Comparison of benthic indices for the evaluation of Ecological Status of three Slovenian transitional water bodies (northern Adriatic).

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Abstract

Benthic indicators are important tools for the classification of coastal and transitional water bodies. The aim of the work was to assess for the first time the Environmental Status (ES) of Slovenian transitional waters, comparing the following biotic indices: richness, Shannon-Weaver diversity, AMBI, M-AMBI, BENTIX and BITS indices. A total of 13 stations were sampled with a Van Veen grab, in three ecosystems in the northern Adriatic. Samples were sieved and sorted, invertebrates identified and counted. The anthropogenic impact was estimated with professional judgement. Richness and diversity showed a good response to anthropogenic pressure. Conversely, indices based on sensitivity/tolerance groups did not showed a clear distinction between more and less impacted ecosystems. In particular BENTIX underestimated the ES, while with BITS there was a overestimation. The best evaluation was obtained with M-AMBI, because even if based on a sensitivity/tolerance approach, it considered also the structural aspect of the community.

Keywords: Water Framework Directive; Benthic indices; Ecological Quality Status; transitional waters; northern Adriatic.

1. Introduction

The European Water Framework Directive (WFD, 2000/60/EC) establishes a framework for the protection and improvement of estuarine and coastal waters. According to the WFD, Member States shall protect, enhance and restore all bodies of surface water, with some exception regarding artificial and heavily modified bodies of water, with the aim of achieving Good Environmental Status (GES).

Macrobenthic communities have been proved to be a biological element that can be reliably used for the classification of coastal and transitional water bodies, thanks to their responsiveness to major environmental or anthropogenic changes. For these reasons they are listed among quality descriptors for the implementation of the European Marine Strategy Framework Directive (MSFD, 2008/56/EC), aiming to provide a mechanism for the protection of the marine environment with the ultimate aim to achieve GES of the European marine water bodies by 2020. For this reason, recently the interest on benthic indicators has increased dramatically, with a long list of new indicators proposed (see Diaz et al., 2004 for a review).

For the analysis of macrobenthic communities, besides classical richness/diversity indices, such as Shannon-Weaver diversity index (Shannon and Weaver, 1948), indices based on sensitivity/tolerance of the different species are currently applied. For the Mediterranean Sea, the most frequently used indices such as AMBI (Borja et al., 2000) and M-AMBI (Muxika et al., 2007), have been developed using the data from the coastal marine areas and are mainly used to assess the organic enrichment. Given the fact that coastal lagoons are naturally organic rich systems, subjected to extreme and variable environmental conditions, a high number of tolerant species are expected, suggesting a prudent approach when these indices are applied (Borja and Muxika, 2005; Munari and Mistri, 2008; Reizopoulou et al., 2014). Other indices have been developed, but their application is still limited to a local scale. The BITS index (Mistri and Munari, 2008) was designed specifically for coastal Mediterranean lagoons. It has been successfully applied to lagoons in Western Adriatic Sea (Munari et al., 2009) and is currently listed among the indices to be used for the Environmental Status (ES) assessment of transitional waters, according to Italian legislation. The BENTIX index (Simboura and Zenetos, 2002), have been developed in the Aegean Sea and is currently applied for ES assessment in Greece and Cyprus.

For Slovenian coastal waters (northern Adriatic) ES has been assessed using Ecological Evaluation Index (EEI-c) based on macroalgae for hard bottom (Orlando-Bonaca et al., 2008) and M-AMBI for soft bottom (Orlando-Bonaca et al., 2010). New indices were also developed, such as MediSkew index for the assessment of the status of *Cymodocea nodosa* (Orlando-Bonaca et al., 2015). Nevertheless, ES for transitional waters has not been assessed to date. The Slovenian coastline host some small transitional areas, more or less impacted by anthropogenic activities. The three water bodies considered for this study are: the estuary of Dragonja river, Stjuža lagoon, a small artificial basin rich of seagrasses, and Škocjan bay, a lagoon separated from the sea by the industrial zone of the city of Koper. Benthic assemblages of these areas are understudied and poorly known.

The aim of the present work is twofold: *i)* to provide the first assessment of the ES for these three transitional water bodies along the Slovenian coast; *ii)* to apply and compare the biotic indices most used for benthic invertebrates in the Mediterranean (richness, Shannon-Weaver diversity, AMBI, M-AMBI, BENTIX and BITS), to test their robustness and/or limitations in their application for ES assessment at the three sampled areas.

2. Material and methods

2.1. Study site

The Slovenian coastal sea is situated in the southern part of the Gulf of Trieste, which represents the northernmost part of the Adriatic and the Mediterranean Sea (Fig. 1). The Gulf of Trieste is a shallow semi-enclosed embayment (maximum depth is approximately 33 m), characterized by the largest tidal differences (semidiurnal amplitudes reach 30 cm) and the lowest winter temperatures (below 10 °C) in the Mediterranean Sea (Boicourt et al., 1999). It is shared by three countries, extending from Croatia (Cape Savudrija) to Italy (Grado) and includes the entire Slovenian coast.

Differently from the Italian coast, mainly characterized by soft bottom, where Grado and Marano lagoons are located, the morphology of the Slovenian coast varies from steep rocky cliffs to gradual sloping beaches consisting of gravel and pebbles (Ogorelec et al., 1991), and there are only three small transitional areas: Stjuža lagoon, Dragonja estuary and Škocjan bay.

Stjuža lagoon is part of the Strunjan Nature Park. More than 200 years ago it was an open sea bay. It was artificially closed with a shallow dyke, and used as a fish farm till the beginning of the 20th century (Sajna and Kaligaric, 2005). Nowadays the lagoon is connected with the sea only through a channel. In the area, due to the properties of flysch, there are underground springs (Sajna and Kaligaric, 2005). Despite its artificial origin it is recognized as a site of great ecological importance, for the presence of halophytic habitat types, classified as priority habitats by the Habitat Directive (Sajna and Kaligaric, 2005). The Dragonja River is the largest river on the Slovenian coast that flows into the Adriatic Sea. It is the only Slovenian river flowing entirely over flysch terrain, and the water at the estuary is brackish (Krivograd Klemenčič et al., 2007). Škocjan bay Nature Reserve is the largest brackish wetland in Slovenia. Situated in the urban fringe of Koper, it had been subjected to heavy anthropogenic impact. It is the result of the transformation of the Škocjan Bay, used as a waste disposal site and heavily polluted, that was gradually closed into a shallow semi-enclosed lagoon with a big freshwater input from streams (Nilsson et al., 2014). Its restoration was completed in 2007.

2.2. Field and laboratory work

A total of 13 stations were sampled (Fig. 1**Errore. L'origine riferimento non è stata trovata.**): 5 stations in Stjuža lagoon, 3 in the inner part and two closed to the mouth, 4 stations in Dragonja river, from the mouth towards the land, and 4 stations in Škocjan bay, the first one in the channel connecting the lagoon with the sea and the others closed to freshwater inputs.

Sampling was performed in 2008 and 2009, twice in each ecosystem, in summer and late fall/winter. At each of the sampling station physical-chemical parameters (salinity, temperature, and oxygen concentration) were measured with a probe, and type of bottom sediment was visually estimated.

Three replicate benthic samples were collected at each station with a Van Veen grab (0.27 and 0.14 m^2). The number of replicates was considered sufficient for the ES assessment using biotic indices (Mavrič et al., 2013). The samples were sieved through a 0.5 mm mesh and preserved in 4%

formalin. In the laboratory, samples were stained with Rose Bengal, macrobenthic invertebrates were sorted, identified to the lowest taxonomic level as possible, and counted. Abundance data were normalised for 1 m^2.

