Report

Ecological Status Classification Schemes for the Upper Prut River basin (Ukraine)

August 2016

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1. INTRODUCTION

This new version of the document includes and update for the analysis of the Ecological Status Classification System. The objective of the ecological classification is to assign rivers into 5 classes of ecological status: high, good, moderate, poor, and bad. High status corresponds to undisturbed conditions showing no or minor deviation from the reference values, whereas in good and moderate status classes the deviation is slight and moderate, respectively. The deviation is measured in relative terms as the Ecological Quality Ratio (EQR = observed value/reference value). The key problems are to set numerical class boundaries for the quality parameters and elements, and to combine the information from many parameters and elements. The classification schemes for the group of water body types, for the macroinvertebrate biological element and for physico-chemical and hydromorphological elements (supporting the biological one) were established.

To establish reference conditions, values and class boundaries data from Joint Field Surveys (JFSs) in the Upper Prut River basin (2013 – 2016) were used. New data from biological (summer 2015 and spring 2016), hidromorphological and physico chemical were used for this new version. Additionally, historical physico chemical data from the period 2000-2015 were analysed in order to improve the boundaries for the different parameters.

2. SELECTION OF THE LOCATIONS WITH TYPE SPECIFIC REFERENCE CONDITIONS IN THE PILOT RIVER BASINS

For the purpose of the JFS and to develop the ecological status classification scheme, three main types of sampling locations were selected in the Upper Prut pilot basin:

- Sampling locations where reference conditions (or insignificant anthropogenic impact) are expected (representing high quality status);
- Sampling locations of lower water quality status, with some human pressure from point and diffuse pollution sources (representing good, moderate, poor and bad status);
- Sampling locations where the river crosses a state boundary.

There are several methods to establish reference conditions such as spatially-based methods, predictive models, palaeo-reconstruction and expert judgement. In the JFSs it was decided to combine a spatially based method with expert judgement. Selection of the potential locations to represent reference conditions involved the following main steps:

- Scoping, using maps and historical literature for preliminary identification;
- Pressure – impact analysis;
- A primary reconnaissance mission by experts (hydrology, hydrobiology, chemistry);
- Sampling for biological elements (macro-invertebrates), hydro-morphological quality elements and physicochemical parameters;
- Reassessment of the selected locations based on the data from earlierJFSs;
- A secondary reconnaissance mission to confirm (or otherwise) the locations of reference sites.

The above steps enabled the selection of sampling locations representative of the specific reference conditions.

In the upper reaches, the Prut is a typical mountain river, with steep slopes, lined with boulders and pebbles. In this section, the river flows in a deep valley, resembling a canyon at times.

The Prut basin in Ukraine is characterized by a very dense hydrographic network, containg 7,192 rivers with combined length of 16.404 km. The main tributaries of the Prut in Ukraine are the *Pistinka, Rybnitsa, Cheremosh, Zhizhiya, Tlumachik, Turka, Chornyava, Cherlena, Ringach* and *Rekitnyanka*.

The Prut River basin as defined by the EU WFD, the pilot river basin was subdivided into these groups of water bodies: Small&Middle mid-altitude rivers, Large mid-altitude rivers and High altitude rivers:

- Mountain “stone” type of river in coniferous forest area
- Flood-plain eroded small river with fine substrata (sand, clay)
- Small plain meandering river with fine substrata (mud, silt, clay)

For those groups the reference conditions locations were selected and analysis of the data from JFS was done to develop the ecological status classification schemes (ESCS).

The waterbodies of the region belong to this three categories:

- 10: Carpathians
- 16: Eastern plains
- 12: Pontic province

For illustration, photographs from three sampling locations expected to be reference conditions locations are given, see Figs. 1, 2 and 3.

Figure 1 riv.Iltsja, vil.Iltsi SW-20

Figure 2 **Zhonka river (water intake of Yaremche settlement (Carpathian Natural Park) (Ivano-Frankivska oblast) SW 43**

Figure 3 SW24 Rybnytsa, downstream of Yavoriv

3. METHODS AND DATA

3.1 Sampling methods

A modified multi-habitat sampling method was used, based on the AQEM/STAR methodology. 11 Sub-samples were collected using a net of 30 x 30 cm mouth size and 0,5 mm mesh size. The replicate sites selected after detailed habitat observation according to the ratio of main bottom substrata (pro-rata multi-habitat approach). Both kick-sampling and wash sampling was made. Usually, a 50:50 ratio of kick-sampling and wash sampling was used for the upper part of the river basin and 100 % kick-sampling for the downstream part (alluvial plain). The total area sampled per location was 1 m^2. Samples were fixed with ethanol 80%, stored in a cooling box and delivered to the laboratory for sorting and identification.

Macroinvertebrate samples were collected by experts from the Institute of Biology of Ukraine.

