OVERVIEW OF COASTAL PHYTOPLANKTON INDICATORS AND THEIR POTENTIAL USE IN SWEDISH WATERS

Helena Höglander, Bengt Karlson, Marie Johansen, Jakob Walve, and Agneta Andersson

WATERS Report no. 2013:5
Overview of coastal phytoplankton indicators and their potential use in Swedish waters

Helena Höglander, Department of Systems Ecology, Stockholm University
Bengt Karlson, SMHI, Oceanographic Unit
Marie Johansen, SMHI, Oceanographic Unit
Jakob Walve, Department of Systems Ecology, Stockholm University
Agneta Andersson, Department of Ecology and Environmental Science, Umeå University

WATERS partners:
Overview of coastal phytoplankton indicators and their potential use in Swedish waters

Cover photos: Mesodinium rubrum, Ceratium tripos, Nodularia spumigena, Cyclotella choctawhatcheeana, Teleaulax sp., and Dinophysis norvegica (by Helena Högländer)

Publisher: Havsmiljöinstitutet/Swedish Institute for the Marine Environment,
P.O. Box 260, SE-405 30 Göteborg, Sweden
Published: August 2013
ISBN 978-91-980646-6-7

Please cite document as:

http://www.waters.gu.se/rapporter
WATERS is a five-year research programme that started in spring 2011. The programme’s objective is to develop and improve the assessment criteria used to classify the status of Swedish coastal and inland waters in accordance with the European Commission (EC) Water Framework Directive (WFD). WATERS research focuses on the biological quality elements used in WFD water quality assessments: i.e., phytoplankton, macrophytes, benthic invertebrates, and fish; in streams, benthic diatoms are also considered. The research programme will also refine the criteria used for integrated assessments of ecological water status.

This report is a deliverable of one of the scientific sub-projects of WATERS focusing on phytoplankton indicators for coastal and transitional waters. The report presents a state-of-the-science review of phytoplankton indicators used in Europe. The results will provide a basis for continued testing and evaluation of phytoplankton indicators in the WATERS programme, including field studies conducted jointly with other sub-projects.

WATERS is funded by the Swedish Environmental Protection Agency and coordinated by the Swedish Institute for the Marine Environment. WATERS stands for Waterbody Assessment Tools for Ecological Reference conditions and status in Sweden. Programme details can be found at: http://www.waters.gu.se.
Table of contents

Summary .................................................................................................................................9
Svensk sammanfattning ........................................................................................................11
List of abbreviations ............................................................................................................13

1 Introduction ........................................................................................................................14
  1.1 Factors influencing phytoplankton biomass and composition in Swedish coastal waters .................................................................................................................................14
  1.2 Seasonal succession of phytoplankton in Swedish coastal waters .........................15
  1.3 Phytoplankton as indicators of environmental change ............................................16
  1.4 Phytoplankton and anthropogenic pressures ..............................................................16
  1.5 Phytoplankton and the Water Framework Directive ...................................................19
  1.6 Other relevant directives, conventions, and environmental objectives ..................23
  1.7 Aim and objective of the report .................................................................................24

2 Current Swedish assessment system for phytoplankton ..............................................25
  2.1 Current Swedish assessment system in coastal and transitional waters ............25
  2.2 Current Swedish assessment system in lakes ............................................................27
  2.3 Comments on current Swedish assessment of coastal phytoplankton: total biomass ........................................................................................................................................28
  2.4 Comments on missing parameters in Swedish assessment of coastal phytoplankton .................................................................................................................................30

3 Review of phytoplankton as indicators of ecological status in Europe .......................32
  3.1 Existing European indicators for the WFD in coastal and transitional waters.....32
  3.2 Potential phytoplankton indicators developed in international contexts ..............36
  3.3 Other potential phytoplankton indicators .................................................................42

4 Monitoring of phytoplankton parameters .....................................................................48
  4.1 Traditional water sampling at fixed stations ...............................................................48
  4.2 Automated systems for water sampling and estimates of phytoplankton biomass .................................................................................................................................53
  4.3 Satellite remote sensing for observing algal blooms .................................................57

5 Potential coastal phytoplankton indicators for Sweden ...............................................59
  5.1 What characterizes a good indicator? .......................................................................59
  5.2 Factors limiting choice of indicators .........................................................................59
  5.3 Potential phytoplankton indicators for Swedish coastal waters ............................60
  5.4 Assessment period considerations .............................................................................64

6 Conclusions: High-priority phytoplankton indicators for Swedish coastal waters ......66

References ............................................................................................................................69

Annex ................................................................................................................................79

Appendix 1 .........................................................................................................................79
Appendix 2 .............................................................................................................................81
Appendix 3 .............................................................................................................................83
Appendix 4 .............................................................................................................................84
Appendix 5 .............................................................................................................................85
Summary

Phytoplankton are one of the Biological Quality Elements (BQEs) used in the EU Water Framework directive (WFD) to assess the ecological status of coastal and transitional waters. To be fully compliant with the WFD, the parameters biomass, taxonomic composition, abundance (or cover), frequency, and intensity of algal blooms should be included in the assessment system. Today only biomass, measured as chlorophyll $a$ and biovolume of autotrophic and mixotrophic species, is used in the Swedish assessment criteria for coastal phytoplankton. Evaluating the existing indicators and developing indicators for the missing parameters are the main objectives of the phytoplankton project being conducted as part of the WATERS research programme.

This report provides an overview of phytoplankton indicators used by other European countries to implement the WFD as well as indicators tested in other contexts. The overview, together with a set of criteria, provides suggested potential indicators for Swedish coastal areas. Three criteria have been crucial for the choice of indicators. First, the indicators should respond to anthropogenic pressures, particularly eutrophication, and be ecologically relevant. Second, since the Swedish coast is very long and the salinity of the coastal areas varies from almost fresh water in the north to almost fully marine in the Skagerrak area, the species composition of the phytoplankton community will change accordingly. Phytoplankton indicators therefore need area-specific considerations. Third, the choice of indicators is also constrained by data availability, both existing and future data that can reasonably be expected to be delivered by monitoring programmes.

We find that the following indicators especially merit evaluation in the WATERS programme. These selected indicators will be evaluated based on analysis of existing data and of data from gradient studies conducted in the WATERS project:

**Total biomass**

- Test the use of the 90th percentile of chlorophyll $a$ measurements for the March–October period (Kattegat and Skagerrak), used by other countries around the North-East Atlantic.
- Evaluate the use of carbon content compared with biovolume (all areas, summer).
Taxonomic composition

- Ratio of nitrogen-fixing cyanobacteria \((Nodularia\ spumigena, Aphanizomenon\ sp.,\) and \(Dolichospermum\ spp.\)) to total biomass (%) (Bothnian Bay, Bothnian Sea, and the Baltic Proper) (summer).
- Ratio of the diatom genera \(Dactyliosolen\) and \(Cerataulina\) to total biomass (%) (Kattegat and Skagerrak) (summer).
- Ratio of potential eutrophication indicator species/groups (e.g., filamentous cyanobacteria and green algae) or of potential oligotrophication indicators (e.g., mixotrophic chrysophyceans and prymnesiophyceans) to total biomass (Gulf of Bothnia and Baltic Proper).
- Biomass of key indicator species/groups: for example, \(Nodularia\ spumigena,\ Aphanizomenon\ sp.,\) and Prymnesiales (Bothnian Bay, Bothnian Sea, and the Baltic Proper) and \(Pseudochattonella\ farcimen\) (spring) and \(Dinophysis\ spp.\) (summer) in the Kattegat and Skagerrak. Screening for the eutrophication response of other species/groups will hopefully reveal other potential indicator species/groups: preferably dominant species, toxic species, and species/groups that respond clearly to a stressor such as eutrophication.

Stations conducting high-frequency sampling in the national monitoring programme are representatively situated in the sea areas around Sweden (i.e., Gulf of Bothnia, Northern Baltic Proper, Kattegat, and Skagerrak) and data from these stations can be used to detect changes in the phytoplankton community that might not be captured by sampling only once per month or only in summer. For high-frequency stations, we suggest testing the following additional indicators:

**Taxonomic composition**

- Seasonal succession of dominant groups (based on biovolume): Dinoflagellates, diatoms, cyanobacteria, and \(Mesodinium\ rubrum\) for the Baltic Sea and diatoms, dinoflagellates and other dominant groups (e.g., Dictyochophytes and Prymnesiophyceans) for the Kattegat and Skagerrak.

**Frequency of blooms**

- Frequency of elevated biovolume, carbon, and chlorophyll \(a\) based on data for the whole year.
Svensk sammanfattning

Växtplankton är ett av flera biologiska kvalitetsfaktorer som används inom EU:s ramdirektiv för vatten (WFD) för att beskryva den ekologiska statusen för ett kustvattenområde. Enligt vattendirektivet ska alla parametrarna biomassa, taxonomisk sammansättning, abundans, frekvens och intensiteten hos algblomningar ingå i bedömningsgrunderna för växtplankton. Idag ingår endast biomassa, mätt som klorofyll a och biovolym av autotrofa och mixotrofa arter, i de svenska bedömningsgrunderna för växtplankton i kustvatten. Utvärdering av de befintliga indikatorerna och utveckling av nya indikatorer för de parametrar där detta saknas är huvuduppgiften inom det växtplanktonprojekt som är del av forskningsprogrammet WATERS och där denna rapport utgör en delrapport.

I den här rapporten sammanfattas de indikatorer som andra europeiska länder använder för att implementera vattendirektivet samt indikatorer som har testats i andra sammanhang. Baserat på dessa indikatorer samt några urvalskriterier ges ett förslag på möjliga växtplanktonindikatorer för svenska kustvatten. Tre kriterier har varit extra viktiga vid valet av indikatorer. För det första ska indikatorer reagera på antropogena påverkansfaktorer, där eutrofiering är den viktigaste, samt vara ekologiskt relevanta. För det andra är Sveriges kust är mycket lång och salthalten varierar från nära sötvatten i norr till full marin salthalt i Skagerrak, vilket gör att även artsammansättningen varierar. De växtplanktonindikatorer som används måste därför anpassas till specifika områden. För det tredje begränsas valet av indikatorer av datatillgängligheten, både av befintliga data och möjliga framtida data som kan tänkas levereras från olika miljöövervakningsprogram.

Vi anser att följande indikatorer är särskilt intressanta för utvärdering inom WATERS-projektet. Dessa indikatorer kommer att utvärderas baserat på existerande data samt data från WATERS-projektets gradientstudier.

Total biomass

- Testa 90:e percentilen för klorofyll a värden för perioden mars-oktober (för Kattegatt och Skagerrak), en indikator som redan används av andra länder runt nordöstra Atlanten.
- Utvärdera användandet av kolinnehåll jämfört med biovolym (för alla områden; sommar).
Taxonomisk sammansättning

- Proportionen av kvävefixerande cyanobakterier (Nodularia spumigena, Aphanizomenon sp., och Dolichospermum spp.) av totala biomassan (%) (Bottniska viken och Egentliga Östersjön) (sommar).
- Proportionen av kiselalgsläktena Dactyliosolen och Ceratulina av totala biomassan (%) (Kattegatt och Skagerrak) (sommar).
- Proportionen av potentiella eutrofieringsindikator-arter/grupper (t.ex. filamentoса cyanobakterier, grönalger) eller proportionen av potentiella oligotrofieringsindikatorer (t.ex. mixotrofa chrysofycéer och prymnesiofycéer) av totala biomassan (Bottniska viken).

Stationer med hög provtagningsfrekvens inom det nationella miljöövervakningsprogrammet finns representativt belägna i havsområdena runt Sveriges kust (Bottniska viken, norra egentliga Östersjön, Kattegatt och Skagerrak) och data från dessa stationer kan användas för att påvisa förändringar i växtplanktonsamhället som kanske inte kan upptäckas vid endast månadssvis provtagning eller då prover endast tas på sommaren. För dessa högfrekventa stationer föreslår vi att följande ytterligare indikatorer utvärderas:

 Taxonomisk sammansättning

- Säsongssuccession av dominerande grupper (baserat på biovolym): dinoflagellater, kiselalger, cyanobakterier och Mesodinium rubrum för Bottniska viken och Egentliga Östersjön samt kiselalger, dinoflagellater, och andra dominerande grupper (t.ex. prymnesiofycéer och dictyochophyta) för Kattegatt och Skagerrak.

Algblomningsfrekvens

- Frekvens av förhöjd biomassa (biovolym), kol och klorofyll a, baserat på data från hela året.
List of abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>BQE</td>
<td>Biological Quality Element</td>
</tr>
<tr>
<td>EQR</td>
<td>Ecological Quality Ratio</td>
</tr>
<tr>
<td>SEPA</td>
<td>Swedish Environmental Protection Agency</td>
</tr>
<tr>
<td>SwAM</td>
<td>Swedish Agency of Marine and Water Management</td>
</tr>
<tr>
<td>CAB</td>
<td>County Administrative Board</td>
</tr>
<tr>
<td>HELCOM</td>
<td>The Helsinki Commission</td>
</tr>
<tr>
<td>OSPAR</td>
<td>The Oslo Paris Commission</td>
</tr>
<tr>
<td>ICES</td>
<td>International Council for the Exploration of the Sea</td>
</tr>
<tr>
<td>WFD</td>
<td>Water Framework Directive</td>
</tr>
</tbody>
</table>
1 Introduction

1.1 Factors influencing phytoplankton biomass and composition in Swedish coastal waters

Phytoplankton are greatly influenced by various environmental factors structuring the water column they live in, and can be used as indicators of environmental change. Light, temperature, and nutrients are factors driving temporal and spatial changes in the phytoplankton community, while salinity has a crucial spatial influence in Swedish marine waters.

Light is a crucial factor affecting the photosynthesis and growth of phytoplankton. Light intensities that are too low or too high, for example, at depth or near the surface, respectively, can limit growth. Freshwater input from rivers can transport both coloured dissolved organic material (CDOM) and suspended particulate material (SPM) to the coast, reducing both water transparency and phytoplankton growth (Andersson in prep.). In addition, resuspension of sediments from the sea floor may reduce available light. However, species can adapt to different light climates by increasing the chlorophyll $a$ concentration in their cells or using other pigments (e.g., Andersson et al. 1989).

Although individual species have typical temperature preferences (Wasmund 1994), the indirect effects of temperature are greater than the direct physiological impact. Temperature influences water stratification, and some phytoplankton groups prefer a stable stratification while others prefer a mixed water column. The stratification affects both the light and nutrient availability for the phytoplankton. The fast-growing and silicified diatoms thrive in strong water mixing conditions (Margalef 1978), while the motile dinoflagellates can be found in more stratified water columns (Margalef 1978).

Salinity is a crucial factor affecting phytoplankton in the Baltic Sea area with its strong salinity gradients ranging from almost freshwater in the north and close to river mouths to almost fully marine environments in the Skagerrak area. The regional species composition changes strongly with the salinity. The diversity seems to be lowest in the intermediate brackish water of approximately 5–8 psu (Hällfors 2004). Few marine and limnetic species survive at this salinity and there are few genuine brackish-water species (e.g., Wasmund and Siegel 2008). However, several cyanobacteria species are adapted to the intermediate salinity of the Baltic Proper, and have rarely been observed in the Kattegat (e.g., Hällfors 2004) and the northern Gulf of Bothnia (Jaanus et al. 2011). Salinity can also vary
temporally, especially the surface salinity in the Kattegat and Skagerrak and in coastal areas affected by variable freshwater runoff. When using phytoplankton composition as a water quality indicator, salinity gradients must be taken into consideration.

Nutrient availability is one of the most important factors affecting phytoplankton growth. Nutrients can be both natural and anthropogenic in origin. In the Kattegat, nitrogen is usually the most limiting nutrient, but co-limitation with phosphorus may occur (HELCOM 2002). In the Baltic Proper (Wasmund et al. 2001) and the offshore Bothnian Sea (Andersson et al. 1996), nitrogen is the main limiting nutrient, but in coastal areas with high nitrogen loads, phosphorus limits phytoplankton growth (Wasmund et al. 2001). In the Bothnian Bay, phosphorus limits phytoplankton growth (Andersson et al. 1996).

Water depth can also have a structuring effect on the phytoplankton community. Sediments in shallow areas function as seed banks for the cysts and resting spores of various species (Godhe and McQuoid 2003, McQuoid 2002), and nutrients released from these sediments contribute to the mentioned nutrient-related effects on the community.

Filter feeders in or on the sea floor (Trottet et al. 2008) or zooplankton may affect phytoplankton biomass by grazing. Grazers may also change the community structure; for example, mussels graze more on larger than smaller phytoplankton (Trottet et al. 2008).

1.2 Seasonal succession of phytoplankton in Swedish coastal waters

During the yearly growth period from spring to autumn, phytoplankton biomass and species composition develop in response to changing environmental conditions. This seasonal succession of phytoplankton can be divided into four major seasons: spring, summer, autumn, and winter.

Spring usually has the highest biomass of the year. Primary production increases quickly as the light conditions improve. Various diatoms and dinoflagellates dominate the Baltic Sea in spring (Edler 1979). In the Kattegat and Skagerrak, diatoms dominate but small flagellates, belonging to the class Dichtyochophyceae, occur together or directly after the spring diatom bloom. The spring bloom period ends when the water column becomes stratified and either nitrogen or phosphorus is depleted. Due to the low zooplankton biomass in spring, much of the algal biomass settles to the seafloor, resulting in important food input for the benthic community.