Pressures were quantified (0: no pressure, 1: low, 2: medium and 3: high) for each ecosystem and sampling station, following the approach proposed by Borja et al. (2011), based upon best professional judgment, and a pressure index was calculated as the sum of partial pressures for each station.

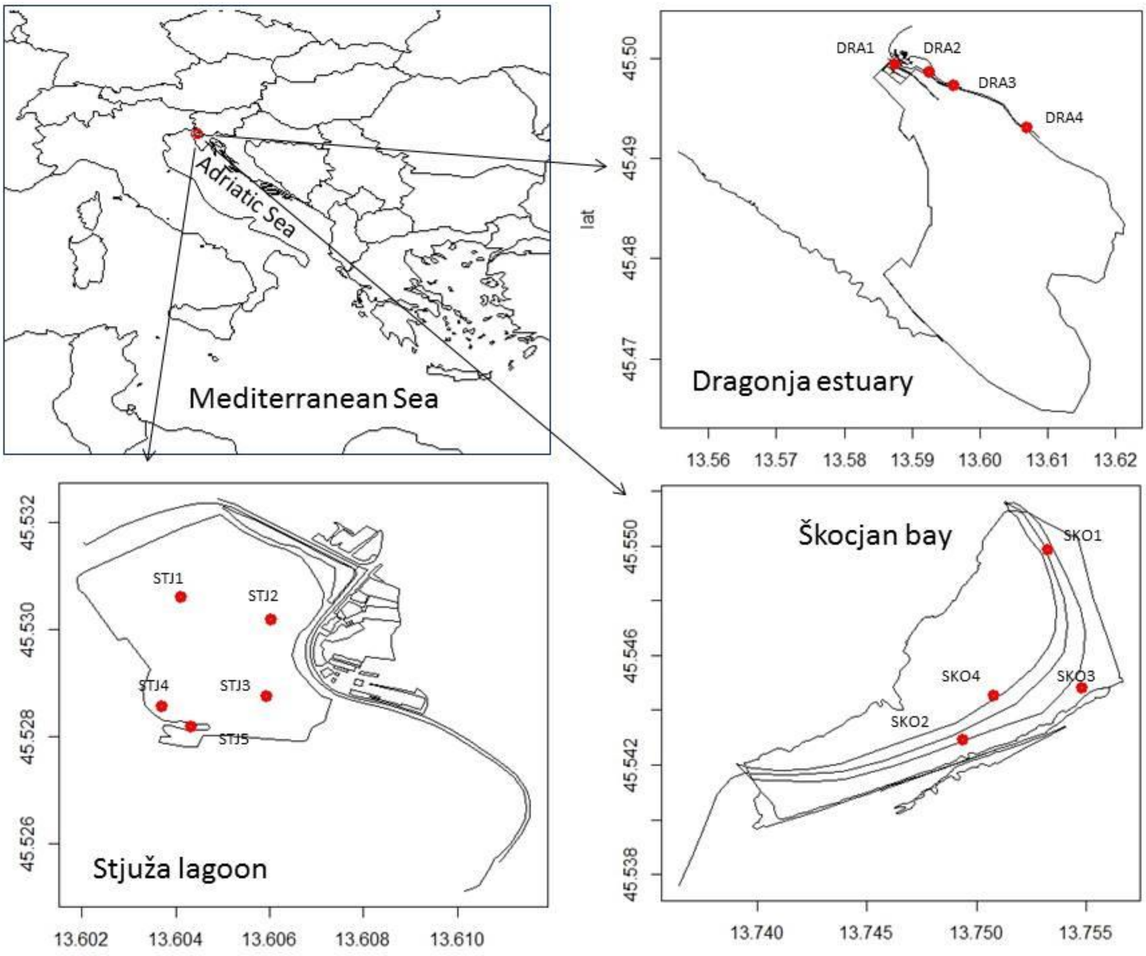

Fig. 1 Map with the three ecosystem and the different sampling stations (DRA : Dragonja estuary; STJ : Stjuža; SKO : Škocjan bay).

2.3 Data analysis

To analyse community composition, the percentage of frequency and abundances were calculated and ranked for each species in each of the three ecosystems.

To observe the spacial distribution of the data a Non-Metric Multidimensional Scaling (MDS) analysis was performed on Bray Curtis similarity matrix (calculated on fourth-root transformed abundance data). One-way Analysis of Similarities (ANOSIM) was performed in order to test the significance of differences among: the different sampling stations, the different sampling periods and the three sampling ecosystems. Species contributing mostly to the dissimilarity among groups were investigated using the SIMPER percentages procedure.

Species abundance data were used to calculate the ecological quality indices most used in the Mediterranean. Among richness/diversity indices we calculated taxa richness (S) and Shannon diversity index on $\log_2$ basis (H).

Multivariate analysis and diversity indices were calculated with PRIMER v6 + PERMANOVA software package, developed in the Plymouth Marine Laboratory (Clarke and Gorley, 2006; Anderson *et al.*, 2008).

Among indices of the sensitivity/tolerance group, the following biotic indices and corresponding Ecological Quality Ratio (EQR) were calculated: AMBI (Borja et al., 2000) and M-AMBI index (Muxika et al., 2007), BENTIX (Simboura and Zenetos, 2002), and BITS index (Mistri and Munari, 2008). AMBI and M-AMBI index were calculated using the free software (http://www.azti.es v.5.0, species list updated in November 2014) along with the guidelines from the authors (Borja and Muxika, 2005); the percentage of invertebrates belonging to the different sensitivity AMBI groups at each sampled station was also calculated. BENTIX was calculated using the free software (Add-in v.1.0 version) for MS Excel (http://www.hcmr.gr/en/articlepage.php?id=141). The BITS index was calculated using the dedicated software (http://www.bits.unife.it). For each index the corresponding EQR was calculated, according to the following reference values: for M-AMBI, H = 3.3, S = 25 and AMBI = 1.85 (non-tidal lagoons), H = 3.4, S = 28 and AMBI = 2.14 (oligo/meso/polihaline lagoons), and H = 4.23, S = 46 and AMBI = 0.63 (eu/iperhaline lagoons); for BITS, 2.8 for non-tidal and 3.4 for microtidal lagoons. The threshold values used were the following: for S ''High/Good'' if S > 25 (non-tidal lagoon), S > 28 (oligo/meso/polihaline), ''High/Good'' if S > 46 (eu/iperhaline); for Shannon index: ''High'' if H' > 4, ''Good'' if 3 < H' ≤ 4, ''Moderate'' if 2 < H' ≤ 3, ''Poor'' if 1 < H' ≤ 2, and ''Bad'' if H' ≤ 1 Vincent et al., 2002; for Ambi ''High'' if BC < 1.2, ''Good'' if 1.2 < BC ≤ 3.3, ''Moderate'' if 3.3 < BC ≤ 5, ''Poor'' if 5 < BC ≤ 6, and ''Bad'' if BC ≥ 6 Borja et al., 2000; 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; for BENTIX index: ''High'' if 4.5 < BENTIX ≤ 6, ''Good'' if 3.5 < BENTIX ≤ 4.5, ''Moderate'' if 2.5 < BENTIX ≤ 3.5, ''Poor'' if 2 < BENTIX ≤ 2.5, and ''Bad'' if BENTIX ≤ 2.5 Simboura and Zenetos, 2002; for BITS ''High'' if BITS ≥ 0.87, ''Good'' if 0.68 < BITS ≤ 0.87, ''Moderate'' if 0.44 < BITS ≤ 0.68, ''Poor'' if 0.25 < BITS ≤ 0.44, and ''Bad'' if BITS ≤ 0.25. Those reference values and thresholds for ES classification were chosen because had been set and used for monitoring of Adriatic lagoons, classified as: oligo/meso/polihaline (Dragonja estuary), eu/iperhaline (Stjuža lagoon) and non-tidal lagoon (Škocjan bay).

