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Review

Challenges to water quality assessment in Europe – Is there scope for improvement of the current Water Framework Directive bioassessment scheme in rivers?

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A B S T R A C T

The EU Water Framework Directive (WFD) assessment scheme has been putting in force the evaluation of freshwater ecosystems in Europe, including a new paradigm of ecological status. After almost 20 years since the WFD implementation, it is imperative to evaluate the efficiency of its standard assessment scheme and to explore the possibility of learning how to improve its effectiveness. That is the spirit of this review, aiming (i) to explore the existing literature on the WFD bioassessment scheme for assessing freshwater ecosystem health, particularly in lotic ecosystems (where the WFD scheme is most consolidated); (ii) to document which paths are suggested by the scientific community to improve the efficiency of the bioassessment in tackling current challenges. In the specific arena of bioassessment, we first identify the major constraints to the WFD full implementation in rivers. Second, we analyse retrospective Ecological Risk Assessment (ERA) as an evaluation approach supporting management actions that could inspire improvements in the WFD bioassessment scheme. Third, we review the advances and debate on complementary metrics to improve WFD evaluation protocols and/or the feasibility of the evaluation outcome. Fourth, a conceptual scheme for an improved evaluation strategy is presented. Our proposal essentially merges the WFD bioassessment scheme with the ERA philosophy, proposing a tiered approach of increasing complexity and spatial resolution, where expert judgement is included surgically at all decision stages. This scheme requires true integration of chemical, ecological and ecotoxicological LoE for a quantitative estimation of risks, and provides a comprehensive framework that accommodates tools and perspectives already suggested by other authors. Besides providing a literature review on the strengths and weaknesses of the current WFD bioassessment scheme, we wish to open way for the scientific discussion towards an improved conceptual scheme for the evaluation of ecosystem health.

1. Introduction

Freshwater ecosystems are essential to human life, as populations depend on the various services provided by freshwaters, whether they are provisioning services (e.g. food, fuel, water), regulating services (e.g. climate regulation, water regulation, natural hazard regulation), cultural services (e.g. cultural diversity, ecotourism) or supporting services (e.g. photosynthesis, nutrient cycling, water cycling). Notwithstanding its instrumental value (anthropocentric perspective), water is an ecosystem holder, which generates a debate on its intrinsic philosophical value (non-anthropocentric perspectives) – see Ghilarov (2000), Jakobsen (2017) or Piccolo et al. (2018). Although the recognizable importance of freshwater, there are many threats to its integrity, thus compromising the availability of the ecosystem services provided by it. This situation represents a major concern in developing countries, although it is happening in developed countries too (Vörösmarty et al., 2010). Most of these services link to a direct benefit of humankind, but the exploitation of freshwaters and associated services and goods keeps increasing (Millennium Ecosystem Assessment, 2005), affecting the social-natural relationship as a whole (Bennett et al., 2009). The recent report on the Environmental Rule of Law (UNEnvironment, 2019) showed that there is a widespread failure on the implementation of the

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ever growing regulations concerning environmental protection, this being one of the hardest obstacles to overcome on the path to improve ecosystem health.

While the presence of a multitude of stressors pressuring and posing risk to freshwaters constitute important threats to water integrity *per se*, presently they must be framed within the scope of climate change (Carpenter et al., 2011). In fact, climate change can modulate the effects of these stressors, thus playing a key role in defining the responses of aquatic ecosystems. Climate change is a global phenomenon but its impacts vary substantially from region to region. Predictions include the increase in the frequency of extreme meteorological events such as floods, heat waves, severe droughts and windstorms, and alterations in the water cycle can also be expected (IPCC, 2014a; Millennium Ecosystem Assessment, 2005). The ongoing uptake of energy by the climate system, enhanced by anthropogenic drivers, potentiates global warming (IPCC, 2014a), which accelerates the decreasing of the extent of glaciers, permafrost degradation and the increase of evapotranspiration. Adding these variations to the hydrologic inputs of freshwater to the increasing variability of precipitation, the levels of oceans, lakes and rivers are also changing. This is likely to originate salinization of water (Cañedo-Argüelles et al., 2013) and soil, and interfere on sediment deposition dynamics (Grove et al., 2015), which also affects freshwater systems. Water warming has been particularly noticeable in lentic ecosystems (IPCC, 2014a), leading to a decrease in the availability of dissolved oxygen for respiration and processing of organic matter and contaminants. However, freshwaters can be somewhat purified during the hydrological cycle of Earth: rivers have a certain degree of capability of regenerating themselves by taking contaminated water downstream and into the ocean and replenishing it with clean inputs from offsprings and rain. The period of time needed to fully replenish the water of a river is estimated in 16 days (Shiklomanov, 2000), but, due to climate change, many large rivers have been facing an increase in these turnover time ranges, hinting that they are becoming less capable of diluting the impacts of industry and human development on the basis of natural processes (Vörösmarty et al., 2010). This is worsened by the position of rivers in the landscape, which often convert them in primary recipients of many types of contaminated runoffs (Dudgeon et al., 2006). In fact, because rivers are usually interconnected systems, the majority of the world's largest rivers are moderately to heavily impacted (Vörösmarty et al., 2010).

In order to sustainably protect ecosystems and the services they provide under such changing scenarios of the interplay between anthropogenic stressors and climate change, it is vital to hold accurate information on stressors and their effects on ecosystem health. This review aims to explore the existing literature on how the bioassessment of freshwater ecosystem health is done and the strengths and weaknesses of each evaluation approach. A special focus is put on European freshwaters, and particularly rivers, where water quality assessment is most consolidated (see below). We then explore which paths are suggested by the scientific community to improve the efficiency of the assessment in tackling the challenges arising from chemical contamination and/or climate change in freshwater ecosystems. In particular, we will focus on the EU Water Framework Directive (WFD) assessment scheme for freshwaters (particularly rivers), which is a benchmark instrument resulting from a cooperative transnational effort to define standard approaches and methodologies to assess the status of waterbodies based on different lines of evidence (LoE), with especial emphasis on ecological data (biological communities). After almost 20 years since the WFD implementation in Europe, it is imperative to evaluate the efficiency of its standard assessment scheme as an ecological evaluation tool and to explore the possibility of learning on how to improve its effectiveness.

Although the full implementation of the WFD is still not complete, the current scheme for the bioassessment of river quality and integrity (ecological status, chemical status and waterbody status, in the WFD nomenclature) is – in our opinion – the most well-succeeded and complete case of an assessment scheme for European freshwaters, following the spirit of the WFD. The current bioassessment scheme for rivers (fully lotic ecosystem) incorporates several biological communities and hydromorphological variables (descriptors), and results in a final holistic exercise of looking at ecological quality (based on multiple descriptors) and chemical quality (based on the quantification of waterborne substances). Working from this starting point, we highlight the benefits that can be extracted from other types of evaluation approaches that include multiple LoE, namely under the Ecological Risk Assessment (ERA) philosophy. Although both water quality assessment approaches (WFD and ERA) integrate information from different LoE, they are conceptually different in a few major points *vis* (i) on the extent of the application of ecotoxicological evidences clarifying on cause-effect relationships; (ii) on the room available for expert judgement in the fine-tuning of sampling methodologies, strategic analysis, data interpretation and decision processes; (iii) on the practical meaning of the concept of LoE integration ("one-out, all-out" principle *versus* integrated risk quantification). This will be further scrutinized in the following sections, and we will address how ERA could inspire the WFD assessment scheme for European rivers.

We also review the weaknesses to the WFD assessment scheme that have been pointed out so far by various authors, and that may pave the way for future improvements. As evidenced in Fig. 1 (see it also as a roadmap of the present paper), our major line of reasoning here stems from the recognition that the broad assessment of water quality (also towards the recovery of impacted ecosystems) under the WFD regulatory requirements may not always be feasible in practice. Nonetheless, we recognize the good will and substantial efforts put up towards its successful implementation. Section 2 hence revises how the assessment of ecological status of water has been done through comprehensive frameworks following the WFD, finishing by highlighting (not comprehensively reviewing) the positive aspects that are covered by the ERA assessment schemes that could be incorporated in the WFD assessment scheme to improve the previously recognised weaknesses. Thus, strengths, weaknesses and threats to the efficiency of the WFD are analysed (see Fig. 1), and the comparative strengths of the ERA approach will be appraised. Section 3 mostly collects the opportunities for optimizing the ecological assessment of freshwaters, especially focused on lotic ecosystems, pointing out possible approaches to mitigate the weaknesses and threats as previously identified. Based on the critical synthesis of the body of knowledge made in previous sections, we finally elaborate on a conceptual model exposing the possibility of integrating a site prioritization system based on ERA principles in a logic of simplification, before advancing to comprehensive ecological assessment as demanded by the WFD (Section 4).

2. Assessment of ecological status of water

Among the existing approaches used worldwide, there are two major comprehensive frameworks that constitute very complete schemes using multiple lines of evidence (LoE) to perform the ecological assessment of aquatic ecosystems and that have actual implications or are actually involved in regulatory acts or legislation towards environmental protection: the Water Framework Directive (WFD) assessment scheme, adopted in Europe through the Directive 2000/06/EC, and Ecological Risk Assessment (ERA), adopted for example by the US Environmental Protection Agency as a basis to regulate hazardous waste sites, the management of watersheds or other ecosystems affected by multiple stressors (e.g. SuterII, 2006). These two evaluation frameworks have two main conceptual differences between them: 1) the WFD assessment/classification scheme defines the water body status based on the element providing the most environmentally protective/conservative indication of status, this being generally known as the "one-out, all-out" principle (see Section 2.1), while ERA always integrates all evidences collected from the available LoE (chemical, ecological and ecotoxicological in a complete approach) in risk calculation, using the uncertainty of that integration to define whether a progression to a more detailed analysis

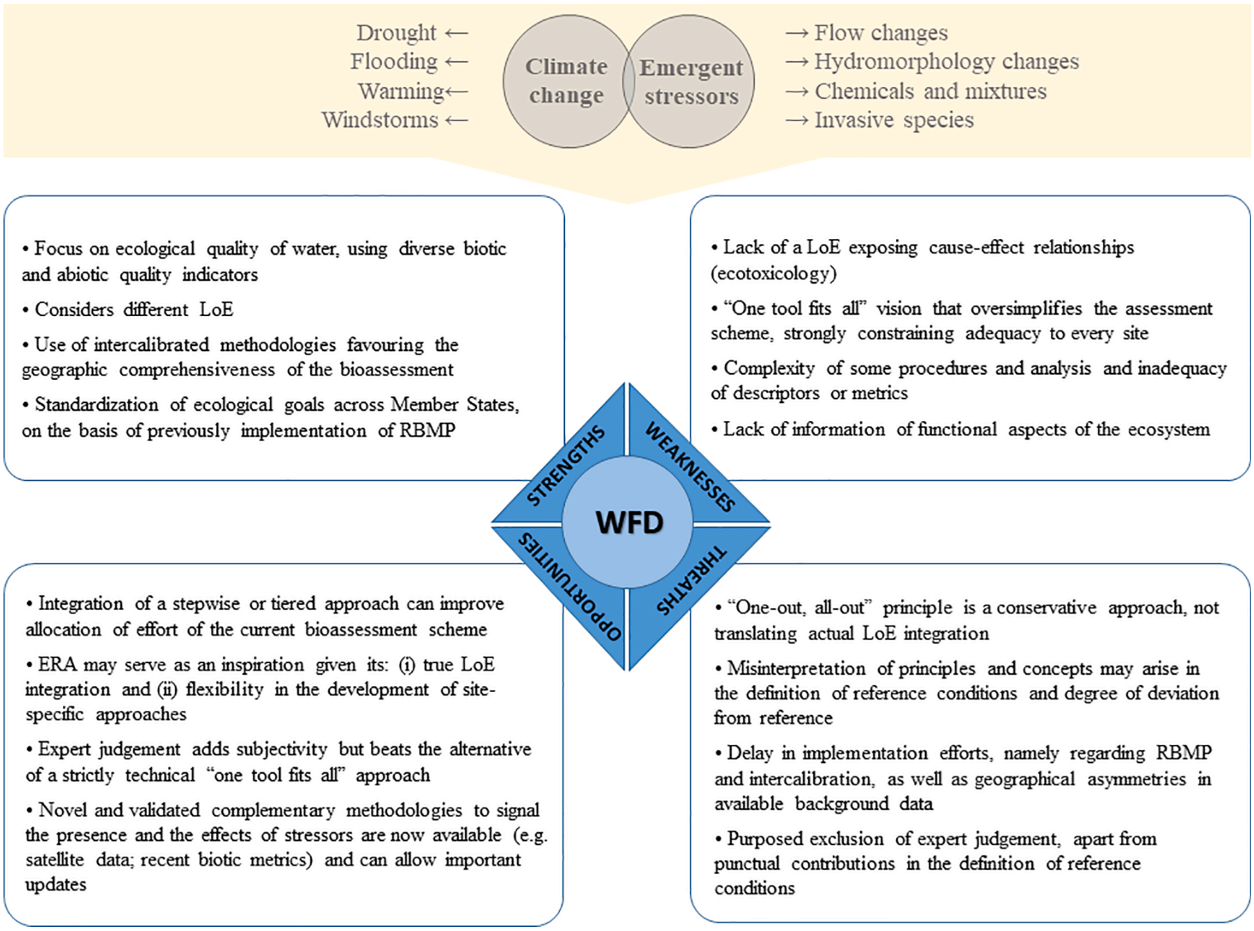

Fig. 1. SWOT analysis exposing the position of ecological quality assessment of freshwaters *sensu* WFD to face current challenges regarding climate change and the range of emerging stressors significantly affecting freshwater ecosystems. This analysis also represents a roadmap of the present review.

should take place or not; 2) ERA integrates the ecotoxicological LoE, basically adding a channel in assessment stages to define cause-effect relationships while there is not such an explicit equivalent in the WFD. Both approaches are complex and present advantages and constraints to their application, as discussed in further sections.

2.1. Evaluation of water quality in Europe with the WFD assessment scheme and constraints to its implementation

Since its adoption in 2000, the Water Framework Directive (WFD; 2000/60/CE) has been one of the most important pieces of legislation concerning the protection, enhancement and restoration of water bodies in Member States of the European Union (EU), establishing a framework for the Community action in the field of water policy (European Commission, 2000). It also changed the paradigm of water management by shifting from an anthropocentric perspective of water (defining it as a resource for direct exploitation by humankind) of previous EU regulations to an ecocentric perspective (where water is seen as an ecosystem holder), establishing ecological status as a new concept and focusing on ecosystem integrity as the foundation of management decisions concerning water quality (European Commission, 2000). The assessment of ecological status of a given water body changed from a general chemical quality assessment into the integration of a range of descriptors concerning biological communities and hydromorphological and physico-chemical quality elements. Moreover, the previous fragmented efforts of evaluation and management, using generalist ecological standards, inadequate legislation or misfit timings (Verdonschot, 2000), were transformed into a more comprehensive approach to the evaluation of ecosystem health (Howarth, 2006), by collecting on different aspects that may constrain the overall ecological quality.

The chemical LoE in the WFD stems from four quality elements: i) chemical and physico-chemical quality elements, ii) specific pollutants, iii) priority substances (Directive 2013/39/EU) and iv) other hazardous substances (defined by national or European quality standards). This is similar to the classical evaluation of water quality that was done prior to the implementation of the WFD, but it was upgraded to a version that is more comprehensive and attempting to meet the contemporary requirements and challenges, imposed by the recent alterations derived from global changes in climate and lifestyle of human communities. For most substances, concentration thresholds (environmental quality standards) were set at the European or national level in the follow-up of the WFD implementation, and are regularly updated. Whenever possible, these limits on concentration of substances were defined based on acute and chronic toxicity data. Thus, the ecotoxicological line of evidence is partially and implicitly considered in the WFD assessment scheme, but no explicit ecotoxicological assays are required to assess the quality of water or sediments (unlike ERA).

The inclusion of the ecological LoE in the evaluation of water bodies constituted an innovation of the WFD. It includes the evaluation of several quality elements for freshwaters: i) benthic macroinvertebrates (abundance and community composition); ii) fish (abundance, community composition and age structure); iii) aquatic flora, including phytoplankton, phytobenthos and macrophytes (abundance and community composition); iv) hydromorphological elements that act as holders of the biological elements (e.g. flow characteristics, channel and bank morphology, riparian vegetation). The results of the evaluation of these quality elements feed ecological, often multimetric indices, which take into account river typology (see Section 2.1.3 for the strategies used to define river typologies). Results are compared with type-specific reference conditions and values, which may include a normalisation procedure and the results are expressed as Ecological Quality Ratios (EQRs). EQRs represent the amount of deviation from a previously defined reference condition (according to the specific river typology), thus translating the ecological status of the evaluated site.

The WFD assessment scheme is highly conservative. As thoroughly illustrated before (see the compilation in European Commission, 2019c), the chemical status relies on a chemical line of evidence focusing on priority substances while the ecological status is underpinned by both the chemical (chemical elements and specific pollutants) and ecological (hydromorphological, physico-chemical and biological elements) LoE. Both chemical and ecological statuses are defined by the quality element with the worst status classification (thus translating the most impacted quality element); this conservative approach is the "one-out, all-out" principle. As the outcome of the assessment, the status of the given focused site under evaluation is also classified according to the worst (more protective; e.g. Caroni et al., 2013) status from ecological and chemical statuses, using the "one-out, all-out" conservative principle too. However, this may ultimately be a source of bias. In fact, the occurrence of an event generated by chance may lead to over or underestimation of the ecological status if the ecological status is (rigidly) defined by the "one-out, all-out" principle. Paradigmatic examples of this type of events are heavy rainfalls that lead to lixiviation of periphyton (Miller et al., 2009) or sampling in low conductivity waters, which substantially reduces electrofishing efficiency (Allard et al., 2014; Pottier et al., 2019); in both cases, underestimation of abundance and diversity of biological communities is likely, giving extra leverage to these descriptors in the assessment of ecological status. Unquestionably, the WFD assessment scheme considers two distinct LoE but, by using the "one-out, all-out" principle, the two LoE are not integrated in the most insightful sense of the word. Of course, some may argue this is semantics, but we propose that real integration can only be achieved by using expert judgment or some sort of multivariate framework that rationally weighs the contribution of the various LoE.

In order to have an effective implementation of the WFD in all Member States and comparable results among them, a pan-European intercalibration exercise was identified as a crucial step prior to evaluation. The main aim of the exercise was to calibrate good ecological status boundaries (obtained with ecological evaluation methodologies) between countries, allowing the further wide implementation of standard, technically easy-to-follow protocols for water quality assessment. An intercalibration network was established through the Common Implementation Strategy and then Member States were divided in groups (Geographical Intercalibration Groups - GIG) according to their geographical region (and expected similarities in general climatic characteristics): Mediterranean, Central, Alpine, Eastern Continental and Northern. Also, four other groups (one working group and three cross-GIG groups) were established: i) the Reference Conditions Working Group, whose task was to assure the intercomparability of reference conditions between Member States; ii) the Very Large Rivers Intercalibration Group, responsible for a harmonised intercalibraton exercise of very large rivers across GIGs; iii) the Lakes Phytobenthos Intercalibration Group, responsible for the intercalibraton of phytobenthos classification methods across GIGs; and iv) the combination of all Member States in a common database, divided in regional groups, to calibrate national ecological quality ratios for the quality element "River fish fauna". These groups were coordinated by the Joint Research Centre of the EU. This multi-phased exercise was recently finished and some objectives were already accomplished: agreement on class boundaries for some quality elements has been met and harmonisation of classification systems is taking place (European Commission, 2018a, 2019a,b; Solheim et al., 2019). The most frequently established methodologies to implement the WFD are those concerning the evaluation of ecological status using benthic macroinvertebrates, fish fauna and phytoplankton (Birk et al., 2012) because they are already intercalibrated for most Member States.

The design and establishment of national River Basin Management Plans, suited to the hydrological reality of each country, is also critical. According to the WFD, the best model of water management is using a river basin approach, since the river basin is the natural hydrological unit. Thus, Member States were enforced to develop River Basin Management Plans for each national river basin, concerning the general and specific ecological objectives for it and whether those goals are being accomplished or not. The plan includes a general description of the river basin, the pressures affecting the water body, the results of monitoring programmes, as well as problems and difficulties encountered in the implementation of the WFD process and delineation of mitigation and restoration measures. Plans should be designed for each river basin irrespective of administrative borders, forcing the collaboration among Member States if necessary (European Commission, 2000).