In the Baltic Sea, summer is usually dominated by various small cyanobacteria species that can efficiently take up nutrients or that can move in the stratified water column and by species that are mixotrophic (i.e., that can shift between being an autotrophic plant and a heterotroph feeding on other organisms). In the Kattegat and Skagerrak, diatoms, dinoflagellates, and small flagellates are the most important groups in summer.

In autumn, when the stratification of the water is broken down and nutrients from the bottom waters are mixed into the water column, various species and groups can dominate. Winter is usually a period of low production.
1.3 Phytoplankton as indicators of environmental change

Phytoplankton are good indicators of environmental change due to their quick response to changes in environmental pressures such as nutrient availability. Changes in the phytoplankton community and biomass greatly affect the rest of the pelagic system as well as the benthic community. The biomass of phytoplankton affects the light climate for benthic macrophytes (Sand-Jensen and Borum 1991) as well as the nutrient availability (Sand-Jensen and Borum 1991) and oxygen conditions for benthic macrophytes through their sedimentation (e.g., Holmer and Bondgaard 2001). High phytoplankton production can lead to high sedimentation rates, resulting in plenty of food for benthic communities (Cederwall and Elmgren 1990). Sedimentation of phytoplankton and subsequent degradation by bacteria also lead to increased oxygen consumption and the risk of oxygen depletion for the benthos (Cederwall and Elmgren 1990). Phytoplankton can also affect water quality, by giving water a bad odour when found in high abundances (Zigone and Oksfeldt Enevoldsen 2000) or by producing toxins that can be released into the water when the phytoplankton degrade or be accumulated in other organisms feeding on the phytoplankton (e.g., mussels) (Zigone and Oksfeldt Enevoldsen 2000). Some phytoplankton species cause damage to fish gills, resulting in the mortality of wild fish and, for example, salmonids in fish farms (Albright et al. 1993).

1.4 Phytoplankton and anthropogenic pressures

1.4.1 Eutrophication

Eutrophication, together with its consequences, is one of the main problems facing aquatic ecosystems. It is also the main pressure studied in the current WATERS project. Coastal areas and semi-enclosed basins such as the Baltic Sea are especially affected by anthropogenic inputs of nutrients (Nixon 1995). Since the 1950s, an increase of nutrients in the surface layers (Nausch et al. 2008) has been observed not only in coastal areas of the Baltic Sea, which are directly influenced by terrestrial inputs, but also in the Central Baltic Sea (Nausch et al. 2008). The coastal phytoplankton community is therefore affected not only by increased coastal loads of nutrients but also by the elevated nutrient concentrations in the open sea.

Increased nutrient availability through eutrophication may have the following effects on the phytoplankton community:
• increased production
• increased biomass
• changes in species composition
• increased bloom frequency
• high abundances that reduce transparency and light availability
• increased sedimentation of cells or detritus

A complicating factor when studying the response of the phytoplankton to eutrophication gradients is that both nutrient availability and salinity can vary along the same gradient (e.g., Gasiumaitė et al. 2005).

1.4.2 Acidification

Carbon dioxide (CO\(_2\)) is the main source material for phytoplankton photosynthesis. In water it exists as dissolved CO\(_3\), as the ions HCO\(_3^-\) and CO\(_3^{2-}\), and as carbonic acid (Gattuso and Hansson 2011). When atmospheric CO\(_2\) dissolves in water, carbonic acid is formed, which dissociates into hydrogen (H\(^+\)) and bicarbonate (HCO\(_3^-\)) ions (Gattuso and Hansson 2011), lowering the pH in the water. Due to the pH-dependent equilibrium between the different forms of carbon, these hydrogen ions will combine with carbonate ions (CO\(_3^{2-}\)) to form bicarbonate (HCO\(_3^-\)) while lowering the CO\(_3^{2-}\) concentration (Gattuso and Hansson 2011).

Until recently, acidification has not been recognized as a problem in marine waters due to their high buffering capacity. However, the rise in the anthropogenic CO\(_2\) emissions to the atmosphere over the past two centuries (IPCC 2007) has led to greater CO\(_2\) uptake in the oceans. As much as one third of anthropogenic CO\(_2\) emissions has been found to be absorbed by the oceans (Sabine et al. 2004). This so-called seawater acidification will enhance CO\(_2\) availability and may increase primary production (Wasmund and Siegel 2008), but will at the same time reduce CO\(_3^{2-}\) concentration, which is disadvantageous for calcareous organisms. Coccolithophores (e.g., *Emiliania huxleyi*), which can form early summer blooms in the Kattegat and Skagerrak, have plates of calcium carbonate and are considered susceptible to this acidification (e.g., Riebesell 2004), although the responses are still not clear (see, e.g., Smith et al. 2012). Some cyanobacteria (e.g., *Nodularia spumigena*, Czerny et al. 2009) also lose competitive advantage in more acid water (preferring higher pH), and other phytoplankton groups may also benefit if the pH decreases (Wasmund and Siegel 2008). Ocean pH has already decreased by approximately 0.1 units, from 8.2 to 8.1, over the last century (Gattuso and Hansson 2011), and if CO\(_2\) emissions do not decrease, the pH might continue to drop an additional 0.3–0.4 units before the end of this century (IPCC 2007). Ocean acidification will clearly be an increasing problem in the future.

1.4.3 Non-indigenous species

Non-indigenous, or alien or non-native, phytoplankton species are species introduced from outside their natural range and dispersal potential by humans, for example, through the exchange of ballast water. Species of unclear origin are classified as cryptogenic. If
non-indigenous species increase in abundance and biomass and spread over large areas, they can affect phytoplankton biodiversity, ecosystem functioning, and socio-economic values (DAISIE 2009). The European Alien Species Database (DAISIE 2009) identifies approximately 50 phytoplankton species as non-indigenous to European coastal waters. Of the twelve non-indigenous or cryptogenic phytoplankton species recorded in the Baltic Sea (Olenina et al. 2010), only one (the dinoflagellate Pterocentrum minimum) has been categorized as an invasive alien species having recognizable environmental effects (Olenina et al. 2010).

Pseudochattonella farcimen, a flagellate belonging to the algal class Dictyochophyceae, may have been introduced into Scandinavian waters. It was first observed in bloom abundances in 1998 in the eastern North Sea and the Skagerrak (Karlson and Anderson 2003 and references therein). In the Belt Sea, Kattegat, and Skagerrak, blooms of Pseudochattonella farcimen are a problem since it is a fish-killing species. It occurs together with the spring diatom bloom or immediately after it. In addition, a diatom species that may cause damage to fish may have been introduced into the area (e.g., ICES 2012); the species has not been described (Skjevik and Edler 2011) but it is similar to Chaetoceros concavicornis.

1.4.4 Morphological alterations and human built structures

The WFD considers not only the effects of eutrophication but also other alterations of the seas and other water bodies. One example is morphological alterations, for example, changes of the sill depth of a fjord or the construction of harbours. Sometimes these exploited areas are defined as “heavily modified water bodies”, for which special environmental goals, i.e., good ecological potential, should be achieved. The authors are unaware of any documented effects of such activities on phytoplankton in Sweden. Harbour construction along the Mediterranean has resulted in enclosed water bodies with small water exchange. Such confined waters favour dinoflagellates, for example, playing a key role as reservoirs accumulating cysts and vegetative cells and aiding the expansion of these dinoflagellates in the region (Bravo et al. 2008).

Blooms of dinoflagellates belonging to the genus Alexandrium are documented in newly constructed harbours in the Mediterranean (Vila et al. 2005). Alexandrium spp. produce paralytic shellfish toxins (PST) and occur also in the Baltic Sea and Kattegat–Skagerrak. Alexandrium spp. have caused elevated PST levels in blue mussels along the Swedish Skagerrak coast (Persson and Karlson 2009). In the Åland archipelago, Alexandrium ostenfeldii has caused bioluminescence and contains PST (Hakanen et al. 2012, Kremp et al. 2009). Effects on co-occurring biota are likely. Effects on zooplankton have been documented (Sopanen et al. 2011).
1.5 Phytoplankton and the Water Framework Directive

According to the Waters Framework Directive (WFD; 2000/60/EC), the ecological status of all surface water bodies should be assessed. Marine surface water is defined as all coastal and transitional water within one nautical mile outside the baseline. Transitional water areas are all bodies of surface water in the vicinity of river mouths that are partly saline in character due to their proximity to coastal waters but that are substantially influenced by freshwater flows.

The Swedish coast is divided into 25 water body types (Table 1.1 and Figure 1.1) of which salinity, stratification, exposure, and ice cover are the leading structuring parameters (NFS 2006:1); 23 of these are coastal areas and two are transitional water body types.

**TABLE 1.1**
Overview of Swedish coastal and transitional (*) water body types, according to NFS 2006:1 (2006).

<table>
<thead>
<tr>
<th>Type no.</th>
<th>Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Inner coastal waters of the west coast</td>
</tr>
<tr>
<td>2</td>
<td>West coast fjords</td>
</tr>
<tr>
<td>3</td>
<td>The Skagerrak, outer coastal waters of the west coast</td>
</tr>
<tr>
<td>4</td>
<td>The Kattegat, outer coastal waters of the west coast</td>
</tr>
<tr>
<td>5</td>
<td>Coastal waters of southern Halland and northern Öresund</td>
</tr>
<tr>
<td>6</td>
<td>Öresund coastal waters</td>
</tr>
<tr>
<td>7</td>
<td>Skåne coastal waters</td>
</tr>
<tr>
<td>8</td>
<td>Blekinge archipelago and the inner coastal waters of Kalmarsund</td>
</tr>
<tr>
<td>9</td>
<td>Blekinge archipelago and the outer coastal waters of Kalmarsund</td>
</tr>
<tr>
<td>10</td>
<td>Coastal waters of eastern Öland, south-eastern Gotland, and Gotska sandön</td>
</tr>
<tr>
<td>11</td>
<td>Coastal waters of western and northern Gotland</td>
</tr>
<tr>
<td>12</td>
<td>Central coastal waters of Östergötland and Stockholm archipelago</td>
</tr>
<tr>
<td>13</td>
<td>Östergötland, inner archipelago</td>
</tr>
<tr>
<td>14</td>
<td>Östergötland, outer coastal waters</td>
</tr>
<tr>
<td>15</td>
<td>Stockholm archipelago, outer coastal waters</td>
</tr>
<tr>
<td>16</td>
<td>South Bothnian Sea, inner coastal waters</td>
</tr>
<tr>
<td>17</td>
<td>South Bothnian Sea, outer coastal waters</td>
</tr>
<tr>
<td>18</td>
<td>North Bothnian Sea, inner coastal waters of Höga kusten</td>
</tr>
<tr>
<td>19</td>
<td>North Bothnian Sea, outer coastal waters of Höga kusten</td>
</tr>
<tr>
<td>20</td>
<td>Inner coastal waters of North Quark</td>
</tr>
<tr>
<td>21</td>
<td>Outer coastal waters of North Quark</td>
</tr>
<tr>
<td>22</td>
<td>Bothnian Bay, inner coastal waters</td>
</tr>
<tr>
<td>23</td>
<td>Bothnian Bay, outer coastal waters</td>
</tr>
<tr>
<td>24 (*)</td>
<td>Stockholm inner archipelago and Hallsfjärden</td>
</tr>
<tr>
<td>25 (*)</td>
<td>Estuaries of the Göta Älv and Nordre Älv rivers</td>
</tr>
</tbody>
</table>
According to the WFD, phytoplankton status should be classified based on the following parameters:

- biomass
- taxonomic composition
- abundance
- frequency and intensity of algal blooms
The assessment methods used in the WFD should use five status classes (i.e., high, good, moderate, poor, and bad) with boundaries set as defined in Annex V of the WFD. For these definitions for phytoplankton, see Table 1.2.

The EU WFD requires that at least good ecological status be achieved in coastal and transitional waters. According to the WFD definition, good ecological status for phytoplankton implies that the composition and abundance of phytoplankton taxa display only slight signs of disturbance. Furthermore, there should be only slight changes in biomass compared with type-specific conditions and such changes should not indicate any accelerated growth of algae resulting in undesirable disturbance of the balance of organisms present in the water body or of the water quality. Only a slight increase in the frequency and intensity of the type-specific planktonic blooms is congruent with good status.

Reference values and class boundaries for the existing phytoplankton parameters biovolume and chlorophyll $a$ were proposed by Larsson et al. (2006). The current Swedish assessment methods for phytoplankton, i.e., chlorophyll $a$ and biovolume, were adopted in 2007 (Naturvårdsverket 2007). Together with other biological quality elements (i.e., macrophytes and benthic invertebrates), they have been used by the Swedish county administrative boards (CABs) to classify the water quality status in Swedish coastal and transitional areas (VISS 2012).
TABLE 1.2
Definitions of high, good, and moderate ecological status in coastal and transitional waters according to phytoplankton (Annex V, WFD 2000).

<table>
<thead>
<tr>
<th>Status</th>
<th>Coastal waters</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>High</strong></td>
<td>The composition and abundance of phytoplanktonic taxa are consistent with undisturbed conditions. The average phytoplankton biomass is consistent with the type-specific physico-chemical conditions and is not such as to significantly alter the type-specific transparency conditions. Planktonic blooms occur at a frequency and intensity which is consistent with the type-specific physico-chemical conditions.</td>
</tr>
<tr>
<td><strong>Good</strong></td>
<td>The composition and abundance of phytoplanktonic taxa show slight signs of disturbance. There are slight changes in biomass compared to type-specific conditions. Such changes do not indicate any accelerated growth of algae resulting in undesirable disturbance to the balance of organisms present in the water body or to the quality of the water. A slight increase in the frequency and intensity of the type-specific planktonic blooms may occur.</td>
</tr>
<tr>
<td><strong>Moderate</strong></td>
<td>The composition and abundance of planktonic taxa show signs of moderate disturbance. Algal biomass is substantially outside the range associated with type-specific conditions, and is such as to impact upon other biological quality elements. A moderate increase in the frequency and intensity of planktonic blooms may occur. Persistent blooms may occur during summer months.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Status</th>
<th>Transitional waters</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>High</strong></td>
<td>The composition and abundance of phytoplanktonic taxa are consistent with undisturbed conditions. The average phytoplankton biomass is consistent with the type-specific physico-chemical conditions and is not such as to significantly alter the type-specific transparency conditions. Planktonic blooms occur at a frequency and intensity which is consistent with the type-specific physico-chemical conditions.</td>
</tr>
<tr>
<td><strong>Good</strong></td>
<td>There are slight changes in the composition and abundance of phytoplankton taxa. There are slight changes in biomass compared to the type-specific conditions. Such changes do not indicate any accelerated growth of algae resulting in undesirable disturbance to the balance of organisms present in the water body or to the physico-chemical quality of the water. A slight increase in the frequency and intensity of the type-specific planktonic blooms may occur.</td>
</tr>
<tr>
<td><strong>Moderate</strong></td>
<td>The composition and abundance of the phytoplanktonic taxa differ moderately from type-specific conditions. Biomass is moderately disturbed and may be such as to produce a significant undesirable disturbance in the condition of other biological quality elements. A moderate increase in the frequency and intensity of planktonic blooms may occur. Persistent blooms may occur during summer months.</td>
</tr>
</tbody>
</table>
1.6 Other relevant directives, conventions, and environmental objectives

Along with the WFD, other directives and national objectives also use phytoplankton as a water-quality indicator.

The Marine Strategy Framework Directive (MSFD; 2008/56/EC) was adopted in 2008. According to the MSFD, Member States should achieve or maintain good environmental status (GES) in the marine environment by 2020. According to Article 3.5 of the MSFD, GES is defined as: “The environmental status of marine waters where these provide ecologically diverse and dynamic oceans and seas which are clean, healthy and productive within their intrinsic conditions, and the use of the marine environment is at a sustainable level, thus safeguarding the potential for uses and activities by current and future generations”. GES is based on 11 descriptors and should be further defined according to criteria outlined in an EC decision document (2010/477/EU). Phytoplankton are one of the organisms groups that should be considered when defining and assessing GES and is relevant to at least four descriptors (from 2010/477/EU):

- Descriptor 1: Biological diversity is maintained. The quality and occurrence of habitats and the distribution and abundance of species are in line with prevailing physiographic, geographic, and climate conditions.
- Descriptor 2: Non-indigenous species introduced by human activities are at levels that do not adversely alter the ecosystems.
- Descriptor 4: All elements of the marine food webs, to the extent that they are known, occur at normal abundance and diversity and levels capable of ensuring the long-term abundance of the species and the retention of their full reproductive capacity.
- Descriptor 5: Human-induced eutrophication is minimized, especially adverse effects thereof, such as losses in biodiversity, ecosystem degradation, harmful algal blooms, and oxygen deficiency in bottom waters.

In July 2012, Sweden adopted a regulation that defines GES in Swedish marine waters and lists a set of indicators to be used when assessing the status of the marine environment (HVMFS 2012:18). Since the coverage of the WFD and the MSFD overlap one nautical mile in coastal areas, Sweden has adopted the WFD indicators in the coastal area. In offshore waters, chlorophyll is the only phytoplankton-related indicator adopted so far.

