Response of different biotic indices to gradients of organic enrichment in Mediterranean coastal waters: implications of non-monotonic responses of diversity measures

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Abstract

According to the Water Framework Directive (Directive 2000/60/CE), assessment methods for the biological quality element benthic invertebrates must include diversity, abundance and proportion of pollution sensitive/indicator taxa as indicative parameters. By one hand, the use of pollution sensitive/ indicator taxa (indicator taxa indices) is criticized due to the lack of a reliable methodology to know the level at which indicator species can be well represented in unaffected communities. By the other hand, it is often remarked in the literature that the response of diversity measures may be biased by several methodological constraints. In the last few years, several multimetrics, combining both types of indices, have been proposed with the aim of providing a better picture of the response of benthic communities to disturbance gradients. In order to understand how different responses of diversity measures may affect the responses of multimetric indices, several biotic indices, including diversity measures, indicator taxa indices and multimetrics, were calculated for a set of Mediterranean coastal ecosystems affected by different ranges of organic matter content. Diversity measures did not show monotonic patterns of response to the gradient of organic content, particularly at the low end of its range, while strong correlations were found between indicator taxa indices and this pressure indicator gradient. The multimetric used in the study (M-AMBI) was more correlated with its diversity components (H' and S) than with its indicator taxa component (AMBI) and consequently, M-AMBI was always less correlated with the gradient of organic content than AMBI. In Mediterranean coastal water ecosystems naturally poor in sediment organic matter content, indicator taxa indices such as MEDOCC, BOPA, AMBI or BENTIX, seem to give a more reliable picture of the response of benthic communities to moderate increments of organic content than diversity indices.

Keywords: diversity, biotic indices, multimetrics, Water Framework Directive, organic enrichment, Mediterranean coastal waters

1. Introduction

The Water Framework Directive (WFD) defines the composition and abundance of benthic invertebrates as one of the biological quality elements (BQE) for the classification of the ecological status in European costal and transitional waters. According to the WFD normative definitions, the values of these descriptors for each water body must be compared against type-specific reference values recorded in undisturbed conditions, in order to allow the classification of the faunal communities in High, Good, Moderate, Poor or Bad status. Assessment methods for the benthic invertebrates must include diversity, abundance and proportion of sensitive/ pollution indicator taxa as indicative parameters.

The increasing need for stable and comparable criteria of environmental quality in European aquatic ecosystems (including coastal zones and estuaries), which followed the promulgation of the WFD, reactivated at the end of 2000 the use and search for pollution biological indicators (Salas et al., 2006). Several authors do not recommend the use of pollution indicator taxa to assess ecological status since these may naturally occur in relative high densities. Besides, there is no reliable methodology to know the level at which indicator species can be well represented in an unaffected community, leading to a significant exercise of subjectivity (Salas et al., 2006 and references therein). Despite these criticisms, indices like AMBI (Borja et al., 2000), BENTIX (Simboura and Zenetos, 2002), MEDOCC (Pinedo and Jordana, 2007) and BOPA (Dauvin and Ruellet, 2007) have gone back to update such pollution detecting tools (Marques et al., 2009).

Alternatively, diversity indices are highly applied in environmental studies but some authors point out that these measures can be highly influenced by different sample sizes, sampling effort, habitat type or complexity, and do not show monotonic behaviour in response to environmental degradation (Gray, 2000; Rogers et al., 1999; Warwick and Clarke, 1998; Wilkinson, 1999). In fact, according to the Pearson and Rosenberg (1978) model, diversity does not show a monotonic trend along both spatial and temporal gradients of pollution. When moving away from the source of pollution, the peak of opportunists is often followed by a maximum value in diversity, which then stabilizes at a slightly lower level. This means that in a gradient of pollution, the highest values for the diversity index may be recorded when the number of species is still low and the community is still at an early stage of recovery.

In order to fulfil the WFD requirements, many authors have developed multimetric indices, through the combination of different parameters and/or indices into a multivariate approach. M-AMBI (Muxika et al., 2007) for example, is a combination of the AMBI index with richness and Shannon diversity. The BAT, proposed by Teixeira et al. (2009), is also a multimetric methodology using three indices (AMBI, Shannon-Wiener diversity and Margalef index) selected from previous works (Bettencourt et al., 2004; Teixeira et al., 2007), which includes ‘abundance’ and ‘composition’ as measurable attributes for macroinvertebrate benthic fauna. Similarly, the DKI and UK indices (Borja et al., 2007) result from the combination of AMBI with other community parameters (H’, S, N, λ).

However, the above-mentioned disadvantages in the use of diversity measures may raise two questions: (i) what kind of responses to pressure gradients can be expected from diversity

measures and (ii) how can those responses affect multimetric indices? With the aim of answering these questions, three objectives were set for the present paper:
(1) To compare the responses of M-AMBI components to gradients of organic enrichment, in different Mediterranean coastal areas.
(2) To compare the effect of increasing organic matter content in the values of biotic indices independent from diversity measures (BOPA, AMBI, BENTIX and MEDOCC), and compare it with the response of M-AMBI and its diversity components.
(3) To assess the relationship between different types of biotic indices for Mediterranean coastal waters datasets.
The only multimetric used in this study was M-AMBI since it is the only one including diversity measures, which has been proposed for the establishment of the WFD ecological status in several countries from the Mediterranean ecoregion.

2. Material and Methods

2.1 Datasets

The present study was based on a dataset of soft bottom macrofauna samples collected in several Mediterranean coastal areas affected by different ranges of organic enrichment. A total of 677 samples from Spain (including Andalusia, Valencia, Murcia, Catalonia and Balearic Islands regions), Greece and Slovenia, were collected in shallow fine soft-bottom communities between spring and summer from 2002 to 2009. Table 1 summarizes the main characteristics of the quantitative samples. Due to the low number of samples available from Slovenia, these were only taken into account in the analysis carried out on the global dataset (all datasets pooled together).
The macrofauna was collected with a van Veen grab (600 cm^2 in Murcia, Valencia, Catalonia and Balearic Islands, 500 cm^2 in Andalusia and 1000 cm^2 in Greece and Slovenia) and sieved through a 0.5 mm sized mesh in Andalusia, Murcia, Catalonia and Balearic Islands, 0.63 mm in Valencia, and 1 mm in Slovenia and Greece. Samples were preserved in a 4% buffered formalin solution and the fauna were sorted and identified to the species level whenever possible.
Organic matter content in the sediment (OC) was measured in order to test the pressure-impact relationship of the assessment methods. This environmental variable was chosen as a surrogate for generalised anthropogenic pressures since it tends to correlate with a wide set of factors causing ecological stress in benthic communities (e.g. low dissolved oxygen, high ammonia and sulphide, chemical contamination) (Hyland et al., 2005).

2.2 Computation of indices

In the present study three general groups of biotic indices were used: 1) diversity measures, sensu Magurran (1989): number of taxa and Shannon-Wiener diversity index; 2) indices based

in pollution-indicative or sensitive taxa: BOPA, AMBI, MEDOCC, BENTIX; 3) multimetrics: M-AMBI.
The values of the BOPA, MEDOCC, BENTIX, AMBI, M-AMBI and Shannon-Wiener indices were calculated on the benthic data series using the following algorithms:

(1) $BOPA = \log (fp/fa + 1) + 1$

where *fp* is opportunistic polychaete frequency, and *fa* is amphipod (excluding G. *Jassa*) frequency. BOPA index varies between 0 (when $fp = 0$) and 0.30103 (when $fa = 0$) (Dauvin and Ruellet, 2007). The assignment of the opportunistic attribute to polychaetes was made according to the available AMBI's list of ecological groups. BOPA was not calculated for samples with total number of specimens < 20 and $fp + fa = 0$.

(2) $MEDOCC = [(0) (\%EGI) + (2) (\%EGII) + (4) (\%EGIII) + (6) (\%EGIV)] / 100$

where *EGI, EGII, EGIII,* and *EGIV* are sensitive, indifferent, tolerant, and opportunistic species, respectively. MEDOCC values can vary between 0 (only sensitive species are present) and 6 (opportunistic species are the 100 % of the total abundance) (Pinedo and Jordana, 2007). MEDOCC was not calculated for samples with > 20 % of non-assigned taxa.

(3) $BENTIX = [(6) (\%GS) + (2) (\%GT)] / 100$

where *GS* and *GT* are all "sensitive" (including the indifferent) and all "tolerant" (including tolerant and opportunistic) species, respectively. BENTIX values range from 6 (only "sensitive" species are present) to 2 ("tolerant" species are the 100% of the total abundance) (Simboura and Zenetos, 2002). BENTIX values with low confidence levels (according to the results given by the BENTIX software) were considered invalid.

(4) $AMBI = [(0) (\%EGI) + (1.5) (\%EGII) + (3) (\%EGIII) + (4) (\%EGIV) + (6) (\%EGV)] / 100$

where *EGI, EGII, EGIII, EGIV, EGV* are sensitive, indifferent, tolerant, second order opportunistic, and first order opportunistic species, respectively. AMBI values vary between 0 (only sensitive species are present) and 6 (first order opportunistic species are the 100 % of the total abundance), being 7 when the sediment is azoic (Borja et al., 2000). AMBI was not calculated for the samples with > 20 % of non-assigned taxa.

