water

MDPI

Review

Benthic Diatoms in River Biomonitoring—Present and Future Perspectives within the Water Framework Directive

Andreas Masouras [1,2,*], Ioannis Karaouzas [1], Elias Dimitriou [1], George Tsirtsis [2] and Evangelia Smeti [1]

[1] Institute of Marine Biological Resources and Inland Waters, Hellenic Centre for Marine Research, 46.7 km Athens-Sounio Ave., 19013 Anavyssos, Greece; ikarz@hcmr.gr (I.K.); elias@hcmr.gr (E.D.); evasmeti@hcmr.gr (E.S.)
[2] Department of Marine Sciences, University of the Aegean, University Hill, 81100 Mytilene, Greece; gtsir@aegean.gr
* Correspondence: a.masouras@hcmr.gr; Tel.: +3-022-9107-6358

Abstract: The European Water Framework Directive 2000/60/EC (WFD) has been implemented over the past 20 years, using physicochemical, biological and hydromorphological elements to assess the ecological status of surface waters. Benthic diatoms (i.e., phytobenthos) are one of the most common biological quality elements (BQEs) used in surface water monitoring and are particularly successful in detecting eutrophication, organic pollution and acidification. Herein, we reviewed their implementation in river biomonitoring for the purposes of the WFD, highlighting their advantages and disadvantages over other BQEs, and we discuss recent advances that could be applied in future biomonitoring. Until now, phytobenthos have been intercalibrated by the vast majority (26 out of 28) of EU Member States (MS) in 54% of the total water bodies assessed and was the most commonly used BQE after benthic invertebrates (85% of water bodies), followed by fish (53%), macrophytes (27%) and phytoplankton (4%). To meet the WFD demands, numerous taxonomy-based quality indices have been developed among MS, presenting, however, uncertainties possibly related to species biogeography. Recent development of different types of quality indices (trait-based, DNA sequencing and predictive modeling) could provide more accurate results in biomonitoring, but should be validated and intercalibrated among MS before their wide application in water quality assessments.

Keywords: phytobenthos; biological quality indices; ecological status; surface waters; water quality

Citation: Masouras, A.; Karaouzas, I.; Dimitriou, E.; Tsirtsis, G.; Smeti, E. Benthic Diatoms in River Biomonitoring—Present and Future Perspectives within the Water Framework Directive. *Water* **2021**, *13*, 478. https://doi.org/10.3390/w13040478

Academic Editor: Jesús R. Aboal
Received: 8 January 2021
Accepted: 9 February 2021
Published: 12 February 2021

1. Introduction

The degradation of water quality in Europe has forced the European Parliament to establish the Water Framework Directive (WFD) (2000/60/EC) which required that EU Member States (MS) should achieve "good ecological status" and "good chemical status" of surface waters by 2015 [1]. This goal was difficult to achieve for a significant proportion of water bodies, so the European Commission allowed the extension of the deadline up to 2027 or beyond [2]. This extension highlights the complexity of the factors ruling "ecological status" and the need to better define the metrics used.

"Ecological status" is expressed as an ecological quality ratio (EQR = observed/reference) into five scale-status classes (high, good, moderate, poor and bad), depending on the scale of deviation from reference conditions, where 0 corresponds to maximum deviation (i.e., bad) and 1 corresponds to no deviation (i.e., high) [3]. Ecological status is based on biological quality combined with physicochemical and hydromorphological quality, for an integrated assessment. In rivers, the main biological quality elements (BQEs) used, so far, are benthic invertebrates, phytobenthos, fish, macrophytes and phytoplankton. The WFD suggests that European MS should use all BQEs in ecological quality assessment of their surface waters, as each group represents specific responses to various pressures related to their habitat requirements and lifecycle [4–7] and expects a reason in the case where this is not true. Biological quality is then derived by implementing a "one-out, all-out" approach, whereby the BQE with the lowest performance should be retained for the assessment [3]. Not all MS apply all BQEs, as this could depend on the water types, the BQEs traditionally used for biomonitoring and the expertise of the involved researchers. Benthic invertebrates and phytobenthos (i.e., mostly benthic diatoms) are the most commonly used indicators for the evaluation of river quality in Europe [8,9].

Undoubtedly, the implementation of the WFD over the last twenty years changed biomonitoring of European aquatic ecosystems significantly. Nevertheless, there is still room for development and improvement of the monitoring system according to the requirements of the WFD. This is essential, as the assignment of a wrong ecological status class to a water body can have significant economic consequences [10]. Therefore, it is imperative to find an accurate approach for each BQE, in order to integrate them in a more holistic ecological assessment.

Herein, we review the implementation of benthic diatoms (the dominant group of the phytobenthos BQE) in river biomonitoring for the purposes of the WFD, focusing on their advantages as bioindicators and the biological quality metrics applied so far. We further discuss the potential of recent approaches, including trait-based metrics, DNA sequencing and predictive modeling as tools of diatom biomonitoring. Towards this aim, we searched for all available peer-reviewed scientific articles (using keywords WFD, diatom quality indices, benthic diatoms, diatoms as a BQE) in Google scholar, ResearchGate, Web of Science and PubMed. We reviewed more than 200 papers that described metrics of surface water quality based on benthic diatoms and almost 100 of them were used for this review paper. Furthermore, we searched in the WFD webpage (https://ec.europa.eu/environment/water/water-framework/index_en.html, last accessed on 29 January 2021) where intercalibration reports of all MS were uploaded and the Water Information System for Europe (WISE) database (https://water.europa.eu, last accessed on 29 January 2021) to retrieve data for the ecological quality of MS after the second river basin management plan.

2. Benthic Diatoms in Biomonitoring

2.1. Importance of Benthic Diatoms as Biological Indicators

Diatoms have a fundamental ecological role in aquatic ecosystems. They are key players in ecosystem functioning, being responsible for up to 20–25% of organic carbon fixation in the planet [11], also supporting primary productivity and nutrient cycling such as phosphorus, nitrogen and silica [9,12–16]. In freshwater ecosystems, although occasionally found in the water column as planktic cells, they are mainly considered benthic species, i.e., attached on substrates such as aquatic plants (epiphyton), stones (epilithon), sediments (epipelon) [17]. In running waters, their benthic nature accounts for responses to nutrients, and organic and inorganic micropollutants [18,19]. The morphological and ecological characteristics of benthic diatoms constitute them as one of the best bioindicators of pressures such as eutrophication, and chemical and organic pollution [11,20–22], revealing, therefore, their importance in water quality assessment.

Their short lifecycle allows them to respond fast to any natural and anthropogenic disturbance, making them more sensitive to environmental changes than other biotic groups [23,24], and highlighting their pivotal diagnostic potential. They rapidly respond to changes of environmental parameters such as temperature, pH, salinity, organic pollutants, inorganic nutrients and heavy metals [25–31], being sensitive both to nonpoint (e.g., agriculture) [7,26] and point-source pollution (e.g., olive mill wastes [28], toxic industrial wastes [29]). Diatoms have the advantage to reveal pollution of heavy metals and toxic elements at the organism level, through the occurrence of teratological forms, whereas assessment of assemblage changes or common biological quality indices could mask possible negative effects [29–33]. Their small size (<10–200 μm in diameter or length) [12] and their diverse life forms, make them vulnerable, and thus potentially good indicators of hydrological alterations on streams and rivers [34], responding faster than other biota [26].

Benthic diatoms could also be a valuable tool in ecotoxicity tests and active biomonitoring, where key species or whole diatom assemblages could be grown on artificial substrates [35–37]. Therefore, whole diatom assemblages could be tested for toxic contaminants or other pollutants in the laboratory or in the field, providing an advantage of diatoms over other taxa. In active biomonitoring, artificial substrates are submerged in a river site and then transferred elsewhere to test for the effect of selected environmental parameters on assemblage structure and composition, also assessing ecological health after remediation [36]. On the other hand, ecotoxicological tests could expose model species from different functional groups to river sediments collected on-site, providing important information that could be more useful in ecological status assessment than time-consuming and costly methods defined by the WFD [35].

2.2. Advantages of Benthic Diatoms over Other Biological Quality Elements (BQEs)

The choice of BQE in water quality assessment depends on river type and the stressor that is known to affect it [19]. Benthic diatoms are advantageous over other BQEs in most habitats or for environmental stressors, making them, thus, more useful in routine biomonitoring [19,38,39] (Table 1). A major advantage is that they can be found everywhere, in almost any type of running water [40] where sufficient light is available, including fresh and marine waters, moist and terrestrial habitats [11,24]. They can be abundant in poor habitats, on hard substrates or in rivers with high flow velocity where macroinvertebrates, macrophytes and phytoplankton (commonly used in lowland rivers), could be absent [7,41–45]. Due to their fast growth rate, benthic diatoms react faster to short-term hydrological changes, as opposed to macroinvertebrates (e.g., in an intermittent river in Greece [26]) and macrophytes (e.g., in rivers in central and southern Poland [46]).