The HELCOM Baltic Sea Action Plan (BSAP) (HELCOM 2007) was adopted by the countries around the Baltic Sea in 2007. BSAP stresses HELCOM's vision for a good environmental status in the Baltic Sea. One of the objectives is that algal blooms should be kept at natural levels. The project HELCOM CORESET has worked with core indicators to enable indicator-based follow-up of the implementation of the HELCOM Baltic Sea Action Plan (BSAP) and also to facilitate the implementation of the EU MSFD by those HELCOM Contracting Parties that are also members of the EU. The project has considered phytoplankton indicators related to taxonomic composition and algal blooms.
but as of November 2012 the only phytoplankton-related indicator regularly used in HELCOM assessments is chlorophyll a.

The Convention for the Protection of the Marine Environment of the North-East Atlantic (henceforth, OSPAR) entered into force on 25 March 1998. The objective of its Strategy for the Protection of the Marine Environment of the North-East Atlantic 2010–2020 (OSPAR Agreement 2010-3) with regard to eutrophication is to combat eutrophication in the OSPAR maritime area, with the ultimate aim of achieving and maintaining a healthy marine environment where anthropogenic eutrophication does not occur. This includes minimizing biodiversity losses, harmful algal blooms, and oxygen deficiency in bottom waters. To measure progress, Ecological Quality Objectives (EcoQO) for eutrophication have been developed that concern phytoplankton (OSPAR 2005). So called ‘OSPAR common indicators’, some including phytoplankton parameters, have additionally been developed to follow up the MSFD.

The Swedish national environmental objectives (www.miljomal.nu) that relate to phytoplankton are mainly no. 7 “Zero eutrophication” and no. 11 “A balanced marine environment, flourishing coastal areas and archipelagos”. Today none of the existing environmental indicators coupled to the targets of these objectives involves coastal phytoplankton.

### 1.7 Aim and objective of the report

This report gives an overview of phytoplankton indicators used in various countries and areas around Sweden and Europe and suggests possible new phytoplankton indicators and revisions of existing phytoplankton indicators for Swedish coastal and transitional waters. The suggested new indicators will be further investigated and evaluated in the future work of WATERS. The primary aim is that the indicators should allow assessment of ecological status according to WFD requirements, but it is also desirable that the indicators can be implemented in the MSFD.
2 Current Swedish assessment system for phytoplankton

2.1 Current Swedish assessment system in coastal and transitional waters

Status of phytoplankton in Swedish coastal and transitional waters is currently classified based on the total biomass of autotrophic and mixotrophic phytoplankton measured as follows (Table 2.1):

- biovolume (mm$^3$ L$^{-1}$)
- chlorophyll a (µg L$^{-1}$)

When both biovolume and chlorophyll data are available, they should be combined into one standardized status classification for phytoplankton (average of both parameters). If data are missing for one of these parameters, the classification is based on the remaining parameter.

Both parameters should be sampled 3–5 times per year over the June–August period. Classification is done based on data from at least three years from the latest six-year period due to the variability between years.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Pressure</th>
<th>How often measurements need to be taken</th>
<th>Sampling period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biovolume (mm$^3$ L$^{-1}$)</td>
<td>Nutrient level:</td>
<td>3–5 times/year. Classification based on data from at least three years from the latest six-year period</td>
<td>June–August</td>
</tr>
<tr>
<td></td>
<td>eutrophication</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlorophyll a (µg L$^{-1}$)</td>
<td>Nutrient level:</td>
<td>3–5 times/year. Classification based on data from at least three years from the latest six-year period</td>
<td>June–August</td>
</tr>
<tr>
<td></td>
<td>eutrophication</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**TABLE 2.1**
Overview of the Swedish assessment system for coastal and transitional waters (Naturvårdsverket 2007, Appendix B).
Phytoplankton biovolume is based on data from integrated samples (hose sampling or composite samples taken using a water sampler at various depths) from the surface layer (0–10 m) or from discrete samples from the surface (0.5 m) if the water depth is under 12 m. Data from other depth intervals can be converted to 0–10 m using conversion factors found in Naturvårdsverket (2007), Table 4.1.

The assessment criterion for phytoplankton biovolume is based on the quantification and species identification of phytoplankton in Lugol’s-preserved samples. The analysis is conducted using an inverted light microscope in accordance with the Swedish EPA’s survey types or HELCOM’s COMBINE manual, both of which are based on the Utermöhl method. The biovolume is obtained using the size classes of Olenina et al. (2006) with the latest version of the Excel file associated to the publication. The latest update of the HELCOM-PEG list of biovolumes of phytoplankton in the Baltic Sea and Kattegat–Skagerrak is available from ICES at: http://www.ices.dk/marine-data/vocabularies/Documents/PEG_BVOL.zip.

Chlorophyll \(a\) is based on data from the same depth as the biovolume samples for the Swedish west coast (types 1–7 and 25) and Gulf of Bothnia (types 16–23). For the Baltic Proper (types 8–15 and 24), the classification should be based on data from a depth of 0.5 m. Chlorophyll data from other sampling depths need to be adjusted according to known empirical relationships to ensure that they correspond to the above-specified depths and depth intervals (Table 4.1 in Naturvårdsverket 2007).

Standard methods are used to analyse chlorophyll \(a\): the Swedish standard (SS 02 81 46) prescribes acetone as an extraction solvent, whereas HELCOM’s COMBINE manual prescribes ethanol for this purpose. In both methods, water is filtered through glass-fibre filters and extracted using the solvent before absorbance is measured in a spectrophotometer, or fluorescence in a fluorometer, calibrated to a spectrophotometer.

The boundaries for both chlorophyll and biovolume in area types 8, 12, 13, and 24 should be corrected for salinity before data classification (Naturvårdsverket 2007).\(^1\) The correction follows the principle for correction of boundaries for total nitrogen and phosphorus, which is applied to all Swedish inner coastal type areas, not just the Baltic Proper. Reference values for nutrients are assumed to follow a simple (i.e., linear) mixing model of naturally high-nutrient freshwater and lower-nutrient open seawater. The reference value for a specific nutrient measurement is calculated according to the measured salinity and the defined linear nutrient–salinity relationship. The boundaries are

---

\(^1\) Excel application for salinity correction:
adjusted according to the reference values and the fixed EQR (Ecological Quality Ratio) values.

In area types 8, 12, 13, and 24 in the Baltic Proper, the adjusted reference values for chlorophyll and biovolume are calculated from the reference values for total nitrogen and defined chlorophyll to nitrogen and biovolume to nitrogen relationships. This means that, for a certain calculated reference value for total nitrogen in a salinity gradient, there is a corresponding reference value for chlorophyll and biovolume.

The method of correcting according to salinity was implemented only for the Baltic Proper but could in principle be extended to all areas. It has the advantage that reference values are flexible for the often large area types. A disadvantage is the more complicated, less transparent procedure to classify status. It should be noted that the procedure only takes into account natural variability correlated with salinity and no other factors that may influence natural variability of the reference conditions, such as water body size and depth.

2.2 Current Swedish assessment system in lakes

The current Swedish assessment system for lake phytoplankton includes the following factors (according to Naturvårdsverket 2007, appendix A):

1. **Total biomass of phytoplankton (mg L⁻¹).** Sampling: once/year, but averaged over three years. Sampling period: July–August. Pressure: Eutrophication. Sampling depth: Integrated sample, above the thermocline.

2. **Trophic Plankton Index (TPI).** Based on indicator species ranked using a scale ranging from −3 to +3. Index numbers 1–3 indicate whether species are tolerant and abundant in the most eutrophic environments, 3 being the most eutrophic, 1 the least. Sensitive species that are abundant in oligotrophic environments are assigned negative numbers, with −3 indicating the most abundant species in oligotrophic environments. Sampling: once/year, but averaged over three years. Sampling period: July–August. Pressure: Eutrophication. Sampling depth: Integrated sample, above the thermocline.

3. **Cyanobacteria as per cent of total biomass.** All cyanobacteria species included (but not picocyanobacteria). Sampling: once/year, but averaged over three years. Sampling period: July–August. Pressure: Eutrophication. Sampling depth: Integrated sample, above the thermocline.

4. **Chlorophyll a.** Mainly used as a screening method when phytoplankton analysis data are missing. Classification based on chlorophyll is used only if other parameters are missing. If classification is moderate or worse, additional phytoplankton analysis is required. Sampling: once/year, but averaged over three years. Sampling period: July–August. Pressure: Eutrophication. Sampling depth: surface (0.5 m).

5. **Number of species.** Sampling: once/year, but averaged over three years. Sampling period: July–August. Pressure: acidity. Sampling depth: Integrated sample, above the thermocline.
Total biomass, Trophic Plankton Index (TPI), and per cent of cyanobacteria should all be used together to classify a lake by averaging all three parameters. TPI can be used only when at least four species have been classified according to the TPI index. In some lakes, the ecological status is based only on total biomass and per cent of cyanobacteria. In lakes where the species *Gonyostomum semen* is abundant, only TPI and per cent of cyanobacteria should be used. Chlorophyll *a* is used mainly as a screening method, only being used for classification if information about total biomass and cyanobacteria is missing. Changes in chlorophyll or outlying results should be followed up by an analysis of the phytoplankton composition.

### 2.3 Comments on current Swedish assessment of coastal phytoplankton: total biomass

The current use of chlorophyll *a* and biovolume as assessment criteria for phytoplankton supplies information only about the total biomass of phytoplankton. No assessment criteria based on the other parameters defined in the WFD (e.g., taxonomic composition, abundance, and frequency/intensity of algal blooms) have yet been developed.

Phytoplankton biomass can be measured as total wet weight or biovolume, since the volume-to-weight ratio is close to one for phytoplankton, or as carbon content. Chlorophyll *a* occurs in all autotrophic and mixotrophic phytoplankton organisms and its concentration is widely used as a proxy for total phytoplankton biomass. However, the relationship between chlorophyll *a* and phytoplankton biomass can vary (e.g., Andersson and Rudehäll 1993), and the relationship is often weak, so it is not an optimal measure of phytoplankton biomass (Kruskopf and Flynn 2006). Many factors influence the relationship between the two parameters. Phytoplankton can adjust their chlorophyll *a* content depending on the light climate (Andersson et al. 1989) and different species can contain different amounts of chlorophyll *a*. Moreover, inactive pigment in dead cells or detritus can influence the chlorophyll measurements. These factors mean that the biomass-to-chlorophyll *a* ratio will not be consistent. One advantage of chlorophyll *a* measurements, however, is that most of the chlorophyll-containing cells are retained on the filter used for analysis (usually a GF/F filter with a pore size of 0.7 µm), while in routine light microscopy, cells under 2 µm in size (i.e., picoplankton) are not counted. In summer, the small picoplankton (e.g., the picocyanobacteria) can constitute much of the biomass (e.g., Hajdu et al. 2007). Chlorophyll *a* measurements are also cheaper than the time-consuming phytoplankton analysis. The WFD does not mention chlorophyll, which does not provide either detailed species or group information as phytoplankton analysis can, but it has been accepted as a proxy for phytoplankton biomass.

Today different depths are used when measuring chlorophyll and biovolume in the Baltic Proper (i.e., 0 m for chlorophyll *a* and 0–10 m for biovolume). It would be of interest to evaluate how different depth intervals affect the status classification and the advantages and disadvantages of different sampling strategies. Depth differences may at least partly
explain why biovolume often indicates a better quality status than does chlorophyll a (e.g., Svealands Kustvattenvårdsförbunds Årsrapport 2012, Lücke 2010).

High concentrations of humic substances have, especially in the northern Baltic Sea, been found to indicate high chlorophyll a concentrations even if the biovolume has not increased. In these areas, correction for light climate might be needed or the class boundaries may need to be revised. This is especially important since chlorophyll a is the most common measurement used, because biovolume analysis is more expensive and fewer biovolume data are available.

Large species of the phytoplankton group diatoms have a very high biovolume. This has been observed, for example, in coastal areas of the Kattegat and Skagerrak (Skjevik et al. 2011). Much of the diatom cell volume is a vacuole that contains very little organic substance, meaning that the total biovolume data may give a skewed biomass value when large diatoms are present. In this case, measures other than biovolume (e.g., carbon content) should be evaluated. This would also make it easier to use the data in the context of carbon flow through the food chain and in biogeochemical and ecological modelling.

Although summer is also a productive period, the highest biomasses of phytoplankton are found in spring, since grazing pressure is low at this time of year. Copepods and other multicellular zooplankton grow more slowly than do phytoplankton. Variability in the biomass of phytoplankton, measured as biovolume or chlorophyll a, is very high in spring and this period would need a high sampling frequency (at least weekly) to resolve natural variability. Summer generally has smaller temporal variability, although shifts in weather may cause rapid changes in some areas, for example, via upwelling. On average, summer has less temporal variability than does spring and was chosen as the assessment period in the 2007 version of the WFD phytoplankton indicators in Sweden.

Today the assessment period is the same (i.e., June–August) for the whole Swedish coast, although the seasonal succession of the phytoplankton community differs between the Swedish west coast and the northern Baltic Sea (Bothnian Bay). After cold winters, the spring bloom can be late in the northern Baltic, which influences the phytoplankton biomass measurements made in June. Adjusting the assessment period so that spring does not affect it is therefore advisable.

Overall, more data are available now than when the assessment was first developed, and the prevailing reference values and class boundaries should be revised according to the new/additional data, and be intercalibrated with those of other countries around the Baltic Sea.
2.4 Comments on missing parameters in Swedish assessment of coastal phytoplankton

2.4.1 Abundance

Since phytoplankton cells range in size from one to several hundred µm, biomass is usually a better measure than cell abundance to describe the phytoplankton community and its composition. Abundances either overestimate or underestimate the importance of different groups in the phytoplankton community (see, e.g., Figure 2.1).

2.4.2 Taxonomic composition

Phytoplankton community structure can be described in various ways, for example, by functional groups, species dominance relationships, size groups, diversity indices, and phytoplankton pigments.

2.4.3 Frequency of algal blooms

There are many different definitions of an algal bloom. In this report, an algal bloom is defined as when the long-term mean biomass or abundance is exceeded by either the whole community or single species/groups.

Bloom frequency can be described as, for example, how often the biomass, either total or of certain phytoplankton species/groups, exceeds the area-specific long-term mean for all or part of the year (e.g., summer). Bloom intensity can be measured as a combination of bloom duration (number of days exceeding a reference value) and coverage (km² covered by blooms) (Hansson 2006), or as a combination of bloom duration and biomass at a single station.
FIGURE 2.1
The abundance (cells L$^{-1}$; above) and biovolume ($\mu$m$^3$L$^{-1}$; below) from analyses conducted at station L9 (Laholm Bay), monthly means 2005-2010. Small Cryptophyceae and Prymnesiophyceae can be abundant but, due to small biovolumes, may constitute little of the total biovolume. Large Diatomophyceae, occurring in moderate abundances in summer, may instead constitute most of the biovolume.
3 Review of phytoplankton as indicators of ecological status in Europe

Along the Swedish coasts, the salinity shifts from almost fresh water to fully marine waters; this shift influences the species composition along the salinity gradient, with many freshwater species in the northern low-saline area and close to river mouths and marine species in the Kattegat and Skagerrak area (Hällfors 2004). Phytoplankton indicators used in fresh, brackish, and marine waters can therefore all be applicable in Swedish coastal areas.

3.1 Existing European indicators for the WFD in coastal and transitional waters

To intercalibrate the phytoplankton assessment systems used in the different EU countries, special Geographical Intercalibration Groups (GIGs) have been created. For coastal and transitional areas, there are four GIGs: the Baltic GIG, North-East Atlantic (NEA) GIG, Mediterranean GIG, and Black Sea GIG.

3.1.1 Baltic Geographical Intercalibration Group

The existing assessment methods for phytoplankton in countries around the Baltic Sea (from Bothnian Bay to the Öresund area) are summarized in Table 3.1 and Appendix 1. None of the Baltic countries has developed a full BQE method, i.e., including all parameters.

Phytoplankton biomass is described as chlorophyll $a$ and, for some countries, total biovolume of autotrophic and mixotrophic species. Only Germany has a method involving taxonomic composition (i.e., biovolume of cyanophytes and chlorophytes, for the eastern part of the German Baltic coast) (Sagert et al. 2008). The sampling period and sampling depth differ between countries (Appendix 1), while the assessment period (summer) varies between May and September. There is no current assessment method for algal bloom frequency, intensity, or abundance in the Baltic Sea area.

3.1.2 North-East Atlantic (NEA) Geographical Intercalibration Group

The existing phytoplankton assessment methods for countries along the North-East Atlantic (in the NEA GIG group) are summarized in Table 3.1 and Appendix 2.
Phytoplankton biomass is approximated as chlorophyll $a$ in all countries. The 90th percentile of chlorophyll concentrations for the growing period (March–October) should remain below thresholds set for type-specific class boundaries. Chlorophyll values exhibit periodicity and episodic change, resulting in asymmetric distribution with a few high and many low values (e.g., Devlin et al. 2007). By using the 90th percentile for the chlorophyll data, as agreed on in NEA GIG, highly skewed values can be omitted. Carstensen et al. (2008) tested the precision of the two chlorophyll $a$ indicators, mean and 90th percentile, the latter being found to be more uncertain than the former. Carstensen et al. (2008) consequently recommend using the mean instead of the 90th percentile; only if the 90th percentile indicator is related more strongly to nutrient status than is the mean indicator do they recommend using it. However, the 90th percentile indicator is still used for chlorophyll in NEA GIG.

