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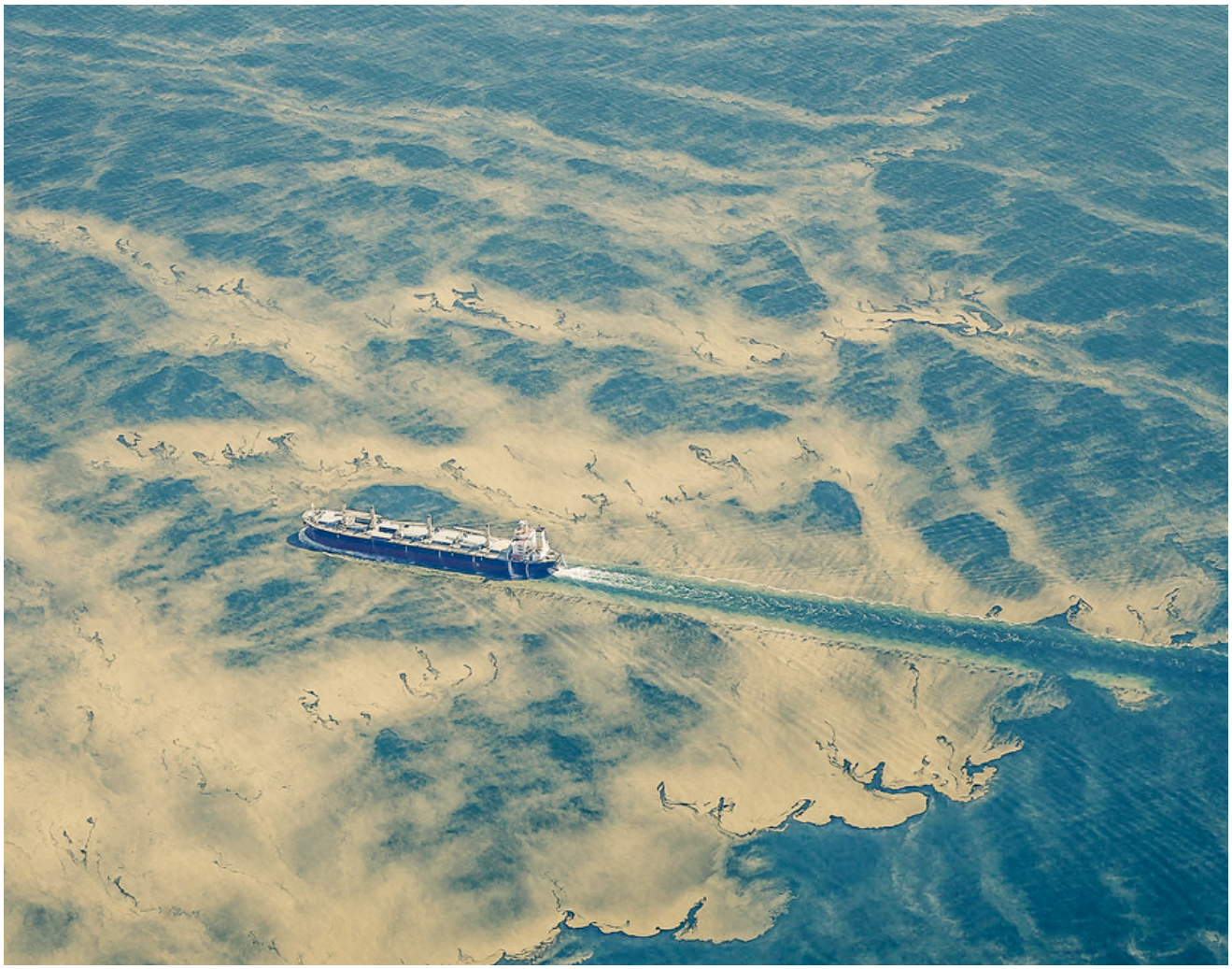
Swedish Agency for Marine and Water Management

Monitoring Methods of Phytoplankton in the Baltic Sea and Kattegat-Skagerrak



Swedish Agency for Marine and Water Management report 2014:26

Monitoring Methods of Phytoplankton in the Baltic Sea and Kattegat-Skagerrak

Bengt Karlson

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The report is written by Bengt Karlson, Swedish Meteorological and Hydrological Institute, oceanographic unit, on commission from the Swedish Agency for Marine and Water Management. The author is responsible for interpretations and conclusions.

