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Performance and Level of Agreement of Macrozoobenthic Indices in the Shallow Coastal Area of the Southern Bulgarian Black Sea (Burgas Bay)

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Abstract: The performance and level of agreement of the macrozoobenthic biotic indices Shannon-Wiener H', AMBI, M-AMBI and BENTIX, in the shallow coastal areas (up to 13 m depth) of the Burgas Bay (South-Western Black Sea, Bulgaria) were compared. All indices detected the community changes in the anthropogenic pressure gradient in the study area. The agreement was the highest between AMBI and BENTIX and between H' and M-AMBI. AMBI and BENTIX performed better, indicated by their significant negative correlations with levels of eutrophication and an integrated pressure index – the Land Use Simplified Index (LUSI). H' and M-AMBI were not as accurate and M-AMBI showed weaker significant correlations with only some of the pressure indicators. The observed differences in the assessments were likely caused by peculiarities of the index design, as well as reference values unsuitable for the specific shallow coastal habitats. H' and M-AMBI are too influenced by community dominance patterns, which are more pronounced in the shallow coastal zone. The community composition and the proportions of the ecological sensitivity groups could be examined in parallel with the index values for a better evaluation of the overall status of the macrobenthic communities in cases where habitat-specific reference values are not yet established.

Key words: benthic macroinvertebrates, biotic indices, agreement, Bulgarian Black Sea, coastal ecosystems, ecological quality assessment

Introduction

In recent years, the intensive coastal land use has exposed the inshore marine ecosystems to high amounts of anthropogenic pressure from both point and diffuse sources. The impacts of human activities, such as agriculture, transport, industry, tourism, dredging, fishing and aquaculture, on the biodiversity and ecosystem processes are most evident in coastal areas (Halpern et al. 2008). To protect marine habitats, different environmental legislative measures and policies have been developed worldwide. In Europe, two of the most prominent ones, which include or are specifically aimed at the marine environment, are the Water Framework Directive (WFD, 2000/60/EC) and the Marine Strategy Framework Directive (MSFD, 2008/56/EC).

Zoobenthic communities integrate stress effects over time and over multiple types of stressors, and can thus provide site-specific indication of habitat conditions (Abbasi & Abbasi 2012). Consequently, these communities have a long tradition of use in benthic ecological studies and water quality assessments. Benthic biotic indices, which synthesize the complex processes and variability of the ecosystems into a single value, are especially relevant to management efforts under the WFD and the MSFD. Most indices are derived from the taxonomic composition and/or abundance of the taxa at a sampling site and are based on their sensitivity to different kinds of disturbance. The most commonly used benthic indices include classical diversity measures (Shannon-Wiener diversity H', Pielou evenness J');

biotic indices based on species sensitivity traits such as AMBI (Borja et al. 2003), BENTIX (Simboura & Zenetos 2002), MEDOCC (Pinedo & Jordana 2007) and BO2A (Dauvin & Ruellet 2009); multimetric indices combining taxa sensitivity with measures of the structural diversity of the communities, e.g. M-AMBI (Muxika et al. 2007) and UKI and DKI (Borja et al. 2007).

Given the framework nature of the WFD and the MSFD, the choice of metrics for determining the Good Ecological Status (WFD) or the Good Environmental Status (MSFD) is left to each Member State (MS). Accordingly, a vast number of ecological quality assessment methods have been developed and adopted by the different MS, leading to the need to intercalibrate and validate them to harmonize the assessments across the European seas (Birk et al. 2012). Based on the experience gained from the implementation of the WFD, it has been suggested that the indicators already developed and tested for the WFD can be adapted for use in the context of the MSFD (Hering et al. 2010).

The intercalibrations of the macrozoobenthic indices for the Black Sea between Bulgaria and Romania were based on standard sampling stations with depths 15-30 m. The MSFD broad habitat type „shallow sublittoral sediments", with a depth <20m, covers a large part of the southern Bulgarian Black Sea coast. The shallow zoobenthic communities are subjected to more unstable conditions than the deeper ones: higher wave action and temperature, salinity and dissolved oxygen variations, patchiness of the suitable substrates and thus often exhibit mozaic distribution, lower richness and higher dominance of a few species (Marinov 1990). These characteristics could influence the performance of the biotic indices used to assess benthic community status and could lead to ambiguous or misleading results. The shallow coastal communities are often the first to be subjected to the adverse effects of anthropogenic coastal activities, and therefore the most vulnerable ones; they also tend to be the first to recover after termination of impacts. Therefore, the correct assessment of their ecological status is relevant and important for a complete understanding of these effects and, ultimately, the effectiveness of management measures.

The aim of the present study is to contribute to the tuning of the tools for evaluation of Good Ecological/Environmental Status under the WFD/MSFD in the shallow coastal Bulgarian Black Sea. For this purpose, we compared the sensitivity and performance of several uni- and multivariate zoobenthic indices in terms of their ability to detect environmental changes and their effects on community structure and produce a comprehensive assessment of the status of the shallow coastal ecosystems.

Materials and Methods

Study area: Burgas Bay is a semi-enclosed bay in the south-western part of the Black Sea (Fig.1). The city of Burgas is a major source of anthropogenic pressure to the benthic communities in the bay, with urban and industrial land uses, maritime transport, recreation and other activities concentrated in the area. The inner parts of the bay are subjected to high eutrophication pressure, exacerbated by longer water residence times due to the prevailing patterns of circulation (Trukhchev et al. 2004). The bay has suffered extreme algal blooms and consequent hypoxic events and massive benthic community losses in the 1980s (Sukhanova et al. 1988). The current eutrophication levels remain high in the inner bay, gradually decreasing to the south, where the coastline is more open and the water circulation increases (Hiebaum & Karamfilov 2005, Berov et al. 2012). According to recent biogeochemical models, the inner Burgas Bay is exporting nutrients to the adjacent areas and could be considered a permanent, stable diffuse source of eutrophication pressure (Miladinova et al. 2015).

Six sampling stations were selected in the shallow coastal zone of the Burgas Bay (10-13 m depth), with a distance gradient from Burgas in order to capture the whole spectrum of anthropogenic pressure in the study area. The stations were situated within three coastal water bodies (WFD): BG2BS000C008 (stations 1-3); BG2BS000C011 (stations 4-5); BG2BS000C012 (station 6). Station 1 was subjected to a direct effluent of untreated urban wastewater from the Kraimorie area and is in close proximity to the Port of Burgas. Station 2 was situated near the Oil Terminal operated in the area. Station 3 was located near a naval base and close to another untreated urban wastewater point source active during this study. At stations 4, 5 and 6 the main anthropogenic pressures resulted from tourism and leisure activities. Station 6 was situated within a Natura 2000 marine protected area and was near the national reference site for pristine marine conditions (Maslen nos). It was supposed to be closest to the natural undisturbed conditions.

