

Joint Position paper on the only use of Chl a for the establishment of ecological status (Phytoplankton element) in coastal waters.

Executive summary

The WFD requirement for assessing ecological status of the phytoplankton quality element includes taxonomic composition, abundance and biomass of phytoplankton as well as bloom frequency to be taken into account for transitional and coastal water bodies.

Experts acknowledge that indicators of phytoplankton bloom frequency and community composition indices may potentially add more information to the phytoplankton quality element than Chl a/biovolume alone, but so far proposed indicators of these sub-elements have only been suggested in some very small parts of the intercalibration area but their wider applicability have not been demonstrated across broader regions. For the overall WFD intercalibration and status assessment the inclusion of these sub-elements have never been successful despite large efforts in various research projects.

In the most intensively monitored regions in Europe where the phytoplankton data, have been thoroughly analysed to investigate the potentials of various indicators for phytoplankton blooms and community structure, experts have observed that the uncertainty associated with these indicators is disproportionately large relative to the responses of these indicators to pressures. Consequently, the use of these indicators to achieve a status classification with a reasonable precision would require unrealistic monitoring efforts, rendering these indicators non-operational as decision support for river-basin management plans.

This position paper presents scientific arguments for the use of the biomass parameter measured as chlorophyll a as the main operational phytoplankton indicator for the majority of the countries at present.

Introduction- BQE Phytoplankton-WFD requirements

Phytoplankton species follow seasonality cycles that depend on latitude and distance to the coast. The fundamental causes of variability in phytoplankton have been studied in detail (Margalef, 1978; Kilham & Kilham, 1980; Harris, 1984; Huisman & Weissing, 1999). Here, we will detail some general points of the features of the phytoplankton communities that are relevant for the WFD requirements.

Causes of variability in phytoplankton communities. The role of nutrients in controlling ocean productivity has long been recognised, as nutrient elements constitute one of the required resources for the growth and survival of organisms that use light to fix carbon. These organisms 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. In addition to nutrients, other main chemical and physical drivers affects the phytoplankton community: nutrient stoichiometry, salinity turbulence, stability of the water column, degree of confinement and water residence times,

temperature, tidal mixing and light (Margalef, 1978; Justic et al. 1995; Vila & Maso, 2005; Arin et al. 2013; Ribera d'Alcala et al. 2004), all this along also with biological factors, such as predators and parasites (Calbet et al. 2003; Garcés et al. 2013). Additionally, the phytoplankton community is made up of many different species with different life strategies. The survival of those species is favoured by biological strategies, such as nutritional diversity (mixotrophy), grazing, and competition strategies, adapted life cycles, etc (Garcés et al. 2013; Steidinger, K. A. & Garcés, E 2006; Stolte & Garcés, 2006). All these features allow phytoplankton to survive in different coastal environments and govern their community structure.

Phytoplankton communities, an indicator without memory. Phytoplankton communities are highly dynamic, which is a consequence of the relatively high growth rates of the different species (Stolte & Garcés, 2006; Garcés et al. 2011). The ability of temperate marine phytoplankton communities to rapidly respond to changes in environmental conditions implies that phytoplankton reflect the physico-chemical properties of their environment at a particular time and space and do not temporally integrate environmental changes.

Relationships between the quality element with the pressures. The relationships between environmental conditions and the abundance and composition of phytoplankton are complex (Cloern & Jassby, 2008; 2010) and the link between certain environmental conditions and phytoplankton community structures is rarely a direct one. These points out one of the main drawbacks of the WDF: because phytoplankton communities are highly diverse and well-adapted not only to the nutrient fluctuations over time, but also to the changing physical parameters, clear-cut relationships between phytoplankton as a BQE and environmental pressures cannot be established (Camp et al. 2015).

The idea of the climax and reference conditions Frederic Clements (1916) developed a simple view of equilibrium in biological communities: *A (climatic) climax community is 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 European Directive suffers from insisting on this view even though the recent scientific literature contains strong evidence that it is an inappropriate model for ecosystem management (Gowen et al. 2012). Rather, to assess the status of marine ecosystems and to quantify disturbances to the balance of organisms—in this case phytoplankton communities—it is necessary to take into account the dynamic nature of ecosystems and communities. In a seasonal cycle, phytoplankton communities will be highly variable. Therefore, the establishment of reference conditions, for the WDF's purposes, a description of phytoplankton communities based on completely or nearly completely undisturbed conditions, with little or no impact from human activities, which is one of the most important steps in the WDF's status assessment, is in practise very difficult to realise (Garmendia et al. 2013; Borja et al. 2012). Consequently, the status of phytoplankton should not, and cannot, be assessed by comparing its composition and relative abundances with a "reference" assemblage of species that is in fact, not at all representative.

