Pros and Cons of Biological Quality Element Phytoplankton as a Water-Quality Indicator in the NW Mediterranean Sea

Jordi Camp, Eva Flo, Magda Vila, Laura Arin, Albert Reñé, Nagore Sampedro, Marta Manzanera, and Esther Garcés

Abstract The Water Framework Directive (WFD) mandates the use of biological quality element (BQE) phytoplankton to assess the ecological status of coastal and transitional water bodies (WB). Here, we present (i) a critique of the general ecological assumptions of the WFD, (ii) a review of the ecological features of coastal phytoplankton dynamics, (iii) several approaches to establish a methodology to assess water-quality along the Catalan coast (NW Mediterranean Sea) based on BQE phytoplankton, and (iv) a critical examination of the use of phytoplankton as a BQE. Since 2005, we have followed several approaches aimed at assessing water-quality based on BQE phytoplankton and linking this indicator to a proxy to a costal pressure index. We have therefore studied phytoplankton communities at three different levels: as potentially harmful species, as functional or taxonomic groups, and with respect to their bloom frequency. Despite intense efforts, none of these fulfilled the WFD's management requirements, which in this context were found to contain several inherent flaws. As an alternative, we propose a methodology to assess water-quality based on the use of chlorophyll-*a* (Chl-*a*), as a proxy of phytoplankton biomass. The Chl-*a* concentration offers a very simple and representative measure of the phytoplankton community, and, importantly, it is used worldwide in water-quality studies, thus allowing not only regional but also cross-country comparisons. Moreover, because Chl-*a* concentrations clearly respond to nutrient enrichment, we were able to establish a BQE-specific typology for water bodies based on salinity, which is linked to nutrient loads. Using a newly developed coastal pressure index (Land Use Simplified Index, LUSI) that also reflects nutrient

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inputs, we demonstrated a significant pressure–impact relationship, as required by the WFD for management purposes. Based on this relationship, we were able to define reference conditions and water-quality boundaries for each type. We conclude our discussion with a consideration of the pros and cons of the use of phytoplankton as a BQE.

Keywords Biological quality element, Chlorophyll-*a*, Coastal waters, Continental pressures, NW Mediterranean Sea, Phytoplankton, Pressure–impact relationship, Water-quality assessment

Contents

1 Ecological Systems, Biological Communities, and the Water Framework Directive

Coastal waters are the most productive and diverse areas of the global ocean. Their unique structural properties include continental shelves, benthic–pelagic coupling, strong gradients, terrestrial inputs, geomorphic effects, and the broad spectrum of oceanographic conditions. Moreover, coastal waters are also strongly influenced by human activities, which result in the enrichment of coastal areas with organic and inorganic nutrients, such as carbon, nitrogen, and phosphorus, and therefore in unprecedented increases in eutrophication. Indeed, the deterioration of water-quality, understood as the loss of desirable (near pristine) conditions, has mainly been due to anthropogenic pressures, most of which originate on land. Identification of the causal links between these pressures and ecosystem status is therefore a fundamental step in any policy aimed at improving the environmental quality of coastal waters.

The purpose of the Water Framework Directive (WFD) is to establish a framework for the protection of freshwaters, marine waters, and groundwater. The main environmental objective of the WFD is the achievement by all European water bodies (WBs) of a Good Ecological Status (GES) by 2015. The ecological status is used to define the water-quality of a WB and is based on hydromorphological and physico-chemical criteria as well as biological quality elements (BQEs) and quantified by an ecological quality ratio (EQR). The EQR is a relative measure that compares the structural and compositional features of an ecosystem with those of a reference system characterized by a low level of anthropogenic pressure and therefore with good water-quality. Any deterioration or improvement in ecological status, and hence in water-quality, is reflected in the responses of these BQEs in the EQR. In the case of coastal and transitional waters, BQEs are presumed to respond to the effects of the main pressures, especially eutrophication. For example, the WFD recognizes that nutrient enrichment and changes in the stoichiometry of nutrient elements can give rise to shifts in the composition and biomass of phytoplankton species and to increases in the frequency, magnitude, and duration of phytoplankton blooms. It has therefore included phytoplankton as a BQE and mandated determinations of its taxonomic composition, abundance, biomass, and bloom frequency to assess water-quality.

While the WFD emphasizes sustainable use, its GES criteria assume that a manageable relationship exists between the structure and function of ecological systems that can be evaluated by quantifying the designated BQEs. However, questions regarding the validity of this assumption and whether a BQE-based approach is robust enough to support the goals of the WFD have generated intense controversy within the scientific community. A major concern is that the proposed methodology for the achievement of GES relies on an outdated interpretation of ecology and on a highly idealized pristine state free of any type of human impact [1]. In addition, the WFD's goals are based on the concept of a balanced (or climax) community and what nowadays is recognized as an overly simplistic view of the equilibrium of biological communities. That line of thinking was developed by Clements [2], who defined a climax community as "*a biological community of plants and animals which, through the process of ecological succession has reached an equilibrium in response to climate, soil and other environmental factors. In the absence of human interference, this state is self-maintaining*." The directive has adopted this view even though the recent scientific literature contains strong evidences that it is an inappropriate model for ecosystem management [1].

The WFD is also founded on several other assumptions, which can be summarized as follows: (1) ecological systems have a clear identity; they are recognizable and spatially clearly delimited. (2) In the absence of pressures, ecological systems achieve a steady state and are both temporally and spatially stable. (3) Ecological systems have "memory" and undergo structural changes in response to sustained pressure. (4) Changes caused by anthropogenic pressures can be distinguished from those resulting from natural causes, which in turn imply known pressure–impact relationships. (5) Ecological systems will return to an initial reference state if the pressures ceased. (6) Any changes in an ecosystem will be reflected by corresponding changes in each of its components or at least, per their definition, in its BQEs.

However, the WFD's assumptions are highly contestable and largely outdated, such that today none would withstand rigorous scientific evaluation. Consequently, the validity of the entire WFD regarding its reliance on BQEs must be questioned. It is beyond the scope of this chapter to discuss the weaknesses of each of the above-listed assumptions in detail. A summary of the opposing arguments would show that the WFD's assumptions are valid only if we impose appropriate spatio-temporal restrictions on the use of a particular BQE. This approach requires in-depth knowledge of the BQE's function in the target ecosystem and a recognition of the limitations and potential artifacts of the various methods used to sample BQEs, neither of which has been sufficiently evaluated.