To test if the biotic indices showed significant different values among water bodies Chi square test applied to Kruskal-Wallis (KW) ranks was run. In order to test which index gave the best response, the correlation between the EQR of each biotic index and the pressure index (PI) was calculated using Spearman rank correlation coefficients (r_s). A p-value < 0.05 was chosen as threshold for significance. These analyses were performed using R version 2.4.0.

3. Results

2.1 Chemical physical parameters

Stjuža lagoon was characterised by wide extension of healthy seagrass meadow, in Dragonja estuary seagrass meadow was limited to the first stations closest to the sea, while in Škocjan bay the sediment was prevalently silty. Shallow waters characterised all the ecosystems, reaching a maximum of 2 m depth in Dragonja estuary (Table 1**Errore. L'origine riferimento non è stata trovata.**). Average salinity values varied from a minimum of 8.9 (±4.6) ‰ in Škocjan bay to a maximum of 40 ‰ in Stjuža lagoon (Table 1). The temperature varied with the sampling period, ranging from a maximum of 31.4 (±1.0) °C in Stjuža lagoon, to a minimum of 7.3 (±2.1) °C Škocjan bay (Table 1). The highest oxygen values, 13.8 (±0.4) mg/l, was observed in Stjuža lagoon in summer, while the lowest in Dragonja estuary, 6.0 (±1.7) mg/l, and Škocjan bay, 6.2±0.7 mg/l, in the same period (Table 1).

Table 1 Average (± SD) values of abiotic parameters of the three ecosystems: depth range (m), season, salinity (‰), temperature (°C), Oxygen (mg/l).

	Dragonja estuary		**Stjuža lagoon**		**Škocjan bay**	
Sediment	silt and seagrasses		silt and seagrasses		silt	
Depth range	0.5 - 2		0.3 - 0.8		0.5 - 1.3	
Season	Summer	fall/winter	Summer	fall/winter	Summer	fall/winter
Salinity	27.0±2.9	39.0±0.0	30.0±0.0	40.0±0.0	35.3±0.5	8.9±4.6
Temperature	27.1±0.8	17.9±0.4	31.4±1.0	20.0±0.1	23.7±0.7	7.3±2.1
Oxygen	6.0±1.7	7.7±0.5	13.8±0.4	8.0±0.1	6.2±0.7	9.9±0.7

The three studied ecosystems were subjected to different anthropogenic pressures, which were summarized in the pressure index (PI) (Table 2). Škocjan bay was the ecosystem more impacted by anthropogenic activities, with pollutants coming mainly from the surrounding urban area, the port and the agricultural area (Table 2). Stjuža lagoon and Dragonja estuary instead showed low levels of pressures, mainly related with domestic and recreational activities (Table 2).

Table 2. Pressures determined for each ecosystem and sampling station. From 0 to 3: 0 = no pressure, 1 = low pressure, 2 = moderate pressure 3 = high pressure.

Station	**Non-point pollution sources**			**Point pollution sources**			**Habitat loss**	**Ports**			**Fisheries**	**Pressure index**
	Agricoltural inputs	**Freshwater inputs**	**Road traffic**	**Domestic**	**Agricultural**	**Industrial**	**Land-claim**	**Port activity**	**Navigation**	**Dredging**		
DRA-01	0	0	0	0	0	0	0	0	1	0	0	**1**
DRA-02	1	1	0	1	0	0	1	0	1	0	0	**5**
DRA-03	1	1	0	1	0	0	1	0	1	0	0	**5**
DRA-04	1	2	0	1	0	0	1	0	1	0	0	**6**
STJ-01	1	0	0	0	0	0	1	0	0	0	0	**2**
STJ-02	1	0	1	1	0	0	1	0	0	0	0	**4**
STJ-03	1	0	1	1	0	0	1	0	0	0	0	**4**
STJ-04	1	0	1	1	0	0	1	0	0	0	0	**4**
STJ-05	1	0	0	1	0	0	2	0	1	0	0	**5**

SKO-01	1	1	0	0	0	0	3	3	0	0	0	**8**
SKO-02	1	3	3	2	2	2	3	3	0	0	0	**19**
SKO-03	1	3	3	3	3	3	3	3	0	0	0	**22**
SKO-04	1	1	0	1	1	1	3	3	0	0	0	**11**

3.2 Community composition

Altogether 42397 specimens were counted: 29863 in Stjuža lagoon and 11015 at Dragonja estuary, 1519 at Škocjan bay. Specimens belong to 10 different phyla: Cnidaria, Nemertea, Anellida, Arthropoda, Mollusca, Platyhelminthes, Sipunculida and Phoronida (Supplementary material, Table A).

In Stjuža lagoon very frequent and abundant species were the polychaetes *Phylo foetida ligustica* (97% of frequency, 13% of abundances), *Neodexiospira pseudocorrugata* (86% of frequency, 13% of abundances) and the gastropod *Bittium reticulatum* (86% of frequency, 13% of abundances). Very frequent but less abundant were the polychaetes *Notomastus latericeus* (86% of frequency, 1.5% of abundances), *Kirkegaardia dorsobranchialis* (83% of frequency, 2.8% of abundances) and *Capitella capitata* (79% of frequency, 0.5% of abundances), the isopod *Cyathura carinata* (79% of frequency, 0.5% of abundances), the gastropod *Gibbula adriatica* (79% of frequency, 1.6% of abundances).

At Dragonja estuary the most frequent and abundant species was the polychaetes *Cirrophorus furcatus* (96% of frequency, 20% of abundances). Very frequent but less abundant were the polychetes *Kirkegaardia dorsobranchialis* (96% of frequency, 4.3% of abundances), *Aphelochaeta marioni* (79% of frequency, 2.1% of abundances) and *Capitella capitata* (75% of frequency, 0.6% of abundances), and the isopod *Cyathura carinata* (96% of frequency, 2.3% of abundances). The polychaete *Neodexiospira pseudocorrugata* was abundant (15% of abundances) but less frequent (37% of frequency).

At Škocjan bay the most frequent and abundant species was the bivalve *Abra segmentum* (96% of frequency, 27% of abundances), followed by the bivalve *Cerastoderma glaucum* (63% of frequency, 18.5% of abundances) and larvae of Chironomidae (63% of frequency, 33.1% of abundances). Frequent but not abundant was the gastropod *Haminoea hydatis* (52% of frequency, 2.9% of abundances).

Multivariate analyses showed no significant difference among different sampling stations within each ecosystem (ANOSIM test, $R = 0.104$, $p > 0.05$, for Škocjan bay; $R = 0.219$, $p > 0.05$ for Stjuža lagoon; $R = 0.784$, $p > 0.05$ for Dragonja estuary).

Conversely there were significant differences between the two sampling periods (ANOSIM test, $R = 0.716$, $p < 0.01$); in particular this difference was significant only in Škocjan bay (ANOSIM pairwise test, $R = 0.937$, $p < 0.01$). The taxa contributing most to this difference (cumulative dissimilarity contribution 45.36) were larvae of chironomids, *Gammarus aequicauda*, *Cerastoderma glaucum* and *Lekanesphaera hookeri*, all more abundant in the warm period.

