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## **Deliverable D3.3-4: Assessment of ecological effects of hydromorphological lake shore alterations and water level fluctuations using benthic macroinvertebrates**

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PP	Restricted to other programme participants (including the Commission Services)	
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## Non-technical summary

The Directive 2000/60/EC, commonly known as the Water Framework Directive (WFD), legally requires EU member states to assess the ecological status of their surface waters. Biological assessment methods should be used based on biological quality elements (BQEs), i.e. fish, phytoplankton, macrophytes, phytobenthos and benthic invertebrates. Benthic invertebrates have been demonstrated to respond to hydromorphological alterations of lakes. However, so far no assessment methods based on this pressure-response-relationship are available at European level.

Within the EU project WISER, it was possible to obtain a methodologically homogeneous dataset on eulittoral invertebrates, this dataset was collected within the Wiser WP3.3 field campaign (see D3.3.2 for details), which allowed for the development of multimetric indices for hydromorphological degradation. Hence, this deliverable documents several approaches for the development of invertebrate based metrics suitable for indicating hydromorphological alterations in (sub-)Alpine and Central Baltic lakes. All information will be made publicly available via the project's website <http://www.wiser.eu>.

## 1 Introduction and aims

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The Directive 2000/60/EC, commonly known as the Water Framework Directive (WFD), legally requires that EU member states complete an ecological assessment of the functioning and structure of aquatic ecosystems. This includes several biological quality elements (BQEs), i.e. fish, phytoplankton, macrophytes, phytobenthos and benthic macroinvertebrates. Benthic invertebrates constitute an important link between primary producers, detrital deposits and higher trophic levels in lake ecosystems and are an integral part within food chains as well as lake productivity, nutrient cycling and decomposition. Hence, benthic invertebrates do not only indicate eutrophication, for which mainly phytoplankton and macrophytes are used, but also hydromorphological degradation.

The habitat of bottom-dwelling, benthic invertebrates in lakes can be divided into 3 major zones: eulittoral, sublittoral and profundal which harbour distinct macrozoobenthos communities. The uppermost eulittoral zone is inundated at high water levels and falls dry at low water levels. Hence, it is especially sensitive to wave action, water level changes and structural lake shore alterations that have detrimental impacts on the littoral zone through the alteration and/or loss of littoral habitats. Anthropogenic water level fluctuations severely affect macroinvertebrates in reservoirs and regulated lakes since their low mobility restricts the ability of benthic organisms to follow the receding water. Ship-induced wave action often substantially exceeds the strength and impact of natural waves and affects the habitat characteristics of macroinvertebrate organisms by influencing sediment particle size distribution and the structure and composition of macrophyte patches. Shore line development (e.g. shore protection by rip-rap or vertical

walls) has been shown to have detrimental impacts on the littoral zone through the alteration or loss of littoral mesohabitats such as stones, sand, macrophytes and woody debris. Such anthropogenic hydromorphological degradation is reflected in the diversity and composition of eulittoral macrozoobenthos communities and demonstrates the use of macrozoobenthos organisms as indicators for this. Furthermore, it has been shown that the trophic state influences the composition of eulittoral macroinvertebrate communities to a lesser extent than has been previously reported for profundal habitats and they are hence weak indicators of eutrophication.

In order to obtain an ecological quality assessment of lakes, benthic macroinvertebrates, as one of the Biological Quality Elements (BQEs), must be analysed in terms of taxonomic and functional composition, abundance, disturbance sensitive taxa, diversity and absence of major taxonomic groups.

This can be achieved by means of metrics and multimetric indices. A metric is a summary measure of a part or process of a biological system which should change in value along a gradient of anthropogenic influence, while a multimetric index is a combination of standardized single metrics. Multimetric indices are often used in assessment systems because they synthesize information on different biological attributes into a single index value.

So far, only methods to assess hydromorphological degradation of lakes exist for several EU member states and have been successfully intercalibrated in the Central Baltic and Alpine Geographical Intercalibration Groups (GIGs). However, these methods differ largely in their used sampling protocols, sampling devices and their taxonomic resolution. Within the EU project WISER, it was possible to obtain a methodologically homogeneous dataset on eulittoral invertebrates from 7 countries in Northern, Western, South and Central Europe which allowed for the development of multimetric indices for hydromorphological degradation. Hence, in this deliverable we present several approaches for the development of invertebrate based metrics and multimetric indices suitable for indicating hydromorphological alterations in lowland lakes of the Northern, Central-Baltic/Atlantic and Mediterranean GIG regions in Europe.

## 2 Methods

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### 2.1 Design of sampling schedule

In contrast to other biological quality elements, there were few databases existing in European member states that contained results from surveys on littoral invertebrates, with related data on potential morphological degradation of the sampling sites. Hence, WP 3.3 was scheduled to analyze on the one hand existing (mostly heterogeneous) data from mostly national monitoring activities, but on the other hand to conduct a comprehensive field sampling campaign within WISER.

According to the WISER Description of Work, ‘the ultimate aim of the field exercise will be to quantify the confidence for the classification of BQE metric results. Variability in metric scores

associated with spatial, temporal and analytical variability will be examined. Spatial variability may include within-type (different lakes), within-lake (different locations) and within-location (sample and sub-sample) variability. Temporal variability (seasonal, inter-annual) will be examined, where possible, through analysis of existing long-term datasets.’

WISER WP 3.3 focused on hydromorphology assessment, and on disentangling the effects of eutrophication and hydromorphological degradation. Hence, the field sampling campaign followed a strategy to produce a dataset that would hold observations within the full range of these variables (independent variation), allowing sound statistical analyses. The resulting homogeneous dataset should enable unbiased analyses on the pressure sensitivity of metrics to hydromorphological alterations, and also the assessment of uncertainty associated with sampling procedures used. The effect of uncertainty produced from various sources of spatial, temporal and analytical variability can also be studied. Using estimates of time (cost) per sample and the uncertainty associated with each technique, it should be possible to quantify the cost associated with varying levels of precision – the cost effective precision of sampling.

It was agreed at the WISER kick-off meeting not only to apply the standard (habitat-specific 1 m<sup>2</sup>) sampling protocol, but to take a number of additional samples with a low-cost method involving composite habitat samples (using 1 min time-limited sampling) at each invertebrate sampling site. The objective was to produce a nested, hierarchically structured dataset that facilitates analyses of uncertainty. It was agreed that sampling should be performed once at 9 sites per lake, with 3 sites representing low hydromorphological pressure (reference/high status), intermediate hydromorphological pressure (good/moderate status), and high hydromorphological pressure conditions (poor/bad status). This sampling scheme was intended to be applied to 9 lakes per country covering the eutrophication range with 3 lakes at reference eutrophication level, 3 at intermediate eutrophication level, and 3 at high eutrophication level. As there were 4 major partners in the WP performing the field campaigns and 36 lakes were planned to be sampled, with a maximum of 5 habitat-specific samples. One additional composite sample was to be collected at each sampling site of each lake, summing up to a maximum of 486 samples to be collected in each country. Of the 36 lakes to be sampled for WP 3.3, 15 lakes were initially planned to be sampled for the cross-BQE exercise to allow harmonisation of assessment results.

However, it transpired during the WISER kick-off meeting that there were few available lakes with existing monitoring data for several Biological Quality Elements. Therefore not many lakes met the requirements for the WP 3.3 field campaign, which focused on within lake variation in hydromorphological pressure, while at the same time meeting the requirements for WPs 3.1, 3.2 and 3.4, which focused on lake eutrophication pressure.

Therefore, it was not possible to identify 15 cross-BQE lakes as part of the WP 3.3 specific lake list. Additional sampling was performed voluntarily by IGB in Denmark, SYKE in Finland and by CEH in the UK. Lakes were selected from three common lake types representing three European regions (GIG regions):

- Northern: Low alkalinity, deep lake (L-N2b)
- Central-Baltic / Atlantic: High alkalinity, shallow lake (L-CB1 / L-A1/A2)
- Mediterranean: High alkalinity, deep reservoir (L-M8)

## 2.2 Implementation of the WISER field sampling campaign

A common WP 3.3 lake macroinvertebrate sampling protocol was agreed among all WP 3.3 partners during a WP 3.3 workshop in Berlin in April 2009. Morphological alterations were classified as “medium alteration” (e.g. riparian clear-cutting, recreational beaches) and “high alteration” (e.g. retaining walls, rip-rap). Macroinvertebrate samples were collected from 3 high alteration, 3 medium alteration and 3 natural sites within each lake. Each sampling site represents a shoreline section of minimum 25 m length representing either high alteration, medium alteration or natural sites. If neither of the two alteration types was present at a lake, the total number of sampling sites per lake was still kept constant (9 sites per lake). Sampling was carried out in the season commonly used for aquatic invertebrate surveys in each ecoregion. At each sampling site one composite sample was collected in addition to a number of habitat specific samples (minimum number of habitats = 3; number of habitat samples kept constant among all sampling sites and lakes in each country, even at sites which only showed one or two habitats) plus one composite sample were collected.

Composite samples comprised a standardised 1 min macroinvertebrate sample involving sampling of all available habitats in proportion to their availability within each sampling site. Habitat-specific samples comprised the collection of 1 m<sup>2</sup> samples per habitat, which is an area that will comprise most of the species present (Schreiber & Brauns 2010). This agreed overall sampling schedule also reflects the outcome of extensive discussions on a balanced sampling scheme held with WP 6.1 (Uncertainty) at the WISER kick-off meeting in Mallorca. Originally it was planned that nine lakes should be selected in Sweden, Ireland, Germany and Italy which should cover a range of trophic pressures (oligotrophic, mesotrophic, eutrophic states represented ideally by 3 replicates each) and ideally show two different shoreline morphological alteration types in each of the selected lakes.

The actually planned number of 36 lakes to be sampled in WP 3.3 increased to 51 lakes, as 15 additional lakes were selected for the WISER uncertainty field exercise, to be sampled for macroinvertebrates. These additional 15 did not always have all necessary morphological alteration types. In Italy, 2 additional lakes were sampled, in order to adequately cover both Italian lake areas in Northern and Central Italy. This totals to 39 lakes which were sampled according to the agreed WISER WP 3.3 sampling protocol. A further 12 lakes were sampled for macroinvertebrates in order to meet the requirements of the WISER uncertainty field exercise, including cross-BQE comparisons. Only those cross-BQE lakes which fitted the WISER WP 3.3 sampling protocol were sampled accordingly. From the additional lakes only composite samples were collected.

During the sampling protocol workshop in Berlin an introduction to the Lake Habitat Survey (LHS) methodology (Rowan et al. 2004, 2006) was given, and it was agreed to conduct a complete LHS for each lake as well as hab-plot/site specific LHS at each macroinvertebrate sampling site.

Sampling for lake benthic macroinvertebrates using the agreed WP 3.3 common sampling protocol was completed in all countries (Finland: 4 lakes, September/October 2009;

Germany/Denmark: 11 lakes, April/May 2010; Ireland: 9 lakes, April/May 2009; Italy: 15 lakes (8 lakes in the subalpine and 7 lakes in the Mediterranean region), August-November 2009; Sweden: 9 lakes, November 2009; UK: 3 lakes, October 2009). In some lakes, the general sampling schedule had to be modified, as not all pressure levels were encountered and sometimes only composite samples were taken in order to keep total number of samples within feasible/resource limits. Whole lake and hab-plot/site-specific LHS has been carried out in all lakes in all countries (Finland: September/October 2009; Germany: August 2010; Ireland: September 2009, Italy: October 2009; UK: October 2009).

Subsequently, the macroinvertebrate samples were processed, which took 1 – 1.5 years at the various institutions. During the WISER kick-off meeting in Mallorca it was agreed to collect additional pooled “composite” macroinvertebrate samples (pooling all habitats) and to test the usefulness of composite sampling as an alternative cost-efficient assessment method. To help assess the costs saved and the possible loss in assessment precision by collecting composite instead of habitat specific macroinvertebrate samples. At each sampling site one composite sample was collected in addition to the three habitat specific samples during the field campaign of the WP 3.3 lake macroinvertebrate team.

The collection of composite samples and habitat samples generally involved the same amount of time, with the average time to collect each composite or habitat sample accounting for 0.4 hours. For the composite sampling method the collection of only 1 sample per site would be necessary, the habitat specific sampling method, however, involves the collection of at least 3 different habitat samples (ideally sand, stones and macrophytes) and is thus more time and cost intensive (composite sampling per site = 0.4 hours; habitat specific sampling per site = 1.2 hours; average over all countries). The time and cost-effectiveness of the composite sampling method is, moreover, supported by time estimates for sorting of macroinvertebrate samples per site (example from lake Werbellinsee, Germany: average time to sort a composite sample per site = 10.2 hours; average time to sort 3 habitat specific sample per site = 30 hours). However, the usefulness of this apparently more cost-efficient composite sampling method for monitoring of lakes, still requires a more thorough analysis of the complete WISER WP 3.3 lake macroinvertebrate data set. It remains to be seen whether or not the results generated using the composite sampling method are as precise as the more time intensive habitat specific sampling method.

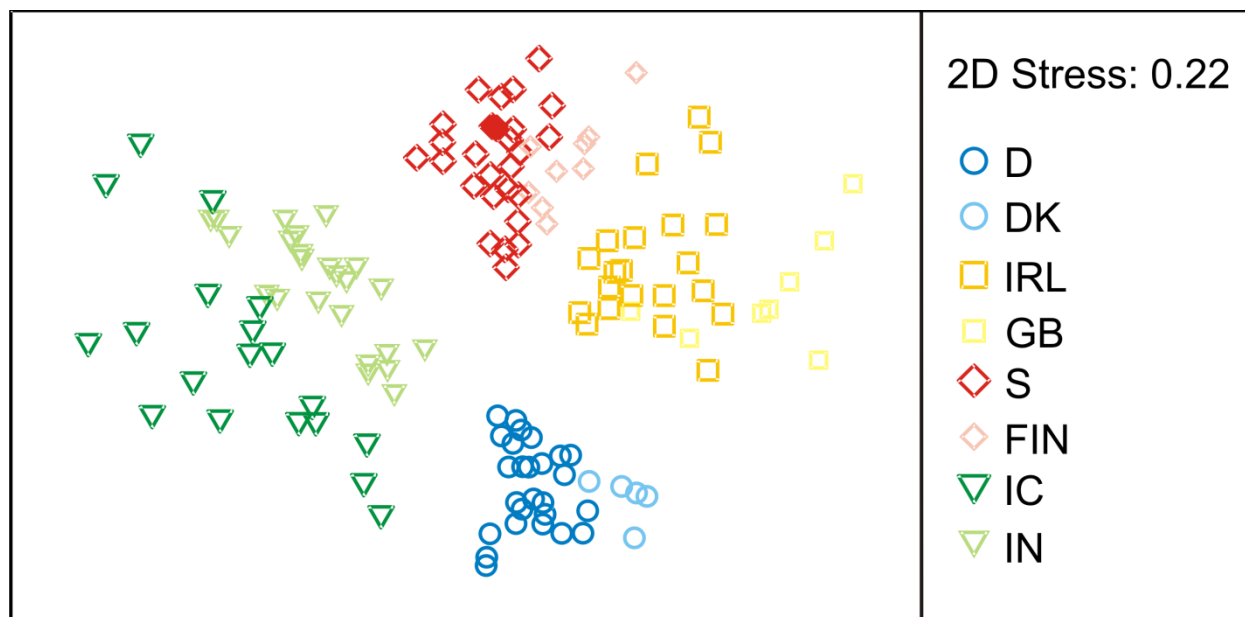
### **3 Stressor index**

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In conjunction with the macrozoobenthos sampling, properties of the physical structure of the lake shores were recorded using the Lake Habitat Survey (LHS) method (Rowan 2008). These data were then used to construct a stressor index representing the degree of hydromorphological degradation, which was needed to calibrate the final multimetric index and its component metrics.