In the following, we examine the limitations of phytoplankton as a BQE. We begin with a review of the ecological features of coastal phytoplankton and its dynamics. We then present some of the findings of our 10-year experience in the use of BQE phytoplankton to assess the water-quality of the Catalan coast (NW Mediterranean Sea) – as required by the WFD – which illustrate several of the problems inherent to the directive. As will become apparent in this chapter, for BQE phytoplankton, none of the tested parameters, i.e., taxonomic composition, abundance, biomass, and bloom frequency, are sufficiently reliable to establish ecological status when used on their own, as they do not fulfill the management requirements demanded by the directive. Instead, we show that the chlorophyll-*a* (Chl-*a*) concentration, which serves as a proxy measure for phytoplankton biomass, clearly responds to nutrient enrichment. Accordingly, we were able to establish a positive pressure–impact relationship between a coastal pressure index (Land Uses Simplified Index, LUSI) and Chl-*a*. Based on this relationship, we developed a methodology, discussed herein, in which water-quality can be determined by measuring phytoplankton biomass. Moreover, our approach complies with the WFD's requirements regarding ecosystem management applications.

2 Is Phytoplankton an Adequate Bioindicator?

2.1 Causes of Variability in Phytoplankton Communities

The growth and distribution of phytoplankton species follow seasonal cycles that depend on latitude and on the distance of the respective community to the coast. The fundamental causes of this variability have been well studied and include nutrient availability [3–8]. Nutrient elements are essential for the growth and maintenance of photoautotrophic organisms, which use light to fix carbon dioxide, and are responsible for the vast majority of primary production in the ocean.

Phytoplankton and other microbes take up nutrients and assimilate them into macromolecules, resulting in the formation of particulate organic matter which is then integrated into food webs and, thus, into higher trophic levels. Along with nutrients and their stoichiometry, several chemical and physical factors affect the phytoplankton community, including salinity, turbulence, the stability of the water column, the degree of water confinement, water residence time, temperature, tidal mixing, and the availability of light [3, 9–12]. Additionally, the phytoplankton community is composed of many different species, whose survival is favored by differences in their ecological requirements and by their distinct life strategies based on nutritional diversity (autotrophy vs. mixotrophy), different modes of competition, adapted life cycles, and differences in growth rates [13–15]. These processes account for the highly dynamic nature of phytoplankton, their rapid response to changes in environmental conditions, and their ability to inhabit a geographically broad range of coastal environments. However, they also underlie the complex relationship between environmental conditions and both the abundance of phytoplankton [16, 17] and the unpredictable structure of their communities. Thus, one of the main drawbacks of the WFD in its designation of phytoplankton as a BQE can be summarized as follows: phytoplankton communities are highly diverse and well adapted not only to nutrient fluctuations but also to physical parameters that change over time, all of which preclude the identification of clear-cut relationships between BQE phytoplankton and environmental pressures.

2.2 Phytoplankton Communities: An Indicator Without Memory

To establish reference conditions, as mandated by the WFD, phytoplankton communities must be described based on their state under completely or nearly completely undisturbed conditions, with little or no impact from human activities. The WFD also assumes that the nature of phytoplankton communities reflects the "memory" of sustained pressure. However, as noted above, even in the absence of anthropogenic pressure, phytoplankton communities are highly dynamic. Marine phytoplankton communities respond to the physico-chemical properties of their environment and, therefore, do not temporally integrate environmental changes. Indeed, even within a single seasonal cycle, phytoplankton communities will be highly variable [18, 19] and will not give rise to a climax community. An effective and accurate assesment of the status of marine ecosystems and the disturbances to them requires recognition of the dynamic nature not only of phytoplankton but also of ecosystems and their communities in general. In other words, the status of a phytoplankton community should not, and cannot, be evaluated by comparing its composition and relative abundances with a static "reference" assemblage of species that, even if it existed, would by no means be representative.

2.3 Phytoplankton Species as a Bioindicator

Among the various methods for evaluating the effects of human perturbations on coastal ecosystems, the use of specific species, rather than assemblages, as indicators of ecological status has been proposed. However, in the context of eutrophication, the proliferation of a particular species of phytoplankton in direct response to a disturbance in the balance of an aquatic ecosystem is by no means certain [20]. For example, species belonging to the genus *Phaeocystis* are regarded as a nuisance in the coastal waters of the North Sea. Yet, following anthropogenically derived nutrient enrichments of Belgian coastal waters, there was little change in the respective ecosystem despite a considerable increase in *Phaeocystis* spp. [21] In the Mediterranean Sea, there is no evidence of opportunistic phytoplankton species or of a significant indicator species in the sense relied upon by the WFD in its definition of a BQE. Similar conclusions have been reached in studies conducted in other European regions.

2.4 Phytoplankton Biomass as a Bioindicator

The immediate biological response to nutrient inputs is an increase in primary production, which manifests as an increase in phytoplankton and/or macroalgal abundances [22–25]. Accordingly, Chl-*a* is commonly accepted as a proxy for phytoplankton biomass, and extensive literature supports its use as an indicator of eutrophication in coastal waters [26–34]. In relation to the WFD, this assertion supports the mandatory inclusion of a pressure–impact relationship in each methodology used to assess water-quality.

There is general agreement on the relationship between the mean concentrations of nutrients and Chl-*a* in coastal waters. However, to assess this pressure–impact relationship, Chl-*a* data must be statistically integrated with respect to time, due to the highly dynamic behavior of phytoplankton communities. Therefore, a suitable temporal database is necessary, which in turn implies the need for sufficiently frequent sampling over an adequate period of time. Thus, the WFD mandates a 6-year period to assess the ecological status of a WB. Nonetheless, in some cases and in certain places, there will be no obvious relationship between the concentrations of nutrients and Chl-*a*. This could be due to a temporal or spatial mismatch of nutrients and Chl-*a*. For example, nutrients reach coastal waters at a certain time, and some time later, Chl-*a* will be generated but it also may be the case that the nutrients are further transported before Chl-*a* can be generated. Therefore, the physical and biological processes that modulate Chl-*a* production in turbulent and dynamic environments, such as coastal waters, may also disrupt its relationship to nutrient levels.

3 The Mediterranean Sea and the Catalan Coast as a Case Study

The Mediterranean Sea is a valuable paradigm to assess anthropic pressure, because of the contrasting nature of its offshore and coastal areas. The offshore waters of the Mediterranean Sea are among the most oligotrophic areas of the world. In these waters, nutrient availability is low and inorganic phosphorus concentrations limit primary production [35]. Consequently, large areas of the sea's surface waters are characterized by low amounts of phytoplankton biomass. Modest late-winter/early-spring increases of biomass are observed in some areas, such as in the northwest basin, associated with increasing daily irradiances and a greater stability of the surface layers after winter mixing brings nutrients to the surface. Relatively high-biomass peaks also occur in fronts, upwellings, and cyclonic gyres. By contrast, coastal areas are nutrient rich, as they receive river discharges, runoff from populated areas, and submarine groundwater, but they are also influenced by offshore oceanographic conditions. The coastal marine zone is therefore a transitional area characterized by strong physical, chemical, and biological gradients that extend from land to sea. Here, biological production is closely coupled to processes that deliver nutrients to surface waters. Anthropogenic forcing clearly influences the absolute availability of these nutrients and their stoichiometry, both of which impact phytoplankton productivity and species composition.