Environmental parameters: Three samplings were conducted during the study: in June and September 2013 and in July 2014. At each station, the total organic matter content of sediments (%TOM, as weight loss on ignition at 520°C); nutrients (P-PO_4^{-3}, N-NO_3^-, N-NH_4^+, total N), chloro-

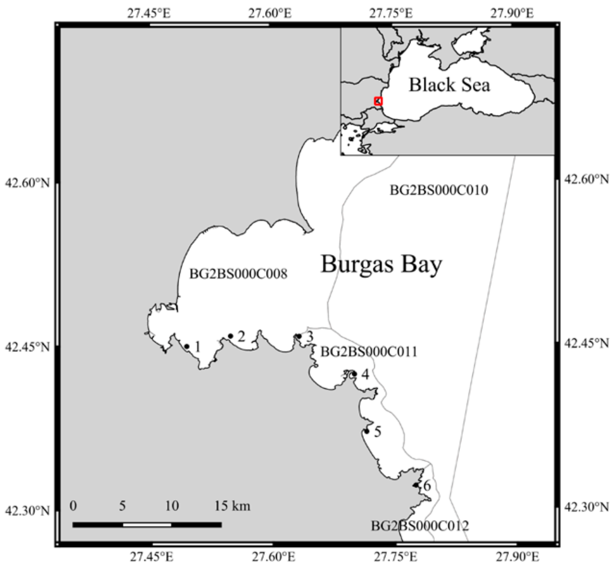

Fig. 1. Map of the study area and sampling stations. 1 - Kraimorie, 2 - Chukalya, 3 - Akin, 4 - Sozopol, 5 - Agalina, 6 - Paraskeva. The codes of the coastal water bodies (WFD) are also shown.

phyll-*a* and suspended particulate matter concentrations in the water column according to Grasshoff (1976); and water transparency (Secchi depth) were measured. The dissolved oxygen concentration near the bottom was measured with a Multi 197i instrument with a CellOx 325 O_2 electrode (WTW). The grain size composition of the sediments was determined by wet sieving as the proportions of gravel (> 2 mm), sand (0.063 – 2 mm), and silt and clay (< 0.063 mm) (Erftemeijer & Koch 2001). The mean grain size (µm) was determined statistically.

The Land Use Simplified Index (LUSI) (Flo et al. 2011) was used to quantify the level of anthropogenic pressure in the study area. LUSI was calculated based on the Corine Land Cover (2012) dataset on land use in the 3000-m wide strip in the catchment area along the coastline, for watersheds adjacent to the sampling sites to account for strong local influence of nutrient inputs from land by wetlands and rivers. Correction factors for indirect impacts (wastewater treatment plants, untreated waters, river inputs), proximity to major ports and tourist centres and proximity to water bodies in a degraded state, were also applied. Higher LUSI values indicate a higher amount of pressure exerted on the marine environment.

Macrozoobenthos: At each station, three replicate macrozoobenthic samples were collected using a Van Veen grab with sampling surface 0.05 m^2. The samples were sieved with a 0.5 mm-mesh sieve and fixed in a 10% formaldehyde-seawater solution. The organisms were hand-sorted in the laboratory under a stereomicroscope, identified to the lowest possible taxonomic level and counted.

Data analysis: The quantitative structure (species richness and specific abundance in individuals.m^{-2}) and taxonomic composition (proportions of the groups Mollusca, Crustacea, Polychaeta and Varia) of the benthic communities at the stations were determined.

The changes in community composition in the study area were explored through non-metric multidimensional scaling (nMDS) and the validity of the grouping was tested through analysis of similarities (Anosim). The environmental variables were fitted to the ordination in order to explore their relationship with the observed grouping. To overcome the characteristic considerable variability of water column parameters, we used long-term averages of monitoring data available for the nutrient, chlorophyll-*a* and suspended matter concentrations and the Secchi depth (Berov et al. 2012). Cluster analysis by k-means clustering and subsequent indicator species analysis (Dufrêne & Legendre 1997) were applied to both abundance and biomass data to determine the most characteristic species for each cluster. Based on the results, the stations were then classified into the national soft sediment biotope types (Todorova 2017).

The ecological status of the benthic communities based on biotic indices was evaluated. The selected indices were those adopted for the national monitoring programs for the WFD and MSFD in Bulgaria and Romania: the biodiversity index Shannon-Wiener H’, the species sensitivity index AMBI and the multimetric index M-AMBI. We also tested the sensitivity index BENTIX, commonly used in the Eastern Mediterranean Sea, which responds effectively to different kinds of anthropogenic pressure (Zenetos et al. 2005).

AMBI classifies species into five ecological groups according to their tolerance to increasing ecological stress: from very sensitive (Group I) to opportunistic (Group V). BENTIX (a modification of AMBI) only considers two ecological groups: a sensitive and a tolerant one. M-AMBI integrates AMBI, H’ and species richness S through factorial analysis. Its calculation requires reference high and bad status values for all component metrics. Here we used the following: for high status H’ = 4, S = 40, AMBI = 1.2 and for bad status H’ = 1.3, S = 15, AMBI = 5.5 (Trayanova et al. 2008).

AMBI and M-AMBI were calculated using AZTI’s free software tool (http://ambi.azti.es). BENTIX was calculated using an add-in for MS Excel 2007 (version 1.1, http://www.hcmr.gr/gr/listview3.php?id=1195).

Table 1. Ecological Quality Status boundaries for the tested indices (EQR).

Ecological Quality Status boundaries (EQR)	AMBI	BENTIX	M-AMBI	H'
High/Good	0.83	0.75	0.85	0.89
Good/Moderate	0.53	0.58	0.55	0.69
Moderate/Poor	0.39	0.42	0.39	0.49
Poor/Bad	0.21	0.00	0.20	0.29

Table 2. Water column and sediment parameters and pressure index LUSI at the sampling stations. Mean values ± SE.

