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Ecological State of Varna Bay in Summer 2009 according to Benthic Invertebrate Fauna

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Abstract: The objective of this research is to assess the ecological state of Varna Bay according to benthic invertebrate fauna, to examine the similarity pattern and to find the best combination of environmental variables explaining the biotic pattern. Three zones with similar community composition are distinguished on abundance dataset – northern, central and southern. Cluster analysis on biomass dataset pools samples in two main groups – the southern zone and the stations dominated by crustacean *Upogebia pusilla* (Petagna, 1792). Ecological state of Varna Bay, assessed by M-AMBI, varies from bad and poor to moderate and improves in parallel with the decrease of average percentage content of organic carbon and concentration of extractable matters in sediments. The best combination of abiotic environmental variables explaining the biotic pattern are found to be the depth, concentration of extractable matters, salinity of bottom water, oxygen content of bottom water and percentage contents of carbon, clay, silt and sand in sediments. In all cases, there is a significant correlation between the biotic indices reflecting the environmental quality and their response to pressures, particularly extractable matters, organic carbon, pH and redox potential.

Key words: macrozoobenthos, Water Framework Directive, coastal waters, biotic indices, environmental quality

Introduction

Varna Bay is located in the northern part of Bulgarian Black Sea coast, locked between c. Galata and c. St. George. It is the second largest bay along Bulgarian Black Sea coast after Burgas Bay (Fig. 1). It has flat bottom sloping towards east. Its maximum depth is 18.5 m. On the west it is artificially linked by two canals to Varna Lake, which has a major impact on it. Varna, the third largest city in Bulgaria, is situated around the bay. Its population is over 300 000 inhabitants which increases considerably during the summer season. Tourism is the fastest growing sector in the region within the past few years. The largest sea ports of Bulgaria are located in Varna and Burgas Bays. Varna Port is a basic hub of the logistical chain of VIII Pan European transport corridor.

In 2000, the Water Framework Directive (more formally the Directive 2000/60/EC of the European Parliament and Council of 23 October 2000) entered into force. The goal of WFD is not only to prevent further deterioration of water bodies but also to protect and enhance the state of water resources to the level of quality defined as ‘good ecological status’. Being an EU member state Bulgaria is obliged to protect, enhance and restore all bodies of surface water with the aim of achieving good surface water status before 2015.

According to WFD criteria Varna Bay is classified as moderately exposed, euhaline, shallow (< 20 m) coastal water body type (Volckaert 2007). The sediments are contaminated as a result of urban and industrial activities and municipal wastewater (Shtereva *et al.* 2004). Point sources of pollution are not identified. The following contaminants in sediments are regarded as a diffuse source of pollution – 4,4'-DDT; 4,4'-DDD, 4,4' – DDE, penthachlorobenzene, hexachlorobenzene, 4-tert-octylphenol, DEHP, Bisphenol A, PCB 153, PAH . Varna Bay is identified as a water body at risk of failing to achieve good ecological status by 2015 (Basin Directorate for Water Management in Black Sea Region 2009).

Material and Methods

Study area

The study area covered Varna Bay, the northern canal connecting Varna Bay with Varna Lake and the eastern part of Varna Lake. Fifteen stations were sampled in June and September 2009 ranging from 3 m to 18 m depth (Table 1, Fig. 1). Sampling was carried out for those abiotic environmental parameters considered necessary to explain the community structure and state of macrozoobenthos (Borja *et al.* 2004).

Bottom water samples

The samples from bottom water were collected using Go-Flow sampling bottles and temperature (ºC), salinity, pH and dissolved oxygen (ml/l) were measured onboard. Dissolved oxygen (ml/l) was determined by Winkler method (Grasshoff *et al.*1983) and pH – by WTW pH/conductivity meter.

Sediment samples

The sediment samples were collected with van Veen grab (0.1 m^2). In sediments the temperature (ºC), pH and redox potential (mV) were measured onboard immediately after the sampling. Organic carbon (%) samples were dried at room temperature and analysed after 'wet aching' of the dried sediments using sulphuric acid mixture of dichromate at high temperature (Romankevich 1980). Samples for extractable matters (mg/kg) determination were stored in glass containers, extracted by n-hexane and analysed using a Gas Chromatograph with Flame Ionization Detector (GC-FID) in a certified laboratory (ISO 16703 2004). Grain-size analysis was performed after the method of Folk (Folk 1966).

Table 1. Coordinates, depth (m) and sediment type of sampled stations.

Station	Latitude	Longitude	Depth	Sediment
1	43º11'49"	27º55'30"	7	sand
2	43º11'10"	27º55'30"	12	silty sand
3	43º10'41"	27º55'12"	7	sand
4	43º12'07"	27º55'47"	7	sand
5	43º11'10"	27º56'13"	15	silty sand
6	43º10'37"	27º55'55"	7	sand
7	43º12'24"	27º56'48"	9	sand
8	43º12'04"	27º58'12"	18	silt
9	43º10'34"	27º57'00"	17	sand
10	43º12'56"	27º58'41"	16	sand
11	43º11'31"	27º55'31"	11	sand
12	43º11'39"	27º53'55"	8	sand
13	43º12'22"	27º53'10"	3.5	sandy silt
14	43º10'48"	27º54'46"	3	sand
15	43º11'42"	27º56'24"	13	sand

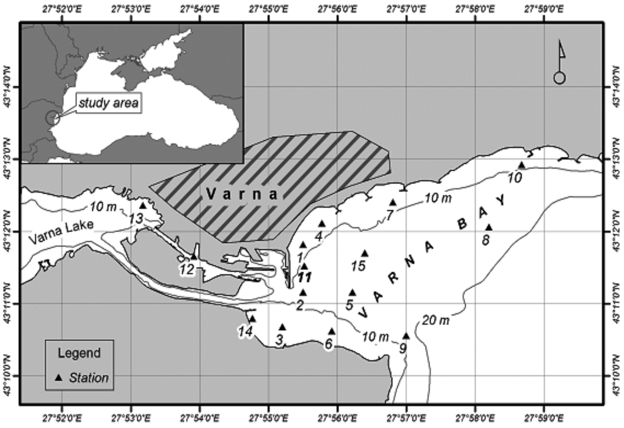

Fig. 1. Sampling network of the study area.