Multivariate analyses showed a significant difference among the three transitional zones in both sampling periods (ANOSIM test, R = 0.906, p = 0.01 for summer; R = 0.931, p = 0.01 for fall/winter). Škocjanki bay clearly differed from the Dragonja (97.99 of dissimilarity) and from Stjuža (98.52 of dissimilarity), as the non-Metric Multidimentional Scaling (MDS) analysis showed (Fig. 2). This distinction was due both to species abundance and species composition. Taxa that mostly contributed to this difference (cumulative dissimilarity contribution 44% for Stjuža and 40% for Škocjan bay) were the oligochaetes, the polychaetes *C. furcatus*, *K. dorsobranchialis*, *A. marioni*, *Cauleriella alata*, *N. pseudocorrugata, Notomastus latericeus,* and *Chaetozone zetlandica,* the isopod *C. carinata,* the gastropods *B. reticulatum* and *Gibbula albida*. All these species were absent or less abundant in Škocjan bay. Conversely chironomid larvae, very frequent and abundant in Škocjanki bay during summer, were present only in one sample in Dragonja estuary and were totally absent in Stjuža lagoon.

Benthic communities differed significantly between Stjuža lagoon and Dragonja estuary (ANOSIM test, R = 0.646, $p < 0.01$), as well. This difference (73.67 of dissimilarity) was mainly due to the different abundances of the dominant taxa, namely oligochaetes, the polychaetes *P. foetida ligustica, C. furcatus*, *N. pseudocorrugata* and the gastropod *B. reticulatum* (cumulative dissimilarity contribution 35.12%).

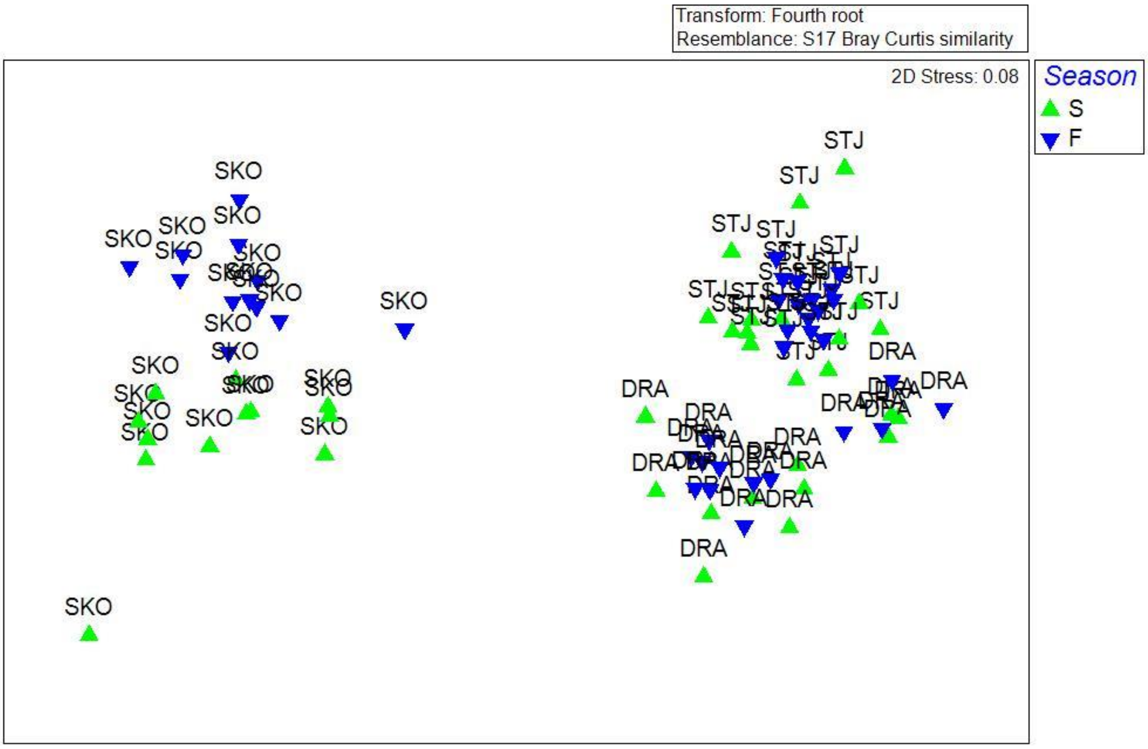

Fig. 2. MDS based on Bray-Curtis similarity computed on fourth root transformed data (DRA : Dragonja estuary; STJ : Stjuža; SKO : Škocjan bay). S=summer, F =fall/winter.

3.3. Richness/diversity indices

Considering taxa richness (S), Dragonja estuary was classified on average as “High/Good” (4 out of the 8 stations reached this score), Škocjan bay as “Moderate/poor/bad” (same score for every stations), while for Stjuža lagoon results from summer and fall samples were clearly different. Considering summer samples the result was “Moderate/poor/bad” (all stations except STJ5), considering fall samples the result was “High/Good” (all stations except STJ5).

In the classification based on overall diversity (H’) instead, Dragonja and Stjuža were both at the border within “Moderate” and “Good”, with average values very close to the threshold. Considering each station separately, 3 out of 8 stations in Dragonja and 3 out of 10 stations in Stjuža were classified as “Good”, the others were “Moderate”. Conversely Škocjan bay was classified as “Moderate/poor”, with only 1 “Good“ station, 3 “Moderate“, 2 “Poor“ and 2 “Bad“.

In Stjuža lagoons and Dragonja estuary (Fig. 3) S and H’ were significantly higher than in Škocjan bay (KW = 16.647, df = 2, p-value < 0.05). In Dragonja estuary values of S and H’ did not differ significantly among seasons but there was a high variability among sampling stations (Fig. 3), with station DRA1 showing the highest values of S, more than twice values of other stations. In Stjuža lagoon values of H’ did not varied with season, but S was on average higher in autumn (Fig. 3). In Škocjan bay values of S did not varied on average, but H’ was higher during the cold season, in particular at stations SKO2 and SKO3.