Station	%TOM	%silt-clay	Mean grain size (µm)	*Chl-*a* (µg.L^{-1})	*Suspended particulate matter (mg.L^{-1})	*N-NH_4^+ (µg.L^{-1})
1	2.67 ± 0.35	7.48 ± 3.40	230.77 ± 6.11	3.23 ± 0.22	2.29 ± 0.19	1.10 ± 0.17
2	1.71 ± 0.04	2.99 ± 0.67	125.38 ± 48.87	3.09 ± 0.23	2.44 ± 0.22	0.97 ± 0.12
3	1.28 ± 0.07	2.68 ± 0.45	75.09 ± 0.01	3.10 ± 0.18	2.21 ± 0.16	0.86 ± 0.08
4	1.51 ± 0.05	3.21 ± 0.82	74.99 ± 0.11	2.85 ± 0.24	1.54 ± 0.10	0.69 ± 0.05
5	2.10 ± 0.14	1.56 ± 0.49	244.29 ± 1.50	2.63 ± 0.21	1.33 ± 0.07	0.75 ± 0.08
6	2.10 ± 0.42	1.10 ± 0.22	75.22 ± 0.01	2.29 ± 0.17	1.25 ± 0.07	0.70 ± 0.06
Station	*N-NO_3^- (µg.L^{-1})	*Total N (µg.L^{-1})	*P-PO_4^{-3} (µg.L^{-1})	O_2 bottom (mg.L^{-1})	*Secchi depth (m)	LUSI-3000
1	2.63 ± 0.39	19.48 ± 1.47	0.34 ± 0.08	5.82 ± 1.40	3.25 ± 0.17	6.25
2	2.37 ± 0.36	19.11 ± 1.55	0.32 ± 0.09	5.11 ± 0.81	4.36 ± 0.23	4.00
3	2.10 ± 0.26	17.39 ± 1.09	0.28 ± 0.06	7.45 ± 0.50	5.10 ± 0.15	4.00
4	2.72 ± 0.44	18.41 ± 0.97	0.18 ± 0.03	7.94 ± 0.10	6.33 ± 0.18	2.00
5	2.66 ± 0.39	18.21 ± 0.97	0.16 ± 0.03	6.98 ± 0.42	6.99 ± 0.19	2.00
6	2.22 ± 0.29	16.33 ± 0.73	0.14 ± 0.01	7.48 ± 0.18	7.35 ± 0.18	0.75

*- parameters for which long-term monitoring data (2009-2011, 2013-2014) were used in order to reduce the bias introduced by their typically high variability.

The index ecological quality ratios (EQR), the ratio between the observed value and the value of the same metric under reference conditions, were calculated and normalised according to van de Bund et al. (2008) to allow a direct comparison between indices. The class boundaries for the index EQRs used in this study are given in Table 1. For H', AMBI and M-AMBI, the boundaries valid for the Bulgarian Black Sea during the study period were used (Trayanova et al. 2008); H' values are those for habitats with predominantly sandy substrate in accordance with the type of substrate at the stations. BENTIX class boundaries are according to Simboura & Zenetos (2002).

The differences in index EQRs between stations, as well as between biotopes, to test for natural variability due to habitat differences at the different stations, were tested using robust regression by iteratively reweighted least squares (IRLS) with covariance estimation for heterogeneous group variances to compensate for the presence of influential observations in the AMBI, H' and M-AMBI series (Herberich et al. 2010).

The level of agreement between indices was determined through the absolute average class difference between assessments, using a threshold of sufficient comparability of <0.5 classes difference (van de Bund et al. 2008). Spearman rank correlations between index EQRs and environmental parameters were calculated in order to analyse the observed behaviour of the indices and the validity of the assessments. If an index functions correctly, its EQR is expected to decrease when the amount of pressure increases (negative correlation with eutrophication parameters and pressure indicators) and vice versa.

All statistical analyses were performed using the software package R (R Core Team 2015) and additional contributed packages.

Results

There was a general trend of decrease in the amount of anthropogenic pressure towards the outer part of the Burgas Bay, clearly indicated by the decrease in LUSI and levels of eutrophication (long-term water column nutrient concentrations, chlorophyll-a and suspended matter concentrations) and the increase in water transparency (Secchi depth) and dissolved oxygen (Table 2). The sediment characteristics at the sampling stations were variable; the percentage of silt and clay was the only parameter that decreased at the outer Burgas Bay stations. The mean grain size at the sampling stations differed: the sediments were coarser at stations 1, 2 and 5 and finer – at stations 3, 4 and 6.

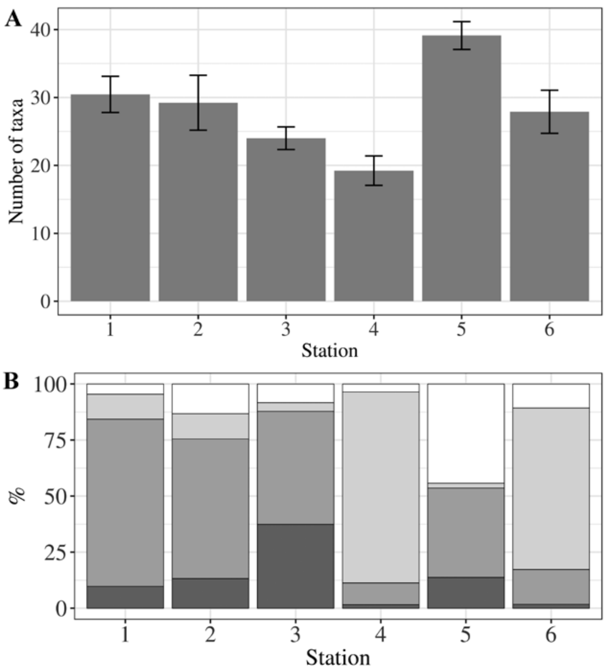

Fig. 2. Structure and taxonomic composition of the macrozoobenthic communities from the Burgas Bay (average for 2013-2014). A: Number of taxa; B: Proportions of the main taxonomic groups.

The structure and composition of the communities changed along the anthropogenic pressure gradient (Fig.2). The number of taxa was higher at the inner Burgas Bay stations 1 and 2, as well as at station 5 and decreased at the outer stations. There was a decrease in the proportion of the more tolerant groups (polychaetes and the group Varia, composed predominantly of oligochaetes) and an increase in the proportion of the more sensitive molluscs and crustaceans, towards the outer Burgas Bay stations. The taxonomic composition of station 5, dominated by crustaceans and polychaetes, differed from all other stations.

Four main groups can be distinguished on the nMDS plot (Anosim, $R = 0.949$, $p = 0.001$) (Fig.3), which is also the optimum number of clusters suggested by the cluster analysis.

