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

1. Introduction

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

In order to estimate the proportion of variability associated with each examined scale and to identify the most relevant spatial scale, the hierarchical sampling approach is considered the 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 (Morrisey 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-Kowalczuk 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-Kowalczuk 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

103 investigation could provide interesting practical implications for future studies, not only in
104 this lagoon, but also in other similar brackish environments.
105
106 ## 2. Materials and Methods
107 ### 2.1 Study area and sampling
108 The Santa Giusta lagoon (Western Mediterranean, Italy) is one of the largest coastal brackish
109 environments of Sardinia island, it is a polyhaline basin located along the central-western
110 coast of Sardinia. The lagoon is included in the Ramsar convention (1971) and belongs to a
111 complex system of transitional waters of high natural and economic value. The Santa Giusta
112 lagoon is approximately circular in shape with an area of 7.9 km^2 and a mean depth of 1 m; it
113 is located near the town of Oristano and Santa Giusta, in the plain of Pesaria, an agricultural
114 area that is intensively cultivated with rice. The lagoon has no natural attributes and is
115 separated from the sea by a longshore bar, it is also connected with two inner small basins
116 called Pauli Maiori and Pauli Figu (Figure 1). Central and peripheral canals have been
117 dredged about 2 m deep in order to facilitate seawater flow into the lagoon. As a consequence,
118 waters of Santa Giusta lagoon are now well mixed as regards circulation and stratification
119 (Sechi et al., 2001). Salinity ranges from 25‰ to 42‰, with a mean annual value of 30‰
120 (Sechi et al., 2001; Luglié et al., 2002). There is a prevalent sandy-muddy bottom, with small
121 patches of both macroalgae and angiosperms (e.g. *Enteromorpha* sp., *Gracilaria* sp., *Ruppia*
122 *cirrhosa*, *Zostera* sp.), which are distributed all over the lagoon.
123 In this context, three sampling zones were randomly selected among the three habitats in the
124 lagoon with different sediment and hydrodynamic properties (Luglié et al., 2002) (Figure 1).
125 Specifically, the alpha zone was in the central-northern part of the lagoon and it was mostly
126 influenced by the urban and industrial wastewaters from Oristano and Santa Giusta areas. It
127 has been considered a low-intermediate hydrodynamic energy environment with a prevalence

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

2.2 Statistical analyses

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

according to a three factors (zone, site, area) nested design through 4999 permutations of residuals under a reduced model (Anderson and Ter Braak, 2003). At species level, data were analysed using the Bray-Curtis dissimilarity measure on untransformed and transformed data (square-root, fourth-root, presence/absence), in order to evaluate if assemblages are mainly driven by compositional or relative abundance changes. Using stronger and stronger transformations, the emphasis of results can be shift from the most abundant species to the rarest ones; in particular, variability measured by presence/absence data reflects only compositional changes, while variation in relative abundances is more important in analyses based on other transformations or untransformed data (Clarke and Gorley, 2001; Anderson et al., 2005b). Furthermore, mean squares calculated by PERMANOVA were used to estimate multivariate variance components associated at each spatial scale, in a way analogous to univariate partitioning using ANOVA (Searle et al., 1992; Benedetti-Cecchi, 2001b). For a better comparison, in y-axis the variability at each spatial scale was expressed as square-root of variance components; therefore, the values could be interpreted as percentages of Bray-Curtis dissimilarity (Anderson et al., 2005a). Separate analyses were performed using the square-root transformed data at species, family and class levels of taxonomic resolution. The family level was chosen because it has been often indicated as the most effective in minimizing the cost-benefit ratio (Lardicci and Rossi, 1998; Karakassis and Hatziyanni, 2000; De Biasi et al., 2003; Lampadariou et al., 2005), while the class level was chosen to assess the effectiveness of a further higher resolution. Since a significant result for a given factor from PERMANOVA could indicate that the groups differ in their location and/or dispersion, PERMDISP analyses were also performed to focus only on dispersion effects, testing the factors “zone” and “site” (Anderson, 2006). Analogous analyses were performed using 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).
189

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

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

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

219 In alpha zone, despite the smallest number of individuals (5 550), the highest number of
220 species (75) was recorded, with five species that accounted for 60.4% of total abundance.
221 This group included species typical of brackish habitats, such as *Monocorophium sextonae*,
222 *Cymodoce truncata*, *Hydroides elegans* and *Corophium acherusicum* (Ruffo, 1998) and a
223 species typical of sandy-muddy bottom such as *Pseudopolydora antennata* (Lardicci et al.,
224 2001). Moreover, SIMPER analyses highlighted the value of other species in determining the
225 dissimilarity among the three zones: a polychaete, *Cirriformia tentaculata*, and a crustacean
226 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 *Phylo foetida* 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.*

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

277 variability at the zone scale were differences in location and dispersion (Table 5); however
278 polychaetes showed the greatest variability among replicate samples and such spatial scale
279 was also important for the other taxonomic groups (Figure 8). Except for molluscs, a small
280 variability was associated with the two intermediate spatial scales (Figure 8); in particular at
281 the site scale, there was a prevalent location effect both for polychaetes and crustaceans
282 although differences in dispersion were also detected especially for crustaceans within the
283 beta zone (Table 5).
284 The nMDS plot based on the species abundance data showed some differences among the
285 three sampled zones. In particular, gamma samples were clearly clustered and separated from
286 the other zones, while alpha and beta samples were partially overlapped (Figure 9a). At the
287 family level, nMDS ordination was very similar to that obtained at the species level (Figure
288 9b). Further aggregation to the class level produced a different ordination pattern, with
289 substantially higher levels of overlapping of the three zones (Figure 9c). Relationships
290 between similarity matrices calculated for the three taxonomic levels were confirmed by
291 RELATE results, which showed $\rho = 0.960$, $p<0.001$ between species and family levels and ρ
292 $= 0.565$, $p<0.001$ between species and class levels.
293