(5) $Shannon\text{-}Wiener\ index\ (H') = -\Sigma p_i \log_2 p_i$

where p_i is the proportion of abundance of species i in a community where species proportions are $p_i, p_2, p_3 ... p_n$ (Magurran, 1989).

As mentioned above, M-AMBI is a multimetric approach including the number of species (S), the Shannon diversity index (H'), and the AMBI index. Its procedure is based on a factor analysis including two virtual samples representing high and bad ecological quality status. The M-AMBI is obtained by calculating the Euclidean distance between the projection of each station to the line connecting both high and bad reference stations (see Bald et al., 2005 for further details). M-AMBI reference conditions were set following two different approaches. In the western Mediterranean (Spain) the M-AMBI software default reference conditions were used (higher H' and S and lower AMBI values from the entire dataset). For the eastern Mediterranean samples, median values of best-available sites were increased (H´, S) or decreased (AMBI) by about 10 % (in Greece) and by 15 % (in Slovenia) of the absolute difference between the lower anchor and the median value (Carletti et al., 2009; Simboura and Reizopoulou, 2008).
AMBI and M-AMBI methods were calculated using the AMBI software (http://www.azti.es). BENTIX index was applied using the Add-In (1.1. version) software package for MS Excel 2007 (http:// bentix.ath.hcmr.gr/).

2.3 Data analyses

Biotic indices, diversity measures and organic matter content in the sediment (OC) were analysed through non-parametric correlation analysis ($p \leq 0.05$). Spearman´s rank correlation coefficients were used in order to know whether the different indices varied monotonically with the pressure indicator or not. Analyses were performed with R v2.9.0 (R Core Team, 2009. http://www.R-project.org). Non metric multidimensional scaling (nMDS) was performed on the triangular matrices of the Spearman correlation coefficients calculated for each pair of biotic indices, obtained for each site (Clarke and Warwick, 2001). This multivariate ordination analysis was carried out with the PRIMER 6.0 (PRIMER-E, Plymouth) statistical package.

3. Results

3.1. Response of M-AMBI and its components to the pressure indicator gradient

The response of the M-AMBI multimetric and each of its components (AMBI, H' and S) to the pressure indicator gradient (OC) was investigated for the five Spanish and the single Greek datasets. Each biotic index was plotted against OC values and the Spearman rank correlation coefficient was calculated for each pair of parameters obtained (Figures 1-5 for Spanish datasets and Figure 6 for Greek dataset).
For the Spanish datasets, the OC ranged from 0.19 % in Murcia to 9.80 % in Andalusia, although half of its values concentrated between 0.8 and 2.0 %. The lowest maximum OC values were recorded in the Balearic Islands (2.8 %), revealing that the pressure indicator gradient for this dataset was clearly skewed towards low OC values (Figure 5). In this low range of pressure values, neither M-AMBI, nor any of its components, was able to detect a

response of the invertebrate benthic communities to changes in the OC content of the sediment. A similar pattern was observed for the Greek dataset (Figure 6), where the M-AMBI multimetric and its component metrics could not be related to variations in OC values (maximum of 3.1 %). However, the results obtained for this last dataset should be interpreted with care since it contained data from only 24 averaged samples.
In the remaining datasets, AMBI showed a significant and positive monotonic response to the OC gradient, although this was stronger for the westernmost regions (Spearman's $\rho = 0.47$ and 0.53 for Andalusia – Figure 1, and Murcia – Figure 2, respectively). These were also the datasets where M-AMBI showed a significant response to the pressure gradient, although much weaker than AMBI (Spearman's $\rho = - 0.25$ in both cases). The single dataset where S correlated significantly with the pressure indicator gradient was Catalonia (Figure 4), although the correlation coefficient was very low and in the unexpected direction (Spearman's $\rho = 0.23$). Similarly, a significant monotonic response of H' was observed only for the Murcia dataset, but with a rather low coefficient value again (Spearman's $\rho = - 0.27$).
From Figures 1 to 6, it is possible to observe that diversity measures did not show predictable patterns in their response to OC, particularly for very low OC values (~ < 3 %). In this range of OC values, and considering all datasets pooled together, S varied from 2 (corresponding to H' = 0.97) to 138 taxa (corresponding to H' = 5.8).

3.2. Responses of different types of indices to the pressure indicator gradient

Given the different ecological concepts behind different types of biotic indices, it is important knowing to which extent such differences affect the ability of responding to gradients of pressure indicators. Table 2 summarizes the range of Spearman's coefficients obtained for the correlations between the different biotic indices and OC, calculated for each region and for the global datasets. Biotic indices were separated in three different types, according to the description provided in Section 2 (Material and methods): 1) Diversity measures, based in community properties such as species richness, diversity and evenness; 2) Indicator taxa indices, based in the proportions of pollution-indicative or sensitive taxa; 3) Multimetrics M-AMBI, which integrates information from types 1 and 2.
Overall, when comparing coefficients from correlations of type 1 (n = 24) and type 2 (n = 12) indices, indicator taxa indices showed stronger correlations with OC than diversity measures ($t = 3.74$, $p < 0.005$). Moreover, M-AMBI correlations with OC (n = 6) were not significantly different from those of the diversity measures ($t = - 0.05$, $p = 0.96$). The use of different sampling sizes in different geographic areas had no influence on the patterns of response of the different indices to the gradient of OC. In general, data with higher sample sizes (0.1 m^2 in Greece and Slovenia) did not stand out in the different data clouds analysed (Figure 7).
In the datasets where OC values were always below 3 %, the pattern shown by the values of the biotic indices could not be linked to OC variations. In Greece, for instance, only BENTIX could be significantly correlated with OC. But as the range of OC values increased, up to 10 %, there was a stronger response of the taxa indicator indices when compared with the response of diversity measures.

In the westernmost areas (Andalusia and Murcia), all taxa indicator indices showed identical responses to the impact pressure gradient, although the strength of the correlation was slightly higher for MEDOCC. In Valencia, AMBI, MEDOCC and BENTIX were able to respond to changes in OC (MEDOCC showing the highest relation), while in Catalonia BOPA showed the strongest correlation with this pressure indicator (Spearman's $\rho = 0.40$, $p \sim 0$). Nevertheless, in Catalonia S and AMBI showed also significant correlations with OC ($p = 0.23$ and 0.20, respectively) and MEDOCC, despite showing a non-significant monotonic behaviour with the pressure gradient, showed in this dataset a significant linear response to OC (Pearson's $r = 0.23$, bootstrapped $p = 0.018$).

3.3. Correlations between different indices

The analysis of the relationship between two different biotic indices may help understanding if both indices are measuring the same aspect of the community and in the same direction. Accordingly, for each of the individual and for the global datasets, all biotic indices were plotted against each other and Spearman rank correlation coefficients were calculated for each pair (Figures 1-5 for Spanish datasets, Figure 6 for Greek dataset and Figure 7 for the global dataset). For the global dataset, the highest correlations were found between AMBI and MEDOCC (Spearman's $\rho = 0.83$, $p \sim 0$) and between M-AMBI, H' and S ($0.71 <$ Spearman's $\rho < 0.77$, $p \sim 0$); this pattern was more or less consistent throughout all datasets. It must be pointed out that in Valencia, Catalonia and Balearic Islands, AMBI and M-AMBI were not significantly correlated.

BOPA was always highly significantly correlated with the remaining indicator taxa indices (AMBI, MEDOCC and BENTIX), except in the Balearic Islands dataset.

In Spain, M-AMBI showed significant, although low correlations with BENTIX and MEDOCC (Spearman's ρ absolute value ≤ 0.50); the correlations with BOPA were weak (maximum Spearman's $\rho = -0.39$ in Andalusia), and for the Catalonia dataset there was no correlation at all. This trend changed in Greece, where BOPA and M-AMBI showed a strong monotonic relationship (Spearman's $\rho = -0.71$), as did BOPA and H' (Spearman's $\rho = -0.64$).

In the global dataset H' showed weak correlations with taxa indicator indices (Spearman's ρ absolute value < 0.10) but in Greek samples, correlation coefficients were always highly significant (> 0.64, absolute value). For S, the stronger correlations recorded were, by far, with H' and M-AMBI in all datasets.

The observations described in the previous paragraphs are quite well summarized in the nMDS diagrams of Figure 8. From this figure it is clear that M-AMBI is more often correlated with H' or S than with AMBI. In fact, AMBI is usually closer to other indicator taxa indices than to M-AMBI. Although based in the same basic concept, the relationship between indicator taxa indices showed a certain variation from region to region. For instance, the four indicator taxa indices grouped together only for the datasets of Andalusia and Catalonia.

