The difficulty of disentangling natural from anthropogenic forcing factors makes the evaluation of ecological quality problematic: a case study from Adriatic lagoons

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Abstract

The complex and dynamic nature of transitional ecosystems pose problems for the assessment of the Ecological Quality Status required by the European Water Framework Directive (WFD; 2000/60/EC). In six Adriatic lagoons, Ecological Quality Status was studied by comparing a biotic index based on macrophytes (MaQI), and three indices based on invertebrates (M-AMBI, M-bAMBI, and ISD). Ecological Status evaluated though MaQI and ISD resulted in quite degraded ecosystems (moderate/poor/bad), with only opportunistic algae and macrobenthic communities dominated by small size classes. Those results were supported by physico-chemical parameters, indicating high nutrients inputs, and anthropogenic pressures related with agriculture and fishery activities. Ecological Status obtained with M-AMBI and M-bAMBI was higher, with some sites reaching even the "good" status. The best response to anthropogenic pressures, in terms of a pressure index, was obtained by M-AMBI and M-bAMBI. Nevertheless, the response of used metrics (such as AMBI and bAMBI) to environmental variables not related to anthropogenic impact, and the high heterogeneity of physical—chemical conditions within lagoons, represent potential problems for the correct evaluation of Ecological Status of transitional waters. When different metrics give different responses it becomes a problem for managers who cannot easily make a decision on the remedial measures. The disagreement among indices arose because of the different response of biological elements to different stressors, and because the different indices based on macroinvertebrates focused on different aspects of the community, providing complementary information. So urge the need to find alternative approaches for a correct assessment of Ecological Status, with the combination of different biological elements, and considering the development of new indices (e.g. M-bAMBI) or refinement of the existing ones.

1. Introduction

The Water Framework Directive (WFD; 2000/60/EC) establishes a framework for the protection of European waters, including transitional waters (estuaries and lagoons). This directive invited the scientific community to undertake specific studies for the assessment of the Ecological Quality Status (EcoQ) of transitional waters (TWs). The legislation requires the quality to be defined in an integrative way using several biological elements, supported by hydro-morphological and physico-chemical quality parameters (Prato et al., 2014). In Italy, the WFD was implemented through the National Act 260/10, which indicates official methods for EcoQ assessment in TWs only for ‘‘macrophytes’’ (MaQI index: Sfriso et al., 2009; 2014a), and ‘‘macrozoobenthos’’ (M-AMBI index, Muxika et al., 2007; BITS index: Mistri and Munari, 2008). Phytoplankton and fish fauna were also indicated by the WFD as biological quality elements (BQEs) but methods to assess EcoQ have not been finalized yet, especially for fish fauna (Prato et al., 2014). For the phytoplankton the MPI - Multimetric Phytoplankton Index, is proposed by the Mediterranean Geographical Intercalibration Group (Mediterranean GIG), to assess the quality of transitional waters according to the composition of phytoplankton populations (Facca et al., 2014)

Despite the plethora of methods to assess the EcoQ developed to date in Europe, studies on the response of assessment indices to human pressures are more scarce, and limited to few types of pressures, such as eutrophication, and other sources of pollution (Borja et al., 2013; Hering et al., 2013). As results of a recent project (WISER project, Borja et al., 2013; Hering et al., 2013) authors evaluated the need to develop new assessment methods, taking into account the lack of methods for some BQEs (e.g. macrophytes) and some ecotypes (e.g. hard substratum, lagoons, etc.). Particular attention should be given to TWs, where the assessment of EcoQ is challenging, due to the complex and dynamic nature of the ecosystem. TWs are the interfaces between the terrestrial and freshwater environments and the sea. It completely fit, as ecosystems, with the concept of ecotone, originally proposed to define ecosystem boundaries rather than true ecosystems (Basset et al., 2013). Coastal lagoons are subjected to natural disturbance which depends mainly on morphodynamics and on climatic factors, such as freshwater flooding and summer drought (Viaroli et al., 2008; Elliott and Whitfield, 2011). First attempts to show the fundamental properties of transitional waters included the Remane diagram (Remane, 1934), a conceptual model designed to show species diversity distribution along a salinity continuum and displays the numbers of species with different salinity tolerances (freshwater, brackish and marine) which comprise the communities across that continuum. Recent papers reviewed and adapted this model in order to suite to estuaries worldwide (Whitfield et al., 2012; Basset et al., 2013). The pressures on the biota and therefore characteristic and diversity of benthic communities, are shaped by the relative influence of the tides and river inputs, determining changes in salinity, nutrients and sediment transport and turbidity (Elliott and Whitfield, 2011). In TWs the effects of anthropogenic pressures are similar to those of natural variability, as expressed with the so called “Estuarine Quality Paradox” (Elliott and Quintino, 2007) making EcoQ assessment problematic and the inconsistencies between the responses of different indicators most pronounced (Borja et al., 2011). Even if a number of different indices have been developed (in particular based on macrobenthic invertebrates), to date few studies have evaluated the equivalency of EcoQ levels obtained through different BQEs for TWs (e.g. Borja et al., 2004; Curiel et al., 2012; García-Sánchez et al., 2012; Christia et al., 2014; Prato et al., 2014; Beiras, 2016).

In the present work the EcoQ of six lagoons, part of a transitional system (Po Delta, Northern Adriatic) heavily impacted by agricultural and fishery activities, have been evaluated through two BQEs (macrophytes and macroinvertebrates) required by WFD, using different indices. The aim was fourfold: (i) compare EcoQ based on the two BQEs: macrophytes and macrozoobenthos represented by MaQI and M-AMBI, respectively; (ii) compare EcoQ based on benthic invertebrates using M-AMBI index with other recently developed indices not required by national law (M-bAMBI and ISD); (iii) compare the response of each method to anthropogenic pressures estimated as a pressure index (PI), and other environmental factors that could affect indices; (iv) for a better understanding of the differences among biotic indices, the response of single metrics (EGs, size classes, AMBI, bAMBI, H, H_b, and S) to anthropogenic and environmental factors was checked.

2. Materials and Methods

2.1 Study area

The Po Delta is a heterogeneous and dynamic complex of lagoons and ponds originating from the deposition of sediment transported by the Po River. It has high social-economic value arising from fishing, tourism and agriculture. The huge nutrient load entering from the river drainage basin, together with human activities related to agriculture, aquaculture and urban development within the Po Delta, have significantly affected the environmental equilibrium of the transitional area (Simeoni and Corbau, 2009).

The six lagoons considered in this study (Caleri, Marinetta, Vallona, Barbamarco, Canarin, and Scardovari, Figure 1) show a range of geomorphological and environmental features, depending on river inflows, seawater exchanges, and depth (Sfriso et al., 2014b). Depth can range from 0.5 to 1m or from 1.5 to 2.5m depending on the basin. Water column parameters, such as temperature and salinity depends on the season and the rate of freshwater and marine inputs.

Sediment parameters were analysed from 2008 to 2009. Sediment samples were collected with corers and retained for grain size and nutrient analyses (total carbon TC, total phosphorus TP, total nitrogen TN) according to the procedures reported in Sfriso and Marcomini (1996). Water column parameters (temperature T, salinity, oxygen saturation %DO, and pH) for the sampling periods (2008 to 2010) were obtained from the archive of the Regional Environmental Protection Agency of Veneto (ARPAV, 2008-2010).

A total of 37 sites were analysed, 20 for macrobenthic invertebrates (4 in Caleri, 4 in Marinetta, 2 in Vallona, 2 in Barbamarco, 3 in Canarin, and 5 in Scardovari) and 17 for macrophytes (3 in Caleri, 2 in Marinetta, 2 in Vallona, 3 in Barbamarco, 3 in Canarin, and 4 in Scardovari). Sampling sites were chosen to be representative of main hydrological conditions of each lagoon. Sampling of both macrophytes and invertebrates were performed during 2008, 2009, and 2010.

Pressures were scored (1: low, 2: moderate , 3: high) for each sampling station, as partial pressure, following an approach close to that proposed by Aubry and Elliott (2006) and Borja et al. (2011) based upon best professional judgment. A pressure index (PI) was calculated as the sum of partial pressures for each station (Table 1), and used as anthropogenic stressor to validate the results obtained with the different biotic indices.

2.2 Macrophyte sampling

Total macrophyte cover of each sampling site was assessed by touching 20 times the soft bottom by a rake in order to detect the presence/absence of macroalgae. Results are reported as a percentage of the cover according to the monitoring protocols by ISPRA, 2011. One touch with the rake accounts for a cover of 5%, the limit considered in MaQI to discriminate areas where macrophytes are able to bloom from areas where the growth is hampered by disturbance factors (Sfriso et al., 2014b). The Rhodophyta/Chlorophyta ratio, another metric considered in MaQI, was obtained by sorting and wet weighting (precision ± 1 g) the macroalgae collected in 6 additional random samples by scraping the bottom for approximately 1 m with the rake all around the boat. Macrophyte subsamples representative of the collected biomass were preserved in 4% formaldehyde seawater and determined at specific and intra-specific level by means of a stereo microscope and a light microscope. Some samples of doubtful identification were also kept fresh for molecular analyses.

2.3 Macrobenthic invertebrate sampling

At each sampling station, three replicates were collected with a Van Veen grab (area: 0.027 m^2; volume: 4 l). Samples were sieved (Ø = 0.5mm mesh) in the field and preserved by using a buffered solution of formaldehyde (8% in brackish water). Specimens were identified to the highest possible taxonomic separation (usually species), and their abundance was quantified as the number of individuals per sample. Animals were dried at 80°C for 48 hours in a hoven, and then incinerated at 450 °C for 4 h in a muffle furnace. Each individual was weighted and the average weight per species was determined. Ash-free dried weight was considered as estimate of biomass.