294 **4. Discussion**

295 The first result that stands out from our work is that the benthic assemblages of Santa Giusta
296 lagoon were extremely variable, with significant differences at all considered spatial scales,
297 from metres up to thousands of metres. This outcome was highly consistent with results
298 reported in studies analysing spatial variability by nested design, whatever the habitat
299 investigated (see Fraschetti et al., 2005 for a review). In the Santa Giusta lagoon most of the
300 variation was associated with the smallest and the largest spatial scale, thus indicating that
301 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; Morrisey et al., 1992; Benedetti Cecchi et al., 2001a; Coleman et al., 2002;
306 Rossi and Lardicci, 2002; Fraschetti 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. Olsgard 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)

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

377 dispersion. Similar spatial patterns were found at the two lowest taxonomic levels, probably
378 because of the high percentage of families represented by a single species as usually occurred
379 in brackish environments (Giangrande et al., 2005). Further aggregation at the class level
380 showed relevant changes in observed spatial patterns; in particular, the overall spatial
381 variability decreased reflecting a more homogenous distribution of class abundances within
382 the lagoon. As a consequence, few significant differences were detected among levels of each
383 investigated spatial scale. Meanwhile, the relatively higher variability among replicates
384 probably increased because of the uneven distribution of some organisms living in small
385 dense patches (e.g. *Mytilaster minimus*).
386 The usefulness of TS method has been evaluated and often promoted in order to streamline
387 expensive and time consuming sampling protocols, like those employed in soft bottom
388 macrofauna analyses (*i.a.* Olsgard et al., 1998; Terlizzi et al., 2003; Lampadariou et al., 2005;
389 Wlodarska Kowalczuk and Kedra, 2007). However, other possibilities were also investigated.
390 Lampadariou et al. (2005) compared results obtained using different mesh-size and type of
391 sampler; they indicated that small samples taken with corers and sieved at 0.5 mm provided a
392 large proportion of benthic spatial distribution, even if data were analysed at the family level.
393 Other studies examined single taxonomic groups as representative of the whole assemblages,
394 but contrasting outcomes emerged. While Olsgard et al. (2003) promoted polychaetes alone as
395 useful surrogates to describe soft bottom macrofauna distribution, in other cases reduced
396 taxonomic resolution was more effective than using a single taxonomic group (Anderson et
397 al., 2005a; Wlodarska Kowalczuk and Kedra, 2007). In Santa Giusta lagoon, different spatial
398 distributions were obtained analysing separately the three main taxonomic groups and none of
399 them reflected results obtained by the whole benthic assemblages. Therefore future studies
400 investigating macrobenthic spatial distribution of this lagoon should prefer the TS method to
401 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 (Morrisey 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

427 and distribution of macrobenthic communities should be avoided, even in small brackish
428 environments usually considered as homogenous habitats. Furthermore, explicitly testing for
429 differences in dispersion among groups has been demonstrated to obtain a more accurate
430 interpretation of the detected spatial patterns and such an approach should be more frequently
431 adopted in future studies. Especially for routine monitoring programs, long term data sets at
432 the finest taxonomic level and large sampling effort are usually the preferred approach for
433 analyses of macrobenthic assemblages. Unfortunately, there are often many practical
434 difficulties such as reduced budgets or lack of well-trained taxonomists, and compromise
435 solutions are unavoidable. However the present study highlighted that reasonable choices and
436 useful advice can be obtained only if the planning of monitoring programs is proceeded by a
437 detailed baseline study (Terlizzi et al., 2003; 2008a), thus avoiding any *a priori* decision. In
438 particular, our results showed that in Santa Giusta lagoon, if resources are limited, analysing
439 different spatial scales considering the whole benthic assemblages at the family level may be
440 more important than classifying all individuals at the species level (Kingston and Riddle,
441 1989; Lampadariou et al., 2005). Although spatial patterns do not necessary remain constant
442 over time and further analyses at several temporal scales are needed, in future routine
443 investigations taxonomic costs can be probably reduced without losing the power to detect
444 macrobenthic spatial patterns, both in terms of location and dispersion effect. Since the most
445 frequent disturbance events (e.g. organic enrichment, eutrophication, chemical pollution) are
446 likely related to changes in spatial patterns of assemblages (Caswell and Cohen, 1991;
447 Warwick and Clarke, 1993; Fraschetti et al., 2001; Terlizzi et al., 2005), monitoring
448 programs, based on periodical surveys and TS, may be useful for a quick environmental
449 assessment. Further detailed analyses, like identification at the species level, should be carry
450 out if changes in spatial patterns are detected, in order to confirm and clarify disturbance
451 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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References

Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecology, 26, 32-46.

Anderson, M.J., 2006. Distance-based tests for homogeneity of multivariate dispersion. Biometrics, 62, 245-253.

Anderson, M.J., ter Braak, C.J.F., 2003. Permutation tests for multi-factorial analysis of variance and regression. Journal of Statistical Computation and Simulation, 73, 85-113.

Anderson, M.J., Diebel, C.E., Blom, W.M., Landers, T.J., 2005a. Consistency and variation in kelp holdfast assemblages: spatial patterns of biodiversity for the major phyla at different taxonomic resolutions. Journal of Experimental Marine Biology and Ecology, 320, 35-56.

Anderson, M.J., Connell, S.D., Gillanders, B.M., Diebel, C.E., Bolm, W.M., Saunders, J.E., Landers, T.J., 2005b. Relationships between taxonomic resolution and spatial scales of multivariate variation. Journal of Animal Ecology, 74, 636–646.

Archambault, P., Bourget, E., 1996. Scales of coastal heterogeneity and benthic intertidal species richness, diversity and abundance. Marine Ecology Progress Series, 136, 111-121.

Barnes, R.S.K., 1991. European estuaries and lagoons, a personal overview of problems and possibilities for conservation and management. Aquatic Conservation: Marine and Freshwater Ecosystems, 1, 79-87.

