

Comparative study based on sediment characteristics and macrobenthic communities in two Italian lagoons

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Abstract The aims of this study were to analyse sediment characteristics and macrobenthic assemblages in two very close Italian coastal lagoons (Lesina and Varano) and to assess the different behaviour between the two basins and the relationship between sediment matrix and benthic organisms within and between the two lagoons. The comparative study was performed in July 2007 at 13 sampling sites in Lesina lagoon and 15 sites in Varano basin for sediment grain size, total organic carbon (TOC), total nitrogen (TN), total phosphorus (TP) and macrobenthic structure analyses. Both lagoons were generally dominated by fine-grained sediments (clay and silt components). The average contents of TOC and TN

measured in Lesina was higher than in Varano (3.31% vs 2.52% for TOC and 5,200 $\mu\text{g}\cdot\text{g}^{-1}$ vs 3,713 $\mu\text{g}\cdot\text{g}^{-1}$ for TN); in contrast, the TP was lower (540 $\mu\text{g}\cdot\text{g}^{-1}$ vs 620 $\mu\text{g}\cdot\text{g}^{-1}$). Based on macrobenthic community patterns, the central zone in Varano lagoon and the eastern area in Lesina lagoon were characterised by the lowest abundance (168.7 $\text{ind}\cdot\text{m}^{-2}$ and 503.2 $\text{ind}\cdot\text{m}^{-2}$, respectively) and by the lowest number of species, as highlighted by the diversity indices (Shannon–Wiener, H' range was 0.47–1.45 for Lesina and 0.00–1.68 for Varano; Margalef species richness, d range was 0.00–1.67 for Lesina lagoon and 0.00–2.38 for Varano basin). Ordination diagrams suggested an influence of marine and freshwater inputs on the sediment distribution in Varano lagoon and on macrobenthic assemblages in Lesina lagoon.

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Introduction

Coastal lagoons constitute important transition zones between the land and the sea. They are enriched by both the marine and continental inputs and are among the most productive aquatic ecosystems (Nixon 1988), playing an important economic role. They are often nutrient rich

(Cauwet 1988; Colombo 1977) as a result of nutrient input by rivers and recycling between sediment and water column (Nowicki and Nixon 1985; Schleyer and Roberts 1987). They take up 13% of the world coastline (Carrada 1990) and more than half of the Mediterranean lagoons are exploited for extensive and semi-intensive aquaculture (both fish and shellfish; Barnes 1991, 1995). The importance of such a system for fisheries and aquaculture at global level, and for Italy in particular, directs research towards the study of the relationships between abiotic environmental factors and the biological productivity. The ecological characteristics of all organisms living in coastal lagoons are related to environmental stress due to the alternating inputs of marine and fresh waters, in addition to the increased soluble nutrients from surrounding watershed (Carrada 1990; Nixon 1982; Perez-Ruzafa et al. 2005; Sfriso et al. 1992; Taylor et al. 1995). Due to their low water turnover and long residence time, many Mediterranean coastal lagoons are greatly affected by nutrient loads from their catchments (La Jeunesse and Elliott 2004). The high variability in chemical, physical and hydrologic parameters make them typically unstable environments (Millet and Guelorget 1994) and the concept of short- or long-term variations is relative for these ecosystems compared to marine environments (Marzano et al. 2003). Urban development and human activity on coastal lagoons have increased considerably in recent years, and the impact on these productive and economically important environments has become a major concern. Depending on their hydrologic and trophic status, these environments are often affected by severe anoxic crises followed by mortality events (Lardicci et al. 1997; Sorokin et al. 1996). These phenomena may be exacerbated by organic enrichment, laced with fine sediments, nutrients and contaminants (Förstner and Wittmann 1979; Birch 2000), resulting from the discharged storm water and sewage from urban and agricultural areas. This has often been linked with the deterioration of floral and faunal habitat characteristics such as water and physicochemical sediment quality (Corbett et al. 1997; Wahl et al. 1997).

In these coastal ecosystems, sediments play an important role in biogeochemical cycles (Pomero-

et al. 1965). Much of allochthonous material is incorporated in the sediments, through assimilation, adsorption and direct sedimentation processes of suspended particulate, so they act as a trap of detritus material and mineral nutrients supply (Lijklema 1986). Organic matter is mineralized and dissolved nutrients exchanged within the interface. Sediment oxygen demand includes bacterial mineralizing activity, oxygen needs for nitrification and benthic fauna respiration (Di Toro et al. 1990). Benthic assemblages are certainly the best tool to describe the ecological conditions of these systems since macrobenthic fauna is highly correlated with the sediment which accumulate the multiple sources of organic enrichment and pollution (Dauer and Alden 1995; Lardicci and Rossi 1998; Le Bris and Glemarec 1996). The present work was performed in two coastal lagoons (Varano and Lesina) located in the south-eastern coast of Italy. It is important to emphasize that the two basins are geomorphologically different and are characterized by very different trophic and biological variables. The aims of this study were: (1) to analyse sediment characteristics and macrobenthic assemblages in each lagoon through a combination of univariate and multivariate analyses; (2) to assess a different behaviour between the two lagoons in relation to both sediment variables and macrobenthic structure; (3) to evaluate the effects of marine and freshwater inputs on distribution patterns of both sediment variables and macrofaunal community; (4) to assess if the distribution of benthic organisms within and between the two lagoons are related to that of sediment characteristics.

Methods

Study areas

Varano lagoon and Lesina lagoon are two Mediterranean coastal lagoons located within Gargano National Park, a protected area and tourist resort of the SE Italy, (Fig. 1). Despite their proximity, the lagoons differ considerably in geomorphological structure, physical characteristics and surrounding land. Table 1 summarises some of the main general features of the two

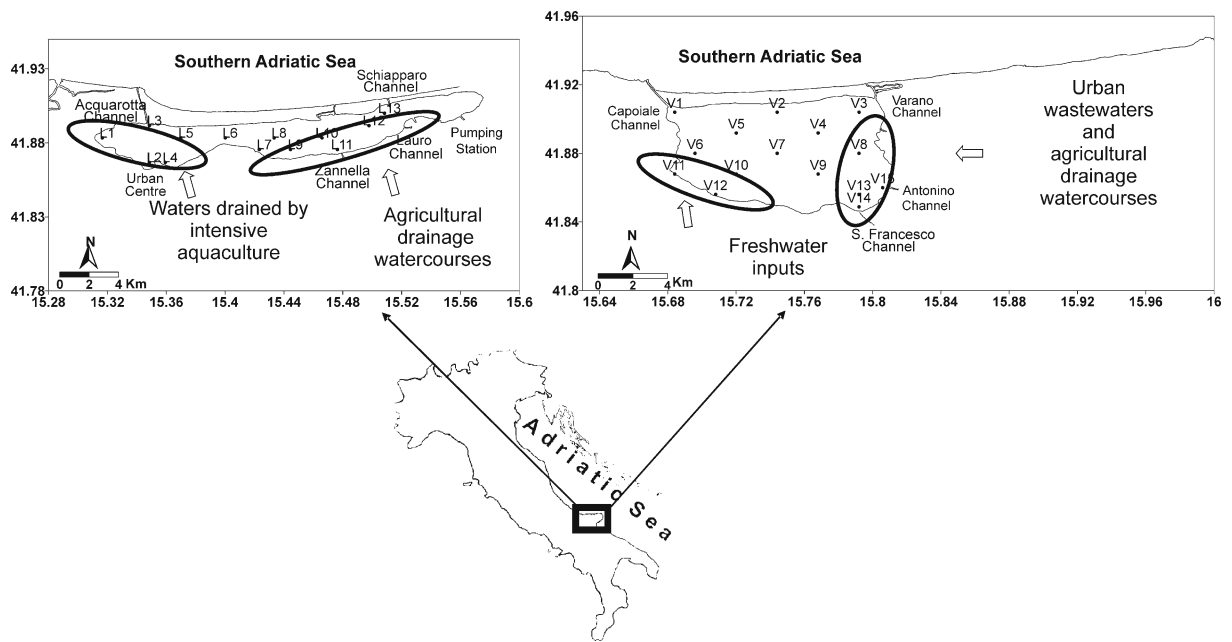


Fig. 1 Location of the two studied lagoons and map showing the sampling sites within each basin

lagoons. Both systems communicate with the Adriatic Sea by means of two artificial channels, located in the western and the eastern side of the two lagoons. Due to the low tide excursion and reduced exchange with the adjacent coastal area, water time residence is very long in both systems (Table 1). North-north-western winds are very frequent in this area, above all during the winter season, helping sea water inputs into the lagoon. Strong seasonal variations of temperature (7°C in winter and 26–32°C in summer) and salinity (from 5 to 34 psu) characterize Lesina lagoon (Marolla et al. 1995; Priore et al. 1994). Moreover,

the western part of the lagoon, which receives water drained by the intensive aquaculture farms, generally exhibits higher salinity values compared to the eastern area; this last zone of the lagoon receives freshwater inputs collecting agricultural drainage water from a pumping station located south of Lesina where the land is lower than the average sea level. Varano lagoon shows a regular seasonal cycle of temperature, with the highest average reached in summer (30°C) and the lowest means in winter (8–10°C), while salinity values are relatively constant, ranging between 23 and 29 psu (Caroppo 2000; Marolla 1980, 1981;

