

# Report

## **Ecological Status Classification Schemes for Lowland Prut in Moldova and small tributaries**

**August 2016**

Project Title : **Environmental Protection of International River Basins Project**

Project Number : Contract No. ENPI/2011/279-666

Countries : Armenia, Azerbaijan, Belarus, Georgia, Moldova, Ukraine

Address : Hulla & Co. Human Dynamics KG  
13, Knjaz Dondukov Blvd. , fl. 3  
Sofia 1000, Bulgaria

Tel. number : +35929359978

Fax number : +35929359970

Contact person Ivan Davidov

Signatures : \_\_\_\_\_

Date of report: 5<sup>th</sup> August 2016

Author of report: Peter Rončák & Romina Álvarez-Troncoso

Acknowledgements: Thanks to William Parr and Paul Buijs for reviewing the draft versions

EC M & E team \_\_\_\_\_  
[name] [signature] [date]

EC Delegation \_\_\_\_\_  
[name] [signature] [date]

ENPI Bureau \_\_\_\_\_  
[task manager] [name] [signature] [date]

## Table of Contents

<b>1. INTRODUCTION</b> .....	4
<b>2. SELECTION OF THE LOCATIONS WITH TYPE SPECIFIC REFERENCE CONDITIONS IN THE PILOT RIVER BASINS</b> .....	5
<b>2.1 Sampling location with water body types specific reference condition</b> .....	5
<b>3. METHODS AND DATA</b> .....	7
<b>3.1 Sampling methods</b> .....	7
<b>3.1 Macroinvertebrates community</b> .....	8
<b>4. ENVIRONMENTAL DATA</b> .....	9
<b>4.1 Selection of metrics</b> .....	9
<b>5. ESTABLISHMENT OF THE TYPE-SPECIFIC REFERENCE CONDITIONS VALUES</b> .....	12
<b>5.1 Effect of sampling season</b> .....	12
<b>5.2 Variation of selected metrics</b> .....	12
<b>5.3 Metrics redundancy</b> .....	13
<b>6. CLASS BOUNDARIES AND CLASSIFICATION</b> .....	15
<b>6.1 Classification schemes for the macroinvertebrates in the Lowland Prut River basin in Moldova</b> .....	16
<b>6.1.1 Comparison of the class boundaries with other regions</b> .....	17
<b>6.2 Class boundaries for physico-chemical parameters in Prut river and small tributaries rivers in Prut River Basin in Moldova</b> .....	18
<b>6.2.1 Background concentrations</b> .....	18
<b>6.2.2 Relationship with stressors</b> .....	19
<b>6.3 Class boundaries for hydromorphological quality elements</b> .....	21
<b>7. RELATIONSHIP BETWEEN BIOLOGICAL, HYDROMORPHOLOGICAL AND PHYSICO-CHEMICAL QUALITY ELEMENTS</b> .....	23
<b>8. CLASSIFICATION EXERCISE</b> .....	24
<b>9. CONCLUSIONS AND RECOMMEDATIONS</b> .....	26

## 1. INTRODUCTION

The objective of the ecological classification is to assign rivers into five classes of ecological status: high, good, moderate, poor, and bad. Note that in case of AWB and HMWB classification is about the ecological potential, distinguishing four classes. 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.

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

In some of the pilot basin to establish the reference sites is becoming a problem because of the lack of suitable sites. Apart from this situation, there are not enough data from the monitoring program needed to calculate the boundaries. In this new approach of the ESCS data from longest period of the project were used for biological calculation (2013-2016). For physico chemical analysis, historical data for all the sites and data since 2000 was used in order to calculate the new boundaries.

### 2.1 Sampling location with water body types specific reference condition.

The WFD provides 3 options to define reference conditions:

- Use of data from physically similar, quasi-pristine locations.
- Modelling.
- Expert judgement.

In the JFSs it was decided to use a method combining elements of the first and third of these options. Selection of the potential locations to represent reference conditions involved the following main steps:

- An initial scoping study using maps and historical literature for preliminary identification.
- Pressure – impact analysis results.
- Reconnaissance mission by experts (hydrology, hydrobiology, chemistry).
- Sampling for macro-invertebrates, hydro-morphological quality elements and physicochemical parameters.
- Reassessment of the selected locations based on the data from the first JFSs rounds.
- A second reconnaissance mission to confirm (or otherwise) the locations for reference conditions.

The above steps allowed for more or less adequate selection of sampling locations representative of the specific reference conditions.