Basset, A., Sabetta, L., Fonnesu, A., Mouillot, D., Do Chi, T., Viaroli, P., Giordani, G., Reizopoulou, S., Abbiati, M., Carrada, G.C., 2006. Typology in Mediterranean transitional waters: new challenges and perspectives. Aquatic Conservation: Marine and Freshwater Ecosystems, 16, 441-455.

Benedetti-Cecchi, L., 2001a. Variability in abundance of algae and invertebrates at different spatial scales on rocky sea shores. Marine Ecology Progress Series, 215, 79-92.

Benedetti-Cecchi, L., 2001b. Beyond BACI: optimization of environmental sampling designs through monitoring and simulation. Ecological Applications, 11, 783-799.

Caswell, H., Cohen, J.E., 1991. Communities in patchy environments: a model of disturbance, competition and heterogeneity. In: Kolasa J., Pickett STA (Eds.) Ecological heterogeneity. Springer-Verlag, New York, pp. 97-122.

Chapman, M.G., 1998. Relationships between spatial patterns of benthic assemblages in a mangrove forest using different levels of taxonomic resolution. Marine Ecology Progress Series, 162, 71–78.

Chapman, M.G., Underwood, A.J., 2008. Scales of variation of gastropod densities over multiple spatial scales: comparisons of common and rare species. Marine Ecology Progress Series, 354, 147-160.

515 Clarke, K.R., 1993. Non-parametric multivariate analyses of changes in community structure.
516 Australian Journal of Ecology. 18, 117-143.
517
518 Clarke, K.R., Gorley, R.H., 2001. PRIMER V6: User Manual/Tutorial. Primer-e, Plymouth,
519 United Kingdom.
520
521 Cognetti, G., 1982. Adaptative strategy of brackish-water fauna in pure and polluted waters.
522 Marine Pollution Bulletin, 13, 247-250.
523
524 Coleman, M.A., Browne, M., Theobalds, T., 2002. Small-scale spatial variability in intertidal
525 and subtidal turfing algal assemblages and the temporal generality of these patterns. Journal
526 of Experimental Marine Biology and Ecology, 267, 55-74.
527
528 De Biasi, A.M., Bianchi, C.N., Morri, C., 2003. Analysis of macrobenthic communities at
529 different taxonomic levels: an example from an estuarine environment in the Ligurian Sea
530 (NW Mediterranean). Estuarine, Coastal and Shelf Science, 58, 99-106.
531
532 Dethier, M.N., Schoch, G.C., 2006.Taxonomic sufficiency in distinguishing natural spatial
533 patterns on an estuarine shoreline. Marine Ecology Progress Series, 306, 41-49.
534
535 Doak, D.F., Bigger, D., Harding, E.K., Marvier, M.A., Omalley, R.E., Thomson, D., 1998.
536 The statistical inevitability of stability-diversity relationships in community ecology.
537 American Naturalist, 151, 264–276.
538
539 Edgar, G.J., Barret, N.S., 2002. Benthic macrofauna in Tasmanian estuaries: scales of
540 distribution and relationships with environmental variables. Journal of Experimental Marine
541 Biology and Ecology, 270, 1-24.
542
543 Ekman, J.E., 1979. Small-scale patterns and processes in a soft substratum, intertidal
544 community. Journal of Marine Research, 37:437.
545
546 Ellis, D., 1985. Taxonomic sufficiency in pollution assessment. Marine Pollution Bulletin, 16,
547 459.
548
549 Ferraro, S.P., Cole, F.A., 1995. taxonomic level sufficient for assessing pollution impacts on
550 the Southern California Bight macrobenthos – revisited. Environmental Toxicology and
551 Chemistry, 6, 1031-1040.
552
553 Foster, M.S., 1990. Organization of macroalgal assemblages in the North Pacific: the
554 assumption of homogeneity and the illusion of generality. Hydrobiologia, 192, 21-33.
555
556 Fraschetti, S., Bianchi, C.N., Terlizzi, A., Fanelli, G., Morri, C., Boero, F., 2001. Spatial
557 variability and human disturbance in shallow subtidal hard substrate assemblages: a regional
558 approach. Marine Ecology Progress Series, 212, 1-12.
559
560 Fraschetti, S., Terlizzi, A., Benedetti-Cecchi, L., 2005. Patterns of distribution of marine
561 assemblages from rocky shores: evidence of relevant scales of variation. Marine Ecology
562 Progress Series, 296, 13-29.
563

