Development, Evaluation & Implementation of a Standardised Fish-based Assessment Method for the Ecological Status of European Rivers - A Contribution to the Water Framework Directive (FAME)

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Defining Reference Conditions (D3)

FINAL REPORT

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SUMMARY

Implementation of monitoring programs aiming to assess the ecological status of European rivers and the degree of deviation from natural conditions requires the establishment of biological reference conditions, with which monitoring information can be compared. This report focuses on the establishment of reference conditions for riverine fish communities, and describes principles, criteria and methodologies for deriving them. The report is organised in three parts. The first part covers the theoretical part of the reference condition issue, with the objective of clarifying concepts and to give an overview about the range of possible methods to characterise references. Biological reference conditions have been used both in spatial and historical contexts and represent the expected status of biological communities. These expectations may range from "pristine" to "best available ambient" conditions, and are usually based on the status of the communities in a population of reference sites, which are relatively undisturbed and homogeneous with respect to the morphological, hydrological, physicochemical and biological conditions. Key approaches for deriving ecological reference conditions, namely spatial methods, modelling, historical evidence and professional judgement are outlined, and the advantages and disadvantages of each are discussed.

The second part concentrates on the interpretation of the WFD requirements and acceptable procedures for defining reference conditions. Reference conditions are equated with the "high ecological status" of the classification system and are meant to represent the structure and functioning of biological communities under no or very minor anthropogenic disturbances. Establishing type-specific reference conditions requires firstly the stratification of the monitored area using zoogeographic features and abiotic variables, with the objective of deriving strata of the highest possible ecological homogeneity. Next, biological attributes of ecological quality (metrics) are selected with respect to their sensitivity to particular types of environmental stress. Finally, reference conditions for the selected metrics and the appropriate strata are specified. Implicit to the WFD conception of reference conditions is that when spatial methods are used, the reference sites should be relatively undisturbed. Some methodologies applied in Europe are based on the "best available site" approach, which does not seem to comply with the WFD. In the case of heavily modified rivers, this approach is likely to be accepted for assessing the maximum ecological potential. Fish introductions generate a problem for the designation of reference conditions as they represent an anthropogenic disturbance but their occurrence in a river is not causing any management measures. Opinions and possible solutions to this problem are discussed. Principles for selecting and scoring metrics are outlined, mainly with respect to the possibility of assigning a reliable reference value to them.

The third part describes the methodological plan adopted for the FAME project. Spatial methods will be preferably employed, using data from sites that can be characterised as reference (they are no or minimally impacted, they are representative for the region and river type, and they exhibit the natural ranges of the biological variation). If no such sites can be identified, alternative methods (modelling, historical, etc.) will be employed. Application of spatial methods requires as a first step the development of impairment criteria for reference site selection. The starting point was the list of criteria developed during the AQEM project, which were refined further from the fish perspective, taking into account the provisions of the WFD for the desired values of the abiotic elements defining the biological communities. One of the problems anticipated is that a site may be affected by anthropogenic disturbances occurring in distant areas. To overcome this difficulty a hierarchical framework for establishing a reference network is developed that takes into account the spatial scale of different pressure types and involves three levels of selection: rivers, segments and sites. Within each level a list of obligatory criteria for minimal impairment is generated, which identify common and important stressors, to be used across geographic boundaries and river types. The obligatory list can be complemented by national teams with a list of optional criteria to be considered for local stressors. This hierarchical selection scheme is guided by the principle that the ecological status of a site or river is determined by the integrated effect of all pressures. Standardisation of field procedures, validation and long-term evaluation of the reference network are important parts of reference characterisation, which must be a continuous process primarily based on monitoring programmes and periodic reviews of the impact of human activity.

CONTENTS

1. INTRODUCTION

The WFD requires that European Union (EU) member states establish monitoring and ecological quality classification systems for the purpose of systematically assessing the ecological status of surface waters, and for determining the level of human impact on ecosystems. The Directive adopts the reference condition approach, through which the classification of ecological status is conducted by means of comparison with reference conditions. A crucial aspect of the ecological assessment is identifying reference conditions, which are perceived as the set of conditions to be expected in the absence of or under minimal anthropogenic disturbances.

In line with these requirements, the FAME project aims to develop and deliver a pan-European ecological status assessment and classification system, using fish-based indicators as a means to substantiate ecological quality. The underlying assumption is that the fish community structure integrates and reflects the functioning of the ecosystem. The characterisation of type-specific reference conditions becomes a critical part of the development of such a system. Only when reliable reference conditions have been defined does it becomes possible to set quality class boundaries and criteria for ecological classification.

This report presents the results of WP2 "Defining reference conditions" of FAME and provides a platform for further elaboration in WP6 "Modelling reference conditions". The first part of the report covers the theoretical part of the reference condition issue, with the objective to clarify concepts and to give an overview about the range of possible methods to characterise reference. The second part focuses on the interpretation of the WFD requirements from the fish perspective. The third part proposes a hierarchical process for setting of reference conditions, including criteria for reference site/river selection.

It was not the objective of WP2 to define reference conditions at the metric level, as this topic will be covered in combination with setting class boundaries and modelling of reference conditions in other WPs. However, some general theoretical considerations are included in this report.

2. THE REFERENCE CONDITION APPROACH

2.1. The basic concept

A crucial part of any scientific research is to establish controls for comparison with test results. The concept behind the reference condition approach is to establish such controls, as a benchmark, representing conditions in unimpaired water bodies, against which the conditions in impaired bodies can be evaluated. In a general context, reference conditions may refer to biological, physical, chemical or hydrologic elements of the environment. In a more specific biological context, they represent the expected status of biological communities in the absence of stress from human activities. They can be applied to a single component of the biota, such as fish, or to a combination of components.

Currently, most existing ecological classification systems take a narrow systematic approach in ecosystem assessment, relying on only one taxonomic group (e.g. fish), for which reference conditions are determined. Future elaboration may involve more integrated approaches that combine various taxonomic groups. This will require merging ideas from various taxonomic,

ecological and physiological fields in order to arrive at a thorough understanding of the processes operating in aquatic ecosystems.

It is clear that when using biological elements to detect the impact of one or more stressors, it is necessary to know the structure and properties of undisturbed communities. Reference conditions for such "reference communities" may be site-specific or be defined at a coarser (regional) level. Site-specific reference conditions represent biological attributes of the reference community in a specific (undisturbed) site. At the regional scale, they represent attributes of the reference community, derived from survey data in a population of sites or estimated by modelling and other methods.

Before proceeding, a clarification of the terms reference site and reference conditions becomes necessary. The glossary of the US Environmental Protection Agency (EPA) provides the following definitions:

"Reference Site is a specific locality on a waterbody which is minimally impaired and is representative of the expected ecological integrity of other localities on the same waterbody or nearby waterbodies".

"Reference Condition is a set of selected measurements or conditions of minimally impaired waterbodies characteristic of a waterbody type in a region".

The actual assessment of ecological status is based on the values of specific biological parameters that describe structural and functional attributes of the biological community (metrics). The metrics are selected with respect to their sensitivity to anthropogenic stress, and are scored relative to the performance of undisturbed communities. For a metric to become an appropriate indicator of ecological quality it must change in a consistent manner with a degradation type, and a reliable assessment of its reference value must be possible.

2.2. Baseline expectations

Reference conditions have been used in a variety of contexts, ranging from the "pristine" to the "best available" state of a water body, in the historical or the spatial context, and may contain only environmental objectives or may also incorporate a broad array of economic, social and political considerations. A variety of terms, sometimes used with equivalent meanings, have been employed to describe reference conditions or sites (e.g. unimpaired, unperturbed, undegraded, unaltered, undisturbed, nearly undisturbed, least affected, etc.).

The key to defining biological reference conditions is to have a clear idea about the desired ecological status of the environment. The definition dictates the restoration targets and has important methodological implications. If the reference conditions are defined as the best available ambient biological conditions, direct derivation from survey data is usually possible. If they are defined as undisturbed conditions in the historical context, no "natural" reference conditions can exist in modern environments. Hence, it becomes necessary to reconstruct the potential reference conditions (e.g. through modelling).

2.3. Accounting for natural variability

One of the problems associated with using the reference condition approach is that high natural variability may be present, making difficult to distinguish between anthropogenic and

environmental effects on biotic communities. For effective use of the reference data in environmental quality assessments, the variability within the monitoring network over which the same reference conditions apply must be sufficiently small, allowing the effects of anthropogenic activities to dominate.

One major source of natural variability is geographical differences. Regional differences among European rivers exist as regards geomorphological, climatic, hydrological and biogeographic conditions that may result in biological differences, because of which the communities of these rivers are not truly comparable. Another source of variability is river type differences. Even in the same geographical area, rivers may vary widely in size, catchment area, elevation and other ecological characteristics. This variation may also reflect to biological differences. Here again, reference conditions derived from biological attributes over a broad spectrum of river types would be misleading.

Both types of variability must be accounted for in the establishment of reference conditions. To minimise the variability associated with geographical differences, a common approach is to organise environmental information on a narrow spatial scale. The ecoregion concept is widely applied for establishing a broad framework for reference conditions. The delineation of ecoregions is based on a combination of abiotic and biotic attributes, such as soil geology, climate, and land use, which support and to a certain extent define biological attributes. Therefore, ecoregion-wide reference conditions have a high probability of representing communities similar in many biological respects. However, ecoregions may still accommodate a high biological variability due to environmental heterogeneity or historical/phylogenetic factors. This is particularly true for Europe, where the established ecoregional classification (Illies 1978) groups together large areas with very variable environmental conditions. A further stratification into smaller geographical units (e.g. sub-ecoregions) becomes necessary to reduce biological heterogeneity within ecoregions. Such stratification may be based, for example, on geomorphological zonation or zoogeography, and provides a rational basis for generating more specific ecological expectations.

To minimise the variability associated with water body-type differences further stratification using typology characteristics is required. In essence, the aim is to partition variability of biological conditions within regional strata by grouping together those rivers that are similar in, for example, size, catchment area, altitude and geology or present similar patterns of flow. The underlying basis for this stratification is that rivers that are similar in such characteristics may also have similar biological communities with respect to ecological structure and performance.

The key task is the identification of appropriate ecoregional (geographic) and typological stratification characteristics that assist in “predicting” biological conditions. The purpose is to attain the highest possible biological homogeneity within strata (and greatest heterogeneity between them). If the composition and functional organisation of communities within a stratum were similar, then similar reference conditions for all communities represented by this stratum would apply.

After allocating the monitored area into appropriate strata, high natural variability may still remain; this may reflect for example, seasonal differences, genetic differences within species, the effects of plasticity, or originate from factors not accounted for in the stratification scheme. It is not possible to reduce the variability originating from intrinsic (e.g. genetic) factors; however, it is possible to reduce the variability originating from external factors by

using additional stratification descriptors. However, a high level of discrimination requires reference conditions to be set in a larger number of strata, generating a number of disadvantages (costs, attainability, data availability, time availability, etc.).

A residual amount of variability will be inevitably included in reference conditions. When the range and cause of this variability is not known, a difference between the test and the reference community may be erroneously attributed to human impacts, whereas the reason is likely to be a difference in ecological, geomorphological or zoogeographical factors. Obviously, an important aspect of reference data quality is that the statistical properties of natural variability are described adequately.

2.4. Potential uses of reference conditions

From the conservation/restoration perspective, the reference conditions serve the following three purposes:

- Provide the 'baseline' against which to measure the effects of anthropogenic activities.
- Describe the biological community potential, which is important for setting direction to restoration efforts.
- Describe the range of spatial and temporal variability that characterises healthy ecosystems.

Environments constantly change and the reference communities change concomitantly for reasons that are unrelated to human impacts. To keep the reference standards updated it becomes necessary to periodically review the biological conditions, and adjust the reference conditions accordingly. Therefore, an additional use of the reference conditions is:

- Through routine monitoring, to detect and describe ecological trends linked, for example, with long-term climatic change.

Once selected, the reference sites should be preserved and protected from deterioration, e.g. through including them in officially designated protected areas. Preservation of the reference sites helps a two-fold objective: to maintain the reference standards and to detect trends associated with global environmental change.

Routine monitoring of the reference sites facilitates the scope of environmental programmes focused on the preservation of habitats or biodiversity. By collecting environmental information from the reference sites it becomes possible to:

- Obtain useful information on habitats, biological assemblages and species ecology and biology, and also to identify ecosystems and species of priority for protection.

2.5. Review of methodologies for establishing reference conditions

An analysis of methods allowing the establishment of reference conditions and a discussion of their strengths and weaknesses is given by Johnson (2001a). A brief review of the principal methods using, in part, material from Johnson's paper is presented below.

A usual practice is the development of a **spatial network** of reference sites. The establishment of spatially based reference networks requires the existence of survey (sample)

data. There are two basic approaches to characterising reference conditions from biological surveys: site-based (selection of minimally impaired natural sites) and condition-based (setting reference conditions as the best available ambient biological conditions, usually on the basis of information from the least impacted sites).

In the **site-specific approach**, data from the reference sites are grouped into homogeneous strata and are used to estimate the natural ranges of values of the selected metrics. Through comparisons with the conditions in reference sites, the degree of deviation of the conditions in the test sites or at a future time can be ascertained. Statistically, this means that the ecological status of a river is judged by comparing the values of metrics from a population of test sites from this river with the values of metrics from the established population of reference sites for this river type in the same area. The reference sites must be carefully selected, because they will be used as "controls", against which other sites or entire rivers will be evaluated. A basic requirement is that they are minimally impaired (the accepted level of impairment depends on the adopted definition of reference condition). Another requirement is that they are representative of the region and water bodies under consideration and display the natural ranges of the biological variation.

A usual practice is to define a priori rule-based and/or quantitative **exclusion criteria** with regard to specific types of impacts (e.g. lack of canalisation, absence of dams, flow and velocity of water being unaffected by water abstraction, lack of point pollution sources, unaltered land-use patterns, etc.). Central to this practice is that sites not satisfying the pre-determined criteria are excluded before the initiation of surveys. Application of this practice requires that undisturbed or relatively undisturbed sites exist in the study area. In a posteriori designs (exclusion criteria are decided after the initiation of surveys) it is important not to exclude sites with low natural variability, as sites with low variability are not uncommon in natural ecosystems. Also, not to introduce circularity in the identification of reference conditions by using, as exclusion criteria, biological response variables that are subsequently used in the ecological status classification (Johnson 2001a).

When sites satisfying an a priori criterion of minimal impairment do not exist, the "**best available**" (= "least disturbed" or "best existing") practice is often used to establish reference conditions on the basis of information from the least impacted sites in the area. Although the selection of reference sites may still be based on an a priori consideration of impacts, the criteria for the selection of reference sites from the entire population of sites are not exclusive. Effectively, the level of disturbance that qualifies a reference site is variable and does not become a limiting factor in site selection. Unless anti-degradation safeguards or environmental objectives in the expectations for restoration are set, the "best available sites" approach may lead to environmental deterioration. Even in the most disturbed watershed, "least disturbed" sites relative to other sites can always be found. If the whole region suffers environmental degradation, these sites too will suffer degradation, causing the new reference conditions to be degraded relative to the conditions at a previous time.

One advantage of the site-specific spatial methods is that reference conditions can be measured directly in an area, another that natural variability can be accommodated in reference. A disadvantage is the high sampling cost, especially when high natural variability must be accounted for by increasing the number of surveyed reference sites. When high natural variability is present, high sampling frequencies are also needed during the monitoring operations to permit distinction from human impacts. Nonetheless, spatially based biological surveys provide the best current information for determining reference conditions and

subsequently the class boundaries of metrics. Both in a priori and a posteriori designs, it becomes of paramount importance to establish methodologies and impairment criteria for reference site selection.

