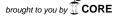
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Benthic macroinvertebrates as indicators of the water quality in Bulgaria: A case-study in the Iskar river basin

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ABSTRACT

Macroinvertebrates were sampled at 15 locations in the Iskar river basin in Bulgaria for the purpose of water quality assessment. Based on the chemical as well as the biological parameters, it was concluded that the water quality was still good upstream of Sofia, however, despite a huge waste water treatment plant, a strong decrease was observed when the river passed Sofia. Due to self-purification and dilution, a gradual amelioration of the water quality was observed 40 and 80 km downstream of Sofia, however, water quality was still insufficient. The Irish Biotic Index (IBI), which is currently used in Bulgaria for the national monitoring of macroinvertebrates for water quality assessment, does not fulfil the requirements of the European Union Water Framework Directive (WFD). The Multimetric Macroinvertebrate Index Flanders (MMIF), on the contrary, is a WFD compliant method developed for the northern part of Belgium, which is based on (1) the total number of taxa, (2) the number of Ephemeroptera, Plecoptera and Trichoptera taxa, (3) the number of other sensitive taxa, (4) the Shannon–Wiener index and (5) the mean tolerance score. The outcome of this MMIF was strongly correlated with the outcome of the Irish Biotic Index. Therefore, it should be possible to develop a similar multimetric index for macroinvertebrates to evaluate the biological water quality in Bulgaria without much effort.

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Introduction

According to the European Union Water Framework Directive (WFD), at least a good status should be achieved for all European surface and ground waters by the end of 2015 (EU 2000). For natural surface waters, this objective is more specifically described as the attainment of a good ecological and chemical status (EU 2000). A good ecological status implies that all biological quality elements show low levels of distortion by human activity and deviate only slightly from those normally associated with this type of surface water under undisturbed conditions (EU 2000).

The WFD requires member states to develop an assessment system for all types of rivers, lakes, transitional and coastal waters based on a number of biological quality elements, including benthic macroinvertebrates (EU 2000). In the national monitoring network of Bulgarian watercourses, a biotic index is used which is adapted from the Irish 'Quality Rating System' (Clabby and Bowman 1979; Mcgarrigle et al. 1992). In this index, selected indicator taxa are assigned to five sensitivity classes (most tolerant forms to sensitive forms based on saprobic sensitivity). Based on the most sensitive taxa present in a sample together with the total number of taxa in that sample, the score of this index is determined, in which five water quality classes are recognised. However, the WFD demands that assessment methods should meet a number of requirements. The Irish Biotic Index (IBI) has proven to be a reliable method; however, it does not meet all requirements of the WFD. At the moment, only one classification system is used for the evaluation of all types of rivers. The taxonomic composition is already used for the calculation of the IBI since it depends on the number of observed taxa, but abundances are not yet taken into account. Therefore, the IBI has to be adapted or a new method has to be developed. The index should be type-specific and abundances should be taken into account. The index has to reflect the relative distance to the reference conditions and it should have a value between 0 and 1, where 1 corresponds to a very good and 0 to a poor ecological status.

Nowadays, a lot of WFD compliant methods consist of multimetric indices (Buffagni et al. 2004; Böhmer et al. 2004a,b; Dahl and Johnson 2004; Gabriels et al. 2010; Hering et al. 2006; Ofenböck et al. 2004; Pinto et al. 2004). A multimetric index describes the state of an ecosystem by means of several individual variables (metrics). These metrics each represent a different component of ecosystem quality and are combined into one index value. Advantages of multimetric indices are that they are flexible and can easily be adjusted by adding or removing metrics or fine-tuning the metric threshold values. The development of a type-specific multimetric