Because no typology was originally undertaken for the Lowland Prut River basin and because the quality and the areas of the waterbodies in the lowland part of the River Basin were very similar the river network was analyzed in one water body into two groups:

- Lowland part of Prut river
- Small lowland tributaries of Prut river basin



**Figure 1 Vilia, Tetcani, Moldova**



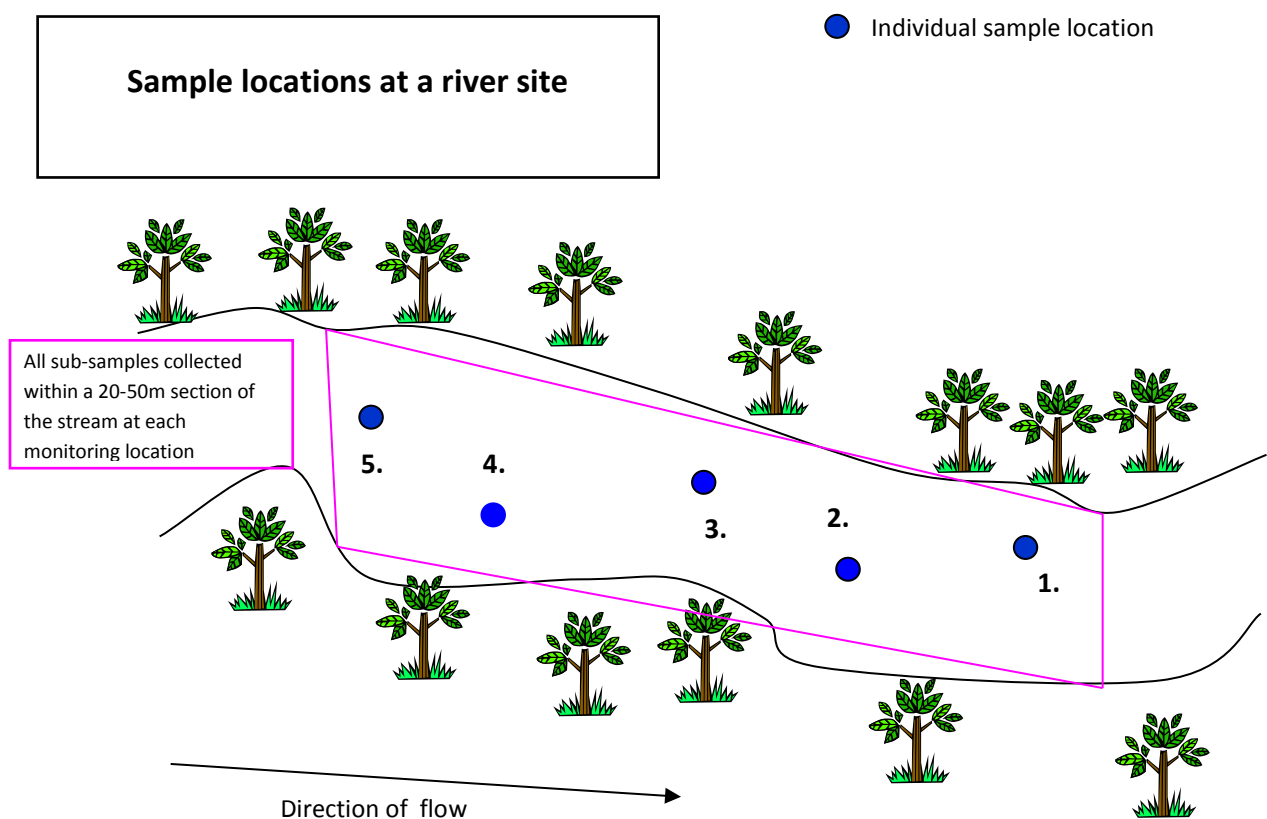
**Figure 2 Lipcani, Prut river, Moldova**

### 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<sup>2</sup>. 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.



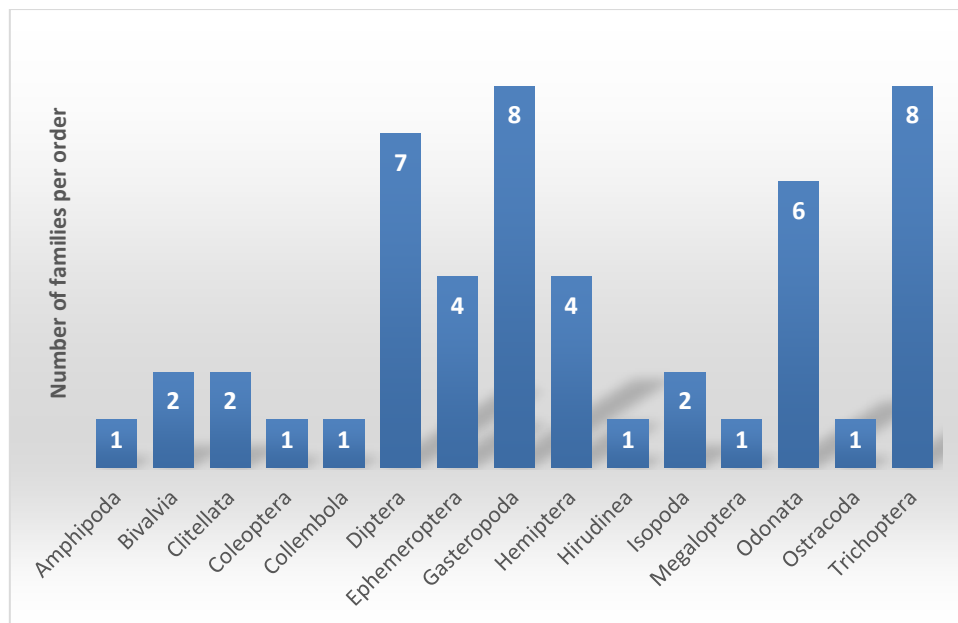
**Figure 3 Scheme of the placement of replicate samples within a single river riffle stretch in relation to direction of flow.**

The Prut river in the lowland part and small tributaries located in the Prut River Basin in Moldova present different problematic for designing the ecological status classification, the anthropic pressures due to the lack of good quantity of water increase the risk of low quality and lack of water in the basin . Apart from this, in the case of the tributaries, additional seasonal influence is present so in most of the fieldworks so it is shown in the flood regime and consequently the quality of the waterbodies is below as expected. Sampling in spring (April-May) is considered to be the most appropriate moment of the year for this lowland waterbodies (JFS IV results were better).

