to develop
 35 strategies of management and conservation (e.g. Lubchenco et al., 1991), as well as to advise
 36 suitable guidelines for periodical monitoring programs (e.g. Underwood, 1997). Soft bottom
 37 environments are usually considered homogeneous habitats; however structural analyses in
 38 marine and brackish systems have repeatedly demonstrated that patchiness of macrofaunal
 39 assemblages is a common feature at both small-medium scales, (Thrush et al., 1989; Hewitt et
 40 al., 2002; Noren and Lindegarth, 2005) and large scales (Morrisey et al., 1992; Edgar and
 41 Barret, 2002; Ysebaert and Herman, 2002). In particular, those previous studies emphasized
 42 the lacking of a single correct scale at which assemblages can be described (Levin, 1992),
 43 since different patterns of distribution can be obtained depending on the spatial scale of
 44 observation. The description of the distributional patterns at multiple spatial scales and
 45 identification of the most relevant ones are needed to formulate possible explanations about
 46 ecological processes, or unnatural impacts structuring ecosystems (Underwood and Chapman,
 47 1996; Underwood et al., 2000; Ysebaert et al., 2003). Furthermore, such information can be
 48 useful for avoiding erroneous interpretations of spatial patterns observed at a particular scale
 49 and also to advise useful guidelines for routine environmental monitoring programs.

50 In order to estimate the proportion of variability associated with each examined scale and to
 51 identify the most relevant spatial scale, the hierarchical sampling approach is considered the
 52 most appropriate method (Underwood, 1997; Hewitt et al., 1998). In hierarchical designs

53 small-scaled sampling units are nested within larger-scaled ones, allowing unconfounded
 54 statistical comparisons among each spatial scale (Underwood, 1981; Kotliar and Wiens,
 55 1990). Nested designs have been successfully used to investigate populations and
 56 assemblages across a wide range of marine habitats and organisms. Most studies focused on
 57 intertidal and subtidal rocky shores (*i.a.* Archambault and Bourget, 1996; Underwood and
 58 Chapman, 1996; Benedetti-Cecchi, 2001a; Fraschetti et al., 2001; 2005; Chapman and
 59 Underwood, 2008), while soft-bottoms have been less explored (Morrissey et al., 1992; Stark
 60 et al., 2003; Noren and Lindegarth, 2005; Terlizzi et al., 2008b).
 61 The increase of soft bottom macrobenthic studies is often hidden because identifying and
 62 enumerating all organisms are time-consuming and labour-intensive processes (Warwick,
 63 1993; Olsgard et al., 1998), that requires taxonomic expertise (Terlizzi et al., 2003). In the
 64 last two decades, many studies have analysed data at several taxonomic resolutions, showing
 65 that results obtained using species or family level are very similar (*i.a.* Warwick, 1988;
 66 Vanderklift et al., 1996; Olsgard et al., 1998; Karakassis and Hatziyanni, 2000; Lampadariou
 67 et al., 2005; Wlodarska-Kowalczyk and Kedra, 2007). Identifying a taxonomic level higher
 68 than species that is sufficient for detecting differences in assemblage composition without
 69 losing important information is a concept termed “taxonomic sufficiency” (TS, Ellis, 1985).
 70 The TS method might have some practical implications, in particular routine monitoring
 71 programs could become less expensive and faster than those conducted at the species level
 72 resolution and therefore macrobenthic assemblages could be analysed more frequently.
 73 However, at present, most studies have usually compared different levels of TS at a single
 74 spatial scale (Vanderklift et al., 1996; Olsgard et al., 1998; Karakassis and Hatziyanni, 2000;
 75 De Biasi et al., 2003; Wlodarska-Kowalczyk and Kedra, 2007), while few researchers have
 76 investigated the effects of TS on the spatial distribution patterns observed at multiple scales
 77 (Chapman, 1998; Anderson et al., 2005; Dethier and Schoch, 2006). Moreover in these

78 previous works, spatial variability was usually not distinguished in relation to differences in
79 location or dispersion among groups of samples, while the ecological heterogeneity is
80 considered a valuable feature of any habitat which can provide important information on
81 biological assemblages (Anderson, 2006; Terlizzi et al., 2008a).

82 In the present study, abundance and composition of soft bottom benthic macrofaunal
83 assemblages in a Western Mediterranean coastal lagoon were described with particular
84 attention to their variability across different spatial scales. The Santa Giusta lagoon can be
85 considered as representative of small microtidal brackish environments characterizing the
86 Mediterranean region (Basset et al., 2006). Coastal lagoons are areas of considerable
87 naturalistic interest but often are located close to urban or industrial centres, therefore they are
88 possibly affected by direct (e.g. sewage discharge, aquaculture) or indirect (e.g.
89 eutrophication) human activities (*i.a.* Barnes, 1991; Lardicci et al., 2001). Given the
90 naturalistic and economic importance of these biotopes, research that may provide appropriate
91 quantitative data is relevant for their conservation and management.

92 In this study, a hierarchical sampling design including four spatial scales (ranging from
93 meters up to thousands of metres) was used *i)* to estimate the relative importance and test
94 statistical significance of macrofauna variability at different spatial scales, in order to identify
95 the spatial scale associated with the highest variability; *ii)* to examine if spatial patterns are
96 influenced mainly by changes in species composition or relative abundances, comparing
97 results obtained from several transformations of species abundance; and *iii)* to analyse if
98 lower levels of taxonomic resolution (family and class) show similar spatial patterns with
99 respect to those obtained at species level, both in terms of location and dispersion effects.

100 Results will allow to increase the knowledge of macrobenthic spatial distribution in Santa
101 Giusta lagoon and to assess the applicability of TS method for decreasing time and cost in
102 subsequent routine surveys. The methodological approach employed in the present

investigation could provide interesting practical implications for future studies, not only in this lagoon, but also in other similar brackish environments.

2. Materials and Methods

2.1 Study area and sampling

The Santa Giusta lagoon (Western Mediterranean, Italy) is one of the largest coastal brackish environments of Sardinia island, it is a polyhaline basin located along the central-western coast of Sardinia. The lagoon is included in the Ramsar convention (1971) and belongs to a complex system of transitional waters of high natural and economic value. The Santa Giusta lagoon is approximately circular in shape with an area of 7.9 km² and a mean depth of 1 m; it is located near the town of Oristano and Santa Giusta, in the plain of Pesaria, an agricultural area that is intensively cultivated with rice. The lagoon has no natural attributes and is separated from the sea by a longshore bar, it is also connected with two inner small basins called Pauli Maiori and Pauli Figu (Figure 1). Central and peripheral canals have been dredged about 2 m deep in order to facilitate seawater flow into the lagoon. As a consequence, waters of Santa Giusta lagoon are now well mixed as regards circulation and stratification (Sechi et al., 2001). Salinity ranges from 25‰ to 42‰, with a mean annual value of 30‰ (Sechi et al., 2001; Luglié et al., 2002). There is a prevalent sandy-muddy bottom, with small patches of both macroalgae and angiosperms (e.g. *Enteromorpha* sp., *Gracilaria* sp., *Ruppia cirrhosa*, *Zostera* sp.), which are distributed all over the lagoon.

