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Assessment of the ecological status of the Mediterranean coastal lagoons using macroinvertebrates. Comparison of the most commonly used methods

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Abstract

Benthic communities were studied twice (during the autumn and spring) in three Mediterranean coastal lagoons located in Greece (Logarou) and Italy (Cesine and Grado-Marano). The species composition and distribution, community diversity, species richness, dominant taxa and their ecological identity, benthic trophic and biomass size structure were investigated in these lagoons and the results were correlated with the environmental variables. The overall similarity based on species composition and abundance among the lagoons was low due to the differences in the dominant environmental factors, whereas the variations in the community diversity and species richness were mainly related to the degree of marine influence, reflecting the natural structure. The benthic classification indices AMBI, M-AMBI, BENTIX, BO2A, ISD and ISS were applied to assess the ecological status of the lagoons studied. The results revealed that the biotic indices AMBI, M-AMBI, BENTIX, and BO2A were not adequately efficient due to the natural dominance of the tolerant and opportunistic species and the correlation of the species diversity with natural stress. The ISD and ISS, on the other hand, based on size distribution frequencies and on size spectra sensitivity, respectively, showed good discrimination power among the impacted and unimpacted sites. The results indicate that except for species sensitivity, other traits of the communities such as the biomass or size structure could be more robust, sensitive and suitable for assessing the ecological quality of lagoons.

Keywords: Coastal lagoons, zoobenthos, biotic indices, functional diversity.

Introduction

Naturally stressed environments, such as coastal lagoons, discourage the settlement of several organisms; therefore, a small number of species and a strong dominance of a few ones have often resulted in low diversity. The natural stress within the lagoons, expressed as the distance from the marine influence and the seasonal fluctuations of the freshwater input are known to dramatically influence the macrobenthic communities. How can zoobenthic communities be used to assess environmental quality in brackish water lagoons?

Several classification indices have been proposed for the assessment of environmental quality in aquatic systems (for instance, reviews by Occhipinti-Ambrogi & Forni, 2004; Borja *et al*., 2009; Birk *et al*., 2012). Many of them are based on the benthic invertebrates, which are one of the basic biological quality elements included in the Water Framework Directive of the EU (EU, 2000/60/EC). Most of these indices have been developed using the data from the coastal marine areas and are mainly used to assess the organic enrichment. Coastal brackish water lagoons, however, are harsh, naturally stressed and organically enriched areas populated by less sensitive (or more tolerant) species. These characteristics would correspond to a polluted situation in the coastal waters. Reizopoulou & Nicolaidou (2007) examined the use of some of these indices formulated in the coastal waters to assess the degree of disturbance in the brackish water lagoons. Similarly, Dauvin (2007) proposed the concept of the “estuarine quality paradox” further developed by Elliott & Quintino (2007) and Dauvin & Ruellet (2009). The latter warned that: “indices based on the abundance of stress-tolerant species… and used to plan environmental improvements will be flawed.”

Biomass structure is a feature of the lagoonal community that may reflect alterations in the benthic ecosystem along a pollution gradient. Shifts in the distribution of the benthic fauna in different biomass size classes under conditions of disturbance have been documented. Smallbodied invertebrate species may characterise the environments with high instability; however, the numerical dominance of small sized individuals could be a consequence of the anthropogenic pressures imposed on the organisms (Reizopoulou & Nicolaidou, 2007; Basset *et al*., 2012)

The aim of this study is to examine the community organisation in three SE European lagoons under different anthropogenic stress and to relate it to their abiotic characteristics. Moreover, the study explores the applicability of six of the most commonly used benthic indices in assessing the environmental quality in these brackish water lagoons. Four of these indices are biotic (among them, the factorial M-AMBI actually combines the biotic index AMBI with the diversity measures) based on the ecological groups theory while two use the size distribution of the individuals.

Material and Methods

Sampling was performed in three Mediterranean transitional water systems (Fig. 1). Cesine is a small transitional water body in the south-western Adriatic Sea, subjected to a slight anthropogenic influence (Ponti *et al*., 2008). Logarou lagoon, is situated in the Amvrakikos Gulf, an organically enriched area subjected to the agricultural activities in Western Greece (Kormas *et al*., 2001). The Grado-Marano lagoon in the North Adriatic Sea, is characterised as one of the most polluted lagoons in Italy, affected both by chemical pollution and eutrophication (Ianni *et al*., 2008; Ponti *et al*., 2008; Facca & Sfriso, 2009; Sfriso *et al*., 2014).

Sampling in the lagoons was performed twice, during autumn 2004 and spring/early summer of 2005. Six stations were sampled in Cesine and Grado-Marano and eight in Logarou. Temperature, salinity and dissolved oxygen were measured just above the bottom using the temperature/salinity and oxygen probes. Grain-size measurement of the sediment was undertaken using the Sedigraph 5100 system after separation of the sand fraction (> 63μm) by wet sieving.

The total surface collected was considered sufficient according to Mavric *et al.*, (2012) in analysing the influence of the sample size on the ecological status assessment using biotic indices. Five replicate benthic samples were taken at each station with a box corer sampling 0.03 m^2 of the bottom. The samples were sieved through a 0.5 mm mesh sieve, stained with Rose Bengal and preserved in 4% formalin. In the laboratory, the macrofauna were sorted, identified up to species level where possible, and counted.