Sweden is the only country in NEA GIG that uses biovolume as a measure of phytoplankton biomass. Norway is developing carbon as a measure of biomass (e.g., Havsforskningsinstituttet 2012).

The marine flagellate Phaeocystis can form mucous colonies and can occur in very high biomasses (Lancelot et al. 1987). The dying blooms can cause oxygen depletion in the bottom waters and foam accumulation on beaches in large quantities (Lancelot et al. 1987). The frequency of elevated counts of Phaeocystis (exceeding $10^6$ cells $L^{-1}$) (Devlin et al. 2007) is used by the United Kingdom, Germany, the Netherlands, and Belgium as a measure of these blooms.

The seasonal succession of four major functional groups, including diatoms, dinoflagellates, microflagellates (excluding Phaeocystis), and Phaeocystis sp., is used by the United Kingdom (Devlin et al. 2007). A shift in functional groups may affect ecosystem function in terms of the carbon available to higher trophic levels or settling to the sediments (Devlin et al. 2007). Counts of the four groups are averaged for each month over a sampling year. Skewed data are accounted for by transforming phytoplankton counts on a natural log scale (Devlin et al. 2007). Phytoplankton counts are averaged over months, and monthly mean and standard deviations calculated for each group. Through normalization, transformation, and calculation of a monthly $z$-score, comparable seasonal distributions can be established for each functional group over one year (Devlin et al. 2007). A positive $z$-score indicates that an observation is greater than the mean and a negative score that the observation is less than the mean, while a $z$-score of zero indicates that the monthly sample is approaching the overall mean for the sampling period.

France uses the frequency of elevated counts of small and large phytoplankton, with the species community divided into the two size fractions (i.e., $>20 \mu m$ and approximately $2–20 \mu m$), as a measure of algal blooms and frequency (Carletti and Heiskanen 2009). The per cent of cell abundances above a specific threshold is used to identify a bloom, the threshold being above 100,000 cells $L^{-1}$ for large species and 250,000 cells $L^{-1}$ for small species.
Spain has also tested the use of elevated counts of size-fractionated phytoplankton divided into the 2–20 µm and >20 µm size classes (Revilla et al. 2009), with two thresholds for the two size classes (Revilla et al. 2009). However, it turned out to be overly time-consuming to divide the phytoplankton community into the two size classes (based on the cell size at analysis), so size fractionation was abandoned and instead a single threshold is used for every phytoplankton taxon (Revilla et al. 2009). In addition, France, Ireland, Portugal, and the United Kingdom use the frequency of elevated counts of any phytoplankton species as a frequency/abundance indicator. This index is defined based on (Carletti and Heiskanen 2009): a) the number of samples in which a single taxon count exceeds a predefined threshold, b) six years of year-round routine monitoring data, c) a recommended minimum of 12 sampling occasions (monthly) per year, and d) the number of sampling occasions when the single taxon counts exceed a threshold, and the classification is calculated as the percentage of the total number of samples collected in a single water body type over the six-year period.

3.1.3 Mediterranean Geographical Intercalibration Group

The existing phytoplankton assessment methods for countries in the Mediterranean Sea area are summarized in Table 3.1 and Appendix 3. The only parameter used in assessing phytoplankton in the Mediterranean is biomass, for which concentration of chlorophyll a is the only existing method. Currently, no assessment criteria exist for the other parameters. None of the other parameters is currently used, but work is in progress in some countries.

3.1.4 Black Sea Geographical Intercalibration Group

The existing phytoplankton assessment methods for countries around the Black Sea are summarized in Table 3.1 and Appendix 4. Total phytoplankton biomass is measured in Bulgaria and Romania as biovolume and as chlorophyll a, in samples collected between June and September. Values are seasonal to reflect the great seasonal variability of the phytoplankton community. Abundance is measured as total abundance (cells L⁻¹). Taxonomic composition is measured as the proportion of total abundance of dinoflagellates and the sum of abundance of species of the three taxonomic groups microflagellates, Euglenophyceae, and Cyanophyceae as a per cent of total summer abundance. Two diversity indices, the Menhinick and Sheldon indices, are also used; the Menhinick index is based on species richness and total abundance (Menhinick 1964).
### TABLE 3.1
Summary of the phytoplankton assessment methods used by European countries.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description of parameter</th>
<th>Countries 1</th>
<th>Geographical area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biomass</td>
<td>Total biomass (biovolume), mean (mg m⁻³), summer</td>
<td>DE², EE, FI², LT², LV², PL², SE</td>
<td>Baltic</td>
</tr>
<tr>
<td></td>
<td>Total biomass (biovolume), 90th percentile (mg m⁻³), summer</td>
<td>BG, RO</td>
<td>Black Sea</td>
</tr>
<tr>
<td>Chlorophyll a, mean, summer</td>
<td></td>
<td>DE, DK, EE, FI, HR³, IT³, LT, LV, PL, SE, SI³</td>
<td>Baltic</td>
</tr>
<tr>
<td>Chlorophyll a, 90th percentile, March–October</td>
<td></td>
<td>BE, BG⁴, CY⁴, DE, DK, ES, FR, IE, NL, NO, PT, RO⁴, UK</td>
<td>North-East Atlantic, Mediterranean, Black Sea</td>
</tr>
<tr>
<td><strong>Taxonomic composition</strong></td>
<td>Biovolume of all cyanophyceae, excluding picocyanobacteria, March–October</td>
<td>DE</td>
<td>Baltic</td>
</tr>
<tr>
<td></td>
<td>Biovolume of chlorophyceae, March–October</td>
<td>DE</td>
<td>Baltic</td>
</tr>
<tr>
<td></td>
<td>Seasonal succession of functional groups (i.e., diatoms, dinoflagellates, microflagellates, and Phaeocystis spp.), whole year</td>
<td>UK</td>
<td>North-East Atlantic</td>
</tr>
<tr>
<td></td>
<td>Total abundance of dinoflagellates, summer</td>
<td>BG, RO</td>
<td>Black Sea</td>
</tr>
<tr>
<td></td>
<td>Sum of abundance of three taxonomic groups (i.e., microflagellates, Euglenophyceae, and cyanophyceae) as a per cent of total abundance, summer</td>
<td>BG, RO</td>
<td>Black Sea</td>
</tr>
<tr>
<td>Abundance/ frequency, and intensity of algal blooms</td>
<td>Total abundance (cells L⁻¹)</td>
<td>BG, RO, UK</td>
<td>North-East Atlantic, Black Sea</td>
</tr>
<tr>
<td></td>
<td>Frequency of elevated counts of any phytoplankton species</td>
<td>ES, FR, IE, PT, UK</td>
<td>North-East Atlantic</td>
</tr>
<tr>
<td></td>
<td>Frequency of elevated counts of small (2–20 µm) and large (&gt;20 µm) phytoplankton species</td>
<td>FR</td>
<td>North-East Atlantic</td>
</tr>
<tr>
<td></td>
<td>Frequency of elevated counts of Phaeocystis spp.</td>
<td>BE, DE, NL, UK</td>
<td>North-East Atlantic</td>
</tr>
</tbody>
</table>

1 BE = Belgium, BG = Bulgaria, CY = Cyprus, DE = Germany, DK = Denmark, EE = Estonia, ES = Spain, FI = Finland, FR = France, HR = Croatia, IE = Ireland, IT = Italy, LT = Lithuania, LV = Latvia, NL = the Netherlands, NO = Norway, RO = Romania, PL=Polgen, PT = Portugal, SE=Sweden, SI = Slovenia, UK = the United Kingdom
2 Biovolume indicator under development
3 March–October
4 Summer
3.2 Potential phytoplankton indicators developed in international contexts

3.2.1 The WISER project

In the first round of the WFD intercalibration, only chlorophyll \( a \) of the biological quality element phytoplankton was intercalibrated for coastal areas (Carletti and Heiskanen 2009). The EU project Water bodies in Europe: Integrative Systems to assess Ecological status and Recovery (WISER; www.wiser.eu) has developed new assessment methods to better capture all aspects of the phytoplankton community, such as composition, abundance, and biomass.

**Index of Size Spectra Sensitivity of Phytoplankton (ISS-Phyto)**

A new phytoplankton index, Index of Size Spectra Sensitivity of Phytoplankton (ISS-Phyto) (Vadrucci et al. submitted), has been developed in the WISER project. This index integrates size spectra metrics, size class sensitivity to anthropogenic disturbance, phytoplankton biomass (chlorophyll \( a \)), and taxonomic richness thresholds (Lugoli et al. 2012). This index is relevant to both the biomass parameter and taxonomic composition. Tests of both transitional and coastal waters indicated that ISS-Phyto consistently discriminated between anthropogenic and natural disturbance conditions (Lugoli et al. 2012). In this index, the phytoplankton community is divided into six size classes based on a \( \log_2 \) scale of individual weight (pgC cell\(^{-1}\)), with a size class width of 1 (Lugoli et al. 2012). However, a full description of the index is found only in Vadrucci et al. (submitted), which was unavailable at the time of writing.

**Other considerations**

Pigment data have been tested unsuccessfully for potential multi-species and assemblage indices (Henriksen et al. 2009). While chlorophyll \( a \) was found to be significantly correlated with total nitrogen (TN), the distribution patterns of pigment samples and communities indicated a major correlation with salinity and temperature and only minor correlation with TN as a measure of eutrophication (Henriksen et al. 2009). The concentration of individual pigments increased with increasing TN, whereas no clear relationships were found for the relative contributions of different phytoplankton groups (Henriksen et al. 2009).

To enhance the precision of estimated phytoplankton composition and density, WISER (2012a) stresses that the single most important measure is the continuous training and intercalibration of the staff involved in counting. For the Baltic Sea area, this is achieved through the work of the HELCOM Phytoplankton Plankton Expert Group, which maintains an updated species and biovolume list (see Olenina et al. 2006, and its updated Excel file) and arranges regular intercalibrations and taxonomy courses (HELCOM PEG 2012).
3.2.2 Northern Lakes Geographical Intercalibration Group

The methods used for phytoplankton assessment in the Northern Lakes Geographical Intercalibration Group (comprising Finland, Ireland, Norway, Sweden, and the United Kingdom) are summarized in Table 3.2 (see also Birk et al. 2010). Chlorophyll a is used as a proxy for biomass by all five countries, while biomass is only used by Finland, Norway, and Sweden.

Cyanobacteria biomass

Cyanobacteria biomass is used by all countries except Ireland, although the method varies between countries (see Table 3.2). A common cyanobacteria biomass method has been developed (Mischke et al. 2010) based on summer mean cyanobacteria biomass for the July–September period. Quantile regression was used to model cyanobacteria responses to total phosphorus. The quantile model was combined with the three WHO thresholds for cyanobacterial abundance (WHO 2003). Medium- and low-risk waters are those in which cyanobacteria cells are at or above 100,000 and 20,000 cells mL⁻¹, respectively (WHO 2003). These abundances are converted to biovolume, giving approximately 2 mm³ mL⁻¹ as a low-risk threshold and 10 mm³ mL⁻¹ as a medium-risk threshold (Mischke et al. 2011).

Phytoplankton Trophic Index (PTI)

All countries have their own phytoplankton trophic index. However, a common Phytoplankton Trophic Index (PTI) has now been developed in the EU WISER project (Phillips et al. 2012). WISER recommends chlorophyll a, cyanobacteria biomass, and PTI as methods for assessing lake phytoplankton (WISER 2012b).

PTI is based on lake phytoplankton biovolume in late summer, defined as July–September (Phillips et al. 2012). The mean late summer biovolume for each taxon is calculated. To reduce differences caused by different taxonomic traditions, etc., all taxa are aggregated at the genus level or at higher taxonomic levels when the genus is unavailable. The phytoplankton data are converted to a proportion of biomass, summing to a value of 1. Lakes containing more than 50% biomass of Gonyostomum are excluded from analysis. The PTI metric is derived from a canonical correspondence analysis (CCA) ordination constrained by a single environmental variable, total phosphorus. Sites and taxa are arranged along the 1st ordination axis of the CCA plot, with negative scores reflecting lower than average phosphorus and positive scores higher than average phosphorus. Taxon optima are obtained from the CCA taxon axis 1 scores (+3 to −3) and are equivalent to the total phosphorus concentration for the mean occurrence for each taxon. The taxa are divided into the groups very sensitive taxa, sensitive taxa, tolerant taxa, and very tolerant taxa, with more negative values indicating very sensitive and higher positive scores very tolerant. The PTI metric scores are converted to an EQR with a scale ranging from 0 to 1 (Phillips et al. 2012).
TABLE 3.2
Phytoplankton indicators used by countries in the Northern Lakes Geographical Intercalibration Group (Birk et al. 2010).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Countries¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biomass</td>
<td>Chlorophyll a</td>
<td>FI, IE, NO, SE, UK</td>
</tr>
<tr>
<td>Total biovolume</td>
<td></td>
<td>FI, NO, SE</td>
</tr>
<tr>
<td>Trophic index</td>
<td>SE and FI: Trophic Plankton Index (TPI) NO: PTIno (Ptacnik et al. 2009) UK: Taxonomic composition (PTIuk) IE = Irish Phytoplankton Composition and Abundance Index (IPI)</td>
<td></td>
</tr>
</tbody>
</table>

¹ FI = Finland, IE = Ireland, NO = Norway, SE = Sweden, UK = the United Kingdom

3.2.3 HELCOM CORESET

The HELCOM CORESET project (2010–2013) is developing a set of core indicators to enable indicator-based follow-up of the implementation of the HELCOM Baltic Sea Action Plan (BSAP). Besides the already existing chlorophyll a indicator, the following phytoplankton indicators have been suggested (HELCOM 2012):

- phytoplankton diversity
- seasonal succession of phytoplankton groups
- ratio of diatoms to dinoflagellates (supplementary indicator)
- ratio of autotrophic to heterotrophic organisms (supplementary indicator)
- zooplankton–phytoplankton biomass ratio

The two first have been tested, especially in the MARMONI project (see below), while the other indicators are still only suggestions.

3.2.4 MARMONI

MARMONI (Innovative approaches for marine biodiversity monitoring and assessment of conservation status of nature values in the Baltic Sea) is a project funded by the European Union LIFE+ Nature & Biodiversity programme (October 2010–March 2015).
The project’s overall objective is to develop concepts for assessing the conservation status of marine biodiversity, including the species and habitats and impacts of various human activities. In 2012, MARMONI project experts elaborated a draft set of marine biodiversity indicators, six of which involve phytoplankton:

- seasonal progression of phytoplankton functional groups
- phytoplankton taxonomic diversity
- phytoplankton species assemblage clusters based on environmental factors
- phytoplankton functional diversity
- spring bloom biomass
- cyanobacterial surface accumulations

The two first indicators have also been suggested in HELCOM CORESET and are further described below. The “phytoplankton species assemblage clusters based on environmental factors” and “phytoplankton functional diversity” indicators are described only briefly in MARMONI (2012). “Spring bloom biomass” and “cyanobacterial surface accumulations” are indicators for more open-sea conditions and are based on remote sensing and ship-of-opportunity data (MARMONI 2012); they are not described further here.

**Seasonal succession of phytoplankton groups: adjusted to the Baltic Sea**

This indicator is based on the seasonal succession index used by Devlin et al. (2007) but modified to represent the Baltic Sea. The coupling between this index and eutrophication is somewhat unclear, although the nutrient status alters the abiotic factors affecting the monitored groups. However, the succession of dominant groups can potentially provide an index that represents a healthy planktonic system, with natural progression of these dominant groups throughout the seasonal cycle (Devlin et al. 2007). The functional groups used in the index should be adjusted to the water area studied. For the northern Baltic Sea, the following groups have been proposed for inclusion in the index (Jaanus unpublished): 1) diatoms, 2) dinoflagellates, 3) cyanobacteria, and 4) *Mesodinium rubrum*. Instead of abundance, wet weight biomass is used. Acceptable deviations from long-term monthly means are ±2 standard deviations. In Jaanus (unpublished), April–October data have been included in the index. Monthly means and standard deviations are calculated for each phytoplankton functional group. The present state is assessed by comparing the present seasonal distribution of each functional group with a reference using a normalized z-score. The monthly z-score establishes comparable seasonal distributions for each functional group for a sampling year. A positive z-score indicates that the observation is greater than the mean and a negative score indicates that it is less than the mean. Data should be from stations monitored weekly or every second week.

**Phytoplankton taxonomic diversity**

This index is outlined in HELCOM (2012) and further described by Uusitalo et al. (2013). The index captures changes in phytoplankton species composition due to eutrophication. The index focuses on dominant phytoplankton species and their diversity using an applied
Shannon’s index, and includes the most abundant species that together constitute >95% of the total biomass. The exact percentage of biomass to be included in the metric can be defined according to the area and season (Uusitalo et al. 2013). Exclusion of rare species (which occur both randomly and uncertainly) from the indicator enhances its robustness. Data should be from stations monitored weekly or every second week (June–September) and are thereby restricted to data from ships of opportunity or possibly high-frequency sampling stations.

