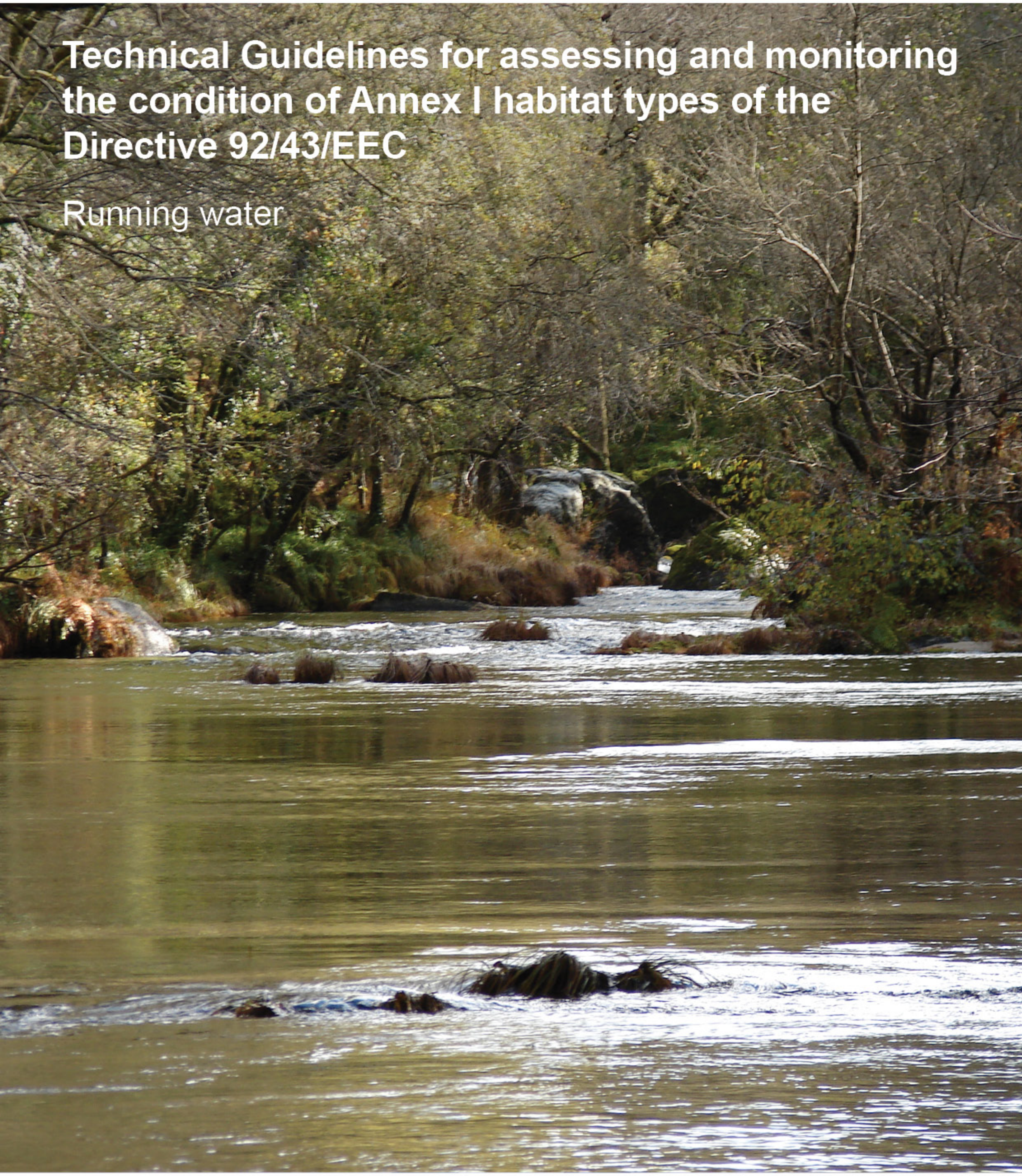
European Commission

Technical Guidelines for assessing and monitoring the condition of Annex I habitat types of the Directive 92/43/EEC

Running water



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EUROPEAN COMMISSION

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Contents

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Glossary and definitions

Habitats

Natural habitats: are terrestrial or aquatic areas distinguished by geographic, abiotic and biotic features, whether entirely natural or semi-natural (Habitats Directive).

Habitat condition: is the quality of a natural or semi natural habitat in terms of its abiotic and biotic characteristics. Condition is assessed with respect to the habitat composition, structure and function. In the framework of conservation status assessment, condition corresponds to the parameter "structure and function". The condition of a habitat asset is interpreted as the ensemble of multiple relevant characteristics, which are measured by sets of variables and indicators that in turn are used to compile the assessments.

Habitat characteristics: are the attributes of the habitat and its major abiotic and biotic components, including structure, processes, and functionality. They can be classified as abiotic (physical, chemical), biotic (compositional structural, functional) and landscape characteristics (based on the Ecosystems Condition Typology defined in the SEEA-EA; United Nations et al., 2021).

Species

Characteristic species: are species that characterise the habitat type, are used to define the habitat, and can include dominant and accompanying species.

Typical species: are species that indicate good condition of the habitat type concerned. Their conservation status is evaluated under the structure and function parameter. Usually, typical species are selected as indicators of good condition and provide complementary information to that provided by other variables that are used to measure compositional, structural and functional characteristics.

Variables

Condition variables: are quantitative metrics describing individual characteristics of a habitat asset. They are related to key characteristics of the habitat that can be measured, must have clear and unambiguous definition, measurement instructions and well-defined measurement units that indicate the quantity or quality they measure. In these guidelines, the following types of condition variables are included:

- **Essential variables:** describe essential characteristics of the habitat that reflect the habitat quality or condition. These variables are selected on the basis of their relevance, validity and reliability and should be assessed in all MSs following equivalent measurement procedures.
- **Recommended variables:** are optional, additional condition variables that may be measured when relevant and possible to gain further insight into the habitat condition, e.g. according to contextual factors; these are complementary to the essential variables, can improve the assessment and help understand or interpret the overall results.
- **Specific variables:** are condition variables that should be measured in some specific habitat types or habitat sub-groups; can thus be considered essential for those habitats, which need to be specified (e.g. salinity for saline grasslands, groundwater level for bog woodlands, etc.).

Descriptive or contextual variables: define environmental characteristics (e.g. climate, topography, lithology) that relate to the ecological requirements of the habitat, are useful to characterise the habitat in a specific location, for defining the relevant thresholds for the condition variables and for interpreting the results of the assessment. These variables, however, are not included in the aggregation of the measured variables to determine the condition of the habitat.

Reference levels and thresholds: are defined for the values of the variables (or ranges) that determine whether the habitat is in good condition or not. They are set considering the distance from the reference condition (good). The value of the reference level is used to re-scale a variable to derive an individual condition indicator.

Condition indicators: are rescaled versions of condition variables. Usually, they are rescaled between a lower level that corresponds to high habitat degradation and an upper level that corresponds to the state of a reference habitat in good condition.

Aggregation: is defined in this document as a rule to integrate and summarise the information obtained from the measured variables at different spatial scales, primarily at the local scale (sampling plot, monitoring station or site).

Abbreviations

EU**:** European Union

HD – Habitats Directive

IAS – Invasive Alien Species

MS: Member State

EU Member States acronyms:

Austria	(AT)	Estonia	(EE)	Italy	(IT)	Portugal	(PT)
Belgium	(BE)	Finland	(FI)	Latvia	(LV)	Romania	(RO)
Bulgaria	(BG)	France	(FR)	Lithuania	(LT)	Slovakia	(SK)
Croatia	(HR)	Germany	(DE)	Luxembourg	(LU)	Slovenia	(SI)
Cyprus	(CY)	Greece	(EL)	Malta	(MT)	Spain	(ES)
Czechia	(CZ)	Hungary	(HU)	Netherlands	(NL)	Sweden	(SE)
Denmark	(DK)	Ireland	(IE)	Poland	(PL)		

SEEA EA – System of Environmental Economic Accounting- Ecosystem Accounting

WFD: Water Framework Directive

Executive summary

These technical guidelines present a general characterisation of running water habitats included in the Habitats Directive, an analysis of methodologies used by EU Member States for assessing and monitoring the condition of these habitats, and provide a framework for harmonisation of these methodologies. The main objective of this guidance is to support Member States in applying a standardized and consistent approach for evaluating and reporting habitat condition in accordance with Article 17 of the Habitats Directive.

Running waters included in subgroup 32 in the Annex I of the Habitats Directive comprise stream and river reaches from all the biogeographical regions, which are described as "Sections of water courses with natural or semi-natural dynamics (minor, average and major beds) where the water quality shows no significant deterioration".

Rivers are highly dynamic ecosystems, and their structure, functions and species composition are strongly influenced by the unidirectional flow. Flow and hydrological regime are considered the main structuring factors of stream biological communities. A functional river with a natural flow exhibits a diversity of structural elements, a strong connection to riparian vegetation as well as other lateral habitats and ecosystems. Sediment transport occurs laterally and longitudinally, facilitating the distribution of patches of aquatic and riparian vegetation.

The document includes a proposal of variables to measure habitat condition, outlines different approaches for setting reference values and threshold to determine good condition and describes methods for the aggregation of results. This framework aims to ensure comparability of assessments across regions and enhance the implementation of national monitoring programmes, which are crucial to inform conservation actions and policy decisions.

The concept of Condition Variables is central to this guidance. These are quantitative indicators that reflect habitat quality and functioning, and are classified as essential, specific and recommended variables, depending on their ecological importance and site specificity. Descriptive or contextual variables (e.g. temperature, precipitation, substrate characteristics) are also proposed to help interpret results and define ecologically meaningful thresholds, though they are not used directly in condition scoring. Condition variables are assessed to determine the degree of habitat condition (good/nor good) relative to defined thresholds.

These guidelines review several approaches to variable aggregation, discussing the merits and drawbacks of each. The alignment and coordination with the assessment of ecological status in the implementation of the Water Framework Directive is also considered and proposed in these guidelines.

1. Definition and ecological characterisation

1.1 Definition and interpretation of habitats covered

Rivers support some of the most biodiverse ecosystems in the world and provide essential ecosystem services to society (Grizzetti et al., 2019). Accordingly, these ecosystems are considered biodiversity hotspots (Strayer & Dudgeon, 2010). However, at the same time, freshwater ecosystems are among the most endangered ecosystems in the world (Dudgeon et al., 2006), and rivers are among the most threatened (Reid et al., 2019; Grzybowski & Glińska-Lewczuk, 2019) due to overexploitation, pollution, regulation, climate change, drainage-basin degradation, non-native species, and synergistic impacts (Dudgeon et al., 2006; Dudgeon, 2013). As a result, this extensive habitat deterioration is causing a decline in biodiversity of freshwater ecosystems that is far more significant than in the most affected terrestrial ecosystems (Sala et al., 2000).

Rivers, as highly dynamic ecosystems, having their structure, functions and species composition strongly influenced by the unidirectional flow. As they drain and shape the landscape, rivers act as a structuring element, but at the same time, in their waters, they detect and reflect every change that occurs within their drainage basin. These waters are loaded with ions from the basin depending on the way in which the flow interacts with the different substrates. As a result, the physicochemical characteristics of the water and its flow regime are both an expression of the basin and determinants of the community and functioning of the river ecosystem.

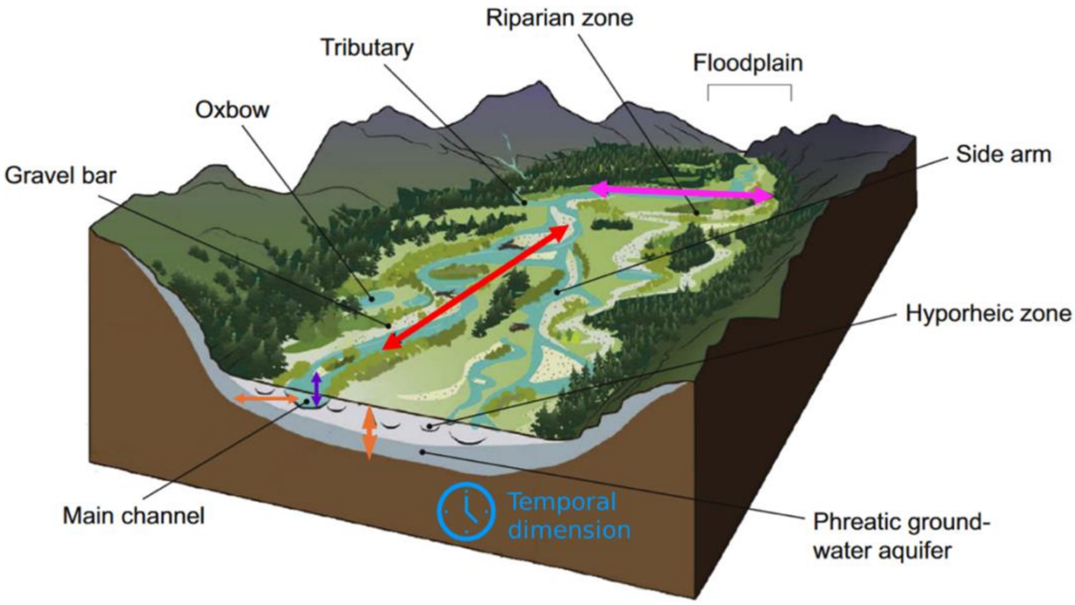
The flow, as a structuring agent, determines the characteristics and processes along the channels. Flow and hydrological regime are considered the main structuring factors of stream biological communities (Poff et al., 1997; Poff, 2018) and several studies have demonstrated a close relationship between the hydrological regime and biological communities (Bunn et al., 2002; Monk et al., 2006; Belmar et al., 2013). Spatial and temporal connectivity play a crucial role in maintaining functional river ecosystems. The longitudinal (from headwater sections to the mouth), lateral (between the river channel and the floodplain) and vertical connectivity (between surface ecosystems and aquifers), as well as its temporal component, which is especially important in Mediterranean ecosystems dominated by temporal systems, are essential for maintaining key ecological processes, such as the transfer of matter and energy throughout the system and seasonal migratory movements. Therefore, the longitudinal axis of the river most clearly determines the succession and changes at any spatial scale and the heterogeneity of the different types of habitat and river ecosystems, which are structured along the other spatial axes: the vertical, of the hyporheic and surface layers of the water; and the lateral or transverse channel and alluvial plain (Junk et al., 1989; Vannote et al., 1980).

This type of ecosystem, in which the abiotic component is the determining factor and where flow regimes define the structure and function of the ecosystem, differs fundamentally from ecosystems such as forests, where floristic composition or the dominant tree species dictate structure and function. In fluvial ecosystems, structure and functions are intertwined, with indistinct boundaries between both.

A functional river with a natural flow regime is simultaneously a well-structured river. It exhibits complexity and a diversity of structural elements, such as bars, islands, and a strong connection to riparian vegetation as well as other lateral habitats and ecosystems. Such rivers are dynamic, with sediment transport occurring laterally and longitudinally, facilitating the redistribution of patches of macrophytes, helophytes, and riparian vegetation. This dynamic interaction ensures the ecological integrity and resilience of the system. As a result, it is not easy to determine which variables are structural or functional and, in fact, the main structural and functional variable is water flow, its volume and pattern.

All these ecological processes are crucial for maintaining the biodiversity and ecosystem services associated with these environments. River ecosystems are also important backbone elements of the landscape, facilitating connectivity at the landscape level for species that are not strictly dependent on water. However, connectivity in all its dimensions has undergone significant modifications over the last century.

Figure 1. A gravel-bed floodplain river with its main elements



The arrows show the river's four-dimensional structure, i.e., its longitudinal (red), vertical (orange and blue), transversal floodplain (purple) gradients and temporal dimension.

Source: modified from Stanford, 1998; Hauer and Lamberti, 2007

On the other hand, the condition of the river must be understood by considering a basin perspective, river ecology and connectivity. In the case of lotic ecosystems, longitudinal connectivity plays a determining role in the maintenance of ecological processes that support freshwater biodiversity (e.g., migrations and gene exchange). However, it also contributes to the spread of threats throughout the system.

The Habitat Directive 92/43/EEC (HD) (European Council, 1992), in its Annex I, lists the Habitat Types of Community Interest (hereinafter HTCIs) that must be subject to protection, evaluation and monitoring. According to the Interpretation manual of European Union habitats (European Commission, 2013), running waters included in subgroup 32 of Annex I comprise stream and river reaches from the Boreal (e.g., 3210), to the Mediterranean (e.g., 3280 and 3290) biogeographical regions and are described as “Sections of water courses with natural or semi-natural dynamics (minor, average and major beds) where the water quality shows no significant deterioration”. This document proposes grouping these habitat types into permanent and temporary rivers, according to their flow regime (Table 1).

The listing of ten river habitats, however, can hardly represent the European diversity of streams. These habitats must be interpreted as rivers or water courses with distinctive characteristics that can include certain types of vegetation, species or lists of species

(European Commission, 2013), but in no case should they be considered as just vegetation patches.

Table 1. Running Water habitats included in the Annex I of the Habitats Directive

Groups	Cod.	Habitat Name
Permanent	3210	Fennoscandian natural rivers
	3220	Alpine rivers and the herbaceous vegetation along their banks
	3230	Alpine rivers and their ligneous vegetation with *Myricaria germanica*
	3240	Alpine rivers and their ligneous vegetation with *Salix elaeagnos*
	3250	Constantly flowing Mediterranean rivers with *Glaucium flavum*
	3260	Water courses of plain to montane levels with the *Ranunculion fluitantis* and *Callitricho-Batrachion* vegetation
	3270	Rivers with muddy banks with *Chenopodion rub*ri p.p. and *Bidention* p.p. vegetation
	3280	Constantly flowing Mediterranean rivers with *Paspalo-Agrostidion* species and hanging curtains of *Salix* and *Populus alba*
	32A0*	Tufa cascades of karstic rivers of the Dinaric Alps
Temporary	3290	Intermittently flowing Mediterranean rivers of the *Paspalo-Agrostidion*

The Water Framework Directive 2000/60/EC (WFD), the main EU legislative act regarding water resource management, seems to have integrated some of the concepts of stream ecology. To evaluate the ecological status of freshwater ecosystems, the WFD considers physicochemical, biological and hydromorphological variables, indices, and parameters that, with some limitations, capture river structure and functionality, i.e. the essence as a stream ecosystem. This is key because the protection and conservation of ecosystems require an in-depth comprehension of their function and structure, and streams are among the most dynamic and complex ecosystems.

Unfortunately, there is no link between habitat types from group 32, “Running waters” in the Habitat Directive Annex I and the types of rivers defined under the WFD. Consequently, it is not possible to establish crosswalks between them. Running waters in Annex I of HD have in most cases been defined based on riverine vegetation and aquatic plant communities rather than on typically fluvial communities (Toro et al., 2009a-h). The different classifications carried out by Member States for streams and rivers in the implementation of the WFD use variables such as altitude, geology, size of the catchment area systems (systems A and B) and water temperature, precipitation, chloride, morphological variables, and others (system B). As a result, the classifications obtained under the Habitat and Water Framework Directives have little in common. The WFD provides a perspective closer to fluvial ecology, whereas the HD mostly relies on a classification based mostly on vegetation. Even though both directives share the same physical space, the rivers or running waters, and the objective of classifying and protecting streams and rivers, their approaches differ significantly.

Nevertheless, both directives, the HD and the WFD, have been adopted inter alia to guarantee the good ecological status of EU lotic ecosystems. They have common objectives and similar monitoring requirements (Schmedtje et al., 2011; Janauer, et al., 2015). Therefore, their integration and coordination seem to be an essential step towards the assessment and monitoring of the condition of river habitats. These guidelines will try to promote further coordination in the monitoring of running water habitats under both the Habitats and the Water Framework Directives.

1.2 Environmental and ecological characterisation and selection of variables to measure habitat condition

One of the main characteristics of fluvial ecosystems is that their structure, functioning and community composition are primarily influenced by the unidirectional flow of water and its characteristics. Flow is a determining factor for the characteristics of the various elements that define the ecosystem and the processes along the river course. However, this unidimensional and unidirectional feature is not the only one. The river's longitudinal axis determines spatial succession or changes and the heterogeneity of different types of habitats or fluvial ecosystems. Still, rivers are in turn structured along other spatial axes: the vertical (from the hyporheic zone to the water surface) and the lateral or transversal (from the channel to the floodplain). These dynamics give rise to processes such as nutrient spiralling (Ensign & Doyle, 2006; Newbold et al., 1981; Vannote et al., 1980) and interactions with riparian vegetation, which must be considered a part of the lotic ecosystem.

On the other hand, rivers cannot be only considered only as sections of water course, but as a complete ecosystem from headwaters to the mouth. Thus, a river can only be understood holistically as an ecosystem that should be analysed in its entirety, across its entire length, according to the River Continuum Concept (Vannote et al., 1980). Lotic ecosystems are among the most structurally unique ecosystems due to their inherently linear nature, where their function and structure are primarily determined by flow. Moreover, rivers are intrinsically linked to their catchment areas both structurally and functionally (Downes et al., 2002) and cannot be perceived as isolated ecosystems but rather as elements interacting with the surrounding riparian forests, the hyporheic zone, groundwater, and floodplains. These interactions generate intricate dynamics related to sediments, the transport and processing of organic matter, and nutrient spiralling (Elosegi & Sabater, 2009; Ensign & Doyle, 2006; Meyer, 1997; Newbold et al., 1981; Vannote et al., 1980; Ward, 1989).

Just as vegetation acts as the structural element in a forest ecosystem, flow serves this role in lotic ecosystems. Therefore, rivers as ecosystems have been defined under a set of premises that must be considered when they are assessed, as they are closely linked to these premises (Dodds, 2002):

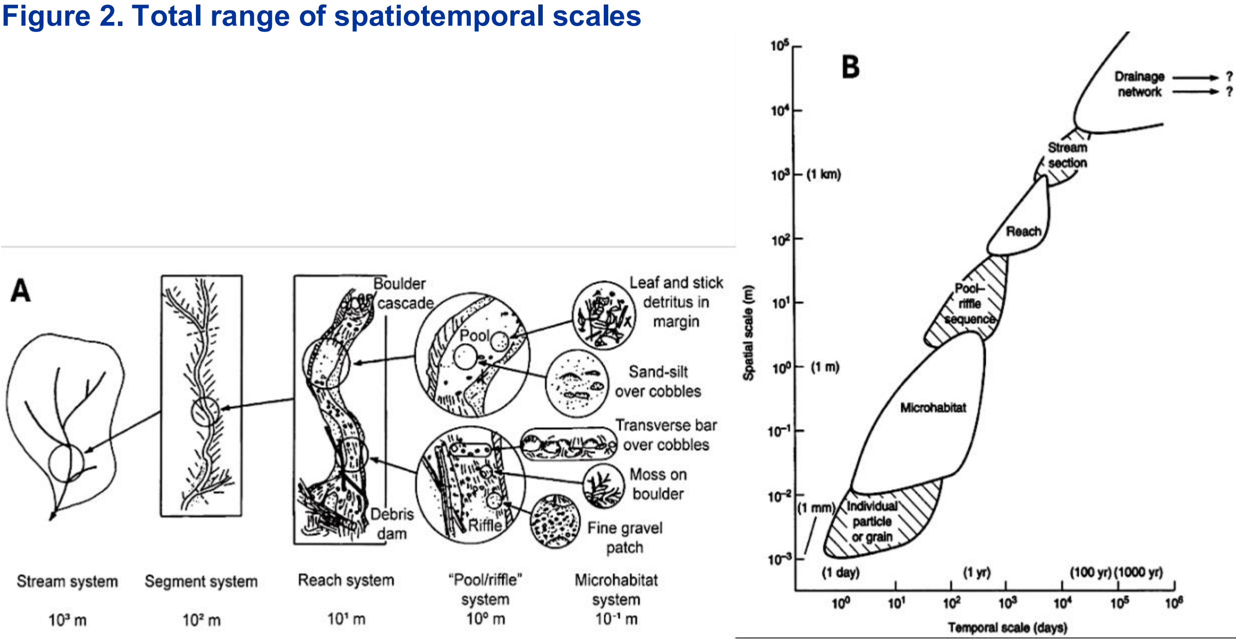
- **The Flood Pulse Concept** (Junk et al., 1989). Far from viewing floods as a perturbations or disturbances that alter or even destroy fluvial ecosystems, they must be considered an intrinsic property of the function of river and stream ecosystems. A functional river has to cover the floodplain regularly. Floods help control primary producers, contribute to fertilizing crops and capture organic matter from surrounding ecosystems.
- **Autochthonous versus allochthonous production**. Streams have a strong influence from terrestrial derived organic material. The source of this organic material determines the composition and characteristics of the macroinvertebrate community (Cummins 1973). However, this contribution of riverside vegetation must not be considered an allochthonous source of organic carbon since the gallery forest, riparian forest, or woodland, is a fundamental part of fluvial ecosystems. Traditionally, it has been considered that respiration is higher than production in rivers, partly because primary production from riparian forests has not been considered.
- **Inverted biomass pyramid** (Allen's paradox). This phenomenon is common in aquatic ecosystems. The problem arises from measuring primary production as the biomass of producers when their removal and growth rates are high, and, as a consequence, their

production per unit of biomass is high enough to support primary, secondary and even tertiary consumers (Allan, 1995; Benke 1984).

- **Nutrient spiralling** (Ensign & Doyle, 2006), Traditionally, the cycle of nutrients has been conceptualized as a wheel in lakes, but in streams, due to drift processes, the cycling of nutrients in unidirectional flow environments forms a spiral. The length of this spiral is determined by the flow velocity.
- **The River Continuum Concept** (RCC) (Vannote et al., 1980) is one of the most important and influential ideas in stream ecosystem theory. According to this theory, stream have to be conceptualised as a connected continuum from small, forested headwater streams to large rivers. The associated gradient in abiotic and riparian characteristics shapes the biological community.

While there are other descriptive concepts of fluvial ecosystems, these exemplify some of the key characteristics of lotic ecosystems (Dodds, 2002) that must be considered when a monitoring programme is implemented. In fact, the River Continuum Concept describes processes from the catchment area to microhabitat scales within the context of highly unstable ecosystems, such as many streams, where the biotic component assumes critical importance for the ecosystem stability (Vannote, 1980). Scales are essential in fluvial ecosystems and physical, chemical and hydromorphological processes take place at spatial scales, ranging from individual particles to the entire drainage basin and over an equally broad temporal scale. Therefore, monitoring and research must consider the total range of spatiotemporal scales (Figure 2).

Figure 2. Total range of spatiotemporal scales



A) Hierarchical and nested organisation of a stream ecosystem. B) Conceptual spatio-temporal scale where physical processes take place in rivers.

Source: Frissell et al. (1986)

Due to these characteristics, assessing the structure and function of these ecosystems is a complex task that cannot be addressed by analysing isolated stretches of a catchment area. A comprehensive holistic approach is needed for evaluating rivers, based on the River Continuum Concept (Ensign &Doyle, 2006; Newbold et al., 1981; Vannote et al., 1980).

However, this approach is not always practical, and when applied, one often finds that the majority of rivers do not function properly or are not structured correctly as ecosystems. Hence, there is a need to adopt an approach based on river stretches.

Monitoring lotic ecosystems requires the integration of multiple data that allows for a comprehensive evaluation of these ecosystems (Barbour et al. 1999). Integrating the information obtained from bioindicators, physicochemical analysis and hydromorphological evaluation will allow generating a diagnosis that closely reflect the reality of the conservation status of river ecosystems (Barbour et al. 1999; Karr & Chu, 1997; Schlosser & Karr, 1981).

Selecting key characteristics and variables to measure habitat conditions in lotic ecosystems involves a multidisciplinary approach that traditionally integrates **physical, chemical, and biological parameters**. This ensures a comprehensive understanding of ecosystem function, species diversity and health. Proper selection of these variables, tailored to the key characteristics that determine the condition of these habitat types, enables their effective assessment and monitoring. The selected variables should consider the following main aspects (Downes et al., 2002):

- From a structural and functional perspective, lotic ecosystems are intrinsically linked to their catchment areas.
- The functionality of lotic ecosystems is strongly influenced by longitudinal processes and predominantly unidirectional interactions (headwater to mouth), but also by lateral processes and interactions (channel to floodplain), and vertical processes and relationships (channel to hyporheic zone).
- Lotic ecosystems support a rich and diverse biota that is specialised and adapted to thrive in these dynamic environments.
- They exhibit a high degree of spatial and temporal heterogeneity, which is particularly pronounced in Mediterranean lotic ecosystems characterised by high intra- and inter-annual variability.

The ecological characterisation of river habitats should consider their main functional and structural characteristics as well as the key processes that are important for ecosystem persistence. These processes should ensure the flux of matter and energy and the conditions needed to maintain water flow, characteristic patterns, continuity and connectivity; otherwise, the system may shift to alternative states depending on its resistance and resilience.

To address the characterisation and monitoring of freshwater ecosystems, a number of variables have traditionally been selected and used. These include variables reflecting the fluvial ecology perspective (Allan et al. 2007; Allan 1995; Hauer & Lamberti, 2007) and applied under the Water Framework Directive, which can be grouped according to the classification proposed by the United Nations (UN, 2021) within the SEEA-EA framework (Table 2).

Table 2. Ecological characterisation and selection of possible variables for the evaluation of river habitats condition, classified according to the SEEA-Framework

Ecological characteristics	WFD Classification	Stream Ecology Classification	Ecosystem Condition Typology	Key characteristics	Example of variables
Abiotic characteristics	Physicochemical and hydro-morphological	Chemical	Chemical state characteristics	Descriptors of the chemical characteristics of the abiotic component of lotic ecosystems: water mineralisation and type of dominant salts, acidity, major ions, alkalinity, other ions, dissolved gases. Inorganic nutrients (nitrate, nitrite, ammonium, sulphate, sulphite). Organic matter (dissolved and particulate organic matter concentrations), oxygen demand., pollutants and heavy metals.	pH; alkalinity; salinity; water electrical conductivity (µS/cm); oxygen (O_2); carbon dioxide (CO_2); nitrogen (N_2) (mg/L; %); nitrates; nitrites; ammonium; sulphate; sulphites; phosphorus; major dissolved ions (Ca^{2+}, Na^{+}, Mg^{2+}, K^{+}, Cl^{-}); Ca^{2+}/Na^{+} ratio; dissolved organic matter (DOM); particulate organic matter (POM); environmental quality standards (EQS); priority substances; Al, Fe, Ca, Mg; biological oxygen demand (BOD); chemical oxygen demand (COD).
		Physical	Physical state characteristics	Physical state descriptors of the abiotic components of the ecosystem, such as climate, which largely determines hydrology. Geology, particularly rock lithology in the river basin and its catchment area, which transfer salts to the runoff water. Water physical features, such as transparency, temperature, and density.	Air and water temperature (T_{air} ºC, T_{water} ºC ºC); precipitation (annual, average and accumulated in mm); water electrical conductivity (µS/cm); light; turbidity.
		Hydromorpho-logica		Watershed (basin or catchment area): surface, description and characterisation. Geomorphological characteristics determine the configuration, structure, and function of each specific lotic ecosystem. Stream flow and its alterations, sediment load, and processes involved in the persistence of the system. Riverside soil (edaphological) characteristics. Connection to groundwater (inflow and outflow), renewal rate, hydroperiod, and water level fluctuations.	Water velocity (m/s), water and sediment flow (m^3/s), substrate description (boulder, cobble, pebble, gravel...), geological characterisation of the watershed, river section (m^2), sinuosity, siltation (mm/year), land use surface area (km^2), riffle-pools sequences, bars, islands, floodplain condition.

