Applicability of the 'Watershed Habitat Evaluation and Stream Integrity Protocol' (WHEBIP) in assessment of the stream integrity in Bregalnica River Basin

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Abstract

This paper reports the assessment of ecological integrity of streams in Bregalnica River Basin with emphasis on river Bregalnica as the biggest and most important watercourse in Eastern Macedonia. The results have principally been derived from remote sensing data and set up in a model build up on Watershed Habitat Evaluation and Stream Integrity Protocol (WHEBIP). WHEBIP effectiveness in predicting ecological integrity of streams has been assessed by correlation analyses derived upon data on macroinvertebrate biotic indices and physico-chemical parameters on 35 localities throughout the basin. The statistical analyses confirmed the capacity of WHEBIP to predict stream site-specific features with great accuracy in case of Bregalnica.

The results obtained in this study contribute towards improvement of the WHEBIP protocol and in general promotes applicability of stream integrity assessment tools in setting priorities for integrated watershed management.

Introduction

Healthy riverine ecosystems are essential in supporting human societal existence. The urge to mitigate the intensive anthropogenic pressure that riverine ecosystems have faced in the past decades has prompted worldwide initiatives and actions for their preservation and management (Bernhardt *et al.* 2005, Palmer *et al.* 2007, Bernhardt and Palmer 2011). In response to the need of integral and hierarchical approach in river conservation and management (Tockner *et al.* 2010, Törnblom *et al.* 2011, Leal *et al.* 2016), conservationists and policymakers have broadened their scope from riverine ecosystem to a river basin-based management. Consequently, assessment tools that allow practical approximation of the ecological integrity of streams on a large scale have been continuously developed and reviewed (Resh *et al.* 1995, Bain *et al.* 2000, Fernández *et al.* 2011).

Freshwater ecologists have recognised that riverine ecosystems are strongly influenced by the land use/cover properties at both reach and catchment-scale (Roth *et al.* 1996, Riseng *et al.* 2011, Clapcott *et al.* 2012, Valle Junior *et al.* 2015). Still, the conclusions on the relative importance of land use in determining stream integrity vary depending on the spatial scale, the interactive nature of the disturbances and the responding indicators (Allan 2004, Bruno *et al.* 2014, Tanaka *et al.* 2016, Segurado *et al.* 2018). The results of these studies are particularly applicable for defining the underlying mechanisms that regulate correlations between landscape indicators and riverine ecosystems (Gergel *et al.* 2002, Burcher *et al.* 2007). Growing interest in exploring the complex interactions between riverine ecosystems and their basins contributes to the complementation and validation of existing, and conceptual definition of new stream integrity assessment methods. In this regard, Goforth and Bain (2010) have put forward a stream-integrity protocol – WHEBIP, that relies on "interpretations of remotely sensed land-cover patterns of riparian and subbasin areas adjacent to and upstream from reaches of interest", primarily for the purpose of guiding watershed restoration priorities.

Freshwater ecologists have given a prodigious consideration to the causal effect of watershed properties and landscape indicators on stream integrity. Even so, regional studies aimed at determining the ecological status of streams are restricted on a specific stream lot and mainly based on physico-chemical parameters and biotic indicators. In Macedonia, stream integrity assessment based on an interpretation of remotely sensed land-cover patterns of riparian and subbasin areas with respect to the applicability of WHEBIP has been considered by Jovanovska *et al.* (2013). Due to lack of adequate comparative field data Jovanovska *et al.*

(2013) give only general discussion of the relevance of the results obtained by the stream integrity assessment, unable to contribute towards validation of the effectiveness of the protocol.

Recent continuous interest of international donors and agencies for Bregalnica River Basin (Basler and Partner 2016, NCP-SDC 2016) allowed freshwater ecologists to carry out detailed research on the ecological status of rivers in Bregalnica River Basin (Hristovski and Brajanoska 2015, Krstić *et al.* 2016). Thus, the existing data on ecological status of the carrying watercourse - Bregalnica derived from biotic indices i.e. macroinvertebrates (Slavevska-Stamenković 2013), fish populations (Kostov *et al.* 2010) and cytological biomarkers in fish (Rebok *et al.* 2010) were complemented and revised. The availability of these field specific data gives a solid basis for constructing a comparative analysis in order to review and determine the effectiveness of WHEBIP stream integrity assessment protocol.

Even though the applicability of the abound of rapid stream integrity protocols has been previously reviewed (Resh *et al.* 1995, Fernández *et al.* 2011) and trialed by many (Hawkins *et al.* 2000, Barquín *et al.* 2011, del Tánago and de Jalón Lastra 2011, Feld, Segurado, *et al.* 2016), their success in predicting the stream integrity still varies with the environmental specifics and differs across ecoregions. Therefore, the goal of this study is to assess the applicability of a multimeric assessment tool - Watershed Habitat Evaluation and Stream Integrity Protocol (WHEBIP) (Goforth and Bain 2010) by using available field specific data on the ecological status of streams derived from biotic indices and physico-chemical parameters in Bregalnica River Basin.

Methodology

Study area

River Bregalnica is the largest river in Eastern Macedonia with a length of 225 km and is also the longest tributary of the river Vardar. Bregalnica River Basin covers an area of ˜4300 km^2 (Gaševski 1979, Hristovski and Brajanoska 2015). The mean longitudinal slope of the river Bregalnica's riverbed is 7‰, while its largest tributaries Kriva Lakavica and Svetnikolska Reka have the lowest relative longitudinal slope of 7.6‰ and 11.6‰, respectively. In the headwater, the bottom of the river Bregalnica is mainly composed by boulders, cobbles and gravel, and the river has a river width and depth of up to 5 m and 0.5 m, respectively. In its middle part, the riverbed is manly represented by cobbles, gravel and sand, and the river is up to 10 m wide and around 1 m deep. In its lower end, the riverbed is mainly represented by fine sand and silt, and the river is up to 20 m wide and its depth reaches up to 1.5 m. The area drained by river Bregalnica and its tributaries is characterised by high geomorphological diversity throughout broad altitudinal gradient (see Fig. S1 in supplementary material). These attributes of Bregalnica River Basin, complemented by the complexity of several variances of climate types', ultimately enabled a great diversity of habitats of different distribution and distinctive organisation (Lazarevski 1993, Zikov 1995, Filipovski *et al.* 1996).