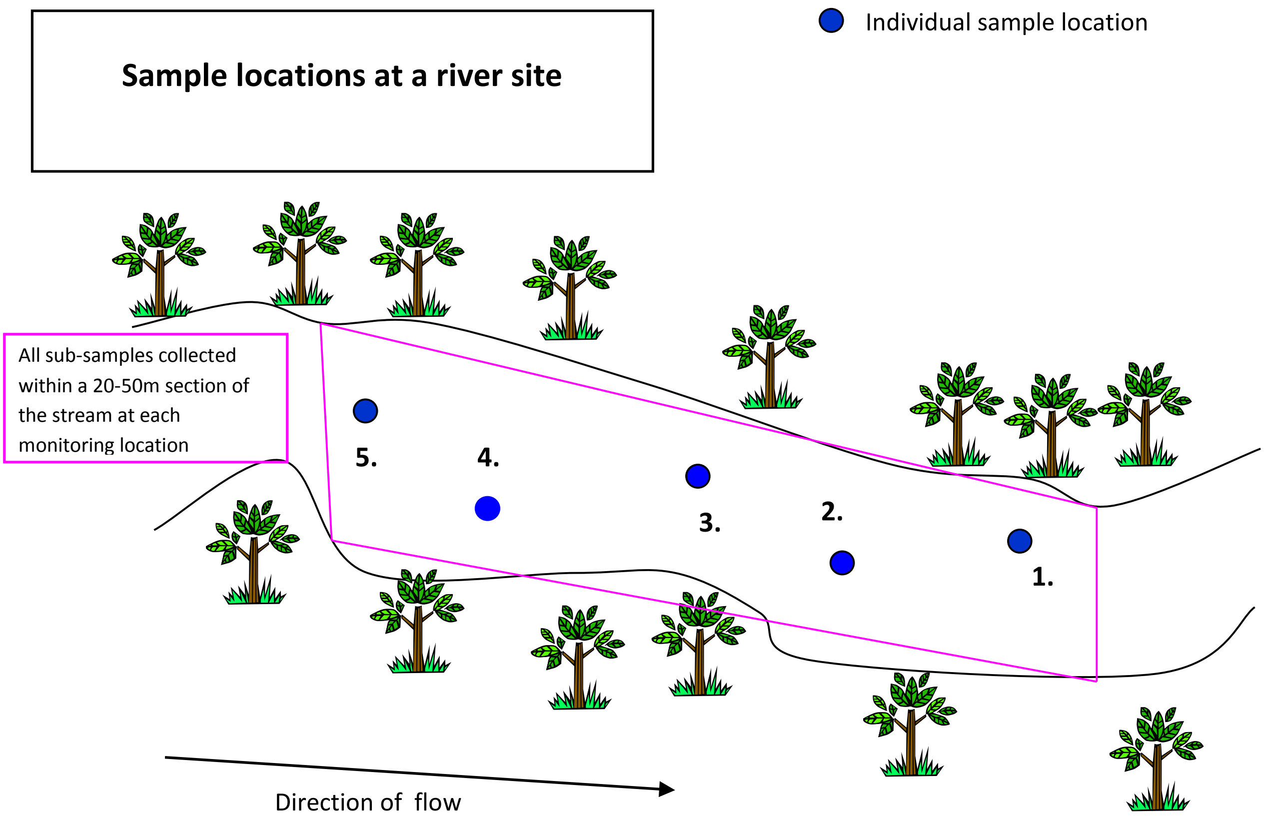

Figure 4 Scheme of the placement of replicate samples within a single river riffle stretch in relation to direction of flow.

3.2 Macroinvertebrates community

66 families were identified in the Prut River basin in Ukraine the most abundant families were Naididae and Tubificidae (Oligochaeta), Chironomidae and Simuliidae (Diptera), Baetidae and Heptagenidae (Ephemeroptera), Nemouridae and Leucridae (Plecoptera), Bithyniidae (Gasteropoda), Sphaeriidae (Bivalvia), Dugesiidae (Turbellaria), Elmidae (Coleoptera) and Limnephilidae, Brachycentridae, Hydroptilidae and Hydropsychidae (Trichoptera). These families are tolerant to some levels of organic pollution and altered basins with human impacts, they are also typical of high and middle mountain areas. There area many representants of scratchers related with the upper part of the river basin and more leaves and branches to be processed into organic materia particulated.For illustration, the overall number of families per order (2016) is presented for the Prut River basin in Fig. 5.

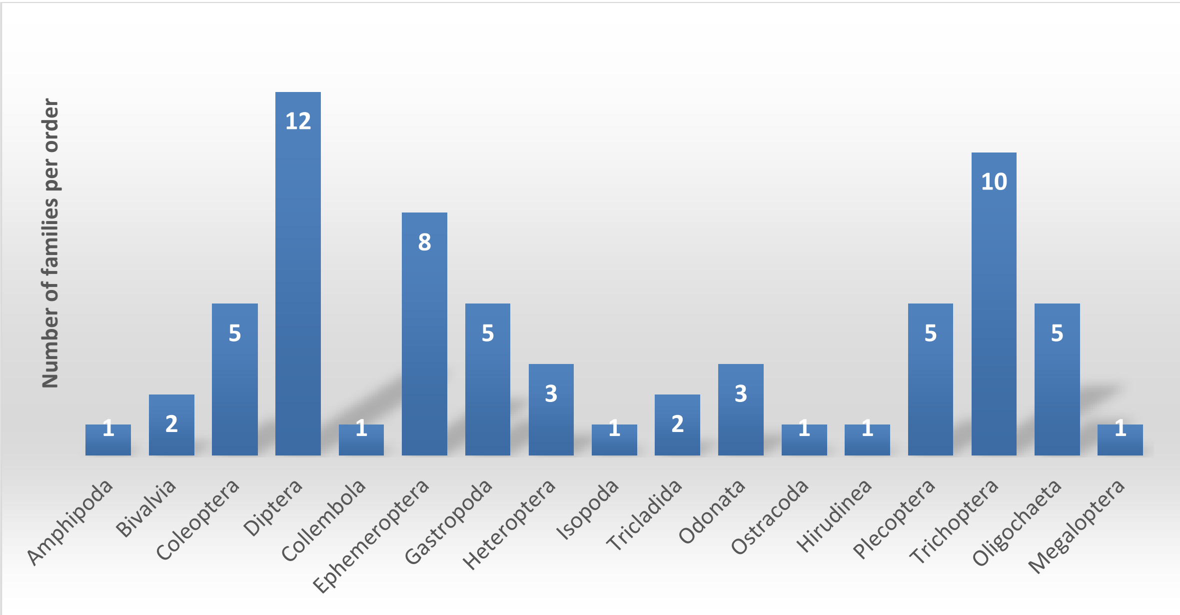

Figure 5 The number of macroinvertebrate taxa (families) per taxonomic group (order) for the Prut River basin (JFS 2016)

4. ENVIRONMENTAL DATA

All sampling locations were surveyed for basic physico-chemical parameters (Table 1).