Ecological characteristics	WFD Classification	Stream Ecology Classification	Ecosystem Condition Typology	Key characteristics	Example of variables
		Hydromorpho-logical		Connectivity	Longitudinal, lateral, vertical and temporal connectivity dams, weirs...); lateral, transversal and longitudinal barriers (number, characteristics, etc.); permeability; transversal barriers; connectivity between subpopulations (genetic flow).
				Substrate	Substrate description (boulder, cobble, pebble, gravel...), Fluvial Habitat Index (FHI)
				Morphology	Stream order (Strahler), river section, sinuosity, shoreline length (m), riffle and pool sequences, reach length and width (m), area (m^2), bars, islands…
Biotic characteristics	Biological	Biotic[2]	Compositional state characteristics	Riverside vegetation	Presence, abundance and composition of riverine vegetation. Index for assessing the quality of riparian vegetation.
					Species and functional diversity of riverine vegetation.
				Species assemblages	Presence, abundance, age class and population structure and composition of bacteria, phytobenthos and plankton, fungi, macrophytes, macroinvertebrates, fish and other animal species (birds, amphibians, reptiles, mammals…).
			Structural state characteristics	Structure of the community	Species diversity (richness, Shannon–Wiener index, etc.) of bacteria, phytobenthos and plankton, fungi, macrophytes, macroinvertebrates, fish and other nektonic fauna, and other species (amphibians, reptiles, birds, mammals…).

Ecological characteristics	WFD Classification	Stream Ecology Classification	Ecosystem Condition Typology	Key characteristics	Example of variables
			Functional state characteristics	Processes involved in the persistence of the system, such as primary production, consumption and food webs, interspecific interactions, colonisation processes, biogeochemical cycles (nutrient spiralling), planktonic chlorophyll-a concentration, and decomposition.	Chlorophyll-a, O_2, O_2%, functional diversity, average energy transfer between trophic levels (%), food web structure, woody debris, and decomposition.
Landscape characteristics	Hydro-morphological	Biotic	Landscape and Ecosystem characteristics	Ecosystem spatial scale and connectivity: metrics describing spatial characteristics at ecosystem level (river basin), mosaics of ecosystem types at broad (landscape) spatial scales (e.g. landscape diversity, connectivity, fragmentation), including processes such as exchange of individuals (immigration-emigration processes), fluxes of materials and energy.	Stream order; longitudinal, lateral, vertical and temporal connectivity (dams, weirs, etc.); lateral, transversal and longitudinal barriers (characteristics, etc.); permeability, transversal barriers; predominant surrounding land use type; channelisation;
					connectivity between isolated subpopulations (genetic flow); connectivity and permeability of transversal barriers.
					Genetic flow between subpopulations.

2 - According to the WFD and stream ecology perspectives, this category can be divided into groups defined by taxonomic criteria (Table 3)

From the stream ecology perspective and the Water Framework Directive approach, which follows the former, biological characteristics should be classified as shown below (Table 3).

Table 3. Classification and selection of biological characteristics and variables linked to the ecological characterisation and evaluation of stream and river ecosystem conditions according to the WFD and stream ecology

Ecological characteristics	WFD Classification	Types	Description	Example of variables
Biotic characteristics	Biological	Bacteria	Bacterial organisms	Presence or abundance, composition and diversity of bacteria
		Fungi	Fungi in lotic ecosystems drive organic matter decomposition and nutrient cycling	Presence or abundance, composition and diversity of fungi
		Phytobenthos*	Phytobenthic organisms especially diatomaceous	Presence or abundance, composition and diversity of phytobentos
		Macrophytes*	Hydrophytes and macrophytes are plants adapted to aquatic ecosystems	Presence or abundance, composition and diversity of macrophytes
		Macroinvertebrates*	Benthic invertebrate organisms	Presence or abundance, composition and diversity of macroinvertebrates (arthropods, molluscs, etc.)
		Fishes*	Fish community	Presence or abundance, composition and diversity of fish
		Amphibians and Reptiles	Herpetofauna, not only strictly aquatic, but linked to the riparian zone	Presence or abundance, composition and diversity of amphibians and reptiles
		Birds	Birds, not only strictly aquatic but linked to the riparian zone	Presence or abundance, composition and diversity of birds
		Mammals	Mammals, not only strictly aquatic, but linked to the riparian zone	Presence or abundance, composition and diversity of mammals
		Riverside vegetation	Riparian vegetation is the community of plants along the river margins	Presence or abundance, composition, structure, and diversity of riverbank vegetation in the riparian zone

* These groups are included in the WFD monitoring system.

From an ecological perspective, classifying organisms by functional or structural characteristics is complicated because they are an integral part of the ecosystem, but at the same time, they play functional roles in these ecosystems, such as producers, predators, etc.

In fact, when we use the term diversity or biodiversity, we are not only referring to richness or composition (structure), but also to functional diversity (function).

Hereafter, we will summarise and explain some of the most important environmental and ecological variables.

1.2.1 Abiotic characteristics

Abiotic variables used to evaluate stream ecosystems can be classified into physical, chemical and hydromorphological categories according to fluvial ecology (Allan 1995; Allan et al. 2007; Dodds 2002; Hauer & Lamberti, 2007; Elosegi & Sabater, 2009).

Physical

Fluvial ecosystems are characterised by distinct physical properties derived from the aquatic environment. Water is a three-dimensional ecosystem and a medium denser than air, whose physical characteristics, such as temperature, can modify these properties, as well as the presence of dissolved gases like oxygen, which are crucial for the presence of living organisms.

The most important physical characteristics for evaluating river habitats are temperature and light. Temperature influences the metabolic rates of aquatic organisms, their reproductive cycles, and the solubility of gases like oxygen. Moreover, bellow 4 °C, the physical characteristics of water change (lowest density) and at 0 °C and below it enters another state (solid). An increase in temperature may have significant effects on the metabolism of many ectothermic organisms, on spawning (the temperature determines the maturation of fish egg, among others), size of organisms, etc. It also has many implications for chemical processes, such as the dissolution of oxygen. Warmer water holds less oxygen, which can stress or harm species sensitive to oxygen levels, while colder waters provide oxygen for some species and reduce the effects of organic matter decomposition. Temperature can determine the occurrence and condition of habitat types. For instance, a high mountain river (habitat type 3220) requires low temperatures, with a maximum of 16 °C. These temperatures are also essential for species like salmon, among others. An increase in temperature for any reason will alter these conditions and can make the habitat unsuitable for these species, as there is less oxygen available, and the eggs may be lost or fail to hatch properly.

In relation to light, water turbidity is linked to the concentration of suspended particles in the water. High turbidity can reduce light penetration, affect photosynthesis, and can clog the gills of fish and other aquatic organisms.

Hydromorphological

The main hydromorphological abiotic variables are streamflow, measured as discharge, velocity, flow variability, inundation frequency, and inundation duration. Flow determines habitat structure, nutrient availability, and sediment transport or sediment load. Seasonal changes in flow (e.g., floods and droughts) create dynamic habitats that are essential for species that d depend on fluctuating conditions. Other relevant hydromorphological variables include substrate composition, which refers to the structure and distribution of sediments (sand, gravels, rocks, wood debris, etc.) and bedrock, the regime of solid discharges or sediment load in rivers and the obstacles that could cause the retention of these sediments. The amount and type of sediment in a river influences the physical structure of habitats (e.g., riverbeds) and can affect water quality. Too much sediment can smother habitats, reduce light penetration, and clog fish gills also increasing turbidity. Excessive sedimentation can significantly impair the habitat of fish, particularly affecting spawning and juvenile stages.

Channel morphology is also considered a hydromorphological aspect. It is measured as stream width, depth, and shape (sinuosity, ponds, etc.) and provides essential information for detecting processes such as incision in the fluvial network and the loss of natural dynamics that increase the variability of the channel structure, complexity and connectivity with lateral and riparian ecosystems. The connectivity of the river with groundwater ecosystems, the spatial distribution of this connectivity and the balance of this connection are other hydromorphological parameters that should be measured to evaluate the state of fluvial ecosystems. As the riparian zone is part of fluvial ecosystems, the evaluation of its structure is also essential for evaluating the state of these ecosystems, so identifying the type, composition and density of riparian vegetation along the stream banks is crucial. When riparian forests are assessed and monitored separately, e.g. as part of the assessment of forest habitats in a country, it is advisable to integrate both assessments of riparian forests and river habitats in the relevant localities.

Chemical

Oxygen, carbon dioxide and nitrogen co-occur as dissolved gases in river waters in significant amounts. Both oxygen and CO_2 occur in the atmosphere and dissolve into water according to partial pressure and temperature. The solubility of oxygen in freshwater decreases with altitude, due to lower atmospheric partial pressure, as well as with increasing salinity and temperature. Oxygen is vital for the respiration of most aquatic organisms. Low oxygen levels (hypoxia) can lead to "dead zones" where life cannot be sustained, while sufficient oxygen supports biodiversity and healthy ecological functions. Typically, dissolved oxygen levels are lower in groundwater, while CO_2 levels are often high due to microbial processing of organic matter as water passes through soil. Localities that receive substantial groundwater inputs may reflect this, but equilibration with the atmosphere usually occurs once hyporheic water enters the stream. Therefore, CO_2 concentrations in stream water are influenced not only by atmospheric diffusion and instream metabolism but also by groundwater inflows, which are often substantially enriched with CO_2 due to soil respiration throughout the catchment. In small streams with turbulent flow, increased diffusion maintains oxygen and CO_2 near saturation. However, in larger rivers, diffusion is less important, and other processes, such as decomposition of organic matter by microorganisms, become more significant.

Nitrogen can be incorporated into nitrogen (N) cycling within stream ecosystems by certain specialized bacteria, but the concentration of dissolved N_2 itself is of little biological importance, except during eutrophication processes.

The dissolved constituents characterise the river water. The sum of total dissolved ions concentrations is referred to as total dissolved solids (TDS). The major dissolved ions include four cations (Na^+, K^+, Ca^{2+}, Mg^{2+}) and four anions (HCO_3^-, CO_3^{2-}, SO_4^{2-} and Cl^-). Salinity refers to the sum of the concentrations of all dissolved ions, and is therefore more inclusive term than TDS, although for practical purposes, conductivity measures the electrical conductance of water and provides an approximate measure of total dissolved ions.

In stream ecosystems, organic matter is an essential source of energy and nutrients. Organic matter, both dissolved and particulate, plays a fundamental role in stream ecosystems, serving as a critical component in energy flow, nutrient cycling, and habitat structure. Dissolved organic matter (DOM) includes small organic molecules, like sugars, lipids, amino acids and other compounds that are readily available to microorganisms, such as bacteria and fungi consumption, making it a vital base for the food web. Particulate organic matter (POM) consists of larger organic particles, such as leaf litter, woody debris, other decaying plant material, and parts of macroinvertebrates or other animals. It forms the basis of the detrital food chain, where decomposers break down POM.

The acidity or alkalinity (pH levels) of water affects the solubility and availability of nutrients and toxins, such as heavy metals. Extreme pH levels can be harmful to aquatic life and affect the bioavailability of metals and chemicals, impacting water quality and organism health. A change in the pH of a river, e.g., from acidic to more neutral would have a significant impact on the bacterial community, among others, within the river.

Nutrients, such as nitrates, phosphates and sulphates, are essential for plant and algae growth, but excessive concentrations can lead to eutrophication processes and harmful algal blooms, which reduce oxygen levels and degrade the ecosystem's overall health.

Finally, pollutants should also be monitored and analysed because their effects, such as acute and chronic toxicity to aquatic organisms, and their accumulation within ecosystems, can cause habitat loss, biodiversity decline, and, moreover, pose a serious threat to human health. The Water Framework Directive 2000/60/EC specifies in Article 16 a list of environmental quality standards (EQS) for priority substances and certain other pollutants aimed at achieving good surface water chemical status, in accordance with the provisions and objectives of Article 4 of that Directive. Directive 2008/105/EC establishes environmental quality standards (EQS).

1.2.2 Biotic characteristics

Physical, chemical and hydromorphological variables are extremely useful for evaluating the state of fluvial ecosystems. These variables interact to shape the physical and biological structure of these ecosystems, influencing species diversity, abundance, distribution and overall ecological balance. Monitoring these biotic variables is also essential for assessing the condition of riverine systems. For instance, a temporary alteration of temperature or a chemical discharge cannot be detected by a discontinuous measuring system. In these cases, the use of biological indicators becomes essential for assessing lotic ecosystems.

Traditionally, algae, macrophytes (aquatic plants), macroinvertebrates and fish communities, their composition, diversity and condition have been used to evaluate lotic ecosystems, and they are core components of the WFD's Biological Quality Elements. Macrophytes and phytobenthos (algae) help assess nutrient levels and water clarity. For example, excessive algal growth can indicate nutrient pollution. Macroinvertebrates are good bioindicators, especially those that are sensitive to pollution (e.g., mayflies, stoneflies), including acidification and oxygen levels and, consequently organic matters. As outlined by both the WFD and the Habitats Directive, monitoring fish species diversity, abundance, and population structure provides insights into ecological status.

Other biological components were not previously included in monitoring programmes and evaluation systems, or they appeared as part of other components. For example, riparian vegetation, due to its close link to water flow, sediment loads and their dynamics, is usually included as part of the hydromorphological variables, and this approach is not incorrect, but it might be reconsidered as part of the biotic component. Bacteria and fungi are essential due to their role in matter cycling through organic matter decomposition, such as woody debris. Their role in the decomposition of dead wood and leaf litter is a key factor in lotic ecosystems, but, until now, they have not been part of the usual monitoring systems. Vegetation debris significantly influences the physical habitat, affecting hydromorphology, because it can alter flow patterns, create different habitats, and influence sediment transport. Moreover, it provides habitat, refuge and food for invertebrates, fish, and other aquatic organisms, which are used as indicators of ecological status. Finally, it plays a key role in nutrient cycling, because decomposing debris releases nutrients, impacting water quality.

In addition to fish species, which are usually monitored in rivers, many species of amphibians, reptiles, birds and mammals, are also strictly linked to aquatic ecosystems, but due to their complexity, mobility, ethology and ecology, are not often monitored and this situation should be reconsidered. Moreover, many of these species (amphibians, bats, etc.) are included in the Habitat Directive annexes, so monitoring these species is also required.

Landscape

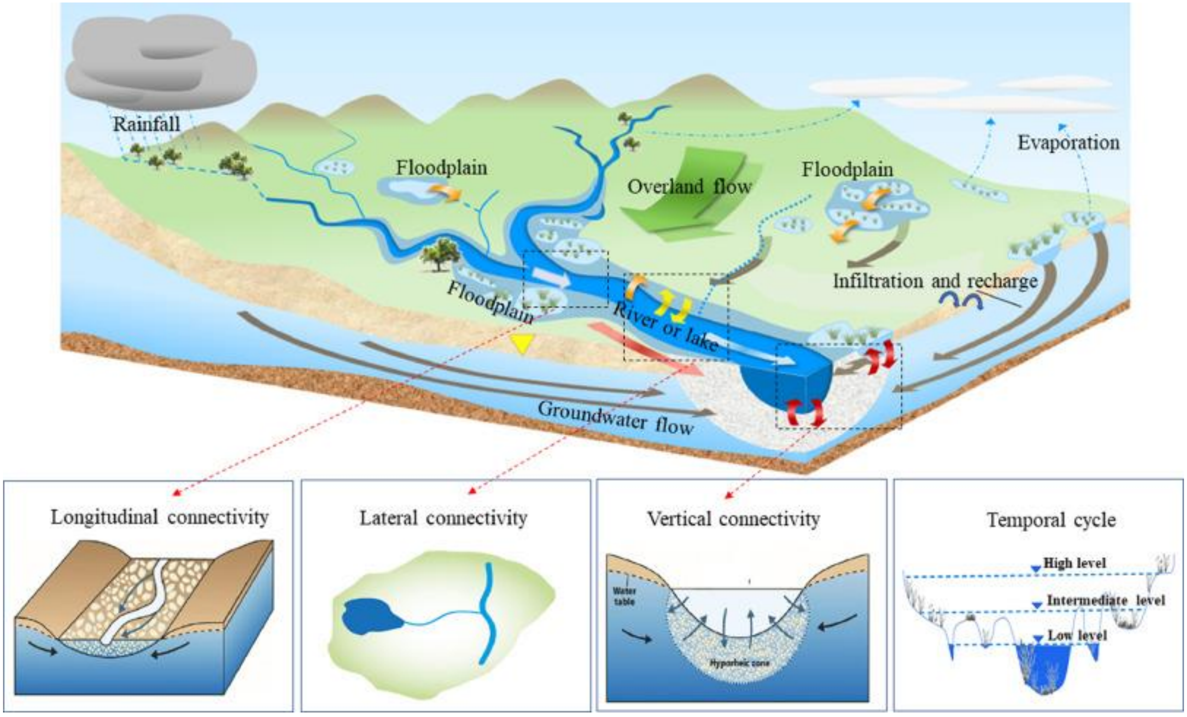
As previously mentioned, stream functionality is strongly influenced by longitudinal processes and predominantly unidirectional interactions (headwater to mouth), but also by lateral processes and interactions (channel to floodplain), and vertical processes and relationships (channel to hyporheic zone). Moreover, rivers should not be considered merely as sections or reaches of water courses but as complete ecosystem from headwaters to the mouth.

Rivers are intrinsically linked to their basins, both structurally and functionally (Downes et al., 2002) and must be perceived as elements interacting with the surrounding riparian forests, the hyporheic zone, groundwater, and floodplains, creating complex processes with flow as the structuring element. Consequently, streams and rivers cannot be considered simply as channels, but as complex ecosystems vertically connected to the interstitial and phreatic levels and to groundwaters and intrinsically linked to the riverine forest and floodplain through lateral connectivity (Junk et al., 1989). Therefore, the evaluation of the conservation status of fluvial ecosystems must consider these three dimensions, as well as the temporal dimension, which is typical of river ecosystems. A functional stream or river is connected to its floodplain and the riverine forest through floods and the river “pulses”.

Consequently, any approach to the landscape ecology perspective must keep these precepts in mind, with most basic principle being continuity and connectivity of stream ecosystems. Barriers, dams and weirs, alter intensively this continuity. At the operational level, scales of work at the national level (e.g. macroscale), or even the basin level may be considered too coarse, as they do not allow the differentiation of particular habitat types or the evaluation of processes taking place at the mesohabitat scale (e.g. drift processes, nutrient exchanges with the hyporheic) or microhabitat (e.g. presence of organic detritus, decomposition of fine and coarse particulate organic matter, etc.). However, at the macroscale or basin level, processes such as the level of fragmentation of river ecosystems can be detected, as well as their disappearance, recovery and global evolution.

Conservation policies have traditionally been focused on terrestrial ecosystems, and this has generated problems that are common throughout the hydrographic network, such as insufficient river connectivity, the absence of a basin perspective and the lack of consideration for river ecology when delimiting protected areas

Figure 3. Representation of hydrological flow paths and the four dimensions of hydrological connectivity



Arrows represent surface water and groundwater flows occurring throughout the whole catchment.
Source: Li et al., 2021, modified from USEPA, 2015.

1.3 Selection of typical species for condition assessment

Typical species of the habitat are used to assess the habitat conservation status. The Habitats Directive uses the term ‘typical species’, but it does not define this term for its use in reporting. For a habitat type to be considered as being at favourable conservation status, the Directive requires that both its structure, functions and its ‘typical species’ are in a favourable status (Art. 1(e)).

According to the guidelines for reporting under Article 17 (European Commission 2023), typical species should be selected from those that occur regularly and with high consistency within a habitat type, or at least within a major subtype or variant. They should include species that are good indicators of favourable habitat quality, e.g., by signalling the presence of a broader group of species with specific habitat requirements.

Typical species should also include those that are sensitive to changes in habitat condition - so-called early warning indicator species. In addition, they should provide supplementary information beyond what is already captured through monitoring of the habitat’s structure and functions.

Typical species may be drawn from any taxonomic group. While vascular plants are the most frequently selected, attention should also be given to lichens, bryophytes, fungi, and animals. Among animals, both vertebrates – such as birds – and invertebrates should be considered. Many ecological functions, such as pollination and decomposition, rely mainly on invertebrates, and their exclusion may lead to incomplete assessments of habitat function.

The species or groups of species that can be used to monitor the condition of lotic ecosystems may differ depending on their geographical location and characteristics. There is a consensus on the use of at least four groups of species for the assessment of fluvial habitats: phytobenthos, macrophytes, macroinvetebrates and fishes (Table 4). However, as previously indicated, other groups of species should be considered before their inclusion as “typical species”, such as amphibians, reptiles, and aquatic birds, etc.

The lists of typical species for each habitat in Group 32 (running waters), for each biogeographical region must be determined within each geographical area. Moreover, ‘typical species’ can also change along the river from headwaters to the mouth, in accordance with the physical and hydromorphological characteristics of stream reaches or river sections (Vannote et al., 1980).

Table 4. Possible groups for the selection of typical species for monitoring running waters habitats

Species Group	Ecological note - Bioindication	Sensitive to changes in quality
Phytoplankton or benthic algae	Influenced by grazers (trophic relations).	Light, nutrients, flow, substrate, temperatures and turbidity, salinity
Macrophytes	Good indicators of flow regulation	water flow, conductivity, nutrient levels
Macroinvertebrates	Insects, crustaceans and molluscs might be included as bioindicators.	water flow, water chemistry, hydrology, nutrient levels, acidification, temperature
Fish	Due to the reduced number of species in some rivers, their use as bioindicators is sometimes difficult. Therefore, parameters related to population structure are essential.	water flow, water levels, habitat disturbance, temperature
Riparian forest	Its main role at freshwater ecosystem is as a source of POM.	water flow, nutrients

There are statistical methods to determine the occurrence or abundance of a small set of indicator species as an alternative to sampling the entire community. This approach provides a list of species that is particularly useful in long-term environmental monitoring for conservation or ecological management. The criteria for selecting a species as indicator must are:

- It should reflect the biotic or abiotic state of the environment;
- It should provide evidence of the impacts of environmental change; or
- It should predict the diversity of other species, taxa, or communities within an area.

To determine indicator species, it is possible to conduct an analysis using the functions included in the package INDICSPECIES (De Cáceres et al., 2009). The MULTIPATT function from the INDICSPECIES library (De Cáceres et al., 2009) was applied to determine lists of species that are associated with particular groups of sites (or combinations of those). Out of the four available indices, the INDICATOR VALUE (INDVAL) (Dufrêne & Legendre, 1997) is used in ecology when the aim is to identify which species can be used as indicators of an ecosystem or type/group of ecosystems. The INDICATOR VALUE (INDVAL) method (Dufrêne & Legendre, 1997) can be applied to identify indicator taxa for the types. This method identifies the indicator taxa that best characterise groups of samples. This methodology has already been used to identify diagnostic species (Chytrý et al., 2002; De Cáceres et al., 2008).

2. Analysis of existing methodologies for the assessment and monitoring of habitat condition

This section summarises the methods used by EU Member States to assess the structure and function of the habitats included in Group 32 Running Waters. This analysis draws on methodologies from 18 Member States covering all river habitats (including a personal communication by SYKE, Finland).

2.1 Variables used, metrics and measurement methods, existing data sources

Table 5 presents a synthetic overview of the main characteristics considered in the reviewed national methodologies, along with the associated variables used for assessing and monitoring river habitats in EU Member States. Further details on the metrics and measurement methods are provided in the following sections as well as in the Annex.

Table 5. Ecological characteristics and variables measured across Member States

Ecological characteristics	Variables used in national methodologies	AT	BE	BG	CZ	DE	DK	FI	FR	GR	HU	IT	LT	NL	PL	RO	SE	SI	SK
Abiotic characteristics																			
Physical characteristics																			
Hydrological conditions	Water flow, velocity	x	x	x				x		x	x	x			x		x	x	
	Natural river dynamics - flooding		x					x								x			
	Sediment load (granulometry/texture)											x							
Hydro-morphological parameters	IDRAIM methodology											x							
	Morphology, width and depth of river			x				x							x		x		
	Habitat Quality Assessment (HQA)														x				
	Dams/barriers to water flow					x		x							x			x	
	River course modifications					x													
	Riverbanks (preservation/ modification)				x	x												x	
	Gravel deposits width														x				
Substrate	Riverbed/ bottom substrate		x	x		x		x		x	x	x			x		x	x	
Water physical characteristics	Temperature (°C)			x				x				x							
	Electric conductivity		x	x				x											
	Water transparency, turbidity, water colour			x	x			x		x			x			x		x	

Ecological characteristics	Variables used in national methodologies	AT	BE	BG	CZ	DE	DK	FI	FR	GR	HU	IT	LT	NL	PL	RO	SE	SI	SK
Abiotic characteristics																			
Chemical characteristics																			
Chemical status parameters	Water quality class	x																	
	Chemical status according to WFD					x		x	x										
	Acidity (pH)		x	x			x	x				x					x	x	
	Dissolved oxygen, oxygen saturation, BDO, CDO		x	x				x										x	
	Other substances							x	x										
	Chloride		x					x											
	Dissolved Gases: oxygen/nitrogen		x					x											x
	Pollutants					x		x										x	
	Nutrients: total N, total P, sulphate, nitrates, N-Kjeldahl/ ammonia		x					x										x	x
Trophic status	Trophic status index															x			
Biotic characteristics																			
Compositional characteristics																			
Plant species	Characteristic/typical plant species	x	x	x	x	x		x			x	x	x	x	x	x	x	x	x
	Macrophytes and phytobenthos, River Macrophyte Biological Index (RMB EQR)			x		x		x	x			x					x		
	Invasive alien species (IAS) presence		x						x	x	x		x		x	x			
	Riparian Quality Index (RQI)								x										
	Eutrophic, nitrophilous species, disturbance indicator species		x			x			x	x	x		x		x	x			
Animal species	Fish (ecological status), River fish index			x		x		x	x	x		x					x		
	Macro-invertebrates (ecological status), GNBI EQR			x		x		x	x								x		
	Other animal species presence (Odonata, Orthoptera, birds, reptiles)								x	x		x							
	Danish Aquatic Fauna Index (DVFI)						x												
Structural characteristics																			
Vegetation cover and structure	Total vegetation cover	x						x			x				x	x	x		
	Cover of helophytes		x					x					x						

Ecological characteristics	Variables used in national methodologies	AT	BE	BG	CZ	DE	DK	FI	FR	GR	HU	IT	LT	NL	PL	RO	SE	SI	SK
	Vertical and horizontal structure	x	x					x								x			
	Age structure - tree and shrub layer				x			x											
	Shrub and tree species cover					x					x				x		x		
	Typical vegetation structures (water course, bank & waterbed)					x													
	Width and zonation of riparian vegetation									x									
	Shrubs height														x				
	Invasive species, weeds, IAS cover		x			x			x		x		x						
Functional characteristics																			
Regeneration	Age structure & regeneration of alpine river characteristic species	x				x									x				
Dynamics	Height of selected species that indicate different dynamic stages								x										
	Dynamics defined by changes in the shrub and tree density										x								
Decomposition	Leaf litter cover										x								
	Dead wood abundance							x									x		
Landscape characteristics																			
Spatial relations	Spatial relations with other habitats (3220, 3240, 91E0, 7240)	x																	
	Land use in surrounding areas							x					x						
Fragmentation	Habitat fragmentation by anthropogenic structures			x											x				
	Distance to similar habitats, patch size, role of the neighbouring habitats										x								
	Spatial analysis of the distance between populations using GIS											x							
Other: disturbance, degradation																			
Habitat deterioration caused by human activity	Modifications of hydrological characteristics (e.g. by dams)							x			x								
	Gravel extraction	x																	
	Recreational use	x				x													
	Removal of sediments, driftwood, trees, scrub					x													
	Habitat damage by land use and disturbance							x	x		x	x							

Note: BE stands for Belgium Flanders.

2.1.1. Abiotic variables

The methodologies from 17 Member States incorporate abiotic variables into the assessment of river habitats condition (see Table 5 below and the Annex), including physical and chemical parameters such as hydrological regime, sediment transport, water temperature, pH, dissolved gases, and nutrients.

Physical variables

The most used physical variables are those related to water flow and substrate. The latter is connected to flow and sediment transport – parameters that are often overlooked.

The flow regime is a key determinant of the structure and function of lotic ecosystems, and most Member States include it in their assessment and monitoring protocols. However, in many cases, the indices used to measure flow, and its alternation, magnitude, temporal pattern, and velocity are not provided. Some countries measure flow only using a qualitative approach. For example, the methodology from Romania (Trif et al., 2015) classifies flow into three levels based on flood recurrence (i.e., no flooding, periodic/seasonal flooding, accidental flooding), while Hungary classifies flow in four categories (good, satisfactory, too dry, or too watery).

Analysis of water flow and other hydrological conditions, such as width and depth, are included in the methodologies of seven MSs. Germany (BfN, 2017) evaluates a relatively broad range of hydromorphological parameters, but their assessment primarily relies on expert judgement. In Italy, on the other hand, hydromorphological aspects are evaluated using standardised methodologies that monitor and classify the quality of river dynamics processes, such as IDRAIM (System for hydro-morphological assessment, analysis, and monitoring of watercourses), the IFF Index (Fluvial Functionality Index), and indices such as the IQM (Index of Morphological Quality) and IDM (Index of Morphological Dynamics), among others (Angelini et al., 2016).

Flow velocity is measured in Bulgaria (MOEW, 2013), using hydrological propellers, and Szoszkiewicz and Gebler (2012) classify the percentage of rapid flows in the river transect being monitored, but no measurement method is indicated. Ludin et al. (2017) recommend measuring water turbidity as a proxy for current velocity using colour and appearance. For each flow class (calm, weak and rushing flow) a number from 0 to 3 is assigned, and during fieldwork, the observer has to indicate the percentage corresponding to each flowing class. A dominant class has also to be assigned.

Several methodologies also measure turbidity, water transparency and colour (e.g. national methodologies available from, Czechia, Greece, Lithuania, Romania and Slovenia).

Bottom substrate characteristics are measured in the methodologies of five MSs. Ludin et al. (2017) note that monitoring of bottom substrates comes with high cost and should only be carried out in areas where their presence is relevant and, thus, it is worth analysing these characteristics. Oosterlynck et al. (2020) propose a qualitative assessment of the presence of disturbances and whether the substrate comes from dredged material or pavement. Angelini et al. (2016) assess the percentage of coverage and type of substrate material. However, no measurement methods or threshold values are provided.

Presence of typical river structures (channels, riverbanks, etc.) is considered in the methodologies from Germany (BfN, 2017) and Bulgaria (MOEW, 2013).

The methodologies from Austria (Ellmauer et al., 2005) and Germany (BfN, 2017) assess hydrological and morphological changes in watercourses by expert visual assessment of the presence of dams and barriers to water flow or by diversions.

The Nature Agency of Germany (BfN, 2017) classifies the water body structure in accordance with the WFD categories. Category 4, which has the lowest conservation value, is assigned to altered habitats that no longer classify as habitat types of Community interest.

Lithuania (GTM, 2015) assesses parameters such as water conductivity, colour, transparency, and provides details on these measurements. In Poland (Mróz, 2012, 2015), hydromorphological aspects including flow, dams and shading are assessed by expert judgement. However, HQA (Habitat Quality Assessment) and HMS (Habitat Modification Score) indicators are also used to evaluate river habitats in the country.

In Slovenia, nine hydromorphological parameters are used (Petkovšek, 2013), but these are assessed qualitatively rather than quantitatively.

Chemical variables

At least ten national methodologies employ chemical variables to assess habitat condition (Table 5). However, there are significant variations in the extent and specificity of these evaluations across countries.

The methodology from Belgium-Flanders (Van Calster et al., 2020) provides a detailed and complete list of chemical variables, along with their metrics and measurement methods. This approach aligns with standard practices in fluvial ecology and reflects a high level of transparency and methodological robustness. Nonetheless, some of the methods used are not fully detailed for certain variables, such as nitrates, which are critical indicators of nutrient dynamics and potential eutrophication.

Slovenia also reports an extensive array of chemical variables (Petkovšek, 2013). However, the evaluation is carried out by consolidating the results into five broad categories. While this likely involves the collection of quantitative data, the process of categorisation obscures granular details, which may limit its utility in detecting subtle changes in habitat quality. Similarly, Italy, Germany, and France report that they evaluate chemical parameters.

