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The European reference condition concept: A scientific and technical approach to identify minimally-impacted river ecosystems

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ABSTRACT

One objective of the European Union (EU)'s Water Framework Directive (WFD: Directive 2000/60/EC) is for all European surface waters to achieve 'good status' by 2015. In support of this objective, the EU has facilitated an intercalibration exercise to ensure harmonized definitions of the status of water bodies, reflecting the deviation of their properties (mainly biotic assemblages) from a minimally disturbed state, termed the "reference condition". One of the major challenges of the WFD has been to find common approaches for defining reference conditions and to define the level of anthropogenic intervention allowed in reference sites. In this paper we describe how river reference sites were selected in the Central-Baltic region of Europe. A list of pressure criteria was provided and 14 Member States (MSs) categorized each criterion according to the method (i.e. measured, field inspection, etc.) used for reference site screening. Additionally, reference land-use and water-chemistry thresholds were agreed among countries in order to base reference site selection on objective criteria. For land-use criteria, a reference threshold and a rejection threshold were established. Sites with all criteria below the reference threshold were considered to be reference sites; sites having most criteria below the reference threshold and only some parameters between the reference and rejection threshold were "possible reference sites". These sites were retained only after carefully checking the cumulative effects of the pressures using local expertise, and a posteriori water-chemistry evaluation was necessary. In general, the most widespread method for defining a reference site was the measurement of pressures, followed by field inspections and expert judgment. However, some major pressures (e.g. hydromorphological alteration) were evaluated in a number of different ways (e.g. measured, field inspection, expert judgment). Our meta-analyses reveal a need to reinforce standardization in the application of pressure criteria by Member States. The pressure criteria identified in this exercise should be refined and tested with biological data to help in the further validation of minimally disturbed sites (i.e. the WFD "reference condition") and to provide a firm foundation for ecological status assessment. This in turn would ensure that there is pan-European comparability when evaluating the achievement of environmental objectives.

1. Introduction

Assessment of water quality in rivers during the 20th century focused on establishing links between pollutants and biota, generally reinforcing the theory that stressors reduced biological diversity (Hynes, 1994). Reversing this logic, the absence of stress equates to the undisturbed condition of the same water body, from which the concept of "reference conditions" arises (Karr, 1981; Reynoldson

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and Wright, 2000; Stoddard et al., 2006; Wright et al., 1984; Wright, 2000). Reference conditions refer to the "naturalness" of the biota, in the absence of human disturbance or alteration (Stoddard et al., 2006) and, as such, represent a target for remediation and restoration. This philosophy underlies the European Union's Water Framework Directive (WFD: Directive, 2000/60/EC), which requires assessment of "ecological status" of a water body; defined as the distance its biota has moved from an undisturbed state. Early attempts to define reference conditions were based on the identification of biological attributes assumed to characterize the undisturbed state (Plafkin et al., 1989; Wright et al., 1984). The concept of reference conditions for quality assessment in water bodies has been widely developed since this point (Bailey et al., 2004; Hawkins et al., 2010; Hughes et al., 1986; Reynoldson et al., 1997; Stoddard et al., 2006; Sweeting et al., 1992; Wright et al., 1993), and has been previously adopted in legislation in various parts of the world, e.g. the Clean Water Act in the USA and the Water Reform Framework in Australia. Strict application of this approach is a challenge in Europe, where pristine or nearly pristine examples of many types of aquatic ecosystems are absent or rare (Nijboer et al., 2004; Nõges et al., 2009).

The WFD defines reference conditions as the values of biological, hydromorphological and physical–chemical quality elements at high ecological status (WFD, Annex II, clause 1.3). "Reference sites" are characterized by minimal changes in their hydromorphological and physical–chemical characteristics so long as these do not have a significant effect on the ecosystem (Wallin et al., 2003). This state is akin to "minimally disturbed condition" (sensu Stoddard et al., 2006) and should be found in places that have escaped all but the broadest-scale human disturbances (Stoddard et al., 2006). Such sites should provide objective benchmarks for ecological status assessment. By selecting reference sites primarily on the basis of non-biological measures, it is possible to ensure the absence of significant human pressures (Wallin et al., 2003; Bailey et al., 2004; Stoddard et al., 2006) whilst ensuring that preconceived notions about the "natural" structure and composition of biotic assemblages do not influence the process (Bailey et al., 2004; Stoddard et al., 2006).

A big challenge for those involved in implementation of the WFD has been to find common approaches for defining reference conditions in aquatic ecosystems (Nõges et al., 2009). This reflects the large natural variability in aquatic ecosystems across Europe, even in the absence of human disturbance (Hering et al., 2010). The WFD partly solves this issue by requiring that water bodies are grouped according to their characteristics and the region they are located in. This enables "type-specific reference conditions" to be defined and should ensure like-with-like comparisons of biological communities. More importantly, there is ambiguity in the operational definition of reference conditions (Moss, 2008), in which the level of anthropogenic intervention that can be allowed is not clearly established. Unclear understanding of what minimally-disturbed conditions represent may lead to the selection of reference sites with some anthropogenic impact (Whittier et al., 2007), precluding comparisons as the denominator in observed/expected equations is not constant. As a result, ecological assessment would not be consistent between Member States (MSs). Early guidance on screening reference sites in Europe (Wallin et al., 2003) provided a list of general types of pressures (i.e. point source pollution, morphological alterations) but lacked recommendations on how these should be specifically evaluated and quantified. As a result, expert judgment was allowed to influence the selection of reference sites (Basset, 2010). Whilst an experienced limnologist should be able to differentiate high/good from moderate from poor/bad status (Davies and Jackson, 2006; Moss et al., 2003), expert judgment can also be a source of error (Whittier et al., 2007), particularly on the large spatial scales necessary for the WFD. Another list of pressure criteria for streams was provided by Hering et al. (2003), and further refined by Nijboer et al. (2004); however, this proved to be too stringent to be practicable and, therefore, a further quantification of the criteria was necessary (Nijboer et al., 2004).

However, whilst agreeing a set of quantitative criteria by which reference sites may be screened should allow all countries to adopt a consistent approach to reference screening, there are, to our knowledge, no studies which provide an indication of the level of pressure that may be acceptable. Ideally, these should be based on the concept of ecological thresholds i.e. the point at which there is a change in an ecosystem quality, property or phenomenon (Groffman et al., 2006) and, on evidence of a lack of significant impact on the biota.

In this paper, we describe an exercise to agree pressure criteria for the identification of riverine reference sites. Our objective was to provide guidelines for Member States (MSs) to select reference sites in a harmonized way, ensuring comparability in the criteria which need to be fulfilled by any water body proposed as a reference site. These have now been agreed by countries within the European Central-Baltic region. We: (i) propose a list of criteria which reference sites should fulfill; (ii) compare how countries have evaluated such criteria in their screening and (iii) set the pressure level (non-impact threshold) that should be used to screen candidate reference sites, taking into account river typology. Non-impact thresholds are proposed both for critical drivers and pressures (i.e. artificial land-use or intensive agriculture in the basins) and stressors that may demonstrate the existence of a pressure not previously identified within the catchment (i.e. nutrients and dissolved oxygen). There is ample knowledge on the impact of land use on stream health (see review in Allan, 2004) and, in particular, on aquatic invertebrate communities (i.e. Donohue et al., 2006; Harding et al., 1998; Hilderbrand et al., 2010; Utz et al., 2009). However, fewer studies have addressed the link between nutrients and the ecological status of macroinvertebrate assemblages (see Townsend et al., 2008; Wang et al., 2007) and there are many other pressures, whose effects still need to be addressed and quantified. The list we present, though no more than a "work in progress" has, at least, been agreed among the participating countries as a workable basis for comparisons between methods.