Quality elements	Indicative parameters
Thermal conditions	*Water temperature*
Oxygen status	*Dissolved oxygen, oxygen saturation* *BOD_5* *COD* *Total suspended solids*
Nutrient conditions	*NO_3* *NH_4* *PO_4 (orthophosphates)*
Salinity	*Conductivity* *Cl* *SO_4* *Total dissolved solids (total mineralization)*
Acidification status	*pH*
Heavy metals	*As, Cd, Cu, Ni, Pb, Zn*

Table 1 Physico-chemical parameters used in the assessment of the ecological status in the Prut River basin

Physico-chemical parameters used in the assessment of the ecological status in the Prut River basin

Standardised methods were used for the subsequent analysis of the physico-chemical quality parameters.

Hydro-morphological degradation of the streams was based on the hydromorphological field protocol and hydromorphology assessment protocol (see Annex 1) and quantified by the hydromorphology quality score (HMQS).

4.1 Selection of metrics

The normative definitions of ecological status classes given in Annex V of theWFD include five characteristics of river benthic invertebrate assemblages to be considered:

(i) Community composition

(ii) Abundance of taxa,

(iii) The ratio of disturbance sensitive taxa to insensitive taxa,

(iv) The level of diversity, and

(v) The occurrence of major taxonomic groups.

If the normative definitions are to be taken strictly, all these features of macroinvertebrate community structure should be measured for the purpose of ecological classification. In practice, a large number of metrics (parameters, variables or indices) commonly used in biological assessment can be regarded to portray them.

In the case of the Prut River basin, metrics were calculated with AQEM (Asterics) software and these were used in the selection of candidate metrics, to the extent feasible with the available limited data. A good metric should have a small within-type variation in reference condition, hence allowing for detection of human impact. This requirement was examined by calculating coefficient of variation for reference sites within a type and by comparing the values between references and monitoring sites (t-tests). The metric values were also correlated (Pearson correlation) with variables indicative of human influence.

The following metrics were selected:

BMWP Score, BBI, IBE, EPT Taxa and Margalef's Diversity Index:

The Biological Monitoring Working Party Score (BMWP) *is a procedure for measuring water quality using macroinvertebrates as biological indicators. The method is based on the principle that different aquatic invertebrates have different tolerances to pollutants. In the case of BMWP, this is based on the sensitivity/tolerance to organic pollution (i.e. nutrient enrichment that can affect the availability of dissolved oxygen). The division of invertebrates into taxonomic groups is undertaken predominantly at family level (81 families), but with one order (oligochaeta). In the case of BMWP, the presence of mayflies or stoneflies, for instance indicate the cleanest river and are given a* ***tolerance score of 10****. The lowest scoring invertebrates are* ***worms (Oligochaeta) which score 1.*** *The number of different macroinvertebrates is also an important factor, because the better quality water is assumed to contain fewer pollutants that would exclude "sensitive" species resulting in a higher diversity.*

BBI *The Belgian Biotic Index is based upon the relative sensitivity of specific indicator species to pollution and the diversity of species present. The index value varies from 0 (extremely bad quality) to 10 (extremely good quality). Initially, the invertebrates present are divided into 7 main faunal groups (at order, family and/or genus level), then the number of systematic units is counted. The division of taxa into systematic units is undertaken predominantly at family and genus level, and results are compared with a pre-determined 5-class look-up table. Overall, the index is based on a combination of taxa diversity (1415 taxa) and presence or absence of specific indicator groups.*

IBE: *The IBE. Method derives from the Trent Biotic Index (Woodiwiss 1964). It is based on two evaluation parameters: taxonomic richness and the presence of pollution-sensitive taxa. This method offers synthetic information about the general conditions of river ecosystems, underlining potential alterations.*

The ETP Taxa (ETP) *displays the richness within the insects groups (Ephemeroptera, Trichoptera and Plecoptera), which are considered to be sensitive to pollution and will increase with increasing water quality. The ETP Index is equal to the total number of families represented within these three orders of in the sample.*

Margalef's Diversity Index *is a simple-to-calculate measure of taxonomic (species) richness, best employed on large sample sizes. It is a combination of the number of taxa (species) counted and the total number of individuals sampled.*

5. ESTABLISHMENT OF THE TYPE-SPECIFIC REFERENCE CONDITIONS VALUES

Type-specific reference conditions values can be established either on the basis of data from reference sites, or, if data are insufficient, using modelling, expert judgement or a combination of these. Reference conditions values from neighbouring countries or catchment areas may also be used. In the case of the Prut River basin, the reference conditions values were established from JFS results (2013–2016) for Mountain and Semi- mountain “stone-gravel” types of river.

For some types (Flood-plain eroded small river with fine substrata (sand, clay) and small plain meandering river with fine substrata (mud, silt, clay)) the reference conditions values will be stablished using the expert judgement because of the lack of field data being that only one sampling location was included in the JFS for this type of water bodies.

5.1 Effect of sampling season

In the Upper Prut river basin it was possible to test this effect using the data from summer (2013, 2014 and 2015) and 2016 spring samples.

Generally, macroinvertebrate fauna data has differed composition due to seasonal changes as for example between spring and summer. Therefore, an analysis of data from the reference sites was undertaken for the metrics described above. Student´s t-tests were used to test the null hypothesis that “the means of spring and summer data sets for the selected metrics in the Upper Prut River basin in Ukraine are equal”. As it is presented in Table 2, the results from the analysis have shown that there is significant difference between spring and summer data for the water body group types in the Upper Prut River basin (p value were higher than 0,05), for all the indexes. However, to confirm the effect of the sampling season on the macroinvertebrate fauna the large data sets to cover different years regarding the climate and precipitation situations should be used. Furthermore, sampling time and identification methods may also affect the results.

