

Benthic Diatoms in River Biomonitoring

Subjects: Ecology

Contributor: Andreas Masouras

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

1. Background

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 ^[1]. 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) ^[2]. 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 ^{[3][4][5][6]} 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 ^[2]. 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 ^{[7][8]}.

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 ^[9]. 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 ^[10], also supporting primary productivity and nutrient cycling such as phosphorus, nitrogen and silica ^{[8][11][12][13][14][15]}. 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 (epilithon) ^[16]. In running waters, their benthic nature accounts for responses to nutrients, and organic and inorganic micropollutants ^{[17][18]}. 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 ^{[10][19][20][21]}, 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 ^{[22][23]}, 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 ^{[24][25][26][27][28][29][30]}, being sensitive both to nonpoint (e.g., agriculture) ^{[6][25]} and point-source pollution (e.g., olive mill wastes ^[27], toxic industrial wastes ^[28]). 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 ^{[28][29][30][31][32]}. Their small size (<10–200 µm in diameter or length) ^[11] and their diverse life forms, make them vulnerable, and thus potentially good indicators of hydrological alterations on streams and rivers ^[33], responding faster than other biota ^[25].

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 ^{[34][35][36]}. 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 ^[35]. 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 ^[34].

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 ^[18]. Benthic diatoms are advantageous over other BQEs in most habitats or for environmental stressors, making them, thus, more useful in routine biomonitoring ^{[18][37][38]}. A major advantage is that they can be found everywhere, in almost any type of running water ^[39] where sufficient light is available, including fresh and marine waters, moist and terrestrial habitats ^{[10][23]}. 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 ^{[6][40][41][42][43][44]}. 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 ^[25]) and macrophytes (e.g., in rivers in central and southern Poland ^[45]).

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 ^[46]. This occurs in both mountain and lowland water bodies in France ^[46], Germany and Austria ^[37], China ^[47] and in a temporary river in Greece ^[25]. 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 ^[46]. Diatoms show to be more affected by toxic wastes (i.e., olive mill wastewaters)

than invertebrates in temporary rivers in Greece [48] and in northwestern Spain because of sensitivity of diatom-based indices to heavy metals [49]. Furthermore, diatoms are more affected by diffuse pollution than benthic invertebrates, providing a stricter ecological status in Mediterranean small-sized streams [50].

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 [20], compared especially to fish sampling, where the commonly used method of electrofishing could lead to fish deaths [51][52], whereas its efficiency is affected by turbidity and conductivity [53]. 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 [54][55]. On the other hand, macroinvertebrate taxonomy under the genus/species level for many groups is practically impossible for routine biomonitoring [53].

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 [56]. 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 [57]. 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 [58][59]. 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][60][61].

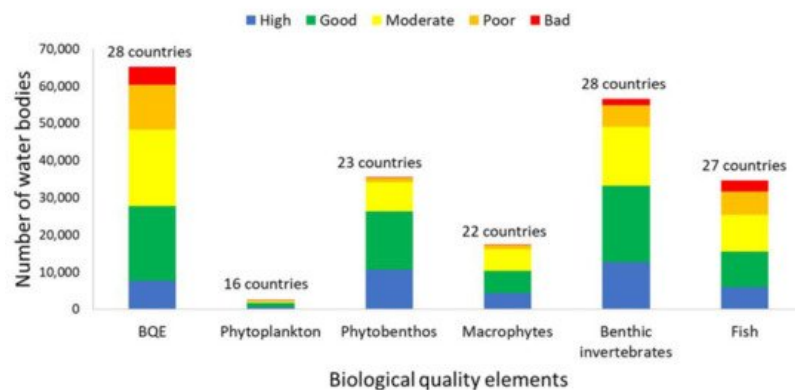


Figure 1. Biological quality elements (BQEs) used in water bodies of European Member States (MS) in accordance with the Water Framework Directive (WFD). Data from one country that uses phytobenthos are not available in WISE Database. Source: WISE Database, 2021.

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 [62], 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 [63][64][65][66][67] following the recovery of chemical parameters compared to other BQEs could result in better biological quality status indicated by phytobenthos.