Benthic invertebrates

The samples for analysis of the benthic invertebrates were collected with van Veen grab (0.1 m^2) and gently sieved through metal gauze sieves with mesh size 1.0 x 1.0 mm and 0.5 x 0.5 mm onboard. The collected samples were fixed with 4% buffered formaldehyde and the containers were appropriately labelled for further identification. Sorting, taxonomic identification, abundance and biomass (wet weight) determination were performed in the laboratory of Marine Biology and Ecology Department.

The procedures of collection, onboard and laboratory processing of samples were accomplished according to Todorova, Konsulova (2005).

Statistical treatment of data

Univariate and multivariate statistical analyses were performed on abundance and biomass datasets. Similarity pattern was examined (Clarke, Warwick 2001). Ecological state was assessed by application of the following indices and corresponding classification scales: diversity index H' (Shannon, Weaver 1949) (Table 2), A Marine Biotic Index (AMBI) (Borja *et al.* 2000; Borja *et al.* 2003) and multivariate AMBI (M-AMBI) (Borja *et al.* 2007). In the process of AMBI calculation the polychaete worm *Aricidea claudiae* Laubier, 1967 was classified as belonging to III ecological group due to statistically demonstrated species similarity (Bray-Curtis similarity based on log (x+1) transformed abundance) with a number of other tolerant species (Todorova *et al.* 2008). In the process of M-AMBI calculation the reference and bad state values for species richness (S) were set sediment dependently. For water bodies with muddy sediments the boundary values for S of high and bad state were ≥ 40 and < 10, and for the stations with sandy and mixed sediments ≥ 50 and < 15 respectively (Trayanova *et al.* 2007). The ecological state of a particular station was assessed using M-AMBI, a combined biotic index including diversity (H'), species richness (S) and AMBI (proportion of opportunistic to sensitive taxa), into a factor analysis multivariate approach (Muxika *et al.* 2007). The ecological class boundaries were those given by BORJA *et al.* (2007). BIOENV procedure on abundance and abiotic matrices was carried out in order to find the best combination of environmental variables explaining the biotic pattern. The combination of variables with the highest significance was subjected to ordination by Principal Component Analysis (PCA) (Clarke, Warwick 2001). PRIMER 6 (Primer-E Ltd) and AMBI 4.0 (AZTI-Tecnalia) software packages were employed for statistical analyses of the data.

Results and Discussion

Abiotic environmental variables

The physicochemical characteristics of Varna Bay-canal-Varna Lake bottom water and sediments varied spatially depending on the location, depth and existing sources of pollution (Table 3). The lake system provided freshwater inflow through the canal forming an area of lower salinity in the western part of Varna Bay. Lower temperatures and salinity at depth 15-18 m resulted in vertical stratification of bay waters associated with reduced mixing of bottom water with upper layers (Shtereva, Dzhurova 2006; 2007). Thus dissolved oxygen measured in bottom water at the central deeper stations was low in both sampling campaigns, ranging between 3.73-4.50 mL/L. Due to being a virtually enclosed area with reduced water exchange and possibly as a result of uncontrolled wastewater discharge in the eastern part of Varna Lake as well as the presence of point sources of pollution from shipbuilding industry and ship piers, organic pollutants reached very high levels in sediments from the canal and Varna Lake, extractable matters exceeded by order of magnitude those encountered in Varna Bay (Hristova, Dzhurova 2011). The spatial distribution of sediment contaminants in Varna Bay was distin-

Table 2. Classification scale for diversity index H' (Trayanova *et al.* 2007).

Water bodies with muddy sediments					
Ecological state	**High**	**Good**	**Moderate**	**Poor**	**Bad**
H' average	3.6	2.9	2.2	1.5	0.7
Range	H' ≥ 3.3	3.3 > H' ≥ 2.5	2.5 > H' ≥ 1.8	1.8 > H' ≥ 1.1	H' < 1.1
Ecological Quality Ratio	≥ 0.92	0.69	0.50	0.31	< 0.31
Water bodies with sandy and mixed sediments					
Ecological state	**High**	**Good**	**Moderate**	**Poor**	**Bad**
H' average	4.5	3.6	2.7	1.8	0.9
Range	H' ≥ 4	4 > H' ≥ 3.1	3.1 > H' ≥ 2.2	2.2 > H' ≥ 1.3	H' < 1.3
Ecological Quality Ratio	≥ 0.89	0.69	0.49	0.29	< 0.29

Table 3. Minimum, maximum and average (SD) values of abiotic environmental variables (AEV) (stations 12 and 13 are not included).