4. Discussion

4.1. Indices response to disturbance

In the framework of ecological status assessment and subsequent environmental management actions, ecosystem degradation must be, as much as possible, linked with its causative stressors. This means that biotic indicators, used as surrogates for biota condition, must show a significant correlation with the targeted anthropogenic pressure indicators (Cairns et al., 1993; Dale and Beyeler, 2001). In the Mediterranean datasets investigated, diversity measures (Shannon-Wiener diversity index and number of taxa) showed a weaker ability to respond monotonically to changes in the organic content of sediments (OC) than did the biotic indices based in the proportion of pollution-indicative/sensitive taxa (taxa indicator indices: AMBI, BOPA, MEDOCC, BENTIX). Consequently, the response of the M-AMBI multimetric to the pollution gradient was always weaker than the response of its AMBI component due to the absence of a consistent monotonic response of the remaining M-AMBI parameters. In fact, in some of the studied regions there was no relationship between AMBI and M-AMBI indices at all.

The response of multimetrics to gradients of pressure may be strongly influenced by the individual responses of each of its components (Quintino et al., 2006). Inconsistent responses of the latter may affect more or less the response of the former, depending on the weight of each component on the final calculation of the multimetric. In all datasets investigated, M-AMBI final values seemed to be more influenced by the values of its diversity components than by AMBI values, which was particularly patent in the ordination plots obtained from the non-parametric correlation coefficients between indices. It is not the first time this performance issue is pointed out to the M-AMBI multimetric. Bakalem et al. (2009), for instance, do not recommend the use of M-AMBI since they consider that it gives too much weight to diversity. Munari and Mistri (2010) reached identical conclusions when applying this index in Mediterranean transitional ecosystems. These authors went even further, suggesting that the double weight given to diversity in M-AMBI (directly as H', and indirectly as S) may have partially explained mismatches in the ecological status assessment of four different biotic indices. In a study on the environmental impact of fin- and shellfish aquaculture, Borja et al. (2009) present two sites (Baie des Veys and Sounion) where, despite identical responses of S and AMBI were detected, M-AMBI responses differed and followed the trend recorded for H' in each of the sites. Identical patterns were found in a study on the effect of oyster farming on the EcoQS of intertidal mudflats; this time, despite the high correlation observed between AMBI and the gradient of OC ($p < 0.001$), the absence of a monotonic relationship between diversity and OC weakened the response of M-AMBI to the anthropogenic gradient ($p < 0.05$) (Bouchet and Sauriau, 2008).

In the present study, increases in OC, within the ranges proposed by Hyland et al. (2005) and Magni et al. (2009) (TOC: 10 to 30 mg g^{-1} ~ OC: 3 to 10 % OC, according to Leong and Tanner (1999)), could hardly be related to diversity measures, although they were significantly related to monotonic responses in indicator taxa indices. These results suggest

that in Mediterranean coastal water ecosystems, naturally poor in sediment organic matter content, moderate increments in organic matter loads are more reliably detected by indicator taxa indices than by indices relying on diversity measures (sensu Magurran (1989)). Unpredictable responses of diversity measures to the OC gradient may be related to the skewness of the latter towards its lower values. In Catalonia, for instance, as OC increased there was also a slight increase in the number of species suggesting that high OC values favour benthic communities, probably owing to an increase in food availability. In accordance with the Pearson and Rosenberg's conceptual model of benthic response to organic enrichment (Pearson and Rosenberg, 1978), benthic faunal variables (number of species, biomass and abundance) are expected to increase in relation to increasing OC, up to a certain point, before they begin to decline. This initial positive response of the communities may be due to a combination of the nutritional value of OC and a low incidence of environmental stressors (Hyland et al., 2005).
For the different datasets analysed in the present study, different sample sizes were reported. Sample size is known to influence diversity measures, while it usually shows neither effect on the response of taxa indicator indices nor on multimetrics such as M-AMBI (Dauvin and Ruellet, 2007; Dauvin et al., 2010; Magurran, 1989; Simboura and Zenetos, 2002). Nevertheless, the differences in sample sizes reported had no influence on the several correlations found between indices and between indices and the pressure gradient. In the several data clouds analysed, when data points were coded in relation to sample size, no pattern of variation was observed which could be attributed to varying sample sizes.

4.2. Comparison of indices performance in the environmental assessment

When comparing the performance of different diversity indices in their responses to gradients of pressure, although a high agreement may be found for bad ecological conditions, a low agreement is usually reported at high ecological status (Grémare et al., 2009; Munari and Mistri, 2010). In degraded environmental conditions, benthic invertebrates' assemblages respond all in a similar manner, showing a reduction in the number of species and diversity and an increment of dominance and opportunistic species (Odum, 1985; Pearson and Rosenberg, 1978). Conversely, as environmental stress diminishes, and assemblages develop a more K-strategist profile, their spatiotemporal dynamics becomes more complex and more dependent on several other abiotic (salinity, sediment properties, food supply and dispersal) and biotic (mainly competition for resources) factors (Anger, 1975; McLusky and Elliott, 2004). However, unexpected results have been observed in the Catalonia dataset where few significant relationships were obtained between biotic indices and OC. The worst classified station located in the near-shore area of Llobregat River (Bad ecological status) showed only 1 % of organic matter content in the sediment but the abundance of *Capitella capitata* (an opportunistic species) was extremely high (2783 individuals in 600 cm^{-2}). Kinoshita et al. (2008) observed that in a process of rapid population growth of this species, the decomposition of organic matter in the sediment was markedly enhanced.

The absence of a monotonic response of H' and S to the target environmental pressure indicator may lead to misclassifications of the ecological condition of the communities assessed by M-AMBI or other indices relying on diversity measures. In such situations, the highest (or lowest depending on the direction of the index) values cannot be associated to the lowest impact situation and the same index value may be observed at different degrees of impact. In an evaluation of the EcoQS of undisturbed soft-bottoms of the Reunion Island, Bigot et al. (2006; 2008) observed a polynomial relationship between AMBI and H', apparently driven by an incoherent classification of sites by H': the highest values of H' were concentrated in the Good class rather than the High, while sites with ~ 2.0 < H' < 3.5 were classified as in High, Good or Moderate conditions. Facing a conceptually identical problem, Muxika et al. (2007) in the paper where M-AMBI is described for the first time, excluded the use of density and biomass as parameters in the multimetric because of their bimodal (non-monotonic) response to a source of disturbance.

Despite departing from the same ecological concept, and being based in the same ecological paradigm, taxa indicator indices correlated with each other in a different way from region to region. For the different datasets investigated in this study, which cover a wide geographical range within the Mediterranean, the best correlations were observed for MEDOCC index with AMBI or BENTIX depending on the region (with the exception of Murcia where AMBI and BENTIX showed the best result). BOPA showed high correlations with AMBI, as expected since it classifies opportunistic polychaetes according to the AMBI's list of ecological groups. Significant correlations were also found between BOPA, MEDOCC and BENTIX. Taking into account that BOPA uses a lower taxonomic resolution level (it only requires the sorting of the invertebrate fauna in amphipods and opportunistic polychaetes, while the remaining indices require identification to the species level) these results suggest that the taxonomic sufficiency principle (Dauvin et al., 2003; Ellis, 1985) also applies to the assessment of the ecological status of Mediterranean coastal waters. Identical results had been previously achieved for several Mediterranean transitional (Forni and Ambrogi, 2007; Munari and Mistri, 2010) and coastal (de-la-Ossa-Carretero et al., 2009) ecosystems.

5. Conclusions

The Mediterranean coastal ecosystems investigated were naturally poor in sediment organic matter content. In such conditions, diversity measures showed a weaker ability to respond monotonically to changes in the organic content than did the biotic indices based in the proportion of pollution-indicative/sensitive taxa (such as AMBI). Moreover, the M-AMBI multimetric was not the best indicator of the benthic response to increases in organic content, since it was strongly influenced by the response of its diversity components and showed always a weaker response than its AMBI component. These results suggest that, for each individual dataset, the suitability of diversity measures to assess the ecological status of benthic communities in coastal ecosystems where the gradient of organic content is clearly skewed towards its lower end, must be carefully investigated. In such circumstances, biotic

indices based in the proportion of pollution-indicative/sensitive taxa (e.g. AMBI, BENTIX, BOPA and MEDOCC) seem to give a more reliable picture of the benthic condition.

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Figure captions

Figure 1. Scatterplot matrix of the different biotic indices (BOPA, AMBI, M-AMBI, S, H', BENTIX and MEDOCC,) and the pressure indicator (OC: organic content) for Andalusia region. In each scatterplot a lowess smooth is shown as a solid grey line, while the linear regression line is shown in dashed grey. The diagonal of the matrix shows the density plots for each variable. Lower-left triangle: values of the Spearman correlation coefficients for each pair of variables, and respective probability values; significant correlations ($p < 0.05$) are shown in bold.