Table 1. Advantages and disadvantages in the use of diatoms as a biological quality element in biomonitoring.

	Advantages	Disadvantages
Biomonitoring	Widespread distribution, even in extreme environments or poor habitats	Heterogeneous distribution (e.g., light/flow dependent)
	Sensitive to any natural or anthropogenic disturbance	Poorly sensitive to habitat alterations
Sampling	Quick and easy collection (scraping, pipetting, using corers for soft sediments and sand)	Risks of loss (e.g., floods) of artificial substrates
	Cost efficient with minimal impact on resident biota	
	Sampling on artificial substrates (when natural substrates missing)	
Taxonomic identification	Numerous identification resources (identification guides, articles, websites)	Difficult and frequently changing systematics
		Taxonomic misidentification (due to endemism and rare species)
		Time consuming
		High quality microscope necessary/skilled taxonomists

Benthic diatoms appear to be more sensitive to nutrient enrichment, responding from low to moderate levels of physicochemical quality degradation, compared to macroinvertebrates and fish, which respond from moderate to high levels of physicochemical quality degradation [47]. This occurs in both mountain and lowland water bodies in France [47], Germany and Austria [38], China [48] and in a temporary river in Greece [26]. This might be a result of sedimentary nature and short lifecycle of benthic diatoms compared to fish that are characterized by stronger adaptability due to their migratory capacities and long lifecycle [47]. Diatoms show to be more affected by toxic wastes (i.e., olive mill wastewaters) than invertebrates in temporary rivers in Greece [49] and in northwestern Spain because of sensitivity of diatom-based indices to heavy metals [50]. Furthermore, diatoms are more affected by diffuse pollution than benthic invertebrates, providing a stricter ecological status in Mediterranean small-sized streams [51].

Important aspects of biomonitoring that should be considered when assessing different BQEs are sampling effort and taxonomy. Sampling of benthic diatoms is relatively easy, cost efficient and with minimal impact on resident biota during field collections [21], compared especially to fish sampling, where the commonly used method of electrofishing could lead to fish deaths [52,53], whereas its efficiency is affected by turbidity and conductivity [54]. Taxonomic identification in diatoms is relatively easy up to genus level and even though it could be considered rather difficult on the species level, there is sufficiently large available literature [55,56]. On the other hand, macroinvertebrate taxonomy under the genus/species level for many groups is practically impossible for routine biomonitoring [54].

2.3. Benthic Diatoms in the Water Framework Directive

Benthic diatoms are the dominant part of phytobenthos, one of the most common BQEs for the purpose of biological assessment in the WFD [57]. During the second river basin management plan, a total of 65,284 water bodies from 28 countries were classified into a biological quality class using the "one-out, all-out" approach on the BQEs used in each water body [58]. The most used BQE was benthic invertebrates, applied in almost 85% of water bodies, followed by phytobenthos (54%), fish (53%), macrophytes (27%) and phytoplankton (4%) (Figure 1). Data for phytobenthos were derived from 23 countries; as for the other five countries—accounting for the 13.5% of the total water bodies assigned to a biological quality class—data were not available in the WISE database. This could be a result of late compliance of these countries to the WFD objectives (e.g., intercalibration reports for Denmark and Latvia were only approved in September 2020). The effort of these five countries to apply benthic diatoms in biomonitoring is also apparent by peer-reviewed studies [59,60]. Despite using data from less MS, phytobenthos was used in almost the same number of water bodies as fish. This highlights the ubiquitous nature of benthic diatoms compared to fish, which may be absent from many water bodies. This could be the case in intermittent rivers, where extreme natural drought events could lead local fish populations to collapse [47,61,62].

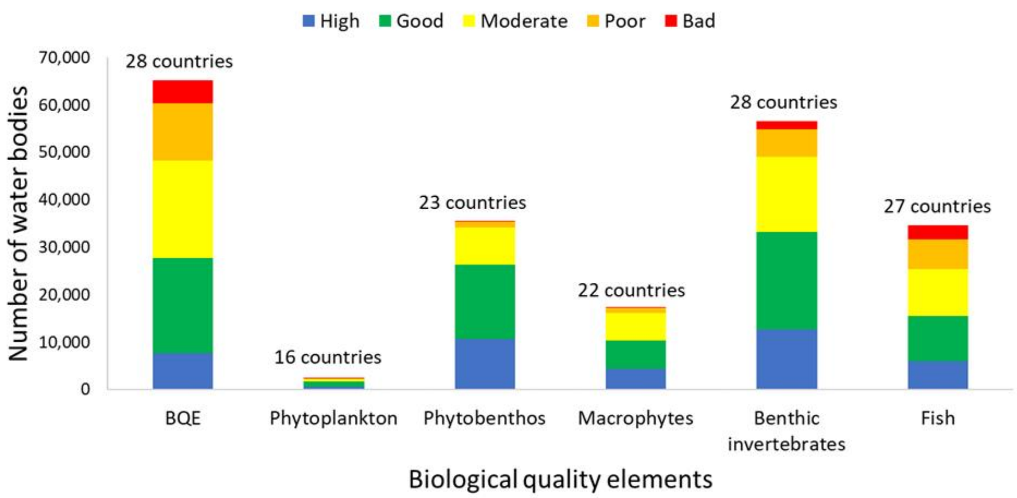

Figure 1. Biological quality elements (BQEs) used in water bodies of European Member States (MS) in accordance with the Water Framework Directive (WFD). Data from one country that uses phytobenthos are not available in WISE Database. Source: WISE Database, 2021.

Almost 40% of river water bodies are classified as high and good, and 60% in moderate, poor and bad ecological status, based on the one-out/all-out principle (Figure 2). Phytobenthos seems to overestimate the ecological status, classifying more than 70% of water bodies to good and high status, whereas fish seem to be the strictest BQE and thus the most influential to the ecological status due to the one-out/all-out principle (Figure 2). This observation does not diminish the importance of benthic diatoms as suitable bioindicators, but could be related to many different types of pressures in water bodies [63], such as long-term hydrological and habitat alterations to which other BQEs respond better. However, it could also be attributed to naturally poor habitats, where other BQEs are poorly represented [7]. Furthermore, the fast recovery of benthic diatoms [64–68] following the recovery of chemical parameters compared to other BQEs could result in better biological quality status indicated by phytobenthos.

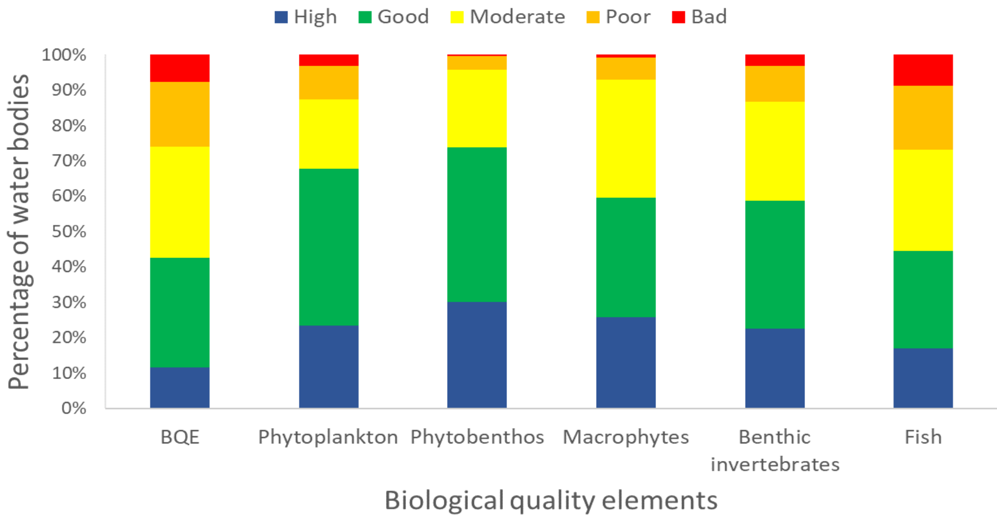

Figure 2. Percentage of water bodies belonging to different quality classes following biological quality assessment in European rivers (https://www.eea.europa.eu/themes/water/european-waters/water-quality-and-water-assessment/water-assessments/quality-elements-of-water-bodies, last accessed on 29 January 2021) based on the one-out/all-out approach (BQEs) and the most common BQEs. Colors show the different quality classes.