	June 2009			**September 2009**		
AEV	**Min**	**Max**	**Average (SD)**	**Min**	**Max**	**Average (SD)**
Bottom waters						
Temperature (ºC)	17.10	24.00	20.07 (2.26)	21.40	23.50	22.08 (0.69)
Salinity	16.30	17.40	17.02 (0.28)	16.40	17.30	17.15 (0.26)
pH	8.02	8.49	8.33 (0.09)	8.02	8.34	8.24 (0.07)
Dissolved Oxygen (ml/l)	3.73	6.67	5.15 (0.89)	4.52	6.21	5.02 (0.54)
Sediments						
Temperature (ºC)	10.50	21.00	14.92 (2.55)	13.80	24.00	20.63 (2.78)
pH	7.66	8.12	7.90 (0.15)	7.04	8.02	7.57 (0.28)
Redox Potential	-118.00	193.00	85.92 (98.21)	-216.00	186.00	-13.15 (158.62)
Extractable Matters (mg/kg)	5.27	62.03	23.17 (17.18)	11.09	29.39	21.89 (5.69)
Organic Carbon (%)	0.06	0.75	0.25 (0.21)	0.08	0.84	0.26 (0.21)

Table 4. Pearson correlation coefficient p between biotic indices species richness (S), diversity (H'), a marine biotic index (AMBI), multivariate AMBI (M-AMBI) and environmental variables depth, extractable matters (EM), temperature of bottom water (T_{bw}), pH of bottom water (pH_{bw}), salinity of bottom water (S_{bw}), dissolved oxygen of bottom water (DO_{bw}), temperature of sediments (T_s), pH of sediments (pH_s), redox potential of sediments (Eh_s), content of organic carbon in sediments (C_{org}), percentage share of clay, silt and sand in sediments (in bold, significant values at the level of significance alpha=0,050 two-tailed test).

	S	AMBI	H'	M-AMBI	depth	EM	T_{bw}	pH_{bw}	S_{bw}	DO_{bw}	T_s	pH_s	Eh_s	Corg	clay	silt	sand
S	1																
AMBI	**-0,57**	1															
H'	**0,48**	-0,16	1														
M-AMBI	**0,87**	**-0,74**	**0,69**	1													
depth	0,16	0,18	0,30	0,16	1												
EM	-0,52	**0,60**	-0,36	**-0,62**	-0,33	1											
T_{bw}	-0,15	-0,12	**-0,39**	-0,19	**-0,56**	0,27	1										
pH_{bw}	-0,12	0,17	0,13	-0,05	-0,09	**0,41**	0,18	1									
S_{bw}	**0,45**	-0,21	**0,49**	**0,49**	**0,74**	**-0,70**	**-0,54**	-0,30	1								
DO_{bw}	-0,24	-0,10	-0,38	-0,21	**-0,65**	**0,46**	**0,70**	**0,49**	**-0,76**	1							
T_s	0,07	-0,29	-0,32	-0,03	**-0,39**	-0,18	**0,67**	**-0,46**	-0,12	0,14	1						
pH_s	0,25	**-0,57**	**0,52**	**0,54**	-0,04	**-0,46**	-0,27	0,12	0,22	-0,08	-0,20	1					
Eh_s	0,33	**-0,64**	0,17	**0,46**	-0,17	**-0,42**	-0,02	0,09	0,10	0,21	-0,06	**0,64**	1				
Corg	**-0,62**	**0,71**	**-0,49**	**-0,75**	-0,29	**0,89**	0,24	0,20	**-0,71**	**0,38**	-0,08	**-0,65**	**-0,56**	1			
clay	-0,32	**0,45**	-0,15	-0,32	**0,55**	0,21	-0,34	-0,10	0,15	-0,34	-0,27	**-0,52**	**-0,60**	**0,36**	1		
silt	**-0,45**	**0,54**	-0,34	**-0,46**	0,28	**0,47**	-0,13	0,05	-0,19	-0,01	-0,30	**-0,64**	**-0,62**	**0,66**	**0,86**	1	
sand	-0,02	**-0,38**	0,10	0,11	**-0,51**	-0,28	0,26	-0,06	-0,05	0,13	**0,38**	**0,43**	0,31	**-0,42**	**-0,54**	**-0,67**	1

guished by differentiation of an area with relatively high concentrations of organic carbon and extractable matters in the central part in correlation with the high content of fine fractions (silt and clay) in sediments at stations 2, 5, 8 and 9 (Fig. 1). The sediments around the shallow perimeter of the bay were characterised by predominant coarse fraction and low organic carbon (0.06 - 0.15%).

The temporal variability of the physicochemical features of Varna Bay bottom water was characterized by higher temperature in September (average for the area 22.08 ºC) than in June (average for the area 20.07 ºC), slightly higher salinity in September than June (Table 3) and lower pH in September (average for the area 8.33) than in June (average for the area 8.24). Dissolved oxygen in bottom water was predictably lower in September (average for the area 5.02) than in June (average for the area 5.15), although minima were observed in June (Table 3). In sediments the average temperature was significantly higher in September (average for the area 20.63 ºC) than in June (average for the area 14.92 ºC) which had implications for the chemical and biochemical processes occurring there. Thus the redox potential was predominantly oxidising in June (average 85.92 mV) and reducing in September (average -13.15 mV).

The relationships between environmental variables were explored by Pearson correlation coefficient p calculated at significance level $\alpha = 0.05$ (Table 4). The redox potential decreased with the increase of extractable matters, organic carbon and percentage share of clayey and silty fractions. The redox potential profiles provide useful information, since the decrease in redox potential is associated with degradation of organic matter (Zobell 1946). The organic carbon content in sediments exhibits very high positive correlation with the extractable matters. A positive correlation between the concentration of organic carbon in sediments and the levels of contaminants is frequently observed in coastal marine sediments. This is due to the fact that organic carbon can react with contaminants through processes such as hydrophobic interactions with non-polar organic contaminants, or formation of complexes at polar functional groups with heavy metals. In all cases, sediments with high organic carbon have higher concentrations of contaminants (Shine, Wallace 2000). Given these relationships, it is clear that the organic carbon content can be an indicator of the presence of contaminants.