564 Giangrande, A., Licciano, M., Musco, L., 2005. Polychaetes as environmental indicators –
565 revisited. Marine Pollution Bulletin, 50, 1153-1162.
566
567 Guelorget, O., Perthuisot, J.P., 1983. Le domaine paralique. Expressions géologiques,
568 biologiques et économiques du confinement. Travaux du laboratoire de géologie, 16, 1-36.
569
570 Hewitt, J.E., Thrush, S.F., Cummings, V.J., Turner, S.J., 1998. The effect of changing
571 sampling scales on our ability to detect effects of large-scale processes on communities.
572 Journal of Experimental Marine Biology and Ecology, 227, 251-264.
573
574 Hewitt, J.E., Thrush, S.F., Legendre, P., Cummings, V.J., Norkko, A., 2002. Integrating
575 heterogeneity across spatial scales: interactions between *Atrina zelandica* and benthic
576 macrofauna. Marine Ecology Progress Series, 239, 115-128.
577
578 Karakassis, I., Hatziyanni, E., 2000. Benthic disturbance due to fish farming analyzed under
579 different levels of taxonomic resolution. Marine Ecology Progress Series, 203, 247-253.
580
581 Kingston, P.F., Riddle, M.J., 1989. Cost Effectiveness of Benthic Faunal Monitoring. Marine
582 Pollution Bulletin, 20, 490-496.
583
584 Kotliar, N.B., Wiens, J.A., 1990. Multiple Scales of Patchiness and Patch Structure: A
585 Hierarchical Framework for the Study of Heterogeneity. Oikos, 59, 253-260.
586
587 Lampadariou, N., Karakassis, I., Pearson, T.H., 2005. Cost/benefit analysis of a benthic
588 monitoring programme of organic enrichment using different sampling and analysis methods.
589 Marine Pollution Bulletin, 50, 1606-1618.
590
591 Lardicci, C., Abbiati, M., Crema, R., Morri, C., Bianchi, C.N., 1993. The distribution of
592 polychaetes along eEnvironmental gradients: an example from the Orbetello lagoon, Italy.
593 Marine Ecology, 14, 35-52.
594
595 Lardicci, C., Rossi, F., Castelli, A., 1997. Analysis of macrozoobenthic community structure
596 after severe dystrophic crises in a Mediterranean coastal lagoon. Marine Pollution Bulletin,
597 34, 536-547.
598
599 Lardicci, C., Rossi, F., 1998. Detection of stress on macrozoobenthos: evaluation of some
600 methods in a coastal Mediterranean lagoon. Marine Environmental Research, 45, 367-386.
601
602 Lardicci, C., Como, S., Corti, S., Rossi, F., 2001. Recovery of the macrozoobenthic
603 community after severe dystrophic crises in a Mediterranean coastal lagoon (Orbetello, Italy).
604 Marine Pollution Bulletin, 42, 202-214.
605
606 Lasiak, T., 2003. Influence of taxonomic resolution, biological attributes and data
607 transformations on multivariate comparisons of rocky macrofaunal assemblages. Marine
608 Ecology Progress Series, 250, 29-34.
609
610 Levin, S.A., 1992. The problem of pattern and scale in ecology. Ecology, 73, 1943-1967.
611
612 Lubchenco, J., Olson, A.M., Brubaker, L.B., Carpenter, S.R., Holland, M.M., Hubbell, S.P.,
613 Levin, S.A., MacMahon, J.A., Matson, P.A., Meliello, J.M., Mooney, H.A., Peterson, C.H.,

614 Pulliam, H.R., Real, L.A., Regal, P.J., Risser, P.G., 2001. The suitable biosphere initiative: an
615 ecological research agenda. Ecology, 72, 371-412.
616
617 Luglié, A., Sechi, N., Oggiano, G., Sanna, G., Tapparo, A., 2002. Ecological assessment of
618 Santa Giusta Lagoon (Sardinia, Italy). Annali di chimica, 92, 239-247.
619
620 Mistri, M., Rossi, R., 2001. Taxonomic sufficiency in lagoonal ecosystems. Journal of Marine
621 Biological Association of U.K., 81, 339-340.
622
623 Morrisey, D., Howitt, L., Underwood, A.J., Stark, J.S., 1992. Spatial variation in soft-
624 sediment benthos. Marine Ecology Progress Series, 81, 197-204.
625
626 Noren, K., Lindegarth, M., 2005. Spatial, temporal and interactive variability of infauna in
627 Swedish coastal sediments. Journal of Experimental Marine Biology and Ecology, 317, 53-
628 68.
629
630 Olsgard, F., Brattegard, T., Holthe T., 2003. Polychaetes as surrogates for marine
631 biodiversity: lower taxonomic resolution and indicator groups. Biodiversity and Conservation,
632 12, 1033-1049.
633
634 Olsgard, F., Somerfield, P.J., Carr, M.R.., 1998. Relationship between taxonomic resolution,
635 macrobenthic community patterns and disturbance. Marine Ecology Progress Series, 172, 25-
636 36.
637
638 Pearson, T.H., Rosenberg, R., 1978. Macrobenthic succession in relation to organic
639 enrichment and pollution of the marine environment. Oceanography and Marine Biology:
640 Annual Review, 16, 229-311.
641
642 Pérez-Ruzafa, A., Marcos, C., Pérez-Ruzafa, I.M., Barcala, E., Hegazi, M.I., Quispe, J., 2007.
643 Detecting changes resulting from human pressure in a naturally quick-changing and
644 heterogeneous environment: Spatial and temporal scales of variability in coastal lagoons.
645 Estuarine, Coastal and Shelf Science, 75, 175-188.
646
647 Ramsar convention, 1971. Convention on wetlands of international importance especially as
648 waterfowl habitat. Ramsar (Iran), 2 February 1971. UN Treaty Series No. 14583. As amended
649 by the Paris Protocol, 3 December 1982, and Regina Amendments, 28 May 1987 (available at
650 www.ramsar.org/key_conv_e.htm)
651
652 Rossi, F., Lardicci, C., 2002. Role of the nutritive value of sediment in regulating population
653 dynamics of the deposit-feeding polychaete *Streblospio shrubsolii*. Marine Biology, 140,
654 1129-1138.
655
656 Ruffo, S., 1998. The Amphipoda of the Mediterranean (959 pp). Memoires de l'institut
657 Oceanographique de Monaco, 13.
658
659 Searle, S.R., Casella, G., McCulloch, C.E., 1992. Variance components. New York, Wiley.
660
661 Sechi, N., Fiocca, F., Sannio, A., Lugliè, A., 2001. Santa Giusta lagoon (Sardinia):
662 phytoplankton and nutrients before and after waste water diversion. Journal of Limnology, 60,
663 194-200.

Snelgrove, P.V.R., 1998. The biodiversity of macrofaunal organisms in marine sediments. Biodiversity and Conservation, 7, 1123-1132.

Stark, J.S., Riddle, M.J., Rodney, D.S., 2003. Human impacts in soft-sediments assemblages at Casey Station, East Antarctica: spatial variation, taxonomic resolution and data transformation. Australian Ecology, 28, 287-304.

Terlizzi, A., Bevilacqua, S., Fraschetti, S., Boero, F., 2003. Taxonomic sufficiency and the increasing insufficiency of taxonomic expertise. Marine Pollution Bulletin, 46, 556-561.

Terlizzi, A., Scuderi, D., Fraschetti, S., Anderson, M.J., 2005. Quantifying effects of pollution on biodiversity; a case study of highly diverse molluscan assemblages in the Mediterranean. Marine Biology, 148, 293-305.

