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Background

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. Within the Baltic Sea area, at present, the EU Member States are using chlorophyll a (Chla) or biovolume as a proxy measure for phytoplankton biomass for the intercalibration, whereas indicators of the other sub-elements (phytoplankton composition and blooms) have not been intercalibrated. The Baltic GIG phytoplankton experts are currently preparing a justification why indicators on phytoplankton composition and blooms could not be successfully intercalibrated. Some of the points the experts make are also of relevance for the indicator development in EUTRO OPER, in particular the difficulty in developing phytoplankton composition indicators of relevance for the whole Baltic Sea and the high variability of the bloom indicators.

Action required

The Meeting is invited to take note of the information, when discussing the development of new eutrophication indicators.

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. Within the Baltic Sea area, at present, the EU Member States are using chlorophyll a (Chla) or biovolume as a proxy measure for phytoplankton biomass for the intercalibration, whereas indicators of the other sub-elements (phytoplankton composition and blooms) have not been intercalibrated.

The Baltic GIG phytoplankton experts acknowledge that indicators of phytoplankton bloom frequency and community composition indices may potentially add more information to the phytoplankton quality element than Chla/biovolume alone, but so far proposed indicators of these sub-elements have only been suggested in some very small parts of the intercalibration area (e.g. taxonomic composition for low salinity waters (between 5 and 10) in Germany), 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 and within the Baltic GIG expert group.

The Baltic Sea is one of the most intensively monitored regions in Europe, and the phytoplankton data, quality assured through standard common procedures in HELCOM PEG, have been mainly investigated in various research projects over the last decades. These data have been thoroughly analysed to investigate the potentials of various indicators for phytoplankton blooms and community structure, but the uncertainty associated with these indicators is disproportionally 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 neighbouring countries at present.

In the annex to this position paper, detailed responses to the comments of the ECOSTAT review panel are given.

Suitability of phytoplankton taxonomic composition as indicator

The phytoplankton community consists of several thousand different species, each having their own optimal 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), the Baltic GIG phytoplankton group submits that 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.

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 such as diatoms.

This ranking is based on a number of studies, globally and from the Baltic Sea.

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 biomass the composition of the phytoplankton community was more governed by 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. 1). 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).

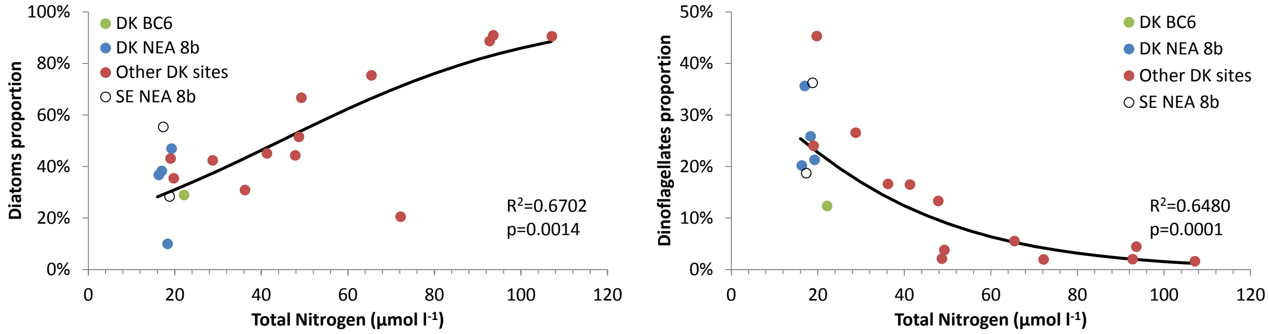

Fig. 1: Estimated changes relative to the TN concentration in the proportion of the dominant phytoplankton life forms from different coastal ecosystems in the western Baltic Sea. Each observation represents mean proportion from long-term phytoplankton monitoring data (typically ~100 observations). Salinity 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 indicative of ecological status. 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. In summary, phytoplankton communities in the Baltic Sea 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 constant change 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. Species sensitive to nutrient fluctuations would have disappeared a long time ago in the evolution process.

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 processes (Carstensen et al. 2007). This implies that blooms, defined as rapid increase in biomass resulting from imbalance between phytoplankton growth and mortality, 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. (subm.). 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.

Suitability of Chla as indicator

The most common measurement of phytoplankton biomass is Chla, which is a pigment in the chloroplasts that is responsible for the photosynthesis. It should be acknowledged that Chla 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. 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. All EU member states in the Baltic Sea region 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 Chla 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 relationship may change over time in response to other signals of global change (see Carstensen et al. 2011). Consequently, 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.

Sampling frequency for using phytoplankton parameters as suitable indicators

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 are smallest for chlorophyll a and largest for phytoplankton composition.

Monitoring requirement can be assessed using power analyses based on estimated variances for the random variations in monitoring data. 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.

Conclusions and future directions

- Indicators of phytoplankton bloom frequency and composition are available and respond to nutrient pressure, but the inherent uncertainty of these 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 precise indicators 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 diverted towards understanding and developing indicators for phytoplankton blooms and composition, and these 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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Annex: Answers to the Review panel evaluation.

Questions for the Review Panel (RP)

1. Do you think that the justifications provided by the experts have been exposed correctly and sufficiently informative to allow decisions on the WFD compliance criteria?
2. If you think that the information provided by the MSs is correct and enough, please, provide your opinion on the no use of all phytoplankton parameters to establish ecological status in Baltic coastal waters.
3. Do you think that due to the existing doubts on the mechanism about the link between eutrophication and phytoplankton bloom frequency and phytoplankton community composition indices, these parameters do not add more information for management than the Chl-a as BQE?
4. Additional question: Do you think that it is useful and to have data about phytoplankton (biomass, composition, abundance) on a 6 month basis?