Germany assesses chemical status in coordination with the WFD. The assessment is conducted according to WFD monitoring protocols or by expert judgement on rivers that are not covered by the WFD. Pollution with chemicals, hormones, etc. is also measured, as far as possible (BfN, 2017). In France, good chemical status according to the WFD is considered the minimum basis for achieving a favourable conservation status, and its rating can be obtained from the Office Français de la Biodiversité (OFB) or the Water Agencies (Mistarz et al., 2018).

When considering the physico-chemical parameters that should be included in the assessment of river habitat conditions, several groups can be identified, including basic variables (conductivity, salinity, pH, and temperature), dissolved gases (O_2, CO_2), nutrients (nitrates, phosphorus, and organic matter), major ions (Ca, Na, Mg, K, etc.), pollutants (priority substances and EQS) and other ions as Al, Fe, etc.

While variables such as conductivity, salinity, pH, and dissolved gases are widely regarded as essential, their systematic assessment remains inconsistent across Member States. Germany, France, and Italy are believed to monitor these parameters, but explicit confirmation exists only for Belgium and Slovenia. In Slovenia's case, however, the previously noted limitations in reporting and categorisation may reduce the clarity and practical usability of the data.

2.1.2. Biotic variables

The biotic characterisation includes compositional, structural and functional properties. All MSs evaluating lotic ecosystems include biotic variables in river habitats condition assessments (Table 5).

Compositional variables are measured in all 17 MSs, while structural variables are included in 11 methodologies and functional variables appear to be measured only in six Member States. Most compositional and structural variables are focused on the presence or coverage of key species and are used to detect zonation patterns, ecological changes, transitions between successional stages, or habitat degradation.

Compositional variables

All the national methodologies reviewed include the assessment of the vegetation composition of river ecosystems by considering their characteristics and typical species in some form. Some countries also consider exotic and invasive species, nitrophilous species, and disturbance indicator species.

Some MSs assess only the presence of species, while others also assess their abundance. The assessment is sometimes done using the Braun-Blanquet scale (r, +, 1, 2m, 2a, 2b, 3, 4, 5) or the AFOR scale (Abundant, Frequent, Occasional, and Rare). In Greece, also the vitality of the species is also assessed, considering the propagation and health of the plants (Dimopoulos et al., 2018).

The parameters and indices used by the WFD to assess ecological status are also applied in some national methodologies, including the River Macroinvertebrate Biodiversity Index – Ecological Quality Ratio (RMBI EQR), the Riparian Quality Index (RQI), the Riparian Plant Index (RPI), and the Global Nitrogen Biomonitoring Index – Ecological Quality Ratio (GNBI EQR).

Bulgaria assesses the ecological status of water bodies based on biological parameters (phytobenthos, zoobenthos, phytoplankton, zooplankton, and macrophytes) according to the WFD's five-point scale (MOEW, 2013). Poland also employs the WFD's ecological status assessment.

Germany conducts inventories of plant species and establishes the presence of certain species as necessary or indispensable, it essentially applies the Water Framework Directive methods but instead of using the five levels inherent in it, it applies three levels (very good, good, and moderate or poor). Characteristic plant species are assessed according to reference lists of vascular plants, algae, mosses, and lichens; however, liverworts, mosses and lichen are not usually included in these protocols (BfN, 2017).

Italy uses the RMBI index and information related to the species of conservation importance or included in Annex II or IV of the HD (Angelini et al., 2016).

In France, the assessment of some river habitats (3260, 3280, 3290) is conducted using indices from the WFD, such as the Riparian Quality Index (RQI), River Fish Index (RPI), Global Standardised Biological Index Ecological Equality Ratio (GNBI EQR) and the River Macrophytic Biological Index Ecological Equality Ratio (RMBI EQR). The River Fish Index measures the difference between the fish population at the station and the reference population, and a list of 34 fish species is considered for its calculation. The index is standardised and takes into account seven parameters: - the total number of species; the number of rheophilic species (i.e. those that live in waters with strong currents); the number of lithophilic species (i.e. species that reproduce on a pebble/gravel substrate); the density of tolerant individuals; the density of insectivorous individuals; the density of omnivorous individuals; the total density of individuals. Furthermore, expert judgment is also used to assess the presence of eutrophic species and to visually estimate of the percentage cover of invasive alien species (Mistarz, 2018).

In addition to fish, the presence of other animal species relevant to the habitat (Odonata, Orthoptera, birds, reptiles, amphibians) is assessed in methodologies from France, Italy and Greece (Viry, 2013; Mistarz, 2018; Angelini et al., 2016; Dimopoulos et al., 2018). In Denmark, a Danish Stream Fauna Index (DSFI) is used (Skriver et al., 2000).

Structural variables

Although there may be some overlaps between compositional and structural characteristics, it is possible to identify structural variables used for the assessment of river conditions in the methodologies considered in this analysis.

Horizontal and vertical vegetation structure is assessed in some countries. In Belgium (Oosterlynck et al., 2020), key species vegetation cover and vertical structure, indicated by cover oh helophytes, are measured. In Romania (Trif et al., 2015), vertical structure is assessed by field observation of the vegetation stratification, based on the height/depth at which the various strata are arranged, and vegetation cover is measured using the Braun-Blanquet scale. In Hungary (Horváth et al., 2021; Csiky et al., 2021), vegetation cover and heterogeneity, considering the diversity of species composition and height are assessed by expert judgment. In Czechia (Lustyk et al., 2023), the vertical and horizontal structure of the vegetation are assessed in three categories, considering the representation of dominant species and the vertical arrangement of the shrub and tree layers (for habitats 3230 and 3240). In Poland (Mróz, 2012, 2015), vegetation cover and height are measured by expert visual assessment, considering the herbaceous layer, shrubs and trees present in the river habitats. The cover of non-native species is also assessed. In Germany (BfN, 2017), the cover of woody species of alpine rivers (in 3220 and 3230) and vegetation structure of water bodies and riverbanks are assessed in some habitats by expert judgment (e.g. habitat 3260).

In France, the visual estimation of bare soil cover by expert judgment is an integral part of the definition of habitats that are typically open formations (e.g. 3220 and 3250) with very low vegetation cover (Viry, 2013). As already mentioned above, a Riparian Quality Index (Indice de qualité de la bande riveraine-IQBR) is also used in the assessment of river habitats in France (Mistarz, 2018). The IQBR provides information on the quality of the riparian zone and is based on four criteria: the total percentage of riparian cover, the structure of the vegetation cover (trees, shrubs), the canopy quality (number of native tree species) and alterations to riparian vegetation.

In Greece, the width and zoning of riparian vegetation are assessed but, no further details are available on how these variables are measured (Dimopoulos et al., 2018). Lithuania qualifies riparian vegetation, the riverbed, and the percentage of shade over it, however, the methodology used is not clear.

Functional variables

Due to the complexity of directly measuring functional characteristics, few national methodologies include true functional variables, while many others rely on the assessment of alterations to functions (see further details below in the section on “Other Variables”).

In Italy, the Fluvial Functional Index (FFI), which is based on the collection of information about main ecological characteristics, is used for the assessment of functional aspects of rivers (Angelini et al., 2015). According to this index, river stretches are classified into five levels of functionality.

Eutrophication is assessed in Bulgaria, Belgium and France, for example through the percentage cover of indicator species associated with this alteration (Oosterlynk et al., 2020, Viry, 2013).

River continuity is assessed in Germany by considering the presence of dams and other obstacles to water flow (BfN, 2017).

Age structure and regeneration of characteristic species are assessed in some methodologies, for example *Myricaria germanica* in the alpine river habitat 3220 (in Austria, Germany and Poland) and *Salix eleagnos* in habitat 3240 in Germany (Ellmauer et al., 2020; BfN, 2017, Mróz, 2012).

Vegetation dynamic characteristics are measured in some methodologies, for example by measuring the height of selected species that indicate different dynamic stages (e.g. in France) and changes in the shrub and tree density (e.g. in Hungary).

2.1.3. Landscape variables

Landscape variables are essential for understanding the influence of regional processes on habitat conditions, as well as their continuity and connectivity. However, few national methodologies include landscape variables (Hungary, Italy, Bulgaria and Poland).

Hungary evaluates the rate of isolation (subjectively), the distance to similar habitats, the size of the habitat patch, and the role of neighbouring habitats (friendly or non-friendly) (Horváth et al., 2021). Italy conducts spatial analysis using GIS to determine the distance between habitat patches (Angelini et al., 2016), identify potential causes when changes are not attributable to riverbed morphology and locate areas of high potential. Bulgaria assesses fragmentation by identifying the presence of infrastructure fragmenting the river habitat (MOEW, 2013). Finally, Poland considers the existence of river habitat complexes, where various river habitats and riparian forests (e.g., 3220, 3230, 3240 and 91E0) occur in a river (Mróz, 2012).

2.1.4. Other variables

Several methodologies include variables primarily focused on disturbances, impacts and damage to river habitats when assessing their condition. In Austria, alterations caused by different activities (e.g., gravel extraction, recreational use, infrastructure) are recorded during field inspection. In Belgium and Germany, recreational activities are also assessed as potential disturbances. In a methodology from France (Viry, 2013), diffuse and heavy damage is assessed by estimating the surface area affected by various impact sources, including artificialisation of banks, heavy machinery passage, material deposition, material extraction, and point source pollution. Italy requires the identification of anthropogenic activities but does not provide specific metrics or measurement methods. In Czechia, the effects of degradation are assessed using qualitative scale. Hungary evaluates the potential effects of land use (agriculture, grazing, tourism, etc.) by describing their intensity at the level of sampled area. Lithuania also considers and describes the predominant land use in the surrounding area (agriculture, forestry, recreation, etc.).

2.2 Definition of ranges and thresholds to obtain condition indicators

The assessment of habitat condition requires setting thresholds to determine whether the results obtained from the measurement of each variable indicate good or poor condition. Reference values and thresholds are not always described in the methodologies analysed and in general the criteria for establishing thresholds are insufficiently documented or completely undocumented.

In many cases, and for many variables proposed in the national methodologies, reference values, thresholds or ranges are not specified. When such values are indicated, there is often not any information about how they were calculated and, in some cases, presumably they were established by expert judgment. and evaluated qualitatively. However, it must be noted that determining reference conditions for habitats is inherently complex, as it often involves consideration of interactions between multiple environmental factors and the ecological requirements of a wide range of species.

In Belgium-Flanders, favourable ranges or thresholds expressed as quantitative values are available for all abiotic chemical variables in the methodology applied (Van Claster et al., 2020). For instance, regarding biological oxygen consumption in the water column, a median value bellow 2 mg/l is considered a favourable value and for total nitrogen in the water column, a favourable average value in summer must be below 4 mg/l for habitat type 3260. The favourable abiotic ranges may be obtained from literature. For biotic variables (Oosterlynck, et al., 2020), a set of threshold values has been determined for the different variables measured. For instance, for variables that measure coverage, whether it is coverage of a certain species or a disturbance, threshold values are used to discriminate between favourable or unfavourable conditions, which are set at 10%, 30%, 50%, or 70% cover depending on the case. For example, 10% is set as the limit value for some disturbance indicators, when a low cover is already indicative of bad condition; for species that naturally have a significant cover, 50% or 70% can be set as threshold values for favourable condition depending on the species.

Other methodologies, such as those from Austria (Ellmauer et al., 2005, 2020) and Germany (BfN, 2017), define the condition status of variables in three categories (A = excellent, B = good, and C = poor) to which quantitative or qualitative values are assigned. For example, for the compositional variable measuring the presence of vegetation structure along riverbanks (BfN, 2017), the following thresholds are considered: A = typical bank vegetation present on the most of the bank length, B = typical bank vegetation on larger sections of bank length, C = typical bank vegetation only on small sections of sampled bank length. For invasive alien species cover, the following thresholds apply: A = IAS cover ≤ 5 %; B = IAS cover > 5 % ≤ 10 %; C = IAS cover > 10%).

In methodologies used in France (Viry, 2013; Mistarz, 2018), a positive or negative score is assigned to each variable’s value obtained during the assessment, considering a threshold and its influence on habitat condition. For instance, in the variable assessing different succession stages based on the height of various riparian species, the following scores are assigned: 1 stage = -10; 2 stages = -5; more than 2 stages = 0). These scores are later aggregated to obtain a final assessment of the ecological condition of the habitat (as explained in more detail in Section 2.3).

2.3 Aggregation methods at the local scale

Ecological assessment involves the integration of various physical, chemical, and biological factors. The choice of an aggregation method for combining partial evaluations into an overall assessment significantly influences the final outcome. Two primary aggregation approaches are commonly employed: the "one-out, all-out" rule, also referred to as minimum aggregation, and additive aggregation, which can take various forms, such as an arithmetic mean or weighted addition (Langhans et al., 2014). Other majority rules and categorical combinations of the results obtained in the measurement of the variables are used in the methodologies considered in this analysis.

In the methodology used in Belgium-Flanders for the Article 17 report on the status of habitat types during the period 2013-2018, a weighting of the importance of the individual indicators was applied, based on their relevance for achieving favourable habitat condition in the long term and the following decision framework was used, which combines the one out-all out rule with a majority rule:

a) when at least one very important indicator scores unfavourably, the local status of the site is classified as unfavourable (one-out-all-out rule);
b) when none of the very important indicators score unfavourably, but half or more of the indicators score unfavourably, the local status of the site is classified as unfavourable;
c) when none of the very Important indicators score unfavourably, but less than half of the indicators score unfavourably, the local status of the site is classified as favourable.

In Poland, the "one-out, all-out" principle also underpins the aggregation methodology. Expert judgement is applied to assess fundamental variables such as shrub and tree coverage in habitats like 3220, 3230, and 3240, the presence of invasive species, and the diversity of characteristic species, particularly in habitat 3270. These fundamental variables are crucial for determining the overall structure and function of the habitat.

In the German methodology (BfN, 2017), the assessment of habitat structure and functions rely on a decision matrix with three possible levels, and a majority rule is applied to aggregate the variables measured. The process is qualitative in nature and requires expert judgement for its application. It is based on three groups of variables: habitat structure, typical species inventory and disturbances. Each variable is rated as A (excellent), B (good), or C (poor), with the overall evaluation corresponding to the most frequently assigned value. However, if a C rating is assigned to any variable, the habitat cannot be evaluated as excellent overall. Aggregation occurs initially within each group of variables and subsequently across the three groups. This system is closely aligned with the Water Framework Directive, which establishes quantitative and specific thresholds for many of the variables it employs. Nonetheless, in many instances, the system relies on expert judgement or applies subjective classification criteria (e.g., minimal to moderate, not significant, only minimally altered).

In Lithuania, advanced statistical techniques are employed to evaluate conservation status. These include the Bray-Curtis index for analysing multidimensional data matrices, descriptive statistics for comparing linear or binary data, and non-hierarchical cluster analysis such as the K-means method. Principal Component Analysis (PCA) is also used to visualise and assess relationships among groups of indicators. Conservation status thresholds, such as A (excellent), B (good), C (poor), and D (bad), are determined based on the statistical analysis of indicator values and their proximity to reference conditions.

In Slovakia, the evaluation of non-forest habitats is conducted using multidimensional ordination methods. Variables such as indicator species, vertical structure, area changes, management influences and future prospects are analysed. To standardise the variability of these variables, they are normalised on a 0–1 scale using the min-max transformation. The position of a monitoring record in multidimensional space is then interpreted based on its Euclidean distance from the optimal reference condition, which represents the best quality record. A suitable coefficient is derived to express the quality of the monitoring record, and thresholds for conservation status categories are defined in equal intervals. These categories include A (excellent), B (good), C (poor), and D (bad).

In France, the aggregation methodology involves comparing data collected for each variable in the field against established threshold values to derive scores. These scores, ranging from 0 to -20, are aggregated and subtracted from 100 to obtain a conservation status value. This value is then compared against thresholds to determine the overall conservation status. The process is repeated for each variable, and the final scores for all indicators are summed. Based on these aggregated scores, conservation status is assigned to each habitat section. The methodology also accounts for indicators related to habitat structure, its eco-complex, and its degree of disturbance. Sites are classified into categories such as more than 70% in good conservation status, between 40% and 70%, or less than 40%, reflecting the overall habitat quality.

In Slovenia, the assessment of ecological conditions in watercourse channels and banks incorporates natural hydromorphological and biotic structures, the influence of water infrastructure, and point source loads on the watercourse and riparian area. A set of 28 indicators is used, with a partially adapted colour scale to align with reporting on conservation status under Article 17 of the Habitats Directive.

In the methodology from Sweden for habitat type 3260 (Lundin et al., 2017), the aggregation of the biological, physico-chemical and hydromorphological factors follows a “one-out-all-out” rule in which all three criteria must be assessed as favourable, starting with the biological factors. If these are assessed as unfavourable, the final assessment is automatically considered unfavourable.

Once the condition is assessed at the local level through these procedures, it can be aggregated to a larger scale, such as the biogeographical region, following methodologies that integrate data across monitoring plots and sites to provide a comprehensive conservation status assessment.

2.4 Aggregation at biogeographical scale

For the 2019–2024 reporting period, the guidelines for reporting under Article 17 (DG Environment, 2023) indicate that the condition of a habitat type at the biogeographical level is determined by the proportion of the habitat area where the "structure and functions" parameter is assessed in good or not good condition. These guidelines specify that the "structure and functions" parameter is deemed "favourable" if at least 90% of the habitat area is in good condition. Conversely, it is considered "unfavourable–bad" if more than 25% of the habitat area is not in good condition, and "unfavourable–inadequate" for intermediate values. The habitat condition can also be reported as unknown, where the proportion of the area in good or not good condition is unknown or the estimate lacks robustness.

However, detailed information on the specific methods used by Member States to aggregate data for this habitat type at the biogeographical level is often lacking. Nonetheless, it is expected that Member States will adhere to the relevant guidance documents. This calculation requires summing the sampling areas of the habitat type assessed in good and not good condition and may involve weighting local-scale assessments according to the corresponding area.

2.5 Selection of monitoring localities

The approaches used for the selection of monitoring localities and sampling sites vary across the national methodologies analysed. For example, Poland relies solely on expert judgment, with the requirement that sampling locations should encompass habitat diversity. In contrast, Belgium employs a more systematic approach using Generalised Random Tessellation Stratified (GRTS) sampling, integrating habitat maps with a 32 x 32 m master sample grid. This method, which appears robust, typically results in approximately 150 samples per habitat type. Belgium's approach involves two steps:

1) A master sample is created across Flanders using the GRTS algorithm, with a density of 32 m x 32 m. Each point is assigned a rank number, which allows for the selection of a sample of the desired size. The master sample forms the basis for selecting samples for various habitat subtypes.

2) The desired number of sample points is then selected within the polygons of a given habitat type, guided by the rank number. Further details can be found in Oosterlynck et al., (2020). Additionally, Belgium ensures that assessments are conducted at the appropriate scale by establishing specific criteria.

In Czechia and Slovakia, habitat status is assessed at permanent monitoring plots (PMPs) or points (PMLs), respectively. These are predefined polygons where a specific habitat occurs, typically homogeneous and managed uniformly. In Czechia, eight permanent monitoring plots for running waters—seven for habitat type 3220 and one for habitat type 3230—were established in 2005 based on expert judgment to represent characteristic or typical occurrences.

The methodologies available from France for Natura 2000 sites (Viry, 2013; Mistarz, 2018) state that the number of samples should be sufficient to allow for meaningful statistical analysis. While a minimum of 30 samples per site is conventionally recommended, this is often unfeasible due to cost and resource constraints. However, it will be necessary to ensure that the sample is representative at the site level. As a compromise, it is suggested that a minimum of 10 sections be sampled. Synergies with the Water Framework Directive have facilitated the identification of 402 monitoring stations within Natura 2000 sites.

In Greece, monitoring locations were chosen based on sampling sites identified during a previous project (the IDH-TACI Project, 1999–2001) and the need to evaluate the conservation status of habitat types in areas where existing data were insufficient. All habitat types are assessed within a 10 km reference grid, except for habitat type 3260, which is assessed using a 5 km grid.

In Hungary, the number of required sampling plots is determined based on factors such as distribution, rarity, conservation significance, and Hungary's role in the preservation of specific habitats. The required number of sampling points for each habitat type is specified and distributed among the national park directorates. For example, 70 sampling sites across nine national park directorates are designated for habitat type 3270, while 12 sites across three directorates are assigned to habitat type 3260. The conditions for relevé preparation, such as the ideal vegetation period and required equipment, are defined. Surveys must be conducted when the habitat is in its optimal state. Rules are also in place for relocating sampling points in cases where a site is destroyed. Both "typical" plots and secondary or degraded sites must be included, though specific selection details are not provided. Sampling outside Natura 2000 sites is also required, but there are no rules regarding the proportion of such samples.

In Romania, monitoring guidelines are primarily designed for application within protected areas where target habitats have been identified, though they may also be applied outside these areas. The guidelines recommend selective sampling, with a minimum of six transects per habitat type (seven for habitats 31A0 and 3260), distributed within and outside protected areas. It is unclear whether this minimum applies to the local, regional, or national scale. Sample plot sizes range from 1 m² to 100 m², and standardised field sheets are used to record data. Vegetation associations are correlated with the national vegetation inventory and descriptive monographs.

According to Ludin et al. (2017), aerial images and GIS data were analysed to select the water courses that correspond to habitat 3260. The absence of dams and other alterations was considered. However, the total number of watercourses monitored is not clear. Regarding the Danish methodology described by Thodsen et al., (2024) a total of 257 time series stations were monitored in 2022.

Finally, in Slovenia, stream sections representing the same qualifying habitat types are identified as the basic spatial units for habitat monitoring. If a stream contains only one qualifying habitat type or if all habitat types are evenly distributed, it is treated as a single section. Otherwise, it is divided into multiple sections according to the distribution of habitat types.

2.6 General monitoring and sampling methods

The methodologies employed by Member States vary significantly with respect to monitoring and sampling protocols, ranging from expert-based assessments, as in Slovakia, to more complex and quantitative approaches, such as those used in France.

In Denmark, a total of 257 time series stations were monitored in 2022 (Thodsen et al., 2024). These were located across different stream sizes classes. The size typology of the streams is categorized as follows:

1) streams with a catchment area of less than 10 km^2;
2) medium-sized streams with a catchment area between 10-100 km^2;
3) large streams with a catchment area greater than 100 km^2.

Ludin et al. (2017) mapped watercourses in stereo models of IRF aerial images within 3 × 3 km^2 square sampling units (boxes) with a buffer zone of 50 m around each box to deal with box edge effects in terms of the smallest mapping unit. Hydrology lines from Lantmäteriet's Property map are used as input data for further processing. The minimum mapping unit for lines is 30 m. Additionally, data from the Damregistret and the Swedish Water Archives (SVAR) are utilised.

According to Vydrova (2014), monitoring of watercourses in the Czech Republic is conducted every 6 years. The monitoring units are sections of 1 km length divided into segments of 100 m in length. These sections are established (and monitored for the first time) only in places where macrophyte vegetation currently occurs, and their location may differ from the original selection. The coordinates of the upper and lower edges of the section are recorded, which are marked in the GIS layer. The upper and lower edges of the monitored section and the

100 m segments are drawn on the map (plot). The monitor inserts the modified map in this way into the photo documentation of the area or instead provides the situation drawing.

With regard to the use of existing data or information from other EU reporting schemes, it is essential to mention the implementation of the Water Framework Directive. According to the review, at least five Member States (Bulgaria, Germany, Italy, Poland and France) have applied or are planning to apply (as is the case of France), methodologies an information obtained from Water Framework Directive monitoring programmes to some extent. In some cases, they simply utilize existing data or protocols available under the WFD, while in others, a broader coordination is sought to take advantage of synergies between the two directives.

As the WFD data expand, it may be possible to demonstrate the utility of WFD indicators for assessing and monitoring the conservation status of habitats. While this is theoretically applicable, its practical relevance for assessing and monitoring the conservation status of running water habitats has not yet been definitively established, however.

2.7 Other relevant methodologies

Monitoring lotic ecosystems around the world

To understand ecosystems comprehensively, it is essential to study their structure, dynamics, and natural functioning. Lotic ecosystems, in particular, interact across four dimensions (Amoros et al. 1987; Elosegi & Sabater, 2009; Hauer & Lamberti, 2007; Meyer, 1997; Ward, 1989; Gordon et al., 2004).

Addressing these issues is essential for understanding the functioning of lotic ecosystems, which is a prerequisite for evaluating their structure and function. Europe is not the first region to attempt to establish monitoring and assessment system for fluvial ecosystems. The United States had already implemented a monitoring system developed by the USGS, as detailed in the work of Barbour et al. (1999). This document, along with the monitoring and assessment framework for fluvial ecosystems in the United States, served as an inspiration for the system established under the implementation of the Water Framework Directive. Indeed, the document by Barbour et al. (1999) formed the basis for the protocols adopted by some Member States, both in terms of sampling methodologies and the biotic and abiotic variables to be measured.

The monitoring of rivers and streams in the U.S. is primarily managed by federal agencies, with the collaboration of state and local governments. The U.S. Geological Survey (USGS) maintains the National Streamflow Information Program (NSIP) through a network of over 8,500 stream gages that measures streamflow, water levels, and related hydrologic data. USGS also develops Water Quality Programs to monitor pollutants, nutrients, and sediment levels in rivers and streams and provide Real-Time Data about streamflow data to predict floods, droughts, and water availability. The Environmental Protection Agency (EPA) conducts periodic surveys of rivers and streams to assess their ecological health, focusing on biological, chemical, and physical conditions, named National Aquatic Resource Surveys (NARS) and the Clean Water Act Programs that mandate states to monitor water quality and report on impaired water bodies. The NOAA (National Oceanic and Atmospheric Administration) monitors water levels, especially for flood prediction, through its National Water Model and weather-related hydrologic forecast. The FEMA (Federal Emergency Management Agency) uses river monitoring data for floodplain management and disaster preparedness. State agencies and local watershed organisations conduct water quality testing, often focusing on specific pollutants such as nitrates, phosphates, heavy metals, and pathogens. Moreover, citizen science initiatives, like volunteer water monitoring, complement governmental efforts.

In Canada, monitoring is also a collaborative effort between federal, provincial, and territorial governments, with Environment and Climate Change Canada (ECCC) playing a leading role. The ECCC has established the Water Survey of Canada (WSC) which operates over 2,800 hydrometric stations to monitor river and streamflow across the country. ECCC has also established a National Long-Term Water Quality Monitoring Program that tracks water quality in major watersheds, focusing on pollutants and ecosystem health and Real-Time Hydrometric Data that provides real-time access to water level and streamflow data. Water quality monitoring is managed by Provincial Agencies addressing local issues like industrial discharges, agricultural runoff, and urban stormwater. Programmes like Alberta's River Water Quality Index or Ontario's Water Quality Monitoring Network assess and report on river health. Indigenous communities play a key role in water stewardship, combining traditional ecological knowledge (TEK) with modern monitoring techniques. There are also collaborative programmes, such as the First Nations Water Initiative, that support Indigenous-led monitoring efforts. In Canada, Academic institutions and NGOs are actively involved in stream and river monitoring by conducting independent research and monitoring or working on freshwater health assessments and advocating for better conservation policies. There is a programme named Streamkeeper that trains volunteers on how to monitor and evaluate freshwater ecosystems with basic protocols (Taccogna & Munro, 1995).

The Water Framework Directive

The development of a protocol for assessing the hydromorphology of water bodies under the framework of the Water Framework Directive represents a significant advancement and the first necessary step toward evaluating the structure of fluvial ecosystems while adhering to the foundational principles of fluvial ecology theory. At least Germany, France and Spain have developed protocols or methodologies for evaluating hydromorphology in streams and rivers (Lamand et al., 2017; MITECO, 2019; MAPAMA, 2017; Quick et al., 2019). These documents and their implementation represent a significant step forward in the study and understanding of lotic ecosystems, despite some acknowledged limitations.

These protocols have only recently been developed, and their implementation has taken place in recent years, so it is too early to discuss their weaknesses and strengths. The lack of experience in their application means that they currently should be regarded as preliminary. That said, monitoring systems can only improve through their implementation. Therefore, despite the identified challenges, their initiation remains both important and necessary.

2.8 Conclusions

Various approaches are implemented across Europe to assess the condition of running eater habitats. The analysis of methodologies available from 18 EU MSs reveals limitations in the use of abiotic variables in the assessment of river habitat structure and function, representing a significant gap in the assessment of these habitats that are greatly influenced by abiotic factors.

The variation in chemical monitoring approaches undermines the comparability of assessments and complicates efforts to address widespread ecological challenges and anthropogenic pressures. Biotic variables, particularly those related to the composition and structure of vegetation, mainly focusing on characteristic and typical species, remain the most commonly used indicators, reflecting the botanical and phytosociological interpretation of river habitats protected under the Habitats Directive. However, this focus neglects an ecosystem-based perspective. While monitoring programmes based on species composition may appear suitable, a monitoring framework based mostly on plant communities is insufficient to address the management and conservation requirements of river habitats, and does not provide a meaningful evaluation of the ecosystem's structure and function.

Integrating chemical monitoring with biological and hydrological assessments would provide a more comprehensive understanding of habitat conditions. Additionally, leveraging data from the WFD would support the Habitats Directive by explicitly linking WFD ecological status with the structure and function conditions required under the Directive.

Moreover, discrepancies between administrative bodies, academic traditions, and technical perspectives contribute to the inconsistent interpretation and evaluation of these methodologies. Combined with local differences, these variations create a fragmented and incoherent network of approaches. This lack of standardisation results in significant data comparability issues, preventing the development of a coordinated network that can effectively address monitoring challenges.

Overall, the lack of harmonisation in habitat assessment methodologies across the EU is evident. Challenges to standardisation include the use of diverse approaches and criteria, as well as a reliance on expert opinions, which introduces variability into assessments. As a result, data quality and availability differ greatly among Member States. Establishing an intercalibration process similar to that of the Water Framework Directive (WFD) seems essential. For "running water" habitats, leveraging the work already done under the WFD would be a logical step.

In many cases, reference values and thresholds for determining good condition are poorly defined and predominantly based on expert judgement, with limited explanation of methodologies grounded in data analysis or the use of reference stations. This weakens the credibility and robustness of the procedures. Additionally, significant variation exists among Member States in determining reference values, thresholds, and condition indicators. Many criteria heavily depend on subjective evaluations rather than quantitative data, with differences in the selection of variables, weighting systems, and aggregation methods. This makes defining optimal conditions for habitats and species a complex and subjective process.

The absence of standardised methodologies, the reliance on expert judgement, and difficulties in establishing reference conditions contribute to uncertainty in habitat quality assessments. Addressing these issues requires greater harmonisation of methodologies, the development of standardised data collection protocols, and the establishment of clear, scientifically validated reference values and thresholds.

To overcome these challenges, there is a need to develop standardised methodologies, promote data sharing and interoperability, carry out intercalibration processes, invest in capacity building, and encourage ongoing research. Standardising the selection of variables, measurement techniques, and reporting formats would improve the comparability of habitat assessments across Europe and support the effective conservation and management of habitats of Community interest.

Many of the methodologies analysed identify and measure the intensity of pressures or disturbances. To avoid circular reasoning, it is necessary to eliminate variables that directly measure pressures.

Promoting the standardisation of methodologies across Member States and the further integration of abiotic variables into habitat assessments would improve the comparability of evaluations across the EU.

The monitoring of "running water" habitats (group 32) under Annex I of the Habitats Directive varies among EU Member States, but common approaches are also evident. Most Member States use WFD standards to evaluate ecological status, focusing on parameters such as water quality, hydromorphology, and biological indicators like fish and macroinvertebrates. Increasingly, remote sensing and GIS technologies are being adopted to monitor larger river stretches efficiently and track changes over time.