Terlizzi, A., Anderson, M.J., Bevilacqua, S., Fraschetti, S., Wlodarska Kowalczuk, M., Ellingsen, K.E., 2008a. Beta diversity and taxonomic sufficiency: Do higher-level taxa reflect heterogeneity in species composition? Diversity and Distributions, in press (doi: 10.1111/j.1472-4642.2008.00551.x).

Terlizzi, A., Bevilacqua, S., Scuderi, D., Fiorentino, D., Guarnirei, G., Giangrande, A., Licciano, M., Felline, S., Fraschetti, S., 2008b. Effects of offshore platforms on soft-bottom macrobenthic assemblages: a case study in a Mediterranean gas field. Marine Pollution Bulletin, 56, 1303-1309.

Thrush, S.F., Hewitt, J.E., Pridmore, R.D., 1989. Patterns in the spatial arrangements of polychaetes and bivalves in intertidal sandflats. Marine Biology, 102, 529-535.

Thrush, S.F., 1991. Spatial patterns in soft-bottom communities. Trends in Ecology and Evolution, 6, 75-79.

Underwood, A.J., 1981. Techniques of analysis of variance in experimental marine biology and ecology. Oceanography and Marine Biology: Annual Review, 19, 513-605.

Underwood, A.J., 1997. Experiments in ecology: their logical design and interpretation using analysis of variance. Cambridge: Cambridge University Press.

Underwood, A.J., Chapman, M.G., 1996. Scales of spatial patterns of distribution of intertidal invertebrates. Oecologia, 107, 212-224.

Underwood, A.J., Chapman, M.G., Connell, S.D., 2000. Observations in ecology: you can't make progress on processes without understanding the patterns. Journal of Experimental Marine Biology and Ecology, 250, 97-115.

Vanderklift, M.A., Ward, T.J., Jacoby, C.A., 1996. Effect of reducing taxonomic resolution on ordinations to detect pollution - induced gradients in macrobenthic infaunal assemblages. Marine Ecology Progress Series, 136, 137-145.

Warwick, R.M., 1988. The level of taxonomic discrimination required to detect pollution effects on marine benthic communities. Marine pollution Bulletin, 19, 259-268.

714
715 Warwick, R.M., 1993. Environmental impact studies on marine communities: pragmatical
716 considerations. Australian Journal of Ecology, 18, 63-80.
717
718 Warwick, R.M., Clarke, K.R., 1993. Increased variability as a symptom of stress in marine
719 communities. Journal of Experimental Marine Biology and Ecology, 172, 215-226.
720
721 Wilson, W.H., 1991. Competition and predation in marine soft sediment communities.
722 Annual Review of Ecology and Systematic, 21, 221-241.
723
724 Wlodarska Kowalczuk, M., Kedra, M., 2007. Surrogacy in natural patterns of benthic
725 distribution and diversity: selected taxa versus lower taxonomic resolution. Marine Ecology
726 Progress Series, 351, 53-63.
727
728 Ysebaert, T., Herman, P.M.J., 2002. Spatial and temporal variation in benthic macrofauna and
729 relationship with environmental variables in an estuarine, intertidal soft-sediment
730 environment. Marine Ecology Progress Series, 244, 105-124.
731
732 Ysebaert, T., Herman, P.M.J., Meire, P., Craeymeersch, J., Verbeek, H., Heip, C.H.R., 2003.
733 Large-scale spatial patterns in estuaries: estuarine macrobenthic communities in the Schelde
734 estuary, NW Europe. Estuarine, Coastal and Shelf Science, 57, 335-355.
735

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 alpha		Av. Transf. Abundance beta		Av. Transf. Abundance gamma
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
Minuspio 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.

		Species		Family		Class	
Source	Pairwise	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**
		Species		Family		Class	
Source	Pairwise	L+D	D	L+D	D	L+D	D
Sites within	1 – 2	0.0016	**0.2030**	0.0006	**0.2612**	0.0124	**0.9206**
alpha zone	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							
beta 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							
gamma 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 groups 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

Figure 1

Figure 1.