Suitability of phytoplankton taxonomic composition as indicator

The phytoplankton community consists of several thousand different species, each having their own life strategy, selected through evolution. Specific characteristic strategies include low affinity to nutrients, fast growth rates, motility, allelopathy, defenses towards grazing, nitrogen fixation, etc. Physical and human perturbations in coastal environments constantly alter the environmental conditions and as a consequence, the phytoplankton community constantly changes to adapt to these dynamic conditions. In addition, biological interactions, most prominent in the form of grazing by both filter feeders and zooplankton as well as phytoplankton phenology, further add to this complexity of factors governing the phytoplankton community. Thus, nutrient enrichment is just one of several factors, and most likely not the most important, structuring the phytoplankton community.

Based on the present general consensus of drivers affecting the phytoplankton community (e.g. Paerl & Justic 2013), experts submit the following ranking of factors governing the phytoplankton community structure (i.e. not the biomass per se):

- 1- *Phenology*. All phytoplankton species have their own specific phenology, which is most typically observed in the seasonal succession of the community.
- 2- *Physical factors*. Salinity, stratification, temperature, tidal mixing, light, turbulence.
- 3- *Nutrient ratios*. Low silica concentrations relative to inorganic nitrogen and phosphorus favour non-silicious species (non-diatoms). Nitrogen depletion during summer may favour nitrogen-fixing cyanobacteria.
- 4- *Nutrient concentrations*. All phytoplankton compete for resources, including nutrients. Increasing nutrient concentrations may favour fast-growing species.

Studies of the seasonality of the phytoplankton in the Mediterranean area (Arin et al. 2013; Ribera d'Alcala et al. 2004; Aubry et al. 2004; Estrada et al. 1999) support the view of phytoplankton communities as being highly dynamic, changing in response to seasonal environments and, throughout the year (succession). Moreover, community composition may change not only in response to the changing seasons but also in response to individual pulses of nutrients, whether from inland, open water, or water masses with a different composition. This implies that the taxonomic composition of phytoplankton is not only linked to current nutrient content but also to the history and characteristics of the water body in which it develops .

Results of the study of functional groups in the Mediterranean sea showed that the index of seasonal succession of functional groups (Iss)¹ cannot be considered as a suitable classification tool, mainly because seasonal succession can hardly be assessed on a monthly basis, given the fast generation times of phytoplankton (differences from year to year are substantial and natural).

Table 1. Values of Iss (in %) for phytoplankton and its functional groups as determined at five Slovenian stations in the period 2007/2008.

¹ The index assesses the shift in the seasonal succession of phytoplankton functional groups with respect to the reference conditions.

	000F	00MA	00C4	0DB2	000K
Nanoflagellates	95.83	91.67	87.50	91.67	95.83
Diatoms	95.83	91.67	87.50	91.67	95.83
Dinoflagellates	100.00	87.50	95.83	91.67	95.83
Coccolithophorids	95.83	87.50	83.33	83.33	83.33
Total	96.88	89.58	88.54	89.58	92.71

Moreover, the diatom: dinoflagellate ratio² did not show differences (Figure 1). Although in summer there was a slight predominance of dinoflagellates over diatoms at the monitored station the data mostly indicated the seasonal pattern of phytoplankton that is typical of the NW Mediterranean (Vila et al., 2005; Margalef et al., 1967).