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Swedish summary/Svensk sammanfattning

Rapporten syftar till att ge underlag för revison av svensk nationell marin miljöövervakning relaterad till växtplankton. Sveriges miljömål, EU:s Havsmiljödirektiv och Vattendirektiv samt Helsingfors- och Oslo-Paris-konventionen ställer krav på växtplanktonövervakning. Speciella krav gällande övervakning av algblomningar av biotoxinproducerande arter gäller i områden där det bedrivs akvakultur (EU:s hygiendirektiv). Dessutom ger förändringar relaterade till ett förändrat klimat ytterligare anledningar till förbättrad växtplanktonövervakning. Förslag i korthet:

1. Var försiktig vid förändringar av existerande långsiktig miljö-övervakning. Ändra inte metodik när det finns långa tidsserier utan lägg till ny metodik och nya parametrar.

Förändringar som kan genomföras från år 2015

2. Fortsätt med samma analysmetodik för växtplankton som idag (Utermöhl-metoden) men lägg till analys av en större volym för att fånga upp ovanliga arter och mikrozooplankton.
3. Använd kol som enhet för växtplanktonbiomassa istället för biovolym.
4. Säkerställ att samma metodik används i alla havsområden runt Sverige.
    a. Lägg till autotrofa picoplankton där det saknas (egentliga Östersjön och Västerhavet).
    b. Klorofyllmätningar bör ske både i slangprover och i prover från vattenhämtare i alla områden (Bottniska viken avviker idag).
5. Högfrekvent provtagning bör ske var fjortonde dag på så kallade vaktpost stationer (sentinel sites), varje vecka är lämpligt under blomningar.
6. Alla större havsbassänger runt Sverige bör ha en högfrekvent utsjöstation och en högfrekvent kuststation för högkvalitativ växtplanktonövervakning av artsammansättning, cellantal och biomassa baserad på cellvolymsmätningar. Dessutom bör högfrekvent provtagning av klorofyll ske vid tre utsjöstationer och tre kuststationer.
7. Använd så kallade FerryBox-system för att höja provtagningsfrekvensen (vattenprover) och för att mäta klorofyllfluorescens, en så kallad proxy för växtplanktonbiomassa.
8. Mät phycocyaninfluorescens vid CTD-kast under miljöövervaknings-expeditioner för att få ett ungefärligt mått på utbredningen av cyanobakterier i djupled.
9. Mät ljus i luft och i vatten vid CTD-kast vid miljöövervaknings-expeditioner för att kunna beräkna ljustutsläckningskoefficient vid utvalda våglängder

Förändringar som bör utvärderas under 1-3 år för att införas t.ex. år 2018

10. Dokumentera växtplankton genom digital fotografering vid mikroskopering. Spara bilder hos den nationella datavärden

11. Spara planktonprover i en provbank för framtida analys med metoder som inte är kända idag.
12. Inför så kallad Automated Imaging Flow Cytometry för analys av växtplankton som komplement till mikroskopi
13. Inför molekylärbiologisk metodik, t.ex. barcoding av 16S och 18S rDNA, som komplement till optiska analyser
14. Använd det nya nätverket av oceanografiska mätbojar runt Sveriges kuster för mätning av klorofyllfluorescens, ljusutsläckning vid utvalda våglängder (~siktdjup), samt för automatisk växtplanktonprovtagning
15. Inför satellitbaserad fjärranalys för mätning klorofyll, utbredning av cyanobakterieblomningar samt blomningar av coccolithophorider som en integrerad del av den nationella marina miljöövervakningen. De nya ESA satelliterna Sentinel 3a och 3b (uppskjutning planerad tidigast i april 2015) bör användas för mätning av så kallad ocean colour. Data-kvalitet måste kontrolleras genom jämförelser med in situ mätningar.

English summary

The aim of the report is to give input to the revision of the Swedish National Marine Monitoring Program with regard to phytoplankton. The Swedish environmental objectives, the EU Marine Strategy Framework Directive, the Water Directive as well as the Helsinki and Oslo-Paris conventions all include requirements for phytoplankton monitoring. In areas where aquaculture is carried out special demands for monitoring harmful algae, i.e. biotoxin producing species, are in effect (EU hygiene directive). Climate change also result in needs for improved phytoplankton monitoring. A summary of suggestions:

1. Use caution when making changes in long term monitoring programs. Do not change methodology if there are long time series based on a certain method; instead add new methods and new parameters.