2.4 Biotic indices

The Ecological Status (ES) of each lagoon of the studied transitional system was assessed by applying one index based on macrophytes (MaQI), and three indices based on invertebrates (M-AMBI, M-bAMBI, and ISD).

Macrophyte Quality Index (MaQI), was developed and validated by Sfriso et al. (2009) and intercalibrated in the framework of the Mediterranean Geographic Intercalibration Group (Med-GIG; European Commission, 2010). After determination, each macroalgal taxon, was associated to a score according to the degree of sensitivity (0 = tolerant taxa, 1 = indifferent taxa, 2 = sensitive taxa). For the EcoQ calculation the following metrics are used: total number of species, number and percentage of sensitive macroalgal taxa, total percentage of macroalgal cover, Rhodophyta/Chlorophyta biomass ratio and percentage of aquatic angiosperm cover. The EcoQ calculation is obtained by two entries, one for macroalgae and the other for angiosperms, if present, following Sfriso et al. (2016). MaQI is a categorical index in order to be applied also in the presence of a very low cover or a few taxa, which is not possible with continuous indices. The sampling sites were classified according the following scheme: “High” if EQR = 1 or 0.85, “Good” if EQR = 0.75 or 0.65, “Moderate” if EQR = 0.55 or 0.45, “Poor” if EQR = 0.35 or 0.25, and “Bad” if EQR = 0.15 or 0 (Sfriso et al., 2014).

Abundance (M-AMBI) and biomass (M-bAMBI) based indices were calculated using AMBI 5.0 software (freely available at http://ambi.azti.es). For both indices invertebrates are assigned a score for I to V, based on their tolerance, following to AMBI library (Borja and Muxika, 2005). M-AMBI index is based on the following metrics: AMBI, Shannon diversity (H_{log2}), and taxa richness (S) (Muxika et al., 2007). For M-bAMBI the same metrics are calculated using biomass data (Mistri et al., 2018). Reference conditions were those reported by the Italian Act 260/10, for microtidal oligo/meso/polyhaline lagoons (AMBI = 2.14; H’ = 3.4; S = 28). The sampling sites were classified according to boundaries required by Italian law for M-AMBI: “High” if > 0.96, “Good” if 0.71 < M-AMBI ≤ 0.96,“Moderate” if 0.57 < M-AMBI ≤ 0.71, “Poor” if 0.46 < M-AMBI ≤ 0.57, and “Bad” if M-AMBI ≤ 0.46, and following Mistri et al. (2018) for M-bAMBI: “High” if > 0.930, “Good” if 0.739 < M-AMBI ≤ 0.930,“Moderate” if 0.632 < M-AMBI ≤ 0.739, “Poor” if 0.548 < M-AMBI ≤ 0.632, and “Bad” if M-AMBI ≤ 0.548.

The ISD is based on the distribution of individuals across geometric size classes (class I: >0 – <0.2 mg, class II: >=0.2 – <0.4 mg, class III: >=0.4 – < 0.4 mg, ... class XII: >=204.8 – <409.6 mg). The percentage of individuals per geometric size class and station was determined and the skewness (type G_1, see Joanes and Gill, 1998) of the distribution was calculated for each station, representing the ISD value (Reizopoulou and Nicolaidou, 2007). The sampling sites were classified according to the classification scale provided by Reizopoulou and Nicolaidou (2007): “High” if -1 ≤ ISD > 1, “Good” if 1 ≤ ISD > 2, “Moderate” if 2 ≤ ISD <3, “Poor” if 3 ≤ ISD < 4, “Bad” ≥ 4. Calculations were performed in R version 5.1 using libraries tidyverse, xlsx and e1017 (R Development Core Team, 2008, Wickham, 2017, Dragulescu and Arendt, 2018, Meyer et al., 2018).

Abundance/Biomass Comparison (ABC) method (Warwick, 1986) was used to determining the level of disturbance of benthic macrofaunal communities at each station combining the two aspect: abundance and biomass. It was used as an additional confirmatory measure of the degree of disturbance affecting macroinvertebrate community. This method involves the plotting of separate k-dominance curves for species abundance and species dominance on the same graph and W statistic (ranging from -1 to +1) was used to compare the forms of these curves. When the stress is severe communities become dominated by few or one small-bodied opportunistic species, dominating the numbers but not the biomass, therefore the abundance curve lies above the biomass curve and W tend to -1. Conversely undisturbed communities are dominated by one or few large species, dominating biomass but not abundance. Thus biomass curve lies above the abundance curve and W tend to +1.

2.5 Statistical analyses

Non-parametric chi-square test applied to Kruskal-Wallis (KW) ranks (Kruskal and Wallis, 1952) was used to check if the abiotic parameters, the biotic indices and the abundance-biomass pattern changed significantly between years and lagoons. When significant differences were encountered, a Wilcoxon rank sum test (W) post hoc comparison test was also carried out.

The response of each index to PI, was calculated separately with the non-parametric Spearman Rank-order coefficient (r_s) (Spearman, 1907). The same coefficient (r_s) was calculated between PI and environmental variables, to check whether anthropogenic activities affected environmental parameters. Collinearity among environmental and biotic variables were checked in order to understand whether the effect of a variable could be masked by another one.

Redundancy Analysis (RDA) was used to show how much of the variance of the biotic indices was related to the environmental variables and PI. RDA was chosen because it explicitly models response variables as a function of explanatory variables (Zuur et al., 2007). Ordination of the data used to calculate the correlation coefficients was performed after log (x + 1) transformation of biotic data, using ‘vegan’ package for R version 3.5.1 (R Development Core Team, 2008; Oksanen et al., 2008). The results was presented as a correlation biplot (Scaling 2) displaying correlations between environmental variables, and biological parameters (EGs calculated on abundance and biomass, size classes, biotic indices: M-AMBI, M-bAMBI, ISD, and MaQI, and metrics used for indices calculation: S, H, H_b, AMBI, b-AMBI). Environmental variables displayed in the biplot were chosen in order to avoid collinearity and provide the best representation of data. Permutation test under reduced model was performed to check the significance of the environmental variables.

3. RESULTS

3.1 Physico-chemical parameters

Sediments composition differed among lagoons. Barbamarco, Canarin, Scardovari and Vallona lagoons were characterized by a dominance of silt (Table 2), and high concentration of total carbon (Table 2), nitrogen (Table 2) and phosphorous (Table 2). Conversely, sediments in Caleri and Marinetta are composed mainly by sand (Table 2), with lower percentages of total carbon (Table 2), nitrogen (Table 2) and phosphorous (Table 2). Higher percentages of shells were observed in Scardovari and Vallona (Table 2). No significant differences of physico-chemical parameters (grain size, TC, TN, TP, temperature, and salinity) were observed among lagoons nor sampling year (KW,

p >0.05), with the exception of pH showing lower values in 2009 (mean 8.0) compared to 2008 (mean 8.2). Conversely, a certain variability in terms of grain size was observed within some lagoons (Table 2), such as Caleri (% of silt and % of sand) and Scardovari (% of shells). TN showed also a marked variability, in particular within the lagoons of Barbamarco, Caleri, and Canarin (Table 2).

The increasing of anthropogenic pressure (PI index, Table 1) was related to an increase of silt content ($r_s = 0.59$) and total carbon content in sediments ($r_s = 0.65$), together with a decrease of sand ($r_s = - 0.62$). Concentration of nutrients, in particular total carbon (TC) and total phosphorus (TP), increased with increasing percentage of silt ($r_s > 0.92$ for TC and $r_s > 0.77$ for TP) and decreasing content of sand ($r_s > - 0.91$ for TC and $r_s > - 0.72$ for TP).

3.2 Macrophytes

The complete absence of aquatic angiosperms in all the water bodies and the almost complete absence of sensitive species (overall only single small thalli of 4 species at Caleri) indicate a severe degradation of the ecological conditions of the entire study area. The most frequent species (Table S1) were typical of polluted areas (score = 0): *Ulva rigida* C. Agardh (73% of frequency) and *Gracilaria vermiculophylla* (Ohmi) Papenfuss (69% of frequency). On average, the number of Chlorophyta exceeded that of Rhodophyta whereas the presence of Ochrophyta was negligible showing a maximum of 3 taxa in Barbamarco in 2008. The translation of the MaQI values into classes of ecological quality results in the majority of stations being classified as “poor”, and few stations classified as “bad” (Table 3). The MaQI index and consequently the overall ecological status was more or less constant throughout the six lagoons and the studied years (KW, $p > 0.05$, Figure 2A), with three stations showing a decrease in ecological quality by one class (from poor to bad) from 2008 to 2009, and then a return to the same proportion of 2008 the following years (Figure 3).

3.3 Macrobenthic invertebrates

Macrobenthic invertebrates community was dominated by annelids with 89% of total abundances, followed by arthropods with 8% and molluscs with 3%. The most frequent and abundant species (Table S2) were the polychaetes *Streblospio shrubsolii* (Buchanan, 1890) (98% of frequency, 52% of total abundances), and *Capitella capitata* (Fabricius, 1780) (87% of frequency, 11% of abundances). Among the most frequent species there were also oligochaetes (83% of frequency), the polychaetes: *Polydora ciliata* (Johnston, 1838) (69%), *Alitta succinea* (Leuckart, 1847) (67%), and *Spio decorata* Bobretzky, 1870 (56%), and the amphipods: *Monocorophium insidiosum* (Crawford, 1937) (58%), and *Gammarus aequicauda* (Martynov, 1931) (56%).