	BMWP Score	BBI	IBE	EPT Taxa	Margalef’s Diversity Index
Upper Prut River Basin in Ukraine	0,60	0,65	0,25	0,49	0,63

Table 2 Student´s t-test (p values, T2) for the spring and autumn data sets of the selected metrics for the Upper Prut River basin (2013 – 2016)

5.2 Variation of selected metrics

Within – type variation

Selected metrics should have low within type variation (less than 30%). In the case of the Prut River basin, just one or two metrics from reference locations had coefficients of variation equal to or less than 30% so that results are not good enough for the classification and some expert judgement should be used to calibrate the results. On the other hand, variations of the metrics values in monitoring locations (impacted sites) should be higher to leave scope for metrics values from impacted locations to deviate substantially from those in reference locations in the same water body type (see Table 3). All metrics had higher coefficient of variation than 30% (except BBI index) that reflect the ability of selected metrics to cover the whole range of disturbances.

This finding means that the within – type variations are quite high for sampling seasons (2013 -2016) in reference locations. This fact can also indicate the natural unstable conditions, probably due to hydrological and hydraulics conditions of the rivers and also that the selection of reference sites flawed and maybe that all of the impacted sites, subject to about the same type and level of pressure.

In the future JFS it has to be ensure that the quality assurance guidance is followed not only in the field works but also in the preparation of the sampling locations and during the taxonomic identification process.

	All (2013-2014)	All (2013-2016)
BMWP Score		
cv – Ref – Con locations	**0,33**	**0,37**
cv – impacted locations	**0,38**	**0,38**
BBI		
cv – Ref – Con locations	**0,18**	**0,16**
cv – impacted locations	**0,21**	**0,25**
IBE		
cv – Ref – Con locations	**0,28**	**0,37**
cv – impacted locations	**0,47**	**0,54**
EPT Taxa		
cv – Ref – Con locations	**0,5**	**0,47**
cv – impacted locations	**0,57**	**0,57**
Margalef's Diversity Index		
cv – Ref – Con locations	**0,43**	**0,36**
cv – impacted locations	**0,27**	**0,38**

Table 3 Coefficients of variation of the selected metrics for different sampling seasons for the reference conditions locations and impacted locations in the Prut River basin (2013 -2014 and 2013-2016).

5.3 Detection of impact

Another important aspect of the selected metrics is that their values should be significantly different between reference condition locations and impacted locations. To evaluate such differences Student´s t-tests were applied to the data sets from the Prut River basin. The analysis shows that 2 of the 5 selected metrics (BBI and IBE) were significantly different ($p \leq 0.05$) between reference and impacted locations in the Prut River basin. These results again reflects that the impacted sites are not particularly heavily impacted compared to the reference sites, so more work related with reference site locations definition should be necessary.

5.4 Metric redundancy

When metrics are selected, it is also necessary to test on the redundancy among them. As it can be seen from the results presented in Table 4, there is a strong correlation between BBI and the other indexes, except correlation between Margalef index with the others.

	BBI	IBE	EPT	Margalef's Diversity Index	BMWP Score
BMWP Score	**0,747**	0,692	**0,905**	0,572	-
BBI	-	**0,801**	0,281	**0,772**	**0,743**
IBE	**0,801**	-	0,233	0,697	0,692
EPT	**0,772**	0,697	-	0,591	**0,905**
Margalef's Diversity Index	0,281	0,233	0,360	-	0,572

Table 4 Pearson correlations between selected metrics ($p < 0.05$). N =95 (2013-2016) for the both small and middle mountainous river types in the Prut River basin (Correlations ≥ 0.7 or ≤ -0.7 are bolded).

Note: in this analysis simple correlation coefficient "r" was used that indicates the relationship and dependence between variables (the close to 1 (or -1) the stronger dependence, the closer to 0 the weaker dependence).

6. CLASS BOUNDARIES AND CLASSIFICATION

Data for 5 selected metrics from three reference conditions locations in the Prut River basin were used to calculate the reference condition values distribution (percentiles).

In most cases the reference locations have, unavoidably, some degree of human influence, and thus do not fully represent true reference conditions. This is often taken into account by setting the High/Good –boundary (see Figure 6) to some percentile (e.g. 25th %tile, 50th %tile) of the distribution of metric values among the reference data from each river type.

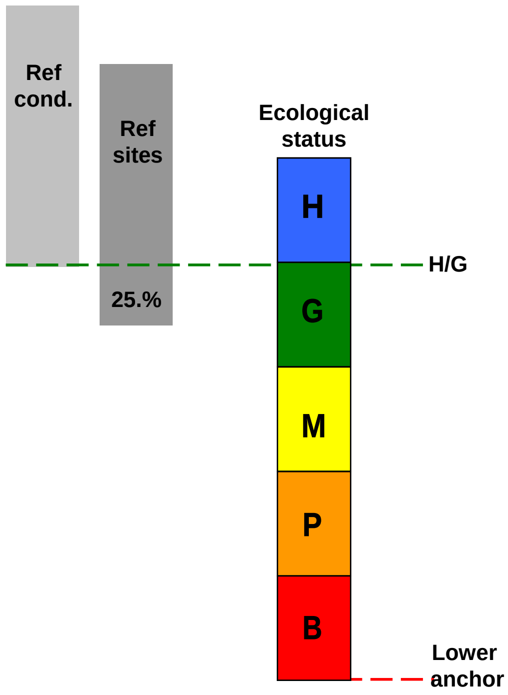

Figure 6 Schematic presentation of the class boundary setting for biological parameters
H = High, G = Good, M = Moderate, P = Poor and B = Bad ecological status
A crucial point in the boundary setting is also the selection the lower anchor (LA) values for metrics. This can be the lowest (i.e. worst) value observed in the data or the theoretical minimum (i.e. worst) value of the metric. The remaining class boundaries (Good/Moderate [G/M], Moderate/Poor [M/P] and Poor/Bad [P/B]) were established, e.g. by dividing the range between the LA and G/M boundary evenly into three bands (but there are other, inevitably equally arbitrary options).

In the case of the Prut River basin, H/G boundary was set to the 25th percentile of the type-specific reference value distribution. To illustrate the effect of the H/G-boundary value, the50th percentile (median) was also used for comparison for the middle mountainous river type. (For the small mountainous river type, the H/G boundary was set to 25th percentile.) The LA was set to the theoretical minimum value (i.e. the lowest attainable value (“0“)) of the metric and quality classes were evenly spaced within the range LA – G/M class boundary was set as the 70% of deviation of reference conditions according to the results from Central Baltic GIG. The results are presented in Table 4.

