ANNEX 2.3.1 – National assessment systems for macroinvertebrates

1.) Overview

A. Officially accepted as a part of national legislation
B. Officially accepted
C. Finalized
D. Under development
E. Not started yet
F. Other (please specify)

QE Macroinvertebrates	*Assessment Method*	*Status (choose A-F)*
AUSTRIA	Multimetric Indices for General Degradation (Structural Diversity, nutrients,…), Saprobic Index (Moog, O. & Ofenböck, T.(2006), in print)	B
FRANCE	French WFD classification Indice Biologique Global Normalisé (IBGN) - norm AFNOR NF T 90 350 (1992) and circular MEDD/DE 05 n°14 (July 05	A Agreed National method and classification (but revision planned to meet WFD requirements)
GERMANY	Handbuch zur Untersuchung und Bewertung von Fließgewässern auf der Basis des Makrozoobenthos vor dem Hintergrund der EG-WRRL, April 2005, www.fliessgewaesserbewertung.de	C Finalized: National method in verification
ITALY	**STAR Intercalibration Common Metric Index (STAR_ICMi), type adapted**	A Agreed national method.
SLOVENIA	Multimetric index (Hydromorphology), Saprobic Index	D In development
SPAIN	IBMWP-Iberian BMWP	B Frequently used method

2.) Relation to Normative Definitions

The WFD sets out in the normative definitions descriptive criteria for the condition of the river invertebrate fauna in the various status classes required by the Directive. The descriptions are based on composition and abundance, major taxonomic groups, ratio of disturbance sensitive to insensitive taxa and level of diversity. In order to be compliant with the Directive, it is necessary to demonstrate that national classification systems reflect the normative definitions in a robust and defensible manner.

Country	**AUSTRIA**
Classification System:	Austrian Quality Assessment System for Macroinvertebrates
General Description	Selection of reference sites according to REFCOND Guidance, National Strategy paper ("Criteria for the identification of potential reference sites") and criteria used by AQEM/STAR. The Austrian classification scheme consists of three modules (figure 1): 1. Module "Organic Pollution" (Saprobic Index in relation to stream type specific reference value) 2. Module "General Degradation" consisting of two sub-modules (2 multimetric indices) 3. Module "acidification" index (Braukmann & Biss, 2004; applied only in bioregions at risk of acidification) Metrics used for the multimetric indices are standardised in relation to the 95th percentile of metric values under stream type specific reference conditions. These standardized values are termed as "scores". Indices are calculated by averaging these scores. The benchmark value between reference (High) and good status conditions is defined as the 25th percentile of index values under reference conditions and set to a value of 0.8. That means, observed index values are divided by the benchmark value and multiplied by 0.8. Values > 1 are set to 1. Class boundaries for the ecological quality classes are defined as follows: Quality Class 1: ≥ 0.8 Quality Class 2: $\geq 0.6 < 0.8$ Quality Class 3: $\geq 0.4 < 0.6$ Quality Class 4: $\geq 0.2 < 0.4$ Quality Class 5: < 0.2 The Final Ecological Quality Class is determined by the worst case applying all relevant modules.

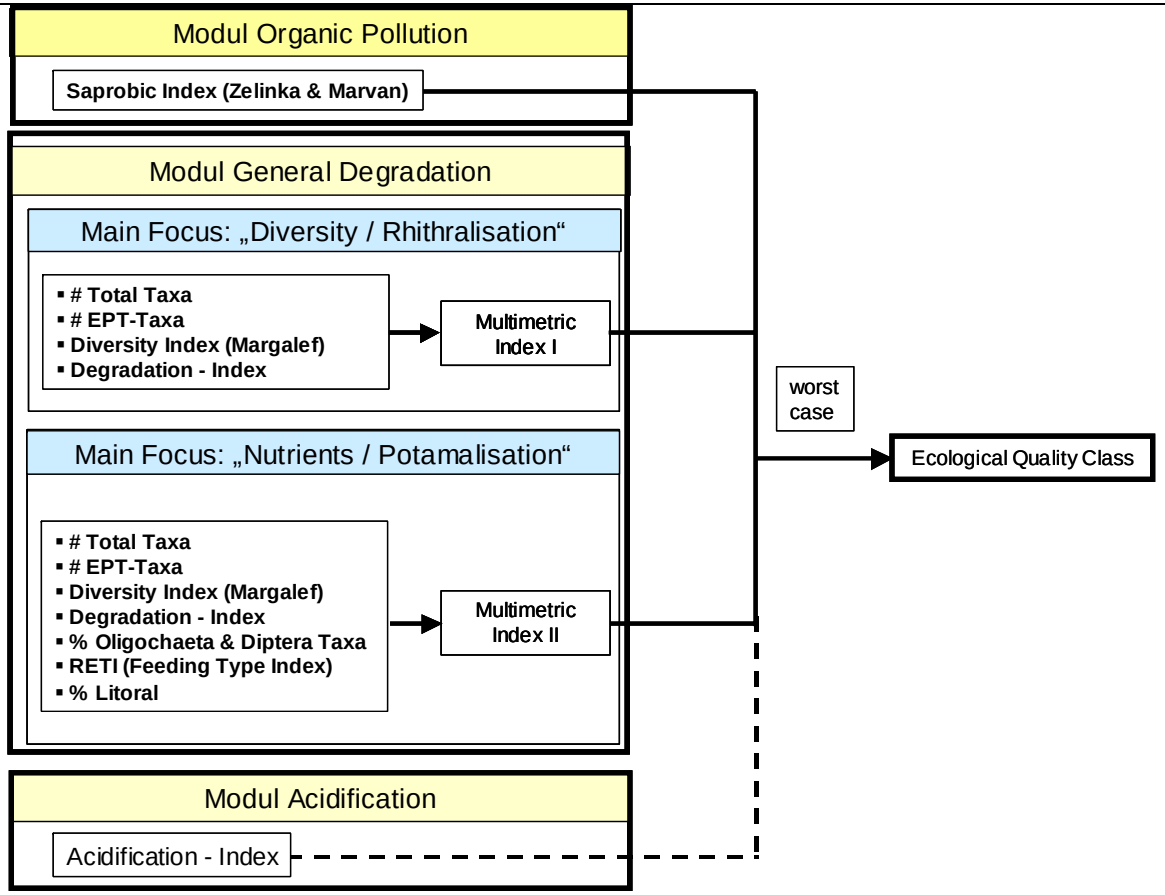
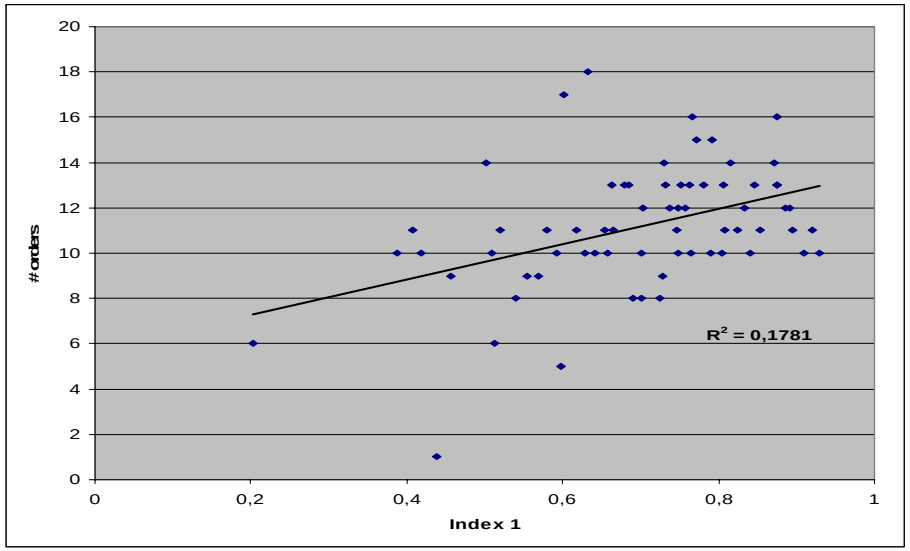


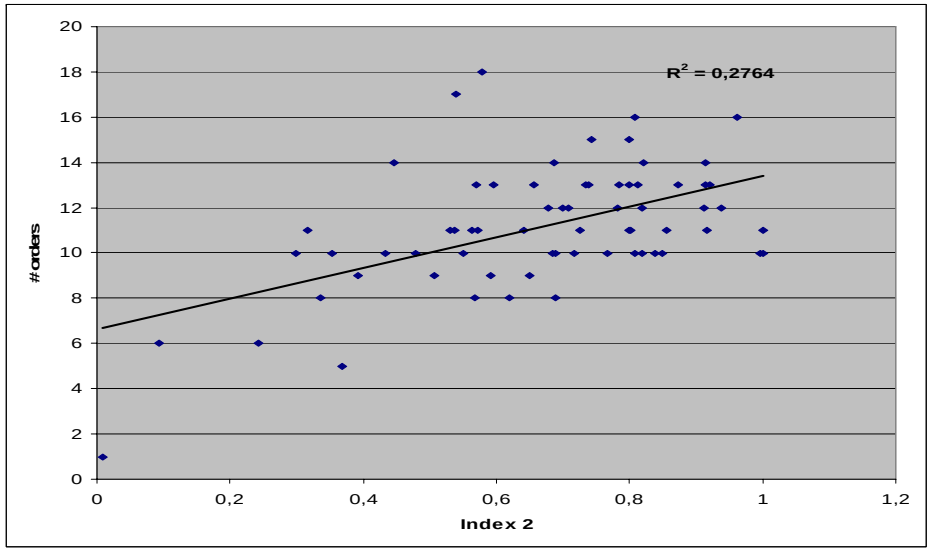
Figure 1: Scheme for the evaluation of ecological quality classes