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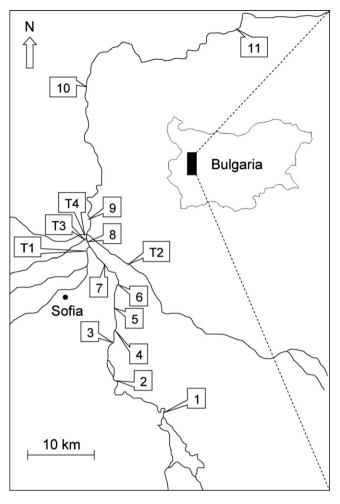


Fig. 1. Location of the sampling sites in the Iskar river basin in Bulgaria. The direction of the water flow in the Iskar River is from south to north. With the exception of the two most upstream sites (1 and 2), the water quality of all sampling sites is affected by effluents of Sofia, especially tributary T1, but the quality starts to improve downstream of Sofia (10 and 11) due to self-purification.

index for macroinvertebrates could therefore be a suitable solution to meet the WFD requirements in Bulgaria.

In the present study, the results are presented from an international collaboration project between Bulgaria and Belgium dealing with the water quality of the Iskar River near Sofia. The Irish Biotic Index, which is developed to evaluate the water quality based on benthic macroinvertebrates, is compared with a WFD compliant method consisting of a multimetric index. It is also indicated how a multimetric index can be developed with little effort, in order to meet the requirements of the WFD in Bulgaria.

Materials and methods

Study area

The Iskar river basin is situated in the southwest of Bulgaria. Sofia, the capital of Bulgaria, is located along the Iskar River and thus causes a strong pressure on its water quality. The population of Sofia is increasing rapidly and is now estimated at about 2 million inhabitants. A waste water treatment plant is operational with a capacity of an equivalent of 1.6 million inhabitants. However, the plant is still working below its capacity because a lot of the waste water from Sofia does not reach this plant. For the assessment of the water quality, 11 stations along the Iskar River were selected (Fig. 1). Samples were taken from 40 km upstream of Sofia, where the water quality is almost pristine to 80 km downstream of Sofia, where the river already has got some opportunity for self-purification after the inflow of waste water in Sofia. In addition, four tributaries were sampled: the Vladayska River (T1) that drains part of Sofia, the Lesnovska River (T2) draining a rural area and some metal processing industry, the Kakach River draining a rural area (T3) and the Blato river draining a rural area and the city of Kostinbrod (T4) (Fig. 1).

Sampling

Sampling took place from 31 March 2008 to 4 April 2008. Macroinvertebrate samples were always collected by the same operator with a standard hand net consisting of a metal frame holding a conical net ($20 \text{ cm} \times 30 \text{ cm}$, $300 \mu \text{m}$ mesh size). Sampling duration was 5 min active sampling. Organisms were collected from the different habitats present at the sampling site. Riffle habitats were sampled by holding the net downstream while the operator disturbed the substrate by kicking directly in front of the net opening. Stream edge habitats were sampled by vigorously sweeping along the stream margins disturbing bottom and bank substratum. The objective of the sampling was to collect the most representative diversity of macroinvertebrates at the site examined. This sampling procedure fulfils the requirements of both the Irish Biotic Index (IBI, Clabby and Bowman 1979) and the Multimetric Macroinvertebrate Index Flanders (MMIF, Gabriels et al. 2010). After separation, macroinvertebrates were identified under a stereomicroscope up to genus level (Plathelminthes, Polychaeta, Hirudinea, Mollusca, Odonata, Megaloptera, Hemiptera, Ephemeroptera, Plecoptera) or family level (Oligochaeta, Crustacea, Diptera, Coleoptera, Trichoptera), which is sufficient to calculate IBI (Clabby and Bowman 1979) as well as MMIF (Gabriels et al. 2010). Current velocity, dissolved oxygen, oxygen saturation, pH, conductivity and water temperature were measured in the field using electrodes. In addition, water samples were collected to measure nitrite, nitrate, ammonium, Kjeldahl nitrogen, phosphate, biological and chemical oxygen demand in the accredited analytical laboratory of Executive Environment Agency (the Ministry of Environment and Water) in Sofia.