2.4. Diatom-Based Indices Used So Far in the Water Framework Directive

The need to monitor water quality has led to the development of standardized sampling protocols and assessment methods, through single, simplified indices. The most widespread diatom-based indices used for water quality assessment are based on classic taxonomy, up to genus and most frequently species level [30,66–69]. These taxonomy-based diatom indices are based on diatom assemblage composition and relative abundance. The main concept behind their development is the fact that each species has specific environmental requirements and has a different extent of occurrence (indicator value) and a different sensitivity to pollution (sensitivity value). Most of the indices that have been developed are based on the Zelinka and Marvan (1961) formula, which accounts for the relative abundance of each species along with its indicator and sensitivity values, based on the saprobic status of the system. Other indices focus on trophic pollution, organic pollution, acidification or heavy metal pollution [30,66,70].

MS are using previously developed taxonomic indices for water quality assessments that have been intercalibrated among the MS (Table 2). This enabled the use of common indices, despite the strong evidence that such metrics are less useful when applied in regions other than those where species–environment relationships were originally assessed [30]. To overcome this biogeographic limitation, certain countries developed new indices, adapted to their own environmental gradients and species presence.

Table 2. Most used species-based indices for water quality assessment in the MS.

Index name	Focus	Member States Using It	References
IPS (Specific Pollution Index)	Organic pollution	Most of EU members Greece, Belgium, Estonia, Luxembourg, Sweden, Finland, Bulgaria, Croatia, Cyprus, Spain, Portugal, Hungary, Slovakia, Poland, Latvia	[66,71]
GDI (Generic Diatom Index)	Organic pollution and trophic status	Belgium, Poland	[67,72]
ICM (Metal Pollution Index)	Heavy metal pollution	NW Spain, Hungary, Italy, Lithuania	[30]
DDI (Duero Diatom Index)	Nutrient pollution	Duero basin at NW Spain	[69]
TDI (Trophic Diatom Index)	Trophic status	UK, Croatia, Ireland, Poland	[68]
BDI (Biological Diatom Index)	Trophic status	France, Romania, Spain, Poland	[73]
EPI-D (Eutrophication Diatom Index)	Trophic status	Italy, Slovakia, Poland	[70]
DIATMIB (Diatom Multimetric Index)	Trophic status	Spain (temporary streams)	[20]
IO (Multimetric Diatom Index, TI: Trophic Index SI: Saprobic Index)	Trophic status and organic pollution	Poland, Slovenia, Germany, Austria, Hungary, Denmark	[74–76]
DAM (Diatom Acidification Metric)	Acidification status	United Kingdom, Ireland	[77]

In Europe, 26 countries have successfully intercalibrated their assessment methods using the BQE phytobenthos (Table 2). From the two remaining countries, Malta appears to be using it; however, no intercalibration report is available and available literature concerning benthic diatoms in rivers of Malta is very limited [78]. The Netherlands, on the other hand, has used the presence of negative indicator species, a metric that could not be successfully compared with other MS, and are now adapting their methods to comply with WFD objectives [79] Almost 60%, 15 out of 26 countries, use the IPS, and nearly 25% (6 countries out of 26) use the multimetric diatom index (IO) to estimate the biological status. The IO is a combination of two indices: the trophic index (TI) [74] and the saprobic index (SI) [75]. Many countries, such as UK, Ireland, Italy, Spain, Croatia and Poland, use multiple indices, depending on river type or source of pollution. To detect heavy metal pollution, four countries out of 26 are using the metal pollution index (ICM) index (Table 2, [30]), whereas for acidification assessment, two countries use the diatom acidification metric (DAM) index (Table 2, [77]).

The most valuable tool for water quality assessment and biomonitoring [68,80–83] based on diatoms is the OMNIDIA software [84], which uses the indicative properties of diatoms and includes information on the tolerance of diatom taxa to environmental parameters [76]. The software is continuously upgraded and extended with new diatom-related data. The latest version 6.0 contains a taxonomical and ecological database that includes 720 genera and 21,000 diatom species, and calculates 18 diatom indices and 33 ecological statistics (www.omnidia.fr, accessed on 8 February 2021).

Regardless of their importance and their wide use, taxonomic indices are related to many uncertainties that could even change the result of the water quality assessment. This could be more important in the case of sites between the good–moderate quality boundaries, where misclassification could result in considerable time and money loss or insufficient conservation [85]. These uncertainties are partly related to the fact that diatom indices are developed for specific geographic regions but have frequently been used in others [69,86,87]. The most prominent example is the case of the most commonly used specific pollution index (IPS) index (developed in France but used in more than half of MS), but also the trophic diatom index (TDI) (developed in UK but used in other three countries) and biological diatom index (BDI) (developed in France but used in other three countries). The broad use of locally developed indices could be an issue as species response to environmental parameters depend on geographic or habitat distributions, with different responses in different ecoregions [88]. This is apparent in the development of indices from different ecoregions that use different ecological profiles for the same species [88]. Another form of uncertainty is related to taxonomic misidentification, where species with similar morphology might present different ecological optima. Furthermore, the presence of rare species or species with updated taxonomy is hard to evaluate, as their ecological profiles are not clearly defined [88].

Soon after the implementation of WFD, its significance prompted countries outside Europe to consider adopting similar legislations and assessment methods. Neighboring countries such as Turkey have developed their own indices for biomonitoring, considering them as more accurate for their ecoregion (Turkey trophic index) [89]. In North America, the US is using biomonitoring through the Clean Water Act, whereas Canada has taken initiatives to implement a more organized framework to bioassessment methods already sporadically applied [90]. In South America, Argentina has developed its own diatom-based indices for assessing water quality (Pampean diatom index), [18]. In Australia, phytobenthos has been used for biological quality assessment for many years before the implementation of WFD in Europe (e.g., [91]); however, there has been no governmental coordination [90]. In Africa, South Africa has a long legacy of diatom research and use in biomonitoring; however, greater effort is needed for organized implementation in other African countries [92]. In Asia, the Asian Pacific Water Summit in 2007 started a new era in water quality assessment in Asian countries [93] The need for global application of bioassessments influenced by WFD is apparent; however, further discussion deviates from the scope of the present review.

3. Recent Approaches and Future Perspectives

To overcome the restrictions of the taxonomy-based indices, nontaxonomic measures emerged recently, taking into consideration functional traits (e.g., cell size, ecological guilds, life forms) and DNA sequences (e.g., operational taxonomic units, exact sequence variant, individual sequence units). Furthermore, assemblage structure methods, such as predictive models and statistical techniques (e.g., machine learning) have been developed to assess water quality using benthic diatom assemblages against different environmental parameters. We present below all of these promising new approaches, including advantages and disadvantages (Table 3), which need further scientific research before they can be implemented for the purposes of WFD.

Table 3. Advantages and disadvantages of taxonomy-based diatom indices applied so far for water quality assessment and new promising tools.

Indices	Advantages	Disadvantages
Taxonomy-based	• Accurate method • Many references • Many indices	• Time consuming • Taxonomic misidentification/skilled microscopists • Expensive optical equipment • Access to specialized literature • Lack of rare species database • Different species response in different ecoregions
Trait-based	• Less time consuming • Less effort • Taxonomy free • Related to environmental stressors	• Need more studies for different ecoregions • Need more generalized environmental gradients
Molecular-based	• Less time consuming • Taxonomy free	• Complementary tool for biomonitoring • Most of species not represented in the molecular databases • Cannot be linked to observed species in the field
Predictive models	• Less time consuming • Taxonomy free	• Need more studies to validate their results
Machine learning techniques	• Less time consuming • Taxonomy free	• Need more studies

3.1. Trait-Based Diatom Indices

In recent years, trait-based approaches have started to emerge as a potential tool for ecological status/quality assessment [94–97]. Traits are defined as "any morphological, physiological or phenological measurable feature at the individual level" [98] and are more tightly linked to ecosystem functioning than species; thus, their study provides a more integrative ecological assessment potential at the ecosystem level [88]. Traits described in benthic diatoms are cell size, ecological guilds and life forms, and have been successfully related to environmental stressors (i.e., pesticides–herbicides, heavy metals, nutrients and organic pollution) [99–105].

Cell size is related to the cell biovolume, or the cell's length to width ratio. Even though cell size is considered as one of the most important functional traits, being related to growth rates and nutrient uptake [103], there is no clear evidence that it is related to trophic status or organic pollution based on organic waste [99]; however, smaller cells have been shown to increase at high pesticide concentrations at the expense of larger cells [101]. Ecological guilds refer to a group of taxa that exploits the same resources and are divided into high profile, low profile, motile and planktic [99–101]. Ecological guilds are connected to physical disturbance due to currents but also to nutrient concentrations. Low profile cells are favored at high flows and nutrient-poor environments, high profile cells are favored at low flows and motile cells are abundant in nutrient-rich environments [99,100,106]. Life forms are related to the species living as solitary cells or colony-forming and their attachment to the substrate [101]. Different life forms are linked to trophic status and organic micropollutants, with tube-forming colonial diatoms being sensitive to trophic and organic micropollutants, and stalked diatoms tolerating increased levels of trophic and organic pollution [99].