The relationship between the organic content and grain size composition of the sediments shows that Corg correlates positively with fine fractions of sediments (clay and silt) and negatively with sand (Table 4). This suggests that organic carbon content of Varna Bay depends on the type of bottom sediment.

Composition and structure of macrozoobenthos

The pool of samples yielded 81 species and 4 taxa identified at higher level (Turbellaria, Nemertini, Nudibranchia and Oligochaeta). The average number of species per sample was 16 (2), the minimum was 9 and the maximum was 31 species. The major groups in the taxonomic structure were polychaetes (29 species), crustaceans (26) and molluscs (23 – 6 gastropods and 17 bivalves). Group Varia included 1 anthozoan, 1 phoronid, 1 cephalochordate and the higher taxa Turbellaria, Nemertini, Nudibranchia and Oligochaeta. The average abundance of macrobenthic fauna in the studied area was 8212 (2941) ind.m^{-2} and varied in wide range with minimum 840 ind.m^{-2} and maximum 34 730 ind.m^{-2}. The average biomass of the benthic invertebrate fauna was 144.945 (81.887) gWM.m^{-2}. The biomass varied from minimum 13.999 gWM.m^{-2} to maximum 597.546 gWM.m^{-2}.

Hierarchical clustering procedure according to log (x+1) transformed abundance data distinguished 3 clusters pooling samples with similar community composition – southern Varna Bay (SVB), northern Varna Bay (NVB) and central Varna Bay (CVB) (Fig. 2).

SVB encompassed 3 stations located in the shallow part of the bay (3 to 7 m depth) which were characterised by sandy sediments (Table 1). Average similarity within the group was 50.89% with the highest contribution of psamophilic bivalves *Lentidium mediterraneum* (O. G. Costa, 1829) and *Tellina tenuis* da Costa, 1778, and the polychaete worm *Spio filicornis* (Müller, 1776). The average abundance 15 930 ind.m^{-2} was the highest compared to the other two groups. It was formed predominately by molluscs (86.9%) and especially by *L. mediterraneum*, which average abundance was 13 295 ind.m^{-2}. The mean organic carbon content in sediments was 0.10%.

NVB included 5 stations with sandy sediments at depth range 7 to 16 m. The polychaetes *Heteromastus filiformis* (Claparède, 1864), *Capitella minima* Langerhans, 1881 and the crustacean *U. pusilla* contributed mostly to the similarity (53.70% average similarity).The average abundance 2029 ind.m^{-2} was dominated by the polychaetes (76.73%). The mean percentage of organic carbon was 0.15%.

CVB was composed of 6 stations with 54.24% similarity at depth ranging from 8 m to 18 m. Sediments varied from silt, silty sand to sand. The highest contributions to similarity belonged to polychaetes *H. filiformis* and *Polydora cornuta* Bosc,

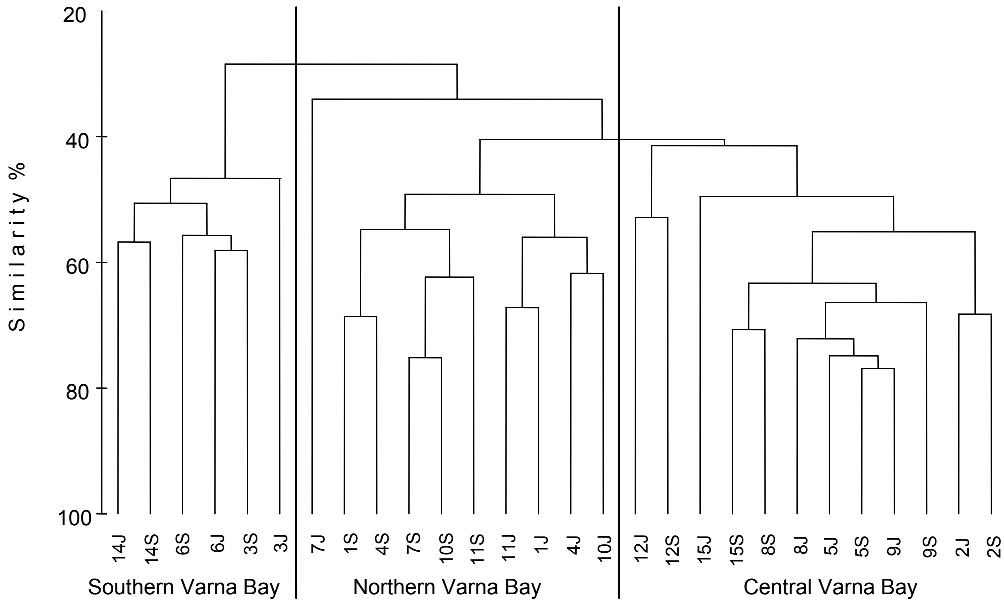

Fig. 2. Dendrogram for hierarchical clustering (group-average linking) of samples based on Bray-Curtis similarities (%) of log (x+1) transformed abundance of macrobenthic fauna.

1802, oligochaetes and crustacean *U. pusilla.* The average abundance was 9271 ind.m^{-2} formed by polychaetes (87.60%) and oligochaetes (9.52%). The organic content in sediments was 0.57%.