### 3.1 Macroinvertebrates community

**Forty nine families** were identified in the Prut River basin in Moldova the most abundant families were Candonidae (Ostracoda), Chironomidae, Ceratopogonidae and Simuliidae (Diptera), Poduridae (Entognata) Gammaridae (Amphipoda), Baetidae and Heptageniidae (Ephemeroptera); Elmidae (Coleoptera), Hydropsychidae and Limnephilidae (Trichoptera), Corixidae (Hemiptera), Asellidae (Isopoda) and Valvatidae (Gasteropoda).

Some of the families presented in the samples are typically available in lowland part of large rivers (Corixidae, Valvatidae and Sphaeriidae)), similar than the rivers studied. Some other taxa as Chironomidae, Simuliidae and Hydropsychidae are tolerant to the pollution.



**Figure 4** The number of macroinvertebrate taxa in taxonomic groups for the Prut RB (MD) River basin River basin identified during the JFS 2016.



## 4. ENVIRONMENTAL DATA

All sampling locations were surveyed for basic physico-chemical parameters and other specific pollutants as it is presented in Table 1. In the general document it is explained.

Quality elements	Indicative parameters
<i>thermal conditions</i>	<i>water temperature</i>
<i>oxygenation conditions</i>	<i>dissolved oxygen, oxygen saturation</i> <i>BOD5</i> <i>COD</i> <i>total suspended solids</i>
<i>nutrient conditions</i>	<i>NO3</i> <i>NH4</i> <i>PO4 (orthophosphates)</i>
<i>salinity</i>	<i>Conductivity</i> <i>Cl</i> <i>SO4</i> <i>total dissolved solids (total mineralization)</i>
<i>acidification status</i>	<i>pH</i>
<i>Other specific pollutants - heavy metals</i>	<i>As, Cd, Cu, Ni, Pb, Zn</i>

**Table 1 Physico-chemical parameters and other specific pollutants used in the assessment of the ecological status in the Lowland 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). The physical and chemical characteristics of the river bed, riparian zones and flood plain were assessed using this method.

### 4.1 Selection of metrics

The normative definitions of ecological status classes given in Annex V of the WFD 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 their combination and also the reference conditions values from neighbouring countries or catchment areas. In the case of the Prut in Moldova and small tributaries River basin, reference conditions values were established from the JFS results (2013 – 2016) for the types. In some locations was different to define the reference sites so expert judgment was used to analyse the boundaries define by the statistical analysis.

### 5.1 Effect of sampling season

Generally, macroinvertebrate fauna data has differed 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 Prut River Basin basin 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 lowland part of Prut River basin (p value were higher than 0,05). 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
Lowland rivers in Prut River Basin (Prut river and small tributaries)	0,99	0,50	0,06	0,44	0,60

**Table 2 Student's t-test (p values, T2) for the spring and autumn data sets of the selected metrics for the Lowland Prut River basin (2013 – 2016)**

### 5.2 Variation of selected metrics

One of the assumption in the process of metrics selection is to test the within type variation. Selected metrics should have low within type variation (less than 30%). In case of the Lowland part of Prut River basin and small tributaries, selected metrics had coefficient of variation among reference locations higher than 30 %, except of BBI and IBE index for reference sites (see Table 3 (in red values)). 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.

	All (2013-2016)
<b>BMWP Score</b>	
cv – Ref – Con locations	<b>0,29</b>
cv – impacted locations	<b>0,53</b>
<b>BBI</b>	
cv – Ref – Con locations	<b>0,19</b>
cv – impacted locations	<b>0,35</b>
<b>IBE</b>	
cv – Ref – Con locations	<b>0,14</b>
cv – impacted locations	<b>0,51</b>
<b>EPT Taxa</b>	
cv – Ref – Con locations	<b>0,43</b>
cv – impacted locations	<b>0,68</b>
<b>Margalef's Diversity Index</b>	
cv – Ref – Con locations	<b>0,26</b>
cv – impacted locations	<b>5,54</b>

**Table 3 Coefficients of variation of the selected metrics for different sampling seasons for the small gravel mountainous water body type for reference conditions locations in Lowland part of Prut River basin and small tributaries, (2013 -2016).**

In IBE and BBI the coefficient of variation is smaller than 30%, for the rest having higher than 30% reflects the ability of selected metrics to cover the whole range of disturbances.

### 5.3 Metrics 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 quite strong correlation among them, except correlation between BBI and IBE with the other indexes. However, the results can be effected by the very high variation and also by the short datasets that has been investigated.