Table 1 Main physical characteristics of Lesina and Varano lagoons

	Lesina lagoon	Varano lagoon
Location	41.88°N; 15.35°E	41.51°N; 15.47°E
Catchment land use	National Park, urban, agricultural and fisheries	National Park, urban, agricultural and mussel farming
Watershed area (km ²)	400	357
Surface area (km ²)	51	65
Mean depth (m)	0.8	4
Maximal depth (m)	1.5	5.8
*Residence time (year)	About 1	1.5
Total length from east to west (km)	22.4	11
Maximum width (km)	3.4	7.2

Specchiulli et al. 2008). It receives freshwater inputs of approximately $87,000 \text{ m}^3 \text{ d}^{-1}$ with an organic content mostly originating from urban and agricultural runoff, fish-farming and zoo-technique activities (Spagnoli et al. 2002; Villani et al. 2000). The freshwater inputs to the lagoon come from groundwater as springs in the southwestern basin of the lagoon, while in the southeastern zone, surrounding urban wastewaters and drainage watercourses (from surrounding agricultural area) discharge into the lagoon.

The economic relevance of both the lagoons is mostly related to fishing activity and extensive aquaculture farming. Moreover, mussels breeding (*Mytilus galloprovincialis*) was carried out into Varano lagoon, especially in the northern side of the basin, but the fish production has been greatly reduced over time and in 1995 it was estimated less than 100 t against 700–800 t of 60–70 years. The mussels, with a production of a few hundred quintals per year, faces significant difficulties in the summer months for the deficiency of oxygen in the water and consequent reduction in growth of shellfish (Breber and Scirocco 1998).

Sampling

The study was performed in summer season (July 2007) at 13 sampling points in Lesina lagoon and 15 stations in Varano basin (Fig. 1). Summer provides the greatest power for trend detection about benthic macrofauna and differences among stressed and non-stressed sites is also greatest in this season (Alden et al. 1997). Recent efforts to develop biocriteria assumed that summer sampling would provide the greatest opportunity for distinguishing degraded from reference sites (Alden et al. 1997; Engle et al. 1994; Weisberg et al. 1997). The differences are probably greatest in summer because it includes the period of greatest hypoxic stress and precedes the fall recruitment period when partial recovery might occur. In addition, summer is also the time of highest metabolic activity for bacteria and therefore the effects of sediment contamination would be most severe. The distribution of sites was not intended to provide representation of the whole lagoon surface but to ensure some correspondence in the

positions of sites along environmental gradients due to the marine, fresh and groundwater influence (Fig. 1). Three core samples were collected at each sampling station, using a box-corer ($15 \times 15 \times 15 \text{ cm}$). One core was used for grain-size determination, the second core for measurements of physicochemical variables and the other one for an estimate of macrobenthic abundance and diversity. Resources did not permit triplicate sampling for each typology of analysis.

Granulometric analysis

The classification of fine and coarse sediments followed the scale of Shepard (1954) where sediments were partitioned into % sand (2000–63 μm), % silt (63–2 μm) and % clay (<2 μm). Grain-size analyses were carried out, after elimination of the organic fraction with H_2O_2 , by wet sieving, to separate sand from the fine fractions. For sandy fractions, a sieve size >63 μm was used. The weight of the sand trapped by the sieves was measured, and the percentage with respect to the total weight of sandy sediment fraction was determined. For fine sediments, a Sedigraph 5100 Micromeritics was employed. This instrument computes the grain size by estimating the transmittance produced by an X-ray beam which crosses the sediment scattered in a water sample.

Macronutrients quantification in sediments

For the purpose of this study, only the 5-cm top layer sediment fraction was considered. The percentage of water content was calculated as the weight difference between wet and dried sediments (dried overnight at 105°C), in three replicates for each sediment sample. Sediment samples for physicochemical analyses were homogenized, dried (70°C , 48 h) in oven until constant weight and weighed. Dried sediments were analysed in order to determine: total carbon (TC, microgram per gram), total organic carbon (TOC, %), total phosphorus (TP, microgram per gram) and total nitrogen (TN, microgram per gram). Samples were analysed in triplicate; average and standard deviation (SD) were calculated. Chemicals and

reagents were analytical grade and glassware was carefully washed to avoid sample contamination. Determinations of TC and TN were performed by direct total flash combustion using a CHNS Elemental Analyzer with a thermo-conductivity detector TCD (Perkin Elmer, mod. CHN/O 200) according with ICRAM methods (2001). TOC analyses were performed by preliminary acid digestion with HCl 19% and total flash combustion. TP determination was carried out according to Aspila et al. (1976) methods, by acid digestion and next quantification using a spectrophotometer UV–VIS (Perkin Elmer, mod 6505) after colorimetric reaction.

Quality assurance/Quality control (QA/QC)

Recoveries and reproducibility were checked by analysing procedural blanks and reference materials purchased from: National Institute of Standard and Technologies (NIST—New York waterway Sediment SRM1944), Institute of Environmental Chemistry Academia Sinica Beijing China (Tibet soil), and NIST (Estuarine Sediment SRM1646a). Analytical blanks were prepared prior to the testing samples using the same analytical procedures. A solvent blank was analysed every 15 samples to check the response of the instrument detection. Standard reference materials were analysed in statistical replicates ($n = 10$) to calculate averages and standard deviation (SD) of recoveries. Measured average recoveries were the follows: TOC 101.4% (0.33 SD, SRM1944), TN 94.4% (0.005 SD, Tibet Soil), TP 98.9% (3.30 SD, SRM1646a). Concentrations were not recovery corrected. Limit of detection (LOD) was defined as the average blank ($n = 10$) plus three standard deviation (SD) and were the following: 0.01% for TC, TOC, and TN while 0.001% for TP.

Macrobenthic organisms

Sediment cores for macrofaunal analysis were taken from each sampling station, sieved (1 mm mesh size) and fauna were preserved in 7% buffered formalin. In the laboratory, samples were sorted, identified to the lowest possible taxonomic level, deemed sufficient to detect anthropogenic changes (Clarke and Warwick 1994; Olsgaard et al.

1998; Sommerfield and Clarke 1995; Warwick 1988) and counted. Results were expressed per m^2 . Benthic response variables regarding total abundance (N , ind m^{-2}), total wet biomass (B , g m^{-2}), diversity (Shannon–Wiener, H') and species richness (Margalef, d) indices were used, as these variables are most often indicative of the condition of the benthos (Weisberg et al. 1997).