2. Methods

The Central-Baltic Geographic Intercalibration Group (CB GIG) and the steering group on reference conditions were facilitated by the Ecological Status working group (ECOSTAT) which was mandated by the Commission and the Member States to coordinate the European intercalibration exercise of WFD.

2.1. Geographical extent

Countries from the Central, Baltic and western parts of Europe, collectively known as the Central-Baltic Geographic Intercalibration Group (GIG), participated in this exercise (Fig. 1). "Intercalibration" is a key component of the WFD, ensuring that concepts of high and good ecological status are harmonized throughout the EU. Seventeen European countries (the two regions of Belgium both have their own assessment method and are considered separately) participated in this study, and fourteen provided data from reference sites: Austria, Belgium-Wallonia, Czech Republic, Estonia, France, Germany, Ireland, Italy, Lithuania, Luxembourg, Poland, Spain, Sweden and United Kingdom. Netherlands, Belgium-Flanders and Denmark acknowledged the absence of reference sites within their territory. Streams and rivers within these countries belonged to six WFD intercalibration common types, defined by their basin size, dominant geology, altitude and substrate (Table 1).

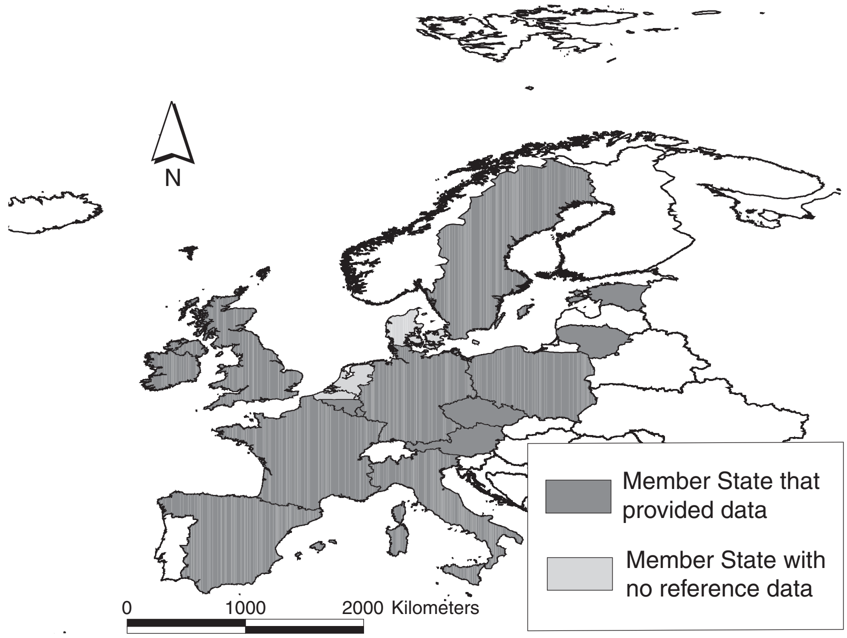

Fig. 1. Countries participating in the Geographic Intercalibration group of the Central and Baltic rivers.

2.2. Data collection and analysis

2.2.1. Pressure criteria used to identify reference conditions

In order to compare views on national reference conditions, we assembled a list of pressures criteria (see the Central-Baltic (CB) GIG reference screening questionnaire in Annex 2.1.1.3 in van De Bund, 2009) that can be used to evaluate anthropogenic impacts in rivers. These criteria covered all pressure types identified in the EU's "Guidance on establishing reference conditions" (Wallin et al., 2003) and allow a thorough and objective characterisation of pressures at different scales (i.e. basin,, reach and site). For some pressure criteria, two thresholds were defined: i) a "reference" threshold, below which a site was considered as "probably reference" and ii) a "rejection" threshold, corresponding to a high likelihood of significant impact, above which a site was eliminated. Sites that have all criteria below the reference threshold were considered as reference sites; sites having most criteria below the reference threshold and a few variables between the reference and rejection threshold were "possible reference sites". For these sites, no more than 10% of the criteria should exceed the reference threshold level. These sites were retained only after careful checks of the cumulative effects of pressures using local expertise together with checks against water chemistry reference thresholds. If a site exceeded the rejection threshold on any one criterion it was eliminated.

Member States were asked to categorize each criterion in the Screening Questionnaire according to the method they used for reference sites screening (Measured, Field inspection, Expert judgment, Alternative criterion or Missing information). The category "measured" implies that a value that has been quantified on a continuous scale using an objective method; "field inspections" corresponds to *in situ* visual evaluations that are either categorical or semi-quantitative; "expert judgment" is based on opinions from the scientific community; "alternative criterion" includes all other ways of evaluating a pressure not indicated in the criteria list; and "missing information" indicates either a lack of information or no response by the countries. A final category ("OK") was added when a country did not specify the method used but just indicated that the specific criterion was applied. To analyze the responses, we selected 47 criteria which were most relevant and generally answered (Table 3), covering all different pressures. We discarded those criteria that were only assessed by a few countries.

The relevance of each method for the evaluation of major pressures for reference site screening across Europe was evaluated by principal components analysis (PCA, command: dudi.pca, package ade4, R software). For this analysis, the list of criteria was considered as study cases and the methods as variables. The raw values in the matrix were the proportion of countries applying a given method to evaluate a criterion. In order to assess differences in the number of required criteria and in the methods used to evaluate major pressures, we conducted a between-class analysis (command: between, package ade4, R software) using major pressure type as the grouping factor. This method represents an ellipse that includes all assessment methods within a given major pressure and computes its centroid.

Table 1
Central-Baltic (CB) rivers common intercalibration types for reference condition (RC). The CB Geographical Intercalibration Group river types were defined within the WFD Common Implementation Strategy (van De Bund, 2009).

Type	River characterisation	Catchment area (km^2)	Altitude and geomorphology	Alkalinity (meq/l)
RC-1	Small lowland siliceous-sand	10–100	Lowland, dominated by sandy substrate (small particle size), 3–8 m width (bankfull size)	<0.4
RC-2	Small lowland siliceous-rock	10–100	Lowland, rock material 3–8 m width (bankfull size)	<0.4
RC-3	Small mid-altitude siliceous	10–100	Mid-altitude, rock (granite)–gravel substrate, 2–10 m width (bankfull size)	<0.4
RC-4	Medium lowland mixed	100–1000	Lowland, sandy to gravel substrate, 8–25 m width (bankfull size)	>0.4
RC-5	Large lowland mixed	1000–10,000	Lowland, barbel zone, variation in velocity, max. altitude in catchment: 800 m, >25 m width (bankfull size)	>0.4
RC-6	Small, lowland, calcareous	10–300	Lowland, gravel substrate (limestone), width 3–10 m (bankfull size)	>2

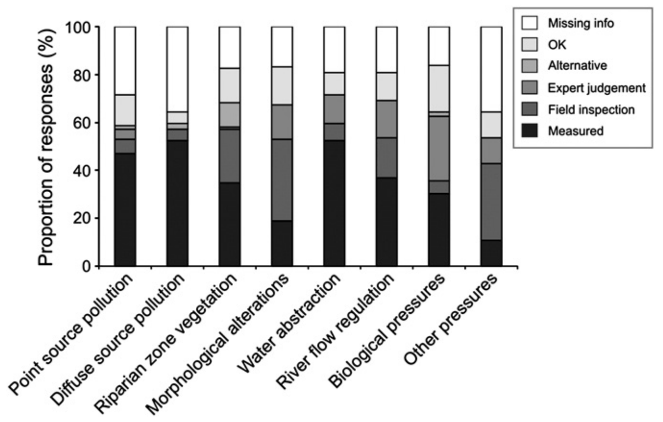

Fig. 2. Proportion of 282 total responses provided in the "Screening Questionnaire" for all major pressures.