Site-specific spatial methods are inappropriate when an area has been so strongly affected by anthropogenic activities that undisturbed or best-available sites, satisfying a pre-set criterion of acceptable impairment, cannot be identified. In such cases, reference conditions are often derived from spatial data using condition-based approaches. One such is the "**excellent conditions**" technique in which reference values of the chosen metrics (usually the highest scores) are determined from an entire population of sites. There are several variations of this technique, e.g. the reference values may be taken from the least impacted sites, from sites determined subjectively (e.g. inclusion of certain habitat types), from sites presenting the highest values of given metrics (species richness, abundance of intolerant species, etc.) or according to statistical design. In the latter case, for example, the determination is usually made in the following way:

(a) a representative sample of sites is taken from the entire population of sites;
(b) the population distribution of each metric is determined;
(c), the 95^{th} percentile of each metric is taken as its reference value.

A central assumption of the "excellent conditions" approach is that at least some sites in the area are relatively unimpaired, which will be reflected in the highest scores of the individual metrics. This approach may introduce unknown bias in the estimation of reference conditions, because the reference conditions are established without sound criteria for impairment. It may also introduce circularity, because the highest metric values are characterised a priori as the best of existing biological conditions, and are taken to represent the appropriate "excellent" community in the area. However, low scores of a metric may be natural, not an indication of degradation. Thus, whenever this approach is chosen, it becomes important to develop methodologies and criteria for setting reference conditions to metrics, e.g. by using independent stress-response relationships. In the absence of an external confirmation of the response of metrics to stressors, assumptions about the sensitivity of metrics to human disturbances should be made with caution.

Prediction of expected reference conditions using **models** is particularly useful when the human impacts are so intense and widespread that reference sites satisfying the criteria for minimal impairment do not exist or cannot be found and/or few records of past conditions are available. In such cases, the reference value of a metric can be approximated either by extrapolating a stress-response relationship established in the area to lower target levels, or by the expected value for an undisturbed community in another area (in the latter case, the two areas must not exhibit important faunal differences).

The first modelling approach requires adequate and suitable local data for the development of functional relationships between human impacts and ecosystem responses. Provided that such data exist or can be obtained, a strength-response relationship can be established for a metric under impacted conditions. Extrapolation then enables the prediction of the expected value of the metric (in the same area) in the absence of human disturbances. Because of the anticipated scarcity of data available from sites in good condition, emphasis should be given during research projects to the detailed ecological description of least influenced sites. Even if these sites do not satisfy some pre-set impairment criteria for reference (see above), these

descriptions may allow the establishment of relationships that can predict reference conditions.

The second approach is to establish relationships (e.g. through regression analysis) between individual metrics and environmental or geographic variables that are not sensitive to anthropogenic impacts (e.g. substrate type, altitude, etc.) using data from reference sites in another, relatively undisturbed area. The established relationships permit the estimation of "theoretical" values (and range of variance) of various metrics in the reference sites, with which comparisons of values from new sites can be made. This approach is less direct than the spatial approaches described earlier but allows, under certain assumptions, application of the reference data from an unimpacted area to an impacted area. Thus, even if none of the existing sites in a watershed satisfies the reference condition criterion "minimally impacted", the determination of reference conditions is still possible by extrapolation of measurements from other similar watersheds. However, the method is sensitive to assumptions about the generality of these relationships. It also requires good quality ecological data and knowledge of the mechanisms structuring natural communities. These two modelling approaches have been reviewed in detail by the REFCOND group (Johnson, 2001a).

For the sake of completeness, the **hindcasting** modelling approach should be mentioned. This approach uses the past as a key to the future, and relies on historical records and paleoecological analysis as a means of reconstructing past ecosystems. However, ancient ecosystems cannot always be assumed to represent present conditions. **Paleoreconstruction** is a method attempting to provide records of the geomorphological, physicochemical or biological changes of the history of a water body, e.g. using sediment cores to assess the abundance of diatoms in past eras. Thus, reference conditions representing a past-unimpacted environment can be established regardless of the severity of present-day impacts. With some exceptions, paleoreconstruction is more applicable to lakes than rivers. Here again, a past reference may not be representative of present conditions. Thus, expert judgement is required for integrating this information with modern ecological information.

Historical data provide insight into past conditions, essential to estimating what the current ecological potential could be. Historical assessment of ecosystem change may examine erosion and sediment transport or deposition processes, or changes of hydrological and morphological characteristics, landscape patterns, human uses, and abundance and distribution of species. Such methods are particularly useful in significantly degraded systems, because they provide a reference for comparison with present conditions and with management objectives. Various sources of data can be used for detecting how ecological conditions have changed (old maps, photos, scientific records, and even anecdotal information or oral histories).

However, historical methods of reconstructing reference conditions, if not applied carefully, suffer from various disadvantages. One is that the conditions in past environments are not precisely known. Reference conditions are not static values in time, but are flexible responses to environmental change. Therefore, changes in ecosystem components, for the purpose of establishing reference conditions, should be examined within the current climatic period, otherwise the historical evidence may be misleading. Another disadvantage is that historical records rarely permit the estimation of natural variability, or identification of the causes. For example, a species appearing in historical data could have been misclassified or may not exist today due to a natural extinction process. On the contrary, a species may be lacking in historical documentation because past studies were not interested in recording its presence.

Moreover, historical data are subject to unknown sources of bias, because insufficiently documented methodologies, different gear and unstandardised sampling techniques may have been employed, or because the data were derived for uses other than assessment of reference conditions. Overall, historical data need to be carefully evaluated by experts and validated using independent data sets.

Expert judgement allows a flexible choice of methods and arguments for defining reference conditions. Also, it is a relatively inexpensive technique, and has the potential to integrate a broad range of relevant information. The method is particularly useful in significantly disturbed areas, as is the case of heavily modified bodies, where no suitable reference sites can be identified. However, the method suffers from subjectivity errors and inability to arrive at quantitative and standardised procedures. Expert judgement is, however, essential in arriving at a balanced and comprehensive assessment of the information derived from other approaches. Even when spatial or modelling methods have been adopted, some kind of expert consensus is often required, for instance in evaluating data quality when the objectives and survey methods have changed over time or when extrapolating results from laboratory experiments to the field.

Each of the above approaches has advantages and disadvantages. In most countries, there is little practical experience and limited knowledge on their potentialities and the drawbacks associated with them. Johnson (2001a) summarised the following strengths and weaknesses of the various methods (Table 2.1).

Table 2.1 Strengths and weaknesses of methods used to determine reference condition. (from Johnson 2001a)

Approach	**Strengths**	**Weaknesses**
Expert opinion or best judgement	May incorporate both historical data/opinion and present day concepts	Bias may be present
Historical data	Often inexpensive to obtain	Variable data, few metrics and data quality may be poor or unknown, static measure
Paleoreconstruction	Incorporate both physico-chemical and biological data	Basically limited to lentic systems, high initial costs.
Direct	Site-specific	Not many metrics
Indirect	Calibration models currently available for modelling a number of stressor variables; pH, TP and temperature reconstruction.	
Modelling	Site-specific	Requires data, calibration and validation
Survey	Region specific	Expensive to initiate

A single method does not yield a fully satisfactory view of the undisturbed situation. Compiling all available information into an integrated picture is the best strategy to derive and calibrate reference conditions (Schmutz *et al*. 2000). Data availability, local factors and type of anthropogenic impacts may influence the choice of methods. For instance, spatial methods may be used to establish the reference state for species abundance and taxonomic composition, but historical methods may be used to assess the past hydrological regime and the distribution of migratory species.

3. REFERENCE CONDITIONS IN THE CONTEXT OF THE WFD

This section of the report focuses on the establishment of ecological reference conditions for European rivers using fish as the biological quality element. It describes the concept of reference conditions from the WFD perspective and the processes for deriving them. Alternative approaches for deriving ecological reference conditions are outlined, and the advantages and compatibility with the WFD of each approach are discussed. To determine the basic requirements that the reference network must satisfy, a brief review of the WFD, including normative definitions, are provided in Appendix I.

3.1. Definition and conceptual clarifications

"Reference conditions" is still an ambiguous term in ecological literature. It is usually perceived as the "pristine" state of the environment prior to, or in the absence of, major human disturbances, and is often used to describe a desired ecological state in environmental management plans. A number of conceptual inconsistencies in the use of the term are prevalent in relevant literature (Johnson 2001a). Some workers base reference conditions upon historic landscapes, linking the reference state with a "former" or "natural" environment, to which it would be good to return. Others define reference conditions in the context of regionally representative conditions that are indicative of minimum or no anthropogenic stress.

The WFD sustains the conceptual ambiguity, vaguely equating the reference state with "high ecological status" in the hydromorphological, physicochemical and biological quality elements (Appendix I). High ecological status is meant to represent the state of no, or only very minor, anthropogenic alterations to the values of these elements for a surface water body type from those normally associated with that type under undisturbed or minimally disturbed conditions. In the case of heavily modified or artificial surface water bodies, high ecological status is meant to represent the "maximum ecological potential". High ecological status and maximum ecological potential define directions in management plans, but the targets of restoration are the "good" status and the "good ecological potential" respectively. In any case, no judgement of optimal condition should be made from the economic or political points of view.

Some degree of ambiguity in the description of reference conditions is not necessarily a bad thing. Given the high heterogeneity of environmental conditions across Europe, and also the diverse nature of the available data set, some flexibility must be allowed in the interpretation of criteria and approaches for deriving reference conditions. Such flexibility may concern, for example, the selection of the biological quality elements to be used for the assessment of high status (e.g. fish, phytoplankton, etc.), or the biological attributes upon which the assessment will be based (e.g. taxonomic composition, age structure, etc.). Also, the description of the benchmark for minimally disturbed conditions (e.g. spatial Vs temporal or the historical

period of relevance) and the methodologies to derive reference conditions require flexibility. This flexibility permits the establishment of reference conditions under various ecological circumstances and with the different types of available data, as the quality elements, benchmarks and methodologies applicable to one region or to one type of data may be different from those applicable to another region or to another type of data.

However, this flexibility does not extend to the basic interpretation of the conservation/restoration objectives of the WFD, as it is clearly determined that the reference conditions must represent no, or very minor, human disturbances. The terms "undisturbed conditions" and "minimally disturbed condition" remain poorly defined in the WFD, and yet their interpretation may have major consequences for the implementation of the WFD's environmental objectives. If the overall goal of the directive is to develop a consistent monitoring and management framework throughout the EU, then it is important to ensure uniformity in the assessment criteria of reference conditions. Inappropriate or unreliable reference conditions could result in failure to detect human impacts on the environment or, on the contrary, to erroneously identifying natural variation as human impact, with a concomitant and unnecessary expenditure on restoration measures.

If the main function of reference conditions is to allow the degree of deviations of the conditions as a result of human activities to be ascertained, then it is important to have a clear understanding of what "high status" represents, what is meant by "undisturbed" and "minimally disturbed" condition, and how to establish the values of quality elements that reflect totally, or nearly totally undisturbed conditions. The crucial issue is the determination of the thresholds separating "high" from "good" status. FAME partners agreed that terms such "very minor" or "minimal" anthropogenic disturbance and "minimal impairment" need more precise definitions to be as objective as possible.

The same issue has repeatedly been raised in workgroups established for the implementation of the WFD. In presenting the results of WP5 REFCOND, Owen (2002) outlined the development of the topic in the context of a number of questions, e.g.:

- is a more precise or a more practical definition of reference conditions required?
- what is meant by "minimally disturbed" condition?
- what are the limits of an acceptable degree of slight change within reference condition?
- what benchmark should be used to determine undisturbed condition?
- does any modification of a water body disqualify it as being in reference
- how much natural variation can be accommodated within waterbody types?
- how can natural variation and anthropogenic impact be differentiated?
- how to segregate impacted from unimpacted sites?

With regard to the definition, the recommendation put forward (Owen 2002), and adopted by the REFCOND Guidance Drafting Group (Wallin et al. 2002a), is that the definition of reference condition should be as precise as possible and should follow the terms of the Directive, containing the basic requirement that "*the values of physico-chemical, hydromorphological and biological quality elements should correspond to totally, or nearly totally undisturbed conditions*".

With regard to the relevance of these quality elements in the classification of ecological status, WP4 REFCOND report (Wilde 2002) considers two possible interpretations, depending on the focus:

(a) the "biological focus", in which the biological elements define the final ecological status of a water body;
(b) the "chemical focus", in which both the biological and the chemical elements define the status.

The report concludes that the WFD favours the biological focus. The other quality elements are treated as conditioners for life.

With regard to the establishment of benchmarks, the REFCOND report (Owen 2002) evaluated alternative options and recommendations put forward in earlier reports, and considered that it is necessary (a) to take into account anthropogenic pressures, and (b) to come to a view on the temporal benchmark to set in respect of anthropogenic pressures. The report cited certain of the conclusions of CEN TC230 WG5, namely that:

> *"Undisturbed" conditions meaning 'completely lacking in any form of human impact' will be virtually impossible to find in any rivers in the EU…. Thus, rivers where 'type-specific reference conditions' are described will include those that show some departure from undisturbed conditions. For hydromorphological features the definition of 'undisturbed conditions' should relate to channels and riparian zones but not extend across the whole catchment area… Four general sets of characteristics must determine rivers in reference condition:*
>
> - *freedom of lateral movement;*
> - *free flow of water and sediment in the channel;*
> - *land-use and vegetation in the flood-plain/riparian zone;*
> - *bed and bank character.*

The WP5 REFCOND report (Owen 2002) considered that some flexibility in the third criterion (land-use and vegetation in the flood-plain/riparian zone) would be permitted but no deviation [from naturalness] should be allowed for the other three criteria.

Bearing in mind that an absolutely pristine, post-glacial state is not realistic, the final recommendation was that "Reference conditions should accommodate a level of impact compatible with the extent of land-use pre-intensification pressures, intensification pressures should be identified as significant step-changes in land management within the recent history (i.e. no longer than 150-200 year ago) of the water body". However, it was pointed out that:

- The temporal benchmark may be flexible and need not be coincidental for each pressure – the concern is to identify reference conditions prior to any environmentally significant step-change. The actual date of such change could vary conceivably from 150 years ago to present day condition.
- For morphological character, changes to which the ecosystem has become adapted and attained equivalence to a natural ecosystem may be acceptable ('naturalised' ecosystems). Thus, "*Reference conditions should accommodate a level of direct morphological alteration compatible with ecosystem adaptation and recovery to a level of biodiversity and ecological functioning equivalent to unmodified, natural water bodies"*.
- For current uses, it is necessary to provide guidance on the degree of acceptable change within the reference condition (pressure, exclusion or avoidance criteria). The validity

or suitability of these criteria should be qualified in each case by the overriding requirement to demonstrate no significant ecological change**.**

3.2. Stratification of the monitored area

The WFD prescribes that the first step in the quantification of the ecological state of rivers requires the definition of appropriate river types. Then, reference fish communities will be identified in each river type to be used in monitoring programmes. According to the adopted typology scheme, the development of river types is based on abiotic criteria. However, the reference conditions and the subsequent classification of the ecological status are based on biotic criteria. The underlying assumption is that the abiotic factors used in river classification can predict biological communities. Biological criteria are not introduced directly in the classification scheme as descriptive variables, however an ecoregional classification based on Illies (1978) ecoregion concept, which incorporates biological parameters, is included in the river typology classification system A as a spatial component.

It appears that the general tendency in workgroups established to support the WFD is to adopt the river classification system B (e.g. Bund 2001). This system does not include ecoregions or any other biological or geographical stratification both in the obligatory and in the optional descriptors. Eventually, the WP4 REFCOND report (Wilde 2002) considers that countries that will adopt this system will not use ecoregions. However, some spatial stratification becomes necessary. Reference conditions need to be set at the local scale.

Provided that an ecoregional (or other spatial classification) scheme will generally be adopted, only two stratification levels are recognised: ecoregions and river types. However, stratification into ecoregions and river types may not provide the specificity needed for water quality assessments and management. A further division into smaller units may be required to classify fish communities into homogeneous assemblages (in effect, to reduce natural variability within strata so that the human impacts will be brought into prominence). A REFCOND report (Bund 2001) concluded that simple typologies are not always optimal for defining reference conditions.

It seems that the historical patterns of fish distribution have not been taken into account in the establishment of ecoregions. Therefore, a possible division into sub-ecoregions based on zoogeography may become necessary. If high natural variability is still present, then it is possible to use additional optional parameters of the river typology system B in order to arrive at finer river strata. An alternative solution is to introduce an additional stratification level based, for example, on habitat types.