Note: The differences in the boundary values for the ecological status classes between spring and autumn are visible only for the BMWP Score metric. Similarly to the results of the Student´s t-test as described in the chapter above.

	All results from 2013-2016									
	BMWP Score	***EQR***	***BBI***	***EQR***	***IBE***	***EQR***	***EPT - Taxa***	***EQR***	***Margalef's Diversity Index***	***EQR***
H/G**	121,50	**0,84**	9,00	**1,00**	8,00	**0,83**	15,5	**0,87**	4,36	**0,95**
G/M***	87,00	**0,60**	5,40	**0,60**	5,78	**0,60**	10,68	**0,60**	2,75	**0,60**
M/P***	58,00	**0,40**	3,60	**0,40**	3,86	**0,40**	7,12	**0,40**	1,83	**0,40**
P/B***	29,00	**0,20**	1,80	**0,20**	1,93	**0,20**	3,56	**0,20**	0,92	**0,20**
Lower anchor	0		0		0		0		0	

Table 5 *Class boundaries for BMWP Score, BBI, IBE, EPT – Taxa and Margalef's Diversity Index for the Prut River basin (based on JFS 2013 – 2016) for high and middle mountainous river water body types.*

*** H/G – 25th %ile; *** other boundaries were distributed equally*

6.1 Classification schemes for macroinvertebrates in the Prut River basin

Based on the previous analysis of the macroinvertebrates data from JFSs in the Prut River basin (2013–2014), classifications schemes were developed for the high-middle mountainous river type and the small mountainous river type. Combined data sets (1 result per year in 2013 and 2016) were used in this process.

Note: The classification scheme for the high-middle river type will be further developed when results from the 2015 JFS become available for the middle lowland river type modelling or expert judgement will be used.

	High-middle mountainous type; Altitude 400–800 m a.s.l.				
Class	**I**	**II**	**III**	**IV**	**V**
EQR	**>0,84**	**>0,60**	**>0,40**	**>0,20**	**≤0,20**
BMWP Score	>121,50	>87	>58	>29	≤29
EQR	**>1**	**>0,60**	**>0,40**	**>0,20**	**≤0,20**
BBI	>9,0	>5,4	>3,6	>1,8	≤1,8
EQR	**>0,83**	**>0,60**	**>0,40**	**>0,20**	**≤0,20**
IBE	>8	>5,78	>3,86	>1,93	≤1,93
EQR	**>0,87**	**>0,60**	**>0,40**	**>0,20**	**≤0,20**
EPT	>15,5	>10,68	>7,12	>3,56	≤3,56
EQR	**>0,95**	**>0,60**	**>0,40**	**>0,20**	**≤0,20**
Margalef's Diversity Index	>4,36	>2,75	>1,83	>0,92	≤0,92
Multimetric Index EQR	**>0,90**	**>0,60**	**>0,40**	**>0,20**	**≤0,20**

Table 6 Classification scheme for the high-middle mountainous of water body type in the Prut River basin

Note: Multimetric Index EQR values were calculated as averages EQRs values for the selected metrics.

6.1.1 Comparison of the class boundaries with other regions

Classification schemes established for the Prut River basin (BMWP Score) were compared with the class boundaries established and used in the Central Baltic GIG group, included the mountains of the south of Poland and some type rivers in the north of Spain very similar for the similar types of the water bodies (see Table 13 and 14).

Compared results show that for the Middle mountainous river type both BMWP Score from GIG Central Baltic have higher values as it in the Prut River basin. On the other hand, for the Small mountainous river types the class boundaries are only BMWP Score from the GIG, so in the future monitoring programs this information will be updated.

	High -Middle mountainous type				
Class	**I**	**II**	**III**	**IV**	**V**
BMWP - Prut	>121,50	>87	>58	>29	≤29
BMWP – GIG CB	>107,88	>81,2	>58	>30,9	<30,9

Table 7 Comparison of the class boundaries for the BMWP Score between the Prut classification scheme and classification scheme in the GIG Central Baltic (high and middle river type)

	Lowland and small river type		
	I	**II**	**III**
EQR BMWP – GIG CB	0,93	0,70	0,46
EQR EPT Taxa - GIG CB	0,93	0,70	0,46

Table 8 According to the EQR in GIG Central Baltic (lowland and small river type) these are the values for the class boundaries for the BMWP Score and EPT Taxa.

6.2 Class boundaries for physico-chemical parameters

Based on the pressure and impact analysis organic pollution from untreated waste waters, nutrients from agricultural activities and heavy metals from mining industry in the Upper Prut River basin are stress factors. Therefore, BOD_5, COD, O_2, NH_4, NO_3, PO_4 and 6 heavy metals were included in the JFS programme and also to the classification system development (correlation analysis).

6.2.1 Background concentrations

It is important to know the background (natural) concentrations of heavy metals in the river in order to assess the contribution of human activities into the total heavy metals load detected. Background concentrations were calculated using the statistical method based on theoretical log-normal distribution defined by two parameters (mean value μ and standard deviation σ). Data sets of heavy metals available for the upstream part of the Prut and tributaries, from the JFs (2013 – 2016) were used in the calculations.

1. The Prut-Prutest, Shibeni, Beliy Cheremosh, Chorniy Cheremosh, Pistinka and Ribnitsya were selected as location with natural, or minimal anthropogenic influence.
2. The whole data set was used to calculate statistical parameters,
3. One value, close to "0" was added to the original data sets (for example detection limit divided by 100 can be used),
4. All values in the data sets were re-calculated as log-values,
5. Both mean value μ and standard deviation σ are calculated from the log-values data sets to create the theoretical log-normal distribution functions,
6. Calculation of the given percentile in the range from 10 to 95-tile was done,

7. Estimation of the background concentration values was made from the log-normal probability curve, as percentile.

Following the above procedure the background concentrations of heavy metals (Cu, Ni and Zn) in the Lowland Prut River basin in Moldova River were estimated (see Table 9). For illustration, background concentration of Zn is shown on the Figure 7 and 8.