2.2.2. Establishment of non-impact threshold values to identify reference sites

The percentage of artificial land use was extracted from level I Corine Land Cover (Coordination of Information on the Environment, Land Cover 2000), including urban areas continuous and discontinuous, industrial and commercial zones, communication infrastructures and networks, mines. Intensive agriculture was the sum of the Corine categories corresponding to a high potential impact from agricultural activities: arable land (including irrigated land), permanent crops (with associated annual crops), vineyards, orchards, olive groves, and complex cultivation patterns. Artificial (reference threshold <0.4% and rejection threshold >0.8%) and intensive agriculture (reference threshold <20% and rejection threshold >50%) thresholds, were derived as agreement within the CB GIG supported by the review of existing studies on the effect on land use on biotic communities at the time of the agreement. The GIG agreed that proposed thresholds for artificial land use 0.4–0.8% were very protective, when compared with results from preliminary studies from France (Wasson et al., 2005). A higher variability in the results obtained by Wasson et al. (2005) on the thresholds for intensive agriculture influencing streams depending on the French region and on the valley floor occupation, led CB GIG to agree in a safe reference threshold (20%), but extended the rejection threshold to the maximum value of 50%. The high upper thresholds should only be accepted provided that other conditions were met (absence of erosion in the basin, the valley floors were mainly occupied by low intensity agricultural area (mainly pastures) and/or semi-natural areas), the riparian corridors were intact at the reach and site scales, and the water chemistry fulfilled their reference thresholds.

Reference thresholds were also agreed for water-chemistry variables related to organic and nutrient enrichment disturbances (N–NH_4, N–NO_3, P–PO_4 (P–PO_4 was converted by consensus to Total-P with an approximate ratio of 2), O_2, and BOD_5) (Table 3). Water chemistry parameters are considered as stressors that indicate pressures within the catchment area that had not otherwise been identified. A water chemistry check was used to evaluate the existence of an impact on water quality for those sites having land use values between reference and rejection thresholds. The methods used to set the national water quality thresholds were:

1. Identification of "no impact thresholds" by means of a linear regression between the macroinvertebrate Intercalibration Common Metric index and the pressure/stressor of interest (Wasson et al., 2006). The non impact threshold corresponds to the maximum pressure level at which the relationship is not significant;
2. Application of normative values from national water quality classifications (France, SEQ Eau, 1999; Italy, DL 11.05.1999, N. 152, 1999; Germany, LAWA 1998) that corresponded to "very minor" biological impact; and
3. Comparison with chemical values from reference sites (or high status sites) in similar river ecosystems (Annex 2.1.1.2. CB Chemical thresholds values in van De Bund, 2009). In some cases, expert judgment suggested that a common threshold might not be adequate for all stream types because of their different environmental characteristics. In these cases, a different value was set (broad river types defined and used for intercalibration, see Table 1). Similarly, in some cases different thresholds were provided for diatoms and invertebrates, based on their different sensitivities to nutrients. Reference thresholds were based on mean and/or spot values (instantaneous measurements), as available, as some countries had scant data and, thus, proposed thresholds had to cover the variety of existing data.

3. Results

3.1. Comparison of pressure criteria used to identify reference conditions

The most common method used to evaluate the level of pressure at a reference site was by a measurable criterion (34% of the total 282 answers), followed by field inspection (19%) and expert judgment (10%). The application of an alternative criterion corresponded to only 2% of the answers. No response (i.e. missing information) accounted for 20% of the answers and an imprecise answer ("OK") was given for 13% of the answers. Major pressures differed in the methods most frequently used for their evaluation (Figs. 2 and 3). The first PCA axis accounted for 37.77% of total variance and discriminated pressures that had been evaluated mainly by measured criteria (e.g. point and diffuse source pollution, Fig. 3) from pressures evaluated by means of field inspections (e.g. morphological alteration (34.2% of the answers) or other pressures). The pressure criteria for riparian zones were clearly differentiated along the second PCA axis (23.55%) (Fig. 3), probably due to the high proportion of answers for "alternative criterion" (Fig. 2).

Most countries used a combination of different methods to evaluate the potential impact on riparian zone vegetation (Fig. 2), which comprised mainly measured criteria (34.7%) followed by field inspections (22.4%). Water abstraction and river flow regulation pressures were measured in 52.4% and 36.9% of cases, respectively although the application of expert judgment and field inspections was notable (Fig. 2). Biological pressures were evaluated predominantly by measured criteria (30.4%) and expert judgment (26.8%). The category "other pressures" was characterized by a high proportion of missing information (35.2%); where it was evaluated, it was predominantly by field inspection (32.1%).

3.2. Criteria used to quantify each major pressure

Criteria used to assess major pressures influencing stream and river ecosystems, defined in general terms by Wallin et al. (2003) are described in Table 2.

Point and diffuse pollution sources were addressed by criteria established mainly at the basin level, indicative of the large scale cumulative influence of land use (Table 2). Similarly, water abstraction and regulation pressures were identified by hydrological criteria, of relevance for the maintenance of near-natural flow regimes, using criteria on water abstraction levels and flow dynamics at the basin scale (Table 2). The local impact caused by any water regulation structure was taken into account by the evaluation of various criteria established at the lower reach scale.

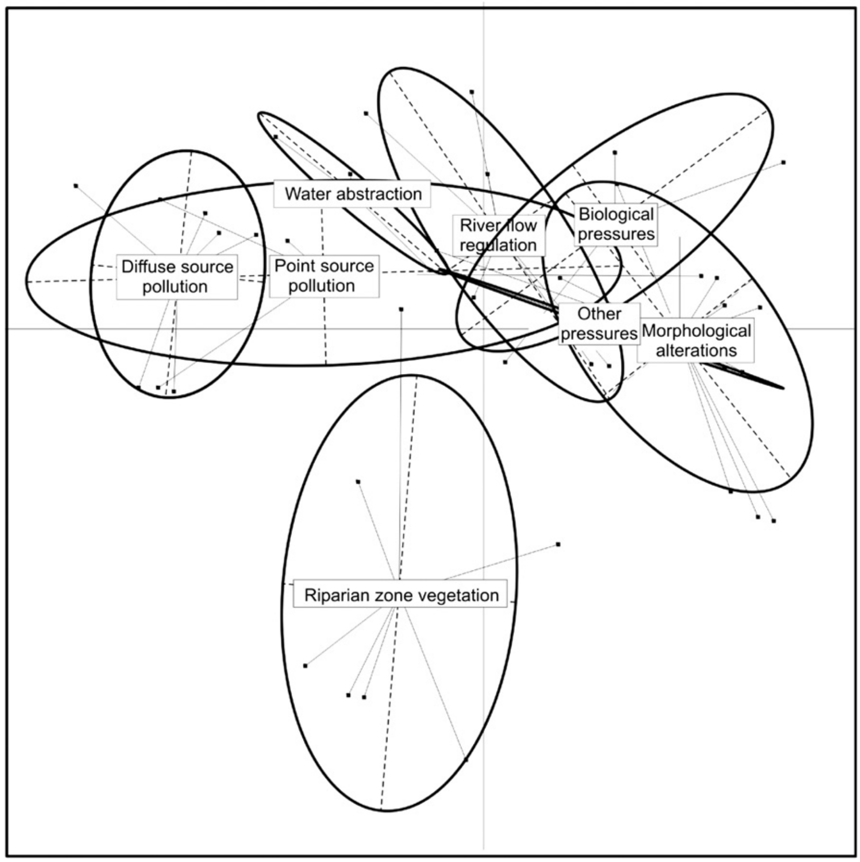

Fig. 3. Results from Principal Component Analysis using the list of the 47 criteria as study cases represented by dots, and the proportion of countries' answers for each assessment method as input variables. Cases related with the same major pressure are grouped, and their centroid and embracing circumference represented.