Albeit all the positive changes the WFD intended to enforce, and the enormous investment in intercalibration made, its implementation has been facing many constraints (as explored in the subsequent subsections) that have been hampering the fulfilment of the main objective of the WFD – all water bodies achieving "good ecological status" (Article 2(22) of the WFD; European Commission, 2000) or higher by 2015. It is noteworthy that this deadline was pushed forward to 2027, with cycles of progress evaluation every six years (European Commission, 2012a). Within this overall context, Carvalho et al. (2019) surveyed the prognoses of a group of experts on the achievement of the WFD objectives for 2027, evaluated their perception on the implementation of the Directive and the receptivity of these experts to some improvements of the WFD framework; then, the most relevant pointed obstacles to the WFD implementation were discussed. Our revision in the subsequent Sections 2.1.1–2.1.5) has naturally some points of contact with the discussion embedded in Carvalho et al. (2019), but it collects further on other relevant constraints to the WFD implementation as recognised by other authors. These sections bear a review nature directed to provide the appropriate support to the proposal on the reorganisation of the WFD bioassessment presented in Section 4.

2.1.1. Misinterpretation of definitions and objectives of the WFD

Misinterpretation of the WFD due to lack of clarity on the explanation of some definitions and objectives has been pointed out by some authors (e.g. Agustsson, 2018; Josefsson and Baaner, 2011; Kelly, 2013; Moss, 2008; Pardo et al., 2012). Dubious definitions become the source of erroneous ecological characterization and setting of expectations, compromising the overall effectiveness of the WFD as a tool to protect the environment. The main doubts arise in the definitions of (i) reference conditions; and (ii) in the degree of deviation from reference conditions defining the boundaries between ecological statuses.

The lack of precision in the WFD while defining what are expected to be reference conditions (pristine-like conditions) for each typology of water body can compromise the accuracy of the evaluation. This is worsened by the fact that pristine sites, with minimal anthropogenic impact (Hering et al., 2003), are now very hard to find at the European scale (Comiti, 2012; Golfieri et al., 2016; Pardo et al., 2012; Reyjol et al., 2014; Vörösmarty et al., 2010). Anthropogenic impacts can even be disguised by time and sometimes they can be traced back to centuries ago, even if the ecosystem is now stable. Moreover, an upstream pristine location may not present the same conditions as a downstream pristine condition, even in the same river. This presents an additional difficulty in the definition of reference conditions, especially in the case of large rivers. When reference sites are defined very upstream in a river, this can lead to erroneous classifications of the ecological status in downstream sites, because conditions are inevitably different in upstream and downstream portions of the river, even in the case of non- (or very slightly) impacted rivers.

Unclear definitions in the range of ecological status categorical classifications (high, good, moderate, poor and bad) in the WFD can compromise the interpretation of the results of the ecological evaluation. For example, the WFD defines a high ecological status as having "no, or only very minor, anthropogenic alterations" compared to the reference values for the evaluated quality elements, reflecting an undisturbed location. But in fact, multiple interpretations can be made regarding this definition, essentially on what is an untouched water body and if subtle anthropogenic impacts are present or not. For example, Finland and Norway reached different conclusions on the ecological status of Tana River, in the Norwegian-Finnish border, due to slightly variable parameters (Finnmark County Council, 2016). To attenuate this problem, Stoddard et al. (2006) suggested a group of terms to better frame the possible meanings of reference condition in the general concept defined in the WFD. Pardo et al. (2012) also made an effort to build a guideline with criteria to ease the selection of adequate reference sites to perform the ecological evaluation of rivers in such a way that comparability across Member States is assured. Adding to the ambiguity of the term "reference condition", some evaluators seem to be also misinterpreting the "one-out, all-out" principle, using it in early levels of the assessment (applying it between the evaluation metrics within each quality element) instead of using it at the level of quality elements, which is the intended approach in the WFD assessment scheme (Borja and Rodríguez, 2010).

This ambiguity in the definitions in the WFD can compromise the whole process of monitoring from early stages onwards. The erroneous classification of a site as a reference site could lead to an under- or overestimation of the ecological status of a given study location, biasing the ecological assessment. Also, in latter stages, the misinterpretation of definitions can contribute to a misfit in the delineation of remediation and mitigation strategies, with inadequate costs. This can even lead to an attempt to restore ecosystems to its pristine condition, which is usually an unattainable objective that may compromise a more realistic objective, like rehabilitating it to achieve a sustainable use of its services and functions (Josefsson and Baaner, 2011).

2.1.2. Challenges in river Basin management Plans design and implementation

Almost all European Member States have been investing in the design and implementation of River Basin Management Plans, promoting cooperation among Water Directors and relevant stakeholders in each country and among Member States (European Commission, 2012a). Some attempts to develop effective assessment methods took place, but the underestimation of the effort required to accomplish this complicated the process and delayed the conclusion of the Plans (European Commission, 2019c; Hering et al., 2010). As a result, many Plans were not fully implemented in many Member States within the expected schedule (European Commission, 2012a) and although second cycle River Basin Management Plans have been adopted in most Member States, the implementation of the WFD is not fully concluded yet (European Commission, 2018b). Challenges to the implementation of the WFD have been reported by some Member States in the cyclic reports. For example, in Portugal it has been difficult to keep updated monitoring networks of river basins (due to challenges in the maintenance of monitoring stations), resulting in information gaps throughout time (European Commission, 2015a). Sweden has been experiencing time delays derived from legislation/regulation and administration barriers to implement monitoring procedures and measures included in the River Basin Management Plans (European Commission, 2015b). Challenges in coordinating the process of implementation of the WFD between different regions and inadequacy of the assessment methods of ecological status have been experienced by Belgium (European Commission, 2015c). Croatia noticed the need of improving second-cycle River Basin Management Plans in order to address newly identified pressures and impacts of relevance for which information is lacking, this being a major cause of delaying the effective implementation of the WFD in the country (European Commission, 2015d). Gaps of knowledge are also a problem in Iceland, which has species and extreme natural conditions that are not fully studied yet (Agustsson, 2018). Overall, Member States rarely met the target of successfully implementing the first River Basin Management Plans because the results of ecological assessment were often not available within the expected time span (Hering et al., 2010). Also, the integration of mechanisms of detection of emergent contaminants and mixtures of chemicals in the water, and further analysis of the effects of their presence, is not fully developed in many second River Basin Management Plans (European Commission, 2019c).

2.1.3. Problems with intercalibration between Member States

The intercalibration exercise was paramount to allow a reliable comparison of results across Member States and thus a uniform enforcement of the WFD across the EU. However, it faced many constraints, despite the support by scientific teams and technical staff specifically directed to the exercise (Poikane et al., 2014). As a result, there are countries that use classification methods for which the comparability assessment could not be complete within their GIG. This happened when there was a lack of common types of water bodies (which was frequent), different pressures were addressed with different methods by each country within a GIG or different assessment concepts were present (European Commission, 2018a, 2019a,b).

The diversity of water body types within each GIG represented a difficulty in the intercalibration exercise, worsened by the fact that some of these water bodies may not be properly characterized. Lack of data sometimes hindered the establishment of accurate water body types and corresponding reference conditions (Birk et al., 2012), preventing a direct picture of what could be the reference scenario to be used in the ecological assessment, adding to the natural variation of most ecological variables in river basins (Josefsson and Baaner, 2011). To cover that variation, the WFD suggested two systems based on abiotic factors to define surface water body types: system A, based on relevant ecoregions and a few fixed physical and chemical descriptors (altitude, river size, geology); and system B, based on more thorough combination of obligatory and optional physical and chemical descriptors (Annex II of the WFD). A thorough revision of the reports on the implementation of River Basin Management Plans revealed that lack of historical data was a problem for many Member States and that many did not provide accurate information on which system was used to classify river types (although most of them opted for system B). Lack of data to support decision-making on typologies and different options on the choice of classification system by the Member States, as well as unclear information about the definition process, may lead to inconsistencies between countries, turning the whole process of intercalibration more difficult. Doubts in enunciation of pristine sites nowadays can also arise from the general lack of historical data, compromising the definition of reliable type-specific reference values for the metrics proposed in the WFD. The confusion in the establishment of typologies and reference conditions (derived from the challenges referred to in subsection 2.1.1) surely compromised the fluidity of the intercalibration exercise.

Other issues that have been compromising the intercalibration exercise are the constraints in the establishment of type-specific reference values for some chemical quality elements that are needed to define good ecological status (e.g. nutrients; van de Bund and Poikane, 2015) and ecological quality elements (e.g. fishes, macrophytes), based on available autoecology and ecotoxicological data. Additionally, a certain level of diversity of methods and approaches used across Europe is expected, due to differences, not only in biodiversity and hydromorphology, but also in country-specific environmental regulations and scientific practice. For example, a wide variety of sampling methodologies for biological assessment between Member States complicated the comparison of ecological classifications (Birk et al., 2012; Solimini et al., 2009). An obvious example of this is the availability of methods that use benthic macroinvertebrates (Birk et al., 2012), which is disproportionally high compared to other quality elements. This represents a considerable concern because intercalibration is, precisely, the way to overcome the possible confusing factors brought by this high variety of methodologies. Moreover, it may lead to asymmetries in the evaluation scheme among countries. The use of all quality elements and the comparability of the obtained results are crucial for a truthful picture of ecological status, as stressors can affect different communities in different ways, leading to different conclusions on statuses. For example, differences in sensitivity have been reported between macroinvertebrate and periphytic communities that translate into distinct ecological status classification (revisited in Johnson et al., 2006a, 2006b; Passy et al., 2004; Roig et al., 2015; Santos et al., 2019). Without an effective inter-correspondence of results among Member States, there is a risk of setting different levels of ambition concerning the ecological status of water bodies among Member States. In fact, the classification of the ecological status of a water body in one Member State may not correspond to the same ecological status in other Member State, defrauding the expectations of correlation across Europe brought by the WFD.

2.1.4. Complexity and/or inadequacy of currently used assessment methodologies

Some of the methodologies that are currently used under the WFD assessment scheme may be too complex or even inadequate, turning the whole process of ecological assessment into a slow and complex scheme of procedures, delaying the ecological evaluation, while the inadequacy of the defined (i.e. standard) methodologies to specific threats can compromise the overall quality of its results. Complex methodologies often slow down the evaluation process and generate unnecessary work. Also, their inadequacy entails deficient information on (i) physical alterations and its consequences on the biota; (ii) effects of certain types of pollutants; (iii) accumulation of contaminants on sediment; and (iv) ecosystem functioning. This complexity and/or inadequacy is frequently present in each phase of the ecological evaluation, including in the sampling methods under use, in sample processing and analysis of quality elements under evaluation as further addressed in the present subsection 2.1.4. The specific case of the inadequacy of the biotic metrics used in the WFD bioassessment scheme will be addressed in the following subsection 2.1.5.

Although the sampling effort may not involve complicated methods, sample processing and the analysis of the results derived from the enforcement of the metrics can be quite challenging. For example, while macroinvertebrate and periphyton sampling is simple, sorting and sample preparation are time-consuming and/or require high level of expertise in terms of the taxonomic resolution required for the calculation of metrics.

Another noteworthy example concerns mandatory chemical quantification of pollutants, requiring costly analysis of fixed lists of chemicals (set by Member State) that are relatively long, regardless of the particular characteristics of the water body under evaluation. For example, in Portugal, the list of chemical substances under mandatory evaluation comprises 45 priority substances + 8 other pollutants (Decree-Law No. 218/2015) + 126 specific pollutants (INAG, 2009) entries. Moreover, various substances are currently under scrutiny in European and National watchlists, potentially increasing the quantity of compounds of mandatory analysis in the future.

A case of inadequacy concerns the evaluation of the physical alterations in the water body under evaluation, and its consequences to the biological communities. Physical alterations of river hydromorphology and flow constrain the suitability/representativeness of the quality elements that are used in the assessment of water quality (Swanson et al., 2017). To address this problem, the EC recommends the development of metrics or approaches that are sensitive to hydrological and physical alterations of water bodies in the Member States (European Commission, 2015e). Also, the importance of lateral connectivity of fluvial systems should not be underrated because these systems are usually complex and suffer multiple impacts (Buijse et al., 2005). Good examples of such metrics exist to monitor changes in flow and in the lentic-lotic character of the aquatic system, including the LRD abiotic index (Buffagni et al., 2009) and the LIFE biotic index (based on macroinvertebrates; Extence et al., 1999). Flow changes in rivers are particularly important in Mediterranean systems, for example, which are prone to large fluctuations in drought and flood cycles (EEA, 2016; IPCC, 2014b). Another example is the PSI mixed-level index, which has been proposed (Extence et al., 2013), improved (Turley et al., 2015) and validated (Extence et al., 2017; Turley et al., 2016) to identify the impacts of fine sediments in temperate rivers and streams.

Concerning pollution, rivers often show (i) point-source contaminant input and (ii) diffuse contamination requiring specific attention (EEA, 2015). Point-source contamination reaches water bodies by identifiable sources. On the contrary, diffuse pollution enters aquatic ecosystems from widespread activities like agriculture, mostly by lixiviation of pesticides and fertilizers into edge-of-field surface waters (Carpenter et al., 2011). Although both contamination processes are fairly known, an effective control of chemical contamination inflow to water bodies at non-harmful levels is difficult. The WFD advices Member States on the establishment of emission control strategies and limit values, as well as best environmental practices, regarding both point-source and diffuse contamination (European Commission, 2000). Even though in some countries (e.g. Finland, Germany, Ireland) the process of emission of allowances to control point-source discharges and diffuse pollution has been working well, this is not the trend across Europe (European Commission, 2012a). To worsen that, by 2012, only 9% of surface water bodies were consistently monitored for priority substances, mostly due to lack of confidence in the monitoring results or inadequacy of monitoring tools (European Commission, 2012a). Also, a growing concern has arisen because of the so-called emergent contaminants (such as pharmaceutical products, licit and illicit drugs, nanomaterials and additives to personal care products), which are chemicals with largely unknown biological effects in aquatic ecosystems, and for which there is scarce information on stability or persistence in the environment (Reid et al., 2019). The impacts derived from point-source and diffuse pollution with both well-known and unstudied contaminants are not easily measurable because they are associated with numerous other pressures (multiple stressor framework; Nõges et al., 2016; Ormerod et al., 2010) that work as confounding factors. However, the identification of the causes leading to environmental degradation is important to support restoration measures, especially towards the fulfilment of a “good” ecological status. Obviously, this requires in-depth analyses and high level of expertise, which was not the purpose while designing the WFD. In fact, the unclear linkage between the pressures that are present in a given ecosystem and their effects to its functioning is considered as a major weakness of the WFD by the participants of a recent international conference on the future of the WFD in Europe (Carvalho et al., 2019).

Another inconsistency that can be clearly identified in the WFD is the main focus on the water column for contaminant quantification in lotic systems, while contaminants are rather likely to accumulate and persist both in sediments and organisms. The sediment capacity for adsorption of some contaminants contributes to the increase in the residence time of these substances in the ecosystems, comparing to the time they would persist in water (especially in running water), because they work as sinks for persistent contaminants. These then re-enter the water column when resuspension of the river bed occurs due to water turbulence, bioturbation or human induced mechanical perturbation. The dynamic relationship between water and sediment, with consecutive resuspension-sedimentation cycles, contributes to turn sediments into secondary sources of contamination, repeatedly reintroducing them back in the water phase, as referred to by many authors (e.g. Heise and Förstner, 2007; Schüttrumpf et al., 2011; Zoppini et al., 2014). Besides this, most riverine inhabitants are intimately dependent on the benthos, either as a substrate or habitat (macrophytes, most macroinvertebrates, periphyton) or as food provider and refuge (fish). Thus, these organisms are permanently exposed to any contaminant incorporated in the sediment. Accordingly, Turley et al. (2016) claim that a sediment-specific biomonitoring approach is highly desirable for rivers and streams. However, rather than mandatory sediment analyses (pollutant substances, other physical and chemical features), the WFD assessment procedure only requires effectively the analysis of chemical contamination in the water column, which in practice neglects the potential of sediments as a sink and source of contaminants. The potential of bioaccumulation and biomagnification of such contaminants has also been neglected, although some important steps to overcome this flaw are being taken, by turning mandatory some quantifications in the biota and in the sediment, as well as by setting corresponding environmental quality standards (EQS) for priority substances (see Directive 2013/39/UE).

Conceptually, monitoring plans *sensu* the WFD are based on the assessment of ecological status of the quality elements individually, discarding the relationships among elements (and using the "one-out, all-out" principle), this practice being contrary to the WFD theoretical recommendations as an assessment scheme (Voulvoulis et al., 2017). For example, the close relationship among macroinvertebrate fauna and vegetation degradation by fungi (e.g. Pascoal et al., 2003) or periphyton and fish (e.g. Jardine et al., 2013) is well known but it is not directly assessed in monitoring plans within the scope of WFD. Therefore, while evaluation elements can denote good ecological quality, this does not necessarily translate into good functioning of the ecosystem. In fact, it can be deceiving, as interactions between elements may not be evident but play important roles in ecosystem functioning (Solimini et al., 2009). A good way of addressing such functional relationships is the use of functional traits and metrics (see subsection 3.3).

This inadequacy of currently used methodologies for an accurate evaluation of ecological status has been effectively exposed by some authors. For example, Ramos-Merchante and Prenda (2017) evaluated the sampling effort needed to rigorously estimate macroinvertebrate richness and concluded that the sampling effort used in an evaluation *sensu* the WFD may be unable to provide reliable information on community composition. Kelly (2013) reviewed phytobenthos assessment under the scope of the WFD and noticed that the methods to do this in 42% of the Member States were exclusively based on diatoms, overlooking non-diatom phytobenthos, which may unintentionally ignore the occurrence of alterations in the dynamic relationships among groups of phytobenthos. Still regarding phytobenthos, there is increasing evidence of the protective role of the biofilm structure, architecture and composition in extracellular polymeric substances, defining the sensitivity of diatom communities depending on the stressors and conditions involved (Admiraal et al., 1999; Gold et al., 2002; Larras et al., 2013). When this protective role is effective, the metrics based on diversity and abundance of diatoms leading to ecological status classification are unlikely to reflect the actual ecological quality as their capacity to appropriately discriminate impacts can be largely impaired; such a bias can be even more critical in cases where periphytic communities are the single available biological element for ecological quality assessment.

The complexity and/or inadequacy of the methodologies used in the WFD assessment scheme is, therefore, evident. Also, this assessment scheme was designed to be used in river basins (European Commission, 2000) and applied in the total extension (or almost) of the river course, thus demanding an enormous sampling effort, although rivers usually (but not always) show an upstream–downstream gradient of threat (Vörösmarty et al., 2010). Besides their complexity, in an effort to standardize the evaluation process, the WFD enforces the application of the same conceptual assessment scheme to every water body, in a "one tool fits all" logic. This leaves little room to the inclusion of particular characteristics of each water body under evaluation, regardless of the specific characteristics of the monitored ecosystem that may contribute to its dynamic equilibrium, turning the whole process in a somewhat rigid scheme of procedures. Because of this rigidity, it is very difficult to accurately perform an ecological evaluation of some rivers with special features, preventing some rivers/water bodies to be evaluated at all. In this way, such an approach for environmental assessment and ultimately protection of freshwater ecosystems may be conceptually flawed.

