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Benthic algae assessments in the EU and the US: Striving for consistency in the face of great ecological diversity

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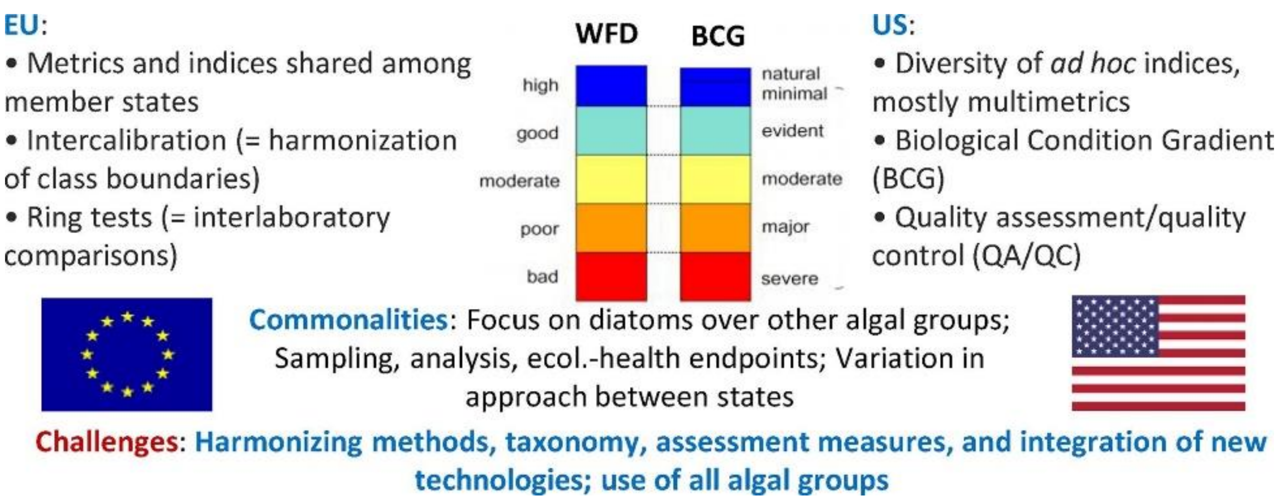
Graphical abstract



Highlights

- Benthic algae assessments in the EU and US share common goals based in policy.
- Diatoms are primary indicators in both EU and US.
- Assessment sampling, analysis, and ecological health endpoints are similar.
- Considerable variation in approach among states in both EU and US.
- Harmonizing taxonomy and endpoints plus new technologies will strengthen programs.

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ABSTRACT

Freshwaters face multiple environmental problems including eutrophication, acidification, salinization, and climate-change, all of which can lead to impairment of ecosystem structure and function. Furthermore, these stressors often act in combination. Benthic algal-based assessments to quantify impairment are used in both the EU and US. In this review, we use case studies, experience, and the literature to compare concepts, approaches, and methods between the EU and US to offer an updated picture of benthic algal-based assessments. Both the US and EU are composed of numerous constituent states having considerable flexibility to adopt individual methods. The goal of this work is to synthesize the various approaches that are used across the EU and US. Specifically, we compare and contrast benthic algal assessment performed in response to core legislation – the Water Framework Directive in the EU and the Clean Water Act in the US, with a particular focus on the steps taken to ensure consistency at different stages of the process. This includes consideration of approaches to sampling design and field methods, taxonomic resolution and laboratory harmonization, metric selection and choice of algal groups, assessment of stressors and stressor/response relationships. A number of commonalities emerged during this process, particularly the focus on diatoms over other algal groups. However, there are also a number of key differences, including more widespread use of multimetric indices in the US compared with the EU. Finally, we consider emerging opportunities, including the potential for using metagenomic approaches for bioassessment in the future.

1. Introduction

Freshwaters are a vital yet threatened resource, providing a range of ecosystem services, including drinking water, fisheries and irrigation along with recreational opportunities that enhance well-being. Ensuring the good condition of freshwaters, therefore, contributes to the overall resilience of societies. Legislation to meet this objective has been developed in many parts of the world with early attempts (e.g. Federal Water Pollution Control Act (FWPCA, 1948)) tackling the most extreme manifestations of ecosystem degradation such as toxic pollution. Later legislation focused on effects-based management to reduce toxic pollution and ensure waters provided at least a basic level of ecosystem services (United States Clean Water Act, 1972), which included protection of ecosystem health.

The application of benthic algae ("*Aufwuchs*", "periphyton", or "phytobenthos") in environmental assessment started over a century ago in Europe (Kolkwitz and Marsson, 1908), and continued throughout the 20th century, with diatoms being key indicators of environmental changes that helped solve problems with organic, nutrient and toxic substance pollution of lakes and streams. Parallel to this was Ruth Patrick's pioneering work assessing diversity responses of diatoms to pollution in the US (Patrick, 1949; Patrick and Strawbridge, 1963).

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Since the 1980's, benthic algal assessments in the European Union (EU) and United States (US) have expanded conceptually because of the essential role algae play in primary production, food webs, biogeochemical cycling, and microbial biodiversity.

This Virtual Special Issue (VSI) is a collection of papers produced from the latest of a series of symposia on the use of algae for monitoring freshwaters that started in 1991. Historically, most of the papers in these symposia were from Europe, but not all. Even a brief comparison of the contents of this volume with those of the early volumes (Whitton et al., 1991; Whitton and Rott, 1996) is enough to highlight a significant shift: the early volumes focus on the potential of algae-based assessments in Europe for water quality, whereas subsequent ones document their gradual development to measure ecosystem health and their incorporation into the regulator's toolkit to, eventually, their enshrinement in Europe's core legislation, the Water Framework Directive (WFD: European Union, 2000).

The track for adoption of benthic algal assessments in the US has been similar to that in the EU, but for different reasons and with less complete adoption across all states. As in the EU, research and application of benthic algal assessments were conducted at smaller scales: watersheds, states, and then regional scales in pilot projects. Early projects established the potential for benthic algal assessments, which then became more widely adopted by states for two reasons. First, the Clean Water Act Action Plan (US EPA, 2009a) called for all states to establish nutrient criteria for all waters and, as benthic algae were highly sensitive to nutrients, they provided complementary biological information. Second, the United States Environmental Protection Agency (US EPA, 2013a) called for all states to use two groups of organisms in assessment of the biological condition of state waters. Macroinvertebrates were the first choice for almost all states; whilst assessments of fish were also well established in many states. However, because some states did not have diverse fish communities in their waters, benthic algae became a good second option for assessing biological condition.

Widespread adoption of benthic algal assessments has brought with it many challenges, several of which are explored in this VSI. In particular, environmental legislation was often enacted and enforced within structures in which individual entities (States in the US, Member States in the EU) have considerable independence to interpret and enforce the primary legislation. Some EU Member States are, themselves, federal or devolved structures, adding a further layer of complication. Furthermore, the legislation applies across large geographical areas, spanning considerable climatic, geological and ecological diversity. These natural gradients, in turn, shape human activities within catchments which, in turn, translate into different types and intensities of pressures facing freshwaters.

One result of this heterogeneity is that a wide range of approaches for assessing benthic algae in freshwaters have been developed, each tuned to a specific set of local or regional factors. Yet, there is also a need for consistency at different levels. Within a (member) state, this might be manifest in the need to ensure consistency among analyses so that index values, as far as possible, reflect the condition of a waterbody rather than variations in analytical methods and natural variability among sites. Consistency of a different sort is necessary when applying the broad principles of legislation to all entities within a federal (or quasi-federal) structure and using benthic algae in sufficiently similar ways to address goals of the legislation. First, this ensures that the ambition of the policy is shared by all; second, having shared benchmarks means that the effectiveness of implementation can be tracked in space and time, authorities can be held accountable and lessons can be learned and shared.

Now is an opportune time to review, synthesize and develop a vision for next steps because of the widespread adoption and implementation of benthic algal assessment programs. Here we compare the many approaches used in benthic algal assessment, their similarities and differences as well as their successes and challenges, to advance the science and value of benthic algal assessments. We compare assessments employed by the countries of the EU and the states of the US (henceforth referred to collectively as "(member) states"). In addition, we evaluate challenges with consistency and aggregating assessments among (member) states within these federations. Many other large and economically-significant countries are also federal structures so lessons learned by the EU and US on the challenges of developing bioassessment programs will need to be understood by these if global environmental objectives are to be achieved.

The VSI for which this review serves as an introduction includes papers on approaches and perspectives in freshwater benthic algae-based assessment and monitoring. Several of these papers were presented at a special session on algae-based assessments during the '11th Symposium for European Freshwater Sciences (SEFS)'. Whilst our focus is on rivers, we also draw on information on the use of benthic algae in lakes, where appropriate.

2. Methods

Our comparison of benthic-algae-based assessments in the EU and US is derived from:

- searches in Scopus, Web of Science, and Google Scholar (June 2019 and July 2020), using as search terms "alg* assessments", "alg* monitoring", "comparison EU-USA", "WFD", "BCG", "QA/QC", "intercalibration", "harmonization", "ring-test" etc. and combinations of these;
- contributions and discussions at a special session (10th UAMRICH – 'Use of algae for monitoring rivers and comparable habitats') organized as part of the '11th Symposium for European Freshwater Sciences (SEFS)' (Zagreb, Croatia, June 30 to July 5, 2019), attended by academics and professionals involved in algal assessments both in the EU and in the US;
- our own scientific-literature collections on the relevant topics and personal experience – all of us have been intensively involved in this type of environmental assessments in the EU or US, and one of us (MC) has experienced both;
- descriptions of biological assessment methods implemented in the WFD across the 27 Member States.

The authors discussed the structure of the paper and the topics of the individual sections during a series of weekly online meetings between June and August 2020.

3. Goals of assessment based in legislation

Goals for ecological assessment are mainly associated with legislation and enforcement of government policies. Whilst assessments can also be part of research related to non-government organizations and businesses seeking new knowledge or solutions to environmental problems, this paper focuses on assessment goals derived from legislation.

3.1. Water Framework Directive (WFD)

The WFD has a generic goal of "achieving good surface water status" (Article 4, clause 1) with Annex V laying out in detail what this entails. In brief, "good surface water status" can be summarized as the state where hydromorphological, hydrological, chemical and ecological criteria show no more than a slight deviation from that expected in the absence of significant anthropogenic alterations. For freshwaters, benthic algae are covered by the "macrophytes and phytobenthos" biological quality element within the ecological criteria and Member States have generally considered "phytobenthos" to refer to microscopic algae although some include larger filamentous and crust-forming algae in their macrophyte assessment systems.

Whilst “good surface water status” is the de facto target, the WFD also includes “no deterioration” clauses (meaning that a water body judged to be at high status cannot be allowed to fall to good status) and recognises that, for some water bodies, other legislation such as the Habitats Directive (EEC, 1992) may require higher standards. Finally, there are provisions for “less stringent objectives” (Article 4 clause 5) in situations where achievement of good status is infeasible or disproportionately expensive. This means that there are many waterbodies where the target is moderate status (or even lower, in a few cases). When good status is not achieved, a “program of measures” is required to address the problems and restore the water body to a state where it can support a healthy biota again. Different criteria apply to artificial or heavily-modified water bodies, but this falls beyond the scope of this review. Ecosystem services are not explicitly acknowledged in the text of the WFD, although they do form an integral part of catchment planning in many EU states (Carvalho et al., 2019).

Whilst Annex V focuses on narrative descriptions (“normative definitions”) of five distinct ecological status classes, practical assessment is based on a continuous scale, the “ecological quality ratio”, in which the observed state is the numerator and the expected state the denominator. This means that all national assessment systems, in theory, depend upon characterisation of “reference conditions” from which the value of metrics when human impacts are absent or minimal can be derived. This was the focus of much attention in the early years of WFD implementation (Pardo et al., 2012; Kelly et al., 2012; Feio et al., 2014) but the long history of human engagement with the European landscape means that in many parts of lowland Europe, true “reference sites” are not available and alternative solutions need to be sought (Birk et al., 2012a; Kelly et al., 2020a).

A further requirement of the WFD is that national assessment systems are subject to a process known as “intercalibration” (Annex V, clause 1.4.1) which ensures that the approach to assessment covers all the criteria mentioned in the normative definitions and that boundaries between high, good and moderate ecological status are consistent amongst Member States (Birk et al., 2013; Poikane et al., 2015). In practice, these exercises proved to be invaluable for the exchange of ideas amongst national experts struggling to implement the WFD against tight deadlines. Where reference conditions were available, the exercise was relatively straightforward but alternative approaches needed to be devised for the many situations where this was not the case (Birk and Hering, 2009; Kelly et al., 2014). Intercalibration enabled Member States to adjust their boundaries to ensure consistency amongst countries that shared similar river or lake types and, over time, consensus views of “high” and “good” status have emerged, against which new methods can be aligned directly, even if no reference sites are available (Kelly et al., 2019).

3.2. Clean Water Act (CWA)

The CWA, more formally known as the Federal Water Pollution Control Act of 1972, states in Section 101(a), “The objective of this Act is to restore and maintain the chemical, physical, and biological integrity of the Nation’s waters.” To achieve this objective, CWA Sections 101(a.1 and a.2) call for the elimination of pollution discharged into navigable waters and “wherever attainable, an interim goal of water quality that provides for the protection and propagation of fish, shellfish, and wildlife and provides for recreation in and on the water (the so-called “fishable/swimmable goal”). These legislated goals needed to be translated into measurable endpoints by the US EPA, which was tasked to interpret and enforce much of the law.

All waterbodies of the US are protected by water quality standards composed of three parts: a designated use, criteria to protect this designated use, and an antidegradation clause. Waterbodies can have more than one designated use. Drinking water and recreational uses such as fishing, swimming, and boating require low microbial contamination and benefit from low algal biomass. Agricultural and industrial water supply as well as navigation are uses that benefit from high water quality, but sometimes call for waterbody alterations that require tradeoffs with other uses. Aquatic life use is also directly related to both the ultimate CWA goal of biological integrity and the interim CWA goal of protecting and propagating fish, shellfish and wildlife. Water quality criteria serve as management targets that will protect or restore designated uses. Antidegradation clauses are usually more generally written and call for protection of waterbodies so that conditions do not get worse.

When standards for a waterbody are not met, waterbodies are put on the 303(d) list, named for the CWA section that describes this process. Waterbodies on the 303(d) list should be restored by reducing pollution and habitat alterations sufficiently to meet designated uses.

In the decade after the CWA was passed, the US EPA employed pollution discharge permits and also convened a panel of experts to determine how to define biological integrity (US EPA, 1977). Biological assessment of waters was a young science at this time and whilst an understanding of the ecological impacts of pollution was emerging (Hynes, 1963), there were few attempts to apply this knowledge to water management. In 1981, Karr developed the concept of multimetric indices of biological integrity which have been used widely with fish, invertebrates, and algae in US streams and rivers. More recently, Davies and Jackson (2006) convened a workgroup of state and federal resource and policy managers, as well as university scientists, to develop concepts for standardizing the conceptual foundation and characterization of biological condition in US streams and rivers. These concepts (which will be explained more fully in Section 5.2) serve as a foundation for characterizing biological condition for many groups of organisms and habitats.

Responsibility for developing methods for managing waters under their jurisdiction lies with States and Native American tribes. However, the methods for assessing waters must be approved by the US EPA, which publishes guidelines for these methods. If the methods do not meet approval of the US EPA, states and tribes are asked to revise and resubmit methods that will meet US EPA approval. If satisfactory methods are not submitted by states and tribes, the US EPA has authority to promulgate methods for states and tribes to use. The result of this, despite the oversight of the US EPA, is considerable variation in methods used to assess the biological condition of their waters.