Criteria for Boundary Setting	**High/Good boundary**	**Good/Moderate boundary**
Taxonomic composition and abundance	25th percentile of reference sites taxonomic composition represented in indices by: # taxa, % Oligochaeta and Diptera taxa, # EPT	25% deviation (of indices) from reference conditions comment: Major taxonomic groups (defined at the level of order - cannot be used for setting good/moderate

	Abundance included in Saprobic Index (# Individuals/m²) and RETI	boundary – see Appendix!): no groups missing
Ratio of disturbance sensitive to insensitive taxa	25th percentile of reference sites sensitive to insensitive represented in MMI by: # EPT, % Oligochaeta and Diptera taxa, RETI, % litoral, degradation index, acidification index	25% deviation (of indices) from reference sites comment: crossover points sensitive/insensitive taxa were not used for setting good/moderate boundary (depending too much on which taxa are selected as sensitive/insensitive)
Level of diversity	25th percentile of reference sites diversity is represented in indices by: Margalef diversity index, # taxa	25% deviation (of indices) from reference sites

Appendix (AT): Figures 2-4: Missing major taxonomic groups: number of orders vs. national MMI's





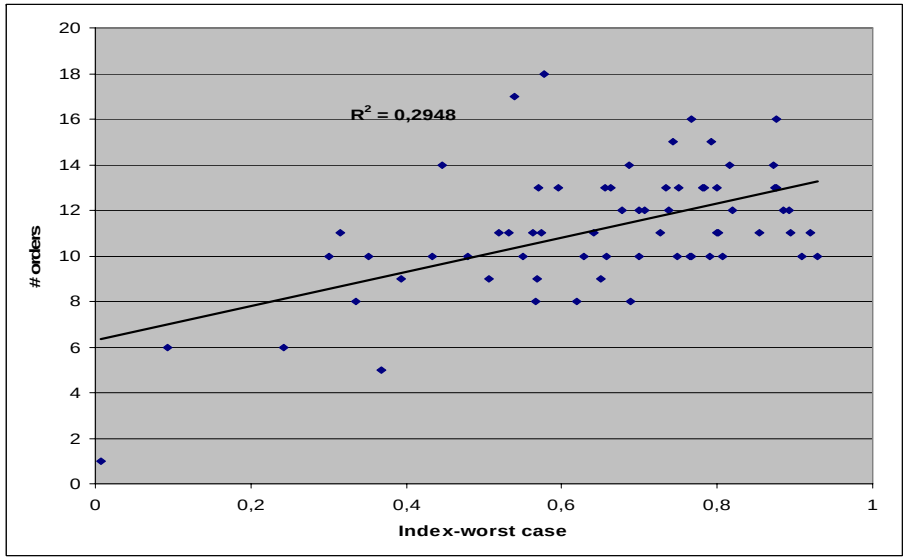
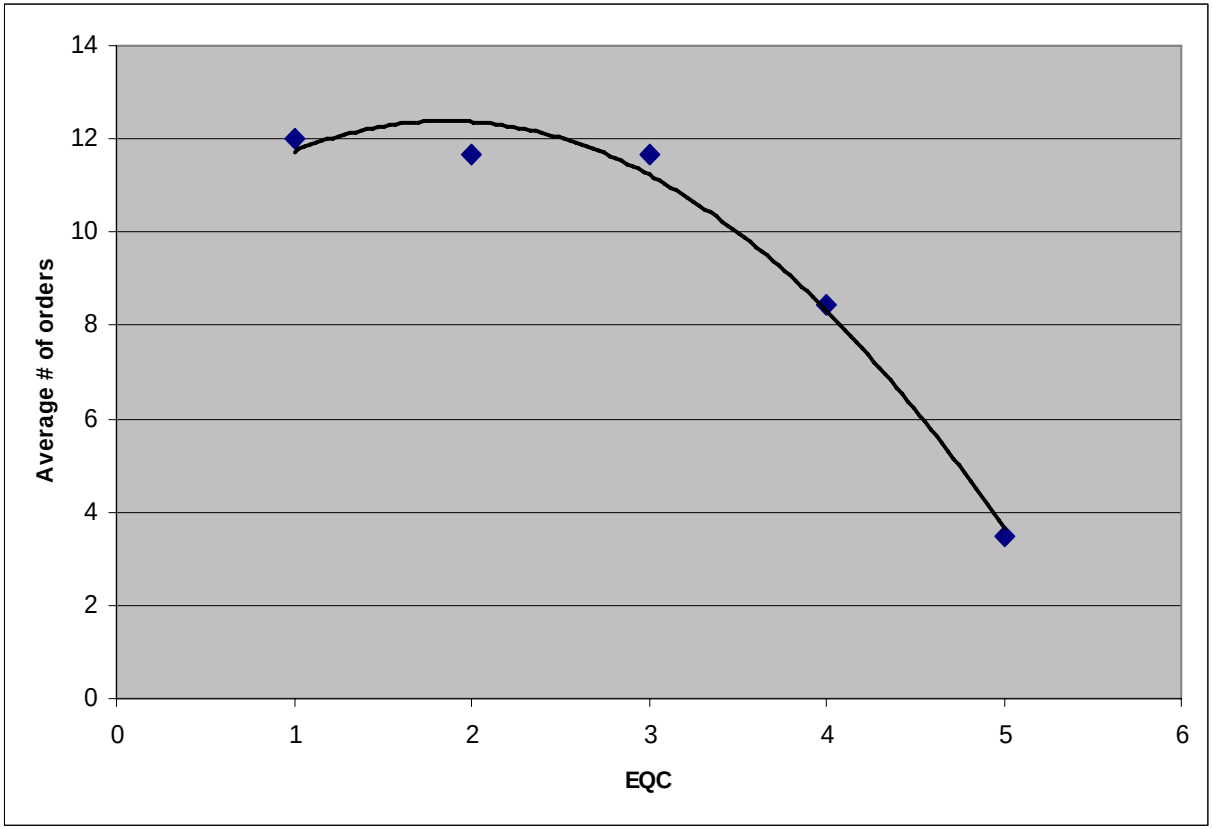


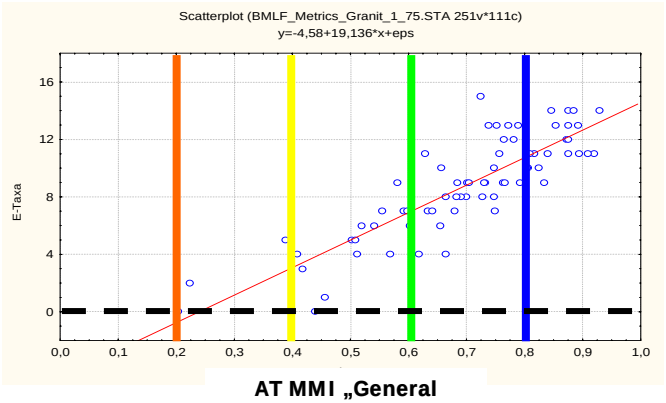
Figure 5: Average number of orders vs. Ecological Quality classes

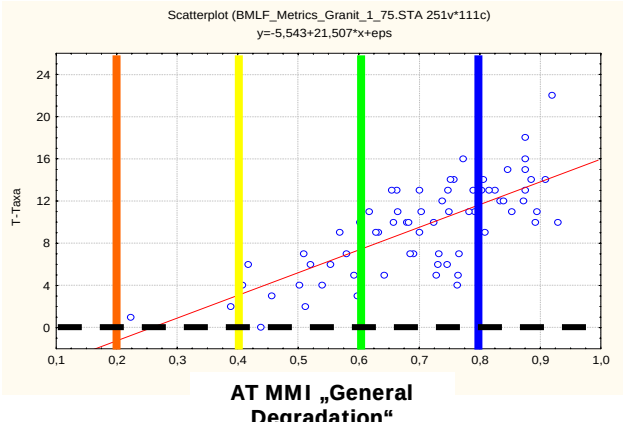


A significant decline in the number of orders can only be observed for poor and bad status class. The number of orders can therefore not be used for setting good/moderate boundaries.

EPT-Taxa can be seen as the most sensitive taxonomic groups in aquatic ecosystems. There is a strong correlation between the number of EPT-taxa and anthropogenic alteration. The decrease in the number of EPT is used in the national Multi-metric-Indices
A total disappearance of E, P and T-Taxa can only be found below good/moderate class boundary. The disappearance of EPT can therefore not be used for defining good/moderate class boundary.