In its largest, Bregalnica River Basin is covered by deciduous forests (45%) ranging zonally from oak to beech and even mixed (3%) and coniferous forests (2%) at a higher altitude (Filipovski *et al.* 1996, Hristovski and Brajanoska 2015). A significant part of the forested area up to ˜1000 m a.s.l. in Bregalnica basin is represented by secondary vegetation - hilly dry grasslands (9%) or secondary mountain grasslands at the high altitudes (1%), and where abandoned, successional vegetation of scrubland or heathlands (4%) prevail.

The riparian vegetation in river Bregalnica basin is represented by riverine willow scrub and willow and poplar galleries (Hristovski and Brajanoska 2015). In the upper parts of the river flows, the willow and poplar galleries are often mixed with alder and fuse with the surrounding forests. In the upper part of river Bregalnica riparian forests dominate over riparian scrub and usually do not exceed 30 m in width. The riparian vegetation along the middle part of river Bregalnica is dominated by riverine willow scrub and willow and poplar belts, while large part of the riparian belt is significantly altered (habitat conversion, hydromorphological alterations). Riparian forests in the lower part of river Bregalnica have no significant breaks in canopy continuity, while the width of riparian forests in most part exceed 50 m in width and in some parts extend 150 m in width.

About one-third of the land in river Bregalnica basin (33%) is used as agricultural land. Most of the wetland areas in Bregalnica River Basin have been drained for the purpose of irrigation as many streams including river Bregalnica have been hydromorphologicaly altered (irrigation channels and hydro-accumulations) (Gaševski 1979, Filipovski *et al.* 1985, Zikov 1988).

According to the State Statistical Office (2012a) Bregalnica River Basin administratively encompasses 19 municipalities and sustains a population of about 180.000 (State statistical office 2002). The region is characterised by poor economic growth, considerable rural-urban migration and high rate of emigration (State statistical office 2012a, 2012b). Human presence is most prominent along the rivers as about 40% of the settlements and villages situated in the area are adjacent to rivers and streams. The main economic activity, especially in the lowland areas is agriculture (State statistical office 2012a).

Materials and methods

Stream integrity was interpreted from available remotely sensed data sources (land cover/use maps (CLC 2012), Google Earth satellite imagery and ASTER GDEM) and digitised vector data coupled by topography maps, scale 1:25,000 (Agency for Real Estate Cadastre of the Republic of Macedonia). Computer processing has been performed with the ArcGIS 10.2.2 software.

For calculation of the stream integrity we followed the concept presented in Jovanovska *et al.* (2013) based on the multimetric assessment tool - the Watershed Habitat Evaluation and Biotic Integrity Protocol (WHEBIP), first elaborated in detail by Goforth and Bain (2010).

All streams were categorised by order of stream (following the Strahler (1952) hierarchy of streams) and consistency of flow (continuous or intermittent). Following Goforth and Bain (2010), a stream segment is considered to be a length of stream bounded by an upstream source or confluence and a downstream confluence or terminal water body (lake, water reservoir). A subbasin is the land area drained by a stream segment as separated topographically from adjacent basins by drainage divide. Finally, upstream stream segments are those that converge to form the stream segment that is being evaluated.

The following streams have been taken into consideration as relevant for the analysis: streams with continuous flow, intermittent streams with noticeable basins and intermittent streams that delineate considerable change in land use along the mainstream basin. Segmentation has been avoided on minor streams or those streams that delineate basins characterised by consistent land use. Each segment's integrity has been calculated separately. All stream segments and basins have been marked with a unique code containing the information about its hierarchical link to the main recipient.

The model includes 12 category metrics (Fig. 1, also Tab. S2 in supplementary material), comprising four groups of riparian and subbasin properties, which, according to Goforth and Bain (2010), significantly influence stream ecological processes and functions: riparian structure, subbasin land-use composition, watershed slope gradient, populated places and conservation enhancements. In order to improve the accuracy of WHEBIP, we made few alterations to the approach applied in Jovanovska *et al.* (2013) in regard of WHEBIP categories 1, 3, 4, 7, 8, 10 and 11. The width of the buffer that is created for calculating WHEBIP scores of categories 1, 3, 4, 7, 10 and 11 can be adapted and should be determined on the basis of the expert assessment. The width of the buffer depends on the character of the features subjected to the analysis, the accuracy of the available vector files and the specifics of the area of interest.

Specifically, for calculating the WHEBIP scores of categories 1, 4 and 7 (Fig. 1 upper left for categories 1 & 7; middle right for category 4) the buffer width ranged from 30, 50 and 100 m depending on whether the analysed stream segment is a portion of the upper, middle or the lower reaches of the watercourse. For calculating the score of WHEBIP category 3 (Fig. 1 middle right) the buffer of the riparian land cover has been increased from 5 m in Jovanovska *et al.* (2013) up to 15 m in the case of Bregalnica in order to enable intersection with the line vector of the stream segment.

WHEBIP category 10 metric (Fig. 1 lower left) has been calculated as the presence or absence of intersection between the union of populated places/settlements vector and point sources pollution vector (digitised polygon-vectors) with a) stream segments (for differentiation between the low and middle score) and b) stream segments' subbasin (for differentiation between middle and high score). The buffer width on populated places/settlements varied from 30 m, 50 m to 100 m depending on the settlement type and impact. The buffer width of other single identifiable sources of pollution ranged from 250 m for industrial centres, factories, disposal sites and dumps going up to 500 m for mines depending on the character and the degree of impact of the pollution source.

