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ASSESS-HKH Methodology Manual describing fundamentals & application of three approaches to evaluate the river quality based on benthic invertebrates: HKH screening, HKH score bioassessment & HKH multimetric bioassessment

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1. Introduction

The principle objective of ASSESS-HKH is to develop and validate a three-tier methodology for assessing the ecological quality of rivers in the HKH region, representing different degrees of precision. The ecological assessment system is using **benthic macroinvertebrates as bio-indicators**, which often provide a deeper understanding of ecosystem dynamics and information on anthropogenic effects than chemical measurements. It will serve as a basis for policy development, trans-national water resource planning and ecosystem management.

As in many Asian countries, river monitoring in the HKH region is presently mostly based on the analysis of chemical data. As proven in Europe, Australia and North America, biological data are a worthwhile addition or substitution for the detection of pollution and for the determination of the ecological status of rivers. Biological data are particularly well suited for the determination of steep pollution- and disturbance gradients.

The use of benthic macroinvertebrates for ecological assessment of running surface waters has been a programme of emphasis in European research since last century and was intensified by the introduction of the EU Water Framework Directive (Directive 2000/60/EC Establishing a Framework for Community Action in the Field of Water Policy). The European partners in the ASSESS-HKH Project have played a major role in developing assessment systems based on biotic indicators within EU-funded research projects for the implementation of the directive.

This ASSESS-HKH Methodology Manual is describing the fundamentals & the application of a standardised sampling procedure and three biological assessment methods, which are:

- HKHscreening: screening method (rapid field bioassesment method)
- HKHbios: score bioassessment method
- HKHindex: multimetric bioassessment method

The HKHscreening (rapid field bioassessment method) is a basic screening method that is useable with or without software. This method also serves as an “early warning” method to quickly identify areas for urgent attention or to set priorities for the selection of sites for detailed investigation.

The HKHbios (score based assessment method) is a score-based assessment method on mostly higher taxonomic bioindication units (genus, family, order) that needs only simple data processing.

Finally, the HKHindex (multi-metric assessment method) is a scientifically sound method for the detailed analysis of biological data based on the best available taxonomic resolution. The calculation of the multi-metric index needs a more sophisticated calculation tool.

2. River quality classes

ASSESS-HKH river quality classes follow the system used in the EU-Water Framework Directive (WFD) with 5 quality classes from best to worst. As general consensus a sixth river quality class, visualised in black colour for river(s) (sections) with no higher life, was implemented in the project.

River quality class

I	high
II	good
III	moderate
IV	poor
V	bad
VI	no higher life

Figure 1: river quality classes

3. Sampling Procedure

3.1. General recommendations for sampling

The sampling site (river reach) should be representative for the river-segment and the purpose of the study. Therefore, the sampled stretch of about 100 m of length should reflect the typical situation (at least two riffle-pools sections) along a section of one to several kilometres. The length of the investigated river reach should increase with the size (stream order) of the river.

If a river to be investigated is new for the researcher, it is recommended to check a river section of about 500 m for selecting a representative investigation site.

Sampling starts at the downstream end of the stretch and proceeds upstream. The river section to be sampled should not be disturbed by physical contact (don't step into the river upstream the sampling area) before sampling starts.

3.1.1. General remarks

In general **no** sample should be taken

- during monsoon season;
- during or shortly after floods; a recovery period of four to six weeks after a spate must be considered;
- during or shortly after droughts (completely dry river sections), (see above);

- during any other natural or man-induced disturbances, e.g. if unnatural turbidity prevents a proper estimation of the habitat composition or sampling of the stream bed;

3.1.2. River quality mapping

For the purpose of river quality mapping samples should be taken

- during low flow conditions; these show worst case in terms of organic pollution and this status is used for colour banding;
- for Lower Gangetic Plains (IMO 120): during low flow conditions; several visits recommended before deciding the optimum time for sampling;

3.2. Description of the sampling approach

The sampling follows the multi-habitat approach (Moog 2007). This means that the distribution of sampling units must follow the distribution of habitats. A sample usually consists of 20 "sampling units" taken from all representative habitat types at the sampling site, each with a share of at least 5 % coverage.

For the purpose of river quality mapping following the Screening method, ten sampling units may be sufficient. More sample units should be taken if some targeted organisms are missed. In case of heavy deteriorated rivers with a poor fauna even less than 10 sampling units can be taken.

A “sampling unit” is a sample performed by positioning the net and disturbing the substrate in a quadratic area that equals the frame-size upstream of the net. Sediments must be disturbed to an adequate depth that ensures capture of all species present depending on substrate diameter, compactness and “shape” (organic substrata). E.g. sediments should be disturbed to a depth of approximately 5-10 cm (finer substrates: psammal, pelal, FPOM), 10-15 cm (intermediate sized substrates: akal, microlithal, CPOM) or 15-20 cm (larger substrates: macrolithal; living parts of terrestrial plants).

Following the AQEM (AQEM consortium 2002) procedure with a square net of 25 x 25 cm, the sampling procedure equals a sampled area of approximately 1.25 m^2 of the stream bottom.

3.3. Sampling gear

The proposed sampling gear to be applied in **wadeable rivers** is the **AQEM/STAR net sampler**:

- Shape of the frame: rectangular. A frame in front of the hand net of 625 cm^2 area is recommended to enable the sampling of a distinct area.
- Dimensions of the frame: 0.25 m width by minimum 0.25 m height. The frame attaches to a long handle, similar to a broom stick.
- Shape of the net: cone or bag shaped for capturing organisms. Mesh size of the net: standard mesh size of 500 µm nytex screen.

The proposed sampling gear **for large rivers** is the **grab sampler** (e.g. Van Veen grab):

- The grab sampler can be used from a boat or the margins of the river.
- The content of the Van Veen grab is about 7 liters and covers a surface of 625 cm^2.

3.4. Sample treatment

3.4.1. General sample processing in the field

Removal of large material and field sorting: Branches, sticks and stones can be removed after being rinsed and inspected for burrowing, clinging or sessile organisms. Any organisms found should be placed into the sample container. Generally, it is recommended not to spend time inspecting small debris in the field.

Storing: Transfer the sample from the net to sample container(s) and preserve with formalin (4% final concentration of formaldehyde in the containers) or in sufficient 95% ethanol to cover the sample completely, immediately after collection. This form of fixation is important to prevent carnivores, particularly stoneflies (Setipalpia [=Systellognatha]), beetles (Adephaga), caddis larvae (e.g. *Rhyacophilidae*), *Sialidae* and certain *Gammaridae* from feeding on other organisms. The final ethanol concentration in the containers should be around 70 %. When using ethanol, water in the sample should be decanted before adding the fixation liquid. Forceps may be needed to remove organisms from the dip net. The sample container should be closed tightly. The samples should be stored cool.

Labelling: Place a label (written in pencil, printed on a laser printer or photocopied) indicating the following information inside the sample container: project (optional); stream name; site name; site code; date of sampling; sampling gear, fraction; investigator's name (optional), number of sample units.

3.4.2. Sample processing in the field for the Screening Method

Each sample unit is regularly emptied into a white tray before the sample unit from the next habitat is added. The specimens are picked out and a variety of them is put into a Petri dish (this will be helpful for writing the taxa list and abundance estimates). The remaining rest of the sample unit is poured into a bucket. Each sample unit is treated the same way. It is not necessary to pick out all specimens but a representative portion should be treated.

The next step is the provision of a taxa list with relative abundance for each taxon. The estimation of abundance is according to the following scheme:

Table 1: Estimation of abundance

Abundance	Verbal description
1	single finding, rare
2	few
3	medium
4	many
5	masses

Intermediate classes (e.g. 2.5) may be used.

The estimated abundance of each taxon needs to be filled in the column "Abd." of the HKHbios scoring list on the backside of the Screening Protocol (see chapter 4.6.7).

Not all HKHbios-taxa can be identified in the field without doubt. It is therefore recommended to give a temporary name to problematic taxa and perform their identification in the lab.

Further processing of the sample in the lab

After at least one week of fixation, samples taken to the lab are sorted, identified, and additional taxa (each with notification of the respective abundance class) are included in the taxalist prepared in the field. **The examination and post-sorting of the sample is essential for quality assurance.**

4. HKHscreening - rapid field bioassessment method

4.1. Introduction

The Screening method provides an overview on the river quality status of an investigation site. This methodology is based on the Austrian method for screening the ecological quality of rivers and stream developed by Moog et al. (1999) which was adapted for ASSESS-HKH by Moog & Sharma (2005). Basically the assessment procedure is based on sensoric criteria and the biota that can be identified in the field with a special focus on the bioindicative value of benthic invertebrates. Nevertheless it is recommended to confirm the field identifications in the lab.

The rationales of a Screening method are (Barbour et al. 1999):

- Cost-effective, but scientifically valid, procedures for biological surveys
- Provisions for multiple investigations of sites in a field season
- Quick availability of results for management decisions
- Scientific reports intelligibly translated to management and the public
- Ecologically-friendly procedures

4.2. Scope

For the development of the ASSESS-HKH assessment methodology two major stressors have been defined (Feld et al. 2005):

1) Water pollution mainly caused by organic load, such as sewage from both private households and industries. The entry of organic material into freshwater ecosystems is linked to an increase of the aerobic metabolism to break down organic compounds. This, in turn, causes severe oxygen depletion and indirectly and directly affects the in-stream fauna.

2) Flow alteration caused by transversal dams or weirs. Dams and weirs not only reduce the flow velocity directly upstream of the structures, but also lead to a strong decrease of habitat variation, such as monotonous substrate compositions with accumulations of fine sediments and a poor current diversity. The reduction of flow causes oxygen depletion and accumulation of organic matter.

The Screening Methodology was primarily developed for estimating the ecological status of a site due to impacts of organic pollution (organic degradable wastes). As the effects of weirs and dams cause a similar pressure as direct organic pollution, the Screening method is applicable for assessing the impact of both pressures (see practical experience in chapter 4.7).

For assessing the river quality of streams two tools are available:

- a narrative description of the saprobic river quality classes and selected river quality criteria
- a rapid field decision support table to estimate the organic pollution status of a river which is based on an assessment of sensory and biotic features.

For assessing the river quality status of a river one starts with tool 2 (field decision support table) and compare the result of that rapid assessment with the narrative description of saprobic river quality classes (tool 1).

4.3. Adjustment of Screening Protocol (decision support table)

The pre-classification of sampling sites for the development of a three-tier methodology was accomplished by the use of a pre-classification scheme (Screening Methodology) (Feld et al. 2005). Overall 390 sites have been pre-classified and sampled within two sampling seasons.

For the purpose of river quality mapping in all Asian partner countries (Shresta at al. 2007) the Screening Methodology has been adjusted due to experiences gained from the extensive field work conducted in the region within the two sampling campaigns.

Based on the field work experiences as well as on data evaluation, the following adjustments of the Screening protocol have been made:

- Preparation of two separate Screening Protocols for *Mountains* (all ecoregions exceptive Gangetic Plains) and *Lowlands* (only Gangetic Plains);
- Use of benthic macroinvertebrates as bioindicators and adaption of scores given to taxa based on experiences from three sampling campaigns;
- Adaptation of scoring and choice of benthic macroinvertebrates based on HKHbios scoring (see chapter 5);
- Implementation of a sixth river quality class (black colour), for rivers (sections) with no higher life;

The rationales of the conducted adaptations were:

- Guarantee of an equal distribution of indicators for all quality classes
- Consideration of only those taxa which can be identified in the field without doubt
- Assurance that the indicators occur in all ecoregions

4.4. Chemical measurement

The collection of water chemistry data is not obligatory for the application of the Screening method. Nevertheless it is recommended to measure the following chemical parameters:

- Water temperature (to be measured in the field)
- Conductivity (to be measured in the field)
- Oxygen content & oxygen saturation (to be measured in the field)
- BOD_5 (to be measured in lab only!)

4.4.1. The role of water chemistry in the ASSESS-HKH project

Pysico-chemical measurements provide only a partial assessment of the health and condition of aquatic ecosystems. An assessment based only on the measurement of physical/chemical parameters may not detect adverse water quality conditions if measurements have not been taken at the right time or under the right conditions. Furthermore, the effects of contamination on an aquatic ecosystem may last long after physical and/or chemical conditions have returned to normal. Physico-chemical parameters are precise, discriminatory and quantitative for the variables to be determined at the moment of sample collection. But there are thousands of chemicals that may be discharged to a stream and only few chemicals are selected for routine chemical analysis and the concentration of pollutant vary with time leaving their effect for many weeks or months. Therefore, in recent years there is a shift in emphasis towards the development of more holistic measures for assessing aquatic ecosystem condition (biological assessment).

Within the frame of ASSESS-HKH physico-chemical date have been used for an orientating pre-classification and a rough calibration of biological results. The according classification of water chemistry results to a river quality class is described in chapter 4.5. Basically, chemical data were not used for river quality assessment.

4.5. Description of saprobic river quality classes (tool 1)

The following narrative of parameters that describe the saprobic aspects of river quality have been used as part of the pre-classification exercise. Please note that these descriptions have been developed in Central Europe and their application in Asia should be done with caution.

Saprobic river quality class I - none to very slight organic pollution

1) clean water with only very little amount of organic matter and only very little concentration of nutrients
2) clear water with exception of natural turbidity (e.g. glacier fed brooks with turbidity caused by weathering processes)
3) waters are **well oxygenated**, 8 or more mg/l oxygen, with frequent small detectable deficits
4) oxygen saturation between 95 and 110%
5) BOD_5 generally below 2 mg/l
6) COD generally below 5 mg/l
7) phosphorous below 35 µg/l (to be discussed later: <80 µg/l)
8) **no reduction phenomena** exist at all; even fine sediments (silt, mud and sand) are of light or brownish colour and of high minerogenic content
9) substrate cover mainly consists of algae (mainly **diatoms**, specific blue-greens and red algae) whereas filamentous algae are not very abundant (exception: specific red algae), **mosses** (several species)
10) **highly diverse insect fauna** of **only few specimens; planarians** and **insect larvae** prevail (Ephemeroptera, Trichoptera, in medium and higher reaches several species of Plecoptera occur)

Saprobic river quality class II - moderate organic pollution

1) moderately polluted water with a higher amount of organic matter and higher concentration of nutrients
2) **clear water** with exception of natural turbidity; in **lowland rivers** suspended solids caused by natural processes may cause a **certain turbidity**
3) oxygen content always over 6 mg/l
4) oxygen saturation between 70-125%
5) BOD_5 frequently between 2-6 mg/l
6) COD generally between 5-12 mg/l
7) phosphorous between 36-81 µg/l (to be discussed later: 80-<200 µg/l)
8) only **little reduction phenomena** may occur in very fine sediments at **lentic sites**; at the surface, the fine sediments (silt, mud, sand) are aerobic, brownish/light coloured, in the deeper interstitial zone sediments may be grey or blackish because of oxygen depleting organic matters
9) substrate cover consists of algae (all groups), mosses (few species) and macrophytes, filamentous algae may be abundant
10) the benthic invertebrate fauna consists of many groups (Mollusca, Crustacea, several insect orders); net-spinning Trichoptera may be numerous at lotic sites; highly diverse insect fauna of high abundance

Saprobic river quality class III - critical organic pollution

1) river reaches with clearly visible load of **nutrients** as well as organic, oxygen-consuming substances
2) the water is in certain circumstances **slightly turbid** (heavier load of organic matter)
3) amount of oxygen 4 mg/l or even less
4) oxygen saturation between 50-150%
5) BOD_5 frequently between 6-10 mg/l
6) COD generally between 12-18 mg/l
7) phosphorous between 82-200 µg/l (to be discussed later: 200-<500 µg/l)
8) fine-grained substrates are aerobic, brown or light coloured at the surface, and in deeper areas sometimes dark (chemically reduced); **black spots** can appear **beneath stones**;
9) **fish kills** are **possible** due to strong **fluctuations** in the **oxygen budget**, but usually these river reaches still produce high yields of fish
10) the diversity of macro-organisms is sometimes reduced; certain species show an abnormal mass development; fauna: sponges, moss animals, crustaceans, molluscs, and insect larvae; leeches clearly increase; net-spinning Trichoptera (especially *Hydropsyche*) often appear in large quantities as well as Chironomidae (tunnel-building forms in fine sediments)
11) **filamentous algae** (e.g. *Cladophora*) and **macrophytes** frequently cover large areas or occur as mass colonies; green algae are more abundant than in river quality class II
12) sewage bacteria can be recognized; ciliates are diverse, but colonies are rare

Saprobic river quality class IV - heavy organic pollution

1) the water shows a **high amount** of **organic matter** and **high concentration** of **nutrients**
2) the water may be **turbid** locally or at given times; due to the effluents a certain water colour may be developed (brownish after paper industries; diverse colours after textile factories, etc.) as well as a peculiar smell
3) putrefactic conditions occur in fine/very fine sediments at lentic sites; at the surface, the finer sediments of lotic sites are muddy, in the deeper interstitial zone the sediments blackish because of oxygen depleting organic matter; **black reduction spots** (ferro-sulphide) occur beneath the stones and **may cover bigger areas**
4) the oxygen content is low and **periodically** causes **fish kills** or injuries to sensitive species; oxygen present in amounts of 2 – 4 mg/l, but drops at times to < 2 mg/l
5) oxygen saturation between 25-200%
6) BOD_5 frequently between 10-15 mg/l
7) COD generally between 18-25 mg/l
8) phosphorous between 201-300 µg/l (to be discussed later: 500-<1000 µg/l)
9) filamentous algae and tolerant macrophytes may grow in high abundances; tolerant blue-greens and diatoms may cover bigger lentic areas; **sewage "fungi"** (e.g. *Sphaerotilus*) visibly grow on hard substrates or cover benthic invertebrates; the ciliates show +/- abundant colonies of sessile species (seen by naked eyes)
10) invertebrate fauna: only of those **few groups** which are tolerant against oxygen deficiency, but leeches and water-hog lice (*Asellus*) may occur in high numbers; in a transition to river quality class V sludge deposits in lentic areas are densely colonized by chironomids of the genus ***Chironomus*** (bloodworms), tolerant Tanypodinae, and **tubificid worms**
11) poor fauna of high abundance

Saprobic river quality class V - very heavy to extreme organic pollution

1) limited conditions for life due to the **very heavy loading** of **organic**, oxygen-consuming **substances** appear; oxygen content sometimes less than 1 mg/l, as a rule not more than a few mg/l
2) oxygen saturation < 10% (deficit) to > 200 (over-saturation)
3) BOD_5 more than 15 mg/l
4) COD generally > 25 mg/l
5) phosphorous > 300 µg/l (to be discussed later: >1000 µg/l)
6) occasionally anoxic conditions prevail; the water is often coloured and **extremely turbid** due to suspended matter from sewage discharges and drifting tufts of sewage bacteria; **fine substrates** in deeper areas are nearly black throughout, sludgy, and occasionally re-lease a clearly detectable odour of **hydrogen sulphide**; in lentic areas even the **upper sides of stones** are **black**
7) sludge deposits in lentic areas are densely colonized by chironomids of the genus ***Chironomus*** (bloodworms), tolerant Tanypodinae, and **tubificid worms**; in cases with extremely low oxygen supply nearly no higher life is possible with the exception of air breathing animals (**breathing-tubes**, e.g. **rat-tailed maggot**)
8) compared to saprobic river quality class III the algal cover is reduced in both qualitative and quantitative terms; in lotic sites blooms of **filamentous sewage-bacteria** occur (typical "sewage fungus-development"), and **sulphur bacteria** can form striking macroscopic layers
9) the micro-benthos mainly consists of **ciliates**, **flagellates** and **bacteria**, which often show mass development
10) the existence of a self-sustaining and balanced fish population is no longer possible

Saprobic river quality class VI – no higher life

1) no or nearly no higher life except air-breathing animals (e.g. rat-tailed maggots) due to lack of oxygen.