As these traits are relatively easy to measure and define, the development of trait-based indices could be advantageous for river quality assessment because they should be less time consuming and require much less effort than taxonomy-based indices [88]. Towards the development of trait-based indices, the same rationale, as for the well-known taxonomic-based indices, was used. Each trait was given an ecological value based on its position along an environmental gradient of nutrient concentration and organic micropollutants, and a model was fitted to the resulting distribution [107]. Correlations between trait-based indices and pollution gradients, and trait-based and taxonomic indices, showed that the former could adequately predict water quality [88,97,107]. Even though trait-based approaches show a great potential for assessing water quality, their use has been only tested thus far in specific rivers (e.g., the Mesta River in Bulgaria and the White Creek, NY, USA [102]), islands (the Mayotte Island) [107] and countries (France) [99]. Therefore, the low number of trait-based indices developed so far is tightly linked to specific environmental conditions and gradients in the study areas. More studies should be conducted in different ecoregions and environmental gradients to ensure robustness of those indices prior to their use for routine biomonitoring.

3.2. Molecular-Based Diatom Indices

Following advances in the field of molecular ecology, high-throughput sequencing (HTS) techniques, especially DNA metabarcoding, seem to be a promising tool for future monitoring and assessment protocols, providing reliable, high quantity and quality standardized data with lower cost and time compared to common taxonomic methods [108–112]. These techniques use gene markers to identify taxa-specific sequences in the cell's DNA, i.e., a barcode [109,111]. These sequences are stored in a database and are assigned to a diatom species. Therefore, the identification of multiple taxa from many samples simultaneously is feasible, speeding up the assessment process [112].

First attempts of HTS applications on benthic diatom biomonitoring were directly linked to taxonomic assignment of sequences in order to apply already available indices on identified sequences. However, the low number of common taxa found in molecular and morphological datasets highlighted the existing gap in reference libraries when considering diatom species [113,114]. Hence, a large part of the biological diversity unraveled by DNA methods was discarded and could not be used for bioassessment purposes [114]. To overcome this obstacle, taxonomy free indices (i.e., molecular indices directly from DNA data without any reference to morphotaxonomy) are now being developed [108].

DNA data used for the development of these indices have been grouped using different methods, resulting in different indices, aiming to overcome bias derived from clustering. Operational taxonomic unit (OTU) is the most common grouping, where sequences are clustered based on their similarity. The similarity threshold (usually 97–99%) is a source of method uncertainty, altering the number of groups in the analysis. However, clustering with a high sequence similarity increases the risk of giving ecological sense to sequence errors and artifacts [115]. Towards a more concrete grouping, allowing reproducibility of analysis and possibility of meta-analysis, exact sequence variant (ESV) and individual sequence units (ISUs) have been proposed. These are unique DNA reads with biological meaning [116–118] and are taxonomy- and clustering-free [94]. For the development of the indices, each group of sequences (OTUs, ESV, ISUs) was positioned along the environmental gradient defined by the given dataset to detect its ecological optimum, thus defining sensitivity and indicator values assigned to the group [108].

Taxonomy-free indices presented similar results concerning water quality assessment when compared to taxonomy-based indices, highlighting their potential in routine biomonitoring [108,113]. However, they were only tested in small datasets from France [108], Switzerland [113] and Portugal [119], and they were directly related to environmental gradients of those datasets. Comparisons between quality classes determined by taxonomy-based and molecular-based indices showed an agreement of over 65% in French rivers [120]. Furthermore, most of the HTS techniques are prone to biases related to factors such as the absence of a complete reference library [110,118], the DNA extraction method [106], the DNA barcode used [121], the bioinformatics treatment [122], the sequences clustering thresholds and the gene copy number per cell [123]. Even though improvements are made towards resolving these issues, they are considered as complementary tools rather than replacements for morphology-based methods [113], suggesting that there is still a long way to go before they can be used reliably for routine WFD biomonitoring.

3.3. Predictive Models

Predictive models are β-diversity metrics, measuring the quality of a site as the alteration of diatom assemblages between impacted and reference sites of comparable environmental conditions [124]. This method forms an integrated approach, since it relies on differences between assemblage structure and composition rather than between individual species. These models have been used in Portugal (DIATOMOD) [125] and Northern Spain (NORTIdiat) [15], with NORTIdiat being intercalibrated as a nonofficial method of the member state [15].

These predictive diatom-based models are a taxonomy-free method for assessing the ecological status in rivers and streams. Since the comparisons in predictive models are made between impacted and reference sites of comparable conditions, it is imperative to define reference sites in different river types. The NORDIdiat was found to be a good predictor of eutrophication and intensive agriculture [15], whereas DIATOMOD was also proved to be a good predictor of hydromorphological alteration [125]. These results show the potential for using predictive modeling in water quality assessment using diatoms; however, its limited use and its dependence on specific river types hinders its wider application in biomonitoring. In the future, more studies should be conducted in different regions to validate these results, with various data types (i.e., species, traits or molecular data).

3.4. Machine Learning Techniques

Another nontaxonomic approach to predict diatom assemblages and water quality was very recently proposed by the use of machine learning techniques [126,127]. The purpose is to develop models that could actually predict diatom assemblages from environmental data [119]. Datasets used to train the models were based on taxonomic data from morphological observations (HYDMORPH) and on OTU lists from molecular analysis (HYDGEN). Water quality is assessed using the observed/expected richness ratio. Both models were accurate and sensitive to anthropogenic disturbance, but the model based on OTUs was sensitive to more stressors compared to the model based on taxonomic data. The combination of taxonomy free datasets and machine learning techniques to assess water quality based on diatom assemblages seems promising; however, more studies need to be done to validate consistent results in order to be used for regular biomonitoring [126].

4. Conclusions

During the last two decades, the WFD has been the main European legislation used for biological quality assessment of surface waters. Benthic diatoms, as the dominant part of phytobenthos, were used and successfully intercalibrated by 93% of EU MS in 54% of the total water bodies during the second river basin management plan. Their sensitivity to natural or anthropogenic disturbance, their ubiquitous nature (present in all types of natural and artificial substrates), their easy sampling and their fast response to environmental changes render the benthic diatoms as valuable bioindicators of biological assessment of aquatic systems.

Diatom quality indices are being implemented for the purpose of WFD, and many advances have been made in their development over the past 20 years. However, it seems that most of these advances were made toward the same direction, by adapting locally the same taxonomy-based indices. This resulted in more than half of the MS using the same index (IPS) irrespective of their ecoregion, raising doubts on the accuracy of the results. Development of HTS techniques have given a new boost in classic taxonomy-based indices, increasing the number of sequences that could be important for water quality status and probably introducing a more accurate classification. Agreement in quality classes has been proven high in cases tested, highlighting their future merits despite their long way before they can be generalized and used as a standalone method rather than a complementary tool in biomonitoring.

It was not until recently that research turned to other aspects of diatom assemblages, such as quality elements (functional traits, ecosystem processes and β-diversity approaches). These new approaches could lead biomonitoring into a new era, by linking water quality assessment to ecosystem structure and function, thus towards the true objective of the WFD, i.e., a holistic ecosystem integrity approach. All of these new approaches should be validated and intercalibrated among MS, however, before their application in future water quality assessments.

Author Contributions: Conceptualization, A.M. and E.S.; formal analysis, A.M.; investigation, A.M.; writing—original draft preparation, A.M., E.S. and I.K.; writing—review and editing, E.D. and G.T.; supervision, E.S. and I.K. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: No new data were created or analyzed in this study. Data sharing is not applicable to this article.

Acknowledgments: The authors would like to thank Nikolao Skoulikidi for his constructive comments.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. European Commission. *Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 Establishing a Framework for Community Action in the Field of Water Policy*; Official Journal of the European Union: Luxemberg, 2000; Volume 164, p. 19.
2. European Commission. *Report from the Commission to the European Parliament and the Council on the Implementation of the Water Framework Directive (2000/60/EC) River Basin Management Plans*; Official Journal of the European Union: Luxemberg, 2012.