Assessments of sustainability call for evaluations of ecosystem services, their valuation, and the costs and benefits of environmental management strategies (NRC, 2011). The CWA also calls for consideration of economic cost and benefit of environmental decisions (CWA 33USC1314), as well as designated uses of water bodies which are themselves ecosystem services. Calls for sustainability analysis and CWA requirements have led to conceptualization and research by US EPA on topics of ecosystem services and their valuation to help resource economists with needed cost-benefit analyses (Boyd and Banzhaf, 2007; US EPA, 2015; US EPA, 2013b).

4. EU and US assessment programs – overview

The EU, US and their (member) state level agencies have primary responsibility for achieving the goals of the WFD and CWA. They implement the stream and river bioassessment programs that provide information on which policies, regulations and other measures are based. Assessment programs are also carried out by other administrative and non-governmental agencies, but they are usually smaller in scale, and use methods similar to those in larger programs. They are not considered in this review. The programs evaluated for this review are described below.

4.1. EU programs

In the EU, all assessment programs are carried out by Member States following the WFD’s requirement that “Member States shall ensure the establishment of programs for the monitoring of water status in order to establish a coherent and comprehensive overview of water status within each river basin district" (Article 8 clause 1). Member States are also responsible for reporting the assessment results to the Water Information System for Europe (WISE) following the WFD Reporting Guidance (EC, 2016). This information is used to derive comprehensive assessments of status and pressures of EU waters (EEA, 2018). Therefore, there is no distinct "EU-level programme"; however, all 27 EU members states 'monitoring programs could be considered as components of an WFD-monitoring network due to the consistency in their monitoring, assessment and reporting guidelines along with intercalibration (see 8.3) which sets a consistent level of ambition among ecological status classifications (Birk et al., 2013).

The WFD sets out three types of monitoring programs: surveillance, operational and investigative, each with different objectives and strategies (Table 1). These programs differ only in their objectives and design (i.e. what to monitor, where and how often) whilst assessment methods are the same for all types of monitoring. The results of monitoring determine whether water bodies are in good status and, if not, what measures need to be included in the river basin management plans in order to reach good status.

In general terms, there is good coverage across the European Union with almost 110,000 surface water monitoring stations (EC, 2019). By far the largest number of monitoring stations are located on rivers (79.5%), followed by lakes (11.8%), coastal waters (6.5%) and transitional waters (2.1%). Across the EU, 67,691 river sites were used for operational monitoring compared with 19,637 for surveillance monitoring. Benthic aquatic flora is assessed at about 40% of these.

4.2. US programs

In the US, there are three main national-level assessment programs that include benthic algae along with several state agency programs. The federal-level algal assessment programs are designed to develop a nationwide understanding of ecological conditions and the relative importance and geographic distribution of water resource issues. Most were mandated by the US Congress to help them develop legislation and set water quality standards. Nationwide assessments led by federal agencies are deemed necessary because the differences among state-level monitoring programs preclude the possibility of combining their results to provide a coherent national picture (e.g., Paulsen et al., 2020; Shapiro et al., 2008).

NARS. The US EPA National Aquatic Resource Surveys (NARS) are designed to sample 1,000 rivers and streams, lakes, wetlands and estuaries on a 5-year rotating basis. The National Rivers and Streams Assessment (NRSA) started sampling algae with the 2004 Wadeable Streams Assessment (Paulsen et al., 2020). It also sampled algae as part of the 2008–2009 (US EPA, 2016b), 2013–2014 (US EPA, 2013c) and 2018–2019 NARS studies (US EPA, 2017a, 2017b, 2019). The main purpose of NARS is to quantify the percent of water bodies in ecological condition categories in the US. Another purpose is to assess the primary stressors causing impairment. Every five years, a new set of study sites are selected from a target population of all stream reaches. This sampling design was first used with the Aquatic Effects Research Program (AERP), which later evolved into the Environmental Monitoring and Assessment Program (EMAP) before being adopted by NARS (Paulsen et al., 2020). (see https://archive.epa.gov/emap/archive-emap/web/html/index.html for EMAP reports on large regional studies in the west and mid-Atlantic highlands). The initial focus of NARS was to provide information in a short-term study-year by study-year time frame. Building capacity and infrastructure to support methods/procedures, especially for benthic algae, was not a priority. Although a goal is to compare results every 5 years to detect trends, this has not been possible for algae due to inconsistency of identifications (e.g., Paulsen et al., 2020). In the past few years, the US EPA has made efforts to build capacity and support improvements in taxonomic accuracy and consistency, including those that might allow comparisons of data from past surveys and lead to revised protocols.

NAWQA. The initial phase of the USGS National Water Quality Assessment Program (NAWQA) (1993–2005) studied streams and rivers in 51 mid-size watersheds (Study Units) located throughout the US (Carlisle et al., 2013). Sites were selected to represent major hydrologic types and the influence of different types of land-use, particularly agriculture and urbanization. The main goals were to determine ecological condition and, more particularly, to understand in detail the mechanisms of how natural and human-influenced factors affect the systems. Considerable effort was devoted at the beginning of the program to developing, testing, and documenting methods, including collecting and analyzing algae samples, with an expectation that sampling would continue for many years and a desire to avoid having to make changes (Berkman and Porter, 2004). Less intensive monitoring continued on 42 Study Units from 2002 to 2012. Beginning in 2013, NAWQA began Regional Stream Quality Assessments (RSQA) (https://webapps.usgs.gov/rsqa/#!/) studying five main regions of the country and focusing on the effects of agriculture and urbanization.

NEON. The NSF supported National Ecological Observatory Network

Table 1
The types of national monitoring programmes set out in the WFD.

Aims of the monitoring programme	Selection of monitoring points and quality elements
Surveillance monitoring programme	
To provide an assessment of the overall surface water status within each catchment or subcatchments within a River Basin District;	Sufficient water bodies to provide an assessment of the overall surface water status within each catchment and sub-catchment of a River Basin District
To provide information on long-term natural changes and long-term changes resulting from widespread anthropogenic activity;	All biological, hydromorphological and general physico-chemical quality elements for a period of at least one year during the period covered by a river basin management plan
To supplement and validate risk assessments, particularly at those sites where the degree of uncertainty is greatest	
Operational monitoring programme	
To establish the status of those bodies identified as being at risk of failing to meet their environmental objectives;	All water bodies that have been identified as being at risk of failing the relevant environmental objectives
To assess whether the measures aimed at achieving environmental objectives are successful	Quality elements most sensitive to the pressures to which the body or bodies are subject
Investigative monitoring programme	
To ascertain the causes of water bodies failing to achieve the environmental objectives (where the reason is unknown);	Water bodies failing to achieve the required environmental objectives
To ascertain the magnitude and impacts of accidental pollution	Quality elements relevant to the specific case or problem being investigated; ecotoxicological monitoring and assessment methods would in some cases be appropriate

(NEON) (2020) project was funded in 2011 and became fully operational in 2019; it is designed to continue for 30 years (www.neonscience.org/). The NEON network includes sites located throughout the US representing many different types of ecosystems. It samples microalgae at all 24 wadeable stream sites in spring, summer and autumn and is designed to produce results compatible with NARS and NAWQA (https://www.neonscience.org/data-collection/microalgae). It also samples aquatic plants (https://www.neonscience.org/data-collection/aquatic-plants). It is unique in that its purpose is only to collect data and make it publicly available so it can be analyzed by interested scientists. Because of its national coverage and long timeframe, its data are well suited to assess the effects of regional and continental-scale stressors (e.g., climate change and other ecosystem change issues). Many of the study locations are undisturbed, so they serve as good reference sites.

States. Several states incorporate algae as part of their bioassessment programs. They vary considerably in purpose and design (Paul et al., 2017). In 1995, only 4 states reported using periphyton as part of biomonitoring programs (Davis et al., 1996). As of 2001 there were 20 (US EPA, 2002). Now, as many as 22 states have included benthic algae (Paul et al., 2017), though only a few have done so for a long period of time (Kentucky, Connecticut, Vermont, Virginia, Maine, Massachusetts, California, Florida).

There is no federal requirement that states monitor algae, though the US EPA encourages the use of at least two groups of organisms (US EPA, 2011) and provides guidance on how to do so (e.g., US EPA, 2011; Stevenson and Bahls, 1999). Most states rely on benthic macroinvertebrates and fish for assessments. They might include algae if they have special problems with nutrients and nuisance algal growth, have many sites with low diversity of macroinvertebrates or fish, or have a state biologist with a background and interest in algae. Several states are using algae to help develop nutrient criteria, while macrophyte monitoring is rare.

In recent years, the USGS and EPA have worked together more closely, and have engaged in joint projects (e.g. Munn et al., 2018). This reflects the recognition of the benefits of consistency and coordination. There is, however, little effort to coordinate methods or share results among states although there are a few cooperative efforts with federal agencies.

About 20,000 sites in the US have been sampled for algae as part of national, regional and state level programs since the 1980s. This includes all USGS programs, NARS and other EPA projects (e.g., Mid-Atlantic Integrated Assessment (MAIA; Stoddard et al., 2006a), Mid-Atlantic Highlands Assessment), and state programs. Some states (e.g., California) have done much more extensive sampling than others.

A major difference between the EU and US algae-based assessment programs is that the EU spends more effort working with Member States to ensure that assessment results are consistent than is the case for the US federal government. On the other hand, the US does not need to rely as much on coordinated results from states because it relies on federal-level monitoring to provide national assessments. Another key difference is that the EU samples about twice as many sites as is the case for the US. Since the US is more than twice the geographic area of the EU, the EU may have a sample site density four times that of the US.

5. Characterizing ecological status and biological condition

5.1. Ecological status

Whilst much of the original thinking about ecological integrity/ecological health took place in the US, active incorporation of these principles into legislation and water management started in Europe with the adoption of the benthic invertebrate-based RIVPACS (Invertebrate Prediction And Classification System: Wright et al., 1989) in the UK. This used the ratio of the measured value of metrics (observed) with the expected value (then called "Ecological Quality Index") which, in time, evolved into the Ecological Quality Ratio, the cornerstone of ecological status assessments throughout the EU. RIVPACS also set a precedent in one other respect: it used an existing index (Biological Monitoring Working Party Score: Hawkes, 1998), optimized for an organic pollution gradient, as the basis of the calculation, rather than developing new metrics specifically to assess ecological integrity. One characteristic of method development in the WFD era has been a tendency to repurpose existing indices (such as the diatom-based Indice de Polluosensibilité, IPS: Coste in CEMAGREF, 1982) rather than develop new metrics from scratch.

5.2. Biological condition

A symposium was held in 1975, early after the CWA passed, to explore characterizations of chemical, physical and biological integrity of water. At that symposium Frey (1977) characterized biological integrity as "the capability of supporting and maintaining a balanced, integrated, adaptive community of organisms having a composition and diversity comparable to that of the natural habitats of the region." Frey's basic concepts of biological integrity were advanced in Karr and Dudley (1981) and persist today in US EPA guidelines for states and in state statutes. Quite commonly, the natural balance of flora and fauna, adaptability of the community, and both structural and functional attributes of biological integrity are included as narrative criteria to support aquatic life use of state's waters.

Biological condition is expressed in terms of the measurements of biological attributes ('metrics') used to determine whether waterbodies meet aquatic life use goals. Biological integrity can be defined as a high level of biological condition. Many papers have been written about what makes a good metric, but typically an emphasis is placed on attributes of the biological assemblages that are affected by human activities and the pollution and habitat alterations they cause. The use of metrics is discussed further in Section 7.

Karr (1991) argued that multiple attributes of biological condition should be measured to ensure a response in the measure of biological condition if any important changes in physical and chemical condition occur. This has led to use of multimetric indices (MMIs) of biological condition, sometimes called multimetric indices of biological integrity (IBIs). MMIs are widely used for fish and invertebrates in the US so it makes practical sense to use MMIs when assessing ecological condition with benthic algae.

So what are the attributes of benthic algae that we should measure for biological condition? The US EPA formed a workgroup that elaborated on existing concepts of biological condition. They delineated 10 attributes of biological condition (Davies and Jackson, 2006). Although a couple do not apply well to benthic algae, diatom metrics could be developed for the other eight attributes: endemic taxa, sensitive rare and sensitive ubiquitous taxa, taxa with intermediate and high tolerance to stress, non-native taxa, organism condition, and ecosystem function.

To further explain biological condition and to establish a standardized scale for measurement, a Biological Condition Gradient (BCG) was proposed (Davies and Jackson, 2006). This model gradient defines six biological condition levels in terms of response to increasing levels of a generalized stressor gradient resulting from human disturbance. They are roughly analogous with the WFD's ecological status classes (see descriptions and comparison in Table 2). Because they apply to any group of organisms and all geographic regions, the BCG provides a basis of consistency for defining biological condition among assessments.

Although the US EPA cannot require states to adopt the BCG approach, it is promoting it and provides guidelines for implementation (US EPA, 2016b). Algae-based BCG systems have been developed for only 2–3 states, but now that these are completed, it should be easier for others. There are several BCG systems in place based on benthic invertebrates and fish, which should mean that agency biologists with a background in macroinvertebrates and fish may better relate (and be more receptive) to algae BCGs in the future.

Historically, the value of biological assessment has been linked to

Table 2
Comparison between WFD status classes and BCG levels.

WFD Ecological status		Biological Condition Gradient	
Class	Description	Level	Description
High	There are no, or only very minor, anthropogenic alterations to the values of the physic-chemical and hydromorphological quality elements for the surface water type from those normally associated with that type under undisturbed conditions. The values of the biological quality elements for the surface water body reflect those normally associated with that type under undisturbed conditions, and show no, or only very minor, evidence of distortion. These are the type-specific conditions and communities.	1	Natural or native condition. Native structural, functional and taxonomic integrity is preserved, ecosystem function is preserved within range of ecological variability
		2	Minimal changes in structure of biotic community; minimal changes in ecosystem function. Virtually all native taxa are maintained with some changes in biomass and/or abundance; ecosystem functions are fully maintained within range of natural variability
Good	The values of the biological quality elements for the surface water body type show low levels of distortion resulting from human activity, but deviate only slightly from those normally associated with the surface water body type under undisturbed conditions.	3	Evident changes in structure of biotic community; minimal changes in ecosystem function. Some changes in structure due to loss of some rare native taxa; shifts in relative abundance of taxa but sensitive ubiquitous taxa are common and abundant; ecosystem functions are fully maintained through redundant attributes of system
Moderate	The values of the biological quality elements for the surface water body type deviate moderately from those normally associated with the surface water body type under undisturbed conditions. The values show moderate signs of distortion resulting from human activity and are significantly more disturbed than under conditions of good status.	4	Moderate changes in structure of biotic community; minimal changes in ecosystem function. Moderate changes in structure due to replacement of some sensitive ubiquitous taxa by more tolerant taxa, but reproducing populations of some sensitive taxa are maintained; overall balanced distribution of all expected major groups; ecosystem functions largely maintained through redundant attributes
Poor	Waters showing evidence of major alterations to the values of biological quality elements for the surface water body type and in which the relevant biological communities deviate substantially from those normally associated with the surface water body type under undisturbed conditions.	5	Major changes in structure of biotic community; moderate changes in ecosystem function. Sensitive taxa are markedly diminished; conspicuously unbalanced distribution of major groups from that expected; organism condition shows signs of physiological stress; system function shows reduced complexity and redundancy; increased buildup or export of unused materials
Bad	Waters showing evidence of severe alterations to the values of the biological quality elements for the surface water body type and in which large portions of the relevant biological communities normally associated with the surface water body type under undisturbed conditions are absent.	6	Severe changes in structure of biotic community; major loss of ecosystem function. Extreme changes in structure; wholescale changes in taxonomic composition; extreme alterations from normal densities and distribution; organism conditioning is often poor; ecosystem functions are severely altered.

assessing the physical and chemical as well as the biological condition of waters. Of course, biological integrity of waters is codified in the CWA, but an additional benefit for biological assessment of ecological conditions is the sensitivity of biological condition to physical and chemical conditions that can be used as a surrogate for the many physical and chemical parameters that are not practical to measure in assessments. Karr has argued that we really do not need to worry about changes in physical and chemical condition if biological condition has not been altered, because those changes would be too small to affect other uses of water (Karr, 1991).