	Cu µg/l	Zn µg/l
Background concentration	1,2	5,3

Table 9 Calculated background concentrations for selected heavy metals in the Lowland Prut River basin in Moldova River basin (based on the results from JFSs 2013 – 2016)

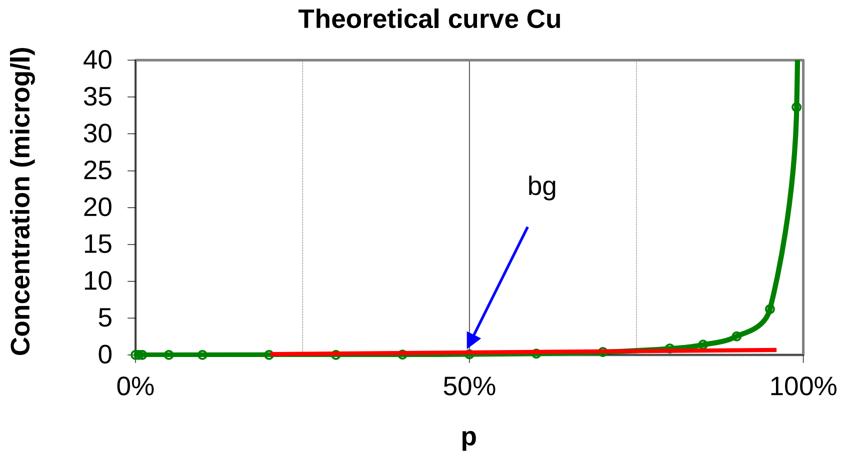

Figure 7 Background concentration of Cu for the Upper Prut River basin (based on data from summer JFS 2013 – 2016)

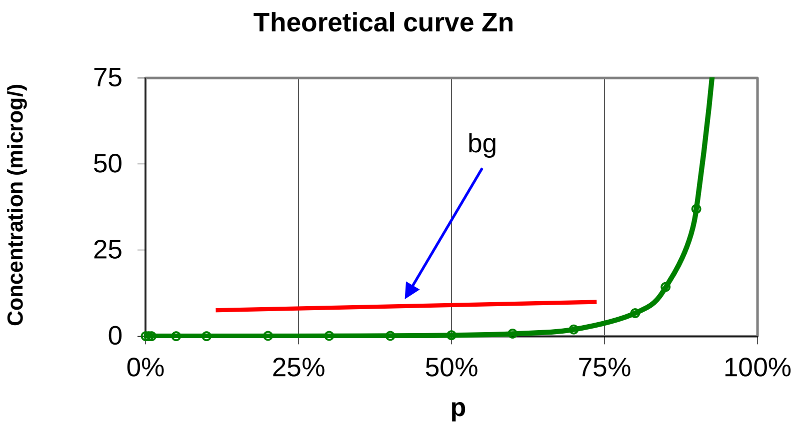

Figure 8 Background concentration of Zn for the Upper Prut River basin (based on data from summer JFS 2013 – 2016)

6.2.2 Relationship with stressors

To find critical threshold or class boundary values for variables indicative of human induced stress (water quality should support biotic elements (Annex V)) they can be correlated with the estimated biological EQRs or biological status. In this case, Pearson correlation was used to analyse the relation between the BOD_5 and other selected water quality parameters and also between the BMWP Score and selected water quality parameters.

Results from the analysis with the data in the last 4 years have shown that there is high correlation relationship among the O_2 and PO_4, but weak with the rest of parameters. Nevertheless, if the long historical data are taken into account, the correlation for BOD $_5$ with the parameters increases (better correlation for COD, NH_4, NO_3, O_2 and PO_4) (Table 10 and 11).

	O_2	COD	NH_4	$N0_3$	PO_4
BOD $_5$	0,513	-0,01	0,179	-0,14	0,602

Table 10 Pearson correlations between selected BOD_5 and selected physico-chemical parameters ($p < 0,05$). N = 94 (from 2013 – 2016) for the Upper Prut River basin.

	O_2	COD	NH_4	$N0_3$	PO_4
BOD $_5$	0,275	0,355	0,563	0,468	0,321

Table 11 Pearson correlations between selected BOD_5 and selected physico-chemical parameters ($p < 0,05$). N = 1000 (from 1993 – 2013) for the Upper Prut River basin.

The correlation between BMWP Score (see Table 12) is weak with almost all the parameter with the exception of O_2. However, the situation is not different using all the historical data for biological data (2013-2016) and for physicochemical parameters (1993-2013). In general, tendencies of the relationships between BMWP Score and tested water quality parameters (with few data and long historical data) are towards expected directions (negative for BOD_5, COD and nutrients and positive for O_2).

	BOD_5	O_2	COD	NH_4	NO_3	PO_4
BMWP – RC	-0,15	0,359	-0,102	-	-0,105	-

Table 12 Pearson correlations between BMWP Score and selected physico-chemical parameters ($p < 0,05$) seasons from 2013 – 2016 for the Upper Prut River basin River basin

	BOD_5	O_2	COD	NH_4	NO_3	PO_4
BMWP RC	0,133	0,664	-	-0,113	-0,107	-

Table 13 Pearson correlations between BMWP Score and selected physico-chemical parameters ($p < 0,05$). N = 1000 (from 1993 – 2013) for the Upper Prut River basin

Mean annual concentrations (in case of oxygen concentration minimum value) of the physico-chemical parameters (general conditions) are recommended to be used to classify ecological status. However, in the case of the EPIRB project for JFS only one sampling round in given year can be used to classify the ecological status. Due to this fact, it was decided to evaluate data (long-term data in the last years) from the national monitoring programme for selected parameters and calculate the mean and standard deviation values for each month. The data from the JFSs sampling round were compared with the given month whether they fit with the long term values (see Fig. 9). This approach was used to illustrate the necessity to use the annual means values of physico-chemical parameters to support the ecological status classification of surface (running) water.