In order to improve the accuracy of WHEBIP for calculating stream integrity, we added another sub-category that complements the basic 12 WHEBIP categories originally given in Goforth and Bain (2010) and is closely related to WHEBIP category 10. WHEBIP sub-category 10a assesses (Fig. 1 lower left) the impact of point source pollution upstream of the analysed stream segment, including tributaries. If there is a presence of a point source pollution adjacent to the stream segment upstream of the analysed stream segment (including point source pollution presence adjacent to its tributaries) 20 points are deducted from the overall WHEBIP score (-20); presence of a point source pollution within the drainage area of the stream segment upstream of the analysed stream segment (including point source pollution presence within the drainage area of its tributaries) (-10); no presence of point source pollution upstream (0). The valuation principles follow-up on those applied in WHEBIP category 10.

The score for WHEBIP category 11 (Fig. 1 middle left) has been calculated as the presence or absence of intersection between a) the stream and the buffer of a vector comprising hydromorphological disturbances and alterations (determining the lowest score) and b) intersection between 30 m buffer of the stream segment and the vector comprising hydromorphological disturbances and alterations (for differentiation between the middle and high score). The width of the buffer for the vector comprising hydromorphological disturbances and alterations (5 m, 10 m and 50 m) depends of the character and the degree of impact of the hydromorphological disturbance. Roads, bridges, sand quarries, canals, river barrages, reservoirs and accumulations have been taken as relevant input data on hydromorphological disturbances. Additionally, if the upstream stream segment riverbed has been hydromorphologicaly altered by dam construction or an accumulation reservoir then WHEBIP categories 7 and 8 are assigned with the lowest score (1). Standing waters such as accumulations, reservoirs and lakes have not been assessed.

A detailed overview of the applied alterations to the original WHEBIP protocol first elaborated by Goforth and Bain (2010) are presented in Table S2 (supplementary material). The table also provides a comparative overview of all WHEBIP category metrics, their descriptive characteristics and calculation specifics applied in the case of Bregalnica River Basin with reference to supporting literature.

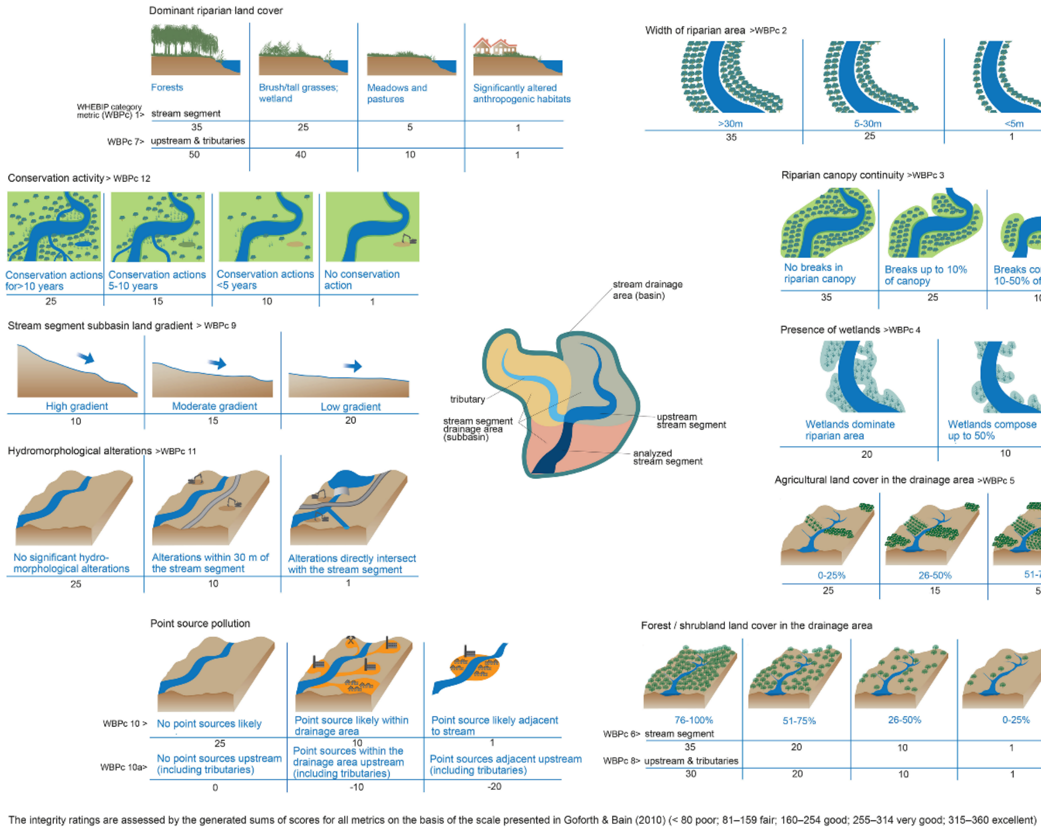

Figure 1. Graphical presentation of the methodological approach used in stream integrity assessment in river Bregalnica Watershed following Watershed Habitat Evaluation and Biotic Integrity Protocol (WHEBIP) first elaborated in detail by Goforth & Bain (2010)

In order to assess the effectiveness of WHEBIP in predicting stream integrity, WHEBIP stream integrity scores and associated integrity ratings were compared with biotic and saprobic indices and associated integrity ratings derived from site-specific survey data. The biotic indices used to determine stream ecological status are macroinvertebrate-based indices and include: BMWP (Biological Monitoring Working Party), ASPT (Average Score per Taxon) in sense of (Armitage *et al.* 1983) and EPT richness (number of taxa in Ephemeroptera, Plecoptera and Trichoptera taxon) in sense of (Bode *et al.* 1997). In addition, the study includes correlation analysis between WHEBIP scores and associated integrity ratings with saprobic index in sense of Zelinka and Marvan (1961) indicating the ecological status of the stream in terms of organic pollution. Macroinvertebrate-based indices (BMWP, ASPT, EPT) and saprobic index final score ranges and associated ratings were adapted in order to respond to ecoregion specifics (Tab. 1). Sampling of macroinvertebrates was carried out in accordance with international standard specified criteria (ISO 10870: 2012). ASTERICS software, version 3.0 (www.aqem.de) was used to calculate above mentioned indices.