5.3. *Comparison of ecological status and Biological Condition Gradient approaches*

Ecological status and the BCG differ in detail but both are, essentially, yardsticks by which ecological health is measured. Both are rooted in an ideal scenario and are underpinned by narrative descriptions of the expectations for different levels of deviation from the natural state (Table 2). In the EU, there are five ecological status classes, compared to six in the BCG. Ecological status extends beyond the biota to encompass physical and chemical conditions which support the biota whilst the BCG applies only to the biota.

In Europe, the WFD prescribes "good ecological status" as the threshold that all water bodies should attain, albeit with caveats that allow lower status in some cases (with a justification) and, via a "no deterioration" clause, it can also require high status to be maintained. In the US, the objective is determined by individual States. Both the EU and US require metrics to be calibrated against the "reference state"; though this calibration is challenging in areas with high population densities or long histories of human intervention. Both also have workarounds to fit the many situations where there are no or insufficient reference sites.

Another major difference between the EU and US is that the former generally relies upon metrics established before the WFD era whereas the US has tended to develop new metrics specifically for evaluation of biological condition. Many (but not all) of the EU methods are based on single metrics (particularly the IPS, Coste in CEMAGREF, 1982) whilst most (but, again, not all) of the US approaches are MMIs. Reliance on existing metrics in Europe allowed backward compatibility and, therefore, a seamless transition into the WFD-era. Use of such metrics also reflects the tight implementation timetable whilst, in the US, there appears to have been more time for reflection and consideration of ecological theory before implementation.

Finally, there appears to be much closer oversight of individual states by the central authorities in the EU compared to the US and, in particular, there has been a major effort to ensure consistent interpretation of the normative definitions between Member States (the "intercalibration exercise") for the full range of biological quality elements (BQEs). By contrast, states in the US are encouraged (but not required) to evaluate the condition of at least two biological assemblages; however, there is no rule akin to the EU's "one out, all out" rule that determines how these measures should be combined to produce an overall assessment of a site. There are situations, nonetheless, where the US EPA can intervene and force a state to set realistic regulations (e.g., establishing nutrient criteria in Florida).

6. Sampling design and field and laboratory methods

Sampling design and collection methods vary substantially among assessment programs involving benthic algae, and they may or may not affect consistency and comparability of ecological condition determinations (e.g., Lowe and Pan, 1996). A key reason for the differences is that the goals and scales of the programs vary, e.g. some are monitoring rather than ecological assessment programs; or they are research programs to evaluate assessment programs.

6.1. Sampling design

Four important aspects of sampling design are: targeted versus probabilistic site selection, inclusion of reference sites, representation of geographic regions and ecosystem types, and timing of sample collection.

One of the biggest differences among sampling designs is whether study sites are probabilistic (statistically-selected), so that results can apply to entire populations of streams and rivers (e.g., US EPA, 2016c), or whether they are targeted to represent specific water bodies with known characteristics (e.g., reference conditions, pollution problems, point source impacts, distribution along an ecological gradient). Estimates of levels of impairment in an area can vary substantially depending on which design is used. Differences in results using the two designs will depend on the proportion of the target population that is sampled and criteria used to select targeted habitats. If most rivers and streams in a region are sampled, there may be little difference in results of the two designs. In the case of statistically-selected sites, sampling design can be stratified to more accurately represent certain classes of sites. Also, statistical selection may be more appropriate if the target region contains a large proportion of smaller sites.

In the EU, study sites are typically selected individually; probabilistic sampling is not widely used. Due to the high number of sites already selected in several regions, there is less need for statistical sampling in the EU. Germany, for instance, has a monitoring network of 11,000 stream and lake sites (Cantonati et al., 2017). However, sampling networks that have evolved over time often embed the preoccupations of the past. The Urban Wastewater Treatment Directive (European Community, 1991), for example, focused attention only on large sewage works creating a de facto "operational monitoring" network (see Table 1) that could be adopted as an off-the-shelf surveillance monitoring network for the WFD era but which, in turn, may have led to an overly pessimistic view of ecological condition.

In the US, most recent US EPA funded projects at the national level (e.g., NARS), and many at the state level, use a randomized sampling design (Paulsen et al., 2020). The USGS NAWQA program uses a targeted sampling approach to provide the best opportunity to study the influence of a wide range of human disturbance on ecological processes. Recently both the US EPA and USGS have used both approaches to meet their individual needs (Munn et al., 2018).

Another key design aspect is how geographic study areas are defined. This is important in terms of how metrics are developed and how results are applied to management decisions. In the EU, several Member States devolve environmental decision-making to regional governments and these political divisions can determine sampling programs as well regardless of whether they are distinct ecoregions. In the US, study regions may be small to large watersheds (e.g. NAWQA), ecoregions (Mazor et al., 2006), major continental areas, entire states, or combinations of these, depending on the program (see Section 3).

Another aspect is the number of samples and seasonal distribution. Most sampling in the EU and US is done between late spring and early fall, though there are regional exceptions. Periods following hydrologic extremes (spates, droughts etc.) are generally avoided. In the EU, only one sample per water body per reporting period (6 years) is required for water quality surveillance, with the timing left to the discretion of the competent authority. However, the WFD also requires Member States to "achieve adequate confidence and precision in the classification of the quality elements" which implies a need for spatial and temporal replication within a water body during this period (Kelly et al., 2009a, 2009b; Clarke, 2013; Carvalho et al., 2013; Moe et al., 2015). However, whilst classification of ecological status is governed by a set of clear rules (EC, 2005), the process by which Member States apply these criteria for "confidence and precision" to decision-making is more opaque.

In the US, most EPA sponsored projects (e.g., EMAP, MAIA, NARS) sample sites one time only, with some repeat and replicate sampling for QA/QC purposes. NARS collects samples from a different set of sites every 5 years; a subset of the sites are sampled every time to help account for long-term trends. The initial phase of the NAWQA program sampled most primary sites once per year for 3 years, though some were sampled once only and some were sampled multiple years to detect long-term trends. The NEON program samples sites three times per year, with the intention of collecting for 30 years.

6.2. Sample collection methods

Differences in sample collection methods among programs may have little effect on final assessment results within a program but will affect comparability of basic algal data between programs. The most important collection-related issues are targeted vs multi-habitat sampling, distribution of subsamples within a site, type of substrate sampled, and collection device.

In the EU, a wide variety of collection methods have been used by different countries. This variety reflects the diversity of common habitats in their stream and river systems. Since the onset of the WFD, however, procedures have become more standardized with most Member States following EU protocols (CEN, 2009, 2014a, 2014b). Typically, at least five representative cobbles or small boulders are sampled using a toothbrush (Kelly et al., 1998). For soft-bodied algae, there is an emphasis on coverage of distinct algal elements assessed in the field with a semi-quantitative sampling approach: 5-point scales are used both for field and for microscope quantifications (the Norwegian soft-bodied alga method relies on presence/absence: Schneider and Lindstrøm, 2011).

In the US, several methods are also employed by federal agencies (e.g., EMAP – Lazorchak et al., 1998; NAWQA – Moulton et al., 2002; NRSA – US EPA, 2009a, 2009b, 2017a, 2017b; NEON – microalgae website) and states (e.g. Stancheva and Sheath, 2018). Many states have adopted NRSA or USGS methods, in whole or in part, though some rely on their own established procedures. There is no common sampling strategy used in the US. EPA-funded programs such as NARS use a multi-habitat (MH) sampling approach, compositing several microhabitat samples collected over a sampling reach into one (US EPA, 2013c, 2017a, 2017b; Stoddard et al., 2006a). The method is designed to sample the different microhabitats in proportion to their occurrence at a site in order to include many species from the site. By contrast, the USGS has used primarily a richest target habitat (RTH) approach, taking samples from only the most common type of substrate in a sampling reach (usually rocks, but also wood and sediment). This minimizes site-to-site variability in taxonomic composition due to substrate type. More recently, the USGS has used the EPA sampling protocols for some studies (e.g., Munn et al., 2018). The NEON sampling design for wadeable streams divides sampling reaches into macrohabitat types (e.g., riffle, run, pool) and collects discrete samples from the two most common microhabitats in each. Individual states use RTH or MH sampling or a combination of these. According to Paul et al. (2017), most states use RTH methods.

Potapova and Charles (2005) concluded that sampling the same substratum at all sites in large-scale assessment programs should be mandatory only if structural metrics (e.g., diversity, % of growth forms) that are not based on the autecology of many species are used. Artificial substrates (e.g., diatometers, Lowe and Pan, 1996) are sometimes used to collect samples, but usually only in smaller studies. From a theoretical perspective, the WFD requires Member States to characterise the biota within water bodies whilst artificial substrates only show the potential for a particular assemblage to develop. For this reason, natural surfaces are generally preferred (though there is no EU-level prohibition on using artificial surfaces).

Whilst samples are usually collected quasi-randomly in the EU, samples in the US may be collected randomly along one or more transects located evenly or randomly along a sampling reach (e.g., US EPA, 2013c, 2017a; Moulton et al., 2002). Sampling sites may be co-located with sampling of other biota (e.g., macro-invertebrates) or be independent. Some aspects of within-site sampling that vary among assessment programs include: number of subsamples; length of sampling reach;

number of transects; random selection or even placement of transects; preference for riffles or other habitat type. There is no commonly accepted procedure. As with other aspects of study design, there is often tension between a desire to account for within-site variability as much as possible versus the need to limit costs.

In both the EU and US, diatoms are usually removed from substrates with brushes; other groups of algae may be removed in the same way but also more selectively using tweezers, knives etc. In the US, collection of quantitative samples can be done by removing algae from the surface of an entire rock with a known surface area, or using modified plastic syringes or other devices to remove algae from specific areas of habitat.

Most diatom samples taken in the EU are qualitative; however, both quantitative and qualitative approaches are used in the US, depending on the program and whether or not subsamples are used for biomass measurements. For soft-bodied algae, quantitative sampling is preferred in US assessments whilst a semi-quantitative sampling approach is employed in those parts of the EU that use these, with separate samples of all distinct algal elements collected. The focus in the EU is on assessing cover of algal elements in the field and the greater time spent assessing cover in the field is compensated by quicker microscopic analyses that estimate abundance using a 1–5 point scale. For soft-bodied algae, the EU approach thus prioritizes the coarse estimate of algal biomass on large surfaces in the field whilst the US approach prioritizes estimates of the density or biovolume based on relatively-small, quantitatively-collected samples by counting algal cells (Stancheva and Sheath, 2016). This approach is, however, seldom used because it is so temporally variable in streams as a result of spates and other weather conditions.

Rapid periphyton surveys (RPS) to assess benthic algal biomass are sometimes carried out in the US (Stevenson and Bahls, 1999), and, depending on the goal, can be a cost-effective way to determine relationships between nuisance benthic algal cover and nutrients (Dodds et al., 2002). Benthic chl a concentration, in particular quick BenthoTorch™ (bbe Moldaenke, Germany) fluorometer readings in the field, which also allow fractionation of total chl a based on the concentrations of marker pigments of the main algal groups, are increasingly used (Niedrist et al., 2018). In the US, NAWQA, NEON and some state programs collect quantitative samples so that chl a, ash free dry weight, and biovolumes of individual taxa can be determined (Charles et al., 2002). By contrast, biomass is rarely measured in the EU although macroalgal cover often forms part of macrophyte assessments (see 7.1).

6.3. *Laboratory methods – sample preparation and counting*

In general, laboratory methods, primarily sample preparation and counting procedures, are similar in EU and US assessment programs. Differences within and among programs can, however, be a significant source of variability when comparing analyses of the same sample made by different laboratories. These observations have been demonstrated via interlaboratory comparisons (Prygiel et al., 2002; Besse-Lototskaya et al., 2006; Kahlert et al., 2009, 2012; Werner et al., 2016). In the EU, countries follow the CEN (2014a) and CEN (2014b) enumeration protocols, which helps maintain consistency to some extent. The protocols are relatively general, however, and Member States supplement these with detailed protocols that differ from each other in some ways (Kahlert et al., 2012). In the US, there have been few rigorous intercomparisons of laboratory methods, but several smaller studies (Alverson et al., 2003; Lee et al., 2019; Tyree et al., 2020a, 2020b).

In both the EU and US, samples for diatom analysis are cleaned to remove organic matter (and carbonates, if any), and permanently mounted on microscope slides. Soft algae samples are generally subsampled and placed in counting chambers (Charles et al., 2002; Stancheva and Sheath, 2016). Some procedures are quantitative so that density and biovolume of individual taxa can be calculated. EU and US procedures typically require that 300–800 diatom valves be counted. More effort is used in the US than in the EU with typically 500–600 valves counted, compared with generally 400 in the EU, with some Member States counting distinct diatom "items" (frustules as well as valves). In the US, counting transects are marked on the slides with a diamond scriber or, at least, start-point and end-point coordinates are taken; this is not common practice in the EU. In the EU, some early diatom indices (e.g., Dell'Uomo, 1996) only required use of a semi-quantitative scale, an idea resurrected by Brabcová et al. (2017).

There are various soft-algae counting procedures, requiring counts of individual cells, natural units, and other characteristics. The NARS and NAWQA programs counted cells of all algae (diatoms and soft forms) in Palmer-Maloney counting chambers until 300 natural units had been observed and identified. Natural units were free independent cells, colonies or filaments. These counts were often dominated by diatoms, and therefore did not provide widely useful metrics for large scale surveys. Another counting approach scans samples for all soft-bodied taxa, which is designed to observe the diversity of soft algal taxa present as a metric.

7. Metrics used to assess ecological quality and biological condition

7.1. *Assemblages included in assessments:*

"Macrophytes and phytobenthos" is one of the BQEs whose condition contributes to evaluations of "ecological status" in lakes and rivers, as defined by the Water Framework Directive. Because macrophytes and benthic algae require fundamentally different approaches to data collection, all countries have developed separate evaluations for each, which are combined into a single assessment either as an average or as the lowest of the individual modules (Kelly et al., 2020b; Poikane et al., 2016; Schaumburg et al., 2004). In total, 29 macrophyte and 37 phytobenthos assessment systems have been developed and intercalibrated in Europe (Tables S1 in Supplementary material). Most countries developed one method for all river types, while a few have different methods for different types (particularly, rivers with catchment exceeding 10,000 km^2) or regions (e.g., Delgado et al., 2010, 2012). EU countries differ in whether they include (1) non-diatoms as well as diatoms in assessments of "phytobenthos" and, (2) macroalgae in assessments of "macrophytes" (see Table 3).