All the specimens were weighed to the nearest 1 mg after drying for 72h at 60^0C; subsequently, the ash content was obtained at individual level (for the larger species) or groups of conspecifics (for the smaller species) after performing muffle furnace combustion for 24h at 500°C. All data regarding the individual body sizes are

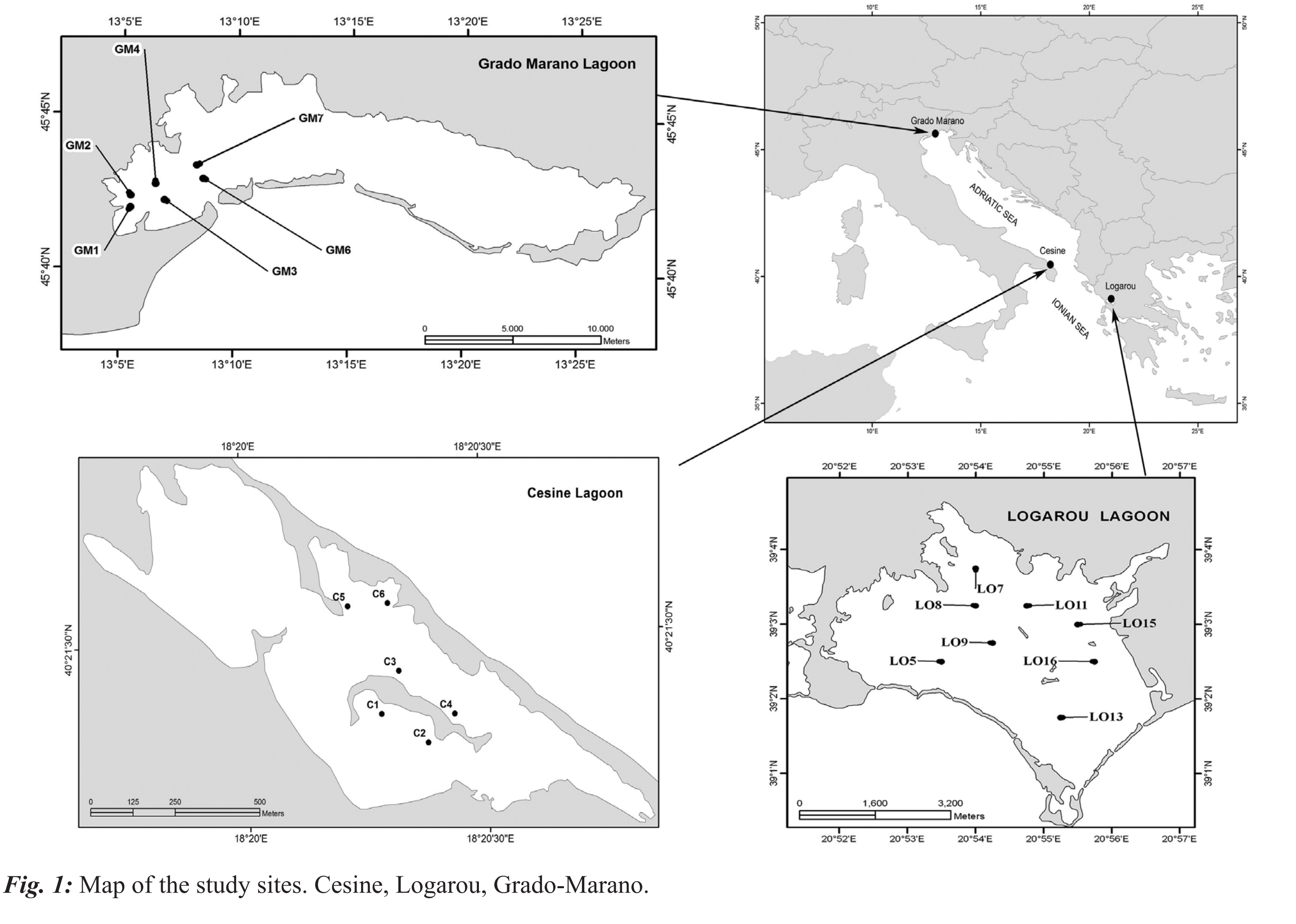

Fig. 1: Map of the study sites. Cesine, Logarou, Grado-Marano.

expressed as individual Ash Free Dry Weight (AFDW).

The following biotic indices and corresponding EQRs were calculated: the AMBI index (Borja *et al*., 2000), BENTIX index (Simboura & Zenetos, 2002), multivariate or factorial method of AMBI with Shannon diversity and Species Richness known as M-AMBI (Borja *et al*., 2004; Bald *et al*., 2005; Muxika *et al*., 2007), Benthic Opportunistic Annelida Amphipod index BO2A (Dauvin & Ruellet, 2007), biomass size structure ISD index (Reizopoulou & Nicolaidou, 2007), and the ISS index (Index of size spectra sensitivity), which integrate size structure metric, size class sensitivity and taxonomic richness, respectively (Basset *et al*., 2012, 2013).

To calculate the AMBI and M-AMBI, the free software (http://www.azti.es v.4) along with the guidelines from the authors (Borja & Muxika, 2005) was used in this study. The reference values for M-AMBI were set as: H = 4, S = 50 and AMBI = 0, as proposed by Simboura & Reizopoulou (2008) for the Mediterranean lagoons. To calculate the BENTIX (Add-in v.1.0 version) the software for MS Excel 2007 has been used, downloaded free from: http://www.hcmr.gr/en/articlepage.php?id=141.

Shannon diversity index (H´; $\log_2$), PCA and MDS analyses were performed using the PRIMER v6 software package, developed in the Plymouth Marine Laboratory.

Results

Environmental variables

The lagoons studied presented significant seasonal fluctuations in the environmental parameters; however, the internal gradients of the physicochemical parameters within each ecosystem persisted during both sampling dates. The seasonal ranges in the abiotic and biotic factors for each lagoon are listed in Table 1.

The lowest salinity, ranging from 4.7 to 6.3, was observed in the Cesine lagoon, the most isolated system, while in Logarou and Grado-Marano the salinity was higher, ranging from 26.4 to 38.8 and from 17.4 to 30.0, respectively; hypoxic conditions were not observed in any of the lagoons; however, high concentrations of Dissolved Inorganic Nitrogen (DIN) and Chlorophyll-α were observed in Grado-Marano.