Statistical analyses

All data were analysed using univariate and multivariate methods in order to evaluate: differences among sampling sites and between the two lagoons, effects produced by marine and freshwater inputs on both sediment characteristics and macrobenthic distribution, and relationships between sediment variables and macrozoobenthic communities. To reach the proposed target, first statistical analyses were separately performed on each system to observe differences among sampling sites and relevance in behaviour, and after data were plotted all together to evidence general trends of variables in the two systems. Principal Components Analysis (PCA) was applied to investigate the similarity of sediment variables. The Euclidean distances resemblance matrix was calculated after fourth square root and $\log(x + 1)$ functions transformation and normalization of sediment variables (Clarke and Green 1988). PCA represents a powerful technique in multivariate statistical analysis (Chatfield and Collins 1980), it is conceptually and computationally simple and its ordination axes are interpretable. In spite of PCA being powerful in defining correlation and similarities, it is less flexible in defining dissimilarities and its distance-preserving properties are poor (Clarke and Warwick 1998). So, in this study, PCA weaknesses were also tested by non-metric Multi-Dimensional Scale (nmMDS) that has, on the other hand, a great flexibility both in the definition and conversion of dissimilarities to distances and its rationale is the preservation of these relationships in the low-dimensional ordination space (Sommerfield and Clarke 1995). Moreover, this procedure allowed us to evidence, in both the systems, different contributions of marine and freshwater inputs in stations segregation. A further analysis of similarities/dissimilarities, the one-way

ANOSIM test (it allows to check for the significance of differences among groups of samples, using permutation/randomisation methods on resemblance matrix) was performed on environmental variables to test the H_0 hypothesis of no difference in variable distributions between the two lagoons.

The statistical analysis of macrobenthic community structure was first performed calculating the univariate diversity indices: abundance (N), total number of species (S), diversity index of Shannon–Wiener (H') and Margalef's species richness (d). These indices were calculated for each site and ecological information at sites based on a geospatial gridding method, known as kriging (Matheron and Armstrong 1987), was obtained. Kriging produced visually appealing contour and surface plots from spaced data. Contours were constructed from data using computer package SURFER version 8.0.

Similarity among sites was analysed by clustering and ordination techniques (nmMDS) based on Bray–Curtis similarity matrix after square root transformation of field data adding a dummy variable of value = 1. Within a community, the relative biomass and abundance of the population can yield useful information related to the condition of the environment (Warwick 1986). By plotting the abundance and biomass of each species ranked on the same axis (ABC method: abundance biomass comparison) as a percentage cumulative figure (k -dominance curves), it is possible to show that for a disturbed benthic community, the abundance curve will rise above the biomass curve and vice-versa for a stable community. Casewell's neutral model was applied to test differences highlighted by the ABC curves while nmMDS was run imposing minimum stress

as 0.01 and restarting the process 9,999 times to evaluate dissimilarities between stress status of sampling stations (Kruskal 1964; Shepard 1962). Obtained nmMDS results were also superimposed on clusters with resemblance levels of similarity of 40, 60 and 120. In order to confirm observed differences in macrobenthic assemblages among sampling sites and between the two lagoons, due to marine or freshwater inputs, the ANOSIM test statistic R (one-way), under null hypothesis that there are no differences among sites, was performed. To evaluate the relationship between sediment variables and macrobenthic communities, univariate community measures were correlated with the main axe of the PCA performed on sediment variables. This BIO-ENV procedure enabled the selection of the abiotic variable subset that maximises the rank correlation between biotic and abiotic (dis)similarity matrices. Statistical analyses were performed using the Primer-E Software package v6.0 (Plymouth Marine Laboratory, UK) according with Clarke and Warwick (2001).

Results

Sediment characteristics

Mean results of sediment characteristics are reported in Table 2, which highlights different benthic environments within the two basins. In Lesina, silt and clay accounted for about half of the western part component, while the remaining area was covered by silt, with a content of 54%, except the eastern side where the sample collected had the coarsest composition (51% sand). In Varano, the clay component prevailed at most sites of the central part (mean of 23%),

Table 2 Mean values and standard deviation (S.D) of grain size and macronutrients in Lesina and Varano sediment samples

	Lesina lagoon		Varano lagoon	
	Mean	SD	Mean	SD
% Sand	21.53	13.84	18.61	22.66
% Silt	50.34	10.43	66.81	17.81
% Clay	28.13	9.15	14.59	7.61
Total organic carbon (%)	3.31	1.14	2.52	1.32
Total nitrogen ($\mu\text{g g}^{-1}$)	5,200.00	2,972.79	3,713.33	2,925.41
Total phosphorus ($\mu\text{g g}^{-1}$)	540.44	133.18	619.80	285.61

the silt component (mean of 10%) covered the entire eastern side and part of the southern shoreline, while the sites located along the northern shoreline, where current dynamics prevent the accumulation of fine particles, had the coarsest composition (88% sand). As regard TOC, in Lesina, the highest concentration was measured in the sample collected near to the urban centre (5.28%, station 2L), although values relatively high (3.80–4.67%) were observed in the central area of the basin; in Varano, the maximum percentage was measured at the station located in the south-western area (5.45%, station 10V), while the south-eastern area was characterised by the lowest values, with a minimum value (0.80%) measured at the station 14V. Lower mean concentrations of TN were measured in Varano rather than in Lesina (Table 2), but the highest value was recorded in Varano lagoon at the station located in the south-western area ($10,900 \mu\text{g g}^{-1}$, station 10V), while in Lesina, TN was abundant in the western and central area ($8,000$ – $9,000 \mu\text{g g}^{-1}$) near

Table 3 Correlation coefficients between the sediment variables and the Principal Components for (a) Lesina lagoon, (b) Varano lagoons and (c) both systems together

	PC1	PC2	PC3	PC4	PC5
Lesina					
% Sand	0.042	<i>0.611</i>	0.426	0.228	0.279
% Silt	−0.158	−0.319	−0.368	0.604	−0.288
% Clay	0.098	−0.304	−0.177	−0.663	0.248
TN	<i>0.830</i>	0.294	−0.462	0.065	−0.086
TP	−0.252	0.093	−0.505	0.195	0.779
TOC	0.46	−0.58	0.429	0.319	0.403
Varano					
% Sand	−0.496	−0.216	0.34	−0.66	0.39
% Silt	<i>0.539</i>	−0.269	−0.285	−0.45	−0.08
% Clay	<i>0.456</i>	0.179	−0.22	−0.158	0.683
TN	0.194	<i>0.459</i>	0.546	0.242	0.343
TP	0.456	−0.44	0.666	−0.014	−0.207
TOC	0.101	<i>0.667</i>	0.118	−0.528	−0.462
Both systems					
% Sand	−0.542	0.042	0.399	0.437	−0.342
% Silt	<i>0.514</i>	−0.359	−0.156	−0.168	−0.266
% Clay	<i>0.468</i>	<i>0.289</i>	−0.063	<i>0.757</i>	0.332
TN	0.199	<i>0.505</i>	0.614	−0.425	0.286
TP	0.389	−0.364	<i>0.623</i>	0.158	−0.344
TOC	0.179	<i>0.631</i>	−0.218	−0.041	−0.709