Almost all countries use diatoms as proxies for the whole benthic algae community (32 methods; Table 3), but four methods also include one (e.g. "filamentous green algae") or more other groups in addition to diatoms (Schaumburg et al., 2004; Pfister and Pipp, 2013). In Norway, assessments are based only on soft algae (Schneider and Lindstrøm, 2011). In addition, macroalgae are frequently included in macrophyte methods (18 methods), typically as indicator taxa in the macrophyte indices (15 countries; Holmes et al., 1999; Haury et al., 2006; Szoszkiewicz et al., 2020) but in some cases also in growth form and algal abundance metrics (Willby et al., 2012). However, six countries rely exclusively on diatoms and do not use macroalgae at all (Table 3).

While no particular algal group is specified for consideration in the CWA, a similar pattern emerges in US algal assessment programs wherein diatoms are the primary indicators included in metric calculations, but soft algae are frequently used as an additional line of evidence (Table 3). Many programs choose to sample and generate taxonomy data on both diatom and non-diatom algae, while using diatoms as the predominant focus of quantitative tools and biological indices. Fifteen states sample both diatoms and soft algae (Table 3), as well as national NRSA (US EPA, 2017a, 2017b), NAWQA (Moulton et al., 2002) and NEON programs, although the NRSA program has recently narrowed focus to primarily diatoms (US EPA, 2019). Six states, including Alaska and New Mexico, focus exclusively on diatoms, while only Massachusetts focuses exclusively on soft algae. Ten states, including California, Connecticut, Florida and New Jersey, include macroalgal taxonomic analyses (Table 3; Supplementary Table S2).

Table 3
Benthic algal groups included in assessments in EU countries and US state and national programs. Biomass includes measure of chlorophyll *a*, ash-free dry mass, and percent algal cover.

Indicator taxa			Biomass, cover indicators, growth form metrics	EU Countries	US States/Programs
Diatoms	Non-diatom microalgae	Macroalgae			
x				Bulgaria*, Denmark, Finland, Romania, Slovenia, Sweden	–
x	x	x		Austria, Czech Republic, Germany	–
x	x	x	x		California, Connecticut, Florida, Kentucky, Maine, New Jersey, North Carolina, Pennsylvania, USGS NAWQA
x		x		Belgium (Wallonia), Croatia, Cyprus, Estonia, France, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Poland, Portugal, Slovakia, Spain	–
x		x	x	Belgium (Flanders), United Kingdom	Montana
x			x	Bulgaria, Netherlands	Alaska, Arizona, Colorado, Georgia, Idaho, Indiana, New Mexico
	x	x		Norway	–
	x	x	x	–	Massachusetts
x	x		x		Minnesota, Nevada, New York, North Dakota, Rhode Island, South Dakota, Tennessee, EPA NARS/NRSA, NEON
			x		Alabama, Arkansas, Kansas, Utah
				*Method for very large rivers	

7.2. Algal abundance

In both the EU and US, the composition of diatoms is mostly expressed as a percentage of the total valve counts whereas macroalgae is expressed as abundance classes. In the EU, only one method (for Mediterranean temporary streams) includes benthic algae biomass (measured as chlorophyll *a*: Delgado et al., 2012), whilst a few others evaluate abundance of *Cladophora* or "filamentous algae" as percent cover (Cheshmedjiev et al., 2010; Willby et al., 2012). Biomass/abundance would seem to be a key component of eutrophication assessment systems (Dodds et al., 2002; Hilton et al., 2006) as it is in lakes (Carvalho et al., 2013), but it is not always addressed explicitly in assessment systems for European rivers. In contrast, most US programs incorporate a measure of algal biomass, including either ash-free dry mass (AFDM), chlorophyll *a*, or percent benthic algae cover (Table 3), frequently using the Rapid Bioassessment Protocol to visually assess benthic cover, thickness, and filament length (Stevenson and Bahls, 1999: see also Kelly et al., 2016). Lastly, in addition to quantitative counts of diatom taxa, a few states, including California (Stancheva and Sheath, 2018), also include a quantitative measure of soft-bodied microalgae (Table 3).

7.3. Metric selection

Although multimetric indices have been advocated in Europe (Hering et al., 2006), and are widely used for some other organism groups (e.g. invertebrates: Mondy et al., 2012), many benthic algae methods in Europe either rely on a single metric (the IPS, in particular) or on much simpler multimetrics than those used in the US (see Paul et al., 2020). This is driven by two factors: first, the requirement to base ecological status classifications on several biological quality elements effectively creates a meta-multimetric spanning all biota simultaneously. Thus, shortcomings in benthic algae assessments should be compensated for (in theory, at least) by assessments of other organisms. Second, the requirement to express assessment outcomes as observed/expected deflected attention (and limited resources) to defining reference conditions and "expected" values of metrics. This all needs to be set into context: monitoring programs were expected to be in place six years after the WFD came into force (Article 8, clause 2) which was an extremely tight schedule bearing in mind that few countries had any prior experience evaluating ecological health (as distinct from inferring levels of pollution) in 2000. In reality, most countries missed this deadline and budgets become even more significant factors in determining method development following the global financial crisis of 2008.

Five basic attributes of metrics are used to select the best metrics: 1) they should vary substantially among sites; 2) they should have values greater than zero for most of those sites; 3) there should be low variation in metrics among reference sites; 4) there should be significant differences between reference and highly disturbed sites; and, 5) metrics should be independent, i.e. not highly correlated with each other. In addition to these, we could add a further goal of capturing as many of the attributes of biological condition as possible. Whilst metrics alone cannot measure the extent of impacts and ecosystem connectivity, the other eight attributes of biological condition (Section 5.2) could be addressed to some extent using metrics (Stevenson, 2014).

Selecting metrics for assessments requires determining metric response to human disturbance and their stressors (defined as pollutants and habitat alterations by humans) and for benchmarks to be set for what is considered a good or bad condition. Whilst methods used to select sites that would qualify as reference sites vary amongst states and programs, they almost always include low stressor levels and/or low levels of human disturbance in the watershed. Stoddard et al. (2006b) clarified the concepts and language to improve consistency by emphasizing the distinction between minimally disturbed reference sites, where stressor and human disturbance levels are low, and least disturbed reference sites which have the lowest levels of stressors and human disturbance in the region. Understanding the criteria used for reference site selection is, therefore, important when comparing assessments over large areas where the extent of human disturbance varies among regions.

7.4. Metrics calculated

For benthic algal assessments in the EU, the diatom index IPS (Coste in CEMAGREF, 1982) is the most widely used index (21 methods, 16 countries), applied both alone and in combination with other indices. Other widely used indices are Trophic Index TI (11 countries; Rott et al., 1999), Saprobic Index SI (8 countries; Rott et al., 1997), and Trophic Diatom Index TDI (5 countries, Kelly et al., 2008a). However, 11 other diatom indices are applied in only one or two countries (see Table S1). In contrast, soft algae metrics are few and include both abundance metrics (cover of *Cladophora*, cover of filamentous algae) and taxonomic indices (non-diatom index PIT). No diversity metrics are used for benthic algae assessments. Twenty assessment systems include just one diatom metric, most commonly IPS, while 11 combine several diatom indices, usually TI combined with either SI or IPS. Other options include combinations of a diatom index with soft algae metrics (2 methods) or indices including all algal groups (2 methods). Most macrophyte indices include macroalgae, but not all (e.g., Pall and Pall, 2018).

In contrast to the EU, US algal programs have few shared indices and tend to favor multimetric approaches (see Supplemental Table S2). With the exception of the Kentucky Diatom Bioassessment Index (KDBI) that is used in three states, biological indices are largely developed independently for each state or national program. While developed independently, these indices (e.g. for Alaska, Florida, Idaho, NAWQA) frequently leverage a popular assortment of autoecological traits, including organic nitrogen tolerance, saprobity, trophic state (Porter et al., 2008; van Dam et al., 1994), pollution class (Bahls, 1993), pollution tolerance (Lange-Bertalot, 1979), nutrients (Potapova and Charles, 2007), salinity and motility (Porter et al., 2008). In addition to published autoecological trait information, California (Theroux et al., 2020), Connecticut (Becker et al., 2018), and Maine (Danielson et al., 2011; Danielson et al., 2012) have also developed state-specific metrics derived from species optima and stressor response modeling.

7.5. Accounting for geographic variability

Both EU and US bioassessment programs have recognized the importance of accounting for regional variability in the development of algal indices. In the EU, the WFD required Member States to use an abiotic typology to account for natural differences in assemblages (European Union, 2000; Wallin et al., 2003). Ecoregions are used in the US (Hughes et al., 1986). However, the advent of predictive biological indices has allowed for the development of site-specific, reference-based expectations of both observed versus expected reference taxa numbers (O/E) or metric values at reference sites. Originally pioneered for use with benthic macroinvertebrate communities, these predictive indices incorporate measures of geographic setting to account for local and regional variation in expected species composition (Wright, 2000). In the EU, efforts to develop predictive algal indices include diatom indices for UK (Kelly et al., 2008a), Portuguese (Feio et al., 2009) and northern Spanish (Pardo et al., 2018) streams, a predictive diatom and macrophyte index (AQUAFLORA: Feio et al., 2012), and a diatom index for Romanian lakes (Kelly et al., 2019). The US EPA uses an ecoregion approach in its NARS studies (Herlihy et al., 2008; US EPA, 2016c). Predictive algal indices for evaluating taxonomic completeness have been developed for California (Mazor et al., 2006; Theroux et al., 2020), Idaho (Cao et al., 2007), Appalachian streams (Carlisle et al., 2008) and the southeastern US (Tyree et al., 2020b). In California, a predictive MMI for both diatoms and non-diatom algae has recently been developed (Theroux et al., 2020). Predictive and non-predictive diatom MMIs for ecoregions across the US were compared using data collected by the US EPA NRSA; results showed that using predictive models was necessary to account for natural variation among sites within ecoregions (Tang et al., 2016, 2020).

Tradeoffs exist between consistently using the same metrics across regions and varying metric selection based on which have the highest performance. Tang et al. (2016) found that MMIs in which metrics are modeled to account for natural variability among sites performed better at the national scale if metrics were varied among ecoregions of the US. But modeled MMIs that had the same metrics for all regions also had high performance.

7.6. Integration of emerging molecular and metagenomic approaches

Both EU and US algal programs will soon face a multitude of decisions related to if, when, and how they decide to collect data using molecular, or DNA-based, approaches. Adherents claim that the transition to molecular methods offers the potential to generate data with greater speed, precision, and accuracy due to computational approaches that are less prone to human bias (Pawlowski et al., 2018). The drawbacks of this transition include the current lack of standardized protocols and approaches for collecting, processing and analyzing DNA samples, thereby hampering broadscale comparisons and adoption. Several studies in recent years have begun to explore the potential to generate data for bioassessment applications using molecular approaches and have highlighted the obstacles currently facing this implementation. These studies have shown that the decisions surrounding how to best generate DNA taxonomy data and DNA-compatible biological indices have substantial impacts on resulting assessment outcomes (Bailet et al., 2020).

European groups are currently outpacing their US counterparts in piloting DNA metabarcode approaches for algal bioassessment. Primarily focused on diatom DNA metabarcode sequencing, there are published and ongoing studies examining sampling, preservation, DNA extraction (Vasselon et al., 2017), target DNA barcode region (Kermarrec et al., 2013; Visco et al., 2015; Zimmermann et al., 2015, 2011), sequencing approach (Loman et al., 2012; Shokralla et al., 2012), gaps in DNA reference libraries (Weigand et al., 2019), and the development of DNA reference libraries (Zimmermann et al., 2014; Rimet et al., 2016). Likewise, multiple studies have now compared bioinformatic approaches, including clustering approaches for reducing datasets and their impacts on assessment outcomes (Keck et al., 2018; Kelly et al., 2020a; Tapolczai et al., 2019b). In 2017, England replaced the diatom morphological approach with a molecular approach for river monitoring (Kelly, 2019), though outcomes are still not used for formal assessments of ecological status.

As discussed above, several biological indices rely on species relative abundance estimates, including the widely-used IPS (Cemagref, 1982), which is popular in Europe (Coste et al., 2009; Kelly et al., 2009c). However, the relationship between DNA sequence read counts and cell counts is not always linear, largely due to variations in cell biovolume and target gene copy number variation (Kelly et al., 2020a; Pawlowski et al., 2018). The use of cell biovolume information for individual species has been proposed as a correction factor (Rivera et al., 2020; Vasselon et al., 2018); however, Kelly et al. (2020a) point out that this "correction" ignores the inherent biases of the traditional microscopy-based approach and, instead, recommend development of new metrics directly from metabarcoding data. Lastly, the advent of "taxonomy-free" biological indices has encouraged the bioassessment community to draw on previously overlooked taxa whose biological signal can be discerned from distributions across stressor gradients so that it is no longer reliant on a priori trait attribute assignments (Apothéloz-Perret-Gentil et al., 2017; Tapolczai et al., 2019a, 2019b). This approach has the potential to leverage taxonomic groups often overlooked by microscopy-based approaches, but it also ignores the autecological information for key indicator taxa that has been collected in the past. Future studies will undoubtedly help to further define the standardized or accepted protocols that are used for generating DNA-based biological assessments, and may choose between the straightforward replacement of microscopy data with DNA-based taxonomy data, a transition to “Biomonitoring 2.0” approach (Baird and Hajibabaei, 2012) wherein novel indices are designed around DNA-based data, or perhaps a hybrid of the two (Hering et al., 2018; Kelly, 2019).

In the US, there have only been a handful of efforts aimed at attempting algal bioassessments with molecular data. Studies funded by the US EPA have looked at diatom assemblage response to an urban stressor gradient (Bagley et al., 2019) and nutrient pollution (Smucker et al., 2020). A study of New Jersey stream biofilms likewise found that molecular taxonomy was able to detect changes in diatom assemblages in response to environmental gradients (Minerovic et al., 2020). However, none of these studies attempted to calculate diatom metrics with DNA-derived taxonomy data. Algal DNA sample collection is now included in New Hampshire, California, NRSA and NAWQA sampling campaigns (US EPA, 2017a, 2017b) and forthcoming comparisons between morphology and molecular algae taxonomy data are expected.

8. Setting benchmarks for assessment

Assessment calls for an evaluation of the condition of a waterbody (Stevenson et al., 2004) and, when performing assessments across a federation, there needs to be a shared understanding. Thus, we need benchmarks as common points of reference for characterizing the ecology, which could be used to classify waterbody status as good, fair, and poor (US EPA, 2009b; US EPA, 2016a; US EPA, 2016b; US EPA, 2016c) or, in Europe, as high, good, moderate, poor or bad. In addition, ecological benchmarks are used to determine whether waterbodies fail to meet management goals, and thus require restoration. In addition, we can use benchmarks for waterbodies that meet management goals but need protection because further pollution or habitat alteration will degrade condition to unacceptable levels. Finally, we can use these benchmarks to indicate progress during restoration, even though restoration may not have improved conditions to a level that would meet management goals (e.g. criteria, Davies and Jackson, 2006).

8.1. Benchmarks for ecological criteria

Benchmarks for characterizing condition are commonly based on composition of benthic algal assemblages. The extent to which assessments of condition equate to management goals and water quality criteria varies (see Tables S1 and S2). Most benchmarks for management goals associated with benthic algae in the US are for nutrient concentrations (e.g. to determine nutrient thresholds that would reduce the risk of nuisance algae to an acceptable level). Benchmarks are also established, although less often, for pollution criteria associated with changes in metrics of benthic algal species composition (Stevenson et al., 2008).