2.1.5. (In)flexibility/Inadequacy of the WFD biotic metrics to realistic stressor scenarios

Albeit the comprehensiveness of the WFD bioassessment scheme, several studies have been emphasising they may not be tuned to a wide range of current stress scenarios, including interacting stressors of similar or different type. This is due to the fact that most of the methods used in the ecological evaluation in the scope of the WFD (56%, according to Birk et al., 2012) consist of biotic indices that derive from previous knowledge about the effects of organic pollution or eutrophication, thus compromising their suitability to detect other types of contamination or stresses (Carvalho et al., 2019). Those measures have been effective, and are widely used, in the detection of pressures like organic enrichment from point and diffuse sources of contamination and also in the study of the effects of the resultant eutrophication. For example, the United Kingdom uses TDI (Trophic Diatom Index) (Birk et al., 2012), Sweden uses the IPS index (*Indice de Polluosensibilité Spécifique*) and other Nordic countries use similar indices that evaluate the status of periphyton according to their tolerance to eutrophication (Andersen et al., 2016). Kalogianni et al. (2017) also used biological indices to evaluate the ecological impacts of nutrient and organic load from diffuse agricultural and industrial pollution (effluents from an olive oil mill and a waste water treatment plant) in a river, confirming their negative impacts on the biological communities. Particularly, macroinvertebrate communities are often studied using indices like ASPT that are sensitive to organic pollution, either applying the indices solely or using them to feed multimetric indices (Martinez-Haro et al., 2015). Van Ael et al. (2015) used a macroinvertebrate-based biotic index (MMIF – Multimetric Macroinvertebrate Index Flanders) to estimate critical metal concentrations for good ecological quality of water, achieving some valuable information on the suitability of biotic indices in the estimation of EQS for metals in the scope of the WFD ecological assessment. But some stressors may have synergic or antagonistic effects rather than additive effects on the local species composition depending on the specific features of the water body, the type of stressors and their individual levels (Altenburger et al., 2015), meaning that the presence of multiple stressors can enhance or reduce the effect expected from the presence of each of them simply added (in terms of concentration) to another one, respectively. So, as indices may be unable to detect these effects, the development of an integrative approach to evaluate the health of the ecosystem is fundamental (Solimini et al., 2009), mostly because these scenarios of multiple stress are becoming more relevant in the context of the ongoing climate change and increasing anthropogenic pressure that directly affect freshwater ecosystems.

Adding on this possible lack of sensitivity of some metrics to certain types of stress, they may also be defective in the detection of functional alterations in the ecosystem. These may be provoked by the loss and/or replacement of species or taxonomic groups with similar or distinct ecological functions in an ecosystem. As functional redundancy can explain different aspects of biodiversity (Rosenfeld, 2002), it is imperative to also take it into account when evaluating the ecological status of a given ecosystem. Some authors (e.g. Bruno et al., 2016; Hering et al., 2010) point out the absence of tools to evaluate the functional elements of the ecosystem as a weakness of the WFD assessment scheme.

Moreover, the indices used in the scope of the evaluation with the WFD assessment scheme largely rely on an accurate knowledge about the sensitivity of the organisms. This may be flawed, because there are: (i) gaps in the historical data that supports the assignment to each species of the numerical scores of the metrics; (ii) gaps in the knowledge of life history of rare species (Dudgeon et al., 2006); (iii) different levels of taxonomic resolution when studying distinct communities, due to the fact that there are regional asymmetries on the access to accurate identification guides of the organisms and the level of expertise of identification specialists may be different from one Member State to another, although some countries like Austria, Finland and Germany have been making an effort to overcome this issue by increasing taxonomic training (Hering et al., 2010).

The effects of stressors such as metals, pesticides and even salinization are relatively well known because they have been studied and reviewed (e.g. Carpenter et al., 2011), but it is questionable whether the WFD bioassessment scheme is sensitive to such impacts. Additionally, there are some “new” stressors whose effects may go unnoticed, including: (i) invasive species, which may provoke negative effects that go unnoticed under biological evaluation using the biological quality elements recommended in the WFD (Pereira et al., 2017b); (ii) emerging toxicants and some xenobiotics, for which the recommended analysis in the WFD is outdated or there still are no reliable and thorough methods available to perform the chemical analyses (Schmidt, 2018); and (iii) flow alterations, like extended drought periods related with climate change (Elias et al., 2015) or water abstraction for irrigation (Skoulikidis et al., 2011). Actually, altered flow conditions may lead to erroneous or eventually prevent conclusions on the ecological status of a river because of the loss of organisms and the biased comparison with unrealistic reference conditions. As an example, Austria recognizes hydromorphological alterations as the main hindrance to reach good ecological status in the river network (Agustsson, 2018).

2.2. Valuable lessons from the ERA framework

Similarly to the WFD, ERA can be used as a management tool for aquatic ecosystems. As previously stated, both the ERA approach and the WFD bioassessment scheme are very complete schemes that use multiple LoE, and both have a history in environmental protection strategies. In our opinion, valuable lessons can be extracted from ERA, some of which could inspire the improvement of the WFD bioassessment scheme. Rather than providing a weighed analysis of the ERA framework, the purpose of this section is to highlight which features could serve such inspiration.

Ecological risk assessment (ERA) is a process that evaluates the likelihood that adverse ecological outcomes occur or are occurring due to the presence of one or more stressors in the ecosystem (SuterII, 2006; U.S.EPA, 1992). It can help identifying environmental threats and establish priorities, this way scientifically supporting the decision making concerning regulatory actions (U.S.EPA, 1992) or appropriate management actions towards the mitigation of the environmental threats and possibly ecosystem recovery (SuterII, 2006). There are two types of ERA: (i) prospective risk assessment and (ii) retrospective risk assessment (Calow and Forbes, 2003; SuterII, 2006). Prospective risk assessment is used to predict the risks posed by stressors that are not yet in the environment. On the contrary, retrospective risk assessment is used to identify existing risks of stressors already present in the ecosystem (Calow and Forbes, 2003; SuterII, 2006; U.S.EPA, 1992). Prospective environmental risk assessment is the elected framework in the EU, for example, to set Environmental Quality Standards for metals in water and sediment (European Commission, 2011) as well as to assess whether plant protection products, biocidal products and other chemicals are environmentally safe to be allowed into the European market (European Commission, 2012b, 2009, 2006). On the other hand, the United States Environmental Protection Agency has also been recommending and using retrospective stepwise risk assessment for two decades (U.S.EPA, 1992) to evaluate ecosystem health and support the establishment of effective and site-specific remediation measures.

Ecological risk assessment comprises three stages, formally or informally recognised. The first is problem formulation, which includes the identification of risks and possible effects, site-specific factors that may influence the assessment and the scope and objectives of ERA. The second comprises stressor and effect characterization, in order to predict or measure the spatial and temporal distribution of the stressors and effects, as well as evaluating cause-effect relationships. The third phase is risk characterization, using the data collected in the previous phases to evaluate the likelihood of the occurrence of adverse ecological effects associated with those stressors. Throughout the whole process there is also monitoring and validation of the process stages and results in order to guarantee the overall effectiveness of the assessment (U.S.EPA, 1992). ERA involves the integration of multiple LoE – chemical, ecological and ecotoxicological – through each stage (Burton et al., 2002) and can be done in steps (tiers). In this stepwise assessment logic, the three lines of evidence are always applied, but the first tiers require simple methodologies and the subsequent ones usually require methodologies with growing complexity (Menzie et al., 2007). The evaluation process proceeds to subsequent (and more complex) tiers only if the uncertainty in risk calculation is too high, making the whole process of evaluation more cost-effective, as the effort is optimized in a progressive allocation of human and financial resources during sampling and in the data analysis (Rial and Beiras, 2012).

Regulatory agencies focusing on environmental protection worldwide have been recognizing and suggesting the adoption of stepwise strategies in ecological risk assessment approaches, towards better environmental protection. The EU recognizes the benefits of this strategy when centred in putative pollutants rather than existent scenarios. Barjhoux et al. (2018) do not directly propose a stepwise risk assessment, but reinforce the importance of integrating multiple LoE in a weight-of-evidence approach in order to have a robust evaluation of the ecological status of rivers. The authors used four LoE (chemical hazard, bioavailability, biological responses and ecotoxicological responses at the organism/cellular level) to assess the ecological status of Seine River and concluded that the integration of this process of evaluation in ecological risk assessment would constitute an improvement in the knowledge of the overall status of biological communities.

A key aspect in ERA is the relevance of expert judgement throughout the process. This expert judgement is crucial to the fluidity and efficiency of the assessment (making adequate and site-specific decisions on the design and conceptualization of the assessment and evaluating the relevance of data obtained throughout the process, while following established guidelines) and, ultimately, to the conclusion of the whole process of evaluation (U.S.EPA, 1992). The knowledge on the state-of-the-art regarding ecosystems and pressures by experts represents an added value to the strength of the assessment and enables the integration of new, relevant information in the evaluation with high degree of reliability (De Lange et al., 2010). A rigorous and careful selection of associated panels of experts supports a truthful and embracing picture of the ecological status of the ecosystem, hence expert judgement has been strongly promoted within ERA (e.g. Artigas et al., 2012). Having a good base of knowledge on the evaluated ecosystem and the functional relationships of the ecosystem components assures the effectiveness of the assessment. Lack of knowledge, mostly in the interpretation of the results, can compromise the seriousness of the evaluation. The inclusion of expert judgement in ecological assessment is successfully used in Canada (by Environment and Climate Change Canada (ECCC)) (Environment and Climate Change Canada, 2018) and USA (by United States Environmental Protection Agency (U.S.EPA)) (e.g. U.S.EPA, 1992).

The importance of the inclusion of expert judgement in the ecological assessment is also recognized by the EU, even if this is not integrated in the WFD assessment scheme. In fact, the EC (2012a) recommended expert judgement in the definition of reference conditions for some quality elements of rivers in the face of lack of robust information derived from spatial or modelling approaches. Actually, this was the trend in the definition of reference conditions, as only four countries had available historical data to define reference conditions (at least in that phase). Expert judgement is also used to assess the impacts of pressures like fishing and presence of exotic species (European Commission, 2012a), which are difficult to assess with the standard methodologies recommended by the WFD. Besides this, in Europe, the Pesticides Unit of the European Food Safety Authority (EFSA) offers scientific advice on risk assessments on pesticide usage to the EC. It is very likely that expert judgement was viewed as an undesirable source of uncertainty and subjectivity, and therefore was purposely left out of the WFD assessment scheme. Indeed, expert judgement prevents the blind-like strategies requiring straight application of assessment recipes. Although such strategies could have been viewed as the solution to allow a broad and accelerated application of assessment frameworks such as the WFD assessment scheme by technical personnel, the complexity of aquatic ecosystems and their regulating variables would hardly be appropriately appraised by such a simplistic approach as highlighted in the previous subsection.

In the literature, retrospective ERA is increasingly believed to be more embracing than the WFD assessment scheme. Some authors have been recognizing the virtues of adopting a stepwise strategy in the evaluation of ecosystem health, rather than limiting it to the analyses recommended by the WFD assessment scheme. Moreover, stepwise ERA is more flexible because the methods to be used are chosen according to the characteristics/necessities of the water body under evaluation, instead of using an inflexible strategy common to all scenarios (as per the WFD assessment scheme). For example, Oost et al. (2017a) and Oost et al. (2017b) suggested a customized 2-step strategy that first assesses the wide-spectrum bioanalytical hazards and then carries out a more in-depth ecological risk assessment only in those sites posing higher concern. Other authors have been proposing similar stepwise approaches to evaluate ecosystem health under specifically identified hazardous scenarios. For example, Macário et al. (2017) proposed a stepwise approach to the evaluation of cyanobacterial blooms in recreational bathing waters as required by the EU (Bathing Water Directive – 76/160/EEC) and proved its reliability and effectiveness in the simplification of the monitoring process. Both den Besten et al. (2003) and Babut et al. (2006) suggested stepwise frameworks to evaluate the effects of the presence of dredged material disposal into freshwater ecosystems.

However, and although being a recognised effective tool, stepwise risk assessment also has flaws, as it may become more or as complex as the ecological assessment scheme of the WFD, especially if higher assessment tiers are necessary (Rial and Beiras, 2012). This complexity essentially relates to the need of expert judgment in each step, which is not necessarily bad as it avoids simplistic and inflexible approaches. Thus, there is always room for optimization of the whole process in order to turn it into an effective ecological assessment tool.

3. Acquisition of complementary data to enhance water quality assessment

A multitude of alternative or complementary approaches or methods (mentioned in subsections 3.1 and 3.2) has been proposed for the assessment of water quality and/or ecosystem health. These allow obtaining data to ease or to complement water quality assessment, and thus have typically been presented as complementary to the ecological evaluation *sensu* WFD (e.g. Martinez-Haro et al., 2015; Pawlowski et al., 2018) or as suitable tools to integrate in stepwise risk assessment frameworks (e.g. Barjhoux et al., 2018). Below we conduct a revision of those that translate into more cost- and time-effectives or illustrate how major gaps of knowledge can be tackled.

3.1. Interviews, photography and GIS tools to identify hydromorphological alterations

Alterations in hydromorphology of freshwater ecosystems usually translate into important implications to inhabiting organisms. Sun et al. (2018) studied the importance of some hydrological variables in the distribution of diatom communities and found a close relationship between hydromorphology and the organisms, concluding that hydromorphological variables like antecedent precipitation index and base flow should be considered in the bioassessment of freshwater. However, hydrological characteristics of a water body can present a challenge to its ecological evaluation. For example, it is difficult to evaluate a temporary river because the fluctuations in flow can compromise the interpretation of data obtained with sampling if the context of evaluation is not well adjusted. For instance, the timings of sampling and the reference conditions must be carefully chosen in order to accurately quantify the risks. This issue deserves significant attention because it is predictable that the number of temporary streams will increase due to global climate change (Millennium Ecosystem Assessment, 2005).

For this specific problem, Gallart et al. (2016) suggested the use of interviews to neighbours of the studied fluvial system and aerial photographs to track flow fluctuations. Interviews can be useful to add important local insights from human communities to scientific knowledge, especially in the definition of river regimes and particular characteristics of the studied water body (Gallart et al., 2016). The use of GIS tools was also recommended by other authors (e.g. Artigas et al., 2012; Beketov and Liess, 2012; Filipe et al., 2019; Liess et al., 2008) for a better understanding of the fate of toxicants in the environment, which is intimately related to flow variations, precipitation and hydromorphology of rivers. Also, GIS can be useful to map risk levels in a very intelligible way. For this reason, they have been used more and more in ERA (Artigas et al., 2012). Lahr et al. (2010) put this in practice by developing vulnerability maps of Denmark in order to have a better idea of the overall vulnerability of the environment to various toxicants. Also in Denmark, and in order to support the management of water bodies according to the WFD, scientists designed integrated hydrological models, which are continuously updated in a stakeholder driven process (Højberg et al., 2013). In Portugal, some researchers have been working on modelling fluvial hydrodynamics and sediment transportation (IST, 2018), which can provide useful information to complement ecological assessment. In the U.S.A., NASA has been using remote sensing for two decades to map vegetation density to detect stress on plants, which enables to infer hydrological alterations driven by e.g. drought and helps in the measurement of environmental threat (NASA, 2018). Vegetation indeed plays a key role in the equilibrium of ecosystems. Concerning freshwater ecosystems, changes in flow and riparian vegetation and alterations in the transformation processes of its products (e.g. logs and leafs that fall in streams and river courses) may have huge influence on the ecosystem health and therefore can be used to assess the impact of stressors (Millennium Ecosystem Assessment, 2005).

While interviews and aerial photographs allow increasing resolution at the local and regional scale, GIS tools and satellite data allow covering a much larger spatial scale. Both are clearly an add-on to the available bioassessment scheme and may provide valuable insight on short-term and long-term trends, as well as a distinction between disturbance and natural fluctuations. This positive potential has been recently recognized in the Fitness Check of the WFD and these methods are pointed out as suitable to improve the robustness of the ecological monitoring as a whole (European Commission, 2019c).

3.2. Alternative indicator biotic community metrics and indices

Community metrics and indices allow the inference of the biological status of a water body through the analysis of the community structure, which may vary in response to the presence of stressors. Sometimes the results on the ecological status obtained with different biological metrics are not consistent with each other (Santos et al., 2019; Solimini et al., 2009). Although this may be interpreted as a weakness of the metrics that were used, this information could work as an early warning of alteration in the ecosystem that, although present, is not fully reflected on the results of all metrics (Carpenter and Brock, 2006). Also, variability in the results of the metrics can provide valuable clues on the relationships between biological and chemical processes, which can work as complementary information to other ecological metrics (Solimini et al., 2009) and thus should be analysed carefully. Some authors have been proposing complementary metrics to the ones recommended in the WFD assessment scheme, also using benthic macroinvertebrates. For example, Turley et al. (2016) suggested the use of PSI (Proportion of Sediment-sensitive Invertebrates index) to fulfil any doubts that may arise in the biological relevance of the results of fine sediment monitoring when using non-biological tools. Golfieri et al. (2016) developed and applied a new multimetric index, the Odonate River Index (ORI), to translate the ecological integrity of the river corridor in an holistic perspective. The authors claim that commonly used bioindicators (diatoms, macrophytes, benthic macroinvertebrates, fish) are not particularly sensitive to hydromorphological degradation; on the contrary, ORI offers a wider view on a threat affecting freshwater ecosystem as odonates seem to be notably suitable to detect alterations in both hydromorphology and vegetation.

Microalgae have also been the subject of studies on novel water quality indices. Wu et al. (2012) successfully developed and tested a multi-metric phytoplankton index of biotic integrity (P-IBI) in Germany to be used in ecological assessment of rivers, complementing the assessment done in the scope of the WFD. The authors focused on phytoplankton because it is not often taken into account on the ecological assessment of lotic ecosystems such as rivers, yet it is recognized as a good indicator of disturbance in lentic water bodies. Moreover, the previous belief that phytoplankton is exclusive to lentic ecosystems has been dismantled by some studies (e.g. Centis et al., 2010; Wu et al., 2011), so it could indeed be a valuable tool in the ecological assessment of rivers. Zalack et al. (2010) specifically focused on periphytic diatoms to assess the impacts of acid mine drainage (AMD) on streams as these organisms provide high level of resolution when it comes to the assessment of the ecological status of ecosystems facing such impact; the authors adapted the Diatom Index of Biotic Integrity (DIBI), which is effective in the evaluation of the effects of eutrophication on biological communities, and created the AMD-DIBI to provide specific information on AMD.

The use of biotic indices that are sensitive to disturbances other than the "classical" organic enrichment paradigm is particularly welcome, because it does not add much work to the ecological integrity evaluation; in fact, such indices stem from the same community data necessary for the computation of the metrics already integrated in the WFD assessment scheme. As such, when the laborious task of sorting, identifying and counting the organisms is done, this same matrix can feed numerous informative indices at almost no additional cost. The development of new multi-metric indices or their combination is of critical relevance because integrating multiple metrics allows describing biotic composition or integrity of one study site more comprehensively, which is most needed as water bodies often face multiple stressors (Zalack et al., 2010). Falasco et al. (2016) advise on the development of a multimetric index that could encompass multiple functional metrics that provide information on the impacts of hydrological disturbance in diatom communities, allowing a more accurate picture of this type of stress.

Moreover, a broader use of the results obtained with the community metrics already in use and the ones proposed in this subsection could be attained applying multivariate analysis, which would contribute to a deeper understanding of the effects of stressors on benthic communities. Multivariate analysis compares large sets of data and reflects the relationships among variables as numerical values (Legendre and Legendre, 2012), reducing data to more understandable dimensions. This approach also provides more information than the analysis of the descriptors separately, because it also analyses the associations and interactions between them (Gauch, 1994), adding to the calculation of multimetric indices and helping to clarify cause-effect relationships to a certain extent.

3.3. *Use of functional traits to assess ecosystem functioning*

Functional traits reflect a species ecological role in the ecosystem – how a species interacts with the environment and with other species (Diaz and Cabido, 2001). The importance of functional traits as biomonitoring and management tools is highlighted in Menezes et al. (2010), who showed how functional approaches have a broader geographic applicability, indicate the stressors more effectively and are more reliable and easy to use than taxonomy-based methodologies. This approach enables gathering information about the ecosystem condition that would be otherwise impossible to measure (Merritt et al., 2017). Other authors share this opinion and some of them suggest the use of functional diversity (based on feeding functional groups of invertebrates, for example) as a complementary method in the bioassessment of ecological status. Again, a major advantage of this approach is that no additional effort is needed for sampling or specimen identification; such functional matrices can be derived from the community data already gathered for the sake of WFD metrics.