### 3.1 Development of a typology based on faunal assemblages

Since the sampled lakes were located in several biogeographical regions, the development of a typology based on dissimilarities in macrozoobenthos assemblages was necessary to account for natural differences in benthic macroinvertebrate community composition. A quantitative biocoenotic differentiation between countries/regions was achieved through an Analysis of Similarities (ANOSIM) in macroinvertebrate community compositions together with a Multidimensional Scaling (MDS) plot (Figure 1). Results showed marked differences among countries (and between the regions of northern and central Italy), indicated by high R values in the ANOSIM analysis (Table 1). The ANOSIM R values for the strength of community composition differences for the pairwise country/region comparisons among either Germany/Denmark, Ireland/United Kingdom, Sweden/Finland or central Italy/northern Italy were lower than 0.85. Samples from lakes in Denmark (2 lakes), United Kingdom (3 lakes) and Finland (4 lakes) could not be analysed specifically because of low observation numbers. Based on this typology, a stressor index and a multimetric index were developed for each of the four country (region) pairs Germany/Denmark, Ireland/United Kingdom, Sweden/Finland and central Italy/northern Italy.



**Figure 1:** Multidimensional scaling (MDS) plot of the macroinvertebrate community composition in the studied lakes. The plot clearly mirrors the geographical distances among the regions and countries sampled (D = Germany, DK = Denmark, IRL = Ireland, GB = United Kingdom, S = Sweden, FIN = Finland, IC = central Italy, IN = northern Italy).



**Table 1:** ANOSIM analysis for biogeographical differences between the macrozoobenthos communities (D = Germany / DK = Denmark, IRL = Ireland / GB = United Kingdom, S = Sweden / FIN = Finland, IC = central Italy / IN = northern Italy).

Comparison	R	p
IN - IC	0.578	0.001
IN - IRL	0.955	0.001
IN - S	0.964	0.001
IN - DK	0.97	0.001
IN - D	0.949	0.001
IN - UK	0.992	0.001
IN - FIN	0.972	0.001
IC - IRL	0.909	0.001
IC - S	0.913	0.001
IC - DK	0.814	0.001
IC - D	0.892	0.001
IC - UK	0.901	0.001
IC - FIN	0.847	0.001
IRL - S	0.868	0.001
IRL - DK	0.847	0.001
IRL - D	0.9	0.001
IRL - UK	0.547	0.001
IRL - FIN	0.847	0.001
S - DK	0.992	0.001
S - D	0.967	0.001
S - UK	0.992	0.001
S - FIN	0.692	0.001
DK - D	0.827	0.001
DK - UK	0.911	0.003
DK - FIN	0.998	0.002
D - UK	0.99	0.001
D - FIN	0.987	0.001
UK - FIN	0.933	0.001

### 3.2 Development of a stressor index

A wide range of Physical Lake Habitat Survey methods to describe the morphology and habitat complexity of lake shores exists, for example in Germany the HML [HydroMorphology Lakes] protocol developed by Ostendorp et al. (2004, 2008). This method is based on the classification of the naturalness of littoral and riparian structures in aerial photographs combined with field assessments. Similar methods have been also developed by the Ministry for Agriculture, Nature Conservation and Consumer Protection Mecklenburg-Vorpommern in Germany (Ministerium für Landwirtschaft, Naturschutz und Verbraucherschutz Mecklenburg-Vorpommern 2004) and in Italy by Siligardi et al. (2010) (SFI = Lake Shorezone Functionality Index).

In the UK, intensive research has been done to develop a Lake Habitat Survey (LHS) method (Rowan et al. 2004, 2005, 2006; Rowan 2008), analogous to the widely applied River Habitat Survey (RHS, Raven et al. 1997, 1998a, 1998b, 2000). The LHS protocol is not based on aerial photographs, but covers an extensive array of environmental parameters that are assessed by field visits. The basic LHS protocol allows the calculation of the Lake Habitat Quality

Assessment (LHQA) Score which assesses habitat diversity, physical structure and the presence of ecologically valuable habitats (Rowan et al. 2004, 2006). However, since the LHQA scores assign a value to the whole lake, Mc Goff & Irvine (2009) developed a sampling site-specific LHQA Score, the HabQA that is derived from 10 parameters at the sampling site (i.e. habplot in the terminology of the LHS manual) level in order to assess the single sites (for a detailed description see McGoff et al. 2009). The HabQA correlated well with taxa richness (Pearson correlation,  $r = 0.62$ ), but its development was based on only one lake (Lough Carra in the Republic of Ireland) and hence required further testing. We tested the general suitability of the HabQA on 11 lakes in Germany and Denmark that were sampled within the WISER WP 3.3 and found a lower correlation with taxa richness (Spearman Rank correlation,  $Rho = 0.4$ ) than in the study of McGoff et al. (2009) (Miler, Porst and Gräwe unpublished results). Furthermore, several values of components of the HabQA differ from the data from Lough Carra. Lough Carra contains a high number and diversity of wetlands, whereas in the 11 German and Danish WISER lakes almost no wetlands occurred. Another parameter is the presence of a trash line that is more frequently present at the medium alteration sites compared with natural sites, and is completely missing at high alteration sites due to the fact that the trash does not gather on riprap or stone walls. This showed the need to develop a new stressor index suitable for indicating hydromorphological differences between the three alteration types that were sampled within the WISER WP 3.3.

First, the LHS results were tested with an Analysis of Variance (ANOVA) for differences among the three pre-defined alteration types. Seven environmental variables were identified that differentiated well between the alteration types, which were hence regarded as potential stressor index components. These variables were: “Number of habitats”, “Habitat diversity”, “Total PVI”, “Sum of macrophyte types”, “Sum of vegetation cover types”, “Sum of Course Woody Debris/roots/overhanging vegetation”, “Pressure index” and “Natural/Artificial dominant land cover type” (for details see Table 2). The calculated variables are either based on presence (= 1) / absence (= 0) or on classified values of areal cover / volume inhabited (tick = 0.5 (> 0 - 1 %), 1 (> 1 - 10 %), 2 (> 10 - 40 %), 3 (> 40 - 75 %), 4 (> 75 %)) of LHS parameters. Table 3 and 4 explain in more detail how the “Pressure index” and the “Natural/artificial dominant land cover type” are composed. These stressor index components were then (separately for each biogeographical region) normalized to values ranging from 0 to 1. Hereby the 5 % percentile value was rescaled to 0 and the 95 % percentile value was rescaled to 1. Values smaller than 0 or larger than 1 were reset to 0 and 1, respectively. In a second step these values were classified in a scale from 1 (best condition) to 5 (worst condition). The scaling for all stressor index components after Hering et al. (2006) and Vlek et al. (2004) was as follows: 0 - 0.2 = 5, 0.21 - 0.4 = 4, 0.41 - 0.6 = 3, 0.61 - 0.8 = 2, 0.81 - 1 = 1. For the definition of the “pressure index” the scaling was then reversed since this variable correlates positively with increasing degradation. All variables were tested for differences between the 3 alteration types, separately for the 4 identified biogeographical regions, and as examples “Habitat diversity”, “Total PVI” and “Pressure index” are presented in Figure 1-3, respectively. The natural sites had almost always significantly lower stressor variable values than either high alteration sites, medium alteration sites or than both of them (Figures 1-3).

**Table 2:** Description of the components of the hydromorphological stressor index developed for the four biogeographical regions (D = Germany / DK = Denmark, IRL = Ireland / GB = United Kingdom, S = Sweden / FIN = Finland, IC = central Italy / IN = northern Italy).

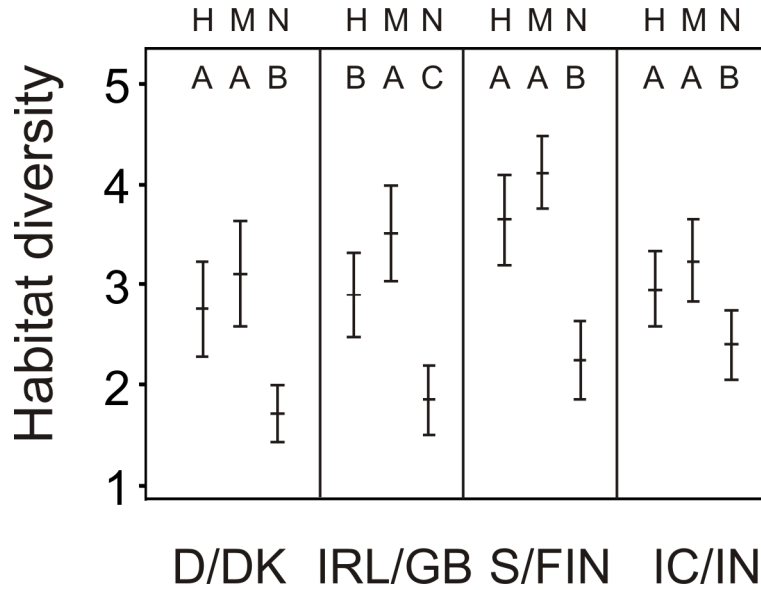
Stressor Index Component	Explanation
<b>Number of habitats</b>	Presence/absence of the following habitat types in the littoral zone: bedrock/boulder, cobbles/pebbles, sand/silt/clay, coarse woody debris, overhanging vegetation, roots, macrophytes
<b>Habitat diversity</b>	Shannon-Wiener diversity of the classified areal cover of the following habitat types in the littoral zone: bedrock/boulder, cobbles/pebbles, sand/silt/clay, coarse woody debris, overhanging vegetation, roots, macrophytes
<b>Total PVI</b>	Classified total percentage volume inhabited by macrophytes in the littoral zone
<b>Sum of macrophyte types</b>	Sum of the classified areal cover of the 12 macrophyte types (according to Rowan 2008) in the littoral zone
<b>Sum of vegetation cover types</b>	Sum of the classified areal cover of the 6 vegetation cover types (according to Rowan 2008) in the riparian zone
<b>Sum of CWD/roots/overhanging vegetation</b>	Sum of the classified areal cover of coarse woody debris (CWD), roots & overhanging vegetation in the littoral zone
<b>Pressure index</b>	Presence/absence of human pressures (assessed over the entire site, see Table 3). Pressure index = Number of Category 1 pressures (next to site) + 2* Number of Category 2 pressures (next to site) + 2 * Number of Category 1 pressures (at site) + 4 * Number of Category 2 pressures (at site). Each human pressure either exists at the site or next to the site, i.e. it can be counted as present/absent only once
<b>Natural/artificial dominant land cover type</b>	Presence/absence of natural/artificial dominant land cover type (according to Rowan 2008, see Table 4) in the riparian zone

**Table 3:** Parameters for the calculation of the stressor index component "Pressure index". Each parameter belongs either to Category 1 (low human pressure) or Category 2 (low human pressure).

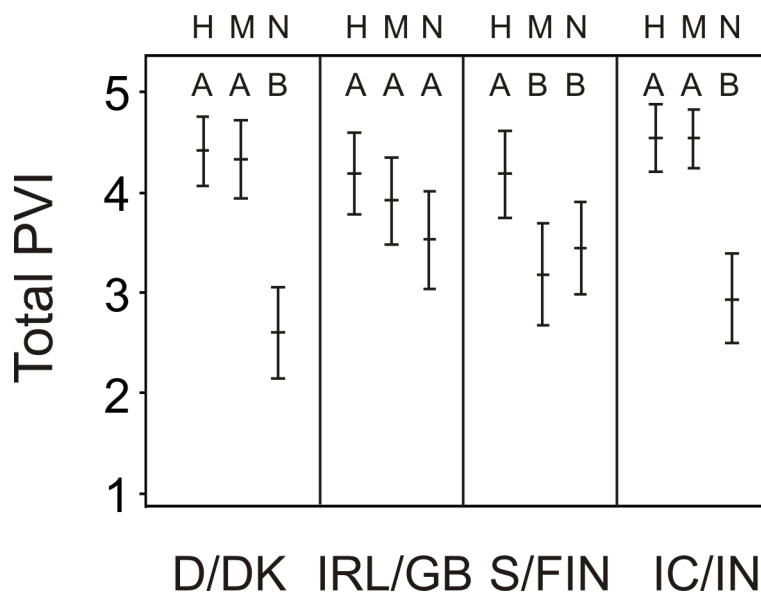
	Human pressure
<b>Category 1 (low human pressure)</b>	Unsealed tracks and footpaths; parks and gardens; coniferous plantation; orchard; improved grassland; other grazed land
<b>Category 2 (high human pressure)</b>	Commercial; residential; roads or railways; camping and caravanning; quarrying, mining, peat extraction; tilled land (arable); docks, harbours or marinas; hard bank engineering; soft bank engineering; flow and sediment control structures; piled structures; outfalls and intakes; flood walls/embankments; land claim; dumping; sediment extraction; floating/tethered structures; macrophyte manipulation; moorings; recreational pressures

**Table 4:** Determination of the “natural/artificial dominant land cover type” according to Rowan (2008).

	Land cover type
<b>Natural</b>	Broadleaf/mixed woodland (semi-natural); coniferous woodland (semi-natural); wetland (e.g. bog, marsh, fen); moorland/heath; rock, scree or sand dunes
<b>Artificial</b>	Broadleaf/mixed plantation; coniferous plantation; scrub and shrubs; orchard, artificial open water; open water; rough/unimproved grassland/pasture; improved/semi-improved grassland/pasture; tall herb/rank vegetation; tilled land; irrigated land; park, lawn or gardens; suburban/urban

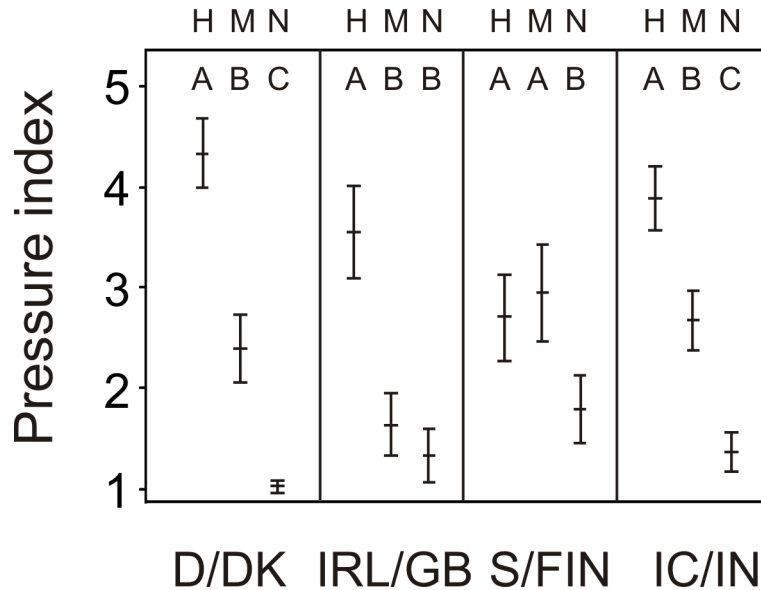


**Figure 1:** Statistical comparison of the stressor index component “Habitat diversity” (for details see Table 1) between the 3 alteration types with an ANOVA (H = high alteration, M = medium alteration, N = natural), performed separately for the 4 biogeographic regions (D = Germany, DK = Denmark, IRL = Ireland, GB = United Kingdom, S = Sweden, FIN = Finland, IC = central Italy, IN = northern Italy). A, B and C indicate significantly different mean values. The “Habitat diversity” is normalized from 1 to 5, 1 indicating the maximum and 5 the minimum value of “Habitat diversity”.