In this context, three sampling zones were randomly selected among the three habitats in the lagoon with different sediment and hydrodynamic properties (Luglié et al., 2002) (Figure 1). Specifically, the alpha zone was in the central-northern part of the lagoon and it was mostly influenced by the urban and industrial wastewaters from Oristano and Santa Giusta areas. It has been considered a low-intermediate hydrodynamic energy environment with a prevalence

128 of clay-silty sediments (Luglié et al., 2002). The beta zone was in the central-southern part of
 129 the lagoon and possibly influenced by the drainage from the surrounding farmlands. This zone
 130 was characterised by an intermediate-high hydrodynamic energy and sand-silty sediments
 131 (Luglié et al., 2002). Then the gamma zone was an area near the Pesaria canal, connecting the
 132 lagoon with the sea; it was characterized mainly by sandy sediments and high hydrodynamic
 133 energy (Luglié et al., 2002), being closer to the sea (Figure 1). Samples were collected in
 134 November 2002, according to a hierarchical sampling design. Within each of the three zones,
 135 four random sites were selected and within each site, four areas were randomly chosen. Two
 136 replicate samples were taken within each area for a total of 96 samples. Spatial variability was
 137 estimated at four hierarchical scales: among zones (10^3 m apart), among sites within zones
 138 (10^2 m apart), among areas within sites (10s m apart) and among replicates (1 m apart). Since
 139 small vegetal patches are widely distributed throughout the lagoon, replicate samples were
 140 carefully placed away from vegetal patches (at least 5 m from the closest patch), in order to
 141 minimise the possible effects of background heterogeneity on macrofaunal composition. Soft-
 142 sediment samples were collected on bare bottom with a box-corer (10×17 cm²), sieved
 143 through a 0.5 mm mesh and preserved in 4 % formaldehyde. All collected macrozoobenthic
 144 organisms were sorted and identified to the species level and abundances (number of
 145 specimens per taxon) were calculated. Time needed for classification and counting of diverse
 146 taxa was recorded. All analyses were performed by researchers, with low experience in
 147 taxonomic identification but supported by skilled taxonomists. The time spent to identify all
 148 organisms at the species, family and class levels was 255, 95 and 5 hours, respectively.

149

150 **2.2 Statistical analyses**

151 Permutational multivariate analysis of variance (PERMANOVA; Anderson, 2001) was used
 152 to test the null hypothesis of no differences among assemblages at different spatial scales,

153 according to a three factors (zone, site, area) nested design through 4999 permutations of
154 residuals under a reduced model (Anderson and Ter Braak, 2003). At species level, data were
155 analysed using the Bray-Curtis dissimilarity measure on untransformed and transformed data
156 (square-root, fourth-root, presence/absence), in order to evaluate if assemblages are mainly
157 driven by compositional or relative abundance changes. Using stronger and stronger
158 transformations, the emphasis of results can be shift from the most abundant species to the
159 rarest ones; in particular, variability measured by presence/absence data reflects only
160 compositional changes, while variation in relative abundances is more important in analyses
161 based on other transformations or untransformed data (Clarke and Gorley, 2001; Anderson et
162 al., 2005b). Furthermore, mean squares calculated by PERMANOVA were used to estimate
163 multivariate variance components associated at each spatial scale, in a way analogous to
164 univariate partitioning using ANOVA (Searle et al., 1992; Benedetti-Cecchi, 2001b). For a
165 better comparison, in y-axis the variability at each spatial scale was expressed as square-root
166 of variance components; therefore, the values could be interpreted as percentages of Bray-
167 Curtis dissimilarity (Anderson et al., 2005a). Separate analyses were performed using the
168 square-root transformed data at species, family and class levels of taxonomic resolution. The
169 family level was chosen because it has been often indicated as the most effective in
170 minimizing the cost-benefit ratio (Lardicci and Rossi, 1998; Karakassis and Hatziyanni, 2000;
171 De Biasi et al., 2003; Lampadariou et al., 2005), while the class level was chosen to assess the
172 effectiveness of a further higher resolution. Since a significant result for a given factor from
173 PERMANOVA could indicate that the groups differ in their location and/or dispersion,
174 PERMDISP analyses were also performed to focus only on dispersion effects, testing the
175 factors “zone” and “site” (Anderson, 2006). Analogous analyses were performed using
176 separate data sets for the three main taxonomic groups.

177 The local species richness was visualised as a function of number of replicate samples in
 178 species-sample accumulation curves based on 999 permutations. To visualize multivariate
 179 patterns in assemblages across the three zones, non-metric multidimensional scaling (nMDS)
 180 ordination plots were produced. A separate plot was done for the overall species community
 181 and also for data aggregated at family and class level of resolution. All plots were done on the
 182 basis of Bray-Curtis dissimilarity matrix of square-root transformed data. To evaluate the
 183 degree of similarity among matrices obtained using different taxonomic aggregations, the
 184 RELATE routine was used to test the null hypothesis of independence of the two similarity
 185 matrices. On the other hand, in order to detect which species contributed most to dissimilarity
 186 among the three different zones, a similarity percentage (SIMPER) routine was performed
 187 (cut off 80%) (Clarke, 1993). All accumulation curves, nMDS plots, RELATE tests and
 188 SIMPER results were obtained using the PRIMER v.6 software (Clarke and Gorley, 2001).

190 **3. Results**

191 **3.1 Faunal composition**

192 A total of 23 878 individuals belonging to 83 species, 43 families, and 4 classes were
 193 collected. The time spent to identify all organisms at the species, family and class levels was
 194 255, 95 and 5 hours, respectively.

195 Considering the number of individuals, crustaceans accounted for 43.5% of total abundance
 196 followed by polychaetes and molluscs representing respectively 30.0% and 26.5% of total
 197 abundance. Instead, considering the number of species, polychaetes were the most
 198 representative group (54 species) followed by crustaceans (19) and molluscs (10). The
 199 number of species per family varied widely (Figure 2), with most of families (29) represented
 200 by only one species. The most species-rich families were within the polychaete class (e.g. 9
 201 Syllidae, 6 Spionidae, 4 Capitellidae, 4 Phyllodocidae and 4 Serpulidae) and to a lesser extent

in the crustaceans (e.g. 3 Corophiidae and 3 Gammaridae). The majority of crustaceans (12 species) and molluscs (7 species) spanned a large part of the lagoon, being recorded in more than 12 of the 48 sampling areas; whereas most polychaetes (24 species) were restricted in less than 12 sampling areas (Figure 3). Seven species (five polychaetes and two crustaceans) were limited to a single area and they were also represented by only one individual. Five species (four polychaetes and one mollusc) were restricted to only two sampling areas and were represented by very few individuals (Figure 3). The mean abundance values for polychaetes and crustaceans were quite similar in all three sampling zones of the study site, while molluscs showed a higher variability being the most abundant taxa in gamma, but almost absent in alpha zone (Figure 4).

The local species richness was higher in the alpha zone than in beta or gamma ones and cumulative samples from each zone were representative reaching an asymptote rather quickly (Figure 5). In particular, the number of species collected would be just slightly reduced, even analysing only 2 sampling sites (namely 8 areas or 16 replicates) in each zone of the lagoon (Table 1). A highly significant difference in the faunal composition of the three zones was detected by SIMPER analysis, with the greatest dissimilarity recorded for the alpha - gamma zone pair (75%), followed by the beta - gamma (69%) and the alpha - beta (68%) ones.

In alpha zone, despite the smallest number of individuals (5 550), the highest number of species (75) was recorded, with five species that accounted for 60.4% of total abundance. This group included species typical of brackish habitats, such as *Monocorophium sextonae*, *Cymodoce truncata*, *Hydroides elegans* and *Corophium acherusicum* (Ruffo, 1998) and a species typical of sandy-muddy bottom such as *Pseudopolydora antennata* (Lardicci et al., 2001). Moreover, SIMPER analyses highlighted the value of other species in determining the dissimilarity among the three zones: a polychaete, *Cirriformia tentaculata*, and a crustacean occurring in areas with abundant algal coverage, *Pseudolirius kroyeri* (Table 2). In the beta

zone 60 species were found and the five most abundant ones (51.3% of total 7 601 individuals) were typical brackish species like *Cymodoce truncata*, *Abra ovata*, *Monocorophium sextonae*, as well as *Minuspio multibranchiata* and *Microdeutopus anomalus*, occurring where macroalgae are present (Ruffo, 1998). Opportunistic species indicating organic enrichment (Pearson and Rosenberg, 1978; Cognetti, 1982), such as *Phylofoetida* and *Neanthes caudata*, were also characteristic of the beta zone (Table 2), as well as suspension feeders (*Loripes lacteus* and *Cerastoderma glaucum*) and grazers (*Cumella limicola* and *Iphinoe serrata*). In gamma zone 10 730 organisms from 59 species were collected and 80% of total abundance was reached with only five species: *Mytilaster minimus* (alone accounting for 33%), *Cymodoce truncata* and *Tanais dulongii*, all typical of brackish environments, besides *Minuspio multibranchiata* and *Naineris laevigata*.