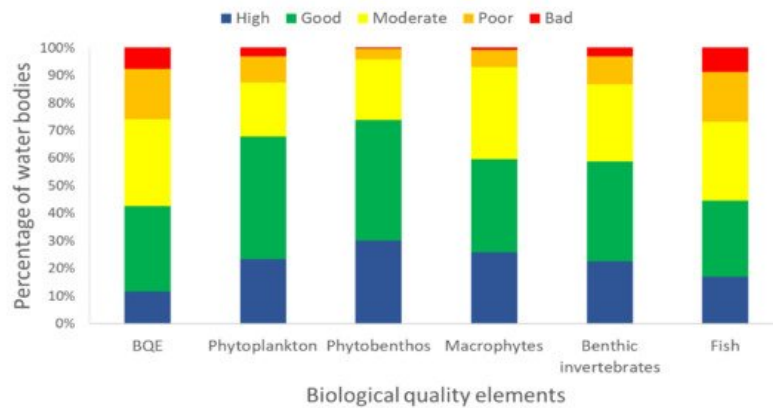


Figure 2. Percentage of water bodies belonging to different quality classes following biological quality assessment in European rivers (<https://www.eea.europa.eu/themes/water/european-waters/water-quality-and-water-assessment/water-assessments/quality-elements-of-water-bodies>, last accessed on 29 January 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][65][66][67][68]. 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][65][69].

MS are using previously developed taxonomic indices for water quality assessments that have been intercalibrated among the MS. 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.

The most valuable tool for water quality assessment and biomonitoring [67][70][71][72][73] based on diatoms is the OMNIDIA software [74], which uses the indicative properties of diatoms and includes information on the tolerance of diatom taxa to environmental parameters [75]. 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 [76]. These uncertainties are partly related to the fact that diatom indices are developed for specific geographic regions but have frequently been used in others [68][77][78]. 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 [79]. This is apparent in the development of indices from different ecoregions that use different ecological profiles for the same species [79]. 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 [79].

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) [80]. 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 ^[81]. 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., ^[82]); however, there has been no governmental coordination ^[81]. 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 ^[83]. In Asia, the Asian Pacific Water Summit in 2007 started a new era in water quality assessment in Asian countries ^[80]. 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.

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 -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.

References

1. European Commission. Report from the Commission to the European Parliament and the Council on the Implementation of the WaterFramework Directive (2000/60/EC) River Basin Management Plans; Official Journal of the European Union: Luxembourg, 2012.
2. European Commission. Carrying forward the Common Implementation Strategy for the Water Framework Directive- Progress and WorkProgramme for 2003/2004; Official Journal of the European Union: Luxembourg, 2003
3. 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, .
4. 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, .
5. 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, .
6. Karaouzas, I.; Smeti, E.; Kalogianni, E.; Skoulidakis, 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, .

7. 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, .
8. 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, .
9. Čirić, 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, .
10. Round, F.E.. The Diatoms; Cambridge University Press: Cambridge, UK, 1990; pp. 747.
11. Mann, D.G.; The species concept in diatoms. *Phycologia* **1999**, *38*, 437-495, .
12. Falciatore, A.; Bowler, C.; Revealing the Molecular Secrets of Marine Diatoms. *Annu. Rev. Plant Biol* **2002**, *53*, 109-130, .
13. 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, .
14. 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, .
15. 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, .
16. Kitner, M.; Pouličková, A.; Littoral diatoms as indicators for the eutrophication of shallow lakes. *Hydrobiologia* **2003**, *506*, 519-524, .
17. Licursi, M.; Gómez, N.; Benthic diatoms and some environmental conditions in three lowland streams. *Ann. Limnol. Int. J. Limnol.* **2002**, *38*, 109-118, .
18. 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, .
19. 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, .
20. 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, .
21. 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, .
22. 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, .
23. 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, .
24. Karaouzas, I.; Smeti, E.; Vourka, A.; Vardakas, L.; Mentzafou, A.; Tornés, E.; Sabater, S.; Muñoz, I.; Skoulikidis, N.T.; Kalogianni, E.; et al. Assessing the ecological effects of water stress and pollution in a temporary river—Implications for water management.. *Total Environ.* **2018**, *618*, 1591-1604,, .
25. Solak, C.N.; Peszek, Ł.; Yılmaz, E.; Ergül, H.A.; Kayal, M.; Ekmekçi, F.; Várbíró, G.; Yüce, A.M.; Canli, O.; Binici, M.S.; et al. Diatoms in Monitoring the Sakarya River Basin, Turkey. *Water* **2020**, *12*, 703, .
26. Cimarelli, L.; Singh, K.S.; Mai, N.T.N.; Dhar, B.C.; Brandi, A.; Brandi, L.; Spurio, R.Int. J. Environ. Res. Public Health; 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, .
27. 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 biodiversity and ecosystem functioning in a Mediterranean temporary river. *Total Environ* **2019**, *647*, 1179-1187, .
28. Laine, M.; Morin, S.; Tison-Rosebery, J; A multicompartiment approach—Diatoms, macrophytes, benthic macroinvertebrates and fish—To assess the impact of toxic industrial releases on a small French river.. *PLoS ONE* **2014**, *9*, e102358, .
29. 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, .