Figures 6-8: Number of E, P and T –Taxa vs. national MMI (EQC)





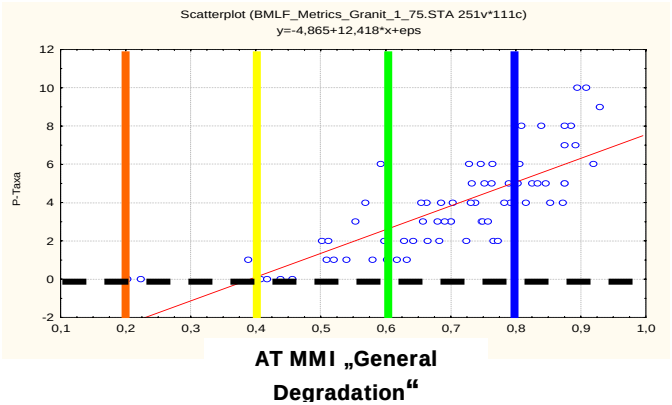
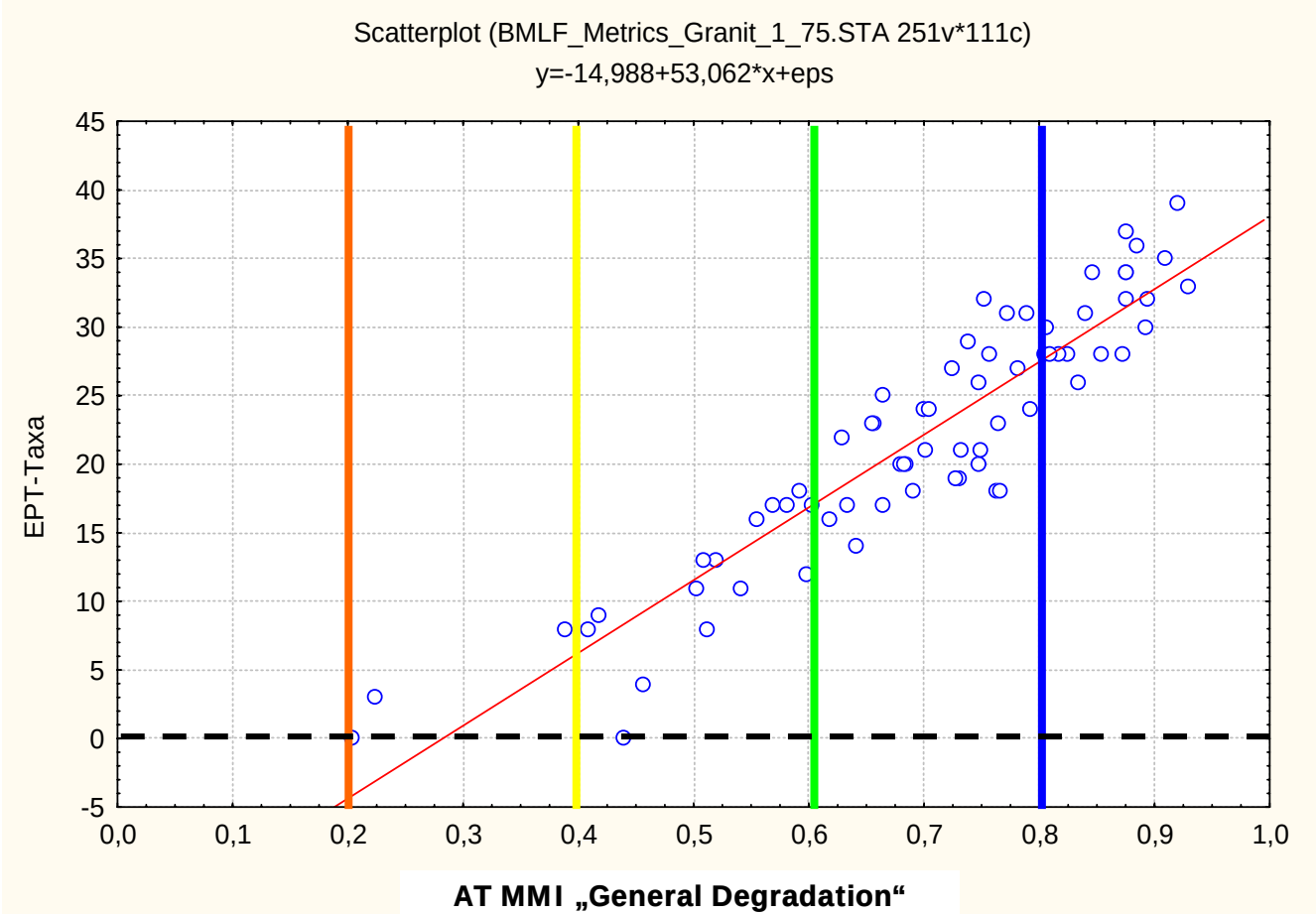


Figure 9: Number of EPT –Taxa vs. national MMI (EQC)



Country	France
Classification System:	**IBGN - Classification française DCE Indice Biologique Global Normalisé**
General Description	Selection of reference sites according to REFCOND Guidance. A set of reference criteria, officially endorsed by the French Ministry of Environment (Circulaire MEDD/ DE/ DCE 08 du 23 décembre 2004) has been applied at the national level. These criteria are compliant with the CB GIG reference criteria, including chemical values when available. (See separate annex on reference criteria). Description of the IBGN Index. (Norme AFNOR NF T 90-350, 1992) The index is based on macroinvertebrate fauna identified at the family level. Eight individual samples are taken from different habitat. The index is semi-quantitative, but quantitative data are available in many cases, allowing the comparison with the ICM index. The IBGN is a combination of 2 metrics : - the total number of taxa (at the family level for Insecta, Crustacea, Mollusca, Acheta; class for the other groups), is expressed in 14 classes of taxonomic richness. The Metric CV : Classes de Variété, varies from 14 (> 50 taxa) to 1 (1-3 taxa) . - the Indicator Faunistic Group representing the presence/absence of 39 indicator taxa, grouped in 9 classes of sensitivity to pollution. The Metric GFI : Groupe Faunistique Indicateur, varies from 9 (very sensitive taxa present) to 1 (only very tolerant taxa remaining). The IBGN index is given by the formula : IBGN = (CV + GFI) -1; it ranges from 20 to 1 in integer values. The index is sensitive to pollution (including toxic), and to general degradation (including habitat alteration). However, in the IC type A2 (Alpes internes), some sensitive taxa used at the national level are naturally missing, and the class 8 of the corresponding metric (GFI=8) cannot be regularly encountered in reference communities. Principles of the classification. A ***provisional*** classification was derived in 2004 and endorsed in 2005 by the French Ministry of Environment (Circulaire MEDD/ DE / MAGE / BEMA 05 / n° 14 / 28 juillet 2005). The same principles were applied to all the river types in France. The classification is based on the level of biological alteration evaluated by the EQR-IBGN values, and compliant with the WFD normative definitions for what concerns a "slight deviation" of taxa richness (CV) and sensitive taxa (GFI), and the disappearance of major taxonomic groups. **1**: definition of Reference values. Reference sites were selected in the monitoring network on the basis of the national reference criteria. For some types, reference data were tested against an independent dataset provided by the Cemagref. The statistical distributions of observed biological values (i.e IBGN index and its two metrics, GFI and CV) were analysed for all the types with a sufficient number of reference sites. For each type, a **reference value** was derived as ***the median of observed values in reference sites*** for the

	IBGN and its separate metrics. All the IBGN values were then transformed in EQR-IBGN; the minimum IBGN value is set at IBGN = 1. **2:** definition of High /Good boundary The H/G boundary is based on the IBGN value corresponding to the combination of the **25th percentile of the metrics values (CV and GFI)** observed in reference sites. **3**: definition of the Good / Moderate boundary The G/M boundary was derived in a two steps procedure: **3-1:** For each type, **the remaining range below the H/G boundary and the minimum IBGN value (=1) was split into 4 equal classes** to derive a preliminary G/M boundary, following a procedure proposed in the REFCOND guidance. **3-2:** Following a pressure / impact analysis at the national scale, this preliminary boundary was then **adjusted at a higher level** (+ 1 point IBGN) for almost all national types, including the IC type A1. However, for the IC type A2 (Alpes Internes), due to the general absence of the GFI class 8 in reference communities, the G/M boundary could not be adjusted. For all types with sufficient reference values, an official table gives IBGN values, corresponding to the reference, H/G and G/M boundaries. Future revision of the classification: A new reference network implemented in 2005 will provide a more consistent reference dataset at the beginning of 2007. At this date, all the reference values will be recalculated for all the types, and a definitive classification will be established. The outcome of the IC process will be taken into account at this time, and the national classification will be adjusted if necessary. The future classification will take into account both - the revision of the preliminary reference values according to the data coming from the new reference network; - the deviation from reference conditions (as EQR-ICM) corresponding to the H/G and G/M boundaries agreed during the IC process. Taking into account that any change in reference values will be reflected in the future classification, the "true" view of the G/M boundary for France represent the **deviation of the EQR-IBGN from the reference values used for the construction of the classification, set at a national level around EQR = 0.75** (range : 0.69 – 0.81 according to the variability of reference conditions of the type).	
Criteria for Boundary Setting	**High/Good boundary**	**Good/Moderate boundary**
Taxonomic composition and abundance	**For the number of taxa,** 25th percentile of the values observed in the reference samples, transformed into the corresponding class of the metric CV.	**For the number of taxa,** the range below the H/G boundary and the minimum value (number of taxa =1) is split into 4 equal classes.
Ratio of disturbance sensitive to insensitive taxa	**For the sensitive taxa**, 25th percentile of the values observed in the reference samples, expressed as the corresponding class of the metric GFI.	**For the sensitive taxa**, loss of one class of the metric GFI from H/G boundary (i.e. GFI H/G minus 1).