238

239 **3.2 Scales of multivariate spatial variability and taxonomic resolution**

At the taxonomic level of species, PERMANOVA showed that there was a highly significant variability at all spatial scales considered (Table 3). The greatest variability occurred at the largest spatial scale, among zones, for which the average Bray-Curtis dissimilarity was around 37%; then, the successive variation component was that among replicate samples (35% of dissimilarity), followed by less variability among areas (22%) and sites (20%) (Figure 6). Furthermore, the relative importance of different spatial scales in the hierarchy did not vary with different data transformations. Similar spatial patterns were obtained for analyses based on untransformed and transformed data (square root, fourth root, presence/absence) (Figure 6).

Highly significant variability at all spatial scales was also detected, at the family and class levels (Table 3). In addition, similar variance components, as well as the relative importance of different spatial scales, were maintained proceeding from species to family analysis (Figure

252 7). Instead, using the class level of resolution, the variance components decreased showing
 253 less dissimilarity among assemblages at all spatial scales analysed; moreover, proportional
 254 amount of variation changed showing the highest variability at the smallest scale, among
 255 replicate samples (Figure 7). At the species level, differences among zones were mainly due
 256 to differences in their location, since a significant dispersion effect was revealed only between
 257 alpha and gamma zones (Table 4). At the scale of site, the source of variability changed
 258 depending on the sampling zone as emerged by pairwise tests, within the alpha zone some
 259 sites were not significantly different from each other (i.e. P-values of both PERMANOVA
 260 and PERMDISP tests were not significant), in some cases sites differed in their location (i.e.
 261 P-values of PERMANOVA significant, P-values of PERMDISP not significant), in other
 262 cases sites differed both in their location and dispersion (i.e. P-values of both PERMANOVA
 263 and PERMDISP tests were significant). Similar results were obtained within the beta zone,
 264 where sites were different also because of their dispersion (i.e. P-values of PERMANOVA
 265 not significant, P-values of PERMDISP significant). Dispersion effect never contributed to
 266 differences among sites in the gamma zone (Table 4). All but two pairwise results were
 267 likewise detected using the family level of taxonomic resolution; while at the class level, the
 268 majority of results were not significant, therefore indicating different relationships compared
 269 to those obtained at finer taxonomic levels, both at zone and site spatial scales (Table 4).
 270 Separate analyses for the three collected taxonomic groups showed a highly significant
 271 variability at all spatial scale; only the variance component for molluscs at the site scale was
 272 found not different from zero because of the greater variability at the smaller spatial scale of
 273 area (Figure 8). Such results matched the pairwise tests which showed that in very few cases
 274 sites differed, mainly in their dispersion (Table 5). For crustaceans and molluscs the greatest
 275 variability occurred at the largest spatial scale (Figure 8) and for both groups it was mainly
 276 due to differences in location among zones (Table 5). For polychaetes, the sources of the high

variability at the zone scale were differences in location and dispersion (Table 5); however polychaetes showed the greatest variability among replicate samples and such spatial scale was also important for the other taxonomic groups (Figure 8). Except for molluscs, a small variability was associated with the two intermediate spatial scales (Figure 8); in particular at the site scale, there was a prevalent location effect both for polychaetes and crustaceans although differences in dispersion were also detected especially for crustaceans within the beta zone (Table 5).

The nMDS plot based on the species abundance data showed some differences among the three sampled zones. In particular, gamma samples were clearly clustered and separated from the other zones, while alpha and beta samples were partially overlapped (Figure 9a). At the family level, nMDS ordination was very similar to that obtained at the species level (Figure 9b). Further aggregation to the class level produced a different ordination pattern, with substantially higher levels of overlapping of the three zones (Figure 9c). Relationships between similarity matrices calculated for the three taxonomic levels were confirmed by RELATE results, which showed $\rho = 0.960$, $p < 0.001$ between species and family levels and $\rho = 0.565$, $p < 0.001$ between species and class levels.

4. Discussion

The first result that stands out from our work is that the benthic assemblages of Santa Giusta lagoon were extremely variable, with significant differences at all considered spatial scales, from metres up to thousands of metres. This outcome was highly consistent with results reported in studies analysing spatial variability by nested design, whatever the habitat investigated (see Fraschetti et al., 2005 for a review). In the Santa Giusta lagoon most of the variation was associated with the smallest and the largest spatial scale, thus indicating that both small-scale and large-scale processes play a major role in shaping benthic community

302 spatial patterns. Variability among replicates at small spatial scale is usually considered a
 303 widespread feature of many different assemblages, being mainly determined by biological
 304 interactions and/or local physical factors (*i.a.* Ekman, 1979; Underwood and Chapman, 1986;
 305 Wilson, 1991; Morrissey et al., 1992; Benedetti Cecchi et al., 2001a; Coleman et al., 2002;
 306 Rossi and Lardicci, 2002; Frascchetti et al., 2005; Chapman and Underwood, 2008). On the
 307 other hand, differences in assemblages at large spatial scales have been mostly related to
 308 abiotic processes (Thrush et al., 1989; Thrush, 1991). Factors such as hydrodynamic energy,
 309 trophic status, seawater and freshwater influence, nutrients supply and confinement could
 310 differently characterise the three sampling zones of the Santa Giusta lagoon, according to the
 311 models of zonation proposed for other Mediterranean coastal lagoons (Guelorget and
 312 Perthuisot, 1982; Lardicci et al., 1993; 1997; Pérez-Ruzafa et al., 2007). In addition, benthic
 313 communities could be unevenly subjected to the two main sources of anthropogenic
 314 disturbance affecting this lagoon at all the study scales. Results of this study showed that the
 315 three sampling zones were clearly distinct and characterised by typical features but analysing
 316 and explaining the effects of abiotic or biotic factors responsible of such differences were not
 317 among the explicit aims of this study.

318 A number of papers reported that results of statistical analyses can be greatly influenced by
 319 the choice of data transformation; in fact, the ability to detect differences along strong
 320 environmental gradients was affected more by changing the data transformation rather than
 321 the level of taxonomic identification (*i.a.* Olgard et al., 1998; Karakassis and Hatziyanni,
 322 2000). This is also consistent with results by Chapman (1998) and Lasiak (2003), who
 323 observed that the type of transformation altered patterns of variability within sites, which may
 324 be important for some research programmes. The choice of transformation determines the
 325 relative contribution of quantitative and qualitative intersample differences in the final
 326 outcome of all multivariate analyses. Strong transformations (fourth root, presence/absence)

327 give little weight to differences in abundance, whereas weak (square root) or null
 328 transformations provide patterns mainly reflecting the differences of the most abundant
 329 species (Olsgard et al., 1998; Karakassis and Hatziyanni, 2000; Clarke and Gorley, 2001). In
 330 particular, the variability estimated by analysing presence/absence data explicitly reflects the
 331 compositional changes of assemblages at different spatial or temporal scales and this can be
 332 compared with analyses based on other transformations (or untransformed data), mostly
 333 describing relative abundance differences (Anderson et al., 2005b). On this basis, some
 334 studies demonstrated that variability of benthic assemblages at larger scales is mainly
 335 “compositional”, as indicated by the presence/absence analyses, while variability at medium
 336 or smaller scales is driven by changes in relative abundance, particularly by numerically
 337 dominant taxa (Archambault and Bourget, 1996; Chapman, 1998; Anderson et al., 2005b).
 338 Conversely, our results showed that the relative importance of examined spatial scales (from
 339 meters up to thousands of metres) was always maintained, despite different transformations
 340 used. These findings underpinned that in Santa Giusta lagoon, large scale processes shaped
 341 three distinct zones characterized by different species. At the other investigated scales,
 342 differences in species composition were possibly caused by the presence of diverse
 343 microhabitats, which created high spatial heterogeneity. For example, small vegetal patches
 344 might possibly influence the faunal composition of the surrounding bare bottoms; however,
 345 such patches are distributed all over the lagoon, thus producing a high spatial heterogeneity at
 346 the smallest spatial scale in all the three sampling zones of Santa Giusta. This outcome
 347 indicated that spatial heterogeneity is not necessarily related to the extent of the study area.
 348 Therefore the unambiguous interpretation of results can be promoted using nested designs
 349 also in small environments, like the Santa Giusta lagoon. The multi-scale approach is
 350 recommended as a basic tool for spatial distribution analyses, especially when such
 351 information is still scarcely known in the investigated environment. In particular for future

352 studies in the Santa Giusta lagoon, single observations should be evaluated in relation to the
 353 proper sampling zone, not being representative of the whole coastal lagoon.