Assessment benchmarks are usually numeric, in order to establish clear management targets. Many US states have narrative criteria, such as “lack of nuisance algae”. In many cases, these narrative criteria become associated with numerical translators which are quantitative benchmarks that are not established in more formal rules or regulations.

In the US, benchmarks for characterizing biological condition or establishing criteria for biological condition are developed using a variety of methods. When relationships among environmental variables are poorly understood, percentiles of a frequency distribution of conditions at either all sites or reference sites have commonly been used. For example, the 75th percentile of an MMI at all sites or its 25th percentile at reference sites have been used to delineate good conditions. The 25th, 10th, and 5th percentiles of an MMI at reference sites have been used in the US NARS to delineate good, fair, and poor condition (US EPA, 2009b; US EPA, 2016a; US EPA, 2016b; US EPA, 2016c). This approach has been avoided in the EU, recognising that the position of percentiles would be very different in a small densely-populated country such as Netherlands, compared with large, sparsely-populated countries such as Sweden or Norway. Some countries in the EU use the point at which a measure of “sensitive” taxa crosses a measure of “tolerant” taxa (e.g. Kelly et al., 2008a). This point has been equated to a shift from “stress-tolerant” to “competitive” taxa (Kelly et al., 2009d; Biggs et al., 1998) and, therefore, provides an insight into changes in function along the stressor gradient. Another approach in the EU is to divide the range of biological conditions into equal segments to establish benchmarks for ecological quality. This approach is, unfortunately, widely used (Birk et al., 2012b) although it was only originally recommended as a last resort in internal EU documentation. Best professional judgement is used in some cases for benthic algal metrics, to detect changes in biological condition (Hausmann et al., 2016; Paul et al., 2020).

8.2. Benchmarks for nutrients and other physical and chemical criteria

Benchmarks for stressor management targets have similarly been developed with frequency distributions of conditions at a large number of sites, with the 25th percentile of all sites (i.e. a low stressor level) or the 75th percentile of stressors at reference sites (US EPA, 2000). Stakeholders have criticized this approach for managing stressors such as nutrients, because the effects of protection at one percentile or another are not taken into account. By contrast, stressor-response relationships provide effects-based criteria, with known levels of protection at different pollution management targets (US EPA, 2009b), although the appropriate degree of protection is still a subject that needs stakeholder consensus. Thresholds, or tipping points, in nonlinear stressor-response relationships are valuable for developing a consensus among stakeholders. For example, the threshold at 10 μg TP/L in the Everglades for great loss of the naturally occurring, floating calcareous algal mat was valuable for developing stakeholder consensus for that TP criterion (Stevenson, 2014). The coupled relationships with non-linear responses between *Cladophora* cover, as determined by TP (Stevenson et al., 2012), and stream aesthetic quality, as determined by benthic algal biomass (Suplee et al., 2009), link phosphorus management targets to a change in a measure of human well-being.

The question of how to better couple the extensive knowledge of the ecological condition of Europe’s water to measures that will drive genuine enhancements has also occupied the minds of those at the EU’s science-policy interface in recent years. As nutrient loading is a key reason for the failure of water bodies to achieve good ecological status in Europe (Carvalho et al., 2019; Poikane et al., 2019b), it has been the focus for much of this work, with a hope that many conclusions will be transferable to other pressures in due course. Each country is responsible for setting standards for physico-chemical “supporting elements” (such as nitrogen and phosphorus) that will ensure that the biota attains good status. However, there is considerable variation between the standards each country sets, even after stream type has been accounted for. In particular, the means by which the standard was set was significant, with countries setting standards by rigorous statistical approaches generally having tighter standards than those that use “expert judgement” (Poikane et al., 2019a, 2020).

This has led to the development of guidance on “best practice” for establishing nutrient concentrations to support good ecological status, particularly when these are just one of a number of pressures impacting on a water body (Phillips et al., 2018; Phillips et al., 2019). This guidance is accompanied by a statistical toolkit that allows Member States to generate threshold concentrations from their own data. Informal feedback is that the guidance and toolkit are being used but the process by which new environmental standards are formally adopted is necessarily slow so it is too soon to see a general move towards more stringent nutrient standards being adopted across Europe. As a general rule-of-thumb, the reputation of certain algae as causing “nuisance” has persisted despite the new focus on good ecological condition. Thus, nutrient thresholds are seen as being necessary to protect against unsightly growths of filamentous algae rather than to support a community that provides essential ecosystem services. As a result, the cost of implementing expensive measures to reduce nutrient concentrations might, for example, be regarded as disproportionate to the benefits that would accrue, especially in situations where there are not obvious algae-related “problems”. Whilst countries in theory play by WFD rules, they may have to prioritise those water bodies where measures are seen to be most beneficial, and to use caveats within the WFD (e.g. “less stringent objectives”) to postpone the implementation of measures elsewhere. The first “measure” that is applied is often to collect more data. This often reflects the very lean sampling programs upon which formal WFD classifications are based. However, it probably also reflects a wariness of going ahead with expensive measures for reasons that few members of the public can really understand.

8.3. Benchmarks for benchmarks?

One of the most significant ways in which the EU and US differ in their use of benthic algae for assessment lies in “intercalibration”. This is the process by which EU Member States harmonize the criteria they use to define boundaries between ecological status classes (see Section 2).

Although EU Member States develop their own ecological assessment methods, they must demonstrate that methods and resulting classifications (boundaries etc.) comply with the normative definitions and are consistent with those used by other Member States (Annex V Clause 1.4). The majority of countries have completed the process for most freshwater BQEs, and only need to repeat it if they change their methods. The intercalibration exercises were facilitated by the European Commission but performed by groups of experts representing the Member States (Birk et al., 2013; Poikane et al., 2015). The process of intercalibration was, in some ways, as important as the formal outcomes, particularly in the early years of WFD implementation, as it brought experts together to discuss approaches. Many Member States adapted their techniques and boundaries, and some adopted aspects of other countries’ approaches. This can be considered an accidental benefit of the WFD: if intercalibration had not been obligatory, then this peer-to-peer sharing is less likely to have happened. Access to this “supermarket” of proven methods was especially helpful for the economically weaker countries. A disadvantage was that intercalibration normalized some weak approaches, such as dividing the EQR scale into equally-spaced classes (see 7.1) rather than forcing Member States to look for ecologically-meaningful criteria with which to define status (Poikane et al., 2016).

In the US, the BCG approach (US EPA, 2016a) is based on the principle that assignments of sites to BCG categories should be consistent from one geographic region to another. The position of BCG level boundaries along key stressor gradients is checked each time a new BCG system is developed. This is done by involving a pool of scientists, experienced with the relevant groups of organisms and aquatic systems, to ensure that normative descriptions of BCG levels are met (see Paul et al., 2020). However, there are no Federal-level attempts to ensure consistency between states. It certainly could be done, however, with one possibility being the intercalibration of methods used by one or more states against those used in a federal program such as NRSA or NAWQA.

9. Which pressures are assessed?

9.1. Terminology

Naturally-occurring factors such as light, temperature and nutrients are considered to be “stressors” when they occur outside their normal range. “Pressures”, by contrast, are the consequences of human activities that lead to these changes in stressors. However, as a single determinand (e.g. total or soluble phosphorus) is often used as a proxy for a pressure, the distinction between “stressor” and “pressure” can become blurred. We have generally stuck to the terminology of the literature that we quote in this section.

Some formal ecological definitions of “stressors” emphasize that they can be naturally occurring factors such as light conditions, temperature, and nutrients as well as factors resulting from human activities. Environmental factors are considered as stressors if they are outside their normal range and cause a change in biological conditions. In practice, a few variables (such as phosphorus determinands) are often used as proxies for both pressures and stressors and terminology is often not applied as rigorously as it should. For example, the relationship between BQEs and total and soluble phosphorus is commonly referred to as a “pressure-response” relationship in the EU, whereas both determinands are actually proxies for the limiting nutrient in all bioavailable forms (the ‘stressor’), and often derive from both point and diffuse sources (two distinct ‘pressures’).

9.2. Pressures assessed in the EU

Benthic algae assessment systems used by EU Member States fall in two general categories. The majority of metrics (e.g. IPS or combination of TI and SI) address combined stressor gradients, incorporating nutrients, organic pollution and/or general degradation. A few respond to nutrient enrichment exclusively (e.g. TDI index originally calibrated against soluble reactive phosphorus or TI calibrated against TP). Nutrient enrichment is targeted by all 37 assessment systems and pressure-response relationships with nutrients have been demonstrated for most of these. Thirty-one assessment systems address organic pollution, but pressure-response relationships have been demonstrated for only a third of these. In contrast, other pressures are addressed by only a few methods, and pressure-response relationships are mostly not documented.

In the EU, proxies are used for the determination of pressure-response relationships. In the case of nutrients, soluble reactive phosphorus (most frequent), total phosphorus, total nitrogen and nitrate-nitrogen are used, typically as annual or seasonal means. Organic enrichment is characterized by dissolved oxygen and BOD, and “general degradation” by land use metrics (Fig. 1).

9.3. Stressors assessed in the US

Similarly, the US algal bioassessment programs use algal metrics as a means to evaluate overall biological health as well as specific stressors, especially nutrient over-enrichment. Broader evaluations of biological impairment are frequently based on algal MMIs. California, Connecticut, Maine and New Jersey have developed algae Biological Condition Gradient models for this purpose. State’s multimetric indices typically include many stressor indicators. The most common are nutrient-related measures, such as saprobity and dissolved oxygen tolerances and trophic state, as well as metrics derived from nutrient response modeling, such as low and high nitrogen tolerant species. Wisconsin, Maine and New Jersey all have diatom indices that are specifically tuned to phosphorus concentrations, while other states (e.g. California) contain individual phosphorus tolerance metrics in their multimetric indices (Supplemental Table S2). National program datasets have likewise helped support the identification of nutrient impairment thresholds, including algal-P thresholds (Stevenson et al., 2008) derived from the NRSA datasets. An increasing number of US states are now using algal metrics to develop narrative and numeric nutrient biocriteria, including Connecticut (Smucker et al., 2013), Maine (Danielson, 2009), and New Mexico (Jessup, 2015). As mentioned earlier, almost all states with an algal bioassessment program collect at least one measure of algal biomass (chlorophyll *a*, ash-free dry mass, or percent algal cover). Additionally, the US EPA Causal Analysis/Diagnosis Decision Information System (CADDIS) (US EPA, 2017a) provides a framework within which algal metrics can be used to help identify the environmental

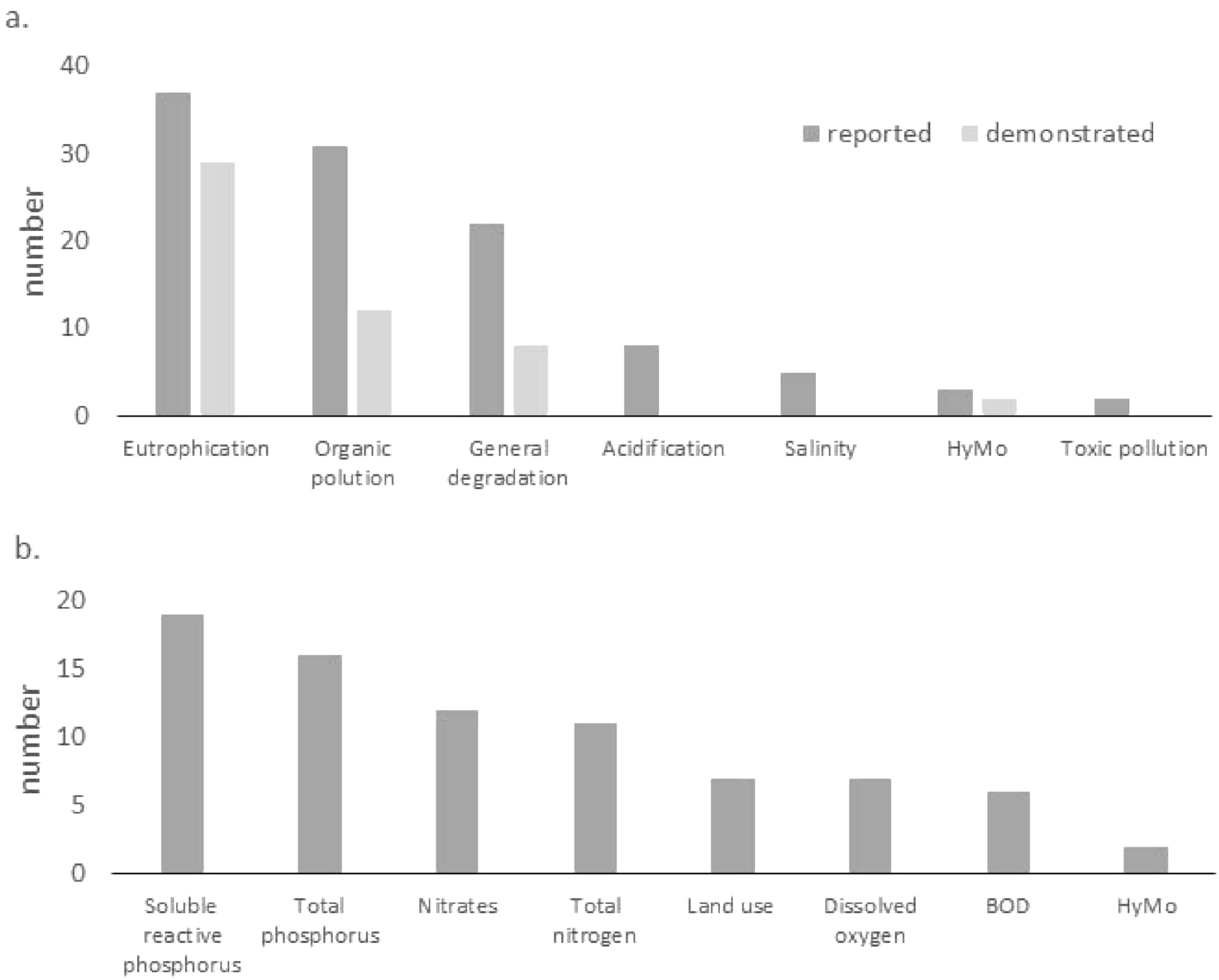

Fig. 1. a) the range of pressures cited by EU Member States as being assessed using benthic algal methods. "Reported" methods is the total number of Member States citing an approach and "demonstrated" is the number who support this with a statistically-significant relationship; b) pressure proxies used in pressure-response relationships. HyMo = hydromorphological pressures.

stressors most relevant to water integrity (i.e. ammonia, dissolved oxygen, flow alteration, herbicides, insecticides, ionic strength, metals, nutrients, pH, physical habitat alteration, sediments, temperature and unspecified toxic chemicals).

In recent years, laboratory and field studies have been undertaken to assess the impact of many stressors, both anthropogenic and natural, on algae communities (e.g. Wagenhoff et al., 2011). Munn et al. (2018), in a unique field study assessing effects of multiple physical and chemical stressors on diatom metrics carried out for a large number of sites at a high frequency over a number of weeks, showed that diatom metrics were influenced to various degrees by all stressors measured in the study (i.e. nutrients, herbicides, fungicides, suspended sediment, temperature and stream flow).