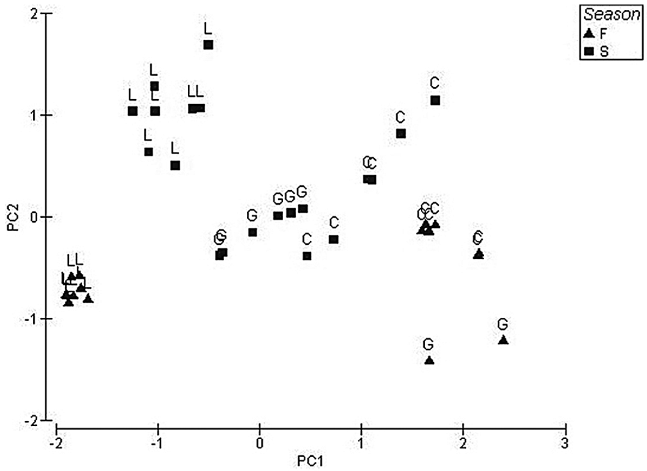

Fig. 2: PCA ordination on the environmental parameters (salinity, temperature and oxygen) (C: Cesine, L: Logarou, G: Grado-Marano; F: Fall S: Spring).

The lagoon sediments were mostly muddy. Sediments in the Cesine and Grado-Marano were mostly muddy sands, often with a high silt content, while in Logarou the sediments were mostly sandy muds, often with a higher proportion of the clay fraction (< 2μ).Generally, Cesine represented the coarser sediment, while a greater range of sediment types was found in Grado-Marano.

The differences among the three lagoons regarding the environmental parameters are illustrated in the PCA (Fig. 2) where, a succession of stations is seen, across the PC1 axis, with the higher salinity in the left part of the graph towards the low salinity stations of Cesine, which are grouped in the right part of the diagram.

Table 1. Seasonal range of the abiotic and biotic factors for each lagoon.

Abiotic and Biotic parameters	Cesine		Logarou		Grado-Marano	
	Fall	**Spring**	**Fall**	**Spring**	**Fall**	**Spring**
Salinity	6.0 – 6.3	4.7 – 4.8	36.3 – 38.8	26.4 – 30.9	23.0 - 30.0	17.4 – 27.2
Temperature (oC)	12.7 – 15.9	18.8 – 20.7	22.2 – 23.5	25.5 - 29.1	5.3 – 7.7	19.9 – 21.9
Oxygen (mg L-1)	8.7 – 9.6	6.4 - 10.3	5.1- 5.6	8.2 - 9.5	9.7 - 10.6	6.3 – 7.9
DIN (μM)	3.30 – 4.38	2.07 – 3.52	0.91 – 3.16	0.85 – 2.38	-	51.21 – 76.30
DIP (μM)	0.09 – 0.18	0.08 – 0.12	0.56 – 2.27	0.12 – 0.19	-	0.06 – 0.14
Chlorophyll-a (mg L-1)	0.88 – 4.26	2.56- 4.29	0.40 – 3.14	0.05 – 2.18	2.45 – 11.74	2.03 – 11.74
Sand (%)	-	64.0 - 92.5	0.6 – 35.3	1.2 - 42.3	17.3 – 27.4	8.2 – 95.5
Clay (%)	-	2.0 - 9.5	32.3 – 65.6	12.3 – 74.9	6.7 – 10.0	6.4 – 9.6
Silt (%)	-	4.5 - 26.5	32.4 – 50.0	20.7 – 74.7	64.4 – 73.1	60.7 – 82.2
Number of species (S)	2 - 7	6 - 9	6-20	8-22	7 - 34	6 - 15
Diversity (H')	0.36 – 1.87	1.17 – 2.11	1.74 – 2.84	0.91 – 2.36	0.45 – 3.50	0.77 – 4.31

Community analysis

In the MDS ordination plot, based on the benthic abundances, the stations of each lagoon were grouped separately (Fig. 3A).

Most of the species were euryhaline and eurythermic with occasional peaks of opportunists. However, differences in the composition and abundance of the dominant species were also evident. The species forming the major part of the community (> 90%) in each lagoon are listed in Table 2. Species enjoying a wide distribution in the lagoons included *Cerastoderma glaucum, Abra segmentum*, *Hediste diversicolor, Nephtys hombergii*, *Heteromastus filiformis,* amphipods of the genus *Gammarus* and *Corophium* and isopods of the family Sphaeromatidae.