Standardised data collection using WFD protocols offers consistency across Member States, enabling cohesive assessments across regions. However, budgetary constraints often result in infrequent monitoring and data gaps, which impede long-term trend analysis. Developing a practical and harmonised protocol is essential to ensure the sustainability of a transnational monitoring system. While WFD methods provide a robust framework, many countries apply arbitrary criteria or inconsistent monitoring frequencies within the context of the Habitats Directive's, creating disparities, particularly for cross-border habitats.

Only through an ecological approach can the structure and function of ecosystems be fully understood and assessed. Incorporating physical and chemical variables alongside biotic factors and integrating all data within the framework of fluvial ecology is essential for the effective evaluation and conservation of "running water" habitats.

3 Guidance for the harmonisation of methodologies for assessment and monitoring of habitat condition

According to Articles 11 and 17 of the Habitats Directive, Member States must undertake surveillance and monitoring of the conservation status of the natural habitats and species included in the directive. As a result, harmonised methods that can provide comparable results on the status of each habitat type across its EU range are required. However, there are important differences in how EU Member States interpret each habitat as well as in the methods, and approaches they use for habitat assessment and monitoring, which represents a significant challenge to achieving this harmonisation.

These circumstances are common to all the habitats and ecosystems in the European Union, but are particularly challenging for rivers, due to the definition and interpretation of freshwater habitats and specifically, the running water habitats protected under the Habitats Directive, mainly based on the presence of vegetation or certain characteristic plant species. As previously mentioned, this is not the most appropriate approach for fluvial ecosystems and does not allow for an ecosystem-based approach for monitoring these habitat types. However, habitat types included in group 32 of the Habitats Directive are defined as “Running water — sections of water courses with natural or semi- natural dynamics (minor, average and major beds) where the water quality shows no significant deterioration.

The Water Framework Directive (WFD) appears to have integrated some of the principles of stream ecology to evaluate the ecological status of freshwater ecosystems, considering physicochemical, biological and hydromorphological variables, indices and parameters that, with some limitations, capture river structure and functionality.

Both directives, the HD and the WFD, have been adopted inter alia to guarantee the good status of EU freshwater ecosystems, so there is an alignment of objectives between them. The WFD's primary objective is to achieve “Good Ecological Status” for water bodies, which involves biological, chemical, and hydromorphological elements (Schmedtje et al., 2011). On the other hand, the HD aims to ensure the favourable conservation status of specific habitat types and species, focusing on the structure, function, and typical species of habitats. Despite their apparently differing focuses, there is considerable overlap. A river that achieves a good Ecological Status under the WFD is likely to exhibit good habitat structure, functioning, and ecological integrity, which are relevant to the assessment of running water habitats. Therefore, their integration and coordination appear to be an essential step towards the assessment and monitoring of river habitat condition. In this document, a coordinated and synergistic approach between both directives is proposed.

The ecological classification developed for the implementation of the Water Framework Directive, is based on the analysis of a comprehensive set of variables and environmental factors including hydrological, geomorphological, geographical and climatological elements. This typology is designed to support the assessment of the ecological status of water bodies, focusing on structural and functional ecosystem components. However, it already provides a comprehensive approach for evaluating the structure and function of lotic ecosystems.

Habitats from Group 32 (Running Water) are mostly classified based on vegetation composition and species assemblages. These include macrophyte communities, riparian vegetation, and sometimes broader biodiversity aspects and the primary goal is to assess the favourable conservation status of specific habitat types, focusing on species typical of the habitat, structural integrity, and functional dynamics.

These fundamental differences result in a mismatch of classification criteria. Under the WFD, rivers are classified into broader functional types, while HD habitats focus on specific ecological niches or vegetation patches along rivers. Consequently, a single WFD river type might overlap with multiple running water habitats of Community interest, and conversely, a single running water habitat might occur in multiple WFD river types, depending on geographic location or hydrological conditions. As a result, there is no one-to-one correspondence between WFD river types and habitats in group 32 of the HD. For example, a WFD lowland river type might include patches of 3260 (Water courses of plain to montane levels with the *Ranunculion fluitantis* and *Callitricho-Batrachion* vegetation) and 3270 (Rivers with muddy banks with *Chenopodion rubri* p.p. and *Bidention* p.p. vegetation), or even no habitat type at all, depending on the presence of vegetation. Similarly, 3260 habitats might occur across different WFD river types (e.g., lowland rivers and calcareous rivers) due to their dependence on species composition, not river type. WFD assessments might overlook habitat-specific features crucial for HD assessments, such as typical species or localised vegetation patches. Conversely, HD habitat mapping might fail to capture the broader ecosystem characteristics evaluated under the WFD. Moreover, there are spatial and methodological gaps because the WFD focuses on ecological and hydrological continuity, whereas HD habitats are often fragmented into smaller patches, complicating joint monitoring and management.

Nevertheless, greater coherence and convergence are possible by using WFD biological monitoring data (e.g., macrophytes and phytobenthos) to inform habitat-specific assessments while establishing monitoring protocols that address both directives' requirements, focusing on overlapping indicators like hydrology, macrophyte composition, and riparian connectivity.

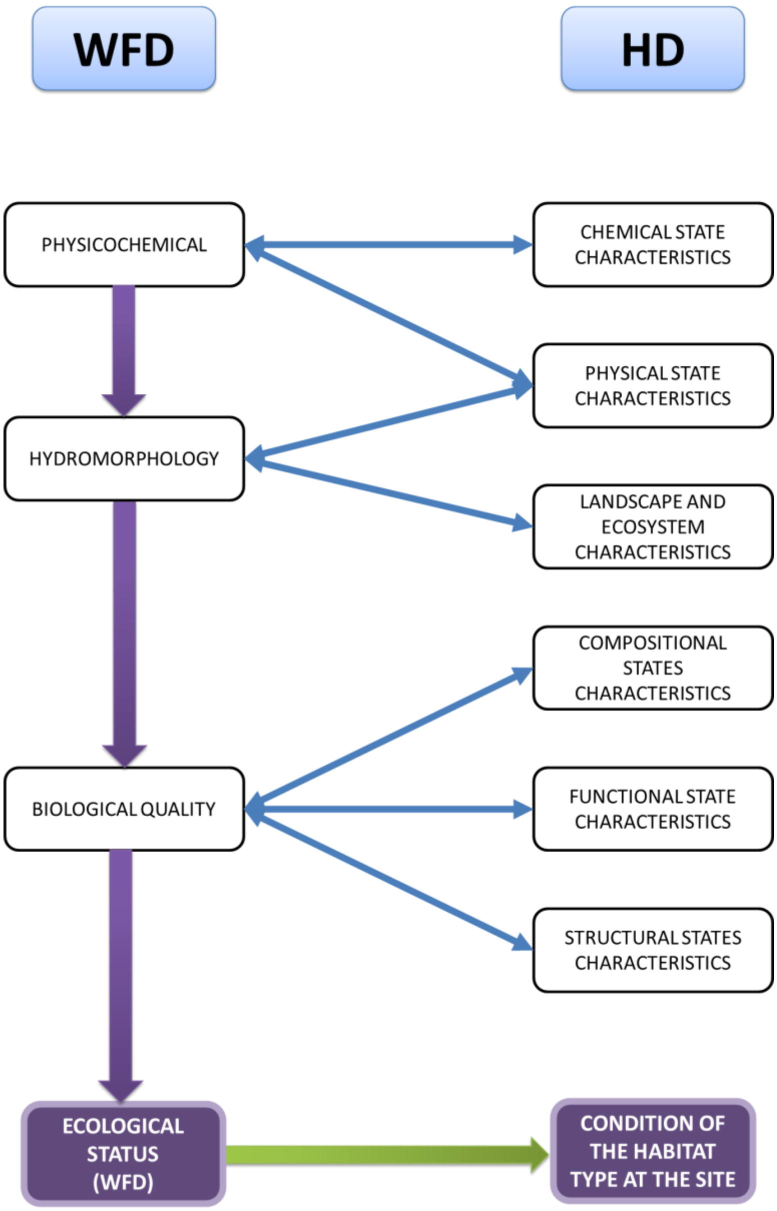
To achieve this integration, there are two possible strategies. The first and theoretically most logical approach involves establishing a monitoring system that uses WFD methodologies, calculating reference values for each habitat type from group 32 and for all variables. The second approach focuses on using and building on the experience and methods developed under the WFD. In this case, a river or reach that corresponds to a water body according to WFD, must be identified as a habitat type under the Habitats Directive and, at the same time, as a type of river according to the WFD and the respective national classification. Then, its "conservation status" would be determined by using WFD criteria, rules, variables, etc.

Nevertheless, although the approaches from the WFD and the HD are not fully equivalent; the assessment of "ecological status" (WFD terminology) could be considered roughly equivalent to the evaluation of the structure and function at the local level, according to the evaluation matrix of the HD. Therefore, although these two systems do not seem to be fully compatible, there are common elements and, with some adjustments and coordination between both approaches, they could be combined to establish a monitoring system for lotic ecosystems within the framework of the Habitats Directive (see Figure 4).

There have been notable attempts to coordinate both directives (Schmedtje et al., 2011; Janauer, et al., 2015; LIFE MedWetRivers project), and results already indicate that such coordination is possible, despite the limited correspondence between the typology of water bodies and the habitat types of Community interest.

However, currently, there is a significant disparity in the variables, metrics, and methodologies used by the different Member States to assess the condition of running water habitats under the Habitat Directive. The challenge lies in establishing common procedures and achieving comparable assessments across habitats at the different biogeographical regions as well as aggregating the data at the Member State and the European Union levels. This section aims to provide basic guidelines to harmonize the MSs approaches to monitoring the condition of running water habitats. It also proposes a framework that could be discussed and further revised by working groups with the participation of EU MSs.

Figure 4. Equivalence between the Water Framework Directive and the Habitat Directive evaluation process



Blue arrows indicate how the different elements interact, purple arrows show the determination of the condition status, and green arrow illustrates the equivalence between the Ecological Status of a water body and the Condition of the Habitat at a site according to the HD.

There are important strengths of the WFD that can be useful for the assessment of running water habitats. For example, the WFD's ecosystem approach and its ecosystem-based methodology align well with the ecological aspects of group 32 habitats, including hydrology, morphology, and connectivity. The WFD evaluates phytobenthos, macrophytes, macroinvertebrates, fish, and to some extent, riparian vegetation to assess ecological status using comprehensive biological indicators, which are directly linked to the structure and function of running water habitats. Moreover, hydromorphological indicators, such as flow regimes, sediment transport, and riparian connectivity, evaluated under the WFD are critical for the physical structure of group 32 habitats. However, the WFD’s approach excludes groups such as amphibians and reptiles, some of which are closely tied to aquatic ecosystems, as well as mammals and birds, whose broader spatial movements may weaken their direct link to fluvial ecosystems. While this could suggest that the WFD is insufficient for informing the HD, it is important to recognise that monitoring and assessment systems are continuously evolving. Including these groups could be an area for future improvement. Furthermore, the HD does not appear to have a systematic monitoring system for these groups, despite isolated efforts.

It is not an easy task, but there are pathways towards Integration between both directives. It is mandatory to use representative stretches as part of the WFD assessment to ensure that findings are extrapolated to both the water body and associated HCI group 32 patches.

Therefore, while not without its limitations, using the WFD's assessment system as a starting point for monitoring lotic ecosystems would represent a significant improvement. These limitations are neither insurmountable nor unacceptable and could be addressed through future joint developments of both monitoring frameworks.

While there is some spatial overlap between WFD river types and HD group 32 habitats, their classifications are conceptually and methodologically distinct. To enhance coherence, greater coordination and integration of monitoring and assessment frameworks are necessary, focusing on shared indicators and aligning the scales of evaluation. This would ensure both directives support each other in achieving their respective goals for aquatic ecosystem conservation and sustainable management. WFD sampling data (e.g., phytobenthos, macroinvertebrates, fish and macrophytes) can contribute to species lists required under the HD. Similarly, incorporating typical species assessments for group 32 habitats into WFD monitoring frameworks could bridge the gap. Shared datasets and harmonised methodologies could improve the effectiveness of both directives and reduce redundancy. Moreover, WFD metrics and indices could be refined to better reflect the requirements of HD assessments, particularly concerning species-specific conservation targets.

To sum up, while the WFD evaluation of rivers cannot completely substitute the HD’s requirements for assessing running waters habitats, it provides a robust starting point. With targeted adaptations—such as integrating species-specific assessments, ensuring spatial alignment, and enhancing coordination between directives, the WFD framework can significantly contribute to evaluating the condition of habitats of Community Interest in Group 32. This integration would also promote efficiency and consistency in meeting the goals of both directives.

3.1 Selection of condition variables, metrics and measurement methods

To evaluate and monitor the structure and function of running water habitats under the Habitats Directive (92/43/EEC), it is essential to choose variables that reflect the ecological integrity, status and overall functioning of the habitat. The main criteria for selecting variables to assess the structure and function of running waters (lotic ecosystems) under the Habitats Directive are described below:

- **Relevance:** Variables must directly measure key habitat characteristics critical for good condition, such as flow pattern, connectivity, and physicochemical attributes.
- **Ecological Sensitivity:** Variables should detect ecosystem changes early (e.g., pollution, climate change). Examples include dissolved oxygen, chlorophyll-a concentration, or

benthic invertebrate diversity are sensitive indicators of water quality and ecosystem health.

- **Representativeness:** Variables should reflect both ecosystem structure (e.g., species composition) and function (e.g., nutrient cycling, energy flow). Monitoring primary productivity (e.g., chlorophyll-a or biomass production) helps assess both structural and functional aspects.
- **Feasibility:** Variables must be practical, cost-effective, and measurable with available tools, ensuring long-term sustainability (Czúcz et al., 2021). For example, remote sensing data for land cover change can be cost-effective and provide continuous monitoring of large areas, making it a practical choice for monitoring landscape-level changes.
- **Minimal set:** A core group of variables should be selected to describe essential habitat characteristics efficiently.
- **Temporal and spatial sensitivity:** Variables should capture short- and long-term changes across different scales, ensuring that both short-term fluctuations and long-term trends are captured. For example, water temperature and flow in a river ecosystem can change rapidly, so frequent, or constant, monitoring might be needed.
- **Standardisation, harmonisation and comparability:** Variables should be harmonised across biogeographical regions and monitoring programmes, using clear, replicable methods and intercalibration processes as done under the WFD. Member States use standard methods for biodiversity and water quality assessment and have conducted intercalibration processes to compare different indices and methodologies. Clear, shared, and replicable descriptions of variables, metrics, and procedures are essential for consistency across countries.
- **Robustness to natural variability:** Variables must distinguish natural seasonal variation from anthropogenic impacts, especially in highly variable ecosystems like Mediterranean rivers.
- **Management relevance:** Monitoring should provide data that can inform practical management strategies and policy decisions, making the programme valuable for stakeholders.
- **Early warning capacity. Variables should detect ecosystem decline promptly, with appropriate measurement frequency.** For example, macroinvertebrates might be evaluated annually to detect the presence on Invasive Alien Species (IAS).
- **Integration with existing monitoring frameworks:** Variables should align with WFD and other monitoring efforts to ensure efficiency and avoid duplication. This ensures that monitoring efforts are efficient, coordinated, and provide a comprehensive assessment of ecosystem health across different policy frameworks.
- **Resilience and adaptive capacity:** Variables should indicate ecosystem resilience and recovery capacity, especially under climate or anthropogenic stressors..
- **Ecosystem services:** Ideally, the variables should include those that reflect ecosystem services (e.g., water purification, carbon sequestration) and have relevance to human well-being. So, monitoring addresses not only ecological aspects but also the services ecosystems provide to society, linking environmental health with human benefits.

By applying these criteria, monitoring programmes can effectively monitor the structure and function of ecosystems, providing the necessary information to assess their condition and guide management and conservation efforts.

The list of selected variables must consider the **main ecosystem characteristics and biophysical control factors**. In general, to assess running waters and to evaluate and monitor their condition, the following key aspects must be considered:

- Climatic factors that largely determine hydrology and physicochemical characteristics.
- Hydrological characteristics, such as water and sediment flow patterns, connections with groundwater bodies, fluctuations and pulses, hydroperiod and water level of fluctuation.
- Geomorphological processes and river basin characteristics forming the basis for the configuration, modelling, and physicochemical characteristics of the lotic ecosystem.
- Geological and edaphic characteristics, especially the lithological features related to the composition of the rocks in the river basin and the drainage basin, as well as soil characteristics.
- Physical-chemical characteristics of water, including mineralisation, type of dominant salts, pH and alkalinity, water transparency, dissolved oxygen and hydrogen sulphide concentrations, inorganic nutrients and organic matter.
- Biological communities typical of these ecosystems that respond to environmental factors, such as submerged or emergent macrophytes, phytobenthos, macroinvertebrates, fish, and other vertebrates.
- Community structural factors, including the physical structure of the community (e.g., the riparian community) and other structural aspects such as diversity and trophic structure.
- Biological processes (primary production, respiration, and ecological interactions).
- Exchanges with other ecosystems in term of energy, matter or organisms.
- Anthropogenic factors, including all types of interactions and effects of human activities.

This last point, anthropogenic factors, is somewhat controversial. When monitoring systems or protocols are developed, we are often tempted to include variables that directly measure pressure or threat. For example, it is common to include Invasive Alien Species (IAS) or pollution as variables or factors in an index, but this could lead to a “tautological” or redundant assessment. If we include an impact value or variable, such as the number of IAS or the volume of water diverted to industrial activities, then, when analysing the effects of these pressures on the ecosystems, a variable describing these pressures and our index will naturally be correlated. As expected, an index that incorporates information about IAS would show a relationship with IAS, but this is not due to the impact of IAS on the macroinvertebrate community itself; rather, it results from circular reasoning, i.e. the index decreases because of the presence of IAS, but not because of the effects of these species. These pressures (IAS, water extraction, etc.) must be analysed as ‘Pressures and Threats’, according to the Habitat Directive, or, as a ‘Pressure’ according to the WFD. This highlights the fact that indices must be designed to capture the effects of human activity independently.

A list of essential, recommended and specific variables is provided in this document (Table 6). **Essential variables** (E) relate to the essential characteristics defining the fundamental nature and distinctness of stream ecosystems, such as water and sediments flow, temperature, dissolved gases and ions, characteristic species, and indicators of habitat condition like nutrients, diversity of species, and the structure of the riparian zone. These variables should be assessed in all MSs following equivalent measurement procedures. **Specific variables** (S) are also linked to the habitat characterisation and condition, but they may need to be measured only in specific habitat types or under certain circumstances, e.g. in temporary and intermittent rivers. **Recommended variables** (R) are generally relevant to habitat assessment but they may not always need to be measured, depending on the specific context of the habitat or region. These variables are recommended in particular cases, according to contextual factors affecting the habitats in specific situations. Finally, a few **descriptive/contextual variables** (D) are also proposed to properly characterise the habitat in a particular location. These variables are also useful for defining relevant thresholds for the condition variables and to interpret the results of the assessment, but they are not included in the aggregation of the measured variables to determine the habitat condition.

The main abiotic variables refer to water flow patterns and sediment load. Moreover, temperature and the physicochemical characteristics of streams and rivers, including alkalinity or pH, conductivity, dissolved gasses and ionic composition, are key determinants of the structure and functions of these ecosystems. Ionic composition, alkalinity, conductivity and dissolved gases inform about the chemical characteristics and the availability of some essential components for the development of life. Therefore, all these variables are considered essential, while those related to nutrients and EQRs are classified as specific. In the case of nutrients and EQRs, they should be evaluated in water bodies at risk not achieving Environmental Quality Objectives (Article 4, WFD).

For compositional characteristics, species richness and (relative) abundance of characteristic species are essential variables. A regional list of species for each habitat type should be developed, using information and knowledge available from the implementation of the Water Framework Directive and in coordination with the work to perform under this directive.

The proposed list of variables is presented in Table 6; further description of their use and measurement methods are described below.

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Table 6. Proposal of condition variables for assessing and monitoring running water habitats

App: Application of the variables (E: Essential, O: optional, R: recommended, D: Descriptive). Metrics may show several options.

Ecological characteristics	Group of variables	Variables and metrics	Main standardized measurement procedures	App
Abiotic characteristics				
Physical state characteristics	Climate related variables	T_{air} ºC Precipitation (mm)	Continuous measurement. Using the nearest meteorological station. Installation of a temperature data logger if required.	D
		Water T in ºC	Continuous measurement, using data loggers.	E
	Stream flow	Discharge Regime Spatial flow variation Temporal flow variation Stream Flow ($m^3 \cdot s^{-1}$) and its alterations Flooding and drought magnitude, duration and frequency	Identification of discharge regime and characterisation of variation in flow over time and space. Regime classification include Glacial, Nival, Pluvial, Nivo-pluvial, Pluvio-nival, etc., based on water source; Perennial, Intermittent, Ephemeral, Seasonal, based on flow permanence. Regulated or natural	E
		Water velocity ($m^2 \cdot s^{-1}$)	Measured with current meters, weirs or pressure data loggers. Continuous measurement	E
	Substrate and sediment	Type and cover of substrate: Boulder, Cobble, Pebbles or Gravel, Sand, Silt, Clay	Granulometry. Visual estimation of percentage of coverage of substrate types in the river stretch. See USGS classification according to Wentworth (1922), and Blair and McPherson (1999).	D
		Sediment load (g/m^3/year)	Sediment traps	E
	Morphology or physical structure	River section: Stream order (Strahler) width, depth (m). Reach length (m), area (m^2), slope (%) Headwater, Middle-order or lowland reaches.	Measured at different parts of the river stretch	D
		Shoreline length and width (m) Shoreline features (modified: length)	Measured at different parts of the river stretch	E

Ecological characteristics	Group of variables	Variables and metrics	Main standardized measurement procedures	App
		Heterogeneity of forms in the riverbed: sinuosity, riffle and pools sequence, bars, islands, meanders…	Measured at different parts of the river stretch. Identification of morphological structures (bars, islands, meadows, riffles, ponds, …)	R
	Light, Solids and Turbidity	Light at river surface: shadow cover	Measured in a river section by calculating the Vegetation Shading Index (VSI), which quantifies the effect of riparian vegetation on the amount of sunlight reaching the water surface, impacting water temperature and aquatic life.	R
		Water transparency or turbidity. Total Dissolved Solids (TDS) and Total Suspended Solids (TSS).	Nephelometric Turbidity Unit (NTU) measured using a turbidity meter, TSS probes and APHA, AWWA & WEF, 2005	R
Chemical state characteristics	Basic chemical parameters	Salinity/Conductivity ($\mu S \cdot cm^{-1}$) pH, Alkalinity ($[HCO_3^-] + 2[CO_3^{2-}] + [OH^-] - [H^+]$) or Inorganic Carbon	Conductometer, pH meter and or a multiparameter probe. Acid/base titration or Gran method (Mackereth et al. 1978)	E
	Dissolved gases	Oxygen (O_2) (Dissolved Oxygen) (mg/l, %) Carbon (CO_2) (mg/l; %)	Multiparameter probe. Oxidation method (adapted from Koroleff, 1983)	E
	Inorganic Nutrients	Nitrogen (N_2), Nitrates (NO_3^-), Nitrites (NO_2^-), Ammonium (NH_4^+), Phosphorous Total (PTD), Orthophosphates, Soluble Reactive Phosphorus	Spectrophotometer. See APHA, AWWA & WEF, 2005	R
	Organic matter	DOM POM	DOM measured through spectrofluorimetry. APHA, AWWA & WEF, 2005	E
	Major ions	Hardness and other major dissolved ions: Ca^{2+}, Na^+, Mg^{2+}, K^+, Ca^{2+}/Na^+ ratio, HCO_3^-, CO_3^{2-}, SO_4^{2-}, Cl^-	APHA, AWWA & WEF. 2005	R
	Pollutants	EQS Priority Substances[1]	Data could be taken from monitoring stations under the WFD, close to the habitat sampling area, when available (measured according to Directive 2008/105/EC, if there is evidence or risk of pollution and contaminants).	R
	Other ions	Al, Fe^+, SiO_2, P inorganic (PRS)	APHA, AWWA & WEF (2005) and Molybdite method for PRS	R

Ecological characteristics	Group of variables	Variables and metrics	Main standardized measurement procedures	App
Biotic characteristics				
Compositional characteristics	Characteristic species - vegetation	Presence of Phytobenthos	Identify presence and number of species using reference lists for each habitat type and region/locality. Data could be retrieved from Phytobentos monitoring under WFD.	E
		Aquatic vegetation (Macrophytes): characteristic vascular plant species richness	Identify presence and number of species using reference lists for each habitat type and region/localities. Data could be retrieved from Macrophytes monitoring under WFD.	E
		Riparian vegetation: characteristic species richness	Identify presence and account number of species using reference lists for each habitat type and locality under monitoring, as appropriate. Data could be retrieved from Riparian vegetation monitoring under WFD.	
	Macroinvertebrates	Presence of macroinvertebrates	Identification of macroinvertebrates that are characteristic of each habitat type. Reference lists should be developed for each habitat type and locality, as appropriate. Data could be retrieved from Macroinvertebrates monitoring under WFD.	E
	Fish species	Presence, composition and number of fish species	Identification of fish species that are characteristic of each habitat type. Reference lists should be developed for each habitat type, river stretch and locality, as appropriate. Data could be retrieved from Fish monitoring under WFD.	E
	Other animal species	Presence, composition and number of bird species	Wildlife monitoring (camera traps, nonlethal traps, eDNA, Line Transects, Netting, Drone surveys, Passive Acoustic Monitoring (PAM)…)	R
		Presence, composition and number of native amphibians		R
		Presence, composition and diversity (number of species) of native reptiles		R
		Presence, composition and diversity (number of species) of native mammals		R

Ecological characteristics	Group of variables	Variables and metrics	Main standardized measurement procedures	App
Structural characteristics	Horizontal and vertical structure of vegetation	Coverage of aquatic vegetation (characteristic species / types: macrophytes, hydrophytes, helophytes, amphiphytes; % and/or m^2)	Visual inspection. Remote sensing / Google Earth (coverage by species %)	E
		Width, zonation and cover of riparian vegetation: herbaceous plants, shrubs and trees, etc	Visual inspection. Remote sensing / Google Earth (coverage by species %)	E
		Height and strata of riparian vegetation	Visual inspection in the river section under monitoring.	E
	Alteration of vegetation structure	Coverage of invasive alien species (%)	Visual determination of coverage: area covered by exotic plant species vs. total habitat area, in %	E
Functional characteristics	Organic load, trophic status	Biological Oxygen Demand: BOD_5 in mg O_2/l	Biodegradable dissolved organic carbon (Servais et al., 1989) or Fluorescence Index (McKnight et al. 2001). APHA, AWWA & WEF, 2005.	E
		Chemical Oxygen Demand: COD in mg O_2/l)	APHA, AWWA & WEF, 2005.	E
		Abundance of Phytobenthos	Sampling according to protocols and indices established by each EU Member State.	R
		Presence of algal growth: filamentous algae or "blooms.	Visual inspection (water surface covered by filamentous algae, in %)	E
		Presence of indicator species for eutrophication	Visual inspection (water surface covered by indicator species for eutrophication, in %)	E
		Woody debris and decomposition	Amount and volume estimated by visual inspection	R

Ecological characteristics	Group of variables	Variables and metrics	Main standardized measurement procedures	App
Landscape characteristics	Catchment characteristics	Drainage basin area (Km^2). Catchment Vegetation (m^2 and composition). Catchment mean slope	Cartography and hydrological projects	E
	Land uses and human activities in surrounding area	Area affected by land use and human activities close to the riparian area in the river section under monitoring	Visual inspection, remote sensing, available maps	R
	River continuity and connectivity	Number, type and characteristics of transversal and lateral barriers (dams, weirds, riverbank protections, etc.). Permeability of barriers, especially transversal barriers. Degree of channelization.	Visual inspection, cartography and hydrological projects	E

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3.1.1 Abiotic variables

The assessment of abiotic parameters is essential in aquatic ecosystems and must include both the physical parameters and the elements and compounds that are part of the water's chemistry (Karr et al., 1986). Abiotic characteristics, such as temperature, flow regime, substrate composition and water quality are fundamental in shaping the structure and function of lotic ecosystems.

Physical variables

Physical characteristics are not only determinants of habitat suitability for specific species but also drivers of ecosystem processes and community dynamics. Their modification, whether through natural events or anthropogenic activities, contributes to the emergence of new characteristics which could modify the composition and structure of the associated biological communities. Flow, substrate, temperature, and, to some extent, oxygen are the four physical variables whose patterns must be analysed to understand the functioning of lotic ecosystems (Allan 1995; Allan et al. 2007).Measuring **climate-related variables** is essential for lotic ecosystems, as these variables directly and indirectly influence the physical, chemical, and biological characteristics of the water. Changes in the climate, particularly those related to temperature and precipitation, can fundamentally alter the health and stability of these environments. Air temperature may determine water temperature, influencing species composition, chemical characteristics such as dissolved oxygen and nutrient levels, biogeochemical cycles and even metabolic rates. Similarly, changes in precipitation intensity or snowmelt occurrence affect water availability and flow, and may cause variations in flooding patterns and drought cycles. As a result, flow regimes can disrupt sediment transport, channel morphology, and habitat availability for aquatic species.

Water temperature is an essential physical variable due to its influence on the metabolic rates and life cycle of aquatic life forms. Most organisms in fluvial ecosystems are ectothermic, making temperature a critical factor that influences not only their activity but also their presence. This variable affects their metabolism, productivity, life cycles, growth rates, and other physiological processes. It also influences the solubility of gases since drastic changes in water temperature can alter the physical state of water (changes in density and solidification). It determines the survival of the riparian vegetation which, in turn, influences the amount of light reaching the water course. Therefore, temperature is proposed as an essential variable to be measured using data loggers.

Flow is a key structuring element of **fluvial ecosystems**, and flow regime — the pattern that describes its dynamics — is central to this. In consequence, the characterisation of the Hydrological Regime is necessary to understand running water habitats. **Flow** is a critical physical factor and the most defining feature of fluvial ecosystems. Its regime, variation, and physical characteristics shape the fluvial habitat. Flow drives processes beneficial to the functioning of lotic communities, such as the transport of nutrients and the removal of waste, as well as some destructive ones, such as drift processes. Both the liquid and solid flow rates characterise a catchment's hydrological regime. Therefore, the characterisation of the hydrological regime of **surface water bodies** should include (MAGRAMA 2017):

1. Analysis of liquid **flow rates**.
2. The hydrogeology of the area as it relates to the connection between surface water bodies and **groundwater bodies**.
3. And the estimation of the potential for **hydrological alteration.**

The first phase in characterising the hydrological regime of a water course is to identify the theoretical hydrological types for the water body, based on the degree of flow permanence and the origin of its inputs. Based on flow permanence, flow regimes can be classified as:

- **Ephemeral rivers**: Water courses where, under natural conditions, surface water only flows sporadically during storm events, for an average period of less than 100 days per year.
- **Intermittent or strongly seasonal rivers**: Water courses that, under natural conditions, have high temporal variation, with water flowing for an average period of between 100 and 300 days per year.
- **Temporary or seasonal rivers**: Water courses that, under natural conditions, show marked seasonality, characterised by low flow or being dry in summer, with water flowing for an average period of at least 300 days per year.
- Permanent rivers: Water courses that, under natural conditions, have water flowing in their channel throughout the year.