Figure 2. Scatterplot matrix of the different biotic indices (BOPA, AMBI, M-AMBI, S, H', BENTIX and MEDOCC,) and the pressure indicator (OC: organic content) for Murcia region. In each scatterplot a lowess smooth is shown as a solid grey line, while the linear regression line is shown in dashed grey. The diagonal of the matrix shows the density plots for each variable. Lower-left triangle: values of the Spearman correlation coefficients for each pair of variables, and respective probability values; significant correlations ($p < 0.05$) are shown in bold.

Figure 3. Scatterplot matrix of the different biotic indices (BOPA, AMBI, M-AMBI, S, H', BENTIX and MEDOCC,) and the pressure indicator (OC: organic content) for Valencia region. In each scatterplot a lowess smooth is shown as a solid grey line, while the linear regression line is shown in dashed grey. The diagonal of the matrix shows the density plots for each variable. Lower-left triangle: values of the Spearman correlation coefficients for each pair of variables, and respective probability values; significant correlations ($p < 0.05$) are shown in bold.

Figure 4. Scatterplot matrix of the different biotic indices (BOPA, AMBI, M-AMBI, S, H', BENTIX and MEDOCC,) and the pressure indicator (OC: organic content) for Catalonia region. In each scatterplot a lowess smooth is shown as a solid grey line, while the linear regression line is shown in dashed grey. The diagonal of the matrix shows the density plots for each variable. Lower-left triangle: values of the Spearman correlation coefficients for each pair of variables, and respective probability values; significant correlations ($p < 0.05$) are shown in bold.

Figure 5. Scatterplot matrix of the different biotic indices (BOPA, AMBI, M-AMBI, S, H', BENTIX and MEDOCC,) and the pressure indicator (OC: organic content) for Balearic Islands. In each scatterplot a lowess smooth is shown as a solid grey line, while the linear regression line is shown in dashed grey. The diagonal of the matrix shows the density plots for each variable. Lower-left triangle: values of the Spearman correlation coefficients for each pair of variables, and respective probability values; significant correlations ($p < 0.05$) are shown in bold.

Figure 6. Scatterplot matrix of the different biotic indices (BOPA, AMBI, M-AMBI, S, H', BENTIX and MEDOCC,) and the pressure indicator (OC: organic content) for Greece. In each scatterplot a lowess smooth is shown as a solid grey line, while the linear regression line is shown in dashed grey. The diagonal of the matrix shows the density plots for each variable. Lower-left triangle: values of the Spearman correlation coefficients for each pair of variables, and respective probability values; significant correlations ($p < 0.05$) are shown in bold.

Figure 7. Scatterplot matrix of the different biotic indices (BOPA, AMBI, M-AMBI, S, H', BENTIX and MEDOCC,) and the pressure indicator (OC: organic content) for the global dataset (pooling data from all datasets, including Slovenia). Triangles and circles represent sample sizes of 0.05 – 0.06 and 0.1 m^2, respectively. In each scatterplot a lowess smooth is shown as a solid grey line, while the linear regression line is shown in dashed grey. The diagonal of the matrix shows the density plots for each variable. Lower-left triangle: values of the Spearman correlation coefficients for each pair of variables, and respective probability values; significant correlations ($p < 0.05$) are shown in bold.

Figure 8. nMDS plots obtained from the triangular matrices of the Spearman's correlation coefficients between every pair of biotic indices, for each individual and for the global dataset. Abbreviations as in Figures 1-7.

Figure 1

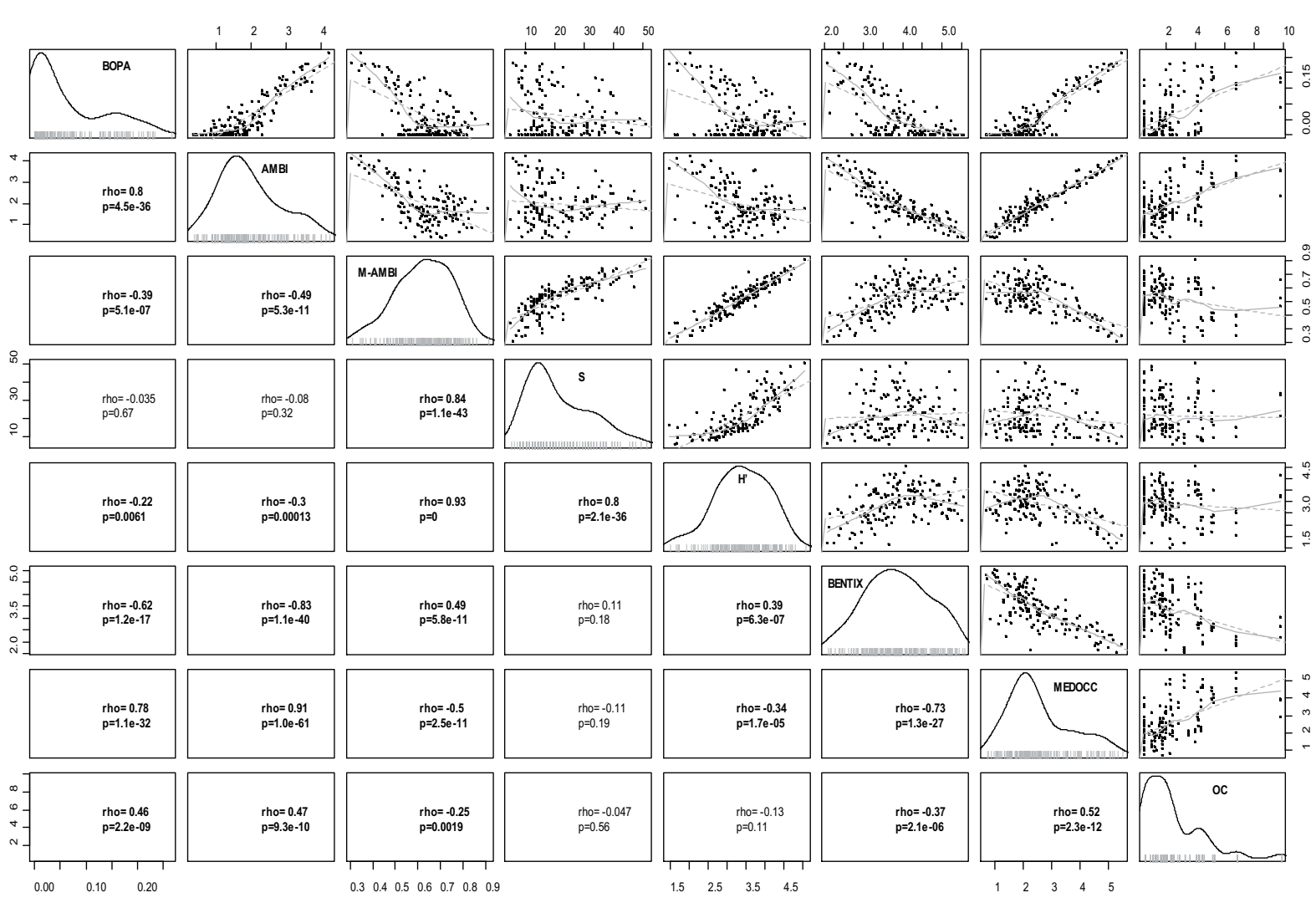
Andalusia
BOPA
AMBI
M-AMBI
S
H'
BENTIX
MEDOCC
OC
rho= 0.8
p=4.5e-36
rho= -0.39
p=5.1e-07
rho= -0.49
p=5.3e-11
rho= -0.035
p=0.67
rho= -0.08
p=0.32
rho= 0.84
p=1.1e-43
rho= -0.22
p=0.0061
rho= -0.3
p=0.00013
rho= 0.93
p=0
rho= 0.8
p=2.1e-36
rho= -0.62
p=1.2e-17
rho= -0.83
p=1.1e-40
rho= 0.49
p=5.8e-11
rho= 0.11
p=0.18
rho= 0.39
p=6.3e-07
rho= 0.78
p=1.1e-32
rho= 0.91
p=1.0e-61
rho= -0.5
p=2.5e-11
rho= -0.11
p=0.19
rho= -0.34
p=1.7e-05
rho= -0.73
p=1.3e-27
rho= 0.46
p=2.2e-09
rho= 0.47
p=9.3e-10
rho= -0.25
p=0.0019
rho= -0.047
p=0.56
rho= -0.13
p=0.11
rho= -0.37
p=2.1e-06
rho= 0.52
p=2.3e-12