	BMWP Score	BBI*	IBE*	EPT	Margalef's Diversity Index
<b>BMWP Score</b>	-	-	-	<b>0,701</b>	<b>0,784</b>
<b>BBI</b>		-	-	-	-
<b>IBE</b>		-	-	-	-
<b>EPT</b>	<b>0,701</b>	-	-	-	0,539
<b>Margalef's Diversity Index</b>	<b>0,784</b>			0,539	

*\*correlation analysis was not done due fact that only several values are available for BBI and IBE index*

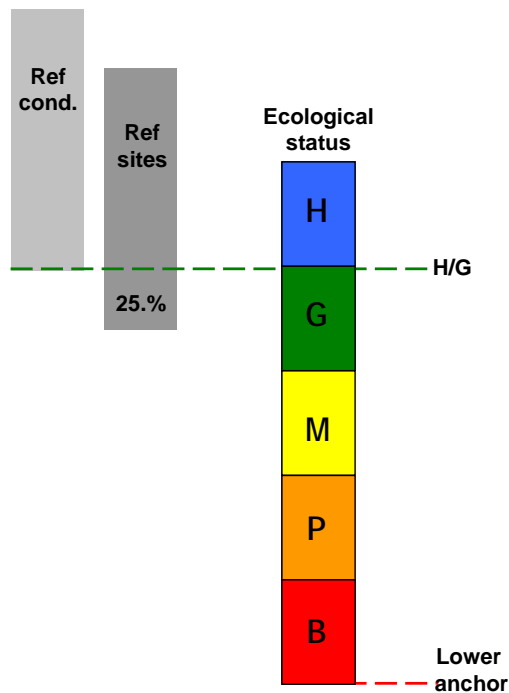
Table 4 Pearson correlations between selected metrics ( $p < 0.05$ ).  $N = 85$  (spring and summer seasons from years 2013 – 2016) for the middle gravel mountainous braided river type in the Lower Prut River basin (Correlations  $\geq 0,7$  or  $\leq -0,7$  are bolded)

*Note: in this analysis simple correlation coefficient “Pearson correlation” 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 Lowland Prut River basin in Moldova River basin were used to calculate the reference conditions 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 %, 50th %) of the distribution of metric values among the reference locations within a type.



**Figure 6** A 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 Lowland Prut River basin and small tributaries, H/G boundary was set to 25th percentile of type-specific reference value distribution and to illustrate the effect of the H/G-boundary value. LA was set to the approximate 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. The results are presented in Tables 5 and 6.

*Note: The differences in the boundary values for the ecological status classes between spring and autumn are visible only for BMWP Score metric, similarly to the results of the Student's t-test (the means of spring and autumn data sets are equal) as it was described in chapter above.*

The following boundaries (table 7) represented the median of the limits calculated in each participant member between the classes for both typologies:

H/G	G/M	M/P	P/B
The 25 <sup>th</sup> ile of the reference sites values for the index	0,6	0,4	0,2

**Table 5 EQR boundaries for river in Prut small tributaries rivers in Moldova**

Data from existent locations in similar rivers in Mediterranean GIG were used to calculate the distribution of metric values among the reference locations within a type.

All results from 2013-2016										
	<i>BMWP Score</i>	<i>EQR</i>	<i>BBI</i>	<i>EQR</i>	<i>IBE</i>	<i>EQR</i>	<i>EPT - Taxa</i>	<i>EQR</i>	<i>Margalef's Diversity Index</i>	<i>EQR</i>
H/G**	62,00	<b>0,88</b>	7,00	<b>0,95</b>	7,00	<b>1,00</b>	3,00	<b>0,75</b>	1,83	0,90
G/M***	42,48	<b>0,60</b>	4,44	<b>0,60</b>	4,20	<b>0,60</b>	1,22	<b>0,60</b>	1,22	0,60
M/P***	28,32	<b>0,40</b>	2,96	<b>0,40</b>	2,80	<b>0,40</b>	0,81	<b>0,40</b>	0,81	0,40
P/B***	14,16	<b>0,20</b>	1,48	<b>0,20</b>	1,40	<b>0,20</b>	0,41	<b>0,20</b>	0,41	0,20
Lower anchor	0		0		0		0		0	

\*\* H/G – 25<sup>th</sup> % ile; \*\*\* other boundaries were distributed equally

**Table 6 Class boundaries for BMWP Score, BBI, IBE, EPT – Taxa and Diversity (Margalef Index) for the Lowland Prut river and small tributaries in Prut in Moldova River basin for small river water bodies' type.**

### 6.1 Classification schemes for the macroinvertebrates in the Lowland Prut River basin in Moldova

Based on the previous analysis of the macroinvertebrates data from the JFS in the Prut River basin (2013 – 2016) the classifications schemes were developed for the Lowland rivers types. Combined data sets (summer and spring) were used in this process and classification schemes are presented in Table 7.