Serra et al. (2017) proved the efficiency of Chironomidae traits in the detection of different levels of disturbance in Mediterranean streams. The discriminative capacity of Chironomidae is typically disregarded since they are frequently associated to lower ecological status suffering from undifferentiated negative impact. However, there are naturally poorly diverse streams that only shelter Chironomidae assemblages. Besides that, numerous studies proved that Chironomidae are sensitive to anthropogenic disturbance (e.g. Lencioni et al., 2012), so their traits can be useful to complement the information provided by the indices used in the ecological assessment in the scope of the WFD (Serra et al., 2017). Other authors have been showing the great utility of macroinvertebrate traits, with a special focus on functional traits, to assess the ecological status of rivers. Pallottini et al. (2017) used functional traits of benthic macroinvertebrate assemblages to assess ecological status in lotic systems, showing that they are important in supporting the discrimination of the level of anthropogenic disturbance, especially when complementing the information given by chemical analyses. Functional approaches based on macroinvertebrate traits has been rarely used in Europe (Merritt et al., 2017), and the WFD does not promote its use. However, Merritt et al. (2017) showed that there are advantages in using this approach, even when there is the need to relate *a posteriori* the quantitative data collected in a bioassessment *sensu* the WFD. In their review of trait-based ecological classifications of benthic algae, Tapolczai et al. (2016) kicked off a new quality evaluation system based on traits and adaptations of these organisms to do the bioassessment instead of being limited to species abundance and diversity. Falasco et al. (2016) investigated if functional metrics of benthic diatoms, such as chlorophyll *a*, could work as an effective indicator of hydrological disturbance in rivers and consequent lentification, in the scope of ecological assessment of these ecosystems. The authors concluded that functional metrics of diatoms are more reliable than diatom indices in indicating the impacts of drought in these riverine communities.

Another functional approach is the measurement of relevant ecological processes or rates in aquatic ecosystems. Gessner and Chauvet (2002) studied the possibility of using ecosystem-level processes in stream assessment, with a focus on leaf litter breakdown as an indicator of disturbance as this is an integrative process that links riparian vegetation to fungi, bacteria and invertebrate activities (Benfield, 1996). The authors concluded that this ecological process would represent a valuable addition to any ecological assessment scheme because it plays a key role in ecosystem functioning; the information provided by studying leaf litter breakdown *per se* can be complemented with other structural and functional parameters related to the breakdown process. The authors also referred that the bioassessment should be complemented not only with leaf litter breakdown data but also with data exposing other ecological processes. Other authors reached similar conclusions. Pascoal et al. (2003) studied the possibility of using leaf breakdown as a measure of the impact of pollution on a river. Leaf breakdown rates and associated aquatic hyphomycete and invertebrate community structures provided information on the effects of contamination on the aquatic system, with leaf breakdown bringing valuable discriminative power in this context (Pascoal et al., 2003; Santonja et al., 2018). Young et al. (2008) also recommend the use of methods for measuring organic matter decomposition and ecosystem metabolism as a complement to already established methodologies of evaluation of ecological status. These authors listed and analysed some factors (e.g. nature of substrate, organic pollution, flow fluctuations) that could be studied to evaluate ecosystem metabolism in order to enhance efficiency of monitoring by augmenting the knowledge of ecosystem processes. Atkinson et al. (2018) used communities of mussels (Bivalvia: Unionidae) to study productivity within ecosystems. The authors studied the rates of consumption and storage of nutrients by the mussels as a measurement of the productivity of the ecosystem, thus hinting on its health.

3.4. Use of molecular tools to improve taxonomic resolution

The most prominent alternative that has been developed recently to overcome (to a certain extent) the limitations of taxonomic identification (see subsection 2.1.4) is based on environmental DNA or DNA from biotic samples (Elbrecht et al., 2017; Pawlowski et al., 2018; Visco et al., 2015). This alternative bears similar or higher costs than the more classical ways of classification of organisms to assess diversity and abundance (so the problems associated to that are similar to the ones pointed out regarding the WFD assessment scheme). However, the use of environmental DNA represents a facilitation of the classification process as a whole, without a massive change of the procedures of sampling, as well as a path to eliminate sampling and/or identification errors. Pilliod et al. (2013) advocate the use of environmental DNA to signal species presence in aquatic ecosystems, especially concerning rare species, that are often hard to detect with more traditional methods. The authors even point out the possibility of constructing species inventories with the aid of this tool in the future. Other authors (see Carvalho et al., 2019 and references therein; Filipe et al., 2019) have also been advocating that DNA-based identification of organisms using environmental DNA barcoding and metabarcoding can apply to eDNA-based monitoring. These methodologies can provide complementary information on the biotic indices and metrics usually used to do the ecological assessment of water bodies in the scope of the WFD, with advantages like the increasing of taxonomic resolution and harmonization of identification, improving the consistency of the calculated indices, or the possibility of identifying the species without killing any organisms (Hering et al., 2018; Pawlowski et al., 2018).

3.5. Ecotoxicological tools to help establishing causal links

Ecotoxicology methods have been increasingly recognized as useful tools to assist the assessment of the ecological status of ecosystems, especially because it is based in dose–response relationships retrieved in biological systems as a measure of the toxicity of chemicals to the test population/individual (Gaddum, 1993), thus aiding in the clarification of cause-effect relationships. A decade ago, Blasco and Picó (2009) accentuated the great potential of studying the relationship between chemical contamination and measurable ecotoxic effects. In order to boost this potential, the authors stressed the need for the development of tools and strategies to obtain data on toxicological endpoints reflecting effect-based key toxicants for ecosystems. The numerous ecotoxicological studies related to water quality done in the last years demonstrate the increasing acceptance of the idea of ecotoxicology as a reliable instrument supporting ecological evaluation. Moreover, it is recognized as a valuable tool to determine and predict the effects of contaminants in the environment as integrated with chemical quantification of suspected contaminants (Schmitt-Jansen et al., 2008; Tousova et al., 2017), especially when reference conditions are not clearly defined (Martinez-Haro et al., 2015).

Ecotoxicological protocols present some advantages: (i) they can be relatively simple and internationally accepted, facilitating validation of results and better allowing comparative approaches; (ii) they can use standard species, which are easy to maintain in laboratory cultures and offer responses which are consistent and can be easily interpreted, while experienced researchers can easily adapt for the use of representative species of the focused ecosystem; (iii) they tend to be time- and cost-effective. However, extrapolation from results obtained with ecotoxicological methods to the community level can be compromised by the oversimplification of methodologies performed in single-species tests. Although the benchmarks retrieved from single species testing are assumed to translate in the protection of ecosystem structure and functions if embracing enough (Forbes and Calow, 2002), the importance of integrating ecological principles in aquatic ecotoxicology to improve ecological realism in assessment strategies of contamination should not be underestimated (Schmitt-Jansen et al., 2008). Adaptation of ecotoxicological methodologies to community tests, addressing biological traits at different degrees of complexity (Artigas et al., 2012), can be a good strategy, although this adaptation of protocols cannot be done lightly. Wood et al. (2014) presented a good example of a simple and quick ecotoxicological test inspired in standard single-species ecotoxicological protocols using natural benthic diatom communities, and generated consistent data on sensitivity of diatom genera to an herbicide. Grantham et al. (2012) performed ecotoxicological tests using a stream mesocosm to evaluate the effects of a wastewater treatment plant effluent on a benthic macroinvertebrate community. By using ecotoxicological tools, the authors were able to distinguish between the multiple stress factors affecting ecological communities, which may be difficult using traditional biomonitoring approaches. Still, the assessment of ecosystem health through single-species tests can be reliable especially when integrated in species sensitivity distributions (Posthuma et al., 2019, 2002) reflecting the expected responses of natural communities as confirmed by e.g. Maltby et al. (2005) and if the selected model organisms are appropriately tuned to the focused environmental compartment (Vidal et al., 2019).

Ecotoxicological assessment based on biochemical markers can be another add-on benefiting ecological assessment. Damásio et al. (2011) suggested the use of a large set of biochemical markers sensitive to water pollution, alongside with biological indices, to provide a complementary and insightful perspective of the ecological status. Prat et al. (2013) also suggest the use of macroinvertebrate metrics combined with studies based on biochemical markers to signal the effects of pollution in the tolerant taxa that comprises the biological communities following contaminant discharges in order to obtain a more effective ecological assessment in the scope of the WFD; indeed, the authors proved that biomarker responses of *Hydropsyche exocellata* can signal the effects of the presence of salinity and ammonia in water and, ultimately, water quality degradation. Similarly, the importance of selected taxa of diatoms as biomarkers of contamination was demonstrated by Lavoie et al. (2017). Çelekli et al. (2017) and Çelekli et al. (2016) showed that the metabolic responses of filamentous algae (such as *Cladophora*, *Spirogyra* and *Ulothrix*) to the presence of xenobiotics in various aquatic ecosystems are important biomarkers to assess ecological health. A more detailed insight on these tools can be found in, for example, Martinez-Haro et al. (2015), Wernersson et al. (2015), Milinkovitch et al. (2019) and Rodrigues et al. (2019).

Information on sediment toxicity is also crucial to a comprehensive ecological evaluation of a water body. Sediment plays a key role in lotic systems as habitat for an array of benthic organisms (fishes, invertebrates, macrophytes, periphyton), so their good condition is paramount. Data on how sediment condition and biological elements health are related could work as complementary information to the analysis of biotic community structure, aiding in the inference of causes of possible or observed biological negative effects. Also, sediment may play the role of a contaminant sink, as already pointed out previously (subsection 2.1.4). Vidal et al. (2012) proved the role of sediment as a secondary contaminant source by performing ecotoxicological tests with elutriates and standard species from different trophic levels. Similar conclusions were reached by Massei et al. (2018), who detected contaminants that are not permitted in Europe in sediment of rivers, reinforcing the knowledge of the legacy role that sediment plays. Again, these authors reinforced the importance of sediment analysis to assess water quality, instead of limiting the evaluation to analysis of contaminants in the water column.

Ecotoxicological evidence can integrate information from the water column and sediment, and should be integrated with other LoE in monitoring programs, complementing the array of information already provided by the current WFD bioassessment scheme. Barjhoux et al. (2018) used a weight of evidence approach and integrated four LoE in order to perform ecological evaluation in three sites along Seine River (France). The authors then concluded that water column and sediment analyses together provide more accurate information than just one of them solely, therefore both should be integrated in the ecological evaluation. They also complemented this study by performing studies with biomarkers (acetylcholinesterase – AChE –, and other enzymatic activity) and bioassays with *Gammarus fossarum*, concluding that the contaminants present in the environment were harmful to the organisms, although undetected in the water column. Barjhoux et al. (2018) suggest the integration of these tools, as well as *in situ* tests (see below), in the scope of biomonitoring and ERA. Similarly, Babut et al. (2006) stressed the importance of using a combination of chemical analysis, bioassays with *Chironomus riparius* and *Hyalella azteca*, and other toxicity biotests in order to assess the ecological risk of dredged sediments. The authors also proposed that this assessment should be done following a stepwise strategy, choosing the tests to perform according to each tier of the assessment. Roig et al. (2015) proved that cost-effective and short-term bioassays are useful to detect contamination in surface water and sediment, thus complementing ecological evaluation with the WFD assessment scheme. The authors used a battery of ecotoxicological tests comprising water and sediment from Ebro River basin (Spain) and model species such as *Aliivibrio fischeri*, *Raphidocelis subcapitata*, *Nitzschia palea*, *D. magna* and *C. riparius*, proving their effectiveness in providing information on the ecological status that was complementary to the data obtained with the WFD assessment scheme.

Similar reasoning has been addressed by large EU-scale projects regarding water quality evaluation. Tousova et al. (2017) proved the effectiveness of a simplified effect-directed analysis (EDA) protocol, as a part of the EU-funded EDA-EMERGE project, which aimed at the development of innovative tools to enhance EDA of emerging toxicants on a European scale. The authors selected bioassays using zebrafish (*Danio rerio*) embryos, algae (*R. subcapitata*) and human and *Xenopus laevis* cell lines, and complemented them with GC–MS screening, to detect and analyse the effects of the presence of expected and non-target contaminants in European surface waters. The authors also pointed out the added value that these effect-based tools would represent in water quality evaluation, to clarify biological effects that otherwise would remain unexplained by simple chemical analysis of target compounds. Other comprehensive EU-funded projects (e.g. MODELKEY, SOLUTIONS) developed new effect-based tools (e.g. biomarkers, *in vivo* and *in vitro* bioassays) in order to thoroughly detect and evaluate the effects of chemical pollution (individually or mixtures of contaminants) in freshwaters. Recent publications in the scope of the EU project SOLUTIONS (e.g. Altenburger et al., 2018; Backhaus et al., 2019; Brack et al., 2019a, 2019b; Faust et al., 2019; Könemann et al., 2018; Schulze et al., 2017) are bringing out new ideas on the use of effect-based tools as feasible methods of ecological quality evaluation and on the importance of the inclusion of multiple LoE in the assessment of the ecological status of European waters. For example, Altenburger et al. (2018) performed 19 different bioassays to evaluate the effect of a group of individual compounds and their mixtures, concluding that the specific measurement of apical endpoints is essential to an effective monitoring of chemicals in freshwaters. The effort done in these projects is expected to lead, in the future, to a more realistic assessment of the ecological status of European freshwaters, if these tools come to be effectively integrated in regulatory assessment scheme. Wernersson et al. (2015) reviewed extensively on how effect-based tools would improve the knowledge on the relationship between the chemical and the ecological statuses in the monitoring programmes in the scope of the WFD. The authors described thoroughly the advantages and the obstacles to the use of *in vitro* and *in vivo* bioassays (e.g. P53 accumulation, growth inhibition of algae and plants), biomarkers in field-exposed organisms (e.g. acetylcholinesterase activity, comet assay) and ecological methods suitable to measure alterations at population and/or community level (e.g. resistance or resilience character of taxa). They then concluded on the importance of an integrated approach to the assessment of water quality, both for the scientific community and stakeholders, leading out the way to the integration of these tools in monitoring programmes.

Also *in situ* tests are often suggested as an efficient methodology to detect negative alterations in the ecosystem. Although presenting higher degree of uncertainty than tests performed exclusively in the laboratory (where every variable can be controlled, thus providing clearer information on cause-effect relationships), *in situ* tests capture quite accurately the natural situation. Damasio et al. (2008) used *in situ* bioassays with *D. magna* to assess post-exposure feeding inhibition and biomarkers (AChE, catalase and glutathione S-transferase activities) to identify environmental stressors and assess their impacts on the ecosystem. The authors obtained good agreement between *in situ* tests and biomarkers, concluding that the latter have a good discriminatory power regarding stressors. By using these tools, it would be possible to obtain a more realistic and holistic perspective of ecological risks present in a given ecosystem. Maltby et al. (2002) also suggested *in situ* bioassays to monitor water quality using a feeding test with *Gammarus pulex*. This gammarid is an important detritivore shredder in stream ecosystems and reductions in its feeding rate should translate into reductions in detritus processing rates, with potential upscaling effects through the food web. The authors proved the effectiveness of the method as an indicator of disturbances over long time periods at community- and ecosystem-levels.

Summing up, several authors have been suggesting ecotoxicological tools that have been continually developed and refined at several levels of complexity depending on the test system used and its representations of each particular study context. Thus, the integration of this type of complementary tools in water quality assessment should not face many constraints.

4. Conceptual optimization of the ecological evaluation

The WFD assessment scheme was conceived with the intention of standardizing and simplifying the ecological evaluation of water bodies across Member States in the EU. This bioassessment scheme changed the paradigm of the ecological evaluation in surface waterbodies, cementing the ecological status concept. In its essence, any subjectivity throughout the process of evaluation is eliminated, offering an assessment approach based on the adoption of multiple quality elements and intercalibrated standardized metrics. Summarising from previous sections, is worth remarking that there is room for improvement of the bioassessment scheme in order to turn it into a more effective and comprehensive process. In our view, the rational route to do it is to follow successful achievements of other assessment frameworks such as ERA (see Section 2.2), holistically considering both current WFD descriptors and metrics (see Section 2.1) and complementary metrics or approaches that have been proposed (see Section 3). Fig. 2 summarizes our thoughts towards a proposal on how such a scheme could be effective in practice, by transforming the assessment scheme into a tiered and more flexible evaluation approach which is based on three fundamental assumptions. These assumptions are critical to promote the efficiency of a wide, yet sensitive assessment of ecological water quality.

The first assumption is that info retrieved from three distinct LoE should be considered while assessing each site of concern, namely the chemical and the ecological LoE (as already appraised by the WFD) but also the ecotoxicological LoE, which essentially clarifies cause-effect relationships (see Fig. 2 for suggestions of adequate methods applying to the three LoE). Importantly, the interpretation of the information retrieved through the three LoE should be integrated (see Section 2.2 for a detailed view on the integrative character of ERA and derived risk calculations), building a realistic view on the ecosystem responses to the

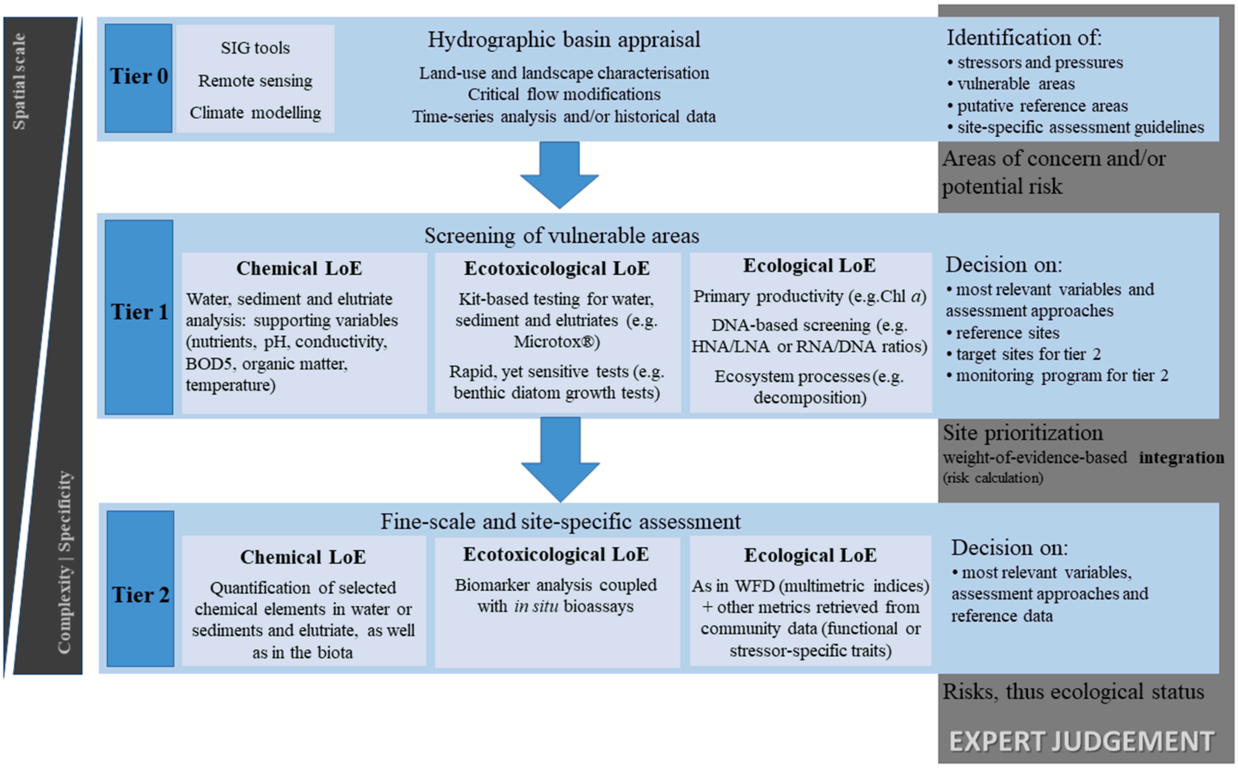

Fig. 2. Conceptual diagram exposing the practical application of a bioassessment scheme alternative to the current practice towards compliance with the WFD. Essential features are the stepwise organization through tiers of increasing complexity as the spatial scale decreases, the value of integration of information provided by three LoE and the critical role of expert judgement in analysis and decision moments through the flow. Examples of methods and parameters that could fit each LoE in each tier are given for clarity purposes.

suspected stressors. This is fundamentally different from the conservative "one-out, all-out" principle of the WFD, which is seen as protective but can lead to an oversimplification of the ecological classification and exclude important aspects such as community resilience to environmental pressure.