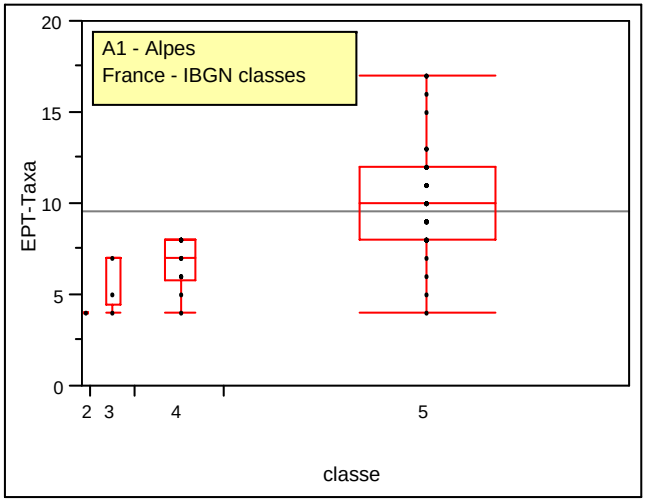
Level of diversity	The diversity is reflected by the **number of taxa (**no quantitative diversity index).	The diversity is reflected by the **number of taxa (**no quantitative diversity index).
Global Index IBGN	The combination of the values corresponding to H/G boundary for the two metrics CV and GFI. Generally equivalent to the 25th percentile of the IBGN values observed in reference samples. EQR-IBGN at H/G boundary: 0.92 to 0.94 for the IC types A1 & A2.	Two steps procedure : 1 - The combination of the values corresponding to G/M boundary for the two metrics CV and GFI as described above. 2- For the A1 type only, following a pressure / impact analysis at the national scale, the boundary was adjusted at a higher level (+ 1 point IBGN). EQR-IBGN at G/M boundary : 0.79 for the IC types A1; 0.81 for the IC type A2 – Pyrénées 0.69 for the IC type A2 – Alpes internes, (due to the lack of GFI class 8)

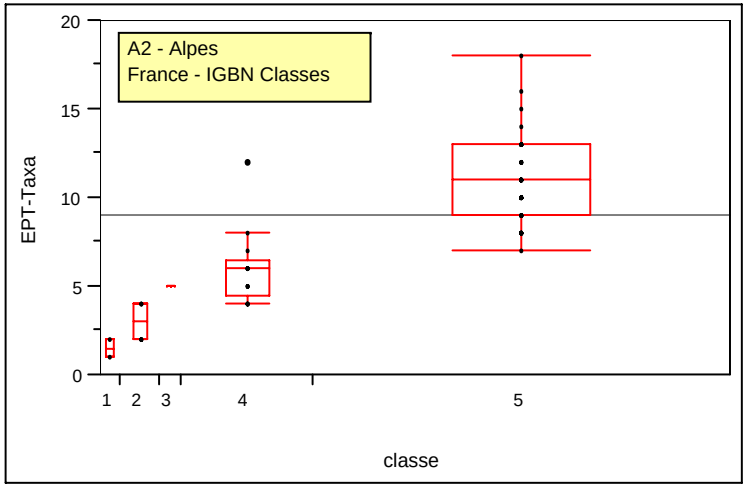
Appendix (FR): Missing major taxonomic groups

EPT-Taxa can be seen as the most sensitive taxonomic groups in Alpine aquatic ecosystems. A positive value of this metric indicates that at least one of the three most sensitive major taxonomic groups is still present in the community.
In the A1 and A2 datasets, a minimum of 4 EPT taxa is encountered at “good satus” (Figures 1 A&B). The disappearance of EPT-Taxa can only be suspected in the classes poor and bad. The disappearance of the most sensitive major taxonomic groups can therefore not be used for defining good/moderate class boundary.

The number of orders represents the total number of “major taxonomic groups”. The mean number of orders per sample observed at different ecological status is represented in figures 1 A, B & C for the different datasets. The total number of order is equivalent at High and Good status for the A1 and A2-Pyrénées dataset, and for A2 Alps, the difference is only one order. Thus it appears clearly that at good status there is no or only very limited loss of “major taxonomic groups”.

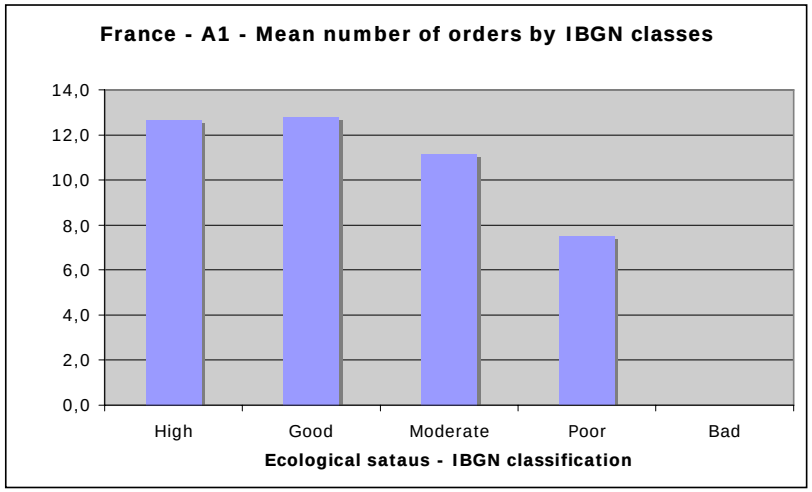
(Note : the figures are provisional – will be updated with the last dataset and completed with E, P, and T analysed separately.)



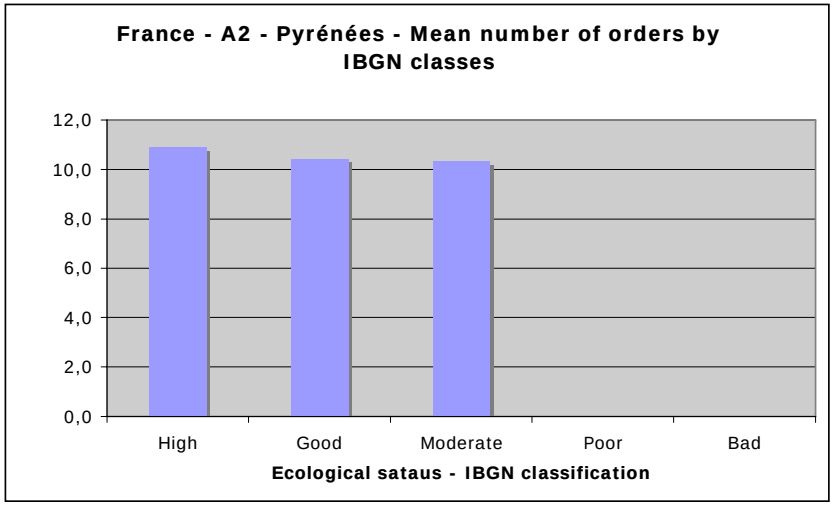


Figures 1 A & B: Distribution of the number of EPT taxa per ecological status classes based on the French IBGN. (1 = bad status to 5 = high status)

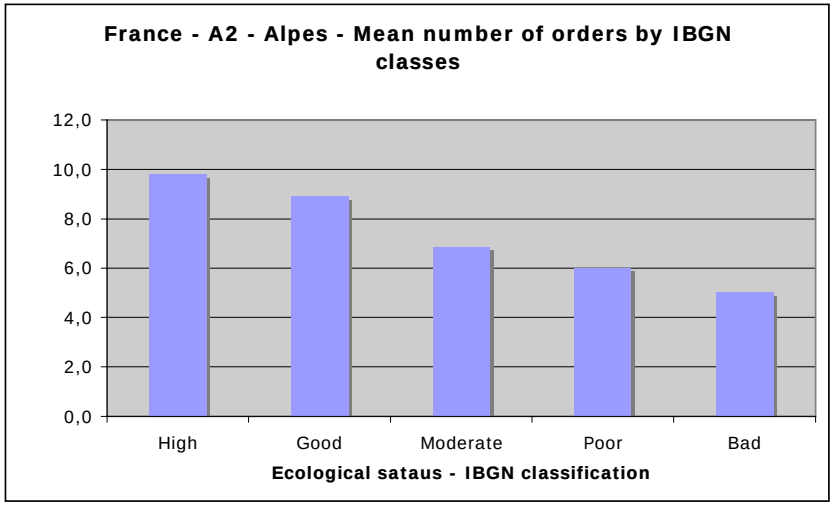
A



B



C



Figures 2 A, B & C : Mean numbers of orders per ecological quality classes in the different datasets