Lowland rivers (Prut river and small tributaries) in Moldova					
Class	I	II	III	IV	V
<i>EQR</i>	>0,88	>0,6	>0,4	>0,2	≤0,2
<i>BMWP Score</i>	>62	>42,5	>28,3	>14,16	≤14,16
<i>EQR</i>	>0,94	>0,6	>0,4	>0,2	≤0,2
<i>BBI</i>	>7	>4,4	>3,0	>1,5	≤1,5
<i>EQR</i>	>1	>0,6	>0,4	>0,2	≤0,2
<i>IBE</i>	>7	>4,2	>2,8	>1,4	≤1,4
<i>EQR</i>	>0,75	>0,6	>0,4	>0,2	≤0,2
<i>EPT</i>	>3	>1,2	>0,8	>,4	≤0,4
<i>EQR</i>	>0,90	>0,6	>0,4	>0,2	≤0,2
<i>Margalef's Diversity Index</i>	>1,8	>1,2	>0,8	>0,4	≤0,4
<i>Multimetrics Index EQR</i>	>0,89	>0,6	>0,4	>0,2	≤0,2

**Table 7 Classification scheme for the Lowland river type in the Prut River basin in Moldova**

### 6.1.1 Comparison of the class boundaries with other regions

Classification schemes established for the Lowland Prut River basin (BMWP Score) were compared with the class boundaries established and used in the Mediterranean areas for the similar types of the water bodies (see Table 8).

Compared results show that class boundaries values of the Spanish Mediterranean classification schemes for the Lowland Mediterranean mineralized river type BMWP Score have higher values as it in the Lowland Prut River basin in Moldova River basin. Such differences might be a signal of the differences in sampling methods (e.g. timing, personnel, site selection, etc.) and also in natural conditions (e.g. hydrological regime and higher water velocities).

	Prut river in Moldova River basin and small tributaries				
	I	II	III	IV	V
<i>BMWP - Lowland Prut River basin in Moldova</i>	>62	>42,5	>28,3	>14,16	≤14,16
<i>BMWP SPAIN</i>	>83	>51	>38	>13	≤13

**Table 8 Comparison of the class boundaries for the BMWP Score between the Lowland Prut River basin in Moldova classification scheme and classification scheme in the Spain (the Lowland Mediterranean mineralized river type)**

## 6.2 Class boundaries for physico-chemical parameters in Prut river and small tributaries rivers in Prut River Basin in Moldova

*Note: The values of the physic-chemical quality elements must be taken into account when assigning water bodies to the high and good ecological status classes.*

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 small tributaries of the *Prut in Moldova River basin* are stress factors. Therefore, BOD<sub>5</sub>, COD, O<sub>2</sub>, NH<sub>4</sub>, NO<sub>3</sub>, PO<sub>4</sub> and 6 heavy metals (Directive 2013/39/EU; classification of metals is ,pass or ,fail) were included in the JFS programme and also to the classification system development (correlation analysis).

Due to the lack of representative data in the period 2013-2016, the historical data was consulted since 2000-2015 in order to know the media values for the periods sampled.

### 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 a statistical method based on theoretical log-normal distribution defined by two parameters (mean value,  $\mu$ , and standard deviation,  $\sigma$ ). Data sets of heavy metals available for the upstream part of the Lowland Prut River basin in Moldova River basin, from the 2013 – 2016 JFs were used in the calculations:

1. Five sampling locations were selected as location with natural, or minimal anthropogenic influence.
2. The data sets from these sampling locations were used to calculate statistical parameters.
3. One value, close to “0” was added to the original data sets (the detection limit divided by 100).
4. All values in the data sets were re-calculated as log-values.
5. Both the mean value and standard deviation are calculated from the log-values data sets to create theoretical log-normal distribution functions.
6. The percentile in the range from 1 to 99% tile was calculated.
7. An estimate of the background concentration values was made from the log-normal probability curve, as a 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 5 and 6.

	Cu µg/l	Zn µg/l
<b>Background concentration</b>	2,6	10,1

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

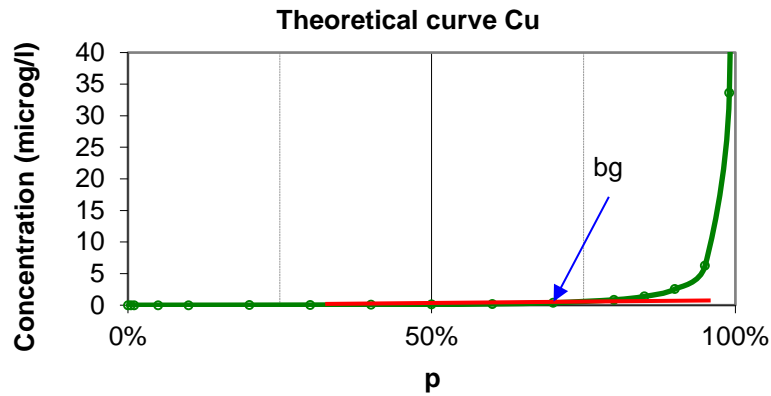


Figure 5 Background concentration of Cu for the Lowland Prut River basin in Moldova River basin (based on data from JFS 2013 – 2016)

