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Rapid Biological Assessment (RBA) based on analysis of benthic macroinvertebrate communities

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Abbreviations

AA	Annual Average
AM	Armenia
AQEM	"The Development and Testing of an Integrated **A**ssessment System for the Ecological **Q**uality of Streams and Rivers throughout **E**urope using Benthic **M**acroinvertebrates" project
ASPT	Average Score Per Taxon
AZ	Azerbaijan
BBI	Belgian Biotic Index
BMWP	Biological Monitoring Working Party (Index)
BOD	Biochemical Oxygen Demand
BY	Belarus
CEN	European Committee for Standardization (European standard / European norm)
DO	Dissolved Oxygen
EC	European Community / European Commission
EEA	European Environmental Agency
EN	European Norm
EP	Ecological Potential
EPA	Environmental Protection Agency
EQR	Ecological Quality Ratios
ES	Ecological Status
EU	European Union
GE	Georgia
GIG	Geographic Intercalibration Group
H_2S	Hydrogen Sulfide
ISO	International Organization for Standardization (international standard)
ITC	Index of Trophic Completeness
MD	Moldova
MI	Macro-invertebrates
MZB	Macrozoobenthos
N	Nitrogen
$N\text{-}NH_4$	Ammonium, as Nitrogen
$N\text{-}NO_2$	Nitrite Nitrogen
$N\text{-}NO_3$	Nitrate Nitrogen
O_2	Oxygen (dissolved / oxygen saturation)
P	Phosphorus
QC/QA	Quality Control/Quality Assurance
RB	River Basin
RBA	Rapid Biological Assessment
RETI	Rhithron-feeding Type Index
RTI	Rhithron-Typie-Index
STAR	**Sta**ndardisation of **R**iver Classifications: Framework method for calibrating different biological survey results against ecological quality classifications to be developed for the Water Framework Directive (EU Project)
SWB	Surface Water Body
T^oC	Temperature (degree Celsius)
TN	Total Nitrogen
TP	Total Phosphorus
UA	Ukraine
UK	United Kingdom
US	United States of America
WB	Water Body
WFD	Water Framework Directive

RAPID BIOLOGICAL ASSESSMENT (RBA) BASED ON ANALYSIS OF BENTHIC MACROINVERTEBRATE COMMUNITIES

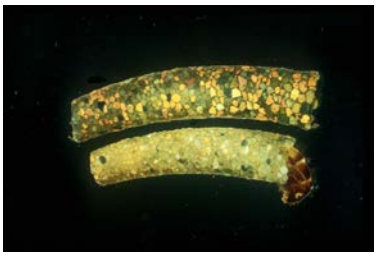
Contribution to: Task 1.4 Assistance to the development of WFD-compliant tools for assessing data from monitoring activities (ecological, chemical, hydro-morphological classifications)

Introduction

Water Framework Directive (WFD) 2000/60/EC in Europe requires monitoring of the benthic macroinvertebrate communities (macrozoobenthos) and ecological status/ potential assessment of rivers. Most of the Geographic Intercalibration Groups (GIG) under the WFD compare macroinvertebrate data sets using as a dimension the number of individuals per square meter based on so-called multihabitat sampling.

A Rapid Bioassessment (RBA) method/index based on analysis of benthic macroinvertebrate communities (macrozoobenthos) has been established for the needs of 6 beneficiary countries (AM, AZ, GE, BY, MD, UA) and their international river basins. The method provides the following provisions:

- ✓ Cost-effective, scientifically valid procedures for biological surveys, risk assessment and integrated monitoring & assessment in compliance with WFD principles;
- ✓ Provisions for multiple site investigations in a field season (June - October);
- ✓ Quick turn-around of results for management decisions; and
- ✓ Monitoring & survey reports easily translated to decision-makers/water managers and the public.



Main features of the RBA can be summarized as follows:

- 5 indicator groups (A, B, C, D, E) sensitive – tolerant taxa;
- 5 abundance groups (approx. 1 sq. m sampling area): Few (1-5), Present (6-20), Common (21-50), Plentiful (51-100), Dominant (100+);
- Special algorithm (Assessment Key) + assessment table (Flanagan, P.J. and Toner, P.F., 1972; modified Clabby & Bowman, 1979; Clabby, 1982; Metcalfe. 1989, Ghetti, 1986; Chandler, 1970; Armitage et al., 1983; De Pauw & Vanhooren, 1983, etc.);
- Taxonomic level of identification is genus (Hirudinea, Mollusca, Megaloptera, Ephemeroptera, Turbellaria) or family (Oligochaeta, Crustacea, Plecoptera, Trichoptera, Odonata, Heteroptera, Coleoptera, Diptera);
- Pro-rata multi-habitat sampling EN 161509:2012 using a handnet - 10 units (0.3 x 0.3 m)+0.1 m^2 = 1 m^2;
- A RBA field protocol in use.

Criteria used to establish assessment method

In adopting or developing a rapid assessment method for use in RB monitoring and assessment programs, the following four considerations are important:

1) The method can be used **to measure ecological status** (benthic macroinvertebrate communities) based on the principles of WFD 2000/60/EC.

   Ecological status can be defined as the ability of a system to support and maintain a balanced integrated, adaptive community of macroinvertebrates having a taxonomic composition (diversity), abundance and functional organization comparable to the natural reference conditions of the surface water type.

2) The method should be **rapid and cost-effective**.

   A rapid method must be able to provide an accurate assessment of condition in a relatively short time period. For this reason we define "rapid" as taking no more than two people, 40 minutes ÷ one hour field work per sampling site, 5-8 sampling site per working day (depends on the travelling distances), and requiring no more than 30 minutes of office preparation and data analysis.

3) The method must be an **on-site assessment**.

   All calculations/scoring and the ecological status assessment as a whole should be preliminary provided in the field. An accurate evaluation using a rapid method requires an *in situ* procedure to ensure that the assessment captures the current ecological conditions of the river water body and does not infer condition based solely on laboratory/office breakdown or the potential of a river to perform certain ecological functions.