Country	Germany	
Classification System:	**Quality Assessment System**	
General Description	Selection of reference sites according to REFCOND Guidance, The German classification scheme consists of three modules: 4. Module “Organic Pollution” (Saprobic Index (DIN 38410) in relation to stream type specific reference value) 5. Module “General Degradation” (multimetric indices: German Fauna Index, EPT_%, EPTCBO_Taxa, Rheoinex) 6. Module “acidification” index (Braukmann & Biss, 2004; applied only in bioregions at risk of acidification) Metrics used for the multimetric indices are standardised in relation to the 95th percentile of metric values under stream type specific reference conditions. These standardized values are termed as “scores”. Indices are calculated by averaging these scores (Fauna Index is weighted 50%). The benchmark value between reference (High) and good status conditions is defined as the 5% deviation of index values under reference conditions and set to a value of 0.8. That means, observed index values are divided by the benchmark value and multiplied by 0.8. Values > 1 are set to 1. Class boundaries for the ecological quality classes are defined as follows: Quality Class 1: ≥ 0.8 Quality Class 2: ≥ 0.6 < 0.8 Quality Class 3: ≥ 0.4 < 0.6 Quality Class 4: ≥ 0.2 < 0.4 Quality Class 5: < 0.2 The Final Ecological Quality Class is to be discussed.	
Criteria for Boundary Setting	**High/Good boundary**	**Good/Moderate boundary**
Taxonomic composition and abundance	5% deviation of reference sites taxonomic composition represented in indices by: EPT_Taxa_%, #EPTCOBAbundance included in Saprobic Index (# Individuals/m²)	25% deviation (of indices) from reference conditions

Ratio of disturbance sensitive to insensitive taxa	5% deviation of reference sites sensitive to insensitive represented in MMI by: EPT_Taxa_%, #EPTCOB Fauna-Index, Rheoindex	25% deviation (of indices) from reference sites
Level of diversity	5% deviation of reference sites diversity is represented in indices by: Fauna-Index	25% deviation (of indices) from reference sites

Description of national assessment method and relation to normative definitions

Country	Italy
Classification System:	**STAR Intercalibration Common Metric Index (STAR_ICMi), type adapted**
General Description	Selection of Reference sites for the IC exercise The selection of reference sites was done according to REFCOND Guidance criteria (see REFCOND criteria table provided) and is based on pressure analysis, which included information on: water chemistry, (hydro)-morphology, general degradation, land use. In CB_GIG, Italy is involved in R-C1 type only, that corresponds to small streams in the Po valley. These streams mainly belong to spring-fed systems and are located in the very lowland areas of Northern Italy. This implies that some chemical compounds (e.g. Nitrates, Phosphates) are usually at higher level than in other geographic contexts. After a detailed and unsuccessful search for sites with lower concentrations, because it is not expected to impact on invertebrate communities, a slightly higher level than fixed for CB_GIG screening of reference sites was accepted for $P\text{-}PO_4$ (average value for reference sites: 0.07 mg/l). All other CB_GIG chemical and pressure criteria are met. In Italy, bio-monitoring based on aquatic invertebrates has a long hystory. The method in use before the Water Framework Directive is the Indice Biotico Esteso (IBE: Ghetti, 1997; APAT-IRSA/CNR, 2004) that only partly satisfies the WFD requirements. New, type-adapted assessment modules are under development to fully comply with the WFD and fulfill the needs of the different WFD monitoring types. Most of them will be ready by the beginning of 2007, to meet the terms of the WFD monitoring plan. Because they are in a refinement stage, i.e. they are being directly related one to each other to guarantee a comparable assessment of ecological quality, the relation to Intercalibration metric(s) (ICMi) is highly beneficial to finally set class boundaries. Thus, for the CB type(s), Italy has decided to adopt the STAR_ICMi, formally in use in the CB_GIG for the IC process, as the official Italian method for setting quality class boundaries. The boundaries for each of these original assessment modules being provided for WFD monitoring – and possibly adapted to monitoring aims, stressor acting, local tradition and expertise – will be derived starting from those set for the STAR_ICMi. Actually, the STAR_ICMi is used by Italy as: a) 'an interim common WFD assessment method for the purposes of the intercalibration exercise' (see the IC Guidance: EC, 2005) and b) the legitimate way to determine class boundaries for any other method more explicitly devoted to standard monitoring for invertebrates. Description of the STAR Intercalibration Common Metric Index (STAR_ICMi) (Buffagni et al., 2005; 2006) The STAR_ICM index was explicitly designed for European IC purposes and it represents one of the indices used in various GIGs for the comparison and harmonization of class boundaries of different MSs. For the CB_GIG, it is presently the only one in use. Its WFD compliancy has been discussed and demonstrated elsewhere (Wasson & Buffagni, 2005). The index was built

	to assess the overall (i.e. general) degradation of a river site, not being aimed at detecting the impact of single stressors on invertebrates (i.e. it is not a stressor-specific system). The STAR_ICMi is directly calculated in the form of Ecological Quality Ratio (EQR), in accordance with WFD requirements for classification systems. Three aspects of the used methodology to derive class boundaries have to be considered for intercalibration purposes and to check compliancy with normative definitions: a) the sampling technique b) the calculation formula c) the conversion of STAR_ICMi values into quality judgement (i.e. class boundaries setting). a) the sampling technique The data used for the Intercalibration exercise were collected by sampling along 1-2 transects across the river, depending on river type, and collecting invertebrates from all major micro-habitats occurring. A preliminary check of taxa found is done in the field, so that the possible absence of taxa which are expected in unaltered conditions can be verified with integrative sampling. A minimum number or specimens, different for each taxon, must be collected to consider valid the taxon for the computation. When a taxon is accepted, three abundance classes are usually reported for the collected taxa: Present, Abundant and Dominant. Such classes are generally interpreted in terms of relative abundance. Alternatively, an AQEM-like approach, as described in Buffagni et al. (2004) can be adopted. Before sampling, a depositional-transport sequence is identified at each site, which roughly corresponds to what is usually referred to as a pool-riffle sequence.The method for the macroinvertebrate collection was then a 'multi habitat sampling' procedure. Ten individual samples are distributed according to microhabitats occurrence in the riffle unit, taken and merged into a sample. A second merged sample is always obtained, following the same criteria, from the pool area for each site.Two taxa lists can thus be attained for each site, for the depositional and transport units respectively. An open Surber sampler is used to collect macroinvertebrates (area 0.05 m^2; mesh size 0.5 mm). All samples are collected in a quantitative way i.e. all specimens for relevant taxa are picked up and brought to the lab for identification. In some cases for particularly abundant taxa, sub-sampling in the field can be used. b) the calculation formula The STAR_ICMi is a multi-metric index and is composed of six metrics, which account for the main aspects present in the WFD Normative definitions (see below): ASPT, Log_{10}(sel_EPTD+1), 1-GOLD, N-taxa, EPT and Shannon-Weiner diversity. The ICMi value is calculated by the sum of all the ICMs, after attributing a weight to each metric. Hereafter, the list and category of each metric is provided (Table 1). After their normalization, the metrics are combined into the ICM index. Metrics are grouped into three groups, providing information on three major response areas: Tolerance, Abundance/Habitat and Richness/Diversity. A different weight is attributed to the metrics within each group, giving greater importance to the metrics based on the whole community (Buffagni et al., 2004). To obtain the final multimetric score, the same weight is attributed to each of the three metric groups (0.333).

Table 1. Intercalibration Common Metrics (ICMs) used in the STAR ICMi

Information type	Metric type	Metric name	Taxa considered in the metric	Literature reference	weight
Tolerance	Index	ASPT	Whole community (Family level)	e.g. Armitage et al., 1983	0.333
Abundance/ Habitat	Abundance	Log_{10} (Sel_EPTD +1)	Log(sum of Heptageniidae, Ephemeridae, Leptophlebiidae, Brachycentridae, Goeridae, Polycentropodidae, Limnephilidae, Odontoceridae, Dolichopodidae, Stratyomidae, Dixidae, Empididae, Athericidae & Nemouridae)	Buffagni et al., 2004; Buffagni & Erba, 2004	0.266
Richness and Diversity	Abundance	1-GOLD	1 - (relative abundance of Gastropoda, Oligochaeta and Diptera)	Pinto et al., 2004	0.067
	Taxa number	Total number of Families	Sum of all Families present at the site	e.g. Ofenböck et al., 2004	0.167
	Taxa number	number of EPT Families	Sum of Ephemeroptera, Plecoptera and Trichoptera taxa	e.g. Ofenbock et al., 2004; Böhmer et al., 2004.	0.083
	Diversity index	Shannon-Wiener diversity index	$D_{S-W} = -\sum_{i=1}^{s}\left(\frac{n_i}{A}\right)\cdot\ln\left(\frac{n_i}{A}\right)$	e.g. Hering et al., 2004; Böhmer et al., 2004.	0.083

c) Accordingly to the WFD requirements, the STAR ICMi class boundaries here presented for High/Good and Good/Moderate status are dedicated to R-C1 rivers i.e. they are type-specific. See next paragraphs for details on technical options used to set class boundaries.