These four groups reflect the natural variability in community composition due to the habitat differences. According to the sediment characteristics and the indicator species analysis (Suppl. 1), they correspond to the following biotopes: Infralittoral medium and coarse sand dominated by *Upogebia pusilla* (station 3); Infralittoral fine and medium sand dominated by *Chamelea gallina*, *Lentidium mediterraneum*, *Tellina tenuis* (stations 4 and 6); Infralittoral sandy muds dominated by *Melinna palmata*, *Anadara kagoshimensis*, *Heteromastus filiformis* (stations 1 and 2); Infralittoral mixed sediments with diverse fauna (station 5). The anthropogenic pressure gradient also contributes to the

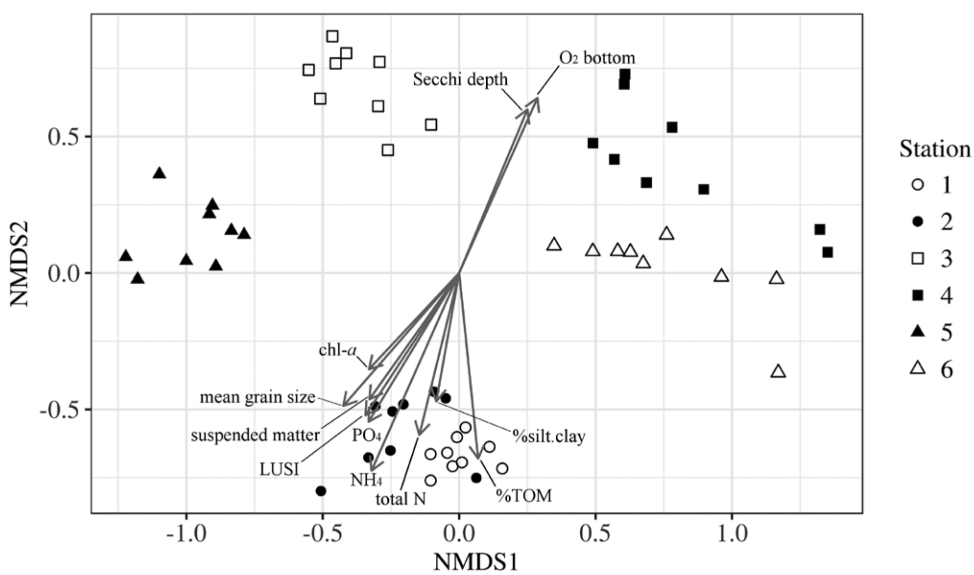

Fig. 3. nMDS plot of the Burgas Bay macrozoobenthic communities (Bray-Curtis distance, stress = 0.16), with fitted vectors of the environmental and pressure parameters that best correlate ($p < 0.05$, based on 999 permutations) with the observed dissimilarities.

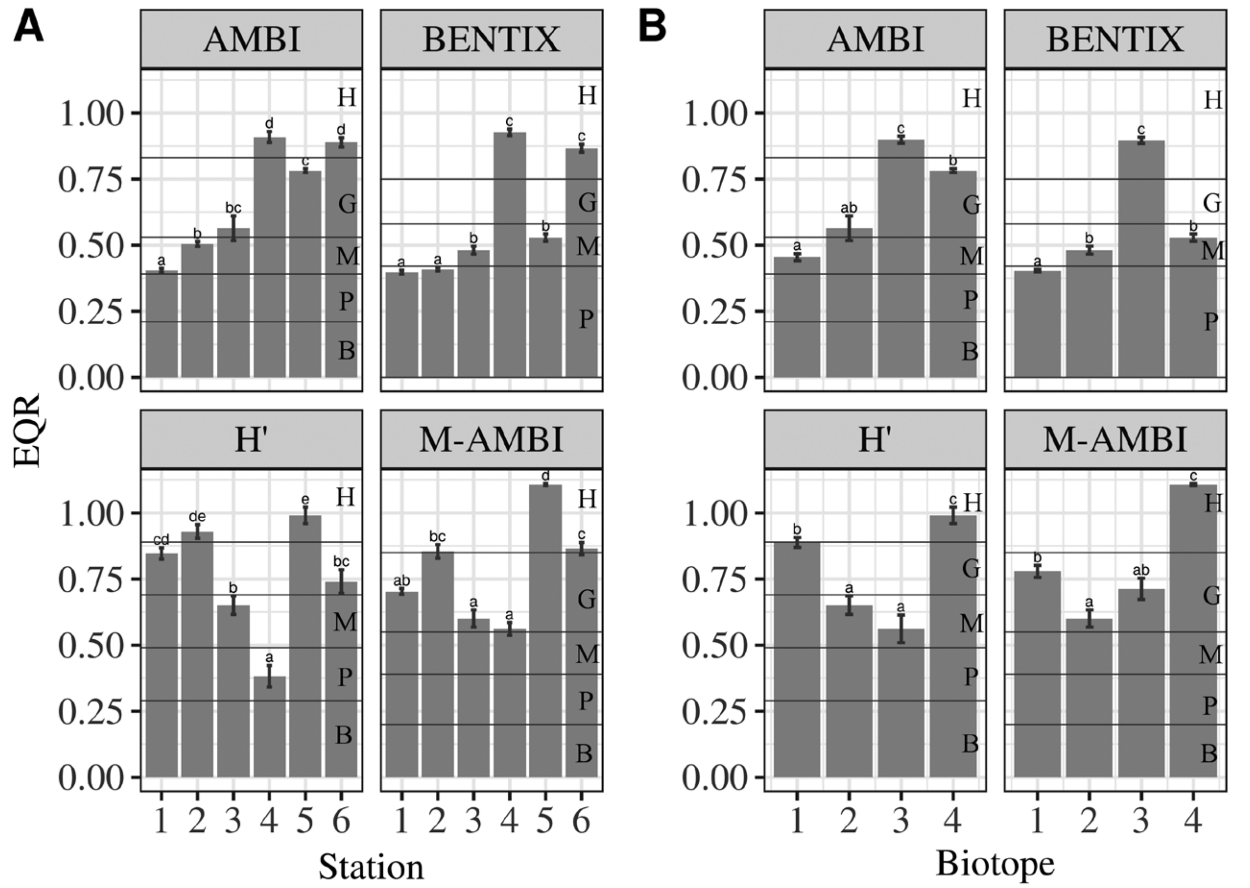

Fig. 4. EQR values of macrozoobenthic biotic indices in the shallow coastal areas of the Burgas Bay (2013-2014); A – by station, B – by biotope. Biotope codes used: 1 – Infralittoral sandy muds dominated by *Melinna palmata*, *Anadara kagoshimensis*, *Heteromastus filiformis*; 2 - Infralittoral medium and coarse sand dominated by *Upogebia pusilla*; 3 - Infralittoral fine and medium sand dominated by *Chamelea gallina*, *Lentidium mediterraneum*, *Tellina tenuis*; 4 - Infralittoral mixed sediments with diverse fauna. Horizontal lines represent ecological status limits for each index; H = high, G = good, M = moderate, P = poor, B = bad. Values are means ± SE. Lowercase letters above bars denote significant ($p < 0.05$) differences between stations/biotopes in IRLS regression with Tukey post-hoc multiple comparison test and covariance estimation consistent with heterogeneous group variances.

separation of the samples along the vertical axis, as evidenced by the fitted vectors of the environmental parameters (Fig.3). The most impacted stations (1 and 2) are grouped together in the lower part of the graph, where the values of LUSI, nutrient concentrations and sediment organic matter content are higher. The least impacted stations (4 and 6) are in the upper part of the graph. Station 3 is close to the less impacted stations and station 5 is close to the impacted stations, at an intermediate level of disturbance.