Figure 2

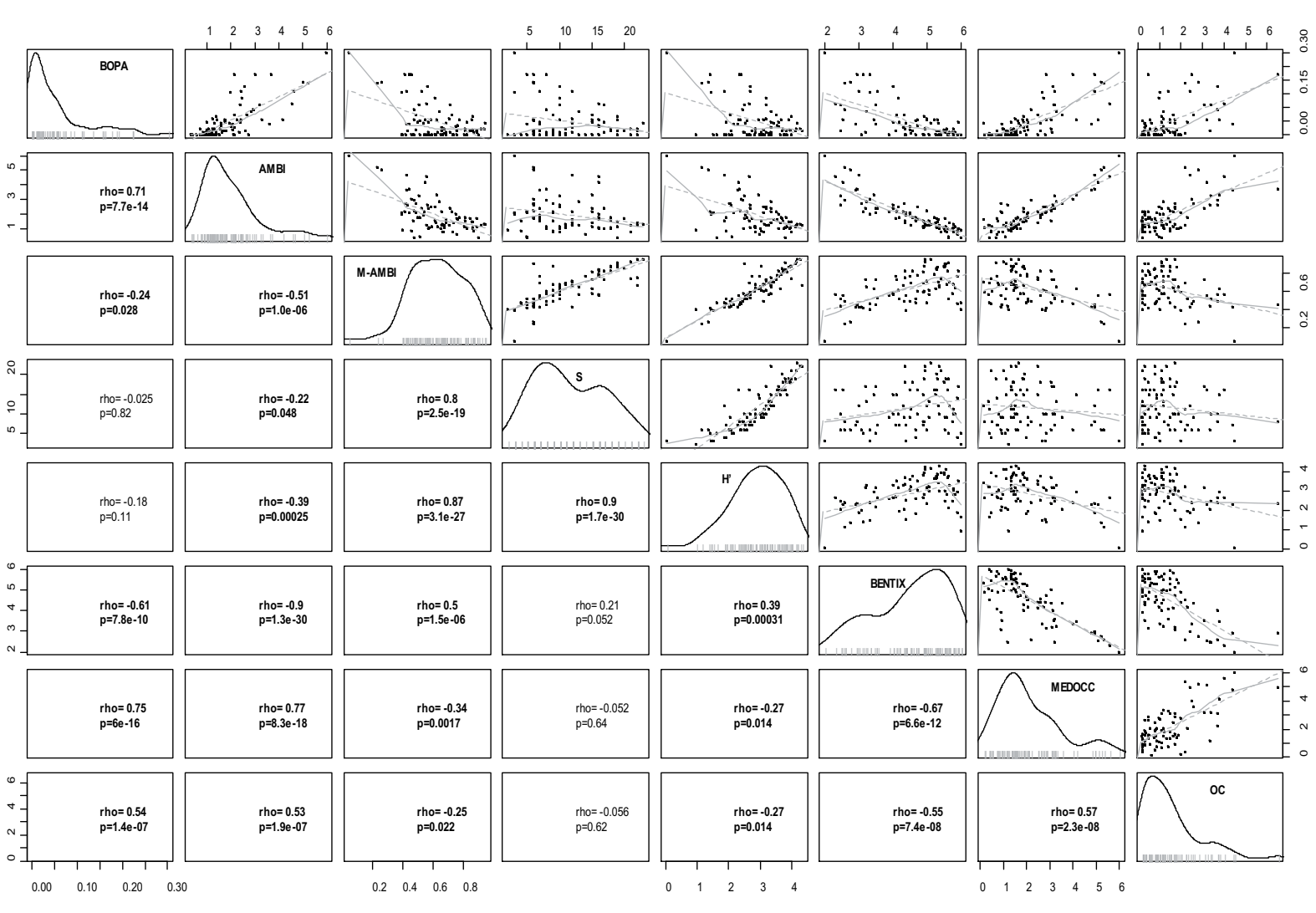
Murcia
BOPA
AMBI
M-AMBI
S
H'
BENTIX
MEDOCC
OC
rho= 0.71
p=7.7e-14
rho= -0.24
p=0.028
rho= -0.51
p=1.0e-06
rho= -0.025
p=0.82
rho= -0.22
p=0.048
rho= 0.8
p=2.5e-19
rho= -0.18
p=0.11
rho= -0.39
p=0.00025
rho= 0.87
p=3.1e-27
rho= 0.9
p=1.7e-30
rho= -0.61
p=7.8e-10
rho= -0.9
p=1.3e-30
rho= 0.5
p=1.5e-06
rho= 0.21
p=0.052
rho= 0.39
p=0.00031
rho= 0.75
p=6e-16
rho= 0.77
p=8.3e-18
rho= -0.34
p=0.0017
rho= -0.052
p=0.64
rho= -0.27
p=0.014
rho= -0.67
p=6.6e-12
rho= 0.54
p=1.4e-07
rho= 0.53
p=1.9e-07
rho= -0.25
p=0.022
rho= -0.056
p=0.62
rho= -0.27
p=0.014
rho= -0.55
p=7.4e-08
rho= 0.57
p=2.3e-08

Figure 3

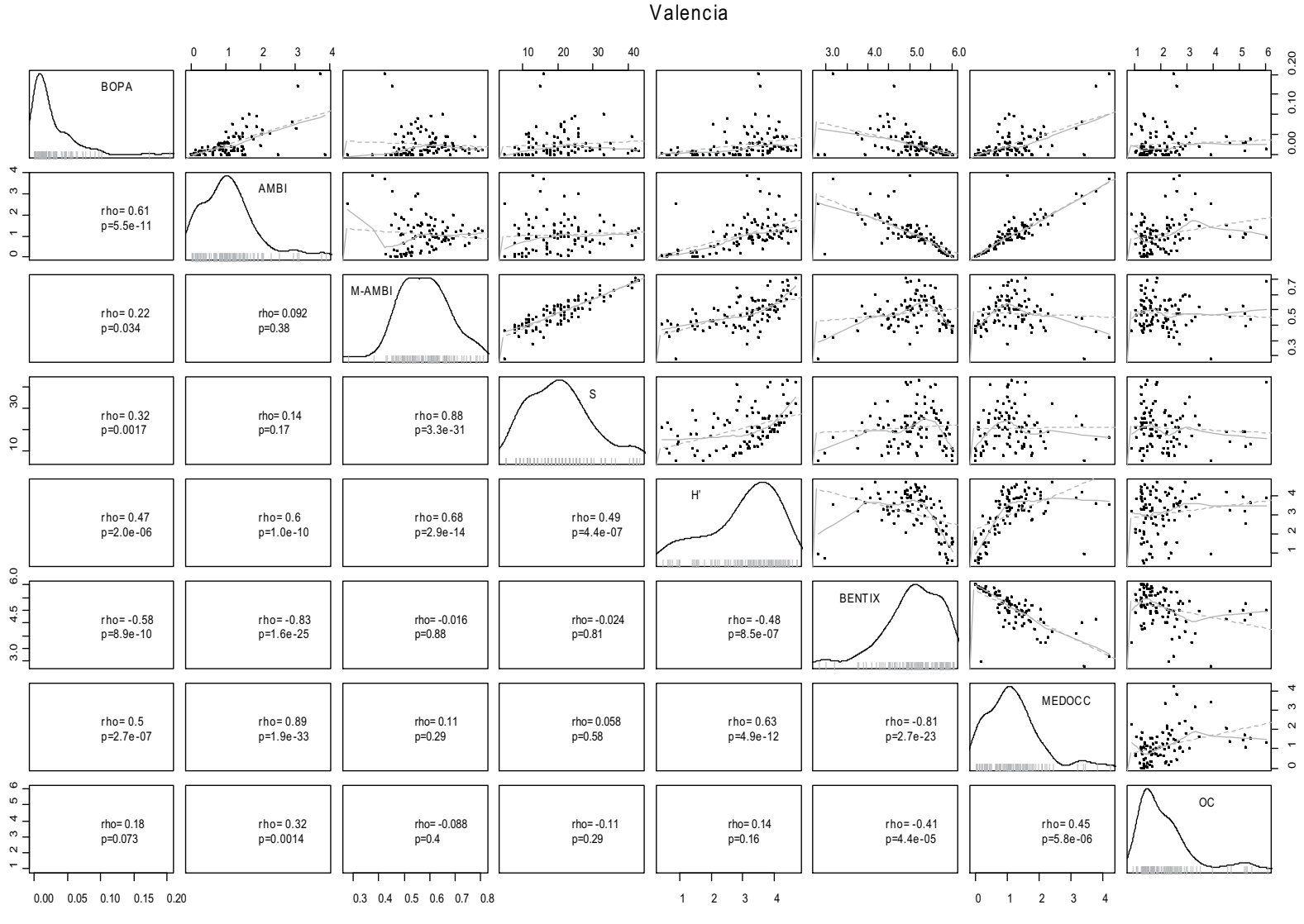
Valencia
BOPA
AMBI
M-AMBI
S
H'
BENTIX
MEDOCC
OC
rho= 0.61
p=5.5e-11
rho= 0.22
p=0.034
rho= 0.092
p=0.38
rho= 0.32
p=0.0017
rho= 0.14
p=0.17
rho= 0.88
p=3.3e-31
rho= 0.47
p=2.0e-06
rho= 0.6
p=1.0e-10
rho= 0.68
p=2.9e-14
rho= 0.49
p=4.4e-07
rho= -0.58
p=8.9e-10
rho= -0.83
p=1.6e-25
rho= -0.016
p=0.88
rho= -0.024
p=0.81
rho= -0.48
p=8.5e-07
rho= 0.5
p=2.7e-07
rho= 0.89
p=1.9e-33
rho= 0.11
p=0.29
rho= 0.058
p=0.58
rho= 0.63
p=4.9e-12
rho= -0.81
p=2.7e-23
rho= 0.18
p=0.073
rho= 0.32
p=0.0014
rho= -0.088
p=0.4
rho= -0.11
p=0.29
rho= 0.14
p=0.16
rho= -0.41
p=4.4e-05
rho= 0.45
p=5.8e-06