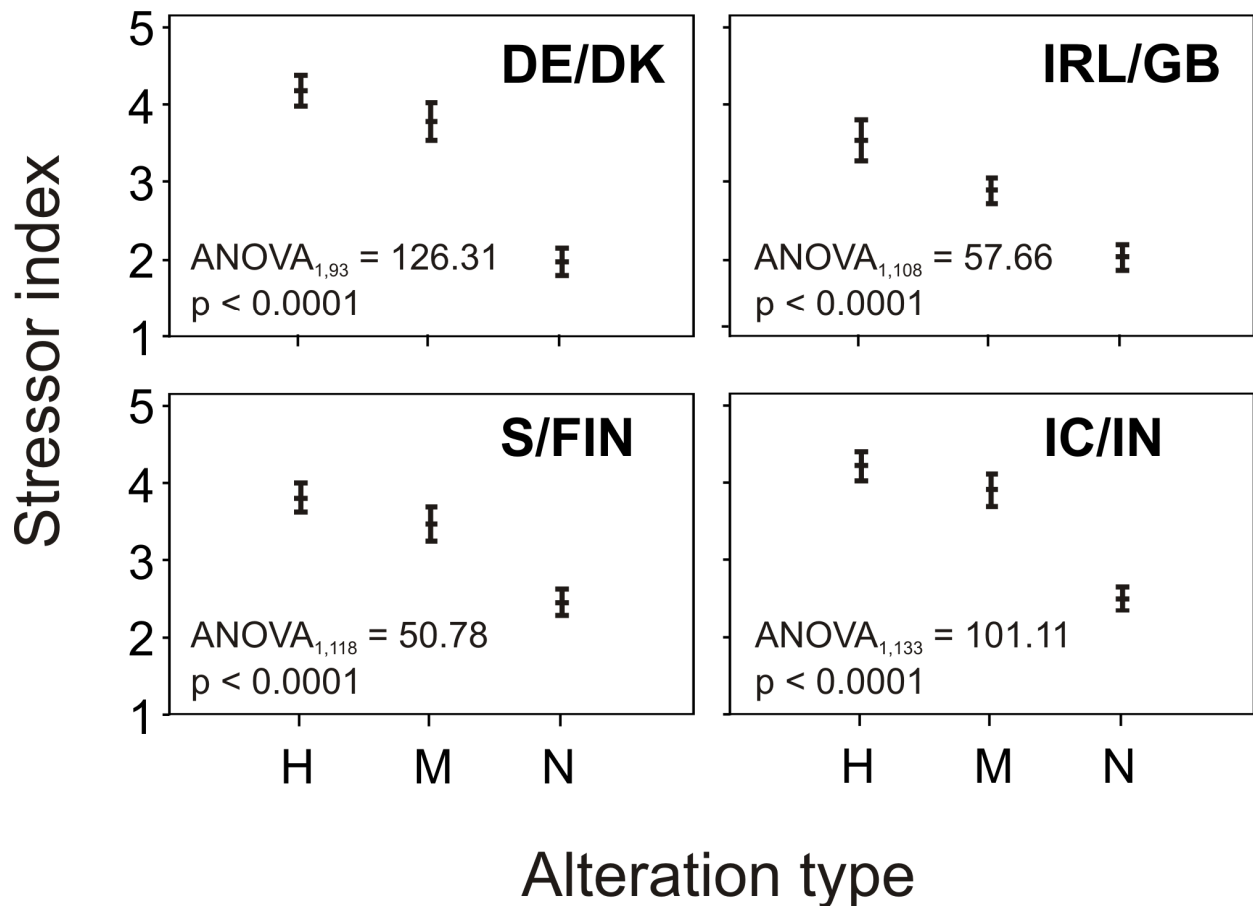
**Figure 2:** Statistical comparison of the stressor index component “Total PVI” (for details see Table 1) between the 3 alteration types with an ANOVA (H = high alteration, M = medium alteration, N = natural), performed separately for the 4 biogeographic regions (D = Germany, DK = Denmark, IRL = Ireland, GB =

United Kingdom, S = Sweden, FIN = Finland, IC = central Italy, IN = northern Italy). A and B indicate significantly different mean values. The “Total PVI” is normalized from 1 to 5, 1 indicating the maximum and 5 the minimum value of “Total PVI”.



**Figure 3:** Statistical comparison of the stressor index component “Pressure index” (for details see Table 1) between the 3 alteration types with an ANOVA (H = high alteration, M = medium alteration, N = natural), performed separately for the 4 biogeographic regions (D = Germany, DK = Denmark, IRL = Ireland, GB = United Kingdom, S = Sweden, FIN = Finland, IC = central Italy, IN = northern Italy). A, B and C indicate significantly different mean values. The “Pressure index” is normalized from 1 to 5, 1 indicating the minimum and 5 the maximum value of the “Pressure index”.

In a next step, several stressor index variants were calculated as the unweighted means from the stressor index components, and tested again with an ANOVA to which degree they mirrored the differences among the 3 alteration types. Hereby variables that showed a cross-correlation (Spearman Rank Correlations) with  $Rho > 0.8$  were not used together in the same stressor index variant, as they describe the same environmental information. This was the case for the variables “Number of habitats”/“Habitat diversity” and for “Total PVI”/“Sum of macrophyte types”. The variant that reflected best the differences among the alteration types, and especially between high and medium shore modification, was chosen for each biogeographical region. Figure 4 shows the best stressor index combinations for the 4 biogeographical regions and in Table 4 the composition of the stressor index variants is displayed. Since the values of the stressor index components were classified in a scale from 1 (best condition) to 5 (worst condition), the values of the stressor index were also in the range from 1 to 5.



**Figure 4:** Statistical comparison of the chosen stressor index variants (for details see Table 1) between the 3 alteration types with an ANOVA (H = high alteration, M = medium alteration, N = natural), performed separately for the 4 biogeographic regions (D = Germany, DK = Denmark, IRL = Ireland, GB = United Kingdom, S = Sweden, FIN = Finland, IC = central Italy, IN = northern Italy).

The development of the stressor index demonstrates the suitability of the LHS method for assessing physical habitat parameters in the littoral, beach and riparian zones displaying hydromorphological alterations. The semi-quantitative scaling of areal cover/volume into 5 levels (1-5) from 0.5 (i.e. tick) to 4 (>75%) proved to be useful and is a cost- and time effective way of quantifying environmental variables for this purpose. However, we only used a small part of the data collected in the LHS survey and suggest a significant reduction of the 7 page long survey protocol to the recording of only those physical habitat parameters that were used for the calculation of the stressor index components listed in Table 5.

**Table 5:** Composition of the hydromorphological stressor index developed for the four biogeographical regions (*D* = Germany / *DK* = Denmark, *IRL* = Ireland / *GB* = United Kingdom, *S* = Sweden / *FIN* = Finland, *IC* = central Italy / *IN* = northern Italy).

Stressor Index Component	Biogeographical Region			
	D/DK	IRL/GB	S/FIN	IC/IN
Number of habitats	X			
Habitat diversity		X	X	X
Total PVI	X		X	X
Sum of macrophyte types		X		
Sum of vegetation cover types	X	X	X	
Sum of CWD/roots/overhanging vegetation	X			X
Pressure index	X	X	X	X
Natural/artificial dominant land cover type				X

## 4 Invertebrate metrics and multimetric index development

*Oliver Miler (IGB)*

Within the WISER WP 3.3. composite samples were taken at all sampling sites (Sweden: 9 lakes; Finland: 4 lakes; Germany: 9 lakes; Denmark: 2 lakes; Ireland: 9 lakes; United Kingdom: 3; Italy (north and central): 15 lakes). In addition, the 3 most commonly occurring habitats, i.e. macrophytes, stones and sand, were separately sampled to allow for an assessment of the single habitats. However, habitat-specific samples were only available for Germany (8 lakes), Denmark (1 lake), Sweden (4 lakes), Ireland (9 lakes) and Italy (14 lakes). In the UK no habitat-specific samples were taken and the Finnish habitat-specific samples were not analysed due to time constraints. We will first describe the multimetric index development for composite samples. Then we will discuss the process for habitat-specific samples, but concentrate here on differences between composite and habitat-specific samples as the development procedure is essentially the same.

### 4.1 Composite samples

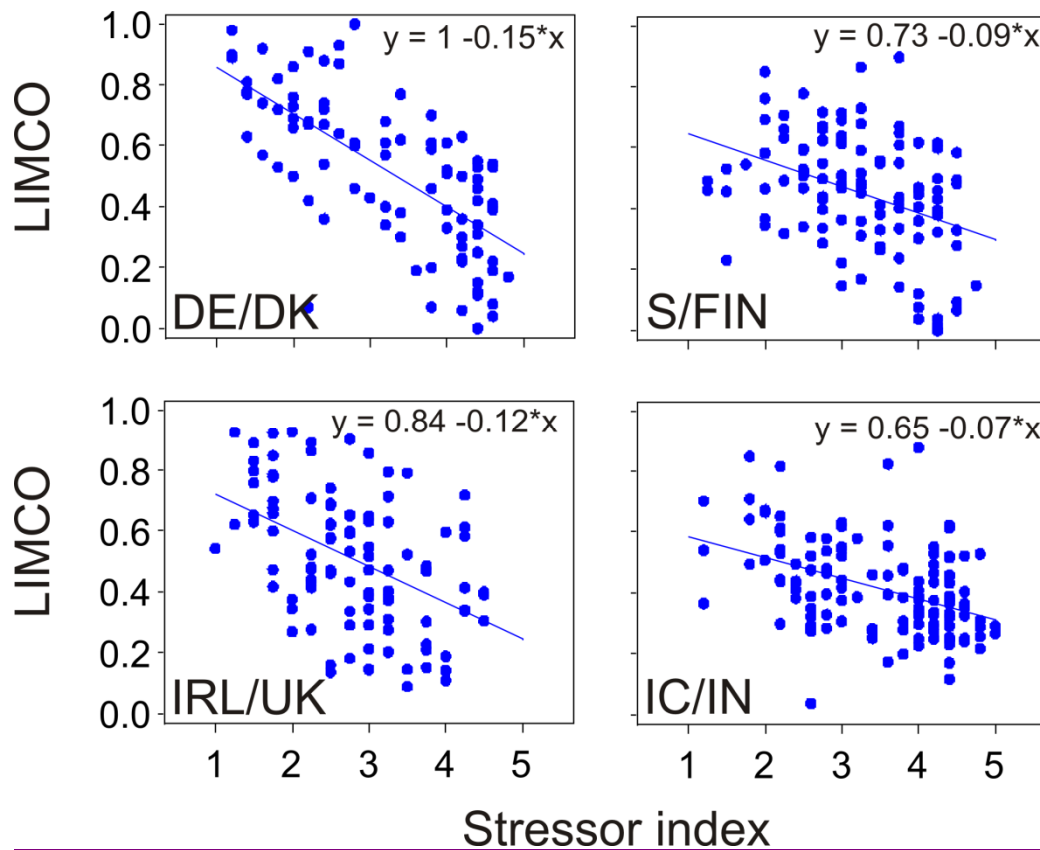
Invertebrate metrics were calculated based on macroinvertebrate abundances and abundance classes (AC): 1-2 = AC 1, 3-10 = AC 2, 11-30 = AC 3, 31-100 = AC 4, 101-300 = AC 5, 301-1000 = AC 6, > 1000 = AC 7. Metrics based on abundance classes have the advantage of being less influenced by a few dominant taxa with very high densities. Metrics were calculated by means of the software program ASTERICS 3.1.1. (developed by the University Duisburg-Essen (UDE)). An a-priori selection of the calculated metrics was carried out to ensure that only metrics that are applicable to and ecologically meaningful for stillwater macrozoobenthos communities are considered later as candidate and finally as core metrics. Table 6 lists the preselected metrics:

**Table 6:** Calculated and preselected metrics. Metric types are classified into 4 (TFC, D, A and DST) groups according to the EU-WFD, Annex V: TFC = taxonomic and functional composition, D = diversity, A = abundance, DST = disturbance sensitive taxa. [AC = abundance class]

Metric	Metric type	Metric	Metric type
No. Taxa	D	No. Plecoptera Taxa	DST
ASPT	DST	No. Heteroptera Taxa	A,TFC
Shannon Wiener Diversity	D	No. Trichoptera Taxa	DST
Evenness	D	No. Coleoptera Taxa	A,TFC
r/K relationship	TFC	No. Diptera Taxa	A,TFC
Type Pel %	TFC	No. EPTCBO Taxa	DST
Type Arg %	TFC	No. Families	D
Type Psa %	TFC	Simpson Diversity	D
Type Aka %	TFC	Margalef Diversity	D
Type Lit %	TFC	No. ETO Taxa	DST
Type Phy %	TFC	r/K relationship AC	TFC
Type Pom %	TFC	Type Pel % AC	TFC
Grazers/Scrapers %	TFC	Type Arg % AC	TFC
Shredders %	TFC	Type Psa % AC	TFC
Gatherers/Collectors %	TFC	Type Aka % AC	TFC
Active Filterfeeders %	TFC	Type Lit % AC	TFC
Passive Filterfeeders %	TFC	Type Phy % AC	TFC
Predators %	TFC	Type Pom % AC	TFC
Swimming/Diving %	TFC	Grazers/Scrapers % AC	TFC
Burrowing/Boring %	TFC	Shredders % AC	TFC
Sprawling/Walking %	TFC	Gatherers/Collectors % AC	TFC
Semisessil %	TFC	Active Filterfeeders % AC	TFC
Turbellaria %	A,TFC	Passive Filterfeeders % AC	TFC
Gastropoda %	A,TFC	Predators % AC	TFC
Bivalvia %	A,TFC	Swimming/Diving % AC	TFC
Oligochaeta %	A,TFC	Burrowing/Boring % AC	TFC
Hirudinea %	A,TFC	Sprawling/Walking % AC	TFC
Crustacea %	A,TFC	Semisessil % AC	TFC
Araneae %	A,TFC	Turbellaria % AC	A,TFC
Ephemeroptera %	A,TFC	Gastropoda % AC	A,TFC
Odonata %	A,TFC	Bivalvia % AC	A,TFC
Plecoptera %	A,TFC	Oligochaeta % AC	A,TFC
Heteroptera %	A,TFC	Hirudinea % AC	A,TFC
Trichoptera %	A,TFC	Crustacea % AC	A,TFC
Coleoptera %	A,TFC	Araneae % AC	A,TFC
Diptera %	A,TFC	Ephemeroptera % AC	A,TFC
EPT Taxa %	DST	Odonata % AC	A,TFC
ETO Taxa %	DST	Plecoptera % AC	A,TFC
EPTCBO Taxa %	DST	Heteroptera % AC	A,TFC
No. Turbellaria Taxa	A,TFC	Trichoptera % AC	A,TFC
No. Gastropoda Taxa	A,TFC	Lepidoptera % AC	A,TFC
No. Bivalvia Taxa	A,TFC	Coleoptera % AC	A,TFC
No. Oligochaeta Taxa	A,TFC	Diptera % AC	A,TFC
No. Hirudinea Taxa	A,TFC	EPT Taxa % AC	DST
No. Crustacea Taxa	A,TFC	ETO Taxa % AC	DST
No. Araneae Taxa	A,TFC	EPTCBO Taxa % AC	DST
No. Ephemeroptera Taxa	DST	Chironomidae % AC	A,TFC
No. Odonata Taxa	DST		