A classic classification of the river regime is based on the origin of the water inputs. This typology, though simple, is widely used and very informative:

- Mountain
    - Glacial
    - Nival
    - Nivo-transitional
    - Nivo-pluvial
    - Pluvio-nival
    - Pluvial
- Atlantic
    - Subtropical Pluvial
    - Oceanic Pluvio-nival
    - Oceanic Pluvial
- Mediterranean
    - Mediterranean Pluvial

Streamflow variables to be measured include discharge, velocity, low variability, inundation frequency, and inundation duration. Water velocity influences processes such as photosynthesis, nutrient assimilation and respiration, and determines the distribution of organisms in these habitats. It is therefore an essential variable to be measured, using current meters or data loggers. Flow can be measured by the area-velocity method as $Q = A * V$, where Q represents flow discharge, A is the cross-sectional area of the river, and V is the average water velocity. Spatial flow variation describes the different ways water moves within a river channel and how it creates a variety of habitats, like pools, riffles, and glides. Monitoring this variation is essential to ensure that a stream can support a diversity of species with different habitat needs. When flow becomes uniform, this habitat heterogeneity is lost, resulting in a decline in biodiversity

Discharge regime is the spatial and temporal flow variation over time. A healthy discharge regime ensures that life stages of aquatic species have the proper conditions for survival. Altering this regime can disturb essential processes of fluvial ecosystems, including breeding cycles, food availability and migration patterns. Discharge regime can be derived from continuous measurements of flow using data loggers or through remote sensing techniques.

To obtain accurate and continuous measurements of Stream Flow ($m^3 \cdot s^{-1}$), specific equipment is required. The simplest and most widely used tool is the current meter, which consists of a mechanical or electronic device that measures water velocity at various points along the stream's cross-section. The stream cross-section is divided into subsections dependent on the river depth, the discharge across each one is calculated and then added together to obtain the total discharge. Notably, the area for each subsection is estimated. An alternative to this method is the use of the Acoustic Doppler Current Profilers (ADCPs), which is a hydroacoustic instrument that uses the Doppler effect to measure water velocity and depth across an entire cross-section, providing a highly accurate discharge estimation. Both methods are useful for discreet measurements, but they cannot be used to perform continuous monitoring.

Gauging stations are the most common and widely used method to monitor flow, its regime and its alterations. Most of the stream gauges do not measure discharge continuously. Instead, they systematically measure the water level, or stage, and periodically measure discharge. A rating curve is hence created with these data points at each stage. Once a reliable rating curve is established, it can be used to calculate the stream's discharge at any point in time.

However, the installation of gauging stations in headwater sections, which are often located in **protected areas** or **nature reserves**, is not feasible. The use of pressure or water column height meters (López-Tarazón & Tena, 2016) is considered an appropriate alternative. When correctly utilised in river sections where the channel's profile is stable, these devices can accurately derive the flow and take continuous readings at a frequency of 5 to 15 minutes. See the discussion for more information on the use of **data loggers**.

The second phase of identifying the hydrological regime of a water course consists of characterising the flow regime, using two types of variables:

- **Theoretical variables**, which characterise the **natural flow regime** at the **water body**'s closure point. These are derived from data available in the integrated precipitation contribution modelling systems and the maps of maximum flows of the natural regime.
- **Actual variables** are measured using data from the gauging stations available throughout the hydrological monitoring networks of the River Basin Management Authorities.

The summary variables of these series are compared over two-time horizons. First, the mean values of the series for the entire available period (with the necessary caveats) is used to characterise the general trends between the natural and actual regimes. In **headwater water bodies** where the natural regime coincides with the actual regime and no gauging stations are present, only the natural regime is recorded.

Secondly, a comparison of the flow regime in the most recent available years is carried out to assess the functioning of the river and the **catchment** in the recent period.

Specifically, the following variables should be collected from the natural regime data:

- Mean monthly flows ($m^3 \cdot s^{-1}$) and mean annual flow ($m^3 \cdot s^{-1}$) for the entire available series.
- Mean monthly flows ($m^3 \cdot s^{-1}$) and mean annual flow ($m^3 \cdot s^{-1}$) for the short series.

From the map of maximum flows in the natural regime the following variables can be collected:

- Instantaneous maximum flows ($m^3 \cdot s^{-1}$) in the natural regime associated with return periods of 2, 5, 10, 25, 100, and 500 years.
- Estimated flow of the ordinary maximum flood ($m^3 \cdot s^{-1}$) and its associated return period.

The actual regime is analysed by selecting the gauging station from the **River Basin Management Authority** which best characterises the reach of the study. The available data from that station is then consulted from the annual gauging data information system. When unavailable, values are extrapolated based on the area of the associated catchments, using gauging stations located upstream or downstream of the water body. If none exist, data on the nearest or most similar water bodies (water bodies of the same type, order, etc.) is used. The following variable values are compiled:

- Mean monthly flows ($m^3 \cdot s^{-1}$) and mean annual flow ($m^3 \cdot s^{-1}$) for the entire available series at the characteristic gauging station.
- Mean monthly flows ($m^3 \cdot s^{-1}$) and mean annual flow ($m^3 \cdot s^{-1}$) for the most recent years that are common between the gauging station data and the natural regime.
- The maximum flow ($m^3 \cdot s^{-1}$) recorded at the gauging station. In cases where only mean daily maximum flows are available, a conversion using Fuller's formula is done.
- The number of times the ordinary maximum is exceeded.

Subsequently, a preliminary assessment of the degree of **hydrological alteration**, and the evolution of the flow in recent years is done by comparing both flow series.

Multiple mechanisms have been developed for the analysis of **hydrological alteration** and the determination of **environmental flows**. Magdaleno (2013) provides a comprehensive review of existing techniques.

The main aim of these methodologies is assessing the most significant environmental changes affecting elements of the flow regime. Furthermore, they enable the evaluation of the alterations that different resource uses and management scenarios would cause to this regime. Additionally, they allow for the interpretation of the environmental consequences of flow regime alteration on the **ecological integrity** of the river and the identification of the aspects of the flow regime that most influence the recovery of a regulated section. Ultimately, they can help establish objective criteria for prioritising the restoration of degraded **fluvial ecosystems**.

According to Magdaleno (2013), the assessment process typically includes the following stages:

1. Selection of the most environmentally significant aspects of the flow regime.
2. Selection of the parameters and variables that allow these aspects to be characterised.
3. Definition of a set of indices that compare the parameter values between situations: **natural regime** versus **altered regime**, and natural regime versus the regimes corresponding to the different scenarios used to define the proposed **environmental flow regime**.
4. Deduction of the environmental implications of the alterations that have been evaluated.

The following quantitative and qualitative methodologies for analysing **hydrological alteration** deserve particular attention:

- IHA (Richter et al., 1996).
- Arthington et al. (2006).
- ELOHA (Poff et al., 2010).
- Delso et al. (2017), specifically for **temporary rivers**.
- Hydrological Alteration Indices for Rivers (IAHRIS method) (Martínez Santa-María & Fernández Yuste, 2006, 2010) for Spain.

Indicators of Hydrologic Alteration (IHA) are a set of 64 indicators developed by Richter et al. (1996, 1997). Beneficially, they do not demand a minimum of 15 years of continuous data as other methodologies. These indicators identify five key elements of the flow regime considered ecologically relevant under the Water Framework Directive's (WFD) that focus on hydromorphology:

1. Magnitude of monthly hydrological conditions: This measures the volume of water flowing in the river on a monthly basis.
2. Magnitude and duration of annual extreme hydrological conditions: This identifies the size and length of high flows (floods) and low flows (droughts) each year, which are critical for species and habitat dynamics.
3. Timing of annual extreme hydrological conditions: This refers to when these extreme events occur, which is important for the life cycles of aquatic organisms and riparian vegetation.
4. Frequency and duration of high and low pulses: This assesses the recurrence and length of high and low flow events, which influences processes like sediment transport and habitat connectivity.
5. Rate and frequency of change in hydrological conditions: This measures how quickly flows rise and fall, which can impact the stability of the river channel and can impact the biodiversity along it.

Regarding **substrate and sediment** characterisation, granulometry analysis through visual estimation of substrate type coverage is proposed. Measurement of the sediment load using sediment traps is also proposed. Sediment not only provides information on a river's characterisation, it also has a relevant role in restricting light availability and influences the distribution of benthic organisms (Elosegi & Díez, 2009).

Measurements of different **structural elements of the river** (width and depth of the river section, reach length, area, shoreline length and width, variety of shoreline forms) are proposed to be performed at different sections of the river stretch through remote sensing techniques and field work.

Light is an important variable to measure as it directly influences primary productivity rates. At the river surface it can be measured using the Vegetation Shading Index (VSI); underwater transparency is measured with the Nephelometric Turbidity Unit (NTU) using a turbidity meter.

Chemical variables

The chemistry of water, its composition, and the total and relative abundance of certain compounds are critical in determining the structure and function of river ecosystems (Butturini et al., 2009). The composition and proportion of major and trace components represent the synthetic result of the water's history. These are determinant factors for the structure and composition of the biological community that a fluvial ecosystem supports, and consequently, for its functioning.

The **l**ithology of the catchment area is the primary source of solutes in river waters. Therefore, the chemical characteristics of the water can vary significantly between catchment areas, to the extent that some rivers exhibit unique and occasionally extreme physico-chemical characteristics (Margalef, 1983). Catchment lithology determines the presence and concentration of the following ions: SiO^{2-}, Fe^{2+}, Mn^{2+}, Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, $CO_3{}^{2-}$, HCO^{3-}, $SO_4{}^{2}$, Cl. This ion load, both in terms of presence and relative proportion of these ions, determines the chemical and metabolic processes that occur in the river.

The initial approach to understanding this ionic composition can be made generally by analysing the **acidity, alkalinity, and ionic load** of the waters. Although these are approximations of the water's overall characteristics, they are highly informative and essential. The assessment of ecological status under the WFD includes the measurement of the following variables:

- Acidification (pH)
- Conductivity (µS·cm-1)
- Alkalinity

Once a general assessment is made, it is necessary to analyse the specific components of the water chemistry. There are a multitude of elements and compounds transported by water which can be studied according to their state in water (dissolved or not), their origin (organic or inorganic) or other relevant criteria. The classification proposed here is based on Allan (1995) and Berner & Berner (1987), and includes:

Basic chemical variables

- Conductivity, pH, alkalinity.
- Dissolved gases: O_2, N_2, CO_2, and others.
- Nutrients: nitrate, nitrite, ammonium, soluble phosphorus, total phosphorus, etc.
- Dissolved organic matter (DOM).
- Particulate organic matter (POM).

These variables are measured in application of the surveillance and monitoring programmes according to WFD.

Complementary, specific chemical variables

- Major dissolved ions: Ca^{2+}, Na^{+}, Mg^{2+}, K^{+}, Cl^{-}.
- Suspended inorganic matter including major elements such as Al, Fe, Si, Na, and P.
- Biological oxygen demand (BOD), chemical oxygen demand (COD).
- Metals (especially copper and zinc).
- Trace elements.

Regarding the assessment and monitoring of running water habitats; **pH, conductivity and alkalinity** are proposed as essential variables. These can be measured with a conductometer and a pH meter, or with a multiparameter probe. **Additionally, dissolved gases in the water** (dissolved oxygen and carbon) can be measured with a multiparameter probe.

Biotic activity, both in the catchment and in the river channel, modulates the availability of **carbon, nitrogen, phosphorus** and other elements. For example, changes in land use, due to human activity, can induce changes in the concentration of HCO^{3-} in groundwater and runoff water. Additionally, photosynthetic and respiration activities influence the concentration of oxygen, dissolved inorganic carbon (and thus alkalinity) and pH (Elosegi & Sabater, 2009).

Dead **organic matter** accounts for most of the chemical energy present in these ecosystems and its decomposition represents the mineralisation pathway in river ecosystems (Butturini et al., 2009). DOM and POM are proposed as essential variables which can be measured through spectrofluorimetry.

Hydrological changes, both seasonal (droughts, thaws) and sudden (floods), determine biogeochemical changes that can provide information on the functioning of the river system and its interactions with the catchment. For example, in a forested catchment, during floods, terrigenous inputs in runoff water cause increases in DOM and nitrate concentrations, while pH and mineral salt concentrations decrease sharply. On the other hand, during droughts, the concentration of salts, DOM and ammonium increase noticeably, while oxygen and nitrate are depleted due to microbial activity (Butturini et al. 2009).

Detecting the presence of **pollutants** in the water is also recommended where contamination coming from agricultural, industrial and urban activities in the surrounding areas is suspected. Monitoring the presence of **other ions** (Al, Fe+, P, SiO2) is also proposed as a recommended variable, especially for rivers possibly affected by regulation through dams or similar structures.

Coordination and alignment with the WFD monitoring programmes for the monitoring of abiotic variables

Most of the physical and chemical variables that have been proposed in this section are monitored in the surveillance and monitoring programmes under the WFD. The measurement methods that can be used to measure the proposed variables in Table 7 are included in the Commission Directive 2014/101/EU of 30 October 2014 that amends the Directive 2000/60/EC of the European Parliament and of the Council establishing a framework for Community action in the field of water policy Text with EEA relevance.

Therefore, MSs could retrieve the required information from the monitoring stations that collect data under the WFD obligations where the water body being monitored is included in the habitat area under assessment. To facilitate convergence between the Water Framework Directive and the Habitat Directive, the proposed methodologies could, when possible and appropriate, be aligned with the Water Framework Directive, in particular the proposed essential variables that are also proposed in Annex V of WFD.

Hydro-morphological variables are part of the evaluation of ecological status under the WFD. To measure hydro-morphological quality elements, standards for hydro-morphological parameters must be followed, such as:

- UNE EN 14614:2004 Water quality — Guidance standard for assessing the hydro-morphological features of rivers.
- UNE-EN 15843:2010 Water quality - Guidance standard on determining the degree of modification of river hydromorphology.

There are some national protocols that could be used as a reference. This is the case of the German (Quick et al., 2019), French (Lamand et al., 2017; Le Bihan, 2023) and Spanish (MITECO, 2019; MAPAMA, 2017) protocols. By following these, most of the hydro-morphological variables used by de Member States can be determined.

Table 7. Hydromorphological indices used by EU Member States in the application of the WFD for rivers (physical, morphological and hydrological)

Index Type	Index Name	Description	Examples of Use by MSs
Physical	River Habitat Index (RHI)	Assesses the quality and structure of river habitats based on physical features, such as substrate, flow, and channel morphology.	Used in DE, AT, UK.
	Channel Morphology Index	Assesses the structural integrity and morphology of the river channel (e.g., width, depth, sinuosity).	Common in FI, DE, UK.
	Flow Regime Assessment	Evaluates the variability and natural regime of river flows, focusing on alterations caused by hydropower or human interventions.	Applied in AT, DE, FR.
Morphological	River Continuity Index (RCI)	Assesses the continuity of river channels, focusing on obstacles to fish migration and sediment transport.	Applied in DE, FR, SE.
	Morphological Quality Index (MQI)	Measures the overall quality of river morphology, considering factors like channel shape, riverbed stability, and substrate diversity.	Used in FR, DE, PL.
	Sediment Quality Index	Measures sediment characteristics (e.g., grain size, substrate composition) to assess riverbed stability and habitat quality.	Used in NL, DE.
Hydrological	Hydrological Quality Index (HQI)	Assesses changes in river flow patterns, such as flow velocity, volume, and seasonality, that can affect river ecology.	Applied in DE, FR.
	Flow Alteration Index (FAI)	Measures alterations in the natural flow regime, focusing on factors like flow variability and seasonal patterns.	Used in IT, DE, ES.
	Flow Modification Index (FMI)	Measures the degree of hydrological change from the natural flow regime due to human activity.	Applied in SE, DE, AT.

Note: This is not an exhaustive list of the indices used by MSs to implement the WFD.

Chemical variables are also measured in the Operational and Investigative Monitoring Programmes established by the WFD (Table 8). The analysis of the main compounds could be conducted following relevant methods and protocols as the following:

- APHA, AWWA & WEF (2005). Standard Methods for Examination of Water and Wastewater. 21st edition. In: Clesceri, Greenberg & Eaton (eds.) American Public Health Association (APHA), American Water Works Association (AWWA) & Water Environment Federation (WEF). Washington.
- CEN EN ISO 5667-3: 2024 Water quality - Sampling - Part 3: Preservation and handling of water samples.
- Guidance on Surface Water Chemical Monitoring under Water Framework Directive (Hanke et al., 2009).
- Elosegi & Sabater (2009). Concepts and Techniques in Riverine Ecology. Fundación BBVA. Bilbao. 444 pp.

- Hauer & Lamberti (2007). Methods in Stream Ecology. 2nd edition. Academic Press. San Diego.
- ISO/IEC 17025, General requirements for the competence of testing and calibration laboratories.
- ISO/IEC GUIDE 43-1: 1997. Proficiency testing by interlaboratory comparisons - Part 1: Development and operation of proficiency testing schemes.
- López-Tarazón & Tena (2016). Medición de procesos hidrosedimentarios. In R. J. Batalla & Á. Tena (eds.) Procesos hidrosedimentarios en medios fluviales, 75–108. Lleida: Editorial Milenio.
- Other Online Catalogues of European Standards. European Committee for Standardisation.
- Catalogue of International Standards: ISO Standards; EN Standards; British Standards; IEC Standards.
- List of methods from the National Environmental Methods Index (NEMI)
- U.S.E.P.A. and USGS methods and guidelines (Barbour et al. 1999; Barbour et al. 2006).

Table 8. Main methodologies for the analysis of carbon, nitrogen and phosphorus, key elements involved in Redfield ratio or stoichiometry of aquatic ecosystems

Element	Solute	Code	Method	Reference
Carbon	Organic	MOD	Dissolved organic biodegradable carbon	Servais et al. (1989)
			Fluorescence Index	McKnight et al. (2001)
	Inorganic	CID	pH indicator change Gran's methods	Mackereth et al. (1978)
Nitrogen	Total	NTD	Oxidation Method	Adapted from Koroloeff (1983)
	Inorganic	NO_2^-	Sulphanilamide method	Mackereth et al. (1978)
		NH_4^+	Salicylate method	Reardon (1966)
		NO_3^-	Nitrite reduction method	Adapted from Mackereth et al. (1978)
Phosphorous	Total	PTD	Oxidation Method	Adapted from Koroloeff (1983)
	Inorganic	PRS	Molybdate method	Murphy & Riley (1962)

3.1.2 Biotic variables

Habitat monitoring programmes under the Habitats Directive must include the monitoring and assessment of the composition, structure and function of biotic components. This is also a requirement for river evaluation under the WFD. The indices used by the WFD include basic information on composition and species assemblage. In consequence, the information obtained in the application of WFD can be useful, at its early stages, to fulfil the HD requirements.

Compositional variables

Most of the **compositional variables** are proposed as essential variables except for the monitoring of fauna groups such as bird, amphibian, reptiles and mammals, which are proposed as recommended variables, when relevant. The presence and number of **characteristic aquatic and riparian plant species** should be recorded based on reference lists specific for each habitat type and locality, through fieldwork monitoring. In addition to plant species, the presence and number of relevant characteristic **macroinvertebrate**, **fish** and **other animal species** for each habitat type should also be monitored. This is relevant to assess the condition of the habitat concerned. Reference lists should be developed for each habitat type, region and locality, as appropriate.

All EU MSs monitor plant composition, especially characteristic plant species, in the river and riparian communities. Presence of fauna is assessed and monitored in at least 6 MSs, including groups such as fish, birds, amphibian, reptiles, orthoptera, odonata, and others.

Structural variables

Coverage of characteristic aquatic vegetation (hydrophytes, helophytes, amphiphytes), **width, zonation, height, strata and cover of riparian vegetation** are proposed as essential variables to assess the structure of running water habitats. Cover and height of characteristic species is included in river habitat monitoring by 11 MSs. Additionally, width, zonation, structure and quality of riparian vegetation is monitored in at least 8 MSs.

Coverage of invasive alien species of aquatic and riparian vegetation is also proposed as an essential variable. Aquatic invasive species are a primary threat to streams and rivers across the globe, impacting biodiversity, disrupting key ecological functions, and compromising ecosystem services (Olden et al., 2022). The assessment of the presence and abundance of these species can be performed along with the monitoring of the native species.

Measuring **abundance of phytobenthos** is also recommended as a useful variable that provides information about the condition of river habitats. Data can be retrieved from WFD monitoring or through sampling carried out according to protocols and indices established by each EU Member State (e.g. IPS measures the abundance of each taxon).

Coordination and alignment with the WFD monitoring programmes for compositional and structural variables

If available, data for the proposed compositional and structural variables could be retrieved from monitoring programmes implemented under the WFD, in particular regarding macrophytes, riparian vegetation, macroinvertebrates and fish. Monitoring of these biotic components, based on WFD methods for ecological status assessment, is implemented in the monitoring methodologies of river habitats by at least 5 MSs. Some of the methods and indices proposed to carry out the assessment and monitoring of running water habitats are presented below.

Macrophytes are important bioindicators under the WFD and offer valuable insights into the ecological health of lotic ecosystems. As primary producers, macrophytes play a central role in riverine food webs, providing shelter and nutrition for aquatic organisms, while influencing water quality and sediment dynamics. Their presence, abundance, and composition are sensitive to a range of environmental factors, such as nutrient levels, water chemistry, hydrological changes, and physical habitat conditions. Macrophytes respond quickly to changes in water quality, especially to nutrient enrichment and eutrophication, which can result from agricultural runoff, wastewater discharge, or other anthropogenic activities. By monitoring macrophyte communities, it is possible to assess the degree of nutrient pollution and the overall trophic state of a river. They also reflect changes in hydrological regimes, such as altered flow patterns or sedimentation.

Table 9. Macrophyte indices used by EU Member States in the application of the Water Framework Directive (WFD) for rivers

Index Type	Index Name	Description	Examples of use by Member States
Compositional	Species Richness	Total number of macrophyte species observed in sample	Common in DE, FR, UK.
	Number of Families	Counts the number of different macrophyte families.	Applied in various Member States (e.g., SE, NL).
	Shannon Diversity Index	Diversity measure based on species abundance and evenness.	Used in various Member States (e.g., UK, FR).
Structural	% Sensitive Species	Proportion of macrophyte species sensitive to nutrient enrichment or pollution.	Common in Northern and Central Europe.
	Macrophyte Index for Rivers (MIR)	Multi-metric index combining species composition and ecological preferences	Widely used in DE, UK, FR.
	Ellenberg Indicator Values (for Nutrient Status)	Measures ecological preferences of macrophytes concerning nutrient levels	Applied in various Member States (e.g., DE, AT).
	% Emergent / Free-floating Species	Proportion of emergent or floating species in river habitats.	Common in riverine ecosystems (e.g., SE, PL).
	Biovolume of Dominant Species	Assesses biomass or volume of dominant species in river macrophyte communities, indicating nutrient conditions.	Applied in IT, FR, DE.
Functional	Functional Group Composition	Classifies species into functional groups based on their roles (e.g., oxygenators, detritivores).	Used in the UK, DE, FR.
	Trophic Level Indicator	Measures dominance of species associated with different trophic conditions (e.g., eutrophic, oligotrophic).	Applied in Mediterranean regions (e.g., ES, PT).
	Life-Form Classification	Classifies species based on morphological traits and ecological roles (e.g., submerged, floating-leaved).	Used in riverine ecological assessments (e.g., NL, SE).

Note: This is not an exhaustive list of the indices used by MSs to apply the WFD.

Riparian vegetation is included in the assessment of hydromorphological features of rivers under the WFD. Several indices are used across MSs which could provide information to assess the proposed compositional and structural variables for assessing and monitoring running water habitats. For instance, France and Spain use a Riparian Quality Index (IQBR), which includes information on the total percentage of riparian cover, the structure of the vegetation cover (trees, shrubs), the canopy quality (number of native tree species) and alterations in riparian vegetation (Munné, 1998; Mistarz, 2018).

Table 10. Riparian vegetation indexes commonly used by EU Member States in the implementation of the WFD

Index Type	Index Name	Description	Examples of Use by MSs
Riparian Vegetation	Riparian Vegetation Index	Assesses the quality and extent of riparian vegetation along riverbanks, which affects water quality and habitat availability.	Used in IT, FR, AT.
	Vegetation Structure Index	Assesses the diversity and structural complexity of riparian vegetation, which is vital for maintaining ecological functions.	Applied in FR, DE, IT.

Note: This is not an exhaustive list of the indeces used by MSs to apply the WFD.

Macroinvertebrates are one of the Biological Quality Elements used to assess the ecological status of water bodies because they play a crucial role as bioindicators due to their sensitivity to environmental changes. These organisms respond to a wide range of stressors, including organic pollution, chemical contaminants, habitat alterations, and hydrological changes. They play key roles in ecosystem functioning processes, such as nutrient cycling, organic matter breakdown and serving as a food source for higher trophic levels. Any changes in macroinvertebrate communities can signal disruptions in these essential ecological processes.

Sampling macroinvertebrates is relatively simple, cost-effective, and does not require sophisticated equipment. Many EU Member States have developed standardised protocols and identification keys, ensuring consistent and reliable monitoring across regions.

Fish are used as bioindicators under the Water Framework Directive (WFD) due to their ecological and biological significance. Fish are indicators of water quality as they are sensitive to a wide range of environmental stressors, including habitat degradation, hydrological changes, pollution and climate variability. For example, the presence or absence of sensitive species, such as salmonids, can indicate the ecological integrity of a waterbody, while an increase in tolerant or non-native species often reflects its degradation. Fish are also migratory and therefore depend on connectivity between habitats, such as spawning grounds and feeding areas. Their populations are directly affected by hydrological alterations, barriers like dams, and habitat fragmentation. Monitoring fish populations helps assess these broader impacts on river continuity and ecosystem functioning (Karr, 1981). However, in certain ecosystems with limited fish diversity – sometimes comprising only two or three species – their effectiveness as bioindicators can be limited.

Fish-based indicators are widely supported by standardised methodologies, such as the European Fish Index (EFI+) (FAME 2004; Consortium 2009), which facilitates consistent and comparable assessments across Member States. These methodologies integrate compositional, structural, and functional metrics to provide a holistic evaluation of ecological status.

Table 11. Macroinvertebrate indices used by EU Member States in the implementation of the WFD for rivers

Index Type	Index Name	Description	Examples of Use by Member States
Compositional	Number of Families	Counts the total number of macroinvertebrate families present in the sample.	Common across Member States (e.g., UK, DE, FR).
	Taxa Richness	Total number of distinct taxa (species, genera, families) in a sample.	Widely used (e.g., IT, NL, PL).
	Shannon Diversity Index	Measures diversity by considering both abundance and evenness of taxa.	Used by countries like ES, PT.
	Margalef Index	A diversity measure that accounts for the number of species relative to the total number of individuals.	Applied in Mediterranean countries.
Structural	BMWP (Biological Monitoring Working Party)	Scores based on macro-invertebrate taxa tolerance to pollution.	Used in the UK, ES, IT, and others.
	ASPT (Average Score Per Taxon)	Mean of BMWP scores for taxa in a sample.	Common in Central Europe.
	% EPT (Ephemeroptera, Plecoptera, Trichoptera)	Proportion of sensitive taxa indicative of good water quality.	Popular in Northern Europe (e.g., SE, FI).
	% Chironomidae	Proportion of Chironomidae (pollution-tolerant) individuals.	Applied in DE, PL, CZ.
	Saprobic Index	Evaluates organic pollution by analysing taxa tolerances to decomposition stages.	Widely applied in DE, AT, PL.
Functional	Biotic Index	Assesses water quality based on the functional role of macro-invertebrates (e.g., tolerance to pollution).	Used in IT and DE.
	Functional Diversity	Examines roles played by organisms in ecosystem functioning.	Rare but growing focus in some countries.
	Functional Feeding Groups (FFGs)	Proportion of taxa groups classified by feeding strategies (e.g., shredders, grazers, predators).	Used in ecological assessments in SE, NL.

Note: This is not an exhaustive list of the indices used by MSs to apply the WFD.

Table 12. Fish indices commonly used by EU Member States in the implementation of the WFD

Index Type	Index Name	Description	Examples of Use by Member States
Compositional	Number of Families	Counts the total number of fish families present in a sample.	Common across Member States (e.g., FR, DE, IT).
	Species Richness	Total number of fish species observed in the waterbody.	Widely used (e.g., UK, ES, PL).
	Shannon Diversity Index	Measures diversity by considering both richness and evenness of species.	Applied in IT, PT, NL.
	Native Species Richness	Counts the number of indigenous fish species present.	Focus in Central and Eastern Europe.
	Fish-Based Index (FBI or EFI+)	Multimetric index combining various metrics (e.g., abundance, composition).	Widely used across the EU (e.g., DE, FR).
Structural	% Sensitive Species	Proportion of species sensitive to habitat and water quality degradation.	Applied in Nordic countries (e.g., SE, FI).
	Abundance, Total Fish Biomass or Individuals (CPUE, BPUE)	Total weight or count of fish captured, reflecting habitat quality.	Used in NL, UK, ES.
	Recruitment Success	Measurement of young-of-the-year (YOY) fish indicating population sustainability.	Common in IT, DE, FR.
	% Tolerant Species	Proportion of species tolerant to pollution or habitat degradation.	Applied in polluted or modified waterbodies.
	Trophic Guild Composition	Proportion of feeding guilds (e.g., piscivores, insectivores, detritivores).	Used in ecological status assessment (e.g., ES, NL).
Functional	Habitat Guild Composition	Proportion of fish adapted to specific habitats (e.g., rheophilic or limnophilic species).	Common in Central Europe.
	Life History Traits	Includes parameters like age structure, growth rates, and reproduction strategies.	Emerging use in advanced assessments.
	Functional Diversity	Examines roles played by fish species in ecosystem functioning.	Rare but growing focus in ecosystem-level analyses.

Note: This is not an exhaustive list of the indices used by MSs to apply the WFD.

Functional variables

The following variables are associated with the organic matter content of the river and its trophic status.

Biological Oxygen Demand (BOD_5) is a measure of the amount of dissolved oxygen (in milligrams of O_2 per litre) that the microorganisms require to break down the organic matter in a water body over a period of five days. This variable quantifies the intensity of respiratory metabolic processes within the ecosystem, which increases with the organic load (both internal and external) and is commonly used as an indicator of organic pollution in aquatic environments.

Chemical Oxygen Demand (COD) represents the total amount of oxygen (in milligrams of O_2 per litre) needed to chemically oxidise both the organic and inorganic substances present in the water. It provides a measure of the total amount of organic matter in water, including autochthonous primary production output (biomass) and external detritus inputs, as well as the load of pollutants that can be chemically oxidised.

Measuring **abundance of phytobenthos** is also recommended as a useful variable that provides information about the condition and functions of river habitats. Phytobenthos are small photosynthetic organisms commonly found on stones in the bottom of rivers and lakes. They are an important part of the aquatic food chain providing nourishment for invertebrates which in turn provide food for fish and birds. When water presents an excess of nutrients, however, growth of opportunistic phytobenthos is accelerated, shifting community composition and, sometimes, reducing dissolved oxygen levels. Therefore, changes in the types and abundance of phytobenthos, particularly diatoms, can signal nutrient enrichment, pollution, and other environmental stressors.

Similarly, **presence of filamentous algae** that can produce “blooms” is commonly the consequence of extreme eutrophication. This variable, as well as the presence of other eutrophication indicator species, can be assessed visually by estimating water surface cover of. This variable is used to determine a river´s trophic status.

Vegetation debris is a natural component of lotic ecosystems, contributing to structural complexity and functioning. It creates diverse microhabitats, supporting a wide range of species, and plays a key role in nutrient cycling and energy flow. Monitoring vegetation debris helps assess the physical habitat conditions and their influence on biological communities. It can indicate lateral connectivity as well as the impact, nature and extent of surrounding human activity, such as deforestation, land use or river management practices. The amount and volume of woody debris estimated by visual inspection is proposed as a recommended functional variable.

Coordination and alignment with the WFD monitoring programmes for functional variables

Some of the proposed functional variables are monitored under the WFD. Biological Oxygen Demand and Chemical Oxygen Demand are monitored as part of the physico-chemical factors supporting the biological elements in the assessment of the ecological status of rivers.

Phytobenthos are one of the Biological Quality Elements used to assess the ecological status of rivers under the Water Framework Directive (WFD). This is due to their sensitivity to a wide range of environmental changes and their integral role in aquatic ecosystems. These organisms respond quickly to variations in water quality, such as nutrient enrichment, organic pollution and changes in pH, making them reliable indicators of ecological conditions.