354 In this study, multivariate analyses revealed that community spatial patterns derived from
 355 species and family abundance data were very similar to each other. Consistent results were
 356 reported in many other works (*i.a.* Warwick, 1988; Ferraro and Cole, 1995; Olsgard et al.,
 357 1998; Lardicci and Rossi, 1998; Mistri and Rossi, 2001; De Biasi et al., 2003; Dethier and
 358 Schoch, 2006), demonstrating redundancy of information in large sets of benthic species data
 359 for identifying significant differences among assemblages, in both polluted and unpolluted
 360 environments. Our results showed that decreasing taxonomic resolution from species to
 361 family was not related to a strong decrease of the overall spatial variability. On the contrary,
 362 lumping species in higher taxonomic groups was usually considered leading to a probable
 363 decrease in estimates of variability as a consequence of an “averaging effect” (Doak et al.,
 364 1998; De Biasi et al., 2003). Analyses based on family abundances were effective in detecting
 365 spatial patterns among the three zones of the lagoon, and they provided estimates of variance
 366 components that were not substantially different from those detected at the species level. In
 367 addition, spatial dispersion of samples was similarly described by both species and family
 368 level and this was a novel finding compared to previous works investigating the TS
 369 applicability. Such works have mainly looked for changes in the location of sample groups in
 370 multivariate space at decreasing taxonomic levels of resolution, while the effects of TS on the
 371 dispersion of sample groups were usually neglected (Terlizzi et al., 2008a). However,
 372 explicitly analysing differences in dispersion among groups is important in order to obtain
 373 more complete information as well as avoid misleading interpretation of results (Anderson,
 374 2006). Thus in Santa Giusta lagoon, PERMDISP results clarified that differences in species
 375 composition concerned almost exclusively spatial differences detected at the zone scale, while
 376 spatial variability observed at site (or even area) scale was mostly due to differences in

dispersion. Similar spatial patterns were found at the two lowest taxonomic levels, probably because of the high percentage of families represented by a single species as usually occurred in brackish environments (Giangrande et al., 2005). Further aggregation at the class level showed relevant changes in observed spatial patterns; in particular, the overall spatial variability decreased reflecting a more homogenous distribution of class abundances within the lagoon. As a consequence, few significant differences were detected among levels of each investigated spatial scale. Meanwhile, the relatively higher variability among replicates probably increased because of the uneven distribution of some organisms living in small dense patches (e.g. *Mytilaster minimus*).

The usefulness of TS method has been evaluated and often promoted in order to streamline expensive and time consuming sampling protocols, like those employed in soft bottom macrofauna analyses (*i.a.* Olsgard et al., 1998; Terlizzi et al., 2003; Lampadariou et al., 2005; Wlodarska Kowalczyk and Kedra, 2007). However, other possibilities were also investigated. Lampadariou et al. (2005) compared results obtained using different mesh-size and type of sampler; they indicated that small samples taken with corers and sieved at 0.5 mm provided a large proportion of benthic spatial distribution, even if data were analysed at the family level. Other studies examined single taxonomic groups as representative of the whole assemblages, but contrasting outcomes emerged. While Olsgard et al. (2003) promoted polychaetes alone as useful surrogates to describe soft bottom macrofauna distribution, in other cases reduced taxonomic resolution was more effective than using a single taxonomic group (Anderson et al., 2005a; Wlodarska Kowalczyk and Kedra, 2007). In Santa Giusta lagoon, different spatial distributions were obtained analysing separately the three main taxonomic groups and none of them reflected results obtained by the whole benthic assemblages. Therefore future studies investigating macrobenthic spatial distribution of this lagoon should prefer the TS method to analysis of a single taxonomic group.

As argued elsewhere, the relationship between time saving and taxonomic level changes from case to case (Olsgard et al., 1998), depends on the number of species within a single family, the taxonomical complexity of families and the availability of taxonomic expertise (Ferraro and Cole, 1995; Dethier and Schoch, 2006). However, it has been calculated that generally the cost of family level identification was 50% to 55% less than species level identification (Ferraro and Cole, 1995; De Biasi et al., 2003). In our case, the time needed for identification at family level was 63% less than the species level identification, considering that 33% of families was represented by two or more species. However, the majority of species belonged to polychaetes and our laboratory team has significant expertise in polychaetes. Resources deriving from such cost reduction could be employed to plan more frequent surveys and/or to adopt more complex spatial sampling designs with a high number of replicates, in order to further minimize spatial variability caused by the dispersion effect. When the distribution of organisms is patchy, it is probably more important to collect many replicates at different spatial and temporal scales than to identify taxa at the finest resolution level (Morrissey et al., 1992; Chapman, 1998). In Santa Giusta lagoon, our baseline detailed multiscale investigation demonstrated that at least two sites (namely 16 samples) for each zone are needed to collect the majority of species and therefore to describe correctly the spatial distribution of benthic assemblages.

Results obtained in this study have important practical consequences for investigations on the distribution of soft bottom macrofauna in brackish habitats, including those concerned with environmental monitoring. In fact, the present study can be considered as a valuable example for a rigorous approach in collecting data for ecological studies, when previous detailed knowledge is scant. The spatial variability observed at all the examined scales indicated that small-scale observations are unlikely to describe the spatial benthic distribution of the whole lagoon (Foster, 1990). As a consequence, any *a priori* statement about composition, structure

and distribution of macrobenthic communities should be avoided, even in small brackish environments usually considered as homogenous habitats. Furthermore, explicitly testing for differences in dispersion among groups has been demonstrated to obtain a more accurate interpretation of the detected spatial patterns and such an approach should be more frequently adopted in future studies. Especially for routine monitoring programs, long term data sets at the finest taxonomic level and large sampling effort are usually the preferred approach for analyses of macrobenthic assemblages. Unfortunately, there are often many practical difficulties such as reduced budgets or lack of well-trained taxonomists, and compromise solutions are unavoidable. However the present study highlighted that reasonable choices and useful advice can be obtained only if the planning of monitoring programs is preceded by a detailed baseline study (Terlizzi et al., 2003; 2008a), thus avoiding any *a priori* decision. In particular, our results showed that in Santa Giusta lagoon, if resources are limited, analysing different spatial scales considering the whole benthic assemblages at the family level may be more important than classifying all individuals at the species level (Kingston and Riddle, 1989; Lampadariou et al., 2005). Although spatial patterns do not necessarily remain constant over time and further analyses at several temporal scales are needed, in future routine investigations taxonomic costs can be probably reduced without losing the power to detect macrobenthic spatial patterns, both in terms of location and dispersion effect. Since the most frequent disturbance events (e.g. organic enrichment, eutrophication, chemical pollution) are likely related to changes in spatial patterns of assemblages (Caswell and Cohen, 1991; Warwick and Clarke, 1993; Fraschetti et al., 2001; Terlizzi et al., 2005), monitoring programs, based on periodical surveys and TS, may be useful for a quick environmental assessment. Further detailed analyses, like identification at the species level, should be carried out if changes in spatial patterns are detected, in order to confirm and clarify disturbance effects on assemblages. However, other disturbance events (e.g. invasion of alien species,

452 climate change) may act gradually and for example change the natural balance of competitive
453 interactions among phylogenetically close species, like species of the same genus or family;
454 in this case, disturbance effects can be detected only analysing the community at the finest
455 taxonomic level.