3. European Commission. *Carrying forward the Common Implementation Strategy for the Water Framework Directive-Progress and Work Programme for 2003/2004*; Official Journal of the European Union: Luxemberg, 2003.
4. Sabater, S.; Guasch, H.; Ricart, M.; Romaní, A.; Vidal, G.; Klünder, C.; Schmitt-Jansen, M. Monitoring the effect of chemicals on biological communities. The biofilm as an interface. *Anal. Bioanal. Chem.* **2007**, *387*, 1425–1434. [CrossRef] [PubMed]
5. Johnson, R.K.; Hering, D. Response of taxonomic groups in streams to gradients in resource and habitat characteristics. *J. Appl. Ecol.* **2009**, *46*, 175–186. [CrossRef]
6. Johnson, S.; Ringler, N. The response of fish and macroinvertebrate assemblages to multiple stressors: A comparative analysis of aquatic communities in a perturbed watershed (Onondaga Lake, NY). *Ecol. Indic.* **2014**, *41*, 198–208. [CrossRef]
7. Karaouzas, I.; Smeti, E.; Kalogianni, E.; Skoulikidis, N.T. Ecological status monitoring and assessment in Greek rivers: Do macroinvertebrate and diatom indices indicate same responses to anthropogenic pressures? *Ecol. Indic.* **2019**, *101*, 126–132. [CrossRef]
8. Poikane, S.; Kelly, M.; Cantonati, M. Benthic algal assessment of ecological status in European lakes and rivers: Challenges and opportunities. *Sci. Total Environ.* **2016**, *568*, 603–613. [CrossRef]
9. Cetin, T.; Demir, N. The use of phytobenthos for the ecological status assessment in Upper Sakarya Basin, Turkey. *Ecol. Environ. Res.* **2019**, *17*, 10155–10172. [CrossRef]
10. Ćirić, M.; Nikolić, N.; Krizmanić, J.; Gavrilović, B.; Pantelić, A.; Petrović, V.M. Diatom diversity and ecological status of the Lasovačka and Lenovačka streams near Zaječar: Consideration of WFD implementation in Serbia. *Arch. Biol. Sci.* **2018**, *70*, 691–698. [CrossRef]
11. Round, F.E. *The Diatoms*; Cambridge University Press: Cambridge, UK, 1990; 747p.
12. Mann, D.G. The species concept in diatoms. *Phycologia* **1999**, *38*, 437–495. [CrossRef]
13. Falciatore, A.; Bowler, C. Revealing the Molecular Secrets of Marine Diatoms. *Annu. Rev. Plant Biol.* **2002**, *53*, 109–130. [CrossRef]
14. Allen, A.E.; Vardi, A.; Bowler, C. An ecological and evolutionary context for integrated nitrogen metabolism and related signaling pathways in marine diatoms. *Curr. Opin. Plant Biol.* **2006**, *9*, 264–273. [CrossRef] [PubMed]
15. Pardo, I.; Delgado, C.; Abraín, R.; Gómez-Rodríguez, C.; García-Roselló, E.; García, L.; Reynoldson, T.B. A predictive diatom-based model to assess the ecological status of streams and rivers of Northern Spain. *Ecol. Indic.* **2018**, *90*, 519–528. [CrossRef]
16. Kelly, M.G.; Chiriac, G.; Soare-Minea, A.; Hamchevici, C.; Birk, S. Defining ecological status of phytobenthos in very large rivers: A case study in practical implementation of the Water Framework Directive in Romania. *Hydrobiologia* **2018**, *828*, 353–367. [CrossRef]
17. Kitner, M.; Poulíčková, A. Littoral diatoms as indicators for the eutrophication of shallow lakes. *Hydrobiologia* **2003**, *506*, 519–524. [CrossRef]
18. Licursi, M.; Gómez, N. Benthic diatoms and some environmental conditions in three lowland streams. *Ann. Limnol. Int. J. Limnol.* **2002**, *38*, 109–118. [CrossRef]
19. Hering, D.; Johnson, R.K.; Kramm, S.; Schmutz, S. Assessment of European streams with diatoms, macrophytes, macroinvertebrates and fish: A comparative metric-based analysis of organism response to stress. *Freshw. Biol.* **2006**, *51*, 1757–1785. [CrossRef]
20. Delgado, C.; Pardo, I.; García, L. Diatom communities as indicators of ecological status in Mediterranean temporary streams (Balearic Islands, Spain). *Ecol. Indic.* **2012**, *15*, 131–139. [CrossRef]
21. Vilmi, A.; Karjalainen, S.M. Freshwater diatoms as environmental indicators: Evaluating the effects of eutrophication using species morphology and biological indices. *Environ. Monit. Assess.* **2015**, *187*, 243. [CrossRef]
22. Giorgio, A.; De Bonis, S.; Guida, M. Macroinvertebrate and diatom communities as indicators for the biological assessment of river Picentino (Campania, Italy). *Ecol. Indic.* **2016**, *64*, 85–91. [CrossRef]
23. Cabecinha, E.; Cortes, R.; Cabral, J.A.; Ferreira, T.; Lourenço, M.; Pardal, M. Ângelo Multi-scale approach using phytoplankton as a first step towards the definition of the ecological status of reservoirs. *Ecol. Indic.* **2009**, *9*, 240–255. [CrossRef]
24. Wu, N.; Faber, C.; Sun, X.; Qu, Y.; Wang, C.; Ivetic, S.; Riis, T.; Ulrich, U.; Fohrer, N. Importance of sampling frequency when collecting diatoms. *Sci. Rep.* **2016**, *6*, 36950. [CrossRef]
25. Solak, C.N.; Peszek, Ł.; Yilmaz, E.; Ergül, H.A.; Kayal, M.; Ekmekçi, F.; Várbíró, G.; Yüce, A.M.; Canli, O.; Binici, M.S.; et al. Use of Diatoms in Monitoring the Sakarya River Basin, Turkey. *Water* **2020**, *12*, 703. [CrossRef]
26. Karaouzas, I.; Smeti, E.; Vourka, A.; Vardakas, L.; Mentzafou, A.; Tornés, E.; Sabater, S.; Muñoz, I.; Skoulikidis, N.T.; Kalogianni, E. Assessing the ecological effects of water stress and pollution in a temporary river—Implications for water man-agement. *Sci. Total Environ.* **2018**, *618*, 1591–1604. [CrossRef]
27. Cimarelli, L.; Singh, K.S.; Mai, N.T.N.; Dhar, B.C.; Brandi, A.; Brandi, L.; Spurio, R. Molecular tools for the selective detection of nine diatom species biomarkers of various water quality levels. *Int. J. Environ. Res. Public Health* **2015**, *12*, 5485–5504. [CrossRef] [PubMed]
28. Smeti, E.; Schiller, D.; Von Karaouzas, I.; Vardakas, L.; Sabater, S.; Tornés, E.; Monllor-alcaraz, L.S.; Guillem-argiles, N.; Martinez, E.; Barceló, D.; et al. Multiple stressor effects on biodi-versity and ecosystem functioning in a Mediterranean temporary river. *Sci. Total Environ.* **2019**, *647*, 1179–1187. [CrossRef]
29. Laine, M.; Morin, S.; Tison-Rosebery, J. A multicompartment approach—Diatoms, macrophytes, benthic macroinverte-brates and fish—To assess the impact of toxic industrial releases on a small French river. *PLoS ONE* **2014**, *9*, e102358. [CrossRef] [PubMed]