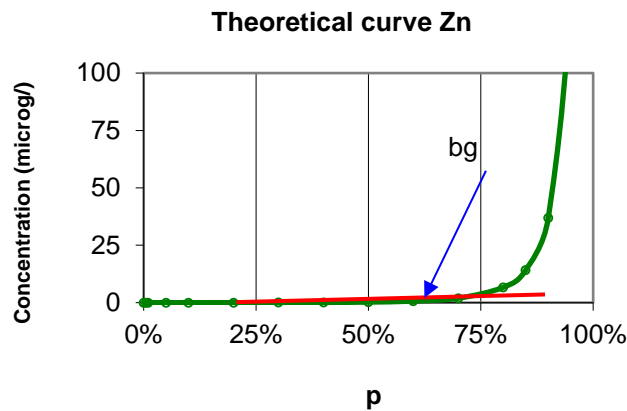


Figure 6 Background concentration of Zn for the Lowland Prut River basin in Moldova River basin (based on data from JFS 2013 – 2016)

### 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 correlations were used to analyse the relation between BOD5 and other selected water quality parameters, and also between the BMWP Score and selected water quality parameters. Results from the analysis have shown that there is only a weak relationship between BOD5 and other parameters (O2, COD, and N-NO3) and strong correlation with the others (N-NH4, P-PO4) (see Table 10) and also between BMWP Score (see Table 11).

	O <sub>2</sub>	COD	N-NH <sub>4</sub>	N-NO <sub>3</sub>	P-PO <sub>4</sub>
<b>BOD<sub>5</sub></b>	0,209	0,074	0,885	0,27	0,966

Table 10 Pearson correlations between selected BOD<sub>5</sub> and selected physico-chemical parameters (p < 0,05). N = 28 (from 2013 – 2016) for Lowland rivers Prut river and small tributaries, Prut River Basin Moldova

	BOD <sub>5</sub>	O <sub>2</sub>	COD	N-NH <sub>4</sub>	Cu
<b>BMWP RC</b>	-0,25	0,13	0,15	0,17	0,4

Table 11 Pearson correlations between BMWP Score and selected physico-chemical parameters (p < 0,05). N = 28 (from 2013 – 2016) for Lowland rivers Prut river and small tributaries, Prut River Basin Moldova

Due to weak correlations it is not possible to indicate the association between the pressure variables and macroinvertebrates metrics. Therefore, it was decided to use expert judgement in setting the EQR boundaries of the selected pressure variables based on the % tiles calculated from the reference condition distributions (see Table 12).

Parameter	unit	I	II	III
Temperature	°C	<26	26-28	≥28
Conductivity*	μS/cm	<500	<700	≥700
pH	-	(7,0; 8,5)	(6,0; 7,0> or <8,5; 9)	≤ 6,0 or ≥ 9,0
Dissolved oxygen	mg/l	>8	>7	≤7
BOD <sub>5</sub>	mg/l	<3	<5	≥5
COD-Cr	mg/l	<10	<25	≥25
N-NH <sub>4</sub>	mg/l	<0,2	<0,5	≥0,5
N-NO <sub>3</sub>	mg/l	<3,5	<6	≥6
P-PO <sub>4</sub>	mg/l	<0,15	<0,3	≥0,3

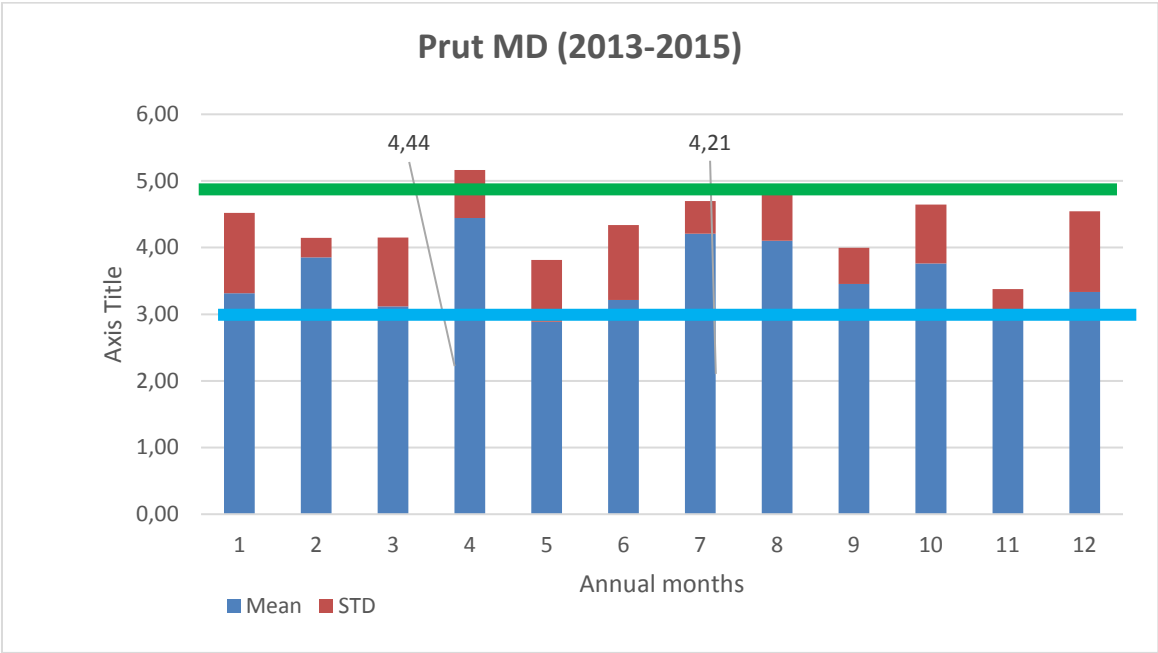
\*There are some tributaries which their conductivity is naturally very high (4.000uS/cm)

#### Table 12 Classification scheme for general physico-chemical parameters

Mean annual concentrations of the physico-chemical parameters (general conditions and other specific pollutants) are recommended to be used to classify ecological status. However,

in the case of the EPIRB project measured concentrations from two sampling rounds in given year are used to classify the ecological status.