The majority of stations showed a strongly left-skewed distribution of size classes, i.e. large individuals were under-represented in the assemblages. The subsequent translation of the ISD values into classes of ecological quality results in most stations being classified as “poor”, some as “moderate”, only in 2009, two stations (Can1 and Ma2) achieved “good” status (Table 3). The calculation of M-AMBI showed overall higher ecological quality and higher variability among stations (Table 3). The majority of stations were classified as “moderate”, “poor”, and “bad”, but with some stations achieving “good” and one (Bar1) even “high” ecological status in 2010 (Table 3). The calculation of M-bAMBI showed overall high variability among stations and even higher ecology quality compared to M-AMBI, with a total of 20 station classified as “good” (Table 3).

The three indices analyzed (ISD, M-AMBI and M-bAMBI) showed no significant differences among the six lagoons (KW, $p > 0.05$). ISD index showed differences (KW, $p < 0.05$) from 2008 to

2009 and from 2009 to 2010 (Figure 2B). The overall ecological status was slightly better in 2009 compared to the previous and following year, where seven stations saw an increase in ecological quality by one class (i.e. from poor to moderate or from moderate to good) from 2008 to 2009; however, in 2010 the ecological status of most stations returned to the conditions of 2008 (with the exception of station Can2 which decreased in quality and station Sca3 which in 2010 achieved a moderate status, having been classified as "poor" in both 2008 and 2009). M-AMBI index (Figure 2C) and M-bAMBI index (Figure 2D) did not show significant differences among years (KW, $p > 0.05$). According to M-AMBI index the ecological status of one station decreased by one class from 2008 to 2009, and signs of improvement from 2009 to 2010, with one station improving ecological status by one class (Table 3). According to M-bAMBI index four stations increased ecological quality by one class (from moderate to good) from 2008 to 2009, but in 2010 the ecological status of most stations returned to the conditions of 2008 (Table 3).

Overall, ISD index classified every lagoon as "poor" or "moderate" (Figure 3). Such a classification was consistent with results of M-AMBI in 2008 and 2009, but in 2010 M-AMBI classified two lagoons (Vallona and Barbamarco) as "good" (Figure 3). M-bAMBI classified Scardovari lagoon as "good" in 2008 and 2009, and "poor" in 2010, whereas indicated an improvement of environmental conditions for Caleri, Marinetta and Vallona lagoon from "poor"/"moderate" status in 2008 and 2009 to "good" status in 2010 (Figure 3).

3.4 Indices validation and relation indices/pressures

Macrobenthic invertebrate communities were mainly represented by tolerant species (EGIII), dominating both in terms of abundances, with values ranging from 69.9% in 2008 to 77.3% in 2010 (Figure 4A) mainly due to polychaetes such as *Streblospio shrubsolii*, *Hydroides dianthus* (Verrill, 1873) and *Ficopomatus enigmaticus* (Fauvel, 1923), both in terms of biomass, with values ranging from 58.1% in 2008 to 61.4% in 2010 (**Error! Reference source not found.**B), mainly due to the bivalves *Ruditapes philippinarum* (Adams & Reeve, 1850) and *Arcuatula senhousia* (Benson, 1842). First order opportunistic species (EGV), mainly represented by the polychaete *Capitella capitata,* followed in terms of abundances (19%-8.8%), with lower percentages of sensitive species (EGI, 7%-4.9%), such as the amphipod *Gammarus aequicauda*, and second order opportunistic species (EGIV, 4.9%-4%), such as the polychaete *Polydora ciliata* and larvae of the insect *Chironomus salinarius* Kieffer, 1915. Conversely, in terms of biomass tolerant species were followed by EGI (19.3%-23.8%), mainly represented by the bivalve *Chamelea gallina* (Linnaeus, 1758) and the amphipod *Gammarus aequicauda*, and EGII (14.7%-7.3%), dominated by species belonging to Actinaria, with lower percentages of EGIV (3.9%-1%), mainly represented by the bivalve *Anadara transversa* (Say, 1822) and EGV (3.5%-1%), mainly represented by the polychaete *Capitella capitata*.

Communities were dominated by the smallest size class (I), ranging from 78.9% of individuals in 2010, to 21.5% in 2008 and 2009. The two biggest size classes (XI and XII) were not represented (Figure 4C).

AMBI index varied significantly from 2008 to 2010 and from 2009 to 2010 (KW and W, $p < 0.05$), but differences among lagoons were at significant level (KW, $p = 0.05$), whereas H and S did not showed any significant difference (KW, $p > 0.05$). Neither bAMBI, nor diversity calculated on biomass (H_b) varied significantly among years, or lagoons (KW, $p > 0.05$)

Abundance/Biomass Comparison (ABC) method (**Error! Reference source not found.**D) showed that communities were subjected to variable levels of stress, with values ranging from $W = -0.342$, indicating more disturbed communities, where the dominant taxa dominated for abundances, to $W = 0.307$ indicating less disturbed communities, where the dominant taxa dominated for biomass. No significant differences were observed among years (KW, $p > 0.05$), but differences were observed among lagoons (KW, $p < 0.05$), in particular Canarin lagoon, showed on average higher W values, indicative of less disturbed communities, compared with Caleri, Marinetta, and Vallona. The variability within lagoons was high, as well.

Redundancy analysis did not show a clear response of biotic parameters to environmental factors (Figure 5). The variability of ecological groups (in terms of both abundance and biomass, Figure 5A), and size classes (Figure 5B) was explained most by variation of salinity (permutation test, $p < 0.05$). The graphs showed a gradient of increasing salinity from right to left. Variation of biotic indices instead (Figure 5C) were explained by both salinity and PI (permutation test, $p < 0.05$). The graph showed from the left to the right an increasing pattern of salinity, corresponding to increasing values of H. From the top right to the bottom left instead there was a decreasing gradient of PI, corresponding to a decreasing ISD, and increasing H_b, S and M-bAMBI.

Comparing the different indices based on macrobenthic invertebrates M-AMBI was strongly correlated with H and S, and moderately with M-bAMBI and H_b; ISD was weakly correlated with M-AMBI, bAMBI and H (Table 4

Table 2. Means (±SD) of sediments and water parameters for each of the six studied lagoons. Water data were obtained from ARPAV archive. TC = total carbon, TP = total phosphorus, TN = total nitrogen), T = temperature, DO = oxygen saturation.

	Barbamarco	Caleri	Canarin	Marinetta	Scardovari	Vallona
Silt (%)	88.4 ± 0.8	44.2 ± 21.3	85.2 ± 11.9	21.2 ± 5.7	61.0 ± 14.2	60.6 ± 3.2
Sand (%)	10.3 ± 0.8	55.4 ± 20.8	14.1 ± 11.8	78.4 ± 5.9	33.9 ± 12.8	37.1 ± 1.6
Shells (%)	1.3 ± 0.0	0.4 ± 0.6	0.7 ± 0.2	0.4 ± 0.2	5.1 ± 1.4	2.3 ± 1.7
TC (mg/g)	35.5 ± 2.7	27.3 ± 6.7	34.7 ± 0.2	24.8 ± 0.2	31.0 ± 0.4	31.8 ± 1.5
TN (mg/g)	1.8 ± 0.6	1.0 ± 0.7	2.1 ± 0.8	0.7 ± 0.0	1.7 ± 0.3	1.3 ± 0.0
TP (µg/g)	647.4 ± 24.4	566.9 ± 70.4	633.1 ± 40.6	513.3 ± 22.9	544.0 ± 16.2	561.2 ± 64.8
Temp (°C)	17.8 ± 0.7	18.9 ± 1.1	18.5 ± 0.3	18.2 ± 1.9	18.8 ± 1.4	18.0 ± 2.0
Salinity	25.6 ± 2.8	26.9 ± 2.7	22.8 ± 0.3	22.8 ± 1.6	26.8 ± 1.9	21.2 ± 0.3
DO (%)	99.8 ± 3.4	112.3 ± 2.5	109.7 ± 15.6	103.2 ± 3.0	108.3 ± 8.8	94.7 ± 4.4
pH	8.3 ± 0.1	8.2 ± 0.2	8.3 ± 0.1	8.0 ± 0.2	8.2 ± 0.1	8.0 ± 0.1

Table 3. Number of stations per Ecological Quality Status in each study year, using different indices.

MaQI			
Status	**2008**	**2009**	**2010**
Poor	15	12	15
Bad	2	5	2
Total	17	17	17
ISD			
Status	**2008**	**2009**	**2010**
Good	0	2	0
Moderate	4	6	2
Poor	16	12	10
Total	20	20	12
M-AMBI			
Status	**2008**	**2009**	**2010**
High	0	0	1
Good	4	3	2
Moderate	7	8	4
Poor	4	4	4
Bad	5	5	1
Total	20	20	12
M-bAMBI			
Status	**2008**	**2009**	**2010**
Good	6	10	4
Moderate	6	2	3
Poor	3	3	1
Bad	5	5	4
Total	20	20	12

Table 4). M-AMBI showed the best response to PI (Table 4), followed by Shannon index calculated on abundances (H) and biomass (H_b), M-bAMBI, S and ISD. MaQI index, AMBI and bAMBI showed no significant correlation with PI (Table 4). S was also correlated with salinity (Table 4), while H was also correlated with oxygen (Table 4). AMBI values increased with increasing salinity (Table 4), while bAMBI was negatively correlated with salinity (Table 4).