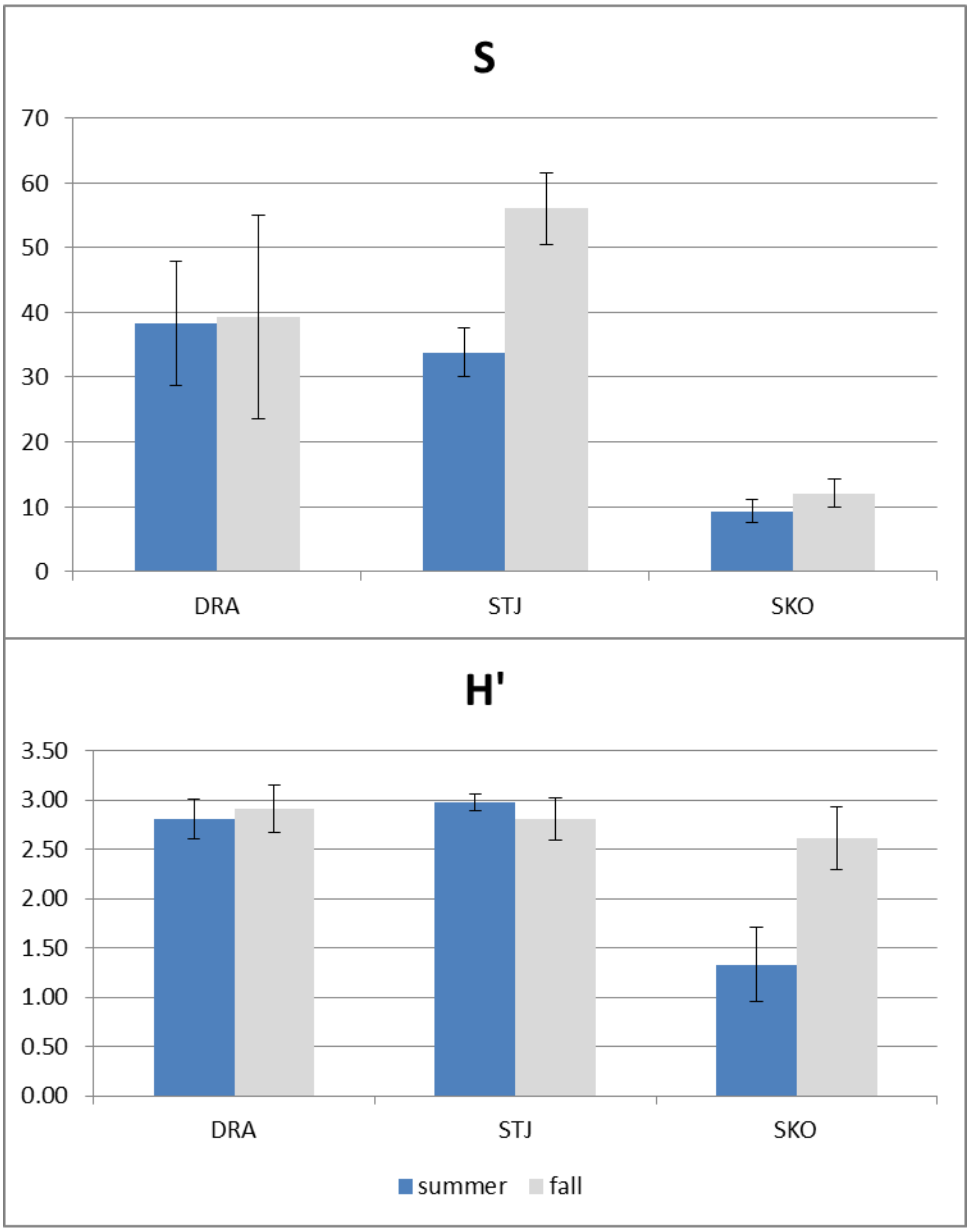

Fig. 3. Average values (±SE) of richness (S) and diversity (H') for each ecosystem in summer and fall (DRA : Dragonja estuary; STJ : Stjuža; SKO : Škocjan bay).

3.4 Sensitivity/tolerance groups-based indices

The evaluation of the ES of the three water bodies differed according to the different biotic indices used (Table 3). Stjuža lagoon showed the most consistent ES, being classified as good by AMBI and BITS, moderate/good by M-AMBI and moderate by BENTIX. Dragonja estuary was classified on average as good by M-AMBI, good/moderate by AMBI, moderate by BITS and poor by BENTIX. Škocjan bay showed the highest variability among indices: it was classified as moderate/high with BITS, good by AMBI, poor/good by M-AMBI and poor/moderate by BENTIX (Fig. 4).

Table 3. Average EQR values (± SE) of biotic indices and respective ES for the three water bodies (DRA : Dragonja estuary; STJ : Stjuža; SKO : Škocjan bay).

	DRA		**STJ**		**SKO**	
	Summer	**Fall/winter**	**Summer**	**Fall/winter**	**Summer**	**Fall/winter**
BITS EQR	1.30 ± 0.19	1.17 ± 0.19	1.61 ± 0.21	1.81 ± 0.15	1.29 ± 0.42	2.95 ± 0.25
BITS ES	M	M	G	G	M	H
AMBI EQR	3.15 ± 0.44	3.31 ± 0.47	2.63 ± 0.39	2.47 ± 0.56	2.79 ± 0.05	2.16 ± 0.23
AMBI ES	G	M	G	G	G	G
M-AMBI EQR	0.86 ± 0.04	0.85 ± 0.09	0.69 ± 0.03	0.75 ± 0.07	0.52 ± 0.06	0.73 ± 0.07
M-AMBI ES	G	G	M	G	P	G
BENTIX EQR	2.26 ± 0.06	2.46 ± 0.23	3.02 ± 0.41	3.05 ± 0.21	2.34 ± 0.07	3.16 ± 0.38
BENTIX ES	P	P	M	M	P	M

Average EQR calculated with BITS index (KW = 5.267, df = 2, p-value > 0.05), AMBI index (KW = 2.820, *df* = 2, p-value > 0.05) and BENTIX index (KW = 4.285, *df* = 2, p-value > 0.05) did not differed significantly among the three ecosystems. The only significant difference was observed with the use of M-AMBI index (KW = 9.265, *df* = 2, p-value = 0.01), which gave an average ES Good for Dragonja estuary and Moderate for Škocjan bay.

EQR calculated with the different indices did not varied significantly with season (BITS: KW = 3.505, *df* = 1, p-value > 0.05; AMBI: KW = 0.632, *df* = 1, p-value > 0.05; M-AMBI: KW = 1.847, *df* = 1, p-value > 0.05; BENTIX: KW = 2.136, *df* = 1, p- value > 0.05).

The most severe classification was obtained with BENTIX index at all the three ecosystems. BITS gave the highest score for Stjuža lagoon and Škocjan bay, while for Dragonja estuary the best score was obtained with M-AMBI.

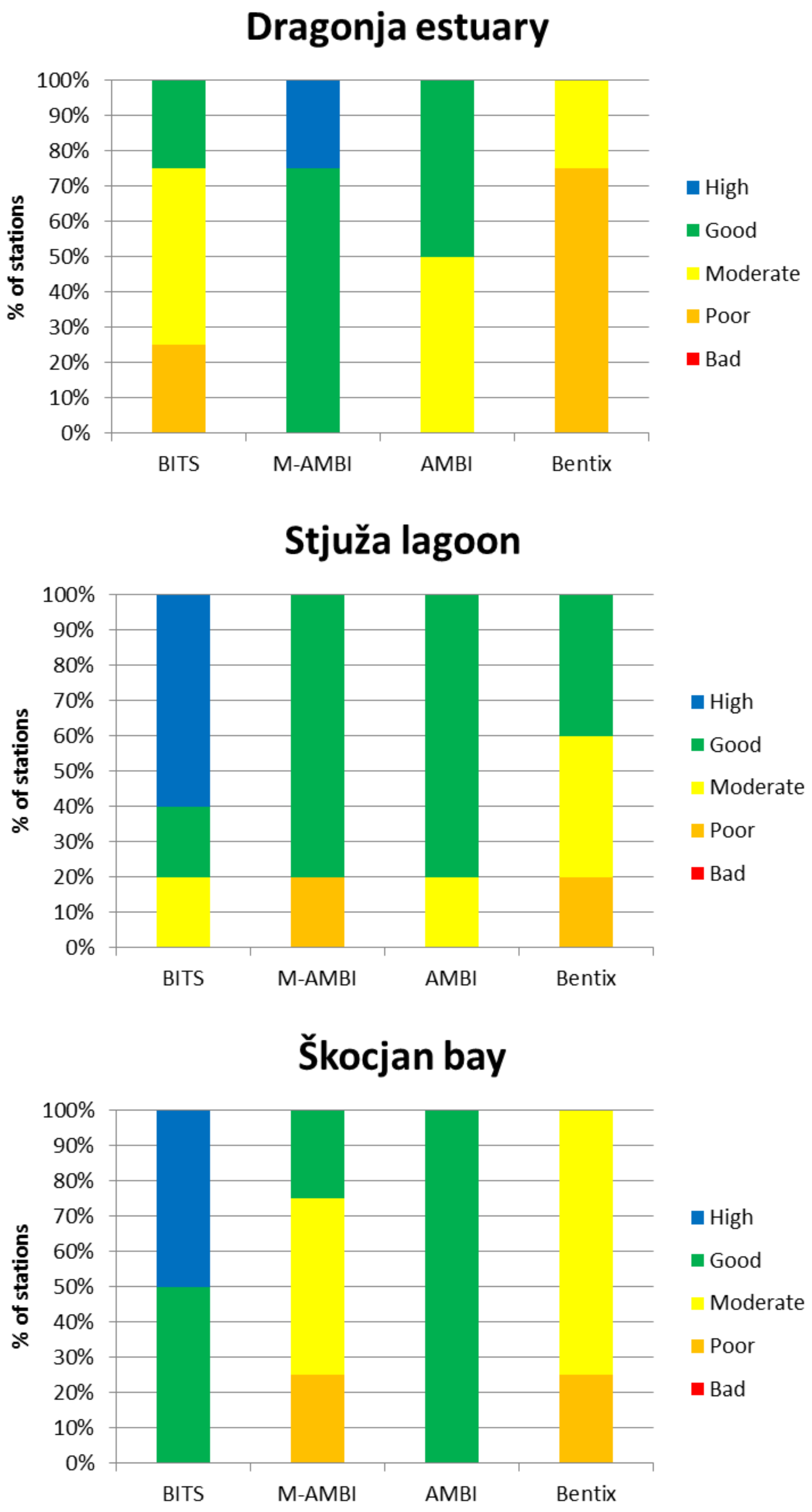

Fig. 4 Comparison of ES classification results derived from the four indices tested in the three ecosystems.