First a boxplot of each metric was plotted to check if the respective metric had a narrow range of values, a highly skewed distribution of values and/or many outliers. If one of these cases was true it would be numerically unsuitable. Subsequently, the metrics were correlated with the stressor index via Spearman Rank Correlations. From this dataset a subset of metrics with  $Rho > 0.2$ , i.e. metrics that correlated well with the stressor index, was chosen and each metric normalized to values from 0 to 1 with the 5 % percentile set to 0 and the 95 % percentile set to 1 again. Values smaller than 0 or larger than 1 were set to 0 and 1, respectively. Finally, for each biogeographical region eight candidate metrics were chosen that (1) correlate with the stressor index with  $Rho > 0.2$ , (2) cross-correlate with each other with  $Rho < 0.8$  and (3) equally represent the four metric types diversity (D), taxonomic and functional composition (TFC), abundance (A) and disturbance sensitive taxa (DST) according to the normative text of the EU WFD. From the eight candidate metrics 32 multimetric index (MMI) variants with the unweighted mean of three or four metrics covering at least the metrics types TFC, D and DST, ideally also A, were constructed. These were correlated using Spearman Rank correlations (Figure 5) with the stressor index and the best correlating MMI variant, denoted LIMCO (**L**ittoral **I**nvertebrate **M**ultimetric Index based on **C**omposite Sampling) was chosen separately for each of the four biogeographical regions (Table 7). The final multimetric index had values from 0 to 1, since the index LIMCO consists of the unweighted mean of four core metrics (Table 7) that were normalized to values from 0 to 1 (see above). In the process of choosing LIMCO, the variants with the unweighted mean of four metrics proved to provide better correlations than those with the unweighted mean of three metrics. However, since the EU WFD requires for the ecological assessment of water bodies a rescaling to values from 1 to 5, representing the 5 ecological quality status classes 1 = “high”, 2 = “good”, 3 = “moderate”, 4 = “poor” and 5 = “bad”, we calculated boundary classes which will be described and discussed in the chapter 4.3 “Boundary Setting Procedure”.



**Figure 5:** Correlation of the type-specific stressor index with the multimetric index LIMCO (Littoral Invertebrate Multimetric Index based on Composite Sampling) for the 4 biogeographical regions (D = Germany / DK = Denmark, IRL = Ireland / GB = United Kingdom, S = Sweden / FIN = Finland, IN = northern Italy / IC = central Italy).

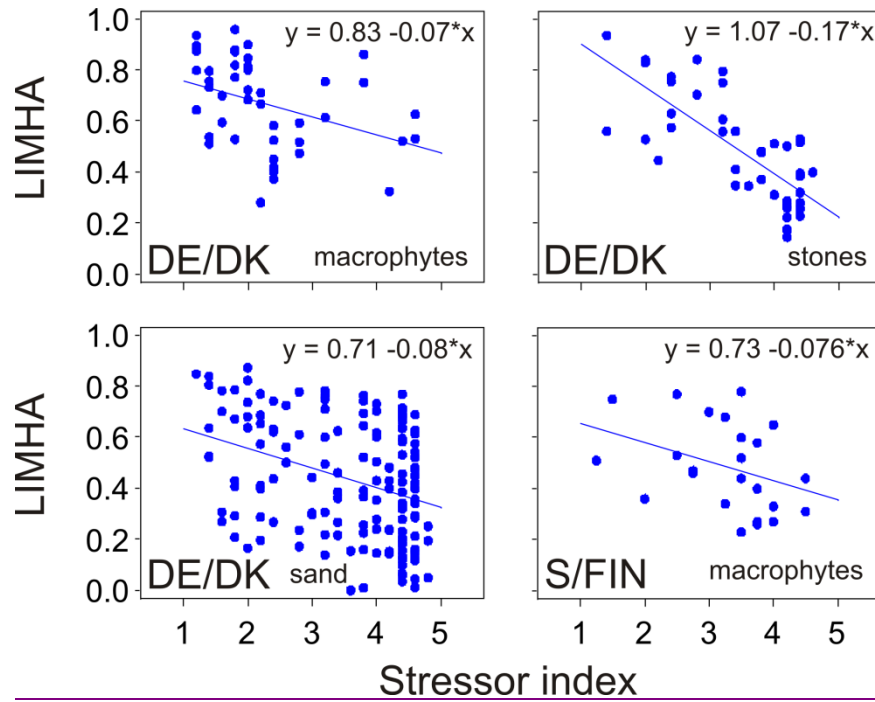
**Table 7:** Selected candidate metrics for the 4 biogeographical regions (D = Germany / DK = Denmark, IRL = Ireland / GB = United Kingdom, S = Sweden / FIN = Finland, IN = northern Italy / IC = central Italy). X denotes core metrics for the final MMI variant: TFC = taxonomic and functional composition, D = diversity, A = abundance, DST = disturbance sensitive taxa. AC = abundance class.]

Biogeographical region		D/DK	IRL/GB	S/FIN	IC/IN
Rho		-0.70	-0.47	-0.39	-0.49
Candidate metric	Metric type				
Margalef Diversity	D	X	X		X
No. Families				X	
Shredders % AC	TFC			X	
Gatherer/Collectors %			X		
Gatherer/Collectors % AC		X			
r/K relationship					X
Odonata %					X
Chironomidae % AC	A, TFC	X			
Diptera % AC			X		
Crustacea % AC				X	
No. ETO Taxa	DST		X		X
No. EPTCBO Taxa		X			
No. Odonata Taxa				X	

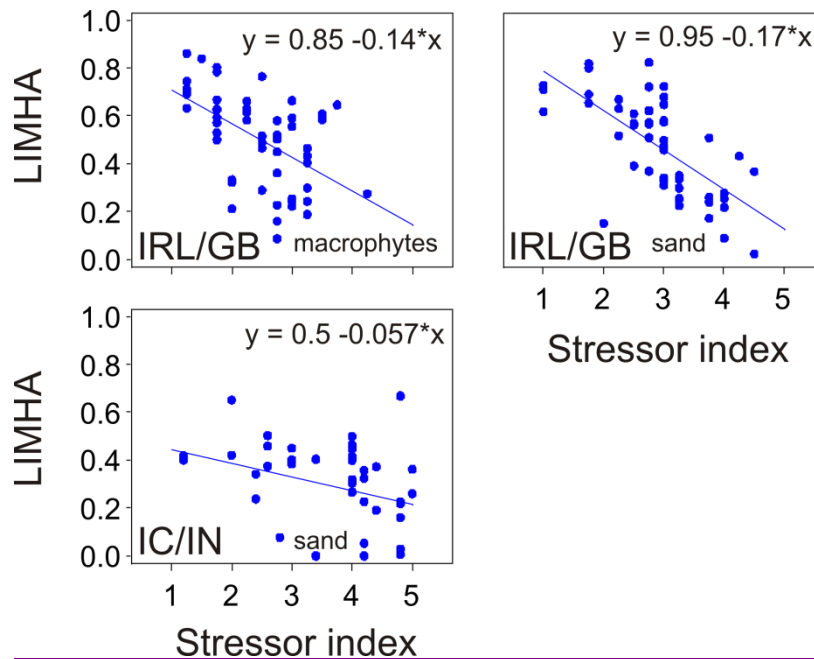
## 4.2 Habitat samples

For some sampling sites, habitat samples in addition to composite samples were available (see details above). Based on these samples, multimetric habitat indices were developed where possible (Table 8, Figures 6 and 7). This was the case for the biogeographic regions Germany/Denmark (macrophytes, stones and sand), Sweden/Finland (macrophytes; no habitat samples for Finland), Ireland/United Kingdom (macrophytes, sand; no habitat samples for United Kingdom) and central Italy/northern Italy (sand). In Italy, habitat samples were more finely divided into sand-gravel/woody debris-CPOM/silt (sand), cobbles-boulders-rock/wood-paling/concrete (stones) and roots/emerged/submerged vegetation (macrophytes) which complicated the index development. In several cases, such as emerged vegetation and submerged vegetation samples, metrics were correlated in opposite directions with the stressor index and did not always show ecologically meaningful relationships with the stressor index due to low sample sizes. For example, when the separate Italian roots/emerged vegetation/submerged vegetation samples were then all pooled together to a macrophyte sample this resulted in no correlations with the stressor index at all. Hence, for the multimetric index development the components of the sand, stones and macrophyte samples, i.e. sand-gravel, woody debris, CPOM, silt, cobbles-boulders-rock, wood-paling, concrete, roots, emerged vegetation and submerged vegetation, had to be regarded as separate habitats. Due to resulting small sample sizes for central Italy/northern Italy only the development of a multimetric index for sand (only silt habitats considered) was possible.

The multimetric index development procedure for habitat-specific samples (LIMHA: **L**ittoral **I**nvertebrate **M**ultimetric **I**ndex based on **H**abitat Sampling) was the same as that used for composite samples (LIMCO) and the same stressor indices as described in chapter 3.2 and the Tables 2-4 were used. However, the results were somewhat inconclusive: Although Rho values were higher for LIMHA in some biogeographic regions (Sweden/Finland, Ireland/United Kingdom), they were lower in others (Germany/Denmark, central Italy/northern Italy). Hence, LIMHA did not correlate consistently better or worse than LIMCO with the respective stressor index.



**Figure 6:** Correlation of the type-specific stressor index with the multimetric index LIMHA (Littoral Invertebrate Multimetric Index based on Habitat Sampling) for the 2 biogeographical regions (D = Germany / DK = Denmark (habitats: macrophytes (MP), stones (ST) and sand (SA)) and S = Sweden / FIN = Finland (habitat: macrophytes (MP)). Please note that there were no habitat samples for Finland within the biogeographical region S/FIN.



**Figure 7:** Correlation of the type-specific stressor index with the multimetric index LIMHA (Littoral Invertebrate Multimetric Index based on Habitat Sampling) for the 2 biogeographical regions (IRL = Ireland / GB = United Kingdom (habitats: macrophytes and sand) and IN = northern Italy / IC = central Italy (habitat: sand). Please note that there were no habitat samples for the United Kingdom within the biogeographical region IRL/GB. IC/IN Sand samples consisted of 2 microhabitats, silt and sand-gravel, of which only silt provided acceptable metric and MMI correlations.

**Table 8:** Selected candidate metrics from habitat samples (MP = macrophytes, ST = stones, SA = Sand) for the 4 biogeographical regions (D = Germany / DK = Denmark, IRL = Ireland / GB = United Kingdom, S = Sweden / FIN = Finland, IC = central Italy / IN = northern Italy). X denotes core metrics for the final MMI variant: TFC = taxonomic and functional composition, D = diversity, A = abundance, DST = disturbance sensitive taxa. AC = abundance class

Biogeographical region		D/DK	D/DK	D/DK	S/FIN	IRL/GB	IRL/GB	IC/IN
Habitat		MP	ST	SA	MP	MP	SA	SA (silt)
Rho		-0.48	-0.72	-0.34	-0.44	-0.55	-0.71	-0.40
% occurrence of habitat		38%	41%	93%	100%	41%	69%	21%
Candidate metric	Metric type							
Type Psa %	TFC							X
Type Pom % AC		X		X				
Gatherer/Collectors % AC			X					
Predators %					X			
Swimming/Diving % AC						X	X	
Shannon Wiener Diversity					X		X	X
Margalef Diversity	D	X	X					
Evenness					X			
No. Families						X		
Coleoptera % AC			X		X			
Diptera % AC	A,TFC			X			X	
Odonata %		X						
Oligochaeta % AC						X		X
No. Trichoptera Taxa	DST			X				
No. EPTCBO Taxa			X					
EPTCBO taxa %					X		X	
No. ETO Taxa		X						
ETO Taxa %						X		
EPT Taxa %								X

### 4. 3 Boundary Setting Procedure

The EU-WFD requires the ecological assessment of water bodies to be expressed in the 5 ecological status classes “high”, “good”, “moderate”, “poor” and “bad”. Since all European water bodies have to be protected or enhanced in order to achieve at least “good” ecological status by 2015, the correct classification of a water body is crucial. A misclassification into a

lower ecological status class would lead to unnecessary implementation and realization of restoration measures and hence to an avoidable consumption of financial resources of the regional and national administrations in EU member states. On the other hand, can the misclassification into a lower ecological status class lead to a situation where a water body that would actually be in need of restoration efforts is not considered and remains in a poorer status than the good ecological status.

We suggest here to use the boundary setting method described for river benthic macroinvertebrates (Intercalibration Common Metric Index (ICMi) from the AQEM/STAR project) in Erba et al. (2009) that is based on the EU Guidance document CIS (2003). Erba et al. (2009) defined reference sites in compliance with the EU WFD (Hering et al. 2003, Nijboer et al. 2004, CIS 2003). The 25th Percentile of ICMi values at the reference sites was set as the High/Good boundary and the Good/Moderate (G/M), Moderate/Poor (M/P) and Poor/Bad (P/B) boundaries were defined as 75 %, 50 % and 25 % of the High/Good boundary value, respectively. This classification was then used to validate the class boundaries determined in the intercalibration exercise performed for streams in the Central-Baltic, Alpine and Mediterranean GIGs (Geographical Intercalibration Groups) and the results were in good agreement with the intercalibration class boundaries.

The calculated boundary classes for LIMCO and LIMHA (MP = macrophytes, ST = stones, SA = sand) are listed in Table 9. We used in each biogeographical region all sites with the a-priori classification “natural” as reference sites. We then calculated the 25th Percentile as the H/G boundary for LIMCO and LIMHA and derived the G/M, M/P and P/B boundaries according to Erba et al. (2009) (see above).