4. Discussion

The lagoons of the Po Delta are heavily affected by different anthropogenic pressures, mainly by high nutrient and pollutant inputs through river outflows and water turbidity due both to the erosion of the riverbanks, always deprived of vegetation, and intense fishing activities to catch the Manila clam (*Ruditapes philippinarum* Adams & Reeve) occurring in many of these areas (e.g. Munari et al., 2010; Sfriso et al., 2016; Maggi et al., 2017; Franzo and Del Negro, 2019). The degraded ecological conditions of these environments were known even without the application of indices of ecological status (Sfriso et al., 2016). The strength of those pressures varied among and within lagoons. Chemical data from the studied period did not suggest any severe dystrophic events, with dissolved oxygen values around saturation levels. Nevertheless, cases of water stratification and hypoxic conditions (oxygen concentration closed to the bottom: 1 mg/l) were reported in some basins in other periods of the year (ARPAV, 2008-2010), and those events could have had a long-term effect on macrobenthic communities. Moreover, recent investigations indicates high nutrient availability in Po Delta lagoons, with seasonal and local variations. In particular dissolved inorganic nitrogen showed maximum values (>30μM) higher than limits proposed in the National Act 260/10, and reactive phosphorous showed a maximum value of 24.9 μM, never recorded previously in any other Italian TWs (Sfriso et al., 2016). In the present work, the relation between PI and total carbon content in sediments confirmed that nutrients inputs represented one of the main anthropogenic pressures. Nevertheless, nutrient content (TC and TP) was higher where silt predominated. Sediments grain size is considered one of the indicators of the potential capacity of the system to react to pollutants: the presence of cohesive sediments maintain water even during emersion, and enable no lateral movement or percolation for water and oxygen, therefore fine sediments are more prone to anoxic condition and sulphide production (Viaroli et al., 2008). The lack of correlation between PI and other parameters, such as oxygen and TN suggests that some components of anthropogenic impacts could be not fully explained by the index PI. Moreover, sediments can also have a direct effect on benthic community, so the variability of environmental parameters (grain size and TN) observed within Barbamarco, Caleri, Canarin, and Scardovari lagoons, can act as confounding factor when comparing the effect of impacts. Salinity is also highly variables within each transitional water body, as result of the combined effects of hydromorphology, river and marine influence (Elliott and Whitfield, 2011, Whitfield et al., 2012; Basset et al., 2013). Other authors have pointed out that a lagoon should not be considered spatially uniform and unique unit but as a mosaic of assemblages when applying the EU Water Framework Directive or assessing environmental impact (Pérez-Ruzafa et al., 2008). Sampling design could help controlling the effect of natural variability, but temporal, together with spatial pattern should be taken into account (Khedhri et al., 2017; Pasqualini et al., 2017). In the present work it was not possible to detect a clear spatial or temporal pattern of abiotic parameters within each lagoon due to the patchiness of environmental conditions and the complexity of the relationship between temporal and spatial changes. Our results are in line with an investigation performed within a hypersaline coastal lagoon in the south-western Mediterranean, where macrophyte assemblages diversity and richness responded more to the frequency, regularity and intensity of environmental fluctuations than salinity or confinement gradients themselves (Pérez-Ruzafa et al., 2008). The numerous natural and anthropogenic stressors in TWs provoke a high level of habitat fragmentation, forcing species and communities to adapt to such heterogeneous conditions (Prato et al., 2014).

In general all indicators used confirmed the general degradation of Po Delta lagoons, with most sample assigned to a EcoQ below the critical Good/Moderate threshold, but in some cases the use of different BQEs and different indices based on the same BQE lead to different EcoQ. Moreover, different biotic indices showed differential response to both environmental parameters and anthropogenic pressures.

Macrophyte composition and MaQI index values lead to classify the ES of all Po Delta lagoons, as "poor" or "bad". Even if in the past the presence of *Ruppia cirrhosa* (Petagna) Grande was reported for Po Delta system (Sfriso et al., 2016), angiosperms have totally disappeared, and were never recorded in the studied period. The presence of very few sensitive algal species, and the dominance of free-floating opportunistic macroalgae (e.g. genera *Ulva* and *Gracilaria*) is a symptom of eutrophication and degradation of the environment (Viaroli et al., 2008). One of the major driver of the shifts from the dominance of angiosperms and sensitive macroalgal species to blooms of opportunistic and nitrophilous macroalgae is the increase of nutrient loads, in particular nitrogen (Viaroli et al., 2008; Sfriso et al., 2016). Some stations were evaluated as "bad", because of the extremely low macroalgal cover (<5%) or their total absence. Such a condition indicates severe degradation: when waters are so turbid that light cannot penetrate to the bottom, macroalgae slow down their growth and phytoplankton and cyanobacteria become the only primary producers (Viaroli et al., 2008; Sfriso et al., 2016). Even if this transitional system is part of the Regional Po Delta Park, the area is completely surrounded by cultivated fields, affecting the transitional system with the high nutrient loads, and likely also with chemicals and other toxic substances. For instance, sediments analyzed in 2008, showed Hg concentration higher that limits established by WFD (> 0.3 mg/kg), with concentration up to three times higher than this threshold in one station in Vallona lagoon (*unpublished results*). Investigation at lower levels of biological organization, with tools such as ecotoxicological biomarkers, could provide more detailed information on the biotic response to this type of stressors (Beiras, 2016). Fishery activities could also be detrimental for macrophytes, destroying the natural sediment texture and resuspending high amounts of fine sediments which dramatically reduce light availability favoring cylindrical and filamentous thalli (Sfriso et al., 2016). The *Poor/Bad* ES evaluated with MaQI enhanced the necessity of a policy of interventions to improve conditions and achieve the *Good* ecological status as requested by the WFD. Those results were consistent with the high level of anthropogenic pressures affecting the studied area, even if the extremely reduced differences of ES between samples lead to no significant correlation between MaQI index and PI, nor between MaQI and other environmental variables.

The indices based on macrobenthic invertebrates in general gave higher scores compared with evaluation based on macrophytes. The disagreement of the EcoQ assessment obtained through different BQEs is particularly marked in TWs (Borja et al., 2011) and arose because of the different response of biological elements to different stressors. Macroalgae were considered more sensitive to eutrophication, seagrasses to hydromorphological changes or habitat loss (Borja et al., 2013), and benthic invertebrates to ''general degradation'' (Hering et al., 2013). ISD index represents the skewness of the distribution of individuals of a benthic community in geometric size (biomass) classes and is an alternative method to investigate benthic community structure (Reizopoulou and Nicolaidou, 2007). Body size abundance distribution is suggested to be related to disturbance pressure through individual energetics, population dynamics, interspecific interactions and species coexistence responses. Even if ISD index did not show the best response to PI, the EcoQ obtained was the most consistent with EcoQ based on macrophytes (MaQI): all analyzed lagoons never reached a *Good* environmental status. In general ISD index gave, with few exceptions, lower scores compared with M-AMBI and M-bAMBI, and this discrepancy was between *Moderate* and *Good* status at some sites. M-AMBI and M-bAMBI indices combine two aspects of macroinvertebrate community: qualitative (sensitivity of each species: AMBI and bAMBI), and quantitative (structural indices: H, H_b, and S). In the present work M-AMBI and M-bAMBI, showed the best response to PI, but they showed a discrepancy with the other two indices, which was more marked for M-bAMBI (higher number of samples classified as *Good*). Since this discrepancy crossed the critical boundary between *Moderate* and *Good* status, the results of those indices should be considered with caution: assessing as *Good* a site which is actually *Moderate* could result in underestimating the necessity of management actions and vice versa.

In general, with few exceptions (Canarin in 2008-2010, and Barbamarco in 2010), ES calculated with M-bAMBI corresponded or was higher than EcoQ calculated with M-AMBI. This was the result of the highest percentages represented by sensitive and indifferent species when biomass was considered, and it's a direct consequence of the biological traits of r- and k-strategists (Pearson and Rosenberg, 1978). All biotic indices considered in the present work are based on the paradigm stating that the increasing organic pollution results in loss of the larger long-lived species (k-strategists) from the community in favor of more tolerant short-lived opportunists (r-strategists) (Pearson and Rosenberg, 1978). Nevertheless, the different metrics used to quantify the alternative states of this phase shift provided different results. While M-AMBI (and AMBI) quantify the effect of organic pollution only in terms of species abundances, ISD, and M-bAMBI (and bAMBI) considered also that k-strategists dominate in terms of biomass, while r-strategists in terms of abundance (Reizopoulou and Nicolaidou, 2007). The theory of changes of benthic community biomass under disturbed conditions, well documented in benthic ecology (Pearson and Rosenberg, 1978), was also at the base of ABC analysis. In the present work, differently from biotic indices ABC method did not discriminate among years, but among lagoons, with also a high within-lagoon variability, suggesting it to be more sensible to local changes, representing the sum of particular conditions, most of them limited in space. It was already pointed out that ABC methods proved to be efficient in defining whether the community was subjected to stress, but it could be biased by recruitment (Beukema, 1988) and it does not always discriminate between natural and anthropogenic causes of such a stress (Clarke and Warwick, 2001; Lardicci et al., 2001). This factor is crucial in TWs, where organisms have to cope with the high natural variability of environmental parameters, resulting in the dominance of tolerant species (EGIII), in terms of both abundances and biomass, a common feature of such environments (e.g. Marchini et al., 2008; Pitacco et al., 2018). Such species did not show any correlation with PI nor environmental variables, but given their dominance, they have a critical role in the assessment of ES, posing problems to the applicability of those biotic indices in TWs. Moreover, the pattern of sensitive and opportunistic species was not consistent with PI and environmental variables, as well. This lack of response could be related to a possible adaptation of local species living in TWs to stressed conditions. Most species living in TWs adapt to such variations (Cognetti, 1992) and become tolerant of changes (Cognetti and Maltagliati, 2000). In fact, analyses on genetic divergence on brackish species showed a high degree of fragmentation in local population, morphologically unidentifiable, with different degrees of adaptability (Cognetti and Maltagliati, 2000). Other authors have pointed out the need to revise the concept of r/K selection concept in TWs. Pérez-Ruzafa et al. (2013) found that estuarine fish species combine r and K characteristics, suggesting lagoonal selection would not necessarily act on all the biological traits of a species but only on some of them, improving the adaptation of local populations to the lagoon environment but upsetting the coherence of all biological traits in an r/K context. Previously Stearns (1977) had observed that in fish assemblages r and K strategists are not necessarily negatively correlated.