30. Fernández, M.R.; Martín, G.; Corzo, J.; de la Linde, A.; García, E.; López, M.; Sousa, M. Design and Testing of a New Diatom-Based Index for Heavy Metal Pollution. *Arch. Environ. Contam. Toxicol.* **2017**, *74*, 170–192. [CrossRef]
31. Falasco, E.; Bona, F.; Badino, G.; Hoffmann, L.; Ector, L. Diatom teratological forms and environmental alterations: A review. *Hydrobiologia* **2009**, *623*, 1–35. [CrossRef]
32. Tornes, E.; Mor, J.R.; Mandaric, L.; Sabater, S. Diatom responses to sewage inputs and hydrological alteration in Mediterranean streams. *Environ. Pollut.* **2018**, *238*, 369–378. [CrossRef]
33. Montesanto, B.; Zille, S.; Coste, M. Diatomees epilithiques et qualite biologique de ruisseaux du mont Stratonikon, Chalkidiki (Grece). *Cryptogam. Algol.* **1999**, *3*, 235–251. [CrossRef]
34. Martínez-Carreras, N.; Wetzel, C.E.; Frentress, J.; Ector, L.; McDonnell, J.J.; Hoffmann, L.; Pfister, L. Hydrological connectivity inferred from diatom transport through the riparian-stream system. *Hydrol. Earth Syst. Sci.* **2015**, *19*, 3133–3151. [CrossRef]
35. Vidal, T.; Pereira, J.L.; Abrantes, N.; Soares, A.M.V.M.; Gonçalves, F. Ecotoxicological Assessment of Contaminated River Sites as a Proxy for the Water Framework Directive: An Acid Mine Drainage Case Study. *Water Air Soil Pollut.* **2012**, *223*, 6009–6023. [CrossRef]
36. Morin, S.; Licursi, M.; Tison-Rosebery, J. Benthic Diatom Monitoring and Assessment of Freshwater Environments: Standard Methods and Future Challenges. In *Aquatic Biofilms: Ecology, Water Quality and Wastewater Treatment*; Romaní, A.M., Guasch, H., Balaguer, M.D., Eds.; Caister Academic Press: Poole, UK, 2016; Volume 6, pp. 111–124.
37. Pandey, L.K.; Bergey, E.A.; Lyu, J.; Park, J.; Choi, S.; Lee, H.; Depuydt, S.; Oh, Y.T.; Lee, S.M.; Han, T. The use of diatoms in ecotoxicology and bioassessment: Insights, advances and challenges. *Water Res.* **2017**, *118*, 39–58. [CrossRef]
38. Dahm, V.; Hering, D.; Nemitz, D.; Graf, W.; Schmidt-Kloiber, A.; Leitner, P.; Melcher, A.; Feld, C.K. Effects of physicochemistry, land use and hydromorphology on three riverine organism groups: A comparative analysis with monitoring data from Germany and Austria. *Hydrobiologia* **2013**, *704*, 389–415. [CrossRef]
39. Mangadze, T.; Bere, T.; Mwedzi, T. Epilithic diatom flora in contrasting land-use settingsin tropical streams, Manyame Catchment, Zimbabwe. *Hydrobiologia* **2015**, *753*, 163–173. [CrossRef]
40. Borojević, K.K.; Udovič, M.G.; Žutinić, P.; Várbíró, G.; Plenković-Moraj, A. Do benthic diatom assemblages reflect abiotic typology: A case study of Croatian streams and rivers. *Acta Bot. Croat.* **2017**, *76*, 80–90. [CrossRef]
41. Janauer, G.; Dokulil, M. *Macrophytes and Algae in Running Waters, Biological Monitoring of Rivers: Applications and Perspectives*; Ziglio, G., Siligardi, M., Flaim, G., Eds.; Wiley Online Library: Hudson County, NJ, USA, 2006.
42. Feipeng, L.; Haiping, Z.; Yiping, Z.; Yihua, X.; Ling, C. Effect of flow velocity on phytoplankton biomass and composition in a freshwater lake. *Sci. Total Environ.* **2013**, *447*, 64–71.
43. Wehr, J.D. Minireview Use of phytoplankton in large river management. *J. Phycol.* **1998**, *34*, 741–749. [CrossRef]
44. Salmaso, N.; Braioni, M.G. Factors controlling the seasonal development and distribution of the phytoplankton community in the lowland course of a large river in Northern Factors controlling the seasonal development and distribution of the phytoplankton community in the lowland course of a large river in Northern Italy (River Adige). *Aquat. Ecol.* **2008**, *42*, 533–545.
45. Chambers, P.A.; Prepas, E.E.; Hamilton, H.R.; Bothwell, M. Current Velocity and Its Effect on Aquatic Macrophytes in Flow-ing Waters. *Ecol. Appl.* **1991**, *1*, 249–257. [CrossRef] [PubMed]
46. Susane, C.S.; Lawniczak, A.E.; Picinska-Faltynowicz, J.; Szoszkiewicz, K. Do macrophytes, diatoms and non-diatom benthic algae give redundant information? Results from a case study in Poland. *Limnologica* **2012**, *42*, 204–211.
47. Marzin, A.; Archaimbault, V.; Belliard, J.; Chauvin, C.; Pont, D. Ecological assessment of running waters: Do macrophytes, macroinvertebrates, diatoms and fish show similar responses to human pressures? Ecological assessment of running waters: Do macrophytes, macroinvertebrates, diatoms and fish show similar responses to human pressures. *Ecol. Indic.* **2012**, *23*, 56–65.
48. Qu, X.; Peng, W.; Liu, Y. Identifying the Impacts of Water Quality on Macroinvertebrate Degration in the Taizi River with a Reconsideration of Water Quality Grades in China. In Proceedings of the 38th IAHR World Congress, Panama City, Panama, 1–6 September 2019.
49. Smeti, E.; Kalogianni, E.; Karaouzas, I.; Laschou, S.; Tornés, E.; De Castro-Català, N.; Anastasopoulou, E.; Koutsodimou, M.; Andriopoulou, A.; Vardakas, L.; et al. Effects of olive mill wastewater discharge on benthic biota in Mediterranean streams. *Environ. Pollut.* **2019**, *254*, 113057. [CrossRef]
50. Blanco, S.; Bécares, E. Chemosphere Are biotic indices sensitive to river toxicants? A comparison of metrics based on diatoms and macro-invertebrates. *Chemosphere* **2010**, *79*, 18–25. [CrossRef] [PubMed]
51. Pace, G.; Bella, V.D.; Barile, M.; Andreani, P.; Mancini, L.; Belfiore, C. A comparison of macroinvertebrate and diatom responses to anthropogenic stress in small sized volcanic siliceous streams of Central Italy (Mediterranean Ecoregion). *Ecol. Indic.* **2012**, *23*, 544–554. [CrossRef]
52. Snyder, D. *Electrofishing and Its Harmful Effects on Fish*; Information and Technology Report, USGS/BRD/ITR; US Government Printing Office: Denver, CO, USA, 2003; p. 13.
53. Dodds, W.; Whiles, M. *Fish Ecology Fisheries and Aquaculture in Freshwater Ecology*, 3rd ed.; Academic Press: London, UK, 2020; Volume 23.
54. Barbour, M.T.; Faulkner, C.; Gerritsen, J. *Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates, and Fish*, 2nd ed.; George Gibson: Washington, DC, USA, 1999.
55. Brabcová, B.; Marvan, P.; Opatřilová, L.; Brabec, K.; Fránková, M.; Heteša, J. Diatoms in water quality assessment: To count or not to count them? *Hydrobiologia* **2017**, *795*, 113–127. [CrossRef]