Where *TN* total nitrogen; *TP* total phosphorus and *TOC* total organic carbon

Correlations significant at the $P < 0.01$ are shown in italics

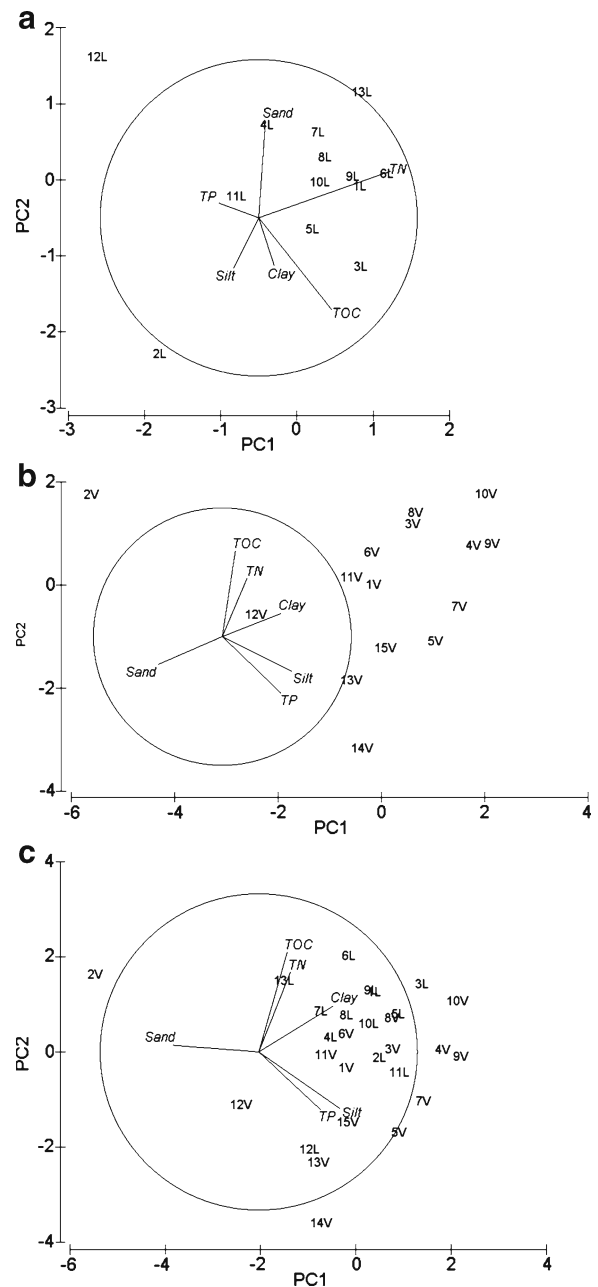


Fig. 2 PCA ordination diagram of sediment variables for **a** Lesina lagoon, **b** Varano lagoon and **c** both systems together. *TP* Total phosphorus, *TN* total nitrogen, *TOC* total organic carbon. Sampling sites are represented by number and letter (*L* for Lesina and *V* for Varano). Arrows (both slope and length) represent the correlations between sediment variables and the principal axes PC1 and PC2. See Table 3 for correlation coefficients

to the western sea inlet. TP showed higher values (mean of $800 \mu\text{g g}^{-1}$) in the south-eastern side of Lesina despite minimum value ($387 \mu\text{g g}^{-1}$) observed at the station 13L close to the eastern inlet; lower values (mean of $478 \mu\text{g g}^{-1}$) were measured at the stations located in the central-western side. In Varano, TP concentrations peaked to $1,131.42 \mu\text{g g}^{-1}$ and $1,070.34 \mu\text{g g}^{-1}$ at the stations close to the wastewater channels in the south-eastern area (14V and 15V, respectively), while the lowest concentrations were measured at the stations located along the northern shore-line (mean of $208 \mu\text{g g}^{-1}$) and along the south-eastern side near the freshwater inputs (mean of $246 \mu\text{g g}^{-1}$).

Differences in sediment variables within and between the two lagoons

PCA analysis was run on transformed and normalized levels of TOC, TN, TP and grain size of sediment samples and produced five principal components for both systems (Table 3). In Lesina lagoon, the first three components (PC1 40.5%, PC2 31.8%, PC3 15.6%) accounted together for 87.8% of the total variance in sediment data. The first component PC1 was positively correlated with sand %, clay %, TN and TOC, and TN had the highest (positive) correlation, while the second component PC2 was positively correlated with sand, TN and TP and strong correlations were found with sand % (positive) and TOC (negative; Table 3). In Varano lagoon, more than half of the total system variability (89.9%) was due to the first axis PC1 (50.6%) compared to the other two ones (PC2 25.9%, PC3 13.2%). The first axis PC1 showed positive correlations with all sediment variables, except for sand % (negative); the second axis PC2 was positively related to clay %, TN and TOC (these last variables had the strongest correlation) and negatively

correlated with sand %, silt % and TP (Table 3). By plotting all data together, the total variability was reduced even if significant and the first three

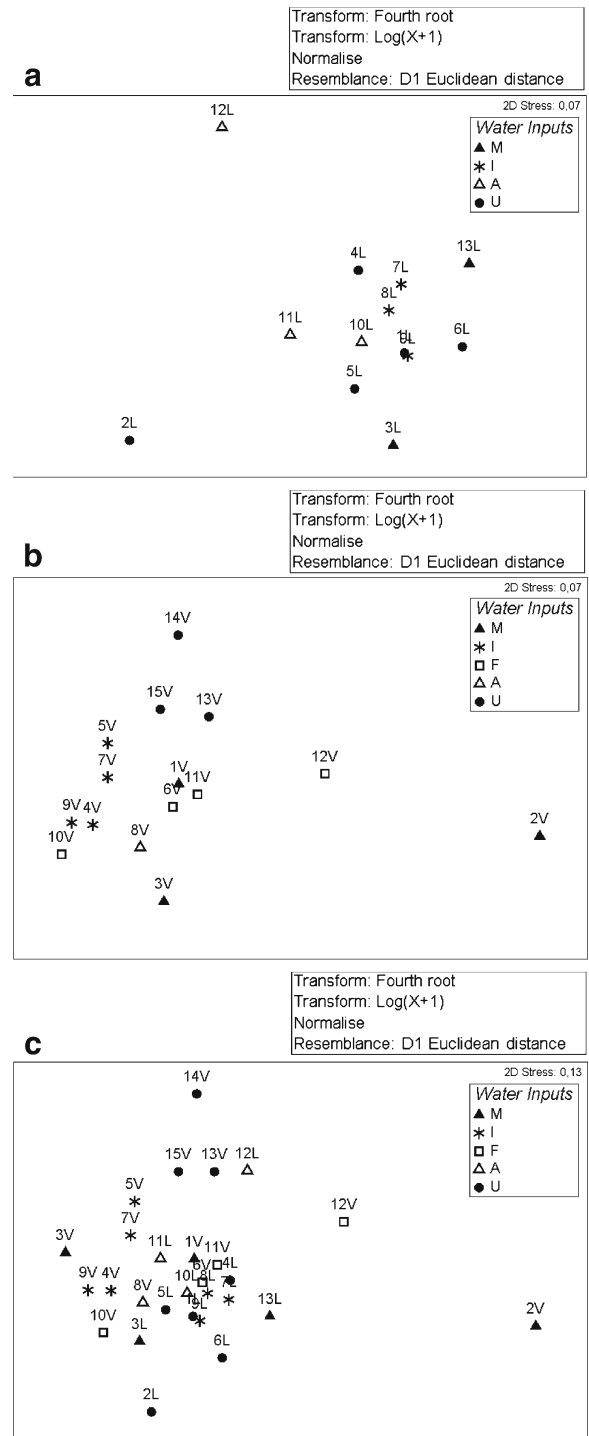


Fig. 3 MDS ordination diagrams of sediment variables in Lesina (a), Varano (b) and both systems together (c). Such a diagram was performed to assess a possible external influence on sediment variables distribution (see the text for explanation). A agricultural, U urban, I internal dynamics, M marine and F freshwater inputs

components (38.4%, 30.1%, 13.8%) accounted for 82.3% of the total variance. The first axis PC1 was characterized by strong negative correlation with sand % and positive correlations with the other variables (silt and clay components had the strongest correlations); silt % and TP had negative correlations with the second axis PC2, while the strongest positive correlations were found with TOC, TN and clay % (Table 3). These results indicated that sediment variables correlated with the first axis had a great significance in explaining the system variability. In Fig. 2, PCA results were plotted for Lesina (a), Varano (b) and both systems (c). In Lesina lagoon (Fig. 2a), the most sites located in the north-western and central parts of the lagoon (1L, 3L, 5L, 6L, 7L, 8L, 9L and 10L) were grouped along the first axis PC1 (strongly associated with concentrations of TN), while they did not show a clear similarity along the second axis PC2 which was correlated with sand % and

TOC; moreover, the Fig. 2a indicated that there was a clear separation of two stations (2L and 12L) from the others sites along both the two axes PC1 and PC2. In Varano lagoon (Fig. 2b) there was a consistent similarity among sites along the first axis PC1 (strongly associated with sediment grain size), except for the station 2V (on the top left of the figure); on the other hand, there was a clear separation of sites along the second axis PC2 (positively correlated with TOC and TN and negatively associated with TP and silt component). Considering the two systems together (Fig. 2c), the two lagoons did not seem to be clearly separated on the basis of sediment variables; only the station 2V was clearly separated from the other sites along the first axis PC1 (negatively correlated with sand component and positively associated with the other variables).