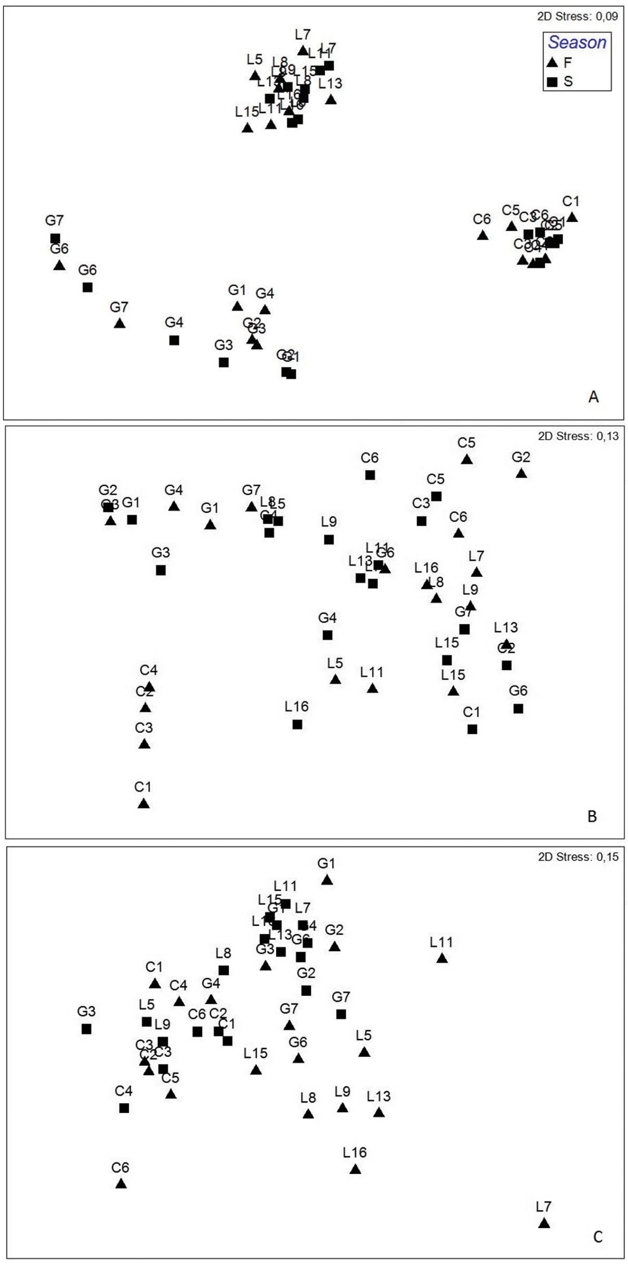

Fig. 3: Multidimensional scaling based on: A) species abundances, B) trophic composition (percentages of trophic groups), and C) body-size distribution of benthic fauna (C: Cesine, L: Logarou, G: Grado-Marano; F: Fall S: Spring).

The total number of species ranged from 14 in Cesine to 67 in Grado-Marano, while the Shannon Index ranged from 0.36 in Cesine to 4.31 in Grado-Marano (Table1). Both metrics were related to the geomorphological characteristics of each ecosystem. In the Cesine lagoon, where low salinity was constantly maintained due to its restricted connection with the sea, low species richness and low diversity were observed; the Grado-Marano, however, with its stronger marine influence, supported a higher number of species and community diversity. Macrofaunal diversity peaked in spring in the Cesine lagoon, and in autumn in Logarou and Grado-Marano. Salinity was found positively correlated with the species richness ($p = 0.01$; $R = 0.56$) and Shannon diversity ($p = 0.00$; $R = 0.44$). No significant correlation between the sediment characteristics, species richness and community diversity was recorded.

The MDS ordination plot based on the trophic composition of the benthic communities is seen in figure 3B. No clear discrimination is seen among the lagoons studied, based on their trophic structure, because in all the study sites the deposit feeders formed the dominant trophic group.

In Cesine, where the benthic communities are characterised by a small number of species, it is the abundance of the variations evident in the very few species that determines the trophic composition of the communities. In autumn, deposit feeders – mainly the Chironomid insect larvae, dominate the lagoon, while in the spring the suspension feeders, such as *Ficopomatus enigmaticus* and *Cerastoderma glaucum* and grazers such as *Gammarus insensibilis,* become equally abundant.

In Logarou, deposit feeders such as *Abra segmentum* and *Monocorophium acherusicum*, and grazers such as *Microdeutopus gryllotalpa* are found in high densities. The predators mainly include *Nephtys hombergii* and occasionally very high numbers of the suspension feeders such as *Cerastoderma glaucum* and *Hydroides dianthus* have been observed. In Grado-Marano, the deposit feeders are dominant during both seasons. These are mainly represented by the group of surface deposit feeders, such as *Cirratulus* sp., *Streblospio shrubsolii* and *Corophium* sp., and the subsurface deposit feeders such as *Heteromastus filiformis* and oligochaetes found in high percentages in autumn.

Body size (biomass) distributions of the benthic organisms were studied in the lagoonal communities during both seasons. In figure 3C the MDS ordination is shown based on the body size of the macrofaunal communities (percentages of individuals in geometric body-size classes). The least impacted Cesine and the most polluted Grado-Marano lagoons form more or less distinct groups, while the Logarou stations are ordinated in an intermediate position largely overlapping both the other lagoons.

The community size structure of each lagoon during both seasons is shown in Figure 4. The frequency distribution of the geometric size classes reflects a clear differ-

Table 2. Most abundant species accounting for at least 90% of the individuals in each station in the three lagoons.

Species	Cesine	Logarou	Grado Marano
Armandia cirrhosa Filippi 1861		+	
Cirratulus sp.			+
Ficopomatus enigmaticus Fauvel 1923	+		
Glycera tridactyla Schmarda 1861			+
Hediste diversicolor Müller 1776	+		+
Hydroides dianthus Verrill 1873		+	
Malacoceros fuliginosus Claparède 1870			+
Mediomastus sp.			+
Micronephthys sp.			+
Naineris laevigata Grube 1855		+	
Nephtys hombergii Lamarck 1818		+	+
Nereis sp.			+
Prionospio caspersi Laubier 1962			+
Spio decoratus Bobretzky 1870			+
Streblospio shrubsolii Buchanan 1890			+
Abra alba Wood 1802			+
Abra prismatica Montagu 1808			+
Abra segmentum Récluz 1843		+	+
Acanthocardia paucicostata Sowerby 1834			+
Cerastoderma glaucum Bruguière 1789	+	+	+
Cyclope neritea Linnaeus 1758		+	
Idotea balthica Pallas 1772		+	
Loripes lucinalis Lamarck 1818			+
Kurtiella bidentata Montagu 1803			+
Ampelisca diadema Costa 1853			+
Ecrobia ventrosa Montagu 1803	+		
Corophium sp.			+
Dexamine spinosa Montagu 1813			+
Elasmopus sp.			+
Gammarus aequicauda Martynov 1931			+
Gammarus insensibilis Stock 1966	+	+	
Gammarus sp.			+
Iphinoe serrata Norman 1867		+	
Lekanesphaera hookeri Leach 1814	+		
Microdeutopus gryllotalpa Costa 1853		+	
Monocorophium acherusicum Costa 1853		+	
Paramysis helleri Sars 1877			+
Phtisica marina Slabber 1769			+
Upogebia pusilla Petagna, 1792			+
Amphiura chiajei Forbes, 1843			+
Chironomidae	+		
Oligochaeta		+	+