Morphological alterations were assessed by criteria ranging from the basin to the site scale (Table 2). At the basin scale the criteria addressed the influence from sediment transportation, whilst at the reach and site scales, morphological modifications had to maintain all longitudinal, lateral and vertical water connections to ensure the integrity of the river channel. At more local scales (reach-site) intensive recreational activities should be avoided. The integrity of the riparian vegetation was evaluated by criteria addressing the maintenance of its natural structure and composition. Local impacts from non-invasive alien species or fish management and/or aquaculture were allowed provided they had no significant impact on native fish populations (Table 2).

3.3. Reference thresholds for water-chemistry to validate land use rejection thresholds

Reference thresholds were set for water-chemistry variables related to organic and nutrient enrichment ($N–NH_4$, $N–NO_3$, $P–PO_4$, O_2, BOD_5) (Table 3). The mean value for all parameters was proposed as this the most robust statistic when few data are available. Additionally, the 90th-percentile was suggested for those with a potential acute toxic effect (BOD_5, dissolved oxygen and $N–NH_4$), but to be used only when enough data were available (at least 12 monthly chemical samples).

A distinction was made for the more sensitive river types RC-2 and RC-3 (small siliceous streams), for which $N–NH_4$, and $P–PO_4$ reference values were more stringent (Table 3). The RC-3 type (mid-steep gradient streams) was considered to be the most sensitive, so $N–NO_3$ and $P–PO_4$ thresholds were most stringent (2 mg/l and 20 μg/l, respectively, see Table 4). For RC-2, $N–NO_3$ thresholds were differentiated for diatoms (4 mg/l) and invertebrates (6 mg/l), recognizing the greater sensitivity of phytobenthos to nutrients.

4. Discussion

4.1. A consistent view of reference conditions across Europe

In ‘Alice's Adventures in Wonderland’ (Carroll, 1865), Humpty Dumpty says ‘ When I use a word.. it means just what I choose it to mean — neither more nor less’. This is roughly the situation that prevailed in Europe with respect to reference conditions in the decade following the adoption of the WFD. Despite the efforts of Nijboer et al. (2004), Wallin et al. (2003) and others, practical implementation of this theoretical concept proved to be difficult. The guidelines presented here represent the “art-of-the-possible”, rather than a definitive account of reference conditions in Europe. They do mean that biologists throughout the EU can now work to a single definition of riverine reference conditions, which translates into explicit and objective criteria, rather than to 27 different variations on a theme. Moreover, outcomes have been discussed and agreed by representatives of the competent authorities in all countries.

A consistent approach to reference conditions, in turn, underpinned the first European intercalibration exercise, which compared national approaches to defining high and good status. The results of these exercise are now legally binding (COM Decision 2008/915/CE; Bennett et al., 2011; Kelly et al., 2009; van De Bund, 2009).

Although these criteria were initially applied only within Central-Baltic GIG, they were later adopted by the other GIGs: Alpine, Eastern, Mediterranean and Nordic, although not in very large rivers (basin area greater than 10,000 km^2), because the pressure criteria and

Table 2
Most relevant pressure criteria selected from the "Screening Questionnaire" for establishing reference conditions. The type of pressure and scale relevant for each criterion is shown. For criteria in which a threshold value is shown, a justification is provided.

Pressure type	Scale	Criterion
Point source pollution	Basin	"Reference" threshold <0.4% of artificial land use in the catchment area (estimated from Corine data). "Rejection" threshold >0.8% of artificial land use in the catchment area. Artificial land use between 0.4% and 0.8%: Validation with physico-chemical parameters at the site scale. Small streams: No known point source discharge, or very localized impact with self purification. Larger streams and rivers: Very low point source discharge level. If point sources are present, a validation with chemical parameters is necessary.
Diffuse source pollution	Basin	"Reference" threshold <20% of intensive agriculture in the catchment area. "Rejection" threshold >50% of intensive agriculture in the catchment area (estimated from Corine data). Intensive agriculture between 20% and 50%: Validation with physico-chemical parameters at the site scale. Cattle breeding: Only non-intensive (outdoor) cattle breeding; <1.25 animal (cattle) units per ha of the catchment area. Vineyards, orchards <1% of the catchment area, and not situated in the riparian zone. Irrigated fields ≤10% Forestry <30% tree plantations (i.e. coniferous, *Eucalyptus*…).
Water abstraction	Basin	No dams or water storage significantly altering the low flow regime; low flow alteration<20% of the monthly minimum flow.
	Reach	Only very minor reductions in flow level changes having no more than very minor effects on the quality elements. No significant water abstraction in the reach. The cumulative effect of water regulation and abstraction at the basin and reach scales is <20% of low flow discharge.
River flow regulation	Basin	No dams which significantly modify the natural hydrological flow regime (i.e. suppression of frequent floods (<5 years) with anomalous development of vegetation in the channel), or low flow alteration (< to + or −20% modification of the natural monthly minimum flow discharge). The total storage capacity of the reservoirs in the catchment is <5% of the mean annual discharge at the site. No change of the natural (type specific) annual flow characteristics (seasonality of high and low flow).
	Reach	No by-passed section with residual flow (legal minimum discharge) No significant hydropower peaking effect (ratio Q hydropeaking/Q baseflow <2) Absence of flow regulation (dam) on the reach itself.
Morphological alterations	Basin	Sediment transport: No dams which significantly modify the sediment regime (sediment retention), evidenced by signs of incision of the river bed (e.g. incision>0.2 m * stream order, bare bed rock appearing…).
	Reach	Flow impedance: < 10% of the reach is affected by flow impedance, due to hydraulic effects of weirs, sluices, etc.… The % of the reach affected by flow impedance can be evaluated by the ratio of the sum of weirs' heights (in meters) to the total difference in height (slope∗length, in meters) between the upper and lower end of the reach. Channelization: <10% of the reach is affected by "hard works" (i.e. modification of longitudinal and/or transverse profiles, narrow embankment, loss of lateral connectivity…), otherwise, bed and banks composed of natural materials Stabilization: <20% of the reach is affected by "soft works" (i.e. bank protection on one side, distant dikes, bank maintenance, not affecting the longitudinal and/or transverse profile, and lateral connectivity globally maintained…). If both types of works are combined ("hard works and soft works") <10% of the reach should be affected. Siltation: Reaches with suspected anomalous siltation, due to agricultural soil erosion, should be avoided. Connection to groundwater: Total lateral and vertical connection to groundwater. Substrate conditions: Correspond to related typology River profile and variation in width and depth: Correspond to related typology River continuity: At the reach scale, the continuity of the river is not disturbed by anthropogenic barriers and allows undisturbed migration of aquatic organisms (including resident fish populations). River continuity: At the reach scale, the continuity of the river is not disturbed by anthropogenic barriers and allows free sediment transport.
	Site	The site is not situated in a zone directly or indirectly impacted by a nearby (upstream or downstream) artificial structure. Lack of instream structural modifications (weirs or dams) that affect the longitudinal and lateral connectivity, and natural movement of river bed, sediment load, water and biota (except for natural waterfalls). Only very small artificial constructions with very minor local effects can be accepted.
Riparian vegetation modification	Reach	In agricultural landscape (intensive agriculture between 20% and 50%): intensive agriculture land cover<10% of the reach scale. Semi natural or low intensity agricultural areas >90% of riparian corridor land use >90. In non agricultural landscape (intensive agriculture <20%): Valley floor and riparian corridor occupied by semi natural or low intensity agricultural areas. Artificial areas <10% of the reach scale.
	Site	The riparian zone of the site is entirely bordered by the type specific natural vegetation or semi-natural land cover, with the possible exception of access to the river site. Riparian vegetation zone continuity: uninterrupted or with few interruptions (i.e. access to the site). The lateral connectivity between river and riparian corridor is maintained along the site. No direct impact of cattle trampling.
Biological pressures	Site	No invasive species, but alien species which are not at the invasive stage are tolerated.
	Reach–Site	No intensive (commercial) fishery. No or very limited direct pollution by aquaculture plants. No biomanipulation.
Other pressures	Site	No intensive use for recreation purposes (no intensive camping, swimming, boating, etc.) No nearby intensive recreational use at the site scale: No regular bathing activities or motor boating. Occasional recreational uses (such as camping, swimming, boating, etc.) should lead to no or very minor impairment of the ecosystem.