The second assumption is that expert judgement must be included surgically at decision stages throughout the assessment scheme (see the right-hand panel in Fig. 2). Although often argued as subjective, expert judgement is critical for the accurate definition of reference conditions and pristine locations, as well as to avoid erroneous evaluations due to inadequate use of indicators unfit to each ecosystem. Also, it may allow overcoming the interference of large regional asymmetries. Besides being our understanding in this proposal, this critical value of expert judgment within risk assessment routines has been recognised worldwide, as detailed in Section 2.2. Expert judgement should come from experienced multidisciplinary teams (biologists, chemists and environmental scientists) who are able to detect subtle changes in the ecosystem even in the absence of conspicuous disturbance and provide valuable insight on the ecological status of a site (Stoddard et al., 2006). Such a team would also be able to provide support during some technical procedures and in the interpretation of results of the ecological evaluation, aiding the decision-making by the stakeholders concerning conservation and remediation measures (Feio et al., 2016). Feio et al. (2016) compared the results of two modes of action in the assessment of the ecological quality of 20 sites located in different Portuguese rivers: (i) experienced experts carrying out the evaluation; and (ii) the WFD assessment scheme. Their results show that an ecological evaluation performed by experienced experts is accurate, when comparing to a technical evaluation directly based on quantitative indices and measurements. They then concluded that both approaches are complementary and should not be dissociated: expert judgement can be highly valuable in river basins for which there is inconsistent data from the ecological evaluation, by gathering scattered information and producing solid advice on the ecological status of the ecosystem, reducing uncertainty. This meets our proposal conceptually, as we indeed include the scoring methods proposed in the WFD assessment scheme in tier 2, while always keeping expert judgment associated to the assessment strategy (Fig. 2).

The third assumption in our proposal is that the assessment scheme for the evaluation of the ecological status of a water body must be tiered, allowing a more efficient management of time, effort and budget. The use of a tiered approach in such a complex scale (ecosystem) is critical for an effective ecological evaluation (De Lange et al., 2010) (see Section 2.2). At each step, the team of experts can iterate the evidence gathered and progressively narrow the focus of the subsequent approaches, both in terms of the spatial resolution as well as the descriptors or endpoints incorporating each LoE. For example, it would be possible to minimize testing of unlikely contaminants, so that the appraisers can dedicate their time and funds to the analysis of chemicals that are more prone to represent a threat to the specific water body under ecological evaluation (Calow and Forbes, 2003). As highlighted in the left-hand panel of Fig. 2, the tiers would proceed in such a way that they would have increasing complexity and consequently specificity of methodologies. Conversely, the prioritization of sites that comes as the result of each tier is expected to provoke a decrease in the spatial scale (and subsequent economical costs) in which the subsequent tier would be applied.

In order to turn the whole process faster and simpler, especially in large hydrographic basins, the inclusion of a tier 0 seems decisive. Tier 0 would be a stepping stone to subsequent levels of the ecological evaluation, contributing to its overall efficiency. U.S.EPA (1992) already recommends a screening tier using readily available data and conservative assumptions to facilitate the subsequent tiers where ecological risk is effectively assessed. This tier 0 should be performed at a hydrographic basin level and would include land-use characterization and the identification of critical flow modifications in subsystems (dams, areas subjected to drought, etc.). Time series analyses and historical data are vital to detect long term changes (e.g. hydromorphological alterations caused by human activities), especially climate change-driven modifications (e.g. identification of drought- or flooding-prone areas). GIS and remote sensing (including satellite and cartographic data) are currently available, as well as climate modelling tools, and have been proven useful (see Section 3.1 and references therein). A very important task within this tier 0 as we conceptualize it is the clear identification of vulnerable areas (based on stressors and pressures identified at such a macroscale), including a definition on the sampling points needed to adequately represent each area, as well as putative reference areas, thus setting-up the basis for tier 1. Primary directions on the specific tuning of assessment frameworks for each vulnerable area should also be identified ("site-specific assessment guidelines" in Fig. 2); for example, while in lotic sub-systems the focus should be primarily the sediment and the benthos, in semi-lotic and lentic sub-systems the water column and the plankton should be targeted.

Tier 1 would comprise an initial and wide screening of the vulnerable areas identified in tier 0, underpinned by the chemical LoE, ecotoxicological LoE and ecological LoE (Fig. 2). At this stage, standardized time- and cost-effective tools should be used, given the still comprehensive spatial dimension of this screening, saving more complex methodologies for the latter stage. First, the chemical LoE of tier 1 should include a geochemical characterization of the study site, including geological typing of the river bed and surroundings, and the measurement of supporting variables (Fig. 2). These are variables already generally considered as physico-chemical elements in the WFD bioassessment scheme, but a fundamental difference exists in this proposal since measurements should be specifically tuned to the focused compartment as defined by the type of sub-system under assessment, thus retrieved on water, sediment and/or sediment elutriates (while the focus of the WFD bioassessment scheme is solely the water). At this stage, monitoring extensive lists of chemical substances as regulated by the WFD would bring uncertainty on how to deal with variation in the data, besides the associated economical costs. More general parameters such as those suggested provide support to interpret the other LoE, as they are normally indirect indicators of specific stressors. For example, conductivity offers clues on the salinity of water and on the presence of metals (e.g. Cañedo-Argüelles et al., 2013; Machado et al., 2016), while nutrients and organic matter indicate on trophic status (e.g. Lacey et al., 2018). Second, the ecotoxicological LoE at tier 1 should be explored with rapid tests targeting water, sediment and/or elutriates with distinct organisms, depending on the relevant compartments under assessment. Available test kits, such as Microtox®, can be a good option as a primary screening method (Abbas et al., 2018) usually applied to all types of matrices because the procedure includes the osmotic adjustment of the test solutions, making it independent of the nature of the test matrix nature (freshwater or saltwater). The recent conclusions of SOLUTIONS project indicate the suitability of effect-based tools that provide rapid answers on toxicological endpoints, e.g. 48-h *Daphnia* immobilization test or specific assays focusing on mutagenicity (Brack et al., 2019b). Other specific and sensitive tests at this stage of the evaluation include assays with diatoms. For example, *Navicula libonensis* was shown to be sensitive to common pollutants (Vidal et al., 2019) and therefore useful in ecotoxicological analysis of freshwater bodies whenever the benthos is the compartment under focus, while growth inhibition tests with standard species would be a good choice to study the impacts of stressors on planktonic diatoms and other algae (e.g. OECD, 2011). Depending on the suspected stressors, standard tests with macrophytes (e.g. OECD, 2006) can be additionally valuable in this context, e.g. when chemicals that may affect producers via systemic uptake are likely to occur. Recent alternatives such as tests based on readily measurable behavioural endpoints may concur to the test battery at tier 1 provided their confirmed high sensitivity to suspected stressors affecting the assessed ecosystem. A good example is the recently developed feeding inhibition test with the benthic widespread bivalve *Corbicula fluminea*, which indicates the presence of very low concentrations of different stressors within a few hours (Castro et al., 2018). Third, the ecological LoE at this stage should focus on simple community level assessments, which may include functional endpoints. We suggest the measurement of Chlorophyll *a* content in plankton (low flow sites) or phytobenthos (high flow sites) as an indicator of ecosystem productivity, either by traditional Chl *a* quantification or high-throughput methods (e.g. PAM fluorescence; Schmitt-Jansen and Altenburger, 2008). Leaf litter decomposition allows a primary understanding on how ecosystem processes regarding nutrient cycling are occurring (e.g. Graça et al., 2015). Also, high-throughput DNA-based screenings could be useful to feed this LoE. For example, measuring Low/High DNA content (LNA/HNA) bacteria without sequencing can rapidly signal and distinguish between different types of bacteria and their physiological state according to their nucleic acid content (high or low), thus turning them into useful bioindicators (see Santos et al., 2019), although further development and especially validation are necessary to confirm the feasibility of the indication. Denaturing Gradient Gel Electrophoresis (DGGE) is a validated alternative to LNA/HNA ratios. Being also sequencing-independent and providing a wide view on the diversity and abundance of aquatic bacteria (traditional indices are used for band patterning analysis), it has been successfully associated to environmental gradients (especially regarding nutrients) in riverine ecosystems (e.g. Liu et al., 2012). Quantification of nucleic acid ratios (RNA/DNA) is also available to provide a short term measurement of condition and growth of the organism or population under analysis, complementing other methods used to evaluate stress (Foley et al., 2016).

Tier 2 is the following step in the proposed assessment scheme and it conceivably consists of a fine-scale and site-specific assessment of defined target sites following LoE integration and risk appraisal in tier 1 (Fig. 2). In our view, it is only at this stage that the full bioassessment scheme of the WFD should be applied, with a few modifications or refinements. First, the chemical LoE should focus only on a set of relevant contaminants and chemical elements, based on suspected stressors identified in tier 0 (e.g. when agriculture fields are present in the vicinities of the river, the presence of pesticides should be verified) and/or following general indication given by the physico-chemical survey done in tier 1. Moreover, quantification of these substances should be done in the sediment and elutriates, particularly in lotic systems, as well as bioaccumulation and biomagnification phenomena (e.g. in the most abundant macroinvertebrate species), instead of focusing solely on the water matrix. This concern has been already lightly referred to in Directive 2013/39/UE but it has not been enforced in practice within WFD bioassessment implementation. Second, the ecotoxicological LoE should be based on the use of biomarker analyses coupled with *in situ* bioassays, focused on the benthos or fish. This combination of tools has been recognized as useful in ecological assessment of aquatic systems (Martinez-Haro et al., 2015). *In situ* bioassays have the advantage of being site-specific, highly relevant (test organisms are subjected to natural environmental fluctuations), and they allow deploying controls in the field (e.g. using clean water or synthetic media), which is useful if reference sites are not representative or abundant (Hopkin, 1998). In most sites, sub-lethal levels of contamination are expected; as such, the use of biochemical or cellular responses (biomarkers) is preferred, as they are sensitive to low concentrations of pollutants and serve as early warning signs of physiological effects on the biota (Prat et al., 2013). This could include stress hormones or biotransformation enzymes, as well as evidence of oxidative stress or neurotoxicity scenarios, but the exact battery could be tuned to specific groups of contaminants. Proper reference sites can be used for comparative purposes, but if difficulties in their validation exist, adequate control treatments could be easily deployed in the field (Correia et al., 2013; Martinez-Haro et al., 2014). Methods have been optimised for biomarker analysis in different freshwater organisms, including macrophytes, diatoms, macroinvertebrates and fish; in the latter case, non-destructive methods should be employed (Akbarzadeh et al., 2018; McCormick, 1993) (see Section 3.5). Another relevant sub-lethal endpoint is feeding inhibition, namely post-exposure feeding inhibition to isolate the effects of stressors directly in the tested organism (Brent and Herricks, 1998; McWilliam and Baird, 2002). Feeding is amongst the most relevant behaviours responding to environmental pressure, configuring an integrated endpoint that concomitantly reflects impairment of ecosystem trophic functioning (Agostinho et al., 2012; Forrow and Maltby, 2000; McLoughlin et al., 2000; Wallace and Webster, 1996). Validated protocols are available both for benthic organisms (e.g. Agostinho et al., 2012; Castro et al., 2018; Crane et al., 1995; Maltby et al., 2002; Satapornvanit et al., 2009; Vidal et al., 2019) and for organisms standing primarily in the water column (e.g. Castro et al., 2004; Vidal et al., 2019). Interestingly, combinations between feeding inhibition and biomarkers have been used successfully to assess the effects of chemical stressors in freshwater ecosystems (see e.g. Castro et al., 2004). Third, the ecological LoE at this stage should incorporate the evaluation of biological elements as defined in the current WFD bioassessment scheme, complemented with additional information that can be retrieved from the community data matrices that are the basis of such assessment scheme (e.g. functional or stressor-specific traits – see Section 3.3).

Although expert judgement is the basis of the options and decisions through the assessment scheme we are proposing, the inherent subjectivity of this process can be toned down by solid reasoning and guidance on how risk indications can be reached. This will depend, however, on the decision-making demands and requirements, since experts are able to provide recommendations based on a weight-of-evidence integration of all three LoE and respective quality benchmarks. The major shortcoming here is obviously the definition of adequate reference sites (see Section 2.1.1 for a detailed view on the recognised problems), which are critical for the calculation of risk quotients for each LoE in ERA and the overall calculation of risk and uncertainty that allows decision regarding the progression through tiers of increasing complexity. Whenever adequate reference sites are unavailable, alternative strategies can be followed.

How LoE-specific hazards can be calculated for further risk and uncertainty derivation is a practical issue requiring in-depth future discussion. In practice, the main output of tier 1 would be a prioritization of the sites within each river under ecological evaluation in order to choose those that need a deeper analysis in tier 2 – target sites. This prioritization would be done by a team of experts on the basis of an unacceptable integrated risk value and/or uncertainty as in traditional ERA (see Section 2.2). In the absence of adequate reference sites, the chemical risk can be calculated using the current quality benchmarks used in the WFD bioassessment scheme (or regionally accepted benchmarks) to define the physico-chemical status as the worst-case reference. In essence, this reflects the philosophy of the ratios between measured environmental concentrations (MEC) and predicted no effect concentrations (PNEC), which can be used in tier 2 (see below), but at a more generalist level (with less specific chemical indicators) than required in tier 1. The ecotoxicological risk calculation can be derived from the internal controls (which also work for test validation) instead of using response data from reference sites (highly variable and potentially not representative). The ratio between the response values obtained in the whole sample (elutriate, sediment or water) treatment and those retrieved in the internal control can be understood as the risk quotient for the ecotoxicological LoE in tier 1. Such an alternative approach was already applied successfully in ERA in extreme environments, where references are difficult to identify (Pereira et al., 2017a). In this approach, the results of a control (culture medium) are artificially set as 0% effect as a surrogate of a natural reference. Risk calculation for the ecological LoE in tier 1 when appropriate reference sites are unavailable is the most challenging. A similar approach to ecological quality ratios could be a solution, provided that metrics for pre-tipified reference conditions are appropriately developed and intercalibrated. However, we have already pointed out some of the problems with this strategy, and the associated perils of inadequate "pristine" benchmarks (Section 2.1). At this stage, a wider input from the scientific community is needed for an alternative view on the definition of benchmarks and risk quotients for the ecological LoE, if one wishes not to leave it all in the hands of experts and their judgment.

Regarding the envisioned outcome of tier 2, chemical risk can be calculated using MEC/PNEC ratios, which have been understood as suitable to appraise real risk associated to contamination by specific compounds (e.g. Bouissou-Schurtz et al., 2014). At this stage, the quantification of specific compounds at the target sites is foreseen, thus MEC will be available for the relevant contaminants as judged by experts. PNEC values are available for a large set of contaminants in open databases by regulatory agencies worldwide, e.g. the Aquatic Life Benchmarks and Ecological Risk Assessments for Registered Pesticides or Freshwater Sediment Screening Benchmarks by USEPA (https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/aquatic-life-benchmarks-and-ecological-risk; assessed on 07/2019). These databases are constantly being populated, given the increased attention on the hazardous potential of chemicals and products; paradigmatic examples are the REACH regulation and the regulation defining requirements and conditions for approval of Plant Protection Products in the EU (European Commission, 2018c, 2009). The appraisal of the targeted mixture of contaminants in risk calculations is an actual challenge, but developments and recommendations have been made at the regulatory level (e.g. More et al., 2019). Similar guidelines as indicated above for tier 1 apply to risk calculation in the ecotoxicological LoE for tier 2, with internal controls deployed in the field in parallel (as often used in *in situ* testing as detailed before) serving as a reference whenever actual reference sites are not established. A straightforward approach for risk calculation regarding the ecological LoE for tier 2 is available when Ecological Quality Ratios (EQR) for biotic communities are calculated as an indicator, following the standard metrics implemented for standard bioassessment *sensu* the WFD. EQR range within 0.00–1.00 and the underlying reasoning involves comparison with reference conditions (European Commission, 2000), which matches the reasoning behind risk calculations. Again (and as stated above for tier 1), further discussion is needed on alternative pathways and/or on the normalisation of non intercalibrated metrics, such as those based on functional traits, etc. (Section 3). If benchmarks for pristine reference conditions are the choice, then new metrics included in the WFD need intercalibration and an enormous effort must be done in setting such benchmarks so that errors from the past can be avoided. Until then, expert judgement may be the most suitable solution.

Beyond the academic exercise of suggesting optimised approaches to address bioassessment towards water quality monitoring, a note is worth on the possible costs of the application of the suggested framework. A serious and quantitative cost-efficiency analysis requires prior maturation and discussion of the proposed assessment scheme, and hence it is logically out of the scope of the present review. However, it is important to remark that (i) monitoring systems delivering a truthful picture of the ecological status of water bodies by providing accurate and reliable information will always represent savings in restoration and mitigation measures; and (ii) the investment in ensuring expert judgement is invaluable to ensure confidence in the implementation of those measures (Carvalho et al., 2019). In our opinion, the cost-effectiveness in the management of freshwaters can only be achieved by an assessment scheme providing integrated quantitative information on the three LoE, that are key to understand the relationships between biological communities and the pressures they are subjected to.

5. Conclusion

Protection of freshwater water bodies as a whole is vital to an effective protection of biodiversity and sustainable use of ecosystem services. However, this is not an easy task, as this requires the management of relatively large areas and it is imperative to take into account the necessities of all biota strictly or partially-dependent on water. The gaps of knowledge when the WFD assessment scheme is applied are evident and need to be circumvented because they are delaying an effective implementation of the WFD and, consequently, hindering the possibility of accurately assess the ecological status of freshwaters. These deficiencies have been pointed out by several other authors and the criticism boosted the proposal of a wide array of methodologies that have been argued to be useful complementary tools to the WFD bioassessment scheme, as revised here. Proposals are scarce on how to incorporate these complementary tools in practice within the WFD assessment scheme, which still represents a very important enhancement of the ecological evaluation of freshwater water bodies. In this context, and structurally inspired by ERA as an alternative bioassessment framework, we are proposing a strategy for the evaluation of the ecological quality of freshwaters that considers some valuable WFD principles and metrics but concomitantly includes complementary methods that have been developed and fills several of its identified flaws. This proposed strategy clearly assumes the benefits of (i) using tiered bioassessment approaches that allow better cost- and time-efficiency; (ii) incorporating effects-based tools (biomarkers in feral organisms; ecotoxicological assays) towards a better appraisal of cause-effect relationships; (iii) effectively integrating distinct LoE (chemical, ecological and ecotoxicological), instead of using the conservative "one-out, all-out" principle; (iv) promoting expert judgement throughout the ecological assessment flow, easing the task of solidly deciding the direction of the evaluation process and meeting the specific environmental agenda of each Member State. Foremost, underlying this proposal is an attempt to promote discussion within the scientific community devoted to the study of freshwater ecosystems (especially rivers) and the negative impacts affecting them, with the expectation of enhancing the quality and comprehensiveness of the future ecological assessment framework for freshwaters.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Abbas, M., Adil, M., Ehtisham-ul-Haque, S., Munir, B., Yameen, M., Ghaffar, A., Shar, G. A., Asif Tahir, M., Iqbal, M., 2018. Vibrio fischeri bioluminescence inhibition assay for ecotoxicity assessment: a review. Sci. Total Environ. 626, 1295–1309. https://doi.org/10.1016/j.scitotenv.2018.01.066.

Admiraal, W., Blanck, H., Buckert-de Jong, M., Guasch, H., Ivorra, N., Lehmann, V., Nyström, B.A.H., Paulsson, M., Sabater, S., 1999. Short-term toxicity of zinc to microbenthic algae and bacteria in a metal polluted stream. Water Res. 33 (9), 1989–1996. https://doi.org/10.1016/S0043-1354(98)00426-6.

Agostinho, M., Moreira-Santos, M., Ribeiro, R., 2012. A freshwater amphipod toxicity test based on postexposure feeding and the population consumption inhibitory concentration. Chemosphere 87 (1), 43–48. https://doi.org/10.1016/j.chemosphere.2011.11.052.

Agustsson, K.M. (Ed.), 2018. Nordic Water Framework Conference 2017 - Towards a better implementation of the WFD from a Nordic perspective. The Norwegian Environment Agency, p. 28.

Akbarzadeh, A., Günther, O.P., Houde, A.L., Li, S., Ming, T.J., Jeffries, K.M., Hinch, S.G., Miller, K.M., 2018. Developing specific molecular biomarkers for thermal stress in salmonids. BMC Genomics 19 (1). https://doi.org/10.1186/s12864-018-5108-9.