To better explore dissimilarities among stations and between the two lagoons, nmMDS was per-

Table 4 Mean values and standard deviation (S.D) of total abundance, wet biomass, class and main species identified in macrobenthic samples of Lesina and Varano lagoons

		Lesina lagoon		Varano lagoon	
		Mean	SD	Mean	SD
Total abundance (ind m ⁻²)		2442.00	2022.19	920.56	799.99
Class	Species				
Polychaete	<i>Perinereis cultrifera</i>	4.56	3.64	5.54	5.43
	<i>Cirratulus chrysoderma</i>			2.67	2.89
	<i>Ficopomatus enigmaticus</i>			1.00	
	<i>Lagisca extenuata</i>			1.00	
Gasteropoda	<i>Cyclope neritea</i>			1.00	
	<i>Haminea navicula</i>			2.00	
	<i>Nassarius</i> spp.			1.00	
	<i>Gastrana fragilis</i>			1.00	
Bivalvia	<i>Abra segmentum</i>	45.09	33.29	7.83	8.06
	<i>Abra prismatica</i>			1.00	
	<i>Cerastoderma glaucum</i>	3.25	1.89	6.00	5.48
	<i>Loripes lacteus</i>			8.00	8.72
	<i>Mytilaster minimus</i>	12.00	15.65	2.00	
	<i>Mytilus galloprovincialis</i>			1.00	
	<i>Musculista senhousia</i>	1.00		7.57	9.59
	<i>Tapes philippinarum</i>			1.00	
	<i>Diadumene luciae</i>			1.00	
	<i>Dispanpeus say</i>			1.50	0.71
Actiniaria	<i>Cyathura carinata</i>	4.67	3.21		
	<i>Sphaeroma serratum</i>	13.00	16.97		
Crustacea	<i>Idotea baltica</i>	1.60	0.89		
	<i>Gammarus aequicaudata</i>	6.00			
	<i>Balanus eburneus</i>	2.00			
Wet biomass (g.m ⁻²)		333.00	259.38	243.61	227.44

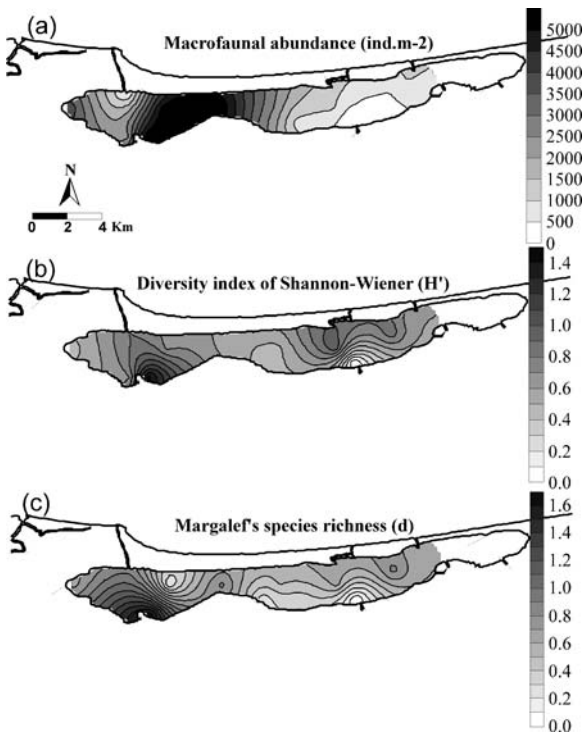


Fig. 4 Spatial distribution of abundance (a), diversity (Shannon–Wiener index) (b) and species richness (Margalef index) (c) in Lesina lagoon

formed, applying the Kruskal stress formula: 1 with Minimum stress of 0.01. Two-dimensional ordination diagrams confirmed the distinct grouping of some sites in Lesina and Varano lagoons. In Lesina, the two stations 2L (near urban centre) and 12L (close to the agricultural inputs) were separated from the others stations that seemed to have the same behaviour and in Varano basin, samples associated with sand component (2V) and freshwater inputs (13V, 14V and 15V) showed a significant difference from the other samples, but the two lagoons seemed not to be clearly separated on the basis of sediment variables. These results suggested that a possible external factor, linked with water inputs, could influence the sediment variables distribution in both the lagoons and contribute to the sites segregation. For this reason, a factor of “water inputs” was considered in according to the location of sites (see Fig. 1) and nmMDS was performed once again to assess the influence of agricultural (A), urban (U), internal dynamics (I), marine (M) and freshwater

(F) inputs on the sediment variables distribution. As indicated by the nmMDS ordination model (Fig. 3), in regard to Lesina lagoon, the sampling sites located near different water inputs were grouped together (e.g. 5L, 8L and 10L in Fig. 3a) while in Varano lagoon, the station segregation due to different external inputs was more evident, and the stations with urban influence (13V, 14V and 15V) were separated enough from those with internal dynamics (4V, 5V, 7V and 9V) or with freshwater inputs (6V, 11V; Fig. 3b). The separation between the two systems was not clear, even if some of sites of Varano lagoon were located in the upper left side of the diagram and some of stations of Lesina in the lower part (Fig. 3c). Observed differences within and between the two lagoons were tested by ANOSIM test one-way (number of permutations of 9,999). Results confirmed that different water inputs produced a significant difference in Varano lagoon ($R = 0.273$, $P = 2.5\%$)

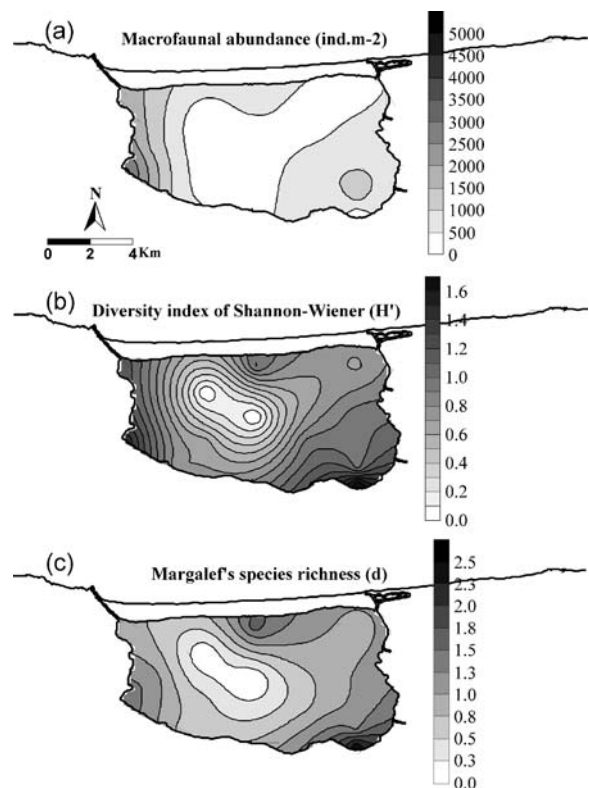


Fig. 5 Spatial distribution of abundance (a), diversity (Shannon–Wiener index) (b) and species richness (Margalef index) (c) in Varano lagoon

and no significant difference in Lesina ($R = 0.051$, $P = 35.7\%$). Differences between the two lagoons were highlighted by ANOSIM test and H_0 hypothesis could be rejected ($R = 0.149$, $P = 0.6\%$) supporting a not marked but evident differences in sediment variables between the two systems.

Univariate variables of macrozoobenthic communities

Mean values of total macrobenthic abundance and biomass, class e the main species for both lagoons are presented in Table 4. Biological data showed that both the abundance and diversity of macrobenthic communities varied within and between the two lagoons. A total of 1,026 organisms were collected, divided in 715 from 13 sites in Lesina and 311 from 15 sites in Varano, and 23 species were identified. In Lesina lagoon, the highest abundances occurred at the stations 4L (5372 ind m^{-2}), 5L (5328 ind m^{-2}) and 6L (5594 ind m^{-2}) located between the central and the western area, while lower abundances were registered in the eastern zone (Fig. 4a). In terms of number of species (S), the highest values were found at the station L4 with a prevalence of Bivalvia (*Abra segmentum*) and Crustacea (*Sphaeroma serratum*); moreover, this site was characterised by the

highest diversity (Shannon–Wiener index, 1.45; Fig. 4b) and species richness (Margalef index, 1.67; Fig. 4c). On the other hand, the station 11L represents the site where no species were identified. As it can be observed from the distributions of abundance and diversity indices, both the eastern area and the southern shoreline were characterised by the lowest number of individuals and lower species diversity. In Varano lagoon, higher abundances were found along the western shoreline at the stations 1V (1,820 ind m^{-2}) and 11V (3,108 ind m^{-2} ; Fig. 5a). Only one species was identified at the central stations 5V and 7V (Bivalvia, *Musculista senhousia*; Fig. 5b, c), while the station 14L was characterised by the highest diversity indices (Shannon–Wiener, 1.68; Margalef, 2.38) and bivalvia were the most representative class.