4.6. Explanations of the decision support table (tool 2)

The decision support table for estimating the river quality is based on:

- *an assessment of* ***sensory features***
- ***periphyton****, bacteria & fungi*
- *macro-invertebrate features*

The decision support table is designed to visualise the personal impression of the surveyor. Each criterion valid for the river stretch under investigation is ticked in the decision support table. Criterions can either be given as words, in percentage or different numbers of points. Completion of the decision support table is explained in chapter 4.6.7.

4.6.1. Sensory features

Non-natural turbidity

Should be ticked, if the river water is turbid (not in accordance with type-specific reference conditions).

natural turbidity *may be caused by:*	***non-natural turbidity*** *may be caused by:*
- *floods, landslips*	- *sewage inflow*
- *snow-fed rivers*	- *construction work*
- *glacier-fed rivers*	- *suspended matter*
	- *pesticide application*
	- *others*

Example: Non-natural turbidity

Figure 2: Ngakhu Chhu, Phunaka, Bhutan

Figure 3: Barandu, NWFP, Buner, Pakistan

Colour

Should be ticked, if the colour of the river is not in accordance with type-specific reference conditions.

***Non type-specific colour** may occur due to e. g. sewage inflow (e.g. direct releases of a carpet factory).*

Example: Colour

Figure 4: Phirke Khola, Kaski, Nepal

Foam

Should be ticked, if foam is detected at the river surface which is not in accordance with type-specific reference conditions. Foam is an indicator of pollution by detergents.

***natural foam** may occur in and may be caused by:*

- *peat waters, bogs, humic acids*
- *rotten pollen*
- *rotten exuviae (e.g. insect skins)*

Example: Foam

Figure 5:unknown river in Pakistan

Odour (water)

Should be ticked, if the water smells not in accordance with the type-specific reference conditions. Some examples of water smells are given below:

smell	caused by
scentless	chemical
aromatic	hydrogen sulphide
spicy	carbon dioxide
earthy	chlorine
turfy	mineral oil
musty	petrol, benzene
moldy	ammonia
rotten	phenol
oily	chlorinated phenol
fishy	tar
cesspool-like	silage
faecal	other

Wastes

Should be ticked, if there are wastes like plastics, bottles etc.. Any other civilisation born material indicates anthropogenic pressure and should be noted.

Example: Waste disposal

Figure 6: Soan, Soan Bridge, Punjab, Islamabad-Pindi, Pakistan

4.6.2. Ferro-sulphide reduction

Ferro-sulphide reduction phenomena are caused by oxygen depletion and are valuable descriptors of river quality classes (RQC). They do not occur in RQC I, but small phenomena may already arise in RQC II. As oxygen content is linked to water current ferro-sulphide reduction phenomena are most likely found in lentic zones and muddy areas or on/beneath stones at bank faces and margins. The dimension of reduction phenomena indicates the degree of oxygen depletion (for allocation of ferro-sulphide reductions to river quality classes see also chapter 4.5).

The occurrence of ferro-sulfide reductions needs to be observed in muddy and stony habitats in three different flow ranges:

ferro-sulphide reduction – lentic zones (current < 0,25 m/s)

- *mud reduced but with aerobic surface*
- *mud reduced but with anaerobic surface*
- *lower surface of stones (% cover black dots)*
- *upper & lower surfaces of stones (-"-)*

ferro-sulphide reduction – lotic zones (current 0,25 - 0,75 m/s)

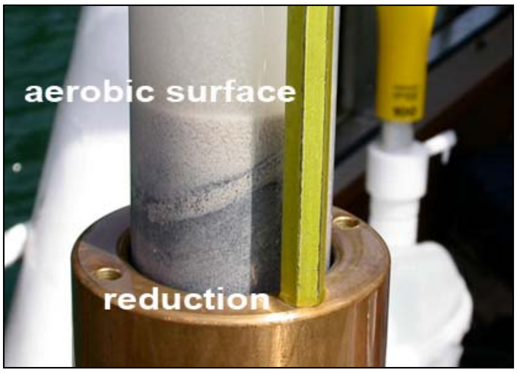
- *mud reduced but with aerobic surface*
- *mud reduced but with anaerobic surface*
- *lower surface of stones (% cover black dots)*
- *upper & lower surfaces of stones (-"-)*

ferro-sulphide reduction – lotic zones (current > 0,75 m/s)

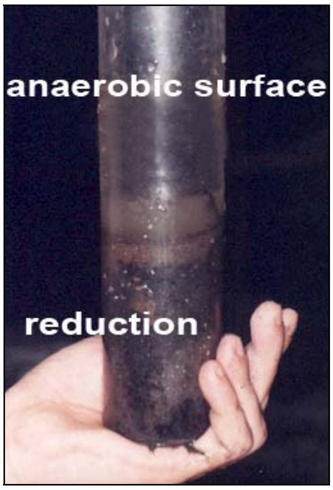
- *lower surface of stones (% cover black dots)*
- *upper & lower surfaces of stones (-"-)*

Examples: Ferro-sulphide reduction – lentic zones

mud reduced with aerobic surface



mud reduced with anaerobic surface



Examples: Ferro-sulphide reduction- lower surface of stones

< 25% cover black dots

25-75% cover black dots

75-100% cover black dots

4.6.3. Bacteria, fungi, periphyton

Bacteria and sewage fungi show mass occurrence in RQC IV and V. They normally cover hard substrates like stones or macrophytes as well as benthic invertebrates.

Periphyton and filamentous green algae reveal the trophic status of a water body with a maximum occurrence of filamentous algae mainly in river quality class III. The degree of coverage as well as the dimension of periphyton are good indicators of nutrient content. The following indicators that must be visible to the naked eyes are used: sewage fungi & bacteria, sulphur bacteria, algae (=periphyton) in thin layers, % periphyton in significant layers, filamentous green algae.

Examples: fungi, bacteria

sewage fungi

sulphur bacteria

Examples: periphyton

thin layers

significant layers

Examples: filamentous algae

filaments, tufts

large tufts

4.6.4. Benthic macro-invertebrates

Taxonomy can cover any level, depending on the possibility to determine a specimen in the field, but must be done consistently among river types.

A site has to be classified with respect to river type specific reference conditions. The following indicator features are used: species richness, abundance, number of sensitive species, number of tolerant species, e.g. “red” chironomids, air-breathing animals (e.g. rat-tail maggots), Oligochaeta/Tubificidae.

Focus is on richness and tolerance/intolerance measure. The score value of benthic macroinvertebrates is given in the HKHbios scoring list (see Table 4). High values, e.g. 9 or 10 indicate very good river quality; low values e.g. 1 or 2 indicate bad river quality.

Examples for clean water species

Peltoperlidae Gen. sp.
HKH-biotic score:10 (Mountains)

Helicopsyche sp.
HKH-biotic score:10 (Mountains & Lowlands)

Iron sp.
HKH-biotic score:9 (Mountains & Lowlands)

Perlidae Gen. sp.
HKH-biotic score:8 (Mountains & Lowlands)

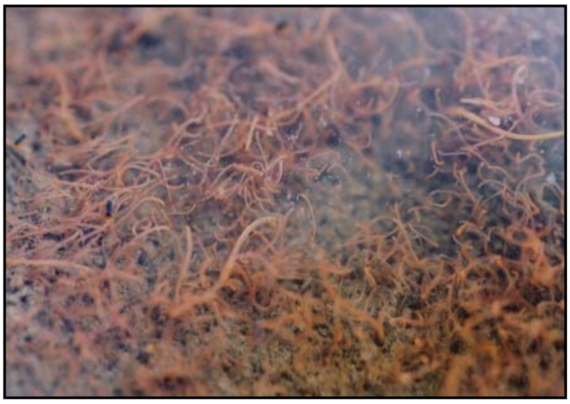
Examples for tolerant species

Chironomids “red”
HKH-biotic score:1 (Mountains & Lowlands)

rat-tailed maggots
HKH-biotic score:1 (Mountains & Lowlands)

Psychodidae Gen. sp. white
(Psychoda sp.)
HKH-biotic score:1 (Mountains & Lowlands)

Tubificidae Gen. sp. (worms)
HKH-biotic score:1 (Mountains & Lowlands)



4.6.5. Screening Protocol (Mountains) for assessing the river quality of streams in the ASSESS-HKH region

Screening Protocol (Mountains) for assessing the river quality of streams in the ASSESS-HKH region						
Decision support table	river quality classes					
Multiple choices possible	I	II	III	IV	V	VI
Sensory features	To be ticked/counted if not in accordance with natural river type					
Non natural turbidity, Suspended solids			+	+	++	
Non natural colour		+	+	+	++	
Foam		+	+	+	+	
Odour (water)		+	++	++	++	
Waste dumping		+	+	+	+	
Ferro-sulphide reduction – (water velocity< 0,25 m/s)	-					
Mud reduced but with aerobic surface		+	+++	++		
Mud reduced but with anaerobic surface				++	+++	+++
Lower surface of stones (% cover black dots)		< 25 %	25-75 %	75-100 %	100%	100%
Upper & lower surfaces of stones				+	++	++
Ferro-sulphide reduction – (water vel.) 0,25-0,75 m/s)	-	-				
Mud reduced but with aerobic surface			+	+++	+	+
Mud reduced but with anaerobic surface				+	++	++
Lower surface of stones (% cover black dots)			< 50 %	50-100 %	100%	100%
Upper & lower surfaces of stones					+++	+++
Ferro-sulphide reduction – (water velocity > 0,75 m/s)						
Lower surface of stones (% cover black dots)			< 25 %	25-50 %	50-100 %	50-100 %
Upper & lower surfaces of stones					+++	+++
Bacteria, fungi, periphyton						
Sewage fungi & bacteria (visible to the naked eyes)	(-)	(-)	few	medium	many +++	many +++
Sulphur bacteria (visible to the naked eyes)	(-)	(-)	(-)	+	+++	+++
Stones with algal vegetation (periphyton) in thin layers	++	++				
% of thick, significant layers of algae	< 25 %	25-75 %	75-100 %	75-100 %	few	
Filamentous green algae	none to few	filaments, tufts	large tufts	(large) tufts	+	
Benthic macro-invertebrates						
Species richness	medium/high	(very) high	medium	few	very few	+++none**
Dominance of very sensitive organisms (8 to 10)*)	+++					
Dominance of sensitive organisms (6 to 8)*)	+	+++	+			
Dominance of medium tolerant organisms (4 to 6)*)			+++	+		
Dominance of tolerant organisms (3 to 4)*)			+	+++	+	
Dominance of extremely tolerant organisms (1 to 2)*)					+++	
Rhithrogena spp.	+++	++				
Perlidae	++	+				
Plecoptera	++	+				
Euphaeidae	++	+				
Stenopsyche spp.	++	+	+			
Rhyacophilidae	+++	++	+			
Caenis spp.	+	++	++			
Heptageniidae	++	++	+			
Psephenidae	++	+++	+			
Glossosomatidae	++	+++	+			
Sphaeriidae	+	+	++			
Simuliidae	+	++	++			
Psychomyiidae	+	+++	++			
Tabanidae	+	++	+++			
Potamidae	+	++	++	+		
Libellulidae / Corduliidae		+	+++	++		
Hydropsychidae (medium to many)		+	+++	+		
Planorbidae		++	+++	+		
Lymnaeidae		+	+++	++		
Physa spp.			+	++		
Leeches (more than naturally occurring)	-	-	+	+++	+	
Chironomids with red colour		very few	few	medium	+++many	none
Bezzia-Group				+	++	
Psychodidae white				+	+++	
Air-breathing animals, e. g. rat-tail maggots					+++	+++
Oligochaeta / Tubificidae (mud-worms)	0 to very few	few	few/ medium	medium/ many	many	none
Sum of columns						
*) check scores in the HKHbios list on the back page **) if only airbreathing organisms						

4.6.6. Screening Protocol (Lowlands) for assessing the river quality of streams in the ASSESS-HKH region

Screening Protocol (Lowlands) for assessing the river quality of streams in the ASSESS-HKH region						
Decision support table	river quality classes					
Multiple choices possible	I	II	III	IV	V	VI
Sensory features	To be ticked/counted if not in accordance with natural river type					
Non natural turbidity, Suspended solids			+	+	++	
Non natural colour		+	+	+	++	
Foam		+	+	+	+	
Odour (water)		+	++	++	++	
Waste dumping		+	+	+	+	
Ferro-sulphide reduction – (water velocity< 0,25 m/s)	-					
Mud reduced but with aerobic surface		+	+++	++		
Mud reduced but with anaerobic surface				++	+++	+++
Lower surface of stones (% cover black dots)		< 25 %	25-75 %	75-100 %	100%	100%
Upper & lower surfaces of stones				+	++	++
Ferro-sulphide reduction – (water vel.) 0,25-0,75 m/s)	-	-				
Mud reduced but with aerobic surface			+	+++	+	+
Mud reduced but with anaerobic surface				+	++	++
Lower surface of stones (% cover black dots)			< 50 %	50-100 %	100%	100%
Upper & lower surfaces of stones					+++	+++
Ferro-sulphide reduction – (water velocity > 0,75 m/s)						
Lower surface of stones (% cover black dots)			< 25 %	25-50 %	50-100 %	50-100 %
Upper & lower surfaces of stones					+++	+++
Bacteria, fungi, periphyton						
Sewage fungi & bacteria (visible to the naked eyes)	(-)	(-)	few	medium	many +++	many +++
Sulphur bacteria (visible to the naked eyes)	(-)	(-)	(-)	+	+++	+++
Stones with algal vegetation (periphyton) in thin layers	++	++				
% of thick, significant layers of algae	< 25 %	25-75 %	75-100 %	75-100 %	few	
Filamentous green algae	none to few	filaments,	large tufts	(large)	+	
Benthic macro-invertebrates						
Species richness	medium/high	(very) high	medium	few	very few	+++none**
Dominance of very sensitive organisms (8 to 10)*)	+++					
Dominance of sensitive organisms (6 to 8)*)	+	+++	+			
Dominance of medium tolerant organisms (4 to 6)*)			+++	+		
Dominance of tolerant organisms (3 to 4)*)			+	+++	+	
Dominance of extremely tolerant organisms (1 to 2)*)					+++	
Heptageniidae	++					
Brachycercus spp.	++					
Anagenesia (Palingeniidae) spp.	++					
Ephemera spp.	++					
Elmidae	+++	++				
Hydraenidae	+++	++				
Prawn (Palaemonidae, Atyidae)	+	++	++			
Planorbidae	+	++	+			
Pleuroceridae (Brotia)	+	++	+			
Thiaridae	+	++	++	+		
Vivparidae	+	++	++	+		
Amblemidae	+	++	++			
Unionidae		++	++	+		
Psychodidae (white)				+	+	
Baetidae different types	3 or more	2 or 3	1 or 2	1		
Chironomids with red colour		single	few	medium	+++many	none
Air-breathing animals, e. g. rat-tail maggots					+++	+++
Tubificidae (mud-worms)	single	few	few/ medium	medium/ many	many	
Sum of columns						
*) check scores in the HKHbios list on the back page **) if only airbreathing organisms						

4.6.7. How to use the table

Score only those criteria that are existent without doubt. The assessment should be oriented on river type specific parameters and on criteria that may potentially occur at the sampling site (e.g. do not evaluate the absence of "red" chironomids, if their habitat (mud, sand) is missing). Each appropriate observation of the listed criteria has to be scored in the **EVALUATION TABLE** (Table 2 & Table 3).

- ***+** or **-*** *accounts for 1 point; "+" means criterion was observed and "-" means criterion was not observed*
- **++** *accounts for 2 points*
- **+++** *accounts for 3 points*
- ***words** (e.g. high, few, many) or percentages (e.g. 25%) accounts for 2 scores each (also in the case of verbal double indications like "few/medium").*

If + or – occur in a row all + or - within the row have to be scored adequately. If "words" or "percentages" occur in a row only those words or figures that meet the observation (e.g. "none to few") have to be scored in that row. For the classification of river quality classes the entries of each column are summed up. The majority of points per column "river quality class" is decisive for the estimation of the river quality class under study.

