The performance of biomass-based AMBI in lagoonal ecosystems

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Abstract

We studied the performance of the AZTI Marine Biotic Index AMBI manipulating input data collected from lagoonal ecosystems. Our data set consisted of macrofaunal abundance and biomass counts gathered at a variety of sites at which the disturbance status was known. Input data were also manipulated using a set of transformations of increasing severity. Biotic indices were calculated using raw and transformed abundance, biomass and production. Among the three categories of AMBI-based indices, medium transformation of data gave the highest correlation with pressures. However, increasing the severity of transformation generally resulted in a decrease of the correlation with environmental factors. The relative importance of ecological groups changed when using abundance or biomass, sometimes leading to an improved ecological status classification. Being biomass and production more ecologically relevant than abundance, using them to derive AMBI-based new indices seems intriguing, at least in lagoonal waters, where the community is naturally disturbed and dominated by opportunists.

Keywords:

AMBI, Biomass, Secondary production, Lagoons, Mediterranean Sea.

1. Introduction

Transitional waters (TWs, lagoons and estuaries) represent important and fragile ecosystems in the coastal landscape, providing key ecosystems services such as water quality improvement, fisheries resources, habitat and food for migratory and resident animals, and recreational areas for human populations. TWs also display distinctive features in terms of their extraordinary history of environmental management, the importance of their productivity and associated economical value, which is reflected on the peculiarity of their fauna (Cognetti and Maltagliati, 2008). For these reasons, TW ecologists experience difficulties in defining and agreeing acceptable quality of a “healthy lagoon” (Ponti et al., 2009). The majority of benthic indices for assessing ecological quality developed in light of the EU Water Framework Directive 2000/60/CE (European Community, 2000) are based on the same paradigm: disturbance/pollution generates secondary successions during which tolerant species are at first dominant and then progressively replaced by sensitive species (Pearson and Rosenberg, 1978). TWs are naturally organic enriched environments, and recent studies (Magni et al., 2009; Munari and Mistri, 2010; Sigovini et al., 2013; Prato et al., 2014) suggested that the use of indices based on species tolerance/sensitivity need to be adapted to such environments, since benthic communities of these ecosystems features low diversity and richness, and high abundance, and it is extremely difficult distinguish between natural or anthropogenic stresses.

The AZTI Marine Biotic Index AMBI (Borja et al., 2000) is probably the most widely used benthic index all over the world. In Europe, for example, many Countries have officially adopted the index for the description of ecological quality of coastal and transitional waters (Bulgaria, France, Germany, Italy, Romania, Slovenia and Spain; Borja et al., 2009; Birk et al., 2012). AMBI relies on the calculation of the biotic coefficient, which is based in turn on the proportion of disturbance-sensitive taxa and is expressed on a continuous scale ranging from 0 (best status) to 6 (worst status). The AMBI approach follows a model (Grall and Glemarec, 1997) which categorizes benthic invertebrates into five ecological groups (from EG-I, sensitive, to EG-V, first order opportunists), depending on their dominance along a gradient of organic enrichment. Recently, Warwick et al. (2010) suggested to estimate AMBI using biomass (BAMBI) and production (PAMBI). This because in an assemblage the abundance of a species can be relatively a poor measure of its functional importance, particularly in stressed situations when the insensitive species tend to be small bodied opportunists (Warwick et al., 2010). Then, since indices based on species abundances can be very sensitive to huge abundance of one or a few dominants (Warwick et al., 2002), they also suggested pre-treatment of data prior to calculating the indices using a set of transformations (square root, fourth root, logarithm, presence/absence) routinely used in multivariate analyses (Clarke, 1993). Recently, Muxika et al. (2012) successfully assessed the proposed modification to AMBI along the Basque coast (northern Spain), and Cai et al. (2014) in Bohai Bay (north of China).

TWs benthic assemblages are naturally characterized by low diversity, low richness, and strong dominance of one or few species (often tolerant or opportunist taxa: EG-III to EG-V), thus often leading to unsatisfactory ecological status classifications (Munari and Mistri, 2010; Prato et al., 2014). Since TWs are often characterized by high productivity (McLusky, 1989), we felt intriguing to manipulate the input data to AMBI through a set of transformations, and to explore the performance of the index using benthic biomass (BAMBI) and productivity (PAMBI) from a variety of TWs from Italy, at which the disturbance status was known.

2. Methods

2.1 Sites description and anthropogenic pressures

Our data set consisted of macrofaunal counts from 9 Italian TWs, occurring along a cline of 7° of latitude (between 45°28'N and 39°56'N). TWs were: Venice Lagoon, Sacca di Scardovari, Sacca di Goro, Valle di Gorino, Valli di Comacchio, and Lesina Lagoon (Adriatic Sea), Orbetello Lagoon, Stagno di Tortolì, and Rio Padrongiano (Tyrrhenian Sea). We intentionally considered only soft-bottom macrofauna due to the reduced distribution of hard bottoms in Italian TWs, where they are mostly represented by wooden piles marking navigable canals. A total of 46 sites have been chosen as representative of the different habitats found within each transitional environment (Fig. 1).

The Venice lagoon is located at the northern end of the Adriatic Sea. It extends for approximately 550 km^2 and has an average depth of 1.2 m. On its east side, two long barrier islands separate the lagoon from the Adriatic Sea. Water exchange occurs through three large entrances (Lido, Malamocco, and Chioggia). Two sites that are representative of the main environmental scenarios and different salinity (poly and euhaline) that occur in the lagoon were sampled. One site was near the Malamocco inlet, and was strongly influenced by the sea. The other site was on the northern side of the translagoon bridge, and was strongly influenced by the urban sewage inflow from the mainland. The Sacca di Scardovari is a large embayment (32 km^2) located between two branches of the Po River delta. The lagoon is connected to the Adriatic Sea through a wide mouth that is partly obstructed by sand banks. It varies in depth from 0.5 to 2.8 m. Its northern area receives nutrient-rich agricultural run-offs, while the southern area hosts extensive bivalve cultures (Munari et al., 2013). Twelve sites were sampled.

The Sacca di Goro is a wide (26 km^2) microtidal lagoon whose maximum depth is 2.0 m. The lagoon receives nutrient-rich freshwater, primarily from Po di Volano. The Sacca is the most important farming grounds for clam *Ruditapes philippinarum* in Italy, and for this reason it is often subjected to management interventions (Munari and Mistri, 2014). Nine sites representative of different microhabitats in the Sacca, were sampled.

The Valle di Gorino (8 km^2) is a *cul-de-sac* of the neighbouring Sacca di Goro. It has a maximum depth of 1.5 m and receives freshwater from the Po di Goro through a gate. Clams are cultured in its westernmost portion. Three sites were established along the major axis of the Valle.

The Valli di Comacchio (average depth: 1 m) are completely surrounded by earthen dikes, and are separated from the sea by the highly anthropogenically impacted, 2.5 km wide Spina spit. The Valli are connected with the Adriatic Sea by 2 marine channels, a third being permanently impounded. Freshwater inputs are derived from the Reno River and a few drainage canals. Marine and freshwater inflows are regulated by sluice gates and dams. Over the last 50 years the Valli di Comacchio have suffered anthropogenic impacts, from land reclamation (the Mezzano reclamation, executed in the

1960s, halved the surface of the Valli), to the effects of contamination of the remaining basin. From the mid-1970s to 1990, intensive aquaculture plants utilized the Valli as receiver and self-purification basin for waste waters. The lagoon is in a permanent state of hyper-eutrophication (Munari and Mistri, 2012). Four sites were sampled.