Allard, L., Grenouillet, G., Khazraie, K., Tudesque, L., Vigouroux, R., Brosse, S., 2014. Electrofishing efficiency in low conductivity neotropical streams: towards a non-destructive fish sampling method. Fish. Manag. Ecol. 21 (3), 234–243. https://doi.org/10.1111/fme.12071.

Altenburger, R., Ait-Aissa, S., Antczak, P., Backhaus, T., Barceló, D., Seiler, T.-B., Brion, F., Busch, W., Chipman, K., de Alda, M.L., de Aragão Umbuzeiro, G., Escher, B.I., Falciani, F., Faust, M., Focks, A., Hilscherova, K., Hollender, J., Hollert, H., Jäger, F., Jahnke, A., Kortenkamp, A., Krauss, M., Lemkine, G.F., Munthe, J., Neumann, S., Schymanski, E.L., Scrimshaw, M., Segner, H., Slobodnik, J., Smedes, F., Kughathas, S., Teodorovic, I., Tindall, A.J., Tollefsen, K.E., Walz, K.-H., Williams, T.D., Van den Brink, P.J., van Gils, J., Vrana, B., Zhang, X., Brack, W., 2015. Future water quality monitoring — adapting tools to deal with mixtures of pollutants in water resource management. Sci. Total Environ. 512-513, 540–551. https://doi.org/10.1016/j.scitotenv.2014.12.057.

Altenburger, R., Scholze, M., Busch, W., Escher, B.I., Jakobs, G., Krauss, M., Krüger, J., Neale, P.A., Ait-Aissa, S., Almeida, A.C., Seiler, T.-B., Brion, F., Hilscherová, K., Hollert, H., Novák, J., Schlichting, R., Serra, H., Shao, Y., Tindall, A., Tollefsen, K.E., Umbuzeiro, G., Williams, T.D., Kortenkamp, A., 2018. Mixture effects in samples of multiple contaminants – an inter-laboratory study with manifold bioassays. Environ. Int. 114, 95–106. https://doi.org/10.1016/j.envint.2018.02.013.

Andersen, J.H., Aroviita, J., Carstensen, J., Friberg, N., Johnson, R.K., Kauppila, P., Lindegarth, M., Murray, C., Norling, K., 2016. Approaches for integrated assessment of ecological and eutrophication status of surface waters in Nordic Countries. Ambio 45 (6), 681–691. https://doi.org/10.1007/s13280-016-0767-8.

Artigas, J., Arts, G., Babut, M., Caracciolo, A.B., Charles, S., Chaumot, A., Combourieu, B., Dahllöf, I., Despréaux, D., Ferrari, B., Friberg, N., Garric, J., Geffard, O., Gourlay-Francé, C., Hein, M., Hjorth, M., Krauss, M., De Lange, H.J., Lahr, J., Lehtonen, K.K., Lettieri, T., Liess, M., Lofts, S., Mayer, P., Morin, S., Paschke, A., Svendsen, C., Usseglio-Polatera, P., van den Brink, N., Vindimian, E., Williams, R., 2012. Towards a renewed research agenda in ecotoxicology. Environ. Pollut. 160, 201–206. https://doi.org/10.1016/j.envpol.2011.08.011.

Atkinson, C.L., Sansom, B.J., Vaughn, C.C., Forshay, K.J., 2018. Consumer aggregations drive nutrient dynamics and ecosystem metabolism in nutrient-limited systems. Ecosystems 21 (3), 521–535. https://doi.org/10.1007/s10021-017-0166-4.

Babut, M.P., Delmas, H., Bray, M., Durrieu, C., Perrodin, Y., Garne, J., 2006. Characterizing the risks to aquatic ecosystems: a tentative approach in the context of freshwater dredged material disposal. Integr. Environ. Assess. Manag. 2 (4), 330–343. https://doi.org/10.1002/ieam.5630020404.

Backhaus, T., Brack, W., Van den Brink, P.J., Deutschmann, B., Hollert, H., Posthuma, L., Segner, H., Seiler, T.-B., Teodorovic, I., Focks, A., 2019. Assessing the ecological impact of chemical pollution on aquatic ecosystems requires the systematic exploration and evaluation of four lines of evidence. Environ. Sci. Eur. 31 (1) https://doi.org/10.1186/s12302-019-0276-z.

Barjhoux, I., Fechner, L.C., Lebrun, J.D., Anzil, A., Ayrault, S., Budzinski, H., Cachot, J., Charron, L., Chaumot, A., Clérandeau, C., Dedourge-Geffard, O., Faburé, J., François, A., Geffard, O., George, I., Labadie, P., Lévi, Y., Munoz, G., Noury, P., Oziol, L., Quéau, H., Servais, P., Uher, E., Urien, N., Geffard, A., 2018. Application of a multidisciplinary and integrative weight-of-evidence approach to a 1-year monitoring survey of the Seine River. Environ. Sci. Pollut. Res. 25 (24), 23404–23429. https://doi.org/10.1007/s11356-016-6993-6.

Beketov, M.A., Liess, M., 2012. Ecotoxicology and macroecology – time for integration. Environ. Pollut. 162, 247–254. https://doi.org/10.1016/j.envpol.2011.11.011.

Benfield, E.F., 1996. Leaf breakdown in stream ecosystems. In: Hauer, F.R., Lamberti, G. A. (Eds.), Methods in Stream Ecology. Academic Press, pp. 579–589.

Bennett, E.M., Peterson, G.D., Gordon, L.J., 2009. Understanding relationships among multiple ecosystem services. Ecol. Lett. 12, 1394–1404. https://doi.org/10.1111/j.1461-0248.2009.01387.x.

Birk, S., Bonne, W., Borja, A., Brucet, S., Courrat, A., Poikane, S., Solimini, A., van de Bund, W., Zampoukas, N., Hering, D., 2012. Three hundred ways to assess Europe's surface waters: an almost complete overview of biological methods to implement the Water Framework Directive. Ecol. Ind. 18, 31–41. https://doi.org/10.1016/j.ecolind.2011.10.009.

Blasco, C., Picó, Y., 2009. Prospects for combining chemical and biological methods for integrated environmental assessment. TrAC, Trends Anal. Chem. 28 (6), 745–757. https://doi.org/10.1016/j.trac.2009.04.010.

Borja, A., Rodríguez, J.G., 2010. Problems associated with the 'one-out, all-out' principle, when using multiple ecosystem components in assessing the ecological status of marine waters. Mar. Pollut. Bull. 60 (8), 1143–1146. https://doi.org/10.1016/j.marpolbul.2010.06.026.

Bouissou-Schurtz, C., Houeto, P., Guerbet, M., Bachelot, M., Casellas, C., Mauclaire, A.-C., Panetier, P., Delval, C., Masset, D., 2014. Ecological risk assessment of the presence of pharmaceutical residues in a French national water survey. Regul. Toxicol. Pharm. 69 (3), 296–303. https://doi.org/10.1016/j.yrtph.2014.04.006.

Brack, W., Ait-Aissa, S., Altenburger, R., Cousins, I., Dulio, V., Escher, B., Focks, A., Ginebreda, A., Hering, D., Hilscherová, K., Hollender, J., Hollert, H., Kortenkamp, A., de Alda, M.L., Posthuma, L., Schymanski, E., Segner, H., Slobodnik, J., 2019a. Let us empower the WFD to prevent risks of chemical pollution in European rivers and lakes. Environ. Sci. Eur. 31 (1) https://doi.org/10.1186/s12302-019-0228-7.

Brack, W., Ait-Aissa, S., Backhaus, T., Dulio, V., Escher, B.I., Faust, M., Hilscherova, K., Hollender, J., Hollert, H., Müller, C., Munthe, J., Posthuma, L., Seiler, T.-B., Slobodnik, J., Teodorovic, I., Tindall, A.J., de Aragão Umbuzeiro, G., Zhang, X., Altenburger, R., 2019b. Effect-based methods are key. The European Collaborative Project SOLUTIONS recommends integrating effect-based methods for diagnosis and monitoring of water quality. Environ. Sci. Eur. 31 (1) https://doi.org/10.1186/s12302-019-0192-2.

Brent, R.N., Herricks, E.E., 1998. Postexposure effects of brief cadmium, zinc, and phenol exposures on freshwater organisms. Environ. Toxicol. Chem. 17 (10), 2091–2099.

Bruno, D., Gutiérrez-Cánovas, C., Velasco, J., Sánchez-Fernández, D., 2016. Functional redundancy as a tool for bioassessment: a test using riparian vegetation. Sci. Total Environ. 566-567, 1268–1276. https://doi.org/10.1016/j.scitotenv.2016.05.186.

Buffagni, A., Armanini, D.G., Erba, S., 2009. Does the lentic-lotic character of rivers affect invertebrate metrics used in the assessment of ecological quality? J. Limnol. 68, 92–105. https://doi.org/10.4081/jlimnol.2009.92.

Buijse, A.D., Klijn, F., Leuven, R.S.E.W., Middelkoop, H., Schiemer, F., Thorp, J.H., Wolfert, H.P., 2005. Rehabilitation of large rivers: references, achievements and integration into river management. Large Rivers 15 (1–4), 715–738. https://doi.org/10.1127/lr/15/2003/715.

Burton, G.A., Batley, G.E., Chapman, P.M., Forbes, V.E., Smith, E.P., Reynoldson, T., Schlekat, C.E., Besten, P.J.D., Bailer, A.J., Green, A.S., Dwyer, R.L., 2002. A weight-of-evidence framework for assessing sediment (or other) contamination: improving certainty in the decision-making process. Hum. Ecol. Risk Assess.: Int. J. 8 (7), 1675–1696.

Calow, P., Forbes, V.E., 2003. Does ecotoxicology inform ecological risk assessment? Environ. Sci. Technol. 146A–151A.

Cañedo-Argüelles, M., Kefford, B.J., Piscart, C., Prat, N., Schäfer, R.B., Schulz, C.-J., 2013. Salinisation of rivers: an urgent ecological issue. Environ. Pollut. 173, 157–167. https://doi.org/10.1016/j.envpol.2012.10.011.

Caroni, R., van de Bund, W., Clarke, R.T., Johnson, R.K., 2013. Combination of multiple biological quality elements into waterbody assessment of surface waters. Hydrobiologia 704, 437–451. https://doi.org/10.1007/s10750-012-1274-y.

Carpenter, S.R., Brock, W.A., 2006. Rising variance: a leading indicator of ecological transition. Ecol. Lett. 9, 311–318. https://doi.org/10.1111/j.1461-0248.2005.00877.x.

Carpenter, S.R., Stanley, E.H., Vander Zanden, M.J., 2011. State of the World's freshwater ecosystems: physical, chemical, and biological changes. Annu. Rev. Environ. Resour. 36 (1), 75–99. https://doi.org/10.1146/annurev-environ-021810-094524.

Carvalho, L., Mackay, E.B., Cardoso, A.C., Baattrup-Pedersen, A., Birk, S., Blackstock, K. L., Borics, G., Borja, A., Feld, C.K., Ferreira, M.T., Globevnik, L., Grizzetti, B., Hendry, S., Hering, D., Kelly, M., Langaas, S., Meissner, K., Panagopoulos, Y., Penning, E., Rouillard, J., Sabater, S., Schmedtje, U., Spears, B.M., Venohr, M., van de Bund, W., Solheim, A.L., 2019. Protecting and restoring Europe's waters: an analysis of the future development needs of the Water Framework Directive. Sci. Total Environ. 658, 1228–1238. https://doi.org/10.1016/j.scitotenv.2018.12.255.

Castro, B.B., Silva, C., Macário, I.P.E., Oliveira, B., Gonçalves, F., Pereira, J.L., 2018. Feeding inhibition in Corbicula fluminea (O.F. Muller, 1774) as an effect criterion to pollutant exposure: perspectives for ecotoxicity screening and refinement of chemical control. Aquat. Toxicol. 196, 25–34. https://doi.org/10.1016/j.aquatox.2018.01.002.

Castro, B.B., Sobral, O., Guilhermino, L., Ribeiro, R., 2004. An in situ bioassay integrating individual and biochemical responses using small fish species. Ecotoxicology 13 (7), 667–681. https://doi.org/10.1007/s10646-003-4427-y.

Çelekli, A., Arslanargun, H., Soysal, Ç., Gültekin, E., Bozkurt, H., 2016. Biochemical responses of filamentous algae in different aquatic ecosystems in South East Turkey and associated water quality parameters. Ecotoxicol. Environ. Saf. 133, 403–412. https://doi.org/10.1016/j.ecoenv.2016.08.002.

Çelekli, A., Kapı, E., Soysal, Ç., Arslanargun, H., Bozkurt, H., 2017. Evaluating biochemical response of filamentous algae integrated with different water bodies. Ecotoxicol. Environ. Saf. 142, 171–180. https://doi.org/10.1016/j.ecoenv.2017.04.008.

Centis, B., Tolotti, M., Salmaso, N., 2010. Structure of the diatom community of the River Adige (North-Eastern Italy) along a hydrological gradient. Hydrobiologia 639 (1), 37–42. https://doi.org/10.1007/s10750-009-0019-z.

Comiti, F., 2012. How natural are Alpine mountain rivers? Evidence from the Italian Alps. Earth Surf. Process. Landforms 37 (7), 693–707. https://doi.org/10.1002/esp.2267.

Correia, V., Ribeiro, R., Moreira-Santos, M., 2013. A laboratory and in situ postexposure feeding assay with a freshwater snail: a feeding assay with freshwater snails. Environ. Toxicol. Chem. 32 (9), 2144–2152. https://doi.org/10.1002/etc.2297.

Crane, M., Delaney, P., Mainstone, C., Clarke, S., 1995. Measurement by in situ bioassay of water quality in an agricultural catchment. Water Res. 29, 2441–2448. https://doi.org/10.1016/0043-1354(95)00085-Y.

Damásio, J., Fernández-Sanjuan, M., Sánchez-Avila, J., Lacorte, S., Prat, N., Rieradevall, M., Soares, A.M.V.M., Barata, C., 2011. Multi-biochemical responses of benthic macroinvertebrate species as a complementary tool to diagnose the cause of community impairment in polluted rivers. Water Res. 45 (12), 3599–3613. https://doi.org/10.1016/j.watres.2011.04.006.

Damásio, J., Tauler, R., Teixidó, E., Rieradevall, M., Prat, N., Riva, M.C., Soares, A.M.V. M., Barata, C., 2008. Combined use of Daphnia magna in situ bioassays, biomarkers and biological indices to diagnose and identify environmental pressures on invertebrate communities in two Mediterranean urbanized and industrialized rivers (NE Spain). Aquat. Toxicol. 87 (4), 310–320. https://doi.org/10.1016/j.aquatox.2008.02.016.

De Lange, H.J., Sala, S., Vighi, M., Faber, J.H., 2010. Ecological vulnerability in risk assessment — a review and perspectives. Sci. Total Environ. 408, 3871–3879. https://doi.org/10.1016/j.scitotenv.2009.11.009.

den Besten, P.J., de Deckere, E., Babut, M.P., Power, B., DelValls, T.A., Zago, C., Oen, A. M.P., Heise, S., 2003. Biological effects-based sediment quality in ecological risk assessment for European waters. J. Soils Sed. 3 (3), 144–162. https://doi.org/10.1065/jss2003.08.084.

Díaz, S., Cabido, M., 2001. Vive la différence: plant functional diversity matters to ecosystem processes. Trends Ecol. Evol. 16 (11), 646–655. https://doi.org/10.1016/S0169-5347(01)02283-2.

Dudgeon, D., Arthington, A.H., Gessner, M.O., Kawabata, Z.-I., Knowler, D.J., Lévêque, C., Naiman, R.J., Prieur-Richard, A.-H., Soto, D., Stiassny, M.L.J., Sullivan, C.A., 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. Biol. Rev. 81 (02), 163. https://doi.org/10.1017/S1464793105006950.

EEA, 2016. Meteorological and hydrological droughts.

EEA, 2015. The European environment — state and outlook 2015: synthesis. Copenhagen, Denmark.

More, S.J., Bampidis, V., Benford, D., Bennekou, S.H., Bragard, C., Halldorsson, T.I., Hernández-Jerez, A.F., Koutsoumanis, K., Naegeli, H., Schlatter, J.R., Silano, V., Nielsen, S.S., Schrenk, D., Turck, D., Younes, M., Benfenati, E., Castle, L., Cedergreen, N., Hardy, A., Laskowski, R., Leblanc, J.C., Kortenkamp, A., Ragas, A., Posthuma, L., Svendsen, C., Solecki, R., Testai, E., Dujardin, B., Kass, G.EN., Manini, P., Jeddi, M.Z., Dorne, J.-L., Hogstrand, C., 2019. Guidance on harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals. EFSA Journal 17 (3), 5634. https://doi.org/10.2903/j.efsa:2019.5634.

Elbrecht, V., Vamos, E.E., Meissner, K., Aroviita, J., Leese, F., Yu, D., 2017. Assessing strengths and weaknesses of DNA metabarcoding-based macroinvertebrate identification for routine stream monitoring. Methods Ecol. Evol. 8 (10), 1265–1275.

Elias, C.L., Calapez, A.R., Almeida, S.F.P., Feio, M.J., 2015. From perennial to temporary streams: an extreme drought as a driving force of freshwater communities. Mar. Freshwater Res. 66 (5), 469. https://doi.org/10.1071/MF13312.

Environment and Climate Change Canada, 2018. Environment and Climate Change Canada 2018-19 Departmental Plan.

European Commission, 2019a. European Overview - River Basin Management Plans, Accompanying the document report from the commission to the European parliament and the council implementation of the Water Framework Directive (2000/60/EC) and the Floods Directive (2007/60/EC). Brussels.

European Commission, 2019b. International Cooperation under the Water Framework Directive (2000/60/EC) - Factsheets for International River Basins, Accompanying the document Report From the commission to the European parliament and the council on the implementation of the Water Framework Directive (2000/60/EC) and the Floods Directive (2007/60/EC). Brussels.

European Commission, 2019c. Fitness Check of the Water Framework Directive and the Floods Directive. Brussels.

European Commission, 2018a. Commission Decision (EU) 2018/229 of 12 February 2018 establishing, pursuant to Directive 2000/60/EC of the European Parliament and of the Council, the values of the Member State monitoring system classifications as a result of the intercalibration exercise and repealing Commission Decision 2013/480/EU.

European Commission, 2018b. Implementation of River basin management plans. Available: <http://ec.europa.eu/environment/water/participation/map_mc/map.htm> [WWW Document].

European Commission, 2018c. Commission Regulation (EU) 2018/675 of 2 May 2018 amending the Appendices to Annex XVII to Regulation (EC) No 1907/2006 of the European Parliament and of the Council concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) as regards CMR substances.

European Commission, 2015a. Report on the implementation of the Water Framework Directive River Basin Management Plans. Member State: Portugal.

European Commission, 2015b. Assessment of Member States' progress in the implementation of Programmes of Measures during the first planning cycle of the Water Framework Directive - Member State Report: Sweden (SE).

European Commission, 2015c. Report on the implementation of the Water Framework Directive River Basin Management Plans - Member State: Belgium (BE).

European Commission, 2015d. Report on the implementation of the Water Framework Directive River Basin Management Plans - Member State: Croatia (HR).

European Commission, 2015e. The Water Framework Directive and the Floods Directive: Actions towards the "good status" of EU water and to reduce flood risks. Brussels.

European Commission, 2012a. Commission Staff Working Document - European Overview, Accompanying the Document: "Report From the Commission to the European Parliament and the Council on the Implementation of the Water Framework Directive (2000/60/EC) River Basin Management Plans." Brussels.

European Commission, 2012b. Regulation (EU) No 528/2012 of the European Parliament and of the Council of 22 May 2012 concerning the making available on the market and use of biocidal products.

European Commission, 2011. Common Implementation Strategy for the Water Framework Directive (2000/60/EC). Guidance Document No. 27. Technical Guidance For Deriving Environmental Quality Standards.

European Commission, 2009. Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC.