RP reply to the questions (1-4)

1. The justifications by the Baltic GIG phytoplankton experts are not sufficient as those are only based on data and observations from one single paper (i.e. Carstensen et al. 2007), and therefore their justifications **are not sufficiently informative to allow decision on the WFD compliance criteria**.

   The argument was based on the fact that developments in the open sea areas of the Baltic Sea indicate that it is possible to have meaningful indicators for assessing phytoplankton bloom frequency and intensity, by using e.g. Algaline data and remote sensing data to develop indices like spring-bloom phytoplankton index, index of cyanobacterial surface accumulations (CSA-index, and Fraction of Cyanobacteria accumulation (FCA).

2. The little information provided by the MSs **is correct but it is not enough**.

   The argument was based on RP's comments presented under question 1.

3. Based on the scientific evidence, RP's opinion is that phytoplankton community composition indices **add more information** than Chl-a as BQE for management of coastal and transitional waters, and support assessing of the ecosystem health of the coastal water bodies.

   RP argued that

   (i) there exists clear evidence of the causal linkage (e.g. Glibert & Burkholkder 2006, Heisier et al. 2008),
   (ii) phytoplankton community composition indices provide more information than just using one single parameter (e.g. Lacouture et al. 2006, Sagert et al. 2008, Tett et al. 2008, Devling et al. 2009, Revilla et al. 2009),
   (iii) the statement in recent HELCOM (2013) report concerning challenges to apply phytoplankton indicators over wider geographical area does not, in RP's opinion, exclude using adjusted /adapted phytoplankton indicators with different reference conditions in different coastal type area,

(iv) suggestion by HELCOM CORESET II project to continue development of three candidate indicators, i.e ratio of diatoms and dinoflagellates, seasonal succession of functional phytoplankton groups and phytoplankton assemblage cluster

(v) Recent work under MARMONI. For example, diversity index (Uusitalo et al. 2013) could be tested in coastal areas provided that sufficiently long and frequent data sets are available.

(vi) Among Baltic countries, large data sets have been complied, harmonized, analysed and published to enable testing within the Baltic GIG work, e.g. by joining the HELCOM CORESET II project.

4. RP suggested three possible solutions for difficulties to elaborate a meaningful indicator on the basis of monitoring in 6 month's interval.
   (i) Phytoplankton experts should review papers concerning sampling frequencies (e.g. Carvalho et al. 2013) and… justify why this is not possible in coastal waters.
   (ii) Multimetric indices would increase reliability of assessment.
   (iii) The monitoring requirements of WFD annexes should be revised and MSs should use novel technologies.

Response from the Baltic GIG experts to RP

Question 1

It is true that bloom indicators can be calculated for many monitoring sites in the Baltic Sea, using among others the statistical approach described by Carstensen et al. (2007). The time series are already long in many places but at the same time, it must be taken into account that in the revised monitoring programs of many countries the sampling frequencies have been reduced. Bloom indicators have been developed based on Algaline data but the data cover mainly the open Baltic Sea. Remote sensing data have also been used to develop bloom indices like CSA for open sea areas. In coastal waters, the interpretations of remote sensing data are, however, uncertain due to shallowness of water bodies, the impact of resuspended particles and CDOM etc. The main problem is that the likelihood of phytoplankton blooms increases with nutrient enrichment, but the triggering mechanism is physical, which adds to variability. Additionally, operational bloom indicators may potentially be developed, but the sensitivity of these in a realistic nutrient management scenario may be too coarse.

Question 2.

See our reply above.

Question 3

We argue against the RP reply that community composition indices and multimetric indicators would add more information and reduce uncertainty. The fact is that this argument has never been demonstrated and documented. It is only a postulation. The composite indicators are only useful when there are clear synergies in the pressure-response relationships.

Although causal linkages between eutrophication and phytoplankton blooms/composition exist, they are not operational within a realistic eutrophication range. For example, the suggested indicators as core indicators of HELCOM CORESET II or developed under MARMONI project have not been documented to be operational to discern different communities in systems with smaller changes in nutrient levels. Moreover, the above mentioned indicators are likely more related to food-web aspect than eutrophication. Some of them also require high frequency data which will be scarce available in future based on traditional monitoring.

Regarding the large phytoplankton Baltic data sets, the data have been analysed in many research publications, but there is a gap between research ideas and operational tools needed for the implementation of the WFD. The GIG phytoplankton experts are ambitious by being involved in many research projects, including HELCOM CORESET, MARMONI and HELCOM PEG, and the standardisation work carried out within HELCOM PEG has ensured large taxonomical comparability of phytoplankton data.

Question 4.

RP suggested the Baltic experts to review papers on uncertainty, such as Carvalho et al (2013). This paper recommended at least 3 samples per year in at least 3 years, which is too simple. The analyses require sensitivity of the indicator as well as confidence and power levels.

As for multimetric indices, this is addressed in the reply to question 3.

All scientists agree on employing novel technologies and getting access to better and more precise data. Novel techniques offer new possibilities, but the techniques need to mature and their reliability needs to be documented before these indicators can be used in an operational WFD status assessment. The Baltic GIG phytoplankton expert group has been involved in several research projects novel techniques, e.g. pigment analyses, FlowCAMs, etc., but the overall applicability of these methods needs to be demonstrated across many different systems. For an operational WFD implementation, it is important that well-proven and sensitive phytoplankton indicators are used only.