Example 1: Sensory features: river water shows unnatural turbidity

EVALUATION TABLE - ORGANIC POLLUTION	**river quality classes**					
Multiple choices possible	**I**	**II**	**III**	**IV**	**V**	**VI**
Non minerogenic turbidity			1	1	2	

Example 2: Ferro-sulphide reduction: lentic zones: lower surface of stones covered 20 % with black dots

EVALUATION TABLE - ORGANIC POLLUTION	**river quality classes**					
Multiple choices possible	**I**	**II**	**III**	**IV**	**V**	**VI**
Lower surface of stones (% cover black dots)		2				

Example 3: Bacteria , fungi, periphyton: filamentous green algae: none to few

EVALUATION TABLE - ORGANIC POLLUTION	**river quality classes**					
Multiple choices possible	**I**	**II**	**III**	**IV**	**V**	**VI**
Filamentous green algae	2					

Table 2: Evaluation table (Mountains) for assessing the river quality of streams in the ASSESS-HKH region

Evaluation table (Mountains) for assessing the river quality of streams in the ASSESS-HKH region						
Evaluation table	river quality classes					
Multiple choices possible	I	II	III	IV	V	VI
Sensory features						
Non natural turbidity, Suspended solids						
Non natural colour						
Foam						
Odour (water)						
Waste dumping						
Ferro-sulphide reduction – (water velocity< 0,25 m/s)						
Mud reduced but with aerobic surface						
Mud reduced but with anaerobic surface						
Lower surface of stones (% cover black dots)						
Upper & lower surfaces of stones						
Ferro-sulphide reduction – (water vel.) 0,25-0,75 m/s)						
Mud reduced but with aerobic surface						
Mud reduced but with anaerobic surface						
Lower surface of stones (% cover black dots)						
Upper & lower surfaces of stones						
Ferro-sulphide reduction – (water velocity > 0,75 m/s)						
Lower surface of stones (% cover black dots)						
Upper & lower surfaces of stones						
Bacteria, fungi, periphyton						
Sewage fungi & bacteria (visible to the naked eyes)						
Sulphur bacteria (visible to the naked eyes)						
Stones with algal vegetation (periphyton) in thin layers						
% of thick, significant layers of algae						
Filamentous green algae						
Benthic macro-invertebrates						
Species richness						
Dominance of very sensitive organisms (8 to 10)*)						
Dominance of sensitive organisms (6 to 8)*)						
Dominance of medium tolerant organisms (4 to 6)*)						
Dominance of tolerant organisms (3 to 4)*)						
Dominance of extremely tolerant organisms (1 to 2)*)						
Rhithrogena spp.						
Perlidae						
Plecoptera						
Euphaeidae						
Stenopsyche spp.						
Rhyacophilidae						
Caenis spp.						
Heptageniidae						
Psephenidae						
Glossosomatidae						
Sphaeriidae						
Simuliidae						
Psychomyiidae						
Tabanidae						
Potamidae						
Libellulidae / Corduliidae						
Hydropsychidae (medium to many)						
Planorbidae						
Lymnaeidae						
Physa spp.						
Leeches (more than naturally occurring)						
Chironomids with red colour						
Bezzia-Group						
Psychodidae white						
Air-breathing animals, e. g. rat-tail maggots						
Oligochaeta / Tubificidae (mud-worms)						
Sum of columns						
*) check scores in the HKHbios list on the back page **) if only airbreathing organisms						

Table 3: Evaluation table (Lowlands) for assessing the river quality of streams in the ASSESS-HKH region

Evaluation table (Lowlands) for assessing the river quality of streams in the ASSESS-HKH region						
Evaluation table	river quality classes					
Multiple choices possible	I	II	III	IV	V	VI
Sensory features						
Non natural turbidity, Suspended solids						
Non natural colour						
Foam						
Odour (water)						
Waste dumping						
Ferro-sulphide reduction – (water velocity< 0,25 m/s)						
Mud reduced but with aerobic surface						
Mud reduced but with anaerobic surface						
Lower surface of stones (% cover black dots)						
Upper & lower surfaces of stones						
Ferro-sulphide reduction – (water vel.) 0,25-0,75 m/s)						
Mud reduced but with aerobic surface						
Mud reduced but with anaerobic surface						
Lower surface of stones (% cover black dots)						
Upper & lower surfaces of stones						
Ferro-sulphide reduction – (water velocity > 0,75 m/s)						
Lower surface of stones (% cover black dots)						
Upper & lower surfaces of stones						
Bacteria, fungi, periphyton						
Sewage fungi & bacteria (visible to the naked eyes)						
Sulphur bacteria (visible to the naked eyes)						
Stones with algal vegetation (periphyton) in thin layers						
% of thick, significant layers of algae						
Filamentous green algae						
Benthic macro-invertebrates						
Species richness						
Dominance of very sensitive organisms (8 to 10)*)						
Dominance of sensitive organisms (6 to 8)*)						
Dominance of medium tolerant organisms (4 to 6)*)						
Dominance of tolerant organisms (3 to 4)*)						
Dominance of extremely tolerant organisms (1 to 2)*)						
Heptageniidae						
Brachycercus spp.						
Anagenesia (Palingeniidae) spp.						
Ephemera spp.						
Elmidae						
Hydraenidae						
Prawn (Palaemonidae, Atyidae)						
Planorbidae						
Pleuroceridae (Brotia)						
Thiaridae						
Vivparidae						
Amblemidae						
Unionidae						
Psychodidae (white)						
Baetidae different types						
Chironomids with red colour						
Air-breathing animals, e. g. rat-tail maggots						
Tubificidae (mud-worms)						
Sum of columns						
*) check scores in the HKHbios list on the back page **) if only airbreathing organisms						

4.6.8. How to fill in the HKHbios scoring list

The estimated abundance of each taxon needs to be filled in the column “Abd.” of the HKHbios scoring list (see Table 4) on the backside of the Screening Protocol. For scheme of estimation see chapter 3.4.2.

The HKHbios scoring list is divided in a list for mountainous areas (Ecoregion IM0301 - Himalayan Subtropical Pine Forests, IM0401- Eastern Himalayan Broadleaf Forests, IM0403 - Western Himalayan Broadleaf Forests, IM0166 - Upper Gangetic Plains Moist Deciduous Forests) and lowland regions (IM0120 - Lower Gangetic Plains Moist Deciduous Forests). Depending on the ecoregion chose either column “mountains” or “lowlands” for indicating the dominance of sensitive/tolerant organisms in Screening Protocol.

Taxa which are not in the HKHbios scoring list or taxa which are given temporary names in the field can be filled in the table “additional taxa”.
A cross in the column “BI” (Binocular) in the HKHbios scoring list indicates the use of a binocular for a reliable identification of an organism.

The distribution of habitats (mineral and organic habitats) needs to be filled in the table “habitats” before sampling. Give only those habitats, which share at least 5 % coverage of the sampling section.

Table 4: HKHbios scoring list

Taxon	Mountain	Lowland	Abd.	BI
SPONGILLIDAE	7	7		
DUGESIIDAE	4	4		x
Nematoda	2	2		
Pila	6	6		
ASSIMINEIDAE		6		x
BITHYNIIDAE	5	5		x
LYMNAEIDAE	4	4		
NERITIDAE		8		
Onchidium		8		
PHYSIDAE	2	2		
PLANORBIDAE	4	6		
Gyraulus	4	6		
Gyraulus	4	6		
PLEUROCERIDAE	6	6		x
POMATIOPSIDAE	8	8		
STENOTHYRIDAE	8	8		
SUCCINEIDAE	5	5		
THIARIDAE	4	4		
VIVIPARIDAE	6	6		
AMBLEMIDAE	7			x
ARCIDAE		8		x
CORBICULIDAE	4	4		
PSAMMOBIIDAE		8		
SPHAERIIDAE	6	6		
Pisidium	7	7		
UNIONIDAE	6	6		
Lamellidens	5	5		
NEPHTHYDAE	5	5		
NEREIDIDAE	6	6		
LUMBRICIDAE	5	5		x
MEGASCOLECIDAE	6	6		x
MICROCHAETIDAE	7	7		
NAIDIDAE	3	3		x
Oligochaeta	2	2		
TUBIFICIDAE	1	1		x
GLOSSIPHONIIDAE	4	4		
SALIFIDAE	3	3		
ATYIDAE	6	6		x
CIROLANIDAE	8	8		x
CYMOTHOIDAE	5	5		x
GAMMARIDAE	8			
HYMENOSOMATIDAE		8		x
MYSIDAE	6	6		x
NEONIPHARGIDAE		10		
PALAEMONIDAE	6	6		
PARATHELPHUSIDAE	6	6		x
POTAMIDAE	7	7		
Himalayapotamon	7			
TALITRIDAE		8		
AMELETIDAE	10	10		x
Acentrella	8			x
Baetiella	8	8		x
Baetis		9		
Cloeoninae	5	5		x
Platybaetis	8	8		x
CAENIDAE	7	7		
Brachycercus		10		
Caenis	7	7		
EPHEMERELLIDAE	7	7		
Cincticostella	8	10		
Drunella	9			
Ephacerella	8	8		x
Torleya	7	7		x
Ephemera	8	10		

Taxon	Mountain	Lowland	Abd.	BI
Epiophlebia	10	10		
HEPTAGENIIDAE	8	10		
cf. Notacanthurus	8			x
Cinygmula	9			x
Ecdyonurus	8	10		
Epeorus	8	10		
Iron	9	9		
Rhithrogena	9	10		x
ISONYCHIIDAE	10	10		
LEPTOPHLEBIIDAE	7	7		
Euthraulus	8	10		x
NEOEPHEMERIDAE	10			
Potamanthellus	10	10		
PALINGENIIDAE	10	10		
POTAMANTHIDAE	10	10		
Prosopistoma	10	10		
AESHNIDAE	7	7		
AMPHIPTERYGIDAE	7	7		x
CALOPTERYGIDAE	8	8		
CHLOROCYPHIDAE	6	6		
COENAGRIONIDAE	5	5		x
CORDULEGASTERIDAE	10	10		
CORDULIIDAE	5	5		x
Macromia	7	7		
EUPHAEIDAE	9	9		
LESTIDAE	7	7		
LIBELLULIDAE	6	6		x
MEGAPODAGRIONIDAE	10	10		
PLATYCNEMIDAE		7		
PROTONEURIDAE	5	5		
SYNLESTIDAE	9	9		
CAPNIIDAE	10			x
CHLOROPERLIDAE	9	9		
LEUCTRIDAE	10	10		x
NEMOURIDAE	9	9		
Amphinemura	9			x
Indonemoura	10	10		x
Mesonemoura	9	9		x
Nemoura	9			x
PELTOPERLIDAE	10			
PERLIDAE	8	8		
PERLODIDAE	9			
TAENIOPTERYGIDAE	10			
Aphelocheirus	7	9		
BELOSTOMATIDAE	6			
CORIXIDAE	6			
Micronecta	5			
Sigara	6	6		
HEBRIDAE	7	7		
MESOVELIIDAE	6	6		x
NAUCORIDAE	7	7		
NEPIDAE	6	6		
NOTONECTIDAE	3	3		
PLEIDAE	4			
VELIIDAE	7			
CORYDALIDAE	7	7		
SISYRIDAE	7	7		
CHRYSOMELIDAE	8	8		
CURCULIONIDAE	5			x
DRYOPIDAE	5	5		x
DYTISCIDAE	5			
ELMIDAE	8	8		
EULICHADIDAE	8	8		
GYRINIDAE	6	7		
HALIPLIDAE	6	6		x
HYDRAENIDAE	7	8		
HYDROPHILIDAE	6			

Taxon	Mountain	Lowland	Abd.	BI
NOTERIDAE	4	4		
PSEPHENIDAE	8	8		
Eubrianacinae	9	9		x
Eubriinae	8	8		
Psephenoidinae	8	8		x
SCIRTIDAE	8	8		
APATANIIDAE	9	9		x
BRACHYCENTRIDAE	8	8		x
Brachycentrus	6	6		
Micrasema	10	10		
CALAMOCERATIDAE	10			
DIPSEUDOPSIDAE	5	5		
Ecnomus	6	6		x
GLOSSOSOMATIDAE	8	8		
Agapetinae	9	9		x
Glossosomatinae	8	8		x
GOERIDAE	9	9		
HELICOPSYCHIDAE	10	10		
HYDROBIOSIDAE	10			
HYDROPSYCHIDAE	7	9		
Cheumatopsyche	7			x
Diplectrona	8			x
HYDROPTILIDAE	7	9		
LEPIDOSTOMATIDAE	8	8		
LIMNEPHILIDAE	7			
Limnocentropus	9			
ODONTOCERIDAE	7	7		
Marilia	7	7		
PHILOPOTAMIDAE	7	7		
PHRYGANEIDAE	8			
Pseudoneureclipsis	8	8		
POLYCENTROPODIDAE	7			
PSYCHOMYIIDAE	7	7		
RHYACOPHILIDAE	8	8		x
Rhyacophila	8			
Himalopsyche	9			
Stenopsyche	8	8		
UENOIDAE	10	10		
PYRALIDAE	7	7		
ATHERICIDAE	9	9		
BLEPHARICERIDAE	10			
Ceratopogonidae type	2	2		
CHIRONOMIDAE red	1	1		
Diamesini	7	8		x
Orthocladiinae		8		x
Tanytarsini		4		x
CULICIDAE	2			
DEUTEROPHLEBIIDAE	8	8		
DIXIDAE	9			
DOLICHOPODIDAE	2	2		
EMPIDIDAE	8	8		
EPHYDRIDAE	1	1		
LIMONIIDAE	8	8		
cf. Antocha	8	8		
MUSCIDAE	1	1		x
PEDICIIDAE	9	8		
cf. Dicranota	8	8		
PSYCHODIDAE black	6	6		
PSYCHODIDAE white =	1	1		
RHAGIONIDAE	9	9		
SCIARIDAE	6	6		x
SCIOMYZIDAE	8	8		
SIMULIIDAE	7	7		
SYRPHIDAE	1	1		
TABANIDAE	6	7		
TIPULIDAE	7	8		
LOPHOPODIDAE	7	7		x

Taxon	Mountain	Lowland	Abd.	BI
PLUMATELLIDAE	7	7		

Additional Taxa		Abd.

Habitats	%
Mineral habitas	
Boulder & Bedrock (Megalithal)	
Cobbles (Makrolithal)	
Stones (Mesolithal)	
Pebbles (Mikrolithal)	
Gravel (Akal)	
Sand (Psammal)	
Sandy mud (Psammopelal)	
Mud (Pelal)	
Clay (Argillal)	
Organic habitats	
Micro algae	
Submerged macrophytes	
Emerged macrophytes	
Living terrest. Plants	
Wood	
CPOM	
FPOM	
Debris	
Sewage bacteria	

4.7. Test of Screening Method for River Damming sites

The following examples show the applicability of the Screening Method for the assessment of the pressure “weirs or dams”. The river quality status of each site was estimated with the new version of the Screening Protocol and was compared with the result of the former pre-classification based on the Pre-classification Guidance Manual (Feld et al 2005).

Example 1: Nepal - Ghatte Khola - Dam at Sukaura (Upstream)

Narrative description:

The water is clear; there are no reduction phenomena. The substrate cover consists of significant layers of algae (25-75 % coverage), also few filaments of green algae are abundant. The species richness is medium with a dominance of sensitive organisms (HKHbios 6 to 8).

- Pre-classification based on Pre-classification Guidance Manual (Feld et al 2005):
  river quality class: II
- Result of the Screening Protocol - Version Nov 07:
  river quality class: II

Figure 7: N03GH023: Nepal - Ghatte Khola - Dam at Sukaura (Upstream)

Example 2: India - Kosi - u/s weir at Dadymkhola village (Almora)

Narrative description:

The water is slightly turbid; there are no reduction phenomena. The substrate cover consists of significant layers of algae (<25% coverage) and only few filaments of green algae. The species richness is medium with a dominance of medium tolerant organisms (HKHbios 4 to 6).

- Pre-classification based on Pre-classification Guidance Manual (Feld et al 2005):
  river quality class: III
- Result of the Screening Protocol - Version Nov 07:
  river quality class: III

Figure 8: I02KO151: India - Kosi - u/s weir at Dadymkhola village (Almora)

Example 3: Nepal - Bagmati Canal - Malahi, upstream of barrage

Narrative description:

The water is turbid with a certain brownish colour; the finer sediments are reduced but with aerobic surface. Black reduction spots (ferro-sulphide) on the lower surface of stones cover bigger areas (50-100%). Filamentous algae (tufts) grow in high abundances. The species richness is medium with a dominance of tolerant organisms (HKHbios 2 to 4).

- Pre-classification based on Pre-classification Guidance Manual (Feld et al 2005):
  river quality class: IV
- Result of the Screening Protocol - Version Nov 07:
  river quality class: IV

Figure 9: N01BG011: Nepal - Bagmati Canal - Malahi, upstream of barrage

5. HKHbios - score bioassessment method

5.1. Introduction

The quality assessment of surface water based on biological methods started about 150 years ago. Kolenati (1848), Hassal (1850) and Cohn (1853) observed that organisms that occur in polluted water are different from organisms that occur in clean water. Since that, hundreds of methods for biological river quality assessment have been developed (e.g. Sládecék 1973a; 1973b; Pittwell 1976; Persoone and De Pauw 1979; Illies and Schmitz 1980; Rosenberg and Resh 1992; De Pauw and Hawkes 1993; Davis and Simon 1995, Sharma & Moog 1996). For further information see also http://starwp3.eu-star.at.

The formulas of biotic indices are based on are closely related to saprobic indices (e.g. Chutter 1972; Hilsenhoff 1977; Tolkamp & Gardeniers 1977; Gonzalez del Tanago and Garcia Jalon 1984; Lang et al. 1989). The Trent biotic index (Woodiwiss 1964) is the origin of standard table based biotic indices (Sharma & Moog 1996). All other indices such as, Graham's index (Graham 1965), Indices Biotiques (Verneaux 1968), Biotic Index (Flanagen and Toner 1972), Danish Biotic Index (Andersen et al. 1984) are modifications of the Trent biotic index. Later, Trent Biotic Index was extended to provide a range of 0-15 in place of 0-10 as the Extended biotic index (Woodiwiss 1978). Spanish modification (Prat et al. 1983) and Italian modification (Ghetti 1986) are based on this Extended Trent biotic index. The score methods were originally differentiated from index methods for involving abundance in calculation (De Pauw and Hawkes 1993). Although this differentiation is no more valid, the calculation procedure of score and index methods are quite different. The origin of all score methods is the Chandler's Biotic Score (Chandler 1970).