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. 7). 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 7 Long-term monthly mean concentrations and standard deviations of BOD (blue line is High status and green line Good status limit values).**

**6.3 Class boundaries for hydromorphological quality elements**

HMQE must be taken into account when assigning water bodies to the high ecological status class. For the other status classes, the HMQE are required to have conditions which are consistent with the biological values set for the biological class for each water body type.

The hydromorphological quality elements (HMQE) assessment system in the EPIRB project is used for the classification in the pilot river basins (see Table 13). This preliminary Hydromorphological Quality Score system was used in Slovakia to classify the water bodies (SHMI, 2004).

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 13** Preliminary boundaries of the hydromorphological quality classes (SHMI, 2004)

## 7. 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. 8). 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.

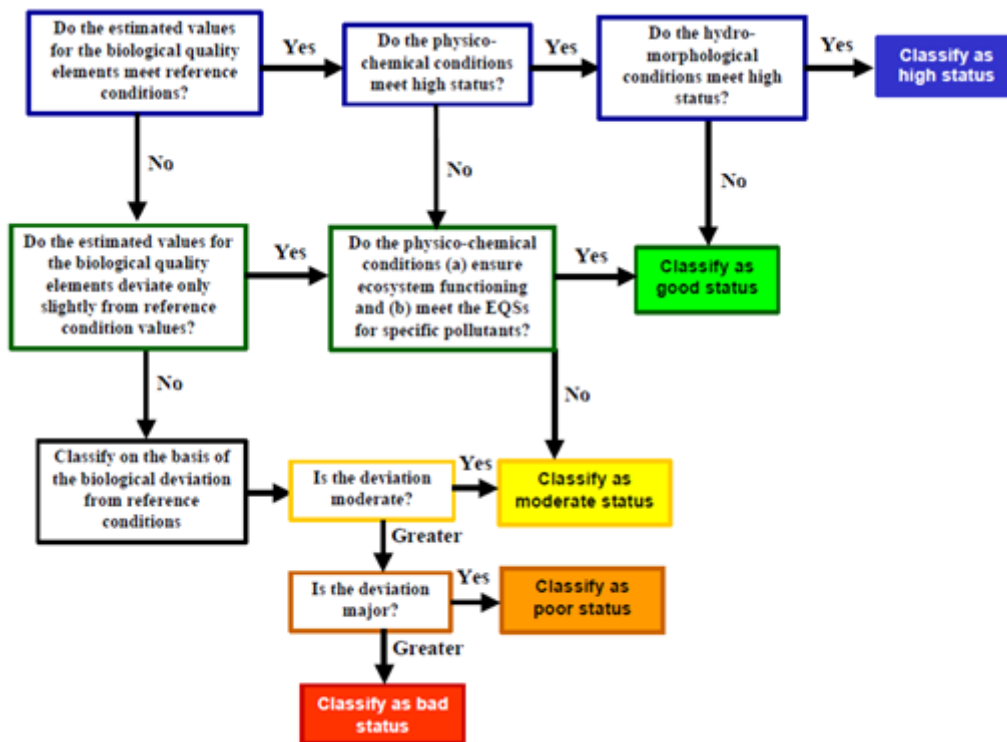


Figure 8 Assessment of the ecological status (reproduced from WFD CIS Guidance documents 10 and 5)

## 8. CLASSIFICATION EXERCISE

The developed ecological status classifications systems for the Lowland Prut River basin in Moldova were used to classify the river basin based on the data from the spring sampling round in 2016 of the JFS.



Table 14 Ecological status classification of the sampling locations in the Lowland Prut River and small tributaries (spring 2016 JFS data used)