**Phytoplankton species assemblage clusters based on environmental factors**

This index is described only briefly in MARMONI (2012). Summer phytoplankton (June–September) have been grouped into seven clusters based on log(biomass) data. The clusters are tested with environmental factors using a Generalised Additive Model (GAM). The following clusters were found suitable for further testing the relationship between these species and eutrophication: a) the cluster of species characteristic of nutrient-rich waters, b) the cluster including *Skeletonema costatum*, and c) summer species positively correlated with temperature and negatively with N-loads. The exact species included in the different clusters were not specified.

### 3.2.5 OSPAR

**Ecological Quality Objectives (EcoQO)**

To measure progress in attaining OSPAR objectives (see also chapter 1), special Ecological Quality Objectives (EcoQO) for eutrophication have been developed that include chlorophyll *a* and level of nuisance and/or toxic phytoplankton species (OSPAR 2005).

**Chlorophyll a**

Maximum and mean chlorophyll *a* concentrations during the growing season (March–October) should remain below elevated levels, defined as concentrations more than 50% above the area-specific background concentration. Background concentrations and elevated levels of chlorophyll *a* for different areas are presented in OSPAR (2005).

**Level of nuisance and toxic species**

The EcoQO for region/area-specific phytoplankton eutrophication indicator species states that these species should remain below their nuisance and/or toxic elevated levels (and there should be no increase in bloom duration).

The phytoplankton indicator species are divided into two groups, nuisance species (forming dense blooms) and toxic species (which are toxic even at low cell concentrations). Nuisance species (e.g., *Phaeocystis* and *Noctiluca*) display elevated cell concentrations and increased bloom duration compared with previous years. The link between toxic phytoplankton species and eutrophication is not consistent. Evidence indicates, however, that elevated levels of some toxic species (e.g., *Prymnesium polylepis* and...
Karenia mikimotoi, synonyms Chrysochromulina polylepis and Gymnodinium mikimotoi) are caused by nutrient enrichment. Elevated levels for a few area-specific nuisances and toxic phytoplankton species are presented in Table 3.3 (OSPAR 2005); area-specific values are found in OSPAR (2008), report 372 Appendix 4, Table 4.4 (see also Håkansson 2003, 2007).

### TABLE 3.3
Toxic phytoplankton indicator species, elevated levels of area-specific nuisance, and effects.

<table>
<thead>
<tr>
<th>Indicator species</th>
<th>Elevated levels¹</th>
<th>Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuisance species</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phaeocystis spp. (colony form)</td>
<td>$&gt;10^6$ cells L$^{-1}$ and $&gt;30$ days duration</td>
<td>Nuisance, foam, oxygen deficiency</td>
</tr>
<tr>
<td>Noctiluca scintillans</td>
<td>$&gt;10^5$ cells L$^{-1}$ and area coverage $&gt;5$ km$^2$</td>
<td>Nuisance, oxygen deficiency</td>
</tr>
<tr>
<td>Toxic (toxin-producing species)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chrysochromulina polylepis</td>
<td>$&gt;10^6$ cells L$^{-1}$</td>
<td>Toxic; fish and benthos kills</td>
</tr>
<tr>
<td>Gymnodinium mikimotoi</td>
<td>$&gt;10^5$ cells L$^{-1}$</td>
<td>Toxic; fish kills, PSP mussel infection</td>
</tr>
<tr>
<td>Alexandrium spp.</td>
<td>$&gt;10^5$ cells L$^{-1}$</td>
<td>Toxic; PSP mussel infection</td>
</tr>
<tr>
<td>Dinophysis spp.</td>
<td>$&gt;10^2$ cells L$^{-1}$</td>
<td>Toxic; DSP mussel infection</td>
</tr>
<tr>
<td>Prorocentrum spp.</td>
<td>$&gt;10^4$ cells L$^{-1}$</td>
<td>Toxic; DSP mussel infection</td>
</tr>
</tbody>
</table>

¹OSPAR (2005) provides no information about how these elevated levels were determined.

### OSPAR common indicators

In June 2013, the OSPAR Commission adopted a first set of common indicators to follow up the MSFD. According to the outcome of the meeting “OSPAR 2013 agreed that these lists of common indicators are to be used where they apply for assessing the status of, and pressures on, the marine environment of the OSPAR maritime area” (meeting document: OSPAR 13/21/1-E, Annex 4). The following adopted common indicators are related to phytoplankton in the Kattegat and the Skagerrak:

‘OSPAR-wide’ common indicators – applicable to all OSPAR regions

- D5 chlorophyll – Chlorophyll concentration

Additional OSPAR common indicators for Region II – Greater North Sea

- D1 Pelagic Habitats 2 – Plankton biomass and/or abundance
- D5 Phaeocystis. Species shift/indicator species: Nuisance species Phaeocystis
A number of candidate indicators are also under development. These indicators may become adopted as common pending the outcome of testing and agreement among Contracting Parties:

- D1 Pelagic Habitats 1. Changes of plankton functional types (life form) index Ratio
- D1 Pelagic Habitats 3. Changes in biodiversity index (s)
- D4 FoodWeb 2. Production of phytoplankton

### 3.3 Other potential phytoplankton indicators

#### 3.3.1 Species index: Indicator species

The following phytoplankton taxa have been tested for use as indicator species for eutrophication in the Baltic Sea, and have a positive linear relationship with nutrient concentration: *Cyclotella choctawhatcheeana* (Jaanus et al. 2009), *Cylindrotheca closterium* (Jaanus et al. 2009), *Planktothrix agardhii* (Carstensen and Heiskanen 2007), and *Mesodinium rubrum* (Samuelsson et al. 2004). While Jaanus et al. (2009) used biovolume measures, Carstensen and Heiskanen (2007) and Samuelsson et al. (2004) used cell abundance. Andersson and Edler (2003) listed several phytoplankton taxa/groups that could be tested as indicators of eutrophication (see Table 3.4).
TABLE 3.4
Summary of phytoplankton species/groups listed in Andersson and Edler (2003) as suitable indicators of water quality.

<table>
<thead>
<tr>
<th>Species/group</th>
<th>Indicates</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Diatoms</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Dactyliosolen fragilissimus</em></td>
<td>Competitive in high nutrient concentrations</td>
<td>Granéli et al. 1989</td>
</tr>
<tr>
<td><em>Thalassiosira nordenskioeldii</em></td>
<td>Can occur at other times than during the spring bloom if high nutrient concentrations are available</td>
<td>Braarud 1962</td>
</tr>
<tr>
<td><strong>Dinoflagellates</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Ceratium furca</em></td>
<td>Growth stimulated by nutrients</td>
<td>Edler and Otsson 1985</td>
</tr>
<tr>
<td><em>Ceratium tripos</em></td>
<td>Growth stimulated by nutrients</td>
<td>Edler 1984, Mahoney and Steimle 1979</td>
</tr>
<tr>
<td><em>Heterocapsa triquetra</em></td>
<td>Forms blooms in nutrient-rich coastal areas</td>
<td>Braarud 1945</td>
</tr>
<tr>
<td><em>Lingulodinium polyedrum</em></td>
<td>Growth stimulated by nutrients</td>
<td>Tangen 1980</td>
</tr>
<tr>
<td><em>Noctiluca scintillans</em></td>
<td>Can form blooms that lead to anoxic events</td>
<td>Lam and Ho 1989</td>
</tr>
<tr>
<td><em>Prorocentrum micans</em></td>
<td>Growth stimulated by nutrients</td>
<td>Braarud 1945</td>
</tr>
<tr>
<td><em>Prorocentrum minimum</em></td>
<td>Forms blooms in nutrient-rich waters</td>
<td>Hajdu et al. 2000, Tangen 1980, Tyler and Seliger 1978</td>
</tr>
<tr>
<td><strong>Prymnesiophyceae</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Chrysochromulina polylepis</em></td>
<td>Potentially toxic species; blooms can indicate eutrophication</td>
<td>Dahl et al. 1989, Hajdu 2002</td>
</tr>
<tr>
<td><strong>Chlorophyceae: green algae</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Monoraphidium contortum</em></td>
<td>Competitive in high nutrient concentrations</td>
<td>Kuosa 1988</td>
</tr>
<tr>
<td><strong>Pyramimonas spp.</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Cyanobacteria</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Aphanizomenon sp.</em></td>
<td>Increased bloom frequency in the Baltic Sea</td>
<td>e.g., Finni et al. 2001</td>
</tr>
<tr>
<td><em>Nodularia spumigena</em></td>
<td>Increased bloom frequency in the Baltic Sea</td>
<td>e.g., Finni et al. 2001</td>
</tr>
<tr>
<td><em>Planktothrix agardhii</em></td>
<td>Occurs in highly nutrient-rich environments</td>
<td>Tinnberg-Kautsky 1979</td>
</tr>
<tr>
<td><strong>Other</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Mesodinium rubrum</em></td>
<td>Increase in eutrophication gradients</td>
<td>Elmgren and Larsson 1997</td>
</tr>
</tbody>
</table>
Several other species have also been tested, revealing no significant correlation with increased nutrient concentration. This includes the biovolume of the most common bloom-forming species (e.g., the cyanobacteria Aphanizomenon sp. and Nodularia spumigena and the dinoflagellate Heterocapsa triqueta) (Jaanus et al. 2009). HELCOM (2006), however, suggests that the abundance of Aphanizomenon sp. (July–August) could serve as an indicator of eutrophication (HELCOM EUTRO results, for the Gulf of Finland). Samue1sson et al. (2004) also tested the abundance of Pyramimonas spp., Dinophysis acuminata, and Monoraphidium contortum, but found no relationship with nutrient concentration. With more data available, however, these species could be tested again.

Andersson and Edler (2003) listed some potentially toxic species that could be used as indicators of water quality: the dinoflagellates Alexandrium minutum, Dinophysis spp., and Noctiluca scintillans, the prymnesiophyte Chrysochromulina polylepis, the diatom Pseudo-nitzschia spp., and the silicoflagellate Dictyocha speculum.

Carstensen and Heiskanen (2007) have developed an application for screening possible eutrophication indicators. This can be used as a tool for selecting potential new indicator species.

Carstensen et al. (2007) propose a statistical approach to identifying bloom observations in long-term monitoring data, defining blooms as chlorophyll a observations deviating significantly from a normal seasonal cycle. They propose using this definition to investigate the underlying mechanisms of summer blooms of various species and their links to nutrient enrichment.

3.3.2 Phytoplankton groups as indicators

Different phytoplankton groups can respond differently to a given pressure. Cryptophyceans have been found to increase in eutrophication gradients (Kononen 1988, Elmgren and Larsson 1997), and species that dominate in spring are also competitive in high nutrient concentrations (e.g., Harris 1986).

Some phytoplankton groups and genera have also been proposed for use as indicators of eutrophication in the Baltic Sea (for a summary, see Table 3.5). Jaanus et al. (2009) found a positive relationship between the summer biovolume of oscillatorian cyanobacteria (mainly Pseudanabaena and Planktolyngbya) and total phosphorus concentrations, and Sagert et al. (2008) found a positive relationship between the biovolume of Chlorophytes and Cyanophytes, and total nitrogen. Samuelsson et al. (2004) found a positive linear relationship between abundance of cryptophyceans and total nitrogen concentration in August.

Samuelsson et al. (2002) demonstrated in an experimental system with water from the Gulf of Bothnia that potentially mixotrophic phytoplankton (Chrysochromulina sp.) became dominant when nutrients were low. One suggestion is therefore to use potentially mixotrophic species as indicators of low nutrient status (i.e., oligotrophic conditions). Mixotrophic algae are normally dominant in phytoplankton communities during the low-nutrient summer period (e.g., Andersson et al. 1996, 2006, Hajdu et al. 1996). Indicators
of oligotrophic conditions could potentially be weighted into an index together with eutrophication indicators such as cyanobacteria. Examples of potentially mixotrophic groups could be chrysophyceans and prymnesiophyceans (Andersson et al. 1989, Samuelsson et al. 2002).

**TABLE 3.5**  
Phytoplankton groups tested for use as eutrophication indicators in the Baltic Sea.

<table>
<thead>
<tr>
<th>Group</th>
<th>Description</th>
<th>Reference</th>
</tr>
</thead>
</table>
| Oscillatorialean cyanobacteria (Pseudoanabaena and Planktolyngbya) | Jaanus et al.: June–September, biomass  
| Cryptophyceae | August, abundance (cells L$^{-1}$) | Samuelsson et al. 2005 |
| Cyanophyceae | May–September, biovolume (mm$^3$ L$^{-1}$) of all cyanobacteria (except picocyanobacteria); used in Germany | Sagert et al. 2008 |
| Chlorophyceae | May–September, biovolume (mm$^3$ L$^{-1}$); used in Germany | Sagert et al. 2008 |
| Bacillariophyceae and flagellates + dinoflagellates | February–May, mean biomass and abundance | Krasniewski et al. 2009 |
| Ratio between Bacillariophyceae and Flagellates + Dinoflagellates | February–May, ratio of mean biomass | Krasniewski et al. 2009 |
| Dinophysis spp. | Spring and summer, mean biomass and abundance | Krasniewski et al. 2009 |

3.3.3 Harmful algae

Harmful algal blooms are considered in the criteria related to descriptor 5 in the MSFD. Monitoring toxin-producing algae is also part of several directives (i.e., EC 852/2004, 853/2004, 854/2004, and 882/2004) related to harvesting farmed or wild bivalves, such as oysters and mussels, for human consumption. In Sweden, the National Food Administration (Livsmedelsverket) is responsible for these directives. Commercial harvesting of mussels currently occurs only in the county of Västra Götaland, i.e., along the Bohus coast (Nordlander et al. 2011).

There are harmful species from many different classes of algae, for example, the dinoflagellates, diatoms, cyanobacteria, dictyochophytes, and haptophytes (or, using the Latin names, Dinophyceae, Diatomophyceae, Cyanophyceae, Dictyochophyceae, and Prymnesiophyceae, respectively). Some species damage fish by affecting their gills. Others
cause shellfish poisoning, since filter feeders such as mussels accumulate the algal toxins when feeding on the phytoplankton, causing, for example, diarrheic shellfish poisoning (DSP), paralytic shellfish poisoning (PSP), and amnesic shellfish poisoning (ASP), while still others release toxins into the water. Some may simply be a nuisance when they occur in high abundances and cause ugly and smelly scums on the water. The Intergovernmental Oceanographic Commission maintains and develops the Harmful Algae Events Database (HAEDAT, www.iode.org/haedat).

OSPAR has used the abundance of selected harmful algal species as part of the common procedure for identifying the eutrophication status (OSPAR 2005). The Swedish reporting of harmful algal species is found in Håkansson (2003, 2007).

The term harmful algal blooms (HABs) is commonly applied to high-biomass blooms, such as blooms of cyanobacteria in the Baltic Sea, and blooms of dinoflagellates, such as those occurring in Laholm Bay in the 1980s. The frequency and size of these blooms are very likely related to eutrophication. However, the term HABs is also applied to low-biomass blooms of toxic species such as *Alexandrium* and *Dinophysis* that produce algal toxins that accumulate in shellfish. These HABs may not be related to eutrophication but to other human activities. It is desirable to have phytoplankton indicators that include HABs in a sensible way.

Cell counts of harmful algae used to be part of the phytoplankton assessment in the Basque Country, northern Spain (Borja et al. 2004), but were removed from the quality index as they provided no relevant information in that area (Bay of Biscay) (Revilla et al. 2009). In the USA, HABs occur in some areas where the coastline has changed rapidly due to the establishment of housing and golf courses. There are indications that blooms are related to both eutrophication and pesticide use.

### 3.3.4 Multi-metric indicators of phytoplankton

Instead of using several individual metrics that are combined according to various combination rules to classify a water area, multi-metric indices have been used for phytoplankton assessment. These can consist of phytoplankton parameters only or a combination of phytoplankton parameters and environmental parameters. However, a combined index of both phytoplankton and environmental parameters is not compliant with the WFD. Lehtinen et al. (2012) have summarized some of these indices, including the metric proposed by Vadrucci et al. (submitted) (see Table 3.6). A multi-metric index does not present the individual metrics, making it difficult to evaluate what causes a potential change. A multi-metric index therefore can only be used when the effects of all the included parameters are properly described.
### TABLE 3.6
Multi-metric phytoplankton indices (summarized by Lehtinen et al. 2012).