9.4. Comparison of EU and US approaches

Leaving issues of terminology aside, there are many similarities between the EU and US approaches. The principal difference is, as we have discussed in earlier sections, a tradition of using tried-and-tested approaches in the EU compared to the development of new tools in the US. Many of the EU approaches arise from an era when a strong correlation between a metric and one or more components of "pressure" was considered to be a good approach. These, then, were repurposed into measures of "ecosystem health" in the WFD era, mainly by assuming that low levels of these pressures equated to the ideal condition. By contrast, the US has seen more efforts to derive measures of ecosystem health from first principles but often with constituent metrics in MMIs borrowing from European approaches. Unsurprisingly, these, too, often show strong correlations with the key "stressors" that arise from anthropogenic drivers and pressures.

10. Taxonomic resolution and harmonization

Taxonomic harmonization is essential for consistency within and among assessment programs. Without it, it is difficult to compare analyst outputs, autecological information and condition assessments. Taxonomic inconsistency has caused significant problems in EU and US assessments, both within and among programs (Kahlert et al., 2016; Paulsen et al., 2020). It has resulted in diatoms/algae being removed from monitoring programs (e.g., EPA lake and wetland NARS programs). Growing recognition of the issue is also stimulating development of alternative methods (Kelly et al., 2020a; Manoylov, 2014).

Because EU assessment programs are carried out by Member States, taxonomic consistency efforts are primarily centered there. The EU, itself, is interested primarily in assessment outcomes, and not the process. Member States vary in the ways that they ensure consistency and, in many cases, the wider profession plays an important role (organising ring tests, for example). There is also collaboration between analysts in neighboring countries.

In the US, taxonomic harmonization has been approached in various ways among Federal and state programs. When the USGS NAWQA program began in 1992, its scientific leadership stressed the importance of taxonomic consistency. They anticipated the program would last many years and recognized the need to document taxa so they could be reliably identified in the future. Among other measures, contract analysts were required to take images and measurements of specimens, and to provide slides with circled specimens of representative taxa. A laboratory unit was established in Denver, Colorado with a taxonomic coordinator responsible for taxonomic consistency.

Taxonomic consistency has been challenging in the US EPA NARS programs. These large national programs produce 1000 samples per year, with contractors selected, at least in part, on a cost per sample basis with little information required about analyst qualifications, past experience with NARS sample analysis, or the capacity to process samples within deadlines. In some NRSA programs, taxonomic harmonization was guided by experienced taxonomists, replicate counts were made during and after all counts were completed, and dominant taxa were documented with images (a common requirement in US assessment contracts). That approach was not as successful in other NARS programs. As a result, taxonomic consistency has been a problem in most NARS programs. Variability in taxonomy used among analysts and laboratories created sufficient concern that diatom data were only reported in the first National Lake Assessment, from which publications documented good MMI performance (Stevenson et al., 2013; Liu and Stevenson, 2017). Benthic diatom metrics and MMIs from the first NRSA (Tang et al., 2016, 2020) were responsive to human disturbance and performed well by standard measures of metric performance (e.g. Stoddard et al., 2008), despite evaluations concluding that diatom identification was inconsistent. Lee et al. (2019) showed a promising harmonization approach that was performed during data analysis and after count harmonization, improving diatom signal sensitivity to total phosphorus. The US EPA continues efforts to improve diatom analyses for the NARS and state applications. They are providing some support and planning to rely more on developing taxonomic resources (see 10.1) as well as testing pre-analysis voucher floras. They are also very interested in the idea of a certification scheme for analysts.

The NEON program also has a strong interest in taxonomic consistency because it is designed to detect trends in biota over a 30 year period. It requires imaging of all new taxa encountered, slide audits, and archiving of all slides and samples in a large repository in Arizona although there is little harmonization amongst contractors.

Harmonization efforts vary widely among states. Most states use outside contractors, but some have staff diatomists who do analysis or at least help provide taxonomic consistency from one project to the next (e.g. Kentucky, Florida, New Jersey, Massachusetts, New Mexico, North Carolina, Wisconsin). Contractors may be required to have a specified level of experience, use a standard list of taxa, meet strict audit criteria, image specimens and archive slides. However, some state contracts include no such requirements. The trend is to include significantly more requirements than in the past.

Achieving taxonomic consistency among multiple algal analysts requires several actions, which are described below. Which ones are applied varies within and between EU and US assessment programs.

10.1. Literature references and taxa lists

In order to maximize consistency among analysts within and among monitoring programs all analysts should use a common set of references for specimen identification, usually supplemented by online resources. It is also important that analysts use a common set of taxa names and concepts to avoid the confusion inherent in trying to match synonyms and applying different criteria for lumping and splitting. These resources, and lumping/splitting criteria should be specified in protocols.

In the EU, there is a long tradition of using algae identification books in the 'Süßwasserflora von Mitteleuropa' series. Cantonati et al. (2017) recently created an updated, English version of these books treating over 800 taxa and designed for use by European analysts involved with ecological assessments. In addition, diatom identification keys have been developed specifically for national monitoring programs (e.g. Poland: Bąk et al., 2012). AlgaeBase (Guiry and Guiry, 2020) and DiatomBase (Kociolek et al., 2020) are widely used on-line sources for currently accepted nomenclature. Jüttner et al. (2020) are developing an online Diatom Flora of Britain and Ireland.

A wide variety of books and papers are used for diatom identifications in the US. Over the past few decades, the use of different combinations by different analysts has been a significant impediment to taxonomic consistency. Books based on diatoms found in European waters are most commonly used, supplemented by taxon-specific papers and regional Floras. Because many of the most up to date Floras are European, there is increased interest in developing North America specific Floras (e.g. 'Diatoms of North America' within the series *Iconographia Diatomologica*: Siver et al., 2005; Antoniades et al., 2008). The web resource 'Diatoms of North America' (DONA; Spaulding et al., 2019; formerly 'Diatoms of the United States') is the fastest growing and most widely used image-based guide. Again, DiatomBase and AlgaeBase are commonly used for determining accepted names, synonyms, correct spelling and original publication. There is no single list of diatom taxa names that is required to be used by funding agencies. In the past, some agencies required that algae names be consistent with the federally supported Integrated Taxonomic Information System (ITIS; itis.gov). However, this list was not up to date and did not include concept references, so it has been little used. The list developed during the NAWQA program and further developed as part of other programs (https://diatom.ansp.org/Taxa.aspx) has been required by some agencies. That list further evolved and is now maintained as part of the USGS BioData database (https://aquatic.biodata.usgs.gov/aboutUs.action) and is used by USGS, US EPA and other agencies. The USGS Biodata taxa list and DONA taxonomic references provide a standardized resource that is used by several US programs, and encouraged for all.

10.2. Voucher floras

These are not as critical in the EU as in the US because many literature references are essentially voucher floras for Europe. In the US, assessment program voucher floras were historically not required, but are becoming increasingly common (e.g., Bishop et al., 2017). They vary widely in the number of images, level of documentation, and ease of use. Some are developed prior to sample analysis to promote consistency in identifications, then supplemented during sample analysis. Others are assembled at the end of a project to document the names used. Many are not publicly available. The USGS NAWQA program required images of taxa, especially of undescribed and difficult taxa. These are available at https://diatom.ansp.org/algae_image/, the site includes images from more recent projects as well. Pre-analysis voucher flora approaches have been promoted (Lee et al., 2019; Tyree et al., 2020a).

10.3. Workshops

In the EU, workshops have been held to discuss algal taxonomy issues in conjunction with ring-test exercises. They are mostly organised by groups of professionals and academics (e.g. Ector, 2011) rather than by government departments. Many taxonomy workshops have been held in the US as part of assessment programs (e.g., NAWQA https://diatom.ansp.org/nawqa/Workshops.aspx), but they are usually not required, and published records are limited. Recently, EPA and USGS have supported workshops in conjunction with creation of regional voucher floras.

10.4. Taxonomic coordinator

Taxonomy coordinators have responsibility for taxonomic consistency within projects or programs involving multiple analysts. In the EU, individual analysts take this issue seriously; however, few would be able to point to formal schemes managed by government agencies. In the US, some assessment programs have had coordinators (e.g., NAWQA, EPA projects) but they are not always well supported. They are typically senior analysts within the organizations doing the analyses.

10.5. Audits

Internal and external audits (more commonly referred to as “Quality assurance/quality control” – QA/QC in the US) are important for discovering and dealing with taxonomic issues, demonstrating minimal levels of competence, and providing estimates of error associated with analyses. In the EU, ring tests are often treated as a form of audit (Kahlert et al., 2016), although they are rarely linked to batches of samples from which assessments are based and their frequency is much lower than that used in chemistry, for example. To the best of our knowledge, there are very few active audit schemes in Europe whilst most federal and state-level algal assessment projects in the US require some auditing (typically 10–15 percent of samples). Percent dissimilarity or similarity is the main measure of acceptance, with acceptable similarity thresholds ranging from 60 to 90%. The difference in the numbers of taxa identified in a sample is also often considered. As an example, in California, 10% of samples are sent out to independent experts for audit. Typically, Jaccard Index and Percent Similarity (PS) are the comparison criteria used. Deviation in biological index score is also used to evaluate performance (Kelly, 2013; Stancheva and Sheath, 2019).

10.6. Ring tests

In the EU, most ring tests originate amongst academics and professionals and are adopted by Member States, rather than organised by state-employed staff. The ring tests are often regional/language based rather than confined to a single country (Francophone/Nordic/UK-Ireland/Dutch-Flemish: Kelly, 2013; Dreßler et al., 2015; Werner et al., 2016; Kahlert et al., 2016). Having many analysts count samples of the same material, and then discussing results helps develop a common understanding of concepts of individual taxa and characteristics separating closely related taxa. Bray-Curtis Similarity can be used to compare analysts counts, but it is sensitive to differences in taxonomic concepts and synonyms. Instead, many ring tests examine variability in calculated metrics (e.g. Prygiel et al., 2002; Werner et al., 2016), the end result of the algal analysis process which is easier to relate to uncertainty associated with classifications. In the UK/Ireland scheme, a “warning limit” is set as 2x the standard deviation obtained by a group of experienced analysts whilst $+/-7$ TDI units is the “action limit”, indicating the point at which an analyst’s deviation exceeds the maximum within-site variability expected (based on previous studies) (Kelly, 2013). However, no-one “fails” the UK/Ireland ring test; rather, everyone learns something and the “action limits” are an indication that there is likely to be an issue with an analysis. Most EU ring-tests are probably best described as “reflective learning” rather than “QA/QC”, with people taking responsibility for their own improvement. Ring-tests have not been performed in the US.

10.7. Taxonomic certification programs

The EU has no taxonomic certification program, per se, but Member States can specify that analysts participate in ring tests or meet certain criteria when letting contracts. A Taxonomic Certification Program is being developed in the US under the auspices of the Society of Freshwater Science, and supported by federal agencies (see https://diatoms.org/practitioners/diatom-taxonomic-certification). No assessment programs currently require that analysts be certified, but the existence of the certification process will raise the likelihood that samples are analyzed by personnel with the required level of competence. Organizers of the Taxonomic Certification program have initiated a series of recorded webinars on taxonomy and ecology topics. These are available at the DONA website.

10.8. Archiving algal samples

There is no central location for archiving EU algal materials. Within individual countries, materials may be placed in museum collections (e.g., Scottish samples were archived at Royal Botanic Garden, Edinburgh), or are kept with the agencies doing the assessments, but they may be discarded after a few years. The US EPA has generally not required, or enforced requirements, that contractors archive their algal materials in a museum or in their institution. Materials are often given to the Academy of Natural Sciences (ANSP) in Philadelphia or California Academy of Sciences (CAS). Diatom materials are kept permanently but soft-algae samples are sometimes discarded after just a few years. At least one set of NAWQA program diatom slides and other algal material are archived in the Diatom Herbarium and Phycology Section at the (ANSP). All NEON diatom slides and algae samples are being archived at the NEON Biorepository at Arizona State University so they will be publicly available. Algal materials from state programs are kept by state agencies, or given to a local depository (e.g., Montana Diatom Herbarium) or national museum (ANSP, CAS). Sometimes they are discarded. However, the ability to archive samples should be treated as one of the unique selling points of diatom samples: how many other biological groups are there where the actual sample itself can be revisited as metrics and objectives change?

10.9. Databases with algal counts

Storing algal counts and metadata in a database is an important way to ensure consistent identification over time and to discover and quantify long-term trends. In the EU, some individual countries or regions have such a database (and, in some cases, this is now publicly accessible via the internet- e.g. England: https://environment.data.gov.uk/ecology-fish/), but there is no all-EU data repository. In the US, data are stored in national and state-level databases, but not in a consistent format, and not always easily available. The EPA has required that data from projects it funds be added to one of its databases, but it can be difficult to retrieve. Data from large projects such as NRSA were available from project websites (e.g., https://www.epa.gov/national-aquatic-resource-surveys/nrsa), but access has recently been ended. The USGS stores its algal study results, including for NAWQA, in its BioData database (McCoy, 2011). NEON algae data are available from it’s website (https://www.neonscience.org/data/samples-specimens/neon-biorepository-asu). Several states maintain websites where algal data can be downloaded.

10.10. Analysis of datasets to detect, describe, and quantify analyst bias

One of the best ways to detect and assess the taxonomic bias among analysts is to examine data they produce (Cao et al., 2007; Tyree et al., 2020a). These analyses are sometimes done specifically for this purpose or are a byproduct of other analyses. This type of analysis is not required by EU/US level agencies. In the EU, “member state” is nearly always a significant “fixed effect” in regressions when datasets are combined (not just for diatoms). This is probably due to a whole raft of methodological and biogeographical factors, of which analytical bias might be one (Kelly et al., 2014). In the US, analysis of surveys that involve multiple analysts or labs can reveal a clear analyst bias. In fact, “analyst” can be the major “environmental factor” explaining variability among algal counts (Tyree et al., 2020a; Cao et al., 2007). Tyree et al. (2020a) outline a four step approach to prevent analyst bias: 1) use of a voucher flora, 2) randomization of sample assignment to analysts, 3) high self-recount and cross-counts, and 4) use of a morphological operational taxonomic unit (mOTU) until a final step of assignment of scientific name.

10.11. Taxonomic resolution

In both EU and US, nearly all algae-based assessment programs require, or at the very least strongly encourage, identification of algae to the lowest practical level; diatoms to species and variety and soft algae to genus. This level of identification maximizes information available to address assessment goals (Rimet and Bouchez, 2012; Poulíčková et al., 2017). It also offers the greatest opportunity for comparing datasets and applying a range of analysis techniques. It is very difficult to harmonize taxonomy among datasets with different levels of taxonomic resolution. The effect of merging taxa that caused taxonomic issues was evaluated during the EU's intercalibration exercise, mostly as a means of determining that analyst bias was not a major factor influencing comparisons between Member State assessment outcomes (Kelly and Ector, 2012; Kahlert et al., 2012) but such exercises are sometimes performed to consider options for potential cost savings. Methods requiring lower taxonomic resolution sometimes perform well, especially if used in a limited geographic region or for projects designed to assess specific stressors/pressures (Hill et al., 2000; Raunio and Soininen, 2007; Lavoie et al., 2009). They may not, however, significantly reduce costs (Bennett et al., 2014).

Not all variation necessary to distinguish species can be resolved easily with the light microscope (Kahlert et al., 2019; Trobajo et al., 2013), so issues of taxonomic resolution are likely to continue into the future. A sensible compromise needs to be found: data that are "split" can be "lumped" but the reverse is not true, so attention to fine taxonomic detail, accompanied by some of the other procedures suggested in this section, is the recommended approach. However, if the costs of (potentially) time-consuming analyses outweigh perceived (short-term) benefits, then archived samples should allow data to be revisited as taxonomic concepts develop in the future.