The zoogeographical factors play a particularly important role in southern Europe. Whilst in northern Europe the small number of species and the relatively high homogeneity of the fish fauna may enable the establishment of reference networks representing wide geographical regions, in southern Europe the larger number of species and the high natural variability in fish community structure among rivers necessitate finer levels of geographical discrimination. A particular problem is the presence of a great number of endemic species, many of which are insufficiently understood. For example, the Greek freshwater fish fauna contains 126 fishes, of which 81 are autochthonous primary freshwater species. Of these, 63 % are endemic either exclusively to Greece or to the southern part of the Balkan Peninsula. Many endemic species have a restricted distribution, and some are limited to only one or few aquatic systems. Sometimes, two otherwise identical systems (from the viewpoint of hydrological

characteristics, elevations, ecological conditions, etc.) may harbour different fish species, because of historical and phylogenetic factors. In such cases, river typology insufficiently predicts fish communities. In view of the fact that the fish community structure is an important metric in the evaluation of impacts, the development of a fish-based assessment method applicable to the Greek situation would require zoogeographic zonation within ecoregions (yet, the zonation must not be so detailed as to consider each river a special case).

It has to be recognised that each stratum will have its own reference conditions. Though a fine stratification using zoogeographic or additional criteria would provide useful distinctions of ecologically comparable regions, the number of strata for which reference conditions should be defined would increase substantially. A REFCOND report estimated that the potential number of river types in only 12 ecoregions of Europe are at least 519 (Wilde 2002). This is a conservative estimation, based on classification scheme A, with only one country per ecoregion (the country with the highest number of rivers) being considered. A high level of discrimination, although biologically desirable for efficiently partitioning natural variation, would increase the number of strata significantly, and the amount of data needed to establish reference conditions for metrics would increase concomitantly.

3.3. Methodological guidance

In accordance with the requirements of the WFD, biological reference conditions may be either spatially based or based on modelling, or may be derived using a combination of these methods. Where it is not possible to use these methods, it is allowed to use expert judgement. The REFCOND Guidance Drafting Group (Wallin *et al*. 2002a) has recommended that one should always try to develop/use site-specific reference conditions as possible.

The WFD does not explicitly require the derivation of reference conditions to be based on survey data. However, the existence of good quality survey data is essential for applying spatial or modelling methods, especially site-specific approaches. The operational monitoring programmes, which are a potential source of such data, will not start before 2006, whereas reference conditions have to be defined by the year 2004 (see Appendix I). The directive states that the member states shall establish surveillance monitoring aiming, among others, to provide information for the efficient and effective design of future operational monitoring programmes, but does not impose an obligation for this. Some countries have not established any kind of monitoring, nor have developed river typologies, at least to the degree of discrimination required for establishing type-specific reference conditions (Section 2.3).

Under such circumstances, the impression is left that reference conditions shall be established on the basis of desk assessments. This seems to be an inconsistency in the WFD, since experience has shown that the establishment of reliable reference conditions requires fieldwork in respect of habitat surveys and fish sampling.

Another anticipated difficulty is that several of the national fish monitoring systems have been based on principles and methodologies incompatible with the WFD, e.g. they do not utilise reference conditions or have adopted the “best available” approach. As is evident in various REFCOND documents, the latter approach will not be accepted for the implementation of the WFD (except perhaps in respect to heavily modified water bodies). However, the data obtained so far may be usefully treated in combination with new data to derive reference conditions through modelling.

3.3.1. Reference criteria

As discussed previously, the question "how should reference conditions be established?" requires the determination of a benchmark for the reference state and the reference criteria. If site-specific spatial methods are used to obtain data (which is a desirable choice), this question prompts a further question "how should reference sites be selected?" In setting criteria for reference site selection two basic requirements dictated by the WFD have to be taken into account: the sites must be minimally impacted, and display the natural ranges of the biological variation.

Both criteria are very demanding. The first requires an *a priori* knowledge of impacts, which means that impact assessments must have preceded the reference site selection process. In the absence of reliable assessments, any site selection should be regarded as interim and subject to future validation. The second criterion requires that observations or surveys of fish habitats have preceded the selection process to ensure representation of different habitat types and fish communities. Ideally, the reference network should include many undisturbed or minimally disturbed reference sites representative of an ecoregion and river type.

Strictly speaking, the normative definition provided by the WFD requires the establishment of reference conditions in relation to the values of the quality elements (*the hydromorphological, the physicochemical and the biological quality elements reflect undisturbed or nearly undisturbed conditions*). At first sight, it would appear that the reference criteria for the biological quality element "fish" could be based on limits of these quality element values. However, prior to establishment of reference conditions, these values are not known. Hence, to avoid circularity, the selection criteria should not include biological variables (e.g. metrics) that will later be used to determine the ecological status. It is only when reference conditions have been reliably established that such variables can be used as manifestation (not selection) criteria. In other words, if the range of values of the biological quality elements in healthy (= entirely undisturbed) ecosystems and the amount of deviation allowed within the concept of reference conditions are known, then these values can be used to validate if a new site qualifies as reference.

Practically, the reference criteria rely on an acceptable degree of change in abiotic attributes due to anthropogenic influences. Impairments to the good status of the water body regarding these attributes result from one or a number of pressures and are considered as impacts. The overall requirement is that "only very minor" human disturbance is allowed. The WFD specifically mentions a number of anthropogenic pressures (pollution, water abstraction, etc.) for which assessment of impacts should be made, and asks for the identification of additional significant anthropogenic impacts (Annex II, 1.4). The interpretation given by various working groups and projects is that certain biological impacts, including introduced species, disease, etc., could form the basis for the formulation of impairment criteria. However, as previously discussed, none of the biological quality elements specified in the WFD should be used to identify pressures, otherwise circularity would be introduced.

Hence, the aim is to develop criteria predicting low (acceptable) levels of alteration of the hydromorphological, chemical and physicochemical elements supporting the biological elements. Due to the great ecological distinction between the taxonomic groups that may be used in the monitoring operations (e.g. fish, phytoplankton, etc.), the criteria that will be used to predict unimpaired conditions need not be the same for different groups. However, it must

be guaranteed that the following elements described in Annex V 1.2.1 of the WFD, each of which can be linked with one or more types of pressures, are basically covered:

Hydromorphological elements supporting the biological elements

- hydrological regime
- quantity and dynamics of water flow
- connection to ground water bodies
- river continuity
- morphological conditions
- river depth and width variation
- structure and substrate of the river bed
- structure of the riparian zone

Chemical and physicochemical elements supporting the biological elements

General

- thermal conditions
- oxygenation conditions
- salinity
- acidification status
- nutrient conditions

Specific pollutants

- pollution by all priority substances identified as being discharged into the body of water
- pollution by other substances identified as being discharged in significant quantities into the body of water

For some types of pressures, setting criteria for impairment is not too difficult and can be approached from more than one standpoint. For example, the intensity of pressures from pollution and the magnitude of impacts can be ascertained by observation of the distribution of point sources in a watershed or by direct measurements of the concentration of pollutants in the water. For some other types of pressures estimation is more difficult, especially if historical data are not available. This is the case with ground water abstraction, which may modify the discharge regime, and thereby affect the distribution and abundance of rheophilic fish species. To assess this kind of pressure information regarding how surface waters are connected with ground waters, information on the past flow regime and the past abundance and compositions of the fish communities are required.

During the formulation of criteria, are non-environmentally-based considerations allowed? In the AQEM a list of criteria, a criterion for political palatability is included. The inclusion of this criterion, which is neither derived from the WFD, nor based on ecological considerations, has invoked some criticism (Owen 2002). The AQEM list is discussed further in section 4.4 and Appendix III.

Should a reference site satisfy all conditions of Annex V? As mentioned earlier, there are views within currently running projects in support of the WFD that rivers satisfying all the requirements of the directive virtually do not exist in Europe. Therefore, suggestions have been made that some departure from undisturbed conditions should be allowed for certain elements (see section 3.1). A similar interpretation is adopted in this report (see section 4.4.5).

What are the acceptable levels of alteration to the values of an element for a site to be considered as reference? Expressing the pressures in quantitative terms remains a largely unresolved issue. In various REFCOND reports there have been proposals for quantitative criteria, but these are usually cited as guidelines or examples aiming to stimulate discussion. For example, the WP5 of REFCOND report (Owen 2002) stated that the degree of acceptable change in water abstraction could perhaps be up to 10 % mean daily flow, adding that negligible effect on ecological structure and functioning should concomitantly be demonstrated. It must be noted, however, that a fixed limit value may not be applicable over a wide area or over a broad spectrum of river types. The same percentage water abstraction may exert a different degree of disturbance on the biological communities of different rivers, depending for example on river size, meteorological factors, seasonal periodicity of flow, local faunas, other concomitant pressures, etc. A case-by-case analysis is required to produce quantitative criteria for the main types of pressures using local information.

Validation, using more recent data and/or independent methods, is an important next step. In the absence of adequate information, a site may be selected preliminarily but its suitability must be evaluated at a latter time.

3.3.2. The case of heavily modified waterbodies (HMWB)

One of the problems anticipated during the implementation of the WFD is that for a large number of seriously degraded European rivers it will not be possible to establish attainable baseline expectations (= reference conditions) within the framework of existing pressures and water use designations. If the impacts are very extensive and the situation irreversible (e.g. due to high costs, social necessities or technical impossibility), the WFD provides the opportunity for a water body to be assigned to the "HMWB" category. The advantage of the HMWB approach is that the WFD allows flexible interpretation of the environmental objectives and equates high ecological status with the maximum ecological potential.

For an unbiased interpretation of the WFD's restoration targets across Europe, it is essential to clarify three issues:

- When does a water body falls under the category "heavily modified"?
- How to fix the limits of the "maximum ecological potential"?
- Can local regulatory necessities and economic and social considerations modify these limits?

The 2.2 working group on HMWB has dealt with some of these issues and has proposed the following procedure for identification and designation of HMWB:

1 assessment of the ecological status based on deviation from reference conditions;
2 definition of measures required to achieve a good status;
3 economic justification that good status cannot be achieved;
4 designation as HMWB;

5 definition of maximum ecological potential (ecological target and restoration measures);
6 definition of good ecological potential (ecological target and restoration measures).

Thus, although no "natural" reference conditions may exist in a "heavily modified" river, the operational determination of reference conditions is the same as that for natural rivers. However, reference conditions need not be described with the same accuracy as in the "normal" approach because of the larger distance between reference conditions and degraded conditions or reference conditions and good ecological potential.

Potentially, there would be two different approaches for establishing reference conditions leading to expectations similar to natural conditions in heavily modified rivers: In the first, if undisturbed conditions still exist in the area, the reference conditions are determined by data from unimpacted sites in adjacent rivers of similar type. In the second, they are reconstructed or conceptualised using predictive models, historical data, expert opinion and any other relevant technique.

In addition to establishing reference conditions, procedures and criteria for the determination of "maximum" and "good" ecological potential are required. Because restoration of the original environment is impossible (and is not demanded by the WFD), the level of acceptable conditions may be based on best existing conditions. On the assumption that the least affected sites reflect the attainable biological potential of the area, the "best available site" approach may be justifiable in this case.

Deciding when a river will be assigned to the "HMWB" category is an issue that has not yet been adequately addressed. The need for specific and precise criteria for designation of HMWB and achieving comparability of "good ecological status" with "good ecological potential" has been stressed in relative discussions within the REFCOND project (Wallin *et al.* 2002b).

3.3.3. The case of introduced species

Implicit to the preservation perspective of the WFD is that the native historic structure of fish communities is maintained within the range of natural variability. In this context, the introduction of new species into a system, either deliberate or unintentional, is an anthropogenic disturbance. It represents some form of "biological pollution" that affects, through trophic and competitive relationships, the structure and function of the ecosystem. On the basis of such considerations, most IBI applications define scoring criteria for a metric describing fish introductions to a system. In these applications, the absence of the introduced species is the reference state (high status), and their presence is seen as a deviation from the original fish community, and scored as degradation.

However, the restoration perspective of the WFD contains the fundamental assumption that if all anthropogenic impacts were removed, the system would return to the high status (= reference) state. Obviously, this is not so with many cases of introduced species. In such cases, the system will never be assigned a high status, even in the absence of any human impact. Hence, there is some conflict between the preservation and restoration perspectives of the WFD.

The presence of alien species is not a problem as far as the establishment of reference conditions is concerned. However, establishment reference conditions is not just a technical matter aiming to materialise a political decision. In deciding whether or not non-native species should be integrated into reference conditions the arguments in support of either option must be considered.

Non-native species should be included in the reference:

- With respect to human impacts, the WFD refers to hydro-morphological stresses.
- The presence of introduced species may not be causing any issues to be considered in the river management plans.
- Many introduced species are now "naturalised", forming concrete components of local fish communities.
- Exclusion of the introduced species generates an ecologically false reference. Even when they are not accounted for in the establishment of reference conditions, they influence through competition and predation the distribution and abundance of native fish species (and other organisms as well) within a water system, and thus that system's structure and function. Thus, in the presence of introduced species, the "apparent" structure and function of the reference community is different than it would be in the absence of them, and hence unnatural, because some links of the food web are not considered.
- Some introduced species may be useful indicators of ecological quality, responding predictably to a particular type of pressure.

Non-native species should not be included in the reference:

- Introduced species represent a disturbance altering the values of biological quality elements (e.g. population structure of a community); harmful impacts on the native fish fauna and natural ecosystems have repeatedly been demonstrated.
- Biodiversity is a key concept of ecological integrity. Inclusion of non-native species in the reference could raise political and legal implications with respect to the preservation of natural biodiversity. On the other hand, however, national and international laws and treaties may protect some of the alien species present in a system.
- Some introduced species may be subject to selective harvesting and removal from natural aquatic systems, thus their inclusion in the reference and metrics (e.g. for presence and abundance) could provide a measure of the success of the efforts for removal.

One view within the FAME consortium holds that reference conditions should be used with a restoration perspective. Since following the introduction of a species the return to the initial fish community structure is almost impossible, what is the purpose of defining reference conditions that represent an unachievable objective? In this context, reference conditions should potentially include non-native species (e.g. those long-established and adapted to a local ecosystem). If such species are excluded, fish-based assessment methods cannot provide a realistic restoration target in management plans (whereas other methods can) and may appear as less applicable to the WFD, except from a theoretical perspective. One of the opinions expressed in a REFCOND report (Wilde 2002) also holds that species introductions should be treated as an anthropogenic process paralleling the natural processes. Therefore, the reference should be flexible, requiring periodic updating (this may necessitate to increment the accepted variation within the reference conditions, thus augmenting the high status definition).

Nonetheless, the strongest tendency within the group is that the alien species should be excluded from the reference. From the management point of view, however, a distinction should be made between ecological impacts and management objectives.

It seems that the problem with introduced species is generated partly from the exclusion of these species from the reference but their inclusion (with a negative score) in the metrics. One possible solution to the problem is to exclude the introduced species both from the reference and from the metrics. This would enable an assessment of the ecological status with a restoration perspective. These issue will be discussed and analysed in the later FAME work packages.

A similar kind of problem appears with species extinction as a result of human impacts (where no re-introduction is feasible or desirable), and similar "comparison tests" could be made.

3.3.4. Principles for selecting and scoring metrics

The reference conditions for each metric represent the "high quality" class in the five-class classification system advocated by the WFD, therefore the width of this class influences the widths of the remaining classes. When data from reference sites are used, the width of this class is determined by the natural variation (Johnson 2001a).

Johnson (2001b) pointed out that the selection of metrics should take into account their discriminatory power with respect to the stressor to be detected and the type of response to the stressor. Also, that high within-site spatial and temporal variability (background noise) may prevent the detection of impacts and should be partitioned (reduced) accordingly by spatial (e.g. habitat) and temporal (e.g. seasonal sampling) stratification. In view of these requirements, three major difficulties are likely to be encountered in metric selection:

Poor information on the prevailing pressures in the area.
Inadequate knowledge of the species ecology, biology and life histories, upon which suitable metrics for local pressures will be selected (e.g. indicator species with regard to habitat type, ecological guild or sensitivity to given stressors).
Insufficient amount and/or quality of sample data (especially in *a posteriori* designs) that will enable assessment of variance and the grading of metrics.