The Lesina lagoon (Apulia, southern Adriatic Sea) is one of the largest (approximately 52 km^2) lagoons in southern Italy. This shallow (maximum depth 0.8 m) basin is connected to the sea by two artificial narrow channels. Freshwater inflows are assured by seasonal streams, which are mostly located in the eastern area of the lagoon. Extensive fishing, mostly for sand smelt and eels, is practised in the lagoon. Domestic waste water from the town of Lesina discharges into its south-western waters. Four sites, reflecting a gradient of impact decreasing from west to east were sampled.

The Stagno di Tortolì is a shallow (average depth: 1.0 m) coastal pond (25 km^2) in central-eastern Sardinia (western Tyrrhenian Sea). It is connected to the sea by two channels, and receives freshwater inputs from the Rio Mannu. Bottoms are sandy-muddy, and large areas are covered with seagrasses (mainly *Z. noltii*). The Stagno hosts a flourishing finfish and shellfish traditional fishery. Six stations were sampled.

The Orbetello lagoon (southern Tuscany, eastern Tyrrhenian Sea) has a surface area of 27 km^2, and is embraced within two sandbars. A third incomplete spit, on which the town of Orbetello lies, is connected with Mount Argentario by a cause-way, which partially divides the lagoon into two basins. The western basin is linked to the sea by the shorter Nassa Channel, and to the mouth of the Albegna river by the longer Fibbia Channel. It is a shallow, non-tidal environment with weak hydrodynamics, which reduces the dilution potential of organic matter and nutrients discharged from urban areas, aquaculture facilities, and agriculture waste waters. Three stations representative of different areas in the western basin were sampled.

The Rio Padrongiano Delta (2.5 km^2) lies in the north-eastern coast of Sardinia (western Tyrrhenian Sea), and has depth of about 0.8 m. It receives marine waters from the Gulf of Olbia, and freshwater from the Rio Padrongiano, whose flow ranges from torrent-like to almost dry behaviour in summer.

It also receives waters from the adjacent Olbia harbour, the most important industrial and tourist port of Western Sardinia. The area is characterized by coarse and poorly vegetated (*Cymodocea nodosa, Z. noltii*) sediments. Padrongiano deltaic area hosts fisheries and mollusc (clams and mussels) aquaculture activities. Three stations were sampled.

At each study site the macrofauna was collected with a Van Veen grab (area: 0.027 m^2; volume: 4 l) in triplicate and sieved through 0.5 mm mesh. Taxonomic identification was carried out to the species level whenever possible. Biomass was assessed as ash-free dry weight, after drying at 80° C for 48 h in a hoven, and incineration at 450° C for 4 h in a muffle furnace.

Pressures (Table 1) were quantified (1: low, 2: medium and 3: high) for each location and sampling station, as partial pressure, total pressure and as a pressure index (PI), following an approach close to that proposed by Aubry and Elliott (2006), based upon best professional judgment. According to Borja et al. (2011) the total pressure was the sum of partial pressures, and the pressure index was calculated as an average value of the pressures.

Comparable environmental data for all 9 TWs were not available. Thus, for the purposes of this analysis we categorized the environmental information as follows: (a) 5 ranks of confinement, 1 (areas close to the sea mouth), 2 (medium confinement from sea), 3 (confined, with reduced hydrodynamism and water inflows), 4 (medium confinement from river), 5 (close to the river mouth); (b) 8 ranks of salinity (%F), where %F was defined as $\%F= [(S_{sea}-S_{site})/S_{sea}]*100$, where S_{sea} is the average salinity of the open sea and S_{site} is the average salinity of the sampling site, 1 (marine influence: %F=0-10%) 8 (river influence: %F>70%); (c) 3 ranks of percentage of organic matter in the sediment, 1 (low, %OM=0-10%), 2 (medium, %OM>10-20%), 3 (high, %OM>20-30%); (d) 7 types of sediment, 1 (coarse sand), 2 (sand), 3 (silty sand), 4 (sandy silt), 5 (clayey silt), 6 (silty clay), 7 (clay); (e) the presence/absence of seagrass, 1 (presence), 0 (absence). Table 2 summarizes the environmental data at the 46 sites.

2.2 Data treatment

Production of each species within communities was approximated using values of abundance (A) and biomass (B) by the Brey's (1990) allometric equation:

$P = (B/A)^{0.73} * A$

where B/A is the mean body size and 0.73 is the average exponent of a regression of annual production on body size for macrobenthic invertebrates (Brey, 1990).

Abundance, biomass, and productivity data were transformed using a set of transformations of increasing severity: square root, double square root, log(1+x), and presence/absence. Biotic indices were calculated using raw and transformed abundance (AMBI), biomass (BAMBI) and production (PAMBI) values (Warwick et al., 2010) using AMBI 5.0 software (freely available at http://ambi.azti.es) and the March 2012 species list. Regression between AMBI-based indices and Pressure Index (PI) was performed to analyse the agreement in the pollution classification, and significance was assessed through regression ANOVA. Community pattern was investigated by means of ordination (nMDS) based on the Bray-Curtis similarity index of untransformed abundance, biomass and production data; the proportion of the five AMBI Ecological Groups was then superimposed on each plot to show the distribution of sensitive-opportunist organisms. The relationships between AMBI and the other indices (BAMBI and PAMBI) were fitted using trend lines. The formulae for the trend lines were then used to calculate values of the various indices corresponding to AMBI values separating status categories defined by Borja et al. (2000). Agreement between classification obtained through AMBI and the other indices was determined by considering only two ecological status: “Undisturbed” and “Disturbed”. The undisturbed status was determined for each index when the derived status, in AMBI's terminology, was Undisturbed/Slightly disturbed, and scored as “1”. Disturbed status corresponded to Moderately/Heavily/Extremely disturbed, and was scored as “0”. The non-parametric Wilcoxon pairs test was used to assess agreement or disagreement between AMBI and other indices on the undisturbed and disturbed status of sites on a statistical basis. This non parametric test is particularly adapted to our data as it allowed comparing related sample classifications based on nominal data (undisturbed *vs* disturbed) and it is as powerful as the t-test (Siegel and Castellan, 1988). Finally, Spearman's rank correlation (through the BIOENV routine) was used to identify how environmental variables correlate with the suite of AMBI-based indices. The PRIMER *v.6* package (Clarke and Gorley, 2006) was used.

3. Results

A total of 268 species from 46 sites was divided among 123 families, 52 orders, 22 classes and 12 phyla (Table 3). Annelids displayed the highest number of species of the total macrofauna (108 species) followed by crustaceans (69 species) and molluscs (52 species). Most species found in all TWs are cosmopolitan (i.e. with a wide geographical distribution, such as *Polydora ciliata, Streblospio shrubsolii,* and *Hediste diversicolor*). Several endemic species were also found (such as *Corophium orientale*, *Microdeutopus algicola*, *Pectinaria koreni* and *Ampihtoe riedli*). Several non-indigenous species have been recorded with a different distribution along the examined TWs: *Paracerceis sculpta* was found in Padrongiano, *Anadara inaequivalvis, Tapes philippinarum Rhithropanopeus harrisii* and *Dyspanopeus sayii* were found in Goro (the latter species also in Comacchio), and *Arcuatula senhousia* was abundant in Goro and Padrongiano.