Basin scale: area of land above a site. Reach scale: part of the river representative of the water body. Site scale: place where sampling occurred.
Reach length: Small rivers (stream order [s.o.] = 1–3) > 1 km; medium size rivers (s.o. = 4–5) > 5 km; large rivers (s.o. > 6) > 10 km
All criteria are reference/rejection thresholds, except for some indicated rejection thresholds.

thresholds are only rarely fulfilled. A revision of the criteria and thresholds is required for very large river basins although it will be difficult to scale-up the results obtained from small reference watersheds as many aspects of ecosystem functioning are quite different (Junk et al., 1989; Thorp and DeLong, 1994; Vannote et al., 1980).

The range of pressure criteria agreed for reference screening addresses all the relevant structural aspects for the preservation of biotic integrity in stream systems. The criteria embrace all major pressures affecting surface water body types (Wallin et al., 2003) although the impact of multiple pressures and the evaluation of

Table 3
Agreed threshold for chemical values in reference sites within the Geographical Intercalibration Group of Central-Baltic rivers.

		RC-1	RC-2	RC-3	RC-4	RC-5	RC-6
BOD_5 (mg/l)	Mean	2.4	2.4	2.4	2.4	2.4	2.4
	90th percentile	3.6	3.6	3.6	3.6	3.6	3.6
O_2 (%)	Mean	95–105	95–105	95–105	95–105	95–105	95–105
	10th–90th percentile	85–115	90–110	90–110	85–115	85–115	85–115
N–NH_4 (mg/l)	Mean	0.10	0.05	0.05	0.10	0.10	0.10
	90th percentile	0.25	0.12	0.12	0.25	0.25	0.25
N–NO_3 (mg/l)	Mean for invertebrates	6.00	6.00	2.00	6.00	6.00	6.00
	Mean for diatoms	4.00	4.00	2.00	4.00	4.00	4.00
P-PO_4 (or SRP) (μg/l)	Mean	40	30	20	40	40	40

synergisms have not been specifically addressed. These criteria were initially developed for benthic invertebrates but should also be applicable to benthic flora (macrophytes and phytobenthos), as the same pressures influence diatoms (organic/nutrients) (Kelly et al., 2009) and macrophytes (organic/nutrients/regulation) (Lacoul and Freedman, 2006; O'Hare et al., 2006; Szoszkiewicz et al., 2006). Despite the different sensitivities to nutrients of invertebrates, algae and macrophytes, a single protective nutrient threshold should be adopted for reference sites, focused on the most sensitive component, in order to protect the entire trophic pyramid. Aquatic invertebrates are suitable indicators of pressures at varying spatial scales (Hering et al., 2006; Johnson et al., 2007). Macrophytes are most suitable at large spatial scales, i.e. river sections (e.g. Robach et al., 1996), but can also indicate changes at a local scale (Armitage and Pardo, 1995). Although these pressure criteria have not been evaluated for fish, those reflecting morphological alterations at the basin scale may be applicable. Fish tend to respond to large-scale hydromorphological pressures (Bain et al., 1988), as they require longitudinal connection within the basin in order to access spawning or nursery areas. However, basin-scale reference conditions for fish probably do not exist anywhere in the EU due to widespread stream regulation and fish stocking.

4.2. Questionnaire results

The most widespread method for reference site screening was measurement of pressures, which allows objective quantification and, as a consequence, reliable comparison of reference sites among countries. Measurements were mainly applied to evaluate diffuse source pollution, point source pollution and water abstraction. Pollution estimation is mainly based on land-use parameters, which may be measured using widely-available Geographic Information Systems (GIS: Basnyat et al., 1999; Wasson et al., 2010). Similarly, water abstraction rates are usually recorded by governments and are readily available. As a consequence, the results of this process suggest that screening of reference sites is biased toward readily available measures of pressures.

Strict quantification may not be necessary for major pressures for which the critical issue is the absence of damaging human intervention, independent of its magnitude. This may be the case for morphological or riparian zone alterations for which field inspection is the most widely used evaluation approach. Despite the large number of criteria provided in relation to alterations of riparian zone vegetation, some countries employed different criteria for its evaluation suggesting further refinement of these criteria may be required. Subjective criteria, such as expert judgment, played an important role in the evaluation of morphological alterations, water abstraction, river flow regulation and water abstraction. Expert judgment should be used in combination with other types of objective criteria, in order to ensure that the process of selecting reference sites is consistent and transparent.

4.3. Land use and water chemistry thresholds

Ecological thresholds show good potential as tools for watershed management. Our proposed thresholds sought to identify non-impact thresholds ("*initiation -of -impact thresholds*", Hilderbrand et al., 2010), i.e. the thresholds above in which there is a detectable negative response in the biota, for pressure variables. Although land use is a key driver of nutrient loading and sediment loadings to surface waters, the extent to which it influences water quality will vary depending on the transport capacity of the watershed (Fraterrigo and Downing, 2008), the influence of riparian buffers (Lowrance et al., 1984a, 1984b), climatic and geomorphological basin features and the existence of additional pressures. Thus a two-fold threshold for land-use was employed: The more conservative threshold being to categorize sites as reference where the level of confidence was highest, and the rejection threshold where sites were substantially different from reference. Sites with values between the reference and the rejection threshold were subject to a posteriori checks against nutrient and oxygen criteria. A posteriori checks using water chemistry data can be assessed both with mean values and 90th percentile values (10th percentile for O_2). Whenever possible, the use of values of a few discrete spot measurements should be avoided in water quality assessments, as they may not be representative of the water body character (Gómez-Rodríguez et al., 2009). Percentiles for N–NO_3 and P–PO_4 were not provided because they do not have acute toxic effects (Wasson et al., 2006), even though other deleterious effects may occur.

