1 **Variance estimate and taxonomic resolution: an analysis of macrobenthic**

2 **spatial patterns at different scales in a Western Mediterranean coastal**

3 **lagoon**

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11

12 **Abstract**

13 The effects of taxonomic resolution on the variance estimates of macrobenthic assemblages

14 were studied at four spatial scales in a Mediterranean coastal lagoon. The assemblages

15 exhibited significant differences at all the investigated scales; however, spatial variability was

16 mainly associated with the smallest and the largest scales. The decrease of taxonomic

17 resolution (from species to family) was not related to a decrease of the overall variability and

18 similar estimates of variance components were obtained using species and family resolution

19 levels. The ordination models derived from species and family abundances were very similar

20 both in terms of location and dispersion effect, while further aggregation to the class level

21 began to alter the observed spatial patterns. In future studies aimed at assessing changes in the

22 lagoon, resources derived from the cost reductions achieved using family level could be

23 employed to plan more frequent surveys and/or to adopt complex spatial sampling designs

24 with a high number of replicates.

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26 **KEY WORDS:** spatial scales - taxonomic resolution - multivariate analysis - macrobenthos -

27 coastal lagoon - monitoring - Western Mediterranean

28

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

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small-scaled sampling units are nested within larger-scaled ones, allowing unconfounded

statistical comparisons among each spatial scale (Underwood, 1981; Kotliar and Wiens,

1990). Nested designs have been successfully used to investigate populations and

assemblages across a wide range of marine habitats and organisms. Most studies focused on

intertidal and subtidal rocky shores (*i.a.* Archambault and Bourget, 1996; Underwood and

Chapman, 1996; Benedetti-Cecchi, 2001a; Fraschetti et al., 2001; 2005; Chapman and

Underwood, 2008), while soft-bottoms have been less explored (Morrisey et al., 1992; Stark

et al., 2003; Noren and Lindegarth, 2005; Terlizzi et al., 2008b).

The increase of soft bottom macrobenthic studies is often hidden because identifying and

enumerating all organisms are time-consuming and labour-intensive processes (Warwick,

1993; Olsgard et al., 1998), that requires taxonomic expertise (Terlizzi et al., 2003). In the

last two decades, many studies have analysed data at several taxonomic resolutions, showing

that results obtained using species or family level are very similar (*i.a.* Warwick, 1988;

Vanderklift et al., 1996; Olsgard et al., 1998; Karakassis and Hatziyanni, 2000; Lampadariou

et al., 2005; Wlodarska-Kowalczuk and Kedra, 2007). Identifying a taxonomic level higher

than species that is sufficient for detecting differences in assemblage composition without

losing important information is a concept termed "taxonomic sufficiency" (TS, Ellis, 1985).

The TS method might have some practical implications, in particular routine monitoring

programs could become less expensive and faster than those conducted at the species level

resolution and therefore macrobenthic assemblages could be analysed more frequently.

However, at present, most studies have usually compared different levels of TS at a single

spatial scale (Vanderklift et al., 1996; Olsgard et al., 1998; Karakassis and Hatziyanni, 2000;

De Biasi et al., 2003; Wlodarska-Kowalczuk and Kedra, 2007), while few researchers have

investigated the effects of TS on the spatial distribution patterns observed at multiple scales

(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

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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 x 17 cm^2), 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,

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

227   zone 60 species were found and the five most abundant ones (51.3% of total 7 601

228   individuals) were typical brackish species like *Cymodoce truncata*, *Abra ovata*,

229   *Monocorophium sextonae*, as well as *Minuspio multibranchiata* and *Microdeutopus*

230   *anomalus,* occurring where macroalgae are present (Ruffo, 1998). Opportunistic species

231   indicating organic enrichment (Pearson and Rosenberg, 1978; Cognetti, 1982), such as *Phylo*

232   *foetida* and *Neanthes caudata*, were also characteristic of the beta zone (Table 2), as well as

233   suspension feeders (*Loripes lacteus* and *Cerastoderma glaucum*) and grazers (*Cumella*