Phytobenthos are primary producers and form the base of the aquatic food web, contributing to energy transfer and nutrient cycling within ecosystems. Their composition and abundance can directly reflect the trophic state of a waterbody, as they are highly influenced by nutrient availability. One of the significant advantages of using phytobenthos as bioindicators is their relatively stationary nature. Unlike fish or invertebrates, phytobenthos remain attached to the substrate, including rocks, sediments, or aquatic plants, offering localised insights into water quality and habitat conditions. This makes them particularly valuable for identifying point-source pollution and assessing the impact of specific stressors. Additionally, their ecological preferences, such as light requirements and tolerance to flow conditions, can provide insights into habitat alterations, sedimentation, and hydrological changes. By analysing phytobenthos communities, water managers can identify not only the current ecological state of a waterbody but also the underlying causes of degradation. Phytobenthos is also cost-effective and efficient to sample, with well-established identification protocols and standardised indices, which allows for consistent monitoring and comparison across MSs. In summary, phytobenthos are indispensable indicators under the WFD due to their sensitivity to environmental changes, ecological significance, and ease of monitoring.

Table 13. Phytobenthos indices used by EU Member States in the implementation of the WFD for rivers

Index Type	Index Name	Description	Examples of use by Member States
Compositional	Species Richness	Total number of phytobenthos species (e.g., diatoms) observed.	Common in DE, FR.
	Number of Families	Counts the number of different phytobenthos families.	Applied in various MSs (e.g., SE, NL).
Structural	Specific Pollution Sensitivity Index (SPI)	Measures the sensitivity of diatoms to organic pollution and water quality.	Applied in DE, AT, FI.
	Eutrophication Pollution Index (EPI-D)	Combines diatom sensitivity to eutrophication and organic pollution.	Common in FR, DE, IT.
	% Motile Diatoms	Proportion of motile diatoms indicating sediment disturbance.	Applied in SE, FI, PT.
	% Sensitive Species	Proportion of phytobenthos species sensitive to pollution or nutrient enrichment.	Common in Northern and Central Europe.
	Functional Group Composition	Classifies phytobenthos into functional groups based on ecological roles (e.g., oxygenators, detritivores).	Used in DE, AT.
Functional	Life-Form Classification	Classifies phytobenthos species based on their life forms (e.g., epilithic, planktonic).	Common in river studies (e.g., NL, FR).
	Trophic Guild Composition	Classifies phytobenthos species into guilds based on their trophic roles.	Applied in studies in DE, ES.
	Diatom Life-Form Composition	Classifies diatoms in relation to their ecological preferences (e.g., epilithic, epiphytic).	Used in DE, IT.
	Trophic Diatom Index (TDI)	Assesses the nutrient enrichment in rivers based on diatom composition.	Used in DE, NL.

Note: This is not an exhaustive list of the indices used by MSs to apply the WFD.

3.1.3 Landscape variables

The river basin concept is central to freshwater ecology and its adoption as the fundamental management unit is strongly advocated for by both the Habitat Directive and the Water Framework Directive. A river basin encompasses the entire area drained by a river and its tributaries, making it a cohesive system where activities in one section can have an impact along the entire area. This hydrological connectivity means that pollutants introduced upstream, for example, can impact water quality and the health of aquatic life downstream.

Beyond its hydrology, the river basin also exhibits ecological interdependence. Various components such as the main channel, tributaries, riparian zones, and wetlands are all interconnected. Organisms move between these habitats, relying on them for different life stages and processes. For example, a point source of nutrient runoff from agricultural land can significantly influence the oxygen levels in the main river.

Recognising the river basin as the basic management unit allows for the integrated consideration of all relevant factors. This includes different land use practices, pollution sources, water abstraction for irrigation or industry and physical habitat modifications, such as dam construction. The main parameters to consider when assessing relevant landscape characteristics influencing river habitat condition include the following:

- **Longitudinal connectivity or fragmentation:** Fragmentation can isolate populations, reduce gene flow, and limit the ability of organisms to move and adapt to changing conditions. Assessing the degree of fragmentation caused by anthropogenic activities is essential and can be done by evaluating the degree of connectivity along the river's length. This is done by considering the number and characteristics of barriers like dams, weirs and other obstacles that can impede fish migration and disrupt natural flow patterns.
- **Lateral connectivity:** This evaluates the connection between the river channel and its floodplain, riparian zones, and wetlands. These areas are important habitats for many species and play a crucial role in nutrient cycling and flood regulation.
- **Land use:** This refers to the types of human activities occurring around the river habitats and in the catchment, such as agriculture, urbanisation, and forestry. Land use can significantly impact water quality, habitat availability, and connectivity. The existence of riparian buffer zones, i.e. of natural habitat, that are maintained around rivers, protects them from the impacts of human activities and can help to filter pollutants, stabilise banks, and provide habitat for wildlife.

Visual inspection and use of cartography to determine the number, type and characteristics of the different barriers present in the river, their permeability and the degree of channelisation are proposed as measurement methods to characterise the rivers. Consideration of the presence, extent and intensity of human activities that can affect the functioning of the river ecosystem is also recommended.

Coordination and alignment with the WFD monitoring programmes for landscape variables

Some national protocols available for the implementation of the WFD could be used for the evaluation of these landscape variables. Namely the German (Quick et al., 2019), French (Lamand et al., 2017; Le Bihan, 2023) and Spanish (MITECO, 2019; MAPAMA, 2017) protocols.

Table 14. Landscape indices used by EU Member States in the implementation of the WFD for rivers

Index Type	Index Name	Description	Examples of use by MSs
Landscape/ Connectivity	Landscape Structure Index	Evaluates the heterogeneity of the landscape surrounding the river, such as the diversity of land uses and natural areas.	Applied in DE, SE.
	Habitat Fragmentation Index	Measures the degree of fragmentation of river habitats due to human activities, such as dams, bridges, or urbanisation.	Common in IT, FR, SE.
	Buffer Zone Integrity Index	Assesses the integrity and width of buffer zones between rivers and surrounding landscapes, which are essential for maintaining ecological functions.	Used in AT, DE, PL.
	River Continuity Index (RCI)	Assesses the continuity of river channels, focusing on obstacles to fish migration and sediment transport.	Applied in DE, FR, SE.
	Connectivity Index	Assesses the connectivity between river habitats and surrounding ecosystems, considering barriers and landscape fragmentation.	Used in DE, AT, SE.

Note: This is not an exhaustive list of the indices used by MSs to apply the WFD.

3.2 Guidelines for the establishment of reference and threshold values, and obtaining condition indicators for the variables measured

The measured values of the condition variables need to be compared with reference values and critical thresholds to assess the condition of each variable. A reference level is the value of a variable under reference conditions, against which it is meaningful to compare past, present or future measurements. The difference between a variable's measured value and its reference level represents its distance from the reference condition.

Reference levels should be defined consistently across different variables within a given ecosystem type, and for the same variable across different ecosystem types. This ensures that derived indicators are compatible and comparable, and that their aggregation is ecologically meaningful (UN, 2021).

Reference levels are typically defined with upper and lower values reflecting the endpoints of a condition variable's range, which can then be used in re-scaling. For instance, the highest value may represent a natural state, while the lowest value may represent a degraded state where ecosystem processes fall below the threshold required to maintain function (Keith et al., 2013, in UN, 2021). For example, pH values in freshwater ecosystems clearly indicate whether biological life can be sustained, while soil nutrient enrichment beyond a certain threshold can lead to the loss of sensitive species.

Establishing reference values and thresholds is essential for determining whether habitats are in good condition or have become degraded. Reference values represent the desired state of an ecosystem, typically reflecting intact or minimally disturbed conditions. These values serve as benchmarks for assessing habitat condition.

These guidelines do not aim to prescribe specific threshold values. Rather, they outline the main criteria and guide for establishing reference values that support the determination of good or not-good condition, while accounting for the ecological variability of habitats across their range.

Concerning the variables, the harmonisation of reference values and thresholds should consider a set of common requirements:

- For a given habitat, the final assessment of its condition and trend over time – based on the reference values and thresholds of the variables characterising the habitat – should be equivalent across Member States (MSs), after accounting for the contextual factors specific to each MS (e.g., climate).
- Thresholds, limits, and reference values should be tested using sufficiently robust datasets that represent the full range of habitat conditions, from degraded to high-quality sites.
- Thresholds must account for the natural variability of habitats across their range. Consequently, different threshold or reference values for the same habitat type may be appropriate in different MSs or in different regions within a single MS
- Establishing reference values requires information external to the evaluated site, which can provide insight into the condition of the habitat and be translated into variable values that characterise that condition.
- Reference values should meet the criteria of validity (ecological relevance), robustness (reliability), transparency, and applicability (Czúcz et al., 2021, Jakobsson et al., 2020).
- Each MS should provide a clear, justified, and comprehensible description of the methodology used to establish threshold and reference values for each variable.
- The methodologies should be designed for regular evaluation and improvement, based on the best available scientific knowledge. Any modifications made – and their implications for past monitoring data – must be communicated transparently.
- A reference library and indicator thresholds should be developed for different habitat types across regions, considering their ecological characteristics and natural variability.
- Joint training or guidance on setting threshold and reference values should be offered to experts from the different MSs to achieve ensure harmonised approaches.

Approaches for setting reference values and thresholds to determine condition

Several approaches have been recognised for estimating reference values to assess habitat condition (Stoddard et al., 2006, Jakobsson et al., 2020, Keith et al., 2020). These can be broadly synthesised into six categories: (1) absolute biophysical boundaries, (2) comparison to reference empirical cases, i.e. areas or communities considered to be in good condition, (3) comparison to undisturbed cases, (4) modelling and extrapolation of variable-condition relationships, (5) statistical assessments, and (6) expert judgement.

All approaches should be grounded in scientific literature. Methods that use values from a single baseline year as a reference for good condition are not recommended, as the selected year may not reflect favourable conditions, and historical data may be unreliable or incomplete (Jakobsson et al., 2020). The use of historical period (e.g., pre-industrial) as a reference state, as proposed by Stoddard et al. (2006) and Keith (2020) aligns with the baseline approach but also overlaps with comparisons to undisturbed cases (see below). If conditions during a specific baseline year are well documented as favourable, they may be useful for trend analyses. Likewise, where historical pristine conditions are clearly documented, they may serve as valid reference states under the undisturbed comparison approach.

Absolute biophysical boundaries

These refer to situations in which observed values of variables exceed the physical and chemical limits (e.g., pH, bare soil cover, critical loads for eutrophication or acidification) or biotic limits (e.g., overdominance of one specie) that define the habitat. When such limits are exceeded, the habitat cannot be in good condition (Jakobsson et al., 2020). These thresholds therefore indicate negative impacts on the favourable condition of the habitat.

- Advantages: This approach provides robust and transparent criteria that are clearly linked to the ecological integrity of the habitat.
- Disadvantages: It is applicable to a limited number of variables, typically those with direct negative impacts on habitat condition.

Comparison to empirical cases considered to be in good condition

This approach is based on identifying areas or communities considered to be in good condition (Stoddard et al., 2006, Jakobsson et al., 2020, Keith et al., 2020). These serve as reference cases from which the reference values can be derived. Therefore, their careful selection – and the availability of a sufficient number of such cases – is essential for ensuring the reliability of the reference value estimates (Soranno et al., 2011). While this method may appear straightforward, it is often limited by the scarcity of suitable sites, especially in landscapes that have been historically modified.

- Advantages: Providing that sufficient data from high-quality cases are available, this approach offers empirical validity and reliability by directly linking variable values to habitat condition.
- Disadvantages: Methodological challenges arise due to the difficulty of identifying enough suitable reference sites in historically altered environments.

Comparison to cases with a natural disturbance regime

This approach is closely related to the previous one, based on the assumption that most human-induced disturbances reduce habitat quality. This assumption is generally valid in human-modified landscapes and can be linked to historical reference conditions when human pressures were less pronounced (Stoddard et al., 2006). However, disturbances that are part of a natural disturbance regime may actually indicate naturalness and thus good habitat condition. In fact, a certain level of disturbance can be beneficial, supporting microhabitat formation, enhancing biodiversity, and promoting regeneration of habitat-characteristic species (Keith et al., 2020).

- Advantages: This approach provides transparent and empirically grounded criteria for defining reference conditions and can benefit from large-scale information on disturbance and land-use history.
- Disadvantages: The assumption that any disturbance reduces habitat quality may not always be valid. Moreover, identifying sufficient undisturbed or naturally disturbed reference areas can be challenging for some habitat types.

Modelling the relationships between variables and condition

This approach assumes a relationship between variable values and habitat condition. When determining threshold and reference values, models that describe these relationships share a conceptual basis with methodologies based on dose-response curves. Such models assume that certain cases of good condition correlate with specific levels of a condition variable.

The advantage of modelling is that it allows reference values to be inferred where empirical examples of good condition or undisturbed condition are lacking. In these situations, information from known empirical examples can be extrapolated to other contexts, such as locations along a climatic gradient.

Various modelling procedures are available. Functional relationships – linear, saturated, or humped – can be applied (Stoddard et al., 2006, Jakobsson et al., 2020). For instance, deadwood volume in pristine forests can be modelled along productivity gradients to establish reference values in climatic conditions where unaltered forests no longer exist (Jakobsson et al., 2020). Correlative climate niche models can also be used to estimate the suitability of species sets (i.e., variables that characterise the habitat) at different points along the climatic gradient (Jakobsson et al., 2020).

Although these approaches offer a functional basis for establishing reference values, they involve several assumptions that often require expert judgement. It is also possible to create models in which condition is inferred from variables other than the condition variable itself – for example, biodiversity-related condition variables may be inferred from pollution levels. However, this approach should be used with caution to avoid tautological inferences involving variables that reflect pressures.

- Advantages: Modelling approaches are flexible, transparent, and encompass a variety of procedures based on functional relationships between variables and condition (validity), drawing on scientific knowledge from multiple disciplines. They can also be applied to obtain reference values when empirical examples of good or undisturbed condition are lacking.
- Disadvantages: The information available to build models is often insufficient or unreliable for many variables. Outputs are highly sensitive to the chosen modelling procedure and underlying assumptions, and expert judgement is ultimately required at multiple stages of the modelling process.

Statistical assessments

This approach is based on quantitative data from databases, such as habitat inventories, which report the distribution of variables within a given habitat. It assumes that higher values of certain variables correspond to good condition when a positive relationship exists, and vice versa. For such variables, high percentile values or confidence intervals (e.g., 95%, Jakobsson et al., 2020), or differences from the maximum observed values (Storch et al., 2018), may be used.

For variables with a negative impact on habitat condition, low (e.g., 5%) or minimum values are applied, while for variables that show a hump-shaped (non-linear) relationship with condition – peaking at intermediate values (e.g., gap occurrence, browsing) – a combination of high and low percentiles may be used.

- Advantages: This approach can be applied with reasonable ease by users with statistical training. It is transparent, replicable, and minimally subjective.
- Disadvantages: The existence of appropriate, quantitative datasets representing the reference state is essential for this method. Its reliability depends on the distribution of condition classes (from bad to good) in the dataset and on how well this distribution corresponds to empirical situations of good condition. As a result, it may lead to under- or overestimation of good condition and may be less reliable for habitats that are poorly represented in the dataset.

Expert judgement

Setting of reference values and thresholds based on expert judgement is common practice, particularly where other sources of information are lacking – for instance, in certain non-abundant habitats where experts have developed empirical knowledge of habitat condition. However, this approach is often criticised for its limited transparency, and the level of expertise may be insufficient in some cases. For this reason, it is sometimes considered a last-resort option for many variables.

Nonetheless, for certain variables – such as assemblages of characteristic species, successional stages, the presence of microhabitats, or regeneration characteristics – expert judgement may be appropriate for establishing thresholds and reference values. In other cases, it can also serve as a complement to other approaches.

In all situations, it is advisable to apply expert judgement through protocols based on consensus and consultation with multiple experts of comparable experience. This should include clear procedures (e.g., standardised questionnaires) and transparent documentation of how conclusions were reached (Stoddard et al., 2006). A further limitation is the lack of available experts for certain habitats, which can hamper the correct application of this approach.

- Advantages: This approach is easy to apply and is commonly used.
- Disadvantages: It entails a high degree of subjectivity and low transparency, which limits replicability and reliability. Its use may also be constrained by the scarcity of suitable experts for particular habitats and Member States.

Given the uncertainties involved in setting reference levels, a combination of approaches is generally recommended to improve reliability. The approaches described are not mutually exclusive, and are often applied in combination. For example, expert judgement is typically required when defining reference cases for good condition or when making modelling decisions about the relationship between variables and condition. Similarly, modelling-based approaches can complement those based on empirical cases of good or undisturbed condition and may also be integrated with statistical methods.

Integration of WFD and Habitats Directive Approaches

This guide emphasises the need for consistency and synergies between the WFD and the Habitats Directive. Under the WFD, reference conditions are established through a combination of:

- Historical data, when available
- Minimally disturbed or undisturbed sites within comparable ecological and geographical settings
- Predictive models simulating conditions without significant anthropogenic pressures.

To assess the condition of running water habitats, it is useful to know whether they achieve good ecological status, according to the terminology of the WFD. The WFD stipulates that the assessment of ecological status should be based on a comparison between the current situation and the reference conditions. For each element considered, the following steps should be taken:

- Define the variables to be measured, based on their capacity to reflect the environmental integrity of the element under analysis.

- Establish the reference conditions, i.e., the values that these variables would have under minimally altered conditions in the fluvial ecosystem.
- Calculate the Ecological Quality Ratio as the ratio between the current value of the variable and its corresponding value under reference conditions.

Thus, within the WFD framework, the ecological status of each water body is assessed based on different parameters and expressed through the EQR, which is calculated by dividing the observed value of the parameter for a specific water body by the median of the reference samples for that type. Therefore, to achieve this, it is first necessary to have reference values. These reference values are derived from reference sites, from which the calculations are then performed.

Annex II from the Consolidated text of the Water Framework Directive defines how to establish type-specific reference conditions for surface water body types. Consequently, based on Commission Decisions 2008/915/EC, 2013/480/EU, 2018/229 and Commission Decision (EU) 2024/721 of 27 February 2024, adopted pursuant to Directive 2000/60/EC of the European Parliament and of the Council, the Member State have established in their monitoring systems, as a result of the intercalibration exercise and to the Directive 2008/105/EC of the European Parliament and of the Council of 16 December 2008 on environmental quality standards in the field of water policy, the following information:

- The reference conditions for each type of water body have been calculated by using the Ecological Quality Ratio (EQR).
- The numerical boundary values for the classes applicable to biological, chemical, physico-chemical, and hydromorphological indicators have been determined.
- The Environmental Quality Standards (EQS) have been calculated for specific pollutants or, where applicable, and the list of priority substances stated.

The reference condition values and class boundaries are unique for each indicator within a given type and will be revised based on the results obtained from the reference subprogramme, or based on the revision of the criteria used to establish them when reference sites are absent, applying the comparability criteria for biological results as defined, particularly those values obtained in the intercalibration exercise.

This section provides a detailed explanation of the entire procedure, describing the methodology, as well as the current limitations and restrictions on its application.

Criteria for the selection of reference sites under the WFD

Selecting a network of reference sites enables the definition of favourable conservation status (reference conditions) for each habitat type and the assessment of deviations across sites. Under the WFD, a Network of Reference Sites for rivers has been established, which could be used,as appropriate, for the monitoring of river habitats under the Habitats Directive to save effort and resources.

Reference sites are chosen based on pressure-screening criteria outlined in the REFCOND Guidance (Working Group 2.3-REFCOND 2003) and the “Guidance Document No. 1043 in Annex Tool 1, titled “Proposed pressure screening criteria for selecting potential reference condition sites or values.” These criteria define reference conditions as areas with negligible or very low pressures, minimal human impacts (urbanisation, industrialisation, intensive agriculture), and minimal physico-chemical, hydromorphological, or biological alterations. It establishes that the following criteria must be met for the selection of reference sites:

- Diffuse pollution from agriculture or any other intensive land use must be completely or nearly absent.
- Specific synthetic pollutants from point sources must be present at concentrations approaching zero, or at least below the detection limits of the most advanced analytical techniques commonly in use.
- Non-synthetic specific pollutants must appear at concentrations within the ranges normally corresponding to unaltered conditions, referred to as background levels.
- There should be no other point sources of pollution, or local discharges should have only minor ecological effects.
- Direct morphological alterations should allow the adaptation and recovery of ecosystems to maintain a level of biodiversity and ecological functionality equivalent to that of natural water bodies.
- Water withdrawals and flow regulations should represent only minimal reductions in flow levels, causing insignificant effects on quality elements.
- The riparian vegetation should be appropriate for the corresponding water body type and its geographical location.
- The introduction of fish, crustaceans, molluscs, or any other types of animals or plants should be compatible with minimal harm to native biota. No impacts from invasive flora or fauna species should be detected.
- Fisheries and aquaculture should maintain the structure, productivity, functioning, and diversity of ecosystems (including the type of habitat and ecologically dependent and related species) upon which the associated industrial activity depends.
- The presence of non-indigenous fish should not significantly affect the structure and functioning of the ecosystem; their presence should be negligible.
- Recreational use should not be intensive (camping, bathing, canoeing, etc.).

Stations meeting the criteria underwent validation to ensure they represent pristine or very well-preserved stretches, lacked significant pressures, had appropriate land use and riparian vegetation, and reflect the ecological types to which they belong. Reference condition values for each type are set out in the national documents.

However, identifying suitable reference water bodies remain difficult. Many lack complete environmental and biological data. For example, in Spain, only 266 sites have environmental data, and just 180 include both. It is a generalised problem that severely limits the availability of data for calculating the EQR.

Finding truly undisturbed rivers is challenging, especially for rare types like saline or acidic rivers. As a result, few reference stations exist, and suboptimal sites or nearby rivers are often used instead. One proposed approach is to adopt the “best attainable condition” (Stoddard et al., 2006), though this still relies heavily on expert judgment. To address these gaps, it is essential to expand the number of reference stations, possibly selecting different sites for different parameters depending on pressures or ecological context.

Calculating the Ecological Quality Ratio (EQR)

The ecological status of each water body is expressed as an Ecological Quality Ratio, calculated by dividing the observed value of a parameter by the median value from reference samples for a specific water body type. Classification of ecological status then uses the boundaries established for each index in the relevant water body type. The EQR essentially measures the spatial distance between the sample of interest and reference conditions unaffected by human activities (Pardo et al., 2012). According to section 1.4 of Annex V of the WFD, “These indices shall represent the ratio between the values of the observed biological parameters in a given surface water body and the corresponding values of those parameters in the applicable reference conditions for the water body. The index shall be expressed as a numerical value ranging from 0 to 1, where high ecological status is represented by values close to or above 1, and poor status by values close to 0."

The optimal monitoring systems, in the case of biological indicators, involve determining the ratio between the observed values and those associated with the reference conditions applicable to the water body. The EQR should range from 0 to 1 and should allow for the establishment of five status classes (high, good, moderate, poor, and bad). Correctly establishing the boundary values between the different ecological statuses is one of the most significant challenges in implementing the WFD.

Final remarks

Habitat condition assessments are based on determining whether the variables used indicate good or not good condition. However, it is common practice to define more than two categories for each variable – e.g., good, medium, and bad – as observed in the analysis of methodologies used by MSs. In such cases good condition is only determined by the first category, while the other two indicate "not good" condition although with some nuances. The criteria for assigning these condition categories vary depending on the characteristics of each variable. For example, categorical variables may involve thresholds such as "no alien species allowed", while quantitative variables may follow linear or non-linear relationships with condition (Jakobsson et al., 2020).

This classification of variable values – whether quantitative or categorical – into condition categories (e.g., good, not good; or good, medium, bad) corresponds to the scaling process needed for joint evaluation through aggregation procedures, as described in the following section. Condition categories can be translated into numerical values (e.g., good = 2, medium = 1, bad = 0|. Alternatively, where quantitative values for the variables are available, these can be directly standardised for use in aggregation.

In habitat condition assessments, each characteristic and its associated variable is likely to be measured in a different unit. Owing to the different metrics and magnitudes used for the variables that characterise habitats, the values obtained from their measurement require some form of standardisation – e.g., through re-scaling – in order to build indicators that combine multiple variables. These values can be normalised to a common scale and aligned direction of change using reference levels and reference conditions, allowing comparison across variables. They can then be combined to form a composite index or used to obtain an overall condition result through appropriate aggregation approaches (see further details in the next section).

3.3 Guidelines for the aggregation of variables at the local level

Ecological assessments require the integration of physical, chemical, and biological quality parameters. The choice of aggregation method for combining these partial assessments into an overall evaluation has been widely discussed within the scientific community, as it can significantly influence the final outcome. Various approaches can be used to integrate the values of measured variables into an overall index reflecting the condition of habitat types at the local scale (e.g., monitoring station, or site).

Monitoring lotic ecosystems requires the integration of multiple data sources that allow for a comprehensive evaluation of these ecosystems (Barbour et al., 1999). Integrating the information obtained from bioindicators, physicochemical analysis, and hydromorphological evaluation will allow generating a diagnosis, more or less, adjusted to the reality of the condition of river ecosystems (Barbour et al., 1999; Karr, 1997; Karr & Chu,1997; Schlosser & Karr, 1981).

Applying appropriate aggregation approaches is essential for categorising conditions at the local scale as good or not good, since the proportions of habitat type area in good/not good condition is the key information needed for evaluating the conservation status of structure and functions at the biogeographical level.

3.3.1 Overview of aggregation methods

Based on the literature (e.g., Langhans et al., 2014, Borja et al., 2014), several aggregation approaches can be distinguished: the one-out, all-out rule (minimum aggregation), averaging approaches (including weighted, non-weighted and hierarchical operations), Conditional rules, Multi-metric indices and High-level integration.

The **one-out, all-out** (OOAO) rule has been recommended for assessing ecological status under the Water Framework Directive (CIS, 2003). The principle behind this minimum aggregation method is that a water body cannot be classified as having good ecological status if any of the measured quality elements fail to meet the required threshold. A precautionary OOAO approach is also used in the aggregation of parameters when assessing conservation status under the Habitats Directives, the IUCN Red List of Species and the IUCN Red List of Ecosystems.

Conditional rules require that a certain proportion of variables meet their respective thresholds for the overall assessment to achieve a good condition rating. For example, the overall status may be considered as not good when a specific number of variables fail to meet their thresholds.

Averaging approaches are among the most commonly used methods for aggregating indicators. These include straightforward calculations such as the arithmetic mean, hierarchical average, weighted average, median, sum, or combinations thereof, to produce an overall assessment value.

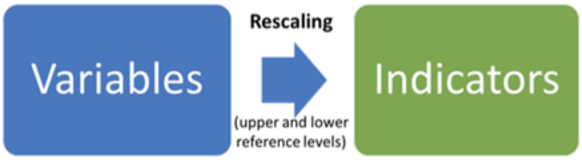
Differential **weighting** of indicators may be applied and the choice of weightings should reflect the relative importance of each indicator in determining the overall condition of the habitat type. Ideally, the approach should be supported by a clear scientific rationale and informed by input from ecologists with expertise in the relevant ecosystem types. However, a robust basis for assigning weights is not always available.

Within the WFD there are many examples of **multimetric indices** developed for different biological elements. Such indices Integrate multiple indicators into one value, which may result in more robust indicators, compared to indicators based on single parameters. However, scaling of a multimetric index may be less straightforward, and ideally the various parameters should not be inter-correlated

Normalisation of variables values (rescaling)

In the assessment of habitat condition, each characteristic and associated variable is likely to involve the use of different measurement units. To ensure comparability, the measured values of variables are often normalised to a common scale (e.g., 0 to 1 or 0 to 100). This involves rescaling the raw data based on reference values or thresholds that define the boundary between good and not good condition for each variable. By rescaling the condition variables, indicators are standardised to the same scale, making it possible to aggregate them into condition indices that reflect the overall condition at a given plot or location.

Figure 5. Example of deriving condition indicators by rescaling the values obtained for variables, based on upper and lower reference levels



$$Condition\ indicator = \frac{(V-VL)}{(VH-VL)} \quad \text{[Equation 1]}$$

Where:

- V is the measured/observed value of the variable,
- VH is the high condition value for the variable (upper reference level),
- VL is the low condition value (lower reference level).

Source: Vallecillo et al. (2022)

Multivariable indices

The application of multivariable indices to aggregate individual variables as part of the assessment of the condition of habitat types at a local scale, is common across EU MSs, especially for inland water habitats.

This aggregation is usually performed through a combination of weighting loads and different approaches such as indices, specific statistics, etc. The aggregation system depends on carefully chosen indicators, well-calibrated weighting, and effective combination algorithms. The weighting for each component is determined by its ecological significance and how accurately its associated metrics reflect the effects of key environmental pressures.

3.3.2 Recommendations for the aggregation of the measured variables to determine the habitat type condition at the local scale

The classification of condition status must be based on the results obtained from the aggregation of indicators related to biotic, abiotic and landscape characteristics. We suggest that in a first step, a set of variables linked to each of these groups of characteristics be aggregated using a conditional rule, whereby a selection of essential variables should meet the threshold values for considering that habitat component (biotic, physicochemical, landscape) in good status. This means that if any of those selected variables does not reach/exceed the minimum thresholds, the condition cannot be considered good for the corresponding component of the habitat (biotic, abiotic, landscape).

Then, in a second step, the results achieved in each the three components (or groups of characteristics) should be aggregated following the “one-out, all-out” rule, i.e. if any of these components does not reach an overall good status, the condition of the river habitat at the local scale cannot be considered good. This aggregation approach is currently used in several EU Member States, as described in section 2.3. It is summarily described below.

Step 1 – Aggregation of the variables measured in each group of characteristics

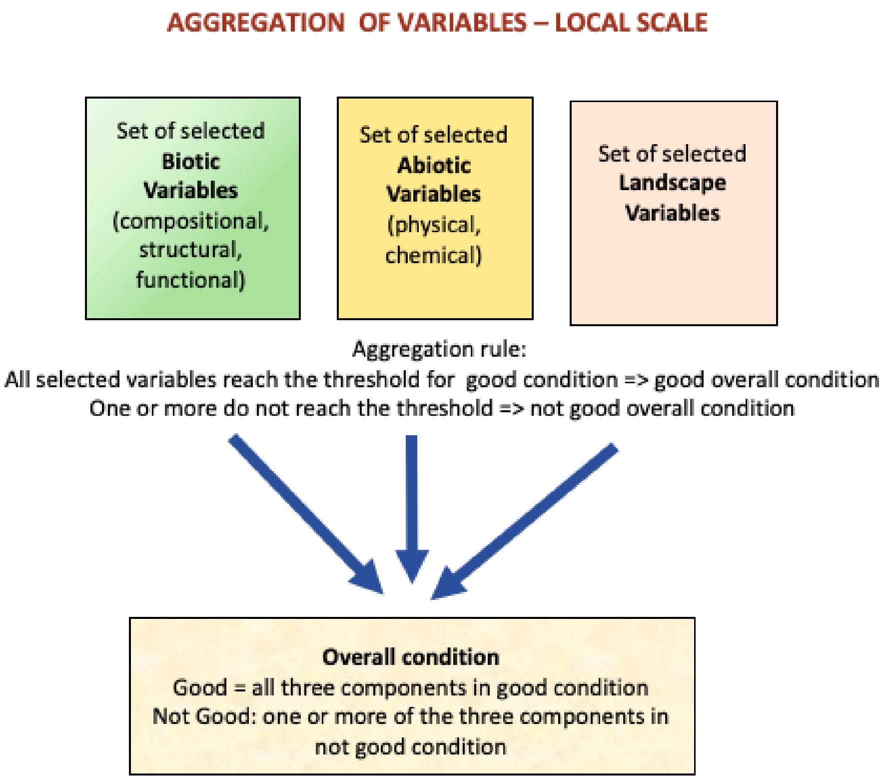
Following a conditional rule, a minimum set of variables in each group must reach/exceed the threshold for good condition. An example of a possible set of selected essential variables is provided below.