456 The present study expanded the current knowledge of macrobenthic assemblages in Santa
457 Giusta lagoon and emphasised the usefulness of multiscale approach to realistically describe
458 spatial patterns of variability. In addition, our results highlighted some helpful methodological
459 procedures, which should be promoted in order to better design future sampling designs in
460 this lagoon, as well as in other similar brackish environments.

461

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Tables

N	Sobs			% Sobs		
	alpha	beta	gamma	alpha	beta	gamma
32	75	60	58	100	100	100
24	72	58	56	96	97	97
16	67	55	53	89	92	91
8	57	48	46	76	80	79

Table 1. The local species richness estimated by accumulation curves (Figure 5) in correspondence with different number of sampling replicates in each zone of the lagoon. N: number of replicate samples analysed; Sobs: number of species observed; % Sobs: percentage of species observed in comparison with the total species number collected.

species	Av. Transf. Abundance <u>alpha</u>		Av. Transf. Abundance <u>beta</u>		Av. Transf. Abundance <u>gamma</u>
Cymodoce truncata	4.26	<	5.11	<	7.01
Monocorophium sextonae	3.33	<	4.20	>	-
Microdeutopus anomalus	0.74	<	4.09	>	0.15
Abra ovata	1.31	<	3.67	>	1.86
Loripes lacteus	1.50	<	2.73	>	1.27
Phylo foetida	0.52	<	3.18	>	0.06
Minuspia multibranchiata	1.57	<	3.60	<	5.28
Cumella limicola	1.38	<	3.11	>	1.01
Hydroides elegans	1.96	>	1.32	<	2.22
Pseudopolydora antennata	1.67	<	2.00	>	0.75
Neanthes caudata	1.12	<	2.85	>	0.82
Cirriformia tentaculata	2.07	>	0.32	<	1.02
Cerastoderma glaucum	0.33	<	1.96	>	0.37
Corophium acherusicum	1.10	<	1.17	>	-
Pseudolirius kroyerii	1.49	>	0.09	<	0.50
Tapes aurea	1.28	>	0.99	<	0.50
Iphinoe serrata	1.00	<	1.49	>	0.15
Mytilaster minimus	0.23	<	0.70	<	9.69
Tanais dulongii	0.27	<	0.91	<	6.83
Nainereis laevigata	0.09	<	0.53	<	2.46
Dynamene bidentata	0.73	>	1.04	<	2.17
Tapes decussata	1.63	>	1.10	>	0.08
Ophiodromus pallidus	0.31	<	0.44	<	1.46
Podarkeopsis capensis	0.59	>	0.46	<	1.83
Cumella limicola	1.38	<	3.11	>	1.01

Table 2. Results of SIMPER analysis. Average abundance of species contributing to most of the Bray-Curtis dissimilarity between zones (cut-off value = 60%) (data square-root transformed). The highest average abundance value is in bold.

Taxonomic Level	Source	df	SS	MS	F	P	Variance Component
Species	Zone	2	77637.7	38818.8	10.977	0.004	1102.6
	Site	9	31829.0	3536.6	2.182	0.002	239.5
	Area	36	58354.7	1621.0	2.071	0.002	419.2
	Residual	48	37561.7	782.5			782.5
	Total	95	205303.1				2543.8
Family	Zone	2	69723.2	34861.6	11.409	0.0004	993.9
	Site	9	27501.4	3055.7	2.699	0.0002	240.5
	Area	36	40756.8	1132.1	1.935	0.0002	273.6
	Residual	48	28077.6	584.9			584.9
	Total	95	166059				2092.9
Class	Zone	2	13944.7	6972.3	5.777	0.0164	180.2
	Site	9	10862.3	1206.9	3.713	0.0002	110.2
	Area	36	11703.0	325.1	1.501	0.019	54.3
	Residual	48	10395.7	216.6			216.6
	Total	95	46905.7				561.2

Table 3. Permutational multivariate analysis of variance based on the Bray-Curtis dissimilarity for square-root transformed data of species (83 variables), families (43 variables) and classes (4 variables) abundance. Analysis was carried out using 4999 permutations of residuals under a reduced model. Estimates of multivariate variation at each spatial scale were included.

Source	Pairwise	Species		Family		Class	
		L+D	D	L+D	D	L+D	D
Zones	alpha - beta	0.0304	0.0564	0.0306	0.1424	0.1102	0.1664
	alpha - gamma	0.0244	0.0272	0.0290	0.0242	0.0290	0.0902
	beta - gamma	0.0266	0.0848	0.0294	0.0646	0.0852	0.7190
Source	Pairwise	Species		Family		Class	
		L+D	D	L+D	D	L+D	D
Sites within <u>alpha</u> zone	1 - 2	0.0016	0.2030	0.0006	0.2612	0.0124	0.9206
	1 - 3	0.1402	0.5904	0.1114	0.4466	0.6660	0.9450
	1 - 4	0.0136	0.0172	0.0034	0.1162	0.0242	0.2228
	2 - 3	0.0012	0.1018	0.0006	0.0894	0.0024	0.8746
	2 - 4	0.0004	0.0008	0.0002	0.0088	0.0006	0.2058
	3 - 4	0.1132	0.1552	0.0492	0.6874	0.2186	0.2880
Sites within <u>beta</u> zone	1 - 2	0.1798	0.0050	0.1332	0.0014	0.5780	0.0106
	1 - 3	0.0090	0.2696	0.0046	0.1976	0.1072	0.6306
	1 - 4	0.0508	0.1624	0.0430	0.2780	0.3208	0.9258
	2 - 3	0.0010	0.1760	0.0004	0.1580	0.0178	0.0584
	2 - 4	0.0052	0.0004	0.0014	0.0002	0.1098	0.0044
	3 - 4	0.0992	0.0216	0.0604	0.0354	0.1446	0.5680
Sites within <u>gamma</u> zone	1 - 2	0.7466	0.1692	0.7482	0.3752	0.4638	0.4212
	1 - 3	0.0004	0.1422	0.0006	0.2778	0.1112	0.3692
	1 - 4	0.0698	0.5336	0.1238	0.4554	0.1380	0.9884
	2 - 3	0.0026	0.9834	0.0018	0.8900	0.1156	0.9268
	2 - 4	0.0014	0.3814	0.0014	0.7870	0.0148	0.4952
	3 - 4	0.0004	0.3526	0.0004	0.6556	0.0022	0.4504

Table 4. P-values for pairwise tests of significant variability among “zones” and among “sites” in each zone, for different levels of taxonomic resolution. “L+D” columns are P-values obtained by PERMANOVA, therefore indicating a “location” and/or a “dispersion” effect. “D” columns are P-values obtained by PERMDISP, therefore indicating only a “dispersion” effect. Results that are not significant at the 0.05 level are given in bold type. Note that the smallest possible P-value with 4999 permutations is 0.0002.