56. Lange-Bertalot, H.; Hofmann, G.; Werum, M.; Cantonati, M. *Freshwater Benthic Diatoms of Central Europe: Over 800 Common Species Used in Ecological Assessment*; English Edition with Updated Taxonomy and Added Species in Freshwater Benthic Diatoms of Central Europe; Cantonati, M., Kelly, M.G., Lange-Bertalot, H., Eds.; Koeltz Botanical Books: Frankfurt, Germany, 2017.
57. Almeida, S.F.; Elias, C.; Ferreira, J.; Tornés, E.; Puccinelli, C.; Delmas, F.; Dörflinger, G.; Urbanič, G.; Marcheggiani, S.; Rosebery, J.; et al. Water quality assessment of rivers using diatom metrics across Mediterranean Europe: A methods intercalibration exercise. *Sci. Total Environ.* **2014**, *476*, 768–776. [CrossRef] [PubMed]
58. European Environment Agency. *European Waters Assessment of Status and Pressures*; Report No 7/2018; EEA: Copenhagen, Denmark, 2018.
59. Wu, N.; Thodsen, H.; Andersen, H.E.; Tornbjerg, H.; Baattrup-Pedersen, A.; Riis, T. Flow regimes filter species traits of benthic diatom communities and modify the functional features of lowland streams—A nationwide scale study. *Sci. Total Environ.* **2019**, *651*, 357–366. [CrossRef] [PubMed]
60. Springe, G.; Sandin, L.; Briede, A.; Skuja, A. Biological quality metrics: Their variability and appropriate scale for assessing stream. *Hydrobiologia* **2006**, *566*, 153–172. [CrossRef]
61. Kalogianni, E.; Vourka, A.; Karaouzas, I.; Vardakas, L.; Laschou, S.; Skoulikidis, N.T. Combined effects of water stress and pollution on macroinvertebrate and fish assemblages in a Mediterranean intermittent river. *Sci. Total Environ.* **2017**, *604*, 639–650. [CrossRef]
62. Vardakas, L.; Kalogianni, E.; Zogaris, S.; Koutsikos, N.; Vavalidis, T. Distribution patterns of fish assemblages in an Eastern Mediterranean intermittent river. *Knowl. Manag. Aquat. Ecosyst.* **2015**, *416*, 30. [CrossRef]
63. Poikane, S.; Salas, F.; Kelly, M.G.; Borja, A.; Birk, S.; Van De Bund, W. European aquatic ecological assessment methods: A critical review of their sensitivity to key pressures. *Sci. Total Environ.* **2020**, *740*, 140075. [CrossRef] [PubMed]
64. Larson, F.; Sundbäck, K. Recovery of microphytobenthos and benthic functions after sediment deposition. *Mar. Ecol.* **2012**, *446*, 31–44. [CrossRef]
65. Calapez, A.R.; Elias, C.L.; Almeida, S.F.P.; Feio, M.J. Extreme drought effects and recovery patterns in the benthic communities of temperate streams. *Limnetica* **2014**, *33*, 281–296.
66. Cemagref. *Etude des Methodes Biologiques Quantitatives d'Appreciation de la Qualite des Eaux*; Agence de l'eau Rhône Méditerranée Corse: Lyon, France, 1982; p. 28.
67. Coste, M.; Ayphasshorho, H. *Etude de la Qualite des Eaux du Bassin Artois-Picardie a l'Aide des Communautes de Diatomees Benthiques (Application des Indices Diatomiques)*; Hal Inrae: Lyon, France, 1991; p. 227.
68. Kelly, M.G.; Whitton, B.A. The Trophic Diatom Index: A new index for monitoring eutrophication in rivers. *Environ. Biol. Fishes* **1995**, *7*, 433–444. [CrossRef]
69. Álvarez-Blanco, I.; Blanco, S.; Cejudo-Figueiras, C.; Bécares, E. The Duero Diatom Index (DDI) for river water quality assessment in NW Spain: Design and validation. *Environ. Monit. Assess.* **2013**, *185*, 969–981. [CrossRef] [PubMed]
70. Dell'uomo, A. Assessment of Water Quality of an Apennine River as a Pìlot Study for Diatom-based Monitoring of Italian Wa-tercourses. In *Use of Algae for Monitoring Rivers*; Institut Fur Botanik, Universitàt: Vienna, Austria, 1996; pp. 65–72.
71. Descy, J.P. A new approach to water quality estimation using diatoms. *Nova Hedwig.* **1979**, *64*, 305–323.
72. Rumeau, A.; Coste, M.; Diatomees, L.E.S.; Generaux, C. Initation a la systematique des diatomees d'eau douce Pour l' utilisation pratique d' un indice diatomique générique for a useful generic diatomic index, bulletin français de la peche et de la protection des milieux aquatiques. *Bull. Fr. Peche Piscic.* **1988**, *309*, 1–69.
73. Coste, M.; Boutry, S.; Tison-Rosebery, J.; Delmas, F. Improvements of the Biological Diatom Index (BDI): Description and efficiency of the new version (BDI-2006). *Ecol. Indic.* **2009**, *9*, 621–650. [CrossRef]
74. Rott, E.; Pfister, P.; van Dam, H.; Pipp, E.; Pall, K. Indikationslisten für Aufwuchsalgen in österreichschen Fliebgewässern. In *Teil 2: Trophiendikation Sowie Geochemische Präferenz, Taxonomische und Toxikologische Anmerkungen*; Bundesministerium Für Land-Und Forstwirtschaft: Vienna, Austria, 1999; p. 248.
75. Rott, E.; Hofmann, G.; Pall, K.; Pfister, P.; Pipp, E. Indikationslisten für Aufwuchsalgen. In *Teil 1: Saprobielle Indikation*; Bundesministerium Für Land-Und Forstwirtschaft: Vienna, Austria, 1997; p. 73.
76. Szczepocka, E.; Żelazna-Wieczorek, J. Diatom biomonitoring—Scientific foundations, commonly discussed issues and fre-quently made errors. *Oceanol. Hydrobiol. Stud.* **2018**, *47*, 313–325. [CrossRef]
77. Juggins, S.; Kelly, M.; Allott, T.; Kelly-Quinn, M.; Monteith, D. A Water Framework Directive-compatible metric for assessing acidification in UK and Irish rivers using diatoms. *Sci. Total Environ.* **2016**, *568*, 671–678. [CrossRef]
78. Saliba, F.M.; Ghobara, M.M.; Attard, E.; Ellul, B. Primary Study of the Non-Marine Epilithic Diatom Communities of Malta and Gozo. *Int. J. Curr. Microbiol. Appl. Sci.* **2016**, *5*, 69–78. [CrossRef]
79. Van de Bund, W. *Water Framework Directive Intercalibration Technical Report, Part 1: Rivers*; European Communities: Luxemberg, 2009.
80. Prygiel, J. Management of the diatom monitoring networks in France. *Environ. Biol. Fishes* **2002**, *14*, 19–26. [CrossRef]
81. Kelly, M.; Bennett, C.; Coste, M.; Delgado, C.; Delmas, F.; Denys, L.; Ector, L.; Fauville, C.; Ferréol, M.; Golub, M.; et al. A comparison of national approaches to setting ecological status boundaries in phytobenthos assessment for the European Water Framework Directive: Results of an intercalibration exercise. *Hydrobiologia* **2009**, *621*, 169–182. [CrossRef]
82. Kelly, M. Role of benthic diatoms in the implementation of the Urban Wastewater Treatment Directive in the River Wear, North-East England. *Environ. Biol. Fishes* **2002**, *14*, 9–18. [CrossRef]

83. Eloranta, P.; Soininen, J. cological status of some Finnish rivers evaluated using benthic diatom communities. *J. Appl. Phycol.* **2002**, *14*, 1–7. [CrossRef]
84. Lecointe, C.; Coste, M.; Prygiel, J. "Omnidia": Software for taxonomy, calculation of diatom indices and inventories man-agement. *Hydrobiologia* **1993**, *269–270*, 509–513. [CrossRef]
85. Kelly, M. Data rich, information poor? Phytobenthos assessment and the Water Framework Directive. *Eur. J. Phycol.* **2013**, *48*, 437–450. [CrossRef]
86. Philibert, A.; Prairie, Y.T. Diatom Inferred Paleolimnological Reconstructions: Do They Work in Nutrient Rich Lakes. In Proceedings of the Sustainable Forest Management Network Conference, Edmonton, AB, Canada, 14–17 February 1999; pp. 155–160.
87. Pipp, E. A regional diatom-based trophic state indication system for running water sites in Upper Austria and its over regional applicability. *Verh. Int. Ver. Limnol.* **2001**, *27*, 3376–3380.
88. Tapolczai, K.; Bouchez, A.; Stenger-Kovács, C.; Padisák, J.; Rimet, F. Trait-based ecological classifications for benthic algae: Review and perspectives. *Hydrobiologia* **2016**, *776*, 1–17. [CrossRef]
89. Toudjani, A.A.; Çelekli, A.; Gümüş, E.Y.; Kayhan, S.; Lekesiz, H.Ö.; Çetin, T. A new diatom index to assess ecological quality of running waters: A case study of water bodies in western Anatolia. *Ann. Limnol. Int. J. Limnol.* **2017**, *53*, 333–343. [CrossRef]
90. McElligott, P. *Developing Biocriteria as a Water Quality Assessment Tool in Canada: Scoping Assessment*; Report; Canadian Council of Ministers of the Environment: North Vancouver, BC, Canada, 2006.
91. Morley, R.J.; Newall, P.R.; Bennison, G.L. *The Biology and Chemistry of Selected Mt. Stirling Sub-Alpine Streams 1986–1988*; Final Report, No. 100; Water Materials and Environmental Sciences Branch, Rural Water Commission: Victoria, Austalia, 1989.
92. Dalu, T.; Froneman, P.W. Diatom-based water quality monitoring in southern Africa: Challenges and future prospects. *Water SA* **2016**, *42*, 551. [CrossRef]
93. Moog, O.; Schmutz, S.; Schwarzinger, I. Biomonitoring and Bioassessment. *Riverine Ecosyst. Manag.* **2018**, *8*, 371–390. [CrossRef]
94. Rimet, F.; Bouchez, A.; Tapolczai, K. Spatial heterogeneity of littoral benthic diatoms in a large lake: Monitoring implications. *Hydrobiologia* **2016**, *771*, 179–193. [CrossRef]
95. Riato, L.; Bella, V.D.; Leira, M.; Taylor, J.C.; Oberholster, P.J. A diatom functional-based approach to assess changing environmental conditions in temporary depressional wetlands. *Ecol. Indic.* **2017**, *78*, 205–213. [CrossRef]
96. Zorzal-Almeida, S.; Soininen, J.; Bini, L.M.; Bicudo, D.C. Local environment and connectivity are the main drivers of diatom species composition and trait variation in a set of tropical reservoirs. *Freshw. Biol.* **2017**, *62*, 1551–1563. [CrossRef]
97. Stenger-Kovács, C.; Körmendi, K.; Lengyel, E.; Abonyi, A.; Hajnal, É.; Szabó, B.; Buczkó, K.; Padisák, J. Expanding the trait-based concept of benthic diatoms: Development of trait- and species-based indices for conductivity as the master variable of ecological status in continental saline lakes. *Ecol. Indic.* **2018**, *95*, 63–74. [CrossRef]
98. Violle, C.; Navas, M.-L.; Vile, D.; Kazakou, E.; Fortunel, C.; Hummel, I.; Garnier, E. Let the concept of trait be functional. *Oikos* **2007**, *116*, 882–892. [CrossRef]
99. Berthon, V.; Bouchez, A.; Rimet, F. Using diatom life-forms and ecological guilds to assess organic pollution and trophic level in rivers: A case study of rivers in south-eastern France. *Hydrobiologia* **2011**, *673*, 259–271. [CrossRef]
100. Larras, F.; Coulaud, R.; Gautreau, E.; Billoir, E.; Rosebery, J.; Usseglio-Polatera, P. Assessing anthropogenic pressures on streams: A random forest approach based on benthic diatom communities. *Sci. Total Environ.* **2017**, *586*, 1101–1112. [CrossRef] [PubMed]
101. Rimet, F.; Bouchez, A. Use of diatom life-forms and ecological guilds to assess pesticide contamination in rivers: Lotic mes-ocosm approaches. *Ecol. Indic.* **2011**, *11*, 489–499. [CrossRef]
102. Passy, S.I. Diatom ecological guilds display distinct and predictable behavior along nutrient and disturbance gradients in running waters. *Aquat. Bot.* **2007**, *86*, 171–178. [CrossRef]
103. Passy, S.I. Differential cell size optimization strategies produce distinct diatom richness–body size relationships in stream benthos and plankton. *J. Ecol.* **2007**, *95*, 745–754. [CrossRef]
104. Wu, N.; Dong, X.; Liu, Y.; Wang, C.; Baattrup-Pedersen, A.; Riis, T. Using river microalgae as indicators for freshwater biomonitoring: Review of published research and future directions. *Ecol. Indic.* **2017**, *81*, 124–131. [CrossRef]
105. Marcel, R.; Berthon, V.; Castets, V.; Thiers, A.; Labat, F.; Fontan, B.; Rimet, F. Modelling diatom life forms and ecological guilds for river biomonitoring. *Knowl. Manag. Aquat. Ecosyst.* **2017**, *418*, 1. [CrossRef]
106. Novais, M.H.; Morais, M.M.; Rosado, J.; Dias, L.S.; Hoffmann, L.; Ector, L. Diatoms of temporary and permanent watercourses in Southern Europe (Portugal). *River Res. Appl.* **2014**, *30*, 1216–1232. [CrossRef]
107. Tapolczai, K.; Bouchez, A.; Stenger-Kovács, C.; Padisák, J.; Rimet, F. Taxonomy- or trait-based ecological assessment for tropical rivers? Case study on benthic diatoms in Mayotte island (France, Indian Ocean). *Sci. Total Environ.* **2017**, *607*, 1293–1303. [CrossRef]
108. Tapolczai, K.; Keck, F.; Bouchez, A.; Rimet, F.; Kahlert, M.; Vasselon, V. Diatom DNA Metabarcoding for Biomonitoring: Strategies to Avoid Major Taxonomical and Bioinformatical Biases Limiting Molecular Indices Capacities. *Front. Ecol. Evol.* **2019**, *7*, 1–15. [CrossRef]
109. Pérez-Burillo, J.; Trobajo, R.; Vasselon, V.; Rimet, F.; Bouchez, A.; Mann, D.G. Evaluation and sensitivity analysis of dia-tom DNA metabarcoding for WFD bioassessment of Mediterranean rivers. *Sci. Total Environ.* **2020**, *727*, 138–445. [CrossRef]
110. Bailet, B.; Bouchez, A.; Franc, A.; Frigerio, J.-M.; Keck, F.; Karjalainen, S.-M.; Rimet, F.; Schneider, S.; Kahlert, M. Molecular versus morphological data for benthic diatoms biomonitoring in Northern Europe freshwater and consequences for ecological status. *Metabarcoding Metagenom.* **2019**, *3*, 21–35. [CrossRef]