11. Discussion

On both sides of the Atlantic, benthic algal assessment is performed in the context of legislation whose goal is to protect and enhance ecological integrity. However, implementation of this legislation in both EU and US is devolved to (member) states with considerable power to tailor approaches to suit their own circumstances. The relationship among (member) states and the European Commission or US Federal Government is akin to that of adolescent children and parents: as long as your actions are within the broad guidelines of acceptable behaviour for assessing the condition of waters, you should be free to behave how you want.

Many of the differences that we see can be traced back to differences in the specific wording of the legislation with the WFD being generally more prescriptive than the CWA. This is reflected in the more widespread use of benthic algae in the EU (where it is mandatory) compared to the US (where it is encouraged but is not obligatory). A further general observation is that EU Member States have tended to rely on established metrics whereas US states have preferred to develop new approaches (albeit sometimes adopting existing metrics as part of MMIs). This partly reflects the short timescale allowed for WFD implementation, combined with the availability in Europe of Omnidia (Lecointe et al., 1993), a software package that allowed diatom metrics to be calculated and compared with ease, and established approaches for sampling and analysing diatoms (CEN, 2014a, 2014b).

Both the EU and US tend to focus on diatoms over other algal groups, although exceptions exist on both sides of the Atlantic. One key difference that emerged is that macrophyte assessment is more common amongst EU Member States than in the US, and macroalgae are often included in these surveys. Thus, whilst many US states include measurement of either algae biomass or cover within their "benthic algae" suite, those EU Member States that do measure algae cover often include it as part of macrophyte, rather than benthic algal assessments. That said, several EU Member States do not have any measure of algal abundance at all which, bearing in mind that it is the quantity of algae rather than, necessarily, the species present, which determines the scale of secondary effects for aesthetics and dissolved oxygen (Suplee et al., 2009; Stevenson et al., 2012) seems curious. The reality is that algal abundance is both spatially and temporally variable, limiting the value of one-off measurements or estimates. At best, most benthic algal methods seem to evaluate the potential for eutrophication rather than the actual risk of secondary effects developing (Schneider et al., 2016).

Overall, however, preparing this review has shown us how much common ground there is between the EU and the US in their use of algae and, as many of the similarities and differences have been addressed earlier in the review, this discussion will focus on shared challenges going forward.

11.1. Challenge 1: communicating the importance of algae

Benthic algae are, generally, little known or understood outside the small groups of specialists who study them. The wider public notices benthic algae only when they form nuisance growths and may not be aware that the thin slippery film of algae on surfaces plays a key role in regulating energy flow and other ecosystem functions in all systems. Thus, whilst it is important to set nutrient criteria that protect against nuisance growths of algae it is, perhaps, more important to emphasize that such criteria are also protecting algal communities that are a necessary part of healthy ecosystems.

Ecological assessments, especially benthic algal assessments, can specifically address ecosystem services (Stevenson, 2014; Stevenson and Smol, 2015). In this paper, we mostly discuss the use of benthic algae to assess biological condition and stressors in habitats. Measuring stressors with benthic algae is valuable because benthic algae can be more accurate and precise measures of stressors that vary in time and space than low frequency measurements of the stressor itself. Measuring (or inferring) stressor levels is important to determine the likelihood (risk) of aquatic ecosystem (designated) use support. However, benthic algae also link more directly to ecosystem services. Naturalness (as biological integrity in the CWA or ecological quality in the WFD), measured as biological condition or ecological quality ratios, can be considered a final ecosystem service because people are willing to pay for moral, religious and ethical benefits. In addition, naturalness could also be an intermediate ecosystem service because it can support other final ecosystem services such as resilience, water clarity, and high-quality drinking water.

Algal biologists need to do more to understand and communicate the importance of algae in healthy/natural ecosystems. Most efforts, to date, have been at a local level, often involving non-government groups (e.g. universities). The WFD's criteria for "less stringent environmental objectives" (i.e. for setting targets below good status) include situations where achievement of good status is "disproportionately expensive" (Article 4, clause 5), implying a value judgement in which the costs of improvements outweigh the benefits attained. If a site is at moderate status due only to a failure of benthic algae to achieve good status, there is a risk that the costs will be deemed "disproportionate" simply because the role of algae in supporting good status is not adequately appreciated by catchment managers and stakeholders.

11.2. Challenge 2: striving for consistency

A recurring theme throughout this review has been the need for consistency in the collection and interpretation of phytobenthos data, with different actors taking a keen interest in distinct stages of the process. Thus, analysts need to be sure that their samples are collected using the same procedures, and they also need to agree on the identities of the organisms present. In some cases, misidentification of a common taxon can lead to the wrong assessment outcome, for example, which has implications for the (member) state. We discuss the ways that this can be achieved in Section 10, differentiating between exercises such as ring tests and certification programmes that ensure that analysts work to the same standards, and formal audits, where the quality of batches of samples is checked and, if necessary, corrective action taken. We note that this adds to the overall cost of analysis and is not always popular with managers whose budgets are under constant scrutiny.

Confidence in assessment outcomes is necessary because, both in the EU and US, ecological assessment is now enshrined in legislation that gives regulators legal powers to enforce changes, possibly leading to substantial investment by major polluters which may affect competitiveness (in the case of some industries) or lead to price rises for consumers (e.g., where costs of wastewater treatment rise to cover the installation of new facilities). The quality of field and laboratory procedures, therefore, should be seen as one component of the fair application of the law within a (member) state. A further component of this is the setting of benchmarks (Section 8) that enable the law to be applied consistently across the whole range of biogeographic, geologic and climatic variation that occurs across a territory. The basis for this lies in narrative descriptions of criteria, seen in both the EU and US (Section 2) and in the processes by which ecological criteria are translated into water quality criteria (Section 7). An upland stream draining igneous geology might, for example, have a tighter phosphorus criterion than a lowland calcareous stream but the rationale behind these can be shared.

A further layer of complication: ensuring consistency in application of legislation across the EU and US, where biogeographic differences may be extreme (both EU and US extend from the Arctic Circle to the subtropics – if French overseas departments are included). Consistent application of the law is difficult under these circumstances yet, at the same time, necessary if no (member) state is to have an unfair advantage. In the EU, the intercalibration exercise has provided a framework for this, but at huge expense; in the US, involvement of several scientists from around the country contributes to consistent application of the BCG (see Paul et al., 2020).

Concern for "horizontal" consistency has prompted much work across the EU and US and, by contributing to an understanding of the natural factors which drive phytobenthos assemblages (e.g. Cantonati et al., 2020; Kelly et al., 2020a, 2020b; Tang et al., 2016, 2020; Theroux et al., 2020), ensures that bias caused by natural spatial variability can be minimised. However, individual (member) states are often more concerned about temporal consistency – ensuring that trends in ecological condition can be followed over time. This can, in turn, create a reluctance to replace existing methods. Kelly (2019) describes one such instance, showing how scientific outputs need to be considered within a broader framework of "change management" within publicly-accountable organisations.

11.3. Challenge 3: adopting new technology

The potential for molecular approaches to supplement or even replace existing light microscopy based analyses of benthic algae is described in 7.6. It is likely that within the next few years metabarcoding-based approaches to benthic algal assessment will be used routinely in both the EU or US. In principle, there is no reason why not as these approaches have a level of performance similar to traditional analyses (Kelly et al., 2020a; Rivera et al., 2020; Vasselon et al., 2017) and there is undoubted potential. We urge caution, however: having stressed throughout the importance of the underlying legislation in determining the way that benthic algae are used in the EU and US, it is important to reflect on this when contemplating new approaches. Makiola et al. (2020), for example, set out the potential for "next generation biomonitoring" but we are working within limits defined by "this generation legislation." Legislation will undoubtedly evolve, but it is important that it enshrines society's aspirations for the environment and that monitoring and assessment are then tailored to those needs. It seems premature to set out a raft of possibilities for monitoring and assessment that do not link explicitly to policy goals.

This does not mean, however, that we are not now in a position to move ahead from the conservative approach of developing molecular analogues of current approaches ("option 1" in Hering et al., 2018), even when working towards WFD and CWA objectives. The WFD, for example, requires assessment of composition and abundance of benthic algae and invertebrates (as well as fish and macrophytes) in rivers. Currently, these are performed as two separate analyses for well-understood practical reasons. There is no reason why these could not be performed as a single analysis using metabarcoding, and that status evaluations consider not just the two distinct organism groups but also the interactions between them (Seymour et al., 2020).

The early tentative steps towards metabarcoding-based assessment in the EU raised anotherquestion: why limit metabarcoding evaluations of benthic algae to diatoms, as has been the preferred option when using traditional approaches? Combined analyses of diatoms and soft algae are possible (Gillett et al., 2009) but the greater taxonomic sensitivity that is achievable when using cleaned diatoms requires separate preparations for soft algae and diatoms, with a concomitant increase in costs. Both diatoms and soft algae behave similarly along the main stressor gradients (Kelly et al., 2008b; Schneider et al., 2013) so there is little added benefit in return, at least from a high-level management perspective.

These justifications, however, no longer apply when using metabarcoding to analyse samples. The reason that metabarcoding studies to date in Europe are largely limited to diatoms is that there is a well curated library of diatom barcodes available (Rimet et al., 2019), allowing confident assignation of Linnaean binomials. In theory, it is possible to extend metabarcoding analyses to other groups of algae if reliable reference libraries were available. In practice, however, primers for rbcL, the preferred barcode (Mann et al., 2010) are optimised for diatoms and the deep evolutionary divisions amongst the algae may require these to be redesigned to embrace other lineages or, alternatively, for rbcL to be replaced by a different barcode such as 18S.

This final prerequisite has been circumvented by Apothéloz-Perret-Gentil et al. (2017) and Tapolczai et al. (2019b) who use raw OTUs rather than assigning these to Linnaean binomials. This approach has the advantage of using all the diversity in a sample, rather than just those taxa represented in the reference library, but it comes at the expense of the extra insights that come from using Linnaean binomial as keys to unlock the wide knowledge base on the ecology of species present. A truly taxonomy-free approach would be, in any case, inconsistent with the WFD's requirement to assess composition. Two possible workarounds are a) to supplement a taxonomy-free approach with a BLAST against a reference library to produce at least a partial taxa list to assist interpretation; and, b) to ensure that all OTUs (or Amplicon Sequence Variants) can be catalogued and are transferable between analyses (which is not the case with current technology). Obviously, the easiest way to catalogue these is to assign a Linnaean binomial; but where this is not possible a higher level assignment (genus, for diatoms, family for other algae, perhaps) along with a numeric code would be a better option than completely ignoring preexisting knowledge.

12. Conclusion

Our review has highlighted many similarities between approaches to benthic algal-based assessments in the EU and US, with key differences driven largely by the wording used in the underpinning legislation. We have also recognized, at several points, that decisions regarding the use of algae are shaped as much by budgets, traditions and (dare we say) the whims of bureaucrats as it is by objective interpretation of evidence. We therefore end this review by considering what advice we would give to the aspiring 51st US State or 28th EU Member State, if asked to develop a benthic algal assessment system that was fully in line with the requirements of the WFD or CWA. This system should be rooted, as far as possible, in strong science and be pragmatic to the extent that it recognizes that bioassessment budgets are finite. The following points should be considered:

- Benthic diatoms will probably address most of the goals set out in current legislation; there is a well-established suite of methods based around light microscopy that can be adapted and adopted. The inclusion of additional algal assemblages, such as macroalgae, can increase diagnostic capabilities when technical expertise and resources allow.
- Simple quantification of biomass in the field (e.g. visual estimates of cover; field estimates of chlorophyll gathered using portable fluorimeters) is a valuable element of benthic algal condition, linking directly to environmental conditions; this should be included in benthic algal assessments if not already included in a macrophyte assessment.
- Sampling a standard, single substrate is a desirable way to eliminate the possible influence of substrate, and is possible in small-scale studies carried out within single water bodies or small watersheds; large-scale studies may require a wider range of substrates to be sampled.
- Ensure that diatom slides are archived in a central herbarium to allow samples to be revisited in the future.
- Both the single-metric approach common in the EU and the MMIs developed in the US have proved effective. Whatever is decided should be tested across all major ecosystems, embracing the widest possible range of stressors and stressor gradients.
- Consider emerging technologies such as metabarcoding: if starting method development from scratch, this may prove to be a more effective option in the long-term.
- Internal and external consistency in application of the rules is important. Do not expect nutrient criteria for high altitude softwater streams to be the same as for low altitude hardwater streams. Calibrate concepts of good aquatic health against those used in neighbouring (member) States using real-time bioassessment data.
- If possible, base site-specific management decisions on several samples from the same water body in order to account for seasonal variation and maximise the statistical power of the assessment.
- Develop a Quality Assurance Framework. Ensure that all analysts are working to the same taxonomic conventions and have access to appropriate literature and online resources. Make sure that taxonomic guidance is updated regularly and provide ring-tests and workshops to refresh analysts' knowledge and to brief them on new developments. Consider a standardized taxonomic list for ecologists that is regularly updated.

Overall, the period since the first workshop on the Use of Algae for Monitoring Rivers (Whitton et al., 1991) has seen the sustained development of an idea into reality. Benthic algae are now a regular part of the bioassessment toolkit, and evidence derived from them contributes to decision-making (and, therefore, to a healthier planet) not just in the EU and US but worldwide.

CRediT authorship contribution statement

Donald F. Charles: Conceptualization, Writing - original draft, Project administration. **Martyn G. Kelly:** Conceptualization, Writing - original draft, Project administration. **R. Jan Stevenson:** Conceptualization, Writing - original draft, Project administration. **Sandra Susanna Poikane Theroux:** Conceptualization, Writing - original draft, Project administration, Conceptualization, Writing - original draft, Project administration. **Aleksandra Zgrundo:** Conceptualization, Writing - original draft, Project administration. **Marco Cantonati:** Conceptualization, Writing - original draft, Project administration.