5.2. Fundamentals of Biotic Indices

Biotic Indices indicate the ecological status of waterbodies based on the study of the biota. Such score-based assessment methods are based on higher taxonomic bio-indication units (genus, family, order) that need only simple data processing facility and promise a quick, less expensive and easy way of making judgments on surface river quality.

As freshwater macroinvertebrates require various physico-chemical conditions (e.g. **dissolved oxygen)** in stream water as well as **specific microhabitats** to survive and to build sustainable populations, the assemblage of species reflects the overall condition of a given site. The number and type of taxa provides an indication of the overall ecological status. Benthic invertebrate taxa are scored according to their sensitivity to stressors. Taxa showing no distinct preferences are not scored.

5.3. Data basis

As described in deliverable 9 (Sharma et al. 2007), in all Asian partner countries sampling was performed in two different seasons (pre- and post monsoon). MHS samples were taken at each site and an elaborated site protocol was completed for each site in both seasons.

Overall, data from 390 MHS-samples were available from five Asian countries. All macro-invertebrates found have been identified to the best level possible. In Bangladesh 68 samples were taken from 34 sites from a single ecoregion (IMO120) divided by tidal and non-tidal influence. In Bhutan 34 samples were taken from 17 sites in the core ecoregion (IM0301), and other 41 samples from 21 sites in Eastern Himalayan Broadleaf Forest (IM0401). In India, sites from two different ecoregions were selected for sampling: (Upper) Gangetic Plains (IM0166), where 32 samples have been taken from 16 sites. In Core ecoregion (IM0301), 17 sites were selected and 34 samples taken. 4 samples were taken in an additional, neighbouring stream type. In Nepal, 17 sites each were selected from three different ecoregions (IM0301, IM0401, IM0120). Thus 102 samples were taken in total. In Pakistan, 41 sites were considered from two different ecoregions, twenty-four of which were from core ecoregion (IM0301) and seventeen from Western Himalayan Broadleaf Forest (IM0403). In total 82 samples were taken.

5.3.1. Pre-classification

For the development of a HKHbios scoring list an estimation of the river quality of sites was necessary to score taxa according to their distribution along river quality classes. Within the project, investigated sites were equally distributed among all five ecological status classes (high=reference, good, moderate, poor and bad) to strengthen the statistical analyses. To achieve this goal a pre-classification process (task 3.3) was used for the selection of the sampling sites to estimate the ecological status of the rivers before sampling. This pre-classification was based on the 'Guidance Manual for Pre-classifying the Ecological Status of HKH rivers' (Moog & Sharma 2005). The pre-classification considered three different stressor types: Organic pollution, river damming for water abstraction and regulation and effects of river engineering. Summarized, the following criteria were used to determine a pre-classification of the ecological status of sites:

Organic pollution:

- *a narrative description of the saprobic river quality classes and selected river quality criteria*
- *a rapid field decision support table to pre-classify the organic pollution status of a river which is based on an assessment of sensory features (non minerogenic turbidity, colour, foam, odour, wastes, ferro-sulphide reduction) and biotic features (bacteria, fungi, periphyton, benthic macro-invertebrates)*
- *chemical and microbiological parameters*

Water abstraction

- *composition of bed sediments and channel features upstream a weir*
- *the amount of residual water in the downstream river section*

Effects of river engineering

- ✓ *composition/alteration of bed sediments*
- ✓ *alteration of current (flow velocity)*
- ✓ *channel features*

5.3.2. Data storage and data management

All data were entered and stored by partners in the HKH-software ECODAT using the tool for data storage (HKHdip). All country specific databases were merged centrally and served as the database for the development of assessment methods and software. In addition to biotic data (taxa lists and related abundances) all site protocol data including ecoregions, river names, site names, dates of sampling, sample codes and pre-classification status are available.

5.4. Methods

5.4.1. Scoring

Within the HKHbios assessment system macroinvertebrates are ranked using a ten point scoring system. The **scores** reflect the sensitivity of taxa to organic pollution (oxygen depletion), chemical pollution as well as on hydromorphological deficits. Taxa with **high scores** indicate **high sensitivity** to stressors and taxa with **low scores** indicate **high tolerance** to stressors.

The procedure for assigning scores follows Sharma (1996), where in a first step a numerical procedure is applied and in a second step the final value is assigned by expert judgement:

a) Numerical procedure

The numerical procedure comprises the calculation of a so called “Guide Score”, obtained from precence/absence of the taxon determining the number of sites represented by the taxon in each pollutional class according to the ***pre-classification***.

Calculation of the guide score

The Guide Score is calculated on the basis of the distribution of taxa along different ecological quality classes. The calculation follows Sharma (1996), adapted to a 5 class system:

Guide Score = $S_I/S_{tot} \times 10 + S_{II}/S_{tot} \times 7.75 + S_{III}/S_{tot} \times 5.5 + S_{IV}/S_{tot} \times 3.25 + S_V/S_{tot}$

S_I, S_{II}, S_{III}, S_{IV}, S_V are the total numbers of sites representing the quality classes according to the pre-classification where a certain taxon was found,

S_{tot} *= total number of sites where a certain taxon was found and 2.25 is the score interval with 10 as maximum (5 pre-classified quality classes) as the scale ranges from 1 point to ten points. Taxa occurring only at pristine sites are assigned to be ranked as 10, taxa occurring only at heavily polluted sites with a score of 1. The interval between 1 and 10 is 9. This interval was subdivided into 5 equal classes, this means that 4 partitioning borders have to be set in between (9/4=2.25):*

1_______3.25_______5.5_______7.75_______10

b) Expert judgement

The expert judgement is based on

- autecological knowledge of benthic invertebrate species
- reference scores assigned by different authors (e.g. GRS-BIOS (Nesemann et al. 2007), NEPBIOS (Moog & Sharma 2005)
- range and distribution pattern of each taxon among quality classes (considering pre-classification and stressor gradients; example Figure 10)
- abundance of taxa within quality classes

Examples:

Family Elmidae

Guide Score: 7.9
Distribution among quality classes: the highest percentage of records was found under reference conditions, followed by quality classes 2 and 3, under poor and bad conditions hardly found (see Figure 10)
Final expert ranking 8

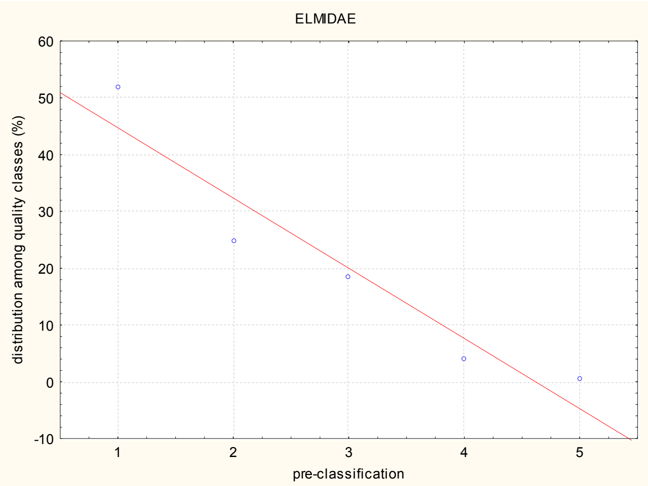


Figure 10: Distribution of Elmidae among different quality classes (pre-classification)

Family Bythiniidae

Guide Score 4.8
Distribution among quality classes: most frequently occuring in river quality 3, scarcely recorded under reference and bad conditions (see Figure 11)
Final expert ranking 5

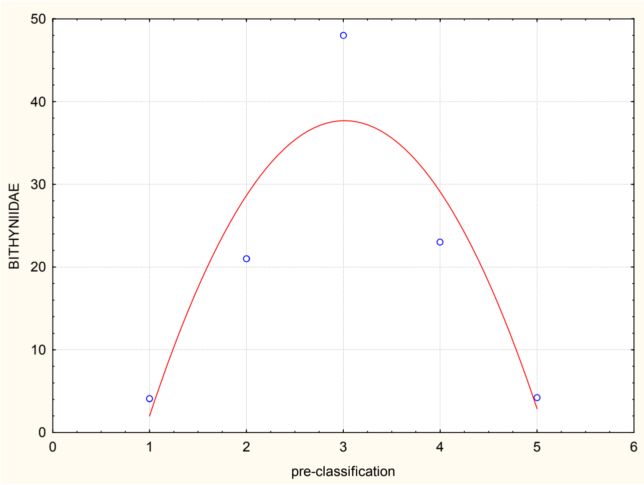


Figure 11: Distribution of family Bythiniidae among different quality classes (pre-classification)

Family Syrphidae

Guide Score 2.2
Distribution among quality classes: occurrence preferably under poor and bad conditions (Figure 12)
Final expert ranking 1

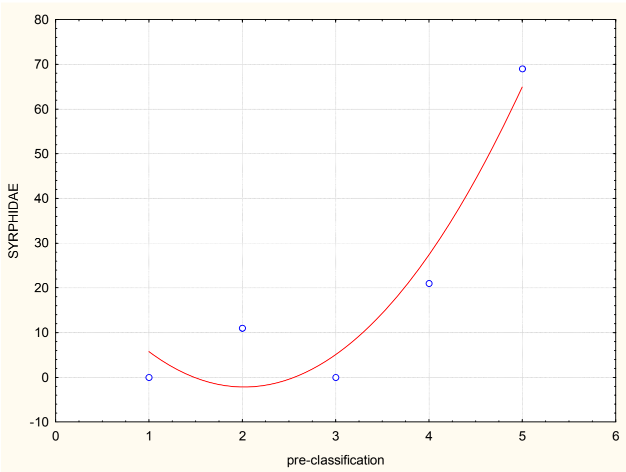


Figure 12: Distribution of family Syrphidae among different quality classes (pre-classification)

Taxa that showed no clear preferences were not scored. In some cases the assignment of scores on genus or family level was split for lowlands and mountains regarding dissimilar ecological requirements.

E.g. Heptageniidae Gen. sp. is scored 7 in mountain rivers, where it also occurring frequently in river quality class II but scored 10 for lowlands, where it actually is an indicator for river quality class I.

5.4.2. Calculation of the HKHbios

The method of HKHbios involves the determination of average score per taxon (ASPT) of the indicator species present in a sample of macro-invertebrates:

$$\mathrm{ASPT} = \frac{\sum_{1}^{i} Score_i}{number\ of\ taxa}$$

$Score_i$…score of taxon i.

During the development process also the weighting of selected taxa was tested. Only taxa that showed a clear preference to very good conditions (score 8-10) or to very bad conditions (score = 1) were considered, whereas a weight of 3 was assigned to strong indicators, a weight of 5 for very strong indicators.

Using different combinations of scores, HKHbios (=ASPT) was calculated and tested against the pre-classification. A first proposal of taxa rankings was sent to partners for comments and all comments and suggestions from partners were collected and included. HKHbios was re-calculated several times to select the best combination of scores. Additionally the effect of including **abundance** values and **weighting** of selected taxa was tested. The HKHbios was calculated and tested against the pre-classification for all ecoregions

- without abundance and weighting
- including abundance
- including weighting of taxa
- including abundance and weighting.

For the integration of abundance classes were used. The original abundance values were transferred into classes by applying the following scheme:

number of individuals	abundance class
1-10	1
11-100	2
101-1,000	3
1,001-10,000	4
> 10,000	5

Weighted average score per taxon ($ASPT_w$) was calculated as

$$\mathrm{ASPT_W} = \frac{\sum_{1}^{i} Score_i * Weight_i}{\sum_{1}^{i} Weight_i},$$

weighted average score per taxon including abundance classes ($ASPT_w$) was calculated as

$$ASPT_{WA} = \frac{\sum_{1}^{i} Score_i * Weight_i * abundance_i}{\sum_{1}^{i} Weight_i * abundance_i}.$$

5.5. Results

5.5.1. Taxa Scoring

Taxa scores as a result of the guide score calculations and expert judgement (including contributions and suggestions) from all partners were tested for calculating the HKHbios. Within an iterative process the best combination of scores was evaluated aiming to achieve the highest accuracy in discriminating different river quality classes (with respect to pre-classification) for each Ecoregion.

The resulting scoring list includes 199 taxa in total. It comprises of 2 taxa on class level, 139 taxa on family level, 4 taxa on subfamily level, 51 genera and 3 taxa on species level. For mountainous Ecoregions (IM0301, IM0401, M0166, IM0403) scores are available for 186 taxa and for lowlands (IM0120) 155 taxa are scored (see Table 6).

In a further step the effect of including abundance and weighting of selected taxa was tested and correlated with the pre-classification. The results show that the integration of abundance (classes) is not appropriate. Especially sites of very good quality are often ranked too low because medium and lower scored taxa - which are also appearing at this sites - in many cases show much higher abundance than high ranked taxa. Thus the HKHbios of pristine sites tends to result in values that are not significantly distinguishable from good and moderate ones.

Table 5: Spearman Rank Correlation of pre-classification vs. HKHbios. r…averages of correlation coefficients from single ecoregions.

HKHbios	r^2
no weight or abundance	***0.60***
including weight	***0.62***
including weight and abundance	***0.48***

The highest accuracy of discrimination was found for HKHbios calculation using only weightings for selected taxa (see Table 5). For mountainous areas (Ecoregion IM0301 - Himalayan Subtropical Pine Forests, IM0401- Eastern Himalayan Broadleaf Forests, IM0403 - Western Himalayan Broadleaf Forests, IM0166 - Upper Gangetic Plains Moist Deciduous Forests) 23 taxa were weighted (see Table 6). For the lowland regions (IM0120 - Lower Gangetic Plains Moist Deciduous Forests) only taxa with high rankings were weighted.

Table 6: HKHbios scoring list.

Taxon	Species	Mountain	Lowland	Weight
SPONGILLIDAE	Gen.sp.	7	7	1
DUGESIIDAE	Gen.sp.	4	4	1
NEMATODA	Gen.sp.	2	2	1
Pila	globosa	6	6	1
ASSIMINEIDAE	Gen.sp.		6	1
BITHYNIIDAE	Gen.sp.	5	5	1
LYMNAEIDAE	Gen.sp.	4	4	1
NERITIDAE	Gen.sp.		8	1
Onchidium	sp.		8	1
PHYSIDAE	Gen.sp.	2	2	1
PLANORBIDAE	Gen.sp.	4	6	1
Gyraulus	labiatus	4	6	1
Gyraulus	convexicusculus	4	6	1
PLEUROCERIDAE	Gen.sp.	6	6	1
POMATIOPSIDAE	Gen.sp.	8	8	1
STENOTHYRIDAE	Gen.sp.	8	8	1
SUCCINEIDAE	Gen.sp.	5	5	1
THIARIDAE	Gen.sp.	4	4	1
VIVIPARIDAE	Gen.sp.	6	6	1
AMBLEMIDAE	Gen.sp.	7		1
ARCIDAE	Gen.sp.		8	1
CORBICULIDAE	Gen.sp.	4	4	1
PSAMMOBIIDAE	Gen.sp.		8	1
SPHAERIIDAE	Gen.sp.	6	6	1
Pisidium	sp.	7	7	1
UNIONIDAE	Gen.sp.	6	6	1
Lamellidens	sp.	5	5	1
NEPHTHYDAE	Gen.sp.	5	5	1
NEREIDIDAE	Gen.sp.	6	6	1
LUMBRICIDAE	Gen.sp.	5	5	1
MEGASCOLECIDAE	Gen.sp.	6	6	1
MICROCHAETIDAE	Gen.sp.	7	7	1
NAIDIDAE	Gen.sp.	3	3	1
OLIGOCHAETA	Gen.sp.	2	2	1
TUBIFICIDAE	Gen.sp.	1	1	1
GLOSSIPHONIIDAE	Gen.sp.	4	4	1
SALIFIDAE	Gen.sp.	3	3	1
ATYIDAE	Gen.sp.	6	6	1
CIROLANIDAE	Gen.sp.	8	8	1
CYMOTHOIDAE	Gen.sp.	5	5	1
GAMMARIDAE	Gen.sp.	8		1
HYMENOSOMATIDAE	Gen.sp.		8	1
MYSIDAE	Gen.sp.	6	6	1
NEONIPHARGIDAE	Gen.sp.		10	1
PALAEMONIDAE	Gen.sp.	6	6	1
PARATHELPHUSIDAE	Gen.sp.	6	6	1
POTAMIDAE	Gen.sp.	7	7	1
Himalayapotamon	sp.	7		1
TALITRIDAE	Gen.sp.		8	1
AMELETIDAE	Gen.sp.	10	10	1
Acentrella	sp.	8		1
Baetiella	sp.	8	8	1