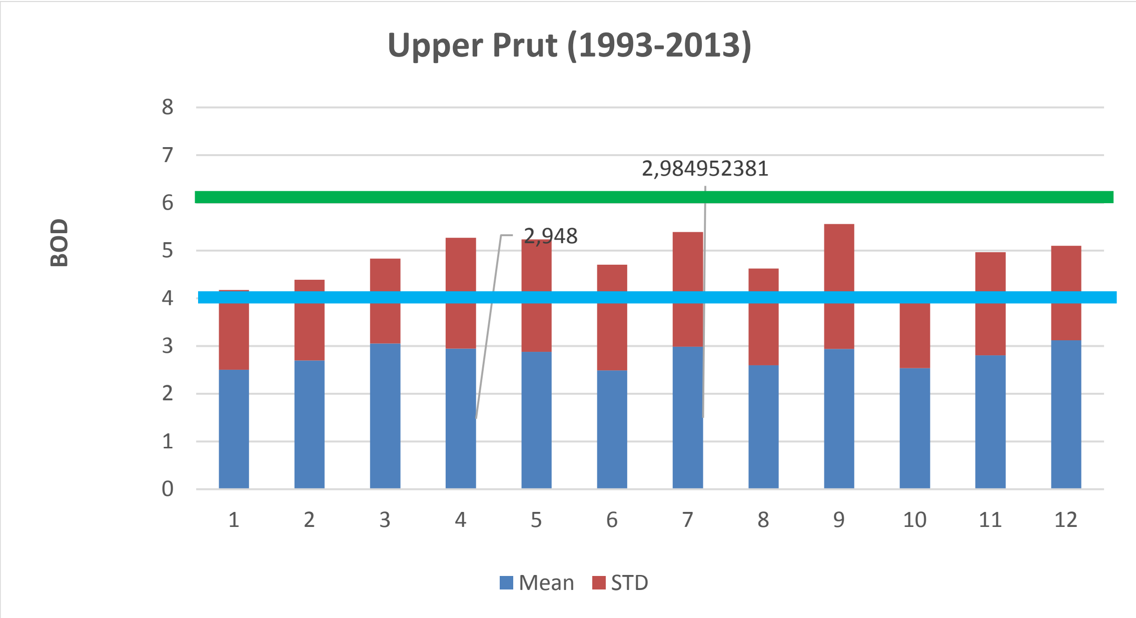

Figure 9 Long-term monthly mean concentrations and standard deviations of BOD (blue line is High status and green line Good status limit values).

Due to weak correlations it is not possible to indicate the association between the pressure variables and metrics (Table 13). Therefore, it was decided to use historical data from 1993-2016 and also expert judgement in setting the EQR boundaries of the selected pressure variables (see Table 14).

Parameter	unit	I	II	III
Temperature	°C	<23	23-26	≥26
Conductivity	µS/cm	<250	250-500	≥500
pH	-	(7,0; 8,5)	(6,0;	≤ 6,0
			7,0>	or
			or	≥ 9,0
		6,5-8,4	<8,5; 9)	
Dissolved oxygen	mg/l	>9	>8	≤8
BOD_5	mg/l	<3	<5	≥5
COD-Cr	mg/l	<10	<20	≥20
N-NH_4	mg/l	<0,25	<1	≥1
N-NO_3	mg/l	<4	<10	≥10
N-PO_4	mg/l	<0,1	<0,3	≥0,3

Table 14 Classification scheme for general physico-chemical parameters

6.3 Class boundaries for hydromorphological quality elements

Presently used HMQE assessment system in the EPIRB project is used for the classification in the pilot river basins.

Hydromorphological quality class		Limit values	Colour
1	High	1,0 – 1,7	
2	Good	1,8 – 2,5	
3	Moderate	2,6 – 3,4	
4	Poor	3,5 – 4,2	
5	Bad	4,3 – 5,0	

Table 15 Preliminary boundaries of the hydromorphological quality classes

6.4 Relationship between biological, hydromorphological and physico-chemical quality elements

There are three quality elements that are used in the assessment of the ecological status of the water bodies: biological, physico-chemical and hydromorphological quality elements (Fig. 10). To classify ecological status, the WFD stipulates that the lower of the values for the biological and physico-chemical monitoring results for the relevant quality elements should be used (Annex V, 1.4.2. (i)). This is consistent with the WFD “one out all out” approach.

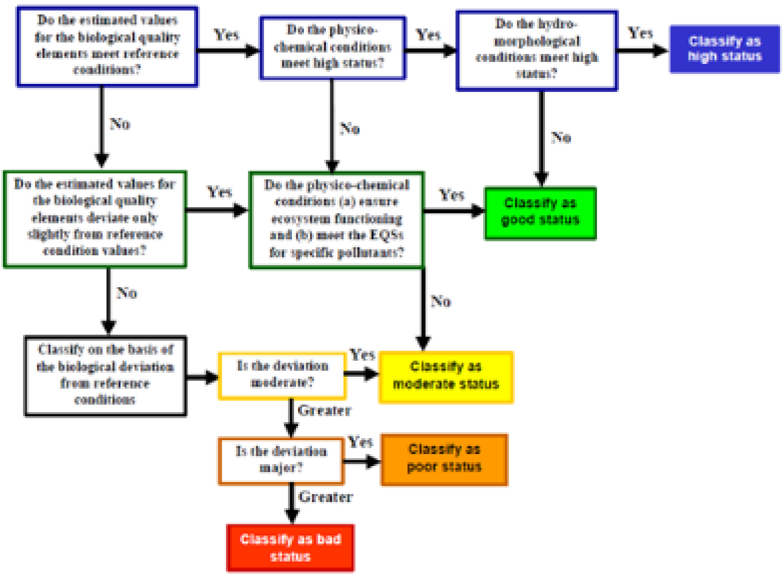

Figure 10 Assessment of the ecological status (reproduced from WFD CIS Guidance documents 10 and 5)

7. CLASSIFICATION EXERCISE

In the next page one first approach of the classification exercise is presented. More accurate exercise will be done later (classification schemes will be applied on the JFS results).