The issue of sampling methodology is also crucial. An inappropriate sampling scheme, e.g. designed in the context of other investigations, may conceal high-amplitude integrated processes or may not reveal the true community structure. The "apparent" structure of a fish community depends not only on its actual structure, but also on the sampling instrument used.

The potential value of the various metrics, with respect to their utility in ecological assessments and sensitivity to stressors, are dealt with in WP3. Here, the interest is centred to whether or not reliable reference conditions for particular metrics can be developed as a validation tool. Obviously, metrics with a high inherent natural variability or showing low or unpredictable response to stress should not be chosen, because they cannot provide a reliable reference value, or cannot be graded adequately against a reference value.

The WFD does not clarify how the reference values of individual metrics would be treated to give the reference value of the biological quality element fish. A WP4 REFCOND report (Wilde 2002) considered two possibilities for the attainment of "good status" (similar principles would apply for attainment of the reference status): all individual biological attributes (metrics) must be at least "good" (named as *sensu lato* approach), or are integrated (by adding or averaging their values) into a single value (*sensu stricto*). The interpretation of that report was that the *sensu stricto* approach is in accordance with the WFD. Nonetheless, most existing fish-based classification systems sum-up the values of individual metrics to arrive at the total value.

For the quality element "fish", the WFD defines the following biological attributes to be used as indicators of ecological condition: species composition, abundance, sensitive species, age structure and reproduction. Each of these can be described by one or more metrics. Reference conditions, representing a fish community in high status waterbodies, should be defined for each individual metric, and for each area and river type. Considering the large number of ecoregional and river typology strata, and the also large number of potential fish metrics applicable over Europe, the number of metrics in all ecoregions and areas where fish-based assessment methods will be applied is large. This issue raises again the problem of data availability/quality.

Another important problem concerns the baseline expectations. For a metric to be an appropriate indicator of water quality, it must respond to environmental degradation in a consistent manner, and the type of response must be known. Although this condition is very demanding, the reference values of various metrics are often assumed or inferred from indirect evidence, rather than calculated from the values in reference sites or stress-response relationships. For example, a usual assumption is that a community with high percentage of a certain ecological groups, e.g. piscivore species, is positively associated with biotic integrity. Conversely, low contribution of piscivores to the community abundance is often taken as evidence of poor ecological quality. Similar assumptions hold for omnivorous species, tolerance, habitat specialisation, etc., where metric values above or below a certain percentage are assigned to the reference state.

On a broad scale, "mature" or "unimpacted" environments may indeed contain high percentages of piscivores, specialists or intolerant species. However, the reverse is not necessarily true. A fish community in a particular site may contain a low percentage of piscivores not because of human impacts, but because of phylogenetic or ecological factors (e.g. high environmental instability in the site). Despite this low percentage, the site may be undisturbed with regards to human impacts, and hence should be regarded as reference.

Similarly, a healthy site may accommodate a lower number of species or individuals than a disturbed site for reasons related, for example, with some habitat properties, which are confounded in coarse groupings of regions or habitats. It may even happen that a disturbed site displays a higher species richness or abundance than an undisturbed site, as happens when local eutrophication attracts fish. Further complexities arise when eutrophication interacts with another form of degradation that has opposite or cumulative effect on abundance, making difficult to identify the predominant agent. In this context, the results of procedures for setting reference conditions to metrics based on the "excellent conditions" approach (metric values or sites exceeding a certain percentage of the total population) must be interpreted with caution.

4. WORK PROGRAMME

Conceptual and methodological issues related to the establishment of reference conditions were discussed during the FAME kick-off meeting at Maastricht in January 2002. A general methodological procedure was adopted, which was elaborated in subsequent background documents and refined further through feedback comments. This section describes the final methodology, as agreed in the Nässlingen meeting (May 2002), focusing on the criteria required to identify reference sites.

4.1. Overview of methods and processes for defining reference conditions in Europe

A REFCOND guidance document (working group 3.1, 12/4/02) has stressed the need for appropriate infrastructure for implementing the reference condition part of the WFD (relevant data, human resources, organisational structure, etc.).

To assess the availability/utility of fish data that can be used for reference description during the FAME project, a review of the national fish data collection systems, and the methods and processes for establishing reference conditions in preparation or use is given in Appendix II. It appears from this review that:

- Several, but not all, countries have established routine monitoring programmes applied to fishes. However, the existing fish data collection systems differ among countries. In the majority of cases these systems do not follow the requirements of the WFD.
- With few exceptions (e.g. Germany, Lithuania, Flanders), river typologies, a pre-requisite for reference description, have not been established. In a number of countries, procedures for describing river typology have been initiated.
- Reference conditions for fish communities and reference networks have rarely been described (e.g. France), but are under construction in some countries (e.g. Austria, UK). The approaches are quite diversified, including spatial or/and historical methods, with or without expert judgement, using best available conditions or other methods. In some cases, reference description is undertaken simultaneously with the establishment of river typology (e.g. Austria, Belgium, and Portugal).

4.2. Agree on a definition

It was imperative that the perception and definition of reference conditions were clarified for the FAME project, as the definition has major consequences for the methodologies and the interpretation of the requirements of the WFD. The definition must follow the rules defined by the WFD and the current interpretations made by the EU working groups. It was proposed that the following definition provided by the REFCOND Guidance Drafting Group (Wallin et al. 2002a) should be adopted:

For any surface water body type Reference Condition is where there are no, or only very minor, changes to the values of the physico-chemical, hydromorphological and biological quality elements which would be found in the absence of anthropogenic disturbance. The following criteria should be met:

i) *the values of physico-chemical, hydromorphological and biological quality elements should correspond to totally, or nearly totally undisturbed conditions.*
ii) *specific synthetic pollutants should have concentrations close to zero or at least below the limits of detection of the most advanced analytical techniques in general use.*

iii) *specific non-synthetic pollutants should have concentrations remaining within the range normally associated with undisturbed conditions.*

4.3. The methodological plan

According to the adopted FAME methodology, reference conditions will be defined using data obtained from existing monitoring or other relevant programmes that will be integrated into a central database. Both spatially-based and modelling approaches will be employed.

An anticipated difficulty is lack of suitable data for applying spatial methods. This is mainly a problem of data quality, not quantity. As noted before (Section 4.1), the existing fish data collection systems differ among countries, depending upon local methodologies and the specific objectives of data collection. Most monitoring programmes do not comply with the WFD, e.g. they do not use the reference conditions approach, or they do not base this approach on the idea of almost negligible disturbances. Therefore, these systems were not designed for collection of reference data from undisturbed sites according to some *a priori* criteria for impairment. In the majority of cases reference networks have not been established. When they have, they include only "best available sites", which are often far worse than a potential high ecological status.

Under such circumstances it may be difficult during the *a posteriori* screening and selection process of the national data sets to identify an adequate number of sites satisfying the criteria of impairment developed during FAME. For small rivers it might be possible to identify some sites representing relatively undisturbed conditions (however, it will be difficult, due to the *a posteriori* choice of sites from the existing population of sampled sites, to ensure the criterion of representativeness). For large or lowland rivers it will almost impossible to apply spatial methods.

The FAME project cannot go beyond the possibilities of the available data sets. In view of these difficulties, the spatial approach may become applicable in only few cases, such as where the national monitoring scheme has been designed according, or close, to a "minimal disturbances" concept of reference conditions. It is important to draw inferences, generalisations and guidance from these few cases for the ecological status assessment and classification system to be developed and delivered by FAME.

With these in mind, the following methodological plan was adopted:

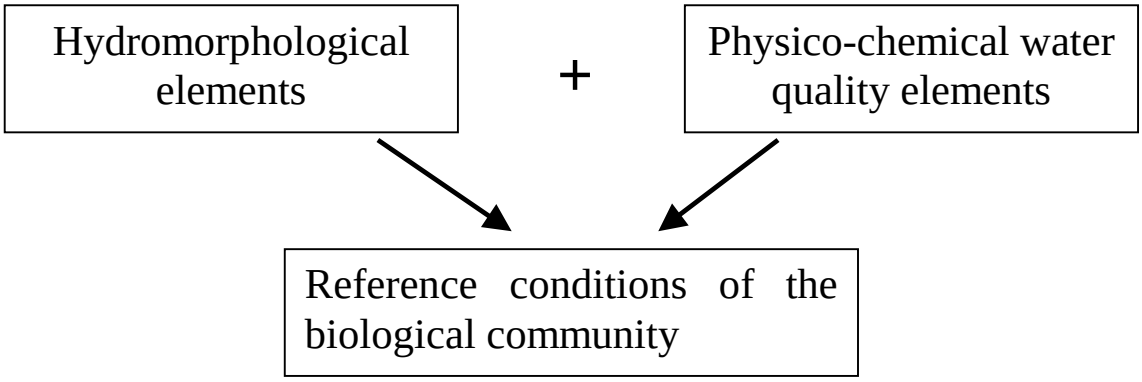
- Set criteria for impairment on the basis of stressors affecting the hydromorphological and physicochemical quality elements supporting the biological communities. It was agreed to accept provisionally the AQEM criteria and refine them further from a fish perspective.
- Investigate during the screening process if there are sites satisfying these criteria.
- If yes, and the sites are representative, spatial methods will be used.
- If no, alternative methods will be used. These may involve the selection of the best existing sites, models, and historical or additional data.

The determination of the reference conditions according to the latter methods suffers from two critical problems: there is no independent determination of the reference biological community, and some of the procedures (e.g. best available sites, ecological models, historical

sources of information) provide no standard way to ascertain the quality of information with a high degree of confidence. Therefore, the validity of the reference conditions must be confirmed with independent tests. Following these confirmations, the final choice is made.

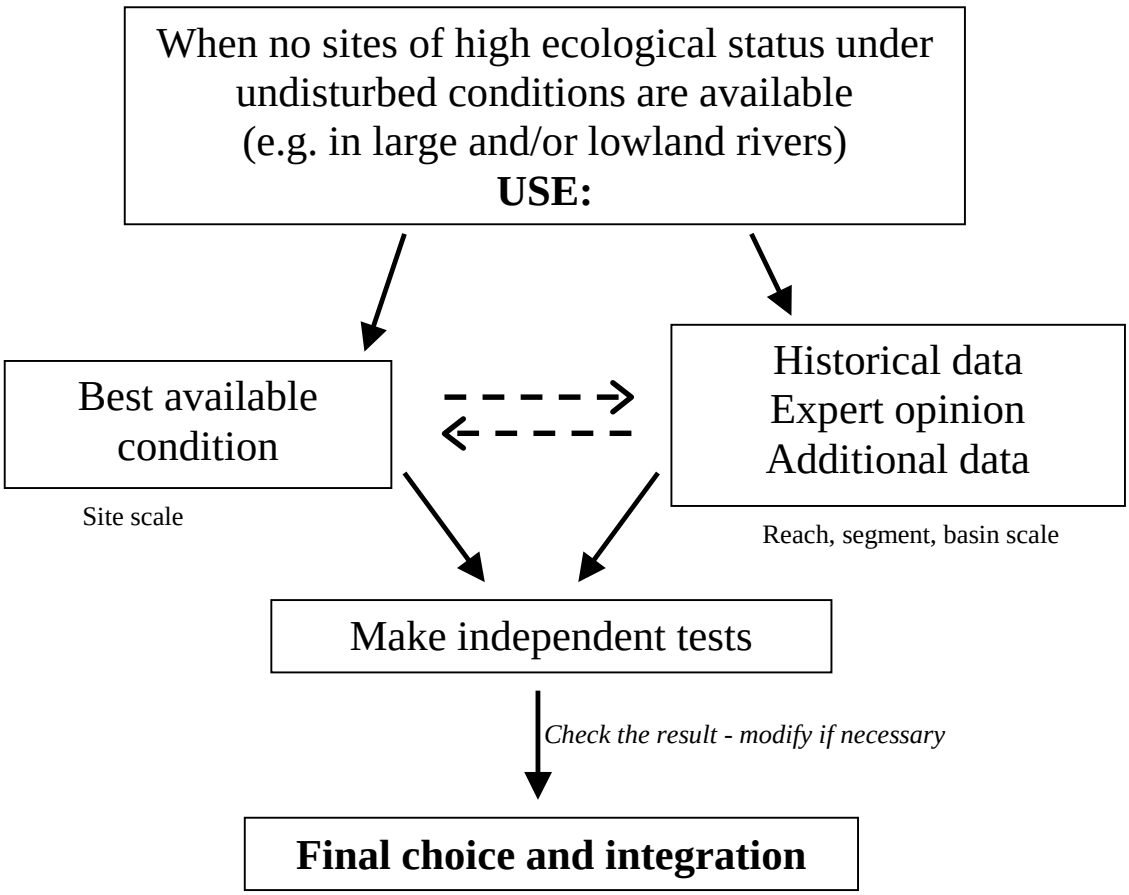
The following scheme summarises the adopted procedures for the characterisation of reference conditions:

Type-specific reference conditions
(as required in Annexes II, 1.3 & V, 1.2 of the WFD)



Notes:

1. *Abiotic criteria are used to predict the expected biological reference conditions, that is, high ecological status under no or minimal impairment (only for the purposes of the WFD and not, for example, for biodiversity purposes).*
2. *This approach is likely to yield satisfactory predictions when relatively undisturbed rivers or river segments are available, such as in the case of many streams and small rivers.*



4.4. Deductions from the analysis of the AQEM criteria

There was consensus within the FAME consortium that the AQEM criteria for identifying invertebrate reference sites could provide a baseline for identifying fish reference sites, but that modifications were required to make them applicable to the FAME project. Opinions diverged as to the utility of some criteria and the kind and extent of modifications necessary, with views ranging from "no problem, most criteria can be used" to "hard to be met".

The AQEM criteria list and an analysis of specific remarks made by the FAME partners, including suggestions for modifications and improvements, are presented in Appendix III. This section summarises the views and suggestions resulting from the analysis of the national reports.

4.4.1. Quality standards

In general it was apparent that the AQEM criteria are very demanding and describe conditions that are difficult to be met in present-day environments. This holds particularly true for medium and large rivers and/or in lowland areas. For example, the "Riparian vegetation and floodplain" group of criteria could effectively exclude all UK rivers, as no systems exist where the floodplain is uncultivated or posses natural climax vegetation. Similar views were expressed for most groups of criteria, especially the "River channel and habitats" and "Hydrologic conditions and regulation" cores, as many large rivers of Europe are now fully controlled, channelised, systems.

Relatively undisturbed sites can still be found in some small rivers and in mountainous areas. However, it will be difficult to identify sites satisfying all criteria simultaneously or to locate a sufficient number of sites representing the whole range of biological conditions in an area/river type.

It must be remembered that the AQEM methodologies have been developed mainly in the context of ecological assessments in small European streams, some of which, in upland areas, are still in pristine condition. Even so, the AQEM research group encountered difficulty identifying an adequate number of candidate reference sites satisfying all criteria.

Part of the problem relates to the wording used in the AQEM list. Some of the criteria are formulated in a strict and absolute sense, generating prohibitive baseline expectations. Examples are: No alterations of the natural hydrography and discharge regime, No hydrological alterations, No known point sources of pollution, eutrophication, nutrients etc., No known impairments due to physical or chemical conditions.

A solution to this kind of problem is to smoothen the formulation of these criteria, which is not incompatible with the WFD. Under the WFD, minor disturbances are acceptable in establishing reference conditions. Therefore, it is possible to replace strict expressions like "no known point sources of nutrients" with ones like "no known significant sources of nutrients". Some members of the FAME consortium have suggested modifications in this direction.