Taxon	Species	Mountain	Lowland	Weight
Baetis	sp.		9	1
Cloeoninae	Gen.sp.	5	5	1
Platybaetis	sp.	8	8	1
CAENIDAE	Gen.sp.	7	7	1
Brachycercus	sp.		10	1
Caenis	sp.	7	7	1
EPHEMERELLIDAE	Gen.sp.	7	7	1
Cincticostella	sp.	8	10	1
Drunella	sp.	9		1
Ephacerella	sp.	8	8	1
Torleya	sp.	7	7	1
Ephemera	sp.	8	10	1
Epiophlebia	sp.	10	10	5
HEPTAGENIIDAE	Gen.sp.	8	10	1
cf. Notacanthurus	sp.	8		1
Cinygmula	sp.	9		1
Ecdyonurus	s.l.	8	10	1
Epeorus	sp.	8	10	1
Iron	sp.	9	9	5
Rhithrogena	sp.	9	10	3
ISONYCHIIDAE	Gen.sp.	10	10	1
LEPTOPHLEBIIDAE	Gen.sp.	7	7	1
Euthraulus	sp.	8	10	1
NEOEPHEMERIDAE	Gen.sp.	10		5
Potamanthellus	sp.	10	10	5
PALINGENIIDAE	Gen.sp.	10	10	1
POTAMANTHIDAE	Gen.sp.	10	10	1
Prosopistoma	sp.	10	10	5
AESHNIDAE	Gen.sp.	7	7	1
AMPHIPTERYGIDAE	Gen.sp.	7	7	1
CALOPTERYGIDAE	Gen.sp.	8	8	1
CHLOROCYPHIDAE	Gen.sp.	6	6	1
COENAGRIONIDAE	Gen.sp.	5	5	1
CORDULEGASTERIDAE	Gen.sp.	10	10	1
CORDULIIDAE	Gen.sp.	5	5	1
Macromia	sp.	7	7	1
EUPHAEIDAE	Gen.sp.	9	9	1
LESTIDAE	Gen.sp.	7	7	1
LIBELLULIDAE	Gen.sp.	6	6	1
MEGAPODAGRIONIDAE	Gen.sp.	10	10	1
PLATYCNEMIDAE	Gen.sp.		7	1
PROTONEURIDAE	Gen.sp.	5	5	1
SYNLESTIDAE	Gen.sp.	9	9	1
CAPNIIDAE	Gen.sp.	10		5
CHLOROPERLIDAE	Gen.sp.	9	9	3
LEUCTRIDAE	Gen.sp.	10	10	1
NEMOURIDAE	Gen.sp.	9	9	1
Amphinemura	sp.	9		5
Indonemoura	sp.	10	10	5
Mesonemoura	sp.	9	9	1
Nemoura	sp.	9		1
PELTOPERLIDAE	Gen.sp.	10		5
PERLIDAE	Gen.sp.	8	8	1

Taxon	Species	Mountain	Lowland	Weight
PERLODIDAE	Gen.sp.	9		1
TAENIOPTERYGIDAE	Gen.sp.	10		5
Aphelocheirus	sp.	7	9	1
BELOSTOMATIDAE	Gen.sp.	6		1
CORIXIDAE	Gen.sp.	6		1
Micronecta	sp.	5		1
Sigara	sp.	6	6	1
HEBRIDAE	Gen.sp.	7	7	1
MESOVELIIDAE	Gen.sp.	6	6	1
NAUCORIDAE	Gen.sp.	7	7	1
NEPIDAE	Gen.sp.	6	6	1
NOTONECTIDAE	Gen.sp.	3	3	1
PLEIDAE	Gen.sp.	4		1
VELIIDAE	Gen.sp.	7		1
CORYDALIDAE	Gen.sp.	7	7	1
SISYRIDAE	Gen.sp.	7	7	1
CHRYSOMELIDAE	Gen.sp.	8	8	1
CURCULIONIDAE	Gen.sp.	5		1
DRYOPIDAE	Gen.sp.	5	5	1
DYTISCIDAE	Gen.sp.	5		1
ELMIDAE	Gen.sp.	8	8	1
EULICHADIDAE	Gen.sp.	8	8	1
GYRINIDAE	Gen.sp.	6	7	1
HALIPLIDAE	Gen.sp.	6	6	1
HYDRAENIDAE	Gen.sp.	7	8	1
HYDROPHILIDAE	Gen.sp.	6		1
NOTERIDAE	Gen.sp.	4	4	1
PSEPHENIDAE	Gen.sp.	8	8	1
Eubrianacinae	Gen.sp.	9	9	1
Eubriinae	Gen.sp.	8	8	1
Psephenoidinae	Gen.sp.	8	8	1
SCIRTIDAE	Gen.sp.	8	8	1
APATANIIDAE	Gen.sp.	9	9	1
BRACHYCENTRIDAE	Gen.sp.	8	8	1
Brachycentrus	sp.	6	6	1
Micrasema	sp.	10	10	5
CALAMOCERATIDAE	Gen.sp.	10		1
DIPSEUDOPSIDAE	Gen.sp.	5	5	1
Ecnomus	sp.	6	6	1
GLOSSOSOMATIDAE	Gen.sp.	8	8	1
Agapetinae	Gen.sp.	9	9	5
Glossosomatinae	Gen.sp.	8	8	3
GOERIDAE	Gen.sp.	9	9	5
HELICOPSYCHIDAE	Gen.sp.	10	10	1
HYDROBIOSIDAE	Gen.sp.	10		1
HYDROPSYCHIDAE	Gen.sp.	7	9	1
Cheumatopsyche	sp.	7		1
Diplectrona	sp.	8		1
HYDROPTILIDAE	Gen.sp.	7	9	1
LEPIDOSTOMATIDAE	Gen.sp.	8	8	5
LIMNEPHILIDAE	Gen.sp.	7		1
Limnocentropus	sp.	9		5
ODONTOCERIDAE	Gen.sp.	7	7	1

Taxon	Species	Mountain	Lowland	Weight
Marilia	sp.	7	7	1
PHILOPOTAMIDAE	Gen.sp.	7	7	1
PHRYGANEIDAE	Gen.sp.	8		1
Pseudoneureclipsis	sp.	8	8	1
POLYCENTROPODIDAE excl. Pseudoneurec-lipsis	Gen.sp.	7		1
PSYCHOMYIIDAE	Gen.sp.	7	7	1
RHYACOPHILIDAE	Gen.sp.	8	8	1
Rhyacophila	sp.	8		1
Himalopsyche	sp.	9		5
Stenopsyche	sp.	8	8	1
UENOIDAE	Gen.sp.	10	10	5
PYRALIDAE	Gen.sp.	7	7	1
ATHERICIDAE	Gen.sp.	9	9	1
BLEPHARICERIDAE	Gen.sp.	10		1
Ceratopogonidae type Bezzia	Gen.sp.	2	2	1
CHIRONOMIDAE red	Gen.sp.	1	1	3
Diamesini	Gen.sp.	7	8	1
Orthocladiinae	Gen.sp.		8	1
Tanytarsini	Gen.sp.		4	1
CULICIDAE	Gen.sp.	2		1
DEUTEROPHLEBIIDAE	Gen.sp.	8	8	1
DIXIDAE	Gen.sp.	9		1
DOLICHOPODIDAE	Gen.sp.	2	2	1
EMPIDIDAE	Gen.sp.	8	8	1
EPHYDRIDAE	Gen.sp.	1	1	5
LIMONIIDAE	Gen.sp.	8	8	1
cf. Antocha	sp.	8	8	1
MUSCIDAE	Gen.sp.	1	1	1
PEDICIIDAE	Gen.sp.	9	8	1
cf. Dicranota	sp.	8	8	1
PSYCHODIDAE black	Gen.sp.	6	6	1
PSYCHODIDAE white = Psychoda	Gen.sp.	1	1	5
RHAGIONIDAE	Gen.sp.	9	9	1
SCIARIDAE	Gen.sp.	6	6	1
SCIOMYZIDAE	Gen.sp.	8	8	1
SIMULIIDAE	Gen.sp.	7	7	1
SYRPHIDAE	Gen.sp.	1	1	5
TABANIDAE	Gen.sp.	6	7	1
TIPULIDAE	Gen.sp.	7	8	1
LOPHOPODIDAE	Gen.sp.	7	7	1
PLUMATELLIDAE	Gen.sp.	7	7	1

5.5.2. HKHbios - Definition of threshold values for ecological quality classes

Different methods have been developed for defining boundaries between quality classes (see Barbour et al. 1999). The derivation of threshold values to discriminate between different stages of stress is based on the index ranges in dependency on the estimated quality class (pre-classification) and is defined for each ecoregion separately. The results of the HKHbios for each ecoregion are shown in Figure 13 to Figure 17 visualised as Box&Whisker-Plots. A five class scheme was used for the delineation of quality classes based on the distribution of reference sites. The proposed class boundaries were defined using unequal class ranges considering that there is not always a linear relationship to stressor intensity. Suggested threshold values are summarized in Table 7.

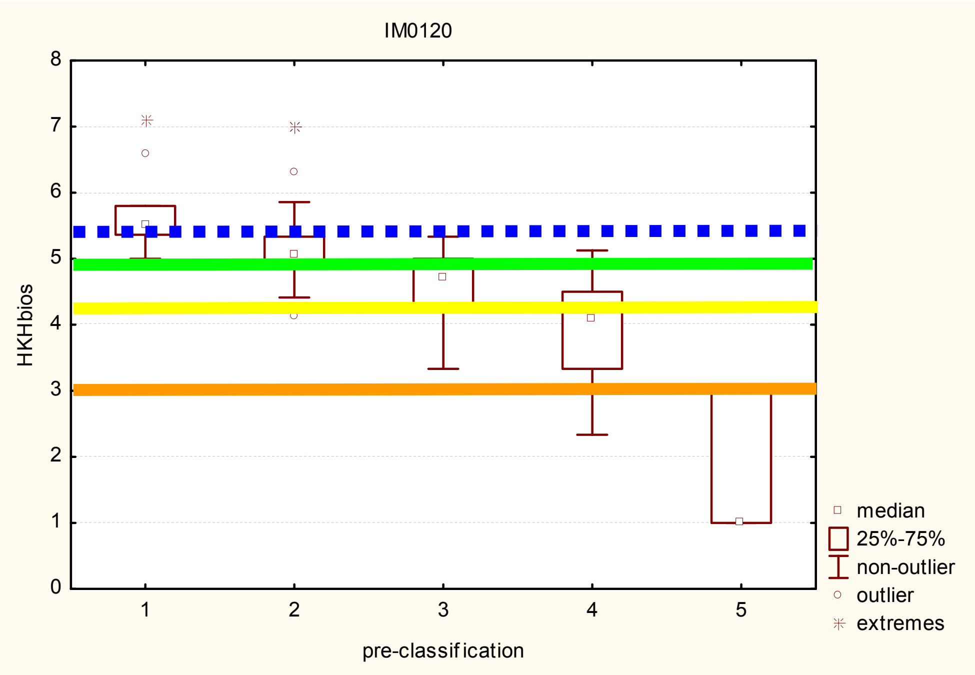


Figure 13: Box & whisker – plots of HKHbios values vs. pre-classification for Ecoregion IM0120. Thresholds: blue dotted line: reference/good, green line: good/moderate, yellow line: moderate/poor, orange line: poor/bad.

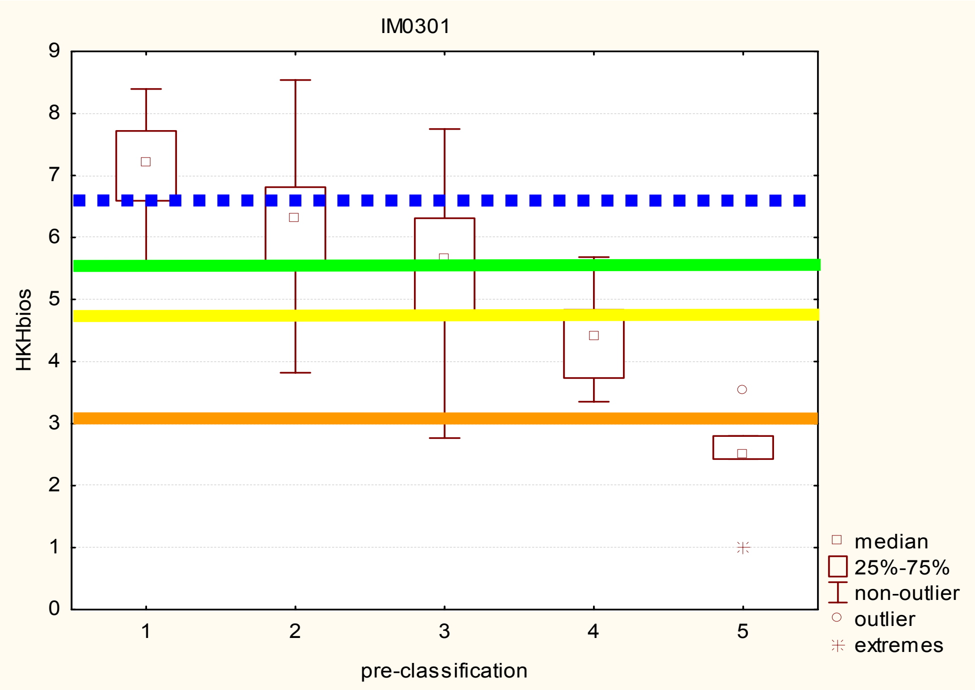


Figure 14: Box & whisker – plots of HKHbios values vs. pre-classification for Ecoregion IM0301. Thresholds: blue dotted line: reference/good, green line: good/moderate, yellow line: moderate/poor,

orange line: poor/bad.

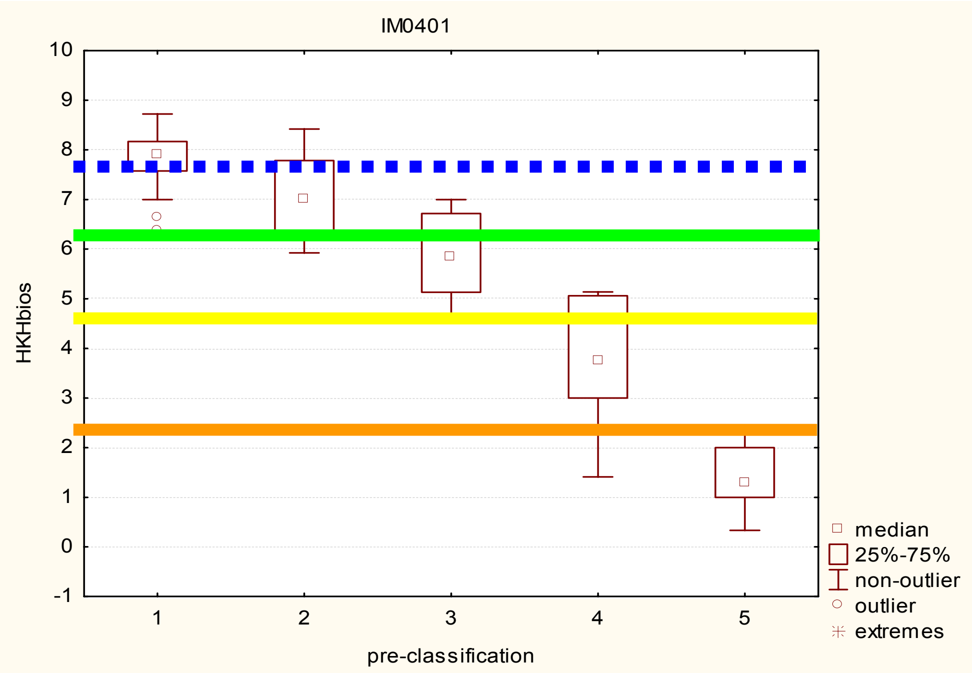


Figure 15: Box & whisker – plots of HKHbios values vs. pre-classification for Ecoregion IM0401. Thresholds: blue dotted line: reference/good, green line: good/moderate, yellow line: moderate/poor, orange line: poor/bad.

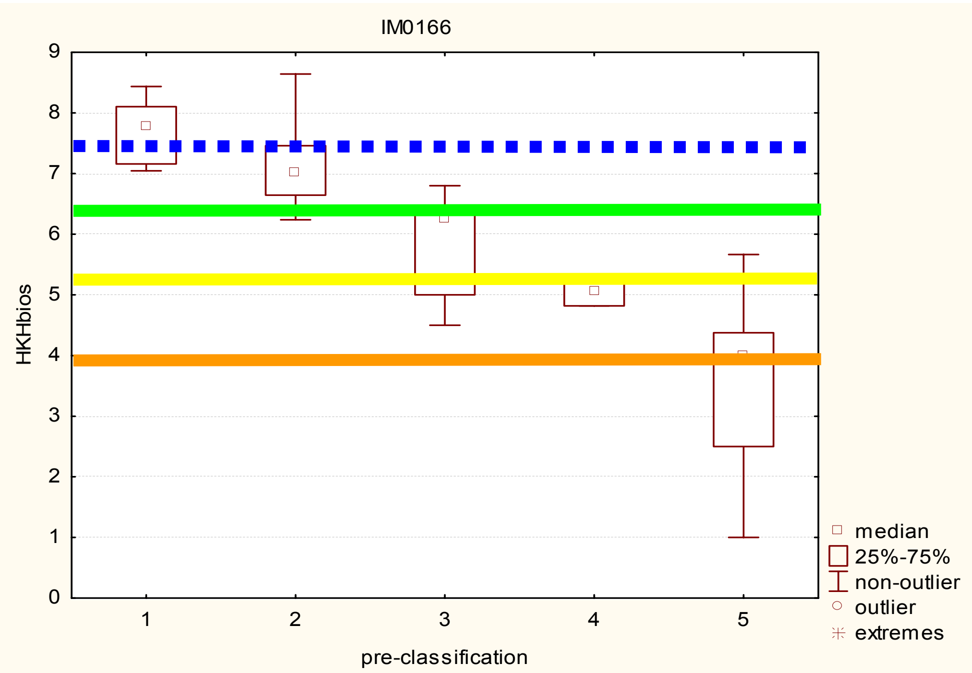


Figure 16: Box & whisker – plots of HKHbios values vs. pre-classification for Ecoregion IM0166. Thresholds: blue dotted line: reference/good, green line: good/moderate, yellow line: moderate/poor, orange line: poor/bad.