The Catalan coast is representative of the NW Mediterranean coast in terms of its geography, demographics, and socio-economic activity [36]. The climate in this area is typically Mediterranean, with moderate temperatures and irregular precipitation throughout the year. The continental topography ranges from rocky and steep to sandy and flat, with deltaic areas, the most important of which is the Ebro delta. The tidal range is small. The sea weather is typically mild but occasionally rough or very rough, with most storms occurring during autumn and winter. Catalan watersheds consist of ephemeral streams, nine medium to small rivers, and the Ebro River in the south, all of which feed directly into the Mediterranean Sea. The Ebro River drains a watershed of 84,230 km^2, with a mean water discharge at the river's mouth of 416 m^3/s [37]. Other major rivers in the region drain an area of 13,400 km^2 and have a mean water discharge of 0.3–16.3 m^3/s [38]. Land use differs along the river basins, with agriculture accounting for 9.6–51%, forests for 18.5–56.6%, and urban areas for 1–19.1%. Agricultural land use is relatively important in southern river basins and urbanization in central ones. In terms of surface area, 10.7% of the total coastal zone is urbanized with 3,9 milions inhabitants [39]. However, the population density along the coast is highly variable, with only 33 inhabitants/km^2 in the Ebro basin but 1,425 habitants/km^2 in Metropolitan Barcelona. During the tourist season, the population density in some areas increases by up to tenfold.

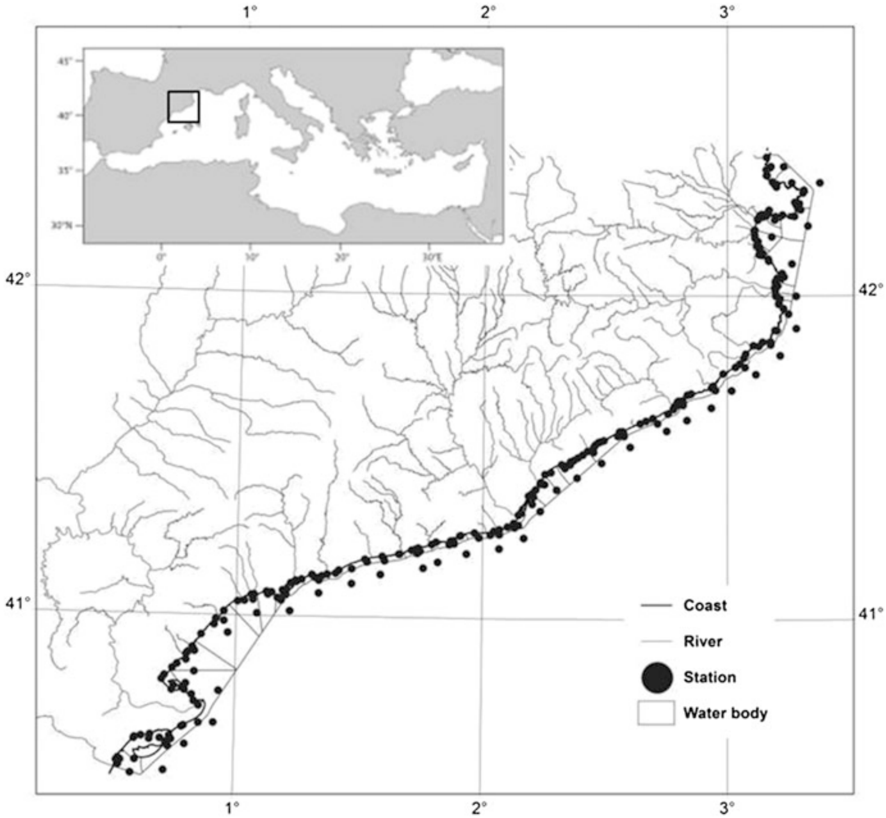

Fig. 1 Map of the Catalan coast. The coastline and main rivers are shown, together with the sampling stations and water bodies

3.1 Water-Quality Surveys at the Catalan Coast

Two time series were carried out along the Catalan coast with the aim of assessing water-quality with respect to phytoplankton: a physico-chemical and biological survey and a survey to monitor phytoplankton. Both were the result of several agreements between the Catalan Water Agency (ACA) and the Institut de Ciències del Mar (ICM-CSIC).

The physico-chemical and biological survey of Catalan coastal waters was initiated in 1990 and is ongoing. It consists of the sampling of 252 stations at specific distances from the shoreline: 35 stations at 5,000 m from the shore, 81 stations at 1,500 m, and 136 stations between 0 and 200 m, depending on the water depth (Fig. 1). The stations nearest to the coast, which are located within the sea, are representative of coastal nearshore waters (CNW) and coastal inshore waters (CIW) [36]. CNW stations are sampled every 3 months and CIW stations every 3 months, monthly, or weekly, depending on the season. At each of these stations, in situ salinity and chemical (inorganic nutrients) and biological (Chl-*a*) parameters are measured in surface waters. Dissolved inorganic nutrient (nitrate, nitrite, ammonia, phosphate, and silicate) concentrations are determined using colorimetric techniques [40] and total Chl-*a* by a fluorometric method [41].

In parallel with the above-described survey, phytoplankton monitoring was initiated in 2000 and is also ongoing. Water samples are obtained weekly, fortnightly, or monthly from 14 to 20 stations, depending on the station and year. The Utermöhl method is used to identify and count phytoplankton species [42].

3.2 WFD Implementation at the Catalan Coast

Implementation of the WFD along the Catalan coast is based on several procedures:

i. Adaptation of surveys. Catalan coastal water time series were modified in 2004 to fulfill the requirements of the WFD. Several stations were added to the surveys and sampling frequencies were in some cases adjusted. The most important change with respect to phytoplankton monitoring was to record the main taxonomic groups and not only the potentially harmful species, as was done at the beginning of the survey.
ii. WB delimitation. Thirty-six WBs located along the coast were defined: 34 coastal waters and two transitional waters (the northern and southern bays of the Ebro delta). The coastal lengths of these WBs and thus the number of stations per WB differ considerably. In the physico-chemical survey, there are between 2 and 30 stations per WB, such that all WBs are covered. For phytoplankton monitoring, 13 WBs are covered, with 1–2 stations per WB.
iii. Definition of a specific typology for BQE phytoplankton. The standard typology of Mediterranean WBs is based on the nature of the bottom substrate and the depth, which are irrelevant for BQE phytoplankton. As an alternative, a specific typology was proposed and subsequently accepted by the Mediterranean Geographical Intercalibration Group (Med-GIG). This typology is based on the degree of freshwater influence that the WB receives from land and is therefore related to nutrient loads. The three WB types are described in Table 1. Their freshwater influences are determined by the annual mean salinity.