Site survey data was obtained from 35 localities throughout Bregalnica River Basin, with 16 localities positioned along river Bregalnica (from the source to its inflow in river Vardar) and 19 localities along the larger tributaries of the river Bregalnica (Fig. 2). Survey scores for both biotic and saprobic indices were compared for associations with WHEBIP scores using Statgraph Centurion XVI by applying simple regression analyses. The relations between the associated integrity ratings were determined using Spearman rank correlation coefficient.

Results

A total of 1421 stream segments of more than 250 rivers and streams have been identified and assessed in Bregalnica River Basin. All analysed stream segments are encompassed in 84 basins covering a total area of 3513 km^2 that combined with the immediate drainage area of river Bregalnica (790 km^2) form river Bregalnica River Basin with a total area of 4303 km^2. The integrity of near 12% of analysed stream segments has been rated as excellent, 23% have been rated as streams segments with very good integrity, 35% as good, 22% as fair and the integrity of 8% of analysed stream segments has been rated as poor. River Bregalnica has been divided into 73 stream segments out of which the integrity of 5 has been rated as excellent, 10 as very good, 28 as good and 22 as fair, whilst 8 of Bregalnica stream segments have been rated as poor. The stream segments of 37 out of 75 analysed tributaries of river Bregalnica, immediately prior their confluence with Bregalnica are rated as streams with fair or poor integrity. The methodology turned to be inapplicable when determining the integrity of mountain streams (26 stream segments out of 1421). Assessed stream segments and their associated integrity ratings are presented on Figure 2. Detailed overview of stream integrity scores and associated integrity ratings category metrics on the 35 stream segments along Bregalnica used in comparison with the site-specific sites are presented in Table S3 (supplementary material).

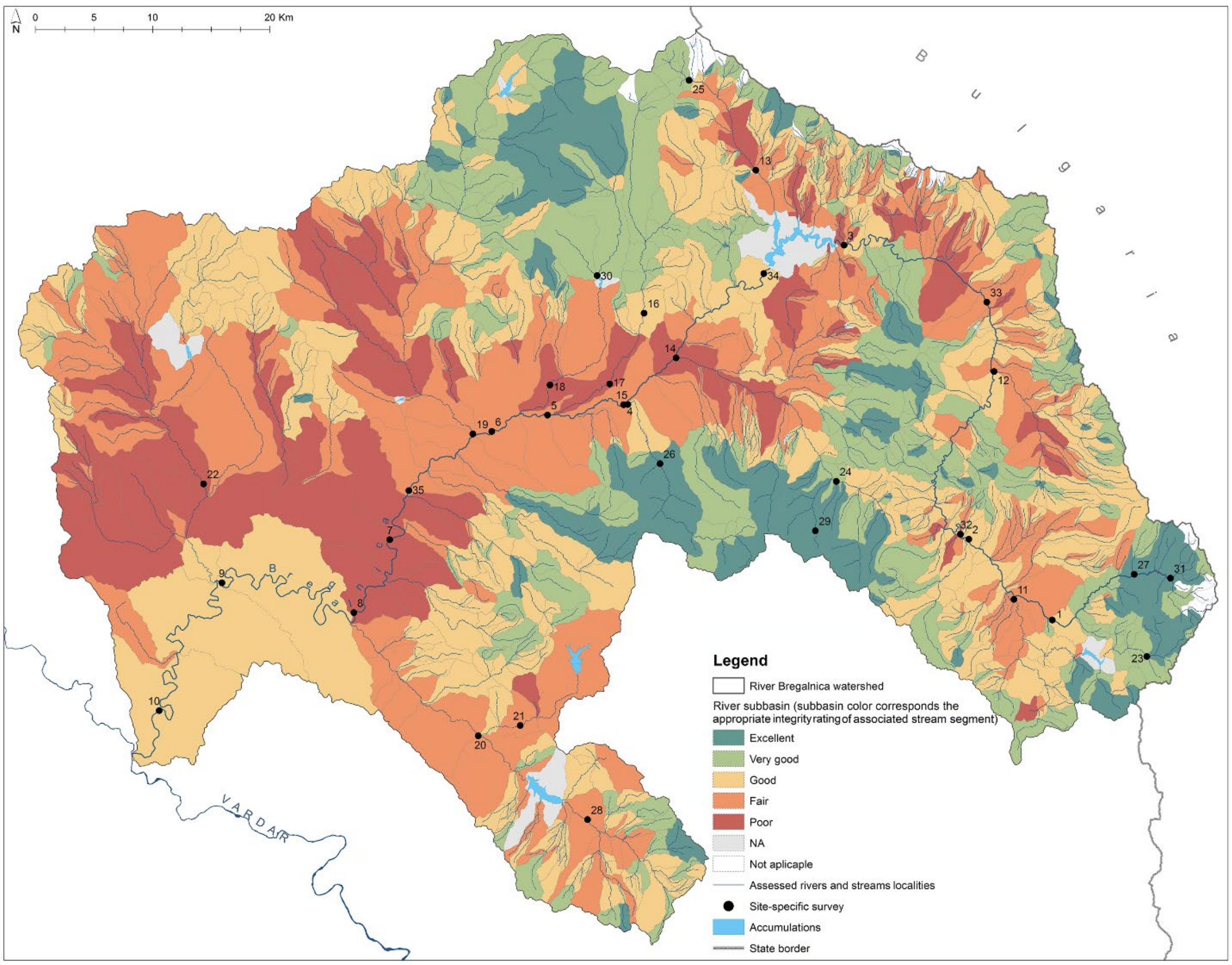

Figure 2. Overview of stream integrity in river Bregalnica watershed including sampling sites layout

The comparison of WHEBIP stream integrity ratings with macroinvertebrate-based stream-site scores showed that WHEBIP integrity ratings were equal to or within ±1 rating class in: 33 cases for BMWP; 23 cases for ASPT and in all 35 cases for EPT. For the saprobic index WHEBIP integrity ratings were equal to or within ±1 rating class in 22 cases (Tab. 1). Spearman Rank correlation coefficients (r) for associations between WHEBIP ratings and site-based integrity ratings varied from 0.63 for the saprobic index, 0.74 for BMWP, 0.64 for ASPT to 0.76 for EPT ($p<0.05$ in all cases).