234   *limicola* and *Iphinoe serrata*). In gamma zone 10 730 organisms from 59 species were

235   collected and 80% of total abundance was reached with only five species: *Mytilaster minimus*

236   (alone accounting for 33%), *Cymodoce truncata* and *Tanais dulongii*, all typical of brackish

237   environments, besides *Minuspio multibranchiata* and *Naineris laevigata.*

238

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

240   At the taxonomic level of species, PERMANOVA showed that there was a highly significant

241   variability at all spatial scales considered (Table 3). The greatest variability occurred at the

242   largest spatial scale, among zones, for which the average Bray-Curtis dissimilarity was around

243   37%; then, the successive variation component was that among replicate samples (35% of

244   dissimilarity), followed by less variability among areas (22%) and sites (20%) (Figure 6).

245   Furthermore, the relative importance of different spatial scales in the hierarchy did not vary

246   with different data transformations. Similar spatial patterns were obtained for analyses based

247   on untransformed and transformed data (square root, fourth root, presence/absence) (Figure

248   6).

249   Highly significant variability at all spatial scales was also detected, at the family and class

250   levels (Table 3). In addition, similar variance components, as well as the relative importance

251   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

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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)

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

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.

402 As argued elsewhere, the relationship between time saving and taxonomic level changes from

403 case to case (Olsgard et al., 1998), depends on the number of species within a single family,

404 the taxonomical complexity of families and the availability of taxonomic expertise (Ferraro

405 and Cole, 1995; Dethier and Schoch, 2006). However, it has been calculated that generally the

406 cost of family level identification was 50% to 55% less than species level identification

407 (Ferraro and Cole, 1995; De Biasi et al., 2003). In our case, the time needed for identification

408 at family level was 63% less than the species level identification, considering that 33% of

409 families was represented by two or more species. However, the majority of species belonged

410 to polychaetes and our laboratory team has significant expertise in polychaetes. Resources

411 deriving from such cost reduction could be employed to plan more frequent surveys and/or to

412 adopt more complex spatial sampling designs with a high number of replicates, in order to

413 further minimize spatial variability caused by the dispersion effect. When the distribution of

414 organisms is patchy, it is probably more important to collect many replicates at different

415 spatial and temporal scales than to identify taxa at the finest resolution level (Morrisey et al.,

416 1992; Chapman, 1998). In Santa Giusta lagoon, our baseline detailed multiscale investigation

417 demonstrated that at least two sites (namely 16 samples) for each zone are needed to collect

418 the majority of species and therefore to describe correctly the spatial distribution of benthic

419 assemblages.

420 Results obtained in this study have important practical consequences for investigations on the

421 distribution of soft bottom macrofauna in brackish habitats, including those concerned with

422 environmental monitoring. In fact, the present study can be considered as a valuable example

423 for a rigorous approach in collecting data for ecological studies, when previous detailed

424 knowledge is scant. The spatial variability observed at all the examined scales indicated that

425 small-scale observations are unlikely to describe the spatial benthic distribution of the whole

426 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

462　**Acknowledgments**

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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.