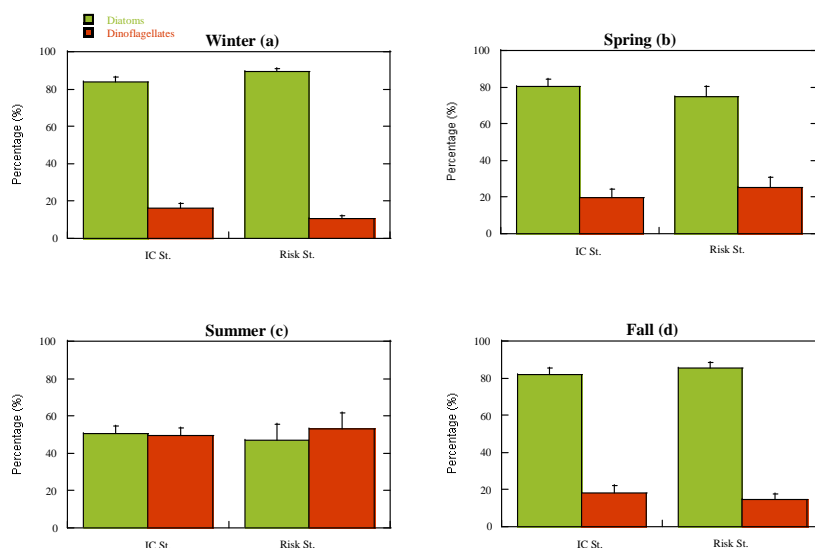


Figure 1. Diatoms and dinoflagellates percentage at the Intercalibration and risk stations for the 4 seasons: a) winter, b) spring c) summer and d) fall. IC stations were defined as completely or nearly completely undisturbed conditions, with little or no impact from human activities and risk stations were defined as impacted ones (for more details see Camp et al. 2015).

Jurgensone et al. (2011) demonstrated in a study from the Gulf of Riga that the biomass of the phytoplankton spring population correlated with the input of phosphorus from land, whereas low silica relative to inorganic nitrogen concentrations would induce a shift from diatoms to dinoflagellates. During summer the phytoplankton biomass changed from being bottom-up controlled (nutrient inputs) to top-down controlled by pelagic grazers (zooplankton). Low inorganic nitrogen relative to inorganic phosphorus induced a shift from diatoms to cyanobacteria, whereas increasing temperature favoured chlorophytes relative to dinoflagellates. Thus, whereas nutrient concentrations are important for the phytoplankton

² The index is used to measure the shift in the ratio of functional phytoplankton types between different types of stations, those completely or nearly completely undisturbed conditions vs. impacted ones.

biomass the composition of the phytoplankton community was more governed by both physical factors and nutrient ratios.

Although it is acknowledged that nutrients also play an important role for the community structure, it is generally difficult to quantify the potential effects of nutrient levels on the phytoplankton community structure using data from a single ecosystem, because variations in nutrients are relatively small compared to the large uncertainties associated with assessing the phytoplankton community from monitoring data. Carstensen et al. (2013) studied the carbon biomass proportions of diatoms and dinoflagellates in the western Baltic Sea and found, on the broad scale, that diatoms were increasingly important in coastal ecosystems with higher nutrient concentrations (Total Nitrogen (TN), in this case), whereas dinoflagellates decreased (Fig. 2). This is consistent with the general theory that diatoms become more dominant in nutrient-rich ecosystems. However, this pattern only emerged over an almost 10-fold range of nitrogen concentrations, whereas it was also observed that the variations around the regression line were large when considering realistic changes in nutrient levels (typically 20-30% from management actions, Carstensen et al. 2006).

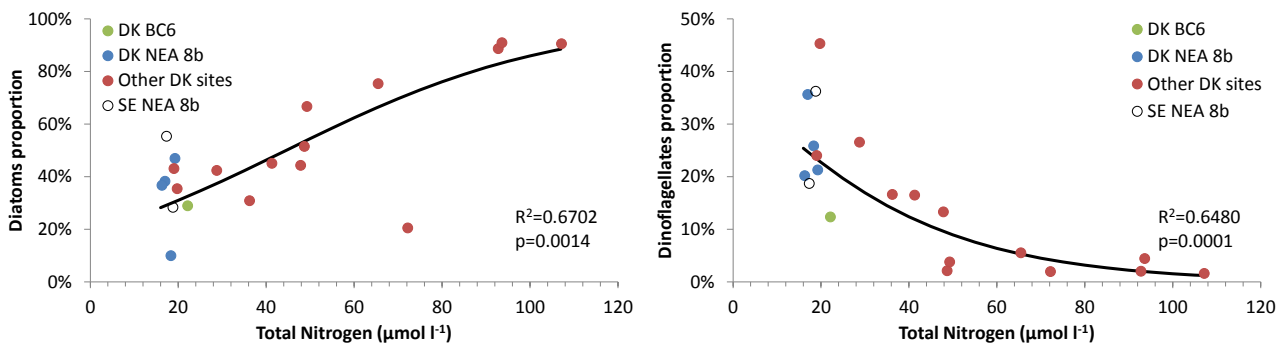


Fig.2. Estimated changes relative to the TN concentration in the proportion of the dominant phytoplankton life forms from different coastal ecosystems in the western Baltic Sea. Each observation represents mean proportion from long-term phytoplankton monitoring data (typically ~100 observations). Salinity was also included as a covariate in the analysis (see Carstensen et al., 2013 for details).