Figure 4

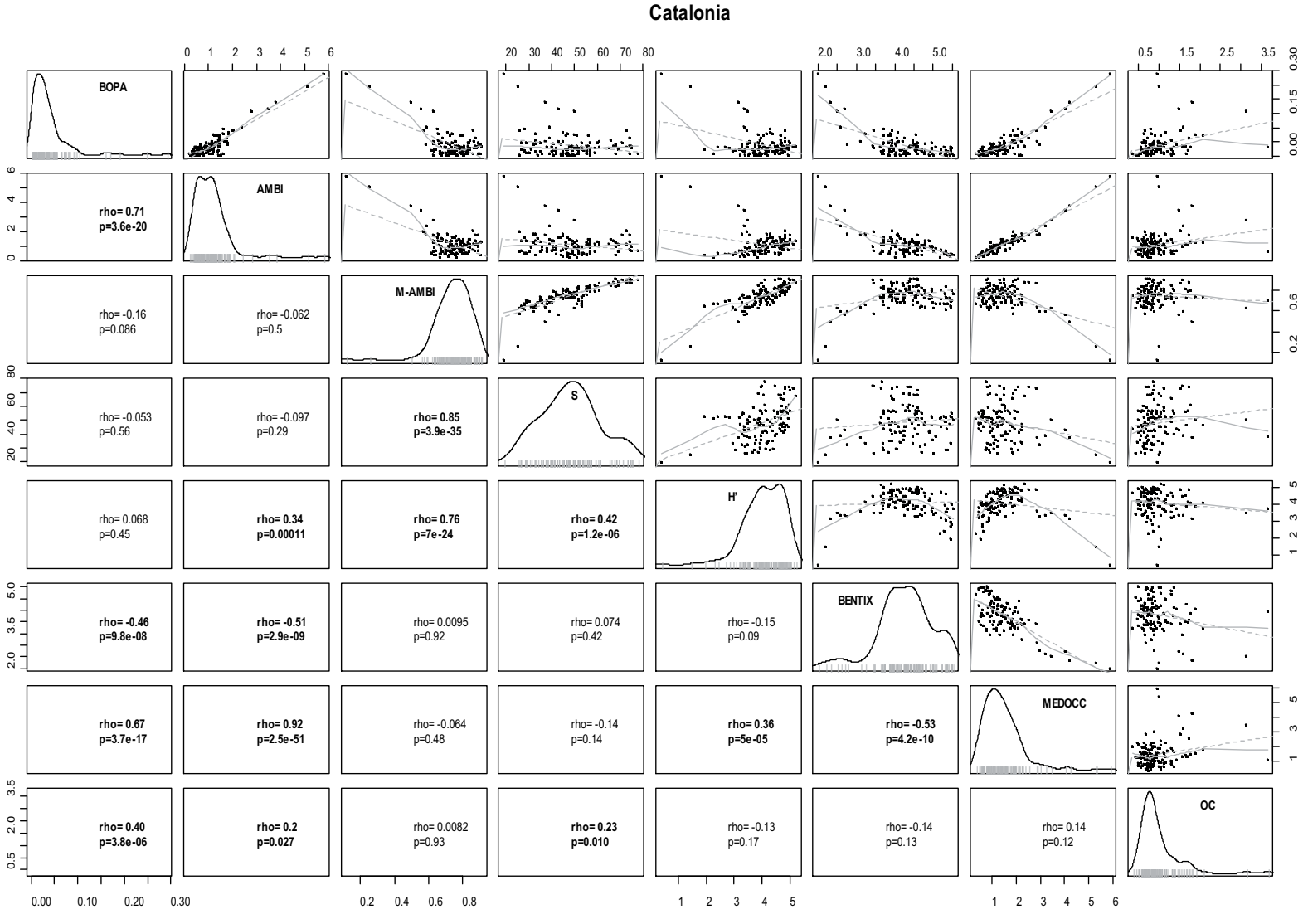
Catalonia
BOPA
AMBI
M-AMBI
S
H'
BENTIX
MEDOCC
OC
rho= 0.71
p=3.6e-20
rho= -0.16
p=0.086
rho= -0.062
p=0.5
rho= -0.053
p=0.56
rho= -0.097
p=0.29
rho= 0.85
p=3.9e-35
rho= 0.068
p=0.45
rho= 0.34
p=0.00011
rho= 0.76
p=7e-24
rho= 0.42
p=1.2e-06
rho= -0.46
p=9.8e-08
rho= -0.51
p=2.9e-09
rho= 0.0095
p=0.92
rho= 0.074
p=0.42
rho= -0.15
p=0.09
rho= 0.67
p=3.7e-17
rho= 0.92
p=2.5e-51
rho= -0.064
p=0.48
rho= -0.14
p=0.14
rho= 0.36
p=5e-05
rho= -0.53
p=4.2e-10
rho= 0.40
p=3.8e-06
rho= 0.2
p=0.027
rho= 0.0082
p=0.93
rho= 0.23
p=0.010
rho= -0.13
p=0.17
rho= -0.14
p=0.13
rho= 0.14
p=0.12

Figure 5

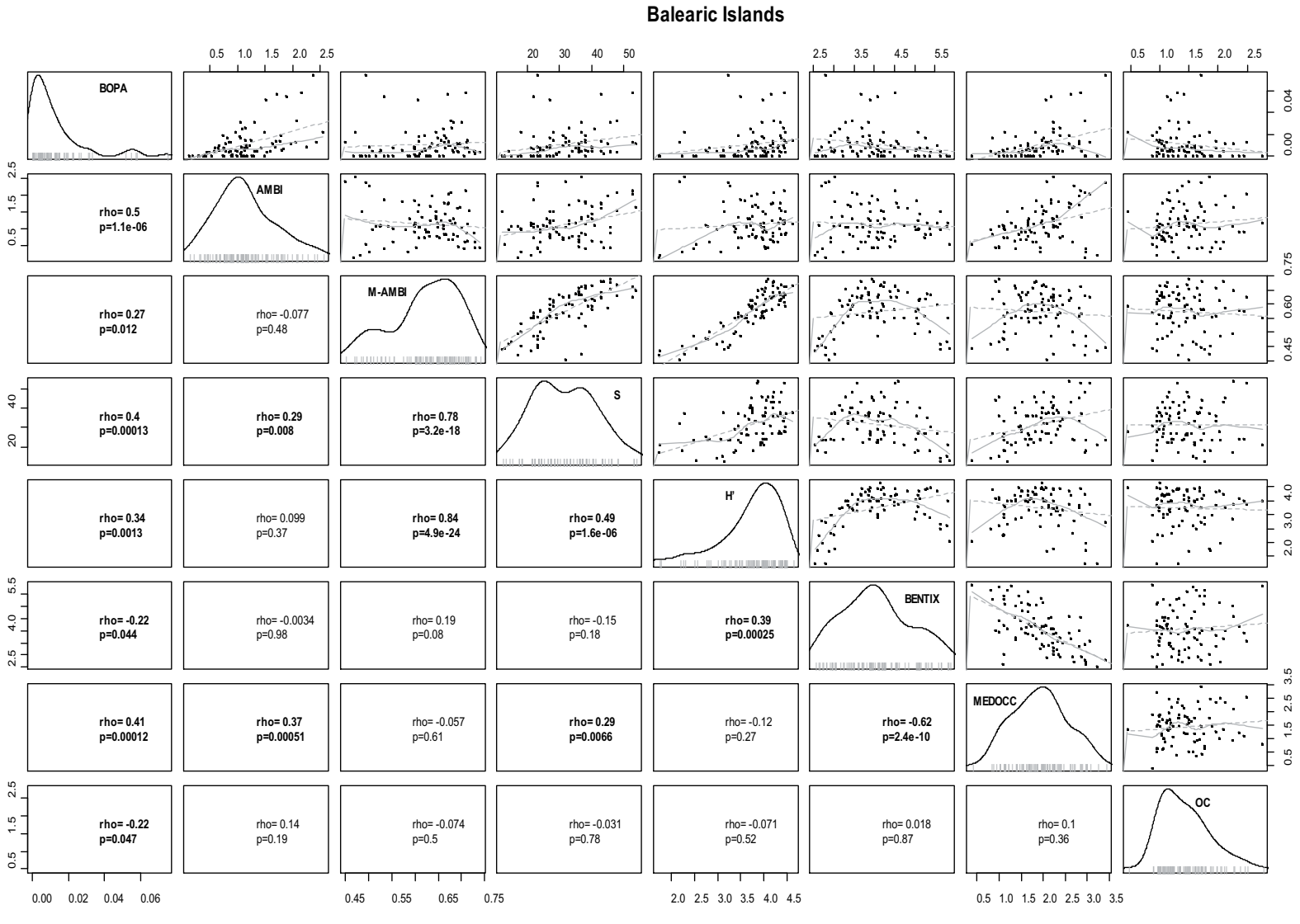
Balearic Islands
BOPA
AMBI
M-AMBI
S
H'
BENTIX
MEDOCC
OC
rho= 0.5
p=1.1e-06
rho= 0.27
p=0.012
rho= -0.077
p=0.48
rho= 0.4
p=0.00013
rho= 0.29
p=0.008
rho= 0.78
p=3.2e-18
rho= 0.34
p=0.0013
rho= 0.099
p=0.37
rho= 0.84
p=4.9e-24
rho= 0.49
p=1.6e-06
rho= -0.22
p=0.044
rho= -0.0034
p=0.98
rho= 0.19
p=0.08
rho= -0.15
p=0.18
rho= 0.39
p=0.00025
rho= 0.41
p=0.00012
rho= 0.37
p=0.00051
rho= -0.057
p=0.61
rho= 0.29
p=0.0066
rho= -0.12
p=0.27
rho= -0.62
p=2.4e-10
rho= -0.22
p=0.047
rho= 0.14
p=0.19
rho= -0.074
p=0.5
rho= -0.031
p=0.78
rho= -0.071
p=0.52
rho= 0.018
p=0.87
rho= 0.1
p=0.36