The WFD (2000/60/EC) requires that any method used to assess the ecological status must detect only anthropogenic pressures, show a clear pressure-response, and avoid the detection of natural variability (Reiss and Kröncke, 2005). In TWs, however, stress of both natural and anthropogenic origin create a variety of conditions that make it difficult to disentangle the effect of anthropogenic activities from naturally induced stress (the so-called ‘‘Estuarine Quality Paradox’’; Elliott and Quintino, 2007; Dauvin, 2007). In the present work biotic indices based on macrobenthic invertebrates showed significant relationships with anthropogenic disturbances (in terms of PI), and seemed robust to natural variability. Some of the metrics used (AMBi, bAMBI and S) for calculation of biotic indices were also related with PI, but also with variation of environmental variables, in terms of salinity and oxygen. Oxygen is one of the elements to be monitored according to National Act 260/10, since its low concentration is an indication of a dystrophic crises, and therefore degraded conditions. Conversely, salinity is related with natural hydromorphological characteristics of the lagoons, not with anthropogenic pressures, and moreover is subjected to daily and seasonal variations. Therefore, this relationship represented a potential problem for the correct evaluation of ES.

The metrics of indices based on macroinvertebrates resulted the most affected by natural variability. In particular the species richness and Shannon–Wiener Index calculated on abundances (H) was the metric showing the highest correlation with environmental parameters. The unreliability of univariate indices for a correct evaluation of EcoQ was already pointed out by other authors. Those indices showed high variability related to seasonality, making than less suitable for the aim of WFD (Reiss and Kröncke, 2005). Indeed, diversity showed also a strong negative correlation with confinement (Reizopoulou and Nicolaidou, 2004), which is a natural situation not always associated with environmental health. Biotic indices such as AMBI resulted more stable with respect to seasonality (Reiss and Kröncke, 2005), but in the present work they responded better to salinity gradient than to PI, probably due to the fact that the percentage of ecological groups did not respond in coherent way to PI as well. The effect of salinity on biotic indices is well known, and in the National Act 260/10 transitional water bodies are classified on the basis of tide and salinity, with the aim of reducing the bias. Nevertheless, the present work showed that the high variability of salinity in transitional waters can influence indices also within the same water typology (the whole Po Delta system fall in the category of “microtidal oligo/poly/mesohaline). M-AMBI and M-bAMBI indices combine two aspects of macroinvertebrate community: qualitative (sensitivity of each species: AMBI and bAMBI), and quantitative (structural indices: H, H_b, and S), and they showed the best response to PI, without bias related to salinity.

The richness of macrophytes at inter-lagoonal level are also known to be influenced by salinity (e.g. Pérez-Ruzafa et al., 2011; Schubert et al., 2011; Janousek and Folger, 2012) generally showing higher values with higher salinity. Nevertheless, in pristine conditions the few species present have high ecological value, such as angiosperms (Sfriso et al., 2016, and references therein), and this makes the macrophytes a more stable BQE in eutrophic areas with high salinity variations, such as transitional areas. Our results highlighted the efficiency of combining different metrics and the necessity of correcting the metrics for salinity and other confounding environmental variables (see for instance Leonardsson et al., 2016).

The metrics used to assess the EcoQ must be able to discriminate between natural and anthropogenic changes otherwise false conclusions may be reached regarding the status being as the result of human pressures. In this view further efforts are still needed to implement the efficiency of EcoQ assessment methods in TWs. A correct classification of transitional ecosystems into discrete categories of ecological status can be best achieved by combining different BQEs, and refining biotic indices in order to improve their efficiency in TWs. The WFD (2000/60/EC) uses the ‘‘one-out, all-out’’ principle to combine assessments from different BQEs. This principle is based upon the assumption that the worst status of the elements used in the assessment determines the final status of a water body (Borja et al., 2004). This method was efficient in determining the ES in the highly impacted systems objected of the present study, but tend to inflate type I error, and resulted in an underestimation of EcoQ (below *Good* status, when it was actually *Good*) in less impacted transitional systems (Hering et al., 2013; Prato et al., 2014). This could become a critical step, in particular when conditions are at the border between *Moderate/Good* status. Further works analyzing the uncertainty of defining boundaries and calculating the confidence of ecological classes, with methods already used within the WFD framework for a UK freshwater macrobenthic dataset (Clarke, 2013), could improve our understanding on those critical boundaries. A further difficulty in disentangling natural and anthropogenic stressors is due to the lack of standard and objective methods to estimate anthropogenic impacts. The quantitative assessment of anthropogenic pressures, commonly expressed as a pressure index (PI), required expert judgment and could be biased by a certain level of subjectivity. In the present work, both biotic and abiotic data clearly support the accuracy of such quantification, but the homogenization of EcoQ in the study system limited the usefulness of the correlation with PI to assess the efficiency of biotic indices. The difficulties inherent to the complex and dynamic nature of transitional ecosystems urge the need to find alternative approaches for a correct EcoQ assessment. In this view the recently developed biomass-based indices seem promising, providing complementary ecologically relevant information with respect to previous ones, downscaling the effects of over abundant small-bodied organisms (Mistri et al., 2018).

5. Summary

The lagoons of the Po Delta are heavily affected by different anthropogenic pressures, mainly by high nutrient and pollutant inputs through river outflows, water turbidity and intense agricultural and fishing activities. The strength of those pressures varied among and within lagoons. It was not possible to detect a clear spatial or temporal pattern of abiotic parameters within each lagoon due to the patchiness of environmental conditions and the complexity of the relationship between temporal and spatial changes. In general the indicators used confirmed the general degradation of Po Delta lagoons, with most sample assigned to a EcoQ below the critical *Good/Moderate* threshold, but in some cases the use of different BQEs and different indices based on the same BQE lead to different EcoQ.

Macrophyte composition and MaQI index values lead to classify the ES of all Po Delta lagoons, as *Poor* or *Bad*. The indices based on macrobenthic invertebrates in general gave higher scores compared with evaluation based on macrophytes. The disagreement arose because of the different response of biological elements to different stressors. In general ISD index gave lower scores compared with M-AMBI and M-bAMBI, and this discrepancy was critical at some sites because it was between *Moderate* and *Good* status. Discrepancies were more marked between ISD and M-bAMBI, and arose because they focused on different aspects of the community, providing therefore complementary information.

MaQI results were consistent with the high level of anthropogenic pressures affecting the studied area. Nevertheless, the extremely reduced differences of ES between samples lead to no significant correlation between MaQI index and PI, nor between MaQI and other environmental variables, suggesting it was not sensitive to minor differences among lagoons. Conversely, biotic indices based on macrobenthic invertebrates were significantly correlated with anthropogenic disturbance expressed in terms of PI, suggesting this BQE is more sensitive to changes also in highly degraded condition. Nevertheless, the discrepancy among indices between the critical boundary *Good/Moderate*, indicate caution: assessing as *Moderate* a site which is actually *Good* could result in unnecessary management actions and vice versa.

Some of the metrics used for calculation of biotic indices were also related with PI, but also with variation of environmental variables, in terms of oxygen and salinity. Salinity is related with natural hydromorphological characteristics of the lagoons, not with anthropogenic pressures. This relationship, together with the infra-lagoon variability, represented a potential problem for the correct evaluation of ES and highlight the need of metrics correction for possible confounding environmental parameters. A combination of different BQEs and different indices, with a refinement of some existing indices, would be therefore crucial to improve EcoQ classification.

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Tables

Table 1. Pressures at the various sampling sites. Modified from Mistri et al., 2018.

	Site	Non-point pollution sources		Point pollution sources			Habitat loss		Ports			Fisheries		PI
		Agricoltural inputs	Freshwater inputs	Domestic	Agricultural	Industrial	Land-claim	Physical alteration	Port activity	Navigation	Dredging	Fin-fisheries	Shell-fisheries	
Caleri	Cal1	2			2							1	3	**8**
	Cal2	2			2							1	1	**6**
	400	2			2							1	1	**6**
	Cal3	1			1								2	**4**
	220	1			1								2	**4**
	Cal4	1		1								1	1	**4**
	210	1		1								1	1	**4**
Marinetta	Ma1	1											2	**3**
	Ma2	1											2	**3**
	410	1											2	**3**
	Ma3	1	3		2								2	**8**
	230	1	3		2								2	**8**
	Ma4	1							2				2	**5**
Vallona	Val1	1	1		1				1				3	**7**
	Val2	1	1		1								3	**6**
	250	1	1		1								3	**6**
	240	1											2	**3**
Barbamarco	420	2	1		2			1					3	**9**
	Bar1	2			2			1					3	**8**
	270	2			2			1	2				2	**9**
	Bar2	2			2			1					3	**8**
	260	2			2			1					3	**8**
Canarin	Can1	1											2	**3**
	430	1											2	**3**
	Can2	1			2							1	3	**7**
	440	1			2							1	3	**7**
	Can3	1	1		2							1	3	**8**
	290	1	1		2							1	3	**8**
Scardovari	Sca1	1						1					2	**4**
	330	1						1					2	**4**
	Sca2	1											3	**4**
	Sca3	1											2	**3**
	340	1	1										2	**4**
	Sca4	1	1		2							1	2	**7**
	450	1	1		2							1	2	**7**
	Sca5	1						1			1		1	**4**
	320	1						1			1		1	**4**

Table 2. Means (±SD) of sediments and water parameters for each of the six studied lagoons. Water data were obtained from ARPAV archive. TC = total carbon, TP = total phosphorus, TN = total nitrogen), T = temperature, DO = oxygen saturation.