4) The method can be **verified**.

   Verification may be achieved based on information gathered through empirical studies using anthropogenic pressure data and results from more intensive integrated monitoring & assessment including deviations of biological type-specific reference conditions and benchmark conditions. In this way the assumptions behind the assessment can be tested using intercalibration procedures and QC/QA mechanisms.

RBA sampling technique

All sampling activities are based on the following European standards:

- Pro-rata multi-habitat sampling EN 161509:2012
- Guidelines for the selection of sampling methods and devices for benthic macro-invertebrates in fresh waters EN ISO 10870: 2012

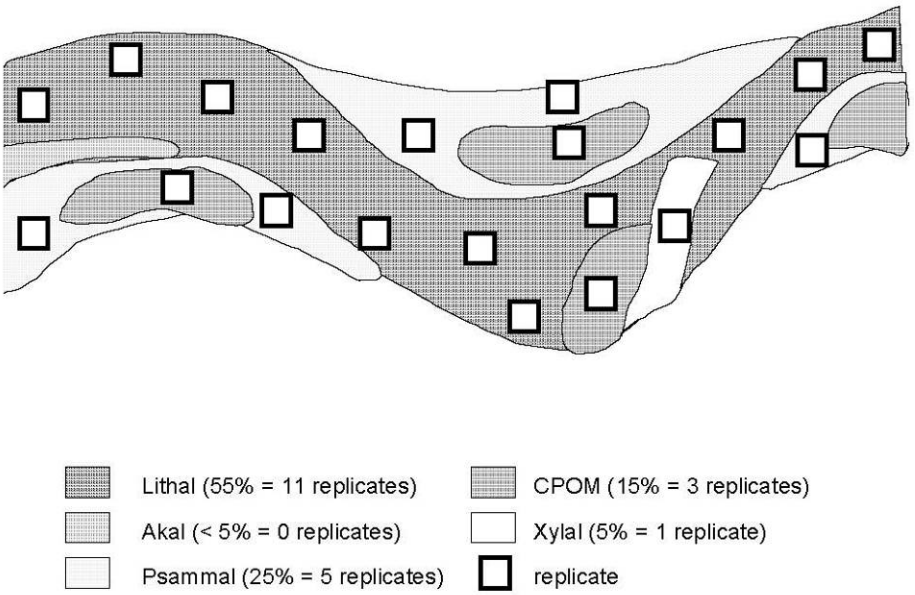
Standard sampling with hand-net (500 µm, rectangular frame: 0.25 x 0.25 m or 0.3 x 0.3 m) for wadeable rivers and light Naturalist's dredge (deeper rivers) should be used.


Sieving-sorting method: (1) Soft net and funnel (Caucasus region); (2) Set of sieves (macrophyte & fine substrata's river types, BY, UA, MD, Metsamor in AM).




Development of coherent sampling techniques to assess the benthic macroinvertebrates in rivers according to the requirements of Water Framework Directive 2000/60/EC is a crucial challenge to each beneficiary country particularly concerning local type-specific river conditions. However, counting all the invertebrates from numerous replicas in the multi-habitat samples that are usually collected for ecological status assessment is time-consuming and costly. In order to standardize biological assessment of surface waters in Europe, a standardized multi-habitat method for sampling, sorting and identification of benthic macroinvertebrates in running waters was developed during the AQEM project (2002). The AQEM method has proved to be relatively time-consuming. Hence, the RBA approach put emphases on a reduction in sample size on efforts/costs and bioassessment results.


A multihabitat sampling method (adapted version) combined techniques of AQEM/STAR methodology as well as the following EN and ISO standards: EN 161509:2012 and EN ISO 10870: 2012, has been developed in compliance of type-specific river conditions in project beneficiary countries. In the process of research work certain specifics of the sampling method have been defined to be distinguished by the original methods (AQEM consortium. 2002; Barbour at al., 1999).



Adapted RBA version	AQEM/STAR method
10 replicas	20 replicas
It can be used a standardized hand-net	Only quantitative samplers, e.g. Surber type
Kick- and wash-sampling together	Mostly wash sampling (stones, cobbles, gravel, etc.)
Washing macrophytes, including when assess the percentage of micro-habitats	Usually macrophytes are not used
Preferring riffle sites when it is possible	No special requirements for riffle or non-riffle sites
Some inappropriate substrata could be avoided, e.g. silt, mud, etc.	It is not allowed to avoid well presented bottom substrata

Multi-habitat sampling method (adapted version) combined the techniques according to AQEM/STAR methodology, US EPA standards was developed in compliance with type-specific river conditions in 6 beneficiary countries (AM, AZ, GE, BY, MD, UA) and their international river basins. A number of specifics with the original sampling method (AQEM consortium. 2002; Barbour at al., 1999) were defined. Ten replicas are sampled per monitoring site with sizes 25 x 25 cm or better 30 x 30 cm. The allocation of these replicas (sampling frames) should be according to the percentage ratio of the main bottom substrata (pro-rata multi-habitat approach). The RBA operator should place the sampling net on the bottom against the velocity covering a little bit more area than the projection of the handnet frame (e.g. from 25 cm x 25 cm to reach 30 cm x 30 cm) to make wash sampling (stones, gravel, macrophytes) or kick sampling (stones, pebbles, gravel, sand, silt, etc.). At the beginning a balance between the number of frames for kick-sampling and wash sampling should be made. Usually the ratio is 50:50 between kick-sampling and wash sampling for mountain and semi-mountain river types, but the ratio could depends on the operator judgment. When washing

macrophytes, the macrophyte types, e.g. emergent/submerged/helophytes (bank vegetation) have to be recorded. Finally the macroinvertebrate results are calculated as number/m^2.