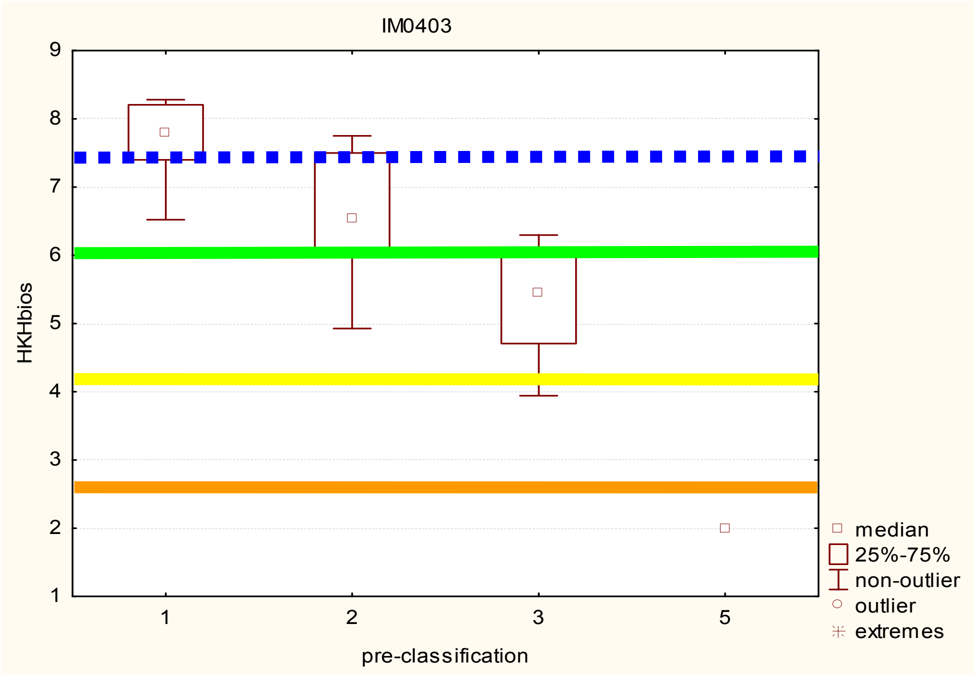


Figure 17: Box & whisker – plots of HKHbios values vs. pre-classification for Ecoregion IM0403. Thresholds: blue dotted line: reference/good, green line: good/moderate, yellow line: moderate/poor, orange line: poor/bad.

Table 7: Class boundaries for individual ecoregions

class boundaries	IM0120	IM0166	IM0301	IM0401	IM0403
reference/good	≥ 5.5	≥ 7.5	≥ 6.8	≥ 7.7	≥ 7.5
good/moderate	≥ 4.9	≥ 6.5	≥ 5.7	≥ 6.3	≥ 6
moderate/poor	≥ 4.3	≥ 5.3	≥ 4.7	≥ 4.6	≥ 4.3
poor/bad	≥ 3.3	≥ 4	≥ 3.4	≥ 2.3	≥ 2.6

5.5.3. Application of the HKHbios: evaluating new streams

First, new streams are assigned to the membership of an ecoregion. The taxalist from a new stream is compared with Table 6 (chose either column “mountains” or “lowlands”, depending on the ecoregion). Those taxa that are ranked are used for calculating the HKHbios of a new site, whereby the following formula is applied:

$$\mathrm{HKHbios} = \frac{\sum_{1}^{i} HKHbios - Score_i * Weight_i}{\sum_{1}^{i} Weight_i}$$

For lowland streams (IM0120, IM0166) the scores in column ’lowland’, for mountain streams (IM0301, IM0401, IM0403) the scores in column ’mountain’ are to be applied. The resulting HKHbios can be allocated to a river quality class according to Table 7.

6. HKHindex – A Multimetric bioassessment method

6.1. Introduction

The aim of a Multimetric Index is to assess ecological river quality with the help of different biotic measures. These biotic measures are called metrics. Metrics are e.g. the number of benthic macroinvertebrate families, number of mayflies within the sample or diversity indices like the Shannon Wiener Index. All metrics are calculated from a taxalist of a given sampling site. The characteristic of a metric is that it is responsive to deterioration of river quality. Hence, a metric value increases or decreases with the increase of river deterioration.

River assessment on the basis of metrics leads to integrative river assessment. Hence, different stressors, which lead to deterioration of river quality, should be considered for the development of the HKHindex. In general two main stressors, namely water pollution mainly caused by organic load and flow alteration caused by transversal dams or weirs were investigated in the ASSESS HKH-Project (see Feld et al. 2005). Based on the fact, that river stretches rarely or almost in no cases suffer from single stressors, a number of possible influences were recorded per site with the help of an elaborate site protocol. From these parameters five different stressor groups were derived for further calculation procedures. These stressor groups are: organic pollution, eutrophication, landuse of the floodplain and catchment area and hydromorphological impact.

The application of ecological river assessment, based on a Multimetric approach, on a given river stretch leads to a single value, the MMI. This single value can be compared with the according MMI from reference condition. Investigations on the degree of impairment and the according response of the MMI lead to the development of different river quality classes. Finally each MMI can be assigned to a river quality class.

The aim of the following analysis was to find metrics which are able to detect and measure river deterioration caused by the five different stressor groups. For each stream type core metrics were derived and combined to build the Multimetric Index from the HKH region, the HKHindex (see Figure Figure 18).

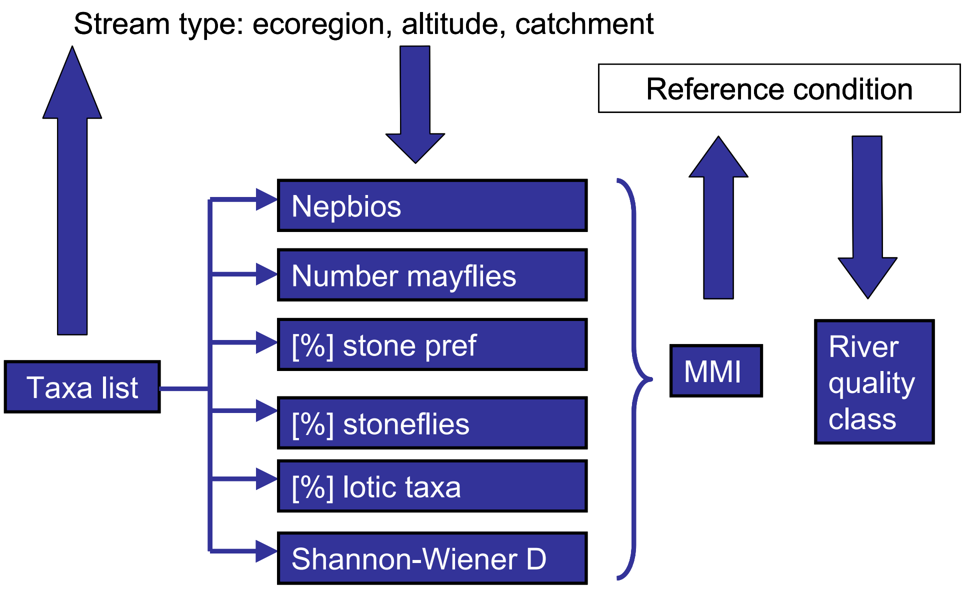


Figure 18: Procedure for the Multimetric index calculation.

6.2. Gradient analysis

In a first step a gradient analysis was carried out to find abiotic parameters which are able to describe deterioration of rivers best. Actually, parameters, which have a large range, e.g. high values at undisturbed river stretches to low values at heavily impacted sites. Hence, the aim was to detect environmental parameters with a long gradient and, which are able to explain the variability within the data set very well. For this analysis the abiotic parameters of the ASSESS-HKH site protocol were used (see Table 8).

Table 8: Environmental parameters from the ASSESS-HKH site protocol which have been considered for gradient analysis. L = Landuse; P = Pollution (eutrophication and organic pollution); H = Hydromorphology.

Parameter	**Lowlands**			**Mountains**		
	L	P	H	L	P	H
Landuse						
Deciduous native forest				x		
Mixed native forest				x		
Open grass-/bushland (natural)	x			x		
Naturally unvegetated				x		
Crop land (tillage, lowland)	x			x		
Pasture				x		
Urban sites (residual)	x					
Villages	x					
Hydromorphology						
Shading at zenith (at noon)			x			x
Removal mineral bed material (yes/no)			x			x
Average density of wooded riparian vegetation			x			x

Parameter	Lowlands				Mountains		
Number bank fixation			x				x
Number bed fixation			x				x
Removal/lack of natural floodplain vegetation (yes/no)			x				x
Pool			x				x
Riffle							x
Run							x
Rapid							x
Number flow types			x				x
Longitudinal impoundments at sampling site (yes/no)			x				
Slack			x				
Pollution / Eutrophication							
Source pollution (yes/no)						x	
Non-source pollution (yes/no)		x				x	
Sewage overflows (yes/no)		x				x	
Eutrophication (yes/no)		x				x	
Water uses		x				x	
Fisheries		x					
Cattle watering place		x				x	
Rubbish		x				x	
Faeces		x				x	
Washing/bathing		x				x	
Foam (yes/no)		x				x	
Turbidity (yes/no)		x				x	
Conductivity [µS/cm]		x				x	
Oxygen saturation (%)		x					
Chloride [mg/l]						x	
BOD_5 [mg/l]		x				x	
Nitrate [mg/l]		x				x	
Ortho-phosphate [µg/l]		x				x	
E-coli [n/100 ml]		x				x	
Ammonium [mg/l]						x	

6.2.1. Selection of abiotic parameters

The first judgement which parameters could detect impact on rivers was done by expert judgement. Altogether 87 abiotic parameters were selected from the ASSESS-HKH site protocol.

6.2.2. Calculation of parameter sums and indices

On basis of the ASSESS-HKH site protocol additional parameters were calculated (see Table 9).

Table 9: Additional parameters, which derive from the site protocol.

Parameter	Explanation
Forest	% share of all kinds of forests
Number bed fixation	% share of all kinds of bed fixation
Number bank fixation	% share of all kinds of bank fixation
Number fixation	% share of all bed and bank fixation
Number waste disposal	Sum of all kinds of waste disposal

In addition different indices were generated to weight the influence of certain abiotic parameters (see Table 10).

Table 10: Indices calculated on basis of site protocol parameters.

Indices	Explanation
Landuse Index of floodplain	4 * urban sites (industrial/residual) + 2 * terraces, crop land, pasture, villages
Landuse Index (Hering et al. 2006b)	% urban sites + 0.5*% cropland
Hydromorphology Index (Hering et al. 2006b)	% shoreline covered woody riparian vegetation + % no bank fixation + % no bed fix – (stagnation*100) - (straightening *100)
Fixation Index bed	4 * (sum concrete without seems+ stone plastering without seems) + 2 * (sum stone plastering with seems + concrete with seems)+ sum remaining fixation bed
Fixation Index bank	4 * (sum concrete without seems + stone plastering without seems) + 2 * (sum stone plastering with seems + concrete with seems) + remaining fixation bank
Fixation Index	Fixation Index bed + Fixation Index bank
Waste Disposal Index	2 * (sum faeces + slaughter waste + industrial water) + remaining parameters waste disposal

6.2.3. Missing data

Mean values were calculated for missing data, actually chemical values. Mean values were calculated according to pre-classification group and ecoregion (seeTable).

Table 11: Sum of missing chemical values.

	Mountain		Lowland	
	available	missing	available	missing
Conductivity	179	1	120	7
Temperatur	179	1	119	8
Chloride	162	18	119	8
BOD_5	180	0	105	22
Nitrate	179	1	122	5
Ammonium	180	0	-	-
Ortho-phosphate	162	18	104	23
E-coli	162	18	105	22

6.2.4. Threshold values

Before a multivariate analysis was applied, which should detect the key environmental parameters a pre-selection was conducted. Therefore, threshold values were defined to detect and remove those parameters that have a small range and gradient, respectively.

Threshold for Chemical values

Regarding chemical values Box- and Whisker-Plots were applied to find those parameters which have a long gradient. The aim was to delete parameters which according Box- and Whisker-Plots exhibit a lot of outlier and extreme values and small boxes (Hering et al. 2006a). Therefore, only those chemical parameters were considered for further analysis that have less than 10% of their values outside the "whiskers" (see Formula 1)

$$\text{Threshold chemical values}: \frac{\sum n > 85\,\text{percentile} + \sum n < 15\,\text{percentile}}{n} \leq 0.1$$

Formula 1: Threshold value chemical parameters

Threshold for ***"yes/no" parameters***

"yes/no" parameters were integrated in the further analysis if the ratio between yes and no was larger than 0.25.

Threshold for relative [%] and nominal parameters

Relative [%] and nominal parameters have been considered if they have been described in at least a quarter of all samples.

6.2.5. Stressor groups

Then, the selected environmental parameters were assigned to four stressor groups characterizing different kinds of impacts, namely:

- Landuse floodplain
- Hydromorphology
- Eutrophication
- Organic pollution

6.2.6. Deletion of inter-correlating parameters

Within each of the five stressor groups Spearman rank correlation was applied to find parameters that were highly correlated; critical values for deletion $R^2 > 0.8$.

Deletion rule:

1. Comparison of Box and Whisker plots and deletion of the parameter that have the smaller gradient.
2. Parameter that was measured (interval scale) was kept. Parameter that was estimated was deleted.
3. Parameter that was additionally correlating with other parameters was deleted.

6.2.7. Principal Components Analysis (PCA):

In a next step a PCA, as multivariate analysis, was applied to find the environmental that a) have with the longest gradient and b) explain the variability within the data set best.

Data preparation for PCA analysis

A stressor specific analysis demands that only sampling sites are considered, which are more or less influenced only by the observed stressor. E.g. if the influence of hydromorphological impact is observed, sampling site should be removed from the analysis which are highly impacted by pollution. Hence, threshold values were defined for excluding sampling sites from gradient calculation for another stressor (see Table 12).

Table 12: Threshold values for excluding sampling sites from calculation for another stressor (according to Hering et al., 2006b).

Stressor	Threshold values
Pollution	Ortho-phosphate [µg/l]: Lowland ≥ 700 µg/l Mountain ≥ 600 µg/l
Landuse	Landuse Index: Lowland > 40 Mountain > 30
Hydromorphology	Hydromorphology Index: Lowland > 0 Mountain > 0

Selection of abiotic parameters

PCA analysis was carried out with the final set of abiotic parameters. The analysis has been carried out for lowland rivers and mountainous rivers and for the different stressor groups separately. Finally, abiotic parameters have been chosen that exhibit the longest gradient and hence explain the variability within the data set best. In addition PCA axis values have also been selected as parameters, since these represent a combination of stress affecting a given sampling site (see Table 13).

Table 13: Abiotic parameters chosen for metric correlation.

Stressor group	Lowland	Mountain
Eutrophication	PCA Axis 1	PCA Axis 1
	PCA Axis 2	PCA Axis 2
	Nitrate	Conductivity
	Ortho-phosphate	Ortho-phosphate
Organic pollution	PCA Axis 1	PCA Axis 1
	PCA Axis 2	PCA Axis 2
	BOD_5	BOD_5
	E-coli	E-coli
Hydromorphology	PCA Axis 1	PCA Axis 1
	PCA Axis 2	PCA Axis 2
	Density wooded riparian vegetation	Density wooded riparian vegetation
	Number bank fixation	Number bank fixation
Landuse	PCA Axis 1	PCA Axis 1
	PCA Axis 2	PCA Axis 2
	Landuse Index	Landuse Index
	Landuse Index of floodplain	Forest
	Villages	

6.3. Selection of candidate and core metric

In the following step the selected abiotic parameters were correlated with the different metrics. The aim was to find metrics which are highly correlated to the increase or decrease of the selected abiotic parameters. In a first step a set of candidate metrics were choosen. Then, core metrics were defined on basis of the candidate metrics. The final sets of core metrics for each stream type and ecoregion respectively, are the basis for the MMI calculation.

Removal of sampling sites from the analysis

Sampling sites containing less that 20 animals were removed from the analysis. This guaranteed that metrics increasing with the increase of impairment respond linear over the whole gradient range of a certain stressor type. Altogether 17 sampling sites were removed from the analysis.

6.3.1. Metric calculation

Table 7 shows the metrics that have been calculated for each sampling site. In the following steps their ability was tested to detect impacts on rivers.

Table 14: Metrics observed to detect impacts on rivers. Metric type: c/a = composition and abundance metrics, r/d = richness and diversity metrics, s/t = sensitivity and tolerance metrics, f = functional metrics. Abundance unit: # = number, nu_ = number individuals transformed into abundance class (Meier et al. 2006), [%] = relative share. EPTC = Ephemeroptera, Plecoptera, Trichoptera, Coleoptera. For further explanation regarding metric types, abundance units and metric types see below "Guideline for the manual calculation of the HKHindex".