Statistics

A basic Prati index, which gives a reflection of the chemical water quality, was calculated on the basis of the oxygen saturation, the chemical oxygen demand and the ammonium content (Prati et al. 1971). Based on the macroinvertebrate community composition, the Irish Biotic Index (IBI) was calculated according to Clabby and Bowman (1979) and the Multimetric Macroinvertebrate Index Flanders (MMIF) according to Gabriels et al. (2010). For the calculation of the MMIF, the Iskar River was regarded as a small river, while the tributaries were regarded as small streams based on their catchment area. In order to model the MMIF as a function of the environmental parameters, forward stepwise multiple linear regression was performed. Linear regressions were applied to assess the relationship between the MMIF and the measured environmental parameters. The Canonical Correspondence Analysis (CCA) option from the program package CANOCO (Ter Braak and Smilauer 2002) was applied in order to get an idea of the environmental parameters affecting the macroinvertebrate community. Prior to CCA analysis, all data were log transformed, except pH, which is already on a log scale. Since dissolved oxygen concentrations and oxygen saturation values were strongly correlated, only the former was retained for analysis.

															К.	Lock
	Flow (m ³ /s)	0.33	1.6	1.7	3.1	4.2	4.2	4.3	11	19	22	27	1.6	3.2	1.0	3.5
ingre measurements (COD: chemical oxygen gemang, bOD: piological oxygen gemang).	Phosphate (mg P/L)	0.042	0.088	0.371	0.138	0.053	0.070	0.066	2.20	1.20	1.78	1.26	2.15	0.088	2.89	0.75
	Kjeldahl N (mg N/L)	0.17	<0.17	0.39	0.36	1.04	0.98	0.45	4.06	4.64	4.61	1.82	8.57	1.26	8.93	1.77
	Nitrate (mg N/L)	0.14	0.22	0.21	0.18	0.54	0.78	0.95	0.91	1.90	1.07	1.72	0.40	2.04	0.86	2.20
	Nitrite (mg N/L)	0:0030	0.0050	0.010	0.011	0.010	0.019	0.025	0.046	0.085	0.091	0.119	0.135	0.667	0.138	0.054
	Ammonia (mg N/L)	0.008	0.014	0.007	0.003	0.18	0.16	0.21	1.90	3.76	3.45	1.06	7.65	0.77	6.05	0.13
	BOD (mg/L)	1.5	3.3	4.4	6.0	7.3	7.1	5.4	7.3	10	12	8.0	11.9	10.0	17.4	7.0
ingie measuren	COD (mg/L)	14.6	21.9	21.0	22.1	24.8	25.4	23.9	23.0	36.8	39.5	21.0	32.0	35.5	51.0	36.3
7	Conductivity (μS/cm)	183	171	193	212	235	270	302	386	494	458	417	419	548	581	621
כם ווו נווב ואנאו וו	Oxygen (%)	96.0	84.1	101.3	98.2	88.4	100.4	89.5	0.69	63.7	74.6	73.5	51.4	84.6	52.6	104.9
בוועווטווווכוונפו לפומוובנכוט וטו נווב טנפנוטוט אמוולובט ווו נווב ואנפו וועבו שמצוו טמטבת טוו	Oxygen (mg O ₂ /L)	9.73	9.94	10.90	10.56	9.79	10.82	10.04	7.12	6.66	7.88	8.05	5.39	8.72	5.50	11.72
וכובוא וטו ר	Ηd	7.8	7.9	8.8	8.8	8.2	8.6	7.9	7.6	7.5	7.7	7.3	7.1	7.9	7.6	8.2
בוורמו המימיוויר	$T(^{\circ}C)$	8.4	9.8	9.8	9.4	8.9	10	8.8	9.5	11.1	10.0	10.1	11.4	10.4	11.1	11.8
EIIVIIUIII		1	2	ę	4	5	9	7	80	6	10	11	T1	T2	T3	T4

Table

Table 2

The basic Prati index (BPi), the number of taxa, the Multimetric Macroinvertebrate Index Flanders (MMIF) and the Irish Biotic Index (IBI) for the stations sampled in the Iskar river basin.