Changes that can be implemented in year 2015

2. Continue using existing analysis method for phytoplankton (the Utermöhl method) but add analysis of large volume samples to get better data on rare species and micro-zooplankton.
3. Use carbon as the unit for phytoplankton biomass instead of bio-volume.
4. Make sure that the same methods are used in all sea areas surrounding Sweden.
    a. Add analysis of autotrophic picoplankton where this is missing (the Baltic Proper, the Kattegat and the Skagerrak)
    b. Chlorophyll analyses should be made both on samples collected using tube sampling and samples collected at discrete depths (the Gulf of Bothnia is the sea area that differ from the others)
5. High frequent sampling should be carried out at sentinel sites every two weeks, weekly during algal blooms.
6. All major sea basins surrounding Sweden should have one high frequent off shore sentinel site and one high frequent coastal sentinel site for high quality phytoplankton monitoring for biodiversity, cell numbers and biomass based on cell volume measurements. In addition high frequent sampling for chlorophyll should be carried out at three off shore and three coastal sites in each major basin.
7. Use FerryBox-systems to increase the water sampling frequency and to measure chlorophyll fluorescence, a proxy for phytoplankton biomass.
8. Measure the fluorescence for phycocyanin when doing CTD-casts during monitoring cruises with research vessels to get information on the vertical distribution of cyanobacteria.
9. Carry out measurements of irradiance in air and in water when making CTD-casts during monitoring cruises to calculate the attenuation coefficient at selected wavelengths.

Changes that should be evaluated during one to three years to be fully implemented e.g. in 2018

10. Document phytoplankton using digital photography during microscopy. Save images at the national data host archive
11. Save phytoplankton in a sample bank for future analysis using methids unknown today.

12. Use automated imaging flow cytometry for phytoplankton analysis as a complement to microscopy.
13. Use molecular biological methodology, e.g. 16S and 18S rDNA barcoding, as a complement to biodiversity analysis methods based on analysing morphology of organisms.
14. Use the new network of coastal instrumented buoys around the coast of Sweden to measure chlorophyll fluorescence, light attenuation at selected wavelength (~Secchi depth) and for automated water sampling for phytoplankton analysis.
15. Integrate satellite remote sensing of ocean colour for estimating chlorophyll a, the distribution of cyanobacteria blooms and blooms of coccolithophorids in the National Marine Monitoring Programme. The new ESA satellites Sentinel 3a and 3b are planned to be launched at the earliest in April 2015. The quality of data must be compared to data from in situ sampling.

Introduction

Aim

The aim of this report is to provide information related to phytoplankton for the revision of the Swedish Marine Monitoring Programme to be implemented in 2015.

Overview of requirements from directives etc.

The Swedish environmental objectives (miljömålen)

Sweden has sixteen environmental objectives decided by the parliament. The following are most relevant for marine phytoplankton monitoring:

- Zero eutrophication
- A balanced marine environment, flourishing coastal areas and archipelagos
- A rich diversity of plant and animal life

Of great importance are also:

- Reduced climate impact
  - e.g. effects of climate change on the marine ecosystems and goods and services
- Natural acidification only
  - e.g. effects of ocean acidification

The EU Marine Strategy Framework Directive (MSFD)

The directive 2008/56/EC and commission decision of 1 September 2010 contains criteria for good environmental status. The following descriptors are highly relevant for phytoplankton:

> *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 ecosystem.
>
> *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.
>
> 4.3. Abundance/distribution of key trophic groups/species
>
> — Abundance trends of functionally important selected groups/species (4.3.1).
>
> 4.3.1— groups with fast turnover rates (e.g. phytoplankton, zooplankton, jellyfish, bivalve molluscs, short-living pelagic fish) that will respond quickly to ecosystem change and are useful as early warning indicators,
>
> Descriptor 5:
>
> Human-induced eutrophication is minimised, especially adverse effects thereof, such as losses in biodiversity, ecosystem degradation, harmful algal blooms and oxygen deficiency in bottom waters.

The EU Water Framework Directive (WFD)

Directive 2000/60/EC contains quality elements for the classification of ecological status. The following are relevant for phytoplankton in marine and transitional waters:

1.1.3. Transitional waters
Biological elements
Composition, abundance and biomass of phytoplankton

1.1.4. Coastal waters
Biological elements
Composition, abundance and biomass of phytoplankton

EU food safety regulations

There are several EU regulations that are related to food safety that have implications for phytoplankton monitoring. One is regulation 854/2004 that includes requirements for monitoring of biotoxin producing microalgae in areas where shellfish are harvested for human consumption. In Sweden this is at present (January 2014) only the part of the west coast from just south of Gothenburg to the Norwegian border.