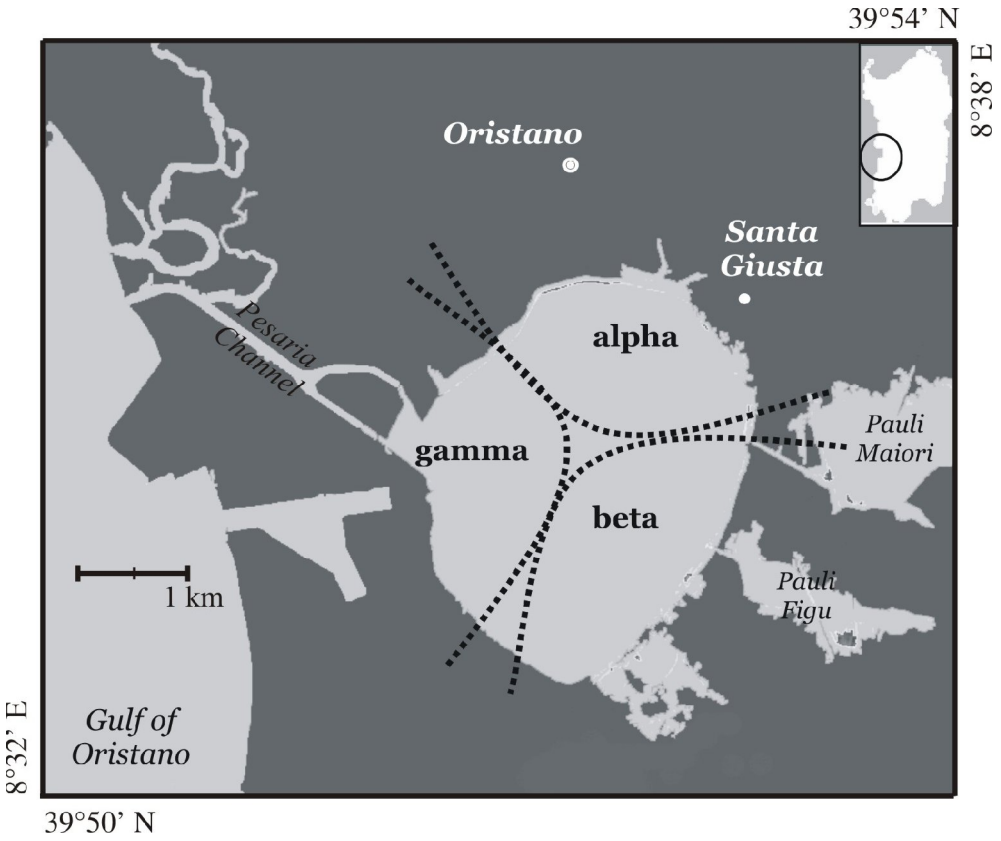


Figure 2

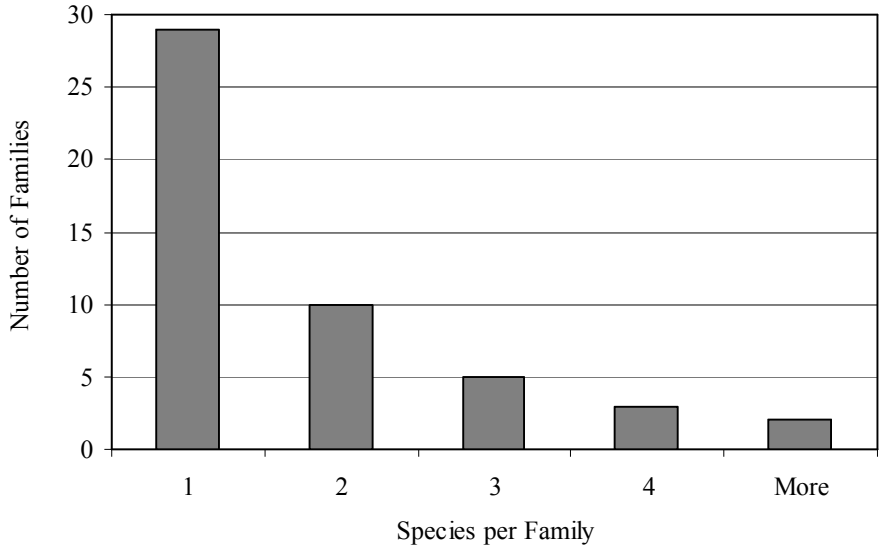


Figure 2. Number of taxonomic families represented in samples and number of species per family. 'More' data points were two, respectively with 6 and 9 species per family

Figure 3

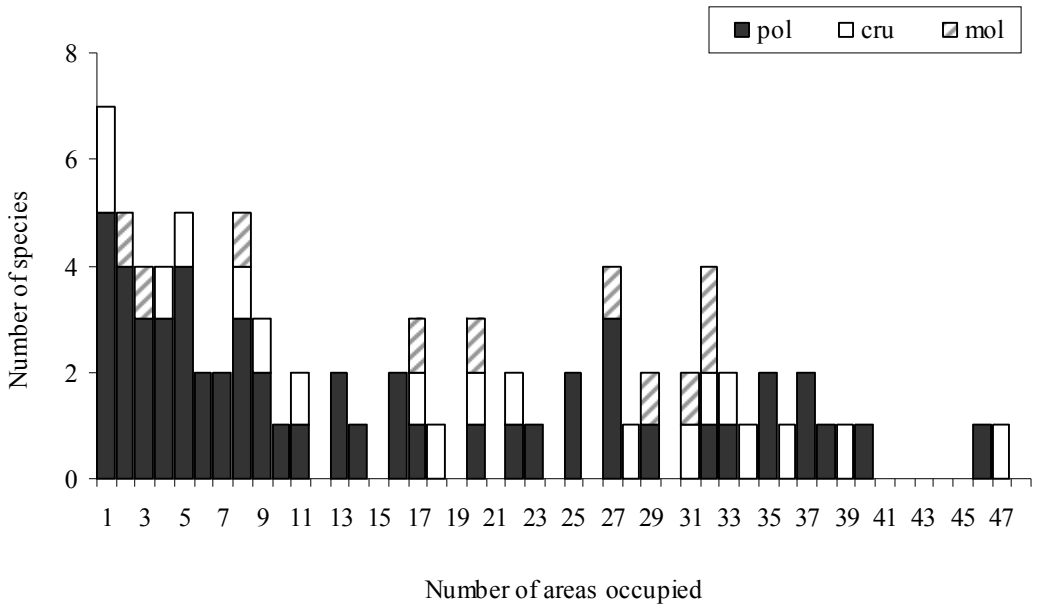


Figure 3. Distribution of species according to the number of areas occupied out of a total of 48 areas. pol: polychaetes; cru: crustaceans; mol: molluscs