		Polychaetes		Crustaceans		Molluscs	
Source	Pairwise	L+D	D	L+D	D	L+D	D
among Zones	alpha - beta	0.0272	0.0290	0.0304	0.0564	0.0294	0.0600
	alpha - gamma	0.0288	0.0278	0.0244	0.0272	0.0286	0.1118
	beta - gamma	0.0274	0.9744	0.0266	0.2794	0.0290	0.0558
		Polychaetes		Crustaceans		Molluscs	
Source	Sites	L+D	D	L+D	D	L+D	D
Sites within alpha zone	1 - 2	0.0146	0.4206	0.0004	0.3502	0.8044	0.8878
	1 - 3	0.0888	0.9636	0.1080	0.2082	0.9148	0.2124
	1 - 4	0.0362	0.1118	0.0014	0.2314	0.9684	0.3426
	2 - 3	0.0010	0.4020	0.0006	0.0590	0.6906	0.1934
	2 - 4	0.0006	0.0062	0.0002	0.0658	0.6342	0.3874
	3 - 4	0.0448	0.1234	0.2136	0.9264	0.8888	0.0720
Sites within beta zone	1 - 2	0.0230	0.0482	0.1104	0.1990	0.8554	0.9824
	1 - 3	0.0002	0.0624	0.0374	0.6736	0.1358	0.0010
	1 - 4	0.0058	0.5592	0.1298	0.0704	0.8174	0.2234
	2 - 3	0.0002	0.7858	0.0002	0.0288	0.1464	0.0018
	2 - 4	0.0004	0.0172	0.0166	0.0016	0.9602	0.2324
	3 - 4	0.0262	0.0222	0.6834	0.0710	0.1396	0.1358
Sites within gamma zone	1 - 2	0.9478	0.9650	0.3334	0.1412	0.3350	0.5506
	1 - 3	0.0010	0.5756	0.0046	0.2748	0.4470	0.7008
	1 - 4	0.0318	0.2308	0.6134	0.0072	0.1774	0.0036
	2 - 3	0.0026	0.6246	0.0264	0.8588	0.3014	0.8382
	2 - 4	0.0048	0.3996	0.0294	0.2960	0.0282	0.0008
	3 - 4	0.0002	0.1702	0.0150	0.2916	0.0606	0.0016

Table 5. P-values for pairwise tests of significant variability among “zones” and among “sites” in each zone, for the three main taxonomic groups. “L+D” columns are P-values obtained by PERMANOVA, therefore indicating a “location” and/or a “dispersion” effect. “D” columns are P-values obtained by PERMDISP, therefore indicating only a “dispersion” effect. Results that are not significant at the 0.05 level are given in bold type. Note that the smallest possible P-value with 4999 permutations is 0.0002.

Figure Captions

Figure 1: The Santa Giusta lagoon. The three sampling zones are delimited by dotted lines

Figure 2. Number of taxonomic families represented in samples and number of species per family. ‘More’ data points were two, respectively with 6 and 9 species per family

Figure 3. Distribution of species according to the number of areas occupied out of a total of 48 areas. pol: polychaetes; cru: crustaceans; mol: molluscs

Figure 4. Mean abundance (number of individuals), with 95% confidence interval, of the three main faunal groups at each of the three sampling zones of Santa Giusta lagoon ($n=32$ per zone)

Figure 5. Species-sample accumulation curves for each zone of the lagoon. Data were based on 999 permutations of replicate samples

Figure 6. Multivariate variance components at each of the four spatial scales for all species, as obtained using mean squares from PERMANOVA performed with different transformations (nt = no transformation; r2 = square root; r4 = fourth root; pa = presence/absence). The values plotted are the square root of the variance components, in order to put the values on the scale of the original Bray-Curtis dissimilarities (expressed as percentage difference between assemblages)

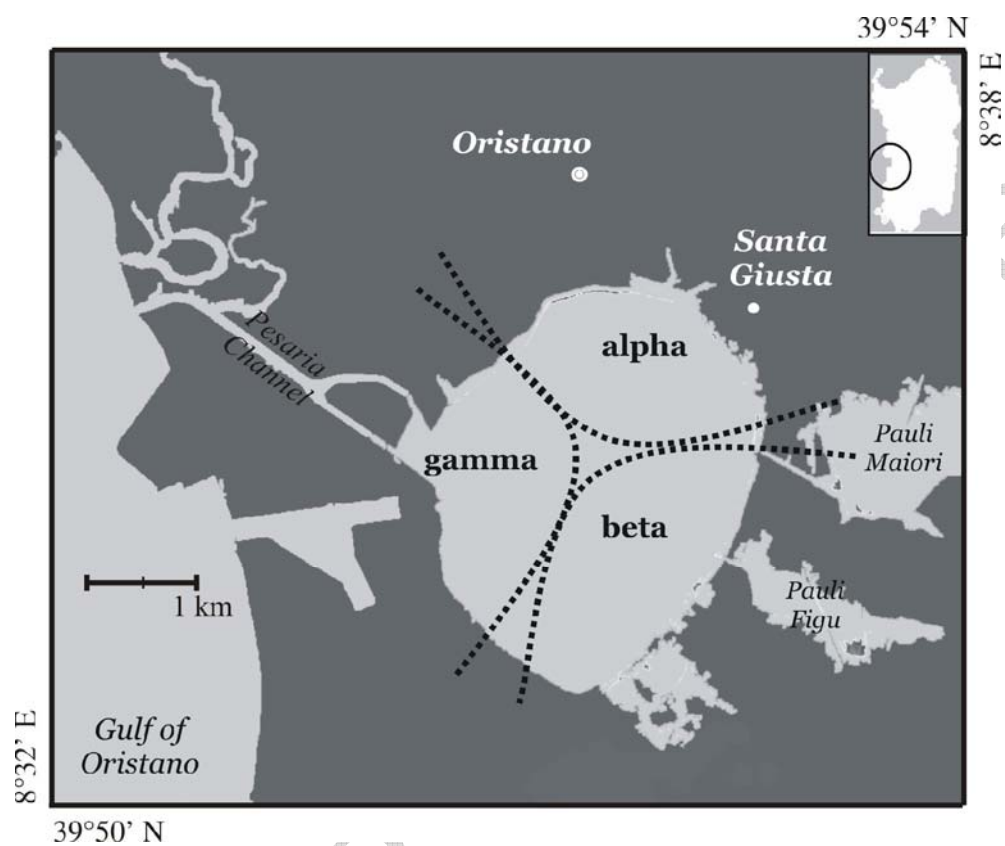
Figure 7. Multivariate variance components at each of the four spatial scales for all organisms collected using species, family and class taxonomic levels. The values plotted are the square root of the sizes of the variance components (Table 3)

Figure 8. Multivariate variance components at each of the four spatial scales for the three taxonomic group collected. The values plotted are the square root of the sizes of the variance components, obtained using mean squares from PERMANOVA performed with square root transformed data. All components were statistically significant at $p<0.001$, except for molluscs at the site scale which were not significant

Figure 9. NMDS plots on the basis of all taxa at species (a), family (b) and class (c) level of taxonomic resolution. Bray-Curtis dissimilarities of square-root transformed abundance values were used

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Figure 1.