Figure 6

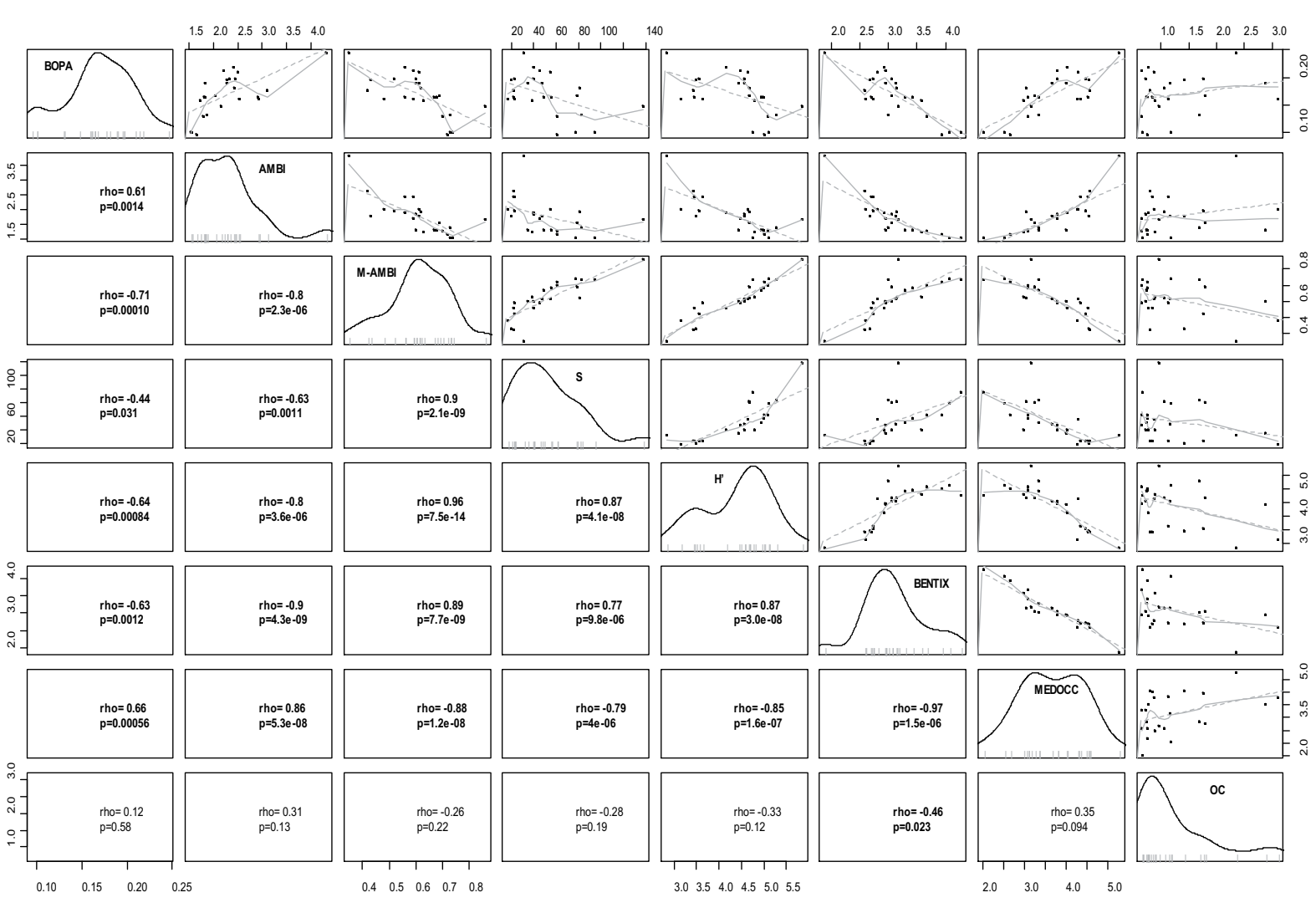
Greece
BOPA
AMBI
M-AMBI
S
H'
BENTIX
MEDOCC
OC
rho= 0.61
p=0.0014
rho= -0.71
p=0.00010
rho= -0.8
p=2.3e-06
rho= -0.44
p=0.031
rho= -0.63
p=0.0011
rho= 0.9
p=2.1e-09
rho= -0.64
p=0.00084
rho= -0.8
p=3.6e-06
rho= 0.96
p=7.5e-14
rho= 0.87
p=4.1e-08
rho= -0.63
p=0.0012
rho= -0.9
p=4.3e-09
rho= 0.89
p=7.7e-09
rho= 0.77
p=9.8e-06
rho= 0.87
p=3.0e-08
rho= 0.66
p=0.00056
rho= 0.86
p=5.3e-08
rho= -0.88
p=1.2e-08
rho= -0.79
p=4e-06
rho= -0.85
p=1.6e-07
rho= -0.97
p=1.5e-06
rho= 0.12
p=0.58
rho= 0.31
p=0.13
rho= -0.26
p=0.22
rho= -0.28
p=0.19
rho= -0.33
p=0.12
rho= -0.46
p=0.023
rho= 0.35
p=0.094

Figure 7

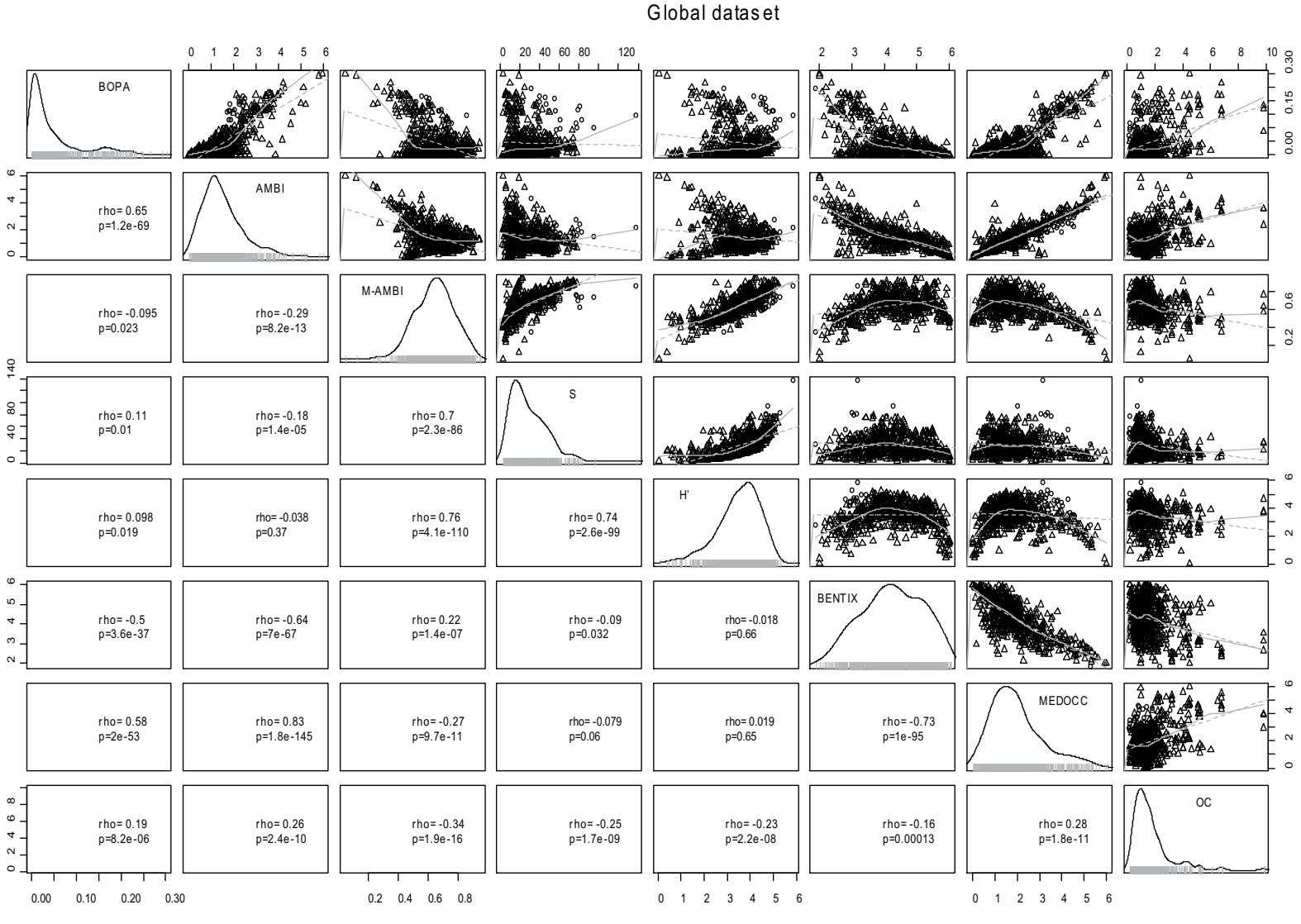
Global dataset
BOPA
AMBI
M-AMBI
S
H'
BENTIX
MEDOCC
OC
rho= 0.65
p=1.2e-69
rho= -0.095
p=0.023
rho= -0.29
p=8.2e-13
rho= 0.11
p=0.01
rho= -0.18
p=1.4e-05
rho= 0.7
p=2.3e-86
rho= 0.098
p=0.019
rho= -0.038
p=0.37
rho= 0.76
p=4.1e-110
rho= 0.74
p=2.6e-99
rho= -0.5
p=3.6e-37
rho= -0.64
p=7e-67
rho= 0.22
p=1.4e-07
rho= -0.09
p=0.032
rho= -0.018
p=0.66
rho= 0.58
p=2e-53
rho= 0.83
p=1.8e-145
rho= -0.27
p=9.7e-11
rho= -0.079
p=0.06
rho= 0.019
p=0.65
rho= -0.73
p=1e-95
rho= 0.19
p=8.2e-06
rho= 0.26
p=2.4e-10
rho= -0.34
p=1.9e-16
rho= -0.25
p=1.7e-09
rho= -0.23
p=2.2e-08
rho= -0.16
p=0.00013
rho= 0.28
p=1.8e-11