	Barbamarco	Caleri	Canarin	Marinetta	Scardovari	Vallona
Silt (%)	88.4 ± 0.8	44.2 ± 21.3	85.2 ± 11.9	21.2 ± 5.7	61.0 ± 14.2	60.6 ± 3.2
Sand (%)	10.3 ± 0.8	55.4 ± 20.8	14.1 ± 11.8	78.4 ± 5.9	33.9 ± 12.8	37.1 ± 1.6
Shells (%)	1.3 ± 0.0	0.4 ± 0.6	0.7 ± 0.2	0.4 ± 0.2	5.1 ± 1.4	2.3 ± 1.7
TC (mg/g)	35.5 ± 2.7	27.3 ± 6.7	34.7 ± 0.2	24.8 ± 0.2	31.0 ± 0.4	31.8 ± 1.5
TN (mg/g)	1.8 ± 0.6	1.0 ± 0.7	2.1 ± 0.8	0.7 ± 0.0	1.7 ± 0.3	1.3 ± 0.0
TP (µg/g)	647.4 ± 24.4	566.9 ± 70.4	633.1 ± 40.6	513.3 ± 22.9	544.0 ± 16.2	561.2 ± 64.8
Temp (°C)	17.8 ± 0.7	18.9 ± 1.1	18.5 ± 0.3	18.2 ± 1.9	18.8 ± 1.4	18.0 ± 2.0
Salinity	25.6 ± 2.8	26.9 ± 2.7	22.8 ± 0.3	22.8 ± 1.6	26.8 ± 1.9	21.2 ± 0.3
DO (%)	99.8 ± 3.4	112.3 ± 2.5	109.7 ± 15.6	103.2 ± 3.0	108.3 ± 8.8	94.7 ± 4.4
pH	8.3 ± 0.1	8.2 ± 0.2	8.3 ± 0.1	8.0 ± 0.2	8.2 ± 0.1	8.0 ± 0.1

Table 3. Number of stations per Ecological Quality Status in each study year, using different indices.

MaQI			
Status	**2008**	**2009**	**2010**
Poor	15	12	15
Bad	2	5	2
Total	17	17	17

ISD			
Status	**2008**	**2009**	**2010**
Good	0	2	0
Moderate	4	6	2
Poor	16	12	10
Total	20	20	12

M-AMBI			
Status	**2008**	**2009**	**2010**
High	0	0	1
Good	4	3	2
Moderate	7	8	4
Poor	4	4	4
Bad	5	5	1
Total	20	20	12

M-bAMBI			
Status	**2008**	**2009**	**2010**
Good	6	10	4
Moderate	6	2	3
Poor	3	3	1
Bad	5	5	4
Total	20	20	12

Table 4. Spearman correlation coefficients (r_s) between pressure index (PI), and environmental parameters (salinity and oxygen), and indices based on macrobenthic invertebrates (MaQI, ISD, M-AMBI, M-bAMBI), and metrics used to calculate them (AMBI, H, bAMBI, h_b, S). Only abiotic parameters significantly correlated with biotic ones are displayed. *NS*: $p > 0.05$, Significant correlations ($p < 0.05$) in bold.

		Indices				Metrics				
		MaQI	**ISD**	**M-AMBI**	**M-bAMBI**	**AMBI**	**H**	**bAMBI**	**H_b**	**S**
Stressors	**PI**	*NS*	**r_s = 0.31**	**r_s = - 0.48**	**r_s = - 0.36**	*NS*	**r_s = - 0.38**	*NS*	**r_s = - 0.38**	**r_s = - 0.35**
	Salinity	*NS*	*NS*	*NS*	*NS*	**r_s = - 0.63**	NS	**r_s = - 0.6**	*NS*	**r_s = 0.61**
	Oxygen	*NS*	*NS*	*NS*	*NS*	*NS*	**r_s = 0.62**	*NS*	*NS*	*NS*
Indices	**ISD**			**r_s = 0.28**	*NS*	*NS*	**r_s = 0.33**	**r_s = 0.29**	*NS*	*NS*
	M-AMBI				**r_s = 0.61**	*NS*	**r_s = 0.86**	*NS*	**r_s = 0.56**	**r_s = 0.71**
	M-bAMBI						**r_s = 0.50**	*NS*	**r_s = 0.76**	**r_s = 0.79**
Metrics	**AMBI**						*NS*	*NS*	*NS*	*NS*
	H							*NS*	**r_s = 0.39**	**r_s = 0.56**
	bAMBI								*NS*	*NS*
	H_b									**r_s = 0.52**

Table 5. Summary of advantages and disadvantages of the use of different indices and metrics, combining results of present work and literature.

		Advantages	Disadvantages
Indices	**MaQI**	effective also with extremely low macroalgal cover (<5%), robust to variation of salinity, consistent with eutrophication	unable to detect changes in heavily degraded conditions (present work)
	ISD	response to PI, and not to environmental parameters (present work)	
	M-AMBI	response to PI, and not to environmental parameters (present work)	critical uncertainty acrossed the *Moderate/Good* boundary
	M-bAMBI	response to PI, and not to environmental parameters (present work)	critical uncertainty acrossed the *Moderate/Good* boundary
Metrics	**H**	response to PI, and oxygen (present work)	high variability related to seasonality (Reiss and Kröncke, 2005), correlation with confinement (Reizopoulou and Nicolaidou, 2004)
	H_b	use of ecollogicallly relevant information (biomass) (present work, Mistri et al., 2018), response to PI (present work)	
	S	response to PI (present work)	response to salinity (present work)
	AMBI	stable with respect to seasonality (Reiss and Kröncke, 2005)	response to salinity (present work)
	bAMBI	use of ecollogicallly relevant information (biomass) (present work, Mistri et al., 2018)	response to salinity (present work)
	W index	sensitive to disturbance (Clarke and Warwick, 2001)	high within-lagoon variability (present work), biased by recruitment (Beugema, 1988), no discrimination between natural and anthropogenic stress (Clarke and Warwick, 2001; Lardicci et al., 2001)

Figure legends

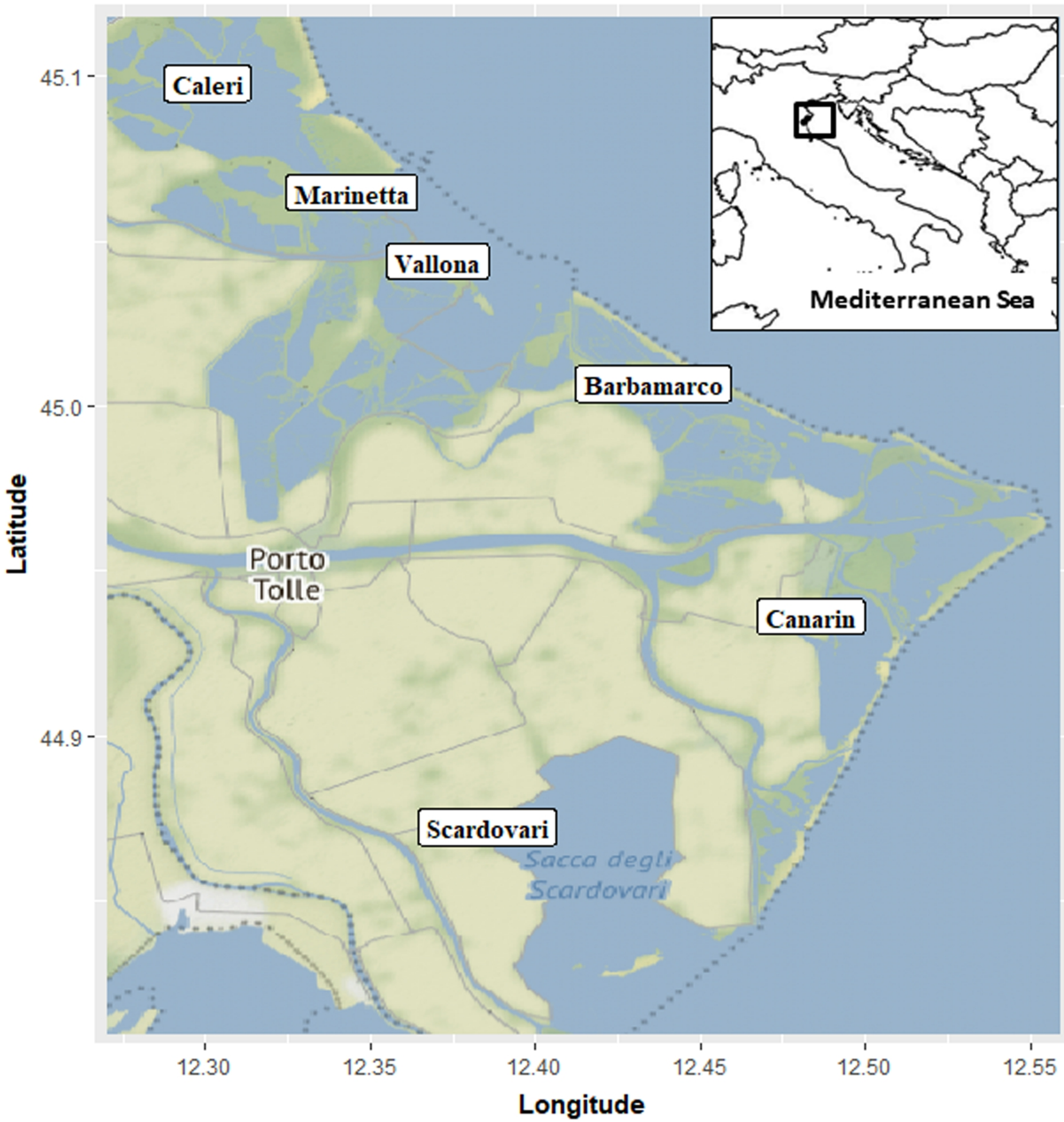
Figure 1. Map of the studied sites

Figure 2. Boxplot showing the distribution of values of MaQI (A), ISD (B), M-AMBI (C), M-bAMBI (D) in each study year. Midline = median; upper limits of the box = third quartile (75th percentile); lower limits first quartile (25th percentile); whiskers = 1.5 times the interquartile range; points = outliers (>1.5 times the interquartile range).