entiation of the macrofaunal communities specific to the lagoons studied, across a rather wide range of human impact. Indeed, a more even-sized distribution is observed in the Cesine lagoon, where small as well as intermediate size classes dominate in high percentages. The size variety of the benthic organisms in the Cesine is mainly determined by the various age-classes of the few species established in this harsh environment. On the contrary, in Logarou and Grado-Marano, a tendency towards smaller size classes is noted. In the Grado Marano lagoon, in particular, in many cases the community was found to be dominated by small-sized brackish water and/or opportunistic species, while the number of individuals assigned to the intermediate size classes was low.

Classification metrics

Table 3 lists the average values of the biotic classification indices (AMBI, BENTIX, M-AMBI, BO2A, ISD and ISS) and the resulting EQS for the three lagoons, while Fig. 5 indicates the number of stations assigned to each EQS (The values of each index and the resulting EQS calculated for each station and lagoon are included in the Annex Tables 1, 2 and 3).

Based on the average values, the Cesine lagoon is classified by AMBI as good with all the stations assigned to that status, by BENTIX as moderate, by M-AMBI as poor, by BO2A as uniformly high, and by ISD and ISS as good.

Table 3. Average values of the classification metrics and respective EQS assessment in the three lagoons.

Index/ EQS	Cesine			Logarou			Grado-Marano		
	Fall	Spring		Fall	Spring		Fall	Spring	
AMBI	2.74	2.53	2.64	1.33	1.99	1.66	3.67	2.06	2.86
AMBI EQS	G	G	G	G	G	G	M	G	M
BENTIX	2.43	2.78	2.6	2.57	2.37	2.47	2.87	3.68	3.28
BENTIX EQS	P	M	M	M	P	P	M	G	M
M-AMBI	0.28	0.32	0.3	0.48	0.45	0.47	0.43	0.44	0.43
M-AMBI EQS	P	P	P	M	M	M	M	M	M
BO2A	0	0	0	0.01	0	0.01	0.19	0.03	0.11
BO2A EQS	H	H	H	H	H	H	G	H	G
ISD	1.34	1.67	1.5	2.09	2.24	2.17	2.94	2.57	2.76
ISD EQS	G	G	G	M	M	M	M	M	M
ISS	2.91	2.98	2.94	2.23	1.96	2.09	2.25	2.54	2.4
ISS EQS	G	G	G	P	P	P	M	M	M

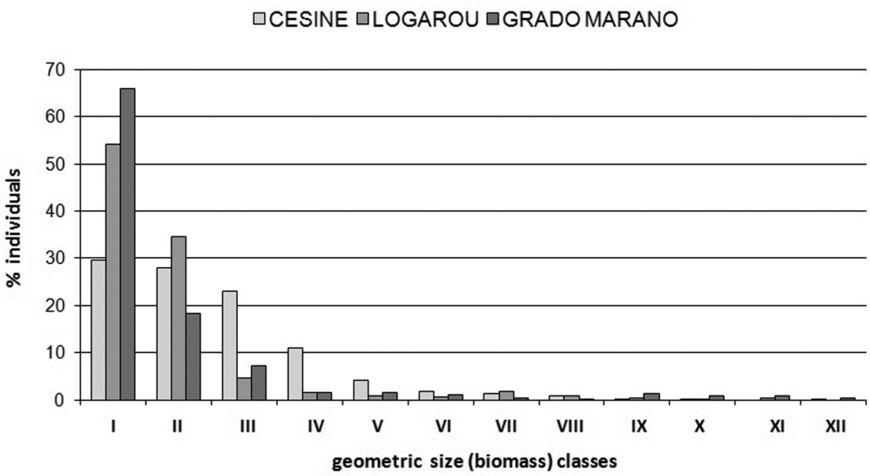

Fig. 4: Integrated body size distributions of benthic communities in the lagoons studied (class I = 0.1 mg, class II = 0.2–0.3 mg, class III = 0.4–0.7 mg, ... class XII = 204.8–409.5 mg).

The Logarou lagoon is classified by AMBI on average as good, by BENTIX and ISS as marginally poor, by M-AMBI and by ISD as moderate, and by BO2A as uniformly high.

In Grado-Marano, a greater variability in the status has been assigned than to the other stations. On average it is classified by AMBI as moderate, with most stations being moderate and good, by BENTIX also as moderate, despite the fact that half of the stations are rated poor, due to some stations being classified as high and as good, and by M-AMBI, ISD and ISS as moderate, with most stations being moderate or poor. BO2A offers a highly confusing assessment, with all five classes being represented. On average it assignes a good status to the lagoon as a large percentage of the stations have been classified as high.

The general performance trend of each index is clearly seen over the whole data set: The BO2A index results in the highest status classification assigning the highest frequency of high status, followed by the AMBI, which also records a high percentage of high and good status. BENTIX and M-AMBI produce, on average similar results, assigning the highest frequency of poor status in the lagoons and generally the most severe classification. The ISD and ISS performances fall in between, in the assessment of the biotic indices.

Although the agreement among the indices is low across the whole data set, when viewed separately for each lagoon, an important agreement is arrived at by at least four among the six indices tested. Thus, in Cesine there is good agreement among the BO2A, AMBI, ISD and ISS, with high and good status assessment. Given the average classification based on all the indices, it is obvious that the Cesine is classified as having the best condition when compared with the others.