<table>
<thead>
<tr>
<th>Name of the multi-metric index</th>
<th>Metrics included</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>A new method for phytoplankton quality</td>
<td>Chlorophyll a and bloom frequency (single taxa counts)</td>
<td>Revilla et al. 2009</td>
</tr>
<tr>
<td>Integrated Phytoplankton Index (IPI)</td>
<td>Chlorophyll a, abundance, diversity</td>
<td>Spartharsis and Tsirtsis 2010</td>
</tr>
<tr>
<td>Phytoplankton Index of Biotic Integrity (P-IBI)</td>
<td>Total and taxa biomass, taxonomic composition, size structure, indicator species abundance, photosensitivity, physiological status, dissolved inorganic carbon</td>
<td>Lacouture et al. 2006</td>
</tr>
<tr>
<td>Phytoplankton Community Index (PCI)</td>
<td>Abundance of &quot;life-forms&quot; plotted in &quot;life-form space&quot;. &quot;Life-forms&quot; are based on taxonomy, biogeochemistry, response to physical environment, and susceptibility to grazing</td>
<td>Tett et al. 2008</td>
</tr>
<tr>
<td>UNTRIX</td>
<td>Chlorophyll a, oxygen deficit, dissolved inorganic nitrogen, total phosphorus</td>
<td>Pettine et al. 2007</td>
</tr>
<tr>
<td>E.I. index of assessing eutrophication</td>
<td>Chlorophyll a, nitrite, ammonia, phosphate</td>
<td>Primpas et al. 2010</td>
</tr>
<tr>
<td>Index of Size spectra Sensitivity of Phytoplankton (ISS-Phyto)</td>
<td>Size spectra metrics, size class sensitivity to anthropogenic disturbance, phytoplankton biomass (chlorophyll a), taxonomic richness thresholds</td>
<td>Lugoli et al. 2012, Vadrucci et al. submitted</td>
</tr>
</tbody>
</table>
4 Monitoring of phytoplankton parameters

4.1 Traditional water sampling at fixed stations

4.1.1 Brief description of method

Currently, phytoplankton are usually sampled using a hose (integrating the water column) or, less commonly, using water samplers located at discrete depths, from ships and boats at specific monitoring stations. The water samples are immediately fixed with preservative (normally acidic Lugol’s solution and an additional sample with neutral Lugol’s solution at stations with calcareous species) and analysed manually in the laboratory using inverted microscopy with the Utermöhl technique. This method allows the counting of phytoplankton larger than approximately 2 μm in size. Today microscopic analysis is the only way to get detailed information about species composition, abundances, and biomasses.

Traditional phytoplankton monitoring is coordinated with the sampling of various supporting parameters, such as temperature, salinity, and nutrient availability, improving the interpretation of the phytoplankton data. Depending on the sampling frequency, succession and trends at specific monitoring stations can be investigated. However, high sample-processing cost often limits both the number of phytoplankton monitoring stations and their sampling frequency, and the present spatial sampling coverage of the coastal zone is low.

4.1.2 Overview of sampling frequency along the coast

The sampling locations for phytoplankton data available at the Swedish National Oceanographic Data Centre at SMHI are shown in Figures 4.1–4.5. Regular phytoplankton monitoring started in Sweden in the mid 1970s in the Öresund (see Skjevik et al. 2011 for more information). When HELCOM started its coordinated sampling programme in the late 1970s, monitoring started to spread. After the *Chrysochromulina* bloom in 1988, several new sampling programmes were started in the Skagerrak and Kattegat. Investigations of the Askö–Himmerfjärden area (south of Stockholm) starting in the late 1970s led to increased phytoplankton sampling and analyses, and interest in the large blooms of cyanobacteria in the Baltic Sea has led to an increased number of sampling locations. Sampling started in the Gulf of Bothnia in 1991. In 1999, several new
sampling locations were established in offshore areas of the Baltic Sea. In 2007, new coastal sampling stations were added, two in the Gulf of Bothnia, one in the Baltic Proper, and one in the Kattegat. Sampling frequency varies substantially, ranging from a few times per year to 24 times per year at a few locations. This can be compared with the sampling frequency in Helgoland, Germany, where sampling is conducted five times a week year-round. In Norway, phytoplankton sampling is conducted three times per week in Flødevigen near Arendal on the Skagerrak coast. On the other hand, the sampling frequency is much lower in other Baltic Sea areas.

4.1.3 Cell abundance versus biovolume

The identification and counting of Baltic Sea phytoplankton has a long tradition, though the large variability in cell-specific biomasses has led to problems interpreting the data. Today, it is an established procedure in the Baltic Sea area to also estimate biovolume per cell, which facilitates the calculation of total biomass and the biomass of the various groups and species of phytoplankton. Biovolume reporting started in the 1970s at some open-sea stations; since then it has gradually increased, spreading to coastal areas and other open-sea stations. It is possible, however, that not all biovolume data is included in the national database. Since 1999, biovolume has been estimated throughout the National Monitoring Programme, and is becoming common in many regional programmes since 2007. A common biovolume file (see Olenina et al. 2006, and its updated appendix) has been produced since 2006, and is updated yearly by the HELCOM Phytoplankton Expert Group. This file, which includes a species list for the Baltic Sea and fixed biovolumes for various species (and size classes), should be used when working in the Baltic Sea area.

4.1.4 Comments about data available in September 2012

The sampling frequency and area coverage have slowly increased since monitoring started in the mid 1970s. Only abundance was recorded in the first decades and biovolume measurements were included starting in 1999 at many locations. The area coverage was adjusted in 2007, and some areas previously not surveyed were included. This has led to better spatial coverage. The National Monitoring Programme, initiated by the Swedish Environmental Protection Agency (SEPA) and now commissioned by the Swedish Agency for Marine and Water Management (SwAM), requires that data be reported to the national data host (SMHI) and that data should be available for anyone to use.

There are also several regional sampling programmes along the coast. Though the results of these programmes are not yet required to be reported to the national data host, an increasing number of such programmes are reporting their data to the national data host. Some of these data have been made available for anyone to use at the national data host website. The lack of financial support for quality control and data importing, however, have hampered the work and some data are still not included.

In 2010 and 2011, a monitoring campaign was initiated by the CABs, commissioned by the SEPA, and several stations along the coast were sampled for abundance and
biovolume. Each campaign was run for a few months in summer or for an entire year. These results were required to be reported to the national data host, as seen in Figure 4.5. Five national monitoring stations take samples at high frequency: the Örefjärden coastal station in Bottenhavet (18 times/year), the Askö coastal station in the northern Baltic Proper (24 times/year), the Landsort Deep open-sea station in the northern Baltic Proper (22 times/year), the Anholt open-sea station in the Kattegat (24 times/year), and the Släggö coastal station in Skagerrak (24 times/year).

### 4.1.5 Phytoplankton data missing from the National Oceanographic Data Centre

The sampling locations for phytoplankton data indicated in the maps in Figures 4.1–4.5 do not show the complete available dataset. For example, some data are missing from BroA and Alsbäck stations in the Skagerrak, Hallands Väderö station in the Kattegat, and from regional sampling programmes such as those of Öresunds Vattenvårdsförbund, Nordvästra Skånes vattenvårdsförbund, Hallands kustkontrollprogram, Bohuskustens Vattenvårdsförbund, Motala Ströms Vattenvårdsförbund, and Svealands Kustvattenvårdsförbund. Data from other regional monitoring, recipient control, and research programmes are also not included in the database. Together with the data host, the Swedish Agency for Marine and Water Management (SwAM) and the data owners will endeavour to fill the database with more data.

*FIGURE 4.1*

Reported phytoplankton abundance between 1978 and 1989 available at the Swedish National Oceanographic Data Centre at SMHI (source: www.smhi.se/sharkweb; accessed 6 September 2012). For this period, only data from Askö area (south of Stockholm) are available in the database.
FIGURE 4.2
Reported phytoplankton abundance between 1990 and 1998 available at the Swedish National Oceanographic Data Centre at SMHI (source: www.smhi.se/sharkweb; accessed 6 September 2012). The number of stations reporting to the database is slowly increasing, although still only abundance is reported.

FIGURE 4.3
Reported phytoplankton abundance (left) and biovolume (right) between 1999 and 2006 available at the Swedish National Oceanographic Data Centre at SMHI (source: www.smhi.se/sharkweb; accessed 6 September 2012). Regional data in the database are not always quality checked; for example, one station is located on land. Abundance is still reported by more stations than is biovolume.
FIGURE 4.4
Reported phytoplankton abundance (left) and biovolume (right) between 2007 and 2009 available at the Swedish National Oceanographic Data Centre at SMHI (source: www.smhi.se/sharkweb; accessed 6 September 2012).

FIGURE 4.5
Reported phytoplankton abundance (left) and biovolume (right) between 2010 and 2011 available at the Swedish National Oceanographic Data Centre at SMHI (source: www.smhi.se/sharkweb; accessed 6 September 2012). Biovolume data have begun to be included in regional data as well. The special 2010–2011 monitoring campaign increased the number of stations reported to the database.
4.1.6 Phytoplankton sampling in coastal monitoring programmes

Coastal monitoring programmes include a varying amount of phytoplankton monitoring. Part of the monitoring is national and is commissioned by the Swedish Agency for Marine and Water Management (SwAM), while other parts of the monitoring are commissioned by regional water quality associations. Appendix 5 presents an overview of existing programmes based on information available from Vattenmyndigheterna (www.vattenmyndigheterna.se) and from the websites of water quality associations in October 2012 and is likely incomplete. In some areas, for example, along parts of the Gulf of Bothnia coast and along the coasts of Kalmar and Blekinge, essentially no phytoplankton monitoring is conducted. Note that the monitoring of harmful algae in areas where mussel farming or wild shellfish harvesting is conducted is not listed in Appendix 5; such monitoring is currently done only along the Skagerrak coast, and is commissioned by the Swedish Food Agency.

4.2 Automated systems for water sampling and estimates of phytoplankton biomass

The present phytoplankton indicators in the Swedish assessment system for phytoplankton are based on ship- and boat-based collection of water samples for phytoplankton microscopic counts and chlorophyll analyses. The following sections present methods that can be used to supplement this type of sampling to increase sampling frequency or reduce costs. Karlson et al. (2009) have extensively reviewed these methods. FerryBox systems, which are cost-effective for high-frequency measurements and for sampling near-surface waters, can cover large sea areas. Buoys can be used for high-frequency measurements and water sampling at several depths. Satellite remote sensing supplements in situ data with estimates of near-surface parameters related to phytoplankton biomass; however, no, or very little, taxonomic information is collected using remote sensing. Buoys and FerryBox systems make it possible to conduct high-frequency water sampling. This reduces the cost of dedicated ship time, though the cost of microscopical analyses is the same as for regular ship sampling. FerryBox systems only sample near-surface waters. A disadvantage of automated systems is that so far they do not allow high-quality analyses of nutrients, which hampers the interpretation of the collected phytoplankton data.

4.2.1 FerryBox systems

The measurement of oceanographic parameters from research vessels has a long history. In addition, instrumentation for automated oceanographic measurements and water sampling has been deployed on ships of opportunity, i.e., ferries and merchant ships traversing the Baltic Sea, Kattegat, and Skagerrak (Figure 4.6 and Table 4.1). This practice started in Estonia and Finland circa 1990 and has subsequently spread to many sea areas in Europe and elsewhere. A basic system consists of a pump, sensors for temperature, salinity, and chlorophyll fluorescence (a proxy for phytoplankton biomass), a GPS unit,
and a computer. Many systems also have water-sampling devices and additional sensors for qualities such as phycocyanin fluorescence (a proxy for cyanobacteria biomass), turbidity, oxygen, pH, and carbon dioxide as well as in-air sensors for irradiance, air pressure, air temperature, and wind. New developments include the possibility of measuring the content of coloured dissolved organic matter (CDOM).

**FIGURE 4.6**
Routes of the main FerryBox systems in the Baltic Sea area.

**TABLE 4.1**
The main FerryBox systems in the Baltic Sea area.

<table>
<thead>
<tr>
<th>No. on map</th>
<th>Ship</th>
<th>Route</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Baltic Princess</td>
<td>Tallinn–Helsinki</td>
<td>EMI</td>
</tr>
<tr>
<td>2</td>
<td>Color Fantasy</td>
<td>Oslo–Kiel</td>
<td>NIVA</td>
</tr>
<tr>
<td>3</td>
<td>Finnmaid</td>
<td>Helsinki–Lübeck–Gdynia–Helsinki</td>
<td>SYKE</td>
</tr>
<tr>
<td>4</td>
<td>MS Bergensfjord</td>
<td>Bergen–Hirtshals</td>
<td>NIVA</td>
</tr>
<tr>
<td>5</td>
<td>Lysbria</td>
<td>Hamburg–Immingham–Halden</td>
<td>NIVA and HZG</td>
</tr>
<tr>
<td>6</td>
<td>Silja Serenade</td>
<td>Helsinki–Mariehamn–Stockholm</td>
<td>SYKE</td>
</tr>
<tr>
<td>7</td>
<td>Stena Spirit</td>
<td>Gdynia–Karlskrona</td>
<td>IMGW–PIB</td>
</tr>
<tr>
<td>8</td>
<td>TransPaper</td>
<td>Gothenburg–Oulu–Kemi–Lübeck–Gothenburg</td>
<td>SMHI</td>
</tr>
<tr>
<td>9</td>
<td>Victoria</td>
<td>Tallinn–Mariehamn–Stockholm</td>
<td>MSI</td>
</tr>
</tbody>
</table>

EMI = Estonian Marine Institute of Tartu University, HZG = Helmholtz-Zentrum Geesthacht – Institute of Coastal Research (Germany), IMGW–PIB = Institute of Meteorology and Water Management – National Research Institute (Gdynia, Poland), MSI = Marine Systems Institute at Tallinn University of Technology, NIVA = Norwegian Institute for Water Research, SMHI = Swedish Meteorological and Hydrological Institute, SYKE = Finnish Environment Institute
4.2.2 Water-sampling devices on ships of opportunity

For example, in Estonia, Finland, Norway, and Sweden, automated phytoplankton sampling using FerryBox systems on ferries and on merchant ships has been conducted for several years. Refrigerated water-sampling devices collect up to 24 one-litre samples from predetermined locations. Sample containers are prefilled with preservative (Lugol’s solution). The sampling frequency depends on the ship schedule, but is often every week or every two weeks. In 2011, phytoplankton were sampled using FerryBox systems by SMHI in cooperation with NIVA in Norway. Six stations around the coast were sampled with the FerryBox system. Preliminary data from SMHI sampling in 2011 are presented in Figure 4.7.
FIGURE 4.7
Phytoplankton data from FerryBox sampling: preliminary data on the biovolume of various phytoplankton groups/classes from 2011. Note the diatom blooms (red) not only in spring but also at other times of the year. Also note the cyanobacteria bloom between Öland and Gotland (TP13) and the bloom of the dictyochophyte *Pseudochattonella farcimen* in the Kattegat and Skagerrak in spring (yellow). Automated sampling devices on the ships TransPaper and Color Fantasy were used for water sampling at a depth of approximately 3 m. Samples were analysed using microscopy. Note that stations FA10+1 and TP21/FA7 (in the Kattegat and Skagerrak) were sampled more regularly than the other stations (where data from some periods are missing).

4.2.3 Buoys

Instrumented oceanographic buoys are often relatively simple devices that measure only waves and sea surface temperature. However, they can also be more advanced systems incorporating sensors at various depths as well as water-sampling devices. SMHI currently operates wave buoys at four locations around Sweden. One more advanced buoy in the Baltic Sea is operated by SMHI and the Swedish Armed Forces. This system has sensors for chlorophyll fluorescence and phycoeryanin fluorescence near the surface. A similar system is to be deployed near the island of Väderöbod in the Skagerrak. A system of coastal oceanographic buoys is being set up by Linné, Gothenburg, Umeå, and Stockholm universities together with SMHI. These buoys have sensors for chlorophyll fluorescence, salinity, temperature, and oxygen. In the United Kingdom, Centre for Environment, Fisheries and Aquaculture Science (Cefas, http://www.cefas.defra.gov.uk/our-services/monitoring-and-mapping/autonomous-monitoring/smartbuoys.aspx) has been using such “SmartBuos” for several years; the buoys have various sensors as well as water-sampling devices for phytoplankton.

For example, in the United Kingdom and the Netherlands, automated sampling devices are used on oceanographic buoys to sample phytoplankton. Two types of devices are used: the Aquamonitr from Envirotech Instruments (Chesapeake, VA, USA) and another device developed by the Centre for Environment, Fisheries & Aquaculture Science (Cefas) (Lowestoft, UK). Both devices collect phytoplankton samples in plastic bags prefilled with preservative, i.e., Lugol’s solution. Up to 50 samples can be collected at predetermined time intervals or triggered in other ways. Samples are analysed using microscopy.

4.3 Satellite remote sensing for observing algal blooms

Algorithms for estimating near-surface chlorophyll $a$, a proxy for phytoplankton biomass, have been developed for several different sensors on satellites. The best-known are the SeaWIFS, Aqua, and Terra satellites (equipped with the MODIS sensor) and EnviSat (equipped with the MERIS sensor). The standard chlorophyll $a$ algorithms are applicable
mainly to the open ocean, where the influence of suspended sediments and riverine input of, for example, humic substances, is small. In coastal water, differences in the atmosphere and land–water adjacency effects add complexity to the measurements. The Algal2 algorithm for coastal chlorophyll has been developed for the MERIS sensor. It has been tested, for example, by Sørensen et al. (2007) and Kratzer and Vinterhav (2010). Unfortunately, the EnviSat ceased operation in May 2012, but the new NPP and Sentinel series satellites will soon be available. The surface accumulations of cyanobacteria in summer months in the Baltic Sea are well suited to satellite remote sensing, and relevant results have been presented by Krahru et al. (2007) and Hansson (2006). SMHI operates the Baltic Algae Watch System (BAWS) (Hansson and Häkansson 2007), which makes daily observations from June to September; results can be found at www.smhi.se and in HELCOM indicator reports (e.g., Hansson and Öberg 2011).