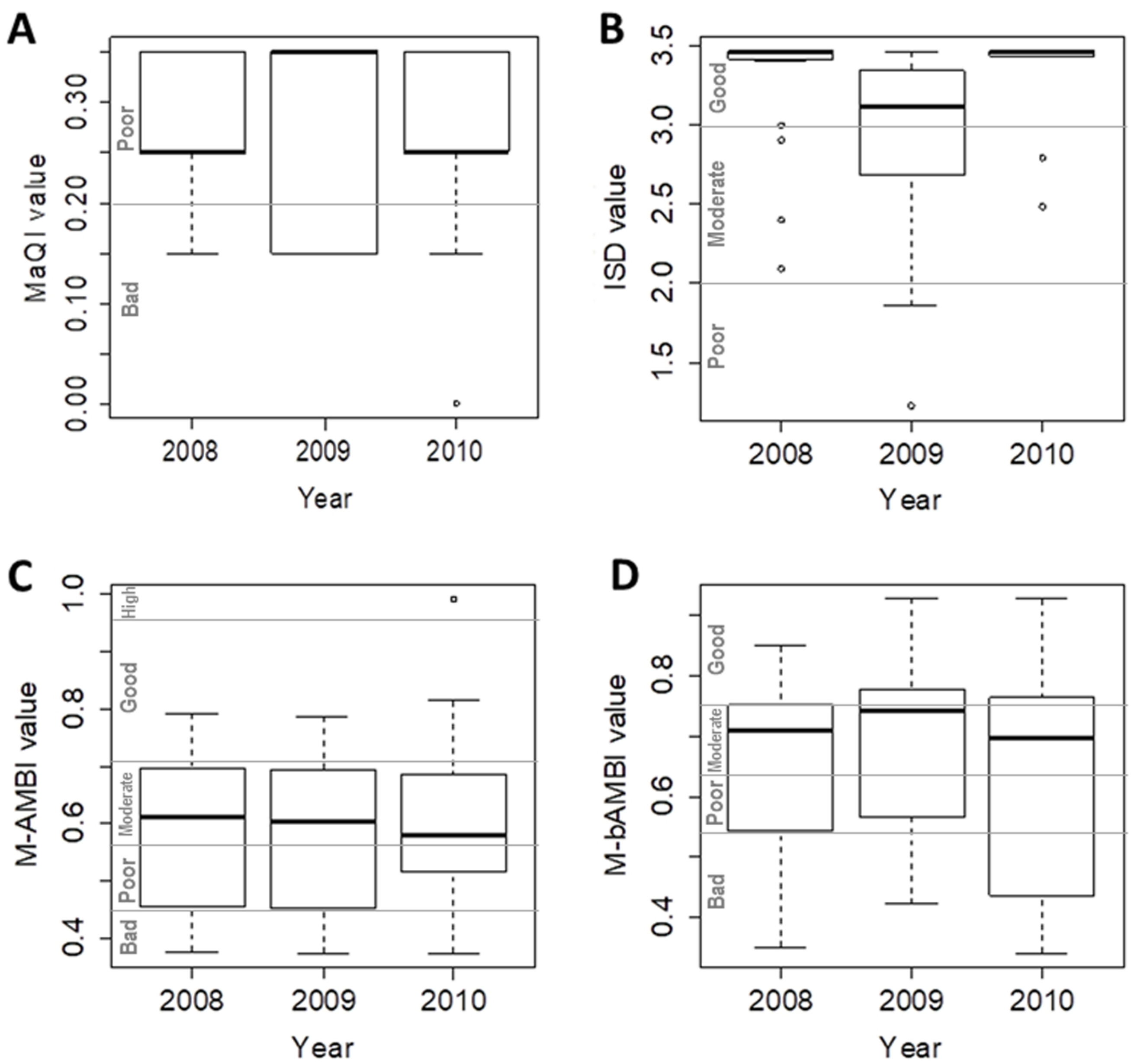
Figure 3. Ecological status (green=good, yellow=moderate, orange= poor, red =bad) of the six analyzed lagoons according to the different biological indices (MaQI, M-AMBI, M-bAMBI, ISD) from 2008 to 2010 (See paragraph 2.4 for thresholds of each index).

Figure 4. Boxplot showing percentage of ecological groups calculated on abundances (A), ecological groups calculated on biomass (B), size classes used for ISD calculation (C), and W statistic (D).

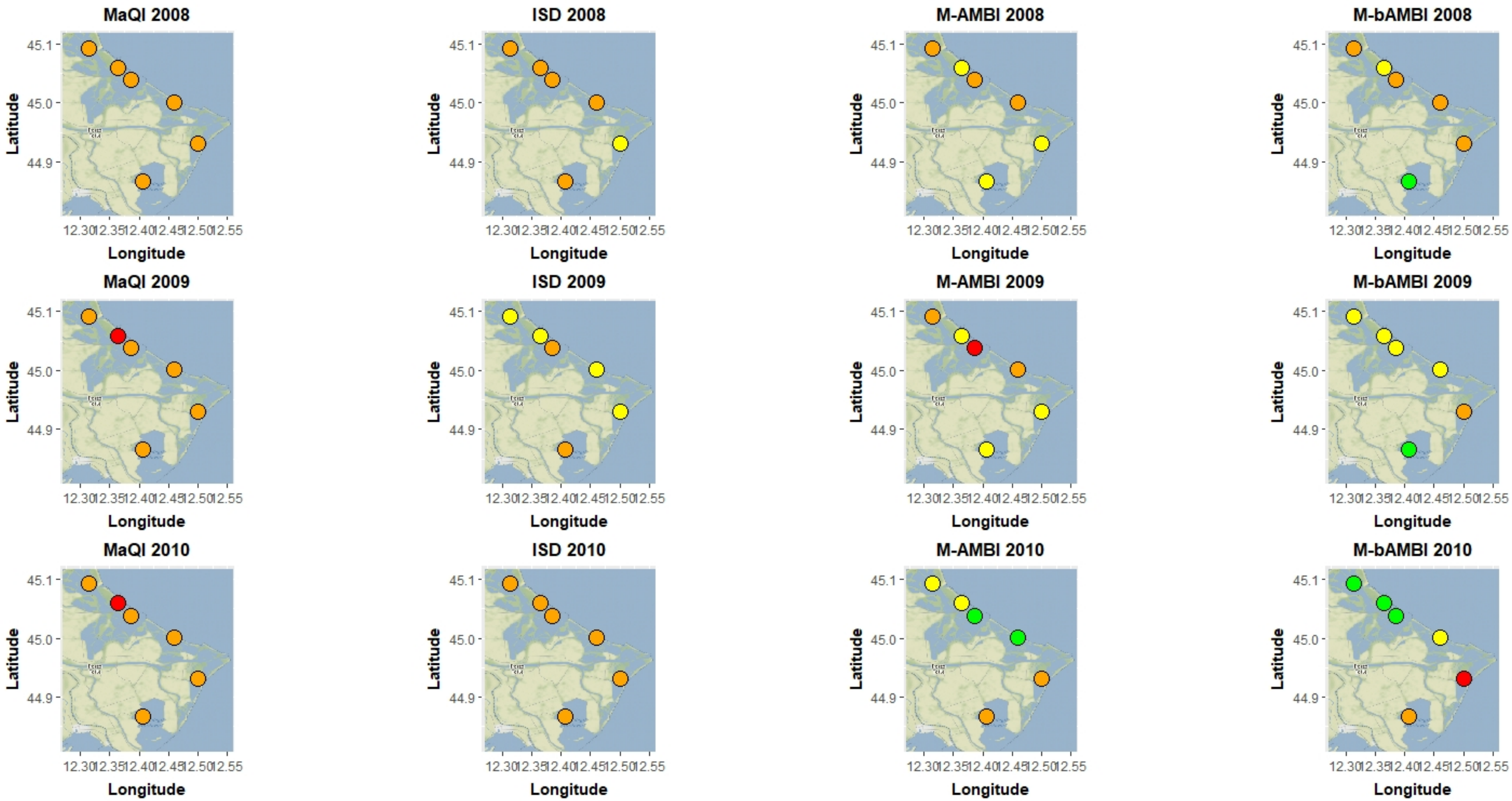
Figure 5. Redundancy analysis showing the relations between environmental factors (blue arrows) and ecological groups (A) calculated on abundances (AB) and biomass (BIO), size classes (B), and biotic indices (C) (red labels). Total inertia: 35.91 (A), 78.283 (B), 1.117 (C); Eigenvalues displayed in figure axes.