Hierarchical clustering procedure according to log (x+1) transformed biomass data distinguished 2 main clusters pooling samples with similar community composition (Fig. 3). The first cluster encompassed virtually the same stations grouped together by the abundance similarity in the southern Varna Bay (SVB) excluding station 3 sampled in June (referred as 3J). Average similarity within the group was 52.69% with the highest contribution of psamophilic bivalves *T. tenuis, L. mediterraneum* and *Chamelea gallina* (Linnaeus, 1758). The average biomass 191.068 gWM.m^{-2} was formed predominately by molluscs (97.17%) and especially by *T. tenuis*, which average abundance was 100.184 gWM.m^{-2}.

The second cluster (*U. pusilla*) pooled stations with an average similarity 53.91% and biomass 124.273 gWM.m^{-2}. The biomass was dominated by crustaceans (69.92%) and particularly by *U. pusilla* (68.54%) having high species-specific weight. The stations 1, 3, 4 sampled in June (referred as 1J, 3J, 4J) and stations 1, 4 sampled in September (referred as1J, 3J, 4J), characterised by higher average biomass (204.657 gWM.m^{-2}), were grouped together due to the co-dominance of bivalves *Anadara inaequivalvis* (Bruguière, 1789) (35.77%) and *T. tenuis* (20.47%) with *U. pusilla* (41.81%). The rest of the stations within the cluster were separated because of the strong dominance of *U. pusilla* (88.51%) and co-occurrence of polychaetes *Nephtys hombergii* Savigny in Lamarck, 1818 and *H. filiformis.*

Generally Varna Bay seabed is occupied by three major biotopes: (i) sands dominated by bivalve molluscs *T. tenuis* and *L. mediterraneum* along the southern coast; (ii) silty sands co-dominated by thallassinid mud shrimps *U. pusilla* and bivalves *A. inequivalvis* and *T. tenuis* in the north-western area, and (iii) sandy silts dominated by thallassinids and polychaete worms in the central and eastern part.

The above biotopes are approximately equivalent to communities identified in previous classifications as follows: biotopes (i) and (ii) are similar to the sandy bottom community described by Marinov (1990), the sand community differentiated by Todorova, Konsulova (2003) and the community of *S. filicornis, Ch. gallina* defined by Trayanova (2008); biotope (iii) is identical to *Melinna palmata* Grube 1870 coastal mud community described by Marinov (1990), the coastal sandy silt community delineated by Todorova, Konsulova (2003) and *A. claudiae, M. palmata,* oligochaetes community outlined by Trayanova (2008).

Station 13 located in the eastern part of Varna Lake was not included in the cluster analysis due to

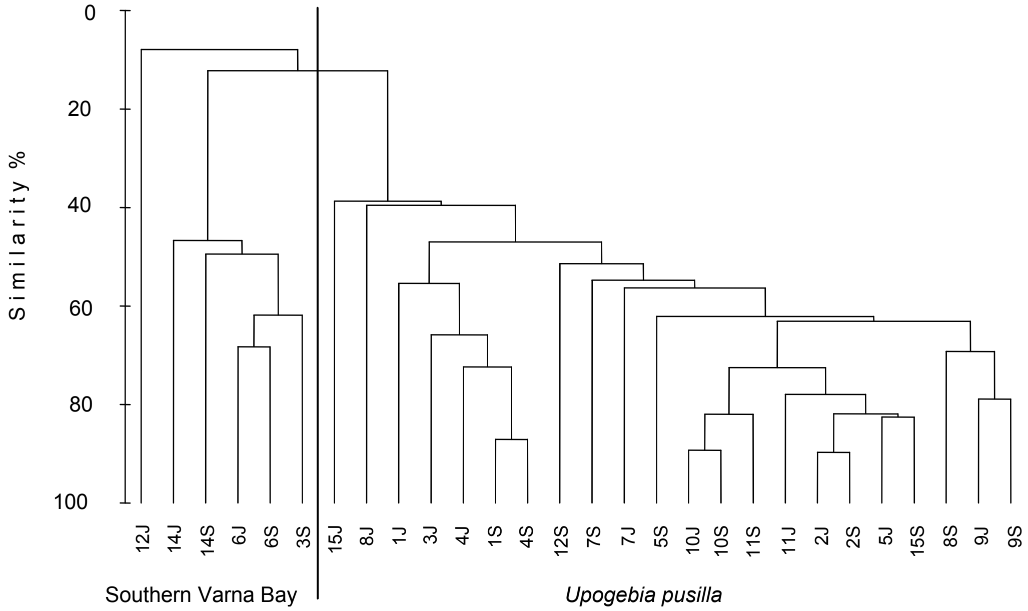

Fig. 3. Dendrogram for hierarchical clustering (group-average linking) of samples based on Bray-Curtis similarities (%) of log (x+1) transformed biomass of macrobenthic fauna.

the lack of macrozoobenthic species (azoic conditions). The sediment was sandy silt. It was characterised by the highest organic load 4.77%.