The average EQR values of the biotic indices for the study period differed significantly between stations (Fig.4A), as well as between biotopes (Fig.4B) (IRLS regression, $p < 0.05$).

All benthic indices detected the changes in community structure and composition in the study area but their performance was affected significantly by the natural variability of the communities between biotopes. AMBI and BENTIX reflected the pressure gradient accurately, with increasing ecological status assessments for the less impacted stations. By contrast, H’ and M-AMBI did not respond to the pressure gradient in the expected manner but indicated a better ecological status for the inner bay stations and a worse ecological status for the outer bay stations. The change in proportions of the ecological sensitivity groups along the gradient, however, demonstrates the effect of disturbance that is not always evident from all index assessments: higher proportions of opportunistic taxa at the inner Burgas Bay stations and an increase in sensitive and indifferent taxa at the outer Burgas Bay stations (Fig.5). Station 5 was situated close to a natural reef and naturally enriched by its primary and secondary production, which could explain its different community composition and the indications of a slight disturbance, and therefore the lower index values of AMBI and BENTIX there.

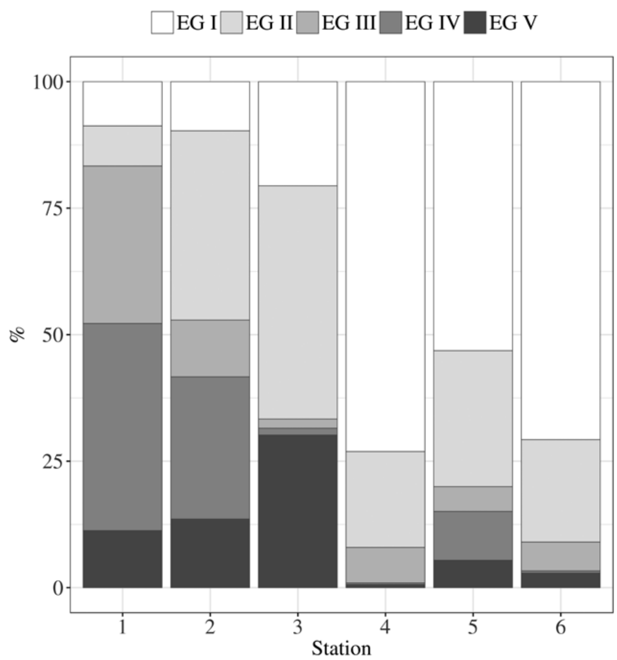

Fig. 5. Proportions of AMBI ecological groups at the Burgas Bay sampling stations (average for 2013-2014). EGI – sensitive species; EGII – indifferent species; EGIII – tolerant species; EGIV – second-order opportunists; EGV – first-order opportunists.

No two indices were in perfect agreement. AMBI-BENTIX and H'-M-AMBI were closest to the 0.5 boundary of acceptable agreement (Fig.6).

AMBI and BENTIX showed strong significant correlations with most environmental and pressure parameters. M-AMBI had significant but weaker correlations with only some of the environmental parameters. None of the significant correlations of H' with environmental parameters were in the expected direction (Table 3).

Discussion

The biotic indices developed during the implementation of the WFD are often calibrated towards a specific region or a type of anthropogenic pressure, outside of which they are not as effective (Abbasi & Abbasi 2012). The performance of an index depends strongly on its structure, specifically the weight assigned to each component relative to the others, and the ecological classification systems, which should be habitat-specific (Simboura & Argyrou 2010).

AMBI and BENTIX performed well in the shallow coastal areas of Burgas Bay, despite being influenced by the natural community variability between biotopes. They responded adequately to the local gradient of anthropogenic pressure and the observed changes in macrobenthic community structure, show-

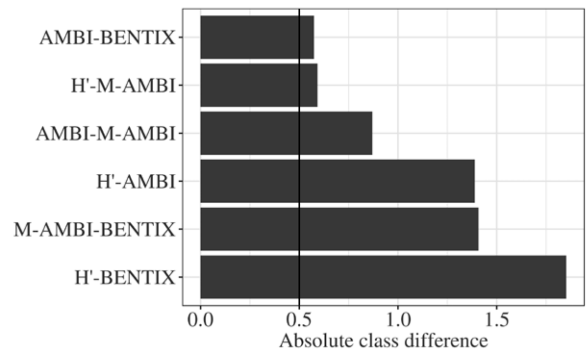

Fig. 6. Pair-wise absolute average class difference between indices. The vertical line marks the acceptable level of disagreement between two metrics.

ing a statistically significant negative correlation with pollution and pressure indicators such as LUSI, the proportion of silt and clay in the sediments, the concentrations of ammonia, chlorophyll-*a* and suspended particulate matter in the water column. Their assessments are also in relatively good agreement – unsurprising because BENTIX is based on AMBI. The discrepancies between the two are probably due to regional specificities in index design. BENTIX is calibrated for the Eastern Mediterranean Sea, where benthic community structure is more balanced (Simboura & Argyrou 2010). The proportion of the tolerant ecological group (group III) is also naturally high in the mesotrophic conditions of the Black Sea (Todorova et al. 2015), while in the Eastern Mediterranean this group is more closely associated with the opportunistic groups. This causes in particular the assessment of station 5 as moderate by BENTIX, when the community composition there actually fits the characteristics of the good status communities in the Black Sea: high abundance and diversity, presence of sensitive to pollution species but dominance of indifferent and tolerant species in the abundance structure (Todorova et al. 2015). The use of only two ecological groups in BENTIX also tends to polarize its assessments and exacerbate any differences with the other indices.

H' and M-AMBI, however, do not react adequately to the pressure gradient in the area. Their performance is significantly affected by the natural differences in community structure between biotopes (Fig.4B). H' also often responds non-monotonically to disturbance: in the beginning of the disturbance, or if it is very slight, an initial increase of species richness and abundance can occur, when opportunists and non-opportunists co-exist in the community, leading to higher H' values (Subida et al. 2012). H' behaved similarly in our study: the most impacted stations (stations 1 and 2) had higher H' EQR values. On the other hand, when the species richness is naturally low but

Table 3. Spearman rank correlation coefficients between index EQRs, and environmental parameters and LUSI. For M-AMBI*n, only the samples from biotopes with developed classification systems and reference values (stations 3, 4 and 6) are included in the calculations. Significant correlations (in bold): *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$.