Principles of the classification

The used approach and thus the proposed values satisfy the requirements of the WFD: type-specific adaptation of reference conditions, use of an EQR scale, REFCOND approach for setting class boundaries. As far as normative definitions in terms of kind of information provided for invertebrates are concerned, i.e. ratio sensitive/insensitive taxa, diversity and abundance, the compliancy is guaranteed by the STAR_ICM index, which directly fulfils such obligations (Buffagni et al., 2005; Wasson & Buffagni, 2005). Also, the level of biological alteration evaluated by the STAR_ICMi values complies with normative definitions

	in terms of: "slight deviation" of taxa richness from reference conditions; presence of sensitive taxa; presence of major taxonomic groups. In Appendix, the relationship between the quality classes based on the proposed STAR_ICMi values and each of the componing biological metrics are shown for Reference and High to Bad quality classes (Box&Whiskers plots). The same principles were applied to most IC river types in Italy. <u>Definition of Reference values and dataset used</u> The invertebrate and pressure data used for R-C1 were provided by Lombardy EPA. Invertebrate samples were collected for standard monitoring purposes at the province scale and cover the full degradation gradient observed in the area (Milan Province, Northern Italy) i.e. from Bad status to Reference sites. Few additional data collected in an adjacent area (Novara Province, Piedmont) were provided by CNR-IRSA. In total, 365 samples collected over a period of ca 10 years ('96 to '06) are being used, including 32 samples from 5 Reference sites. Reference sites were selected on the basis of the REFCOND and CB_GIG criteria. <u>Boundary setting approach</u> *Definition of High /Good boundary* The High/Good class boundary was set accordingly to a 3-step procedure. 1) First, a possible value for the H/G boundary is set to correspond to the 25th percentile of STAR_ICMi values observed at reference sites, which is considered to be a minimal and simple approach in line with WFD requirements → REFCOND approach 2) A second potential value for the boundary is calculated after testing against an independent, benchmark dataset, the AQEM/STAR Benchmark dataset (as described in Buffagni et al., 2005; 2006; Buffagni & Erba, 2006). The value obtained according to this approach should guarantee the similarity to scientifically set (and thus ecologically sound) boundaries. 3) If needed, an intermediate value comprised between the two possible boundary values defined in 1) and 2) is selected as the final H/G boundary, taking care of: a) the percentage of classification of samples from reference sites into classes lower than High (i.e. aiming at lowering this percentage); b) the pre-WFD boundary should at least be maintained (i.e. lower river protection not allowed); c) a balanced positioning between values as defined in 1) and 2) should be preferred. *Definition of Good/Moderate boundary* The Good/Moderate class boundary was set accordingly to a 3-step procedure as well. 1) The G/M boundary is set to correspond to the H/G boundary (see above) multiplied by 0.75. I.e., the range covered by STAR_ICMi values comprised between 0 and the 25th percentile of STAR_ICMi observed at reference sites was partitioned into 4 equally spaced classes, Good status being the highest in terms of STAR_ICMi. A 25% deviation from reference sites value is assumed to be, in general terms, a slight deviation → REFCOND approach

2) The boundary is refined after testing against an independent, benchmark dataset, the AQEM/STAR Benchmark dataset (as described in Buffagni et al., 2005; 2006; Buffagni & Erba, 2006) (same as for H/G boundary).
3) If needed, an intermediate value comprised between the two possible boundary values defined in 1) and 2) is selected as the final G/M boundary (same as for H/G boundary).

Point 1) for preliminary H/G and G/M boundary setting does not need any further explanation, because it simply makes use of a percentile and of an arithmetical separation into 4 equal classes. Nonetheless, such a simple option risks generating numerical results (i.e. boundaries) that are not ecologically sound. That's why point 2) and 3) cheking was adopted (see also Buffagni & Furse, 2006). The values obtained in point 1) ('REFCOND approach') represent one limit (for each of the two boundaries) for the range to be considered to finally set the class boundaries. Point 2) does require some more clarification, which is reported hereafter. The ICMi as accepted by European MSs for a standard use in the CB_GIG for comparison and harmonization, i.e. the STAR ICMi (Buffagni et al., 2005; 2006), was used in statistical testing for deriving a second value, for each boundary (G/M and H/G).
Firstly, the ICMi value for each invertebrate sample was calculated. A statistical comparison was then executed between the ICMi values found in the AQEM/STAR benchmark dataset - which is assumed to enclose WFD compliant classifications (Buffagni & Erba, 2006; Buffagni et al., 2006) - and the same observed in R-C1 dataset for the High status class as defined by using point 1) approach. The procedure is explained in details in Buffagni et al., 2005; 2006. Because R-C1 samples did not significantly differ from benchmark ones i.e showed values not lower than benchmark data, the H/G boundary obtained in point 1) was confirmed. Point 3) adjustment is thus not needed. and the boundary for STAR_ICMi for H/G class is thus finally set.
In the same way as for the adjustment of the H/G boundary, the boundary G/M was considered and the procedure of statistical comparison between Good status classes, as it was carried out for High status, was repeated. Because no statistically significant differences were observed with values obtained at Point 1), the boundary value there set was adopted.

In Table 1, the calculated values by 1), 2) and final boundaries (STAR_ICMi) for all classes are provided.

Table1. EQR scale. Boundary values reported are the lowest limit of each quality class.

Class boundary	STAR_ICMi values according to REFCOND approach	STAR_ICMi values after testing with benchmark	**Final STAR_ICMi boundaries**	final decision criteria
High/Good	0.959	0.959	**0.959**	REFCOND and benchmark testing
Good/Moderate	0.719	0.719	**0.719**	REFCOND and benchmark testing
Moderate/Poor	0.479	nc	**0.479**	REFCOND
Poor/Bad	0.240	nc	**0.240**	REFCOND

	The ecological soundness of the two selected boundaries, H/G and G/M, was then validated, in terms of Normative definitions, by looking at the distribution of WFD compliant metrics values as a function of the final classification. A few examples are reported below, which support the adherence of the classification to Normative definitions for invertebrates. Future revision of the classification A new list of reference sites is being derived in Italy, for all GIGs and types, to be further sampled during 2006 and 2007. This will possibly supply a larger set of invertebrate samples and pressure data. Thus, also in relation to the results of the ongoing Intercalibration activity for other Italian stream types (i.e. from the Mediterranean and Alpine GIGs) and to the definition of an improved typology for Italian rivers, some refinements of boundaries might be required and/or desirable. Essential bibliography APAT-IRSA/CNR, 2004. 'Indice Biotico Esteso (I.B.E)'. In: APAT, Manuali e linee guida 29/2003. APAT-IRSA/CNR, Metodi analitici per il controllo della qualità delle acque, Roma 3: 1115-1136. Buffagni, A., S. Erba, M. Cazzola & J. L. Kemp, 2004. The AQEM multimetric system for the southern Italian Apennines: assessing the impact of water quality and habitat degradation on pool macroinvertebrates in Mediterranean rivers. Hydrobiologia 516: 313-329. Buffagni A. & Erba S. 2006. Contribution to the CentralGIG Intercalibration activities. Notes on a possible Benchmark /Independent dataset for the IC process. CB_GIG Document, 36pp. Buffagni A. & M. T. Furse. 2006. 'Intercalibration and comparison – major results and conclusions from the STAR project'. Hydrobiologia 566: 357-364. Buffagni A., Erba S., Birk S., Cazzola M., Feld C., Ofenböck T., Murray-Bligh J., Furse M. T., Clarke R., Hering D., Soszka H. & W. van de Bund, 2005. 'Towards European Inter-calibration for the Water Framework Directive: Procedures and examples for different river types from the E.C. project STAR'. 11th STAR deliverable. STAR Contract No: EVK1-CT 2001-00089. *Quad. Ist. Ric. Acque* 123, Rome (Italy), IRSA, 468 pp. Buffagni A., Erba S., Cazzola M., Murray-Bligh J., Soszka H. & Genoni P. 2006. 'The STAR Common Metrics approach to the WFD Intercalibration Process: full application across Europe for small, lowland rivers'. Hydrobiologia 566: 379-399. European Commission, 2005. Guidance on the Intercalibration Process 2004-2006. Water Framework Directive Common Implementation Strategy (200/60/EC), Guidance Document n° 14, 2005, 31pp. Ghetti, P. F. 1997. Indice Biotico Esteso (IBE). I macroinvertebrati nel controllo della qualita` degli ambienti di acque correnti. Provincia Autonoma di Trento, 222 pp. REFCOND Guidance - Wallin, M., Wiederholm, T. & R. K. Johnson. 2003. Guidance on establishing reference conditions and ecological status class boundaries for inland surface waters. Produced by CIS working group 2.3 – REFCOND. 2003-03-05, 93 pp. Wasson J.G. & Buffagni A., 2005. Does the ICMi approach ensures the consistency with the WFD normative definitions? River Intercalibration - Discussion paper for the Central/Baltic GIG. Steering group & GIGs coordinators meeting, Lyon, 18-19th May 2005, 8pp.	
Criteria for Boundary Setting	**High/Good boundary**	**Good/Moderate boundary**
Taxonomic composition and abundance	The Total Number of taxa, the number of EPT taxa, 1-GOLD, and Sel EPTD_taxa show - in High status samples - values	For the same metrics, the deviation from reference sites values is slight.