However, it is recognised that the wording is a minor part of the difficulty in meeting the AQEM quality standards. The main difficulty stems from the demanding provisions of WFD. There are views within the FAME consortium that less emphasis should be placed on certain

cores of criteria for which some departure from relatively undisturbed conditions must be allowed. Even so, any system that basically complies with the WFD will effectively exclude most European rivers, unless these rivers are classified as heavily modified (criteria for designation of a river as HMWB and defining reference conditions to HMWB are still needed). This issue is elaborated further in the part of this section that deals with pressures.

Under these circumstances, approaches for deriving reference conditions alternative to the spatial ones have to be utilised in many cases. The "best available" site approach must be excluded as a direct method for deriving reference conditions, as it does not conform to the WFD. However, this approach can be used in combination with modelling and/or other methods (historical data, etc.) (see section 3.3.4). This requires the development of suitable techniques for setting reference conditions to metrics, and the use of expert judgement to determine quality levels and targets.

4.4.2. The fish perspective

The view was expressed within the FAME consortium that the AQEM criteria are not very suitable for fish monitoring programmes, which address a different taxonomic group, function over a different spatial scale and often seek to assess different types of impacts than the invertebrate monitoring programmes. Three basic differences between invertebrate- and fish-based ecological assessment methods, with implications for the criteria formulation, can be identified:

1. Most invertebrates lack considerable mobility, and their sampling spatial scale is small in comparison to the fish sampling scale. At the same time, being relatively immobile, invertebrates are more sensitive to localised deterioration of water quality or habitat degradation than fish. By contrast, fish sampling has the potential to cover many biotic microhabitats in the same round and to integrate processes occurring over relatively large geographical areas. Hence, there is less need for detailed characterisation of microhabitats and adherence to strict water quality and habitat criteria at the narrow site level. Rather, emphasis is shifted to criteria identifying larger scale morphological, physicochemical or hydrological degradations.
2. The invertebrate-based assessment methodologies currently available in a usable form apply to small rivers, whereas fish-based methodologies must also apply to large rivers, where the conditions are often degraded. Criteria for large rivers may be different than for small ones with respect to emphasis. For example, factors such as woody debris and local point sources of pollution may be less important for large rivers and/or from the fish perspective than river management works.
3. Invertebrates and fish are not equally sensitive to the same range of anthropogenic stresses. In fact, according to recently released AQEM deliverables the project concentrated on three types of stressors: acidification, organic pollution and morphological degradation. However, fish-based assessment methods can also detect impacts from stressors that influence the flow regime, including water abstraction, periodic releases and disruption of connectivity.

Overall, the conceptual development of the AQEM list was based on a different systematic group and focused on the disturbances invertebrates are most sensitive to. Generally, the spatial scale of relevance is "site". As noted by the Netherlands FAME partners, the AQEM criteria have been developed for small streams with "habitat" as the spatial level considered. Special attention was paid to criteria identifying pollution, in various forms. The chemical

"core" contains criteria for pollution, nutrients, and eutrophication and diffuse inputs, but not for oxygen, pH, etc., which are included in Annex V of the WFD. Basically, however, the AQEM criteria comply with the elements of the Annex.

As a consequence of this taxonomic specialisation, and the focus on local disturbances, none of the AQEM core groups of criteria clearly refers to the catchment area, with the possible exception of the "Biological conditions" core (which includes species introductions). Practically, all the AQEM criteria refer to impairment at the site level, and they are often followed by the clarification: "*no recognisable effect on the biota of the sampling site*". Even the core entitled "land use practices in the catchment area" is mostly considered from the site perspective: "*…the degree of urbanisation, agriculture and silviculture should be as low as possible for the reference site*" "*…the least influenced site with the most natural vegetation is to be chosen*".

The establishment of reference site selection criteria requires consideration of various types of impacts. The criteria developed by AQEM are based on invertebrates, which show a high sensitivity to some types of impacts, especially pollution. However, fish-based assessment methods are also sensitive to other types of impacts, such as water abstraction, dam operation, channelisation and river fragmentation. With these types of impacts becoming increasingly important, the important issue is to evaluate the capacity of fish-based assessment methods to provide useful ecological classifications.

It may be wrong to ask if fish-based or invertebrate-based assessment methods are better at providing ecological assessments. Each type of method has its own advantages and shortcomings, and both may be selected as complementary tools to increase the precision of assessments and to expand the range of impacts to be evaluated. From the fish-based perspective, however, it seems obvious that more emphasis should be given to criteria identifying large-scale or high-amplitude impacts, and impacts that fish are potentially good indicators of, such as water regulation at a basin level.

4.4.3. The spatial scale

The preceding discussion addresses the spatial scale question. As was highlighted by several FAME partners, one of the difficulties of establishing a reference network is that certain anthropogenic perturbations exert influence on habitats and biota over a large geographical area. Even if a site is unaffected by local disturbances, this site may be affected by disturbances occurring in distant areas, or even by processes affecting the entire basin. Another difficulty occurs when the undisturbed sites are fragmented and separated by heavily disturbed sites. Since fish are mobile organisms and frequently perform shifts between habitats (ontogenetic, feeding, reproductive), the fish community in the apparently undisturbed sites is likely to be affected by the ecological quality of all sites within the travelling range of fishes. Hence, the reference sites in a river system with many (local) disturbances will display a lower ecological quality than in a system without disturbances.

In this general context, the report by the UK FAME partners considered that the AQEM site-specific criteria do not adequately cope with diverse river typology, the river continuum concept and the ecological characteristics of fish populations. Impacts to the hydromorphological regime, e.g. barriers reducing connectivity, and anthropogenic biotic changes e.g. stocking, introduction, translocation, incidence of disease and parasites, may

significantly alter community structure in a large area, and, therefore, should be considered both when establishing criteria and choosing reference sites.

The Dutch FAME partners proposed a distinction between disturbance types (and respective criteria) requiring either a watershed or a site consideration. The scope of the former is to define selection criteria for reference rivers, and of the latter to define criteria for reference sites/reaches. Note that some national or regional monitoring programmes (Sweden, Flanders) already incorporates the idea of reference rivers (see Appendix II). The Dutch partners also proposed that the river selection criteria could contain a minimum group of impairment factors, to make the selection practical and achievable, whereas the site/reach selection criteria could be more extensive and detailed. At the catchment level, the criteria pertaining to "land use", "riparian vegetation and floodplain", and "hydrologic conditions and regulation" were regarded as most relevant. With some exceptions (e.g. introduction of new species), the criteria of most other cores were regarded as important only for sampling site selection. These issues are intimately linked with the question of the spatial scale over which environmental assessments are to be made, or controls applied (longitudinally: site, reach, river section or total river; laterally: channel, riparian, watershed).

The important suggestion was that the procedure for establishing reference sites must combine information on processes occurring at, and disturbances affecting, several spatial scales, and give appropriate consideration to the extent of integration between them. This notion implies some hierarchy in the establishment of the reference network, starting with the selection of reference rivers, or sections of them, and proceeding to the selection of reference sites. Therefore, impairment criteria pertaining to a "large" spatial scale (e.g. catchment, segment) must be set as a first selection level in the process that leads to the designation of reference sites. Within the framework of this "top-down" selection process, the FAME consortium considered two alternative options: to make separate lists for criteria at the site or higher levels; or to make a single list, indicating for each criterion whether it is river-specific, site-specific, or both. The idea of separate lists was adopted.

Each spatial level may incorporate different physical, chemical, morphological etc. criteria, depending on their relative importance at the respective spatial scale. It is not controversial that the same criterion (or criteria) may appear at different spatial scales – however, the emphasis may differ. For example, criteria related to riparian vegetation, floodplains and land use patterns may be put both at the river (or river segment) level and at the site level, the former aiming to identify and exclude rivers (or river segments) with likely extensive impacts on the fish communities, and the latter containing stricter ecological requirements for site selection.

4.4.4. The scales of relevance

A practical problem is the determination of appropriate spatial scales for developing reference criteria. The scales depend on the prevailing pressure types. There is consensus that certain pressures generate impacts over a wide area, while other pressures generate local impacts. Also, that the same pressure may sometimes have widespread and sometimes localised impacts. For example, under certain circumstances impacts on longitudinal connectivity generated by a barrier may extend over the entire basin, while under other circumstances the impacts may be limited to certain river segments. These circumstances may relate to a variety of morphological, technical or biological factors (e.g. river size, position of the barrier, historical presence of migratory species) and make the delineation of the spatial scale of

influence of this pressure type difficult. In other words, it is difficult to devise common rules for deciding if the entire river or certain portions of must be dam free in order to be characterised as reference. Such a decision must be based on local expert knowledge.

Similarly, impacts from discharge fluctuations due to the presence of a hydroelectric dam may be spread over the entire river or may be limited to only a segment of it, depending on the position of the dam, its storing capacity and the characteristics of river flow. In a small low-order river the impact may be important over the entire river basin, but in a large river with many tributaries located below the dam, the influence of dam operation may be limited to the immediately downstream section, because further downstream considerable volumes of water entering from unimpaired tributaries may dampen discharge variations. Analogous considerations arise with respect to sediment transport, surface water abstraction, ground water lowering, etc.

Despite these uncertainties with respect to the scale of pressures, the definition of common scales for setting reference criteria was important for the future work of the FAME project. It was agreed that the river-specific criteria (or some of them) should not necessarily be addressed in an absolute "whole-river" sense. In this context, the simplest version is to distinguish between the site level and a higher level compatible with the river-type level. River basin criteria are included in the framework only for types of **human impacts with widespread effects over most of the basin (e.g. lowland dams preventing the ascent of locally important anadromous species).** It was also agreed to adopt a common river classification, derived in work package 1. In this, the "pool/riffle scale" would more or less reflect a single sampling occasion, the "reach scale" would be equivalent to the site level, and the "segment scale" would be a sub-element of a river comparable with the river-type level or a recognisable division of it. Reference selection criteria should be developed for the site, the segment and the river scale (Frisell 1986, Naiman 1992).

Whilst the "site scale" can be delineated with reasonable accuracy, the "segment scale" must be defined more precisely, taking care to ensure consistency with the river typology system recommended by WP1 to be adopted for FAME. This system contains four classification levels:

1) Ecoregion level (basic abiotic variables and coarse zoogeographic features)
2) Sub-ecoregion level (tier features accounting for natural zoogeography)
3) River catchment level (similar whole-river basin types grouped by their catchment size)
4) River-zone level (more or less homogenous abiotic or biotic characteristics).

This river typology proposes that 'river-type' refers to the whole-river (river catchment level). Hence, the reference segment criteria should cope with the river zone level. It is recognised, though, that interpreting river scale is difficult. The preconceived nomenclature of rivers (e.g. River Danube etc) does cause a problem, as a "river" may be only equivalent of a zone or segment within a larger basin.

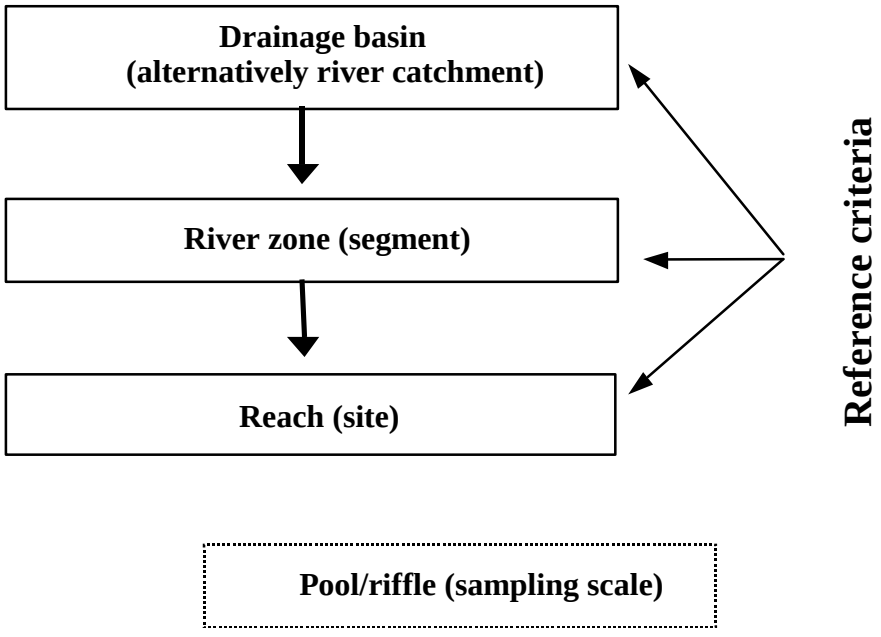
Although some national river typology schemes have been develop for the purpose of assessment of reference condition and ecological quality, no harmonised river typology classification system has been developed so far in Europe to serve as guidance for a more detailed definition of the river zone scale. Thus, whilst it would be desirable to have a typology with prescriptive boundaries for segments (zones), at present only a conceptual approach to zone typology has been defined, at least with respect to the needs of FAME.

According to the results of WP1, the primary abiotic features that usually drive and predict the distribution and composition of fish communities within a zone are altitude, wetted width, depth, flow, gradient, distance from source and width of active floodplain. The length of zones may be variable, depending primarily on stream order and catchment size. The following lengths for river segments (zones) were agreed for FAME:

- Small rivers (catchment < 100 km^2): 1 km
- Medium-sized rivers (catchment 100 - 1000 km^2): 5 km
- Large rivers (catchment > 1000 km^2): 10 km

As previously mentioned, criteria for a higher spatial scale (e.g. catchment or basin) may be important with respect to impacts extending to a scale larger than the zone level. As noted by the UK FAME partners, if impacts to connectivity are regarded as critical to selecting reference sites, then presence of barriers downstream would exclude the whole river, even if the upper segments appear to fit the criteria for reference. However, the criteria for this level should not be very severe, otherwise the selection process will come to a halt, and should be carefully designed to account for meaningful impacts. To this event, the hierarchical framework for developing site-specific reference involves three levels of selection and proceeds according to the following scheme:

A three-level selection system for developing river-type specific reference conditions



4.4.5. Evaluating impacts

During the process of defining reference sites, it was important to what extent would deviation from the "no or minimal disturbances" concept adopted by the WFD be allowed? This required consideration of:

- What kind of disturbances may disqualify a reference site?
- Are the effects of decisive importance in the alteration of the ecological status?
- Is the situation reversible?

Partners in the FAME project expressed the following views:

- The AQEM list is complex, and should be simplified to become workable.
- Criteria of local interest should not be included in the general list.
- Not all kinds of pressures should be given equal weight.
- The criteria that determine reference sites depend upon the type of impact and the level on which it acts e.g. basin, catchment, reach or site.
- "Proximity of impairments" must be defined.
- Make a list of (most) important perturbations to complete the list of selection criteria.

There seems to be no problem or disagreement to reduce the number of the AQEM criteria that justify a reference site if these are of no relevance to the locality (e.g. liming is not important for most countries) and/or from the fish perspective (e.g. woody debris). The crucial question is whether moderate (not just "minor") influences from some kind disturbances are allowed or accepted without disqualifying a reference site.

At first sight, reducing the number of criteria and/or allowing some flexibility for some criteria entails the risks of subjectivity and lowering the water quality standards. Keeping in mind that FAME must adhere to the "high status" standard required by the WFD, the basic requirements for reference river or site selection must comply with the provisions of Annex V of the WFD (see section 3.3.1).

Then, the question becomes: Is the ecological status of a river determined by the character (morphological, chemical, etc.) that is in the poorest condition, or it is determined as an integration of the condition of all characters? In the former case, no significant impairment is allowed for any character. In the latter case, a departure of some characters from nearly undisturbed conditions is allowed, provided that this is not causing significant alterations to the overall ecological status. This question parallels the question of determination of the reference value of the biological quality elements according to the *sensu lato* and *sensu stricto* approach (Section 3.3.4).

This paper adopts the integrated interpretation, that is, a character is allowed to deviate from the "nearly undisturbed conditions", provided that the "high ecological status" is maintained, and this must be proved with independent, alternative methods. This interpretation relies on the following reasoning. Each metric describes a structural or functional aspect of the community and identifies one or more pressures. If integration of the values of metrics to a single value describing the ecological status is allowed (Section 3.3.4), then integration of the (theoretical) values of pressures to a single value must also be allowed.