Ecological state

The linking of the biological patterns to environmental variables carried out by BIOENV procedure revealed that the best combination of variables explaining the biotic pattern (ρ=0.629) included depth, concentration of extractable matters, salinity of bottom water, oxygen content of bottom water and percentage contents of organic carbon, clay, silt and sand in sediments (Table 5). The combination of variables with the highest significance was subjected to principal component analysis (PCA). The first PC axis (PC1) reflected the decreasing concentration of extractable matters and percentage of organic content from clayey and silty to sandy sediments (equation 1). The second axis (PC2) captured the variability of the natural factors: increasing depth and salinity, and decreasing oxygen content, which according to the correlations examined (Table 5) was determined primarily by depth, temperature and salinity (natural factors) but was also influenced by organic carbon content (anthropogenic pressure involved) (equation 2) (Fig. 5). Most of the variability of the selected variables was captured in this 2-d approximation: 82.4% of it, with 46.3% on the first PC axis and 36.0% on the second.

$PC1 = -0.148\ \text{depth} -0.420\ \text{EM} +0.125\ \text{Sbw} + 0.078\ \text{DObw} -0.450\ \text{Corg}$

$-0.444\ \text{clay\%} -0.495\ \text{silt\%} +0.368\ \text{sand\%}$

(equation 1)

$PC2 = +0.525\ \text{depth} -0.238\ \text{EM} +0.556\ \text{Sbw} - 0.478\ \text{DObw} -0.253\ \text{Corg} +0.207\ \text{clay\%} +0.043\ \text{silt\%} -0.150\ \text{sand\%}$

(equation 2)

The relationships between diversity and biotic indices of benthic community used for ecological state assessment and the environmental variables were examined by Pearson correlation coefficient p at significance level $\alpha = 0.05$ (Table 4). Species richness S correlated negatively with the percentage content of extractable matters, organic carbon and silty fraction of sediments, i.e. species richness declined when the content of the above variables increased. Diversity H' was positively affected by rising salinity and pH of sediments and negatively by increasing organic load. Our results were in agreement with a similar study carried out in five distinct environments across Europe, which findings also confirmed that species richness and diversity tended to increase with the decreasing pressure gradient, being very consistent in the pattern (Borja *et al.* 2011).

The value of AMBI increased in parallel with the percentage content of extractable matters, organic carbon, clay and silt fractions of sediments and de-

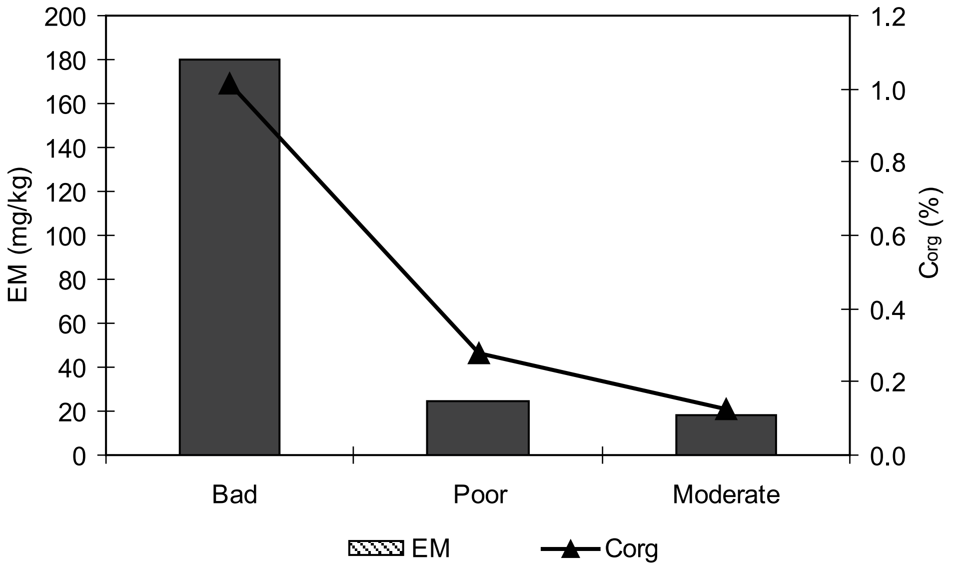

Fig. 4. Average percentage content of organic carbon (C_{org}) and concentrations of extractable matters in sediments (EM) per ecological state category.

creased with increasing pH, redox potential and sandy content of sediments. Borja et al. (2011) also found out that the absolute values of AMBI (this metric has decreasing values with increasing quality status) tended to decrease with the decreasing pressure gradient.

Among all indices used for ecological evaluation M-AMBI showed the strongest negative correlation with organic carbon, extractable matters, and less strong with the percentage of silt fraction. The index increased with the rise of salinity, pH and redox potential. Similar to our results Borja *et al.* (2011) found the highest correlations between M-AMBI and the pressure index, which combined a range of environmental pressures. In another study, carried out by Borja *et al.* (2011a), from 15 coastal cases, 11 presented significant response of M-AMBI to human pressures, 1 was unclear, 1 was not significant and 2 were focused at spatial and temporal variability (showing low variability in both cases).

The results showed that all benthic community indices employed in this study reflected both natural environmental conditions (sediment type, salinity) and impact of anthropogenic pressures (organic load, extractable matters). The strongest correlations were manifested between the biological indices and the content of organic carbon, followed by concentration of extractable matters. Less strong but significant correlations were evident between biological indices and the natural factors salinity and type of sediment. The boundary values for AMBI were set sediment independent, which was in some controversy with our findings. Nevertheless, the ecological state assessment was deemed valid in our study due to the fact that M-AMBI was governed primarily by the anthropogenic pressures and less so by the natural factors as suggested by the strength of Pearson correlations. On Fig. 4 it is seen that the ecological state improves in parallel with the decrease of average organic carbon percentage content and concentrations of extractable matters in sediments.

According to M-AMBI the ecological state of Varna Bay varied spatially, as well as temporally, among the categories bad, poor and moderate (Table 6).