Similar results have been found from analysing an even larger data set (including ~30000 counted samples) within the framework of SCOR WG137 on phytoplankton dynamics (see <http://wg137.net>). Analyses from this working group show, when accounting for differences in salinity, temperature and mixing patterns, that the proportion of chlorophytes increases with higher Total Phosphorus (TP) concentrations and the proportion of diatoms increases with higher TN levels, but these patterns are only apparent over orders of magnitude in nutrient levels. Furthermore, the analyses also documented that variations in physical conditions (most pronounced differences in salinity, temperature and mixing patterns) were more important for structuring the phytoplankton community than nutrient levels. Thus, the analyses from SCOR WG137 confirm that nutrient levels are important for structuring the phytoplankton community, but these effects operate at scales (orders of magnitude difference in nutrient levels) larger than a single ecosystem will experience, rendering the use of indicators based on phytoplankton composition non-applicable as an operational tool for assessing ecological status.

It has also been proposed that specific species could be used as indicators of ecological status. In the context of eutrophication, the assumption that a particular species of phytoplankton will proliferate as a direct result of a disturbance to the balance of an aquatic ecosystem has been strongly questioned (Gowen et al. 2012).

This hypothesis was investigated in Carstensen & Heiskanen (2007) using a large data set from the eastern Baltic Sea spanning broadly in nutrient levels. Out of 76 potential indicator species investigated, half of these responded to changing nutrient levels (mostly weak responses), four species responded strongly over the entire nutrient gradient and only one species was identified as having potential as indicator species, and this potential could only be exploited in a narrow salinity range. Thus, based on the results from Carstensen & Heiskanen (2007) we conclude that it is unlikely to identify specific indicator species (“litmus species”) that respond to nutrient pressure.

German studies have found that the biomass of the different taxonomical groups increase with nutrient enrichment on a broad scale, but these investigations did not reveal if increased nutrient status resulted in relative changes between groups. In waters of higher salinity (>10) proposed indicators based on biovolumes of cyanobacteria and chlorophytes were not applicable, since these groups have low abundances. In areas of higher salinity total biovolume is used in addition to chlorophyll and is intercalibrated with Denmark.

For some coastal regions, the occurrence of a particular species has been used as an indicator of human perturbation, such as members of the genus *Phaeocystis*, which are regarded as a nuisance in coastal waters of the North Sea. Interestingly, however, the authors of that study (Lancelot et al. 2009) concluded that there had been little change in the ecosystem of Belgian coastal waters despite the considerable increase in *Phaeocystis* spp. associated with anthropogenic nutrient enrichment.

In summary, phytoplankton communities have evolved over time to optimally exploit the environmental niches that the different coastal environments provide. The environmental conditions and the biological interactions in coastal ecosystems change constantly resulting in a highly dynamic phytoplankton community, and as a consequence the phytoplankton community can be characterised as a constantly changing complex entity comprised on many different species with different life strategies, sometimes portrayed as chaotic (Beninca et al. 2008). The tenet that the phytoplankton community is sensitive to smaller changes in nutrient pressure (and hence could be useful for the WFD implementation) is flawed, as all phytoplankton species are well-adapted to the ever-changing nutrient fluctuations over time and have developed different strategies in their competition for resources.

Suitability of bloom frequency as indicator

Nutrient enrichment fuels coastal ecosystems with “potential energy” for outburst of phytoplankton blooms, but the mechanisms leading to the actual bloom formations are complex and mediated through physical and biological processes (Carstensen et al. 2007). This implies that blooms, defined as rapid increase in biomass resulting from imbalance between phytoplankton growth or advection and mortality or

dilution, occur when this energy is released through various physical processes, e.g. mixing of nutrient-rich bottom waters into the surface layer or decoupling of benthic grazers during periods of stratification. On the other hand, when the same physical processes conducive to phytoplankton blooms occur in an ecosystem with low nutrient levels, the likelihood of a bloom formation is substantially lower. Thus, there is a clear causal link between nutrient pressure and the probability of blooms occurring (bloom frequency), but this link is indirect since it is mediated through a complex suite of physical processes resulting in noisier quantitative relationships than observed for the more direct causal relationship between nutrient inputs and phytoplankton biomass (see below).