The pressure index (PI) was significantly correlated with taxa richness (S) (Fig. 5E), M-AMBI (Fig. 5C) and Shannon diversity index (H') (Fig. 5F). The best correlation was between PI and S, followed by M-AMBI and H'. Other indices (BITS, AMBI and BENTIX) were not significantly correlated with PI (Fig. 5A,B,D).

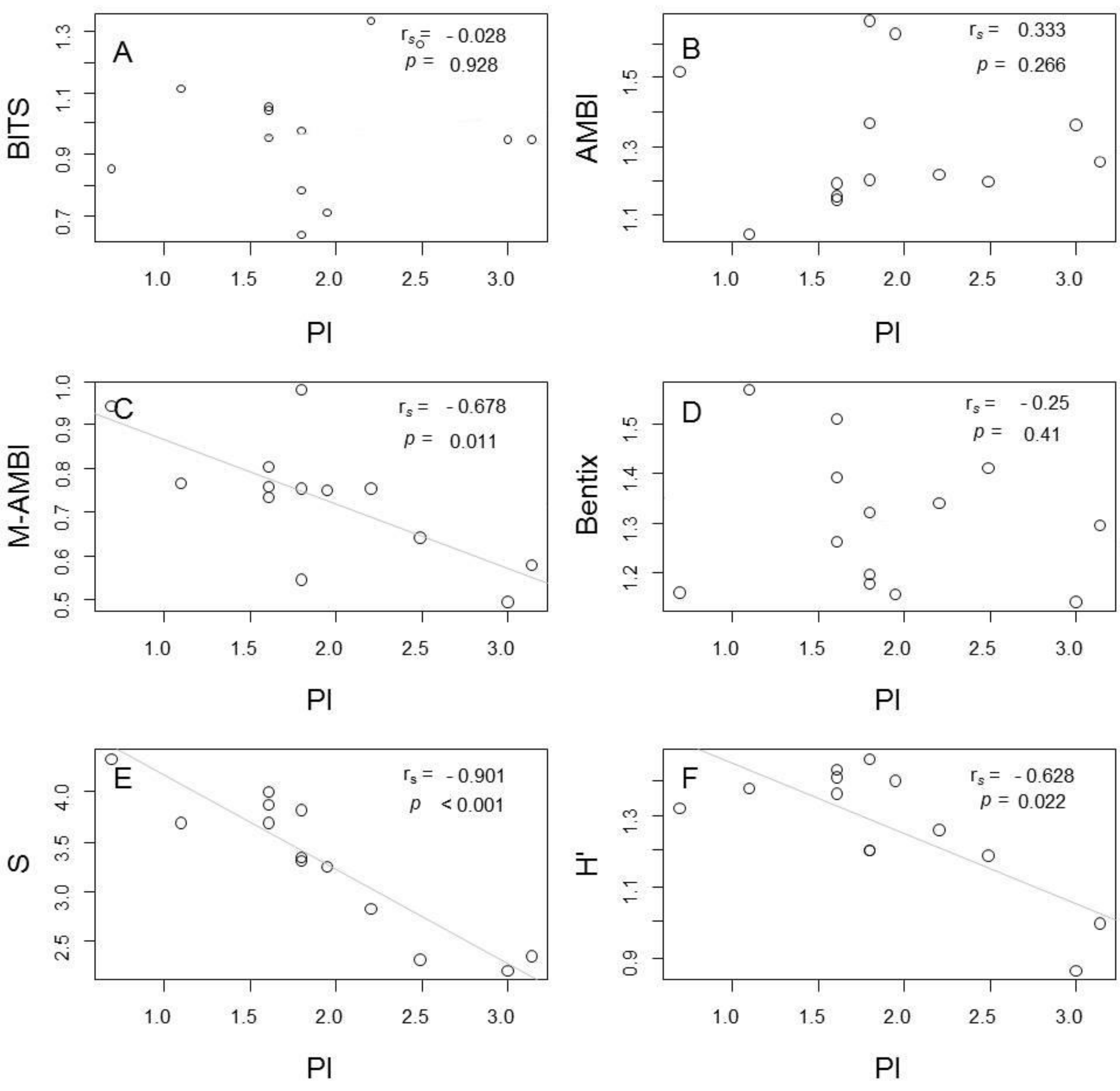

Fig. 5. Spearman correlation coefficient (r_s) and p-value (*p*) between the Pressure Index (PI) and biotic indices: BITS (A), AMBI (B), M-AMBI (C), BENTIX (D), Richness (E), Shannon diversity index (F).

The percentage of invertebrates belonging to the different sensitivity AMBI groups differed among the three ecosystems (Fig. 6). In Škocjan bay there was a dominance of tolerant species (group III), with few species belonging to the other groups. In Dragonja estuary and Stjuža lagoons tolerant species were less abundant, with high percentage of sensitive, indifferent and opportunistic species. The high percentage of first order opportunistic species (group V) in these ecosystems was mainly due to the high abundance of oligochaetes (Table A, Supplementary material). The highest percentages (up to 50%) of sensitive species (group I) were found in Stjuža lagoon (Fig. 6) and were mainly due to the polychaete *Phylo foetida ligustica* and the gastropod *Bittium reticulatum*. Dragonja estuary (Fig. 6) instead was dominated by indifferent species (group II), represented mainly by the spirorbid polychaete *Neodexiospira pseudocorrugata.*