Table 1. Detailed overview of BMWP, ASPT, EPT richness, Zelinka & Marvan saprobic index and WHEBIP scores and integrity ratings. Indices ratings in the table are encoded in order to provide better visibility: excellent (1); very good (2); good (3); fair (4); poor (5). The scores ranges and associated ratings for used indices are: BMWP: >100 (1); 80-100 (2); 50-79 (3); 25-49 (4); <25 (5); for ASPT: ≥6.01 (1); 5.01-6.0 (2); 4.01-5.0 (3); 3.01-4.0 (4); <3 (5); for EPT: >15 (1); 10-14 (2); 6-9 (3); 2-5 (4); <2 (5) and for Zelinka & Marvan saprobic index: < 1,5 (1); 1,5 ≤ SI < 1,8 (1.5); 1,8 ≤ SI < 2,3 (2); 2,3 ≤ SI < 2,7 (2.5); 2,7 ≤ SI < 3,2 (3); 3,2 ≤ SI < 3,5 (3.5 i.e. 4) and 3,5 ≤ SI 4 (4 i.e 5)

Site survey locality code	*SI Z&M score*	*SI Z&M rating*	*BMWP score*	*BMWP rating*	*ASPT score*	*ASPT rating*	*EPT score*	*EPT rating*	*WHEBIP score*	*WHEBIP rating*
1	1.7	1.5	81	2	6.3	1	8	3	189	3
2	3	3	35	4	3.9	4	7	3	174	3
3	2.6	2.5	52	3	5.3	2	5	4	222	3
4	2.5	2.5	37	4	5.3	2	2	5	130	4
5	2.6	2.5	27	4	3.9	4	2	5	120	4
6	3	3	38	4	4.8	3	3	4	97	4
7	2.5	2.5	40	4	5.7	2	6	4	30	5
8	3.4	4	50	3	5	3	5	4	77	5
9	3.3	4	31	4	3.9	4	5	4	169	3
10	3.2	4	56	3	4.25	3	6	4	218	3
11	1.2	1	52	3	6.5	1	4	4	103	4
12	1.9	2	48	4	5.3	2	5	4	106	4
13	2.5	2.5	30	4	6	2	4	4	87	4
14	2.7	3	32	4	4	4	4	4	30	5
15	3.1	3	31	4	4.2	3	5	4	190	3
16	1.3	1	139	1	7.3	1	8	3	160	3
17	2.4	2.5	41	4	5.9	2	5	4	25	5
18	3.6	5	3	5	1.5	5	0	5	15	5

19	2.7	3	40	4	4.4	3	2	5	93	4
20	3.6	5	5	5	2.5	5	0	5	91	4
21	4	5	2	5	2	5	0	5	101	4
22	3.6	5	3	5	1.5	5	0	5	69	5
23	1.3	1	112	1	7.2	1	13	2	297	2
24	1.5	1.5	105	1	6.9	1	11	2	291	2
25	1.2	1	56	3	7.3	1	7	3	307	2
26	1.2	1	133	1	7	1	14	2	331	1
27	1.6	1.5	128	1	6.9	1	13	2	331	1
28	2.5	2.5	47	4	5.2	2	5	4	149	4
29	1.4	1	111	1	6.9	1	12	2	321	1
30	1.4	1	105	1	7	1	13	2	272	2
31	1.34	1	136.4	1	6.96	1	19.25	1	331	1
32	2.71	3	35.2	4	3.98	4	4.00	4	119	4
33	2.20	2	55.3	3	5.10	2	6.67	3	92	4
34	2.17	2	55.4	3	5.47	2	6.67	3	165	3
35	2.54	2.5	60.4	3	5.66	2	6.25	3	87	4

The correlation coefficients (r) for WHEBIP scores and site survey scores for both biotic and saprobic indices varied from 0.62 to 0.81 with p<0.05 in all cases (Fig. 3). All models describing the relationship between WHEBIP scores and site survey indices scores are linear.

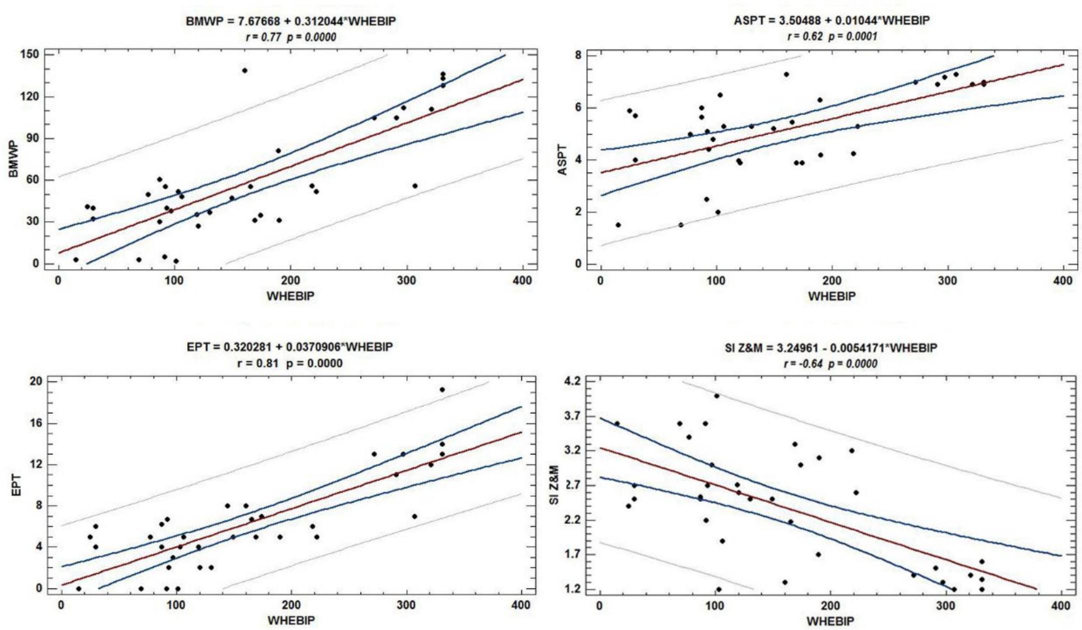

Figure 3. Overview of the correlation results (simple linear regression) between WHEBIP stream integrity scores and macroinvertebrate-based indices: BMWP (Biological Monitoring Working Party), ASPT (Average Score per Taxon) and EPT richness (number of taxa in Ephemeroptera, Plecoptera and Trichoptera taxon) as with Zelinka & Marvan saprobic index scores derived from site-specific survey data