Quotes from 854/2004:

Regulation (EC) No 854/2004 of the European Parliament and of the Council of 29 April 2004, laying down specific rules for the organisation of official controls on products of animal origin intended for human consumption.

B. MONITORING OF CLASSIFIED RELAYING AND PRODUCTION AREAS

1. Classified relaying and production areas must be periodically monitored to check:

for the presence of toxin-producing plankton in production and relaying waters and

biotoxins in live bivalve molluscs; and

4. Sampling plans to check for the presence of toxin-producing plankton in production and relaying waters and for biotoxins in live bivalve molluscs must take particular account of possible variations in the presence of plankton containing marine biotoxins. Sampling must comprise:

(a) periodic sampling to detect changes in the composition of plankton containing toxins and their geographical distribution. Results suggesting an accumulation of toxins in mollusc flesh must be followed by intensive sampling;

IMO Ballast Water Convention

The IMO Ballast Water Convention is likely to become ratified in the next few years. Monitoring of invasive species, including phytoplankton, in harbours is one of the requirements of the convention.

Climate change

Climate change has, at least in part, replaced eutrophication as the major concern for the seas. This is not reflected in the MSFD and the WFD. Global change is likely to affect phytoplankton in general and the distribution and intensity of harmful algal blooms but the problem is complex. Changes in temperature have direct effects on growth and distribution of species but also on the stratification of the seas. Indirect effects of changes in nutrient supply from the deep water and from river runoff also will be important, along with the direct riverine effects of increased stratification and decreased water transparency in coastal waters from input of humic substances. Ocean

acidification will also affect phytoplankton, with some species being favoured by increased CO_2, which also can increase cellular toxicity.

Ongoing phytoplankton monitoring programmes

National Marine Monitoring Programme

Phytoplankton is sampled approximately monthly at the stations indicated with red dots (see map in Fig. 1). At the high frequency stations B1, BY31, Släggö and Anholt E sampling is made approximately 24 times a year. A 10 m long tube is used for sampling, except for at stations B1 and BY31 where the sampling carried out by the Stockholm University is made using a 20 m tube. The HELCOM standard is 10 m. Acid Lugol's solution is used for preservation in general. Alkaline Lugol's is used for coccolithophorids in the Kattegat-Skagerrak during part of the year. Samples for autotrophic picoplankton are at present only collected in the Gulf of Bothnia and analysed by the Umeå University.

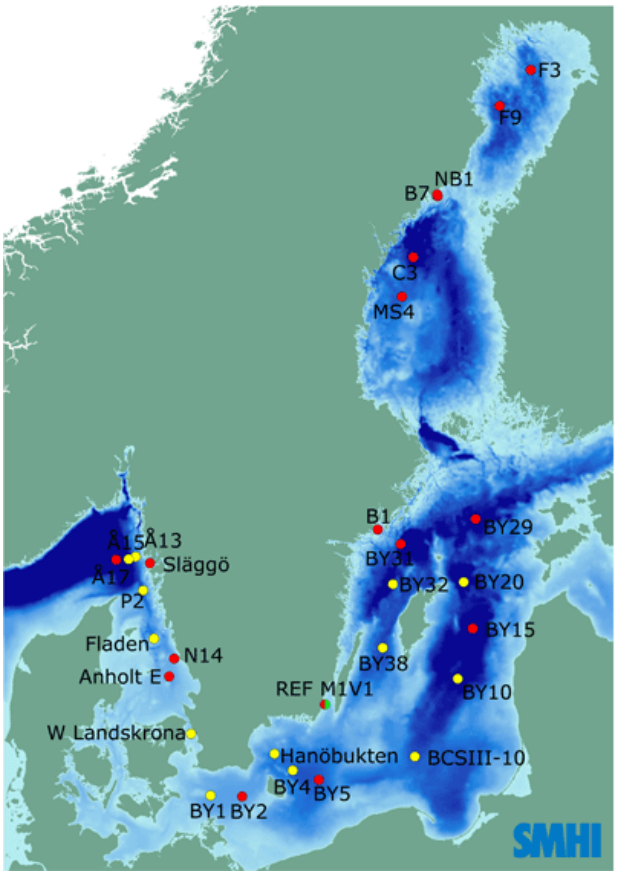

Fig 1. Map shows sampling locations in the National Marine Monitoring Program in 2013. Stations with red markers are funded by SWAM while those labelled with yellow markers are funded directly through SMHI.