AMBI, calculated on raw abundance data, ranged between 1.559 (site GORM) at a marine site in the Sacca di Goro, and 5.34 (site SCA38) at a confined site in the Sacca di Scardovari. Changing the input data (biomass and production) lead the values of the indices to change. BAMBI ranged from 0.775 at a site (LES4) in the Lesina Lagoon, and 4.388 at a site (SCA33) in the Sacca di Scardovari, while PAMBI from 0.929 (again LES4) and 4.875 (again SCA33). Differences between raw and transformed AMBI, BAMBI, and PAMBI were relatively small, but in all cases the same trend in relation to the severity of the transformation was apparent: raw data transformation (square root, s; double square root, ds; logarithmic, log; presence/absence, p/a) reduced the value of the score. At site SCA33, for example, raw AMBI scored 4.993, sAMBI 4.617, dsAMBI 4.402, logAMBI 4.395, and p/aAMBI 4.2.

The nMDS ordination on abundance (a), biomass (b), and production (c) data, with the distribution of sensitive (EG-I) and first order opportunists (EG-V) is shown in Fig. 1 and 2. A gradient of stress is evident on the plots. The overall index score increased from right to left (AMBI), and from left to right (BAMBI and PAMBI), due in large measure to the contrast between the distributions of groups I and V. The proportions of species in EG-I, the most sensitive to environmental stress, were concentrated at the undisturbed (at north-east on abundance plot; at south-west on biomass and production plots) end of the configuration (Fig. 2). The proportions of species in EG-V, the most tolerant to environmental stress, were concentrated at the disturbed (south-west on abundance plot; north-east on biomass and production plots) end (Fig. 3). The concentrations of EG-II to IV (plots not shown) moved sequentially across the configurations. Figure 4 shows the concentration of EG-I-to V at each site, considering abundance (a), biomass (b), and production (c) data.

Regression between AMBI-based indices and the pressure index (PI) allowed us to assess whether the different indices displayed similar tendency in the classification of sites. Among the three categories of AMBI-based indices (abundance, biomass, production), medium transformation of data (logarithmic for AMBI, double square root for BAMBI and PAMBI) gave the highest correlation with PI. The ANOVA of all regressions was, however, highly significant (Tab. 4). In Figure 5, the relationship between PI and raw AMBI, BAMBI, PAMBI, and between PI and transformed AMBI-based indices (only the three best correlated) are shown. Each transformation had the clear effect to reduce the gap between values of the three indices.

Comparisons between AMBI and the different AMBI-based indices resulted in highly significant correlations (Table 5). We used the equations calculated from these correlations to estimate the boundaries between disturbance levels for AMBI, BAMBI and PAMBI calculated using raw and transformed data corresponding to the predefined (Borja et al., 2000) boundaries for AMBI (Table 6). Our 46 sites were then classified using these new boundaries, and the Wilcoxon test showed a good agreement between the classification obtained by AMBI calculated from raw data and all other AMBI-based indices (Table 6).

In order to investigate how the different AMBI-based indices correlate to different environmental variables, the BIO-ENV routine was applied. Significant Spearman's rank correlations with combinations of environmental variables were found (Table 7). All the best-matching combinations of variables included the presence of seagrass, the amount of organic matter in the sediment, and the type of sediment. Increasing the severity of transformation generally resulted in a decrease of the correlation, except for logBAMBI and logPAMBI whose *rho* were almost similar to those exhibited by BAMBI and PAMBI.

4. Discussion

We used macrobenthic data from 46 sites from 9 different Italian TWs to assess the response of the AMBI index (Borja et al., 2000) to different input data (abundance, biomass and production), and different severity of transformation of the data. Despite large variations in the form and nature of the input data, all variations of AMBI were correlated. Regardless of whether units of species abundance, biomass or production were used in the calculation of AMBI, increasing the severity of transformation generally resulted in a down-weighting of the importance of the dominant taxa, but also in a decrease of the correlation with environmental factors. AMBI bases its functioning on dividing benthic species into previously defined ecological groups (EG-I to EG-V), and then determining the respective proportion of the different groups in the benthic community. AMBI calculation rely on the relative decrease of sensitive species (EG-I) confronted with increasing disturbance in the sediment or, conversely, the increase of species that are resistant or indifferent to disturbance (EG-II and EG-III), or that are even encouraged by such conditions like the opportunist species (EG-IV and EG-V) that proliferate when the sediment is rich in organic matter. In our 46 study sites, a clear gradient of impact was evidenced by the multivariate analysis, reflected by the relative abundance of ecological groups, with EG-I dominant at Tortolì, Lesina and the marine site in Venice Lagoon, and EG-V dominating the benthic communities at Scardovari, Goro, and Comacchio. The different distribution of EG-I to EG-V organisms in our 46 sites was obviously reflected by AMBI scores. The usefulness of AMBI in detecting anthropogenic impact gradients has been demonstrated in many coastal areas all over the world. However, some authors (e.g. Ponti et al., 2009) pointed out that AMBI, considering the abundances of stress-tolerant species to detect anthropogenic impacts, does not take into account the fact that tolerant species may also be tolerant of natural stressors. Dauvin and Ruellet (2009) called it the "estuarine quality paradox". Transitional waters (estuaries and lagoons) constitute naturally stressed, highly variable ecosystems that are also exposed to high levels of anthropogenic stress (Elliott and Quintino, 2007). The latter authors stated that: "The dominant estuarine faunal and floral community is adapted to and reflects the high spatial and temporal variability of highly naturally-stressed areas. However, this community has features very similar to those found in anthropogenically-stressed areas, thus making it difficult to detect anthropogenically-induced stress in estuaries". Other difficulties in detecting anthropogenically-induced stress for such transitional waters are related to the high level of heterogeneity of habitats in lagoonal and estuarine ecosystem: salinity for example changes gradually along the freshwater/estuarine/coastal continuum.