For the majority of the stations the assessments made in June and September were in good agreement, M-AMBI falling within the same ecological class. In several cases there was a difference of one ecological class at different sampling campaigns, however the direction of change alternated towards either worse or better state. This result raises the question which month is best suited for ecological state assessment surveys to be carried out. The answer could be different depending on the reason for the observed change. For instance high abundance and dominance of *L. mediterraneum* and *Ch. gallina* recruit at station 3 in September resulted in low diversity index H' reflected in lower value of M-AMBI, therefore poor ecological state in September, while moderate in June. Thus high abundance of juveniles

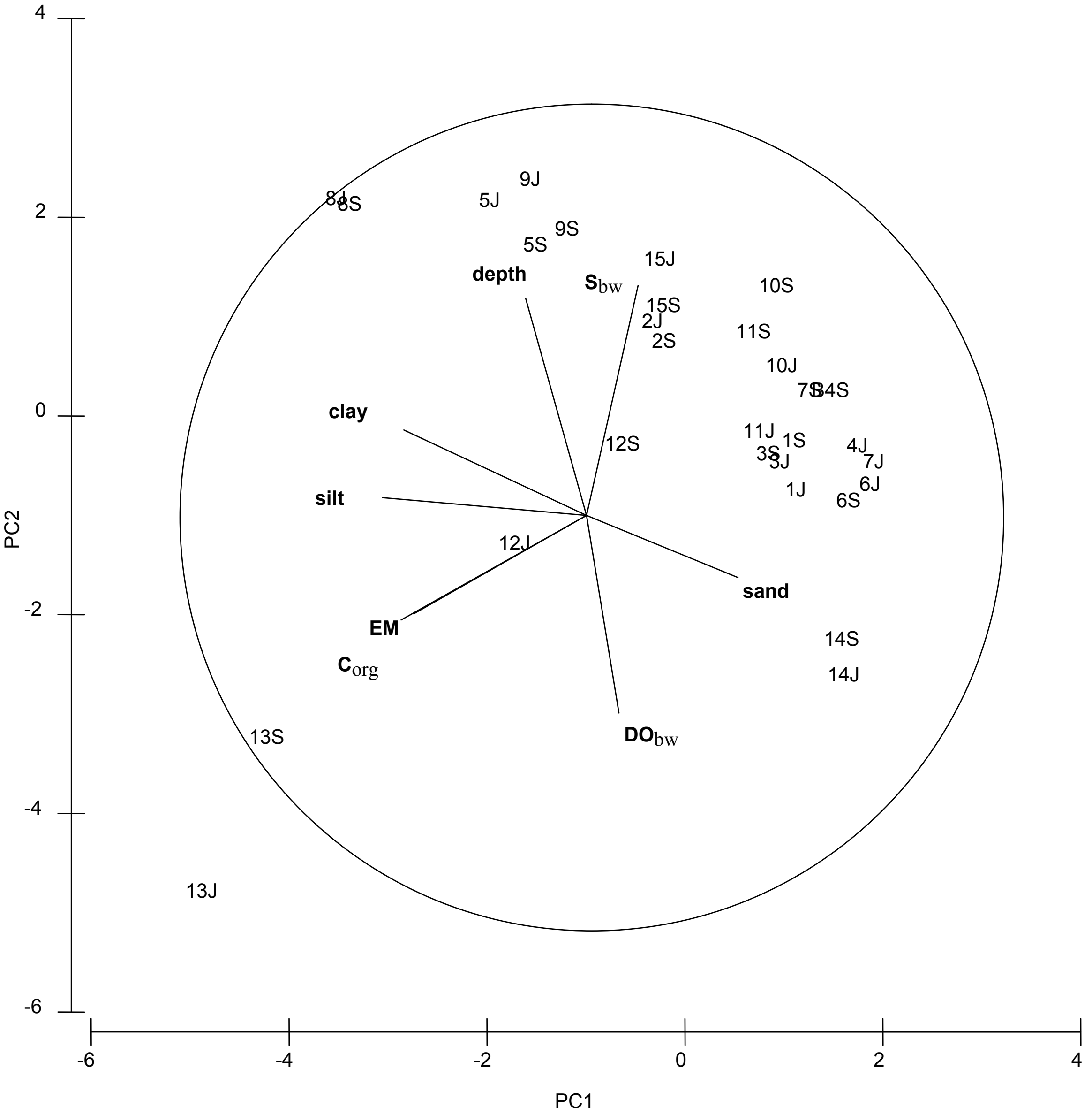

Fig. 5. Principal Component Analysis (PCA) plot of environmental variables (depth, extractable matters (EM), salinity of bottom waters (S_{bw}), dissolved oxygen of bottom waters (DO_{bw}), content of organic carbon in sediments (C_{org}), percentage share of clay, silt and sand in sediments) selected by BIOENV analysis: on x-axis principal component 1 (PC1), y-axis principal component 2 (PC2).