**Table 9:** Boundary classes for LIMCO and LIMHA (MP = macrophytes, ST = stones, SA = sand) for the 4 biogeographical regions (D = Germany / DK = Denmark, IRL = Ireland / GB = United Kingdom, S = Sweden / FIN = Finland, IC = central Italy / IN = northern Italy). The class boundaries are indicated as follows: H/G = High/Good, G/M = Good/Moderate, M/P Moderate/Poor, P/B = Poor/Bad.

Biogeographical region	MMI	n	H/G	G/M	M/P	P/B
D/DK	LIMCO	33	0.57	0.43	0.29	0.14
D/DK	LIMHA (MP)	34	0.53	0.40	0.26	0.13
D/DK	LIMHA (ST)	6	0.35	0.26	0.18	0.09
D/DK	LIMHA (SA)	41	0.28	0.21	0.14	0.07
S/FIN	LIMCO	43	0.45	0.33	0.22	0.11
S/FIN	LIMHA (MP)	8	0.47	0.35	0.23	0.12
IRL/UK	LIMCO	36	0.45	0.34	0.23	0.11
IRL/UK	LIMHA (MP)	23	0.53	0.40	0.26	0.13
IRL/UK	LIMHA (SA)	18	0.57	0.43	0.29	0.14
IC/IN	LIMCO	49	0.38	0.28	0.19	0.09
IC/IN	LIMHA (SA)	10	0.40	0.30	0.20	0.10

## 5 Composite versus habitat specific samples

*Oliver Miler (IGB)*

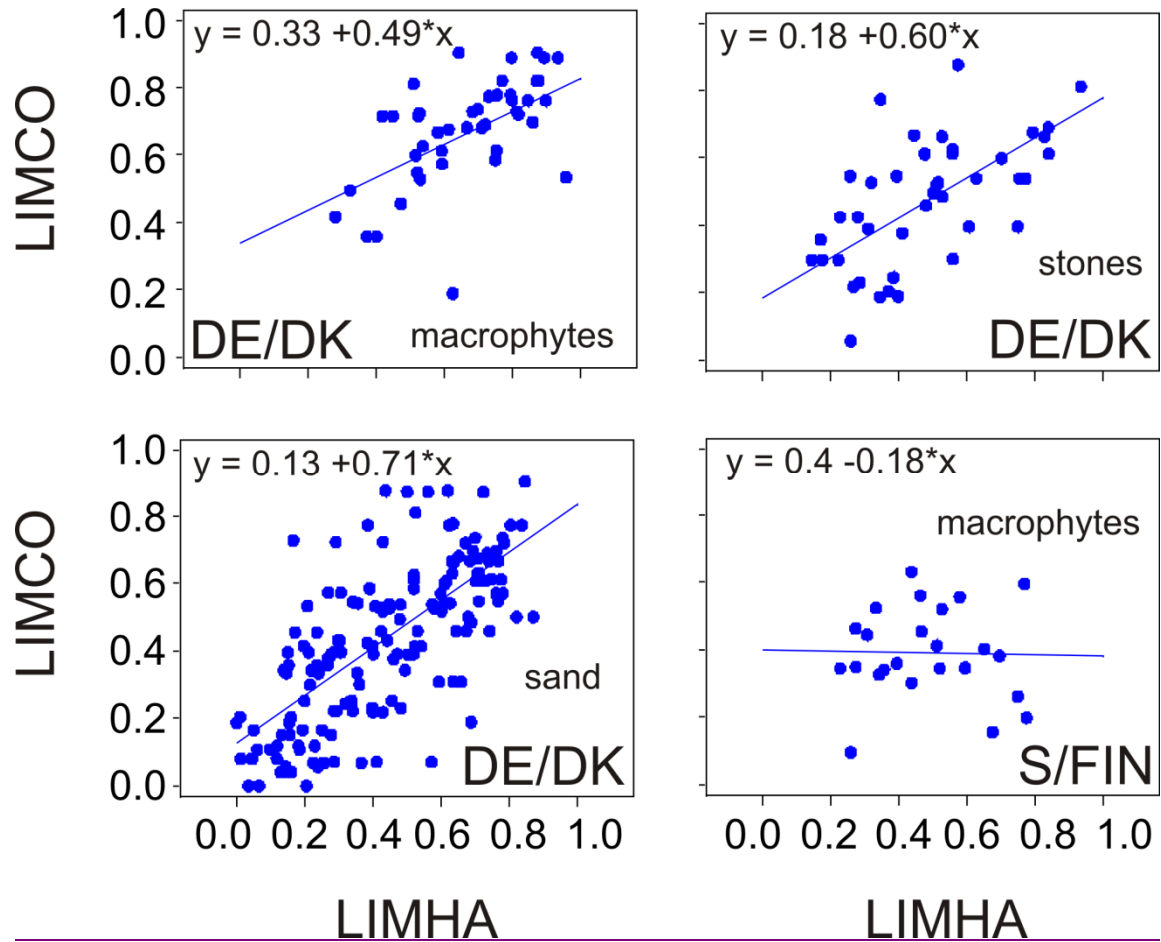
Before an assessment system can be fully utilized, it has to be validated with a dataset other than the one used to build it (Vlek et al. 2004). However, a pre-requisite for the validation dataset is that the organisms (in this case macrozoobenthos organisms) are sampled and processed according to the same or a comparable sampling protocol and the data are taxonomically harmonized to the same levels.

Since such data are presently not available we used a different approach to validate the assessment indices developed in the WISER WP 3.3. Habitat and composite samples were taken from the same sampling sites, so for a certain number of sampling sites both types of samples exist. As both MMI, LIMCO and LIMHA, indicate hydromorphological pressures, they should show a positive correlation to each other, with high Rho values. Originally, it was planned to use only the extreme percentiles, such as the 10 % and 90 % percentiles, for each habitat and biogeographical region, to cover only the most natural and the most degraded sites. However, due to the overall low number of habitat samples (see Table 10), we used all available habitat samples for the correlation analyses. Furthermore, analyses were conducted separately for the 4 biogeographical regions and for each habitat.

In general, LIMCO and LIMHA correlated well with each other (Table 10, Figs. 8 and 9), with Rho values ranging from minimum 0.55 to maximum 0.73. There was no correlation between LIMCO and LIMHA in S/FIN (habitat: macrophytes) alone. This may be owing to the very low overall number of habitat samples (24) from only 4 lakes on which the LIMHA for S/FIN (habitat: macrophytes) is based.

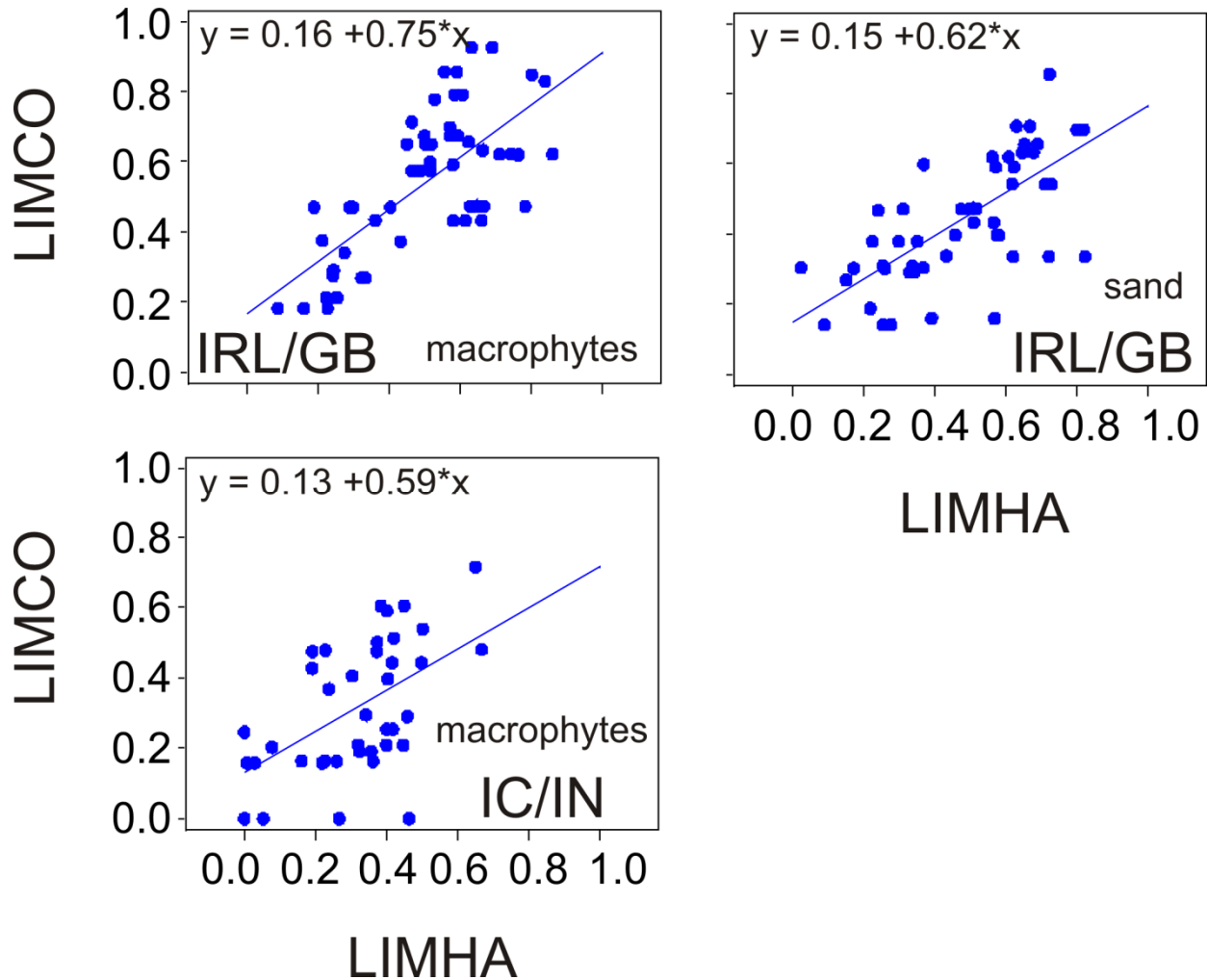
**Table 10:** Correlation coefficients (Rho) of the multimetric index LIMCO (Littoral Invertebrate Multimetric Index based on Composite Sampling) with the multimetric index LIMHA (Littoral Invertebrate Multimetric Index based on Habitat Sampling) in the 4 biogeographical regions D/DK, S/FIN, IRL/GB and IC/IN (habitats: macrophytes (MP), stones (ST) and sand (SA)).

Biogeographical Region	DE/DK	DE/DK	DE/DK	S/FIN	IRL/GB	IRL/GB	IC/IN
Habitat	MP	ST	SA	MP	MP	SA	SA
Rho	0.57	0.60	0.69	-0.01	0.63	0.73	0.55
No. of samples	57	59	167	24	62	53	42



**Figure 8:** Correlation of the multimetric index LIMCO (Littoral Invertebrate Multimetric Index based on Composite Sampling) with the multimetric index LIMHA (Littoral Invertebrate Multimetric Index based on Habitat Sampling) for the 2 biogeographical regions (D = Germany / DK = Denmark (habitats: macrophytes (MP), stones (ST) and sand (SA)) and S = Sweden / FIN = Finland (habitat: macrophytes (MP)). Please note that there were no habitat samples for Finland within the biogeographical region S/FIN.





**Figure 9:** Correlation of the multimetric index LIMCO (Littoral Invertebrate Multimetric Index based on Composite Sampling) with the multimetric index LIMHA (Littoral Invertebrate Multimetric Index based on Habitat Sampling) for the 2 biogeographical regions (IRL = Ireland / GB = United Kingdom (habitats: macrophytes and sand) and IN = northern Italy / IC = central Italy (habitat: sand). Please note that there were no habitat samples for the United Kingdom within the biogeographical region IRL/GB. IC/IN Sand samples consisted of 2 microhabitats, silt and sand-gravel, of which only silt provided acceptable metric and MMI correlations.

## 6 Sources of variation in lake assessment & cost efficiency

Ralph Clarke (Bournemouth University)

### 6.1 Sources of uncertainty in lake bioassessments

Any estimate of an index, metric or multi-metric used to assess the ecological quality and WFD ecological status of a water body is of little value unless without some quantitative knowledge of the uncertainty associated with the estimate due to sampling variation and other methodological errors (Clarke et al 1996, Clarke and Hering 2006).

For lake water body assessments based on macroinvertebrate sampling, the sampling errors in a biological metric estimated from the observed sample macroinvertebrate taxonomic composition can arise from (i) natural spatial variability within the lake, (ii) temporal variability over the period for which the assessment is to represent and/or (iii) sample processing, sub-sampling and taxonomic identification errors. In addition, estimates of WFD Ecological Quality Ratios based on standardising observed metric values with water body type-specific reference condition (RC) values may involve additional sources of uncertainty due to the need to estimate RC values of each metric for the water body.

## 6.2 Estimating sampling variances in metric values from WISER field data

Within the resource constraints of the WISER project, the principal aims of the WISER WP3.3 lake invertebrate field sampling programme were to assess:

- (i) the spatial variance between sites around a lake margin and especially this size of this within-lake variance relative to the variance between lakes (and countries/regions)
- (ii) the relationship between metrics and lake margin modification type (classified as natural (N), medium (M) (e.g. riparian clear-cutting, recreational beaches) and high (H) modification (e.g. retaining walls, rip-rap).

Sampling involved collecting a 1-minute timed composite habitat sample and, where feasible, three habitat-specific samples, from (usually) nine samples sites (each representing min. 25 m shoreline) from each of 51 lakes (15 Italy, 9 Germany, 9 Ireland, 9 Sweden, 4 Finland, 3 UK, 2 Denmark). The nine sampling sites per lake were chosen to include, where available, all three lake margin modification types (natural (N), medium (M) and high (H) modification), ideally with three sites per modification type. 44 of the 51 lakes had at least one sampling site from each of the three modifications to help provide information on the consistency of metric differences between modification types across lakes.

46 metrics (values calculated from the ASTERICS software) were assessed for sampling variances, with 29 based on the percentage (%) of all sampled individuals in specific (feeding, locomotion or taxonomic groups), 13 based on general or specific taxonomic richness and 4 others (Table 11).

Where appropriate, statistical transformations of metric values were made to remove skewness in distributions and to make sampling variance between sites within lakes more equitable across all lakes. Thus metrics which represent the percentage (x) of all individuals in a sample which were of one taxonomic grouping (names denoted with a % in Table 11 and subsequent) were transformed using the arcsine square root transformation ( $\text{asin}(\sqrt{x/100})$ ) usually recommended for variables which are percentages or proportions. Metrics which were number of taxa in a certain taxonomic grouping (metric name begins NTAXA) were transformed to their square roots to make variances more constant regardless of the number of taxa within a lake (as used by Clarke et al (2002) and Clarke et al. (2006) for river macroinvertebrate metric assessments). The other metrics (ASPT, Shannon-Wiener Diversity, Evenness, r/K relationship) were analysed on the untransformed scale. Both of these transformations are catered for in the WISERBUGS

software (Clarke, 2011) and so the derived variance estimates could be used to help assess confidence of class in any lake assessment scheme based on the EQRs derived from one or more of these metrics (although this is beyond the scope of this WP).