Figure 4

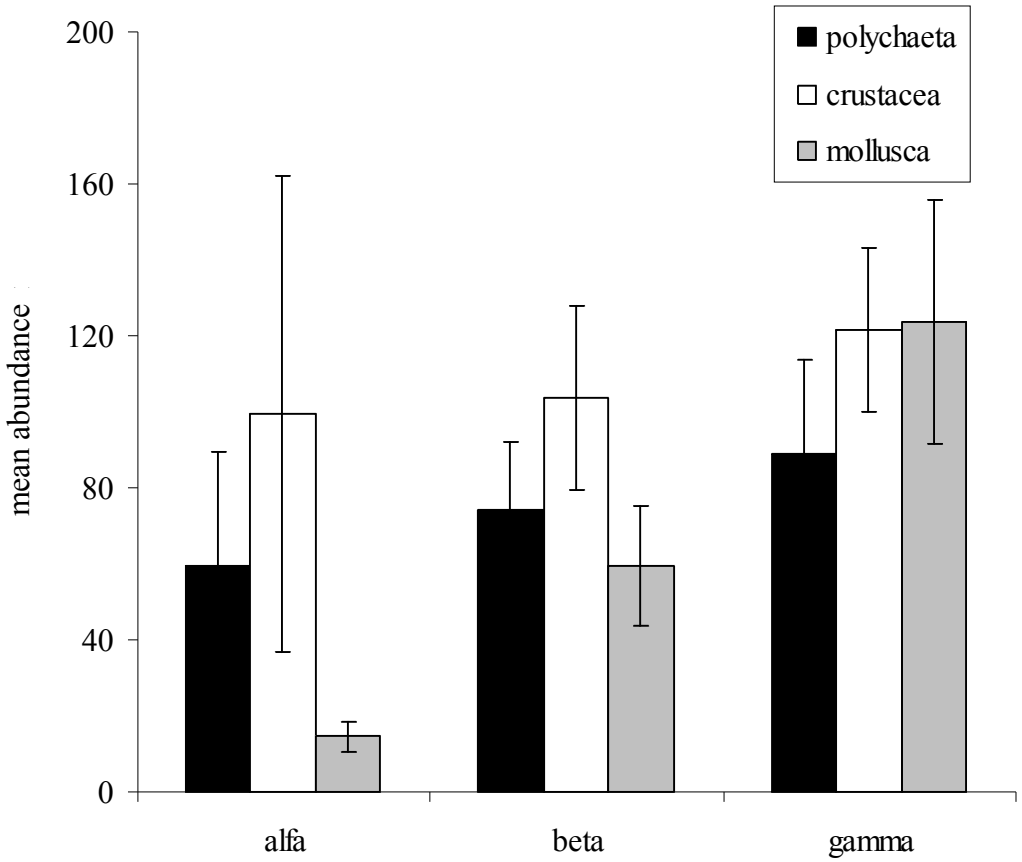


Figure 4. Mean abundance (number of individuals), with 95% confidence interval, of the three main faunal groups at each of the three sampling zones of Santa Giusta lagoon (*n*=32 per zone)

Figure 5

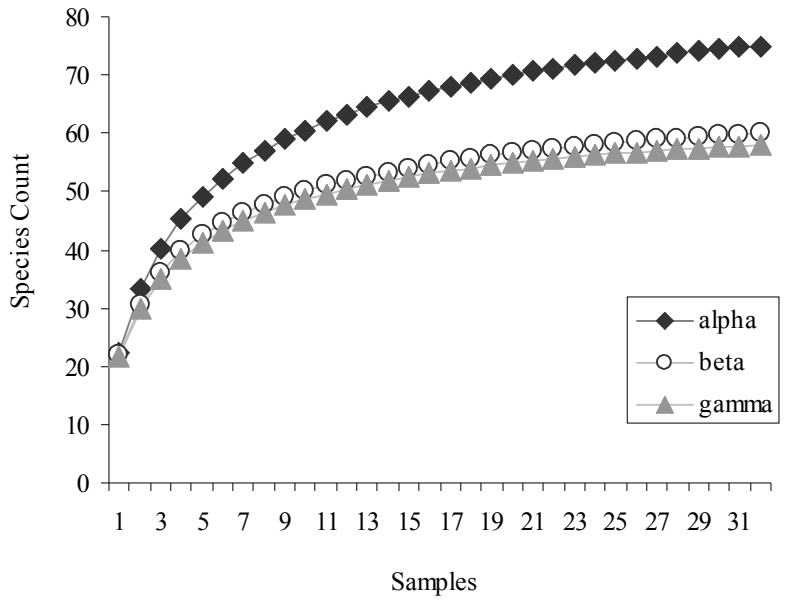


Figure 5. Species-sample accumulation curves for each zone of the lagoon. Data were based on 999 permutations of replicate samples.

Figure 6

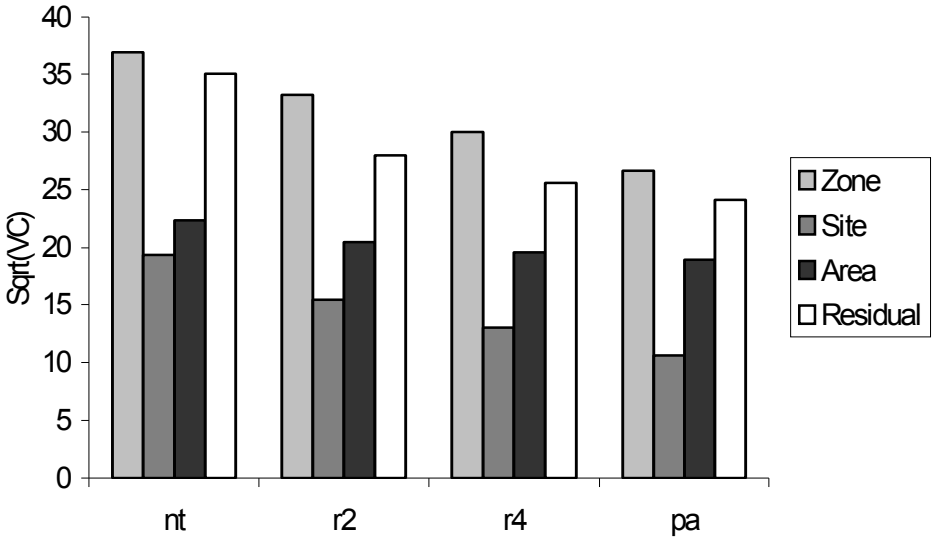


Figure 6. Multivariate variance components at each of the four spatial scales for all species, as obtained using mean squares from PERMANOVA performed with different transformations (nt = no transformation; r2 = square root; r4 = fourth root; pa = presence/absence). The values plotted are the square 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