		Metric type				**Abundance unit**			**Explanation**
Metric	**Abbr.**	**c/a**	**r/d**	**s/t**	**f**	**#**	**nu_**	**[%]**	
Taxa sum	Tax		X			x			Metric counts all taxonomical units
Families sum	Fam		X			x			Metric counts all families
Genus sum	Gen		X			x			Metric counts all genus
Species sum	Sp		X			x			Metric counts all species
Individuals sum	Indiv		X			x			Metric counts all individuals
Bivalvia	Bival		X			x	x		Metric counts mussels
Bivalvia families	Bival fam		X			x			Metric counts mussel families
Crustacea	Crust		X			x	x		Metric counts Crustacea
Crustacea families	Crust fam		X			x			Metric counts Crustacea families
Gastropoda	Gast		X			x	x		Metric counts snails
Gastropoda families	Gast fam		X			x			Metric counts snail families
EPTC			X			x	x		Metric counts Ephemeroptera, Plecoptera, Trichoptera and Coleoptera
EPTC individuals	EPTC ind	X						x	Metric calculates the relative share of Ephemeroptera, Plecoptera, Trichoptera and Col-eoptera individuals
EPTC families	EPTC fam		X			x			Metric counts Ephemeroptera, Plecoptera, Trichoptera and Coleoptera families
EPTC families	EPTC fam	X						x	Metric calculates the relative share of Ephemeroptera, Plecoptera, Trichoptera and Col-eoptera families
EPTC genus	EPTC gen		X			x			Metric counts Ephemeroptera, Plecoptera, Trichoptera and Coleoptera genus
EPTC species	EPTC sp		X			X			Metric counts Ephemeroptera, Plecoptera, Trichoptera and Coleoptera species
EPT	EPT ind		X			x	x		Metric counts Ephemeroptera, Plecoptera and Trichoptera individuals
EPT individuals	EPT ind	X						x	Metric calculates the relative share of Ephemeroptera, Plecoptera and Trichoptera indi-viduals
EPT families	EPT fam		X			X			Metric counts Ephemeroptera, Plecoptera and Trichoptera families
EPT families	EPT fam	X						x	Metric calculates the relative share of Ephemeroptera, Plecoptera and Trichoptera fami-lies

		Metric type				Abundance unit			Explanation
Metric	**Abbr.**	**c/a**	**r/d**	**s/t**	**f**	**#**	**nu_**	**[%]**	
EPT genus	EPT gen		X			X			Metric counts Ephemeroptera, Plecoptera and Trichoptera genus
EPT species	EPT sp		X			X			Metric counts Ephemeroptera, Plecoptera and Trichoptera species
Ephemeroptera individuals	Eph		X			x	x		Metric counts Ephemeroptera individuals
Ephemeroptera individuals	Eph	X						x	Metric calculates the relative share of Ephemeroptera individuals
Ephemeroptera families	Eph fam		X			X			Metric counts Ephemeroptera families
Ephemeroptera families	Eph fam	X						x	Metric calculates the relative share of Ephemeroptera individuals
Ephemeroptera genus	Eph gen		X			X			Metric counts Ephemeroptera genus
Ephemeroptera species	Eph sp		X			x			Metric counts Ephemeroptera species
Trichoptera	Trich		X			x	x		Metric counts Trichoptera individuals
Trichoptera individuals	Trich ind	x						x	Metric calculates the relative share of Trichoptera individuals
Trichoptera families	Trich fam		X			x			Metric counts Trichoptera families
Trichoptera families	Trich fam	X						x	Metric calculates the relative share of Trichoptera families
Trichoptera genus	Trich gen		X			x			Metric counts Trichoptera genus
Trichoptera species	Trich sp		X			x			Metric counts Trichoptera species
Plecoptera	Plec		X			x	x		Metric counts Plecoptera individuals
Plecoptera individuals	Plec ind	x						x	Metric calculates the relative share of Plecoptera individuals
Plecoptera families	Plec fam		X			x			Metric counts Plecoptera families
Plecoptera families	Plec fam	x						x	Metric calculates the relative share of Plecoptera families
Plecoptera genus	Plec gen		x			x			Metric counts Plecoptera genus
Plecoptera species	Plec sp		X			x			Metric counts Plecoptera species
Coleoptera	Cole		X			x	x		Metric counts Coleoptera individuals
Coleoptera individulas	Cole ind	X						x	Metric calculates the relative share of Coleoptera individuals
Coleoptera families	Cole fam		X			x			Metric counts Coleoptera families
Coleoptera families	Cole fam	X						x	Metric calculates the relative share of Coleoptera families
Coleoptera genus	Cole gen		x			x			Metric counts Coleoptera genus
Coleoptera species	Cole sp		X			x			Metric counts Coleoptera species
Diptera	Dip		X			x	x		Metric counts Diptera individuals
Diptera individuals	Dip ind	X						x	Metric calculates the relative share of Diptera individuals
Diptera families	Dip fam		X			x			Metric counts Diptera families

		Metric type				**Abundance unit**			**Explanation**
Metric	**Abbr.**	**c/a**	**r/d**	**s/t**	**f**	**#**	**nu_**	**[%]**	
Diptera families	Dip fam	x						x	Metric calculates the relative share of Diptera families
Diptera genus	Dip gen		X			x			Metric counts Diptera genus
Diptera species	Dip sp		X			x			Metric counts Diptera species
Simuliidae	Si		X			X	x		Metric counts individuals of Simuliidae
Hydropsychidae	Hy		X			X	x		Metric counts individuals of Hydropsychidae
Baetidae	Ba		X			X	x		Metric counts individuals of Baetidae
Shannon Wiener Diver-sity Index	D (S-W)		X			x			D (S-W) explains the homogeneity of taxa distribution $D\ (S\text{-}W) = -\sum_{i=1}^{s} \left(\frac{ni}{A}\right) * \ln\left(\frac{ni}{A}\right)$ ni = number individuals of i-taxon A = number all individuals
Evenness Diversity In-dex	D (E)		X			x			D (E) gives the maximum diversity of a given D (S-W) $D(E) = \frac{D(S-W)}{\ln(t)}$ t = number taxa
Margalef Diversity Index	D (M)		x			x			Relation between all taxa to total amount of individuals $D\ (M) = \frac{(i-1)}{\ln(A)}$ i = sum taxa A = total amount individuals/m²
Maximum Diversity	D (HMAX)		X						H (MAX) = ln (t)
Oligochaeta	Olig			X		x	x		Metric counts Oligochaeta individuals
Oligochaeta individuals	Olig ind	x						x	Metric calculates the relative share of Oligochaeta individuals
Oligochaeta families	Olig fam			X		x			Metric counts Oligochaeta families
Oligochaeta families	Olig fam	x						x	Metric calculates the relative share of Oligochaeta families
Oligochaeta species	Olig sp			x		x			Metric counts Oligochaeta individuals
Chironomidae	Chiro			x		x	x		Metric counts individuals of Chironomidae
Batidae-Simuliidae-Hydropsychidae-Chironomidae	BaSiHyCh			x		x	x		Metric counts Baetidae (Ba),Simuliidae (Si), Hydropsychidae (Hy) and Chironomidae (Ch)

Metric	Abbr.	Metric type				Abundance unit			Explanation
		c/a	r/d	s/t	f	#	nu_	[%]	
									$BaSiHyCh = \sum Ba_{ind} + \sum Si_{ind} + \sum Hy_{ind} + \sum Ch_{ind}$; instead of individuals abundance classes may be summed
BMWP	BMWP			x		x			Biological Monitoring Working Party. Taxa were classified according to their sensitivity to organic pollution. Animals which are sensitive to pollution have a high score and vice versa. The BMWP is the sum of sensitivity values from all families of a given taxalist. $BMWP = \sum_{1}^{i} Score\ i$ Score i = Score of taxon i
ASPT				x		x			Average Score Per Taxon. The ASPT equals the BMWP divided through the number of all families of a given taxalist. $ASPT = \frac{\sum_{1}^{i} Score\ i}{number\ taxa}$
ASPT_weighted	ASPT_w			x		x			ASPT including taxa abundance
Pelal preference	Pel				x	x	x		Metric counts the taxonomical units with mud preferences $Pel = \sum Pel_i + Pel_j + ... + Pel_n$ Pel_i = individuals of taxon i with mud preference; instead of individuals abundance classes may be summed
Lithal	Lith				x	x	x		Metric counts the taxonomical units with stone preferences $Lith = \sum Lith_i + Lith_j + ... + Lith_n$ $Lith_i$ = individuals of taxon i with mud preference; instead of individuals abundance classes may be summed
Lithobiont	Lbio				x	x	x		Metric counts the taxonomical units only living on stones $Lbio = \sum Lbio_i + Lbio_j + ... + Lbio_n$ $Lbio_i$ = individuals of taxon i with mud preference; instead of individuals abundance classes may be summed
Lithophil	Lphi				x	x	x		Metric counts the taxonomical units living preferably on stones but also on other sub-

		Metric type				Abundance unit			Explanation
Metric	Abbr.	c/a	r/d	s/t	f	#	nu_	[%]	
									strates $$Lphi = \sum Lphi_i + Lphi_j + ... + Lphi_n$$ $Lphi_i$ = individuals of taxon i with mud preference; instead of individuals abundance classes may be summed
Lotic	Loti				x	x	x		Metric counts the taxonomical units with preferences to high flow velocities $$Loti = \sum Loti_i + Loti_j + ... + Loti_n$$ $Loti_i$ = individuals of taxon i with mud preference; instead of individuals abundance classes may be summed

6.3.2. Selection of candidate metrics

Graphical analyses have been applied to reveal the ability of a given metric to detect impacts on rivers (Fore et al. 1996; Karr & Chu 1999). Graphical analysis have the advantage that one is able to detect immediately the response of a given metric towards increase or decrease of an environmental stressor, or in case of PCA axes values, a combination of environmental stressors. For the developing of a Multimetric index, which assigns a given sampling site into a five class system, ranging from reference to bad conditions, metrics which respond monotonic increasing or decreasing were most suitable. Therefore, frequency scatterplots in combination with the Spearman rank correlation test were found as the most suited method. A metric was evaluated as a candidate metric for the Multimetric index if it showed a more or less monotonic response over the whole gradient range of a given stressor and exhibits a Spearman rank correlation coefficient (r) > 0.5.

In addition sampling sites were divided into stressed and unstressed sites with respect to a given stressor. Therefore, the 33rd and 67th percentiles of all stressor gradients were calculated. The latter percentile values were used as threshold values to divide the sampling sites into stressed and unstressed sites. Then, box plots have been applied showing a given metric and its response to stressed and unstressed sampling sites. In this case a metric was selected as a candidate metric if the two interquartile boxes (25th/75th percentile) of stressed and unstressed sampling sites were not overlapping (see Figure 19). These metrics were assigned to candidate metrics (Vlek et al. 2004; Ofenböck et al. 2004). The aim was to find at least 3 core metrics per metric type within a given stressor group (according to Hering et al. 2006a). Where this criterion was not fulfilled "non-overlapping" of interquartile ranges was decreased to 30th/70th percentile.

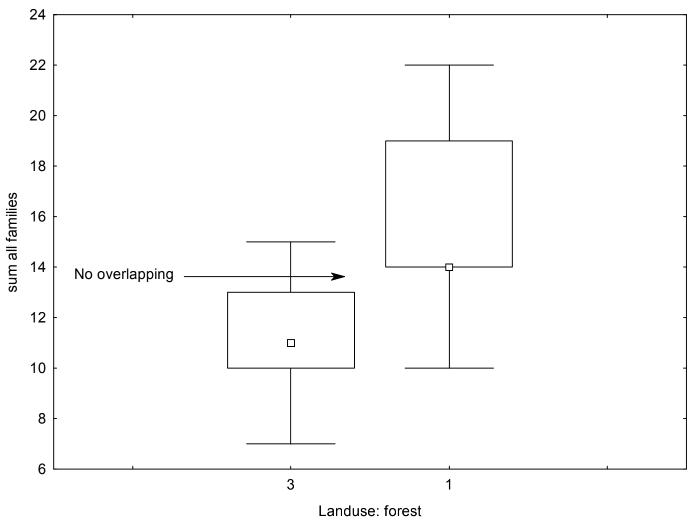


Figure 19: Example of "non-overlapping" interquartile range (25th /75th) for metric "sum all families". Metric is suiTable as candidate metric. 3 = sampling sites stressed by landuse (deforestation), 1= sampling sites unstressed.

6.3.3. Selection of core metrics

Only metrics which scatterplot showed a unidirectional response to increase of impairment were considered as core metrics. Inter-correlation tests between candidate metrics were carried out to detect redundant metrics. Arbitrarily, a threshold value for Spearman's r > 0.75 was considered as a high correlation between two metrics. This observation was conducted for each stream type and within each metric group separately. Deletion criteria were as follows: 1. Deletion of the metric which shows a higher over all correlation (according to Hering et al. 2006a). 2. Comparison of Box- and Whisker-Plots of metrics which were highly correlating: The metric was kept which showed the best separation between stressed and unstressed sampling sites. 3. Comparison of abundance information. Metrics which were based on individual numbers were evaluated weaker than metrics based on abundance classes or relative share values (for the explanation of abundance units see below "Guideline for the manual calculation of the HKHindex"). Hence, in a case of inter-correlation the latter metrics were considered for the selection as core metric.

Calculation of upper and lower anchor values

The range of a metric was fixed through upper and lower anchor values. 95th and 5th percentile were defined as upper and lower anchor values for the metrics of stressors organic pollution and eutrophication. Regarding the stressor hydromorphology and landuse maximum and minimum values of the metrics were defined as upper and lower anchor values. The upper anchor value reflects reference conditions and the lower anchor reflects the heavily impacted situation. Hence, the upper anchor value of metrics, which values increase with the increase of impairment, is low and vice versa (upper anchor value < lower anchor value).

Standardization of metrics values:

In a next step all core metric values were transformed to a value between 0 and 1 (see Formula 2). Hence, all metric values are comparable with each other.

$$\text{Standardized metric value} = \frac{\text{Metric value} - \text{Lower Anchor}}{\text{Upper Anchor} - \text{Lower Anchor}}$$

Formula 2: Standardization of metric values

(Hering et al. 2006a)

Upper and lower anchor values for the final set of core metrics are listed in Table 15. Standardized metric values greater than one were set back as one and metrics values smaller than zero were set back as zero.

Final selection of core metrics

Finally, a metric was choosen as core metrics if the according 25 percentile value in reference conditions was greater than 0.5. These metrics were considered as robust metrics, since they obtain in 75% per cases values greater than 0.5 in reference conditions. In addition the coefficients of variance (C.V.) for the latter metrics were calculated to measure their variability relative to the mean. Metrics were selected as core metrics which have a variability C.V. < 1. These metrics were considered as most effective for river quality assessment, and hence build the metrics for the HKHindex calculation (see Table 15).

The streams observed in the Lower Gangetic Plains did not exhibit core metrics in reference conditions which 25 percentiles obtain values greater than 0.5 (exception: metric Oligochaeta). Since, we have done an extensive pre-selection of sampling sites based on a pre-classification scheme to detect reference conditions, one interpretation is, that this stream type does not have any reference conditions any more. Only ubiquitous taxa were left which are able to tolerate a certain degree of impairment. Another interpretation is, that the identification level, mainly on genus and family level, is too "high". Hence, future investigations in this stream type should focus more on species level.

Table 15: Final set of core metrics per ecoregion (stream type) respectively. UA = upper anchor value (reference), LA = lower anchor value(heavily impacted). UA > LA = metric decrease with increase of river deterioration. UA < LA = metric increase with increase of river deterioration. For explanation of further abbreviations see Table 14

Himalayan Subtropical Pine Forest	**c/a**	**UA**	**LA**	**r/d**	**UA**	**LA**	**s/t**	**UA**	**LA**	**f**	**UA**	**LA**
	[%] EPT fam	68.8	0	D (E)	0.86	0.23	ASPT	7.5	4.8			
				D (M)	7.4	1.07						
Eastern Hima-layan Broad-leaf Forest	**c/a**	**UA**	**LA**	**r/d**	**UA**	**LA**	**s/t**	**UA**	**LA**	**f**	**UA**	**LA**
				D (S-W)	3.09	0.03	ASPT	7	1	nu_Lphil	12	0
				nu_Dip	2	6	nu_Olig	0	7			
				# EPT gen	16	0						
Western Hima-layan Broad-leaf Forest	**c/a**	**UA**	**LA**	**r/d**	**UA**	**LA**	**s/t**	**UA**	**LA**	**f**	**UA**	**LA**
	[%] EPT ind	90.6	4.9	D (M)	3.92	0.8	nu_BaSiHyCh	4	16			
	[%] Plec fam	14.2	0				ASPT_w	7.3	6.35			
Lower	**c/a**	**UA**	**LA**	**r/d**	**UA**	**LA**	**s/t**	**UA**	**LA**	**f**	**UA**	**LA**

Himalayan Subtropical Pine Forest	c/a	UA	LA	r/d	UA	LA	s/t	UA	LA	f	UA	LA
Gangetic Plains												
	[%] EPT fam	33.3	0	# EPT gen	6	0	nu_Olig	0	6	nu_ Pel	22	0
							BMWP	101	5			
Upper Gangetic Plains	**c/a**	**UA**	**LA**	**r/d**	**UA**	**LA**	**s/t**	**UA**	**LA**	**f**	**UA**	**LA**
	[%] EPT fam	58.8	0	D (S-W)	2.73	0.9	BMWP	146	0	nu_ Lith	26	0
							ASPT	7.07	1			
							nu_BaSi HyCh	2	13			

6.4. Calculation of the HKHindex

Calculating the mean value from the set of core metrics which derive from a given sampling site gives the HKHindex. The set of core metrics that have to be calculated for the HKHindex is depended on the stream type (ecoregion) in which the sampling site is situated (see Table 15).

6.5. Setting class boundaries

The assignment of a given sampling site to a certain river quality class is based on the comparison of the MMI of a given sampling site with the according MMI in reference conditions. The degree of alteration from the reference conditions gives the river quality class. Consequently, the class boundary for class "high" must be clearly defined. Our approach is to take the mean which derives from the 25 percentile values of the core metrics from reference conditions as boundary for class "high". This value takes the natural variability of core metric values in reference conditions into account. Since, only metrics were considered as core metrics, which obtain a value greater than 0.5 (see above "Final selection of core metrics") the minimum value for class boundary "high" could be 0.5. The following class boundaries, river quality class good (2) to bad (5), were evenly spread over the range that was left after setting the boundary for class "high" (see

Table 16).

Table 16: Class boundaries

Ecoregion / river quality class	1 "high"	2 "good"	3 "moderate"	4 "poor"	5 "bad"
Lower Gangetic Plains	≥ 0.47	≥ 0.35 < 0.47	< 0.35 ≥ 0.24	< 0.24 ≥ 0.12	< 0.12
Upper Gangetic Plains	≥ 0.65	≥ 0.49 < 0.65	< 0.49 ≥ 0.33	< 0.33 ≥ 0.16	< 0.16
Himalayan Subtropical Pine Forest	≥ 0.58	≥ 0.44 < 0.58	< 0.44 ≥ 0.29	< 0.29 ≥ 0.15	< 0.15
Eastern Himalayan Broadleaf Forest	≥ 0.77	≥ 0.58 < 0.77	< 0.58 ≥ 0.39	< 0.39 ≥ 0.19	< 0.19
Western Himalayan Broadleaf Forest	≥ 0.78	≥ 0.59 < 0.78	< 0.59 ≥ 0.39	< 0.39 ≥ 0.2	< 0.2

6.6. Seasonal Aspect

We observed if the core metric values of reference sites differ according to season. Therefore, Mann-Whitney U-test was applied to detect significant differences between the pre-monsoon metric value and the according post-monsoon value. Only three metric values of all core metrics show significant differences (see Table 17). Consequently the HKHindex can be applied both during post-monsoon season and pre-monsoon season. Nevertheless, the effect of river pollution will increase during dry season due to concentration processes we recommend sampling in the pre-monsoon season.