Number of Site	CODE:	River/Sampling site	Location	BMWP	BBI	IBE	EPT	Margal ef's Diversit y Index	Multimetris Index EQR	Physico-chemical parameters	HM Quality Score	Overall ecological status
1	53-AS-16	r. Zelenaia	s. Drepcăuți	58	6	7	3	1,888	Good	COD	Good	MODERATE
2	54-AS-16	r. Medveja	s. Lipcani, amonte	56	5	7	3	1,681	Good	pH, COD, NO3	High	GOOD
3	55-AS-16	r. Prut	s. Lipcani	42	6	7	2	1,556	Moderate	COD	High	MODERATE
4	56-AS-16	r. Larga	s. Slobozia-Șireuți	42	6	6	4	1,433	Moderate	pH, COD	High	MODERATE
5	57-AS-16	r. Prut	s. Pererîta	68	7	6,6	4	1,821	Good	pH, COD	High	GOOD
6	58-AS-16	r. Vilia	s. Tețcani	54	6	5	3	1,706	Good	COD, NO3	Good	MODERATE
7	59-AS-16	r. Lopatnic	s. Lopatnic	51	6	7	3	1,578	Good	COD	High	GOOD
8	60-AS-16	r. Draghiște	s. Fetești, aval	27	4	4,6	1	0,882	Poor	O2, NH4	Good	POOR
9	61-AS-16	r. Racovat	s. Gordinesti	24	6		1	1,066	Poor	COD	Poor	POOR
10	62-AS-16	r. Ciuhur	s. Stolniceni	11	2	2	1	0,641	Bad	COD	Poor	BAD
11	63-AS-16	r. Camenca	s. Camenca	26	5	5,6	2	1,103	Poor	COD	Poor	POOR
12	64-AS-16	r. Glodeanca	s. Dușmani	13	5	4	1	0,631	Poor	pH, PO4, COD	Good	POOR
13	65-AS-16	r. Girla Mare	s. Blindesti	43	6	6	2	1,146	Moderate	COD	Moderate	MODERATE
14	66-AS-16	r. Prut	or. Ungheni	17	4		0	1,144	Poor	COD	High	POOR
15	67-AS-16	r. Prut	s. Valea Mare	11		2	0	0,867	Bad	COD	Good	BAD
16	68-AS-16	r. Varșava	s. Valea Mare	14	4	3,4	1	0,908	Poor	COD	NO DATA	POOR
17	69-AS-16	r. Lăpușna	s. Lăpușna	16	4	2,4	0	1,155	Poor	COD	Poor	POOR
18	70-AS-16	r. Sarata	s. Vozneseni	82	6	8	3	2,209	Good	COD	Moderate	MODERATE
19	71-AS-16	r. Valea-Galmage	s. Zîrnești	17	3	4	1	0,463	Poor	NO3, COD	Poor	POOR
20	72-AS-16	I. Belev	Girila Manolescu-pod	LAKE								

## 9. CONCLUSIONS AND RECOMMENDATIONS

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 Lowland Prut River basin with small tributaries in Moldova. 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 phytobenthos 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.

Table 1 Physico-chemical parameters and other specific pollutants used in the assessment of the ecological status in the Lowland Prut River basin .....	9
Table 2 Student's t-test (p values, T2) for the spring and autumn data sets of the selected metrics for the Lowland Prut River basin (2013 – 2016) .....	12
Table 3 Coefficients of variation of the selected metrics for different sampling seasons for the small gravel mountainous water body type for reference conditions locations in Lowland part of Prut River basin and small tributaries, (2013 -2016). .....	13
Table 4 Pearson correlations between selected metrics (p < 0.05). N = 85 (spring and summer seasons from years 2013 – 2016) for the middle gravel mountainous braided river type in the Lower Prut River basin (Correlations $\geq 0,7$ or $\leq -0,7$ are bolded) .....	14
Table 5 EQR boundaries for river in Prut small tributaries rivers in Moldova .....	16
Table 6 <i>Class boundaries for BMWP Score, BBI, IBE, EPT – Taxa and Diversity (Margalef Index) for the Lowland Prut river and small tributaries in Prut in Moldova River basin for small river water bodies' type.</i> .....	16
Table 7 Classification scheme for the Lowland river type in the Prut River basin in Moldova .....	17
Table 8 Comparison of the class boundaries for the BMWP Score between the Lowland Prut River basin in Moldova classification scheme and classification scheme in the Spain (the Lowland Mediterranean mineralized river type) .....	17
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) .....	18
Table 10 Pearson correlations between selected BOD5 and selected physico-chemical parameters (p < 0,05). N = 28 (from 2013 – 2016) for Lowland rivers Prut river and small tributaries, Prut River Basin Moldova.....	20
Table 11 Pearson correlations between BMWP Score and selected physico-chemical parameters (p < 0,05). N = 28 (from 2013 – 2016) for Lowland rivers Prut river and small tributaries, Prut River Basin Moldova.....	20
Table 12 Classification scheme for general physico-chemical parameters.....	20
Table 13 Preliminary boundaries of the hydromorphological quality classes (SHMI, 2004) .....	22
Table 14 Ecological status classification of the sampling locations in the Lowland Prut River and small tributaries (spring 2016 JFS data used) .....	25
Figure 1 Vilia, Tetcani, Moldova .....	6
Figure 2 Lipcani, Prut river, Moldova .....	6
Figure 3 Scheme of the placement of replicate samples within a single river riffle stretch in relation to direction of flow. ....	7
Figure 4 The number of macroinvertebrate taxa in taxonomic groups for the Prut RB (MD) River basin River basin identified during the JFS 2016. ....	8
Figure 5 Background concentration of Cu for the Lowland Prut River basin in Moldova River basin (based on data from JFS 2013 – 2016) .....	19
Figure 6 Background concentration of Zn for the Lowland Prut River basin in Moldova River basin (based on data from JFS 2013 – 2016) .....	19
Figure 7 Long-term monthly mean concentrations and standard deviations of BOD (blue line is High status and green line Good status limit values). ....	21

Figure 8 Assessment of the ecological status (reproduced from WFD CIS Guidance documents 10 and 5)..... 23