Discussion

Results indicate that Watershed Habitat Evaluation and Biotic Integrity Protocol (WHEBIP) provides a sufficient insight into Bregalnica River Basin streams integrity. In general, as assessed with WHEBIP category metrics, the integrity of streams in Bregalnica River Basin gradually declines as the anthropogenic impact increases. This pattern is principally notable along middle and lower part of river Bregalnica and its major tributaries along which the anthropogenic pressure is most evident (Fig. 2). The pattern of a general decline in community richness and ecological conditions as a response to the increasing intensity of human pressures, from headwaters to lowlands, was also observed by Bruno *et al.* (2014). Most often referred anthropogenic pressures are changes in natural cover (Valle Junior *et al.* 2015), increase of land use intensity and impervious surfaces (Miserendino *et al.* 2011, dos Santos and Esteves 2015, Leal *et al.* 2016, Segurado *et al.* 2018) and hydromorphological disturbances (Belmar *et al.* 2013, Aguiar *et al.* 2016). The increase of human pressures impairs water quality and physical habitat quality and ultimately leads to change in stream communities (Burcher *et al.* 2007, Riseng *et al.* 2011). In the case of Bregalnica River Basin, the changes in stream integrity are reflected by the changes in the applied macroinvertebrate indices and follow on the WHEBIP results (Tab. 1, Fig. 3, also Fig. S1 in supplementary material).

Statistically EPT and BMWP biotic indices were found to be most closely related to WHEBIP. Namely, WHEBIP stream integrity increases with the naturalness of the land cover and with the decrease in disturbances. Its final score is strongly influenced by the properties of the riparian (WHEBIP 1, 2, 3 and 7) and basin (WHEBIP 5; 6 and 8) land use. The macroinvertebrate biotic indices are generally found to be strong respondents to the increase in land use intensity. The changes in the macroinvertebrate community are particularly evident with increase of agricultural land use (Doll *et al.* 2016, Segurado *et al.* 2018) and percent of impervious surfaces (Wang and Kanehl 2003, Wilkins *et al.* 2015). This is also because intense agricultural activities are often related to hydromorphological alterations, fragmentation of riparian zones and high nutrient loading (Riseng *et al.* 2011). The effects of these disturbances on the stream integrity are better reflected by the EPT communities. The Ephemeroptera, Plecoptera and Trichoptera orders have high habitat requirements and the EPT biotic index tends to decline with the decline of natural cover (Miserendino *et al.* 2011, Valle Junior *et al.* 2015).

BMWP index reflects the differential tolerance of aquatic macroinvertebrate families to the organic pollution (Blanco *et al.* 2007). Though WHEBIP does not directly address the organic pollution, its final score is strongly influenced by the properties of the riparian (WHEBIP 1, 2, 3 and 7) vegetation, thus its resulting correlation with BMWP. In the case of BMWP, higher values related to macroinvertebrates communities are determined by the riparian width and canopy structure of the riparian vegetation even in watersheds dominated by intense agriculture (Tanaka *et al.* 2016). The buffering effect of riparian areas and their mitigation effect of human pressures (agricultural land-use in the basin) was also observed by Riseng *et al.* (2011) and Bruno *et al.* (2014). Miserendino *et al.* (2011) found that even areas dominated by pastures can still support rich communities of invertebrates if the functions of the riparian belt are preserved. The importance of riparian vegetation for the EPT community structure mostly lies in its role as a trophic resource and source of shading (Vimos-Lojano *et al.* 2017) whilst for BMWP riparian vegetation acts as a buffer for disturbances (Riseng *et al.* 2011).

When analysed closely, both EPT and BMWP tend to differ in their response (Tab. 1, also Fig. S1 in supplementary material). Generally, WHEBIP integrity ratings tend to overestimate stream integrity when compared to EPT and underestimate stream integrity in case of BMWP (1 rating class deviations).

In the case of EPT deviations are mostly observed along the middle and lower parts of the river courses. This trend may be associated with the longitudinal change of microhabitat properties (river substrate, temperature and trophic resources) that are not assessed with WHEBIP. According to Slavevska-Stamenković *et al.* (2011) the effectiveness of the EPT index decreases along the river continuum and in the case of typical lowland rivers, the metric is not a good indicator of environmental stress. This is because the EPT taxa within lowland river habitats naturally occur with a lower number of species and lower population densities. Furthermore, Vimos-Lojano *et al.* (2017) found that taxonomic changes of EPT are closely associated with the change in microhabitat factors. In their case, EPT specific taxa exhibited decrease in density with raising water temperature, decrease in cobble substrate and increase of velocity (with exemption of Plecoptera). As expected under the river continuum concept (Vannote *et al.* 1980), another significant determinant of the EPT (that also strongly affects microhabitat factors) is the size of the river (Vimos-Lojano *et al.* 2017). Due to its remote assessment character, WHEBIP does not directly reflect the changes in river substrate (that in the case of Bregalnica gradates from stony with gravel (in the headwaters) to sandy with slit (downstream)). Still, when considering the findings of Barquín *et al.* (2011), WHEBIP scores for categories that reflect the riparian properties (WHEBIP 1, 2 and 3) could be seen as indicative to river habitat heterogeneity.

In the case of BMWP, the underestimating trend (mainly observable in the lower parts of river course) may be a result of the buffering properties of the riparian vegetation in predominantly agricultural basins. Again, considering the remote character of WHEBIP, it cannot assess the full range of riparian natural characteristics (del Tánago and de Jalón Lastra

2011). WHEBIP capacity to assess riparian properties (given its linear character) depends on the scale of input layer and using a small scale datasets (like CORINE land cover) reduces its precision.