Multivariate macrobenthic assemblages and relationship with sediment variables

Based on the diversity observed for both the number of individuals and species, Casewell's neutral model (W statistic) was adopted, in order to evaluate how the observed diversity was dissimilar from natural predicted one. A zero value for the W statistic indicates neutrality, while positive

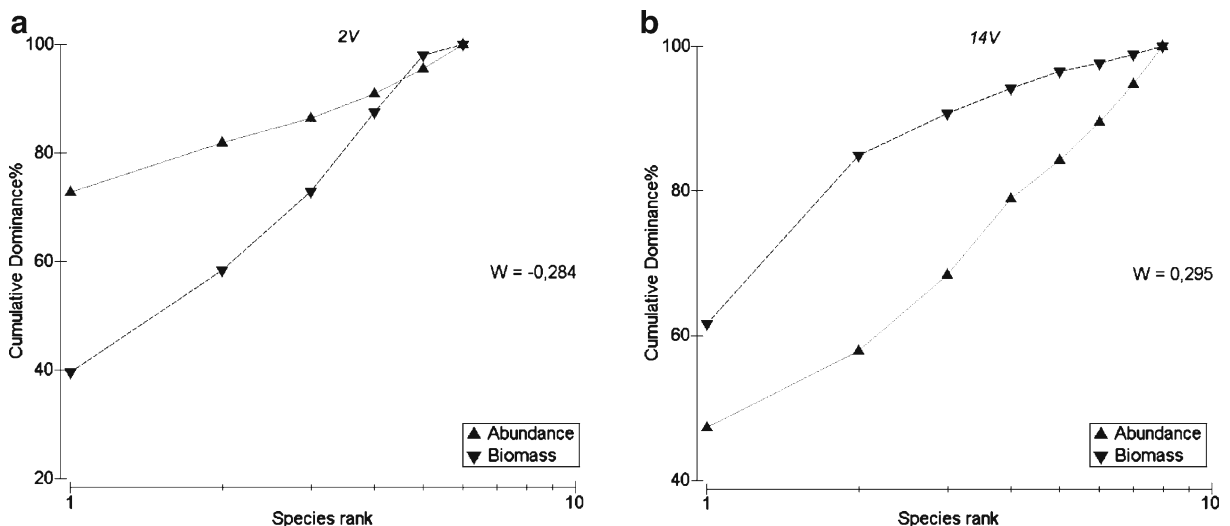


Fig. 6 Results of k -dominance curves (ABC plots) showing differences between stressed (a) and non-stressed (b) sampling sites based on both abundance and biomass curves. The example represents different stress status in Varano lagoon

values suggest greater diversity than predicted one. Casewell's model was calculated for each site in both the lagoons and the results highlighted a diversity generally lower than predicted one, exception made for two stations in Lesina and two stations in Varano, where W positive values were found ($10L = 1.21$; $12L = 0.07$; $9V = 0.99$; $11V = 0.49$). It is important to notice that at the stations 2V (Varano lagoon) and 2L (Lesina lagoon) the W value was lower than -2 , suggesting an evident stress status for the macrobenthic community. A further analysis, the application of abundance/biomass comparison techniques (ABC), was performed. It aimed to determine the levels of disturbance on benthic community. In Fig. 6, an example of stressed (2V) and non-stressed (14V) stations for Varano lagoon, is reported. As it can be observed, for stressed stations, represented by a great number of small size individuals, the ABC plot is characterised by the abundance curve which lies above the biomass one (Fig. 6a). In contrast, for an undisturbed community, the biomass is dominated by one or few species represented by rather few individuals, leading to a curve of abundance which lies below biomass one (Fig. 6b).

To explore the macrobenthos diversity in each lagoon and the different biological behaviour between the two lagoons, a nmMDS was performed and obtained results were superimposed on clusters with levels of similarity of 40 and 60. This analysis indicated a variation in macrobenthic assemblages within and between the two lagoons (Fig. 7). In Lesina, it was possible identify three main clusters (A1, A2 and A3) and two segregated stations (3L, close to the main sea inlet, and 10L) with a similarity of 60, while the station 11L was removed from nmMDS representation, because of its great diversity reduced the differences among the other sites (Fig. 7a). Group A1 combines stations within the western area, 1L and 2L in the extreme side with low abundance and low diversity indices and 4L, 5L and 6L in the middle western part, presenting medium to high number of individuals belonging to high number of species; group A2 comprises three stations mainly in the central zone of the lagoon (7L, 8L and 9L), characterised by a prevalence of *Bivalvia*; group A3 composed of two stations mainly close to the

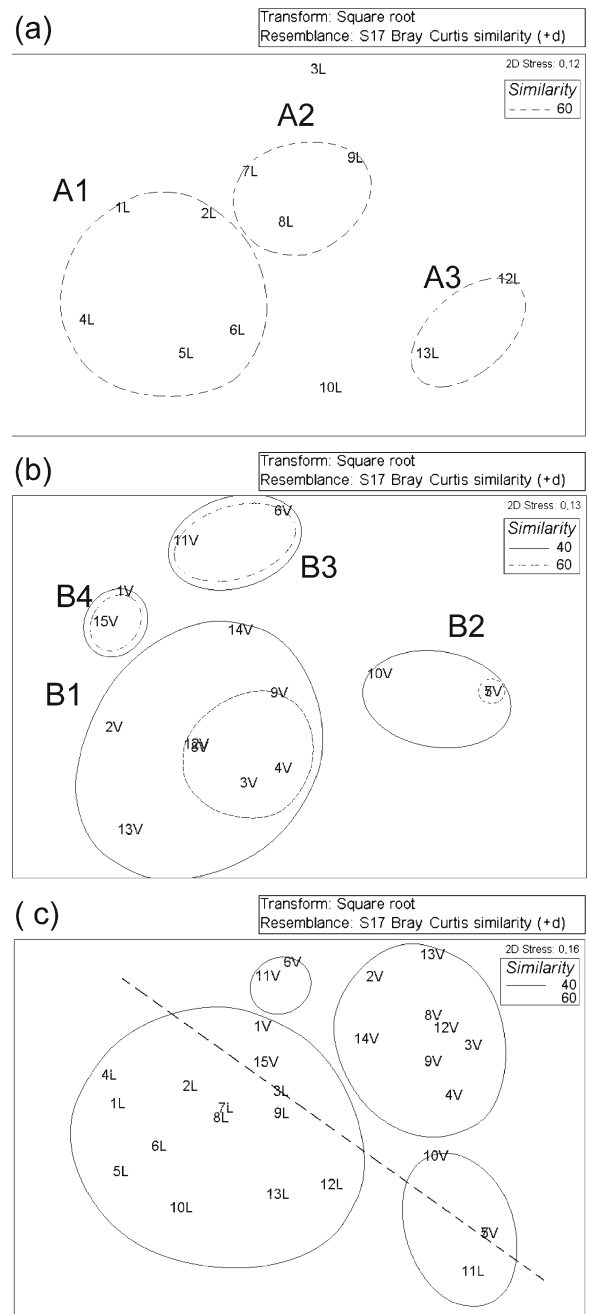


Fig. 7 Ordination diagrams superimposed on clusters with levels of similarity of 40 and 60. Kruskal stress formula: 1 minimum stress 0.01. A1, A2 and A3 (dashed lines) are the main three clusters obtained in Lesina lagoon with a similarity of 60 (a); B1, B2, B3 and B4 (continuous circles) are the main four clusters obtained in Varano lagoon with a similarity of 40 (b); the dash diagonal separates the Varano sites (represented by letter V) from Lesina sites (letter L), showing a clear difference in macrobenthos assemblages between the two lagoons (c)