111. Hebert, P.D.N.; Cywinska, A.; Ball, S.L.; Dewaard, J.R. Biological identifications through DNA barcodes. *Proc. R. Soc. B Biol. Sci.* **2003**, *270*, 313–321. [CrossRef] [PubMed]
112. Taberlet, P.; Coissac, E.; Pompanon, F.; Brochmann, C.; Willerslev, E. Towards next-generation biodiversity assessment using DNA metabarcoding. *Mol. Ecol.* **2012**, *21*, 2045–2050. [CrossRef]
113. Apothéloz-Perret-Gentil, L.; Cordonier, A.; Straub, F.; Iseli, J.; Esling, P.; Pawlowski, J. Taxonomy-free molecular diatom index for high-throughput eDNA biomonitoring. *Mol. Ecol. Resour.* **2017**, *17*, 1231–1242. [CrossRef]
114. Keck, F.; Vasselon, V.; Rimet, F.; Bouchez, A.; Kahlert, M. Boosting DNA metabarcoding for biomonitoring with phylogenetic estimation of operational taxonomic units' ecological profiles. *Mol. Ecol. Resour.* **2018**, *1*, 1299–1309. [CrossRef] [PubMed]
115. Chen, W.; Zhang, C.K.; Cheng, Y.; Zhang, S.; Zhao, H. A Comparison of Methods for Clustering 16S rRNA Sequences into OTUs. *PLoS ONE* **2013**, *8*, e70837. [CrossRef] [PubMed]
116. Callahan, B.J.; McMurdie, P.J.; Holmes, S.P. Exact sequence variants should replace operational taxonomic units in markergene data analysis. *ISME J.* **2017**, *11*, 2639–2643. [CrossRef]
117. Elbrecht, V.; Vamos, E.E.; Steinke, D.; Leese, F. Estimating intraspecific genetic diversity from community DNA metabarcoding data. *Bioinform. Genom.* **2018**, *6*, e4644. [CrossRef] [PubMed]
118. Forster, D.; Lentendu, G.; Filker, S.; Dubois, E.; Wilding, T.A.; Stoeck, T. Improving eDNA-based protist diversity assess-ments using networks of amplicon sequence variants. *Environ. Microbiol.* **2019**, *21*, 4109–4124. [CrossRef] [PubMed]
119. Feio, M.J.; Serra, S.R.; Mortágua, A.; Bouchez, A.; Rimet, F.; Vasselon, V.; Almeida, S.F. A taxonomy-free approach based on machine learning to assess the quality of rivers with diatoms. *Sci. Total Environ.* **2020**, *722*, 137900. [CrossRef]
120. Vasselon, V.; Rimet, F.; Domaizon, I.; Monnier, O.; Reyjol, Y.; Bouchez, A. Assessing pollution of aquatic environments with diatoms DNA metabarcoding: Experience and developments from France Water Framework Directive networks. *Metabarcoding Metagenom.* **2019**, *3*, 101–115.
121. Rivera, S.F.; Vasselon, V.; Ballorain, K.; Carpentier, A.; Wetzel, C.E.; Ector, L.; Bouchez, A.; Rimet, F. DNA metabarcod-ing and microscopic analyses of sea turtles' biofilms: Complementary to understand turtle behavior. *PLoS ONE* **2018**, *13*, 1–20. [CrossRef] [PubMed]
122. Kermarrec, L.; Franc, A.; Rimet, F.; Chaumeil, P.; Humbert, J.F.; Bouchez, A. Next-generation sequencing to inventory taxonomic diversity in eukaryotic communities: A test for freshwater diatoms. *Mol. Ecol. Resour.* **2013**, *13*, 607–619. [CrossRef] [PubMed]
123. Rivera, S.F.; Vasselon, V.; Bouchez, A.; Rimet, F. Diatom metabarcoding applied to large scale monitoring networks: Optimization of bioinformatics strategies using Mothur software. *Ecol. Indic.* **2020**, *109*, 105775. [CrossRef]
124. Vasselon, V.; Bouchez, A.; Rimet, F.; Jacquet, S.; Trobajo, R.; Corniquel, M.; Tapolczai, K.; Domaizon, I. Avoiding quanti-fication bias in metabarcoding: Application of a cell biovolume correction factor in diatom molecular biomonitoring. *Methods Ecol. Evol.* **2018**, *9*, 1060–1069. [CrossRef]
125. Feio, M.J.; Almeida, S.F.; Craveiro, S.C.; Calado, A.J. A comparison between biotic indices and predictive models in stream water quality assessment based on benthic diatom communities. *Ecol. Indic.* **2009**, *9*, 497–507. [CrossRef]
126. Almeida, S.F.; Feio, M.J. Diatmod: Diatom predictive model for quality assessment of Portuguese running waters. Algae for monitoring rivers. *Hydrobiologia* **2012**, *695*, 185–197. [CrossRef]
127. Schiller, D.; Von Acuña, V.; Aristi, I.; Arroita, M.; Basaguren, A.; Bellin, A.; Boyero, L.; Butturini, A.; Ginebreda, A.; Kalogi-anni, E.; et al. River ecosystem processes: A synthesis of approaches, criteria of use and sensitivity to environmental stressors. *Sci. Total Environ.* **2017**, *597*, 465–480. [CrossRef]