Logarou being classified as moderate by ISD and M-AMBI, and as marginally poor by BENTIX and ISS shows good concurrence among those indices. However, the AMBI and BO2A classify the lagoon as having good and high status, respectively, probably overestimating the conditions prevailing in this lagoon.

Grado-Marano is assessed by all the indices, except for BO2A as being, on average, moderate, showing the best agreement among most of the indices and indicating the most severe disturbance among the lagoons studied. Also, the mean ISD value in Grado-Marano is lower

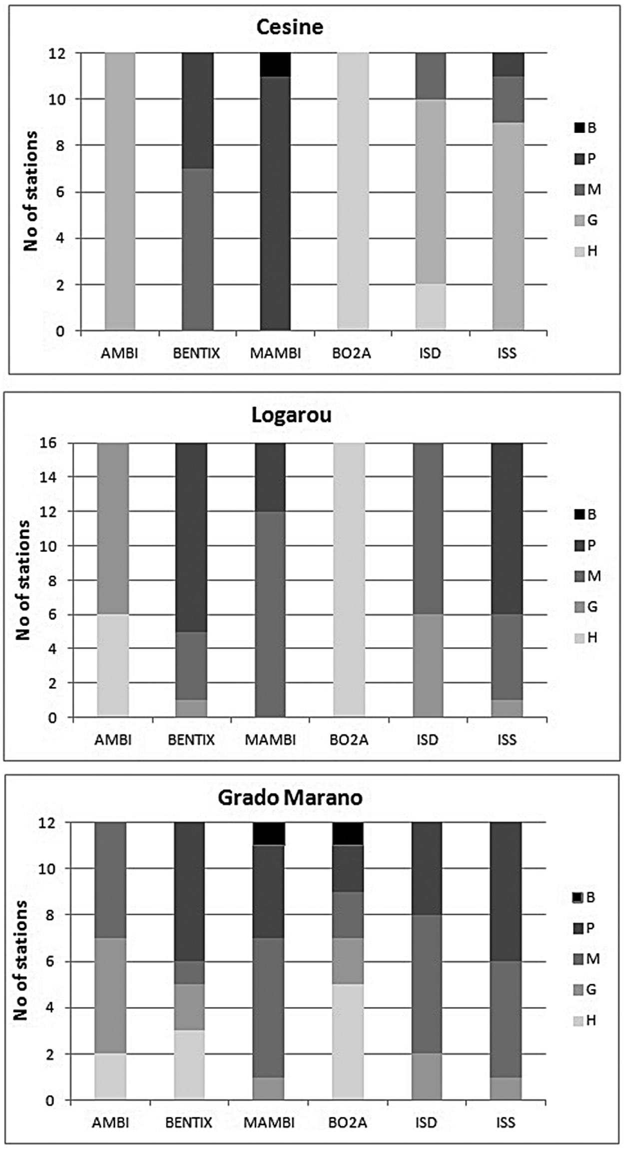

Fig. 5: Ecological quality status classification results derived from the indices tested in the three lagoons.

than that of the Logarou, assigning a mostly moderate and poor EQS in Grado-Marano, while in Logarou the ISD index indicates mostly a moderate and good assessment.

Over the whole data set, it seems that the ISD index performs well for the lagoonal environment, assigning an ecologically relevant status, according to the abiotic and pressure information available, showing a close category agreement or complete matching in each case with at least three of the five biotic indices tested.

Discussion

Soft bottom benthic communities were studied at two seasons, in three Mediterranean transitional ecosystems with varying degrees of human impact. Salinity varied remarkably among the lagoon bodies, while differences in the environmental parameters were also reflected in the biota.

Salinity played a major role in the community structure of the lagoons. Indeed, the correlation analyses between the species diversity indices and environmental parameters showed a positive relationship between species richness and salinity. The lowest number of species diversity was found in the low salinity (4.7-6.3) Cesine lagoon although this was the least anthropogenically disturbed. In systems with salinity fluctuations below 15, the salinity acts as a fundamental limiting factor for species colonization from the marine environment (Cognetti, 1992). Therefore, species richness in the coastal lagoons is not dependent on salinity alone but is the result of a complex of factors, described by the term ‘confinement’ - the time required to renew the marine element (Guelorget & Perthuisot, 1983). The higher the water exchange of a coastal lagoon with the sea, the more stable are its benthic communities (Bachelet *et al.*, 2000).

Trophic analysis of the communities showed deposit feeders to be clearly dominant. In most cases surface deposit feeders prevailed numerically, while seasonally, the grazers and filter feeders became equally abundant. The MDS plot based on the trophic composition of the communities, demonstrated no spatial or seasonal discrimination. This is due to the fact that trophic composition is strictly dependent on the relative roles of a few species. Indeed, it is the variation in the relative abundance of a few, or even one dominant species that triggers drastic changes in the trophic composition of the communities, reflecting the natural instability of these ecosystems. In the MDS, based on size composition, a clear differentiation in the communities studied is evident. The unimpacted lagoons presented a different biomass pattern, compared to the impacted Grado-Marano. Body size structure can be a trait sensitive to the eutrophication and pollution phenomena, as it is well documented that with the increasing anthropogenic pressure, shifts to a smaller body size have been observed (Pearson & Rosenberg, 1978; Warwick, 1986; Weston 1990; Basset *et al*., 2004) Non-impacted communities are characterized by an even distribution of their size structure, because the benthic individuals are assigned to a larger variety of size classes, whereas in the disturbed communities an uneven biomass distribution has been observed. Under pollution effects (e.g. eutrophication), a dominance of small sized individuals was detected, causing the relative dominance of the smaller sized classes at the expense of the intermediate sized classes. In many cases, the variety of the biomass classes and the even size of the distribution observed in the Cesine lagoon, is determined by the different age-classes of one or few lagoonal organisms, implying that the biomass or size is the key parameter in this case. On the contrary, in Grado-Marano, the community was dominated by small sized individuals, lagoonal and/or opportunistic species, while Logarou presented an intermediate condition. Also, in other Mediterranean lagoons, e.g. Papas in Western Greece, the anoxia and prolonged hypoxic conditions can strongly affect the large sized and long-lived species (Reizopoulou & Nicolaidou, 2007).