The European reference condition was defined as a state in the present or in the past corresponding to very low pressure, without the effects of major industrialization, urbanization and intensification of agriculture, and with only very minor modification of physical–chemical, hydromorphology and biology (Wallin et al., 2003). The proposed reference and rejection thresholds for artificial land use (0.4% and 0.8%, respectively) appear somewhat conservative in comparison with those previously established for North American streams (5% of urbanization following Baker and King, 2010; 5–10% of impervious cover following Brabec et al., 2002). However, it has been shown that invertebrate deterioration occurred at lower thresholds along urbanization gradients in Maryland (0.8%–3.4% of watershed urban land following Baker and King, 2010; 0.5%–2% impervious cover, following King and Baker, 2011). Such protective thresholds were agreed in this study because of the historical extent and development of urban areas in Europe. Subsequent checks using biological data from reference sites around Europe indicated that the proposed 0.4–0.8 artificial thresholds did not result in significant ecological impact (Pardo et al., 2011).

Agricultural systems can either be intensive (i.e. agricultural yield is increased due to the use of fertilizers and irrigation), or extensive (i.e. agriculture practices increase the land under cultivation and rely on natural soil nutrients). Although both types of agriculture may affect stream properties, only intensive agriculture was considered in this process because of its greater potential to alter stream water quality. A review of the effects of agriculture in streams concluded that streams in agricultural basins usually remain in good condition until the extent of agriculture (including intensive and extensive) is greater than 30%–50% (Allan, 2004). Both reference and rejection thresholds for intensive agriculture were agreed within the GIG, but there was a clear understanding that the use of the rejection threshold (up to 50%) was only applicable if other pressure criteria and water chemistry showed that the ecological status was not likely to be impaired. The intensive agriculture thresholds were the weakest of all pressure criteria established, as the subsequent check with biological data indicated a significant reduction in EQR below both reference (20%) and rejection (50%) thresholds (Pardo et al., 2011). Future revisions of the reference pressure criteria must address the lowering of the thresholds for agricultural activities within basins, based on such evaluations. Most European monitoring programs are targeted at impacted sites and thus there was a lack of long term monitoring data for minimally disturbed sites from which water chemistry thresholds could be derived. Nutrient reference values were instead compiled from existing water quality standards from several European countries (Austria, Germany, France and UK) and then compared with available data from reference sites. In some cases, the thresholds were adjusted using expert judgment. For total phosphorus, the proposed threshold values ranged from mean values of 40 to 80 μg TP/l. The upper value (80 μg TP/l) for mixed and calcareous rivers corresponds with the reported range of natural background concentrations for the U.S. (e.g. 12–84 μg TP/l in Robertson et al., 2006; 6–80 μg TP/l in Smith et al., 2003) for arid basins. The lowest TP value (40 μg TP/l) for siliceous gradient streams is comparable to the median value reported by Robertson et al. (2008) (35 μg TP/l) for reference conditions in non-wadeable rivers in Wisconsin. However, these thresholds may not be stringent enough to protect diatoms in reference condition waters: Stevenson et al. (2008) advised thresholds of 10 (average value) −12 (75th percentile) μg TP/l to protect high quality biological conditions in streams of the Mid-Atlantic U.S. Highlands.

Reference thresholds for $N–NO_3$ varied from a mean value of 2 mg $N–NO_3$/l for the RC-3 river type (diatoms and invertebrates), to 4 mg $N–NO_3$/l (diatoms) and 6 mg $N–NO_3$/l (invertebrates) in the other RC types. Nitrate–N thresholds used results from an ecotoxicology review paper by Camargo et al. (2005); where long term exposures to 10 mg/l $N–NO_3$ were shown to adversely affect some invertebrates and fish (mainly salmonids in low alkalinity water). Consequently, a protective threshold of 2 mg $N–NO_3$/l was used in this exercise accounting for the most sensitive freshwater species (Diatoms) in low alkalinity streams and rivers. The mean reference values proposed for $N–NH_4$ varied between 0.05 and 0.1 mg/l. In general, the thresholds agreed for nitrogen compounds are less stringent than those proposed in a study of this type carried out by Robertson et al. (2008) in Wisconsin (U.S.). They defined median reference values of 0.061 mg/l for dissolved nitrite plus nitrate, 0.022 mg for $N–NH_4$/l, and 0.514 mg/l for total nitrogen (TN). Natural background concentrations for TN in the U.S., range from 0.02 mg/l in the xeric west ecoregion to more than 0.5 mg/l along the south-eastern coastal plain (Smith et al., 2003). Validation by Pardo et al. (2011) in invertebrate independent datasets showed a significant but weak effect of nitrate below the threshold set here, although nutrient thresholds still need to be checked on larger datasets from minimally impaired sites covering all biological quality elements.

Defining different nutrient threshold for different types of rivers addresses natural spatial variation in geomorphology and climatic conditions across Europe. A similar approach exists in the U.S., where the regional variations in nutrient criteria are used to divide the nation into 14 nutrient ecoregions based on climate, physiography and vegetation cover (Omernik, 1987). Within the Central Baltic European region, regional variation was accounted for by classifying rivers into types based on underlying geology, altitude and river size. Different reference nutrient thresholds for lowland siliceous streams and small, mid-steep gradient siliceous streams were defined because of their oligotrophic character compared to calcareous or mixed geology stream types. The lowest nitrate and phosphorous limits were set for lowland siliceous streams because of their highly transient character.

The definition of reference thresholds for this exercise constitutes an initial step in the process of setting quantitative pressure criteria to aid the management of stream ecosystems. Further refinement for some of the proposed pressure criteria thresholds is needed, ideally based on sound ecological evidence to better understand the links between drivers, pressures, stressors and status in European rivers and streams. Current scientific knowledge on these ecological links lacks practical integration in management measures.

One omission from the present list of criteria is an appreciation of the role of atmospheric deposition in a few regions. This may be an issue in soft water regions, particularly in northern Europe where surface water acidification is a problem. At present, ad hoc solutions are being developed, based on MAGIC, and similar models (de Vries and Posch, 2003) and these could be incorporated into future schemes.

5. Conclusions

This study represents an initial attempt to establish comparable reference condition criteria for the selection of minimally disturbed rivers in 17 countries from the Central, Baltic and western parts of Europe ("Central-Baltic Geographic Intercalibration Group"). The reference condition concept has proved extremely difficult to translate from theory to practice: the absence of truly pristine freshwaters in Europe necessitates establishment of no-effect thresholds for pressures which, themselves, are limited by a lack of strong relationships due to interactions with confounding variables. At a more basic level, even apparently simple tasks such as merging national datasets of chemical and biological variables are complicated by methodological differences. The outcome is, necessarily, a mixture of evidence-based science and expert judgment, facilitated by a group of scientists closely involved in the EU's intercalibration exercise. As such, this represents the "art-of-the-possible" rather than a definitive account of conditions associated with pristine rivers in Europe. Having been agreed by representatives from all EU Members, we believe that, for the sake of transparency, publication will ensure that others working on ecological status in Europe are aware of the thresholds and criteria.