Nationellt mätprogram (utförare: SMHI, UMF, SMF)
SMHIs utsjöprogram

Source

http://www.smhi.se/klimatdata/oceanografi/Havsmiljodata

Regional monitoring programmes

At present phytoplankton monitoring is ongoing in some of the regional monitoring programmes in Sweden. The focuses of the programmes differ considerably. Some include phytoplankton sampling year around. Others focus on the summer period only and some include phytoplankton sampling only if a discoloration of the surface water is detected during monitoring cruises. In general there is more frequent chlorophyll sampling than sampling of phytoplankton for species composition, abundance and biomass determination.

Table 1. Regional monitoring programmes listed at the National data host for marine biological and oceanographic data in January 2014. Note that the regular sampling in the Svealand Water Quality Association is missing. Only some of the regional monitoring programmes include phytoplankton monitoring. Source: http://www.smhi.se/klimatdata/oceanografi/Havsmiljodata

Bohuskustens VVF & Gullmarens KKP
Halland KKP
Nordvästskånes kustvattenkommitté
Öresund VVF
Sydkustens VVF
V Hanöbuktens VVF
Blekinges VVF
Kalmar läns KKP
Motala Ströms VVF
RMÖ Södermanland, Stockholm och Uppsala län Syd
RMÖ Södermanland, Stockholm och Uppsala län Mellan & Stockholm Vatten
RMÖ Södermanland, Stockholm och Uppsala län Nord
Dalälvens VVF
Gästriklands VVF
Ljusnans/Voxnans VVF
NÖ Hälsinglands VVF
Sundsvallsbuktens VVF & SRK Skatan
SRK Nedre Ångermanälven
Gaviksfjärdens KKP , RK Omnefjärden, Ullångersfjärden, Domsjö, Husum & Nätrafjärden
Ume- & Vindelälvens VVF & SRK i Västerbottens län
RK i Västerbottens län
SRK i Norrbottens län

Map of all phytoplankton sampling in 2010

A map of phytoplankton sample data reported to the Swedish National Oceanographic Data Centre is shown in Fig. 2. It includes data from both national and regional monitoring programmes as well as data from short term projects. In 2010 additional sampling funded by the Swedish Environmental Protection Agency through County Administrations Boards (Länstyrelser) was made.