Figure 8

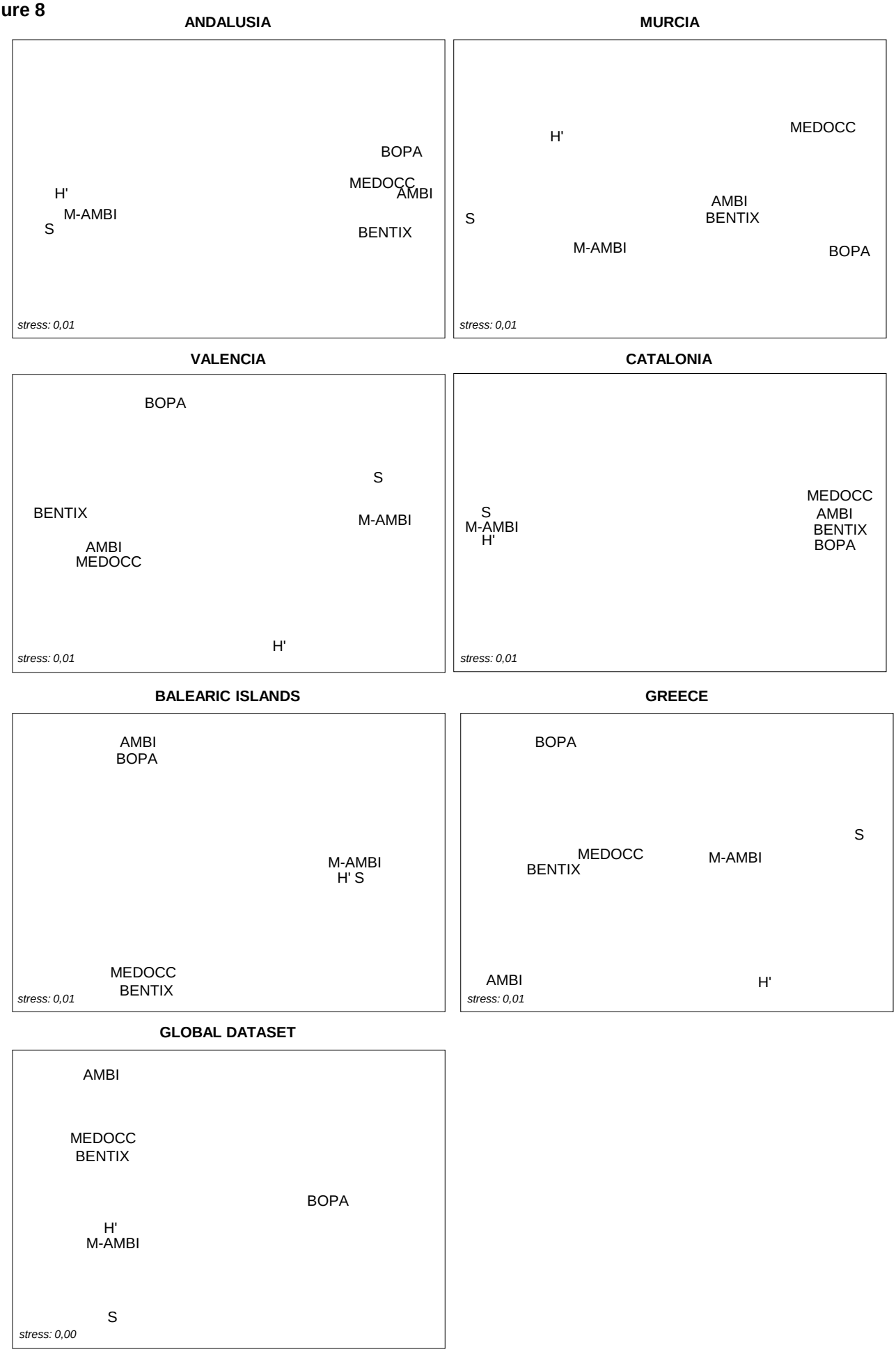

Figure 8

Table 1. Main characteristics of the datasets used in the present study

OSNMCA: operational and surveillance network monitoring in coastal areas

Sampling zone in the Mediterranean coastal areas	Sampling dates	Number of samples	Sampling gear and sampling surface	Sieving mesh	Data source
Spain (Andalusia region)	June-July 2007, 2009	60 replicated samples (156 replicates in total)	van Veen grab (500 cm^2)	0.5 mm	OSNMCA of Regional Government in Andalusia
Spain (Murcia region)	April 2003, July 2006, 2007, 2009	83 averaged samples	van Veen grab (600 cm^2)	0.5 mm	OSNMCA of Regional Government in Murcia
Spain (Valencia region)	June 2005, 2006	95 averaged samples	van Veen grab (600 cm^2)	0.63 mm	OSNMCA of Regional Government in Valencia
Spain (Catalonia region)	June-July 2002, 2003	122 averaged samples	van Veen grab (600 cm^2)	0.5 mm	OSNMCA of Regional Government in Catalonia
Spain (Balearic Islands)	June-July 2005	85 averaged samples	van Veen grab (600 cm^2)	0.5 mm	OSNMCA of Regional Government in Balearic Islands
Greece	July 1996 May 2000, 2003 Dec. 2000, 2002 Apr. 2002	24 averaged samples	van Veen grab (1000 cm^2)	1 mm	HCMR monitoring projects in coastal areas
Slovenia	Feb. 2004 May, Aug.-Sep. 2007, 2008	6 averaged samples	van Veen grab (1000 cm^2)	1 mm	OSNMCA of the Republic of Slovenia

Table 2. Range of spearman's correlation coefficients (absolute values) obtained for each pairwise comparison between biotic indices and OC content, in each dataset. Only significant correlations are included.

Dataset: see Table 1 for further details; Indicator taxa: indices based in pollution-indicative and sensitive taxa; Community measures: number of species and Shannon-Wiener diversity index; n.s.: non significant correlations; n: number of observations used in correlations

Dataset	Indicator taxa	Community measures	Multimetric M-AMBI	n
Andalusia	0.37 - 0.52	n.s.	0.25	156
Murcia	0.53 - 0.57	0.27	0.25	83
Valencia	0.32 - 0.45	n.s.	n.s.	95
Catalonia	0.20 - 0.40	0.23	n.s.	122
Balearic Islands	0.22	n.s.	n.s.	85
Greece	0.46	n.s.	n.s.	24
Global dataset *	0.16 - 0.28	0.23 - 0.25	0.34	571

* obtained by pooling all the remaining datasets, plus Slovenia dataset, together