Index	**%TOM**	**%silt-clay**	**mean grain size (µm)**	**O_2 bottom (mg.L^{-1})**	**Secchi depth (m)**	**chl-a (µg.L^{-1})**
AMBI	-0.24	**-0.63*** **	**-0.33***	**0.38****	**0.82*****	**-0.82*****
BENTIX	-0.25	**-0.56*****	**-0.51*****	**0.53*****	**0.78*****	**-0.78*****
H'	**0.38***	-0.09	**0.73*****	**-0.54*****	-0.12	0.12
M-AMBI	**0.49****	**-0.57*****	**0.67*****	**-0.55*****	**0.35****	**-0.35****
S	**0.51****	-0.23	**0.74*****	**-0.48*****	0.04	-0.04
M-AMBI*n	0.28	**-0.77*****	**0.51****	**-0.81*****	**0.56****	**-0.56****
Index	suspended particulate matter (mg.L^{-1})	N-NO_3^- (µg.L^{-1})	N-NH_4^+ (µg.L^{-1})	total N (µg.L^{-1})	P-PO_4^{-3} (µg.L^{-1})	LUSI-3000
AMBI	**-0.75*****	**0.31***	**-0.90*****	**-0.55*****	**-0.82*****	**-0.88*****
BENTIX	**-0.77*****	0.26	**-0.90*****	**-0.59*****	**-0.78*****	**-0.82*****
H'	0.17	0.02	**0.38****	0.24	0.12	0.18
M-AMBI	-0.27	0.02	-0.09	-0.14	**-0.35****	**-0.31****
S	-0.06	0.06	0.23	0.09	-0.04	0.05
M-AMBI*n	**-0.56****	-0.19	**-0.56****	**-0.75*****	**-0.56****	**-0.56****

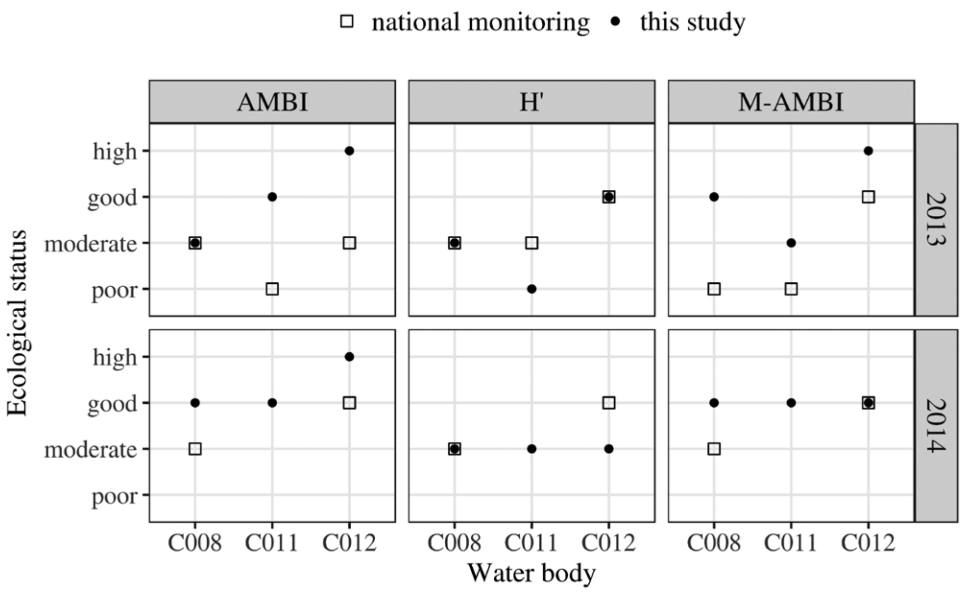

Fig. 7. Ecological status (WFD) of the water bodies in the study area based on macrozoobenthos (2013 and 2014), according to the national monitoring and according to this study. Water body BG2BS000C011 was not assessed during the national monitoring in 2014.

there is a high dominance of some taxa, H' is low even if the community dominants are highly sensitive taxa (stations 4 and 6). Because of this bias, H' alone is not a good biotic index for environmental quality assessments, a conclusion also supported by its lack of correlation with the environmental parameters.

H' as an M-AMBI component apparently has more weight in the final M-AMBI value, as suggested by their similar behaviour and overall level of agreement and the much lower agreement between AMBI and M-AMBI. This also causes the fewer and weaker significant correlations between M-AMBI and environmental parameters.

During the study period, M-AMBI was the macrozoobenthic index used in the national assessment and monitoring of Black Sea waters for the WFD and the MSFD. The present study uses the original ecological status classification system for the sake of comparability with the results of the national monitoring. At the level of the water bodies, our results tend to overestimate the ecological status based on the macrozoobenthos compared to the national monitoring and M-AMBI follows the same trend as H' (Fig.7).

Moreover, in our study, the one out – all out

(OOAO) method of integration of individual station assessments at the water body level is unsuitable, because the metrics (M-AMBI) exhibit high levels of uncertainty (Caroni et al. 2012). OOAO has also been criticised as inconsistent with the ecosystem-based approach of the MSFD, because it effectively excludes ecosystem components from the final assessment, and thus may not adequately represent the overall status of the ecosystem (Hering et al. 2010). Neither of the status assessments was significantly correlated to LUSI calculated at the water body level, further supporting this conclusion.

The demonstrated shortcomings have been recognised and new, habitat-specific classification systems have since been developed for some of the soft-bottom habitats (Todorova 2017). The „classical" M-AMBI used in this study has also been replaced since 2015 in the national monitoring by a simplified version, M-AMBI*n (Sigovini et al. 2013), independent of sampling effort and seemingly more responsive to benthic community changes. When applied to the present dataset, M-AMBI*n with the new habitat-specific classifications indeed showed a better relationship with the pressure indicators (Table 3). However, only two of the biotopes encountered in this study – the infralittoral medium and coarse sand dominated by *Upogebia pusilla,* and the infralittoral fine and medium sand dominated by *Chamelea gallina*, *Lentidium mediterraneum*, *Tellina tenuis,* have specific ecological status classifications (Todorova 2017). In order to limit the uncertainty of the assessments, in cases where habitat-specific reference values in the shallow coastal zone of the Black Sea are not established, ecological group proportions could be examined parallel to the index values, because sometimes they reflect better the actual state of the macrozoobenthic communities (Sampaio et al. 2011).

For a more comprehensive assessment of environmental status, consistent with the MSFD philosophy of functioning marine ecosystems, an assessment of functional community diversity could be incorporated in the monitoring programs. This approach complements well the classical studies focused mainly on structural parameters and provides additional insights into how the communities are reacting to changes under anthropogenic pressure (Bolam 2014).