    These types can also be subdivided into subtypes to differentiate among biogeographic areas with similar freshwater influence. For example, type II is subdivided into type II-A and type II-B to differentiate the moderate influence

Table 1 Specific WB typologies relevant for BQE phytoplankton and their annual mean salinity

Type	Description	Annual mean salinity
I	Highly influenced by freshwater inputs	<34.5
II	Moderately influenced by freshwater inputs	≥ 34.5 and <37.5
III	Not affected by freshwater inputs	≥ 37.5

of freshwater due to continental inputs from inputs coming from the Atlantic Ocean; and type III is subdivided into western (W) or eastern (E) basins of the Mediterranean Sea. More detailed information can be found in the European technical reports [43].

iv. Establishment of a method to assess continental pressures on coastal waters. Every methodology to assess water-quality must be supported by a clearly defined pressure–impact relationship whose underlying mechanisms are known. The establishment of such relationships, and therefore the assessment of anthropogenic pressures, is crucial for the development of the River Basin Management Plans required by the WFD. In coastal systems, these assessments must be focused on inland pressures, as the directive requires the assessment of pressures outside the WBs. However, while human activities are known to cause multiple pressures on different components of the marine ecosystem [44], their quantification and proof of their impacts required sophisticated, integrated tools that currently are not available. Instead, several indices have been proposed to estimate the quantity and distribution of anthropogenic pressures and their potential impacts, including BiPo [45, 46], BSPI, and BSII [47], but their use is either very complicated or requires large amounts of data. We therefore developed the Land Use Simplified Index (LUSI) [48] to simply and cost-effectively assess continental pressures on coastal waters. The rationale for the LUSI is based on the following assumptions: (1) Coastal waters receive pressures only from continental fluxes. (2) Coastal land uses determine the amount and the nutrient richness of continental fluxes. (3) An area of coastal water receiving river flows is therefore influenced by the respective watershed. (4) Coastal morphology has an effect on coastal water confinement and therefore on received pressures. LUSI integrates information regarding the specific continental pressures that influence a WB with information about the morphology of the coastal region involved, which can enhance or diminish those pressures once they reach the coast. The nature of these continentally derived inputs reflects the main characteristics of the land and its uses: urban, industrial, or agricultural. The intensity of the effects of each one on a given WB depends on the amount of land involved, which can be estimated using land use maps. The degree of riverine pressure is estimated based on the specific WB typology for BQE phytoplankton, as described in Table 1. Depending on these characteristics, a score is assigned to each WB and then combined within an algorithm to obtain a unique unitless LUSI value. A low LUSI value indicates that the coastal water is not or only slightly influenced by continental pressures, whereas a high LUSI value indicates a very strong influence of continental pressures on coastal waters. This distinction was validated by measurements of dissolved inorganic nutrient concentrations in coastal waters of the Catalan coast. All these characteristics make LUSI an important tool not only within the WFD but also within the context of DPSIR (driving forces–pressures–states–impacts–responses) models in general. To demonstrate a significant pressure–impact relationship for BQE phytoplankton within the Catalan coast, LUSI values were calculated for the entire WB by using the CORINE land cover map from 2006.

4 Sustainability of the BQE Phytoplankton to Assess the Water-Quality at Catalan Coast

We tested four different phytoplankton-related approaches to evaluate the water-quality of the Catalan coast: (i) the harmful algal bloom (HAB) index; (ii) the diatom/dinoflagellate ratio; (iii) the bloom frequency index; and (iv) measurement of Chl-*a* concentrations. These approaches meet the WFD's requirements with respect to the taxonomic composition and abundance (i and ii), bloom frequency (iii), and biomass (iv) of phytoplankton.

Each approach complied with the WFD's intercalibration process (IC), the aim of which is to ensure the comparability of biological monitoring results obtained by the member states, as required by the directive. Our group was assigned to the Med-GIG from 2005 to 2015. All four approaches were statistically tested following the guideline of the WFD and the directions of the Joint Research Centre. The statistical tests were carried out by selecting several subsets of the two Catalan coastal water time series databases, depending on the BQE parameter. One of the subsets belonged to the common dataset from Med-GIG, which was used to establish a method to assess water-quality based on Chl-*a* (iv). To test the sustainability of the diatom/dinoflagellate ratio (ii), selected stations were classified into two groups, impacted and reference stations (sites with undisturbed conditions), depending on the degree of human disturbance. All the approaches were tested against LUSI values.

i. HAB index. As previously discussed, the assumption that the presence and/or abundances of a particular species of phytoplankton can be used as an indicator of water-quality has been strongly questioned [20]. However, because HAB species are toxin producers or cause other harmful environmental effects, their presence is considered as an environmental disturbance. The HAB index [49] integrates data on the taxonomic composition and abundances of HAB species. In a study conducted at 17 stations sampled monthly during two annual cycles (2005–2006) and covering 13 WBs along the Catalan coast, HAB species were grouped into six different categories based on their toxicity and harmful effects: paralytic shellfish poisoning, diarrheic shellfish poisoning, amnesic shellfish poisoning producers, benthic species, bloom-forming microplankton, and bloom-forming nanoflagellates. Depending on the cellular abundances of these species, they were assigned a score of 0, 1, or 2 such that the cumulative scores ranged from 0 to 12 for each sample. The HAB index was then calculated for each station according to the following formula:

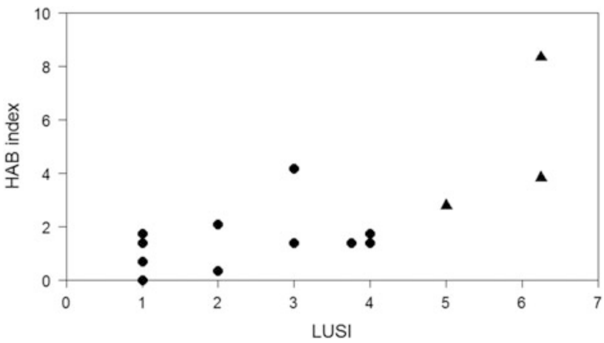

Fig. 2 HAB index and LUSI relationship for the 17 stations studied. *Triangles* represent stations with riverine influences (types I and II in Table 1) and *dots* represent type III stations (as defined in Table 1)

$$\text{HAB index } (\%) = [\text{Sum of scores}/(\text{Number of samples} \times \text{F})] \times 100$$

where $F = 12$, which is the maximum potential score for each sample. Next, the water-quality of each WB was assigned a value according to its HAB index. To confirm the accuracy of the classification, HAB index results were compared with the anthropogenic pressures defined by LUSI for each WB (Fig. 2).

The correlation between the HAB index and LUSI was significant ($R^2 = 0.5366$; $p < 0.001$) when all of the data were included. Stations with higher LUSI values were those affected by river inputs (types I and II), which in turn were prone to developing high-biomass blooms. However, because these stations clearly dominated the HAB index vs. LUSI relationship, it was no longer significant when they were excluded. These results clearly demonstrated that the HAB index is not an accurate indicator of eutrophication, given that the presence and abundances of toxic species were not significantly related to the degree of anthropogenic pressures. In fact, the proliferative potential of a harmful-producing species depends not only on the eutrophication of the WB in which that species resides but also on the species' physiological characteristics, its life cycle, the presence of its competitors and predators, and the properties of the WB itself, such as water motion, the stability of the water column, and the water residence time.