(see Appendix below)	that correspond totally or nearly totally to those observed at reference sites.	**(see Appendix below)**
Ratio of disturbance sensitive to insensitive taxa **(see Appendix below)**	The sensitive to insensitive taxa ratio is reflected by the ASPT metric, by the presence/absence of indicator taxa (Sel EPTD_taxa and 1-GOLD) and by the number of EPT taxa. In High status samples, they show values that correspond totally or nearly totally to those observed at reference sites.	For the same metrics, the deviation from reference sites values is slight **(see Appendix below)**
Level of diversity **(see Appendix below)**	The diversity is reflected by the Total Number of taxa, number of EPT taxa and by the Shannon-Wiener metric. In High status samples, they show values that correspond to those observed at reference sites (they are even higher).	For the same metrics, the deviation from reference sites values is slight **(see Appendix below)**
STAR ICMi, in general	The High/Good boundary was set according to the procedure described in the text above, which is performed on the index values after the combination of the composing metrics. Nonetheless, even the relationship of single metrics included in the index show a good disciminatory power among quality classes (see Appendix). The REFCOND approach was used to set class boundary (25^{th} %ile value of REF samples) and it was further validated by comparing to totally independent, benchmarking system (i.e. AQEM/STAR European dataset)	The Good/Moderate boundary was set according to the procedure described in the text above, which is performed on the index values after the combination of the composing metrics. Nonetheless, even the relationship of single metrics included in the index show a good disciminatory power among quality classes (see Appendix). The REFCOND approach was used to set class boundary (equal classes repartition starting from the 25^{th} %ile value of REF samples) and it was further validated by comparing to totally independent, benchmarking system (i.e. AQEM/STAR European dataset)

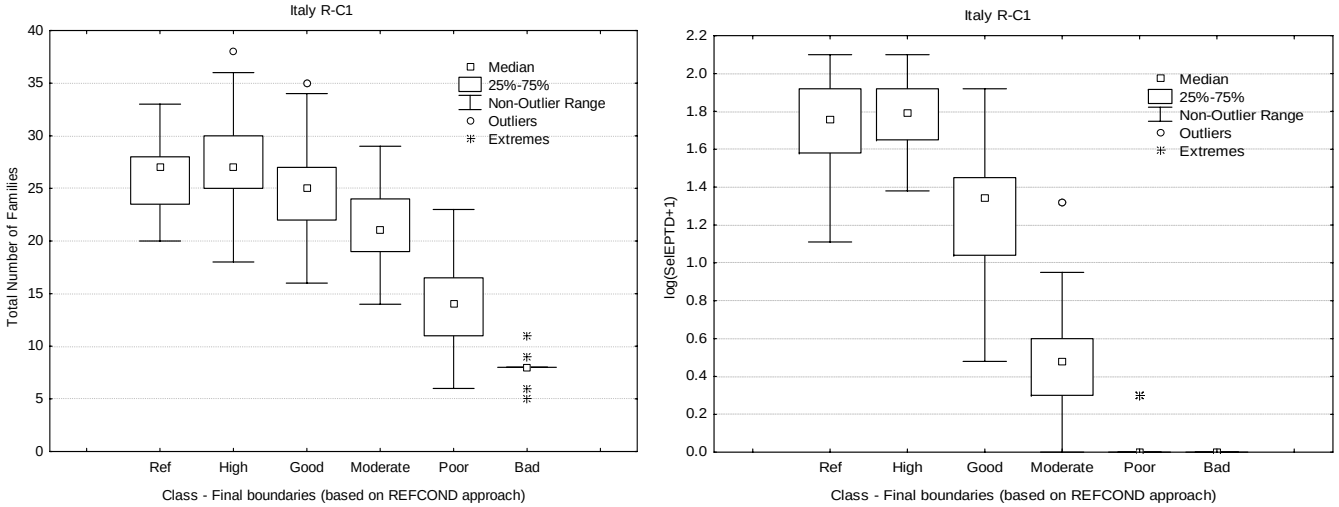
Appendix (IT)

The response of the individual biological metrics included in the STAR_ICMi, which fulfill the WFD definitions for aquatic invertebrates in rivers to the final classification (i.e. boundaries) obtained for R-C1 type, is reported hereafter, according to the main definition categories in the WFD. The distribution of values for each metric in the 5 Ecological Status classes based on the STAR_ICMi boundaries and at Reference sites is shown in the form of Box&Whiskers plots.

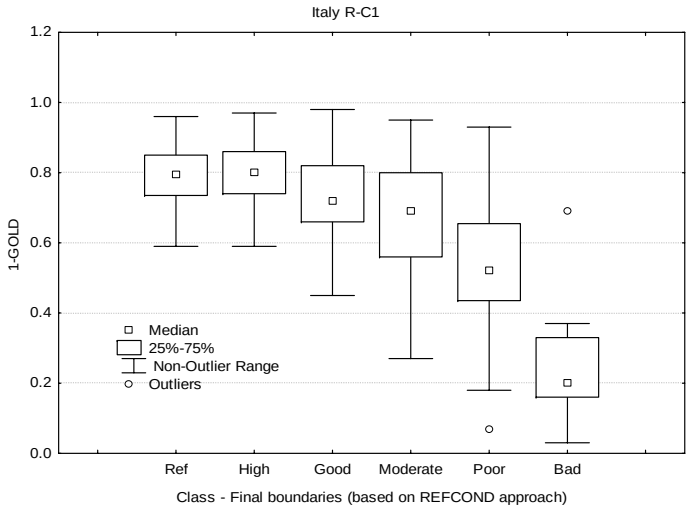
Taxonomic composition and abundance

The Total Number of taxa (here Families) found in a sample can be considered one of the major indicators for taxonomic composition (see figure below, left). The shift from 27 (REF) to 25 (Good status) in the Number of Families (median value) is considered a slight change in the composition of the invertebrate community.

The EPT taxa metric as well contributes to taxonomic composition of the community (see graph in 'Ratio of disturbance sensitive to insensitive taxa').



The abundance-based metric included in the STAR_ICMi (= CB_GIG ICMi) i.e. Sel EPTD_taxa (Buffagni et al., 2005; 2006) accounts for invertebrate abundance in R-C1 (see above, right). The shift from 1.75 (REF) to 1.35 (Good status) in Sel EPTD_taxa is considered a slight change in the composition and abundance of the invertebrate community. In fact, the taxa enumerated in this metric are absent in Bad and Poor status samples (i.e they are sensitive taxa, expected to disappear at altered sites), present in only 50% Moderate status samples (usually with a value below 0.5). In Good status samples, these combined taxa usually reach an abundance corresponding to a metric value around 0.75 that observed at REF sites. The overall trend of the 1-GOLD metric, which is also abundance based, is shown below.

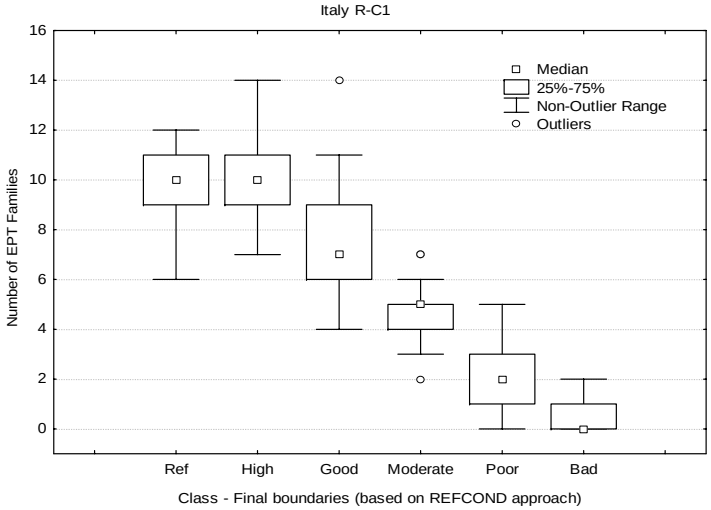


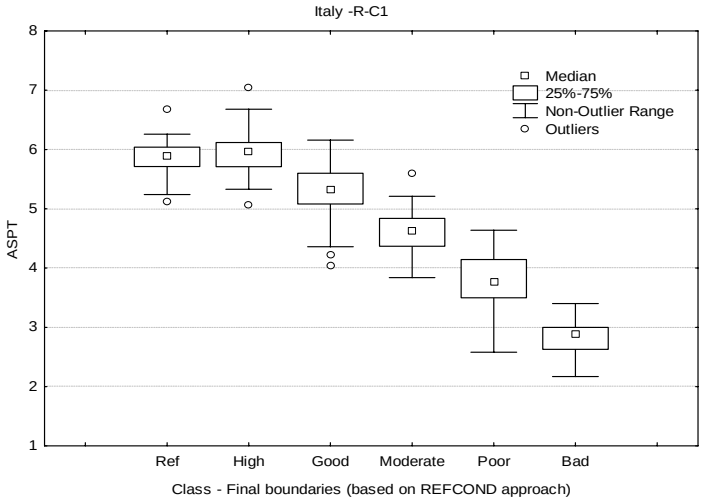
Abundance (in terms of abundance classes) is also used in Shannon-Wiener index calculation (see 'Level of diversity').

Ratio of disturbance sensitive to insensitive taxa (and missing major taxonomic groups)

EPT-Taxa can be seen as the most sensitive taxonomic groups in CB rivers types. A positive value of this metric indicates that at least one of the three most sensitive major taxonomic groups is present in the community.