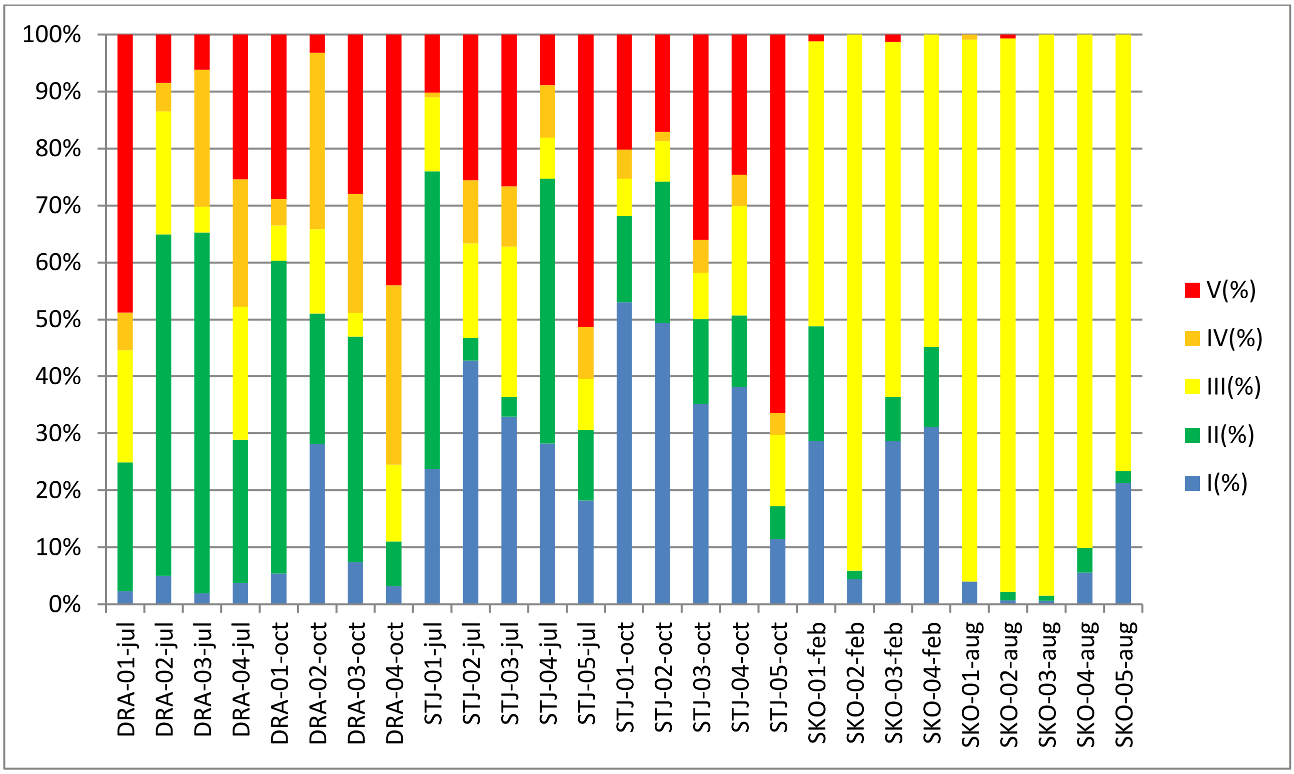

Fig. 6. Percentage of invertebrates belonging to AMBI groups (V = first order opportunistic species, IV = second order opportunistic species, III = tolerant species, II = indifferent species, I = sensitive species) at the different sampling stations.

4. Discussion

4.1 Community composition

The present work represents the first detailed analysis of macrobenthic community of Slovenian transitional waters. Despite the centennial tradition in biological oceanography research in the Gulf of Trieste, the great majority of published works describing macrobenthic communities, were performed in the northern part of the Gulf, which is rather different from the southern part, including the Slovenian coast (Mavrič et al., 2010).

Our results showed that macrobenthic communities clearly differed among the three analysed water bodies. The biggest difference was observed between Škocjanki bay and the other two ecosystems. This difference involved both species composition and distribution and was mainly related with the absence of seagrass meadow and the higher level of confinement in Škocjan bay.

The species contributing most to this difference were typical of Stjuža lagoon and Dragonja estuary, but almost totally absent in Škocjan bay. Some of those species are associated with the presence of seagrasses, such as the spirorbid polychaete *N. pseudocorrugata* and the grazer gastropods *B. reticulatum* and *Gibbula albida*, or with sediment among seagrasses, such as the polychaetes *C. furcatus* (Castelli, 1987). Oligochaetes also heavily contributed to this difference. Seagrass meadows have a great ecological importance in supporting diversity (Boström and Bonsdorff, 1997; Fredriksen et al., 2010), but they are also naturally organic enriched environments for the production of dead leaves (Borja and Muxika, 2005). This explain the extreme frequency and abundance of oligochaetes (Boström and Bonsdorff, 1997; Boström et al., 2010; Fredriksen et al., 2010).

The communities of Stjuža lagoon and Dragonja estuary, showed marine characters mixed with species typical of brackish areas. The isopod *Cyathura carinata*, typical of brackish areas, was found together with species typically associated with seagrasses, such as *B. reticulatum* and *G. adriatica*. Most of the dominant species were present both in Stjuža lagoon and Dragonja estuary, even if with different abundances.

Invertebrate community in Škocjan bay was markedly different from the other two water basins. It was dominated by species characteristic of eurythermal and euryhaline biocoenosis (LEE), namely the bivalves *Cerastoderma glaucum* and *Abra segmentum* (Pérès and Picard, 1964). Moreover, in summer chironomid larvae, typical of the more inner part of the lagoons (fourth zone), were also extremely frequent and abundant. These results were consistent with the high level of confinement and freshwater influence in Škocjan bay.

4.2 Richness / diversity indices

The richness (S) of the community in Dragonja estuary was very high, corresponding to High/Good score, while for Stjuža lagoon High/Good score was reached only in fall. For these two ecosystems the score for diversity (H’) was at the border between “Moderate” and “Good”. The low H’ values, was due to the dominance of oligochaetes and some species of polycheates (*P. foetida ligustica, C. furcatus*, *N. pseudocorrugata*). The high abundance of dominant species in fall also explained seasonal difference in Stjuža lagoon for what diversity is concerned. The higher S value in fall samples did not resulted in a higher H’ value, because dominant species in fall were much more numerous than in summer (in some cases more than one order of magnitude), thus lowering the equidistribution of individuals among species and consequently overall diversity.

Values of S and H’ for Dragonja estuary and Stjuža lagoon were higher than in Škocjan bay. For the classification of Škocjan bay, both indices gave the same result “Moderate/Poor/Bad”. This was due to the low number of species present, together with the dominance of few species typical of eurythermal and euryhaline environments. The higher H’ during the cold season was related not only to a slightly higher species richness, but resulted mainly from the higher abundance of dominant species, in particular chironomid larvae, during the warm season. It is therefore more a reflection of seasonal variation, than an indication of water quality.

In general the values of S and H’ could be related with natural variability, in particular with marine influence. In fact, the highest richness values were found in Dragonja estuary at the most external stations, and the lowest in Škocjan bay, where there was the lowest marine influence. Similar observations have been reported from other Mediterranean lagoons (Reizopoulou et al., 2014). The presence of seagrass meadows in Stjuža lagoon and some stations in Dragonja, was also important in supporting a high diversity of invertebrate community.

4.3 Sensitivity/tolerance groups-based indices

Results of sensitivity/tolerance groups-based indices did not showed a clear distinction between the different water bodies, as was observed with the other analyses. The most problematic aspect, was the fact that in most cases there were uncertainties in the distinction between “good” and “moderate” status. This creates confusion regarding whether remediation measures are needed or not. Moreover there was discordance of ES evaluated with the different indices.

ES based on AMBI index was good for all the three ecosystems, even if the highest percentage of sensitive and indifferent species (groups I and II) was found in Stjuža and Dragonja respectively. In particular in Stjuža lagoon, there were high percentages of sensitive species, which are the first to disappear in case of disturbance (Koutsoubas et al., 2000). High AMBI values for these ecosystems were mainly related with the high abundance of oligochaetes (group V). Conversely the low scores for Škocjan bay, were related to the dominance of tolerant species (group III) and the scarce percentage of opportunistic species, resulting in “Good” ES even with low percentages of sensitive and indifferent species.