"Reference conditions", as defined in the WFD, are open to various interpretations, from the stringent view espoused by Moss (2008), to the more pragmatic views outlined here. Our view is that a concept that is rooted in an understanding of how a truly pristine freshwater ecosystem functions needs to be balanced by the need to have enough sites that meet the desired state – for each ecoregions and stream/river type – to allow meaningful statistical relationships to be developed (without resorting to "pseudoreplication"). We respect the view of Moss (2008) and others but believe that the approach outlined here is the only way to gather sufficient data from the entire EU to ensure that ecological status assessment can be calibrated and, therefore, that results can be compared within and between countries.

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References

Allan JD. Landscapes and riverscapes: the influence of land use on stream ecosystems. Annu Rev Ecol Evol Syst 2004;35:257–84.
Armitage PD, Pardo I. Impact assessment of regulation at the reach level using macroinvertebrate information from mesohabitats. Regul Rivers: Res Manage 1995;10:147–58.
Bailey RC, Norris RH, Reynoldson TB. Bioassessment of Freshwater Ecosystems: using the reference condition approach. New York: Kluwer Academic Publishers; 2004.
Bain MB, Finn JT, Booke HE. Streamflow regulation and fish community structure. Ecology 1988;69:382–92.
Baker ME, King RS. A new method for detecting and interpreting biodiversity and ecological community thresholds. Methods Ecol Evol 2010;1:25–37.