Recently, Warwick et al. (2010) suggested to manipulate AMBI using biomass and production data instead of abundance data. Until now, only two other studies have considered the possibility of using biomass instead of abundance to calculate AMBI. Muxika et al. (2012), using data from the Basque coast and estuaries (northern Spain), found that biomass-based AMBI gave the same results of the abundance-based index. Cai et al. (2014), using data from Bohai Bay (west of the Bohai Sea, north of China), found that the ecological status as assessed using density and biomass was quite similar. Using biomass or production to detect impacts in aquatic systems has great sense. Production is the most common measure of ecosystem function; however, given the difficulty of its assessment, standing biomass is often used as a proxy measure (terHorst and Munguia, 2008). Since biomass and production are more ecologically relevant than abundance, their use to derive AMBI-based indices is intriguing, especially in transitional waters where the community is "naturally disturbed" and dominated by EG-III to EG-V species. In the Sacca di Goro, for example, except for the marine site (GORM), whose community is numerically dominated by EG-II species, EG-IV and EG-V taxa are very abundant at all the other sites, and coexist with EG-III, and even with EG-II and EG-I species. The lagoon is undoubtly disturbed (Munari and Mistri, 2014), however it is difficult to determine how much disturbance is due to anthropogenic causes (e.g. the discharge of nutrient rich waters from surrounding agricultural fields), and how much to natural causes (e.g. the reduced hydrodynamism due to the morphodynamics of the sea mouth). In their study on the Basque Coast, Muxika et el. (2012) found that the distributions of ecological groups' dominances were very similar when biomass was used instead of abundance. Conversely, in our lagoonal data set, the proportion of ecological groups into the community varied greatly if we considered abundance or biomass-based data. In certain cases the use of biomass (or production) instead of abundance even resulted in a different classification of the ecological status. For example, the site COM4 in the Valli di Comacchio (an Adriatic lagoon) was classified "Moderately polluted" by AMBI, but "Slightly polluted" by BAMBI and PAMBI; in the Stagno di Tortolì (a Tyrrhenian lagoon), sites TRT1, TRT2 and TRT4, changed from "Slightly polluted" through AMBI to "Unpolluted" through BAMBI and PAMBI. Emblematic was the case of site PDGC, at Rio Padrongiano (a Tyrrhenian estuary), that was classified "Moderately polluted" by AMBI, "Unpolluted" by BAMBI (with a jump of even two quality classes), and "Slightly polluted" by PAMBI. At this site, considering abundance, the benthic community was numerically dominated by EG-IV and EG-V (23.2 and 28.4%, respectively), followed by EG-II (17.7%), EG-I (15.8%), and EG-III (14.9%). Numerically dominant species were *Schistomeringos rudolphii* (10.2% of the whole community abundance), *Grubeosyllis tenuicirrata* (7.7%), *Capitomastus minimus* (5.6%), and *Microdeutopus obtusatus* (5.1%). These figures changed when we considered biomass, since *S. rudolphii* constituted 0.7% of the total biomass, *G. tenuicirrata* 0.03%, *C. minimus* 0.14%, and *M. obtusatus* 0.4%. Conversely, *Tapes decussatus* constituted 63.9% of community biomass, but only 1.2% of community abundance. As a matter of fact, at site PDGC, considering biomass EG-I constituted 65.3% of the community, EG-II 5.8%, EG-III 23.9%, EG-IV 4.6% and EG-V 0.4%. In transitional ecosystems, the unit of measurement adopted (abundance or biomass) can lead to important changes in results just because of the coexistence of many species spanning all ecological groups: depending on the adopted unit, their relative contribution can vary greatly.

A transformation of intermediate severity gives the best compromise between the scenarios of a community structured on abundances of few dominant taxa, and a community influenced by the occurrence of the rarest taxa (Clarke and Warwick, 1994). The set of transformation chosen (none, square root, double square root, logarithmic, presence/absence) corresponds to a progressive down-weighting of the common species. Because of the down-weighting of the relative abundance or biomass of dominant taxa, the transformation of the data led to lower index values. At PDGC, for example, the set of transformations applied lead the value of AMBI to change from 3.46 (none), to 2.83 (square root), 2.49 (double square root), 2.48 (logarithmic) and 2.18 (presence/absence). Consequently also the new boundaries between disturbance levels (Table 6) were systematically lower than the boundaries for AMBI. Using those new boundaries to classify sites on nominal data (undisturbed *vs* disturbed) through each of the AMBI-based indices, a good level of agreement was found between them. Severe transformation of the data, culminating in presence/absence, degraded the relationship with disturbance, expressed by the pressure index. This finding suggests, however, that if only simple species lists are available, these may still be used in making an environmental assessment through AMBI. All AMBI variations responded basically to the same environmental factors: seagrass presence, sediment organic matter and type of sediment (Table 7). Seagrass presence is related to anthropogenic disturbance: it is well known the role of man-induced eutrophication on the regression and even disappearance of seagrass prairies in transitional ecosystems (Boudouresque et al., 2009). Type of sediment is related to the morphology of the lagoon, with sandy bottom where hydrodynamism is high and mud where it is low. Sediment organic matter depends both on hydrodynamism and eutrophication. Again, it is difficult to ascertain if, in transitional ecosystems, community abundance and/or biomass (and thus AMBI-based indices) respond to anthropogenic or natural stress. These results suggest that, at least at our 9 TWs, both contribute to determining the structure and composition of benthic assemblages.

5. Conclusions

This study suggests that, in transitional waters, it is actually difficult to say if it is better to calculate AMBI from abundance, biomass or production data. In fact, good agreement was found between the response of all AMBI-based indices and disturbance (expressed by the severity of pressures) at the 46 study sites. If, on the one hand, biomass and production data are ecologically and functionally much more relevant than abundance data, on the other, production is, inevitably, calculated in a very empirical manner, with the real risk of introducing large errors in the estimate. However, since the benthic communities of transitional ecosystems are naturally stressed and therefore dominated by tolerant and opportunist species, a biomass-based index could downscale the effects of over abundant small-bodied organisms. More work along gradients of stronger disturbance is needed to ascertain the effective usefulness of a biomass-based AMBI in transitional ecosystems. However, the difficulty of distinguishing the weight of natural to human disturbance in these particular systems remains, and makes it difficult to define gradients of purely human-induced disturbance.

Acknowledgements

We wish to thank an anonymous reviewer for constructive criticism.

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Figure Legend

Fig. 1. Study sites location.

Fig. 2. Ordination plots of a) abundance, b) biomass, c) production data with concentration of sensitive (EG-I) species (VEN: Venice; SCA: Scardovari; GOR: Goro; GRN: Gorino; COM: Comacchio; LES: Lesina; ORB: Orbetello; TRT: Tortolì; PDG: Padrongiano).

Fig. 3. Ordination plots of a) abundance, b) biomass, c) production data with concentration of tolerant (EG-V) species (acronyms as in Fig. 2).

Fig. 4. Concentration of EG-I-to V at each sampling site, for AMBI, BAMBI and PAMBI (acronyms as in Fig. 2).

Fig. 5. Relationship between Pressure Index and untransformed AMBI, BAMBI, PAMBI, and logAMBI, dsBAMBI and dsPAMBI.