In the Italian R-C1datasets, a minimum of 4 EPT Families is encountered at Good status, with 75% of samples bearing 6 or more EPT Families (below, left). The disappearance of EPT Families is only experienced in few cases in Poor status, while it becomes quite common (50% of samples) in Bad status. It appears than clearly how the most sensitive major taxonomic groups are always present in Good status samples according to the proposed boundaries.

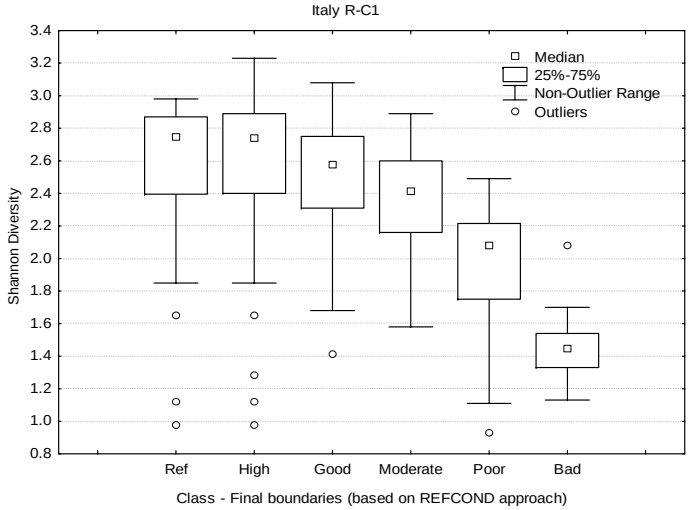




The ASPT metric is also shown, which undoubtedly accounts for the Ratio of disturbance sensitive to insensitive taxa (figure above, right). For the Good status class, it shows a slight deviation from the level observed at Reference sites (far less than 1 unit of variation).

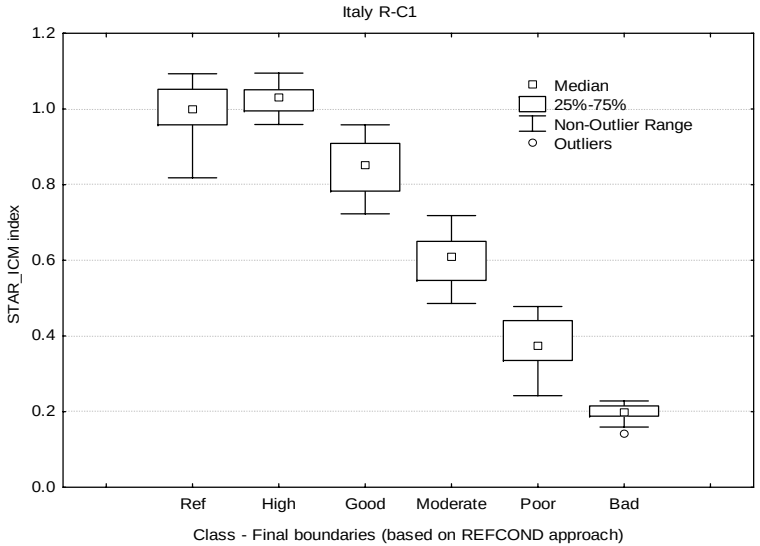
Level of diversity

The level of diversity of invertebrate taxa is present in the STAR_ICMi in terms of Shannon-Wiener diversity index (see graph below). The values of this metric in Good status samples show only slight signs of alteration from reference sites levels (Median of REF: 2.77; Median of Good status: 2.59, with a range going to values lower than 1.4).



Diversity, in terms of Richness of the community, is presented in ‘Taxonomic composition and abundance’ (Total Number of Families).

Overall trend of STAR_ICMi classes



The distribution of the values of the STAR_ICM index (which incorporates all the metrics shown above) in the 5 Ecological Status classes based on the boundary setting procedure explained in the text and at Reference sites is shown to describe the general trend of values into quality classes.

Country	SLOVENIA	
Classification System:	Slovenian Ecological Quality Assessment System	
General Description	Selection of reference sites according to REFCOND Guidance, National Strategy paper ("Criteria for selecting river and lake reference sites in Slovenia"). The Slovenian classification scheme consists of two modules: 7. Module "Organic Pollution" (Saprobic Index in relation to stream type specific reference value) 8. Module "Hydromorphology" (1 multimetric index) Metrices used for the multimetric indices are standardised in relation to the 95th percentile of metric values under stream type specific reference conditions. These standardized values are termed as "scores". Indices are calculated by averaging these scores. The benchmark value between high and good status conditions is defined as the 25th percentile of index values under reference conditions and set to a value of 0.8. That means, observed index values are divided by the benchmark value and multiplied by 0.8. Values > 1 are set to 1. Class boundaries for the ecological quality classes are defined as follows: Quality Class 1: ≥ 0.8 Quality Class 2: ≥ 0.6 < 0.8 Quality Class 3: ≥ 0.4 < 0.6 Quality Class 2: ≥ 0.2 < 0.4 Quality Class 2: < 0.2 The Final Ecological Quality Class is determined by the worst case applying all relevant modules.	
Criteria for Boundary Setting	**High/Good boundary**	**Good/Moderate boundary**
Taxonomic composition and abundance	25th percentile of reference sites taxonomic composition represented in the MMI by: German Fauna Index D04, % Type RL, % Type Pel, # Oligochaeta, # Lumbricidae	25 % deviation of indices values from high/good boundary

	Abundance included in Saprobic Index and the MMI by: % Type RL, % Type Pel, # Oligochaeta, # Lumbricidae	
Ratio of disturbance sensitive to insensitive taxa	25^{th} percentile of reference sites sensitive to insensitive represented in MMI by: German Fauna Index D04, % Type RL, %Type Pel, # Oligochaeta, # Lumbricidae	25 % deviation of indices values from high/good boundary
Level of diversity		

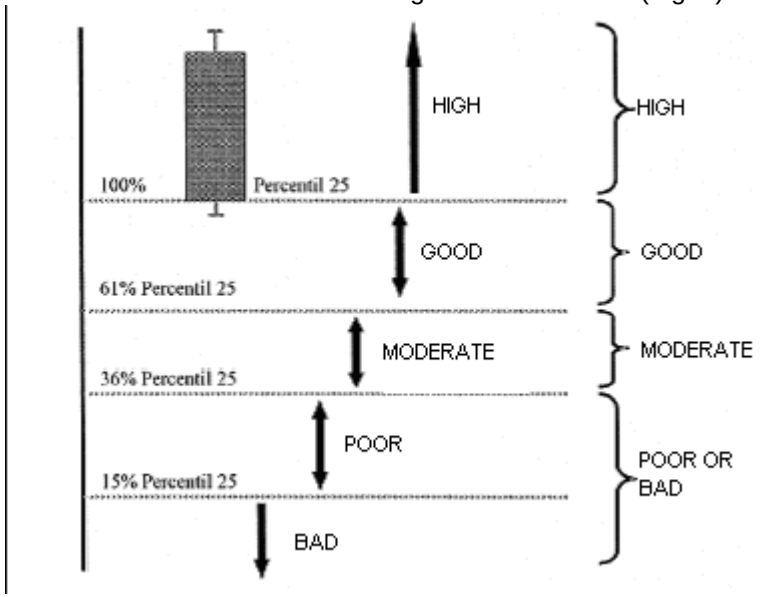
Country	Spain
Classification System:	IBMWP
General Description	Selection of reference sites according to REFCOND Guidance. Reference sites: “those where human pressure and impact in ecosystem are zero or the minimum possible”. Results from Guadalmed project were used, what includes criteria for reference sites identification, based on uses of the basin, characteristics and status of riparian forest, channel and fluvial bed, reach regulation and physico-chemical characteristics (nutrients) (Bonada et al., 2002) Biotic index IBMWP is used (ALBA-TERCEDOR y SÁNCHEZ-ORTEGA 1988, JAIMEZ-CUELLAR *et al.* 2002). It is the adaptation of British BMWP (ARMITAGE *et al.* 1983) to the peninsular fauna. And also IASPT (IBMWP/number of taxa). **Ecological Quality Class**: To set quality levels, the universe of values adopted by the biological index are studied, establishing the quality classes depending on the deviation degree from data obtained in the reference sites previously defined. So to each type, quality classes are established following WFD criteria. Intervals that fulfill defined conditions would be obtained, considering the percentages corresponding to 25th percentile: 100%, 61%, 36% y 15%, respectively, the boundaries between the five ecological status classes(Fig. 1). 

	Fig. 1 Proposal for the ecological status boundary setting protocol using values found in the reference sites following WFD criteria (Alba-Tercedor *et al.* 2002)	
Criteria for Boundary Setting	**High/Good boundary**	**Good/Moderate boundary**
Taxonomic composition and abundance	25^{th} percentile of reference sites IBMWP	61% 25^{th} percentile of reference sites IBMWP
Ratio of disturbance sensitive to insensitive taxa	25^{th} percentile of reference sites IBMWP	61% 25^{th} percentile of reference sites IBMWP
Level of diversity	25^{th} percentile of reference sites IASPT	61% 25^{th} percentile of reference sites IASPT

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