Similar conclusions were reached by other authors [50], who in devising a quality index removed the cell counts of harmful species as they did not provide any relevant information about the study area (Basque coast, NEA region). Other authors have similarly concluded that "HABs are not related to eutrophication of the Mediterranean zone" given that some toxic species are mixotrophic and can bloom even in areas with nutrient limitations [51].

ii. Diatom/dinoflagellate ratio. A second approach was based on the taxonomic composition of phytoplankton, specifically, on the ratio of the two main groups of phytoplankton: diatoms and dinoflagellates. This ratio was expected to be

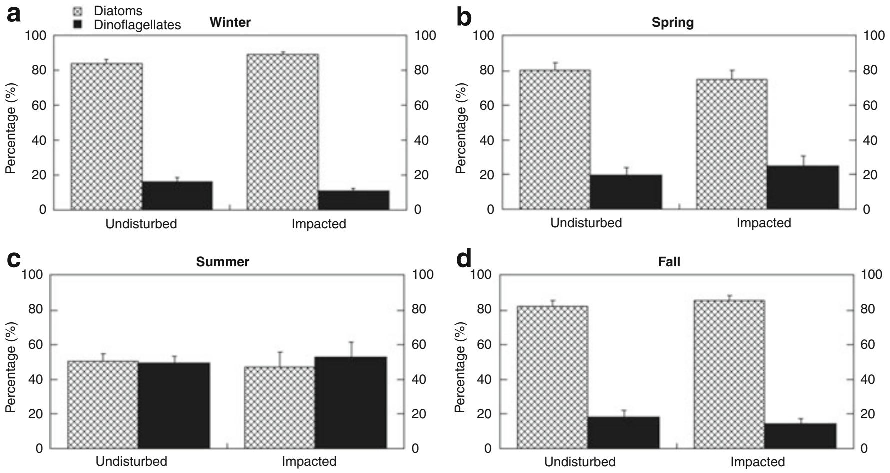

Fig. 3 Diatoms and dinoflagellates percentage at eight undisturbed (median LUSI = 1) and six impacted (median LUSI = 4) stations during (**a**) winter, (**b**) spring, (**c**) summer, and (**d**) fall. These stations were sampled monthly during a period of 2 years

responsive to changes in nutrient ratios induced by human eutrophication, as reported by some authors [52]. Anthropogenic activities increase nitrogen and phosphorous inputs into coastal waters but have little impact or even diminish silicate levels. Nitrogen and phosphorous are inorganic nutrients essential to the growth of all phytoplankton groups, whereas silicate is required by diatoms for the elaboration of their frustules. Accordingly, the growth of diatoms, but not dinoflagellates, is limited when silicate is deficient. We calculated the diatom/dinoflagellate ratio for a total of 14 stations previously evaluated using LUSI. Thus, eight of the stations were undisturbed (little or no impact from human activities; median LUSI = 1) and six were strongly impacted (high pressure from human activities; median LUSI = 4). Our hypothesis was that their LUSI-defined differences would be reflected in the diatom/dinoflagellate ratio. However, despite the clear difference in their LUSI values, there were no differences in the diatom/dinoflagellate ratios of the undisturbed vs. impacted stations (Fig. 3).

Instead, the changes in the diatom/dinoflagellate ratio mainly indicated the seasonal pattern of phytoplankton that is typical of the NW Mediterranean [53]. Therefore, the presence of diatoms and dinoflagellates depends not only on anthropogenic pressures related to nutrient inputs into WBs but also on the hydrographic characteristics of the respective water column and the general patterns of the seasonal succession of phytoplankton [3]. The inability of the diatom/dinoflagellate ratio to serve as a measurement tool of water-quality is consistent with published reports on similar problems encountered during attempts to implement other taxonomic-based indicators specified by the WFD [54–58].

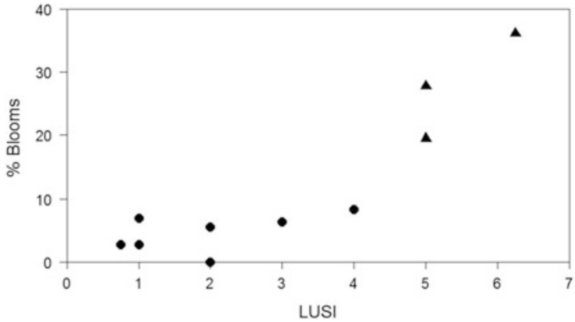

Fig. 4 Relationship between bloom frequency (%) and LUSI for the ten stations analyzed. *Triangles* represent stations with riverine influence (types I and II) and *dots* represent type III stations (see Table 1)

iii. Bloom frequency index. We measured phytoplankton bloom frequency at ten selected stations subject to different levels of anthropogenic pressure. Abundance thresholds were defined for the major phytoplankton groups (diatoms, dinoflagellates, coccolithophorids, and nanoflagellates) to estimate bloom frequency as a percentage of the total number of samples. These percentages were then used to assign water-quality categories. The correlation between the bloom frequency index and LUSI was significant ($R^2 = 0.795$; $p < 0.001$) when all of the data were included (Fig. 4). As in the case of the HAB index, the stations with higher LUSI values were those affected by river inputs, which in turn gave rise to high-biomass blooms. This sequence of events dominated the relationship such that when the respective data were excluded, the relationship was no longer significant. These results clearly demonstrate a nonlinear relationship between bloom frequency and anthropogenic pressures as well as an as-yet undefined pressure–impact relationship. Therefore, the bloom frequency index is not a good indicator of eutrophication.

In summary, the first three BQE-based approaches, which considered phytoplankton community composition and bloom frequency, are inadequate in terms of achieving the objectives of the WFD. Moreover, they reflect the broader problems regarding the use of phytoplankton indexes that rely on phytoplankton composition (whether of species or of functional groups) to classify water-quality in terms of eutrophication pressure. The absence of a direct relationship between blooms or HABs and eutrophication is in line with the current view of the scientific community, that algal blooms, including those that are toxic, can also be natural phenomena [59]. Our findings are also concordant with those reported by researchers in other European Union member states, in which national indices were developed to comply with the WFD but failed to demonstrate a relationship between BQE phytoplankton and water-quality [31, 50, 60].

iv. Chlorophyll-*a*. We followed several approaches to establish and test a methodology to assess water-quality using Chl-*a* as a proxy of phytoplankton biomass. All of them were based on the same assessment concept, in which nutrient concentrations are linked with those of Chl-*a*, which are higher in coastal areas that receive freshwater discharges of nutrients than in those that do not receive continental nutrient loads. On a practical level, this assumption is translated using the BQE-specific typology based on salinity, which recognizes at least three possible degrees of riverine influence on a WB. Thus, a WB with high river influence (type I) will have higher Chl-*a* concentrations than a WB without riverine influence (type III). In addition, all of the methodologies tested determine whether the water-quality of a WB is acceptable by comparing its Chl-*a* concentrations with a reference level, taking into account the typology. Thus, the water-quality of a WB with a Chl-*a* concentration similar to its reference, which in turn implies similarity with respect to salinity and nutrient concentrations, will be acceptable. Conversely, the water-quality of a WB with a Chl-*a* concentration that is much higher than the reference level will be unacceptable. In the latter, the difference between the measured WB and the reference WB is presumably due to an extra nutrient load related to human activities, i.e., eutrophication. In such cases, actions should be implemented to achieve the GES of that WB.