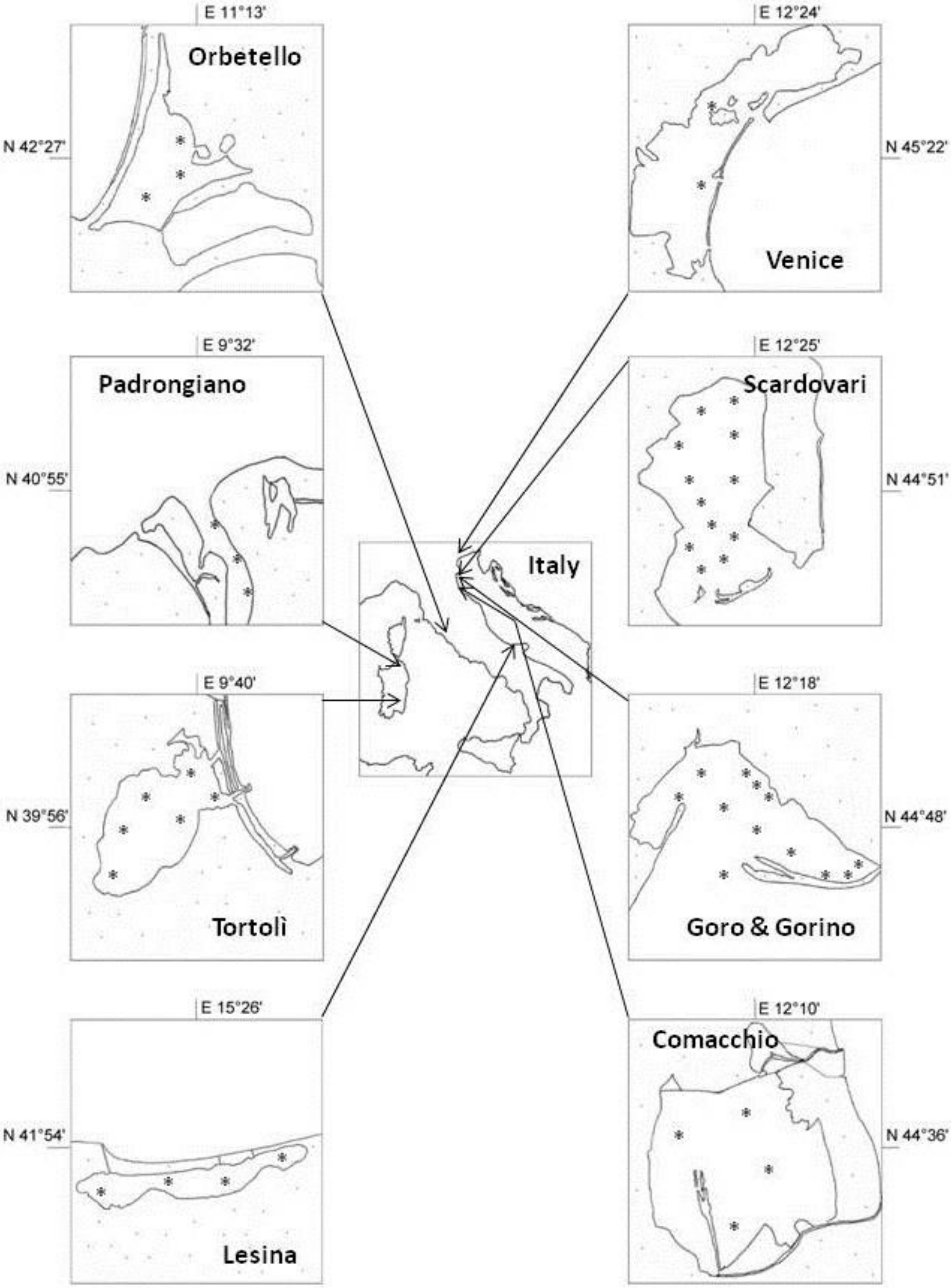
E 11°13'
Orbetello
N 42°27'
E 12°24'
N 45°22'
Venice
E 9°32'
Padrongiano
N 40°55'
E 12°25'
Scardovari
N 44°51'
Italy
E 9°40'
N 39°56'
Tortolì
E 12°18'
N 44°48'
Goro & Gorino
E 15°26'
N 41°54'
Lesina
E 12°10'
Comacchio
N 44°36'

Fig. 1

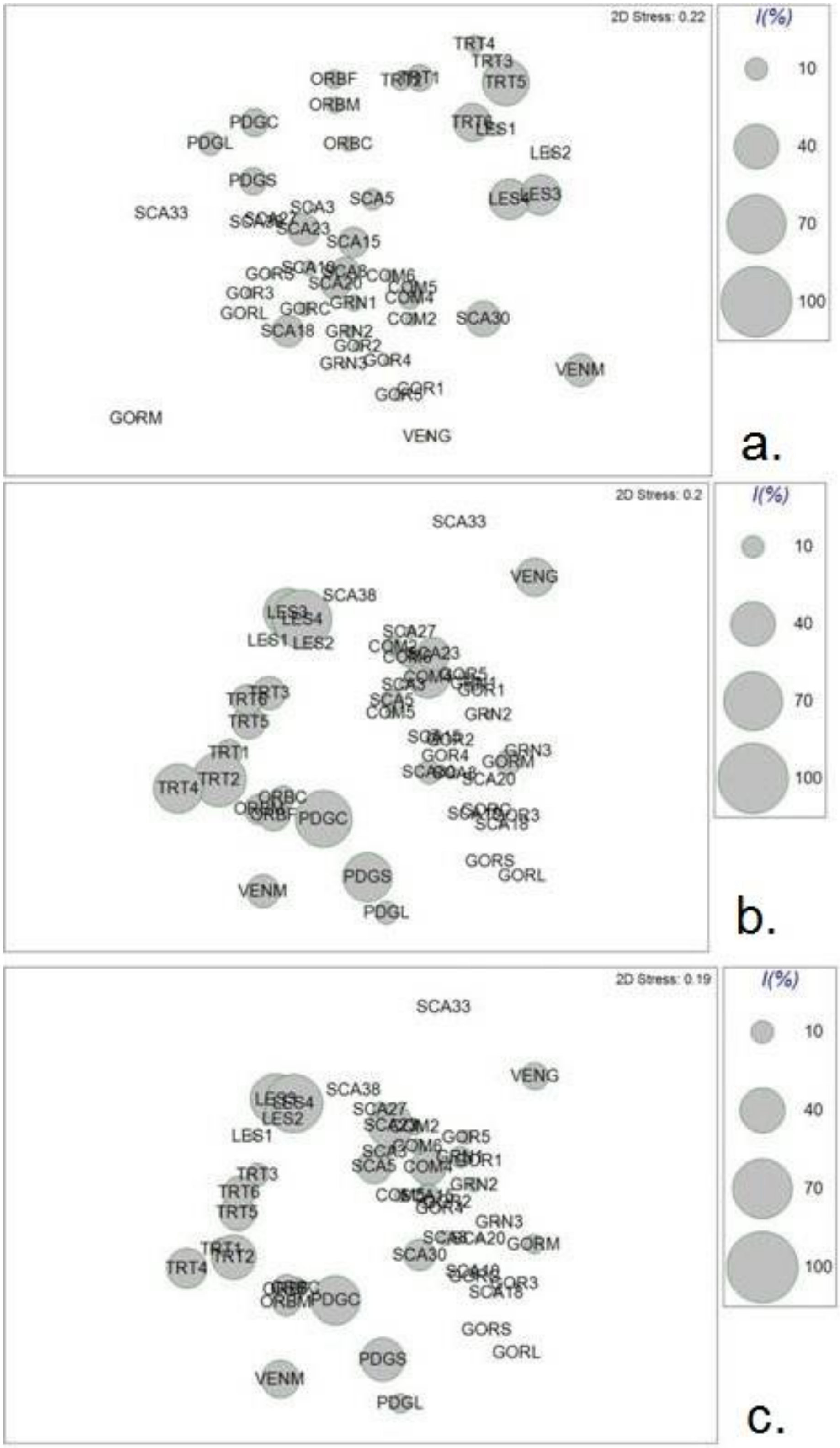
2D Stress: 0.22
I(%)
10
40
70
100
a.
2D Stress: 0.2
I(%)
10
40
70
100
b.
2D Stress: 0.19
I(%)
10
40
70
100
c.
SCA33
VENG
SCA38
VENM
PDGS
PDGL
GORS
GORL
GORM
TRT4