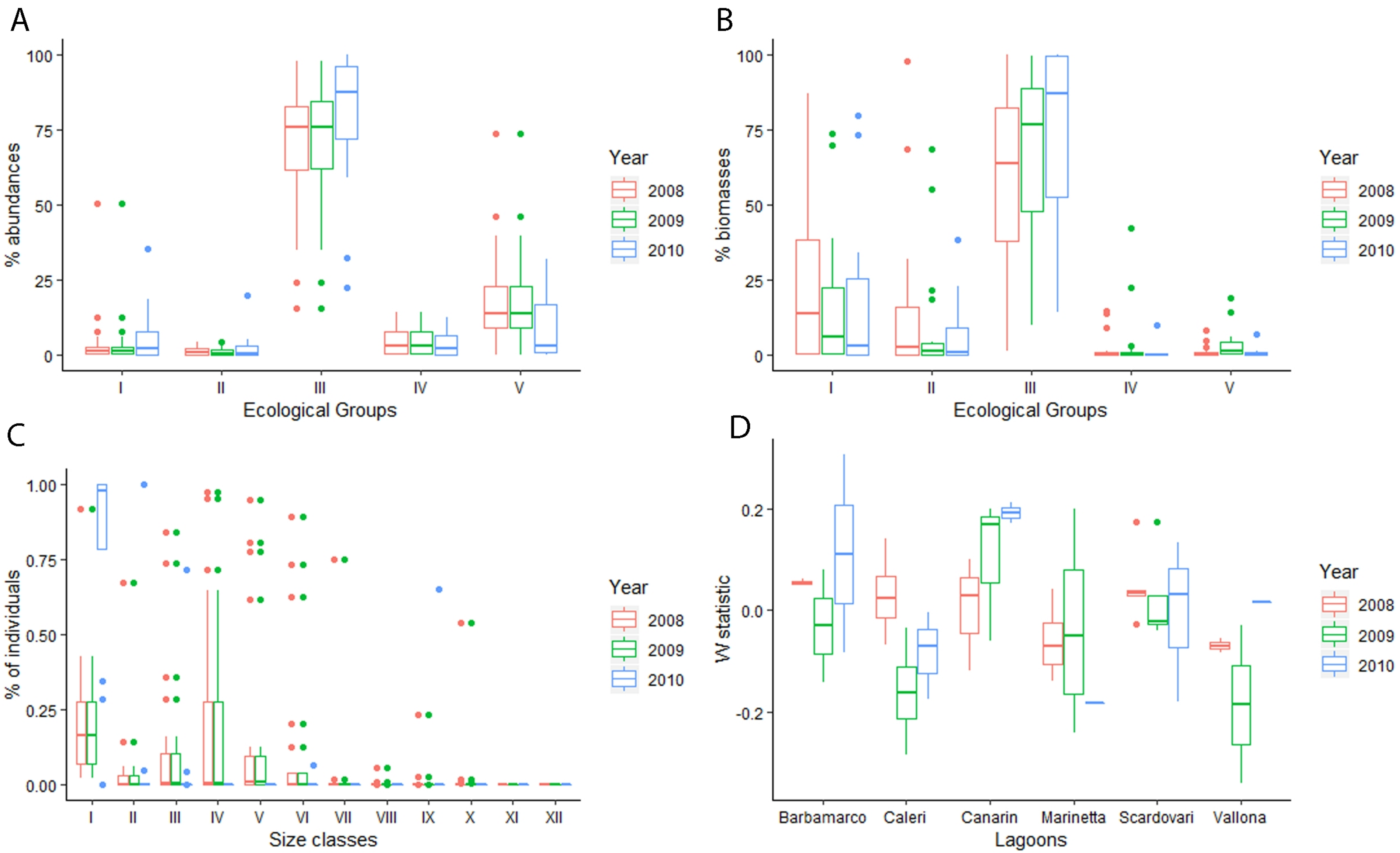


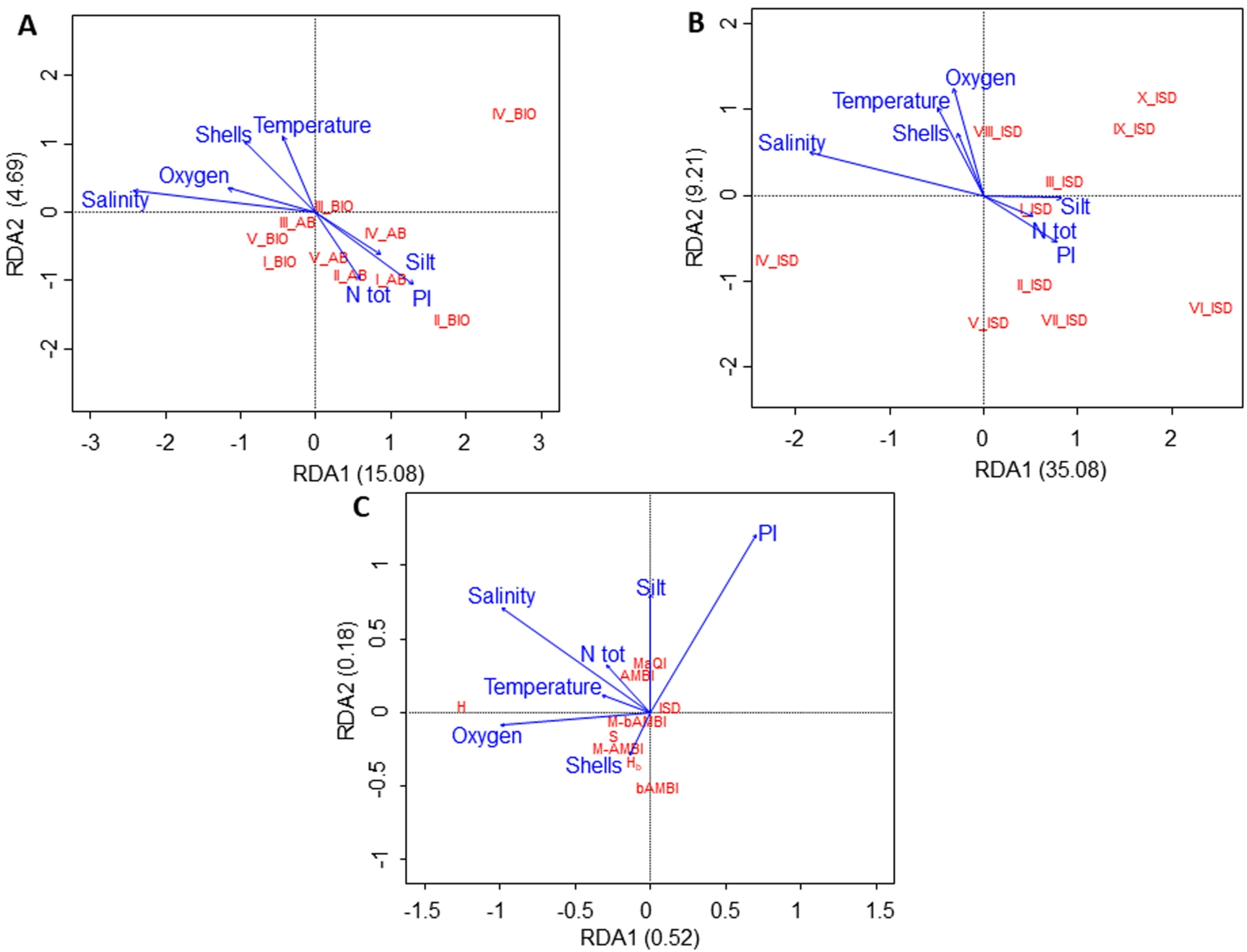
Caleri
Marinetta
Vallona
Barbamarco
Porto Tolle
Canarin
Scardovari
Sacca degli Scardovari
Mediterranean Sea
45.1
45.0
44.9
Latitude
12.30
12.35
12.40
12.45
12.50
12.55
Longitude



A
MaQI value
0.00
0.10
0.20
0.30
Poor
Bad
2008
2009
2010
Year
B
ISD value
1.5
2.0
2.5
3.0
3.5
Good
Moderate
Poor
2008
2009
2010
Year
C
M-AMBI value
0.4
0.6
0.8
1.0
High
Good
Moderate
Poor
Bad
2008
2009
2010
Year
D
M-bAMBI value
0.4
0.6
0.8
Good
Moderate
Poor
Bad
2008
2009
2010
Year


MaQI 2008
ISD 2008
M-AMBI 2008
M-bAMBI 2008
MaQI 2009
ISD 2009
M-AMBI 2009
M-bAMBI 2009
MaQI 2010
ISD 2010
M-AMBI 2010
M-bAMBI 2010
Latitude
Longitude
45.1
45.0
44.9
12.30
12.35
12.40
12.45
12.50
12.55


A
B
C
D
% abundances
% biomasses
% of individuals
W statistic
Ecological Groups
Size classes
Lagoons
Year
2008
2009
2010
Barbamarco
Caleri
Canarin
Marinetta
Scardovari
Vallona


A
B
C
RDA1 (15.08)
RDA2 (4.69)
RDA1 (35.08)
RDA2 (9.21)
RDA1 (0.52)
RDA2 (0.18)
Salinity
Oxygen
Shells
Temperature
Silt
N tot
PI
IV_BIO
III_BIO
III_AB
V_BIO
I_BIO
V_AB
IV_AB
II_AB
I_AB
II_BIO
X_ISD
IX_ISD
VIII_ISD
III_ISD
I_ISD
IV_ISD
II_ISD
V_ISD
VII_ISD
VI_ISD
MaQI
AMBI
H
ISD
M-bAMBI
S
M-AMBI
bAMBI