In archipelagos, problems with land and seafloor influences affecting estimates of phytoplankton biomass have not been resolved. The influences of resuspended sediments and of humic substances make the method unsuitable for quantitative work. Attempts to estimate phytoplankton biomass using satellite remote sensing in lakes and coastal archipelagos have been made and the results are available on the Internet. However, to the author’s knowledge, the quality of these data has yet to be independently evaluated. Casual comparisons of data from Envisat/MERIS with in situ data from the Water Quality Association of the Bohus coast indicate that the estimates of chlorophyll content are not well correlated.

To summarize, measurements of chlorophyll fluorescence and estimates of chlorophyll made from satellites may be used for information on the frequency and distribution of high-biomass blooms in offshore waters under cloud-free conditions. However, essentially no information on the composition of blooms can be acquired using these methods. Another limitation is that only near-surface water is observed by satellites, and phytoplankton often grow deeper in the water column. Satellite remote sensing of ocean colour is sensitive to cloud cover, which is frequent over the waters surrounding Sweden. In coastal waters, satellite remote sensing may supplement other methods, keeping in mind severe methodological problems due to humic substances, resuspended sediments, and effects of the sea floor and land. Kratzer et al. (2011) have reviewed satellite remote sensing of the Baltic Sea.
5 Potential coastal phytoplankton indicators for Sweden

5.1 What characterizes a good indicator?

Ecological indicators need to capture the complexity of the ecosystem, while remaining simple enough to be easily and routinely monitored (Dale and Beyeler 2001). The following criteria characterize a good phytoplankton indicator according to Dale and Beyeler (2001) and Andersson and Edler (2003):

1. It is sensitive to changes in the environment and responds to environmental pressures in a predictable manner; for example, a phytoplankton indicator should respond clearly to increased nutrient concentrations.
2. It is analysed using a robust and accurate method; for example, it is an easily identified species with distinct structures. It should also be easy to prepare the indicator data, so that even non-phytoplankton experts can prepare and understand them.
3. It has low spatial variability, i.e., it is homogenously distributed in the water column.
4. It displays great statistical strength, i.e., a trend should be detectable even with relatively small changes, and it should respond clearly to a stressor such as nutrient addition.
5. It is a key part of the ecosystem, i.e., has a large biomass, is an important functional group, or is potentially toxic.
6. Monitoring the phytoplankton indicator is economically sustainable, i.e., the costs of sampling and analysis are low.

5.2 Factors limiting choice of indicators

An assessment system for phytoplankton fully compliant with the WFD should include all the following parameters: biomass, taxonomic composition, abundance (or cover), frequency, and intensity of algal blooms. However, several factors may restrict the number of parameters that can be included or that should be considered in the process of developing and testing various indicators:

- Although the situation is improving, limited data availability (Figures 4.1–4.5) will restrict both the search for and testing of possible indicators.
• There will always be a trade-off between the data requirements of an assessment system and the data that can reasonably be expected to be delivered from monitoring programmes. Some indicators may be more data intensive than others; for example, a trophic index (e.g., the lake PTI index) or an index based on comparative size class data is more data intensive than is total biovolume. This must also be weighed against the robustness of the index.
• Due to large differences in latitude, salinity, and other factors affecting species distribution, indicators specific to different sea areas may have to be considered.
• Once an index is demonstrated to respond satisfactorily to eutrophication gradients, boundary definition is an important issue. Unless more or less obvious class boundaries can be defined, for example, from the sudden appearance of problematic species in a gradient, boundary setting may have to rely on principles that need general discussion. Existing assessment systems for nutrients and phytoplankton, now including reference values and boundaries for chlorophyll and biomass, can serve as guidelines, but may need revision.
• Depending on the parameter choices, the construction of indices, and their merging into a final EQR value, the inclusion of more parameters may make the overall assessment more robust or add additional uncertainty. This also has to be tested.

These criteria should be considered when proposing potential new indicators for Swedish coastal waters and when evaluating the existing parameters used in the WFD.

5.3 Potential phytoplankton indicators for Swedish coastal waters

5.3.1 Total biomass

Phytoplankton biovolume and chlorophyll $a$

In the current assessment system, the salinity and nutrient-related reference values for chlorophyll $a$ and biovolume are applied to the Baltic Proper only. These may need revision, and their possible implementation in other areas needs evaluation.

The use of mean or 90th percentile for chlorophyll $a$ should be tested and evaluated. This is especially important for the Kattegat and Skagerrak area, since other countries in the North-East Atlantic Intercalibration Group use the 90th percentile for the March–October period and the methods used should be intercomparable.

The chlorophyll $a$ content can vary between species and the amount of humic substances and/or suspended particulate material (SPM) has been found to increase the chlorophyll $a$ content in cells, without simultaneously increasing the biomass. The use of correction factors for chlorophyll $a$ in humic waters should be tested. Chlorophyll and biomass could be divided into two components: a) mean for summer (or March–October) and b) a frequency component, by totalling how often the total phytoplankton biomass/chlorophyll $a$ exceeds an area-specific long-term mean for all or part of the year.
(e.g., summer). If only summer is assessed, this latter component can be included only at stations that are sampled at least every second week. Using at least three years’ data will also increase the data availability for the parameter.

Automated water sampling and measurement of chlorophyll fluorescence as a proxy for chlorophyll $a$ should be evaluated, because increased sampling frequency may improve results and reduce ship costs. Suitable buoys, etc., are not yet available, but when they are, data from these should be evaluated for use in the assessment system. Funding for research infrastructure from the Swedish Science Council will be used for setting up a system of coastal oceanographic buoys for the next few years. Buoys will be deployed near Umeå, at a station in the Stockholm archipelago (station B1, near Askö), on the east coast of Öland (near Kårehamn), and in fjords on the Swedish Skagerrak coast.

A more fundamental evaluation of the present chlorophyll and biovolume reference values and class boundaries, and the relationships with salinity and other factors, is also needed.

**Phytoplankton carbon**

It should be evaluated whether the biomass proxy biovolume should be replaced by or complemented with carbon, based on carbon formulas presented by Menden-Deuer and Lessard (2000) and biovolume measures contained in the updated biovolume file of the HELCOM PEG group. This would be especially useful in areas dominated by large diatoms, where the biovolume parameter would give overly high biovolume values while the carbon content would give more balanced carbon values, since the vacuole of the diatoms (which contains very little carbon) would be compensated for.

5.3.2 Abundance

To avoid comparing the abundance of small cells with that of large ones, biomass is usually a better measure than abundance to describe the phytoplankton community and its composition, due to the large cell-size differences. However, abundance can be used when following a single species that does not vary in size, for example, when using a toxic species as an indicator, in which case the warning levels are usually based on abundance values. (See also the section below about indicator species). However, we recommend using biomass instead of abundance.

5.3.3 Taxonomic composition

**Indicator species and groups**

Nitrogen-fixing cyanobacteria (mainly the genera *Aphanizomenon*, *Nodularia*, and *Dolichospermum*) are a key part of the Baltic summer phytoplankton community (Sivonen et al. 2007). The ratio of the summer biomass of these cyanobacteria to the total phytoplankton biomass is a possible indicator that should be tested for the Baltic Proper, Bothnian Sea, and possibly Bothnian Bay. However, it should be noted that these species
should be relatively abundant in low-nitrogen-load areas and decrease in abundance with high nitrogen loads. *Nodularia* blooms are generally developing in offshore areas and may be a poor indicator for coastal areas. It might also be of interest to test an indicator based on all cyanobacteria (picocyanobacteria excluded), equivalent to the lake cyanobacteria biomass index, or on only non-nitrogen-fixing filamentous species of the order Oscillatoriales (e.g., Jaanus et al. 2009).

Selected harmful algal bloom species should be evaluated as indicator species, i.e., *Nodularia spumigena*, *Pseudo chatttonella farcimen*, *Dinophysis* spp., *Chrysochromulina* (Prymnesiophyceae), and *Pseudo-nitzschia* spp. Promising species displaying a general response to eutrophication for the Baltic Sea are *Mesodinium rubrum*, *Cylindrotheca closterium*, *Cyclotella cunctatahataveana* (recommended by Jaanus et al. 2009), and *Planktothrix agardhii* (Carstensen and Heiskanen 2007). *Ceratium tripos* and *Dactylosolen fragilissimus* (recommended by Andersson and Edler 2003) could be tested for the Kattegat and Skagerrak. Using new data, a general search for species or taxa displaying a clear relationship with nutrient levels may lead to more candidate species for species- or taxon-based indicators. However, although no significant relationship has been found between nutrient gradients and a species or phytoplankton group in one study, it would still be useful to test for the relationship in other areas or using other datasets.

The coccolithophorid *Emiliania huxleyi* occurs in the Kattegat and Skagerrak mainly in May–June but sometimes also later in summer. It can form blooms that are even visible from satellites. Coccolithophorids might be negatively affected by ocean acidification (e.g., Riebesell 2004). Following the biomass and species composition of the coccolithophorids allows a possible ocean acidification effect to be monitored. The proportion of potential eutrophication indicator species/groups (e.g., cyanobacteria and green algae) or the proportion of potential oligotrophication indicators (e.g., mixotrophic chrysophyceans and prymnesiophyceans) would also be interesting to evaluate for the Gulf of Bothnia and Baltic Proper.

**Seasonal succession index**

The seasonal succession index, first developed by Devlin et al. (2007) and then modified for the northern Baltic Proper by Jaanus (unpublished) could be tested for the Baltic Sea by including: 1) diatoms, 2) dinoflagellates, 3) cyanobacteria, and 4) *Mesodinium rubrum*. Instead of abundance, wet weight biomass should be used in the index (as in Jaanus unpublished). Species groups included in index should reflect the main phytoplankton groups in an area (in biomass) and the index should be adapted to the area studied. For the Kattegat and Skagerrak, diatoms and dinoflagellates should be included and other possible groups (e.g., Dictyochophytes and Prymnesiophyceans) should be evaluated depending on the data available.

**Size structure of phytoplankton community**

Several indices based on size spectra are being developed. The basic idea is that small phytoplankton dominate under oligotrophic conditions while larger species dominate
under eutrophic conditions. This is based on the concept of the microbial loop versus the classic food chain (Fenchel 1986).

The new Index of Size Spectra Sensitivity of Phytoplankton (ISS-Phyto) (Vadrucci et al. submitted), developed in the WISER project, could be evaluated once detailed information about the method has been published (Vadrucci et al. submitted). Since WISER was an EU project working on the development of new indicators for the WFD, it would be especially interesting to test the indicators developed and suggested by WISER. Several size-based methods have been tested (Lugoli et al. 2012, Revilla et al. 2009, Vadrucci et al. submitted) and the method chosen should be easy to apply to available data using the updated biovolume file of Olenina et al. (2006). It is important that the definitions of the phytoplankton groups be clear and it is desirable that neighbouring countries have a common system.

**Phytoplankton trophic index for coastal species?**

It would be useful to develop a phytoplankton trophic index for coastal species equivalent to the PTI index (Phillips et al. 2012) developed for lakes by the Northern Lakes Geographical Intercalibration Group. This would be very time consuming, however, and a very large dataset would be needed. Since a phytoplankton trophic index has indicator values that are weighted by abundance or, preferably, by biomass, the correct identification of the dominant species is most crucial (Nõges et al. 2010). The use of only the genus level or even higher taxonomical levels, as in the PTI index (Phillips et al. 2012), would reduce the risk of misidentification but also “even out” species-specific responses to nutrients or other pressures.

**5.3.4 Frequency of blooms**

**Frequency of elevated biomass and/or chlorophyll a**

When considering the frequency of high biomass, the question usually is: How often does the total phytoplankton biomass or chlorophyll $a$ exceed an area-specific long-term mean for all or part of the year? This can be evaluated with data from high-frequency stations (at least ~20 times/year) or for the whole year at stations sampled at least once per month. Here the instrumented oceanographic buoys described above and FerryBox systems may be used to increase sampling frequency in the near future. The Kattegat, Baltic Proper, and Gulf of Bothnia are sampled twice a week by the FerryBox system on the ship TransPaper, which traverses the Gothenburg–Kemi–Oulu–Husum–Lübeck–Gothenburg route every week. Water is currently sampled every other week, mainly in offshore areas.

**Frequency of elevated biomass of any single species or small and large phytoplankton**

The frequency of elevated counts of small and large phytoplankton as used in France, with the species community divided into the two size fractions >20 $\mu$m and 2–20 $\mu$m, as a
measure of algal blooms/frequency (Carletti and Heiskanen 2009), could be tested on a Swedish dataset as well (with the modification that biomass is used instead of abundances). However, it is not as straightforward as it may seem to divide different species into general size classes, because of their variable geometry.

Probably of greater interest is the frequency of elevated biomass of important phytoplankton groups and species, i.e., dominant species/groups or harmful algal bloom species (e.g., *Nodularia spumigena*, *Chrysochromulina* spp., *Dinophysis* spp., *Pseudochatonella* spp., and *Pseudo-nitzschia* spp.).

### 5.3.5 Intensity of blooms

Bloom intensity might be the most difficult parameter to achieve due to the lack of high-frequency and extensive spatial coverage in phytoplankton data. At stations where sampling was conducted at least every second week during the growing period, intensity could be measured as a combination of bloom duration (biomass exceeding a long-term mean) and the actual biomass increase above the long-term mean (measured as the integral below the biomass/time curve). The instrumented oceanographic buoys and FerryBox systems may also be useful here, to increase sampling frequency. Estimates of total phytoplankton biomass measured as chlorophyll *a* from satellites should be evaluated further.

For the open sea, surface accumulations of cyanobacteria in the Baltic Sea have been monitored using satellites since 1997 (Hansson 2006, Hansson and Öberg 2011). The monitored cyanobacteria are a mixture of mainly *Nodularia spumigena*, *Aphanizomenon* sp., and *Dolichospermum* spp. (verified by water sampling). Parameters monitored are bloom duration (days), extent (area coverage in km²), and intensity (area × duration = km² days) (Hansson and Öberg 2011). Satellite data are sampled at a high frequency under cloud-free conditions and cover large areas. Monitoring is limited, however, to open-sea areas due to shallow water effects (e.g., shallow depth and high concentration of suspended material) and land contamination of pixel data (Hansson and Öberg 2011). Furthermore, only near-surface accumulations are detected and not algae suspended deeper in the water column, and the species composition will always need to be validated by water samples. However, the new method used since 2010 enables monitoring closer to land than previously and it is now possible to detect blooms through scattered clouds (Hansson and Öberg 2011). In the future, despite its limitations, satellite surveillance might, when corrections for coastal waters have been refined, coarsely approximate the frequency and intensity of surface accumulations of cyanobacteria in the Baltic Sea.

### 5.4 Assessment period considerations

Summer was used as an assessment period, as it generally is today, because it was considered a period with small variability, meaning that fewer samples are needed to detect changes than, for example, in spring (Samuelsson et al. 2004). Other assessment periods, all or parts of the year, may be of interest in studies of seasonal succession and
bloom frequencies at high-frequency stations. To have an assessment system directly intercomparable with those of other countries in the North-East Atlantic area (which includes the Skagerrak and Kattegat), chlorophyll values for the March–October period should be assessed.

In the Bothnian Sea and Bothnian Bay, the spring bloom can influence the existing assessment system when the month of June is included, suggesting that the period included in the method should be adjusted, at least for this part of the coast.

To enlarge the dataset and reduce the uncertainty, data for several years should be used. The assessment should therefore be based on mean values for three years to reduce the influence of extremes (Samuelsson et al. 2004).
6 Conclusions: High-priority phytoplankton indicators for Swedish coastal waters

Not all the above indicators can be evaluated in the WATERS research project. We plan to focus on the following phytoplankton indicators (see also Table 6.1). The selected indicators will be evaluated by analysing existing data (i.e., from national and regional monitoring programmes) as well as data from the gradient studies (one in the Tjörn–Orust area on the Swedish west coast and one in the Östergötland coastal area, Baltic Proper) conducted in the WATERS project (summer 2012 and 2013).

Total biomass indicators

- The optimal assessment period for the existing biovolume and chlorophyll $a$ parameters will be evaluated in the phytoplankton work package of WATERS.
- Test the 90th-percentile chlorophyll $a$ values for the March–October period (Kattegatt and Skagerrak) used by other countries around the North-East Atlantic.
- Evaluate the use of carbon content compared with biovolume (all areas, summer).

Taxonomic composition

- Ratio of nitrogen-fixing cyanobacteria (i.e., *Nodularia spumigena*, *Aphanizomenon* sp., and *Dolichospermum* spp.) to total biomass (%) (Bothnian Bay, Bothnian Sea, and Baltic Proper, summer).
- Ratio of potential eutrophication indicator species/groups (e.g., filamentous cyanobacteria and green algae) or of potential oligotrophication indicators (e.g., mixotrophic chrysophyceans and prymnesiophyceans) to total biomass (%) (Gulf of Bothnia and Baltic Proper).
- Ratio of the diatom genera *Dactyliosolen* and *Cerataulina* to total biomass (%) (Kattegat and Skagerrak, summer).
- Biomass of key indicator species: for example, *Nodularia spumigena*, *Aphanizomenon* sp., and Prymnesiales (Bothnian Bay, Bothnian Sea, and Baltic Proper) and *Pseudoactinocyclus farcinus* (spring) and *Dinophysis* spp. (summer) (Kattegat and Skagerrak). Other species/genera might also be tested depending on available data, preferably dominant species, toxic species, and species/groups that respond clearly to a stressor such as eutrophication.
Stations conducting high-frequency sampling in the national monitoring programme are representatively situated in various sea areas around Sweden, i.e., the Gulf of Bothnia, Northern Baltic Proper, Kattegat, and Skagerrak. Data from these stations can be used to detect changes in the phytoplankton community that might not be captured by sampling only once per month or only in summer. For these stations, we suggest testing the following additional indicators:

**Taxonomic composition**

- Seasonal succession of dominant groups (biovolume): dinoflagellates, diatoms, cyanobacteria, and *Mesodinium rubrum* for the Baltic Sea, and diatoms, dinoflagellates, and dominant groups for the Kattegat and Skagerrak.