Biological assessment

For the purposes of the RBA procedure benthic macroinvertebrates have been divided into five arbitrary 'Indicator Groups' as follows: Group A (the sensitive forms), Group B (the less sensitive forms), Group C (the tolerant forms), Group D (the very tolerant forms), and Group E (the most tolerant forms). These groups and their relationships with the Rapid Biological Assessment (RBA) are set out below.

Biological material for examination is obtained by sampling in the shallower, faster-flowing areas (e.g. riffles) and the assessment of ecological status is made on site. Having determined the relative proportions of the various organisms in the sample, ecological status is inferred by a comparison of this data with that which might be expected from reference conditions of the river type under investigation. Other relevant factors such as the intensity of algal and/or weed development, water turbidity, bottom siltation, substratum type, current speed (velocity), water depth, DO saturation, electrical conductivity and pH, are also taken into account in the assessment procedure.

Georgia (Chorokhi & Adjaristskali RB District)

Indicator Group	No of taxa in the indicator group	Total number of taxa				
		0 - 1	2 - 3	4 - 7	8 - 10	11+
		Value of RBA Index (%)				
A	4+	n.a.	n.a.	80	90	100
	2/3	n.a.	60	75	80	95
	1	5	40	60	75	85
B	3+	n.a.	40	60	75	80
	1 - 3	5	25	50	55	70
C	All above indicator groups absent	5	25	35	45	65
D	All above indicator groups absent*	0	20	25	30	n.a.
E	All above indicator groups absent*	0	10	15	n.a.	n.a.

(*) – Few specimen of above indicator groups could present in the sample

Armenia, Azerbaijan, Prut River Sub-basin (UA, MD)

Indicator Group	No of taxa in the indicator group	Total number of taxa				
		0 - 1	2 - 5	6 - 10	11 - 15	16+
		Value of RBA Index (%)				
A	3+	n.a.	75	80	90	100
	2	n.a.	60	75	80	95
	1	5	40	60	75	85
B	3+	n.a.	40	60	75	80
	1 - 3	5	25	50	65	70
C	All above indicator groups absent	5	25	35	45	55
D	All above indicator groups absent*	5	20	25	30	n.a.
E	All above indicator groups absent*	0	10	15	n.a.	n.a.

(*) – Few specimen of above indicator groups could present in the sample

Upper Dnieper River Basin (BY, UA)

Indicator Group	No of taxa in the indicator group	Total number of taxa				
		0 - 1	2 - 6	7 - 13	14 - 19	20+
		Value of RBA Index (%)				
A	3+	n.a.	75	80	95	100
	2	n.a.	60	75	90	95
	1	5	45	75	85	90
B	6+	n.a.	40	65	80	85
	5	n.a.	30	60	75	80
	1 - 4	5	25	50	65	70
C	All above indicator groups absent	5	25	35	45	55
D	All above indicator groups absent*	5	20	25	30	n.a.
E	All above indicator groups absent*	0	10	15	n.a.	n.a.

(*) – Few specimen of above indicator groups could present in the sample

Observations on RBA Determination Scheme (RBA Algorithm)

High Status (90-100%) with reference conditions assigned if:

[1] Group A at least common: typically with either 3-4 or more taxa such as *Heptageniidae* species, *Ephemera sp*., *Plecoptera*, etc.;
[2] Group B ranging from few/absent to plentiful;
[3] Group C is usually common or less, but some taxa may be occasionally dominant. e.g. *Baetis*, *Gammaridae*, *Hydropsyche*, etc.;
[4] Groups D and E scarce (few) or absent;
[5] Macrophytes, if present, diverse and not excessive in development;
[6] Filamentous algae (*Cladophora*, etc.) if present not excessive;
[7] *Sphaerotilus* complexes (filamentous bacteria) and other slime growths/complexes absent;
[8] Substrata clean and unsilted;
[9] DO close to 100% at all times.

High Status (80-85%) close to reference conditions assigned if:

[1] At least one Group A taxon present in, at least 6 individuals in the sample. Usually 1-3 taxa of Group A;
[2] Group B taxa may be plentiful, common, few or absent. Rarely Group B taxa may be dominant (slow velocity, 'macrophyte' river types, etc.);
[3] *Baetis*, *Gammaridae*, *Hydropsyche* often dominant but never over 300+ individuals. Other Group C taxa never excessive;
[4] Group D and E may be present in small numbers (few) or absents;
[5] Macrophyte & algal growths not excessive;
[6] *Cladophora*, if present, not excessive;
[7] *Sphaerotilus* complexes (filamentous bacteria) and other slime growths/complexes absent;
[8] Substrata may be lightly silted
[9] DO ranging typically from 80 to 120%.

Good Status (70-80%GE or 60-75%Prut/Dnieper) assigned if:

[1] At least one Group A taxon present in, at least few individuals in the sample (more than 2 individuals);
[2] Group B taxa may be plentiful, common, few or absent. Often Group B taxa may be dominant (slow velocity, 'macrophyte' river types, etc.);
[3] Group C plentiful, dominant or even excessive/super-dominant (over 300+ individuals);
[4] Group D common, present, few or absents. Some *Chironomidae* Gen sp. or specific *Mollusca* species may be plentiful or dominant;
[5] Group E always few or absent;
[6] Macrophyte & algal growths usually luxuriant, often excessive;
[7] *Cladophora*, usually well developed;
[8] *Sphaerotilus* complexes (filamentous bacteria) and other slime growths/complexes sometimes present in small amounts;
[9] Substrata may be considerably silted (especially in slow velocity);
[10] DO ranging typically from <80 to >120%.