Conclusions

The present study shows that in the shallow coastal areas of the Burgas Bay the performance of the biotic indices is influenced by index design peculiarities and/or the properties and structure of coastal communities. The latter prevent some of the indices from accurately responding to the anthropogenic pressure gradient and result in lower agreement between indices. The national index for macrozoobenthos during this study, the classical M-AMBI, performs well in deeper areas but not in the nearshore zone, where it is too biased by more pronounced community dominance patterns. Where available, the new habitat-specific classification systems for M-AMBI*n, H', and S under the MSFD improve index performance and response to pressure in the shallow coastal areas of the Burgas Bay.

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References

Abbasi T. & Abbasi S. A. 2012. The biotic indices. In: Abbasi T. & Abbasi S.A. (Eds.): Water Quality Indices. Amsterdam: Elsevier, pp. 219–247.

Berov D., Deyanova D., Georgieva I., Gyosheva B. & Hiebaum G. 2012. Cystoseira sp.-dominated macroalgal communities in the SW Black Sea (Burgas Bay, Bulgaria). Current state and possible long-term effects of eutrophication. Comptes Rendus de l'Académie Bulgare des Sciences 65 (6): 821-830.

Birk S., Bonne W., Borja A., Brucet S., Courrat A., Poikane S., Solimini A., van de Bund W., Zampoukas N. & Hering D. 2012. Three hundred ways to assess Europe's surface waters: An almost complete overview of biological methods to implement the Water Framework Directive. Ecological Indicators 18: 31–41.

Bolam S.G. 2014. Macrofaunal recovery following the intertidal recharge of dredged material: A comparison of structural and functional approaches. Marine Environmental Research 97: 15–29.

Borja A., Josefson A.B., Miles A., Muxika I., Olsgard F., Phillips G., Rodriguez J.G. & Rygg B. 2007. An approach to the intercalibration of benthic ecological status assessment in the North Atlantic ecoregion, according to the European Water Framework Directive. Marine Pollution Bulletin 55 (1-6): 42–52.

Borja A., Muxika I. & Franco J. 2003. The application of a Marine Biotic Index to different impact sources affecting soft-bottom benthic communities along European coasts. Marine Pollution Bulletin 46 (7): 835–845.

Caroni R., van de Bund W., Clarke R. T. & Johnson R. K. (2013). Combination of multiple biological quality elements into waterbody assessment of surface waters. Hydrobiologia 704 (1): 437–451.

Dauvin J.-C. & Ruellet T. 2009. The estuarine quality paradox: Is it possible to define an ecological quality status for specific modified and naturally stressed estuarine ecosystems? Marine Pollution Bulletin 59 (1-3): 38–47.

Dufrêne, M. & Legendre, P. 1997. Species assemblages and indicator species: the need for a flexible assymetrical approach. Ecological Monographs 67: 345–366.

EC. 2000. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for community action in the field of water policy (Water Framework Directive). Official Journal of the European Union L327: 1–73.

EC. 2008. Directive 2008/56/EC of the European Parliament and of the Council of 17 June 2008 establishing a framework for community action in the field of marine environmental policy (Marine Strategy Framework Directive). Official Journal of the European Union L164: 19–40.

Erftemeijer P.L.A. & Koch E.W. 2001. Sediment geology methods for seagrass habitat. In: Short F.T. & Coles R.G. (Eds): Global Seagrass Research Methods. Amsterdam: Elsevier Science, pp. 345-367.

Flo E., Camp J. & Garces E. 2011. Assessment pressure methodology: land uses simplified indeks (LUSI). In: BQE Phytoplankton, Work Document MEDGIG Meeting, Rome.

Grasshoff K. (Ed.). 1976. Methods of seawater analysis. Weinheim and New York: Verlag Chemie. 317 p.

Halpern B.S., Walbridge S., Selkoe K.A., Kappel C.V., Micheli F., D'Agrosa C., Bruno J.F., Casey K.S., Ebert C., Fox H.E., Fujita R., Heinemann D., Lenihan H.S., Madin E.M.P., Perry M.T., Selig E.R., Spalding M., Steneck R. & Watson R. 2008. A global map of human impact on marine ecosystems. Science 319 (5865): 948–952.

Herberich E., Sikorski J. & Hothorn T. 2010. A robust procedure for comparing multiple means under heteroscedasticity in unbalanced designs. PLoS One 5 (3): e9788.

Hering D., Borja A., Carstensen J., Carvalho L., Elliott M., Feld C.K., Heiskanen A.-S., Johnson R.K., Moe J., Pont D., Solheim A.L. & van de Bund W. 2010. The European Water Framework Directive at the age of 10: A critical review of the achievements with recommendations for the future. Science of the Total Environment 408 (19): 4007–4019.

Hiebaum G. & Karamfilov V. 2005. Regime shifts in the annual dynamics of primary production and chlorophyll-a concentrations in the coastal zone of the Bourgas Bay (Western Black Sea). In: Large-Scale Disturbances (Regime Shifts) and Recovery in Aquatic Ecosystems: Challenges for Management towards Sustainability. Varna: Bulgarian Academy of Sciences Publishing House, pp. 143–158.

Marinov T. 1990. Zoobenthos from the Bulgarian sector of the Black Sea. Sofia: Bulgarian Academy of Sciences Publishing House. 195 p. (in Bulgarian)

Miladinova S., Marinov D., Krastev V. & Marinski J. 2015. Multi-compartment water quality assessment of Port Burgas and Burgas Bay. In: Stylios C., Floqi T., Marinski J. & Damiani L. (Eds): Sustainable Development of Sea-Corridors and Coastal Waters. Switzerland: Springer International Publishing, pp. 95-102.

Muxika I., Borja A. & Bald J. 2007. Using historical data, expert judgement and multivariate analysis in assessing reference conditions and benthic ecological status, according to the European Water Framework Directive. Marine Pollution Bulletin 55 (1-6): 16–29.

Pinedo S. & Jordana E. 2007. Spain (Catalonia and Balearic Islands). In: Carletti A. & Heiskanen A.S. (Eds.): Water Framework Directive Intercalibration Technical Report, Part 3: Coastal and Transitional Waters. JRC Scientific and Technical Reports. JRC, pp. 62–70.

R Core Team. 2015. R: A language and environment for statistical computing.

Sampaio L., Rodrigues A.M. & Quintino V. 2011. Can biotic indices detect mild organic enrichment of the seafloor? Ecological Indicators 11 (5): 1235-1244.

Sigovini M., Keppel E. & Tagliapietra D. 2013. M-AMBI revisited: looking inside a widely-used benthic index. Hydrobiologia 717 (1): 41-50.