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 alpha 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 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
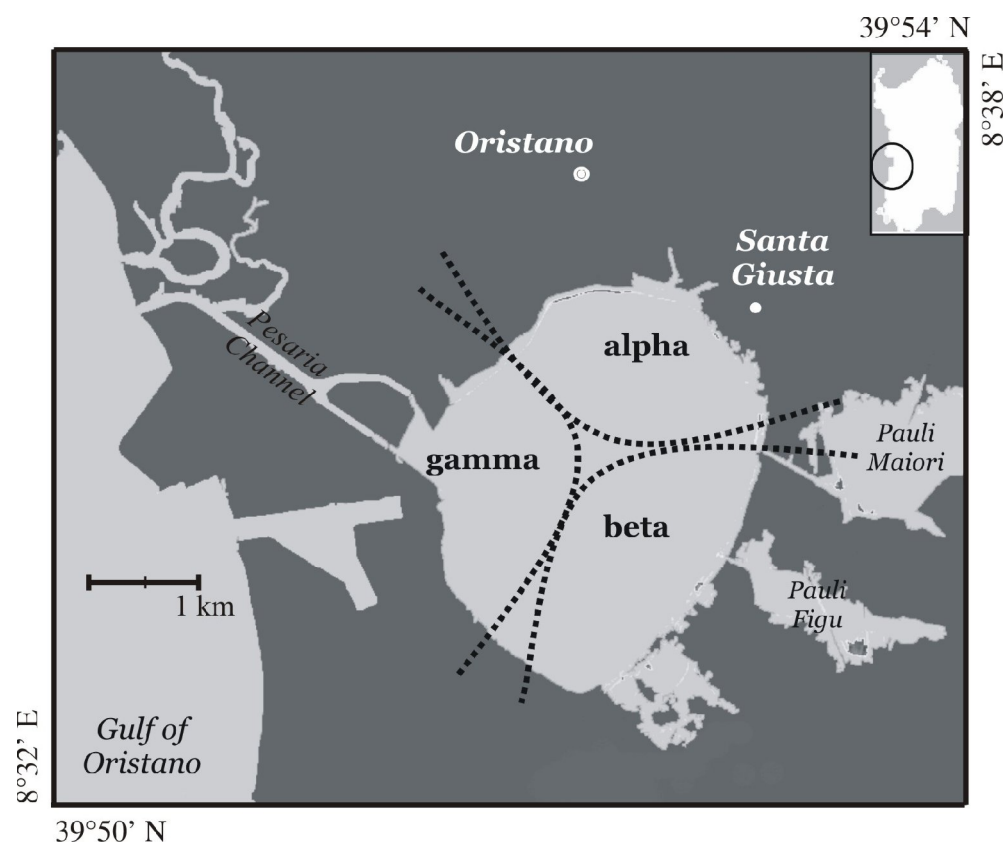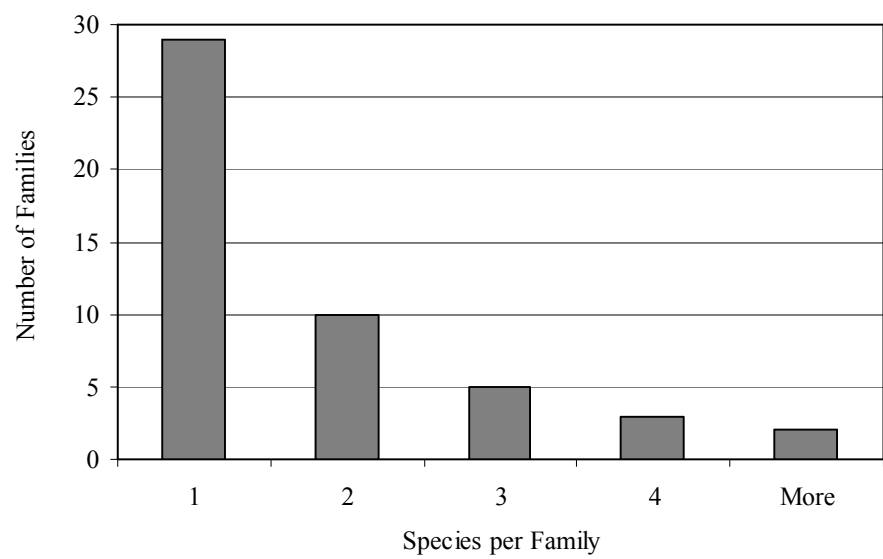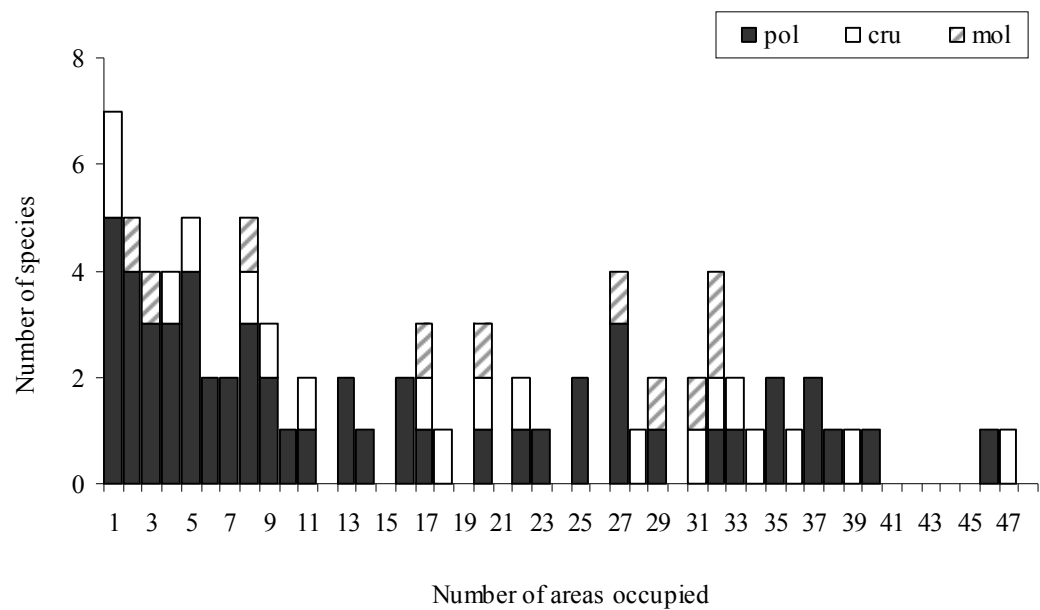