Moderate Status (50-65%GE or 40-55%Prut or 45-55%Dnieper) assigned if:

[1] Group A taxon absent;
[2] Group B present, few or absent (rarely plentiful);
[3] Group C usually excessive/super-dominant (over 300+ individuals). (Some *Gammaridae*, *Hydropsyche*, etc. may be fungus infested);
[4] Group D (excl. *Asellus aquaticus* <21 ind.) common, present, few or absents. Some *Chironomidae* Gen sp. or specific *Mollusca* species may be plentiful or dominant;
[5] Group E few or absent;
[6] Macrophytes, if present often silted and/or infested with epiphytic algae;
[7] *Cladophora*, usually excessive;
[8] *Sphaerotilus* complexes (filamentous bacteria) and other slime growths/complexes sometimes may be considerable;
[9] Substrata may be heavily silted;
[10] DO ranging typically from <80 to >120%.

Poor Status (30-45%GE or 25-35%Prut or 25-40%Dnieper) assigned if:

[1] Groups A and B absent;
[2] Group C present, few or absent;
[3] *Asellus aquaticus* common to excessive. Other Group D taxa may be common, plentiful, dominant or excessive.;
[4] Group E may be common;
[5] Macrophytes, if present often silted and/or infested with epiphytic algae, phycomycetes or filamentous bacteria (*Sphaerotilus natans*);
[6] *Cladophora*, not usually apparent;
[7] *Sphaerotilus* complexes (filamentous bacteria) and other slime growths/complexes sometimes usually considerable;
[8] Substrata usually heavily silted. Often high turbidity and smells of sewage/detergent;
[9] DO usually quite low (20 – 50%).

Bad Status (<30%GE or <25%Prut / Dnieper) assigned if:

[1] Groups A, B and C absent;
[2] Group D present, few or absent (rarely plentiful – *Chironomidae* Gen. sp.; *Oligochaeta* Fem. sp. except *Tubificidae*);
[3] Group E plentiful or dominant;
[4] Macrophytes absent;
[5] *Cladophora* absent;
[6] *Sphaerotilus* complexes (filamentous bacteria) and other slime growths/complexes present (organic load) or absent (toxic waters);
[7] Substrata usually heavily silted with anaerobic deposits. Often smells of H_2S;
[8] DO usually very low, sometimes zero.

Notes to the RBA Determination Scheme:

N1: The above scheme (RBA algorithm) outlines the typical macroinvertebrate composition (ecological status) of rivers and streams unaffected (RBA=90-100%) or variously affected (RBA=0-85%) by anthropogenic pressure (mostly by organic waste inputs or general degradation).

N2: Where possible all available habitats should be sampled by kick sampling, stone washing and weed sweeping with emphasis on riffle sites and macrophytes (if present). Some of fine substrata, e.g. sand/clay/organic silt[1] should be just checked for macroinvertebrates by the RBA operator because they can seriously impede the real assessment of ecological status.

N3: Single specimens may be ignored as they are likely to have drifted from upstream. It depends on expert judgment, and it should be noted in the RBA protocol.

N4: Reference conditions (RBA=90-100%) only ascribed in absolutely or very close to pristine conditions with diverse and functionally balanced faunal community.

N5: Specific conditions can influence on the RBA. Usually 20-30% of sampling sites are specific cases (or RBA exemptions). Sometimes faunal criteria for high or good status are not met due to:

N5-1: Significant groundwater input;
N5-2: Very hard, calcareous conditions;
N5-3: Ultra oligotrophic conditions;
N5-4: Specific substrata – bedrock, peat;
N5-5: Very specific river types or sub-types, e.g. temporary (seasonal) rivers, rivers with huge sedimentation and changeable river bed, very slow velocity with excessive water macrophytes (close to swamp/wetland conditions), etc.;
N5-6: Toxic and other pollutant at low concentrations, e.g. heavy metals, some pesticides, POPs, some hydrocarbons, etc.;
N5-7: Other relevant factors e.g. influence of some invasive water species, influence of beavers on the small rivers within Upper Dnieper RB, after ponds/reservoirs, etc.

All these specific conditions should be carefully described as annexes to the RBA methodology with some corrections in the index or as official exemptions. The data record of specific conditions should be upgraded at least each 6 years.

[1] Organic silt & mud should be distinguished by natural fine detritus in the rivers.

N6: The terms “Taxon/Taxa” are defined by the level of identification as follows:

Turbellaria:*	genus*
Oligochaeta:	family
Hirudinea:	genus
Mollusca:	genus
Crustacea:	family
Plecoptera:	family
Ephemeroptera:	genus
Trichoptera:	family
Odonata:	genus
Megaloptera:	genus
Heteroptera:	family
Coleoptera:	genus
Diptera:	family
Rheotanytarsus sp. or Chironomus sp. or other *Chironomidae*	
Hydrachnidia (*Hydracarina*)	presence
Other water ‘groups’:**	presence

(*) – Dugesia considers as three separated taxa, i.e. *Dugesia gonocephala*, *Dugesia lugubris/polychroa* and *Dugesia tigrina*.
(**)- Other water ‘groups’ may be Nematomorpha (*Gordiidae, Gordius aquaticus*), Nematoda (*Mermithidae*, etc.), Nemertini (*Tetrastemmatidae, Prostoma sp*.), *Urnatella gracilis* (*Urnatellidae*, Kamptozoa), Araneae (*Cybaeidae, Argyroneta aquatica*; *Pisauridae*, *Dolomedes sp.*; etc.), Branchiobdellida (*Branchiobdellidae*, *Branchiobdella sp*., *Xironogiton instabilis*), Cestoda, Coelenterata (*Clavidae*, *Cordylophora caspia*; Hydrozoa, *Hydridae*, *Hydra sp*., *Microhydra sowerbyi*; *Protohydridae*, *Protohydra leuckarti*; *Olindiidae*, *Craspedacusta sowerbyi*), Collembola (*Poduridae*, *Podura aquatica*), Ostracoda (Crustacea, *Cytheridae*), Anostraca (*Branchinectidae*, *Branchinecta sp*.; *Branchipodidae*, *Branchipus sp*., *Tanymastix sp*., etc.; *Chirocephalidae*, *Chirocephalus sp*., *Eubranchipus sp*.), Conchostraca (*Cyzicidae, Imnadia sp., Imnadiidae, Leptestheriidae, Limnadia lenticularis, Lynceus brachyurus, etc.*), Notostraca (*Triopsidae*, *Triops sp*., *Lepidurus apus*), Copepoda (*Cyclopidae*; Harpacticoida, *Ectinosomatidae*), Cladocera, *Argulidae* (*Argulus sp*., Crustacea), *Ligiidae* (*Ligidium hypnorum*, Isopoda), Hypogean Crustacea such as *Bathynelidae* (*Bathynella sp*., *Antrobathynella sp*.), *Bogidiellidae* (Amphipoda, Crustacea), etc., Hymenoptera (*Braconidae*, *Ceraphronidae*, *Chalcididae*, *Eucoilinidae*, *Ichneumonidae*, *Mymaridae*, *Pteromalidae*, *Scelionidae*), Plannipenia (*Neurorthidae*, *Osmylidae*, *Sisyridae*), Rotatoria, Tardigrada, Trematoda, etc.