It is reminded that opinions expressed within the REFCOND working groups (see Section 3.1) support the idea that some flexibility must be allowed for certain types of impacts (e.g. land-use and vegetation in the flood-plain/riparian zone), and also that certain morphological changes may be acceptable, provided that the ecosystem has become adapted and attained an equivalence to a natural ecosystem ('naturalised' ecosystems). However, changes due to current uses are acceptable only if a negligible effect on ecological structure and functioning can be demonstrated.

The integrated interpretation is supported by an additional argument. In evaluating the relative importance of the biological and physicochemical quality elements in the establishment of the ecological status, the WP4 REFCOND favoured the Biological focus. In analogy, it can be claimed that moderate (not minor) physicochemical changes that can be demonstrated to induce insignificant ecological change are acceptable within reference conditions (see section 3.1). Admittedly, this argument introduces some cyclicity in the reference identification process. How can the changes of an abiotic element be demonstrated to have "no significant" effects on the biological communities before the establishment of a reference community? Future work should focus on the identification and evaluation of "alternative methods".

As an outcome of this analysis it is proposed that:

- When deciding impairment criteria for a system or an area, appropriate consideration should be given to pressures that exert overwhelming influence in the particular system or area.
- The criteria for different areas or river types may be different, mainly with respect to the emphasis and the acceptable levels of deviation, depending on the prevailing pressures; the criteria for sites, rivers or higher levels may also be different, depending on the spatial scale of pressures.
- When selecting reference sites or zones (or entire rivers), the integrated effect of all pressures should be evaluated and used for the assessment of status.

It is not meant to imply that the evaluation of a reference site must not be based on the whole range of pressures potentially affecting the biological community. The essential proposition is that moderate deviations of certain impairment criterion from the undisturbed condition may be allowed, provided that this impairment is not causing a significant change to the local community. In other words, the benchmark for certain criteria may be flexible within the perception of the high overall ecological status, in the same sense that the "temporal" benchmark may also be flexible (see Section 3.1). Imagine that major ecological impacts in a certain area are generated by a specific impairment (e.g. acidification in Sweden, water abstraction in the Mediterranean), and that a river is unaffected by this impairment type. It may be improper to exclude this river from the reference network simply because another criterion for impairment, of little importance for this area, is not satisfied.

Strict coherence to a fixed list of criteria may underestimate the importance of principal factors and processes determining the high ecological status in an area, and does not necessarily improve ecological assessments. If all criteria are very demanding and the selection process is too severe, it may result in no suitable reference sites and an switch unnecessary switch from spatial methodologies to alternative (and perhaps less accurate) ones may be required. Nevertheless, the idea is testable once adequate data are available, for example by comparing the biological communities under the absence and the moderate presence of a pressure type.

The practical implication of this reasoning is that once a global list of impairment criteria has been generated to serve as guidance, the selection of the specific criteria to be applied in a given area, river zone or even river basin can be made in a somewhat liberal manner, based on knowledge of factors that exert primary control over the ecological processes operating there.

Evaluation of certain criteria requires detailed information for the site/river and locally important anthropogenic factors that may not be immediately available. Also, it may be difficult to discriminate between anthropogenic factors directly affecting the site and those located elsewhere in the watershed. These might be initial problems, but the establishment of reference sites is a continuous process that is primarily based on monitoring programmes and periodic reviews of the impact of human activity. A site may be characterised and selected as reference but later rejected, but other sites may be nominated and included in the network on the basis of updated information. Therefore, any site selection must be considered as interim and subject to re-evaluation (see below).

It was suggested within the FAME consortium to adopt water quality standards as being more practical in establishment of chemical conditions in a site. Consideration of physicochemical attributes must indeed be an important part of the process leading to the establishment of reference sites, and for some attributes it is demanded by the WFD to take into account the background levels. However, it is also important to determine aquatic life impairment based on site characteristics and the prevailing pressures, e.g. point sources, physicochemical alterations, etc. The reference condition concept is based on the premise that the best way of for sustaining ecosystems is for management to mimic as closely as possible the natural processes, which implies that the reference must represent the structure and function of communities in apparently undisturbed sites.

Nevertheless, the selection of reference sites on the basis of both inspection of the field characteristics and analysis of water quality parameters could well be two complementary processes, as happens in some countries. In France, the selection of reference sites was done on the basis of both water quality map inspection and field reconnaissance. Factors considered in the field inspection included the amount of stream channel modification, channel morphology, substrate character and condition, and the general representativness of the site within the region. However, the sites selected should also belong to the water quality classes “Excellent” or “Good” of the five qualitative classes defined by the Water Quality Index (WQI) developed by the French Water Agencies.

Overall, the water quality analysis parameters may be a process following field inspections, aiming to verify the suitability of the candidate reference sites, or may be a preliminary process, based on available national physicochemical data.

4.5. Proposed reference criteria for FAME

On the basis of the general principles developed in previous sections, this section develops a proposal for reference site selection criteria and methodologies. The overall objective is to develop rule-based processes for deciding, during the screening process of national data, if a site qualifies as a reference site. However, the approach and criteria developed may also be used as guidance in future assessment projects.

The proposed scheme is guided by the following principles:

1. There are separate categories of site-specific, river-segment-specific and river-basin-specific criteria. The last two categories identify impacts with respect to land use, longitudinal connectivity and large-scale physical alterations.

   During discussion within in the group, the importance of longitudinal connectivity was stressed as one of the essential features of river integrity. However, it was recognised that setting a restrictive criterion for connectivity at the basin scale would virtually exclude most European rivers. It was agreed that large scale effects of impounded have to be considered, but only when long-distance migratory species (anadromous and catadromous) are ecologically and quantitatively important in a river. Historical data should be consulted to determine the distribution of such species and expert judgement should be used to identify if barriers to their migration do have impacts on the basin scale or at a smaller section/site scale, and/or whether these impacts cause major deviation from the original community structure and function.

2. The AQEM list was modified to include criteria that are dictated by the WFD (Annex V) or are important from the fish perspective. At the same time, some of the AQEM criteria that are of little relevance to fish-based methods and/or refer to political palatability were excluded.

   Modifications were not extensive, in order to ensure compatibility with AQEM (and its follow-up STAR). However, more emphasis was given to criteria that identify the types of stress fishes are best indicators of.

3. Within each spatial category, two lists of criteria may be generated, more or less in the logic of the obligatory and optional factors of river typology B: a list of obligatory criteria, that refer to common and important stressors, to be used across geographic boundaries and river types; and a non-exclusive list of optional criteria that refer to stressors to be considered whenever they are locally important.

   The logic of this separation is to simplify desk assessments and field decisions by excluding criteria of little importance and regional relevance. The obligatory list contains criteria identifying elements of Annex V 1.2.1 of the WFD. The optional list will be constructed by national experts and may be consulted by country or river management teams to select additional criteria that are applicable for certain cases.

At a later stage the lists generated could be expanded to contain criteria that are important for invertebrates and/or other taxonomic groups. This would facilitate the clustering of the FAME and STAR projects (and/or permit designation of common reference sites for fish and invertebrates).

Ideally, the fish-based and invertebrate-based criteria should be harmonised in the same list. Thus, a rule-based reference site selection system will be generated for field inspections, which takes the fish and invertebrate site selection systems as special cases. An appropriate grouping of the criteria into logical categories facilitates both database standardisation, which is essential for relating site characteristics with impacts, and further analysis and modelling of the relationships between biotic parameters and stressors.

The final lists of criteria, with links to the site-specific criteria of the elements referred to in the Annex V of the WFD, is as follows (only the obligatory site-specific criteria are described):

RIVER BASIN CRITERIA

No extensive urbanisation, agriculture and silviculture in the basin.

< 10% cultivated land (intensive, crops)
< 1% urban land
< 5% of available forested area is used for commercial forestry

No disruption of connectivity with widespread effects on migratory aquatic organisms typical for the river or affecting sediment transport over large portion of the river.

No migration barriers within the river basin (down to confluence in another river or the sea) or functioning bypass/similar device

RIVER SEGMENT-SPECIFIC CRITERIA

Only moderate anthropogenic influence from cultivation and other land use practices. As a guidance, the following values define "almost totally undisturbed" conditions:

< 10% cultivated land (intensive, crops)
< 1% urban land
< 5% of available forested area is used for commercial forestry

If it is typical for the segment, riparian vegetation and floodplains must still exist, ensuring lateral connectivity.

> 90% of shore length (both sides) with vegetation in natural state (normally 30-50 m on each shore)
> 90% of the segment with floodplains in natural state, all floodplain water body types present (only for floodplain rivers)

No migration barriers in the segment. Only minor influences on transportation and composition of sediments/substrates and on the biota caused by upstream or downstream weirs, reservoirs and other barriers.

No migration barriers or functioning bypass/similar device preventing free migration of potamodromous species to segment
No significant deviation from natural sediment load

Only minor disturbance on the bank character, channel width and depth, flow velocities, and substrate conditions by anthropogenic activities.

SITE-SPECIFIC CRITERIA

General criterion: ensure representativeness

The sites to be chosen as reference must represent the range of biological, physical, and chemical conditions of the ecoregion and the specific river-type. Typical habitats and typical natural substrates should be adequately included.

Impairment criteria

WFD (Annex V 1.2.1)	FAME criteria
Hydromorphological quality elements	
Hydrological regime	
The quantity and dynamics of flow, and the resultant connection to groundwater, reflect totally, or nearly totally, undisturbed conditions.	No significant alteration (< 10%) of the natural flow regime (current velocity, water quantity, periodic patterns) due to: water diversion surface water abstraction groundwater abstraction pulse releases
River continuity	
The continuity of the river is not disturbed by anthropogenic activities and allows undisturbed migration of aquatic organisms and sediment transport.	No migration barriers upstream or downstream affecting the occurrence of migrating species in the site. No significant disruption of longitudinal connectivity effecting the bedload transport and/or the biota of the sampling site e.g. due to: upstream impoundment reservoirs weirs
Morphological conditions	
Channel patterns, width and depth variations, flow velocities, substrate conditions, and both the structure and condition of the riparian zones correspond totally or nearly totally to undisturbed conditions.	The floodplain in the reference site is not too extensively cultivated Floodplain-river connectivity is maintained (Only moderate alteration to floodplain natural climax vegetation; anabranches, backwaters, off-channels, wetlands, if typical for the area, are still present) Stream bottoms are not be cleared or fixed Preferably no bridges or other constructions with effects on morphological conditions in proximity to the site. Minimal alteration to natural erosion and deposition processes No significant abstraction of gravel or other alterations of the natural substrate in the broader area of the site No significant anthropogenic effects on the bank character
Physico-chemical quality elements	
General conditions	
The values of the physico-chemical elements correspond totally or nearly totally to undisturbed conditions.Nutrient concentrations remain within the range normally associated with undisturbed conditions.Levels of salinity, pH, oxygen balance, acid neutralising capacity and	No significant impairments due to the physical and chemical conditions. The following characters must be close to natural levels for the area: temperature salinity oxygen balance pH acid neutralising capacity

temperature do not show signs of anthropogenic disturbance and remain within the range normally associated with undisturbed conditions.	No significant impairments due to: point sources of eutrophication nutrients diffuse inputs
Specific synthetic pollutants	
Concentrations close to zero and at least below the limits of detection of the most advanced analytical techniques in general use.	Concentrations of specific synthetic pollutants close to zero
Specific non-synthetic pollutants	
Concentrations remain within the range normally associated with undisturbed conditions (background levels).	Concentrations of specific non-synthetic pollutants near to background levels
Anthropogenically effected biotic changes	
	Only moderate impairment due to: invasive species, fish introduction, fish stocking or fish farming fishing activities introduced disease and parasites weed cutting, changes in vegetation

4.6. Conclusions

It becomes clear that the establishment of a spatial reference site network requires first the monitored area to have been appropriately stratified using geographic criteria, and secondly a standardised categorisation of river types. Desirably, the assessment of impacts must have been completed, following the development of a hierarchical selection and long-term evaluation scheme that is guided by the following principles:

Ensure representativeness. Minimum requirement is the site to be representative of the natural biological community for the river and/or river segment. Sites with unusual environmental characteristics should not be selected.

Set criteria for impairment. After accounting for representativeness, reference sites are best predicted by combinations of regionally relevant variables that describe undisturbed conditions and include information from several spatial scales (channel, riparian and basin).

- Different lists of criteria for reference rivers, segments (zones) and sites are formulated.
- Different criteria may be used in different areas.

Standardise field procedures. To facilitate the processes of reference site selection and future re-evaluation, guidelines for fieldwork, including relevant documentation and protocols, need to be developed.

- What kind of information is necessary to decide reference sites?

- What kind of data must be recorded from reference sites?

Select reference rivers, segments and sites. The process begins with the identification of reference rivers and river segments on the basis of information about large- and medium-scale anthropogenic impacts. The process proceeds with the selection of reference sites on the basis of information about anthropogenic factors directly affecting the site and those located elsewhere.

- Select reference rivers
- Select reference river segments using a minimum set of criteria
- Select reference sites using a more detailed list of criteria

Verification. Since the selection of reference sites is made on the basis of implicitly or explicitly defined types of impairment, sufficient safeguards must exist to ensure exclusion of sub-optimal sites due to unknown (at the time of site selection) impacts. For an independent confirmation, physicochemical parameters have to be analysed to verify high ecological status, according to WFD.

Long-term evaluation. The reference sites must be re-examined periodically and the reference standards updated. Periodic examination of the reference standards may also reveal trends of climatic or other natural origin.

It must be realised though, that the above selection and evaluation procedure may not be applicable to the FAME project, which relies on existing data sets and will utilise an a posteriori procedure for selection of reference sites. However, steps of the proposed procedure may be used as guidance for reference site identification and/or verification of the existing reference sites. This a posteriori verification must be applied not only to the reference site selection but also to the reference site representativeness (number and location of sites allowing the expression of the regional diversity within river types).

5. LITERATURE

Bund van de W. (2001). Summary and conclusions from the group discussions at the REFCOND workshop in Ispra, 5-6 December 2001, 10 pp.

Johnson R. (2001a). Defining reference conditions and setting class boundaries in ecological monitoring and assessment. Background Document, REFCOND project, 13 pp.

Johnson R. (2001b). Indicator metrics and detection of impact. In: monitoring and assessment of ecological status of aquatic environments (ed. K. Karttunen), pp. 41-44. ordic Council of Ministers, Temaord 2001: 563. 8 pp.

Owen R. (2002). Definition and establishment of Reference Conditions, Background Document, REFCOND project, 20 p.

Wallin M., Wiederholm T., Johnson R. & Owen R. (2002a). Minutes from REFCOND Guidance Drafting Group meeting 4 March 2002 at SLU.

Wallin M., Wiederholm T., Johnson R. & Johansson C. (2002b). Notes from meeting with WG 2.3 (REFCOND) lead group (10-4-2002).

Wilde de A.J. (2002). Setting Class boundaries for the classification of rivers and lakes in Europe, Analysis of questionnaires, Final report, Royal Haskoning DG RWS - RIZA, Netherlands, REFCOND project (WP4), 22 p.

Schmutz S., Kaufmann M., Vogel B., Jungwirth M & Muhar S. (2000). A multi-level concept for fish-based, river-type-specific assessment of ecological integrity. *Hydrobiologia* 422/423, 279-289.

APPENDIX I

THE WFD CONCEPT OF REFERENCE CONDITIONS

General

The main objective of the WFD is to achieve good status for all European surface water by the year 2015. The classification of the ecological status will be accomplished by means of comparison of the observed values with reference values. Monitoring will be undertaken based on three classes of environmental components, termed "quality elements": Biological, Hydromorphological and Physicochemical.

The establishment of a sound water body typology is a decisive step towards establishing biological reference conditions, because they have to be established for each body type (two typological schemes are proposed by the WFD, type A and type B). In the context of the WFD, reference conditions for a quality element are equated with the set of conditions that are to be expected for each body type under no or very minor minimal anthropogenic disturbances (= high status). The restoration target, however, is the "good status". In the case of heavily modified or artificial surface water bodies, the reference is the maximum ecological potential and the target is the good ecological potential.