Basnyat P, Teeter LD, Flynn KM, Lockaby BG. Relationships between landscape characteristics and nonpoint source pollution inputs to coastal estuaries. Environ Manage 1999;23:539–49.
Basset A. Aquatic science and the water framework directive: a still open challenge towards ecogovernance of aquatic ecosystems. Aquat Conserv Mar Freshwater Ecosyst 2010;20:245–9.
Bennett C, Owen R, Birk S, Buffagni A, Erba S, Mengin N, et al. Bringing European river quality into line: an exercise to intercalibrate macro-invertebrate classification methods. Hydrobiologia 2011;667:31–48.
Brabec E, Schulte S, Richards PL. Impervious surfaces and water quality: a review of current literature and its implications for watershed planning. J Plann Lit 2002;16:499–514.
Camargo JA, Alonso A, Salamanca A. Nitrate toxicity to aquatic animals: a review with new data for freshwater invertebrates. Chemosphere 2005;58:1255–67.
Carroll L. Alice's Adventures in Wonderland. London: Macmillan; 1865.
Davies SP, Jackson SK. The biological condition gradient: a descriptive model for interpreting change in aquatic ecosystems. Ecol Appl 2006;16:1251–66.
de Vries W, Posch M. Critical levels and critical loads as a tool for air quality Management. In: Hewitt CN, Jackson AV, editors. Handbook of atmospheric science: principles and applications. Malden: Blackwell Publishing; 2003. p. 562–602.
Directive 2000/60/EC of the European Parliament and of the Council establishing a framework for the Community action in the field of water policy.
COM Decision 2008/915/CE. Commission decision of 30 October 2008, establishing, pursuant to Directive 2000/60/EC of the European Parliament and of the Council, the values of the Member State monitoring system classifications as a result of the 25 intercalibration exercise (notified under document number C(2008) 6016) (2008/915/EC). Official Journal of the European Union, L332. ; 2008. p. 20–44.
DL 11.05.1999, N. 152: Disposizione sulla tutela delle acque dall'inquinamento e recepimento della Direttiva 91/271/CEE concernente il trattamento delle acque reflue urbane e della Direttiva 91/676/CEE relativa alla protezione delle acque dall'inquinamento provocato dai nitrati proveniente da fonti agricole, Suppl. Ord. n. 101/L alla G.U. 29.05.1999, 124.
Donohue I, McGarrigle ML, Mills P. Linking catchment characteristics and water chemistry with the ecological status of Irish rivers. Water Res 2006;40:91–8.
Fraterrigo JM, Downing JA. The influence of land use on lake nutrients varies with watershed transport capacity. Ecosystems 2008;11:1021–34.
Gómez-Rodríguez C, Díaz-Paniagua C, Serrano L, Florencio M, Portheault A. Mediterranean temporary ponds as amphibian breeding habitats: the importance of preserving pond networks. Aquat Ecol 2009;43:1179–91.
Groffman P, Baron J, Blett T, Gold A, Goodman I, Gunderson L, et al. Ecological thresholds: the key to successful environmental management or an important concept with no practical application? Ecosystems 2006;9:1-13.
Harding JS, Benfield EF, Bolstad PV, Helfman GS, Jones EBD. Stream biodiversity: the ghost of land use past. Proc Natl Acad Sci U S A 1998;95:14843–7.
Hawkins CP, Olson JR, Hill RA. The reference condition: predicting benchmarks for ecological and water-quality assessments. J N Am Benthol Soc 2010;29:312–43.
Hering D, Borja A, Carstensen J, Carvalho L, Elliott M, Feld CK, et al. The European Water Framework Directive at the age of 10: a critical review of the achievements with recommendations for the future. Sci Total Environ 2010;408:4007–19.
Hering D, Buffagni A, Moog O, Sandin L, Sommerhauser M, Stubauer I, et al. The development of a system to assess the ecological quality of streams based on macroinvertebrates — design of the sampling programme within the AQEM project. Int Rev Hydrobiol 2003;88:345–61.
Hering D, Johnson RK, Kramm S, Schmutz S, Szoszkiewicz K, Verdonschot PFM. Assessment of European streams with diatoms, macrophytes, macroinvertebrates and fish: a comparative metric-based analysis of organism response to stress. Freshwater Biol 2006;51:1757–85.
Hilderbrand RH, Utz RM, Stranko SA, Raesly RL. Applying thresholds to forecast potential biodiversity loss from human development. J N Am Benthol Soc 2010;29:1009–16.
Hughes RM, Larsen DP, Omernik JM. Regional reference sites — a method for assessing stream potentials. Environ Manage 1986;10:629–35.
Hynes HBN. Historical perspective and future direction of biological monitoring of aquatic systems. In: Loeb SL, Spacie A, editors. Biological monitoring of aquatic systems. Boca Raton: Lewis Publishers; 1994. p. 11–22.
Johnson RK, Furse MT, Hering D, Sandin L. Ecological relationships between stream communities and spatial scale: implications for designing catchment-level monitoring programmes. Freshwater Biol 2007;52:939–58.
Junk WJ, Bayley PB, Sparks RE. The flood pulse concept in river-floodplain systems. In: Doge DP, editor. Proc. Int. Large River Symp (Lars) — Can. Spec. Publ. Fish. Aquat. Sci., 106. ; 1989. p. 110–27.
Karr JR. Assessment of biotic integrity using fish communities. Fisheries 1981;6:21–7.
Kelly M, Bennett C, Coste M, Delgado C, Delmas F, Denys L, et al. A comparison of national approaches to setting ecological status boundaries in phytobenthos assessment for the European Water Framework Directive: results of an intercalibration exercise. Hydrobiologia 2009;621:169–82.
King RS, Baker ME. An alternative view of ecological community thresholds and appropriate analyses for their detection. Ecol Appl 2011. doi:10.1890/10-0882.1.
Lacoul P, Freedman B. Environmental influences on aquatic plants in freshwater ecosystems. Environ Rev 2006;14:89-136.
LAWA. (L¨anderarbeitsgemeinschaft Wasser): Beurteilung der Wasserbeschaffenheit von Fließgew¨assern in der Bundesrepublik Deutschland – Chemische Gew¨asserg¨uteklassifikation. Berlin: Kulturbuchverlag; 1998.
Lowrance R, Todd R, Fail J, Hendrickson O, Leonard R, Asmussen L. Riparian forests as nutrient filters in agricultural watersheds. Bioscience 1984a;34:374–7.
Lowrance RR, Todd RL, Asmussen LE. Nutrient cycling in an agricultural watershed.1. Phreatic movement. J Environ Qual 1984b;13:22–7.
Moss B. The Water Framework Directive: total environment or political compromise? Sci Total Environ 2008;400:32–41.
Moss B, Stephen D, Alvarez C, Becares E, van de Bund W, Collings SE, et al. The determination of ecological status in shallow lakes — a tested system (ECOFRAME) for implementation of the European Water Framework Directive. Aquat Conserv Mar Freshwater Ecosyst 2003;13:507–49.
Nijboer RC, Johnson RK, Verdonschot PFM, Sommerhauser M, Buffagni A. Establishing reference conditions for European streams. Hydrobiologia 2004;516:91-105.
Nõges P, Van De Bund W, Cardoso AC, Solimini AG, Heiskanen AS. Assessment of the ecological status of European surface waters: a work in progress. Hydrobiologia 2009;633:197–211.
O'Hare MT, Baattrup-Pedersen A, Nijboer R, Szoszkiewicz K, Ferreira T. Macrophyte communities of European streams with altered physical habitat. Hydrobiologia 2006;566:197–210.
Omernik JM. Ecoregions of the Conterminous United States. Ann Assoc Am Geogr 1987;77:118–25.
Pardo I, Poikane S, Bonne W. JRC scientific and technical reports. Revision of the consistency in Reference Criteria application in the phase I of the European Intercalibration exercise. Luxembourg: Office for Official publications of the European Communities; 2011.
Plafkin JL, Barbour MT, Porter KD, Gross SK, Hughes RM. Rapid bioassessment protocols for use in streams and rivers: Benthic macroinvertebrates and fish. Washington: U.S. Environmental Protection Agency, Office of Water Regulations and Standards D.C.; 1989. EPA 440-4-89-001.
Reynoldson TB, Norris RH, Resh VH, Day KE, Rosenberg DM. The reference condition: a comparison of multimetric and multivariate approaches to assess water-quality impairment using benthic macroinvertebrates. J N Am Benthol Soc 1997;16:833–52.
Reynoldson TB, Wright JF. The reference conditions: problems and solutions. In: Wright JF, Sutcliffe DW, Furse MT, editors. Assessing the biological quality of fresh waters: RIVPACS and other techniques. Ambleside: Freshwater Biological Association; 2000. p. 293–303.
Robach F, Thiébaut G, Trémolières M, Muller S. A reference system for continental running waters: plant communities as bioindicators of increasing eutrophication in alkaline and acidic waters in north-east France. Hydrobiologia 1996;340:67–76.
Robertson DM, Saad DA, Heisey DM. A regional classification scheme for estimating reference water quality in streams using land-use-adjusted spatial regression-tree analysis. Environ Manage 2006;37:209–29.
Robertson DM, Weigel BM, Graczyk DJ. Nutrient concentrations and their relations to the biotic integrity of nonwadeable rivers in Wisconsin. Reston: Professional paper 1754U.S. Geological Survey; 2008.
Smith RA, Alexander RB, Schwarz GE. Natural background concentrations of nutrients in streams and rivers of the conterminous United States. Environ Sci Technol 2003;37:3039–47.
Stevenson RJ, Hill BE, Herlihy AT, Yuan LL, Norton SB. Algae-P relationships, thresholds, and frequency of distributions guide nutrient criterion development. J N Am Benthol Soc 2008;27:259–75.
Stoddard JL, Larsen DP, Hawkins CP, Johnson RK, Norris RH. Setting expectations for the ecological condition of streams: the concept of reference condition. Ecol Appl 2006;16:1267–76.
Sweeting RA, Lowson D, Hale P, Wright JF. Biological assessment of rivers in the UK. In: Newman PJ, Piavaux MA, Sweeting RA, editors. River water quality ecological assessment and control. Proceedings of the international conference on river water quality — ecological assessment and control, Brussels, held at the Palais des Congrès. EUR 14606 EN-FR.Luxembourg: Office for Official Publications of the European Communities; 1992. p. 319–26.
Szoszkiewicz K, Ferreira T, Korte T, Baattrup-Pedersen A, Davy-Bowker J, O'Hare M. European river plant communities: the importance of organic pollution and the usefulness of existing macrophyte metrics. Hydrobiologia 2006;566:211–34.
Thorp JH, DeLong MD. The riverine productivity model: an heuristic view of carbon sources and organic processing in large river ecosystems. Oikos 1994;70:305–8.
Townsend CR, Uhlmann SS, Matthaei CD. Individual and combined responses of stream ecosystems to multiple stressors. J Appl Ecol 2008;45:1810–9.
Utz RM, Hilderbrand RH, Boward DM. Identifying regional differences in threshold responses of aquatic invertebrates to land cover gradients. Ecol Indic 2009;9:556–67.
van De Bund WJ, editor. Water Framework Directive intercalibration technical report-Part 1: Rivers, JRC Scientific and Technical Reports; 2009.
Vannote RL, Minshall GW, Cummins KW, Sedell JR, Cushing CE. The river continuum concept. Can J Fish Aquat Sci 1980;37:130–7.
Wallin M, Wiederholm T, Johnson RK. Guidance on establishing reference conditions and ecological status class boundaries for inland surface waters. Produced by CIS Working Group 2.3 — REFCOND. . Available via the internet athttp://www-nrciws.slu.se/REFCOND/7th_REFCOND_final.pdf2003.
Wang LZ, Robertson DM, Garrison PJ. Linkages between nutrients and assemblages of macroinvertebrates and fish in wadeable streams: implication to nutrient criteria development. Environ Manage 2007;39:194–212.
Wasson JG, Villeneuve B, Mengin N, Pella H, Chandesris A. Modèles pressions/impacts. Approche méthodologique, modèles d'extrapolation spatiale et modèles de diagnostic de l'état écologique basés sur les invertébrés en rivière (IBGN). Rapport, Cemagref Lyon BEA/LHQ, Lyon; 2005. 61 pp + annexes.
Wasson JG, Villeneuve B, Mengin N. Proposals for deriving "no effect thresholds" of selected chemical parameters on the Invertebrate ICM Index for the Central-Baltic GIG intercalibration. Work paper for the CB GIG Steering group; 2006.
Wasson JG, Villeneuve B, Iital A, Murray- Bligh J, Dobiasova M, Bacikova S, et al. Large-scale relationships between basin and riparian land cover and the ecological status of European rivers. Freshwater Biol 2010;55:1465–82.

Whittier TR, Stoddard JL, Larsen DP, Herlihy AT. Selecting reference sites for stream biological assessments: best professional judgment or objective criteria. J N Am Benthol Soc 2007;26:349–60.

Wright JF. An introduction to RIVPACS. In: Wright JF, Sutcliffe DW, Furse MT, editors. Assessing the biological quality of fresh waters: RIVPACS and other techniques. Ambleside: Freshwater Biological Association; 2000. p. 1-23.

Wright JF, Furse MT, Armitage PD. RIVPACS — a technique for evaluating the biological quality of rivers in the U.K. Eur Water Pollut Control 1993;3:15–25.

Wright JF, Moss D, Armitage PD, Furse MT. A preliminary classification of running-water sites in Great Britain based on macro-invertebrate species and the prediction of community type using environmental data. Freshwater Biol 1984;14:221–56.