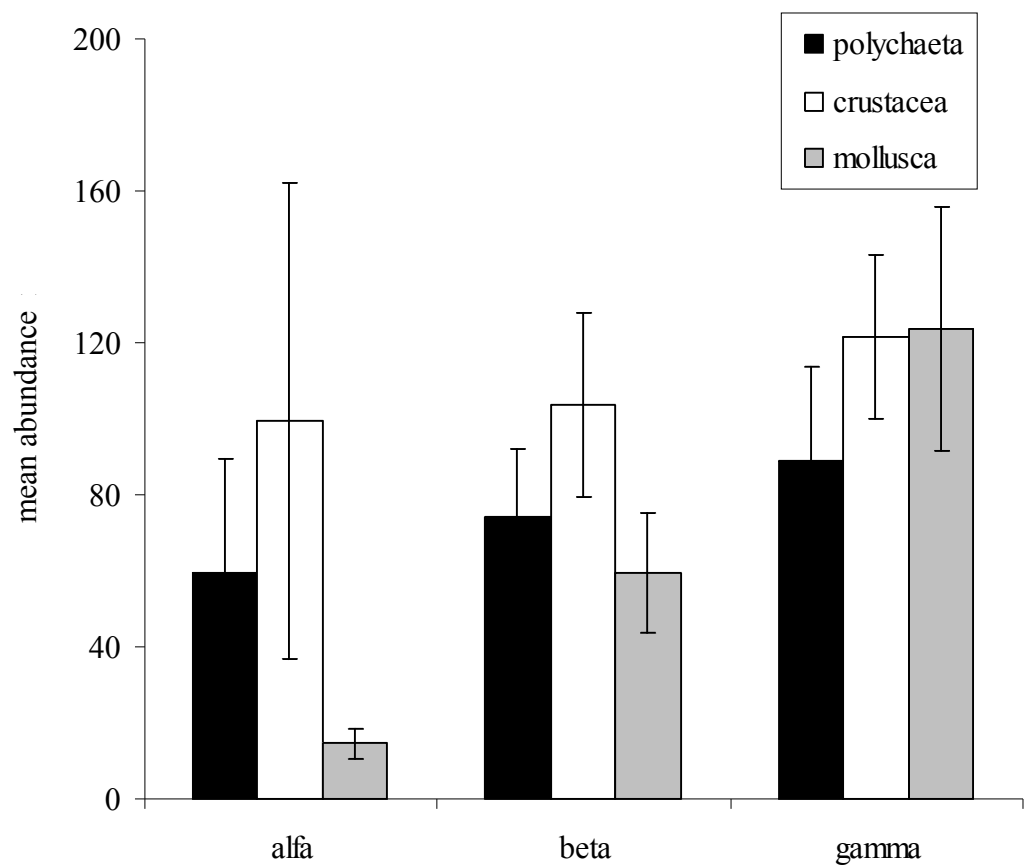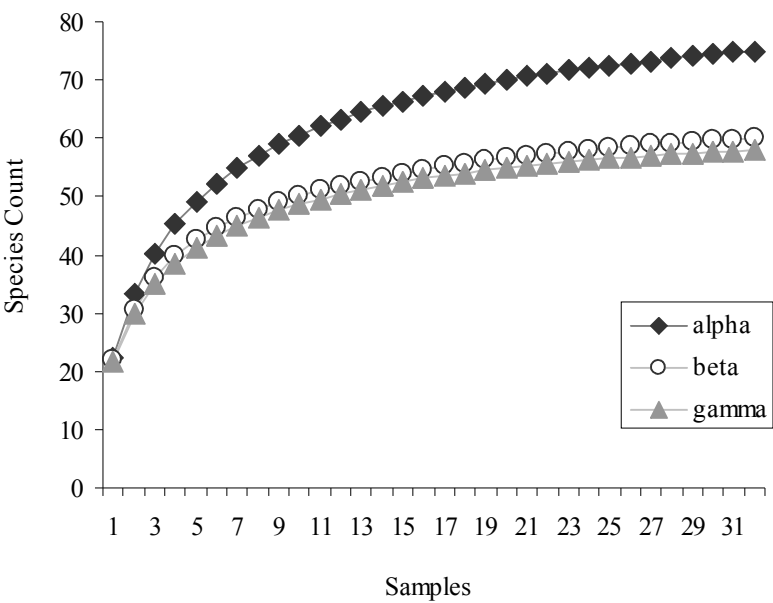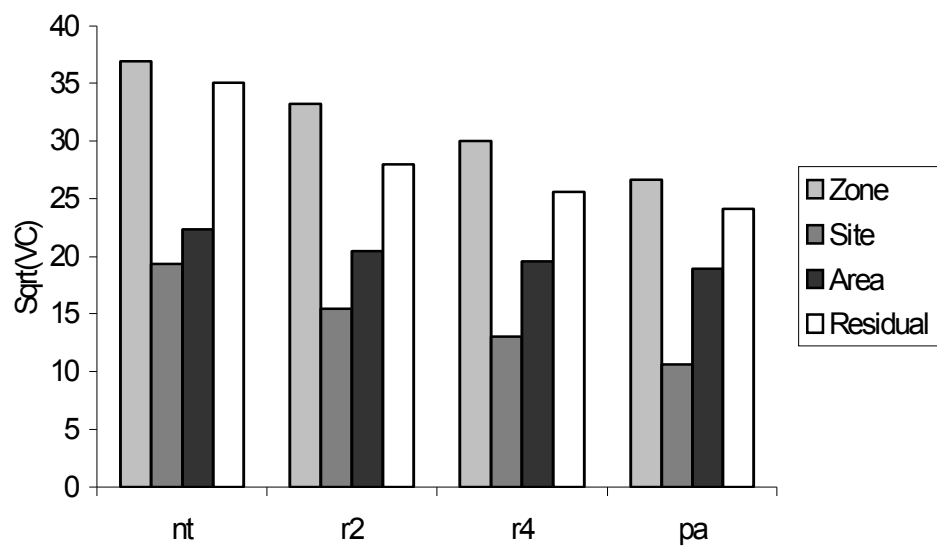
Figure 1