Simboura N. & Argyrou M. 2010. An insight into the performance of benthic classification indices tested in Eastern Mediterranean coastal waters. Marine Pollution Bulletin 60 (5): 701-709.

Simboura N. & Zenetos A. 2002. Benthic indicators to use in ecological quality classification of Mediterranean soft bottom marine ecosystems, including a new biotic index. Mediterranean Marine Science 3: 77–111.

Subida M.D., Drake P., Jordana E., Mavric B., Pinedo S., Simboura N., Torres J. & Salas F. 2012. Response of different biotic indices to gradients of organic enrichment in Mediterranean coastal waters: Implications of non-monotonic responses of diversity measures. Ecological Indicators 19: 106-117.

Sukhanova I.N., Flint M.V., Hibaum G., Karamfilov V., Kopylov A.I., Matveeva E., Ratkova T.N. & Sazhin A.F. 1988. Exuviaella cordata red tide in Bulgarian coastal waters (May to June 1986). Marine Biology 99 (1): 1–8.

Todorova V., Abaza V., Todorov E. & Wolfram G. 2015. Intercalibration technical report. Black Sea GIG - Coastal waters – BQE invertebrate fauna. Report for WG ECOSTAT, EC. 21 p.

Todorova V. 2017. Zoobenthic indicators for assessment of the sandy biotopes state. In: Todorova V. & Milkova T. (Eds.), Final report of the project „Investigations on the State of the Marine Environment and Improving Monitoring Programs developed under MSFD (ISMEIMP)". Institute of oceanology – BAS. Black Sea Basin Directorate, pp. 23-47 (in Bulgarian).

Trayanova A., Todorova V. & Konsulova T. 2008. Bulgaria. In: Carletti A. & Heiskanen A.S. (Eds.), Water Framework Directive Intercalibration Technical Report, Part 3: Coastal and Transitional Waters. JRC Scientific and Technical Reports. JRC, pp. 35–43.

Trukhchev D., Ivanov D., Ibrayev R., Ivanova D., Patzireva T. & Rabie A. 2004. Hydrophysical study of the Bourgas Bay modelling the synoptic circulation patterns. Comptes Rendus de l'Académie Bulgare des Sciences 57 (3): 29-38.

van de Bund W., Poikane S. & Romero J.R. 2008. Comparability of the results of the intercalibration exercise – Summary of responses and way forward. Report ENV-COM240108-5 to the EC, Brussels, Belgium, 17 p.

Zenetos A., Bellou N. & Abousamra F. 2005. The use of BENTIX in assessing ecological quality of coastal waters across the Mediterranean. In: IOC Workshop Report No.195. Paris, France: UNESCO-IOC, pp. 19-22.

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Supplement 1. Biotopes at the study stations in the coastal area of the Burgas Bay (Todorova 2017) and indicator value (IndVal) of the characteristic species for each biotope, by abundance and by biomass, Species with IndVal > 0.500 are shown. All values are significant ($p < 0.05$).

Note: station 3 is classified as Infralittoral medium and coarse sand dominated by *Upogebia pusilla* despite the absence of the latter species in the current dataset, by the characteristic community composition, the depth and the sediment properties.

Species	**IndVal_abundance**	**IndVal_biomass**
Infralittoral sandy muds dominated by *Melinna palmata, Anadara kagoshimensis, Heteromastus filiformis* – stations 1, 2		
Anadara kagoshimensis (Tokunaga, 1906)	1.000	1.000
Heteromastus filiformis (Claparède, 1864)	0.996	0.997
Melinna palmata Grube, 1870	0.943	0.944
Lagis koreni Malmgren, 1866	0.875	0.904
Polydora ciliata (Johnston, 1838)	0.765	0.764
Prionospio cirrifera Wirén, 1883	0.739	0.747
Iphinoe tenella Sars, 1878	0.722	0.722
Parvicardium exiguum (Gmelin, 1791)	0.610	0.694
Leiochone leiopygos (Grube, 1860)	0.578	0.597
Cytharella costulata (Dunker, 1860)	0.509	0.495
Infralittoral medium and coarse sand dominated by *Upogebia pusilla* – station 3		
Ophelia limacina (Rathke, 1843)	0.841	0.598
Bathyporeia guilliamsoniana (Bate, 1857)	0.796	0.797
Oligochaeta	0.790	0.832
Branchiostoma lanceolatum (Pallas, 1774)	0.696	0.666
Protodorvillea kefersteini (McIntosh, 1869)	0.695	0.718
Protodrilus flavocapitatus Uljanin, 1877	0.656	0.662
Nototropis guttatus Costa, 1853	0.585	0.627
Infralittoral fine and medium sand dominated by *Chamelea gallina*, *Lentidium mediterraneum*, *Tellina tenuis* – stations 4, 6		
Chamelea gallina (Linnaeus, 1758)	0.901	0.947
Spisula subtruncata (da Costa, 1778)	0.871	0.866
Lentidium mediterraneum (O. G. Costa, 1830)	0.833	0.833
Magelona papillicornis F. Müller, 1858	0.713	0.724
Pseudocuma (Pseudocuma) longicorne (Bate, 1858)	0.633	0.755
Nephtys cirrosa Ehlers, 1868	0.558	0.376
Spio filicornis (Müller, 1776)	0.551	0.553
Donax venustus Poli, 1795	0.500	0.500
Tritia neritea (Linnaeus, 1758)	0.500	0.500
Infralittoral mixed sediments with diverse fauna – station 5		
Melita palmata (Montagu, 1804)	1.000	1.000
Polycirrus jubatus Bobretzky, 1869	1.000	1.000
Streptosyllis bidentata Southern, 1914	1.000	1.000
Microdeutopus versiculatus (Bate, 1856)	0.999	0.998
Acari	0.968	0.954
Syllis hyalina Grube, 1863	0.889	0.889
Eunice vittata (Delle Chiaje, 1828)	0.857	0.886
Eurydice dollfusi Monod, 1930	0.826	0.877
Schistomeringos rudolphi (Delle Chiaje, 1828)	0.756	0.760
Caecum armoricum de Folin, 1869	0.749	0.685
Sphaerosyllis hystrix Claparède, 1863	0.735	0.778
Hirudinea	0.704	0.770
Polygordius neapolitanus Fraipont, 1887	0.701	0.653
Dinophilus gyrociliatus O. Schmidt, 1857	0.700	0.622
Bodotria arenosa Goodsir, 1843	0.667	0.664
Polycirrus caliendrum Claparède, 1869	0.667	0.667
Syllis gracilis Grube, 1840	0.657	0.745
Holothuroidea	0.556	0.556