30. Falasco, E.; Bona, F.; Badino, G.; Hoffmann, L.; Ector, L.; Diatom teratological forms and environmental alterations: A review.. *Hydrobiologia* **2009**, 623, 1-35, .
31. 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., .
32. Montesanto, B.; Zille, S.; Coste, M.; Diatomees epilithiques et qualite biologique de ruisseaux du mont Stratonikon, Chalkidiki (Grece). . *Cryptogam. Algal.* **1999**, 3, 235–251., .
33. 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, .
34. 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, .
35. 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*; Romani, A.M., Guasch, H., Balaguer, M.D., Eds.; Caister Academic Press: Poole: UK, 2016; pp. 111-124.
36. Pandey, L.K.; Bergey, E.A.; Lyu, J.; Park, J.; Choi, S.; Lee, H.; Depuydt, S.; Oh, Y.T.; Lee, S.M.; Han, T.; et al. The use of diatoms in ecotoxicology and bioassessment: Insights, advances and challenges. *Water Res.* **2017**, 118, 39-58, .
37. 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, .
38. Mangadze, T.; Bere, T.; Mwedzi, T.; Epilithic diatom flora in contrasting land-use settings in tropical streams, Manyame Catchment, Zimbabwe.. *Hydrobiologia* **2015**, 753, 163-173, .
39. Borojević, K.K.; Udovič, M.G.; Žutinić, P.; Várbiro, 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, .
40. 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; pp. ..
41. 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, .
42. Wehr, J.D.; Minireview Use of phytoplankton in large river management. *J. Phycol* **1998**, 34, 741-749, .
43. 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 Italy (River Adige). *Aquat. Ecol* **2008**, 42, 533-545, .
44. P. A. Chambers; E. E. Prepas; H. R. Hamilton; M. L. Bothwell; Current Velocity and Its Effect on Aquatic Macrophytes in Flowing Waters. *Ecological Applications* **1991**, 1, 249-257, [10.2307/1941754](https://doi.org/10.2307/1941754).
45. 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.. *Limnologia* **2012**, 42, 204-211, .
46. 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, .
47. Qu, X.; Peng, W.; Liu, Y. Identifying the Impacts of Water Quality on Macroinvertebrate Degradation 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*.
48. Evangelia Smeti; Eleni Kalogianni; Ioannis Karaouzas; Sofia Laschou; Elisabet Tornés; Núria De Castro-Català; Evangelia Anastasopoulou; Maria Koutsodimou; Argyro Andriopoulou; Leonidas Vardakas; et al. Effects of olive mill wastewater discharge on benthic biota in Mediterranean streams. *Environmental Pollution* **2019**, 254, 113057, [10.1016/j.envpol.2019.113057](https://doi.org/10.1016/j.envpol.2019.113057).
49. S. Blanco; E. Bécares; Are biotic indices sensitive to river toxicants? A comparison of metrics based on diatoms and macro-invertebrates. *Chemosphere* **2010**, 79, 18-25, [10.1016/j.chemosphere.2010.01.059](https://doi.org/10.1016/j.chemosphere.2010.01.059).
50. Giorgio Pace; Valentina Della Bella; Mariachiara Barile; Paolo Andreani; Laura Mancini; Carlo Belfiore; A comparison of macroinvertebrate and diatom responses to anthropogenic stress in small sized volcanic siliceous streams of Central