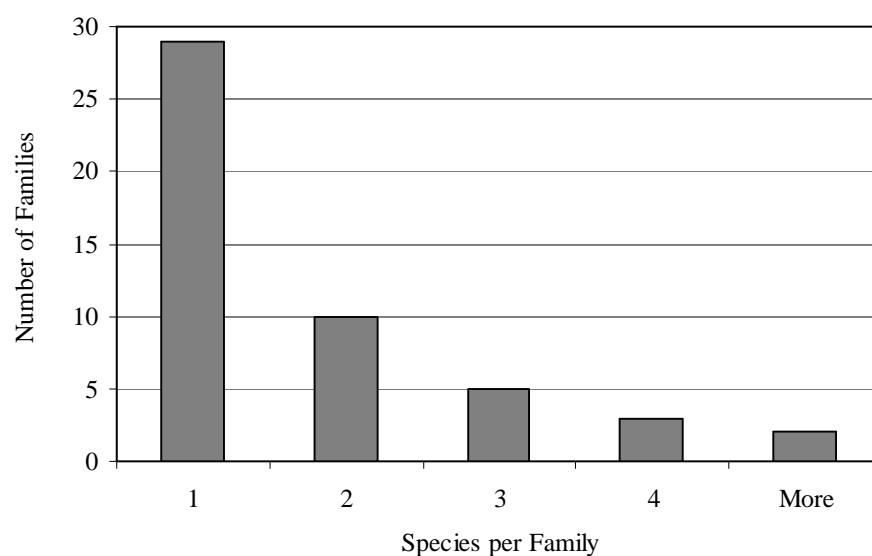


Figure 2. Number of taxonomic families represented in samples and number of species per family. 'More' data points were two, respectively with 6 and 9 species per family

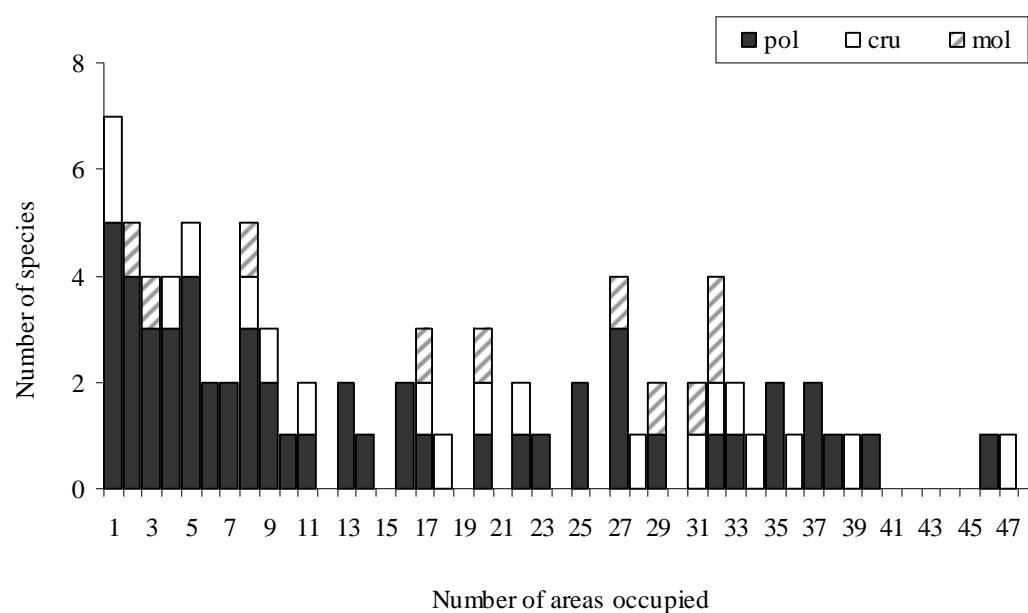


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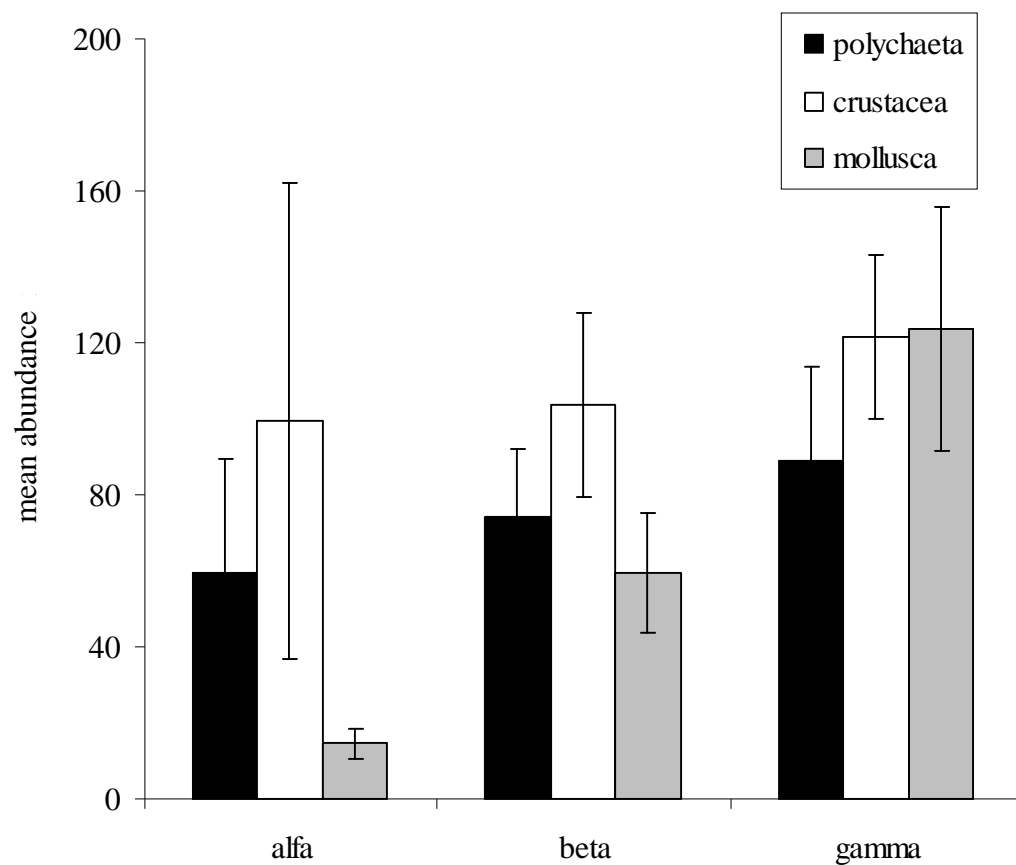


Figure 4. Mean abundance (number of individuals), with 95% confidence interval, of the three main faunal groups at each of the three sampling zones of Santa Giusta lagoon ($n=32$ per zone)

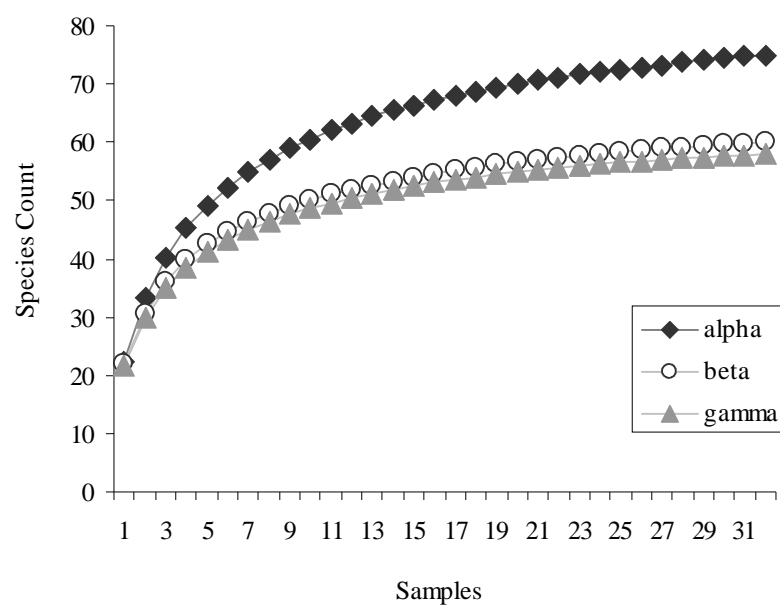


Figure 5. Species-sample accumulation curves for each zone of the lagoon. Data were based on 999 permutations of replicate samples.