Within the framework of the WFD, many authors have presented several classification systems for the biological and physicochemical elements used in the assessment of the ecological status of the water bodies. We compared the efficiency of the classification metrics based on species sensitivity and size composition. Testing the indices is a task aimed not only at selecting the best appropriate index for each case and water body type, but also to ensure that the results are comparable among two or more indices. Moreover, an effective and integrated management strategy, should be based on the sensitivity of the classification tools in assessing appropriately the response of the ecosystem to the varying degrees of impact.

Our results combined with the evaluation of the abiotic and pressure information and previous assessment work in the study areas (Ianni *et al*., 2008; Ponti *et al*., 2008), showed that ISD and ISS could correctly assess, in a robust and integrative way, the overall EQS of the coastal lagoons. The biotic indices tested in our study areas either underestimated (as BENTIX or M-AMBI) or overestimated the ecological status (as AMBI and BO2A) according to their structure. Indices that give equal weight to all opportunistic (ecological groups EG4, EG5 as defined by Grall & Glémarec, 1977, and Borja *et al*., 2000) and tolerant taxa (ecological group EG3) which naturally dominate the lagoons, in their formula as BENTIX, or indices that include diversity indices (that could also reflect natural stress) such as M-AMBI, underestimated the EQS. On the other hand, indices such as BO2A and AMBI that exclude or undervalue the significant - in our opinion - tolerant species group EG3, overestimated the status. In another case of EQS assessment in the dystrophic Papas lagoon, the high percentage of the tolerant EG3 group of the species (Simboura & Reizopoulou, 2008) gave weight to the good class in the AMBI formula resulting in an overestimated 'good' EQS assessment by AMBI.

In his paper on the "Estuarine Quality Paradox" suggested by Elliot & Quintino (2007), Dauvin (2007) critisized the performance of biotic indices in cases of naturally stressed ecosystems where the disturbance-tolerant species naturally dominate. As was well documented in the previous works, the biotic indices in transitional ecosystems tend to either underestimate, as do BENTIX and M-AMBI, or overestimate the EQS, such as AMBI. Indeed, in an internal intercalibration exercise of the performance of various indices in the coastal and transitional ecosystems, Simboura & Reizopoulou (2008) observed that in the coastal lagoons the AMBI tended to clearly demonstrate the status of the slightly and moderately disturbed lagoons, while it overestimated the EQS in the heavily polluted ones. However, the BENTIX and M-AMBI demonstrated poor EQS in the heavily polluted lagoons but underestimated the condition in the less disturbed ones. The results are in accordance with those of the present set of data originating from a wider geographical scale.

Regarding the application of the BO2A index, which is an index also based on the sensitivity/tolerance of groups of benthic species, the results produced an overestimated assessment for at least two of the lagoons, Logarou and Grado-Marano, assigning them to the high status, and an averagely good status in the most polluted one of Grado-Marano. It is noteworthy that by applying the BO2A index (although not shown in the results), adding the polychaetes belonging to EG3 group (the species simply tolerant to organic enrichment) together with the EG4 and EG5 groups, the opportunistic polychaetes, the results were more relevant to the actual situation, thus assigning a good status in the Cesine and Logarou and an averagely bad status in Grado-Marano.

This observation may indicate that the 'tolerant' EG3 species group, which are weighted equally with the opportunistic species in the BENTIX formula, have a specific value in reflecting the anthropogenic stress in the lagoonal as well as coastal ecosystems of the Mediterranean ecoregion.

However, the species groups that mainly constitute the major part of a lagoonal community are the opportunistic or tolerant lagoonal species (EG3, EG4, EG5); therefore, the boundary limit within which a lagoonal community dominated by one or another group of pollution indicators, should be classified as polluted remains highly unpredictable.

The results given above generally demonstrate the weakness of the biotic indices to reflect and discriminate among the anthropogenic and natural stress in the lagoonal ecosystems. The results also demonstrate that species sensitivity, richness and diversity as benthic community traits do not seem to function well in assessing the EQS in lagoonal ecosystems. They also indicate that other traits of the communities such as biomass or size structure could be more integrative, sensitive and effective for assessing the ecological quality in the lagoons.

Biomass structure is an attribute of the community that may reflect the alterations in the benthic ecosystem along a pollution gradient. The integrated plot of size distributions over the whole benthic ecosystem in the lagoons studied, incorporating results from a large sampling area, showed clear differences between the communities studied in a more definitive way than did the biotic indices. Reduced sampling effort could underestimate the presence of the intermediate and larger size classes.

Biotic indices have not been proved adequately efficient, and areas of 'optimum' performance based on their structure and design were demonstrated. Due to the natural dominance of the tolerant and opportunistic species, the transitional waters present strong analogies with the polluted coastal waters, while the biodiversity trends are mainly related to the degree of natural stress (Reizopoulou *et al*., 2014). In testing the functional diversity in order to discriminate the pollution phenomena, further research is required to detect which trait or set of traits can better evaluate the human impact and the relationships of these traits to the environmental constraints of these particular ecosystems.