	BPi	# Taxa	MMIF	IBI
1	1.8	36	0.85	5
2	3.6	28	0.75	5
3	2.3	7	0.25	2.5
4	2.4	16	0.65	3.5
5	3.9	10	0.25	2.5
6	3	9	0.3	2.5
7	3.8	17	0.5	3.5
8	6.9	5	0.1	1.5
9	9.9	7	0.2	2.5
10	9.1	7	0.3	2.5
11	5.7	8	0.35	2.5
T1	12	3	0	1
T2	6	11	0.45	3
T3	13	4	0.15	1.5
T4	4.4	9	0.35	2.5

Results

The measured values of the environmental parameters are listed in Table 1. The highest pH values and oxygen concentrations were observed downstream of the lake situated between sampling points 2 and 3, which contained algae blooms. The lowest oxygen concentrations were measured in the tributaries draining Sofia and in the Iskar River immediately downstream of Sofia. The conductivity, chemical as well as biological oxygen demand and nutrient concentrations, gradually increased from upstream to downstream with a slight amelioration in the most downstream stations. Also tributaries possessed rather high values for these parameters, especially those draining Sofia. The basic Prati index increased from 1.8 at the most upstream station to 9.9 at station 9, which once again reflected a deterioration of the chemical water quality (Table 2). At the most downstream station, the basic Prati index decreased somewhat to 5.7, which indicated a slight improvement 80 km downstream of station 9.

During the sampling campaign, more than 15,000 macroinvertebrates were observed belonging to 51 taxa of which 38 were insects. Ephemeroptera (9 taxa), Trichoptera and Diptera (both 7 taxa), Plecoptera, Coleoptera and Hirudinea (each 5 taxa) had the highest diversity. The number of taxa decreased from 36 in the most upstream sampling station to 5 at station 8 and even less in the tributaries draining Sofia (Table 2). The MMIF decreased from 0.85 at the most upstream sampling station to 0.1 at station 8, however, a slight improvement was observed more downstream, with a value of 0.35 at the most downstream sampling station (Table 2). Since the hydromorphological quality of the Iskar River was still good at all the sampling stations, the deterioration of the ecological water quality was exclusively attributable the chemical quality. The IBI, which ranges from 1 to 5, is highly correlated with the MMIF, which ranges from 0 to 1 ($R^2 = 0.96$, p < 0.0001) (Fig. 2). The basic Prati index (Bpi) is negatively correlated with both the IBI (R = -0.70, p = 0.0034) and the MMIF (R = -0.68, p = 0.0052).

Forward stepwise multiple linear regression was applied with the environmental variables as independent variables and the MMIF as the dependent variable. Kjeldahl nitrogen turned out to be the only parameter with a significant contribution $(R^2 = 0.47, p = 0.0030)$. Based on linear regressions, not only Kjeldahl nitrogen $(R^2 = 0.63, p = 0.00040)$ was correlated with the MMIF, but also temperature $(R^2 = 0.31, p = 0.030)$, oxygen concentration $(R^2 = 0.36, p = 0.018)$, conductivity $(R^2 = 0.34, p = 0.023)$, COD $(R^2 = 0.32, p = 0.028)$, BOD $(R^2 = 0.59, p = 0.00085)$, ammonium $(R^2 = 0.48, p = 0.0043)$ and phosphate $(R^2 = 0.50, p = 0.0030)$, however, the environmental parameters were strongly intercorrelated.

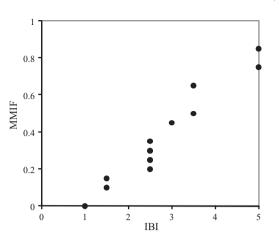


Fig. 2. Relationship between the Irish Biotic Index (IBI) and the Multimetric Macroinvertebrate Index Flanders (MMIF) for the samples from the Iskar river basin.