Fig. 2

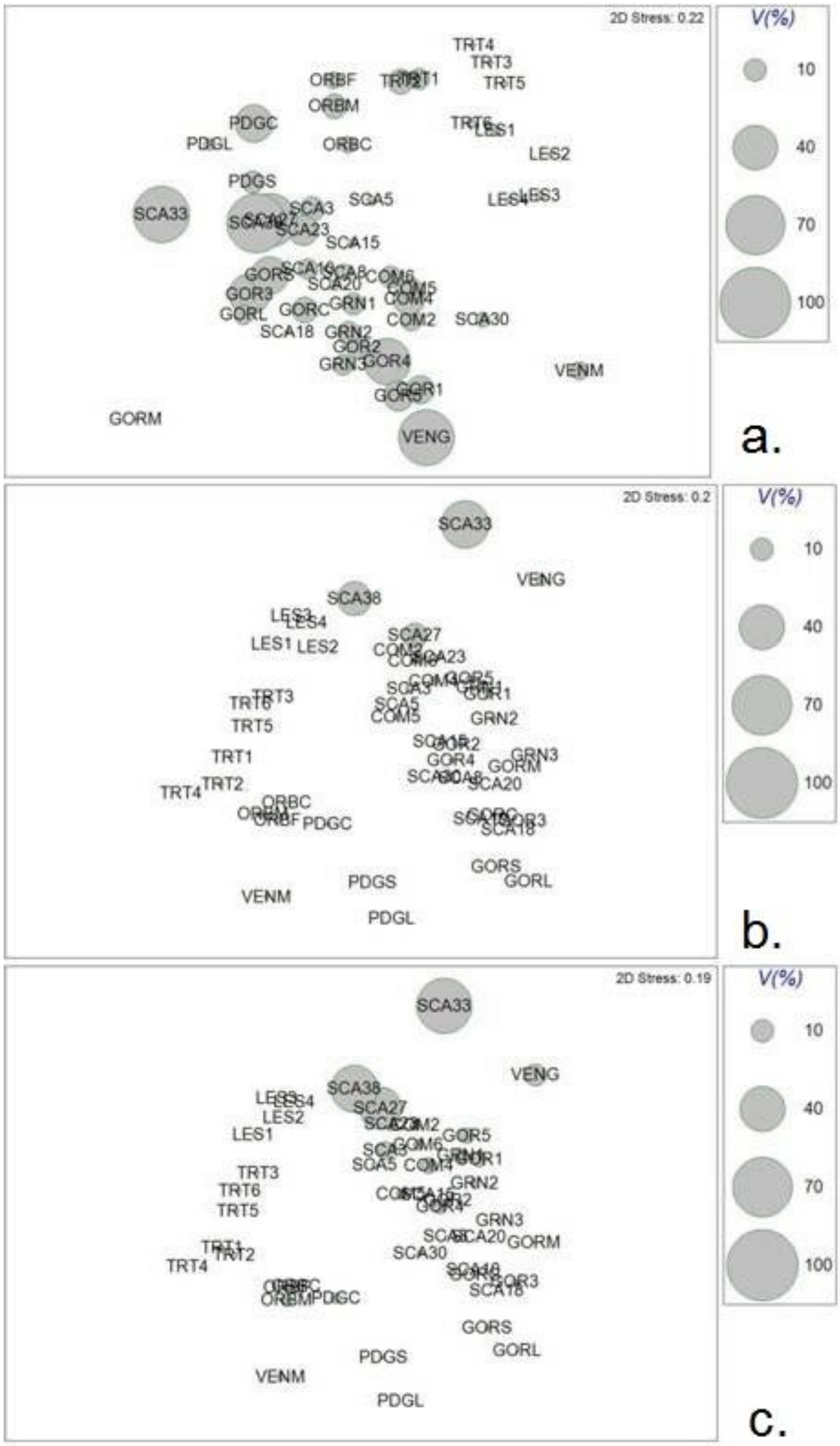

Fig. 3

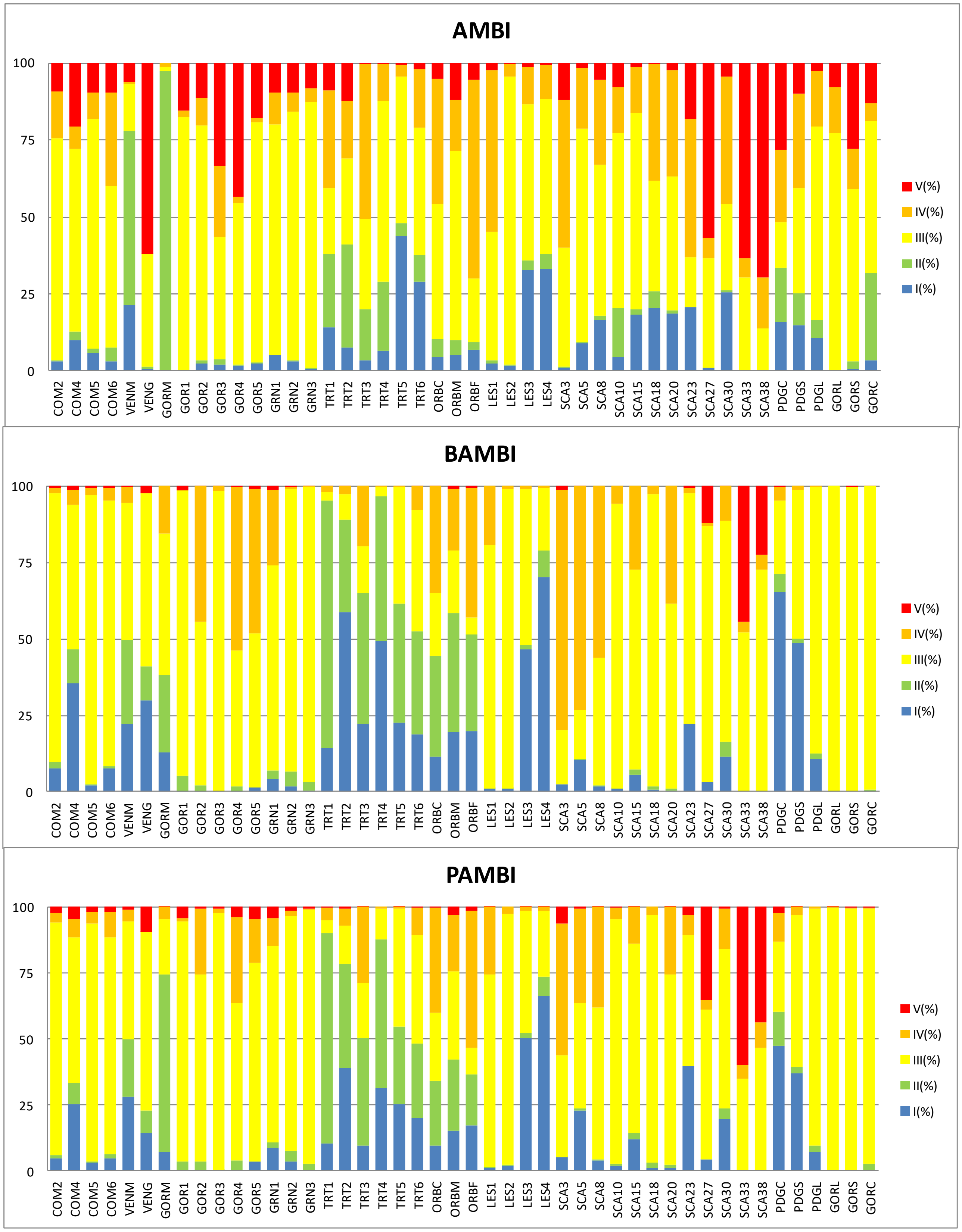
AMBI
BAMBI
PAMBI
V(%)
IV(%)
III(%)
II(%)
I(%)
100
75
50
25
0
COM2
COM4
COM5
COM6
VENM
VENG
GORM
GOR1
GOR2
GOR3
GOR4
GOR5
GRN1
GRN2
GRN3
TRT1
TRT2
TRT3
TRT4
TRT5
TRT6
ORBC
ORBM
ORBF
LES1
LES2
LES3
LES4
SCA3
SCA5
SCA8
SCA10
SCA15
SCA18
SCA20
SCA23
SCA27
SCA30
SCA33
SCA38
PDGC
PDGS
PDGL
GORL
GORS
GORC