Table 17: Core metrics, which exhibit significant differences according to season.

Ecoregion	p-level < 0.05	p-level < 0.01
Lower Gangetic Plains	nu_Pel	
Western Himalayan Broadleaf Forest		[%] EPT ind
		nu_BaSiHyCh

6.7. Application and guideline for the calculation of the HKHindex

In the following a description is given for the manual calculation of metrics and the HKHindex. The aim of the guideline is to clarify the calculation procedure behind the application of the ECODAT management tool, which was developed in the ASSESS-HKH project. However, the ECODAT management tool will do all the necessary calculations automatically (for download see: assess-hkh.at). Actually, for the calculation of the HKHindex for a given sampling site via the ECODAT management tool (HKHdip software), one only has to enter a taxalist into the HKHdip programme. Then, the programme will give the values of the core metrics, the HKHindex and the river quality class automatically.

The guideline is divided into four parts. At the beginning, in part one and two, basic terms are explained. These terms are important since they assign specific taxonomical units and abundance units to metrics. This information is necessary for the metric calculation. In the third part a division of metrics based on Hering et al. (2006a) is introduced to show the different biological "scales" metrics belong to. In this part new findings about habitat and current preferences of selected taxa from the HKH region are introduced. In the following part, exemplarily several metric calculations are presented. Finally, the HKHindex is calculated manually for a fictive taxalist.

6.7.1. Taxonomical units

Metrics can be calculated on different taxonomic units. You may calculate for the example the metric EPT taxa on the family level or on the genus level. In the first case you count all families of EPT families in your sample. In the second case you count all EPT genres.

For the calculation of MMI metrics you have to consider the following taxonomical units:

1. Species – level (spec): Calculations are done on species level of the according metric name, e.g. EPT spec. You have to consider the number of Ephemeroptera, Plecoptera and Trichoptera species in your sample.

2. Genus – level (gen): Calculations are done on genus level of the according metric name, e.g. EPT gen. You have to consider the number of Ephemeroptera, Plecoptera and Trichoptera genres in your sample.

3. Family – level (fam): Calculations are done on family level of the according metric name.

6.7.2. Abundance units

Metrics can be calculated on different abundance levels. There are three abundance units for metric calculations:

1. Total number of individuals without any transformation.

symbol: #

2. Number of individuals transformed into a abundance class (see Table 18)

symbol: nu_

3. Number of individuals tranformed into a relative share.

symbol: [%]

Table 18: Abundance classes (based on Meier et al. 2006).

Abundance class	Number individuals
1	0 < n < 2,5
2	2,5 </= n < 10,5
3	10,5 </= n < 30,5
4	30,5 </= n < 100,5
5	100,5 </= n < 300,5
6	300,5 </= n < 1000
7	1000,5 </= n

6.7.3. Metric groups

According to Hering et al. (2006a) metrics can be compiled to four different metric groups:

1. Composition and abundance metrics

2. Richness and diversity metrics

3. Sensitivity and tolerance metrics

4. Functional metrics.

Composition and abundance metrics

Composition and abundance metrics give the relative proportion of a taxon or taxonomic group with respect to its total number or abundance, respectively. Definition taken from Hering et al. 2006a.

Richness and diversity metrics

All metrics giving the number of species, genera, or higher taxa within a certain taxonomical entity, including the total number of taxa, all diversity indices. Definition taken from Hering et al. 2006a.

Sensitivity and tolerance metrics

All metrics related to taxa known to respond sensitively or tolerantly to a stressor or a single aspect of the stressor, respectively. Definition taken from Hering et al. 2006a.

Functional metrics

Functional metrics reflect certain overlaying ecological features of animals, e.g. substrate or current preference. Consequently, taxonomic units could be summarized to functional groups. Functional groups considered for the calculation of the HKHindex are "Pelal preference", "Lithal preference", "Lithobiont", "Lithophil" and "Lotic preference". These metrics have been developed within the ASSESS-HKH project.

Definition of functional metrics:

"Pelal preference": Taxonomic units assigned to this group show a significant preference for the substrate type mud (= Pelal).

"Lithal preference": Taxonomic units assigned to this group show a significant preference for stony substrate types. Stony substrate types (= Lithal) comprise size groups from fine sized gravel to blocks and bedrock.

"Lithophil": Taxonomic units assigned to this group show a significant preference for stony substrate types, but can be found also on other substrate types.

"Lithobiont": Taxonomic units assigned to this group have been found only on Lithal.

"Lotic preference": Taxonomic units assigned to this group prefer higher flow velocities (lotic). Actually, lotic animals prefer moderate (flow velocities > 10 cm / s) to very fast current velocities (flow velocities > 100 cm / s).

Tables 12 and 13 show the assignment of selected taxa from the Hindu Kush-Himalaya region to certain current velocities and substrate types.

Table 19: Assignment of selected taxa from the HKH region to high flow velocities.

Lotic animals
Ephemeroptera
Acentrella sp.
Baetiella sp.
Drunella sp.
Rhithrogena sp.
Heptageniidae
Epeorus bi-spinosa
Trichoptera
Glossosomatidae
Glossosomatinae
Agapetinae
Hydroptilidae
Goera sp
Cheumatopsyche sp
Rhyacophila sp
Setodes sp
Psychomyiia sp
Uenoa sp
Plecoptera
Perlidae
Perlinae
Diptera
Antocha sp
Hexatoma sp

Table 20: Substrate preferences of selected taxa from HKH region.

Lithal		**Mud**	**Sand**
Lithobiont	**Lithophil**		
Ephemeroptera	**Ephemeroptera**	**Bivalvia**	**Bivalvia**
Cincticostella sp.	Baetidae	*Corbicula striatella*	*Corbicula striatella*
Crinitella sp.	Baetinae	*Lamellidens consobri-nus*	**Gastropoda**
Drunella sp.	*Baetiella sp.*	*Lamellidens corrianus*	*Thiara lineata*
Epeorus sp.	*Acentrellla sp.*	*Lamellidens narain-porensis*	
Epeorus "bispinosus"	Hepatgeniidae	*Pisidium clarkeanum*	
Rhithrogena sp.	*Cinygmina sp.*	*Radiatula caerulea*	

Lithal		Mud	Sand
Lithobiont	**Lithophil**		
Trichoptera	*Notacanthurus sp.*	*Radiatula occata*	
Glossosomatidae	*Choroterpes sp.*	**Gastropoda**	
Agapetinae	**Coleoptera**	*Bellamya (Filo-paludina) bengalensis*	
Glossosomatinae	Eubrianacinae	*Digoniostoma pul-chella*	
Rhyacophila sp.	Scirtidae	*Melanoides tubercula-tus*	
Setodes sp.	**Odonata**	*Thiara lineata*	
Stenopsyche sp.	Gomphidae	*Thiara scabra*	
Uenoa sp.	**Plecoptera**	**Oligochaeta**	
	Nemouridae	*Branchiura sowerbyi*	
	Trichoptera		
	Goera sp.		
	Brachycentridae		
	Hydropsychinae		
	Hydropsyche "white stripe"		
	Hydropsyche sp.		
	Cheumatopsyche sp.		

6.7.4. Examplary calculation of metrics

You will find exemplary explanations and calculations of metrics in the following. All metric calculations are based on the taxalist given in Table 21.

Table 21: Fictive taxalist that derived from a fictive sampling site in the stream type Himalayan Subtropical Pine Forest.

Taxa list	Order	Family	#	nu_	[%]	HKH Bios Score (Nepbios)
Ephemeroptera	Ephemeroptera	-	50	4	1.7	
Baetidae	Ephemeroptera	Baetidae	25	3	0.9	
Baetis species 1	Ephemeroptera	Baetidae	20	3	0.7	
Baetis species 2	Ephemeroptera	Baetidae	10	2	0.4	
Baetiella sp	Ephemeroptera	Baetidae	1	1	0.03	8
Acentrella sp	Ephemeroptera	Baetidae	15	3	0.5	8
Leptophlebiidae	Ephemeroptera	Leptophlebiidae	20	3	0.7	7
Heptageniidae	Ephemeroptera	Heptageniidae	30	3	1	8
Epeorus sp	Ephemeroptera	Heptageniidae	20	3	0.7	8
Epeorus bispinosa	Ephemeroptera	Heptageniidae	15	3	0.5	
Plecoptera	Plecoptera	-	15	3	0.5	
Perlidae	Plecoptera	Perlidae	10	2	0.4	8
Perla sp	Plecoptera	Perlidae	5	2	0.2	
Hydropsychidae	Trichoptera	Hydropsychidae	250	5	8.7	7
Cheumatopsyche sp	Trichoptera	Hydropsychidae	25	3	0.9	7
Hydropsyche sp	Trichoptera	Hydropsychidae	300	5	10.4	
Rhyacophilidae	Trichoptera	Rhyacophilidae	25	3	0.9	8
Rhyacophila sp	Trichoptera	Rhyacophilidae	45	4	1.6	8
Uenoa sp	Trichoptera	Uenoidae	5	2	0.2	10
Elmidae	Coleoptera	Elmidae	25	3	0.9	8

Taxa list	Order	Family	#	nu_	[%]	HKH Bios Score (Nepbios)
Simuliidae	Diptera	Simuliidae	500	6	17	7
Chironomidae	Diptera	Chironomidae	550	6	19.1	1
Antocha sp	Diptera	Tipulidae	25	3	0.9	
Tipulidae	Diptera	Tipulidae	90	4	3.1	7
Oligochaeta	Oligochaeta	-	800	6	27.8	2
SUM		**11 families**	**2876**			**112**

Metric name: # fam

Number of all families in the sample

Calculation of the metric:

Example derived from Table 21: Baetidae, Leptophlebiidae, Heptageniidae, etc.

Result: # fam = 11

Metric name: nu_Eph

Number of all Ephemeroptera individuals transformed into a single abundance class

Calculation of the metric:

Example derived from Table 21: 50 ind Ephemeroptera + 25 ind Baetidae + 25 ind *Baetis species 1* + 10 *Baetis species* 2 + *Baetiella* 1+ *Acentrella* 15 = 206; transformed into abundance class = 4 (see Table 18)

Result: nu_Eph = 4

Metric name: [%] Dip ind

Relative share of Diptera individuals to total amount of all individuals of the sample

Calculation of the metric:

Example derived from Table 21: 500 Simuliidae + 550 Chironomidae + 25 *Antocha* +90 Tipulidae = 1165 Diptera individuals; number all individuals = 2876; consequenty: $\frac{1165}{2876} * 100 = 40.5\%$

Result: [%] Dip ind = 40.5

Metric name: EPTC

The abbreviation EPTC stands for Ephemeroptera (Mayflies), Plecoptera (Stoneflies), Trichoptera (Caddiesflies) and Coleoptera (Water beetles). In the following different examples are given how to calculate the metric with different taxonomical and abundance untis, respectively.

Calculation of the metric:

a) # EPTC fam: sum of all EPTC families

Example derived from Table 21: E = 3 (Baetidae, Leptophlebiidae, Heptageniidae), P = 1, T = 3, C = 1

Result: # EPTC fam = 8

b) [%] EPTC fam: relative share of EPTC families to sum of all families

Example derived from Table 21: sum all families 11; # EPTC fam 8; consequently: $\frac{8}{11} * 100 = 72.7$ %

Result: [%] EPTC fam = 72.7

c) # EPTC ind: sum of all EPTC individuals

Example derived from Table 21: Ephemeroptera 50 + Baetidae 71 + Leptophlebiidae 20…+…+… Perlidae 10 + Perla 5 +…..Elmiidae 25 = 911

Result: # EPTC ind = 911

d) [%] EPTC ind: relative share of EPTC individuals

Example derived from Table 21: # EPTC ind 911, sum all ind 2876; consequently: $\frac{911}{2879} * 100 = 31.6$ %

Result: [%] EPTC ind = 31.6

Metric name: BaSiHyCh

The abbreviation of the metric stands for Baetidae, Simuliidae, Hydropsychidae and Chironomidae

Calculation of the metric:

a) # BaSiHyCh: Sum of all individuals belonging to the four families.

Example derived from Table 21: Baetidae (Baetidae + *Baetis species 1* + *Baetis species 2* + *Baetiella* + *Acentrella*) 71 + Simuliidae 500 + Hydropsychidae 575 + Chironomidae 550 = 1696

Result: # BaSiHyCh = 1696

b) nu_ BaSiHyCh: individuals of <u>each</u> family are transformed into abundance class value (see Table 11). Then, the new values for the four families are summed up giving the metric value.

Example derived from Table 21: Baetidae 4 (= 71 ind transformed into abundance class) + Simuliidae 6 (500 ind) + Hydropsychidae 6 (575 ind) + Chironomidae 6 (550 ind) = 22

Result: nu_ BaSiHyCh = 22

Metric name: nu_Lithal

Sum of taxonomic units that have a significant preference to stony substrate types. Abundances transformed into abundance classes.

Calculation of the metric:

Example derived from Table 21: Taxa in the taxa list that are assigned to stony substrate types (see Table 3) are: Baetidae 3 (= 25 ind transformed into abundance class, see Table 18), *Acentrella* 3, Heptageniidae 3, *Epeorus sp* 3, *Epeorus bispinosa* 3, *Hydropsyche* 5, *Rhyacophila* 4, *Uenoa* 2

3 + 3 +3 +3 +3 + 5 + 4 +2 = 26

Result: nu_Lithal = 26

Metric name: ASPT

ASPT = BMWP, which is the sum of scoring taxa, divided through the number of all families. The score reflects the sensitivity of a given taxon to pollution (see Table 14). The scores of the taxa in the NEPBIOS derived from the BMWP. The HKHBios scores were developed on basis of the NEPBIOS.

Calculation of the metric:

Example derived from Table 21: ASPT = Baetiella (8 = HKHBios score) + Acentrella 8 + Leptophlebiidae 7+ Heptageniidae 8 +…+…+Tipulidae 7 + Oligochaeta 1 = 112, number of families 11; consequently: $\frac{112}{11} = 10.18$

Result: BMWP = 10.18

6.7.5. Calculation of the HKHindex

In the following the HKHindex is calculated based on the taxalist given in Table 21. The according metric values are presented in Table 226.

Table 23: MMI calculation for the stream type (ecoregion) Himalayan Subtropical Pine Forest. For abbreviations see Table 14

Metric	**Metric value**	**Metric value standardized (0 and 1,see Formula 2); Upper and Lower anchor values have been taken from Table 15**
[%] EPT fam	63.6	$= \frac{63.6 - 0}{68.75 - 0} = 0.92$
D (E)	0.67	0.7
D (M)	3.14	0.33
ASPT	10.18	1
MMI (mean of all metric values):		**0.74**

In a final step the calculated MMI is assigned to a river qualtiy class (see

Table 16). River quality classes have been defined for each stream type seperately. In the example (see Table 14) we assumed that the sample was taken in the stream type (ecoregion) Himalayan Subtropical Pine Forest. Hence, the sampling site is assigned to a river quality class "high", i.e. reference conditions.

7. Summary

Within the workpackages 4 and 5 of the ASSESS-HKH project a sampling design and assessment methods for the evaluation of the river quality in the HKH-Region were developed.

Sampling and sample treatment

A sampling design and procedure was developed that follows the multi-habitat approach (Moog 2007). The distribution of sampling units reflects the distribution of habitats. A sample consists of 20 "sampling units" taken from all representative habitat types at the sampling site. If samples for the HKHscreening method (e.g. for water quality mapping) are taken, ten sampling units are sufficient. In case of heavily deteriorated rivers with a poor fauna even less than 10 sampling units are allowed. Samples should be taken using an AQEM/STAR net sampler for wadeable rivers and a grab sampler for large rivers. The sample treatment, including processing in field and lab are described in detail.

Assessment methods

The assessment systems developed within the project are using benthic macroinvertebrates as bio-indicators and can serve as a basis for policy development, trans-national water resource planning and ecosystem management. A three-tier methodology representing different degrees of precision was developed and validated for the ecoregions under study.

The **HKHscreening** method provides an overview on the river quality status of an investigation site. This methodology is based on sensoric criteria and the biota that can be identified in the field with a special focus on the bioindicative value of benthic invertebrates.

The **HKHbios** is a score-based assessment method on mostly higher taxonomic bioindication units (genus, family, order) that needs only simple data processing. Taxa were scored in dependency of their distribution along different quality classes and expert judgement. The final scoring list includes 199 taxa in total. It comprises of 2 taxa on class level, 139 taxa on family level, 4 taxa on genus subfamily level, 51 genera and 3 taxa on species level. For mountainous Ecoregions (IM0301, IM0401, M0166, IM0403) scores are available for 186 taxa, for lowlands (IM0120) 155 taxa were scored. The HKHbios is calculated as a weighted mean of scores. The resulting value (HKH-biotic score) can directly be allocated to the corresponding river quality class. Threshold values for river quality classes are defined individually for ecoregions.

At last, based on the best available taxonomic resolution, the **HKHindex** (multi-metric assessment method) was designed. A stressor-specific PCA analysis was performed to detect those metrics that discriminate best between different degrees of deterioration. Metrics were selected individually for ecoregions. For the calculation of the Multimetric Index the involved metrics are transformed into unitless “scores” with values between 0 and 1. Finally, the Multimetric Index is calculated by averaging the scores and can directly be assigned to a river quality class. As the HKH Index is based on higher taxonomical resolution, the application is also restricted to adequate datasets (20 MHS samples, determination to genus/species level). Due to the lack of further data (only datasets from the HKH-Project are available up to now) and difficulties in taxonomy the significance is probably limited at the moment. To improve the accurateness and explanatory power of the developed index further data will be needed and increased autecological knowledge as well as larval taxonomy are urgently needed prerequisites. The data at hand only allow a rough estimation of ecological quality class and have to be calibrated in concordance with results of HKHscreening and HKHbios.

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