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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Appendix A. Supplementary data

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Supplementary material

Table S1 Description of metrics used in the Member State benthic algal assessments (phytobenthos and macrophyte assessment methods). VLR - index used for very large rivers

	Algal groups			References	
Member State	**Diatoms**	**Non-diatom microalgae**	**Macroalgae**	**Phytobenthos assessment**	**Other algae assessment**
Austria	Trophic Index (TI); Saprobity Index (SI); Reference Index (RI) including all algal groups			Pfister and Pipp, 2013; Rott et al., 1997, 1999	
Belgium Flanders	PISIAD index	-	Macroalgae included in the indicator list of macrophyte indices and in the growth form metrics	Hendrickx and Denys, 2005; Leyssen et al., 2006; Kelly et al., 2009	Leyssen et al., 2005; Birk et al., 2011
Belgium Wallonia	IPS index	-	Macroalgae taxa included in the IBMR index indicator list	Coste, in CEMAGREF, 1982; Lenoir and Coste, 1996; Kelly et al., 2009	Haury et al., 2006; Birk et al., 2011
Bulgaria	IPS index	-	Abundance of Cladophora	Cheshmedjiev et al., 2010; Coste, in CEMAGREF, 1982	
Bulgaria (VLR)	IPS index	-	-	Coste, in CEMAGREF, 1982; Hlúbiková et al., 2016a, 2016b	
Czech Republic	Czech saprobic-trophic index, including all algal groups			Marvan et al., 2011; Schöll et al., 2012	
Croatia	Modified Rott's TI	-	Macroalgae taxa are included in the RI index indicator list	Žutinić et al., 2020 Rott et al., 1999	Alegro, 2020a, 2020b
Croatia (VLR)	TI and SI indices	-		Hlúbiková et al., 2016b	

Cyprus	IPS index	-	Macroalgae taxa are included in the IBMR index indicator list	Coste, in CEMAGREF, 1982; Almeida et al., 2014	Haury et al., 2006; Aguiar et al., 2014
Denmark	TI and SI indices	-	-	Rott et al., 1997, 1999, 2003	
Estonia	IPS index	-	Macroalgae taxa are included in the MIR index indicator list	Lenoir and Coste, 1996 Kelly et al., 2009	Pall, 2016; Szoszkiewicz et al., 2020
Estonia (VLR)	IPS, TDI, Watanabe index	-		Vilbaste et al., 2004; Schöll et al., 2012	
Finland	IPS index	-	-	Coste, in CEMAGREF, 1982; Eloranta and Soininen, 2002	
France	IBD index		Macroalgae taxa are included in the IBMR index indicator list	Coste et al., 2009; Almeida et al., 2014	Haury et al., 2006
Germany	Diatom module: TI, SI, RI	Non-diatom Module: RI Index. Charophytes are included in the macrophyte index		Rott et al., 1997, 1999; Kelly et al., 2009 Schaumburg et al., 2004, 2012	Schaumburg et al., 2004, 2012
Greece	IPS index	-	Macroalgae taxa are included in the IBMR index indicator list	Smeti and Karaouzas, 2016; Coste, in CEMAGREF, 1982;	Haury et al., 2006; Aguiar et al., 2014
Hungary	IPS, SI and TI indices	-	Macroalgae taxa are included in the RI index indicator list	Várbíró et al., 2012; Coste, in CEMAGREF, 1982; Rott et al., 1997, 1999	Lukács et al., 2015
Hungary (VLR)	IPS index	-		van Dam et al., 2011; Schöll et al., 2012; Coste, in CEMAGREF, 1982;	
Ireland	Revised form of Trophic Diatom	-	Macroalgae taxa are included in the MTR index indicator list	Kelly et al., 2008; Kelly et al. 2009	Holmes et al., 1999

	Index (TDI)				
Italy	IPS index, TI index	-	Macroalgae taxa are included in the IBMR index indicator list	Mancini and Sollazzo, 2009, Almeida et al., 2014	Haury et al., 2006; Aguiar et al., 2014
Latvia	IPS index	-	Macroalgae taxa are included in the MIR index indicator list	Jēkabsone, 2019; Lenoir & Coste, 1996	Uzule and Jēkabsone, 2016; Szoszkiewicz et al., 2020
Lithuania	Trophic Index, Saprobic Index	-	Macroalgae taxa are included in the RI index indicator list	Rott et al., 1997, 1999	Virbickas, 2016; Schaumburg et al., 2012
Luxem-bourg	IPS index	-	Macroalgae taxa are included in the IBMR index indicator list	Coste, in CEMAGREF, 1982 Kelly et al., 2009	Haury et al., 2006
Netherlands	EKR index based on % of positive and negative indicators	-	Cover of floating algae bed (% cover) included in the growth form metric	Van der Molen, 2004; Kelly et al, 2009	Pot and Birk, 2015
Netherlands (VLR)	IPS index	-		Van den Berg et al., 2004; Schöll et al., 2012	
Norway	-	Periphyton Index of Trophic Status (PIT)		Schneider and Lindstrøm, 2011	
Poland	TI and SI indices	-	Macroalgae taxa are included in the MIR index indicator list	Picińska-Fałtynowicz, 2009; Rott, 1997, 1999	Szoszkiewicz et al., 2020
Portugal	IPS index	-	Macroalgae taxa are included in the IBMR index indicator list	Coste, in CEMAGREF, 1982; Almeida et al., 2014	Haury et al., 2006; Aguiar et al., 2014
Romania	IPS index, TI index	-	-	Kelly, 2016, 2018; Kelly et al., 2019	

Slovakia	IPS, CEE, EPI-D indices	-	Macroalgae taxa are included in the IBMR index indicator list	Hlúbiková et al., 2007; Schöll et al., 2012	Haury et al., 2006; Baláži and Tóthová, 2010
Slovenia	Saprobic index and Trophic index	-	-	Rott et al., 1997, 1999; Almeida et al., 2014; Schöll et al., 2012	-
Spain (north-west)	SHE, SLAD, IDG, TDI, IPS, L&M indices; sensitive taxa metrics PFSS, PABSS	-	Macroalgae taxa are included in the IBMR index indicator list	Delgado et al., 2010; Kelly et al., 2009	Haury et al., 2006; Aguiar et al., 2014
Spain (south and VLR)	IPS index	-		Coste, in CEMAGREF, 1982; Almeida et al., 2014	
Spain (Balearic islands)	Sensitive taxa metrics PABSS, PABST, chl-a	-		Pardo and Costas, 2018; Delgado et al., 2012	
Sweden	IPS index	-	-	Coste, in CEMAGREF, 1982 Kelly et al. 2009	
Sweden (VLR)	IPS, TDI, %PT, ACI	-	-	Schöll et al., 2012	
UK	Revised form of Trophic Diatom Index (TDI)	-	Macroalgae included in the macrophyte index, number of functional groups and number of taxa; Percentage cover of green filamentous algae included as one of the metrics	Kelly et al., 2008; Kelly et al., 2009	Willby et al., 2012

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Supplemental Table 2. Summary of state and national algal bioassessment programs in the US. EPA = periphyton included in bioassessment program for wadeable streams according to EPA website (US EPA, n.d., p. 2); Chl a = chlorophyll *a* concentrations; AFDM = ash-free dry mass; MMI = multimetric index; IBI = index of biotic integrity; Predictive = predictive metric or index includes a measure of site-specific reference expectations (see main text); BCG = state has an algal Biological Condition Gradient model; Nutrient criteria = algal metrics have been used to develop numeric or narrative nutrient criteria for the state water quality programs. Information presented is considered the best available at the time of publication and is not assumed to be comprehensive. Information on biomass and algal assemblages was derived from water quality department websites and standard operating procedures, in addition to technical reports and published literature. Information on indices, metrics, and predictive metrics is provided if researchers (state government or academic) have explored, developed, or published indices for use in that state. Where multiple indices have been developed, the most recent effort is listed.

State	**EPA**	**Biomass**	**Diatoms**	**Non-diatom microalgae**	**Macroalgae**	**Indices**	**Metrics**	**Predictive**	**BCG**	**Nutrient criteria**	**References**
Alabama	No	Chl a, percent benthic cover									(Alabama Department of Environmental Management, 2017)
Alaska	Yes	Chl a	x			Diatom MMI in developm ent	% Motile;Organic nitrogen tolerance Saprobity;Number of species Trophic state				(Alaska DEC, 2015; Rinella and Bogan, 2007)
Arizona	Yes	Chl a, percent cover	x			Diatom index in developm ent					(ADEQ, 2018)
Arkansas	Yes	AFDM, chl a									(Arkansas DEQ, 2012)
California	Yes	AFDM, chl a, percent benthic cover	x	x	x	Algal Stream Condition Index: Diatom, SBA, and hybrid (diatoms and SBA) MMI.		Yes (Theroux et al., 2020)	Yes (Paul et al., 2020)		(Ode et al., 2016; Paul et al., 2020; Theroux et al., 2020)

Colorado	Yes	AFDM, chl a	x							Diatom nutrient response models under development	(CDPHE, 2015)
Connecticut	Yes	AFDM, chl a	x	x	x	Diatom tolerance metrics	Species tolerances categories developed by Potapova and Charles (2007); indicator species analysis (Dufrene and Legendre, 1997) and weighted averaging using CT collected data and CT derived cutoffs (Smucker et al., 2013a) to define high and low TP sites. GAMs models classification described by Yuan (2006, 2004) to categorize species responses.		Diatom BCG under development		(Becker, 2017; Becker et al., 2018; CT DEEP, 2015; Smucker et al., 2013)
Florida	Yes	Chl a, percent benthic cover	x	x	x	Stream Diatom Index (not recommended)	Percent sensitive cells, percent tolerant cells, percent cells that prefer high oxygen, percent cells that prefer oligotrophic conditions, and van Dam's weighted index for trophic status (TSI).				(FL DEP, 2014; Fore, 2010; Stevenson and Wang, 2001)
Georgia	Yes	Chl a	x							Diatom nutrient response models	(Georgia DNR, 2015)

										under development	
Idaho	Yes	AFDM, chl a	x			River Diatom Index (RDI)	Tolerance and intolerance;% Sensitive;% Very tolerant Autecological guild Eutrophic species richness % Nitrogen heterotrophs;% Polysaprobic;Alkaliphilic species richness % High oxygen Morphometric guild;% Very motile Individual condition % Deformed cells	Yes (Cao et al., 2007)			(Cao et al., 2007; Fore, 2010; Grafe, 2002; Idaho DEQ, 2016)
Indiana	Yes	AFDM, Chl a	x			Diatom IBI in development					(IDEM, 2019; Kevin Gaston, 2016)
Kansas	Yes	Chl a									(KDHE, 2020)
Kentucky	No	Chl a, AFDM, percent benthic cover	x	x	x	Diatom Bioassessment Index (KDBI)	Total Number Diatom Taxa (TNDT); Shannon Diversity, Kentucky Pollution Tolerance Index; Cymbella group richness, Fragilaria Group Richness, % Navicula Nitzschia, Surirella				(KDEP, 2009a, 2009b; Kentucky Division of Water (KDOW), n.d.; Kentucky NREPC, 2002)
Maine	Yes	Chl a, percent benthic cover	x	x	x	Diatom and SBA Metrics	Weighted-average optima for common taxa for total P, total N, specific conductance, %		Yes	Narrative, with quantitative implement	(Danielson, 2014, 2010; Danielson et al., 2012, 2011; Maine DEP, 2014, 2009)

							impervious cover, and % developed watershed. Assigned Maine stream tolerance values and categories (sensitive, intermediate, tolerant) to taxa based on their optima and responses to watershed disturbance.			ation procedures or translators (stream algae)	
Massachusetts	Yes	Chl a, percent benthic cover		x	x					Wadeable rivers: benthic chlorophyll a samples >200 mg/m2 , filamentous algal cover >40%, recurring and/or prolonged algal and/or C-HAB blooms Deep rivers: phytoplankton Chlorophyll a >16 ug/L, recurring and/or	(Massachusetts Division of Watershed Management, 2018)

										prolonged algal and/or C-HAB blooms	
Minnesota	No	Chl a	x	x						Nutrient criteria	(Heiskary et al., 2013)
Montana	Yes	AFDM, chl a, percent benthic cover	x		x	Diatom metrics (for nutrients and sediment)	Nutrient Increaser Taxa: Biometrics based on stressor-specific increaser diatom taxa, as described in (Teply, 2010a, 2010b)			In transition	(Montana DEQ, 2011; Suplee, 2004; Suplee et al., 2009, 2008; Suplee and Sada, 2016; Teply, 2010a)
Nevada	Yes	Chl a, percent benthic cover	x	x		Diatom Bioassessment Index (DBI)	A Diatom Bioassessment Index (DBI), based on the Kentucky Diatom Bioassessment Index (Kentucky 2008) and utilizing investigations of metrics performed by the Desert Research Institute (Davis and Fritsen 2006) for the Program, analyses periphyton function and structure.				(NDEP, 2015, 2009)
New Jersey	No	AFDM, chl a, percent benthic cover	x	x	x	Diatoms			Yes (Hausmann et al., 2016)	Diatom nutrient response models under development	(Charles et al., 2019; Hausmann et al., 2016; Ponader, Karin C. and Charles D. McGee, 2005; Ponader et al., 2008, 2007)

New Mexico	Yes	Chl a	x			Diatom metrics	Porter et al. (2008), Stevenson et al. (2008), and periphyton indices developed by Potapova and Charles (2007).			Diatom metrics used in nutrient response modeling for TN and TP	(Jessup, 2015; New Mexico Environment Department, 2016; Tetra Tech, 2013)
New York	Yes	Percent benthic cover	x	x		Diatom metrics	1) Pollution Tolerance Index (PTI) 2) the Trophic Index (TRI) 3) the Salinity Index 4) the Acidity Index 5) the Siltation Index and 6) the Diatom Model Affinity (DMA). A description of these individual metrics and calculation procedures follows.				(New York State DEC, 2019; Passy and Bode, 2004)
North Carolina	No	Chl a	x	x	x						(NC Department of Environmental Quality, 2016)
North Dakota	Yes	Chl a	x	x		Diatom MMI	Number of species in the old Cymbella genus; percent of total taxa that are highly mobile; percent of total taxa in the oxygen class 1 or 2; number of Gomphonema species; and percent abundance of individuals in the genus Fragilaria				(North Dakota Department of Health, 2013)

Pennsylvania	No	AFDM, Chl a, percent benthic cover	x	x	x	Diatom MMI in development					(Pennsylvania DEP, 2018)
Rhode Island	No	Chl a, percent benthic cover	x	x							(Rhode Island DEM, 2012)
South Dakota	No	AFDM, Chl a	x	x		Periphyton IBI (for some rivers)	11 metrics, including chlorophyll a and biomass. Diatom metrics for pH, salinity, organic nitrogen, oxygen requirement, saprobity, trophic state, % silt tolerant taxa, species richness, % dominant				(SDDENR, 2017, 2007)
Tennessee	Yes	Percent benthic cover	x	x		Kentucky Diatom Bioassessment Index (KDBI) diatom MMI	Total Number Diatom Taxa (TNDT); Shannon Diversity, Kentucky Pollution Tolerance Index; Cymbella group richness, Fragilaria Group Richness, % Navicula Nitzschia, Surirella				(Tennessee DEC, 2010, 2001)
Utah	No	AFDM, Chl a								Diatom TP response models in development	(Utah DEQ, 2018; Utah DWQ, 2019)
Virginia	No	AFDM, chl a	x	x		Diatom metrics	Sensitive-tolerant species, functional composition including			Virginia DEQ collecting	(USEPA, 2019)

							motility, and species diversity (same as EPA NRSA)			and building database	
Washington	Yes	AFDM, chl a	x	x		Diatom metrics	Pollution Index, Siltation Index, Bahls 1993 disturbance index; Van Dam diatom metrics; Non-diatom metrics dominant genera and richness.				(Larson and Collyard, 2019)
West Virginia	No	Chl a, percent benthic cover	x	x							(West Virginia DEP, 2015)
Wisconsin	No	Chl a, percent benthic cover	x			Diatom Nutrient Index (DNI)				The DNI may be used to assess the response of biota in streams to nutrients	(Garrison and LaLiberte, 2007)
Wyoming	Yes	Chl a, percent benthic cover	x	x							(Wyoming DEQ, 2018, 2017)
EPA NARS/NRSA	NA	AFDM, chl a	x	x		Diatom MMI under developm ent	Sensitive-tolerant species, functional composition including motility, and species diversity				(US EPA, 2016; USEPA, 2019, 2017)
NEON	NA	AFDM, chl a, percent	x	x							(Parker, 2015)

		benthic cover									
USGS NAWQA	NA	AFDM, chl a	x	x	x	Diatom metrics (SBA metrics weak)	Eutrophication impacts; biological condition and a suite of stressor-specific metrics and reference/disturbed indicator taxa				(Moulton et al., 2002; Potapova and Carlisle, 2011; Potapova and Charles, 2007)

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