Fig. 4

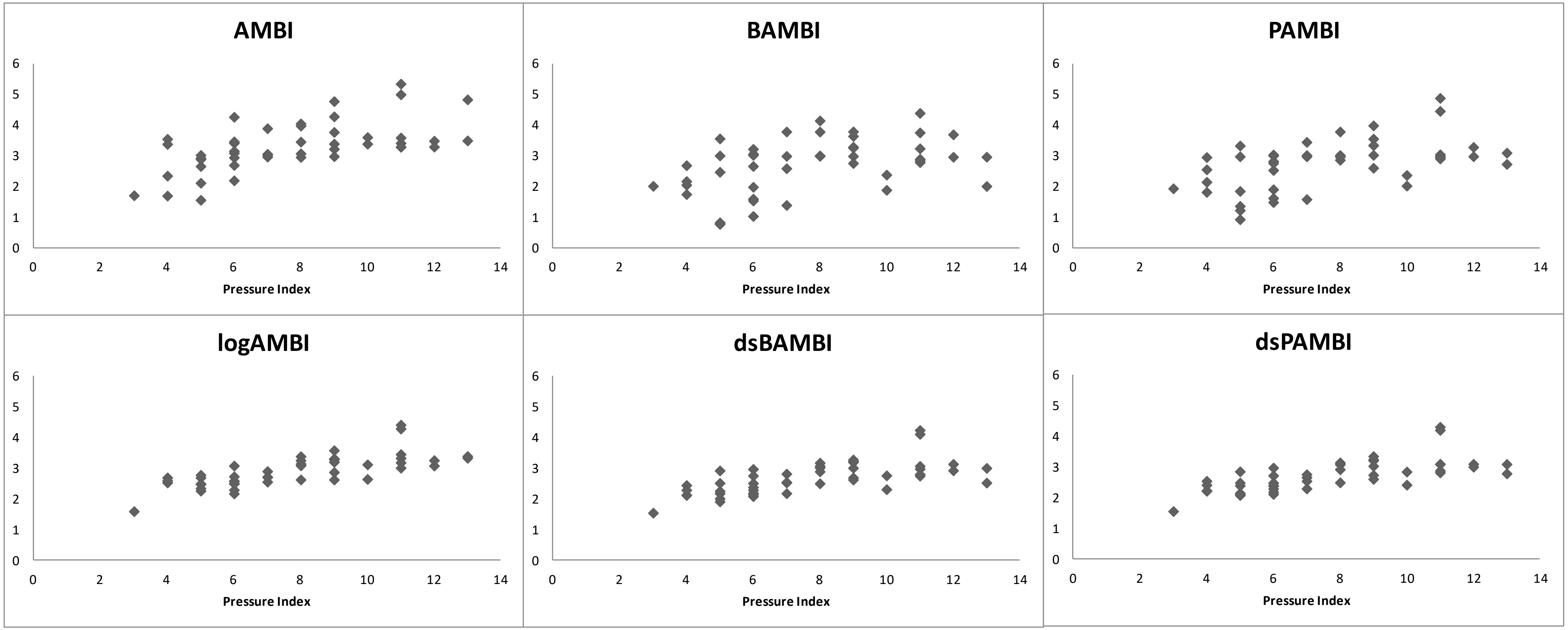
AMBI
BAMBI
PAMBI
logAMBI
dsBAMBI
dsPAMBI
Pressure Index
0 1 2 3 4 5 6
0 2 4 6 8 10 12 14

Fig. 5

Table 1.
Pressures at the various sampling sites.

Site	Non-point pollution sources		Point pollution sources			Habitat loss	Ports			Fisheries		PI
	Agricultural inputs	Freshwater inputs	Domestic	Agricultural	Industrial	Land-claim	Port activity	Navigation	Dredging	Fin-fisheries	Shell-fisheries	
COM2	2	1		2		3				3		11
COM4	2			2		3				3		10
COM5	2	1				3				3		9
COM6	3			1		3			1	3		11
LES1	2		2	1	1					3		9
LES2	2	1		1						3		7
LES3	1	1		1						3		6
LES4	1	1								3		5
ORBC	2	1								1		4
ORBM	2	1								1		4
ORBF	2	1	1	1				1		1		7
TRT1	2	1	1	1						1	1	7
TRT2	2	1								1	1	5
TRT3	2	1		1						1	1	6
TRT4	1	1								1	2	5
TRT5	1	1								1	1	4
TRT6	1	1								1	1	4
VENM	1							1		1		3
VENG	2		2	2	1		1	2		1	1	12
SCA3	2	1		1						1	3	8
SCA5	2			1						2	3	8
SCA8	2	1								2	2	7
SCA10	2	1								1	2	6
SCA15	2	1								1	2	6
SCA18	2									1	2	5
SCA20	2									1	2	5
SCA23	2	2		1						2	3	10
SCA27	2	2		1						2	2	9
SCA30	2	1		1						2	3	9
SCA33	3	2		2						2	2	11

SCA38	3	2		2						2	2	11
GRN1	2	2	1	2						1	3	11
GRN2	2	2	1	2						1	3	11
GRN3	2	3	1	3						1	2	12
GOR1	3	3	1	1	1					1	3	13
GOR2	2	1	1	1				1		1	2	9
GOR3	1	1						1			3	6
GOR4	2	2						1		1	3	9
GOR5	3	3	1	1						1	3	12
GORM	1							1	2		1	5
GORL	2	1	1	1						1	2	8
GORS	2	1	1	1						1	2	8
GORC	2	1	1	1						1	2	8
PDGC	1	3	1								1	6
PDGS	1	3	1								1	6
PDGL	1	3	1								1	6

VEN: Venice; SCA: Scardovari; GOR: Goro; GRN: Gorino; COM: Comacchio; LES: Lesina; ORB: Orbetello; TRT: Tortolì; PDG: Padrongiano

Table 2.
Environmental data at the 46 sites.