Abiotic - Physicochemical variables	Biotic - compositional, structural, functional	Landscape variables
- Water Temperature - Stream Flow - Sediment load - pH - Dissolved gases - Organic matter	- Characteristic species: aquatic and riparian vegetation. - Macroinvertebrates - Width, zonation and cover of riparian vegetation - Biological Oxygen Demand, Chemical Oxygen Demand - Abundance of Phytobenthos - Presence of algal growth or indicator species for eutrophication	River continuity and connectivity: transversal and lateral barriers; permeability of barriers

Step 2 – Aggregation of the variables measured in each group of characteristics

The results obtained in each group of characteristics (abiotic, biotic, landscape) in the above step are aggregated following the "one-out, all-out", which requires that all the three components have been assessed in good status in the previous step for the overall condition of the river habitat at the local scale to be considered good. Otherwise, the condition of the river habitat at the local scale cannot be considered good if any of these components does not reach an overall good status

Figure 6. Aggregation approach for running water habitats



Considering the WFD Ecological status in the evaluation of river habitats' condition

The 'ecological status', as defined in the WFD can be considered an expression of the quality of the structure and function of aquatic ecosystems associated with surface waters (Schmedtje et al., 2011). It is classified according to Annex V of the directive, which defines biological, hydromorphological, and physicochemical quality elements and the status classes.

Under the WFD, the ecological status of a river or stream is assessed by examining the mentioned quality elements: Biological, Hydromorphological and Physicochemical quality elements and Specific pollutants. Each quality element is classified into one of five status classes: high, good, moderate, poor, or bad.

The ecological status is determined by using the “one-out, all-out rule”, which is a key concept used in the WFD to assess the ecological status of rivers and streams that essentially means that the overall ecological status of a water body is determined by the lowest-scoring quality element. For example, if a river has "high" status for most biological and physico-chemical elements but only "moderate" status for hydromorphology due to a dam, the overall ecological status of the river will be classified as "moderate." The “one-out, all-out rule” provides a holistic approach that emphasizes the interconnectedness of different components within a freshwater ecosystem, and it recognizes that even if most aspects of a river are in good condition, a single factor can significantly impact the overall ecological health. Moreover, it follows the Precautionary Principle, acknowledging that it's crucial to address all potential threats to a river's ecosystem, even if they seem minor on their own. It implies setting a high bar for achieving good ecological status, as it requires all quality elements to be at least "good”.

On the other hand, the 'conservation status,' as defined under the Habitats Directive, refers to the combined influences acting on the natural habitat type and its typical species that may affect its long-term distribution, structure, and functions, as well as the survival of its typical species.

The WFD has developed an ecological classification system for the different river ecosystem types. Consequently, the WFD ecological status assessment procedures are considered useful for assessing the condition of river habitat types, enabling more efficient use of resources for assessments under both directives.

The partial compatibility between the WFD and the Habitats Directive has already been recognised (Schmedtje et al., 2011; Janauer, et al., 2015). The WFD aims to achieve good ecological status, while the Habitats Directive seeks to achieve favourable conservation status.

The WFD defines ecological status based on the biological elements of the aquatic ecosystem, while also considering physicochemical and hydromorphological factors that influence them. However, its biological indicators focus less on specific species and more on the overall biological community characteristic of each water body type. This marks the main difference between the two directives. Therefore, the conservation status of fluvial habitat types cannot be assessed solely through the correspondences between the five ecological status levels of river water bodies and the three values (favourable, unfavourable-inadequate, unfavourable-bad) of the conservation status of habitat types.

Nevertheless, the ecological status under the WFD can be useful to inform the assessment of the condition of a river habitat type at the monitoring site. The equivalence between ecological status and condition status implies that the biological, chemical, and hydromorphological elements are in good or very good condition, meaning the specific structure and functions necessary for long-term maintenance exist and are likely to persist (Table 15).

Where the ecological status is available in the river stretch where a running water habitat type is to be assessed, it can be used to inform the condition of that habitat type. Thus, a river habitat should not be considered in good condition if the ecological status determined in the site where the habitat occurs has been assessed in moderate, poor or bad ecological status.

However, in case the river stretch is assessed in good or high ecological status, the compositional and structural characteristics of the particular habitat type, determined by its characteristic species and biological communities, will still need to be assessed to obtain the overall condition at the monitoring site.

Table 15. Equivalences between ecological status classes (Water Framework Directive) and the condition of the habitat type at the site (Habitats Directive)

Ecological Status	Possible condition of the habitat type at the site
High	Good – subject to the assessment of the specific biotic characteristics of the habitat type
Good	
Moderate	Not Good-Inadequate
Poor	
Bad	Not Good -Bad
Unknown	Unknown

Source: adapted from Sánchez-González et al. (2019).

3.4 Guidelines for aggregation at the biogeographical region scale

Once the site condition has been assessed, the “structure and function” status of the habitat is evaluated at the biogeographical region scale based on its total area. If representative sampling of the habitat types has been conducted, it is sufficient to calculate the percentage of the habitat area assessed into each condition category.

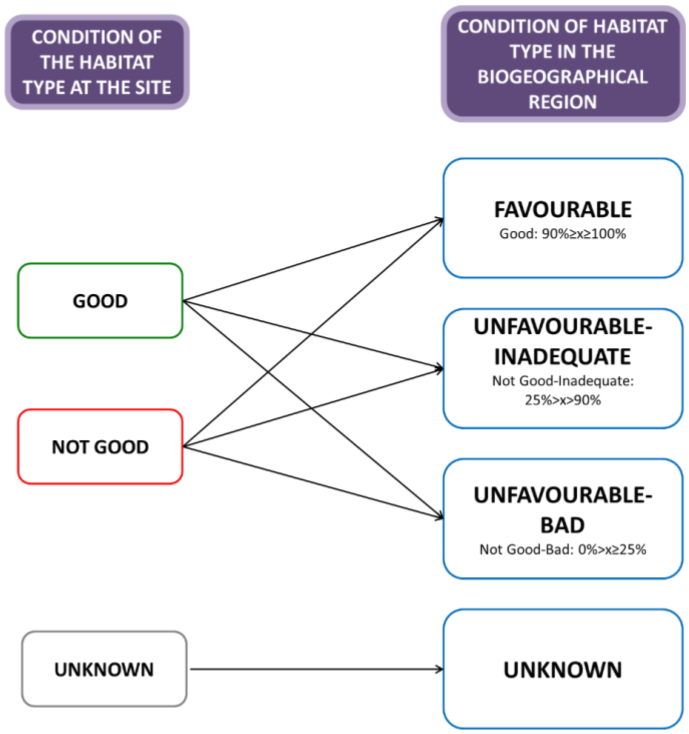
As a minimum requirement, Member States must follow the Article 17 reporting guidelines for the 2019-2024 period. These guidelines specify that the habitat type’s condition at the biogeographical region level must be classified based on the proportion of habitat in “good” or “not good” condition. The calculation must be based on statistically robust estimate from a representative selection of water bodies. The total habitat area classified as good or not good is summed, and the percentage in each category determines the final classification:

- Favourable: more than 90% of the area is in good condition.
- Unfavourable-inadequate: between 25% and 90% of the area is in not-good condition.
- Unfavourable-bad: more than 25% of the area is in not-good condition.
- Unknown: If the area in each condition category is unknown or if the estimate is not robust.

Because it is not possible to assess all water bodies of a given habitat type, the evaluation must rely on a sampling network designed following the protocol outlined in Section 3.6.

However, Member States may adopt stricter thresholds, and the “one-out, all-out” principle (minimum aggregation), widely applied under the WFD, may also be considered appropriate.

Figure 7. Framework to follow for assessing habitat type condition



Source: Sánchez-González et al. (2019).

3.5 Guidelines on general sampling methods and protocols

Before starting any assessment of the conservation status of a water body or a section of a lotic ecosystem, it is essential to collect comprehensive information about both the section and its draining catchment area. First, the station, or sampling point, must be identified, its location determined, and the most suitable access routes mapped. The selected section should be delineated by recording the coordinates in either sexagesimal degrees or UTM (Universal Transverse Mercator) for both the starting and ending points using a GPS (Global Positioning System) device, ensuring the correct datum is registered. Extensive photographic documentation should accompany these records to support future sampling, identify pressures and impacts, and document bed structures. A schematic map of key structural elements is also recommended. The sampling point should be characterised by its length, proportional to the channel width, following the guidelines of Barbour et al. (1999):

- Estimate the section length (m): Essential for quantitative sampling and density estimates.
- Estimate the section width (m): Measure the channel width at multiple points for accuracy.
- Estimate the section area (m²): Calculate from length and width, section can even be divided into segments if needed.
- Estimate the average depth (m): Measure the distance from the water surface to the riverbed.

In priority sections (e.g., river reserves, National Parks, or sites of scientific interest), multiple transects may be used to determine the river cross-sections (Bain & Stevenson, 1999; Elosegi & Sabater, 2009; Hauer & Lamberti, 2007).

Each station should be identified with a standard code and number, coordinated with the WFD coding system. The section must be classified according to both the WFD lotic ecosystem typology and the Habitats Directive typology.

Weather conditions at the time of sampling, as well as recent events affecting flow rates or turbidity, must be documented. Local climate data (e.g., precipitation, temperature) from the nearest meteorological station should also be collected.

After sampling, record the names of the personnel involved, time taken to reach and sample the site, and any incidents, errors, or recommendations to improve future sampling. The frequency suggested for the evaluation of the various parameters is shown in Table 16.

It is proposed to use the protocols described in the following standards and protocols:

- UNE- EN 5667-1: 2023. Water quality - Sampling - Part 1: Guidance on the design of sampling programmes and sampling techniques.
- UNE-EN 14996:2007. Water quality - Guidance on assuring the quality of biological and ecological assessments in the aquatic environment.

Hydromorphological characterisation might be carried out at least once per cycle of the River Basin Management Plan (RBMP), i.e., once a sexennial and conducted at a time of year that allows for a reliable description of the hydromorphological characteristics of water bodies.

Table 16. Frequency of evaluation of the relevant parameters in the selected section

Indicators		Frequency
Biological	Macrophytes	1 year
	Macroinvertebrates	1 year
	Diatomaceous	1 year
	Fish	1 year
	Phytoplankton[1]	6 months
Physicochemical	Thermal Conditions	continuous-monthly
	Oxygenation	Quarterly
	Salinity	Quarterly
	Nutrients	Quarterly
	Acidification	Quarterly
	Other Contaminants	Quarterly
	Priority substances	1 month
Hydromorphological	Continuity	3 years
	Hydrological Regime	Continuous
	Morphology	2 years
	Riverbank Vegetation	2 years
	Groundwater	2 years[2]

[1] In water bodies tending towards eutrophication. [2] It could be considered once every 6 years.

Source: Sánchez-González et al. (2019) modified from Toro et al. (2009i)

The optimal moment of year for fieldwork depends on the morphological variable to be assessed. For instance, to characterise the riverbed and bedforms, it would be advisable to select a period when flow is low (but not when the channel is completely dry). To assess river continuity and the barrier effect of existing weirs, surveys should ideally be conducted during fish migration periods and floods. Regarding vegetation, assessments should take place when the type and structure of vegetation in the channel, along the banks, and in the riparian zone can be recorded accurately, avoiding the winter months.

3.6 Selecting monitoring localities and sampling design

One of the main challenges in experimental design and sampling is ensuring that the selected group is truly representative of the entire population. A representative sample accurately reflects the characteristics of the population as a whole. In other words, the traits of the chosen sites or individuals should mirror those of the entire population, making representativeness the most critical feature of any sample.

Sampling design matters because it must ensure that the observed characteristics in the sample adequately express those in the population, allowing reliable generalisation.

It is important to highlight that random sampling is not automatically representative (Underwood, 1997). In random sampling, each member of the population has an equal, independent chance of being selected. This means that the likelihood of selecting one individual does not affect the likelihood of selecting another. Random samples are generally representative on average. If many random samplings are conducted, a representative sample will eventually emerge. However, a single random sample may not always be representative.

Random sampling offers the advantage of greater independence among observations, reducing bias in parameter estimated. One possible strategy is to select sampling points or observations entirely at random (Downes et al., 2004). However, subsamples must also be spatially representative (Downes et al., 2004). In some cases, simple random sampling may fail to include all habitat types or sections, even though it may be representative on average. Therefore, it is better to ensure a minimum number of observations or sampling points in each subset or subgroup.

For example, if a basin contains three sub-basins of equal size, area, and drainage network, one-third of the total sample points for which we have budgeted, should be allocated to each sub-basin. Within each sub-basin, sampling points can be randomly selected and distributed accordingly. This approach prevents some sub-basins from being underrepresented by chance.

Moreover, representativeness depends on both spatial and temporal scale. The size of the sampled section, as well as timing and frequency of sampling, must be appropriate for the biological group under study. For example, sampling 1 m² might be sufficient for periphyton (complex community of organisms that grows on submerged surfaces in aquatic environments), but inadequate for macroinvertebrates or fish communities. Additionally, the frequency of sampling, as well as the time of day and year, are determinants of the sample's representativeness. Clearly, sampling macroinvertebrates in winter cannot provide representative information for the area. Therefore, spatial scale, different taxonomic groups, temporal scale, and other factors together determine the representativeness of a sample.

Care must be taken in selecting sampling groups and their number, as a high number of subsamples could lead to excessive costs and sampling effort, making the monitoring programme unfeasible (Downes et al., 2004). Despite these considerations, Bolstad (2008) notably highlights that random sampling remains the most effective method for obtaining representative samples, as it provides more representative samples than any non-random system. Furthermore, random sampling not only minimises inference error in but also shows probabilistic measurement of the remaining error. This holds true if the sampling is repeated multiple times and if the sample size (n) represents a significant proportion of the total population (N). Although Bolstad's (2008) assertion is valid, this work advocates the use of stratified random sampling (Snedecor & Cochran, 1989; Southwood & Henderson, 2000) with allocation proportional to the number of water bodies in each group or typology. Stratified sampling divides the population into segments called strata, containing relatively homogeneous units, from which samples are taken randomly or systematically within each stratum independently (Freund & Wilson, 2003). This sampling is justified by the existence of relatively homogeneous and well-defined subgroups within many populations (Freund & Wilson, 2003). This methodology has been widely used and supported (Quinn & Keough, 2002; Manly, 2009; Hurford et al., 2010; Legendre & Legendre, 2012). For more details, see Quinn & Keough (2002).

In line with this design, the first step is to identify the number of water bodies with habitat occurrence in each biogeographical region and determine the proportional number to be sampled in each region, based on the total number of water bodies with the habitat and the calculated number of samples. Finally, the number of water bodies with habitat to be sampled by typology in each biogeographical region will be determined using the same procedure.

In addition to this selection, and to ensure coordination with the WFD activities, consideration should be given to sections included in biological monitoring networks, headwater sections or low-flow river sections listed in the National Catalogue of Natural River Reserves, and sections declared as being of interest for the protection of fish life under Directive 78/659/EEC (1978), among others. The stations where this protocol will be applied could be part of the surveillance monitoring programme, including the reference sub-programme, operational control programme, and investigative monitoring programme.

Finally, a series of sections will be sampled to meet the Habitats Directive requirements. The calculation of water bodies to be sampled by typology in each biogeographical region will guide this selection. In the sections to be coordinated with the WFD, the Water and River Basin Authorities will be identified, with the remaining sections randomly selected until the target number is reached.

Number of reaches or waterbodies to be sampled

The number of samples and the desire to obtain a high number of them is a topic that has received abundant attention in the literature on statistics and experimental design. It is evident that a larger number of samples leads to greater robustness in parameter estimation. Ideally, one would sample the entire population. However, this is often impractical due to resource constraints and, in some cases, unnecessary, as a well-chosen sample can provide sufficient information.

Sample size influences two statistical properties: 1) the precision of the estimates and 2) the power of the assessment to draw meaningful conclusions. The number of plots must be statistically sufficient to detect changes and trends with the desired level of confidence, and appropriate statistical methods should be applied to determine an adequate sample size.

So, how many samples should be taken? The sample size refers to the number of observations, elements, or individuals comprising the sample extracted from a population (Sokal & Rohlf, 1987), which are necessary for the data to be representative of the population.

Nevertheless, setting general rules for determining the number of samples is not straightforward. This is due to multiple factors, such as differences between various statistical techniques, variable distribution, missing data, their variability, and the strength of the relationships between variables (Muthén & Muthén, 2002; Raykov & Marcoulides, 2008). Researchers must balance the maximum possible number of observations with time, costs, and number of variables, aiming to minimise all factors except for the number of observations. In other words, it is considered better to sample fewer variables, which reduced time and costs, rather than decrease the number of observations (Quinn & Keough 2002). Therefore, it is essential to carefully select variables to be measured and the sampling units and consider accessiblity before heading into the field.

There are many rules regarding the sample size, including some unwritten ones. Crawley (2005) suggests that thirty is a sufficient and appropriate sample size, though it is important to note that this rule is not suitable in all cases, but it is a useful figure in cases of doubt.

The reality is far more complex. For instance, fewer replicates are needed when sampling homogeneous population than when sampling attributes with a high heterogeneity and diversity. Understanding the population and its characteristics is therefore essential to selecting an appropriate sample size. Experience and previous studies are therefore indispensable.

Box 1. Key elements for statistical representation

Sample size and distribution:

- The number of localities/transects etc. should be sufficient to provide a statistically robust sample size. This ensures that the data collected can be generalized to the entire habitat type within the region.
- Statistical methods such as stratified random sampling are often used to ensure that all habitat subtypes and environmental gradients are adequately represented.

Sampling design:

- Within each sampling area or locality, multiple plots are established to collect detailed data on benthos, infauna, mobile species and other ecological indicators. The distribution and number of sampling stations depend on the variability and size of the habitat patch. Sampling areas (plots, transects) are laid out considering the existing main ecological gradients, e.g., exposure to waves/currents/tides, depth, sediment characteristics.

Replication and randomisation:

- Replication of sampling units within each locality and randomisation of sampling plots location help to reduce bias and increase the reliability of the data.
- Randomized plot locations ensure that the sampling captures the natural variability within the habitat.

Calculating the sample size

Since the number of bodies of water of a given type is known (N) and assuming a normal distribution of one variable, the sample size can be calculated using the following two formulas.

It is most appropriate to use the following formulas for calculating the sample size when the Population size (N) is known for those samples with a normal distribution. Thus:

Method 1A and 1B:

$$n = \frac{Z^2 \times N \times p \times q}{e^2\,(N) + Z_{\alpha}^2 \times p \times q}$$

$$n = \frac{Z^2 \times N \times p \times q}{e^2\,(N-1) + Z_{\alpha}^2 \times p \times q}$$

Method 2:

$$n = \frac{(Z^2 \times p \times q)/\alpha^2}{1 + \big((((Z^2 \times p\ \times q)/\alpha^2) - 1\big)/N\big)}$$

where:

n is the sample size, *p* proportion of individuals in the population who possess the characteristic of the study, *q* proportion of individuals who do not possess that characteristic, i.e., *q =1-p*, *Z* is the standard normal random variable and is used to calculate the confidence level, N is the Universe, *α* is the level of significance, *e* is the desired sampling error, as a percentage. Acceptable limit of sampling error that, in general, when its value is not available, a value that varies between 1% (0.01) and 9% (0.09) is usually used, a value that is left to the discretion of the surveyor, σ is the standard deviation of the population. If it is not known, a constant value of 0.5 is usually used.

The explained method shows how to estimate sample size by using one variable. However, monitoring programmes have many different variables with different characteristics, properties and attributes. So, different variables would provide different results and sample sizes. These circumstances might be considered when the global sample size is determined.

Criteria for selecting monitoring localities

Therefore, in addition to the randomly selected sections through stratified random sampling, a series of sections chosen based on specific criteria could be added. These criteria have been widely agreed upon with other working groups in other habitat monitoring projects, although some are specific to lotic ecosystems, and all have been adapted to these particularities. The selected criteria are as follows:

- **Ecological variability**: Localities must represent the full range of ecological diversity and variability within the habitat type. Selection should include different ecotypes or subtypes, successional stages, and reflect key environmental gradients such as altitude, soil type, moisture levels, geomorphological features, and topography.
- **Spatial coverage**: Adequate spatial coverage is essential to capture habitat heterogeneity. Localities should be selected across the full geographical range of the habitat type within the region, ensuring they are well distributed and represent a significant proportion of the habitat's total occupied area. This criterion should be considered as a correction factor for the random preselection of selected localities. Suppose a clustering of localities occurs by chance for monitoring. In that case, this criterion should be applied, within the flexibility allowed by the other criteria, to achieve the most comprehensive possible coverage of lotic ecosystems and biogeographical regions.

- **Presence inside and outside Natura 2000 sites and protected areas:** The assessment and monitoring of habitat conservation status must be carried out both inside and outside Natura 2000 sites. This requires selecting localities – and an appropriate number of plots – that reflect the proportion to the habitat's distribution within and outside the Natura 2000 network.
- **Habitat fragmentation at landscape scale**: Localities should be selected based on landscape metrics such as patch size and connectivity. Including both isolated and well-connected sites allow for the assessment of fragmentation effects on habitat conditions. Understanding these patterns is essential for developing strategies to mitigate the negative impacts of habitat fragmentation.
- **WFD water bodies**. In coordination with the relevant authorities, it should be decided which water bodies will be monitored by different organisations based on their competencies and capabilities, while always avoiding duplication of sampling efforts. The sections to be sampled must fall within the operational control, surveillance, and investigative networks. However, the definition of water body may need to be reconsidered or revised.
- **Reference water bodies (Annex III).** As many reference water bodies as possible should be included for each type, provided there are locations considered as reference ecosystems. Many of these water bodies are designated as Natural River Reserves or are located in protected areas, so their selection should be coordinated with criterion 2.
- **Degree of conservation**. The selection of monitoring localities should include areas with varying degrees of conservation and degradation to capture the full range of habitat conditions across its distribution. Besides, lotic ecosystems evaluated as "at risk of disappearance" should be prioritised. According to Member States' evaluations for the different habitat types of community interest and biogeographical regions, available on the European Commission's website, ecosystems of Group 32 (flowing waters) require significantly increased monitoring efforts. As conditions improve, focus should shift on habitats in unfavourable-bad status while maintaining representation across different conservation levels.
- **Exposure to pressures and threats**. This includes both well-preserved areas with minimal human impact, and areas affected by degradation and subject to various pressures. To reflect the diversity of pressures acting on the habitat, localities should span a range of intensity levels – from low to high – and account for different sources of disturbance, such as urbanisation, agriculture, and climate change. Once the pressure has been mitigated or removed, a specific monitoring frequency should be established for a defined period. The frequency and duration of subsequent monitoring should be determined based on type and severity of pressure. For example, the removal of a weir might not require monitoring beyond six years, whereas contamination by heavy metals may necessitate monitoring for several decades.
- **Water bodies of special interest**. Water bodies of special interest include those located within protected areas that require additional control measures. The categories of protected areas are defined in each river basin district by the river basin management plans in accordance with national and/or European legislation. For example, the hydrological plan of each district should include a summary of the protected areas register with indicative maps showing the location of each area, relevant environmental

information, and a description of the EU, national, or local legislation under which it was designated. This register typically includes:

- Water abstraction zones for supply: Protected zones where water is abstracted for human consumption, including future abstraction sites identified in the river basin management plans.
- Water bodies designated for the protection of economically significant aquatic species.
- Water bodies designated for recreational use, including bathing water zones.
- Designated vulnerable zones: In line with Directive 91/676/EEC on protecting waters from nitrate pollution originated from agriculture.
- Water bodies containing sensitive areas: As defined under urban wastewater treatment regulations.
- Habitat or species protection zones: Including Sites of Community Importance (SCIs), Special Protection Areas (SPAs), and Special Areas of Conservation (SACs) integrated into the Natura 2000 network, designated under Directive 92/43/EEC (Habitats Directive) and Directive 2009/147/EC (Birds Directive).
- Protected perimeters for mineral and thermal waters established under specific legislation.
- Surface water bodies identified as river natural reserves in the river basin management plan.
- Special Protection: Zones, basins, or water bodies declared as such due to their natural characteristics or ecological interest, as recorded in the hydrological plan.
- Edge sections and/or sections with significant pressures and threats.
- Sections where pressures have been removed, such as sections where dams or dykes have been dismantled.
- Sections already sampled for various reasons and purposes (scientific, previous impacts, etc.).
- Wetlands of international/national importance, including Ramsar sites and wetlands listed in the National Inventory of Wetlands.

- **Ecological significance** and regional, national, and European Uniqueness. Belonging to the most typical regional, national, and European lotic ecosystems within the European context will be used as a selection criterion. This applies, for instance, to temporary lotic ecosystems of the Mediterranean basin.
- **Lack of information**: Including areas with limited or no data contributes to building a more comprehensive dataset. Selecting localities in historically under-sampled regions ensures a more balanced and complete understanding of habitat condition across its range. This helps to address data gaps and supports more informed conservation planning.
- **Accessibility and practicality**: Monitoring localities should be accessible for regular field visits, considering logistical factors such as distance from roads and ease of access. Practical considerations also include the safety of field personnel and the feasibility of transporting equipment to and from the site.

- **Historical data and existing monitoring sites**: Using existing monitoring sites with long-term historical data strengthens the understanding of trends and changes in habitat condition. Such sites provide valuable baselines for comparison and support more robust trend analyses over time.
- **Scientific Monitoring Sections**: These are sections monitored by universities or research institutions, with data that must be shared with River Basin Districts authorities and integrated into respective databases.
- **Statistical significance**: The minimum number of localities for each running water type (Group 32) will be determined using statistical methods. A relative error estimate based on sample size must fall below the acceptable threshold. The sample size must align with the desired margin of error and available resources. Once the number of samples is determined and water bodies are assigned based on the criteria mentioned earlier, the remaining water bodies will be selected through stratified random sampling.

The list includes the water bodies located within habitat or species protection zones within the Natura 2000 network. Water bodies located in these habitats or species protection zones must be included in the operational monitoring programme if they are at risk of failing to meet their environmental objectives. The information on protected areas under the WFD is aggregated at the national level to respond to the mandatory reporting requirements to the European Union under the WFD. The goal of this work is to monitor lotic ecosystems within the framework of the Habitats Directive. Finally, the selection of sections based on the above criteria and coordinated with the WFD (Article 5) should result in a comprehensive, representative and well-structured network.

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3.7 Use of available data sources, open data bases, new technologies and modelling

Available data sources and open databases

The use of multiple types of data sources, such as satellite remote sensing, in-situ sensor networks, environmental DNA (eDNA) sampling, and hydrological modelling, provides comprehensive datasets on various ecosystem parameters. Each data type offers unique insights:

- Remote Sensing and Satellite Imagery provides high-resolution imagery from satellite platforms (e.g., Copernicus Sentinel satellites) to track water quality indicators such as turbidity, chlorophyll concentration, and land cover changes near water bodies, floodplains and catchment areas. These data could provide essential information to assess anthropogenic impacts, seasonal changes, and long-term habitat trends.
- Unmanned Aerial Vehicles (UAVs commonly named as Drones) have become invaluable in monitoring fluvial ecosystems due to their flexibility, high-resolution imaging, and ability to reach areas that are otherwise difficult to access. When combined with other technologies, they significantly enhance the ability to achieve objectives of the Habitat Directive and the Water Framework Directives by enabling detailed, frequent, and cost-effective ecosystem monitoring.
- LiDAR (Light Detection and Ranging) is another powerful tool for monitoring and managing fluvial ecosystems, particularly under the Habitats and Water Framework Directives. It can map river morphology and floodplain dynamics, analyse riparian zones and identify IAS, monitor erosion and channel stability, and to model hydrology and sediment transport.
- *In-Situ* Sensors: Recent studies (Rodríguez-Castillo et al., 2019) highlight the importance of using data loggers and real-time instruments. Networks of physical sensors measure flow rates, dissolved oxygen, temperature, pH, and nutrient loads. Real-time *in-situ* data provide continuous information on local water quality, which, when combined with other datasets, helps detect early signs of ecological stress and issue flood alerts.

Open databases, such as the European Environment Agency's Waterbase, which is the generic name for the EEA's databases on the status and quality of Europe's rivers, lakes, groundwater bodies, on the quantity of Europe's water resources, and the Global Biodiversity Information Facility (GBIF), a global data infrastructure providing open access to biodiversity data worldwide, offer essential repositories for water quality and biodiversity information across Europe and globally. Another example is HydroSHEDS, a database offering a suite of global digital data layers in supporting hydro-ecological research and applications worldwide, providing various hydrographic data including catchment boundaries, river networks, at multiple resolutions and scales.

These databases must meet the following requirements:

- Raw and processed data: Result should be reported to the European Environmental Agency. Shared data should not be limited to processed datasets; raw data must also be provided. Indices and some parameters required and calculated under the WFD might not fully meet the needs of the HD, particularly those related to riverine vegetation. In consequence, not only indices or processed data might be reported, but also raw data. Reporting raw data allows additional parameters specific to the Habitats Directive to be calculated.

- Data Sharing and Standardisation: Open databases enable cross-border data sharing, allowing multiple stakeholders (e.g., researchers, policymakers, and conservation agencies) to work with standardised datasets. This is crucial for consistent monitoring under the WFD and the Habitat Directives, both of which require comparable data across EU member states.
- Collaboration Platforms: Initiatives such as the European Open Science Cloud (EOSC) provide platforms where researchers can access and contribute data, facilitating the integration of new datasets, including eDNA and remote sensing data, supporting a more cohesive understanding of ecosystem health.

New Technologies for Monitoring and Data Collection

The deployment of advanced monitoring technologies improves the detection of environmental stressors, offering real-time insights that are critical for proactive ecosystem management.

- Environmental DNA (eDNA) and Metabarcoding: eDNA is emerging as a key tool for detecting species presence through genetic material found in water samples, offering a non-invasive method for biodiversity monitoring. eDNA can detect elusive, cryptic, or invasive species with high sensitivity and has substantial potential in verifying ecological status and tracking species' changes in real-time. When combined with metabarcoding (a technique that sequences DNA from complex samples to identify multiple species), eDNA has revolutionised aquatic biodiversity monitoring. In fluvial ecosystems, eDNA samples collected at different locations along a river can provide comprehensive species inventories that are more time and cost-effective compared to traditional survey methods. Metabarcoding allows researchers to simultaneously detect multiple species within a water sample, including those that are rare, difficult to observe, or invasive or alien species in early stages. The high sensitivity of eDNA supports the Habitat Directive application by providing detailed biodiversity data essential for habitat protection and species monitoring. Moreover, metabarcoding can help assess ecosystem status and biodiversity, identifying changes in species composition that may indicate water quality issues.
- Internet of Things (IoT) Devices and Sensor Networks can continuously measure physical and chemical parameters, providing a detailed picture of water quality variations and habitat conditions over time. This real-time data is invaluable for tracking fluctuations and identifying pollution sources quickly.
- Artificial Intelligence and Machine Learning: AI and machine learning algorithms can analyse large datasets from remote sensing, eDNA, and in-situ sensors, detecting patterns that might be missed through manual analysis. For instance, AI models can predict the movement of pollutants based on flow data, identify potential ecological impacts, and prioritise intervention areas.

Modelling Techniques for Predictive and Ecological Assessment

Modelling is essential for evaluating conservation status, forecasting ecosystem responses, assessing impacts of management actions, and testing different conservation strategies. Within this context, techniques such as Machine Learning, Bayesian statistics or Artificial Intelligence have become essential tools.

Hydrological and Habitat models can simulate water flow, sediment transport, nutrient loading, and habitat suitability under different scenarios (e.g., land use changes, climate change), helping to define theoretical reference conditions. By integrating real-time data from in-situ sensors and eDNA, these models provide insights into how changes may affect species distributions and ecological balance.