eastern zone (12L, 13L), characterised by a very low number of individuals and species. In Varano lagoon, four groups of stations (B1, B2, B3, B4) were identified with a similarity of 40 (Fig. 7, continuous lines), while a greater sites segregation was found with a similarity of 60 (Fig. 7, dash circles). Taking into account the similarity of 40, the groups B1 and B2 constitute the central community, as they occupy the central part of the lagoon, characterized by a very low number of individuals and species, dominated by *Bivalvia* (*Musculista senhousia*). Plotting Lesina and Varano lagoons together, different macrofauna assemblages between the two systems was highlighted with a similarity of 40 and a distinct segregation of the two lagoons was evident (Fig. 7c, the dash diagonal separates all Varano sites from those of Lesina lagoon). ANOSIM test was also performed on biological variables to support the different segregation in macrobenthic assemblages between the two systems, producing a global R value of 0.499 ($P = 0.01\%$). As for sediment variables, a factor “water inputs” was taken into account to evaluate the possible effects of fresh, urban and marine waters on macrobenthic distributions within the two lagoons. Ordination diagrams were run once again, introducing the factor “water inputs” for each lagoon (Fig. 8a, b). As shown in this figure, the external input had a great influence in the

stations clustering within Lesina lagoon (similarity of 60), where the stations near the agricultural input (12L and 13L) segregated from those near urban and aquaculture influence (1L, 2L, 4L, 5L and 6L) and from those located in the central zone with an internal dynamic (7L, 8L and 9L; Fig. 8a). In Varano basin, stations located near different external inputs were grouped in the same cluster (similarity of 40); for instance, 1V (close to the main sea inlet) and 15V (close to urban inputs; Fig. 8b).

The BIO-ENV procedure was applied to better evaluate the relationship between macrobenthic assemblages and sediment characteristics in both lagoons. The rank correlation method BIO-ENV was based on weighted Spearman rank correlations between the similarity matrices of the two data sets. The results of this analysis showed that macrobenthic assemblages were most strongly correlated with the distribution of sand %, clay % and TOC. In Fig. 9, the superimposition of sand and silt components on nmMDS ordination diagrams based on Bray–Curtis similarity matrix for Varano lagoon is reported. Sediment grain size were superimposed as bubbles whose sizes reflect the magnitude of these variables and it can be noted the relationship between macrobenthic grouping and sand component (Fig. 9a), while there was no correlation level between organisms

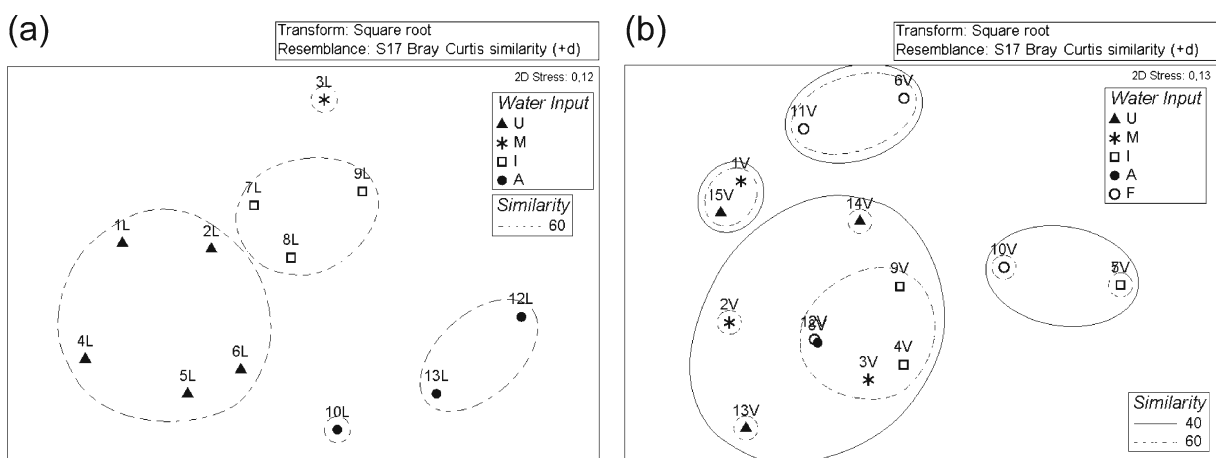


Fig. 8 Ordination diagrams superimposed on clusters for Lesina lagoon with similarity of 60 (a) and Varano lagoon with similarity of 40 (b), taking into account the factor

“water input” in order to assess the external influence on macrobenthos communities distribution (see the caption of Fig. 3 for explanations on symbols U, M, I and A)

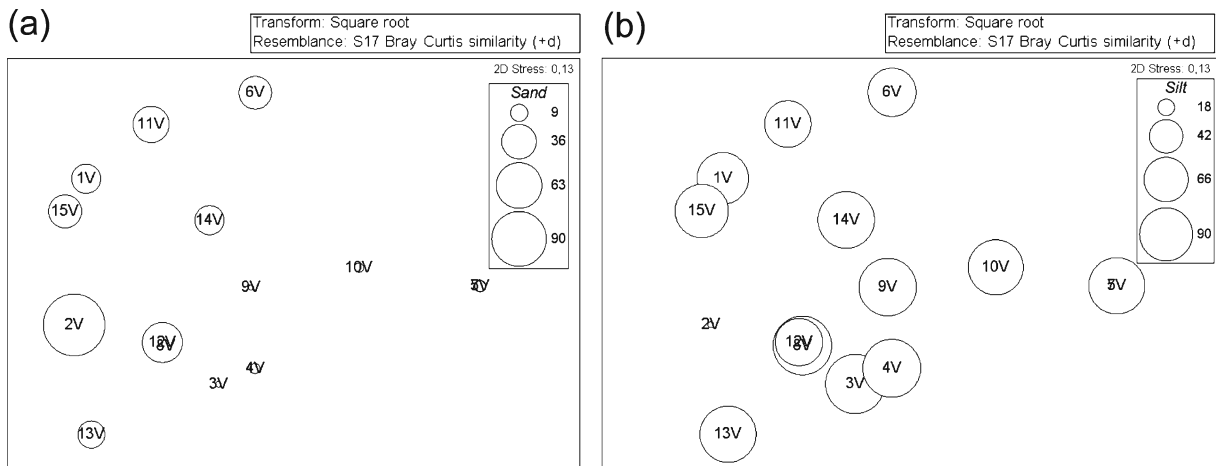


Fig. 9 MDS superimposed on circles, which represent the sand fraction (a) and the silt fraction (b) in Varano lagoon. The circle sizes reflect the magnitude of the two sediment variables

and silt fraction (Fig. 9b, the stations 10V and 15V, biologically different, had the same content of silt %).

Discussion

Based on sediment characteristics, a wide spatial variability was observed in both Lesina and Varano lagoons, which were generally dominated by fine-grained sediments; in particular, silt component was predominant in Lesina lagoon, while the clay fractions were found at most of central sites in Varano. With regard to TOC content, the results of this study were comparable or even lower than those typically found in organic enriched or eutrophic Mediterranean lagoons with economic rating due to fisheries and aquaculture exploitations, such as Venice lagoon (0.5 to >30% TOC, Picone et al. 2008), Fattibello-Spavola (1.2–3.9% TOC, Frascari et al. 2002), Orbetello lagoon (2–7% TOC, Lardicci et al. 2001; Lenzi et al. 2005), Santa Giusta lagoon (0.48–3.9% TOC, Magni et al. 2008b), Cabras lagoon (3.4–6.28% TOC, Magni et al. 2005; Como et al. 2007). Higher value of TOC were also found in Pabellon lagoon on the north west coast of Mexico, surrounded by intensive agricultural area (0.5–11% TOC, Paez-Osuna et al. 1998) or Rio Formosa in southern Portugal which has increased nutrient