The following section provides a description of our methodology to assess water-quality based on Chl-*a*. The method was accepted by the European Commission in 2015, within the third phase of the WFD IC. After a description of the characteristics of the database, we describe the three steps that comprise the methodology: (i) establishment of the pressure–impact relationships, (ii) calculation of the reference conditions, and (iii) setting of the boundaries between water-quality categories. Finally, we provide an example by applying this approach to the Catalan coast.

4.1 Database

The methodology was developed using a subset of the common dataset from the Med-GIG, specifically, data from the NW Mediterranean (the coastal waters of France and Spain). As Spain has data from both CIW and CNW, all CIW data were transformed to CNW data, according to the first IC Med-GIG Technical Report, Section 3 Annex I Spain (Mediterranean Geographical Intercalibration Group, 2007), as shown in Eq. (1):

$$\text{CNW Chl-}a = 1/2^{*}\text{CIW Chl-}a \qquad (1)$$

The subset contained information from 71 WBs (23 of type II-A and 51 of type III-W). Type I was omitted from the subset as it was only present in Spanish waters, which prevented its intercalibration with similar data from France. The subset included information from each WB regarding estimated anthropogenic pressures (LUSI), their potential impacts (90th percentile of Chl-*a* values, in μg/L), and salinity (annual mean values), in order to specify its typology. Chl-*a* and salinity statistics were calculated over a 2 to 6-year period depending on the region and are representative of the CNW of each WB. As Chl-*a* values do not show a normal distribution, they were transformed according to Eq. (2):

$$y' = \log10(y + 1) \quad (2)$$

More detailed information on the sampling and analytical methods and on the common data set can be found in European technical reports [43].

4.2 Pressure–Impact Relationships

This is the first step in obtaining a valid methodology to assess water-quality, as stated by the WFD. Thus, a linear model was fit for each WB type, using a data doubling step and the *R* software package [61]. Due to the data doubling step, the goodness of fit values, but not the *p*-values, was reliable. The linear models for type II-A and type III-W are described by Eqs. (3) and (4):

$$\text{Type II-A}: \quad \text{Chl-}a \text{ Transformed} = 0.05{*}\text{LUSI} + 0.26 \quad (3)$$

$$\text{Type III-W}: \quad \text{Chl-}a \text{ Transformed} = 0.06{*}\text{LUSI} + 0.19 \quad (4)$$

Both show a positive relationship between LUSI and Chl-*a*, indicating that the greater the continental anthropogenic pressure received by a WB, the higher the Chl-*a* concentration, and therefore the stronger the impact on that WB (Fig. 5). Differences between the linear model coefficients of both types are consistent with

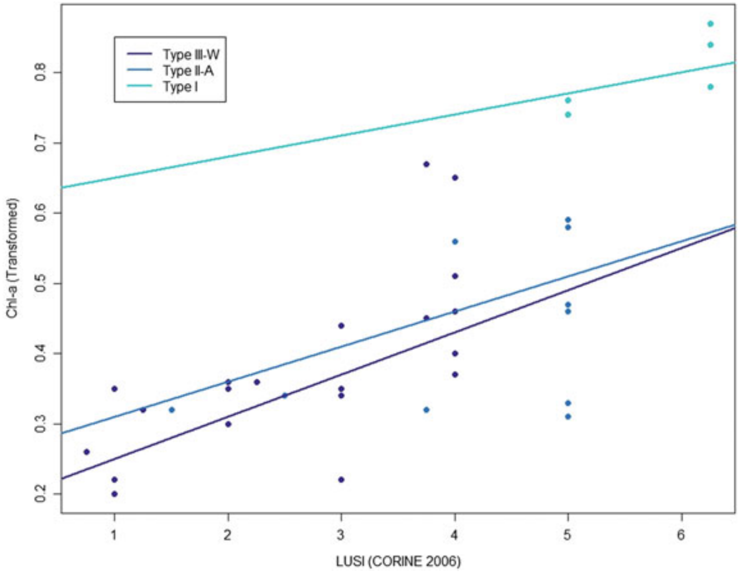

Fig. 5 Relationship between pressure (LUSI) and impact (transformed Chl-*a*) for type III-W, type II-A, and type I WBs. Linear models for type III-W and type II-A were obtained from the Med-GIG data subset. The *dots* represent data from Catalan WBs

Table 2 Reference conditions and boundaries for the assessment of water-quality based on Chl-*a* determinations along the Catalan coast

Type		Type III-W	Type II-A	Type I
Reference conditions (90th percentile Chl-*a*, μg/L)		0.79	1.28	4.13
Boundaries (90th percentile Chl-*a*, μg/L)	H/G	1.18	1.92	6.19
	G/M	1.89	3.5	13.01
	Failed	>1.89	>3.50	>13.01
Boundaries (EQR)	H/G	0.67	0.67	0.67
	G/M	0.42	0.37	0.32
	Failed	<0.42	<0.37	<0.32

Note that type II-A and type III-W also apply to the NW Mediterranean

the assessment concept. Thus, type III-W WBs have a higher slope and lower intercept than type II-A. In other words, for the same amount of pressure, a type III-W WB generates more Chl-*a* than a type II-A WB and is therefore more sensitive to pressures and will be impacted more rapidly. The intercepts provide information about the lowest Chl-*a* concentrations in the absence of pressures (theoretical value of LUSI = 0, which in practice does not exist) in type III-W WBs but not in type II-A WBs, since the latter are naturally affected by freshwater inputs and will, therefore, have a higher Chl-*a* concentration. Goodness of fit values (R^2) are 0.25 and 0.40 for type II-A and type III-W, respectively. These values are not high but they are acceptable, as they reflect the variability of the NW Mediterranean coast.

4.3 Reference Conditions

According to the WFD CIS Guidance Document No. 5, the reference condition must be derived from an undisturbed site or a site with only very minor disturbances. In accordance with this rule and considering that pressure is measured by means of the LUSI values, the minimum LUSI values for each type were selected to calculate the corresponding reference condition by using the previously established pressure–impact relationship. For a type II-A WB, the minimum LUSI value is 2 (regardless of the shape of the coastline); thus, the reference condition for this type is 1.28 μg Chl-*a*/L, expressed as a 90th percentile value. For a type III-W WB, the minimum LUSI value is 1 (regardless of the shape of the coastline), and its reference condition is therefore 0.79 μg Chl-*a*/L (Table 2).