Based on the Canonical Correspondence Analysis (CCA), the sampling stations could be divided into three groups (Fig. 3). Along the first axis (Eigenvalue 0.55), sampling stations 1 and 2, which had low conductivities, water temperatures, biological and chemical oxygen demands and nutrient concentrations, were separated from the other stations. Along the second axis (Eigenvalue 0.27), the stations with the lowest oxygen concentrations (8, 9, T1 and T3) were separated from the other stations. Since only a limited number of samples was included in the present study, the performed CCA only indicates the major trends in the dataset.

Discussion

According to the WFD Intercalibration Technical Report April 2008 (CB-GIG 2008), only two countries from the Eastern Continental Geographical Intercalibration Group developed WFD compliant national methods for benthic macroinvertebrates in rivers: the Slovak Republic (Krno et al. 2007) and Austria (Ofenböck et al. 2004). For the Czech Republic, Hungary, Romania and Bulgaria, WFD compliant methods are still under development. The methods for the Slovak Republic and Austria are both procedures developed within the AQEM research project ('The Development and Testing of an

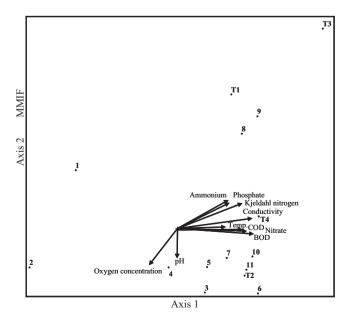


Fig. 3. Biplot of the sample scores and the environmental variables.

Integrated Assessment System for the Ecological Quality of Streams and Rivers throughout Europe using Benthic Macroinvertebrates') carried out under the 5th Framework Programme of the EU (Hering et al. 2006). The AQEM sampling method is based on a multi-habitat scheme designed to sample major habitats with more than 5% coverage in proportional representation within a sampling reach (Ofenböck et al. 2004). Currently, the sampling method in Bulgaria does not allow the calculation of AQEM.

MMIF is a multimetric index that consists of five metrics: the number of taxa, the number of EPT taxa (Ephemeroptera, Plecoptera and Trichoptera), the total number of sensitive taxa (other than EPT), the Shannon–Wiener Index and the mean tolerance score (Gabriels et al. 2010). Despite the fact that the MMIF was not developed to evaluate the river types occurring in Bulgaria, it could be easily applied to the samples from the Iskar river basin, which is not surprising since the MMIF was included in the European Union WFD intercalibration exercise (CB-GIG 2008). A good ecological status, which is attained with a MMIF of at least 0.7, was only observed in the two most upstream sampling stations. Keeping in mind that at least a good status should be achieved for all European surface and ground waters by the end of 2015, there is still need for a serious improvement of the water quality in the Iskar river basin.

The IBI, which ranges from 1 to 5, is highly correlated with the MMIF, which ranges from 0 to 1 ($R^2 = 0.96$, p < 0.0001) (Fig. 2). It should therefore be possible to develop a multimetric index for Bulgaria, which resembles the MMIF, without much effort. This would have the additional advantage that, in contrast to methods using the AQEM sampling method, samples that were taken for the calculation of the Irish Biotic Index during the last decade can still be used to calculate the MMIF (Gabriels et al. 2010). Only a few adaptations of the MMIF are needed for its application in Bulgaria: type-specific threshold values for the five different metrics should be assigned for all river types occurring in Bulgaria, some extra taxa occurring in Bulgaria should be added and tolerance scores should be assigned. In comparison with Flanders, additional river types can be found in Bulgaria. However, since almost pristine watercourses can be easily found in Bulgaria, it should not be to difficult to determine typespecific reference conditions and the mentioned threshold values for the five metrics could therefore easily be assigned based on samples taken in each river type.

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