Statistical mixed (fixed and random level) models (based on function 'lme' in the 'R' free-available software) were used to estimate the various metric variance components, namely the variances due between sites within lakes, between lakes within countries and between countries.

### **6.3 Variance components for composite samples**

The estimates of all variance components, expressed as a percentage of the total variance (countries variance plus lakes variance plus sites variance) are summarised for all 46 metrics in Table 11. If needed to assess the uncertainty of lake mean metric values, the estimates of individual between site variances can be obtained by multiplying the total variance by the appropriate percentage within lake variance estimate.

A second model allowing for differences due to modification type separately within each lake was fitted purely to estimate the average variance between sampling sites with the same modification type (N, M or H) and this variance was expressed as a percentage of the total variance across all countries (%Site/ModType in Table 11). This percentage variance between sites within modification type (%Site/ModType) will always be less than or equal to the overall between site within lake variance.

Amongst the 46 metrics, on average around half (52%) of the total variance in metric values was due to variability between sampling sites within lakes and the other half (48%) is due to differences between lakes, split, on average, roughly equally between country differences (27%) and between lake within country differences (21%). However, metrics varied enormously; the metrics with the lowest percentage total variance due to within lake variability were %Swimming/diving (26%), %Sprawling/walking (34%) and several taxa richness (NTaxa) metrics, including total 'Number of taxa' (37%) and Number of Families (41%) (Table 11).

**Table 11:** Estimates of components of variance (between Countries, between Lakes within Country, between Sites within Lake), each expressed as a percentage of their sum (the Total variance in metric values within the whole dataset); a final column %Site/ModType gives the percentage of total variance explained by average variance between sites within a modification type within a lake

Metric	Metric Variance				
	Total	%Country	%Lake	%Site	%Site / ModType
asin(sqrt(%individuals))) square root (NTaxa) metrics					
ASPT	0.4610	12	24	64	53
Shannon Wiener Diversity	0.2838	9	22	69	64
Evenness	0.0238	6	21	73	72
r/K relationship	0.0065	14	14	72	60
Pel MicroHab %	0.0233	15	28	56	49
Arg MicroHab %	0.0064	12	31	57	48
Psa MicroHab %	0.0169	11	25	64	59
Aka MicroHab %	0.0118	27	17	55	51
Lit MicroHab %	0.0122	0	22	78	72
Phy MicroHab %	0.0213	28	16	55	48
Pom MicroHab %	0.0166	20	28	52	44
Grazers and scrapers %	0.0144	28	12	60	51
Shredders %	0.0262	26	30	44	36
Gatherers and Collectors %	0.0514	21	17	62	60
Active filter feeders %	0.0304	33	20	47	47
Predators %	0.0192	16	11	73	72
Swimming/diving %	0.0384	62	12	26	26
Burrowing/boring %	0.0123	43	9	47	43
Sprawling/walking %	0.0543	53	13	34	26
(Semi) Sessil %	0.0271	26	23	50	44
Turbellaria %	0.0130	22	16	62	56
Gastropoda %	0.0601	34	21	45	43
Bivalvia %	0.0340	28	24	48	41
Oligochaeta %	0.0941	24	20	55	54
Hirudinea %	0.0067	31	12	57	57
Crustacea %	0.0997	38	23	40	35
Ephemeroptera %	0.0757	32	25	43	35
Plecoptera %	0.0010	20	42	38	32
Heteroptera %	0.0271	12	30	58	55
Trichoptera %	0.0165	13	27	60	51
Coleoptera %	0.0095	39	15	47	38
Diptera %	0.0742	18	24	58	52
EPT Taxa %	0.0697	18	31	51	43
Number of Taxa	1.3074	44	19	37	29
NTaxa Turbellaria	0.4253	35	16	49	49
NTaxa Gastropoda	1.0109	36	17	47	41
NTaxa Bivalvia	0.4070	41	20	39	36
NTaxa Hirudinea	0.7540	47	17	36	38
NTaxa Crustacea	0.3528	32	25	43	37
NTaxa Ephemeroptera	0.5663	42	23	35	32
NTaxa Odonata	0.4880	5	29	66	53
NTaxa Trichoptera	0.8284	34	20	46	40
NTaxa Coleoptera	0.7876	52	11	37	32
NTaxa Diptera	0.1230	11	27	62	57
NTaxa EPTCBO	0.9360	30	25	44	34
Number of Families	0.7678	39	20	41	33
Average %		27	21	52	46

**Table 12:** Estimates of components of variance for habitat-specific samples (sand, stones or macrophytes) for 46 metrics; average variance between sites within a lake is expressed as a percentage of the total dataset variance equal to the sum of between sites within lake, between lakes within country and between country variance components

Metric asin(sqrt(%individuals))) square root (NTAXa) metrics	Total variance			% Total variance within lakes			
	Sand	Stones	Macro- phytes	Sand	Stones	Macro- phytes	Composite samples
ASPT	1.0217	1.1461	1.2074	66	54	47	64
Shannon Wiener Diversity	0.4029	0.2644	0.2466	47	64	50	69
Evenness	0.0511	0.0327	0.0205	83	81	69	73
r/K relationship	0.0199	0.0080	0.0046	82	61	68	72
Pel MicroHab %	0.0019	0.0017	0.0017	4	3	5	56
Arg MicroHab %	0.0005	0.0003	0.0004	26	14	15	57
Psa MicroHab %	0.0012	0.0010	0.0011	9	6	7	64
Aka MicroHab %	0.0008	0.0007	0.0008	10	7	8	55
Lit MicroHab %	0.0012	0.0011	0.0011	7	4	5	78
Phy MicroHab %	0.0016	0.0015	0.0014	6	4	5	55
Pom MicroHab %	0.0008	0.0007	0.0008	18	9	14	52
Grazers and scrapers %	0.0164	0.0253	0.0166	60	65	41	60
Shredders %	0.0295	0.0264	0.0165	27	62	60	44
Gatherers and Collectors %	0.0735	0.1064	0.0480	66	42	52	62
Active filter feeders %	0.0336	0.0918	0.0556	39	21	27	47
Predators %	0.0225	0.0302	0.0304	86	52	45	73
Swimming/diving %	0.0342	0.0292	0.0202	51	60	45	26
Burrowing/boring %	0.0030	0.0054	0.0030	36	74	56	47
Sprawling/walking %	0.0511	0.0389	0.0337	48	80	52	34
(Semi) Sessil %	0.0428	0.0734	0.0447	47	19	28	50
Turbellaria %	0.0118	0.0248	0.0256	63	59	55	62
Gastropoda %	0.0557	0.0500	0.0500	49	69	43	45
Bivalvia %	0.0307	0.0706	0.0645	50	21	25	48
Oligochaeta %	0.1823	0.1434	0.0882	50	62	47	55
Hirudinea %	0.0036	0.0042	0.0019	32	51	53	57
Crustacea %	0.1267	0.0996	0.0833	20	57	36	40
Ephemeroptera %	0.0321	0.1027	0.1199	60	26	19	43
Plecoptera %	0.0000	0.0000	0.0002	99	63	75	38
Heteroptera %	0.0364	0.0121	0.0112	55	80	69	58
Trichoptera %	0.0114	0.0251	0.0305	70	56	51	60
Coleoptera %	0.0063	0.0081	0.0060	18	54	54	47
Diptera %	0.1414	0.0871	0.0805	61	38	42	58
EPT Taxa %	0.0381	0.1011	0.1283	59	33	20	51
Number of Taxa	1.7865	1.2923	1.3602	20	30	21	37
NTAXa Turbellaria	0.2965	0.4413	0.4717	60	67	51	49
NTAXa Gastropoda	0.9864	1.0489	1.3827	29	38	31	47
NTAXa Bivalvia	0.6284	0.3904	0.4508	15	46	26	39
NTAXa Hirudinea	0.4077	0.4921	0.4821	34	51	47	36
NTAXa Crustacea	0.5235	0.4272	0.4295	27	25	31	43
NTAXa Ephemeroptera	0.4972	0.7428	0.7696	34	24	26	35
NTAXa Odonata	0.2466	0.3937	0.5611	69	49	43	66
NTAXa Trichoptera	1.0375	1.0439	0.9977	27	33	23	46
NTAXa Coleoptera	0.4567	0.5158	0.6236	27	42	39	37
NTAXa Diptera	0.1760	0.1794	0.1504	64	47	64	62
NTAXa EPTCBO	1.6389	1.2791	0.9919	22	33	23	44
Number of Families	1.2738	0.8707	0.7641	19	35	23	41

## 6.4 Variance components for habitat-specific samples

Equivalent mixed models were used to estimate the same components of variance for the single habitat (sand, stones or macrophytes) samples; the percentage of the total dataset variance (between sites within lakes plus between lakes within country plus between countries) due to between site within lake variability in each metric are summarised in Table 12. If needed to assess uncertainty of lake mean metric values, the estimates of individual between site variances can be obtained by multiplying the total variance by the appropriate percentage within lake variance estimate.

Averaged across the 46 macroinvertebrate metrics assessed, the average percentage of the total data variance due to between sampling site variance was 42%, 43% and 38% for the sand, stones, and macrophyte habitat specific samples respectively. These percentages are all slightly less than the equivalent figure of 46% for the composite samples, indicating that, relatively to the amount of between lake variability in metric values, samples from individual habitats are relatively less variable within a lake than those for composite samples. Thus, single habitat samples have relatively slightly more of their total variability in metric values between lakes giving slightly greater potential power to detect differences in metric values between lakes, perhaps related to lake-wide differences in stress.

## 6.5 Sampling precision of metrics, sampling costs and power to detect biotic response to stress

In general, if a very large percentage of the total variance in sample metric values amongst all lakes of variability in ecological quality is due to spatial variability between sampling sites within any one water body (i.e. lake), then with only small sample sizes per water body that metric will not have great statistical power to discriminate between water bodies of different quality and status. Large numbers of sites would need to be sampled per lake to get adequately precise estimates of the lake-wide mean metric value, in order to discriminate between lakes of different levels of stress, such as from lake-wide eutrophication.

To explain this further, consider a set of lakes of the same broad natural physical type (and hence set the same reference condition (RC) values of biological metrics) but subject to varying levels of anthropogenic stress. If a proportion  $Q$  of the total variance in sample metric values across all sites and lakes within the RC type is between sites within any one lake, then with a single sample and sampling site per lake, the strength of relationship (as measured by regression R-squared) between observed lake metric value and any driving pressure variables cannot be greater than  $(1-Q)$ . More interestingly, as the lake mean metric values are based on the average of  $N$  samples, the maximum R-squared of the lake biota-stress relationship is  $(1-Q)/(1-Q+Q/N)$ .

As an example, we consider composite samples and the metric 'Number of Taxa' (Table 11). As a relatively large percentage (44%) of total variance is between countries, suggesting countries differ naturally in macroinvertebrate richness, we assume each country will have its own RC value. Within a country, on average, the proportion  $Q$  of within lake variance in (the square root

of) Number of taxa is  $37/(37+19)$ , namely 0.66. For this situation, the maximum possible R-squared for the lake mean metric versus stress variable relationship increases from 0.34 for a single sample, to 0.51 for two samples, up to 0.84 when the lake mean metric value is based on the average of 10 sampling sites (Table 13).

**Table 13:** Illustrative effect of sampling sites per lake on precision of observed lake mean value and the maximum possible strength (R-squared) of its relationship with lake stress variables, example based on metric 'Number of taxa' for composite samples

Sampling sites per lake	1	2	3	4	5	10
Max possible R-squared for lake mean metric value v stress variable relationship	0.34	0.51	0.61	0.67	0.72	0.84

In section 2.2, it was reported from tests in Germany, that it takes about the same amount of time to collect (0.4 hours) and sort and process (10 hours) each single habitat sample as it does to collect and sort/process a composite habitat sample. The proportion of total variance that between sites within a lake is slightly lower on average for single habitat samples. Therefore if single habitat samples (such as stones only) could be used to assess a lake, estimates might be slightly more cost effective purely in terms of cost to achieve a specific sampling precision for a lake mean metric value than those based on composite samples. However, individual habitats may be relatively rare around some lakes and thus poorly represent the whole lake, prompting the use of averages of metric values for each major habitat (weighted by the estimated length around the lake of each habitat). The relative cost effectiveness of each sampling approach depends on the relative actual strength of relationship between metric values and stressors in each approach.

In summary, metrics which have a relative large ratio of within-lake to between lake variance will usually only have a detectably strong between lake relationship with any underlying driving pressure variables, if the mean metric value for each lake can be based on sufficient samples and sampling sites to adequately improve individually the sampling precision of the lake mean metric values. Incidentally, the same logic of sampling precision can apply to the estimation of the lake-wide stressor variables.

## 6.6 Rank consistency of modification type differences in metric values across lakes: towards selecting metrics for common multi-metric indices

*Ralph Clarke (Bournemouth University)*

A desirable property of any metric to be used for WFD ecological assessment of European lakes is that it shows a consistent directional response to a particular type of stress across as wide a range of European lakes as possible. Obviously, for different physical types of lake and maybe different countries, the actual values of the metric may differ naturally, requiring the need for different reference condition values to standardise the observed metric values to Ecological Quality Ratio (EQR) values. However, if the response in metric values to a particular or general stress is in the same direction, then that metric has the potential to be suitable as a European-wide common metric.

With this objective in mind, we devised a robust approach to assess the rank consistency of individual macroinvertebrate metric directional responses to both medium (M) and high (H) types of lake margin modification across the WISER field sampled lakes.

Specifically, for any particular metric, for each lake the mean metric value was calculated for the sampled sites in each of the three types (natural (N), medium alteration (M), high alteration (H)) and the three means were ranked from 1 (lowest) to 3 (highest) with tied values sharing tied ranks (e.g. if two types had joint highest means, they were both ranked 2.5). This was done independently for each of the 44 WISER lakes for which there were one or more sampled sites for all three modification types. A Friedman two-way analysis of ranks statistical test (adjusted for tied ranks) was then used to test whether there were statistically significant consistent rank orders of the modification type metric values across the lakes dataset as a whole.

Table 14 gives the Friedman test results, the overall median metric value of each modification type and the average within-lake rank for each type mean value across all 44 lakes. In the absence of any consistency of association between metric and modification type, the average rank for each type should be roughly 2.0, an average rank of 3.0 for a modification type would indicate it had the highest mean metric value for every lake, while an average rank of 1.0 would indicate lowest values for every lake. Fifteen metrics had Friedman test probability p values less than 0.01 (highlighted in bold in Table 14) and all these cases except for ‘% Oligochaeta’ the natural type of site had the highest average value and rank (highlight shading). For all except one of these 14 metrics, the sites subject to medium (M) modification had the lowest value and rank when average across lakes.