**Frequency of blooms**

- Frequency of elevated biovolume, carbon, and chlorophyll *a* based on data for the whole year.
TABLE 6.1
Possible phytoplankton indicators for Swedish coastal waters (see chapter 5) and what could be evaluated in the WATERS project (marked with X).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Indicator</th>
<th>WATERS</th>
<th>WATERS, high-frequency stations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biomass</td>
<td>Chlorophyll <em>a</em></td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>Biovolume</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>Carbon</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Abundance</td>
<td><em>Nodularia spumigena, Pseudoachattonella farcimem, Dinophysis spp., and Pseudo-nitzschia spp.</em></td>
<td>_¹</td>
<td>_¹</td>
</tr>
<tr>
<td>Taxonomic composition</td>
<td>Proportion of cyanobacteria</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Proportion of diatom genera <em>Dactyliosolen</em> and <em>Cerataulina</em></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Proportion of groups/species characteristic of eutrophication or oligotrophication</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Seasonal succession</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>Size structure indices</td>
<td>—</td>
<td>(X)_²</td>
</tr>
<tr>
<td></td>
<td>Index species (biomass): e.g., <em>Nodularia spumigena</em>, <em>Pseudoachattonella spp.</em>, <em>Dinophysis spp.</em>, <em>Pseudo-nitzschia spp.</em></td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>Coccolithophorids</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Harmful algae (see above index species)</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>Phytoplankton trophic index for Baltic and Kattegat–Skagerrak species</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Frequency of blooms</td>
<td>Frequency of elevated biomass or chlorophyll <em>a</em></td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>Frequency of elevated chlorophyll fluorescence (based on automated systems)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Frequency of elevated biomass of small and large phytoplankton</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Intensity of blooms</td>
<td>Biomass exceeding a long-term mean and the actual biomass increase above the long-term mean</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

¹ Instead of abundance we propose using biomass.

² The Index of Size spectra Sensitivity of Phytoplankton (ISS-Phyto) is suggested by the WISER project. When the original description of the index is published (Vadrucci et al. submitted) we will evaluate whether it should be tested in the WATERS project with data from the frequently sampled stations.
References


2010/477/EU (2010). EU Commission decision of 1 September 2010 on criteria and methodological standards on good environmental status of marine waters


Kratzer S., Vinterhav C. (2010.) Improvement of MERIS level 2 products in Baltic Sea coastal areas by applying the Improved Contrast between Ocean and Land processor (ICOL) – data analysis and validation. Oceanologia, 52:211–236.


OSPAR (2005). Ecological Quality Objectives for the Greater North Sea with regard to nutrients and eutrophication effects. OSPAR Background Document on Eutrophication, OSPAR Commission.


Annex

**Appendix 1**

Existing phytoplankton assessment systems for countries in the Baltic GIG (WFD Intercalibration Phase 2: Milestone report 5, Birk et al. 2010 and Carletti and Heiskanen 2009).

<table>
<thead>
<tr>
<th>Member State</th>
<th>Full BQE method</th>
<th>Taxonomic composition</th>
<th>Abundance (or cover)</th>
<th>Frequency and intensity of algal blooms</th>
<th>Biomass</th>
<th>Combination rule of metrics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Denmark</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>• Summer (May–September) mean chlorophyll a concentration or 90th percentile chlorophyll a concentration (March–September)</td>
<td>• No combination</td>
</tr>
<tr>
<td>Estonia</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>• Median chlorophyll a concentration • Total median wet weight autotrophic biomass (including autotrophic ciliate <em>Mesodinium rubrum</em>) (mg/L) (June–September)</td>
<td>• Average of chlorophyll a and biovolume</td>
</tr>
<tr>
<td>Finland</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>• Mean chlorophyll a • Total biomass (mg/L) (July–September)</td>
<td>• Total biomass is not yet officially accepted as a national classification metric • Combination rule will be average of the EQR values of chlorophyll and biovolume</td>
</tr>
<tr>
<td>Country</td>
<td>Starting</td>
<td>Biovolume of Cyanophytes (only in the eastern part); biovolume of Chlorophytes (only in the eastern part)</td>
<td>No</td>
<td>No</td>
<td>• Chlorophyll a concentration (µg/L) • Total biomass (biovolume [mm³/L]) (May–September)</td>
<td>• Weighted average if taxonomic composition can be included; otherwise, the average of total biovolume and chlorophyll a concentration</td>
</tr>
<tr>
<td>----------</td>
<td>----------</td>
<td>-------------------------------------------------------------------------------------------------</td>
<td>----</td>
<td>----</td>
<td>--------------------------------------------------------------------------------------</td>
<td>------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Germany</td>
<td>No</td>
<td></td>
<td>No</td>
<td>No</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Latvia</td>
<td>No</td>
<td></td>
<td>No</td>
<td>No</td>
<td>• Mean chlorophyll a concentration • Mean biovolume (mg/m³) (June–September)</td>
<td>• Average of chlorophyll a and biovolume</td>
</tr>
<tr>
<td>Lithuania</td>
<td>No</td>
<td></td>
<td>No</td>
<td>No</td>
<td>• Mean Chlorophyll a concentration • Mean total biomass (mg/L) (June–September)</td>
<td>• No official rules for combination • Average of chlorophyll a and total biomass is considered</td>
</tr>
<tr>
<td>Poland</td>
<td>No</td>
<td></td>
<td>No</td>
<td>No</td>
<td>• Mean Chlorophyll a concentration • Mean total biomass (mg/L) (June–September)</td>
<td>• Mean of chlorophyll a and biovolume</td>
</tr>
<tr>
<td>Sweden</td>
<td>No</td>
<td></td>
<td>No</td>
<td>No</td>
<td>• Chlorophyll a concentration (µg/L) • Biomass of autotrophic and mixotrophic phytoplankton expressed as total biovolume (mm³/L) (if available) • June–August mean of at least three of the last six years</td>
<td>• Weighted classification average • As biovolume and chlorophyll data are available, they should be cofactored into one standardized status classification for phytoplankton. If there are no data for any of these parameters, the classification is based on the remaining parameters.</td>
</tr>
</tbody>
</table>
## Appendix 2

Existing phytoplankton assessment systems for countries in the North-East Atlantic GIG (WFD Intercalibration Phase 2: Milestone report 6, Birk et al. 2010 and Carletti and Heiskanen 2009).

<table>
<thead>
<tr>
<th>Member State</th>
<th>Full BQE method</th>
<th>Taxonomic composition</th>
<th>Abundance (or cover)</th>
<th>Frequency and intensity of algal blooms</th>
<th>Biomass</th>
<th>Combination rule of metrics</th>
</tr>
</thead>
<tbody>
<tr>
<td>UK</td>
<td>Yes</td>
<td>Seasonal succession of functional groups (diatoms, dinoflagellates, microflagellates, and Phaeocystis)</td>
<td>Used in frequency parameter</td>
<td>Frequency of elevated counts of 1) any single taxa 2) of Phaeocystis 3) total cell count</td>
<td>90th-percentile chlorophyll a</td>
<td>Average of metrics</td>
</tr>
<tr>
<td>Ireland</td>
<td>No</td>
<td>Not planned due to high natural variability</td>
<td>Used in frequency parameter</td>
<td>Frequency of elevated counts of any single taxa</td>
<td>90th-percentile and median chlorophyll a (worst class taken)</td>
<td>Average of biomass and bloom frequency metric</td>
</tr>
<tr>
<td>Sweden</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Chlorophyll a (mean summer) Biovolume (mean summer)</td>
<td>Weighted average metric scores (chlorophyll a and biomass)</td>
</tr>
<tr>
<td>Norway</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>90th-percentile chlorophyll a and cell carbon</td>
<td>No (under development, weighted average of metrics)</td>
</tr>
<tr>
<td>Denmark</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>90th-percentile chlorophyll a</td>
<td>No combination yet</td>
</tr>
<tr>
<td>Germany</td>
<td>No</td>
<td>No</td>
<td>Only one species in frequency parameter</td>
<td>Frequency of elevated counts of Phaeocystis</td>
<td>90th-percentile chlorophyll a</td>
<td>Phaeocystis cannot enhance the assessment results derived from chlorophyll a concentrations</td>
</tr>
<tr>
<td>Country</td>
<td>Frequency in frequency parameter</td>
<td>Frequency of elevated counts of <em>Phaeocystis</em></td>
<td>90th-percentile chlorophyll (a)</td>
<td>Conditional average of both metrics; if the chlorophyll (a) metric has a score lower than <em>Phaeocystis</em>, then only the chlorophyll (a) is determined</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-------------</td>
<td>----------------------------------</td>
<td>---------------------------------------------</td>
<td>----------------------------------</td>
<td>------------------------------------------------------------------------------------------------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>The Netherlands</td>
<td>No</td>
<td>Only one species in frequency parameter</td>
<td>90th-percentile chlorophyll (a)</td>
<td>Conditional average of both metrics; if the chlorophyll (a) metric has a score lower than <em>Phaeocystis</em>, then only the chlorophyll (a) is determined</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Belgium</td>
<td>No</td>
<td>Only one species in frequency parameter</td>
<td>90th-percentile chlorophyll (a)</td>
<td>&quot;One out–all out&quot;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>France</td>
<td>No</td>
<td>Used in frequency parameter</td>
<td>90th-percentile chlorophyll (a)</td>
<td>Average of biomass and bloom frequency metric</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spain</td>
<td>No</td>
<td>Used in frequency parameter</td>
<td>90th-percentile chlorophyll (a)</td>
<td>Average of biomass and bloom frequency metric</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Portugal</td>
<td>No</td>
<td>Used in frequency parameter</td>
<td>90th-percentile chlorophyll (a)</td>
<td>Average of biomass and bloom frequency metric</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Appendix 3

Existing phytoplankton assessment systems for countries in the Mediterranean GIG (WFD Intercalibration Phase 2: Milestone report 5, Birk et al. 2010 and Carletti and Heiskanen 2009).

<table>
<thead>
<tr>
<th>Member State</th>
<th>Full BQE method</th>
<th>Taxonomic composition</th>
<th>Abundance (or cover)</th>
<th>Frequency and intensity of algal blooms</th>
<th>Biomass</th>
<th>Combination rule of metrics</th>
</tr>
</thead>
<tbody>
<tr>
<td>France</td>
<td>Yes¹</td>
<td>Work in progress</td>
<td>No</td>
<td>Not presented in IC work</td>
<td>Chlorophyll a</td>
<td>No combination</td>
</tr>
<tr>
<td>Spain</td>
<td>Yes¹</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Chlorophyll a</td>
<td>No combination</td>
</tr>
<tr>
<td>Italy</td>
<td>Yes¹</td>
<td>Work in progress</td>
<td>No</td>
<td>Work in progress</td>
<td>Chlorophyll a</td>
<td>No combination</td>
</tr>
<tr>
<td>Slovenia</td>
<td>No</td>
<td>Work in progress</td>
<td>Work in progress</td>
<td>Work in progress</td>
<td>Chlorophyll a</td>
<td>No combination</td>
</tr>
<tr>
<td>Cyprus</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Chlorophyll a</td>
<td>No combination</td>
</tr>
<tr>
<td>Croatia</td>
<td>No</td>
<td>Work in progress</td>
<td>Work in progress</td>
<td>Work in progress</td>
<td>Chlorophyll a</td>
<td>No combination</td>
</tr>
</tbody>
</table>

¹ The countries in the Mediterranean GIG have agreed to use only chlorophyll a as the parameter for phytoplankton BQE.
Appendix 4

Existing phytoplankton assessment systems for countries in the Black Sea GIG (WFD Intercalibration Phase 2: Milestone report 4b, Birk et al. 2010 and Carletti and Heiskanen 2009).

<table>
<thead>
<tr>
<th>Member State</th>
<th>Full BQE method</th>
<th>Taxonomic composition</th>
<th>Abundance (or cover)</th>
<th>Diversity (non-mandatory parameter)</th>
<th>Frequency and intensity of algal blooms</th>
<th>Biomass</th>
<th>Combination rule of metrics</th>
</tr>
</thead>
</table>
| Bulgaria and Romania  | No, but nearly complete  | 1) C strategy species colonists, as a proportion of total abundance of Dinoflagellates (summer)  
2) The sum of the abundance of species of three taxonomic groups (microflagellates + Euglenophyceae + Cyanophyceae) as a per cent of the total abundance (summer) | Total abundance (cells L⁻¹) | Menhinick index Sheldon index       | No                                     | • Total biomass (mg m⁻³), chlorophyll a (µg L⁻¹)  
• Values are seasonal to reflect the great seasonal variability of phytoplankton development. | Average metric scores |
Appendix 5

Overview of coastal (i.e., regional) monitoring programmes (based in information from www.vattenmyndigheterna.se).

**Bothnian Bay**

*National monitoring, commissioned by SwAM*

Phytoplankton monitoring is conducted at stations Råneå-1 and Råneå 2; phytoplankton are analysed as biovolumes at station Råneå-1 only.

*Regional monitoring*

No regional monitoring by water quality associations.

**Bothnian Sea**

*National monitoring, commissioned by SwAM*

Phytoplankton monitoring is conducted at stations B7, NB-1, and Gavik 1; phytoplankton are analysed as biovolumes.

*Regional monitoring*

Water quality association of Ljusnan-Voxnans Vattenvårdsförbund is conducting monitoring along the coast near river mouth of the rivers Ljusnan and Voxnan; no phytoplankton monitoring is conducted.

Water quality association of Dalälvens Vattenvårdsförening is conducting monitoring at four stations in the Bothnian Sea near the river mouth of River Dalälven; no phytoplankton monitoring is conducted.

Water quality association of Gästriklands Vattenvårdsförening is conducting monitoring in Gälve fjärdar and Norrsundet; no phytoplankton monitoring is conducted.

**Northern Baltic Proper**

*National monitoring, commissioned by SwAM*

Phytoplankton monitoring is conducted at station B1 near Askö; phytoplankton are analysed as biovolumes.

*Regional monitoring*

Water quality association of Svealands Kustvattenvårdsförbund is conducting monitoring along the coast of Svealand twice every summer (July and August); phytoplankton are analysed as biovolumes at some of the monitoring stations and chlorophyll $a$ is measured at all stations.

The water quality association of Motala ström is conducting monitoring along the coast of Östergötland, phytoplankton are sampled at three stations and biovolumes are analysed.
Southern Baltic Proper

National monitoring, commissioned by SwAM

Phytoplankton are monitored at station REF M1V1 near Kalmar; phytoplankton are analysed as biovolumes.

Regional monitoring

The water quality association of Kalmars Kustvattenvårdsförbund – Samordnad kustvattenkontroll i Kalmar län is conducting monitoring along the coast of the county of Kalmar; no phytoplankton monitoring is conducted.

The water quality association of Blekingekustens Vattenvårdsförbund is conducting monitoring along the coast of the county of Blekinge; no phytoplankton monitoring is conducted.

The water quality association of Vattenvårdförbundet för västra Hanöbukten is conducting monitoring in the Bight of Hanö; no phytoplankton monitoring is conducted.

The water quality association Sydkustens Vattenvårdsförbund monitors phytoplankton at the Falsterbo and Abbekås stations; phytoplankton are analysed as biovolumes.

The Sound, Kattegat, and Skagerrak

National monitoring, commissioned by SwAM

Phytoplankton monitoring is conducted at station N14 near Falkenberg and at station Släggö near Lysekil; phytoplankton are analysed as biovolumes and chlorophyll a is measured.

Regional monitoring

The Öresunds Kustvattenvårdsförbund water quality association monitors phytoplankton at four stations in the Sound, but no biovolumes are analysed.

The Nordvästskånes Kustvattenkommitté water quality association monitors phytoplankton at station S1 in Skälderviken; phytoplankton are analysed as biovolumes.

The Hallands kustvattenkontroll water quality association monitors phytoplankton at station L9 in Laholm Bay and at station N7 near Nidingen, Falsterbo. Phytoplankton are analysed as biovolumes.

The Bohuskustens Vattenvårdsförbund water quality association monitors phytoplankton at six stations; phytoplankton are analysed as biovolumes.
Overview of coastal phytoplankton indicators and their potential use in Swedish waters

An assessment system for phytoplankton fully compliant with the Water Framework Directive (WFD) should include all of the following parameters: biomass, taxonomic composition, abundance (or cover), frequency and intensity of algal blooms. Today only biomass, measured as biovolume and chlorophyll $a$, is used in the Swedish assessment system for coastal phytoplankton. This report summarizes the phytoplankton indicators other European countries use and have tested, and suggests potential phytoplankton indicators for Swedish coastal areas. Some of these will be further explored in the WATERS programme based on existing phytoplankton data and from new field studies along eutrophication gradients.