loading due to land runoff and sewage discharges (0.15–8.3% TOC, Mudge et al. 1998). The relationship between sediment grain size and organic matter has been well documented (Bordovskiy 1965; Colombo 1977; De Falco et al. 2004; Keil et al. 1994; McCave et al. 1995; Magni et al. 2008a; Mayer 1994; Nguyen et al. 1997). Previous studies from other Mediterranean coastal lagoons (Bellucci et al. 2002; Carvalho et al. 2005; Como et al. 2007; Magni et al. 2008a) indicated that TOC concentrations were mainly correlated with fine particles of sediments than with coarse components. Consistent with these studies, TOC contents obtained from the Lesina sediments had the greatest affinity with clay component. On the other hand, in Varano lagoon, the results showed that as the proportion of fine particles increased, the amount of total phosphorus also increased, above all in response to point-source inputs (south-eastern side of the lagoon), indicating that fine particles had a stronger adsorption capacity for phosphorus and play an important role in determining the phosphorus content in sediments. Similar results have been also observed by other recent studies (Tian and Zhou 2008; Lukawska-Matuszewska and Bolalek 2008; Owens and Walling 2002; Selig 2003; Vermont et al. 2002). On the basis of these observations (highlighted by PCA analysis also), a not marked but evident difference between the two lagoons

was observed and supported by ANOSIM test that suggested a greater influence of external sources on sediment macronutrient distributions in Varano rather than in Lesina lagoon. In particular, these results indicate that the primary sources of organic matter in the sediments of Lesina lagoon (particularly in the western area) could be related to a high primary production (phanerogams) (Cozzolino 1995; Marzano et al. 2003) rather than a loading of high amounts of urban waste waters, as highlighted by ordination diagrams (Fig. 3, no influence of external water input). In contrast, in Varano, the greater accumulation of TP in the south-eastern zone rather than in the deeper central zone, could be due to the external input from the two main wastewater channels, the S. Francesco and Antonino channels, which drain waters from the surrounding agricultural areas (Specchiulli et al. 2008) and phosphorus enters this lagoon in a particulate state, adsorbing to silt component; otherwise, in the central area, the organic matter decomposition, enhanced by higher temperatures, could be prevalent compared to deposition processes.

It is well known that lagoon systems are affected by water salinity fluctuations due to seawater exchanges throughout the sea inlets and to freshwater and urban wastewaters inflows. The combination of these factors allows the exchange of water masses with different physical and chemical characteristics (Millet and Guelorget 1994), while ensuring the renewal of water in lagoon (Guélorget and Perthuisot 1992) and determining a selection of macrobenthos community. Salinity changes were considered by literature as the major factor (confining factor) able to determine a spatial segregation in benthic macrofauna assessment (Guélorget and Perthuisot 1983; Kiener 1978; Vatova 1953). Many studies performed on lagoon systems (not only in Mediterranean areas) showed that the distribution and abundance of macrobenthos followed the salinity gradient (Bazairi et al. 2003; Dye and Barros 2005; Koutsoubas et al. 2000; Maggiore and Keppel 2007; Mistri et al. 2001; Mistri 2002). Consistent with these studies, in Lesina lagoon, salinity appeared to play an important role in the macrobenthos community pattern within the basin, even if sediment characteristics seemed to affect

stations distributed near urban and intensive aquaculture inputs. Multivariate analysis (ordination diagrams, Fig. 8) indicated that the macrobenthos assemblages in stations close to the urban inputs were different from those located in both the central zone (with internal dynamics) and the eastern zone close to the agricultural effluents; this pattern followed the salinity gradient present in Lesina lagoon. Nevertheless, a secondary disturbance factor was found in the western zone of the lagoon, close to the urban and intensive aquaculture influence, where the high sediment organic load (TOC), associated with high fine-fraction percentage affect the macrobenthos community's structure, leading to decreased values for both abundance and diversity indices (fewer different species with a prevalence of *Bivalvia-Abra segmentatum*; Fig. 4). These results are consistent with those obtained by Marzano et al. (2003) for Lesina lagoon and, similarly, many studies have shown a marked influence of organic enrichment on qualitative composition of macrobenthos community (Gravina et al. 1989; Labruno et al. 2007; Mucha and Costa 1999; Sardà et al. 1995; Sordino et al. 1989).

Recent papers showed as also macronutrient enrichment and sediment grain size could determine macrobenthic stress phenomena and consequently produce changes in organisms assessment (Bazairi et al. 2003; De Biasi et al. 2003; Labruno et al. 2007; Lardicci et al. 1997). Consistent with these studies, granulometry and physicochemical properties of sediment explained much of the spatial variability of macrobenthos in Varano lagoon, while the significance of salinity in determining spatial distribution was not highlighted in this lagoon (Fig. 8). Stations 5V, 7V and 10V were all located in the central part of the lagoon with high content of clay % and both the lowest abundance and diversity indices; in addition, in this area, an extreme reduced abundance of *Musculista senhousia* with respect to the both western and eastern sides of the lagoon, was observed. Summer hypoxia and anoxia is known to greatly reduce *M. senhousia* abundance (Mistri 2002; Munari 2008). This result suggested that decomposition processes of organic matter, coupled with low water exchange and high temperatures during the summer, could promote quicker

oxygen depletion in the central part of Varano lagoon rather than in other zones. The eastern side of the lagoon (3V, 4V, 8V and 9V) was characterized by finer sediments (silt %), higher content of TN and an increased species richness with a dominance of Polychaetes (*Perinereis cultrifera*) on Bivalvia (*Loripes lacteus*). One important result obtained in Varano lagoon was that the effect of the two wastewater channels in the south-eastern area on benthic macrofauna is effective and restricted to the two stations 13V and 14V (close to the drainage waters from the surrounding agricultural areas) which were characterized by the highest values of TP, finer particles, decreased organic matter and both the highest species richness and diversity (Fig. 5). These observations are consistent with the model proposed by Pearson and Rosenberg (1978) which postulates an increase in species richness as organic matter availability decreases.

The macrobenthos assemblages structure and composition found in both Lesina and Varano lagoons were very different, with a higher number of species in Varano (18) than in Lesina (10). This result could be related to higher salinity fluctuations in Lesina rather than in Varano, as proposed by Wolff (1983). Moreover, dominant species found in Lesina lagoon were *Abra segmentum* and *Mytilaster minimus* and this agrees with results obtained by Marchini et al. (2008).

ABC method and values of *W* statistic were used to identify community disturbance within each lagoon. The results of this analysis showed that in Lesina lagoon, the effects of urban centre on the sites close to the external input was evident (finer sediments, the highest TOC, fewer different species and high negative value of *W*), according to the Pearson and Rosenberg model (Pearson and Rosenberg 1978). In contrast, comparing community structure at the different sampling sites in Varano lagoon, it was possible to observe the existence of disturbance cases not directly related to organic loads gradients. The organic load gradient established a sampling station ordination (the highest values of TOC located in the south-western side of the lagoon), while the biological disturbance indicators (*k*-dominance curves) established another ordination, with the more stressed sites located along the northern

shoreline (in contrast with the response of diversity indices), associated with sediment sand component. Similar results were obtained, with the same approaches, in other Mediterranean lagoons (Koutsoubas et al. 2000; Lardicci and Rossi 1998; Reizopoulou et al. 1996).

Conclusion

The results of this study have provided the different behaviour between the two very close coastal lagoons. A different source for sediment variables emerged during the study in the two lagoons; for Lesina, primary sources were related to internal dynamics, while in Varano, the influence of external input was predominant. The macrobenthic community assemblages seem to reflect the environmental gradients due to marine and freshwater inputs in Lesina rather than in Varano lagoon, where macrobenthic pattern variations arose from physicochemical properties of sediment, in particular from sand and clay components. A stress status for macrobenthic community was observed for each site within the two lagoons and the results showed no clear difference among sites with urban effluents and sea-freshwaters inputs in Varano lagoon, while in Lesina lagoon, the effects of urban centre on the sites close to the external input was evident. Although the sampling has been carried out during a critical season (summer), when severe anoxic crises could be frequent, the observations performed on macrobenthic response variables, regarding total abundance, biomass, diversity and species richness indices, allow us to assess that stressed areas were confined to few sites in both the lagoons.

The relationships between macrobenthic communities and sediment variables in Lesina and Varano lagoons could be considered as a preliminary survey for supporting further studies on the other ecosystem components of these two lagoons, including contaminants such as polycyclic aromatic hydrocarbons and some pesticides, as these factors could also affect benthic organisms (Long et al. 1995). Moreover, these results indicate specific correlations between environmental and biological variables in only one season (summer). For this reason, a temporal and spatial

variation of macrobenthos assemblages in relation to the chancing of ecosystem components for both the lagoon will be taken into account.

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