Macroinvertebrate fauna (relative abundance: individuals/m^2)				
Few (1-5 ind.)	**Present (6-20)**	**Common (21-50)**	**Plentiful (51-100)**	**Dominant (100+)**
6372 Perla sp. (Perlidae, Plecoptera) - 3; 4642 Chironomidae Gen. sp. (Diptera) - 1;	5119 Epeorus sp. (Heptageniidae, Ephemeroptera) - 18; 6780 Rhyacophila sp. (Rhyacophilidae, Trichoptera) - 11;	5605 Hydropsyche sp. (Hydropsychidae, Trichoptera) - 26; 6747 Rhithrogena (Heptageniidae, Ephemeroptera) - 46; 4419 Baetis sp. (Baetidae, Ephemeroptera) - 29;	4465 Blephariceridae Gen. sp. - 59;	6842 Simuliidae Gen. sp. (Diptera) - 339;

Indicator Groups of Macroinvertebrates

Group A Sensitive taxa	**Group B** Less Sensitive taxa	**Group C** Tolerant taxa	**Group D** Very Tolerant taxa	**Group E** Most Tolerant taxa
Crenobia, Polycelis *(Turbellaria)* ***All Plecoptera*** *(*except *Leuctridae)* ***Heptageniidae*** *(*some species of *Ecdyonurus & Heptagenia* are more tolerant A-B or B) ***Siphlonuridae*** ***Ephemeridae*** (*Ephemera*) ***Palingeniidae*** (*Palingenia*) (large rivers, e.g. Prut River) ***Polymitarcyidae*** (*Ephoron*) ***Oligoneuriidae*** (good indicators for semi-mountain 'gravel' type of rivers) ***Cordulegastridae*** (*Cordulegaster*) (small rivers) ***Hydrobiidae*** (*Gastropoda*) ***Blephariceridae*** (*Diptera*)	***Dugesiidae*** (except invasive species of *Dugesia tigrina*) (*Turbellaria*) ***Leuctridae*** (*Plecoptera*) ***Baetidae*** (except *Baetis*) ***Ephemerellidae, Leptophlebiidae, Potamanthidae*** (*Potamanthus luteus*) (*Ephemeroptera*) ***Trichoptera cased, Ecnomidae, Philopotamidae, Polycentropodidae, Psychomyiidae, Rhyacophilidae*** (Trichoptera) ***Odonata*** ***Sialidae*** (*Megaloptera*) ***Aphelocheirus*** (*Heteroptera*) ***Jaera*** (*Janiridae, Isopoda, Crustacea*) ***Rheotanytarsus*** (*Chironomidae*), ***Athericidae, Limoniidae, Pediciidae, Tipulidae*** (*Diptera*) ***Bithyniidae*** (*Bithynia, Gastropoda*) ***Porifera*** (*Spongilidae*)	***Turbellaria*** *(*except *Crenobia, Polycelis, Dugesia gonocephala/lugubris/ polychroa)* ***Baetis*** (Baetidae), ***Caenidae*** (*Ephemeroptera*) ***Hydropsychidae*** (*Trichoptera*) ***Heteroptera*** (except *Aphelocheirus*) ***Coleoptera*** ***Simuliidae*** (some genus are sensitive, e.g. *Prosimulium* gr. B, or more tolerant *Wilhelmia* subgenus – gr. D) ***Ceratopogonidae, Chaoboridae, Culicidae, Cylindrotomidae, Empididae, Tabanidae, Muscidae, Dixidae, Thaumaleidae*** (*Diptera*) ***Lepidoptera*** ***Hydrachnidia (Hydracarina)*** ***Gammaridae*** (some species are good indicators; others like *Niphargus*, etc. are indicators for springs/GW)***,*** ***Corophiidae*** (*Amphipoda, Crustacea*) ***Mysidae*** (*Crustacea*)	***Asellus*** (*Asellidae, Isopoda, Crustacea*) ***Chironomidae*** *(*except *Rheotanytarsus , Chironomus)* – widespread in all indicator groups at species level ***Psychodidae, Stratiomyiidae, Ephydridae, Sciomyzidae*** (*Diptera*) ***Gastropoda*** *(*excluding *Neritidae, Planorbidae)* ***Bivalvia*** (except Unionidae) ***Hirudinea*** (some species are more sensitive, *Piscicola*, *Dina*, etc.) ***Oligochaeta*** (except red *Tubificidae*)	***Tubificidae*** (red coloured small *Oligichaeta*; usually super-dominant) ***Chironomus*** (red coloured large *Chironomidae*, *Diptera*) ***Eristalis*** (*Syrphidae*, *Diptera*)