Typology	Station	%F	Confinement	%OM	Seagrass	Sediment type
non-tidal	COM2	0-10%	confined-reduced hydrodynamism	>20-30%	no	clay
	COM4	0-10%	medium confinement from sea	>20-30%	no	silty clay
	COM5	0-10%	medium confinement from river	>10-20%	no	silty clay
	COM6	0-10%	confined-reduced hydrodynamism	>20-30%	no	clay
	LES1	>50-60%	confined-reduced hydrodynamism	>10-20%	no	clay
	LES2	>40-50%	medium confinement from sea	>20-30%	no	silty clay
	LES3	>60-70%	river mouth	>20-30%	yes	clayey silt
	LES4	>60-70%	river mouth	>10-20%	yes	clayey silt
	ORBC	0-10%	confined-reduced hydrodynamism	>20-30%	no	silty sand
	ORBM	0-10%	medium confinement from sea	>20-30%	no	silty sand
	ORBF	0-10%	medium confinement from sea	>10-20%	no	silty sand
	TRT1	0-10%	medium confinement from sea	>20-30%	yes	sandy silt
	TRT2	0-10%	medium confinement from sea	0-10%	no	sandy silt
	TRT3	>10-20%	confined-reduced hydrodynamism	>20-30%	no	silty clay
	TRT4	0-10%	medium confinement from sea	>20-30%	yes	sandy silt
	TRT5	0-10%	medium confinement from river	>20-30%	yes	sandy silt
	TRT6	0-10%	medium confinement from river	>20-30%	no	sandy silt
microtidal	VENM	>10-20%	sea mouth	>10-20%	yes	silty sand
	VENG	>40-50%	confined-reduced hydrodynamism	>20-30%	no	clay
	SCA3	>10-20%	confined-reduced hydrodynamism	>20-30%	no	silty clay
	SCA5	0-10%	confined-reduced hydrodynamism	>20-30%	no	silty clay
	SCA8	0-10%	medium confinement from sea	>10-20%	no	silty sand
	SCA10	0-10%	sea mouth	0-10%	no	sand
	SCA15	0-10%	medium confinement from sea	>10-20%	no	silty sand
	SCA18	0-10%	sea mouth	0-10%	no	sand
	SCA20	0-10%	sea mouth	>10-20%	no	sandy silt
	SCA23	>10-20%	confined-reduced hydrodynamism	>20-30%	no	silty clay
	SCA27	>10-20%	confined-reduced hydrodynamism	>20-30%	no	silty clay
	SCA30	>10-20%	confined-reduced hydrodynamism	>10-20%	no	silty clay
	SCA33	>10-20%	confined-reduced hydrodynamism	>20-30%	no	silty clay
	SCA38	>10-20%	confined-reduced hydrodynamism	>20-30%	no	silty clay
	GRN1	>30-40%	medium confinement from river	>20-30%	no	silty sand
	GRN2	>40-50%	medium confinement from river	>20-30%	no	silty clay
	GRN3	>50-60%	medium confinement from river	>20-30%	no	silty clay
	GOR1	>70%	medium confinement from river	>20-30%	no	silty clay
	GOR2	>30-40%	medium confinement from sea	>10-20%	no	silty clay
	GOR3	>20-30%	sea mouth	0-10%	no	sand
	GOR4	>30-40%	medium confinement from river	>10-20%	no	clayey silt
	GOR5	>70%	river mouth	>20-30%	no	silty clay
	GORM	>20-30%	sea mouth	0-10%	no	sand
	GORL	>20-30%	confined-reduced hydrodynamism	>20-30%	no	sand
	GORS	>20-30%	confined-reduced hydrodynamism	>20-30%	no	sand
	GORC	>20-30%	confined-reduced hydrodynamism	0-10%	no	sand
	PDGC	>10-20%	medium confinement from sea	0-10%	no	coarse sand
	PDGS	>10-20%	medium confinement from sea	>20-30%	no	coarse sand
	PDGL	>10-20%	medium confinement from sea	>20-30%	no	coarse sand

Table 3
Taxonomic composition of benthic communities at each TW.

Lagoon	Total taxa	Mollusca	Annelida	Arthropoda	Cnidaria	Others
Venice	85	18	30	24	3	10
Scardovari	67	18	19	26	1	3
Goro	55	16	17	19	1	2
Gorino	19	5	5	8	1	0
Comacchio	43	3	16	15	3	6
Lesina	46	6	14	18	2	6
Orbetello	45	10	22	9	2	2
Tortolì	69	10	36	11	4	8
Padrongiano	116	17	62	25	1	11

Others: Turbellaria, Nemertea, Sipuncula, Tunicata.

Table 4
Regression ANOVA for the relationship between AMBI-based indices and Pressure Index. Key: s, square root; ds, double square root; log, logartithm; p/a, presence/absence.

	r	df	F	p
AMBI	0.602	1, 44	25.072	<0.0001
sAMBI	0.724		48.555	<0.0001
dsAMBI	0.724		48.423	<0.0001
logAMBI	0.736		51.922	<0.0001
p/aAMBI	0.61		26.110	<0.0001
BAMBI	0.446		10.910	0.0019
sBAMBI	0.557		19.826	0.0001
dsBAMBI	0.652		32.474	<0.0001
logBAMBI	0.53		17.186	0.0002
p/aBAMBI	0.61		26.110	<0.0001
PAMBI	0.513		15.739	0.0003
sPAMBI	0.632		29.320	<0.0001
dsPAMBI	0.682		38.290	<0.0001
logPAMBI	0.63		28.967	<0.0001
p/aPAMBI	0.61		26.110	<0.0001

Table 5.
Correlation and significance between AMBI and the other AMBI-based indices.

	r	df	F	P
sAMBI	0.941	1, 44	339.1	<0.001
dsAMBI	0.841		106.5	<0.001
logAMBI	0.804		80.5	<0.001
p/aAMBI	0.688		39.6	<0.001
BAMBI	0.485	1, 44	13.6	<0.001
sBAMBI	0.647		31.7	<0.001
dsBAMBI	0.751		56.9	<0.001
logBAMBI	0.649		32.1	<0.001
p/aBAMBI	0.688		39.6	<0.001
PAMBI	0.721	1, 44	47.8	<0.001
sPAMBI	0.793		74.9	<0.001
dsPAMBI	0.797		76.8	<0.001
logPAMBI	0.805		81.2	<0.001
p/aPAMBI	0.688		39.6	<0.001

Table 6.
Boundaries for undisturbed/slightly disturbed (1.2), slightly/moderately disturbed (3.3), moderately/heavily disturbed (5.0) and heavily/extremely disturbed (6.0).
Significancy of Wilcoxon test between AMBI and each other AMBI-based index is also shown.

	Boundaries				Wilcoxon test	
	1.2	3.3	5.0	6.0	Z	p-level
sAMBI	1.57	3.10	4.33	5.06	0.0	1.0
dsAMBI	1.75	2.90	3.83	4.38	0.561	0.575
logAMBI	1.80	2.88	3.75	4.27	0.296	0.767
p/aAMBI	1.82	2.66	3.35	3.75	0.0	1.0
BAMBI	1.54	2.68	3.60	4.14	0.784	0.433
sBAMBI	1.55	2.65	3.55	4.07	0.956	0.339
dsBAMBI	1.64	2.66	3.49	3.97	0.227	0.820
logBAMBI	1.46	2.63	3.57	4.13	0.956	0.339
p/aBAMBI	1.82	2.66	3.35	3.75	0.245	0.807
PAMBI	1.22	2.75	3.98	4.71	1.408	0.159
sPAMBI	1.48	2.74	3.76	4.35	0.213	0.831
dsPAMBI	1.65	2.72	3.58	4.09	0.245	0.807
logPAMBI	1.42	2.76	3.85	4.49	0.213	0.831
p/aPAMBI	1.82	2.66	3.35	3.75	0.471	0.638

Table 7.
Results of BIO-ENV analysis. Variables (Vars): 1, salinity; 2, confinement; 3, organic matter; 4, seagrass; 5, type of sediment.

	Rho	Significance level	Vars
AMBI	0.307	0.4%	4
sAMBI	0.225	1.3%	4, 5
dsAMBI	0.156	12.4%	1, 4, 5
logAMBI	0.157	12.6%	1, 4, 5
p/aAMBI	0.05	68.5%	1, 4, 5
BAMBI	0.372	0.1%	3, 4
sBAMBI	0.358	0.1%	4, 5
dsBAMBI	0.23	0.9%	4, 5
logBAMBI	0.369	0.1%	4, 5
p/aBAMBI	0.05	68.5%	1, 4, 5
PAMBI	0.424	0.1%	3, 4
sPAMBI	0.39	0.2%	4, 5
dsPAMBI	0.207	2.9%	4, 5
logPAMBI	0.384	0.1%	4, 5
p/aPAMBI	0.05	68.5%	1, 4, 5