Ecological Network Models: Network-based models simulate interactions within the ecosystem, such as predator-prey relationships and competition between species. eDNA data, which offers detailed biodiversity information, strengthens these models by accurately representing species presence and dynamics. These models can predict how species interactions shift under various environmental stressors.

Data-Driven Machine Learning Models: Using machine learning algorithms, ecological data from eDNA and other sources can be analysed to reveal trends and classify the ecological status of water bodies. For example, models trained on eDNA datasets can predict biodiversity trends, detect invasive species early, or assess ecosystem recovery following restoration efforts.

Implications for the Habitat and Water Framework Directives

The integration of open data, eDNA, other technologies, and advanced modelling has transformative potential for meeting the requirements of the Habitats and WFD Directives. Using eDNA and metabarcoding significantly expand the ability to track species diversity, including non-native or endangered species. This capability is vital for the Habitat Directive's goals to protect natural habitats and biodiversity for its capacity by enhancing species monitoring.

The use of these new technologies and datasets allows for more accurate ecological status assessments by integrating a broader range of data, from chemical pollutants to biological health indicators, thereby improving the ecological status assessment.

Real-time data and predictive modelling empower resource managers to respond rapidly to emerging threats, such as pollution spikes or invasive species, providing a more effective management and conservation actions. Additionally, these tools support the Habitat Directive's goals by providing data to guide conservation and restoration actions in sensitive or degraded habitats.

By combining open data, eDNA, innovative monitoring technologies, and advanced modelling, a holistic approach to managing fluvial ecosystems under the Habitats and Water Framework Directives becomes possible. This integration enables more precise, efficient, and proactive ecosystem monitoring, promotes resilient aquatic habitats, and supports sustainable water management across Europe. As these technologies continue to advance, they will likely play an increasingly central role in environmental policy and ecosystem conservation.

4 Guidelines to assess fragmentation at appropriate scales

Spatial and temporal connectivity plays a key role in maintaining functional and well-structured river ecosystems. It is widely known that longitudinal connectivity indeed plays a determining role in the maintenance of ecological processes that support freshwater biodiversity (e.g., migrations and gene exchange). However, as already mentioned in section 1.1, stream connectivity acts at 4 dimensions:

- Longitudinal (from headwaters to the mouth),
- Lateral (between the river channel and the floodplain),
- Vertical connectivity (between surface and groundwater), and
- Temporal (particularly relevant in Mediterranean ecosystems dominated by seasonal and intermittent systems).

Together, these dimensions sustain key ecological processes, such as the transfer of energy and matter or seasonal migratory movements (Ward, 1989). These processes are essential for biodiversity conservation and the ecosystem services provided by river systems. Furthermore, rivers act as structural backbones in the landscape, facilitating ecological connectivity even for species not strictly dependent on aquatic habitats (Gilles & Clair, 2008; Sánchez-Montoya et al., 2023). However, they can also spread threats throughout the system.

Most of the protected areas of the Natura 2000 Network were designated with a terrestrial ecosystem focus, creating issues across hydrographic networks, such as:

- Interrupted river connectivity,
- Lack of a basin-wide perspective, and
- Inadequate integration of river ecology in boundary delineation (e.g., protecting only middle reaches without headwaters, using channels as limits so only one bank lies inside the protected area).

Beyond freshwater ecosystems, rivers and streams are vital connectors, enabling the movement of terrestrial species (Sánchez-Montoya et al., 2016; Zimbres et al., 2017) and the transfer of energy and material along landscapes (Tysmans et al., 2013). Consequently, fluvial ecosystems are essential for building a well-connected, coherent and functional network, extending far beyond their direct role in freshwater biodiversity.

However, river connectivity across all spatial and temporal dimensions has undergone important perturbations over the last century and has been deeply transformed. The proliferation of barriers such as dams, weirs, and culverts has fragmented river networks longitudinally (Januchowski-Hartley et al., 2020); the drainage and conversion of floodplains to intensive land uses (e.g., agriculture, urban and industrial) has disconnected riverbeds from their associated wetlands (Reis et al., 2017); the over-exploitation of aquifers has altered the vertical component of connectivity (e.g., Wang, 2023; Green et al., 2024); and, the flow regulation has modified natural flow patterns (including minimum, maximum, and seasonal flow) and flood frequency, thereby altering freshwater ecosystems and the temporal dimension of connectivity (Jaeger et al., 2014). These modifications are now widespread across river networks worldwide and have deeply transformed the structure and functioning of these ecosystems (Grill et. al., 2019). Therefore, restoring connectivity across all dimensions is essential for recovering these ecosystems, along with the biodiversity and services they provide.

The maintenance of biodiversity and river ecosystem services requires a management effort that guarantees these ecosystems retain their value as connectors. The poor conservation status of freshwater ecosystems has triggered global responses and commitments to restoration. Although often treat as part of terrestrial systems (Gonçalves & Hermoso, 2022), recent policy advancements, such as the Kunming-Montreal Global Biodiversity Framework (Conference of the Parties to the Convention on Biological Diversity, 2022; Cooke et al., 2023), or the EU Biodiversity Strategy for 2030 and EU Nature Restoration Law (European Commission, 2024; European Commission, 2020; Stoffers et al., 2024), explicitly recognise the need for targeted restoration efforts to improve freshwater ecosystems and recover their connectivity and biodiversity. The latter initiatives go a step further and set quantitative targets for restoring connectivity in EU river systems, aiming to recover at least 25,000 km of free-flowing rivers by 2030.

To help operationalize these objectives in a standardised way, guidelines for assessing connectivity and identifying free-flowing conditions have been developed (e.g., Van De Bund et al., 2024). Additional opportunities to restore freshwater ecosystems and connectivity arise at a continental scale from the EU Green Infrastructure Strategy (European Commission, 2013; Portela et al., 2021). Green Infrastructure is conceived as a tool “for providing ecological, economic and social benefits through natural solutions” and “mobilise investments to sustain and enhance the value of the benefits that nature provides to human society” (Maes et al., 2015). The Green Infrastructure network also aims to enhance the connectivity among protected areas, contributing to a more coherent and effectively connected network of protected areas (European Commission, 2020). Member States were encouraged to formulate their own green infrastructure strategies and to undertake assessments of ecosystem conditions and services at the national level within the existing political, legal, and financial frameworks of the EU. In response, several Member States incorporated green infrastructure into their biodiversity and nature conservation policies and legislation. Although, only a few have adopted dedicated national strategies for green infrastructure (European Commission, 2019).

Freshwater ecosystems are recognised as important providers of services such as flood mitigation (disaster reduction) or carbon sequestration. Elements such as river courses, wetlands, or habitats at risk of disappearance must be given special consideration. To ensure these services are maintained, the restoration of freshwater ecosystems has been identified as a priority. Such restoration efforts can also benefit biodiversity by recovering habitats and improving connectivity along river networks and between river channels and their adjacent floodplains. This is especially important for river courses and other linear, continuous elements of the landscape, as well as for restoring lateral connectivity between protected Natura 2000 network sites and other areas of high biodiversity value.

Identifying key elements is essential when designing a coherent and continuous network that maximized the contribution that freshwater ecosystems to ecological connectivity and biodiversity conservation. A functional network of freshwater ecosystems or corridors should connect populations of freshwater species and be integrated into the broader ecological network. Given the current fragmentation status of rivers, special attention should be given to the spatial distribution of barriers, with the goal of minimising their impact on ecological corridors and ensuring their functionality. Due to the widespread presence of barriers, restoration efforts will often be necessary to re-establish connectivity and recover the ecological integrity of these corridors.

In the following lines, a methodology is proposed to identify a network of priority river corridors. This methodology aims to design a network of river corridors that enhance connectivity across all its dimensions (longitudinal, lateral, vertical, and temporal), supporting the ecological processes associated with these components of connectivity.

This methodology seeks to guide the identification of key elements to consider when designing a connected fluvial network, focusing on the contribution that freshwater ecosystems can make toward creating a well-connected network and maximising opportunities for the conservation and recovery of freshwater biodiversity. The goal is to design a network of freshwater corridors that link populations of freshwater species, especially those used to evaluate conservation status (see Section 3.1). Given the current fragmentation of rivers, special attention will be paid to the spatial distribution of barriers, to minimise their impact on corridor functionality. However, transversal barriers are not the only connectivity elements addressed by this methodology.

The Marxan software (Ball et al., 2009) is free and open-source and it can be applied to a wide range of problems, such as reserve design and natural resource management in terrestrial, freshwater, and marine systems. The Marxan spatial planning tool was selected to design the network of river corridors because its free accessibility ensures the future use of the proposed methodology. Based on an optimisation algorithm, Marxan identifies a set of patches (or river sections) that connect populations of species or aquatic habitats throughout the hydrographic network, maximising connectivity within the network of corridors. To achieve this, three sources of information are required:

i) a hydrographic network on which all other elements will be mapped,
ii) the distribution of the species/habitats considered in the corridor network design (e.g., possible beneficiaries of the network), and
iii) structural elements that may constrain network functionality (e.g., dams, weirds, etc.).

As mentioned previously, the presence of infrastructure such as transversal, (e.g., dams, weirs) or lateral barriers (e.g., levees, dykes) can compromise longitudinal or lateral connectivity, respectively. The proposed methodology does not aim to identify priority barriers for their removal (although it could also serve this purpose), but rather to integrate the spatial distribution of the mentioned infrastructures into the functionality of the network. Moreover, this methodology can be applied in many different ecosystems, such as forests, scrubs, grasslands, and others, not only in lineal or azonal ecosystems. Thus, it can be used transversally across all habitats of Community interest listed in Annex I of the Habitat Directive 92/43/CEE.

To implement the proposed methodology, various resources are necessary, including georeferenced data and cartography. The essential resource is a base map serving as the spatial framework for analyses, in this case, a hydrologic network derived from digital elevation models, which are available for most of the Member States. It is mandatory to delineate minimum planning units (i.e., intrinsically coherent reaches or river sections).

Additionally, a map of all elements implied in the connectivity, such as barriers, obstacles (lateral or transversal), and infrastructures, is required. Therefore, any monitoring system for river connectivity must compile the following information:

- Transversal barriers, (e. g., dams, weirs).
- Lateral barriers (e. g., levees, dykes)
- Permeability and connectivity with phreatic (groundwater) level
- Connectivity and integrity with floodplains and riverine forest
- This compilation provides sufficient information to evaluate the connectivity.

The basic planning units are defined by the hydrological network segments between barriers. Accordingly, the hydrologic network is divided into stretches separated by barriers. Information about the distribution of species linked to fluvial ecosystems is also required. Both actual and modelled distributions can be used, the later derived from Species Distribution Models developed using tools such as Biomod2 (Thuiller et al., 2009), which has shown high accuracy in predicting species presence and absence. The continuous habitat suitability values must then be converted into presence-absence binary distributions by setting a species-specific threshold that maximises the sensitivity and specificity of the models, using the presence-absence R package (Freeman & Moisen, 2008). These binary distributions are then mapped into the network of planning units described above, resulting in occupancy maps showing the length of each planning unit occupied by each species. This approach can be applied not only to fish but also to other species with varying degrees of dependence on aquatic ecosystems.

Then, Marxan can be applied to design an optimal network of corridors that maximises connectivity among the populations of the species described above. It uses a heuristic optimisation algorithm to identify an optimal set of planning units that collectively achieve a defined representation target (i.e., species coverage) while minimising the cost of the selected units and connectivity penalties associated with fragmented solutions (see more details in Marxan manual; Serra et al., 2020).

Different planning scenarios for designing the network of corridors can be tested. These scenarios aim to depict different planning objectives and constraints, such as

i) a scenario where the distribution of the Natura 2000 network (N2K) is used as the backbone of the corridor network,

ii) an unconstrained scenario where all planning units were equally available (all planning units had a status = 0 in the planning unit file),

iii) a no dams or weirds are allowed scenario (NDA),

iv) a scenario where different types of transversal and/or lateral barriers are considered.

Different indicators can be used to compare the best solutions obtained for each scenario: the number of planning units needed to achieve the target, the number of dams included, the length of continuous units selected for the solution, and the length of continuous units selected for each species individually. The number of planning units serves as surrogate for the efficiency of the solution, assuming that the fewer planning units indicate greater efficiency. Because planning units were defined using barriers as spatial breaks, this indicator also shows how many barriers would be included in the corridor network. Among these barriers, dams are particularly relevant, so we also measured the number of dams included in the solution. The length of continuous units selected was calculated as the total length of planning units with at least one contiguous neighbour (either upstream or downstream) included in the best solution. Isolated planning units without selected neighbours did not contribute to this indicator. This indicator was also measured specifically for planning units where each species was present. In this case, we summed the length of planning units with contiguous neighbours selected when both units contained populations of a given species, reporting this as the proportion of the species' total distribution range. This indicator could be interpreted as the proportion of the species' distribution extent included in neighbouring planning units selected in the best solution.

This methodology has been applied in Spain as a demonstration exercise, initially including only the longitudinal component of river connectivity, the distribution models of fish species, and the inventories of reservoirs and transversal barriers. In this case, a hydrologic network was used to generate a linear structure and scenario. However, this methodology can also incorporate riverside vegetation, floodplains, and surrounding ecosystems.

As a result of this exercise, a network of river corridors was designed that maximizes connectivity across all its components, enabling connections between populations of species or habitats while minimising the impact of structural elements that could compromise its functionality. This approach can be replicated elsewhere to design river corridors beyond the focus on Green Infrastructure planning and to guide future restoration efforts.

The results demonstrated the effect of three decision-making parameters used in the tool on the spatial configuration and extent of the network of priority corridors. These effects were analysed, and recommendations were provided for future users on how to calibrate and define these parameters. Furthermore, the analysis revealed that most protected areas have been designed from the perspective of terrestrial ecosystems. This generates problems across the hydrographic network, such as the lack of river connectivity, the absence of a basin-scale perspective, and the lack of consideration of river ecology when delimiting protected areas (e.g., protecting a middle reach without protecting its headwaters). As a result, the protected reaches become subject to disturbances originated upstream. Likewise, using channels to define protected area boundaries frequently results in only one riverbank falling within the protected area, leaving the opposite bank unprotected.

These circumstances have caused the usual problems of coherence and connectivity within the Natura2000 Network (Hermoso et al., 2015). For lotic ecosystems, the situation has worsened, despite their inherently linear structure, which could otherwise facilitate the development of connectivity networks. Moreover, in river systems, longitudinal connectivity indeed plays a determining role in maintaining the ecological processes that support freshwater biodiversity (e.g., migrations and gene exchange). However, it also contributes to the spread of threats throughout the system. This work has been published as a guide (VV. AA. 2024).

The application of the methodology at any spatial scale (ranging from entire river basins to smaller tributaries) may require a specific assessment of the objectives pursued by the network of corridors and the information necessary for its identification. Once these objectives are defined, they can be integrated into the tool for the designing the corridor network at any chosen spatial scales.

5 Next steps to address future needs

Promoting implementation of these guidelines

These guidelines recommend standard methods for assessing and monitoring running water habitats' condition with the goal of promoting harmonised procedures across the EU Member States. To ensure that habitat condition assessments are comparable across countries, it is essential to define a common set of variables/indicators with well-defined metrics and standard measurement procedures. These should include physical, chemical, compositional, and functional variables to comprehensively evaluate the health of mire habitats.

To implement these guidelines, the following next steps are suggested:

- **Test the proposed set of variables** with agreed measurement procedures and monitoring methods. Use common protocols for sampling, while considering the particularities of specific habitats and existing contextual factors at local and country level; this testing would be useful to identify gaps of knowledge, flaws of applicability and robustness and reliability of results. The evaluation should provide recommendations to be further integrated in the harmonised procedure, as needed.
- Develop further, test and standardise the methods for the establishment of **reference values and thresholds** to determine good condition. Defining ecological thresholds based on proper habitat characterisation is essential. These thresholds will indicate the health and quality of these rocky habitats, aiding in the monitoring of changes over time. They will also facilitate the assessment of impacts of climate change, human activities, and invasive species, providing critical insight for conservation efforts.
- Develop further, test and standardise the methods for the **aggregation of results** obtained from all the variables measured at the local scale and for each biogeographical region.
- **Develop further and test the criteria for the selection of monitoring localities and sampling design** to ensure a sufficiently representative sample that allows for proper implementation of the aggregation of results at the biogeographical region level.
- **Promote harmonised methods for the use of typical species:** Typical species provide a practical way to evaluate habitat status, reflecting specific ecological conditions. Clear criteria should be defined for selecting these species, along with the methodologies to assess their status and integrate the results into overall condition assessment for each habitat.

The current proposal should be viewed as a starting point and may be adapted where more suitable alternatives are identified based on national experience or ecological requirements.

Promote coordination with the WFD

Aligning the assessment of running waters under the Habitats Directive with the monitoring and evaluation of the Water Framework Directive is recommended, and it should consider the following main issues.

- **Integration of habitat conservation objectives**: The WFD's focus on achieving good ecological status should explicitly incorporate the conservation objectives of protected habitats and species listed under the Habitats Directive. This would ensure that river monitoring addresses not only general ecological health but also the specific needs of priority habitats and species.

- **Enhanced hydromorphological monitoring**: Hydromorphological elements, such as flow regimes, sediment transport, and river-floodplain connectivity, are critical for supporting habitats and species of community interest. The WFD should place greater emphasis on detailed monitoring of these elements to meet the requirements of both directives.
- **Inclusion of protected sites in River Basin Management Plans** (RBMPs): The WFD's RBMPs should explicitly account for Special Areas of Conservation (SACs) and Sites of Community Importance (SCIs). This would strengthen the link between water management and habitat conservation planning, ensuring that measures under the WFD contribute to achieving favourable conservation status. This is essential because the Water Framework Directive explicitly incorporates these objectives in articles 4 and 8.
- **Improved assessment of dynamic river processes**: The Habitats Directive emphasises the role of natural dynamic processes (e.g., lateral migration, sediment deposition) in sustaining habitats. The WFD should develop and apply metrics and methodologies to assess these processes more effectively, recognising their importance for habitat restoration and resilience.
- **Adaptation of Biological Quality Elements** (BQEs): The biological quality elements under the WFD (e.g., macrophytes, invertebrates, fish) should be broadened and refined to include indicators directly relevant to the conservation of species and habitats listed under the Habitats Directive.
- **Stronger alignment of monitoring frameworks**: Monitoring frameworks under both directives should be harmonised to reduce duplication of efforts and ensure data compatibility. Joint monitoring programmes could also assess ecological and conservation status simultaneously, increasing efficiency and optimising resource allocation.
- **Increased Stakeholder Collaboration**: Strengthening collaboration between water authorities, conservation bodies, and local stakeholders would facilitate the implementation of measures that benefit both river ecosystems and the protected habitats/species they support.
- **Integration of HD and WFD monitoring systems**: Both directives need to better account for the effects of climate change on hydrology, habitat connectivity, and species distribution. Coordinated adaptation strategies are essential to ensure that monitoring and management remain effective under changing conditions. Moreover, in the rivers of many Member States, multiple administrative bodies with differing competences often converge. It is not uncommon for water management to fall under the jurisdiction of a different authority than the one responsible for the conservation of fluvial ecosystems. Typically, the authority in charge of water management is tasked with implementing the WFD and is therefore responsible for assessing fluvial ecosystems in line with its requirements and achieving Good Ecological Status for rivers. Conversely, the implementation of the Habitats Directive is usually entrusted to the administrative bodies responsible for nature conservation. This division of responsibilities often leads to conflicts, inconsistencies, and inefficiencies.

  To address these issues, this guide proposes enhanced coordination among the various authorities involved to minimize overlaps and redundancies, thereby reducing unnecessary expenditure of public resources. Given that this document has demonstrated that, broadly speaking, the assessment of fluvial ecosystems under the WFD is largely applicable, albeit with some limitations, for evaluating the conservation status of habitats on-site, it seems logical ton coordinate the implementation of both directives at all levels.

This coordination should encompass everything from the selection and allocation of rivers, stretches, or water bodies to be monitored through the development of shared databases containing raw data and indices. This would enable both administrative bodies to access the information required to fulfil their respective obligations more effectively.

- **WFD blind angle:** According to WFD, a water body can only be defined when certain criteria are achieved (e.g., minimal catchment area, minimum water flow). As a result, many small headwater streams and temporary rivers are not included on the hydrographical networks and, in consequently, from WFD monitoring programmes, representing a significant blind spot. However, many of these rivers and streams are part of Natura 2000 network or are identified as Habitats of community Interest from Group 32: Running waters. Therefore, the WFD hydrological networks must be improved and extended to fully meet the objectives of both directives. It is also necessary to revise and reinterpret the group 32 habitat classification to better reflect ecosystem dynamics described under the WFD, ensuring that both structural and functional coherence are emphasised.

Enhance research and methodology development

- **Identify knowledge gaps and prioritise research areas**: Future assessments should start by identifying specific knowledge gaps in the current understanding of running water ecosystems. This includes studying ecosystem dynamics, such as nutrient cycling, species interactions, and habitat connectivity, all of which influence the health of running waters.
- **Develop and refine methodologies for reference conditions**: A critical step in effectively monitoring running waters is defining reliable baseline or reference conditions. Future work should focus on improving these methodologies, taking into account local hydrological, geological, and ecological characteristics. Such methods should be adaptable to both pristine and impacted sites, using indicator species, hydrochemical profiles, and geomorphological markers as baselines.
- **Expand ecological indicators and metrics**: Traditional indicators, such as chemical parameters and biological species indices, should be complemented with more holistic, ecosystem-based metrics. Indicators of ecosystem resilience, biodiversity health, and trophic interactions could provide a richer picture of ecosystem status. Validating these metrics under different seasonal and geographical conditions would make them more robust and widely applicable.
- **Assessment of flow regimes**. One of the most significant gaps identified is the systematic assessment of flow regimes. Magdaleno (2013) highlighted that numerous methodologies have been developed to analyse hydrological alterations and determine environmental flows. These methodologies aim to objectively and efficiently evaluate the most environmentally significant changes affecting flow regime components, making them essential tools for analysing hydrological alterations (Magdaleno, 2013). Additionally, they allow for the evaluation of how various scenarios of water resource use and management scenarios might alter flow regimes, interpret the environmental consequences of such alterations on a river's ecological integrity, identify the aspects of flow regimes that most constrain the rehabilitation or recovery of regulated sections, and establish objective criteria for prioritising the restoration of degraded river ecosystems (Martínez Santa-María

& Fernández Yuste, 2008, cited in Magdaleno, 2013). According to Magdaleno (2013), the evaluation process generally involves the following steps:

i. Selection of the most environmentally significant aspects of the flow regime.

ii. Selection of parameters and variables to characterise these aspects.

iii. Definition of a set of indices to compare parameter values across different situations: natural regime versus altered regime, and natural regime versus scenarios designed to define the proposed environmental regime.

iv. Derivation of the environmental implications of the assessed alterations.

Leverage and integrate existing data sources

- **Utilize national and regional monitoring programmes:** Many countries already conduct extensive environmental monitoring through forest inventories, biodiversity surveys, and water quality databases. Future efforts should aim to harmonize these sources with water monitoring programmes to provide a broader integrated dataset that captures interactions between forests, soils, and water systems, which are particularly relevant for the headwaters and smaller tributaries.
- **Create a centralized data platform for accessibility and analysis**: Integrating diverse data sources would benefit from a centralized, open-access data platform using compatible data formats. By merging information from various sectors (forestry, agriculture, urban planning), researchers could analyse cumulative impacts and regional trends that would otherwise remain invisible in isolated datasets.
- **Encourage cross-border data sharing and interoperability:** Water bodies cross national borders, transboundary data exchange is essential. Establishing EU-wide standards for data interoperability would facilitate cross-country collaboration, allowing a cohesive, catchment-wide approach to monitor rather than limiting assessments to national segments.

Develop and expand monitoring programmes

- **Implement comprehensive, long-term monitoring initiatives**: Long-term monitoring programmes are essential for detecting ecological trends, seasonal variations, and emerging threats, especially in response to climate change. Such programmes should include periodic biological and physical sampling, remote sensing, and continuous in-situ monitoring for real-time water quality and flow measurements.
- **Use technology to enhance data collection**: Technological advancements can transform monitoring efforts. Drones and satellites can provide high-resolution imagery and environmental data from hard-to-reach river sections, while in-situ sensors can continuously collect chemical and biological parameters. Integrating these technologies would allow more accurate, fine-scale datasets that are continuously updated.
- **Adopt adaptive monitoring strategies**: Water ecosystems are highly dynamic, and environmental conditions can change rapidly due to increasing pressures. Adaptive monitoring strategies should allow for responsive and flexible sampling efforts. For example, if data shows sudden declines in water quality in a particular region, monitoring intensity in that area could be temporarily increased to pinpoint causes and mitigate impacts.

Promote stakeholder engagement and capacity building

- **Engage local communities in monitoring efforts**: Local communities, especially those directly dependent on water resources, can be valuable partners in monitoring. Citizen science programmes, where citizens contribute data, can expand spatial coverage while building public awareness and stewardship.
- **Collaborate with industry and agriculture stakeholders**: Industries and agriculture are among the main water users and potential polluters. Collaborating with these stakeholders to promote best practices and reduce environmental impacts can help prevent negative trends at the source. Encouraging industries to monitor their environmental impacts can complement governmental monitoring and improve overall data quality.
- **Build local capacity for monitoring and data analysis:** Effective monitoring requires skilled personnel trained in modern data collection and analytical techniques. Future plans should include training and resources for national and regional agencies to carry out and interpret sophisticated analyses. This includes capacity-building in statistical analysis, GIS mapping, and remote sensing to produce accurate and actionable insights.

Strengthen policy and funding support

- **Align monitoring efforts with EU Directives and initiatives**: Ensuring that monitoring initiatives are aligned with directives as the WFD would streamline efforts, facilitate access to EU funding, and enable the integration of results into EU level policy actions. This alignment ensures that water monitoring remains consistent with the goals of "good status" for all EU waters.
- **Secure long-term funding for continuous monitoring**: One of the most significant barriers to effective monitoring is the lack of stable funding. Unlike short-term projects, continuous monitoring requires stable, long-term financial support. Policymakers should establish dedicated funding mechanisms or create incentives to support ongoing assessment, particularly those involving emerging technologies and innovative methodologies.
- **Develop a legal framework for cross-border monitoring and data integration**: Because many rivers cross political boundaries, monitoring efforts must be coordinated at the transboundary level. Establishing a legal framework for cooperation would promote data sharing, standardise methodologies, and provide a comprehensive picture of the health of cross-border waters.

By addressing these steps comprehensively, future assessment and monitoring efforts for running waters can become more effective, data-rich, and responsive to the challenges posed by changing environmental conditions. This approach not only strengthens the scientific foundation for managing running waters but also enhances resilience against environmental stressors such as pollution, habitat fragmentation, and climate change impacts.

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ANNEX 1 – Summary overview of main characteristics and variables measured in EU MSs to assess river habitat condition (examples)

Ecological characteristics	Types	Description	Examples of variables measured by the Member States	Notes
Abiotic characteristics	**Physical state characteristics**	Hydrology, hydromorphology, substrate, water transparency	- Flow rate, speed, (BE, BG, GR, HU, IT, PL, RO, SI) - Natural, unaltered hydrology, hydromorphology (AT, BG, GR, HU, IT, LV, PL, SI) - Bottom substrate type and cover (BE, BG, DE, HU, IT, PL, SI) - Water temperature (BG, SI) - Water colour, water transparency (BE, BG, CZ, LT, RO, SI) - Shading by riverine vegetation (%) (PL, HU)	- At least twelve Member States monitor physical parameters. - Water flow is monitored by ten Member states. - Eight MSs monitor water transparency, turbidity or colour. - Only two Member States monitor temperature. - Only three Member States monitor morphological or hydromorphological variables.
	Chemical state characteristics	Water chemical status, chemical elements and compounds, nutrients	- pH, salinity (BE, IT, LT, SI) - Dissolved gases (oxygen, nitrogen), BOD (BE, BG, SI) - Pollutants (DE) - Nutrients (BE, SI) - Chemical status according to WFD (DE, FR) - Chemical elements and compounds (P, Cl^-, sulphate, ammonia, etc. (BE)	- At least ten MSs are monitoring chemical variables. - pH, salinity and temperature are monitored by 3 MSs. - Pollutants are monitored by DE as part of WFD. - Only three MSs include dissolved gases in their monitoring. Dissolved oxygen is determinant for life development in aquatic ecosystems.
Biotic characteristics	**Compositional state characteristics**	Characteristic and typical species, composition of riverine vegetation, fauna and invasive alien species	- Characteristic species (BE, BG, CZ, DE, FR, GR, HU, IT, LT, LV, PL, RO, SI, SK) - Riparian vegetation (CZ, DE, FR, GR, HU, LT, LV, PL, SK, SI) - Invasive alien species (BE, GR, HU, LT, PL, RO) - Presence of fauna: fish, birds, amphibian, reptiles, Othoptera, Odonata (BG; DE, FR, GR, IT, SI)	- All MSs monitor plant composition, especially characteristic species and riverine vegetation. - River banks and their vegetation are monitored by eight MSs, showing its traditional importance. - Six MSs monitor IAS presence and abundance. - Monitoring macrophytes, macroinvertebrates and fish based on WFD methods is used in at least four MSs.
	Structural state characteristics	Horizontal and vertical structure of vegetation, cover of riparian vegetation, alien species cover	- Vegetation cover and height (IT, HU, PL, RO) - Cover of characteristic species (AT, BE, DE, FR, HU, GR, IT, LT, PL, RO) - Vegetation structure in water body (submerged and floating vegetation, aquatic mosses) (DE) - Cover of woody species, tree and shrub cover (DE, HU, PL, SK)	- Eleven Member States assess structural characteristics. - Most of the MSs focuses on vegetation cover and cover of characteristic species or groups of species. - The characteristics of riparian vegetation are also assessed in many MSs.

			- Cover of helophytic species (BE, LT) - IAS cover (AT, FR, HU, LT, PL) - Width, zonation, structure and quality of riparian vegetation (DE, GR) - Riparian quality index (IQBR) (FR) - Percentage of bare soil cover (FR)	
	Functional state characteristics	Riverine vegetation, naturalness, vegetation, phytobenthos, fauna	- Natural river dynamics: flooding, sediment deposition is undisturbed (BE, LT). - Eutrophication of the habitat indicated by the presence of indicator species (BE, BG, FR, LT) - Spatial and age structure of tree and shrub layer (3230, 3240) (CZ) - Height and phenology of selected species that indicate different dynamic stages (pioneer, evolved stages) (FR) - Age structure and regeneration of characteristics species (*Myricaria germanica, Salix eleagnos*) (DE, PL) - Leaf litter (HU) - River flow continuity (IT) - Water flow altered by dams or other barriers (DE)	- Functional variables are relatively infrequent in the methodologies considered in this review. - Eutrophication is assessed in at least four MSs based on the presence of indicator species. - Age structure, phenology and regeneration of characteristic species and communities are assessed in four MSs. - River continuity is assessed in few MSs.
Landscape/seascape characteristics		Fragmentation, connectivity, neighbouring habitats	- Rate of isolation, distance to similar habitats, role of neighboring habitats (HU), - Size and spatial structure of habitat patch (HU, PL) - Land use in surrounding areas (HU, LT) - Landscape characteristic (IT) - Habitat fragmentation by infrastructure (BG) - River habitats complex, spatial structure of habitat patches (PL)	- Only four Member States monitor landscape characteristics (DE, HU, IT, PL) - Connectivity is the most important parameter and is monitored by three MSs.
Other		Alterations	- Alterations to riverbed and modification of riverbanks, % of artificial banks (DE) - Degradation by human activities: gravel extraction, recreational use, etc. (AT) - Surface is affected by various activities from a list (FR).	- Several MSs consider different types of alterations and impacts when assessing habitat conditions.

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