European Commission, 2006. Regulation (EC) 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation,

Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/E.
European Commission, 2000. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. Official Journal of the European Communities L 327.
Extence, C.A., Balbi, D.M., Chadd, R.P., 1999. River flow indexing using British benthic macroinvertebrates: a framework for setting hydroecological objectives. Regul. Rivers Res. Manag. 15, 543–574. https://doi.org/10.1002/(SICI)1099-1646(199911/12)15:6<545::AID-RRR561>3.0.CO;2-W.
Extence, C.A., Chadd, R.P., England, J., Dunbar, M.J., Wood, P.J., Taylor, E.D., 2013. The assessment of fine sediment accumulation in rivers using macro-invertebrate community response. River Res. Appl. 29, 17–55. https://doi.org/10.1002/rra.1569.
Extence, C.A., Chadd, R.P., England, J., Naura, M., Pickwell, A.G.G., 2017. Application of the Proportion of Sediment - sensitive Invertebrates (PSI) biomonitoring index. River Res. Appl. 33, 1596–1605. https://doi.org/10.1002/rra.3227.
Falasco, E., Piano, E., Bona, F., 2016. Suggestions for diatom-based monitoring in intermittent streams. Knowl. Manag. Aquat. Ecosyst. 417, 1–12. https://doi.org/10.1051/kmae/2016025.
Faust, M., Backhaus, T., Altenburger, R., Dulio, V., van Gils, J., Ginebreda, A., Kortenkamp, A., Munthe, J., Posthuma, L., Slobodnik, J., Tollefsen, K.E., van Wezel, A., Brack, W., 2019. Prioritisation of water pollutants: the EU Project SOLUTIONS proposes a methodological framework for the integration of mixture risk assessments into prioritisation procedures under the European Water Framework Directive. Environ. Sci. Eur. 31 (1) https://doi.org/10.1186/s12302-019-0239-4.
Feio, M.J., Calapez, A.R., Elias, C.L., Cortes, R.M.V., Graça, M.A.S., Pinto, P., Almeida, S. F.P., 2016. The paradox of expert judgment in rivers ecological monitoring. J. Environ. Manage. 184, 609–616. https://doi.org/10.1016/j.jenvman.2016.10.004.
Filipe, A.F., Feio, M.J., Garcia-Raventós, A., Ramião, J.P., Pace, G., Martins, F.MS., Magalhães, M.F., 2019. The European Water Framework Directive facing current challenges: recommendations for a more efficient biological assessment of inland surface waters. Inland Waters 9 (1), 95–103. https://doi.org/10.1080/20442041.2018.1494973.
Finnmark County Council, 2016. Joint water management of the Finnish-Norwegian river basin district (2016-2021).
Foley, C.J., Bradley, D.L., Höök, T.O., 2016. A review and assessment of the potential use of RNA:DNA ratios to assess the condition of entrained fish larvae. Ecol. Ind. 60, 346–357. https://doi.org/10.1016/j.ecolind.2015.07.005.
Forbes, V.E., Calow, P., 2002. Extrapolation in Ecological Risk Assessment : Balancing Pragmatism and Precaution in Chemical Controls Legislation 52, 249–257.
Forrow, D.M., Maltby, L., 2000. Toward a mechanistic understanding of contaminant-induced changes in detritus processing in streams: direct and indirect effects on detritivore feeding. Environ. Toxicol. Chem. 19 (8), 2100–2106. https://doi.org/10.1002/etc.5620190820.
Gaddum, J.H., 1993. Reports on Biological Standards: 3. Methods of Biological Assay Depending on a Quantal Response. Medical Research Council.
Gallart, F., Llorens, P., Latron, J., Cid, N., Rieradevall, M., Prat, N., 2016. Validating alternative methodologies to estimate the regime of temporary rivers when flow data are unavailable. Sci. Total Environ. 565, 1001–1010. https://doi.org/10.1016/j.scitotenv.2016.05.116.
Gauch Jr., H.G., 1994. Multivariate Analysis in Community Ecology. Cambridge University Press, Cambridge.
Gessner, M.O., Chauvet, E., 2002. A case for using litter breakdown to assess functional stream integrity. Ecol. Appl. 12 (2), 498. https://doi.org/10.2307/3060958.
Ghilarov, A.M., 2000. Ecosystem functioning and intrinsic value of biodiversity. Oikos 90 (2), 408–412. https://doi.org/10.1034/j.1600-0706.2000.900222.x.
Gold, C., Feurtet-Mazel, A., Coste, M., Boudou, A., 2002. Field transfer of periphytic diatom communities to assess short-term structural effects of metals (Cd, Zn) in rivers. Water Res. 36 (14), 3654–3664. https://doi.org/10.1016/S0043-1354(02)00051-9.
Golfieri, B., Hardersen, S., Maiolini, B., Surian, N., 2016. Odonates as indicators of the ecological integrity of the river corridor: development and application of the Odonate River Index (ORI) in northern Italy. Ecol. Ind. 61, 234–247. https://doi.org/10.1016/j.ecolind.2015.09.022.
Graça, M.A.S., Ferreira, V., Canhoto, C., Encalada, A.C., Guerrero-Bolaño, F., Wantzen, K. M., Boyero, L., 2015. A conceptual model of litter breakdown in low order streams: litter breakdown in low order streams. Internat. Rev. Hydrobiol. 100 (1), 1–12. https://doi.org/10.1002/iroh.201401757.
Grantham, T.E., Cañedo-Argüelles, M., Perrée, I., Rieradevall, M., Prat, N., 2012. A mesocosm approach for detecting stream invertebrate community responses to treated wastewater effluent. Environ. Pollut. 160, 95–102. https://doi.org/10.1016/j.envpol.2011.09.014.
Grove, M.K., Bilotta, G.S., Woockman, R.R., Schwartz, J.S., 2015. Suspended sediment regimes in contrasting reference-condition freshwater ecosystems: implications for water quality guidelines and management. Sci. Total Environ. 502, 481–492. https://doi.org/10.1016/j.scitotenv.2014.09.054.
Heise, S., Förstner, U., 2007. Risk assessment of contaminated sediments in river basins—theoretical considerations and pragmatic approach. J. Environ. Monit. 9 (9), 943. https://doi.org/10.1039/b704071g.
Hering, D., Borja, A., Carstensen, J., Carvalho, L., Elliott, M., Feld, C.K., Heiskanen, A.-S., Johnson, R.K., Moe, J., Pont, D., Solheim, A.L., van de Bund, W., 2010. The European Water Framework Directive at the age of 10: a critical review of the achievements with recommendations for the future. Sci. Total Environ. 408, 4007–4019. https://doi.org/10.1016/j.scitotenv.2010.05.031.
Hering, D., Borja, A., Jones, J.I., Pont, D., Boets, P., Bouchez, A., Bruce, K., Drakare, S., Hänfling, B., Kahlert, M., Leese, F., Meissner, K., Mergen, P., Reyjol, Y., Segurado, P., Vogler, A., Kelly, M., 2018. Implementation options for DNA-based identification into ecological status assessment under the European Water Framework Directive. Water Res. 138, 192–205. https://doi.org/10.1016/j.watres.2018.03.003.
Hering, D., Buffagni, A., Moog, O., Sandin, L., Sommerhäuser, M., Stubauer, I., Feld, C., Johnson, R., Pinto, P., Skoulikidis, N., Verdonschot, P., Zahrádková, S., 2003. The development of a system to assess the ecological quality of streams based on macroinvertebrates – design of the sampling programme within the AQEM project. Int. Rev. Hydrobiol. 88, 345–361. https://doi.org/10.1002/iroh.200390030.
Højberg, A.L., Troldborg, L., Stisen, S., Christensen, B.B.S., Henriksen, H.J., 2013. Stakeholder driven update and improvement of a national water resources model. Environ. Modell. Software 40, 202–213. https://doi.org/10.1016/j.envsoft.2012.09.010.
Hopkin, S.P., 1998. In situ biological monitoring of pollution. In: Calow, P. (Ed.), Handbook of Ecotoxicology. Blackwell Science Ltd, Oxford, UK, pp. 397–427.
Howarth, W., 2006. The progression towards ecological quality standards. J. Environ. Law 18, 3–35. https://doi.org/10.1093/jel/eqi049.
INAG, 2009. Critérios para a Classificação do Estado das Massas de Água Superficiais - Rios e Albufeiras. Ministério do Ambiente, Ordenamento do Território e do Desenvolvimento Regional.
IPCC, 2014a. Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part A: Global and Sectoral Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.
IPCC, 2014b. Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part B: Regional Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.
IST, 2018. Fluid Mechanics for Built and Natural Environments [WWW Document]. URL <http://www.civil.ist.utl.pt/~dconde/FMBNE/?page_id=249>.
Jakobsen, T.G., 2017. Environmental ethics: anthropocentrism and non-anthropocentrism revised in the light of critical realism. J. Crit. Realism 16 (2), 184–199.
Jardine, T.D., Hunt, R.J., Faggotter, S.J., Valdez, D., Burford, M.A., Bunn, S.E., 2013. Carbon from peryphiton supports fish biomass in waterholes of a wet-dry tropical river. River Res. Appl. 29, 560–573. https://doi.org/10.1002/rra.2554.
Johnson, R.K., Hering, D., Furse, M.T., Clarke, R.T., 2006a. Detection of ecological change using multiple organism groups: metrics and uncertainty. Hydrobiologia 566 (1), 115–137. https://doi.org/10.1007/s10750-006-0101-8.
Johnson, R.K., Hering, D., Furse, M.T., Verdonschot, P.F.M., 2006b. Indicators of ecological change: comparison of the early response of four organism groups to stress gradients. Hydrobiologia 566 (1), 139–152. https://doi.org/10.1007/s10750-006-0100-9.
Josefsson, H., Baaner, L., 2011. The water framework directive–a directive for the twenty-first century? J. Environm. Law 23 (3), 463–486. https://doi.org/10.1093/jel/eqr018.
Kalogianni, E., Vourka, A., Karaouzas, I., Vardakas, L., Laschou, S., Skoulikidis, N.T., 2017. Combined effects of water stress and pollution on macroinvertebrate and fish assemblages in a Mediterranean intermittent river. Sci. Total Environ. 603-604, 639–650. https://doi.org/10.1016/j.scitotenv.2017.06.078.
Kelly, M., 2013. Data rich, information poor? Phytobenthos assessment and the Water Framework Directive. Eur. J. Phycol. 48 (4), 437–450. https://doi.org/10.1080/09670262.2013.852694.
Könemann, S., Kase, R., Simon, E., Swart, K., Buchinger, S., Schlüsener, M., Hollert, H., Escher, B.I., Werner, I., Aït-Aïssa, S., Vermeirssen, E., Dulio, V., Valsecchi, S., Polesello, S., Behnisch, P., Javurkova, B., Perceval, O., Di Paolo, C., Olbrich, D., Sychrova, E., Schlichting, R., Leborgne, L., Clara, M., Scheffknecht, C., Marneffe, Y., Chalon, C., Tušil, P., Soldàn, P., von Danwitz, B., Schwaiger, J., Becares, M.I.S.M., Bersani, F., Hilscherová, K., Reifferscheid, G., Ternes, T., Carere, M., 2018. Effect-based and chemical analytical methods to monitor estrogens under the European Water Framework Directive. TrAC, Trends Anal. Chem. 102, 225–235. https://doi.org/10.1016/j.trac.2018.02.008.
Lacey, J.H., Leng, M.J., Vane, C.H., Radbourne, A.D., Yang, H., Ryves, D.B., 2018. Assessing human impact on Rostherne Mere, UK, using the geochemistry of organic matter. Anthropocene 21, 52–65. https://doi.org/10.1016/j.ancene.2018.02.002.
Lahr, J., Münier, B., De Lange, H.J., Faber, J.F., Sørensen, P.B., 2010. Wildlife vulnerability and risk maps for combined pollutants. Sci. Total Environ. 408 (18), 3891–3898. https://doi.org/10.1016/j.scitotenv.2009.11.018.
Larras, F., Montuelle, B., Bouchez, A., 2013. Assessment of toxicity thresholds in aquatic environments: does benthic growth of diatoms affect their exposure and sensitivity to herbicides? Sci. Total Environ. 463-464, 469–477. https://doi.org/10.1016/j.scitotenv.2013.06.063.
Lavoie, I., Hamilton, P.B., Morin, S., Tiam, S.K., Kahlert, M., Gonçalves, S., Falasco, E., Fortin, C., Gontero, B., Heudre, D., Kojadinovic-Sirinelli, M., Manoylov, K., Pandey, L.K., Taylor, J.C., 2017. Diatom teratologies as biomarkers of contamination: are all deformities ecologically meaningful? Ecol. Ind. 82, 539–550. https://doi.org/10.1016/j.ecolind.2017.06.048.
Legendre, P., Legendre, L., 2012. Numerical Ecology, third ed. Elsevier.
Lencioni, V., Marziali, L., Rossaro, B., 2012. Chironomids as bioindicators of environmental quality in mountain springs. Freshwater Science 31 (2), 525–541. https://doi.org/10.1899/11-038.1.
Liess, M., Schäfer, R.B., Schriever, C.A., 2008. The footprint of pesticide stress in communities—species traits reveal community effects of toxicants. Sci. Total Environ. 406 (3), 484–490. https://doi.org/10.1016/j.scitotenv.2008.05.054.

Liu, Z., Huang, S., Sun, G., Xu, Z., Xu, M., 2012. Phylogenetic diversity, composition and distribution of bacterioplankton community in the Dongjiang River, China. FEMS Microbiol Ecol 80 (1), 30–44. https://doi.org/10.1111/j.1574-6941.2011.01268.x.

Macário, I.P.E., Castro, B.B., Nunes, I.M.S., Pizarro, C., Coelho, C., Gonçalves, F., de Figueiredo, D.R., 2017. Stepwise strategy for monitoring cyanobacterial blooms. Environ. Monit. Assess. 189 https://doi.org/10.1007/s10661-017-6292-9.

Machado, R.M., Monteggia, L.O., Arenzon, A., Curia, A.C., 2016. Assessment of the toxicity of wastewater from the metalworking industry treated using a conventional physico-chemical process. Environ Monit Assess 188 (6). https://doi.org/10.1007/s10661-016-5361-9.

Maltby, L., Blake, N., Brock, T.C.M., Van den Brink, P.J., 2005. Insecticide species sensitivity distributions: importance of test species selection and relevance to aquatic ecosystems. Environ. Toxicol. Chem. 24, 379–388. https://doi.org/10.1897/04-025R.1.

Maltby, L., Clayton, S.A., Wood, R.M., McLoughlin, N., 2002. Evaluation of the Gammarus pulex in situ feeding assay as a biomonitor of water quality: robustness, responsiveness and relevance. Environ. Toxicol. Chem. 21, 361–368. https://doi.org/10.1897/1551-5028(2002)021<0361:eotgpi>2.0.co;2.

Martinez-Haro, M., Beiras, R., Bellas, J., Capela, R., Coelho, J.P., Lopes, I., Moreira-Santos, M., Reis-Henriques, A.M., Ribeiro, R., Santos, M.M., Marques, J.C., 2015. A review on the ecological quality status assessment in aquatic systems using community based indicators and ecotoxicological tools: what might be the added value of their combination? Ecol. Ind. 48, 8–16. https://doi.org/10.1016/j.ecolind.2014.07.024.

Martinez-Haro, M., Moreira-Santos, M., Marques, J.C., Ribeiro, R., 2014. A short-term laboratory and in situ sediment assay based on the postexposure feeding of the estuarine isopod Cyathura carinata. Environ. Res. 134, 242–250. https://doi.org/10.1016/j.envres.2014.07.013.

Massei, R., Busch, W., Wolschke, H., Schinkel, L., Bitsch, M., Schulze, T., Krauss, M., Brack, W., 2018. Screening of pesticide and biocide patterns as risk drivers in sediments of major European river mouths: ubiquitous or river basin-specific contamination? Environ. Sci. Technol. 52 (4), 2251–2260. https://doi.org/10.1021/acs.est.7b04355.

McCormick, S.D., 1993. Methods for Nonlethal Gill Biopsy and Measurement of Na + , K + -ATPase Activity. Can. J. Fish. Aquat. Sci. 50 (3), 656–658. https://doi.org/10.1139/f93-075.

McLoughlin, N., Yin, D., Maltby, L., Wood, R.M., Yu, H., 2000. Evaluation of sensitivity and specificity of two crustacean biochemical biomarkers. Environ. Toxicol. Chem. 19 (8), 2085–2092. https://doi.org/10.1002/etc.5620190818.

McWilliam, R.A., Baird, D.J., 2002. Application of postexposure feeding depression bioassays with Daphnia magna for assessment of toxic effluents in rivers. Environ. Toxicol. Chem. 21 (7), 1462–1468. https://doi.org/10.1002/etc.5620210718.

Menezes, S., Baird, D.J., Soares, A.M.V.M., 2010. Beyond taxonomy: a review of macroinvertebrate trait-based community descriptors as tools for freshwater biomonitoring. J. Appl. Ecol. 47, 711–719. https://doi.org/10.1111/j.1365-2664.2010.01819.x.

Menzie, C.A., MacDonell, M.M., Mumtaz, M., 2007. A phased approach for assessing combined effects from multiple stressors. Environ. Health Perspect. 115 (5), 807–816. https://doi.org/10.1289/ehp.9331.

Merritt, R.W., Fenoglio, S., Cummins, K.W., 2017. Promoting a functional macroinvertebrate approach in the biomonitoring of Italian lotic systems. J. Limnol. 76, 5–8. https://doi.org/10.4081/jlimnol.2016.1502.

Milinkovitch, T., Geffard, O., Geffard, A., Mouneyrac, C., Chaumot, A., Xuereb, B., Fisson, C., Minier, C., Auffret, M., Perceval, O., Egea, E., Sanchez, W., 2019. Biomarkers as tools for monitoring within the Water Framework Directive context: concept, opinions and advancement of expertise. Environ. Sci. Pollut. Res. 26 (32), 32759–32763. https://doi.org/10.1007/s11356-019-06434-x.

Millennium Ecosystem Assessment, 2005. Ecosystems and Human Well-being: Synthesis. Island Press, Washington, DC.

Miller, M.P., McKnight, D.M., Cullis, J.D., Greene, A., Vietti, K., Liptzin, D., 2009. Factors controlling streambed coverage of Didymosphenia geminata in two regulated streams in the Colorado Front Range. Hydrobiologia 630 (1), 207–218. https://doi.org/10.1007/s10750-009-9793-x.

Moss, B., 2008. The water framework directive: total environment or political compromise? Sci. Total Environ. 400 (1-3), 32–41. https://doi.org/10.1016/j.scitotenv.2008.04.029.

NASA, 2018. Measuring Vegetation (NDVI & EVI) [WWW Document]. URL <https://earthobservatory.nasa.gov/Features/MeasuringVegetation/measuring_vegetation_1.php>.

Nõges, P., Argillier, C., Borja, A., Garmendia, J.M., Hanganu, J., Kodeš, V., Pletterbauer, F., Sagouis, A., Birk, S., 2016. Quantified biotic and abiotic responses to multiple stress in freshwater, marine and ground waters. Sci. Total Environ. 540, 43–52. https://doi.org/10.1016/j.scitotenv.2015.06.045.

OECD, 2011. Test No. 201: freshwater alga and cyanobacteria, growth inhibition test. In: OECD Guidelines for the Testing of Chemicals, Section 2. OECD Publishing. https://doi.org/10.1787/9789264069923-en.

OECD, 2006. Test No. 221: Lemna sp. growth inhibition test. In: OECD Guidelines for the Testing of Chemicals, Section 2. OECD Publishing. https://doi.org/10.1787/9789264016194-en.

Oost, R. van der, Sileno, G., Janse, T., Nguyen, M.T., Besselink, H., Brouwer, A., 2017a. SIMONI (Smart Integrated Monitoring) as a novel bioabalytical strategy for water quality assessment: Part II – field feasibility survey. Environ. Toxicol. Chem. 36, 2400–2416. https://doi.org/10.1002/etc.3837.

Oost, R. van der, Sileno, G., Suárez-Muñoz, M., Nguyen, M.T., Besselink, H., Brouwer, A., 2017b. SIMONI (Smart Integrated Monitoring) as a novel bioanalytical strategy for water quality assessment: Part I-model design and effect-based trigger values: SIMONI: Smart bioanalytical monitoring of micropollutants. Environ. Toxicol. Chem. 36 (9), 2385–2399. https://doi.org/10.1002/etc.3836.

Ormerod, S.J., Dobson, M., Hildrew, A.G., Townsend, C.R., 2010. Multiple stressors in freshwater ecosystems. Freshw. Biol. 55, 1–4. https://doi.org/10.1111/j.1365-2427.2009.02395.x.