The reference conditions for type II-A and type III-W WBs are similar to those measured in WBs of the Catalan coast that receive less continental pressure, that is, WBs located within a marine and terrestrial natural park, in the NE of Catalonia. Regarding type III-W, the Cap Norfeu WB has a LUSI value of 0.75 and its 90th percentile Chl-*a* concentration is 0.80 μg/L. For type II-A, the Cap de Creus WB has a LUSI value of 1.50 and its 90th percentile of Chl-*a* concentration is 1.09 μg/L.

This agreement between the calculated and real natural minimum values of Chl-*a* supports the validity of both the previously determined linear models and the reference conditions of the methodology.

4.4 Boundaries Between Water-Quality Categories

The final step of the methodology is to establish the boundaries between water-quality categories. As water-quality shifts from high to nonacceptable, the boundaries should reflect an increase in the difference between the Chl-*a* concentration and the reference condition. The WFD proposes five water-quality categories, but for management reasons, only the boundaries between two of them are of practical interest: between high and good (H–G) and between good and moderate (G–M). The latter defines the limit between an acceptable and a nonacceptable water-quality.

The H–G boundaries and G–M boundaries were established taking into account the variability within each WB type in the dataset. Thus, for a type II-A WB, 50% of the reference condition was added to the same reference condition to establish the H–G boundary and 82% of the H–G boundary was added to this boundary to obtain the G–M boundary. For a type III-W WB, the H–G boundary was obtained following the same procedure as for type II-A, but the G–M boundary was obtained by the addition of 60% of the H–G boundary. Once these boundaries in terms of the 90th percentile of the Chl-*a* concentration (μg/L) were established, boundaries in terms of the EQR could be defined by applying Eq. (5):

$$\mathrm{EQR} = \frac{\text{Chl-}a \quad \text{reference}}{\text{Chl-}a \quad \text{WB}} \tag{5}$$

The boundaries set in terms of the 90th percentile of Chl-*a* (μg/L) and EQR are shown in Table 2.

In summary, to assess the water-quality of a WB based on phytoplankton biomass requires data on the annual mean salinity and on the 90th percentile of Chl-*a* (μg/L). First, the typology of the WB is established according to Table 1. Second, the WB's reference condition is selected depending on its typology, as shown in Table 2. Third, the EQR of the WB is calculated using Eq. (5). And finally, the WB is assigned to a water-quality category with respect to the boundaries, defined in terms of the EQR (Table 2).

Regarding the IC, when a methodology is established, Member States can compare and harmonize boundaries. For the NW Mediterranean, the results show that France and Spain can use the H–G and G–M boundaries to assess the quality of their type III-W and type II-A WBs. In the case of Spain, this assessment can be made directly, without the need for specific correction coefficients. More information on the comparison and harmonization of boundaries between France and Spain can be found in their third phase IC Working Document regarding BQE phytoplankton, presented to the European Commission in 2014.

4.5 Water-Quality Based on Phytoplankton Biomass of the Catalan Coast

The quality of Catalan coastal waters, based on Chl-*a*, was assessed using the above-described methodology and Med-GIG dataset. Concretely, we used the subset corresponding to the Catalan coast since 2007 to 2010. Since with this intercalibrated methodology only type II-A and type III-W WBs can be assessed, the same procedure was applied to the data corresponding to a type I coastal WB of the Catalan coast. First, a linear model was established. Its goodness of fit (R^2) value was 0.81 and its linear equation was described by Eq. (6):

$$\text{Type I}: \quad \text{Chl-}a \text{ transformed} = 0.03{*}\text{LUSI} + 0.62 \tag{6}$$

The slope and intercept of this linear model were congruent with those of the linear models of type III-W and type II-A WBs (Fig. 5). The slope of the type I linear model was the lowest of the three linear models; thus, as an indicator of the magnitude of the pressure on Chl-*a*, a type I WB is the least sensitive to pressure. Since the value of the intercept of the type I linear model will be the highest of the three linear models, then in type I WBs the theoretical Chl-*a* concentration in the absence of pressures will also be the highest, consistent with this type being the one most influenced by freshwater inputs. Second, type I reference conditions were calculated. For this type, the minimum LUSI value is 3; therefore, the reference condition for this type is 4.13 μg Chl-*a*/L, expressed as a 90th percentile. Finally, boundaries for type I were set at 6.19 μg Chl-*a*/L for the H–G boundary and 13.01 μg Chl-*a*/L for the G–M boundary, taking into account that 110% of the H–G boundary is added to this boundary. For the EQR, the values were 0.67 and 0.32 for the H–G boundary and the G–M boundary, respectively. These boundaries were in agreement with those of type III-W and type II-A WBs.

All WBs from the Catalan coast, including transitional waters, were assessed by assigning each one a typology using Table 1 and a water-quality category following Eq. (5) and by applying the reference conditions and boundaries listed in Table 2. The results are shown in Fig. 6.

Our assessment of all Catalan WBs showed that 89% have an acceptable (high or good) water-quality based on their Chl-*a* concentrations, with 47% having a high water-quality. By typology, the water-quality of 81% of the type III-W WBs is high or good, whereas for type II-A and type I WBs, this percentage is 100%. Only four WBs failed to achieve an acceptable water-quality (Barcelona, El Prat de Llobregat-Castelldefels, Vilanova i la Geltrú, and Tarragona-Vilaseca). Our results are in agreement both with previous assessments of Catalan coastal waters carried