An indicator of bloom frequency was proposed in Carstensen et al. (2007) and has been revised in Carstensen et al. (submitted). This indicator has been applied to 40 coastal long-term time series around the Baltic Sea as well as 45 coastal time series from other parts of the world. Although the bloom identification algorithm is not applicable to all phytoplankton time series in the Baltic Sea region, there are quite a number of waterbodies where this is possible and the bloom frequency can be assessed. However, due to the complex interplay with the physical perturbations the bloom indicator is not regarded as operational because the relationship to the nutrient pressure is too “noisy” to apply the indicator as discriminator between good and moderate ecological status. Both the bloom indicator and indicators of phytoplankton composition are useful for reporting status and trends, taking their uncertainties into account, but at present not applicable as operational tools for management decisions.

In the Mediterranean Sea, with the available data from different countries, experts applied three phytoplankton indices: i) the index of elevated phytoplankton abundances (*I_e*), which assesses the presence, abundance and frequency of occurrence of elevated counts of phytoplankton; ii) bloom frequency, which assesses the frequency of occurrence of elevated counts of functional groups of phytoplankton and iii) the HAB index, which assesses the abundance and frequency of the occurrence of toxic and harmful species. The results showed that there are still many gaps in the knowledge regarding the use of indexes of elevated counts (both of species and of functional groups) to classify the ecological status of phytoplankton in relation to eutrophication pressure. According to the third index, blooms and harmful algal blooms have no direct relation to eutrophication (Reñé et al. 2007), a finding in line with the current view of the scientific community, that algal blooms, including toxic events, can be natural phenomena (Garcés and Camp, 2012). Moreover, these results are concordant with the different national indices that have been developed in the EU member states for assessing phytoplankton blooms, as part of the phytoplankton quality element under the WFD's requirements. All of them with similar conclusions (Devlin et al. 2007; Revilla et al. 2009).

Suitability of Chl a as indicator

The most common measurement of phytoplankton biomass is chlorophyll a (Chl_a), which is a pigment in the chloroplasts that is responsible for the photosynthesis. It should be acknowledged that Chl_a is not a

perfect measure of phytoplankton biomass, since the amount of Chla in the cell varies seasonally and with prevalent light conditions. Nevertheless, there is a direct causal link between nutrient enrichment and enhanced growth of phytoplankton, which will also lead to increasing levels of Chla. The relationship between phytoplankton and Chla derives from the fact that the main limitation for phytoplankton growth is nutrient concentrations and the average nutrient supply is related to the average chlorophyll content in any marine water body. This relationship is valid if data are collected within a suitable spatio-temporal framework, with sufficiently frequent sampling over a reasonable period of time.

Considering the uncertainties associated with indicators of bloom frequency and phytoplankton composition and their weaker responses to nutrient pressure, Chla is the only practically applicable indicator for the intercalibration at present.

In fact, most eutrophication assessment methods recognise that the immediate biological response to nutrient inputs is an increase of primary production, reflected as an increase in chlorophyll a and/or macroalgal abundance (Ferreira et al., 2007; HELCOM, 2009). Chlorophyll a, used as a proxy for phytoplankton biomass, is commonly accepted as an indicator of eutrophication and there is extensive literature on its use for this purpose for coastal waters (Devlin et al., 2007; Bricker et al., 2008; Garmendia et al., 2011).

All EU member states have Chla data from shared types, whereas biovolume data calculated from counted samples impose much stronger data limitations for the intercalibration. In addition, variability in biovolume data is larger than chla for assessing status, suggesting that more counted samples are needed to obtain the same indicator precision (Carstensen 2007).