Pallottini, M., Cappelletti, D., Fabrizi, A., Gaino, E., Goretti, E., Selvaggi, R., Céréghino, R., 2017. Macroinvertebrate functional trait responses to chemical pollution in agricultural-industrial landscapes: macroinvertebrate functional trait responses to chemical pollution. River Res. Appl. 33 (4), 505–513. https://doi.org/10.1002/rra.3101.

Pardo, I., Gómez-Rodríguez, C., Wasson, J.-G., Owen, R., van de Bund, W., Kelly, M., Bennett, C., Birk, S., Buffagni, A., Erba, S., Mengin, N., Murray-Bligh, J., Ofenböeck, G., 2012. The European reference condition concept: a scientific and technical approach to identify minimally-impacted river ecosystems. Sci. Total Environ. 420, 33–42. https://doi.org/10.1016/j.scitotenv.2012.01.026.

Pascoal, C., Pinho, M., Cássio, F., Gomes, P., 2003. Assessing structural and functional ecosystem condition using leaf breakdown: studies on a polluted river. Freshwater Biol 48 (11), 2033–2044. https://doi.org/10.1046/j.1365-2427.2003.01130.x.

Passy, S.I., Bode, R.W., Carlson, D.M., Novak, M.A., 2004. Comparative environmental assessment in the studies of benthic diatom, macroinvertebrate, and fish communities. Internat. Rev. Hydrobiol. 89 (2), 121–138. https://doi.org/10.1002/iroh.200310721.

Pawlowski, J., Kelly-Quinn, M., Altermatt, F., Apothéloz-Perret-Gentil, L., Beja, P., Boggero, A., Borja, A., Bouchez, A., Cordier, T., Domaizon, I., Feio, M.J., Filipe, A.F., Fornaroli, R., Graf, W., Herder, J., van der Hoorn, B., Jones, J.I., Sagova-Mareckova, M., Moritz, C., Barquín, J., Piggott, J.J., Pinna, M., Rimet, F., Rinkevich, B., Sousa-Santos, C., Specchia, V., Trobajo, R., Vasselon, V., Vitecek, S., Zimmerman, J., Weigand, A., Leese, F., Kahlert, M., 2018. The future of biotic indices in the ecogenomic era: integrating (e)DNA metabarcoding in biological assessment of aquatic ecosystems. Sci. Total Environ. 637-638, 1295–1310. https://doi.org/10.1016/j.scitotenv.2018.05.002.

Pereira, J.L., Pereira, P., Padeiro, A., Gonçalves, F., Amaro, E., Leppe, M., Verkulich, S., Hughes, K.A., Peter, H.-U., Canário, J., 2017a. Environmental hazard assessment of contaminated soils in Antarctica: using a structured tier 1 approach to inform decision-making. Sci. Total Environ. 574, 443–454. https://doi.org/10.1016/j.scitotenv.2016.09.091.

Pereira, J.L., Vidal, T., Mendes, C., Ré, A., Santos, J.I., Gonçalves, F., Castro, B.B., 2017b. Invasive Asian clam distribution pattern reveals minimal constraints to downstream dispersal and imperceptible ecological impacts. Aquatic Conserv: Mar Freshw Ecosyst 27 (5), 953–964. https://doi.org/10.1002/aqc.2777.

Piccolo, J.J., Washington, H., Kopnina, H., Taylor, B., 2018. Why conservation scientists should re-embrace their ecocentric roots: conservation and Ecocentrism. Conserv. Biol. 32 (4), 959–961. https://doi.org/10.1111/cobi.13067.

Pilliod, D.S., Goldberg, C.S., Arkle, R.S., Waits, L.P., 2013. Estimating occupancy and abundance of stream amphibians using environmental DNA from filtered water samples. Can. J. Fish. Aquat. Sci. 70 (8), 1123–1130. https://doi.org/10.1139/cjfas-2013-0047.

Poikane, S., Zampoukas, N., Borja, A., Davies, S.P., van de Bund, W., Birk, S., 2014. Intercalibration of aquatic ecological assessment methods in the European Union: lessons learned and way forward. Environ. Sci. Policy 44, 237–246. https://doi.org/10.1016/j.envsci.2014.08.006.

Posthuma, L., van Gils, J., Zijp, M.C., van de Meent, D., de Zwart, D., 2019. Species sensitivity distributions for use in environmental protection, assessment, and management of aquatic ecosystems for 12 386 chemicals. Environ. Toxicol. Chem. 38, 905–917. https://doi.org/10.1002/etc.4373.

Posthuma, L., SuterII, G.W., Traas, T.P. (Eds.), 2002. Species Sensitivity Distributions in Ecotoxicology. Lewis Publishers, Boca Raton, Florida, USA.

Pottier, G., Beaumont, W.R., Marchand, F., Le Bail, P.-Y., Azam, D., Rives, J., Vigouroux, R., Roussel, J.-M., 2020. Electrofishing in streams of low water conductivity but high biodiversity value: challenges, limits and perspectives. Fish Manag. Ecol. 27 (1), 52–63. https://doi.org/10.1111/fme.12384.

Prat, N., Rieradevall, M., Barata, C., Munné, A., 2013. The combined use of metrics of biological quality and biomarkers to detect the effects of reclaimed water on macroinvertebrate assemblages in the lower part of a polluted Mediterranean river (Llobregat River, NE Spain). Ecol. Ind. 24, 167–176. https://doi.org/10.1016/j.ecolind.2012.06.010.

Ramos-Merchante, A., Prenda, J., 2017. Macroinvertebrate taxa richness uncertainty and kick sampling in the establishment of Mediterranean rivers ecological status. Ecol. Ind. 72, 1–12. https://doi.org/10.1016/j.ecolind.2016.07.047.

Reid, A.J., Carlson, A.K., Creed, I.F., Eliason, E.J., Gell, P.A., Johnson, P.T.J., Kidd, K.A., MacCormack, T.J., Olden, J.D., Ormerod, S.J., Smol, J.P., Taylor, W.W., Tockner, K., Vermaire, J.C., Dudgeon, D., Cooke, S.J., 2019. Emerging threats and persistent conservation challenges for freshwater biodiversity. Biol Rev 94 (3), 849–873. https://doi.org/10.1111/brv.12480.

Reyjol, Y., Argillier, C., Bonne, W., Borja, A., Buijse, A.D., Cardoso, A.C., Daufresne, M., Kernan, M., Ferreira, M.T., Poikane, S., Prat, N., Solheim, A.-L., Stroffek, S., Usseglio-Polatera, P., Villeneuve, B., van de Bund, W., 2014. Assessing the ecological status in the context of the European Water Framework Directive: where do we go now? Sci. Total Environ. 497-498, 332–344. https://doi.org/10.1016/j.scitotenv.2014.07.119.

Rial, D., Beiras, R., 2012. Prospective ecological risk assessment of sediment resuspension in an estuary. J. Environ. Monit. 14 (8), 2137. https://doi.org/10.1039/c2em30225j.

Rodrigues, C., Guimarães, L., Vieira, N., 2019. Combining biomarker and community approaches using benthic macroinvertebrates can improve the assessment of the ecological status of rivers. Hydrobiologia 839 (1), 1–24. https://doi.org/10.1007/s10750-019-03991-7.

Roig, N., Sierra, J., Nadal, M., Moreno-Garrido, I., Nieto, E., Hampel, M., Gallego, E.P., Schuhmacher, M., Blasco, J., 2015. Assessment of sediment ecotoxicological status as a complementary tool for the evaluation of surface water quality: the Ebro river basin case study. Sci. Total Environ. 503-504, 269–278. https://doi.org/10.1016/j.scitotenv.2014.06.125.
Rosenfeld, J.S., 2002. Functional redundancy in ecology and conservation. Oikos 98 (1), 156–162. https://doi.org/10.1034/j.1600-0706.2002.980116.x.
Santonja, M., Pellan, L., Piscart, C., 2018. Macroinvertebrate identity mediates the effects of litter quality and microbial conditioning on leaf litter recycling in temperate streams. Ecol Evol 8 (5), 2542–2553. https://doi.org/10.1002/ece3.3790.
Santos, M., Oliveira, H., Pereira, J.L., Pereira, M.J., Gonçalves, F.J.M., Vidal, T., 2019. Flow cytometry analysis of low/high DNA content (LNA/HNA) bacteria as bioindicator of water quality evaluation. Ecol. Ind. 103, 774–781. https://doi.org/10.1016/j.ecolind.2019.03.033.
Satapornvanit, K., Baird, D.J., Little, D.C., 2009. Laboratory toxicity test and post-exposure feeding inhibition using the giant freshwater prawn Macrobrachium rosenbergii. Chemosphere 74 (9), 1209–1215. https://doi.org/10.1016/j.chemosphere.2008.11.033.
Schmidt, T.C., 2018. Recent trends in water analysis triggering future monitoring of organic micropollutants. Anal. Bioanal. Chem. 410 (17), 3933–3941. https://doi.org/10.1007/s00216-018-1015-9.
Schmitt-Jansen, M., Altenburger, R., 2008. Community-level microalgal toxicity assessment by multiwavelength-excitation PAM fluorometry. Aquat. Toxicol. 86 (1), 49–58. https://doi.org/10.1016/j.aquatox.2007.10.001.
Schmitt-Jansen, M., Veit, U., Dudel, G., Altenburger, R., 2008. An ecological perspective in aquatic ecotoxicology: approaches and challenges. Basic Appl. Ecol. 9, 337–345. https://doi.org/10.1016/j.baae.2007.08.008.
Schulze, T., Ahel, M., Ahlheim, J., Aït-Aïssa, S., Brion, F., Di Paolo, C., Froment, J., Hidasi, A.O., Hollender, J., Hollert, H., Hu, M., Kloß, A., Koprivica, S., Krauss, M., Muz, M., Oswald, P., Petre, M., Schollée, J.E., Seiler, T.-B., Shao, Y., Slobodnik, J., Sonavane, M., Suter, M.J.-F., Tollefsen, K.E., Tousova, Z., Walz, K.-H., Brack, W., 2017. Assessment of a novel device for onsite integrative large-volume solid phase extraction of water samples to enable a comprehensive chemical and effect-based analysis. Sci. Total Environ. 581-582, 350–358. https://doi.org/10.1016/j.scitotenv.2016.12.140.
Schüttrumpf, H., Brinkmann, M., Cofalla, C., Frings, R.M., Gerbersdorf, S.U., Hecker, M., Hudjetz, S., Kammann, U., Lennartz, G., Roger, S., Schäffer, A., Hollert, H., 2011. A new approach to investigate the interactions between sediment transport and ecotoxicological processes during flood events. Environ. Sci. Eur. 23, 1–5. https://doi.org/10.1186/2190-4715-23-39.
Serra, S.R.Q., Graça, M.A.S., Dolédec, S., Feio, M.J., 2017. Chironomidae traits and life history strategies as indicators of anthropogenic disturbance. Environ. Monit. Assess. 189 (7) https://doi.org/10.1007/s10661-017-6027-y.
Shiklomanov, I.A., 2000. Appraisal and assessment of world water resources. Water Int. 25 (1), 11–32. https://doi.org/10.1080/02508060008686794.
Skoulikidis, N.T., Vardakas, L., Karaouzas, I., Economou, A.N., Dimitriou, E., Zogaris, S., 2011. Assessing water stress in Mediterranean lotic systems: insights from an artificially intermittent river in Greece. Aquat. Sci. 73 (4), 581–597. https://doi.org/10.1007/s00027-011-0228-1.
Solheim, A.L., Globevnik, L., Austnes, K., Kristensen, P., Moe, S.J., Persson, J., Phillips, G., Poikane, S., van de Bund, W., Birk, S., 2019. A new broad typology for rivers and lakes in Europe: development and application for large-scale environmental assessments. Sci. Total Environ. 697, 134043. https://doi.org/10.1016/j.scitotenv.2019.134043.
Solimini, A.G., Ptacnik, R., Cardoso, A.C., 2009. Towards holistic assessment of the functioning of ecosystems under the Water Framework Directive. TrAC, Trends Anal. Chem. 28 (2), 143–149. https://doi.org/10.1016/j.trac.2008.10.015.
Stoddard, J.L., Larsen, D.P., Hawkins, C.P., Johnson, R.K., Norris, R.H., 2006. Setting expectations for the ecological condition of streams: the concept of reference condition. Ecol. Appl. 16, 1267–1276. https://doi.org/10.1890/1051-0761(2006)016[1267:SEFTEC]2.0.CO;2.
Sun, X., Wu, N., Faber, C., Fohrer, N., 2018. Effects of hydrological variables on structuring morphological trait (cell size) of diatom community in a lowland river. Ecol. Ind. 94, 207–217. https://doi.org/10.1016/j.ecolind.2018.06.044.
SuterII, G.W., 2006. Ecological Risk Assessment, second. ed. CRC Press.
Swanson, S., Kozlowski, D., Hall, R., Heggem, D., Lin, J., 2017. Riparian proper functioning condition assessment to improve watershed management for water quality. J. Soil Water Conserv. 72 (2), 168–182. https://doi.org/10.2489/jswc.72.2.168.
Tapolczai, K., Bouchez, A., Stenger-Kovács, C., Padisák, J., Rimet, F., 2016. Trait-based ecological classifications for benthic algae: review and perspectives. Hydrobiologia 776 (1), 1–17. https://doi.org/10.1007/s10750-016-2736-4.
Tousova, Z., Oswald, P., Slobodnik, J., Blaha, L., Muz, M., Hu, M., Brack, W., Krauss, M., Di Paolo, C., Tarcai, Z., Seiler, T.-B., Hollert, H., Koprivica, S., Ahel, M., Schollée, J.E., Hollender, J., Suter, M.J.-F., Hidasi, A.O., Schirmer, K., Sonavane, M., Ait-Aissa, S., Creusot, N., Brion, F., Froment, J., Almeida, A.C., Thomas, K., Tollefsen, K.E., Tufi, S., Ouyang, X., Leonards, P., Lamoree, M., Torrens, V.O., Kolkman, A., Schriks, M., Spirhanzlova, P., Tindall, A., Schulze, T., 2017. European demonstration program on the effect-based and chemical identification and monitoring of organic pollutants in European surface waters. Sci. Total Environ. 601-602, 1849–1868. https://doi.org/10.1016/j.scitotenv.2017.06.032.
Turley, M.D., Bilotta, G.S., Chadd, R.P., Extence, C.A., Brazier, R.E., Burnside, N.G., Pickwell, A.G.G., 2016. A sediment-specific family-level biomonitoring tool to identify the impacts of fine sediment in temperate rivers and streams. Ecol. Ind. 70, 151–165. https://doi.org/10.1016/j.ecolind.2016.05.040.
Turley, M.D., Bilotta, G.S., Krueger, T., Brazier, R.E., Extence, C.A., 2015. Developing an improved biomonitoring tool for fine sediment: combining expert knowledge and empirical data. Ecol. Ind. 54, 82–86. https://doi.org/10.1016/j.ecolind.2015.02.011.
U.S.EPA, 1992. Framework for Ecological Risk Assessment.
UNEnvironment, 2019. Environmental Rule of Law - First Global Report.
Van Ael, E., De Cooman, W., Blust, R., Bervoets, L., 2015. Use of a macroinvertebrate based biotic index to estimate critical metal concentrations for good ecological water quality. Chemosphere 119, 138–144. https://doi.org/10.1016/j.chemosphere.2014.06.001.
van de Bund, W., Poikane, S., 2015. Water Framework Directive scientific and technical support related to ecological status. https://doi.org/10.2788/071200.
Verdonschot, P.F.M., 2000. Integrated ecological assessment methods as a basis for sustainable catchment management. Hydrobiologia 422 (423), 389–412. https://doi.org/10.1023/A:1017094905369.
Vidal, T., Pereira, J.L., Abrantes, N., Soares, A.M.V.M., Gonçalves, F., 2012. Ecotoxicological assessment of contaminated river sites as a proxy for the water framework directive: an acid mine drainage case study. Water Air Soil Pollut. 223 (9), 6009–6023. https://doi.org/10.1007/s11270-012-1335-x.
Vidal, T., Santos, J.I., Queirós, L., Ré, A., Abrantes, N., Gonçalves, F.J.M., Pereira, J.L., 2019. Environmental benchmarks based on ecotoxicological assessment with planktonic species might not adequately protect benthic assemblages in lotic systems. Sci. Total Environ. 668, 1289–1297. https://doi.org/10.1016/j.scitotenv.2019.03.067.
Visco, J.A., Apothéloz-Perret-Gentil, L., Cordonier, A., Esling, P., Pillet, L., Pawlowski, J., 2015. Environmental monitoring: inferring the diatom index from next-generation sequencing data. Environ. Sci. Technol. 49 (13), 7597–7605. https://doi.org/10.1021/es506158m.
Vörösmarty, C.J., McIntyre, P.B., Gessner, M.O., Dudgeon, D., Prusevich, A., Green, P., Glidden, S., Bunn, S.E., Sullivan, C.A., Liermann, C.R., Davies, P.M., 2010. Global threats to human water security and river biodiversity. Nature 467 (7315), 555–561. https://doi.org/10.1038/nature09440.
Voulvoulis, N., Arpon, K.D., Giakoumis, T., 2017. The EU Water Framework Directive: from great expectations to problems with implementation. Sci. Total Environ. 575, 358–366. https://doi.org/10.1016/j.scitotenv.2016.09.228.
Wallace, J.B., Webster, J.R., 1996. The role of macroinvertebrates in stream ecosystem function. Annu. Rev. Entomol. 41 (1), 115–139. https://doi.org/10.1146/annurev.en.41.010196.000555.
Wernersson, A.-S., Carere, M., Maggi, C., Tusil, P., Soldan, P., James, A., Sanchez, W., Dulio, V., Broeg, K., Reifferscheid, G., Buchinger, S., Maas, H., Grinten, E Van Der, O'Toole, S., Ausili, A., Manfra, L., Marziali, L., Polesello, S., Lacchetti, I., Mancini, L., Lilja, K., Linderoth, M., Lundeberg, T., Fjällborg, B., Porsbring, T., Larsson, D.J., Bengtsson-Palme, J., Förlin, L., Kienle, C., Kunz, P., Vermeirssen, E., Werner, I., Robinson, C.D., Lyons, B., Katsiadaki, I., Whalley, C., den Haan, K., Messiaen, M., Clayton, H., Lettieri, T., Carvalho, R.N., Gawlik, B.M., Hollert, H., Di Paolo, C., Brack, W., Kammann, U., Kase, R., 2015. The European technical report on aquatic effect-based monitoring tools under the water framework directive. Environ. Sci. Eur. 27 (1) https://doi.org/10.1186/s12302-015-0039-4.
Wood, R.J., Mitrovic, S.M., Kefford, B.J., 2014. Determining the relative sensitivity of benthic diatoms to atrazine using rapid toxicity testing: a novel method. Sc. Total Environ. 485-486, 421–427. https://doi.org/10.1016/j.scitotenv.2014.03.115.
Wu, N., Schmalz, B., Fohrer, N., 2012. Development and testing of a phytoplankton index of biotic integrity (P-IBI) for a German lowland river. Ecol. Ind. 13 (1), 158–167. https://doi.org/10.1016/j.ecolind.2011.05.022.
Wu, N., Schmalz, B., Fohrer, N., 2011. Distribution of phytoplankton in a German lowland river in relation to environmental factors. J. Plankton Res. 33 (5), 807–820. https://doi.org/10.1093/plankt/fbq139.
Young, R.G., Matthaei, C.D., Townsend, C.R., 2008. Organic matter breakdown and ecosystem metabolism: functional indicators for assessing river ecosystem health. J. North Am. Benthol. Soc. 27 (3), 605–625. https://doi.org/10.1899/07-121.1.
Zalack, J.T., Smucker, N.J., Vis, M.L., 2010. Development of a diatom index of biotic integrity for acid mine drainage impacted streams. Ecol. Indic. 10, 287–295. https://doi.org/10.1016/j.ecolind.2009.06.003.
Zoppini, A., Ademollo, N., Amalfitano, S., Casella, P., Patrolecco, L., Polesello, S., 2014. Organic priority substances and microbial processes in river sediments subject to contrasting hydrological conditions. Sci. Total Environ. 484, 74–83. https://doi.org/10.1016/j.scitotenv.2014.03.019.