Most significant deviations are noticeable when WHEBIP integrity ratings are compared with those derived by calculation of ASPT index. When compared to ASPT index WHEBIP underestimates stream integrity and deviates of more than 2 ratings in even 12 cases. The higher discrepancies between ASPT and WHEBIP scores may be result of the index specifics, as ASPT represents the average tolerance score of all taxa within the community. Generally, ASPT is not frequently used as an indicator of streams biotic integrity (Resh *et al.* 1995). Roche *et al.* (2010) found that ASPT is less indicative than BMWP of the decreases in environmental quality in the wet season, though ASPT could be advantageous when comparing environmental quality between seasons (Álvarez-Cabria *et al.* 2010).

An additional factor that could contribute to the deviations in ratings among WHEBIP and biotic indices is "coarse" segmentation. Namely, "coarse" segmentation and large sub-basin areas relativize the scores of category WHEBIP 5, 6 and 8 metrics which have a significant share in stream integrity final score. In this regard, in some cases there is an abrupt transition of stream integrity rating between two bordering stream segments. In this case biotic indices and saprobic index results should be interpreted while considering the actual stream integrity scores and also while considering the stream integrity scores and ratings upstream (including tributaries) and downstream. After all, rivers are continuous by their nature and lines and boundaries are set only to enable us humans to rationalise and calculate.

There are also observable deviations amongst the macroinvertebrate indices. This suggest that when assessing the stream integrity in a vast watershed, as in the case of Bregalnica, shifts in abiotic and biotic patterns and processes occurring naturally along the river continuum (Vannote *et al.* 1980, Ward 1998) and the dynamics of the river floodplain (Ward and Stanford 1995) are not evenly recognised even by applied macroinvertebrate indices. The Multiple Variable analysis (correlations) coefficient between the macroinvertebrate-based indices varies from 0.76 to 0.91 for BMWP, ASPT and EPT as the biotic indices ratings deviate amongst by ± 1 rank (Tab. 1, also Fig. S1 in supplementary material). Such deviations may also result in the fact that the ASTERICS software is originally developed for calculation of biotic indices in Eastern European countries and lacks data for 50.88 % of the total abundance of macroinvertebrate fauna from river Bregalnica (Slavevska-Stamenković 2013). This has resulted with a slight shift in the integrity ratings of the biotic indices, especially with relevance to the upper sections of the river flows and with reference to categories 1 and 2 (Excellent and Very Good). Aptly, used biotic indices were adapted in accordance with the general natural features of macroinvertebrate fauna present in Bregalnica River Basin. The scores and the integrity ratings of the biotic indices were modified to respond to the Bregalnica River Basin specifics. Still, there is a possibility that the lack of software performance, with regards to Bregalnica River Basin, may have reflected on the final results of the comparative correlation analysis. Thus, future trials of integrity assessment should consider modifying indicator lists for saprobic system and BMWP/ASPT for the wider area, as for example for Western Balkan area. In this regard, freshwater ecologists in the region should follow with further calibration for EPT, BMWP and ASPT biotic indices integrity ratings considering the ecoregion specifics and the specifics of the macroinvertebrate fauna of upper, middle and lower section of the river flows.

Regarding the saprobic index, WHEBIP integrity ratings were equal to or within ±1 rating class in 22 cases (where needed ratings are subtracted to whole numbers). Its correlation coefficient (-0.64) with WHEBIP scores is weaker than in the case of biotic indices (-0.76 to -0.91). This variance is due to the complex ecosystem interactions that preconditions the characteristics of the substrate and organic matter intake in the immediate drainage area and on a catchment scale (Allan *et al.* 1997, Riseng *et al.* 2011, Clapcott *et al.* 2012, Segurado *et al.* 2018). According to many researchers (Allan 2004, Miserendino *et al.* 2011, Riseng *et al.* 2011, Nõges *et al.* 2016) the naturalness of the riparian and basin land use is very closely related to the organic matter content and its deposition as with the ecosystem processes in the riverine ecosystems. In this regard, the observed correspondence between WHEBIP and the saprobic index could result from WHEBIP metrics tendency to assess the naturalness of the riparian and basin land use.

What further contributes to this correlating trend is the modification made on original WHEBIP metrics by complementing the point source pollution metric by including settlements, industrial centres, factories, disposal sites and dumps. Various studies have confirmed that settlements (Paul and Meyer 2001, Wang and Kanehl 2003, Miltner *et al.* 2004), mines (Alderton *et al.* 2005, Ramani *et al.* 2014) and industrial centres (Imoobe and Koye 2011, Walakira and Okot-Okumu 2011) impact the stream biotic integrity. The negative impact of the urban settlements on biotic integrity of rivers is noticeable when only 7% of urban cover is in the immediate drainage basin (Snyder *et al.* 2003). In that context, WHEBIP metric 10 – point source pollution has been complemented by sub-category 10a-point source pollution upstream (including tributaries) that allows consideration of the impact that point source pollution along and in the basin of upstream segment and its tributaries has on the downstream stream segment.

The amendment of WHEBIP metric 10 in the case of Bregalnica River Basin is further imposed by the characteristics of the area and it follows on the indications of several authors including: Stavreva-Veselinovska and Živanović (2006, 2011), Stafilov *et al.* (2014), Ramani *et al.* (2014).

Even if WHEBIP provides an insight into the saprobity of the riverine system, the indicative results should be interpreted with caution. Being assessed remotely, WHEBIP does not allow full consideration of the effects of potential non-point sources of nutrients or contaminants that are not necessarily related or can be derived from land cover types. Also, WHEBIP does not provide adequate insight of the pollution effect to which the river as a continuum is exposed more than one stream segment upstream. Even if the river ability for auto-purification is taken into account, if the river is under a significant impact of a pollution sources interacting upstream the effects linger in the lower sections of flow. More important, the type of pollution is not assessed in this way. Pollution of toxic chemicals may have a greater impact than pollution by equal number of point pollution sources of different type, e.g. heavy metals from mines. The protocol limitations in this regard potentially distorts the final result of the stream integrity score and thus strongly reflects on the WHEBIP correlation with the saprobic index and might potentially affect its correlation with the macroinvertebrate indices too. For this reason, and following the prospective technological advance in pollution monitoring and control, future applications of the WHEBIP protocol should trial to consider and integrate the effect of the integrative non-point pollution on stream integrity.