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)
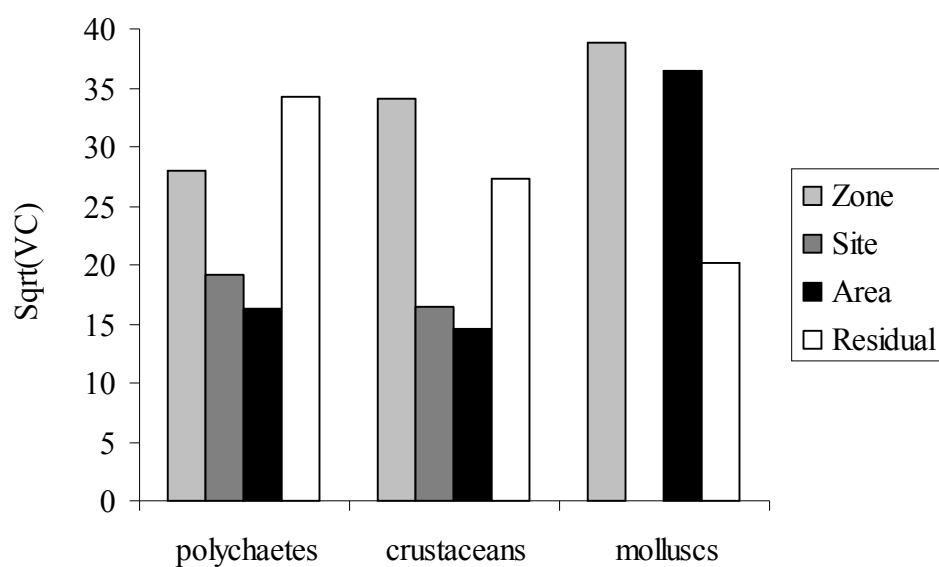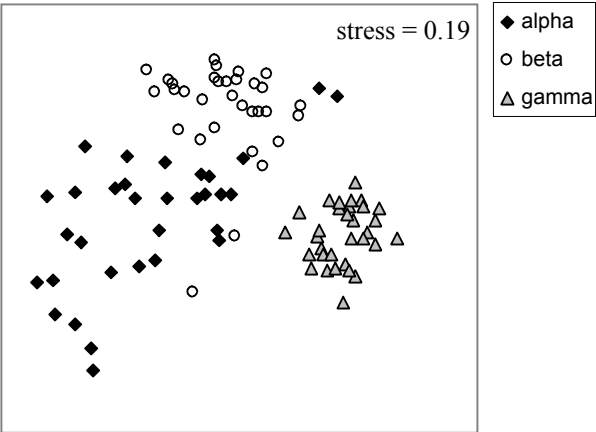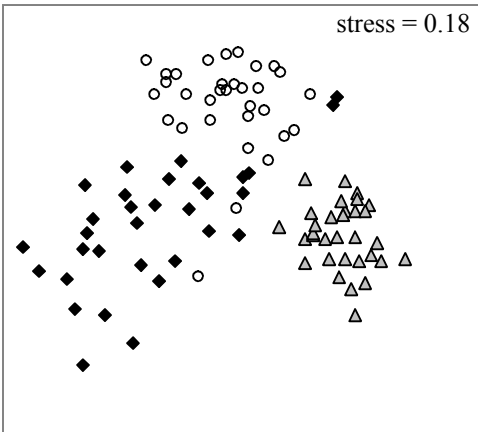
Figure 8

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
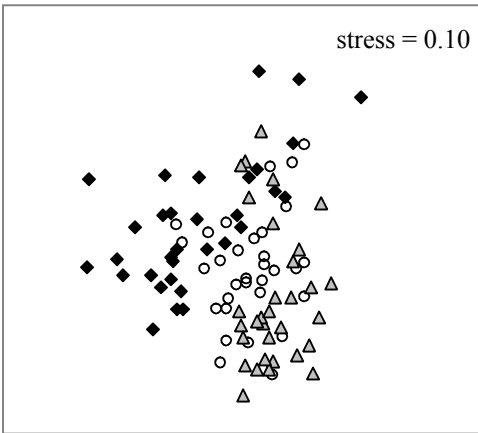
a. Species

b. Family

c. Class

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