**Table 14:** Friedman test p value for consistency across lakes of the within-lake rank order of the site modification types (H,M,N) mean metric values, together with the overall median metric value and mean rank of each modification type across all 44 WISER lakes with composite samples for each site type; metric with  $p < 0.01$  in bold, type with highest value and rank shaded.

Metric	Friedman test p value	Median value of metric			Mean Rank		
		H	M	N	H	M	N
<b>ASPT</b>	<b>0.002</b>	<b>4.82</b>	<b>4.77</b>	<b>4.99</b>	<b>1.84</b>	<b>1.73</b>	<b>2.43</b>
<b>Shannon</b>	<b>Wiener 0.001</b>	<b>1.64</b>	<b>1.61</b>	<b>1.76</b>	<b>1.97</b>	<b>1.56</b>	<b>2.48</b>
Evenness	0.063	0.59	0.59	0.62	2.11	1.70	2.18
r/K relationship	0.047	0.16	0.14	0.13	2.20	2.09	1.70
Pel MicroHab %	0.110	26.95	28.32	25.41	1.82	2.25	1.93
Arg MicroHab %	0.359	0.98	0.84	1.25	2.11	1.83	2.06
Psa MicroHab %	0.016	9.91	12.26	8.67	2.02	2.30	1.68
Aka MicroHab %	0.086	5.38	6.22	4.39	2.14	2.14	1.73
Lit MicroHab %	0.016	10.43	8.56	8.91	2.34	1.91	1.75
Phy MicroHab %	0.012	14.73	13.56	19.42	1.86	1.77	2.36
<b>Pom MicroHab %</b>	<b>0.001</b>	<b>3.76</b>	<b>2.02</b>	<b>5.18</b>	<b>2.05</b>	<b>1.48</b>	<b>2.48</b>
Grazers and scrapers %	0.070	11.18	10.64	12.46	1.93	1.80	2.27
Shredders %	0.020	3.82	2.47	4.81	1.95	1.73	2.32
Gatherers and Collectors	0.006	50.92	52.78	41.14	2.14	2.25	1.61
Active filter feeders %	0.376	7.94	8.75	8.89	1.86	1.98	2.16
Predators %	0.913	8.32	8.77	9.65	1.95	2.00	2.05
Swimming/diving %	0.853	10.56	9.77	11.79	1.95	1.98	2.07
<b>Burrowing/boring %</b>	<b>0.001</b>	<b>0.54</b>	<b>0.37</b>	<b>0.87</b>	<b>1.89</b>	<b>1.66</b>	<b>2.45</b>
Sprawling/walking %	0.148	10.96	10.56	17.19	2.05	1.77	2.18
(Semi) Sessil %	0.328	9.05	8.91	10.76	1.93	1.89	2.18
Turbellaria %	0.254	0.10	0.12	0.27	2.01	1.82	2.17
Gastropoda %	0.288	1.86	2.19	2.99	1.92	1.89	2.19
Bivalvia %	0.194	1.63	1.80	2.25	2.00	1.81	2.19
<b>Oligochaeta %</b>	<b>0.001</b>	<b>13.91</b>	<b>18.12</b>	<b>10.50</b>	<b>2.11</b>	<b>2.32</b>	<b>1.57</b>
Hirudinea %	0.849	0.17	0.20	0.20	2.06	2.00	1.94
Crustacea %	0.106	10.46	3.95	10.38	1.83	1.92	2.25
Ephemeroptera %	0.049	9.39	8.54	8.98	2.14	1.70	2.16
Plecoptera %	0.519	0.00	0.00	0.00	2.01	1.94	2.05
Heteroptera %	0.744	0.92	1.34	0.47	1.97	2.09	1.94
Trichoptera %	0.005	2.61	1.38	2.83	2.25	1.60	2.15
Coleoptera %	0.010	0.23	0.11	0.35	1.97	1.73	2.31
Diptera %	0.126	21.20	29.08	27.02	1.89	2.25	1.86
<b>EPT Taxa %</b>	<b>0.009</b>	<b>13.41</b>	<b>13.34</b>	<b>14.26</b>	<b>2.09</b>	<b>1.64</b>	<b>2.27</b>
<b>Number of Taxa</b>	<b>0.001</b>	<b>18.17</b>	<b>16.83</b>	<b>19.00</b>	<b>1.90</b>	<b>1.55</b>	<b>2.56</b>
<b>NTaxa Turbellaria</b>	<b>0.007</b>	<b>0.33</b>	<b>0.33</b>	<b>0.67</b>	<b>1.84</b>	<b>1.86</b>	<b>2.30</b>
<b>NTaxa Gastropoda</b>	<b>0.003</b>	<b>2.00</b>	<b>1.67</b>	<b>2.33</b>	<b>1.85</b>	<b>1.75</b>	<b>2.40</b>
NTaxa Bivalvia	0.271	0.67	0.83	1.00	1.90	1.93	2.17
NTaxa Hirudinea	0.861	0.50	0.58	0.67	2.02	1.94	2.03
NTaxa Crustacea	0.013	1.33	1.00	1.50	2.05	1.73	2.23
<b>NTaxa Ephemeroptera</b>	<b>0.007</b>	<b>1.83</b>	<b>2.00</b>	<b>2.33</b>	<b>1.92</b>	<b>1.74</b>	<b>2.34</b>
<b>NTaxa Odonata</b>	<b>0.001</b>	<b>0.33</b>	<b>0.33</b>	<b>0.67</b>	<b>1.82</b>	<b>1.74</b>	<b>2.44</b>
<b>NTaxa Trichoptera</b>	<b>0.002</b>	<b>2.00</b>	<b>1.67</b>	<b>2.33</b>	<b>2.01</b>	<b>1.64</b>	<b>2.35</b>
<b>NTaxa Coleoptera</b>	<b>0.003</b>	<b>0.61</b>	<b>0.50</b>	<b>0.72</b>	<b>1.98</b>	<b>1.69</b>	<b>2.33</b>
NTaxa Diptera	0.939	2.00	2.00	2.00	1.97	2.00	2.03
<b>NTaxa EPTCBO</b>	<b>0.001</b>	<b>8.00</b>	<b>7.17</b>	<b>9.17</b>	<b>1.91</b>	<b>1.61</b>	<b>2.48</b>
<b>Number of Families</b>	<b>0.001</b>	<b>15.50</b>	<b>14.17</b>	<b>16.33</b>	<b>1.82</b>	<b>1.58</b>	<b>2.60</b>

These metrics showing some significant consistency of highest values for natural sites and lowest for medium modified sites are ASPT, Shannon-Wiener diversity, % individuals preferring detritus micro-habitats (Pom Microhab %), '% Burrowing/boring', % individuals which are EPT Taxa and nine taxonomic richness metrics of which 'Number of taxa' and 'Number of families' are the best at responding consistently to lake margin modification (Table 14).

In summary, a range of taxonomic richness metrics and several taxonomic composition metrics based on percentage of all individuals in particular taxonomic groups (all as highlighted in Table 14) show significant consistency across a range of European lakes in response to lake margin morphological modification. These should be investigated further, in conjunction with the country/region specific multi-metric indices reported in Section 4 of this report, with the aim of developing a Europe-wide common set of metrics for use in multi-metric assessments of macroinvertebrate response to lake margin morphological modification.

## 7 Rules to combine invertebrate scores

*Francesca Pilotto, Oliver Miler, Martin Pusch*

Since the EU-WFD requires the assessment of the ecological quality at lake level scale, the up-scaling of the assessment from site to whole water body level is an important task. Within the WISER WP3.3, the LHS method was used to describe the naturalness and anthropogenic / hydromorphological degradation of the lake shore. Hereby 2 sets of data were collected, the complete "Whole Lake" LHS (section 1 to 4 of the LHS protocol) and a "Site-specific" LHS (section 2 of the LHS protocol). The main LHS assessment unit is the hab-plot, a 15 m wide part of the shore that extends 15 m landwards into the riparian zone and 10 m lakewards into the littoral zone. The Whole Lake LHS covers 10 evenly spaced hab-plots, with the first hab-plot placed randomly. However, since the macrozoobenthos sampling scheme required 3 sites with high, 3 sites with medium and 3 natural sites with no hydromorphological degradation, these requirements could not always be met by the randomly distributed Whole Lake hab-plots. Hence, to describe also the very sites, where the macrozoobenthos samples were taken, the part of the LHS that assesses environmental variables at the hab-plot level (section 2 of the LHS protocol) was conducted additionally at the 9 macrozoobenthos sampling sites at each lake. For the Whole lake LHS characteristics of the Whole lake (section 1, 3.2 and 4 of the LHS protocol) and also parameters describing the spaces between the 10 hab-plots (section 3.1 of the LHS protocol) were recorded.

From the site-specific LHS a stressor index was calculated for each biogeographic region (see Chapter 3) that is used to determine and calibrate multimetric Makrozoobenthos-Indices (see Chapter 4). However, this provides an ecological assessment of the sampling sites, whereas the EU-WFD requires a Whole Lake Assessment. To calculate a multimetric assessment for the Whole Lake, we propose here 2 procedures that will be described in more detail in the following.

## 7.1 Pressure index extrapolation

As mentioned above, section 3.1 of the Whole Lake LHS protocol describes environmental variables between the 10 hab-plots. These belong to the 4 categories “Shore/littoral pressures”, “Riparian land use pressures”, “Wetland habitats” and “Other habitats” (see screenshot in Fig. 10). The human pressures in the categories “Shore/littoral pressures” and “Riparian land use pressures” that are assessed between the habplots are almost identical to those assessed at the habplots in section 2.4 (see screenshot in Fig. 11). Hence, we can calculate a pressure index analogous the one that constitutes a component of the stressor index in all 4 biogeographical regions (see Chapter 3). A slight modification is that there is no distinction between “at site” and “adjacent/behind site” (marked in section 2.4 as a tick and “B”, respectively) when the pressure indices for the spaces between the habplots are calculated. Since the pressure index is normalized from 0 to 1 and subsequently scored from 1 to 5 (1 indicating no pressure and 5 indicating high pressure) it is comparable to the pressure index calculated as a stressor index component in Chapter 3 (Tables 2 and 3).

In a first step the pressure index at each section between the habplots (A-B, B-C ... until J-A) was calculated. Then, using regressions (derived from the correlations between the pressure index as a stressor index component and the LIMCO/LIMHA index for each biogeographic region and habitat) that were performed *separately for each lake* a LIMCO/LIMHA value at each section between the habplots was calculated.

Since each between hab-plot section covers 10 % of the lake shore, the pressure index and hence LIMCO/LIMHA value in each shoreline section accounts for 1/10 of the Whole Lake LIMCO/LIMHA value. In some lakes in Italy and Finland, where a lower number of between hab-plot sections was assessed, these represented each more than 10 % of the shoreline and this deviation was noted in the protocol and taken into account during calculations.

For the 4 Finnish lakes Iso-Jurvo (IJ), Jyvasjärvi (JY), Sääksjärvi (SÄ) and Vuojärvi (VU) no Whole lake LHS habplots were assigned and the respective environmental variables in section 2.4 were not recorded within a Whole Lake Habitat Survey. However, the section 2.4 was instead filled out by percentages of shoreline between site-specific LHS habplots which were approximately evenly distributed and hence were treated in the same way as Whole Lake LHS habplots.

3. WHOLE LAKE ASSESSMENT (carry out in consultation with large scale colour e.g. 1:25,000 topographic map and recent colour air photograph if at all possible)													
3.1 LAKE PERIMETER CHARACTERISTICS Complete in two belts, the first extends from 10 m into the littoral zone to 15 m landwards of the bank edge (e.g. Hab-Plot length), the second > 15 - 50 m landwards of the bank edge (extra-riparian)													
Complete table from either a boat-based survey (cruising and observing between Hab-Plots) OR by viewing visible perimeter sections from each Hab-Plot (these must be shown on sketch map). Observe progressively from Hab-Plots A, B, C, etc. Observe 100% if possible, but always observe at least 75%. <b>MARK ON SKETCH MAP OR ON ATTACHED TOPOGRAPHIC MAP / AIR PHOTO THE EXTENTS OF ALL SECTIONS</b>													
EXTENT OF LAKE PERIMETER SECTION AFFECTED BY (OR COMPRISED OF) EACH PRESSURE OR LAND COVER TYPE													
Estimate extent (0 (0%), ✓ (>0-1%), 1 (>1-10%), 2 (>10-40%), 3 (>40-75%), 4 (>75%)). Ring entry if known to affect 'critical' area.													
Perimeter section number		1	2	3	4	5	6	7	8	9	10		
Circle option used	Boat: viewed between Hab-Plots	A-B	B-C	C-D	D-E	E-F	F-G	G-H	H-I	I-J	J-A		
	Shore: viewed from Hab-Plots	A	B	C	D	E	F	G	H	I	J		
Section as % of total shore*													
% shoreline (0-15 and 15-50 m)		15	50	15	50	15	50	15	50	15	50	15	50
Shore / littoral pressures	Impounding structures <i>Docks, harbours or marinas*</i>												
	Hard bank engineering (closed)												
	Hard bank engineering (open)												
	Soft bank engineering												
	Flow and sediment control												
	Piled structures												
	Floating & tethered structures												
	Moorings (high density)												
	Outfalls & intakes												
	Floodwalls / embankments												
	Land claim												
	Dumping												
	Sediment extraction												
	Recreational beaches												
Bank erosion													
Riparian land use pressures	Commercial activities												
	Residential areas												
	Roads or railways												
	Unsealed tracks and pathways												
	Parks and gardens												
	Camping and caravanning												
	Educational recreation												
	Quarrying or mining												
	Coniferous plantation												
	Evidence of recent logging												
	Tilled land												
	Improved grassland												
	Soil poaching (trampling)												
	Orchard												
Wetland habitats	Reed-bed												
	Wet woodland (carr)												
	Bog												
	Fen or marsh												
	Floating vegetation mats Other												
Other habitats	Broadleaf/mixed woodland												
	Broadleaf/mixed plantation												
	Coniferous woodland												
	Scrub and shrubs												
	Moorland/heath												
	Open water												
	Rough grassland												
	Tall herb/rank vegetation Rock, scree or dunes												

Figure 10: LHS Field form (latest version from December 2008), section 3.1, with the environmental variables between the habplots A to J that are recorded for the Whole Lake Assessment.