Timetable

The monitoring programmes must become operational six years after the entry into force of the directive that is in the year 2006. By the year 2004 the following works must have been completed by the member states:

A characterisation of water body categories (rivers, lakes, transitional waters, coastal waters, artificial surface water bodies or heavily modified surface water bodies). For each category, water body types will be defined.
The final establishment of reference conditions for each water type with regard to hydromorphological, physicochemical and biological elements.
A review of the impact of human activity on the status of surface waters aiming to identify pressures and to assess the type and magnitude of human impacts.

The monitoring framework

Monitoring will be undertaken for all water body types identified on the basis of the previously mentioned impact assessments as being at risk. Three types of monitoring programmes are specified:

- **Surveillance** monitoring, which aims to validate the desk impact assessments and to provide information for the efficient design of future operational monitoring programmes, especially with regard to the need to identify suitable biological quality elements to be used as monitoring tools. Also, to provide information on long-term changes in natural conditions and anthropogenic activity.
- **Operational** monitoring, which is designed to measure the impact of specific, identified pressures upon a water body with the two-fold objective of establishing the status of these bodies and the evaluation of changes in status.

- **Investigative monitoring**, which aims primarily to detect the impact of unidentified pressures. This type of monitoring will be undertaken when the surveillance monitoring indicates that the quality objectives are not likely to be achieved.

Ecological status classifications will be carried out in the context of the operational monitoring programmes and can be based on one or more biological quality elements. For rivers, four specific biological quality elements (taxonomic groups) are specified: macrobenthic fauna, fish fauna, phytoplankton and macrophytes.

Each group is described by a number of biological attributes, which are based on measurable characteristics and focus on structural and functional aspects. Whenever fish will be used as a monitoring tool, the WFD defines the following five attributes as indicators of ecological condition: species composition, abundance, sensitive species, age structure and reproduction.

A basic requirement: inclusion of natural variability in reference conditions

Given the high natural variability present in natural ecosystems, the WFD stipulates that whenever spatial methods of deriving reference conditions are used, there must be "a sufficient number of sites of high status to provide a sufficient level of confidence about the values of the reference conditions" (Annex II, 1.3.iv).

Methodologies

From the methodological point of view, the WFD provides four potential methods for deriving reference conditions: spatially-based, modelling, combination of the spatial and modelling approaches, and expert judgement.

Reasons for exclusion of a biological quality element

Despite the general obligation of the member states to establish operational monitoring programmes, the WFD permits flexibility in selecting monitoring parameters. Actually, it provides two possibilities for excluding a particular biological quality element in assessments of ecological quality of a surface water type or basin.

- When this element is not sensitive enough to the identified pressures. Effectively, only those quality elements that provide the most useful classifications and assessments of the identified pressures in a surface water type or basin will be included in the monitoring programme. This approach avoids the difficult theoretical question of how to characterise the status of a water body when the assessments based on different taxonomic groups (e.g. fish and invertebrates) differ.
- When it is not feasible to establish reliable type-specific reference conditions for a particular biological quality element due to high degrees of natural variability, but not just as a result of seasonal variations. In such circumstances the member states must state the reasons for the exclusion of this quality element from the river basin management plan (Annex II. 1.3.(vi)).

Definitions of reference conditions

For each waterbody type, the basic requirement that the reference conditions must satisfy is that the hydromorphological, the physicochemical and the biological parameters reflect undisturbed, or nearly undisturbed conditions. The following description of the reference conditions is given in Annex II 1.3 (i):

Establishment of type-specific reference conditions for surface water body types

> *For each surface water body type characterised in accordance with section 1.1, type-specific hydromorphological and physicochemical conditions shall be established representing the values of the hydromorphological and physicochemical quality elements specified in point 1.1 in Annex V for that surface water body type at high ecological status as defined in the relevant table in point 1.2 in Annex V. Type-specific biological reference conditions shall be established, representing the values of the biological quality elements specified in point 1.1 in Annex V for that surface water body type at high ecological status as defined in the relevant table in section 1.2 in Annex V.*

Obviously, this definition of reference conditions relies on the definition of the "high ecological status". General definitions of the ecological quality classes, including the definition of high ecological status, are provided for all quality elements and for all surface water categories in Annex V 1.2 (Table 1.2).

General definition of high ecological status for rivers, lakes, transitional waters and coastal waters

- *There are no, or only very minor, anthropogenic alterations to the values of the physico-chemical and hydromorphological quality elements for the surface water body type from those normally associated with that type under undisturbed conditions.*
- *The values of the biological quality elements for the surface water body reflect those normally associated with that type under undisturbed conditions, and show no, or only very minor, evidence of distortion.*

More specific definitions of the ecological quality classes for each quality element and each surface water category are given in tables 1.2.1 to 1.2.4. The definition of high ecological status for the fish fauna for the water category "rivers" is provided in Annex V 1.2.1.

Definition of high ecological status for the fish fauna in rivers

- *Species composition and abundance correspond totally or nearly totally to undisturbed conditions.*
- *All the type-specific disturbance-sensitive species are present.*
- *The age structures of the fish communities show little sign of anthropogenic disturbance and are not indicative of a failure in the reproduction or development of any particular species.*

In addition to satisfying these criteria for the fish fauna, the "high ecological status" reference conditions must additionally satisfy certain criteria for the hydromorphological and physicochemical quality elements, also provided in Annex V 1.2.1.

Definition of high ecological status for hydromorphological quality elements in rivers

- ***Hydrological regime****. The quantity and dynamics of flow, and the resultant connection to groundwater, reflect totally, or nearly totally, undisturbed conditions.*
- ***River continuity****. The continuity of the river is not disturbed by anthropogenic activities and allows undisturbed migration of aquatic organisms and sediment transport.*
- ***Morphological conditions****. Channel patterns, width and depth variations, flow velocities, substrate conditions and both the structure and condition of the riparian zones correspond totally or nearly totally to undisturbed conditions.*

Definition of high ecological status for physicochemical quality elements in rivers

- ***General conditions***
  - *The values of the physico-chemical elements correspond totally or nearly totally to undisturbed conditions.*
  - *Nutrient concentrations remain within the range normally associated with undisturbed conditions.*
  - *Levels of salinity, pH, oxygen balance, acid neutralising capacity and temperature do not show signs of anthropogenic disturbance and remain within the range normally associated with undisturbed conditions.*
- ***Specific synthetic pollutants***
  - *Concentrations close to zero and at least below the limits of detection of the most advanced analytical techniques in general use.*
- ***Specific non-synthetic pollutants***
  - *Concentrations remain within the range normally associated with undisturbed conditions (background levels).*

APPENDIX II

The situation in Europe - Overview of processes for defining reference conditions in the countries participating in FAME

The FAME database, that will provide data for reference description, includes data obtained during national monitoring programmes and biological surveys. Some of the current systems for the collection and compilation of fish and environmental data have been shaped by different areas of interest and environmental or other objectives, and the output results differ as far as quality and coverage of the parameters of interest are concerned. A review of the methods for obtaining data and the processes used in different countries for establishing reference conditions is given below. The review covers the state of river typology characterisation, which is an important step towards reference establishment, but does not include modelling issues and procedures for setting reference conditions to metrics.

AUSTRIA. The development of type-specific reference conditions in Austria is still under construction. The methodological concept follows an integrated approach using the following types of information: reference sites, historical fish data, historical maps, reference models and expert judgement. Most of the data sources are integrated into a countrywide habitat and fish database (HaFiDat) comprising 2760 fish samples of 281 rivers. Austria follows a biocoenotic river type classification based on System B and extended by fish regions and their subtypes. However, a sound river typology has not yet been established.

Results of the analysis conducted so far show the importance of developing a countrywide fish-based river typology as the basis for establishing reference conditions. Also, that only a combination of different methods and integration of all sources of information yields in a sufficient accuracy of reference characterisation. Best available sites fail to reflect natural conditions of larger and lowland rivers.

BELGIUM FLANDERS. A routine monitoring programme, involving system B (river width, slope, and distance to source and altitude) for classifying rivers has been established. Because accurate historical data on fish populations are incomplete and undisturbed sites are scarce, the “best available” approach has been selected for defining reference conditions.

A number of river sections suffering least disturbance belonging to the bream and barbel zones have been selected as reference rivers. The criteria for identifying reference are water quality (basically oxygen concentration) and habitat variables such as presence of pools and riffle, the degree of meandering and the natural state of banks. In 1957 the water quality was relatively good so this year’s data were used as reference.

In upstream waters, belonging to the grayling and trout zones, no specific references were taken. The medium scores of the metrics used in the IBI (from trend line through all values = average condition) were taken to define threshold (reference) values for the selected metrics. For the IBI of the Schelde estuary, historical data (1945) were used to establish reference for this river. From data recordings a serious impact in water quality was observed in 1930. Data on fish assemblages close to this period were taken as reference. Other estuaries in the same ecoregion (e.g. Ooster Schelde and Eems estuary) could not be used as reference, because the conditions were too different (turbulence, food resources, salinity for Ooster Schelde and pollution in the Eems).

BELGIUM WALLONIA. With the exception of a recent research project (IBIP) there is no systematic monitoring programme over the fish population at the moment in Wallonia (such a programme is under consideration). Although a series of sample data exist (going back to 1953), it is doubtful if these data can be used as a basis for monitoring purposes in the framework of WFD.

River typology has not yet been decided. The methodology for establishing reference networks involves spatially based river-specific methods using the "best available" approach (selection of least degraded sites on the basis of a global indicator of ecological quality).

For setting reference conditions to metrics, a qualitative approach was used. Due to that only few undisturbed reaches can be sampled in Belgium, the highest metric values (95 %) for a given catchment area class were used as estimates of the reference condition, which were usually obtained at the least disturbed sites.

FRANCE. Based on the results of extensive sampling in French rivers covering a period of 10 years, a reference network consisting of 738 reference sites has been constructed.

The selection of reference sites was done by regional experts (fish biologists) on the basis of (a) water quality criteria already available from a national survey programme and/or short-term studies (the highest two out of five water quality classes using 12, predominantly chemical, variables, were acceptable), (b) field inspections, using impairment criteria (amount of stream channel modification, channel morphology, substrate character and condition), and (c) the general representativeness of the site within the region. Reference sites should suffer from minimal habitat perturbations (as measured by the factors used during the field inspections).

The initial data set containing 738 reference sites (RS738) was further randomly divided in two subsets: one set of 650 sites (RS650) that was used to calibrate the models, and one set of 88 sites (RS88) that was used to validate the models. The size of this last data set (RS88) was defined to match the size of the data set of disturbed sites (DS88). Disturbed sites were selected to cover a range of well identified human disturbances including discharge of sewage effluents, urban runoff, channel and bank modifications, presence of weirs.

Reference sites were not totally undisturbed but were those that have suffered the least impact within a particular biogeographical region during the last 20 years. Most of the upstream sites selected were virtually undisturbed but it was impossible to identify completely undisturbed sites for large rivers (at large catchment-level scales).

GERMANY. Every Federal Land has its own river monitoring programme and uses its own database. A standardised German river monitoring system has not been established. However, proposals have been made to establish river-type specific sampling standards and develop a national monitoring programme in accordance to the requirements of the WFD.

Regarding river typology, 20 river types have already been designated, based on system B, using mainly geomorphological criteria. Reference conditions have not yet been elaborated, but will be treated within a currently running national joint project using the outlined river-typology as a basis. It is anticipated that the characterisation of reference conditions will be based both on historical and actual fish data, where such are available. The method has not yet

been fixed but it will likely be a combination of descriptive and predictive methods as well as expert judgements (the "best available" approach will not be adopted). An anticipated difficulty is that unimpaired sites are not likely to be found in many rivers, especially the large ones.

GREECE. There are no operational monitoring programmes in Greece. Some ichthyological data exist, which are the product of small-scale and usually short-term investigations that were not designed for ecological monitoring and assessments. Therefore, neither are the techniques properly standardised nor are the recorded parameters in line with the requirements of the WFD. A series of ichthyological data from investigations in western and southern Greece, undertaken mostly in the framework of fish conservation projects, is available in an electronic database. An analysis of anthropogenic impacts on the water bodies of the same area has also been conducted.

River typologies have not yet been defined. Initial attempts have been in the framework of the AQEM project to develop a typology system, with the whole country been separated into three core river types. However, the characterisation is incomplete and can be regarded as a preliminary zonation within ecoregions. Undisturbed or nearly undisturbed sites can still be found in mountainous areas but such sites are scarce in lowland areas. Reference conditions have not been defined and a reference site network has not been established. However, the existing database contains sites that can be regarded as undisturbed and used as reference. There is also a small reference site network designated during the AQEM project, but from the perspective of the FAME requirements, only a few of the chosen sites coincide with sites from which ichthyological data are available.

LITHUANIA. A monitoring network exists in Lithuania that extensively covers the salmon rivers. Other rivers are insufficiently covered (samples are taken sporadically) or are not covered at all.

A national river typology has been established that includes some descriptors from both – A and B classifications of the WFD. Reference conditions have been estimated according to scientific data collected over a period of about 20 years. The estimation method is descriptive, with the reference conditions for each river type representing the average maximum ratings of all metrics used in ecological assessments (species composition, indicator species, trophic composition, etc.). Spatially based methods, requiring the establishment of reference sites, have not yet been adopted.

POLAND. There is no systematic monitoring programme for fish in Polish waters but such a system is under consideration. Up to now the Polish Anglers Association is the main institution which collects fish fauna data from the whole of Poland. However, not all data collected so far can be easy used as a basis for monitoring according to purposes of WFD framework. Before 1965 data on fish composition and distribution in rivers were collected sporadically. Since 1965 co-operation between several Polish scientific institutions and the Polish Anglers Society has helped develop an inventory of the fish fauna in the main Polish rivers. Electric fishing is the main sampling method, both in wadable rivers and in non-wadable rivers (from a boat).

Reference conditions have not yet been established. There is no common river typology, although both types A and B (mostly B) have been used in typology assessments. A number

of physical and chemical factors are used to determine the characteristics of the river, or part of the river, and then the biological population structure and composition (altitude, latitude, longitude, geology, size, distance from river source, energy of flow, function of flow and slope, mean water width, mean water depth, mean water slope, form and shape of main river bed, river discharge category, valley shape, transport of solids, acid neutralising capacity, mean substratum composition, river width, macrophytes, organic matter (coarse and fine), riparian vegetation, bank modifications, presence of floodplain / land use of floodplain, chloride, air temperature range, mean air temperature and precipitation).

Two networks exist for monitoring surface waters on a nation-wide and a regional level. This monitoring system provides extensive data useful for WFD monitoring. Since July 2000 a Polish "Water Law" was enacted to implement the WFD.

NETHERLANDS. Although good historical data on the fish fauna are lacking, except for commercially exploited species, present monitoring programmes (started in 1992, adjusted in 1996) do include many of the parameters (species composition, abundance, length/age structure etc.) specified in the WFD. A river type classification system, which is in line with the requirements of the Directive, has not been agreed. Attempts to define (semi-) pristine conditions in a semi-quantitative manner have been made, but there is still a lack of good reference conditions. References from other river systems (e.g. the lower Danube) are used on an expert-judgement basis, though they have not (yet) led to the development of a yardstick for the status of rivers in the Netherlands.

PORTUGAL. A monitoring system has not yet been established. Progress in developing river typology has been made, applying system A, and steps towards establishing spatially based reference conditions are taken concurrently using physical-chemical information and expert judgement. This is done in the framework of the IBI already developed for Portuguese rivers. Reference sites were selected on the basis of:

a) the available information from the national water quality survey programme;
b) field reconnaissance of water quality parameters and habitat characteristics;
c) the biological quality of the water, expressed as the index BMWP', an Iberian adaptation of the original UK index using macroinvertebrates.

Reference sites were considered to be those representing the top two classes 'Excellent' and 'Good' of all the indexes, cross-checked by other field observations. It is anticipated that a pragmatic approach using water quality data and field reconnaissance of habitat characteristics, riparian features and instream biota, will be the best available methodology for selecting reference sites. Historical and land use information can be used when and where available.