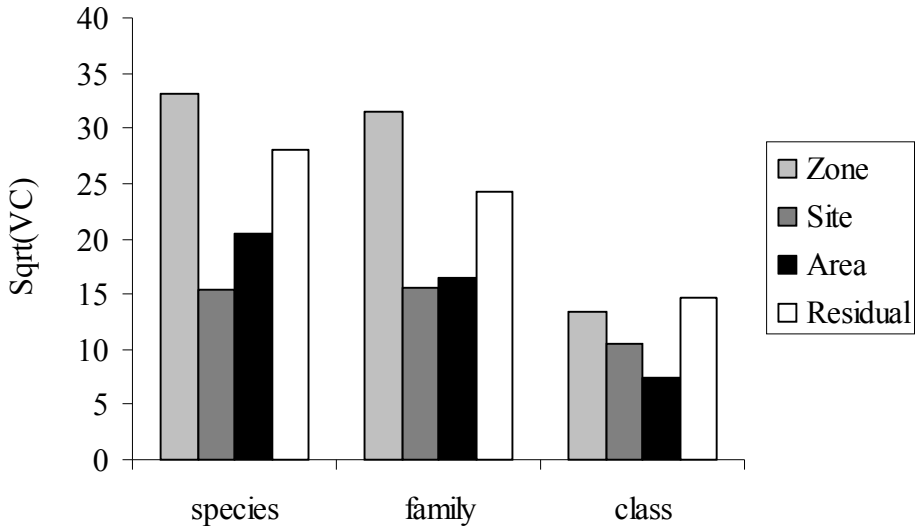


Figure 7. Multivariate variance components at each of the four spatial scales for all organisms collected using species, family and class taxonomic levels. The values plotted are the square root of the sizes of the variance components (Table 3)

Figure 8

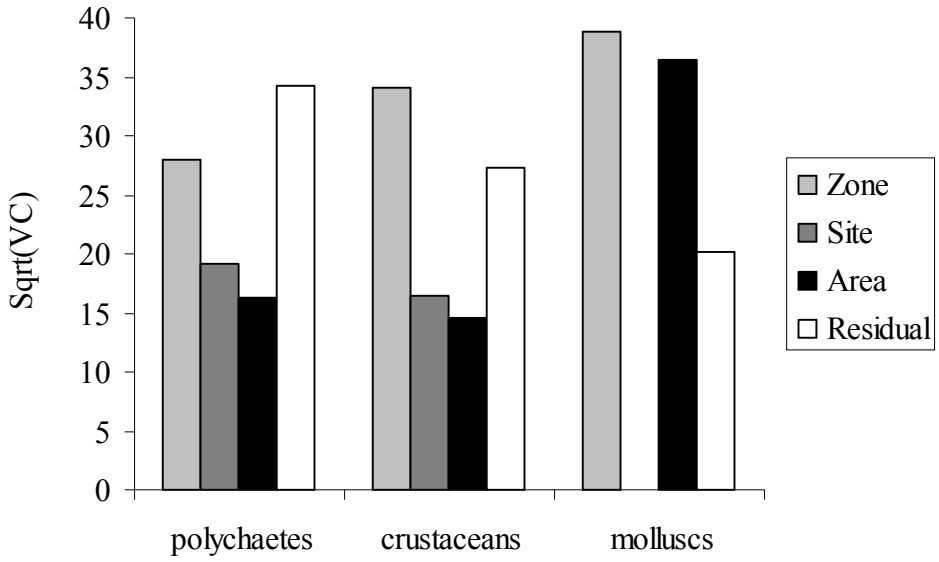
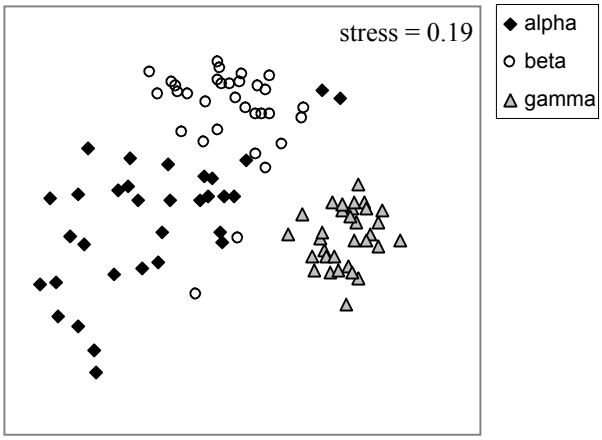


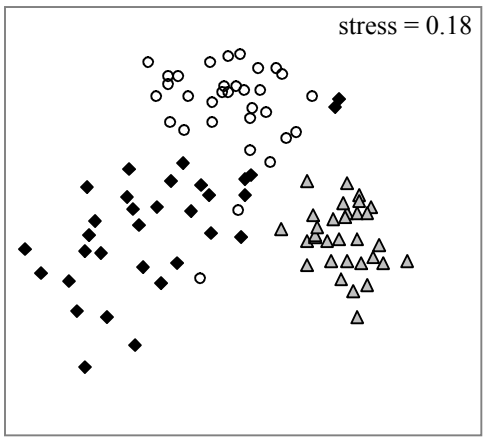
Figure 8. Multivariate variance components at each of the four spatial scales for the three taxonomic group collected. The values plotted are the square root of the sizes of the variance components, obtained using mean squares from PERMANOVA performed with square root transformed data. All components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

Figure 9

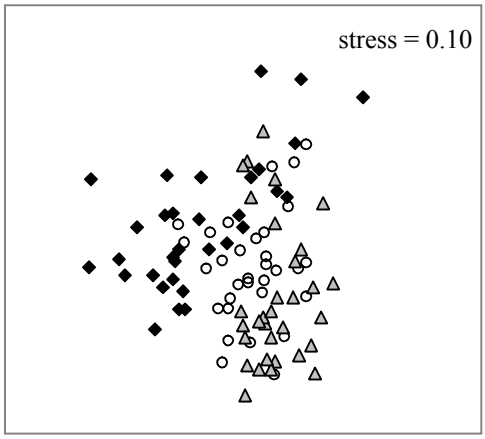
a. Species



b. Family



c. Class



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