51. 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.
52. Dodds, W.; Whiles, M.. Fish Ecology Fisheries and Aquaculture in Freshwater Ecology, 3rd ed.; Academic Press: London, UK, 2020; pp. Volume:23.
53. 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; pp. ..
54. 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; pp. ..
55. Blažena Brabcová; Petr Marvan; Libuše Opatřilová; Karel Brabec; Markéta Fránková; Jiří Heteša; Diatoms in water quality assessment: to count or not to count them?. *Hydrobiologia* **2017**, 795, 113-127, [10.1007/s10750-017-3123-5](https://doi.org/10.1007/s10750-017-3123-5).
56. Salomé F.P. Almeida; Carmen Elias; João Ferreira; Elisabet Tornés; Camilla Puccinelli; François Delmas; Gerald Dörflinger; Gorazd Urbanič; Stefania Marcheggiani; Juliette Tison-Rosebery; et al. Water quality assessment of rivers using diatom metrics across Mediterranean Europe: A methods intercalibration exercise. *Science of The Total Environment* **2014**, 476-477, 768-776, [10.1016/j.scitotenv.2013.11.144](https://doi.org/10.1016/j.scitotenv.2013.11.144).
57. European Environment Agency. European Waters Assessment of Status and Pressures; Report No 7/2018; EEA: Copenhagen, Denmark, 2018.
58. Naicheng Wu; Hans Thodsen; Hans Estrup Andersen; Henrik Tornbjerg; Annette Baattrup-Pedersen; Tenna Riis; Flow regimes filter species traits of benthic diatom communities and modify the functional features of lowland streams - a nationwide scale study. *Science of The Total Environment* **2019**, 651, 357-366, [10.1016/j.scitotenv.2018.09.210](https://doi.org/10.1016/j.scitotenv.2018.09.210).
59. Gunta Springer; Leonard Sandin; Agrita Briede; Agnija Skuja; Biological quality metrics: their variability and appropriate scale for assessing streams. *Hydrobiologia* **2006**, 566, 153-172, [10.1007/s10750-006-0099-y](https://doi.org/10.1007/s10750-006-0099-y).
60. Eleni Kalogianni; Aikaterini Vourka; Ioannis Karaouzas; Leonidas Vardakas; Sofia Laschou; Nikolaos Th. Skoulidakis; Combined effects of water stress and pollution on macroinvertebrate and fish assemblages in a Mediterranean intermittent river. *Science of The Total Environment* **2017**, 603-604, 639-650, [10.1016/j.scitotenv.2017.06.078](https://doi.org/10.1016/j.scitotenv.2017.06.078).
61. L. Vardakas; E. Kalogianni; S. Zogaris; Nicholas Koutsikos; T. Vavalidis; D. Koutsoubas; N. Th. Skoulidakis; Distribution patterns of fish assemblages in an Eastern Mediterranean intermittent river. *Knowledge & Management of Aquatic Ecosystems* **2015**, 416, 30, [10.1051/kmae/2015026](https://doi.org/10.1051/kmae/2015026).
62. Sandra Poikane; Fuensanta Salas Herrero; Martyn G. Kelly; Angel Borja; Sebastian Birk; Wouter van de Bund; European aquatic ecological assessment methods: A critical review of their sensitivity to key pressures. *Science of The Total Environment* **2020**, 740, 140075, [10.1016/j.scitotenv.2020.140075](https://doi.org/10.1016/j.scitotenv.2020.140075).
63. F Larson; K Sundbäck; Recovery of microphytobenthos and benthic functions after sediment deposition. *Marine Ecology Progress Series* **2012**, 446, 31-44, [10.3354/meps09488](https://doi.org/10.3354/meps09488).
64. 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, .
65. 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.
66. Coste, M.; Ayphasshorho, H. Etude de la Qualite des Eaux du Bassin Artois-Picardie a l'Aide des Communautés de Diatomées Benthiques (Application des Indices Diatomiques); Hal Inrae: Lyon, France, 1991; p. 227.
67. M. G. Kelly; B. A. Whitton; The Trophic Diatom Index: a new index for monitoring eutrophication in rivers. *Environmental Biology of Fishes* **1995**, 7, 433-444, [10.1007/bf00003802](https://doi.org/10.1007/bf00003802).
68. Irene Álvarez-Blanco; Saúl Blanco; Cristina Cejudo-Figueiras; Eloy Bécares; The Duero Diatom Index (DDI) for river water quality assessment in NW Spain: design and validation. *Environmental Monitoring and Assessment* **2012**, 185, 969-981, [10.1007/s10661-012-2607-z](https://doi.org/10.1007/s10661-012-2607-z).
69. Dell'uomo, A. Assessment of Water Quality of an Apennine River as a Pilot Study for Diatom-based Monitoring of Italian Waters. In Use of Algae for Monitoring Rivers; Institut Fur Botanik, Universität: Vienna, Austria, 1996; pp. 65-72.
70. Prygiel, J.; Management of the diatom monitoring networks in France. *Environ. Biol. Fishes* **2002**, 14, 19-26, .
71. Martyn Kelly; Cathy Bennett; Michel Coste; Cristina Delgado; François Delmas; Luc Denys; Luc Ector; Claude Fauville; Martial Ferréol; Malgorzata Golub; 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* **2008**, 621, 169-182, [10.1007/s10750-008-9641-4](https://doi.org/10.1007/s10750-008-9641-4).