Group A Sensitive taxa	**Group B** Less Sensitive taxa	**Group C** Tolerant taxa	**Group D** Very Tolerant taxa	**Group E** Most Tolerant taxa
		Potamidae (*Crustacea*) ***Astacidae*** (B-C) (*Crustacea*) ***Planorbidae (incl. Ancylus)*** ***Neritidae*** (*Theodoxus fluviatilis*, some species may be more sensitive) ***Unionidae*** (*Anodonta* species may be more sensitive) ***Polychaeta*** *(Hypania invalida*, etc.*)* ***Bryozoa*** (some species indicate good quality)		

Ecological assessment of main river types

Ecological status assessment and biological reference conditions are estimated using the following type-specific classification systems including EQRs:

CAUCASIAN BLACK SEA RIVER TYPES	EQR	Rapid Biological Assessment (%)
Chorokhi-Adjaristskali RB (GE)	0.85 ÷ 1.0	85 ÷ 100
	0.70 ÷ 0.80	70 ÷ 80
	0.50 ÷ 0.65	50 ÷ 65
	0.30 ÷ 0.45	30 ÷ 45
	0.00 ÷ 0.25	0 ÷ 25

CAUCASIAN KURA-ARAS RIVER TYPES / PRUT RIVER TYPES	EQR	Rapid Biological Assessment (%)
Akhurian and Metsamor river sub-basin (AM)	0.80 ÷ 1.0	80 ÷ 100
The right tributaries of the Central Kura (AZ)	0.60 ÷ 0.75	60 ÷ 75
Prut river sub-basin (UA)	0.40 ÷ 0.55	40 ÷ 55
Prut river sub-basin (MD)	0.25 ÷ 0.35	25 ÷ 35
	0.00 ÷ 0.20	0 ÷ 20

UPPER DNIEPER RIVER TYPES	EQR	Rapid Biological Assessment (%)
Upper Dnieper RB (BY)	0.80 ÷ 1.0	80 ÷ 100
Upper Dnieper RB (UA)	0.60 ÷ 0.75	60 ÷ 75
	0.45 ÷ 0.55	45 ÷ 55
	0.25 ÷ 0.40	25 ÷ 40
	0.00 ÷ 0.20	0 ÷ 20

Total taxa number (family & genus level of identification)

Ecological Status	River basins / Total taxa number		
	Prut river sub-basin (UA, MD), Akhurian and Metsamor river sub-basin (AM), right tributaries of the Central Kura (AZ)	Chorokhi-Adjaristskali RB (GE)	Upper Dnieper RB (BY, UA)
High	16+	11+	20+
Good	11 - 15	8 - 10	14 - 19
Moderate	6 - 10	4 - 7	7 - 13
Poor	2 - 5	2 - 3	2 - 6
Bad	0 - 1	0 - 1	0 - 1

Rapid Biological Assessment (RBA) of rivers in Caucasus Region (AM, AZ, GE), Prut River Sub-basin (UA, MD) and Upper Dnieper RB (BY, UA)						
RBA classes and typical associated Macro-invertebrate community structure						
Macroinvertebrate Indicator Groups*	**High Status (90-100%) ref. conditions****	**High Status (80-85%) close to ref. conditions*****	**Good Status (70-80%GE or 60-75%Prut/Dnieper)**	**Moderate Status (50-65%GE, 40-55% Prut, 45-55%Dnieper)**	**Poor Status (30-45%GE, 25-35%Prut, 25-40%Dnieper)**	**Bad Status (<30%GE, <25%Prut / Dnieper)**
Group A	Richness; At least 3 taxa (4+) well represented	At least 1 taxon (1-3) in reasonable numbers	At least 1 taxon Few - Common	Absent	Absent	Absent
Group B	Few to Plentiful	Few to Plentiful	Few/Absent to Dominant	Few/Absent	Few/Absent	Few/Absent
Group C	Few/Common	Common to Plentiful; Some dominant but never Excessive (300+)	Common to Excessive (usually Dominant)	Dominant to Excessive	Present, Few or Absent	Absent
Group D	Few or Absent	Few or Absent	Few/Absent to Common	Few/Absent to Common; Some *Chironomidae* or *Mollusca* may be Plentiful/Dominant	Dominant to Excessive (300+)	Present, Few or Absent
Group E	Few or Absent	Few or Absent	Few or Absent	Few or Absent	Few / Absent to Common	Plentiful to Dominant
Additional Qualifying Criteria						
Filamentous algae (*Cladophora sp.*) Abundance	Trace only or None	Moderate growths (if present)	May be Abundant to Excessive growths	May be Excessive growths	Few or Absent	None
Macrophytes (typical abundance if present)	Normal growths or absent	Enhanced growths	May be Luxuriant growths	May be Excessive growths	Absent to Abundant	None
***Sphaerotilus* complexes**	Never	Never	Trace to None	May be Abundant	May be Abundant	None

(filamentous bacteria) or other slime growths						
Oxygen Saturation	Close to 100% at all times	80 – 120%	Fluctuates from <80% to > 120 %	Very unstable. Potential fish-kills in Trout/Barbel Zones	Low (but >20%)	Very low, sometimes zero
Substratum Siltation	None	May be light	May be light	May be considerable	Usually heavy	Usually very heavy and anaerobic

Note: The above RBA short scheme outlines the typical macroinvertebrate composition (ecological status) of rivers and streams unaffected or variously affected by anthropogenic pressure (mostly by organic waste inputs or general degradation). Single specimens may be ignored. Specific conditions and other relevant type-specific factors must be taken into account.