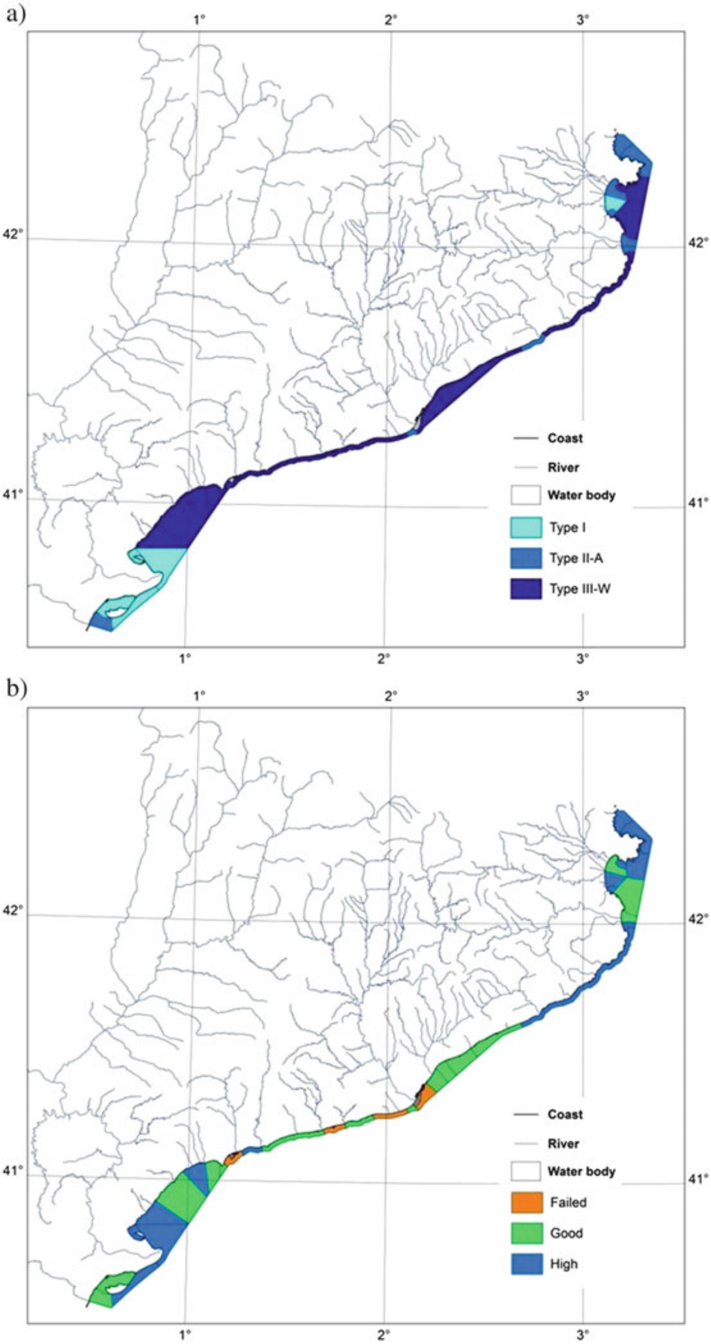

Fig. 6 Maps of the Catalan coast showing the typology of each water body (**a**) and its water-quality based on phytoplankton biomass (Chl-*a*) (**b**). Water bodies that correspond to harbors were not assessed and are indicated in *grey*

out before the implementation of the WFD and with parallel studies. Therefore, our linear models, selected reference conditions, and boundaries are appropriate and the methodology is valid. With this water-quality assessment approach based on BQE phytoplankton, the ecological status of Catalan WBs will be assessed and included within the third River Basin Management Plan.

5 Final Discussion on the Use of Phytoplankton as a BQE

We conclude this chapter by again addressing the general assumptions of the WFD and by considering the knowledge gained by the use of BQE phytoplankton to assess water-quality.

Ecological systems usually exist as a continuum such that their spatial delimitations are difficult, if not impossible, to recognize. This is the first challenge in the implementation of the WFD because its basic management unit is the WB, whose spatial delimitation is an artificial condition that must be fulfilled by Member States. As defined by the WFD, coastal waters are those within "a distance of one nautical mile on the seaward side from the nearest point of the baseline from which the breadth of territorial waters is measured." The absence of clear hydromorphological quality elements makes the definition of a marine WB much more challenging than is the case for other surface waters, such as rivers or lakes. For BQE phytoplankton, the establishment of the offshore limits of the WB is crucial, because phytoplankton communities are present and can be sampled within the whole WB and not only along the coast, as is the case for other BQE such as macroalgae or phanerogams. The results of the first attempt to define Catalan coastal WBs are now available, and the definitions have been used within the first and second River Basin Management Plans; however, a revision of the limits of those WBs should be considered.

Other weaknesses in the implementation of the WFD became apparent during the testing of the phytoplankton-related approaches to evaluate the water-quality here, in the Catalan coast, and elsewhere. These were linked to the temporal and spatial samplings of the WBs. The directive allows Member States to select sampling sites and sampling frequencies within each WB. However, coastal WBs are characterized by spatial heterogeneity and asymmetry, with continental anthropogenic pressures being most obvious near the coastline, especially in the tideless Mediterranean Sea. To adequately characterize Mediterranean WBs, they must be sampled at different distances from the coastline. The failure to include sampling points near the coastline will result in values that are not representative and therefore in large biases regarding spatial heterogeneity. But even before the WFD, water-quality surveys in the Catalan coast included sampling sites at different distances from the coastline [36]. Regarding the time frame for sampling, the directive allows the assessment of water-quality based on BQE phytoplankton with a minimum of two samples per year. Yet, as pointed out in this chapter, because phytoplankton communities are highly dynamic, a higher sampling frequency is necessary to define the temporal heterogeneity of a given WB. Moreover, only a wide dataset will allow the necessary statistical integration needed to reveal changes in parameters of interest, such as Chl-*a*. For the water-quality surveys conducted along the Catalan coast, sampling is carried out weekly, fortnightly, monthly, and quarterly, depending on the degree of variability of the sampling point. As is the case for phytoplankton, the inadequate sampling of Chl-*a* could lead to a misrepresentation of the spatio-temporal heterogeneity of the respective WB and therefore to erroneous quality assessments based on BQE phytoplankton.

The WFD assumes that changes caused by anthropogenic pressures can be distinguished from those resulting from natural causes, which in turn implies known pressure–impact relationships. However, current scientific knowledge, including our own efforts to relate anthropogenic pressures to the parameters proposed for BQE phytoplankton, is too limited to reveal the nature of this relationship; consequently, its translation into management actions is not possible. Nonetheless, Chl-*a* is widely used as a bioindicator of eutrophication in coastal waters. At the management level, some insight has been gained regarding the changes in Chl-*a* caused by anthropogenic pressures (in the form of nutrient inputs), but distinguishing them from changes resulting from natural causes is often difficult. For the purpose of defining and implementing management actions, water-quality assessments based on phytoplankton biomass should be evaluated with respect to the general conditions defined by the WFD. These conditions are defined by physical and chemical quality elements, which in the case of the Catalan coast include dissolved inorganic nutrient concentrations. An evaluation of this type could provide insights into the origin of the detected nutrients, i.e., whether they are due to anthropogenic activity inputs into coastal waters, and thus of Chl-*a*. The results will allow decisions to be made regarding the need for management actions, since naturally high levels of Chl-*a* may not warrant external correction. Along the Catalan coast, these evaluations have already been conducted and the findings taken into account within River Basin Management Plans.

Finally, a much better understanding of nutrient-phytoplankton relationships is needed before the effects of eutrophication based on BQE phytoplankton can be fully understood and the appropriate measures taken. The complexity of the interactions between physical, chemical, and biological factors and phytoplankton hinders the establishment of well-defined impact-pressure relationships, and therefore effective management strategies. Until these challenges are overcome, we recommend the implementation of good practices aimed at nutrient load reduction in coastal areas in order to achieve the GES of all European water bodies, which is the main goal of the WFD.

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