72. M.G. Kelly; Role of benthic diatoms in the implementation of the Urban Wastewater Treatment Directive in the River Wear, North-East England. *Journal of Applied Phycology* **2002**, 14, 9-18, [10.1023/a:1015236404305](https://doi.org/10.1023/a:1015236404305).
73. Pertti Eloranta; Janne Soininen; Ecological status of some Finnish rivers evaluated using benthic diatom communities. *Environmental Biology of Fishes* **2002**, 14, 1-7, [10.1023/a:1015275723489](https://doi.org/10.1023/a:1015275723489).
74. C. Lecointe; M. Coste; J. Prygiel; ?Omnidia?: software for taxonomy, calculation of diatom indices and inventories management. *Hydrobiologia* **1993**, 269-270, 509-513, [10.1007/bf00028048](https://doi.org/10.1007/bf00028048).
75. Szczepocka, E.; Z' elazna-Wieczorek, J.; Diatom biomonitoring—Scientific foundations, commonly discussed issues and frequently made errors. *Oceanol. Hydrobiol. Stud* **2018**, 47, 313-325, .
76. Martyn Kelly; Data rich, information poor? Phytobenthos assessment and the Water Framework Directive. *European Journal of Phycology* **2013**, 48, 437-450, [10.1080/09670262.2013.852694](https://doi.org/10.1080/09670262.2013.852694).
77. 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.
78. 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, .
79. Kálmán Tapolczai; Agnès Bouchez; Csilla Stenger-Kovács; Judit Padisák; Frédéric Rimet; Trait-based ecological classifications for benthic algae: review and perspectives. *Hydrobiologia* **2016**, 776, 1-17, [10.1007/s10750-016-2736-4](https://doi.org/10.1007/s10750-016-2736-4).
80. Otto Moog; Stefan Schmutz; Ilse Schwarzingler; Biomonitoring and Bioassessment. *Riverine Ecosystem Management* **2018**, 8, 371-390, [10.1007/978-3-319-73250-3_19](https://doi.org/10.1007/978-3-319-73250-3_19).
81. Assane Anabi Toudjani; Abuzer Çelekli; E. Yonca Gümüş; Seda Kayhan; H. Ömer Lekesiz; Tolga Çetin; A new diatom index to assess ecological quality of running waters: a case study of water bodies in western Anatolia. *Annales de Limnologie - International Journal of Limnology* **2017**, 53, 333-343, [10.1051/limn/2017012](https://doi.org/10.1051/limn/2017012).
82. 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.
83. Tatenda Dalu; P. William Froneman; Diatom-based water quality monitoring in southern Africa: challenges and future prospects. *Water SA* **2016**, 42, 551, [10.4314/wsa.v42i4.05](https://doi.org/10.4314/wsa.v42i4.05).
84. Tatenda Dalu; P. William Froneman; Diatom-based water quality monitoring in southern Africa: challenges and future prospects. *Water SA* **2016**, 42, 551, [10.4314/wsa.v42i4.05](https://doi.org/10.4314/wsa.v42i4.05).
85. Otto Moog; Stefan Schmutz; Ilse Schwarzingler; Biomonitoring and Bioassessment. *Riverine Ecosystem Management* **2018**, 8, 371-390, [10.1007/978-3-319-73250-3_19](https://doi.org/10.1007/978-3-319-73250-3_19).