(*) Macroinvertebrate criteria should be apply having in mind biological & hydromorphological type specific reference conditions.
(**) Reference conditions (RBA=90-100%) only ascribed in absolutely or very close to pristine conditions with diverse and functionally balanced faunal community.
(***) Caucasian river types in AM, AZ and GE have such assessment close to ref. conditions at RBA=85%. Other river types (Prut – UA & MD; Upper Dnieper RB – BY & UA): 80 – 85% RBA

General characteristics of the 5 ecological classes based on the RBA with benthic macro-invertebrates*

Ecological Status	High Status	High Status	Good Status	Moderate Status	Poor Status	Bad Status
RBA Ratings	**90 – 100%**	**80 – 85%**	**70-80% GE or 60-75% Prut/Dnieper**	**50-65% GE, 40-55% Prut, 45-55% Dnieper**	**30-45% GE, 25-35% Prut, 25-40% Dnieper**	**<30%GE, <25%Prut / Dnieper**
Ref. Conditions	Ref. conditions	Near to ref. conditions	Benchmark conditions	Benchmark conditions	Benchmark conditions	Benchmark conditions
Organic Waste Load	None	None	Light	Considerable	Heavy	Excessive
Maximum BOD_5	Low (< 3 mg/l)	Low (< 3 mg/l)	Often elevated (but usually < 5 mg/l)	High at times (usually < 10 mg/l)	Usually high (10 – 20 mg/l or more)	Usually very high (> 25 mg/l)
Dissolved Oxygen Regime	Close to 100% or typical for ref. cond.	80% - 120%	<80% to >120% (e.g. 60% - 140%)	Very unstable (but > 40%)	Low (but >20%)	Very low, sometimes zero
Annual Median Ortho-Phosphate	≈ 0.015 mg P/l	< 0.030 mg P/l	Usually < 0.050 mg P/l	Usually < 0.075 mg P/l	Usually > 0.1 mg P/l	Usually > 0.1 mg P/l
Total Phosphorus (TP) Annual Average (AA)	Always < 0.025 mg P/l or Trace	Always < 0.055 mg P/l or Trace	Usually < 0.075 mg P/l	Usually < 0.1 mg P/l Risk for eutrophication of receiving 'lake' WBs	Usually < 0.2 mg P/l Serious risk for eutrophication of receiving 'lakes'/Sea	Sometimes > 0.2 mg P/l Great risk for eutrophication of receiving 'lakes'/Sea
Ammonia & Nitrites ($N\text{-}NH_4$ and $N\text{-}NO_2$)	None or close to zero	None or close to zero	Trace to None	Variable, Sometimes $N\text{-}NH_4$ <1 mg N/l, and $N\text{-}NO_2$ <0.09 mg N/l	Variable, Sometimes $N\text{-}NH_4$ <1.5 mg N/l, and $N\text{-}NO_2$ <0.09 mg N/l	Variable, Sometimes $N\text{-}NH_4$ >1.5 mg N/l, and $N\text{-}NO_2$ >0.09 mg N/l
Nitrates ($N\text{-}NO_3$) Annual Average (AA)	Always < 1 mg N/l or Trace	Always < 1 mg N/l or Trace	Usually < 2.5 mg N/l	Usually < 5 mg N/l	Usually < 12 mg N/l	Sometimes > 12 mg N/l
Total Nitrogen (TN) Annual Average	Always < 1 mg N/l or Trace	Always < 1 mg N/l or Trace	Usually < 2.5 mg N/l	Usually < 5 mg N/l Risk for	Usually < 12 mg N/l Serious risk for	Sometimes > 12 mg N/l

Ecological Status	High Status	High Status	Good Status	Moderate Status	Poor Status	Bad Status
(AA)				eutrophication of receiving 'lake' WBs	eutrophication of receiving 'lakes'/Sea	Great risk for eutrophication of receiving 'lakes'/Sea
Organic Siltation	None	May be light	May be light	May be considerable	Usually heavy	Usually very heavy and anaerobic
Filamentous Bacteria complexes	Never	Never	Trace to None	May be Some or Abundant	May be Very Abundant	None (or Abundant)
Filamentous algae	Limited development	Diverse communties (if present)	*Cladophora* may be Abundant	*Cladophora* may be Excessive	Few, Absent (rarely Abundant)	Usually None
Macrophytes (typical abundance if present)	Good diversity Limited growths (normal for the river type)	Considerable growths	Reduced diversity Luxuriant growths	Limited diversity Excessive growths	Tolerant species only May be Abundant	Usually None or tolerant species only
Macroinvertebrates	Diverse communities compared to ref. cond. Normal density. Sensitive taxa usually numerous.	High diversity. Increased density. Sensitive taxa scare or common.	Very high diversity. Very high density. Sensitive taxa scarce.	Sensitive taxa absent. Tolerant taxa common. Low diversity.	Tolerant taxa only. Very low diversity.	Most tolerant taxa. Minimal diversity.
Water Quality / Pollution Status	Pristine, Unpolluted	Unpolluted	Slight Pollution	Moderate Pollution	Heavy Pollution	Gross Pollution
Abstraction Potential	Suitable for all	Suitable for all	Potential problems for drinking water supply (after treatment); Good for irrigation & industrial water supply	Advanced treatment	Low grade abstractions (mostly some industrial purposes)	Extremely limited
Fishery Potential	Game fisheries	Good game	Game fish at risk in	Coarse fisheries	Fish usually absent	Fish absent

Ecological Status	High Status	High Status	Good Status	Moderate Status	Poor Status	Bad Status
		fisheries	Trout rivers	Potential fish-kills	Fish kill events	
Amenity & Tourist Value	Very high Ecotourism	High Ecotourism	Considerable but good for tourism	Reduced Usually not good for tourist activities	Low No tourist potential	Zero No tourist potential
Ecosystem & Water Resource conditions	Satisfactory	Satisfactory	Satisfactory	Transitional	Unsatisfactory	Unsatisfactory

(*) Above Table is intended to be used by decision-makers, water managers and experts from other water disciplines (e.g. hydro-engineers, chemical engineers, etc.) as well as by specialists from other sectors (fishery, tourism & eco-tourism, landscape management, natural resources & nature protected areas, etc.).