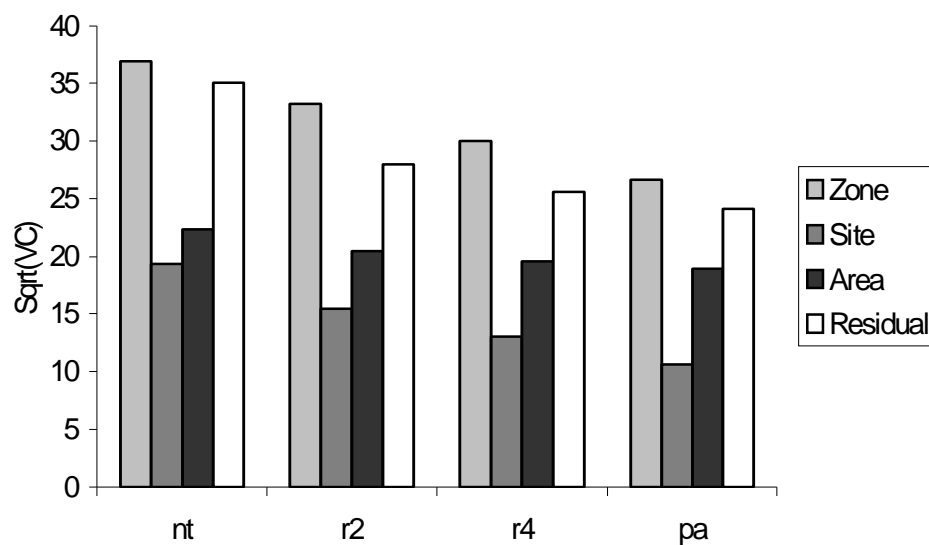


Figure 6. Multivariate variance components at each of the four spatial scales for all species, as obtained using mean squares from PERMANOVA performed with different transformations (nt = no transformation; r2 = square root; r4 = fourth root; pa = presence/absence). The values plotted are the square root of the variance components, in order to put the values on the scale of the original Bray-Curtis dissimilarities (expressed as percentage difference between assemblages)

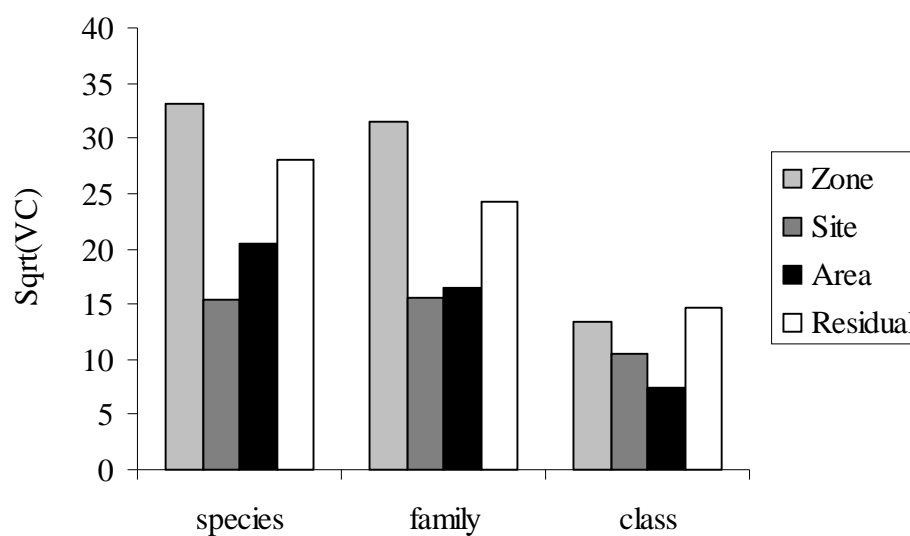


Figure 7. Multivariate variance components at each of the four spatial scales for all organisms collected using species, family and class taxonomic levels. The values plotted are the square root of the sizes of the variance components (Table 3)

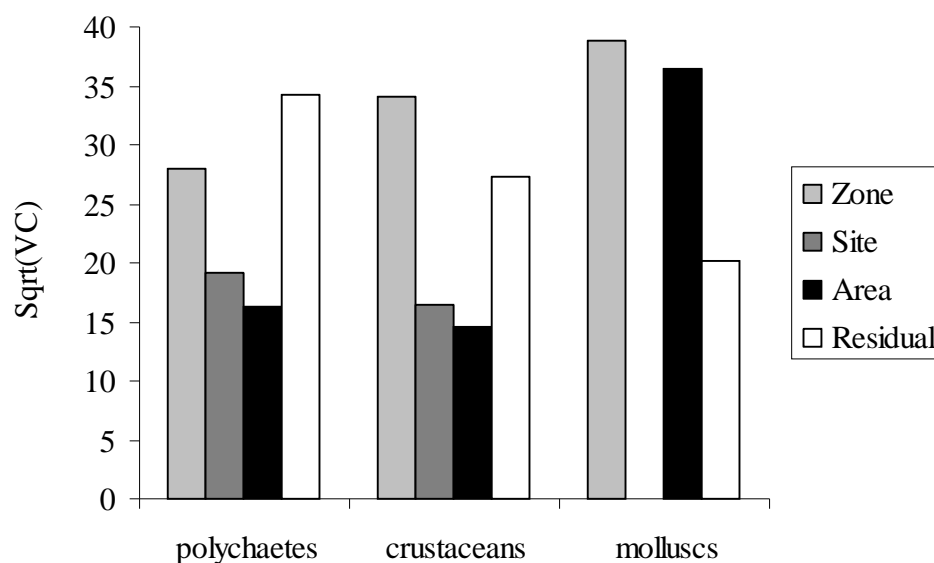
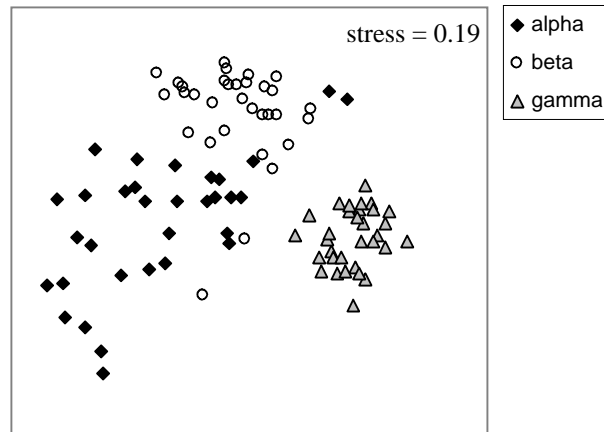
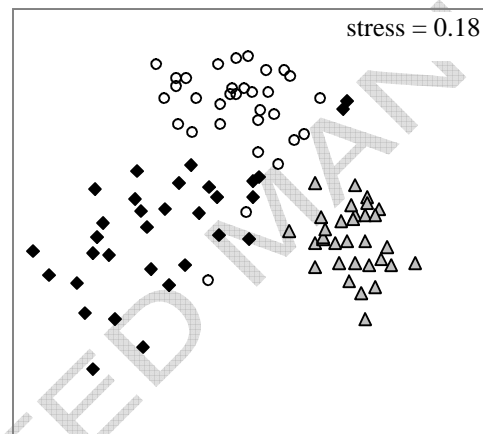


Figure 8. Multivariate variance components at each of the four spatial scales for the three taxonomic group collected. The values plotted are the square root of the sizes of the variance components, obtained using mean squares from PERMANOVA performed with square root transformed data. All components were statistically significant at $p < 0.001$, except for molluscs at the site scale which were not significant.

a. Species



b. Family



c. Class

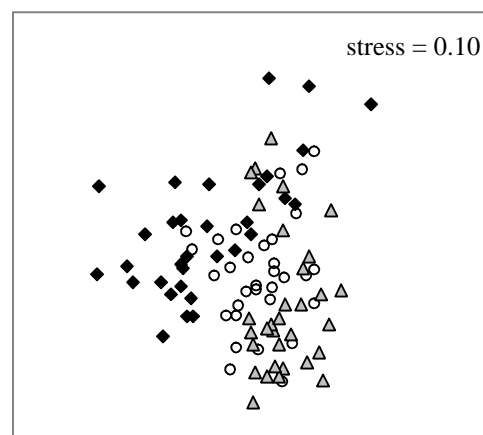


Figure 9. NMDS plots on the basis of all taxa at species (a), family (b) and class (c) level of taxonomic resolution. Bray-Curtis dissimilarities of square-root transformed abundance values were used