Relatively undisturbed reaches can still be found in some small permanent rivers from northern and western Iberian areas; however, it will be nearly impossible to identify undisturbed sites in medium to large rivers. Therefore, the majority of reference sites are not totally undisturbed but represent the least impacted sites within a particular river type or region (the best available condition approach). This approach is likely result to the inclusion of some sub-optimal sites, which will be discarded later on the basis of independent tests and data analyses.

SWEDEN. Fish monitoring in Sweden has a long history (25 years) and forms part of a more general environmental quality assessment scheme that includes other biological quality elements as well. Activities are under the responsibility and co-ordination of the Swedish Environmental Protection Agency (Swedish EPA). The main objective is to detect large-scale changes of the environment, connected for example with climatic change or changed deposition of air-borne substances, via developing time-series data.

Fish surveys and monitoring programmes were stimulated by the alarming decline of salmonid stocks due to acidification, and have led to the development of an acidification index. A specific national monitoring programme has been designed to follow the effects of liming, a countermeasure for acidification. More generally, three ecological quality assessment methods are used in Sweden. All methods potentially incorporate fish-based indicators, but only one method (EQC), primarily designed to distinguish acidification, routinely utilises fish as part of the assessment procedure (the FIX index).

To determine ecological status according to the WFD specifications (assess the effect of all types of human impacts using the conditions in relatively undisturbed sites as a benchmark) was not the initial concern, but is now becoming of primary importance. The environmental objectives of the currently used system reflect the perception of the reference status. In fact, the ecological status is evaluated in relation to specific disturbances and is measured as deviations of the values of the biological and/or other quality elements in a disturbed site from the values in site that are not affected by this specific disturbance. As a consequence, there are reference conditions (and reference rivers/sites respectively) for acidification, liming and probably other types of disturbances as well. However, a reference site, for liming for example, may be affected by other types of disturbances.

It was decided to broaden the existing acidification index to an index of ecological quality according to the WFD using the reference condition approach. Two main problems are currently encountered in attempts in this direction: a national river typology has not yet been fully developed, and localities unaffected by humans to serve as reference are rare, especially in southern Sweden and/or lowland areas. Other important drawbacks are that the current system does not depend on any classification of the physical or chemical state, and has not been shaped and calibrated to evaluate some types of human impacts, for which there is lack of appropriate indicators and hydromorphological quality classifications to support biological assessments. Nevertheless, the vast amount of data collected from the monitoring operations so far offer good opportunities for developing models to establish reference conditions (though the time-series data for most of the reference watercourses are not very long).

UK. Whilst the "UK" has no national fish monitoring programme, three regional groups with different member countries each undertake monitoring: England and Wales (Environment Agency), Scotland (Scottish Environment Protection Agency) and Northern Ireland. Monitoring programmes in England and Wales have a wide coverage but analysis of the data is simplistic and does not use an integrated IBI approach, merely reporting species composition, abundance and age structure.

In the frame of a research project at the Hull International Fisheries Institute, an attempt has been made to establish an IBI model and associated reference condition using data from various monitoring programmes, available scientific literature and fish stock assessment data from other R&D programmes. The model was based on survey data from relatively few rivers, however, data for these study rivers were from extensive data sets.

The problems encountered during establishment of the reference conditions were:

- the poor freshwater fish fauna of Britain,
- the restricted distribution of many species,
- the intensity and widespread nature of anthropogenic activities,
- the number of local fish extinctions, introductions of exotic species and translocation of native species.

These problems, coupled with the rapidly changing fish community structure and ecological status of many rivers in recent years as a result of rehabilitation efforts, create enormous difficulties in attempts to identify reference sites satisfying the criteria for hydromorphological and physico-chemical conditions set by the WFD. Even if relatively unimpaired sites could be found, the biotic characteristics of the resident fish fauna are unlikely to be representative of a stable climax community.

Because of all these problems, the research project employed a combination of methods to establish reference conditions for English lowland rivers, using a guild approach. This approach required information on guild variables (origin, habitat preference, diversity, density, trophic ecology, reproductive guild, and tolerance to degradation of fishes), which was obtained using data from collection records. Available monitoring data was corroborated or refined using historical data, paleoecological data regional ichthyological texts, published reports, scientific papers and expert judgement. The overall objective was to assess the maximum expected number of species in each of the guild categories and the maximum expected values for some metrics.

The hypothesis adopted was that, “least impacted” sites contain the best attainable conditions possible for a watershed within a region. This hypothesis is questionable in various respects; for example, the survey and literature data used to establish the reference status for each of the guild concept categories may actually represent fish communities under perturbed conditions. Therefore, this approach to determining a reference condition has yet to be validated by field trials or assessed with new monitoring data sets and so must be treated with some caution. Ultimately the design of a reference condition and IBI scoring system will be based on the desired concept of excellent quality or ecological integrity and the purpose of the assessment.

Concluding remarks

- Monitoring programmes operating on a routine basis and river typologies have been established in a relatively few number of countries.
- Reference conditions have been described in few only countries. Data needed for reference characterisation are not always available in a readily usable form. This is especially true when the data have been obtained in the context of investigations not compatible with the scopes of the WFD, and therefore unsuitable survey designs and sampling methodologies were employed.
- Rarely the reference description is compatible with the WFD. In many cases the best available approach has been adopted, or has been proposed as more applicable, or even as the only possibility.
- For correctly defining reference conditions, the level of understanding of the processes operating within the framework of the watershed must increase substantially.

APPENDIX III

REMARKS ON THE AQEM CRITERIA

During the January 2002 FAME meeting in Maastricht the consortium discussed the kind of information that is necessary to decide and describe reference sites. There was a broad exchange of ideas on habitat quality assessment variables but no final decision was reached regarding the criteria required to identify reference sites .The participants agreed to accept provisionally the AQEM criteria and refine them further from a fish perspective, taking into account the existing national monitoring schemes and country specific requirements.

Through intranet communication, the group members proposed modifications and improvements that are necessary for the designation of reference site conditions in the framework of the FAME project. This section of the report presents an analysis of remarks on specific criteria and suggestions for modification provided by the partners. The results of the analysis are treated further in the main report, together with views and ideas of general interest, to develop a proposal for minimum reference site requirements and selection methodologies.

THE AQEM LIST OF CRITERIA[1]

Basic statements

- The reference condition must be politically palatable and reasonable.
- The reference condition for a general type of water body should represent large numbers of defined populations of water bodies.
- A reference site, or process for determining it, must represent important aspects of 'natural' conditions.
- The reference conditions must reflect minimal anthropogenic disturbance.
- Whenever possible, states should share reference condition information when they share interstate or boundary water bodies.

Land use practices in the catchment area

- In most countries there is anthropogenic influence within the catchment area. Therefore, the degree of urbanisation, agriculture and silviculture should be as low as possible for the reference site. No absolute minimum or maximum values have been set for the reference condition (e.g. % arable land use, % native forest); instead the least influenced site with the most natural vegetation is to be chosen.

River channel and habitats

- The reference site floodplain must not be cultivated. If possible, it should be covered with natural climax vegetation or unmanaged forest, respectively.
- Coarse woody debris must not be removed (minimum demand: presence of coarse woody debris).

[1] AQEM. The Development and Testing of an Integrated Assessment System for the Ecological Quality of Streams and Rivers throughout Europe using Benthic Macroinvertebrates. *2nd deliverable: Reference biocoenoses and deviations: structure and tools for description,* February 2002.

- Stream bottoms and stream margins must not be fixed.
- No migration barriers (effecting the bedload transport and/or the biota of the sampling site).
- Only moderate influence due to flood protection measures.

Riparian vegetation and floodplain

- Riparian vegetation and floodplains must still exist, making lateral connectivity possible. Example: riparian buffer zone greater or equal 3 x channel width (depending on the stream type).

Hydrological conditions and regulation

- No alterations of the natural hydrograph and discharge regime.
- No or minor upstream impoundment, reservoirs, weirs and reservoirs retaining sediments must be present (no recognisable effect on the biota of the sampling site).
- No hydrological alterations such as water diversion, abstraction or pulse releases.

Physical and chemical conditions

- No known point sources of pollution affecting the site.
- No known point sources of eutrophication affecting the site.
- No known or expected diffuse inputs.
- Near to natural background levels describing the baseload of a specific catchment area.
- No sign of acidification.
- No liming activities.
- No known impairments due to physical conditions, especially the thermal conditions must be close to natural conditions.
- No known local impairments due to chemical conditions, especially no known point sources of significant pollution, taking into account the dilution capacity of the water body.
- No known point sources of nutrients.
- No sign of salinity. - No known or expected diffuse inputs. Minimum: near to natural background levels describing the baseload of a specific catchment area.

Biological conditions

- No significant impairment of the indigenous biota by introduction of fish, Crustacea, mussels or any other kind of plants and animals.
- No significant impairment of the indigenous biota by fish farming.
- No significant impairment by invasive plant or animal species (Neophyta, Neozoa).

SPECIFIC REMARKS BY THE FAME PARTNERS

(General remarks pertaining to the whole range of criteria are provided in the main report).

The AQEM list is comprised of 23 criteria, organised in six "core groups", plus one group containing basic statements, such as that the reference condition must be politically palatable and reasonable, and that they must represent large numbers of defined populations of water bodies for the specific water body type or important aspects of 'natural' conditions.

The five core groups for reference site designation are (number of criteria in parenthesis):

- Land use practices in the catchment area (1)
- River channel and habitats (5)
- Riparian vegetation and floodplain(1)
- Hydrological conditions and regulation (3)
- Physical and chemical conditions (10)
- Biological conditions (3)

All group members made suggestions on the AQEM list. Most suggestions were concerned with deletion or modification of existing criteria, but there were also suggestions for addition of new criteria.

An analysis of the six cores of criteria, with the inclusion of comments from the members of the group, is presented below.

Land use practices in the catchment area

All members seem to believe that this criterion is important. Though land use practices are mostly considered at a site level, suggestions to be considered at the river level as well have been made (Netherlands, Lithuania, Poland, Greece, etc.). Some reports have further suggested that when referring to the site-reach scale, the land use criteria should be dealt with again, together with the riparian - floodplain criteria.

River channel and habitats

The starting point was five criteria generated by the AQEM group. One of these criteria (presence of coarse woody debris) was considered by some members as relatively unimportant, or at least difficult to apply to specific river types.

All four remaining criteria were generally accepted. "*Absence of migration barriers*" (in a site-specific sense) and "*unfixed stream bottoms and stream margins*" were thought by most members as the primary physical habitat variables that influence site selection. However, it was repeatedly stressed that this core must be carefully designed, otherwise it could effectively exclude most rivers as heavily modified.

Some confusion may arise from the fact that some of the criteria of the core also seem to fit in other core categories. For example, the criterion "*absence of migration barriers*" was indicated as also fitting in the core "hydrological conditions and regulation". The criterion "*only moderate influence due to flood protection measures*" was stressed by some members as important, but was not indicated as so by others, perhaps because it does not fit all local cases.

Through their suggestions, the group members added a number of other criteria potentially to be included, or proposed to complement/modify existing ones. Some of the added criteria concern channel or substrate condition and modification, which are attributes inadequately covered by the AQEM list. Examples are:

- Stream bottoms must not be cleared nor be dredged.
- No excessive sedimentation or embeddedness.
- No bridges or other constructions in close proximity to the site.
- No other significant human impacts reducing the diversity of instream habitats must be present.
- No abstraction of gravel or other alterations of the natural substrate in the broader area of the site.
- The sites selected must be representative of the range of substrate material and habitat types within the river type and ecoregion.
- All natural substrates should be available.
- (Fish) typical for connected and isolated water bodies should be present in the flood plain.
- All life cycle typical habitats should be available in the site/reach/river chosen.

Riparian vegetation and floodplain

There is only one criterion in this core, namely that "*riparian vegetation and floodplains must still exist, making lateral connectivity possible…*". From the evaluation of replies, it appears that for some members, this criterion is not applicable, or is not important, or difficult to be met, or even that it overlaps with land use criteria, and therefore it should be treated there. For example, as indicated by the British partners, no systems exist in the UK were the floodplain is uncultivated or posses a natural climax vegetation.

On these grounds, suggestions have been made to reformulate the criterion. One of the suggested reformulation, for example, is: "*If it is typical for the considered river, riparian vegetation and floodplains must still exist, ensuring lateral connectivity*" (Germany). There have also been suggestions (Lithuania, Poland, Portugal) to split the criterion into subcategories and/or to include additional requirements. An example (Poland) is:

Riparian vegetation:
- *consists of natural vegetation*
- *width of riparian vegetation zone is no less than minimum value estimated for different size of rivers*

Floodplain:
- *floodplain-river connectivity (with flooded bank vegetation, anabranches, backwaters, off-channels, wetlands) is present*
- *floodplain is not cultivated*
- *floodplain posses a natural climax vegetation*

Hydrological conditions and regulation

There seems to be common agreement within the group that all three criteria of this core are important determinants of the reference sites. The following suggestions have been made:

- To consider at least some of these criteria at the river basin level. For example, impacts on connectivity to be assigned primary importance for selecting reference rivers. Upper

reaches of a river may appear to fit the criteria for reference sites, whilst impacts to connectivity down stream would exclude the whole river (UK).

- To loosen-up the formulation of some criteria with the acceptance of moderate hydrological alterations.

Physical and chemical conditions

Ten criteria comprise this core, of which at least five are concerned with pollution, nutrients, eutrophication and diffuse inputs. The criterion for liming seems to be of local importance (Sweden) and can be included only in the respective national list. Most of the remaining criteria were indicated as important by at least some members, however other members excluded certain criteria from their lists. A common suggestion was to accept minor disturbances, taking into account the river flow regime, the proximity to the source of the disturbance and the water dilution capacity (protocols and guidance cannot be developed at this stage; some degree of subjectivity is unavoidable; expert judgement is an inevitable resort).

To avoid repetitions and to simplify the description of the criteria of the core, the various factors of interest can be listed individually, and maybe some more biologically important criteria could be added. There has been a suggestion in this direction (Germany) than can form the basis for a re-formulation, e.g.

No significant impairments due to

- *physical conditions, especially the thermal conditions…*
- *chemical conditions, especially point sources of eutrophication, nutrients, pollution…*
- *diffuse inputs*

  etc.

Biological conditions

Results indicated that all three criteria of the core (referring to introductions, fish farming and invasive species respectively) should be maintained. Equal emphasis should not be assigned to them, especially with regard to the spatial scale of consideration. There were suggestions for additions of some new criteria, namely stocking, fishing (professional or recreational), introduced diseases and parasites, land predators and boating.

The inclusion of a criterion for “land predators” raises two objections: it could give rise to political issues e.g. cormorants; the presence of predators is not necessarily an anthropogenic disturbance (these are part of a natural ecosystem).

With regard to the criterion for invasive species, it has to be elucidated if their presence is the result of accidental transfer by man (that probably went unrecorded), in which case it is an anthropogenic biotic change, or a natural process associated with the colonising capacity of some species, in which case it is a physical change. With regard to the criterion for introduced species, one question that remains is if introductions that occurred a long time ago (e.g. in past centuries) must disqualify a river or site as reference.

Views within the group maintained that "fish farming" could be omitted as it may be covered by other criteria like "Only moderate impairment due to accidental escape of fish" or "nutrient concentrations within the range normally associated with undisturbed conditions". However, the criterion is maintained as more practical to check and providing an independent and

additional confirmation of site quality. Besides, fish farms may attract free-living fish, altering the community structure. Views also maintained that the criterion "introduced diseases and parasites" should also be omitted, as this is too difficult to check in the field. Possibly this criterion could be regarded as optional, and used only when both the presence of diseases and parasites and their effect on the biota can be verified.

For the sake of simplification, the variables that can be used as biotic criteria can be split into subcategories. This would also give more weight to the respective impacts and facilitate database standardisation (more criteria can be added locally if necessary). A possible formulation based on the Polish and German reports is as follows:

Only moderate impairment due to:

- *invasive species, fish introduction, fish stocking or fish farming*
- *fishing activities*
- *introduced disease and parasites*