EPIRB PROTOCOL – BENTHIC MACROINVERTEBRATE FAUNA IN RIVERS

RIVER NAME **Kintrishi River** PLACE: *Kobuleti*			**CODE** SW-24
River type: VI			Waterbody name/code: *SWB*
Municipality: *Kobuleti District*		Watershed : *Kintrishi RB*	
2. SAMPLING Date and time: 14/6/2013 13:50		Sampling device: Handnet	Agency: EPIRB
No. of replicas / area: 10 (0.3 x 0.3 m) + 0.1 m2		Sampling area covered [m^2] : 1	
Sampling strategy No: Kick sampling: 5		Washing stones: 5	Wash macrophytes: 0
3. FIELD OBSERVATIONS Surveyor name: *Irakli Kordzaia Svetoslav Cheshmedjiev*			
GPS coordinates	**N:** 41.801601	**E:** 41.8782005	**Altitude (m):** 81

Substrata (%)		**Transparency**	**Water surface**	**Riffle / pool**
Bedrock/rocks [> 4 m]	0	Transparent: ☑	Clean: ☑	Riffles: ☐
Large boulders [256 mm - 4 m]	40	Turbid: ☐	Foam: ☐	Non-riffle site: ☐
Boulders / stones [64-256 mm]	30	Colour: ☐	Litter: ☐	Riffle-pool: ☑
Cobbles [16-64 mm]	10	**Velocity**	**Shading**	**Land use / land cover**
Pebbles [2-16 mm]	10	Very slow: ☐	Open: ☑	Urban area: ☑
Sand [0,06-2 mm]	10	Slow: ☐	Low: ☐	Deciduous forest: ☑
Silt / mud	0	Moderate: ☑	Moderate: ☐	Coniferous: ☐
Coarse detritus (leaves, etc.)	0	Fast: ☐	High: ☐	Mixed forest: ☐
Naturally organic / fine detritus / peaty	0	Very fast: ☐	Very high: ☐	Shrubs: ☐
Tree branches+stems	0	Turbulent: ☐	**O_2 (mg/l):** 8.8 **%:** 97	Pasture/meadows: ☐
Artificial	0	**Width (m):** 25	**pH:** 7.5 **T°C:** 20	Agricultural fields: ☐
Other:	0	**Depth (m):** 0.1-0.4	**EC (µS/cm):** 75	Others: ☐

Other fauna/flora		**Macrophyte description** – abundance acc. to Kohler (1÷5) + % cover (50 m section)		
Filamentous algae (%):	0	**Submerged MPH**	**Floating MPH**	**Helophytes / amphiphytes**
Other algae:	0	0	0	0
Sphaerotilus (%):	0			
Amphibians:	not observed			
Others:	Astacus astacus colchicus, Potamon ibericum			
Abundance				
Low	☐			
Moderate	☑			
High	☐			
Total abundance, m2 684				

Macroinvertebrate fauna (relative abundance)

Few (1-5 ind.)	**Present (6-20)**	**Common (21-50)**	**Plentiful (51-100)**	**Dominant (100+)**
9112 Odontocerum sp. (Odontoceridae, Trichoptera) - 1; 5293 Gammarus sp. (Gammaridae, Amphipoda, Crustacea) - 1; 4480 Brachycentrus sp. (Brachycentridae, Trichoptera) - 1;	5456 Heptagenia (Heptageniidae, Ephemeroptera) - 8; 6780 Rhyacophila sp. (Rhyacophilidae, Trichoptera) - 11; 10626 Coleoptera Gen. sp. Lv. - 13; 5605 Hydropsyche sp. (Hydropsychidae, Trichoptera) - 15; 6747 Rhitrogena (Heptageniidae, Ephemeroptera) - 17;	5119 Epeorus sp. (Heptageniidae, Ephemeroptera) - 35; 4465 Blephariceridae Gen. sp. - 29; 8437 Leuctridae Gen. sp. (Plecoptera) - 37; 5021 Dugesia sp. (Dugesidae, Turbelaria) - 45;	4528 Caenis sp. (Caenidae, Ephemeroptera) - 55; 9981 Goeridae Gen. sp. (Trichptera) - 62; 8825 Hydrachnidia Gen. sp. (Hydracarina) - 62;	4419 Baetis sp. (Baetidae, Ephemeroptera) - 131; 6842 Simuliidae Gen. sp. (Diptera) - 161;

No of MZB taxa 17	**Biodiversity**	**Comments:**	**RBA Index (0 – 100%)**	**100**
Fish	Poor ☐	BS river type - gravel-stone substrata; riffle-pool structure; 8 km before the Black Sea (estuary)! River important for conservation of migratory fish species - Black Sea Salmon (Salmo labrax) and other rare/endemic fish species: Capoeta sieboldii, Capoeta tinca, Chondrostoma colchicum, Barbus tauricus, Alburnus derjugini, etc. or crayfish - Astacus astacus colchicus. Reference conditions.	**EQR**	**1**
Capoeta sieboldii, Capoeta tinca, Chondrostoma colchicum, Squalius cephalus, Salmo labrax, Barbus tauricus, Alburnus derjugini	Medium ☐		**MZB ecological status**	**High**
	High ☑		**BMWP-ASPT**	**107**
	RETI 0.419		**RTI**	**10.3**
	BBI 10		**ITC**	**21.16**

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SOFTWARE USED:

- ☑ ASTERICS (version 3.3.1) with ASTERICS Software manual (version 3.3.1) calculates the ecological status of rivers based on benthic invertebrate taxa lists.
  (developed by AQEM Project http://www.aqem.de)
  http://www.fliessgewaesser-bewertung.de/en/download/berechnung/
- ☑ BMWP' индекс (BMWP-coma.exe)
  Компьютерная реализация: Тимур Павлюк, Екатеринбург, 2005 г. (Only in Russian)
- ☑ Бельгийский биотический индекс (BBI.exe)
  Компьютерная реализация: Тимур Павлюк, ФГУП РосНИИВХ, Екатеринбург, 2005 г. (Only in Russian)
- ☑ The MaTroS program (Macrozoobenthos Trophic Structure) http://macro.nemi-ekb.ru