Table 5. Number of variables, Pearson rank correlation coefficient and best variable combination of environmental variables (depth, extractable matters (EM), salinity of bottom waters (S_{bw}), dissolved oxygen of bottom waters (DO_{bw}), redox potential of sediments (Eh_s), pH of sediments (pH_s), content of organic carbon in sediments (C_{org}), percentage share of clay, silt and sand in sediments.

variables	correlation	best variable combinations
8	0.629	depth, EM, S_{bw}, DO_{bw}, Corg, clay, silt, sand
8	0.628	depth, S_{bw}, DO_{bw}, Eh_s, Corg, clay, silt, sand
9	0.627	depth, EM, S_{bw}, DO_{bw}, pH_s, Corg, clay, silt, sand
10	0.626	depth, EM, S_{bw}, DO_{bw}, pH_s, Eh_s, Corg, clay, silt, sand
9	0.621	depth, S_{bw}, DO_{bw}, pH_s, Eh_s, Corg, clay, silt, sand

Table 6. Values of indices species richness (S), diversity (H'), a marine biotic index (AMBI), multivariate AMBI (M-AMBI) and ecological state according to M-AMBI (ES $_{\text{M-AMBI}}$) in June and September 2009.

Station	S	H'	AMBI	M-AMBI	ES$_{\text{M-AMBI}}$
B1 June	10	2.61	3.38	0.22	Poor
B1 September	15	2.64	2.67	0.33	Poor
B1 average	13	2.63	3.02	0.27	Poor
B2 June	10	1.14	4.66	-0.01	Bad
B2 September	14	1.78	4.71	0.10	Bad
B2 average	12	1.46	4.68	0.05	Bad
B3 June	21	3.16	3.13	0.42	Moderate
B3 September	22	1.34	1.45	0.37	Poor
B3 average	22	2.25	2.29	0.40	Moderate
B4 June	13	2.90	3.67	0.26	Poor
B4 September	16	2.63	2.98	0.32	Poor
B4 average	15	2.76	3.33	0.29	Poor
B5 June	12	1.64	4.16	0.10	Bad
B5 September	13	1.21	4.25	0.06	Bad
B5 average	13	1.43	4.20	0.08	Bad
B6 June	19	0.93	1.70	0.28	Poor
B6 September	20	0.73	1.49	0.29	Poor
B6 average	20	0.83	1.60	0.28	Poor
B7 June	18	2.18	3.65	0.26	Poor
B7 September	13	1.15	4.38	0.05	Bad
B7 average	16	1.66	4.02	0.15	Bad
B8 June	10	1.40	3.56	0.18	Bad
B8 September	18	1.87	3.88	0.33	Poor
B8 average	14	1.63	3.72	0.26	Poor
B9 June	11	1.95	4.12	0.12	Bad
B9 September	22	2.36	3.97	0.31	Poor
B9 average	17	2.15	4.04	0.21	Poor
B10 June	14	2.67	3.66	0.25	Poor
B10 September	12	2.04	4.43	0.12	Bad
B10 average	13	2.36	4.04	0.19	Poor
B11 June	10	1.27	4.03	0.04	Bad
B11 September	9	0.42	4.45	-0.08	Bad
B11 average	10	0.84	4.24	-0.02	Bad
B12 June	15	2.64	4.75	0.19	Bad
B12 September	13	1.29	3.20	0.14	Bad
B12 average	14	1.97	3.97	0.17	Bad
B13 June	0	0.00	7.00	-0.40	Bad
B13 September	0	0.00	7.00	-0.40	Bad
B13 average	0	0.00	7.00	-0.40	Bad
B14 June	17	0.94	1.81	0.25	Poor
B14 September	16	0.52	1.60	0.21	Poor
B14 average	17	0.73	1.70	0.23	Poor
B15 June	31	2.76	4.30	0.44	Moderate
B15 September	25	1.74	4.24	0.27	Poor
B15 average	28	2.25	4.27	0.35	Poor

may distort the assessment and lead to misrepresentation of the ecological state. In the latter case June is probably better suited for ecological assessment unbiased by bivalve molluscs recruitment during the preceding spring-summer. Stations 7 and 10 were in poor state in June and in bad state in September in correlation with decreasing oxygen and increasing organic carbon content. Ecological state at stations 8 and 9 was worse in June compared to September because of the lower oxygen content and higher concentrations of extractable matters.

The average value of M-AMBI from June and September was used to assess the overall state of a particular station. Bad state was characteristic of Varna Bay area adjacent to the canal, the canal itself and Varna Lake, explicable with the pressures originating from industries based around Varna Lake. Poor ecological state prevailed over the rest of Varna Bay area except for station 3, which was in moderate state in June. The latter result is ambiguous due to lack of sampling replication.

Conclusions

Varna Bay sedimentary seabed is partitioned among three major biotopes: sands dominated by bivalve molluscs *T. tenuis* and *L. mediterraneum* along the southern coast, silty sands co-dominated by thallassinid mud shrimps *U. pusilla* and bivalves *A. inequivalvis* and *T. tenuis* in the north-western area and sandy silts dominated by thallassinids and polychaete worms in the central and eastern part.

The best combination of variables explaining the biotic pattern includes the natural variables depth, salinity of bottom water, oxygen content in bottom water and sediment grain size, as well as the anthropogenic pressures – organic carbon and extractable matters. The biotic indices show strong correlations with the pressures organic carbon, extractable matters, pH and redox potential and moderate with salinity and sediment grain size. The observed relationships determine the range of important environmental parameters needed for understanding of benthic invertebrate fauna state and need to be included in monitoring programmes in order to assess the sensitivity and reliability of biotic indices to reflect the anthropogenic pressures.

The prevailing ecological state of Varna Bay in summer 2009 was poor, declining to bad in the vicinity of Varna Lake canal. The ecological state deterioration is associated with the increasing content of organic carbon and extractable matters in sediments, which are identified as major pressures on Varna Bay benthic ecosystem at present.

The results of this study 'ring the bell' that Varna Bay is a water body at risk of failing to meat the WFD requirements for achieving good ecological status of coastal waters by 2015.

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