Further studies on community analysis based on a range of biological traits, and not just those currently tested, could better contribute to the study of ecosystem functioning (Sigala *et al.*, 2012). However, the effect of the natural instability is also reflected in the functional diversity of the lagoons, as most of the species dominating these habitats can be considered as being less specialised as regards food, as shorter lived, with large population fluctuations and shorter life cycles, high reproductive rates, and greater dispersal potential. Therefore, the use of single trait approaches, could provide information on which biological trait is the most suitable and sensitive key variable to reflect the pollution conditions. Our data suggest that body size is a sensitive key parameter in impact assessments of transitional waters. Comparative ecosystem studies are necessary in order to ensure a common basis of understanding and evaluation of the ecological assessments. Research on the Ecological Quality Indices should be carried out on a large-scale level (Ecoregion level), without excessive emphasis on the local conditions, and incorporating the high spatial and temporal variability of such complex ecosystems using integrated models.

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Annex Table 1. Classification metrics values and respective EQS assessment in Cesine lagoon (F: Fall S: Spring).

Cesine	C1F	C2F	C3F	C4F	C5F	C6F	C1S	C2S	C3S	C4S	C5S	C6S
AMBI	2.8	2.75	2.98	3	2.6	2.33	2.64	2.36	2.49	2.81	2.22	2.68
AMBI EQS	G	G	G	G	G	G	G	G	G	G	G	G
BENTIX	2.27	2.42	2.14	2.06	2.63	3.06	2.78	2.98	2.88	2.31	3.2	2.51
BENTIX EQS	P	P	P	P	M	M	M	M	M	P	M	M
M-AMBI	0.19	0.28	0.25	0.25	0.35	0.36	0.29	0.3	0.37	0.28	0.36	0.32
M-AMBI EQS	B	P	P	P	P	P	P	P	P	P	P	P
BO2A	0	0	0	0	0	0	0	0	0	0	0	0
BO2A EQS	H	H	H	H	H	H	H	H	H	H	H	H
ISD	1.81	1.31	1.74	1.49	0.91	0.75	2.25	2.07	1.8	1.52	1.17	1.21
ISD EQS	G	G	G	G	H	H	M	M	G	G	G	G
ISS	1.23	3.30	3.31	3.10	3.24	3.25	2.73	2.78	3.11	3.40	2.91	2.96
ISS EQS	P	G	G	G	G	G	M	M	G	G	G	G

Annex Table 2. Classification metrics values and respective EQS assessment in Logarou lagoon (F: Fall S: Spring).

Logarou	LO5F	LO7F	LO8F	LO9F	LO11F	LO13F	LO15F	LO16F	LO5S	LO7S	LO8S	LO9S	LO11S	LO13S	LO15S	LO16S
AMBI	0.89	2.42	0.71	0.98	2.29	0.73	1.28	1.32	1.7	2.93	2.69	2.61	2.72	0.88	1.12	1.29
AMBI EQS	H	G	H	H	G	H	G	G	G	G	G	G	G	H	H	G
BENTIX	2.34	4.21	2.19	2.09	2.24	3.26	2	2.2	2.09	2.35	2.53	2.21	2.67	2.55	2.36	2.17
BENTIX EQS	P	G	P	P	P	M	P	P	P	P	M	P	M	M	P	P
M-AMBI	0.45	0.55	0.47	0.49	0.41	0.57	0.41	0.5	0.44	0.37	0.39	0.38	0.52	0.55	0.5	0.46
M-AMBI EQS	M	M	M	M	M	M	P	M	M	P	P	P	M	M	M	M
BO2A	0.0000	0.0007	0.0051	0.0108	0.0277	0.0023	0.0303	0.0077	0.0033	0.0002	0.0000	0.0010	0.0023	0.0087	0.0005	0.0083
BO2A EQS	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
ISD	1.23	2.32	2.15	1.8	1.43	2.65	2.52	2.62	2.29	2.56	1.59	1.84	2.28	2.86	2.05	2.46
ISD EQS	G	M	M	G	G	M	M	M	M	M	G	G	M	M	G	M
ISS	2.14	2.09	1.45	1.81	3.54	2.42	2.53	1.83	2.73	1.24	2.28	1.65	2.01	1.72	1.71	2.31
ISS EQS	P	P	P	P	G	M	M	P	M	P	M	P	P	P	P	M

Annex Table 3. Classification metrics values and respective EQS assessment in Grado-Marano lagoon (F: Fall S: Spring).

Grado-Marano	GM1F	GM2F	GM3F	GM4F	GM6F	GM7F	GM1S	GM2S	GM3S	GM4S	GM6S	GM7S
AMBI	4.44	3.93	4.38	3.97	1.7	3.57	3.17	2.93	3.08	2.05	0.29	0.82
AMBI EQS	M	M	M	M	G	M	G	G	G	G	H	H
BENTIX	2	2.09	2.16	2.28	4.66	4.04	2.09	2.38	2.95	4.2	5.66	4.81
BENTIX EQS	P	P	P	P	H	G	P	P	M	G	H	H
M-AMBI	0.17	0.37	0.29	0.42	0.75	0.56	0.3	0.39	0.51	0.48	0.42	0.54
M-AMBI EQS	B	P	P	M	G	M	P	P	M	M	M	M
BO2A	0.2918	0.1978	0.2534	0.1784	0.0605	0.1585	0.0270	0.0322	0.0948	0.0275	0.0022	0.0000
BO2A EQS	B	P	P	M	G	M	H	H	G	H	H	H
ISD	2.89	3.28	2.83	2.05	3.24	3.33	2.3	2.79	1.62	2.99	2.62	3.12
ISD EQS	M	P	M	G	P	P	M	M	G	M	M	P
ISS	2.02	1.91	2.76	2.70	2.02	2.09	2.00	2.48	3.40	1.86	2.76	2.75
ISS EQS	P	P	M	M	P	P	P	M	G	P	M	M