The BENTIX index instead gave the lowest scores (moderate/poor) at all sampling stations. BENTIX index underestimates the ES, giving in their formula equal weight to all opportunistic (AMBI groups IV and V) and tolerant taxa (AMBI group III), which naturally dominate the lagoons. This result is consistent with previous investigations in other Mediterranean transitional areas. The fact that tolerant and opportunistic species are weighted equally in the BENTIX formula, leads to underestimation of ES in particular in less disturbed sites (Munari and Mistri, 2010; Reizopoulou et al., 2014). Moreover, there were some species considered indifferent or even sensitive by AMBI classification that were classified as tolerant according to BENTIX classification. This was the case of the polychaete *C. furcatus* (AMBI group II, BENTIX group 2) dominant in Dragonja estuary, the polychaete *P. foetida ligustica* (AMBI group I, BENTIX group 2) dominant in Stjuža lagoon and the spirorbid polychaetes *N. pseudocorrugata* and *S. marioni* (AMBI group II, BENTIX group 2) abundant in both ecosystems. This was the reason for the flattening of ES evaluation towards the “Moderate/Poor” condition, which was particularly strong for Dragonja.

Conversely, BITS index gave higher scores. In particular the ES of Škocjan bay was overestimated with respect to the other ecosystems. This was due to the absence of oligochaetes and other opportunistic species belonging to the third group (III). The low score for Dragonja was mainly due to the characteristic of the index. Since it is based on taxonomic sufficiency, this index gave to the polychaete *C. furcatus* (AMBI group II, BITS group III), dominant in this ecosystem, the same score of other species belonging to the family Paraonidae, which are mainly opportunistic. The result was an underestimation of the ES of Dragonja estuary.

The combination of richness/diversity and sensitivity/tolerant indices, resulting in development of M-AMBI index, discriminated between Dragonja estuary (ES Good) and Škocjan bay (ES Moderate), but failed to classify clearly Stjuža lagoon, with a difference between “good” and “moderate” status in the two different sampling periods. It is important to highlight the fact that the score for summer period was borderline between “good” and “moderate” status. This could be related with the weight given in this index to diversity, which was at the border between “good” and ”moderate” status, as well.

4.4 Response of indices to anthropogenic pressures

The three studied ecosystems were subjected to different levels of anthropogenic pressures. Stjuža lagoon and the Dragonja estuary showed a low level of anthropogenic disturbance, mainly related with domestic and recreational activities. Conversely Škocjan bay was the ecosystem most impacted by anthropogenic activities. Even if the restoration completed in 2007 succeeded in ameliorating physical chemical values (Lipej and Oven, 2009), the degree of confinement, the heavy freshwater influence and the high variability in physical chemical parameters, still represented an extremely stressful environment for macrobenthic fauna. Moreover, rivers Rižana and Badaševica supply the bay with a significant amount of nutrients and some pollutants, namely faecal and industrial wastewaters, fertilizers, pesticides, and toxic elements, such as Cd, Sb, Pb and Hg (Bajt et al., 2006; Frančišković-Bilinski et al., 2007). Road traffic from the nearby highway also affect the area with water runoff, resulting in high levels of hydrocarbons in sediments (Bajt, 2008).

There was no general agreement among the response of the different indices used and this led to contrasting assessments of the ecological status of the same ecosystem, in particular in Škocjan bay. Such a discrepancy among indices has been observed also in other Mediterranean transitional areas (Munari and Mistri, 2008, 2010; Pollice et al., 2014; Reizopoulou et al., 2014), because different indicators, even if based on the same notion, do not interpret the same information in the same way (Ruellet and Dauvin, 2007). Not all indices gave a good response to anthropogenic pressure, in terms of correlation with the pressure index (PI). Overall the best evaluation of ES for Slovenian transitional water bodies was obtained with M-AMBI index. It was significantly correlated with PI and it clearly discriminated between Škocjan bay and the other ecosystems, even if values for Stjuža lagoon were borderline between good and moderate status. The higher suitability of M-AMBI for the analysed dataset was related with the fact that it takes into consideration also the structural aspect of the community (i.e. S and H'). Even if originally based upon sensitivity to organic enrichment and oxygen depletion, M-AMBI have been successfully used to detect different anthropogenic impacts worldwide (e.g. dredging, pollutants...) in both coastal and estuarine ecosystems (e.g. Borja et al., 2000, 2009, 2011, Lopes et al., in press).

An analysis limited to the sensitivity/tolerance of the different species failed to catch the complexity of the community in Stjuža lagoon and Dragonja estuary. In fact, AMBI index alone did not showed a significant correlation with the pressure index and did not discriminated between ecosystems. This failure was mainly related with the characteristics of sensitivity/tolerance based indices and the peculiarity of transitional waters themselves. Transitional waters are naturally characterised by freshwater inputs, and, consequently, low salinity, high organic production and organic inputs resulting in low diversity and high abundances of tolerant species. Following the models based on Pearson–Rosenberg paradigm (Pearson and Rosenberg, 1978), those characteristics coincide with those of anthropogenic stress, and represent a limitation for environmental impact assessment in such ecosystems. The difficulty to discriminate between anthropogenic induced and natural disturbance in such ecosystems was termed "Estuarine Quality Paradox" (Elliott and Quintino, 2007). Borja and Muxika (2005) themselves warned against the potential reduction of robustness of the AMBI index in naturally-stressed ecosystems, such as *Zostera* beds, and pointed out that a combination of different metrics and analyses (such as multivariate), is necessary to establish a good overview of the benthic community health. Despite the generalised success of this index, some authors pointed out that, in transitional waters, analyses of ecosystem structure in relation to human impacts are not sufficient and ecosystem function has to be given more importance (Elliott and Quintino, 2007). Consequently, metrics considering also other parameters, such as biomass, production or size classes data, could improve ES assessment (Basset et al., 2012; Mistri and Munari, 2015). Nevertheless, to date, the response of M-AMBI to natural and anthropogenic disturbance is generally consistent with other indices, also non-taxonomically based (Pollice et al., 2005; Borja et al., 2011), supporting the accuracy of M-AMBI in evaluating lagoon ecological status.

In the present work, diversity-based indices (S and H') showed a good response to anthropogenic pressure, with S showing a correlation with PI even higher than M-AMBI. Nevertheless, the drawback of diversity-based indices is that they depend on the sampling effort, while sensitivity/tolerance-based indices are invariant with the sample size (Dauvin et al., 2010). Moreover, they are influenced by the natural variation of abiotic factors typical of transitional waters (e.g. salinity and water confinement), and consequently often did not provide a proper ES assessment in lagoon ecosystems (e.g. Munari and Mistri 2008; Reizopoulou et al., 2014). Therefore, S and H' alone cannot be used for a correct ES assessment.

BENTIX and BITS indices did not showed a significant correlation with pressure index. BENTIX index underestimated the ES of Stjuža and Dragonja**,** while BITS index overestimated the ES of Škocjan bay. BENTIX index provided good responses in Hellenic transitional waters, in particular for heavily polluted lagoons (Simboura and Reizopoulou, 2008). Nevertheless, as it was already observed for the Italian coasts (Munari and Mistri, 2010), BENTIX resulted inappropriate for eutrophic Adriatic coastal transitional ecosystems, since it was set on oligothrophic Aegean waters, where the benthic fauna is usually very diverse and evenly distributed, with no species dominating over 10% (Simboura and Reizopoulou, 2008). BITS, which was set on Adriatic lagoons, provided a response more similar to AMBI, despite the different level of taxonomic identification needed (at the species level for AMBI and at the family level for BITS), supporting the applicability of the taxonomic sufficiency principle. BITS provided good response to anthropogenic pressure in some Adriatic lagoons (Munari et al., 2008; Munari et al., 2010;) but showed low performances in others (Borja et al., 2011; Pollice et al., 2005). Therefore these two indices still need more investigation and refinement to become satisfactorily operational in transitional environments. Moreover, since they are both based mainly on a sensitivity/tolerance groups approach, the use of other indices (i.e. diversity H' and richness S) in combination could be useful for a correct ES assessment, as suggested by Simboura and Zenetos (2002) for BENTIX index.

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