Table 16 **Ecological status classification of the sampling locations in the Upper Prut River basin (spring 2016 JFS data used)**

Sampling site number	River	Location of sampling site	BMWP	BBI	IBE	EPT	*Margalef's Diversity Index*	*Multimetris Index EQR*	*Multimetris Index EQR*	Physico-chemical parameters	HM Quality Score	Overall ecological status
2	Prut (Прут)	Low resort of Zaroslyak	154	9	10	18	4.591	0,876	I	COD	II	**MODERATE**
3	Prutec Chemygivskiy	Upper of v. Mykulichin (Выше с. Мыкулычин)	149	10	10	22	4.992	0,96	I		I	**HIGH GOOD**
6	Belyi Cheremosh	v. Yablunitsa (с. Яблуница)	133	9	9,6	17	4.035	0,876	II		III	**MODERATE**
8	Ryngach (Рынгач)	Road Hotyn – Novoselytsa	58	7	6	6	2.295	0,378	III	COD; PO4	II	**MODERATE**
9	Rokytna (Рокитна)	Low of road Chernivtsy - Hotyn	61	7	7	7	2.867	0,432	III	Conductivity	II	**MODERATE**
12	Lopatynka (Лопатинка)	v. Shyshkovtsy (с. Шишковцы)	59	4	5	3	2.854	0,324	III	Conductivity; COD; NO3	IV	**MODERATE**
14	Korovya (Коровыя)	v. Chagor (с. Чагор)	41	5	6	3	1.023	0,324	IV	Conductivity; PO4	II	**POOR**
18	Prutec Chemygivskiy	Low of v. Mykulichin	135	9	8,6	19	4.456	0,834	I		III	**MODERATE**
20	Iltsya (Ильця)	v. Iltsy (с. Ильци)	160	10	10.4	20	5.364	0,96	I	pH	III	**MODERATE**
21	Prut (Прут)	s. Lugany (Лужаны)	122	7	8,4	11	3.931	0,654	II	pH	III	**MODERATE**
23	Beleluya (Белелуя)	v. Krasnostavtsy (с. Красноставцы)	87	9	7,6	6	3.455	0,558	II	COD;PO4	III	**MODERATE**
24	Rybniysa (Рыбница)	v. Yavoryv (с. Яворив)	144	10	10	20	4.122	0,78	II	Conductivity; NO3	II	**GOOD**
27	Kamenka (Каменка)	v. Dora (с. Дора)	86	7	6.4	11	3.749	0,582	**III**	Conductivity; pH	III	**MODERATE**
28	Prut (Прут)	Low of r. Kamenka, v. Dora	129	9	8	19	5.161	0,876	I	Conductivity; pH	III	**MODERATE**
32	Prut (Прут)	Ниже г. Ворохта	107	8	7	12	3.325	0,666	II	Conductivity; pH	III	**MODERATE**
33	Vyhrynets	Upper Bukovel	123	8	6	13	3.003	0,612	II	Conductivity; pH	IV	**POOR**

Sampling site number	River	Location of sampling site	BMWP	BBI	IBE	EPT	*Margalef's Diversity Index*	*Multimetris Index EQR*	*Multimetris Index EQR*	Physico-chemical parameters	HM Quality Score	Overall ecological status
34	Prutets (Прутец)	Low of Bukovel	90	9	7,4	13	3.919	0,708	II	pH, Conductivity	I	**MODERATE**
35	Prut (Прут)	Low of v. Tatariv (нижче с. Татарів)	117	8	6	14	4.515	0,708	II	pH	III	**MODERATE**
36	Prut (Прут)	s. Snyatyn, low of WWTP	88	6	7	8	2.333	0,42	III	Conductivity	III	**MODERATE**
37	Prut (Прут)	Low of v. Zabolotyv (нижес. Заболотив)	104	9	8.4	13	2.821	0,6	II	Conductivity; COD;	III	**MODERATE**
38	Prut (Прут)	Low of s. Kolomyya (Нижер. Коломыя)	66	5	6	6	1.735	0,324	IV	Conductivity; COD;	III	**POOR**
43	Gonka (Жонка)	Upper s. Yaremche	146	10	10.4	24	4.389	0,918	I	Conductivity; COD;	II	**GOOD**
45	Prut (Прут)	Low of s. Chernivtsy (Нижер. Черновцы)	109	7	8	7	2.266	0,504	III	COD	II	**MODERATE**
46	Prut (Прут)	Low of v. Lunka (before broad of Romania)	112	6	7	14	3.249	0,57	II	Conductivity; pH	II	**GOOD**
47	Prut (Прут)	WWTP of s. Yaremche	110	8	7.6	13	3,42	0,792	II	pH	II	**GOOD**

8. CONCLUSIONS AND RECOMMEDATIONS

During the EPIRB Project 2012-2016 many data have been collected in the field and that information was used to develop this first approach of Ecological Status Classification System for the Upper Prut River basin. The system used methodologies applied in the EU Member State during the implementation of the WFD. Reference condition values were established for 5 metrics (BMWP Score, BBI, IBE, EPT-Taxa and Margalef's Diversity Index) to assess the river benthic community based on the normative definitions of the WFD.

The results of the assessment have shown certain seasonality and also very weak correlations between biotic indices and physico-chemical parameters. This fact can be caused by insufficient data sets and also hydrological and hydraulics conditions (natural conditions) in the pilot river basin and uncertainties of the results from the laboratory analysis (heavy metals variations).

On the other hand, classification system reacted on the human induced alterations in the pilot river basin and classified sampling locations into several classes from high to bad.

This ESCS includes macroinvertebrates for the biological evaluation but it is necessary to include more biological elements as macrophytes and phytobethos in the future.

This is a first approach for rivers but also for other water categories it is necessary to develop a system for classification: lakes, artificial waters and heavily modified water bodies.

9. REFERENCES

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