Consistent relationships between Chl a and nutrient levels have been demonstrated in the literature (e.g. Guildford & Hecky 2000; Hoyer et al. 2002; Smith 2006; Carstensen & Henriksen 2009), although these relationships may change over time in response to other signals of global change (see Carstensen et al. 2011). Of course, because the relationship is a statistical one, it may be that within a particular sample or for certain number of samples collected at a specific time point there is no relationship between nutrients and chlorophyll. This reflects the fact that when nutrients reach the medium (coastal waters in this case), chlorophyll must first be generated with a time delay, but at the same time other physical and biological processes characteristic of a turbulent and dynamic environment can interfere, thereby obscuring the relationship between nutrient inputs and Chla concentrations. Nonetheless, Chla is the most promising indicator at present and it is crucial to demonstrate the applicability of this indicator for assessing phytoplankton ecological status within waterbodies as well as intercalibration between waterbodies sharing the same type. The Chla a boundaries harmonization and interpretation require appropriate sampling strategies and appropriate sample handling. For example, in systems under the influence of various gradients, such as occurs along the coastal waters, the main source of variability is the distance to the land. Accordingly, the sampling stations must be appropriately selected.

Sampling frequency for using phytoplankton parameters as suitable indicators

A potential link between certain environmental conditions and phytoplankton community structures can only be interpreted statistically, and requires a large number of samples in a suitable space-time

framework. Statistical integration could evidence some stages of its natural succession but not assures to detect changes due to differences in environmental parameters. Statistical integration could provide evidence of a natural succession in a specific time and space whereas the detection of changes due to differences in environmental parameters is not assured.

Temporal and spatial variations in phytoplankton properties are large, which is manifested by generation times on the order of days, and sampling according to current monitoring programs provides snapshots of this highly dynamical component of coastal ecosystems. The traditional belief has been that a water sample (typically ~10-50 mL analysed) is representative of a larger water mass, which assumes complete mixing, but the emergence of high-resolution sampling in both time and space has revealed substantial patchiness in biomass and composition. The implication of these large sources of variation is that many observations are needed to characterise a given waterbody with sufficient precision. Additional uncertainty arises from the method of analysis, particularly pronounced in counted phytoplankton samples. Uncertainties associated with phytoplankton sampling and analysis is smallest for chlorophyll a and largest for phytoplankton composition.

Many experts consider that the he ideal sampling frequency for using phytoplankton parameters would be every 3–5 days. Currently, no Member State has data based on this sampling frequency. This data can be extracted from remote sensing methods, but only for chlorophyll, and, moreover, a higher sampling frequency does not assure the determination of an impact-pressure relationship for the BQE phytoplankton.

Only few studies have been carried out to assess how much data is actually required for the WFD implementation.

Carstensen (2007) examined data requirements for the WFD implementation based on nutrient and Chla concentrations as well as phytoplankton biomass, and found that if the “true” chlorophyll a mean deviated by 20% from the G-M boundary, 93 samples would be needed for a correct classification with a power of 80% and a confidence level of 95%. Similarly, 245 counted phytoplankton samples would be required under the same conditions. If the “true” value deviated more than 20% from the G-M boundary less observations would be required and similarly, if the “true” value deviated less than 20% from the G-M boundary more observations would be required. These results suggest that at least monthly sampling in all six years of the WFD assessment cycles would be required for chlorophyll a and biweekly sampling would be required for phytoplankton counted samples. These results stress the need for setting up adequate monitoring programs and improve sampling and analysis procedures to reduce sources of uncertainties where possible, but leading to an extraordinary cost.

Conclusions and future directions

- Indicators of phytoplankton bloom frequency and composition are available and respond to nutrient pressure, but the inherent uncertainty of present indicators relative to their sensitivity to

changing nutrient levels render such indicators non-operational for setting boundaries according to the WFD.

- Chlorophyll a, used as a proxy for phytoplankton biomass, is the most useful indicator and most sensitive to nutrient pressure. It is therefore recommended to focus efforts on intercalibrating this indicator and demonstrate its applicability for status assessment and decision support.
- A minimum monitoring requirement of monthly sampling of chlorophyll a is recommended to achieve indicators with sufficient precision to allow status classification with a low probability of misclassification.
- Monitoring of phytoplankton bloom frequency and composition should continue in order to assess and report changes over time. These indicators may constitute supporting elements for the assessment of phytoplankton status. Efforts should still be made towards understanding and developing new indicators for phytoplankton blooms and composition, and these new indicators should be tested for their practical applicability in relation to WFD. Furthermore, this approach is in particular important considering that under the MSFD phytoplankton indicators are under development that address biodiversity and food web aspects.
- Efforts should be directed towards improving sampling and analysis procedures for phytoplankton to reduce sources of uncertainty, and new cost-effective techniques for monitoring phytoplankton should be considered, when these are documented to be sufficiently mature for operational monitoring *sensu* the WFD.

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