Do macrophytes extend lakewards (YE = Yes, NO = No)										
Notable non-native species (NO=None, NP=Nuttalls pondweed, AS=Australian swamp stonecrop, PF=Parrots feather, FP=Floating pennywort, OT=Other) * If animals or fish use OT, explain Section 7										
Extent of non-native species (0 (0%), 1 (>0-1%), 2 (>1-10%), 3 (>10-40%), 4 (>40-75%), 5 (>75%)										
Surface film (NO=None, SC=Scum, AM=Algal Mat, OL=Oily, OT=Other)										
<b>2.4 HUMAN PRESSURES (to be assessed over entire plot)</b> NO=None, ✓ (tick) if present, B = behind or adjacent to plot (within 50m radius)										
<p>Any other pressures or comments for this section (indicate which Hab-Plots affected):</p> <hr/> <p>Note Docks, harbours and marinas are included as a separate category to capture locally significant boating activity</p>	Commercial									
	Residential									
	Roads or railways									
	Unsealed tracks and footpaths									
	Parks and gardens									
	Camping and caravanning									
	Quarrying, mining, peat extraction									
	Coniferous plantation									
	Tilled land (arable)									
	Orchard									
	Improved grassland (ring if grazing observed)									
	Other grazed land (ring if grazing observed)									
	Docks, harbours or marinas*									
	Hard bank engineering									
	Soft bank engineering									
	Flow and sediment control structures									
	Piled structures									
	Outfalls and intakes									
	Flood walls / embankments									
	Land claim									
Dumping										
Sediment extraction										
Floating/tethered structures (includes aquaculture cages)										
Macrophyte manipulation										
Moorings										
Recreational pressures (moderate intensity or more)										

Figure 11: LHS Field form (latest version from December 2008), section 2.4, Human pressures.

Table 15: Extrapolation of LIMCO and LIMHA (MP = macrophytes, SA = sand, ST = stones) for the 4 biogeographical regions (D = Germany / DK = Denmark, IRL = Ireland / GB = United Kingdom, S = Sweden / FIN = Finland, IC = central Italy / IN = northern Italy) via the pressure index component of the stressor index: Whole lake LIMCO and LIMHA values are calculated by summing up the weighted pressure indices of the spaces and correlating these values through the relationship between LIMCO/LIMHA and the pressure index. Ecological quality classes are based on the class boundaries set in chapter 4.3 and on LIMCO.

Biogeographical region	Lake abbreviation	Ecological quality class	LIMCO	LIMHA (MP)	LIMHA (ST)	LIMHA (SA)
D/DK	FU	High	0.81	-	-	-
D/DK	GI	Moderate	0.39	0.77	0.01	0.37
D/DK	GW	High	0.58	0.68	0.43	0.58
D/DK	MU	Moderate	0.36	0.44	0.31	0.21
D/DK	NO	High	0.67	0.62	0.75	0.69
D/DK	ROB	Good	0.54	0.81	0.45	0.42
D/DK	ROF	High	0.85	-	-	-
D/DK	SD	High	0.74	0.84	0.58	0.63
D/DK	STI	Moderate	0.41	0.51	0.39	0.47
D/DK	UN	Good	0.49	0.61	0.50	0.38
D/DK	WE	Good	0.51	0.67	0.49	0.38
IRL/GB	BR	High	0.82	0.66	-	0.24
IRL/GB	CA	High	0.49	0.74	-	0.61

IRL/GB	CU	High	0.50	0.50	-	0.56
IRL/GB	GA	High	0.60	0.47	-	-
IRL/GB	GR	High	0.61	-	-	-
IRL/GB	LO	High	0.88	-	-	-
IRL/GB	MUC	Moderate	0.29	-	-	-
IRL/GB	OU	Good	0.35	0.33	-	0.30
IRL/GB	RE	High	0.56	0.60	-	0.74
IRL/GB	RI	Good	0.36	0.22	-	0.69
IRL/GB	RO	High	0.55	-	-	-
IRL/GB	SC	High	0.54	0.50	-	0.43
IC/IN	AL	High	0.43	-	-	0.32
IC/IN	ALB	Poor	0.09	-	-	0.37
IC/IN	BO	Moderate	0.20	-	-	-
IC/IN	BRA	High	0.42	-	-	-
IC/IN	CAN	High	0.51	-	-	0.02
IC/IN	IS	High	0.40	-	-	0.46
IC/IN	MAR	Good	0.29	-	-	-
IC/IN	MO	Good	0.33	-	-	0.28
IC/IN	MON	Moderate	0.20	-	-	0.12
IC/IN	NE	Moderate	0.24	-	-	-0.35
IC/IN	PI	High	0.54	-	-	-
IC/IN	PU	High	0.42	-	-	0.41
IC/IN	SE	High	0.56	-	-	0.48
IC/IN	VAR	High	0.46	-	-	0.39
IC/IN	VI	Moderate	0.20	-	-	-
S/FIN	FF	Moderate	0.32	0.62	-	-
S/FIN	HF	High	0.66	-	-	-
S/FIN	IJ	High	0.62	-	-	-
S/FIN	JY	not applicable	-0.18	-	-	-
S/FIN	MA	High	0.52	-	-	-
S/FIN	OJ	Good	0.42	0.49	-	-
S/FIN	RU	Good	0.35	-	-	-
S/FIN	SF	High	0.54	0.46	-	-
S/FIN	SS	Moderate	0.32	-	-	-
S/FIN	SV	Good	0.33	-	-	-
S/FIN	SÄ	High	0.65	-	-	-
S/FIN	VA	High	0.50	0.53	-	-
S/FIN	VU	High	0.74	-	-	-

## 7.2 Correlation with stressor indices of the Whole Lake LHS hab-plots

Since the parameters in the section 2 of the LHS protocol, which are the basis for the stressor index calculations, have been recorded for the Site-specific as well as for the Whole Lake LHS, stressor index values can be calculated also for the 10 Whole Lake LHS hab-plots. Under the assumption that each of these represents 10 % of the shoreline, Whole Lake values for LIMCO and LIMHA can be inferred from correlations with the stressor index (see Figs. 5, 6 and 7) that were performed **separately for each lake**.

For the 4 Finnish Lakes Iso-Jurvo (IJ), Jyvasjärvi (JY), Sääksjärvi (SÄ) and Vuojärvi (VU) this method could not be applied since no Whole lake LHS habplot parameters were assessed (see comment in chapter 7.1). Instead a different approach was chosen. Since the habplots from the site-specific LHS were approximately evenly distributed around the lake and the percentages of shoreline between them were known, these were treated in the same way as Whole Lake LHS habplots. For Lake Muckno (MUC) in Ireland only 9 Whole lake LHS habplots were assessed so that an extrapolation of LIMCO and LIMHA to lake level was not possible.

*Table 16: Extrapolation of LIMCO and LIMHA (MP = macrophytes, SA = sand, ST = stones) for the 4 biogeographical regions (D = Germany / DK = Denmark, IRL = Ireland / GB = United Kingdom, S = Sweden / FIN = Finland, IC = central Italy / IN = northern Italy) via the stressor index: Whole lake LIMCO and LIMHA values are calculated by summing up the weighted stressor indices of the Whole lake hab-plots and correlating these values through the relationship between LIMCO/LIMHA and the stressor index (see Figs. 5, 6 and 7). Ecological quality classes are based on the class boundaries set in chapter 4.3 and on LIMCO.*

Biogeographical region	Lake abbreviation	Stressor index	Ecological quality class	LIMCO	LIMHA (MP)	LIMHA (ST)	LIMHA (SA)
D/DK	FU	2.60	High	0.74	-	-	-
D/DK	GI	2.58	Moderate	0.42	0.77	-0.26	0.38
D/DK	GW	2.72	High	0.60	0.71	0.42	0.62
D/DK	MU	3.40	Moderate	0.35	0.36	0.42	0.22
D/DK	NO	2.78	High	0.60	0.63	0.71	0.63
D/DK	ROB	2.82	High	0.60	0.67	0.42	0.58
D/DK	ROF	1.92	High	1.00	-	-	-
D/DK	SD	2.76	High	0.72	0.87	0.55	0.62
D/DK	STI	3.24	Good	0.45	-0.31	0.38	0.51
D/DK	UN	2.98	Good	0.54	0.58	0.46	0.32
D/DK	WE	2.58	Good	0.53	0.09	0.45	0.44
IRL/GB	BR	2.23	Good	0.75	0.57	-	0.24
IRL/GB	CA	2.30	Good	0.54	0.68	-	0.71
IRL/GB	CU	1.95	High	0.54	0.69	-	0.72
IRL/GB	GA	2.30	High	0.57	0.54	-	-
IRL/GB	GR	2.38	High	0.58	-	-	-

IRL/GB	LO	2.33	High	0.81	-	-	-
IRL/GB	OU	1.85	Good	0.33	0.33	-	0.38
IRL/GB	RE	2.20	High	0.56	0.60	-	0.74
IRL/GB	RI	2.60	Good	0.34	0.21	-	0.44
IRL/GB	RO	2.85	High	0.51	-	-	-
IRL/GB	SC	2.00	High	0.56	0.52	-	0.43
IC/IN	AL	2.98	High	0.44	-	-	0.32
IC/IN	ALB	3.06	Moderate	0.25	-	-	0.37
IC/IN	BO	3.64	Good	0.36	-	-	-
IC/IN	BRA	3.46	High	0.40	-	-	-
IC/IN	CAN	2.20	High	0.57	-	-	-0.28
IC/IN	IS	3.26	High	0.38	-	-	0.46
IC/IN	MAR	3.44	Good	0.35	-	-	-
IC/IN	MO	2.50	High	0.44	-	-	0.32
IC/IN	MON	2.62	Good	0.32	-	-	0.20
IC/IN	NE	2.92	High	0.45	-	-	2.40
IC/IN	PI	3.12	High	0.61	-	-	-
IC/IN	PU	3.14	High	0.55	-	-	0.48
IC/IN	SE	2.74	High	0.59	-	-	0.44
IC/IN	VAR	2.78	High	0.49	-	-	0.41
IC/IN	VI	3.22	High	0.42	-	-	-
S/FIN	FF	1.90	Moderate	0.32	0.62	-	-
S/FIN	HF	2.30	High	0.50	-	-	-
S/FIN	IJ	3.35	High	0.57	-	-	-
S/FIN	JY	3.71	Good	0.33	-	-	-
S/FIN	MA	2.25	High	0.52	-	-	-
S/FIN	OJ	2.95	Good	0.39	0.56	-	-
S/FIN	RU	2.48	High	0.47	-	-	-
S/FIN	SF	2.00	High	0.53	0.56	-	-
S/FIN	SS	2.00	Good	0.43	-	-	-
S/FIN	SV	2.58	Good	0.36	-	-	-
S/FIN	S†	3.22	High	0.62	-	-	-
S/FIN	VA	1.73	High	0.61	0.54	-	-
S/FIN	VU	2.76	High	0.76	-	-	-

The ecological assessment with Whole Lake LIMCO in Table 15 and 16 seems to be plausible for some lakes, e.g. for the lakes in Ireland and the UK (IRL/UK). Furthermore, in Germany the near natural (reference) lake Roofensee (ROF) has a much higher value than the hydromorphologically clearly more degraded Lake Müggelsee which is located within the area of the city Berlin.



However, for some lakes the ecological status class does not seem to fit as e.g. the lakes Albano (ALB, IC/IN), Martignano (MAR, IC/IN) and Färnebofjärden (FF, S/FIN) should have better classifications due to the high naturalness of their shoreline. Bracciano (BRA, IC/IN), Iserio (IS, IC/IN), Pusiano (PU, IC/IN), Magelungen (MA, S/FIN) and Jyvasjärvi (JY, S/FIN) should have a worse classification as their shorelines are highly altered, e.g. through hard bank modifications.

The resulting whole lake values for LIMCO and LIMHA show strong differences between each other for some lakes. Due to the low number of only 2 or 3 samples of a specific habitat in a lake, LIMHA values are not always reliable and diverge from LIMCO. Hence even sometimes incorrect, negative values occur. For reliable Whole Lake LIMCO and LIMHA assessments even more than the 9 samples taken for LIMCO are clearly needed, as the unconvincing assessments for some lakes show. An additional explanation is that in the correlations between LIMCO/LIMHA and the pressure index (section 7.1) or stressor index (section 7.2) sometimes not the whole gradient of hydromorphological stress is covered or is strongly biased, e.g. when the highly modified shoreline in one lake is close to the hydromorphological degradation status of a natural site in another lake. For each lake to be studied a solid LIMCO/LIMHA correlation with the pressure index or stressor index has to be established on a large number of sampling sites before a reliable Whole lake assessment based on Whole LHS results can be achieved.

## 8 Synthesis of WP 3.3

*Martin Pusch (IGB)*

With the help of significant additional funding acquired externally, WISER workpackage 3.3 happily succeeded to reach its ambitious aims, i.e.

- to identify responses of eulittoral benthic invertebrates in lakes to hydromorphological pressures based on existing data and new data obtained during the joint field sampling campaign,
- to recommend techniques to sample and process benthic invertebrates in lakes in order to minimise sources of uncertainty influencing the final assessment score,
- to support harmonisation work of ECOSTAT,
- to develop and validate an indication tool based on benthic macroinvertebrates for hydromorphological alterations for lakes in different regions of Europe,
- to recommend under which conditions low-cost monitoring methods on lake shores - such as lake habitat survey - may partially replace indication by lake invertebrates.

For that purpose, a sampling campaign of benthic invertebrates was conducted in the eulittoral zone of lakes in order to produce a methodologically homogeneous dataset. The sampling campaign included 51 lakes in 7 countries from three trophic levels (eutrophic, mesotrophic and oligotrophic), which were sampled at shoreline sections representing three hydromorphological degradation levels (unmodified, moderately modified and highly modified). Each alteration type

was replicated three times per lake, resulting in nine samples for each lake. More specifically, benthic invertebrates were sampled in Germany (9 lakes), Denmark (2 lakes), Ireland (9 lakes), United Kingdom (3 lakes), Sweden (9 lakes), Finland (4 lakes) and Italy (15 lakes altogether; 8 lakes in the subalpine and 6 lakes in the Mediterranean region). Partially these lakes were sampled for other biological quality elements, too.

Hydromorphological pressures to lake shores were parameterized using the Lake habitat Survey (LHS) method. Parameters obtained by the LHS method were used for the development of a stressor index which was needed to calibrate the developed biotic multimetric indices LIMCO and LIMHA.

Based on macrozoobenthos data, a biological typology of European lakes was established based on littoral benthic invertebrates. Based on that, the hydromorphological stressor index and the biological multimetric index were developed separately for each of the four biogeographical regions Germany/Denmark, Ireland/United Kingdom, Sweden/Finland and central Italy/northern Italy.

Candidate metrics were selected and multimetric indices were developed for several European biogeographical regions, based on the newly acquired homogeneous dataset on benthic lake invertebrates covering 7 European countries. The indices may be used to assess hydromorphological lake shore alterations based on benthic macroinvertebrate surveys.

A multimetric index based on composite macroinvertebrate samples (LIMCO) was developed that is adapted specifically to four biogeographical regions across Europe from a dataset that has been obtained with a unified, standardised sampling scheme.

Furthermore, a multimetric index based on habitat samples (LIMHA) was developed, which was also adapted specifically to four biogeographical regions across Europe. It shows that an ecological assessment based on habitat samples is feasible, when a sufficient number of samples is obtained.

The assessment of the ecological effects of hydromorphological alterations to lake shores can be assessed at whole-lake level by interpolation of site-specific biological scores. Interpolation may be supported by physical habitat surveys of lake shores, which can be recalculated into a stressor index closely correlating with the biological metrics.

Hence, WISER WP 3.3 succeeded to establish a second approach to assess the ecological status of Europe's natural lakes, which allows to estimate the ecological effects of morphological alterations to lake shores, which consists the second important human pressure to lakes after eutrophication.

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