In the case of Bregalnica River Basin, WHEBIP fails to provide adequate insight of the stream integrity of mountain brooks flowing throughout mountain pastures (mountain grasslands). As a result, 26 stream segments out of total 1241 were excluded from the overall results. This is mainly due to the fact that 2 out of 12 WHEBIP category metrics (WHEBIP 6 and

8) assess the forest cover along the stream segment and in its sub-basin, five WHEBIP metrics (WHEBIP 1, 2, 3, 4 and 7) rate riparian cover properties or presence/absence of wetlands. Also, originally WHEBIP category metric 5 estimates percentage of land cover beyond riparian zone as cropland or pasture treats the two land cover categories as equal (Goforth and Bain 2010), while pastures (grasslands) in the mountainous region are natural vegetation and has more or less equal significance for the stream integrity as forests in the lower altitudinal belts. Thus, adaptation of WHEBIP protocol should be considered when assessing streams and brooks that flow throughout mountain grasslands or extensively managed mountain pastures.

This is specifically relevant for the territory of Macedonia as in the countries of South-East Europe where even though mountain pastures have a secondary origin, these are characterised by high degree of naturalness, as are the brooks that "feed" them. Namely, mountain pastures in this region would have been potentially distributed over 2200 m a.s.l. but the millennia long lasting tradition of extensive grazing (herds of sheep and cattle during summer period) has artificially lowered the forest line by about 300-500 m (Melovski *et al.* 2015). Considering this, when assessing WHEBIP metrics 6 and 8 the vegetation cover that is considered to be climax or typical for the vegetation zone in question should be granted with the highest score for each metric accordingly. Also, assessing the properties of riparian habitats and peat bogs (WHEBIP metrics 1, 2, 3, 4 and 7) would inquire additional data processing and/or combined application of high-resolution raster imagery. Today, there is a wide array of available free source raster datasets to use e.g. see datasets available from European Space Agency (ESA) and USGS Global Visualization Viewer (GloVis) (see also overview of remote sensing instruments provided by (Mertes 2002)). Still, considering the significant share that these WHEBIP category metrics have in the final WHEBIP score, the WHEBIP protocol could still face limitations when determining the riparian properties of high-mountain streams. This suggestion should be supported by additional research.

Overall, WHEBIP protocol allows adjustment and if ecoregion specifics are considered, WHEBIP protocol provides a sufficient insight into stream integrity. The confirmed parallels between the WHEBIP stream integrity ratings and those obtained from calculation of macroinvertebrate-based indices in Bregalnica River Basin case is confronting Goforth and Bain (2010) indications that WHEBIP is "not as successful in predicting specific measures of stream integrity at local sites (i.e., benthic invertebrate community measures)" which again may be ecoregion specific. Still, this study does not provide comparative results on correlations of WHEBIP stream integrity ratings and scores with fish index of biotic integrity. Fish biotic index for Bregalnica River Basin, though calculated as part of "Bregalnica River Watershed Management Plan" project, was not included in this study. The results were considered as inadequate since EFI biotic index used is specified to be inappropriate for Mediterranean rivers and South-East Europe (Fame Consortium 2005, Krstić *et al.* 2016). Nonetheless, it is noteworthy to add that the results provided by application of WHEBIP protocol are generally in accordance with changes registered in qualitative and quantitative characteristics of fish community along Bregalnica presented in Kostov *et al.* (2010).

When assessing stream integrity in all manner, one must consider a number of indicative site-specific features in relation with landscape indicators in order to respond to the complex interactions in riverine ecosystems (Burcher *et al.* 2007, Tockner *et al.* 2010, Feld, Birk, *et al.* 2016, Segurado *et al.* 2018). The correlations in responses also vary in dependence of the scale against which all these features are interpreted (Roth *et al.* 1996, Allan *et al.* 1997, Allan 2004, Gieswein *et al.* 2017) and are species-specific (Cheimonopoulou *et al.* 2011, Miserendino *et al.*

2011, Clapcott *et al.* 2012, Tanaka *et al.* 2016). Thus, the applicability of stream integrity assessment methods as their relevance in setting priorities for conservation and management is still being reviewed across regions. Given that this "reviews" rely on comparisons with biotic indices derived for "few" localities as calculation of biotic indices is time consuming and site-specific, affirmative studies of stream integrity assessment methods across watersheds are still scarce.

In this regard, results presented in this study can be interpreted as an assertive reference of Watershed Habitat Evaluation and Biotic Integrity Protocol (WHEBIP). Furthermore, the possibility of a separate evaluation and interpretation of the landscape indicators allows distinction of those environmental features that have greatest impact on stream integrity. Moreover, the results obtained by using WHEBIP stream integrity assessment in Bregalnica River Basin can also guide selection of areas for conservation and management in the watershed or be used as a reference to guide site-specific research.

However, there is an imminent need for profound and more constructive studies that will determine the particularities in the underlying mechanisms that regulate correlations between landscape indicators and riverine ecosystems on a different scales. Even today, we still have a lot to learn about the pathways of stressors interaction and floodplain ecology (Gergel *et al.* 2002, Tockner *et al.* 2010, Segurado *et al.* 2018). But, as freshwater ecologists continuously contribute towards better understanding of the patterns of dynamic ecological processes, it is expected that new insights in the applicability of stream integrity assessments are yet to be provided. In that regard, the results of this study are an important contribution towards the confirmation of the applicability of stream integrity assessment methods that according to Sweeney *et al.* (2013) in the near future will have a significant contribution in setting priorities for river conservation and management in vast watersheds.

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