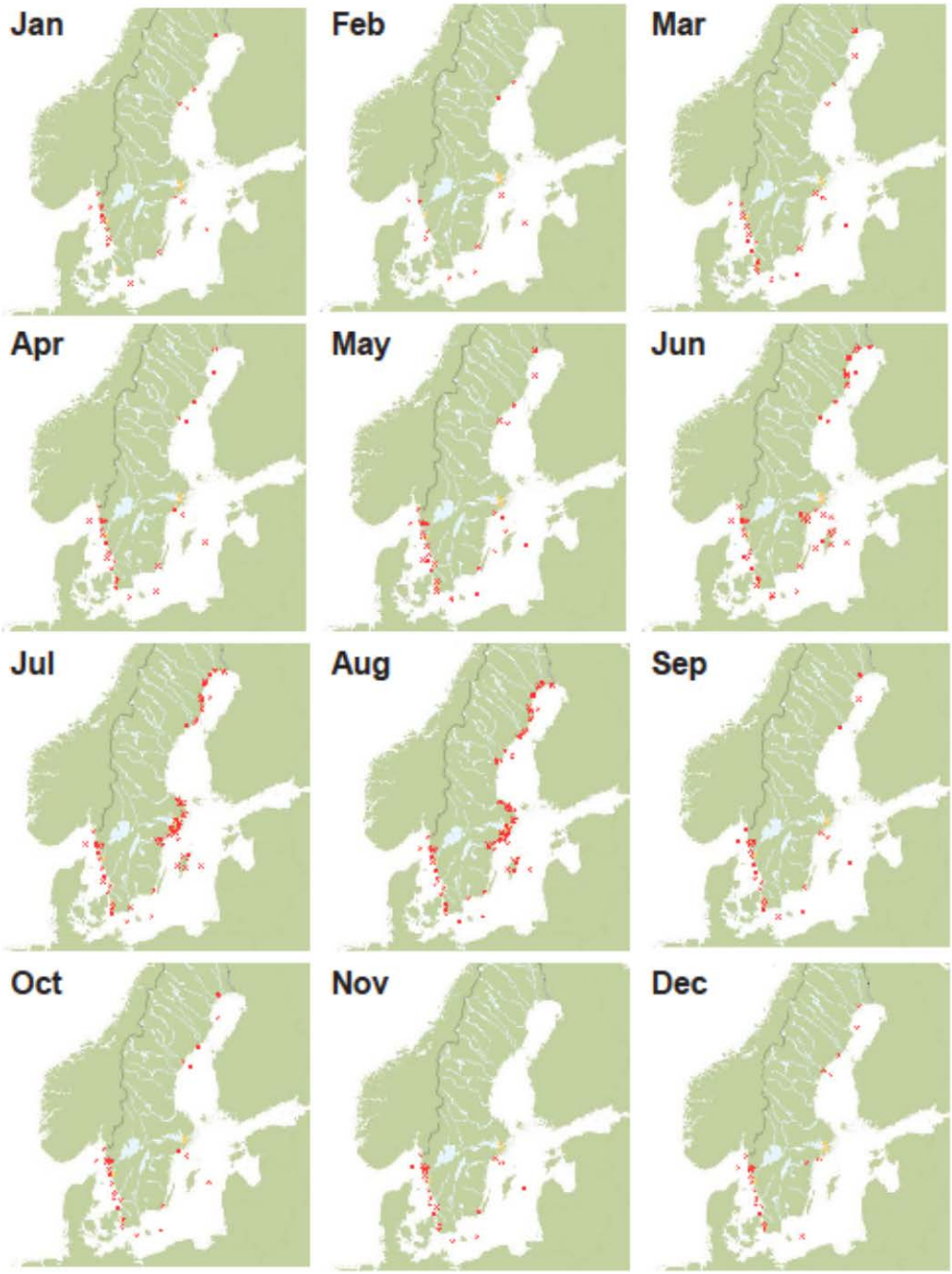

Fig. 2. Sampling locations for phytoplankton in 2010. Maps include samples from the national marine monitoring program, most regional monitoring programmes and short term project sampling. In 2010 additional sampling was carried out in summer compared to normal years. Source: www.shark.smhi.se

Swedish National Food Administration - monitoring for microalgae producing biotoxins

SMHI oceanographic unit in Gothenburg carries out analyses of phytoplankton samples collected in the *National monitoring program for marine biotoxins and fecal contamination in live bivalve molluscs* on commission from the National Food Administration. In Fig. 3 a map shows locations for samples collected in 2012.

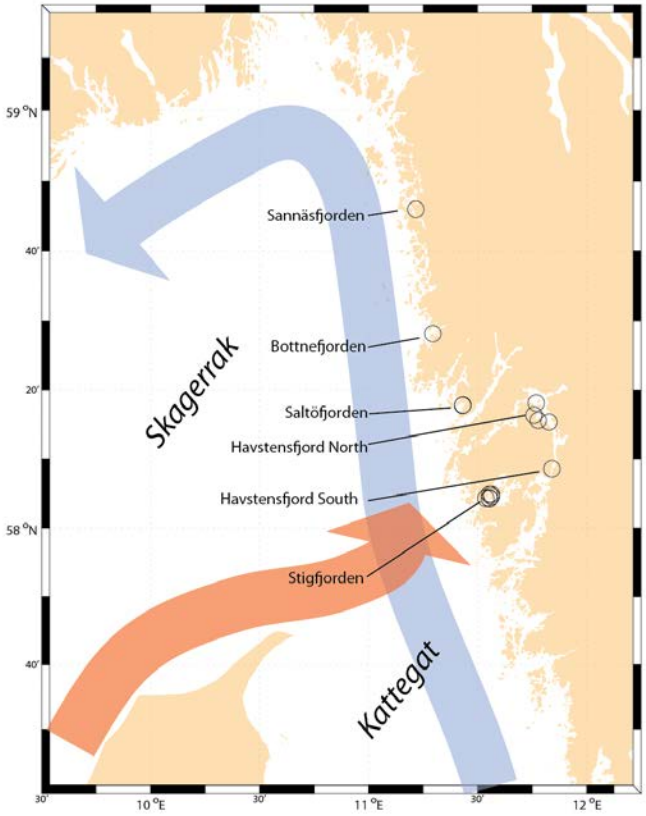

Fig. 3.Sampling locations for phytoplankton in the *National monitoring program for marine biotoxins and fecal contamination in live bivalve molluscs* in 2012.

Remote sensing of algal blooms

The only operational Swedish monitoring programme of phytoplankton using satellites is the Baltic Algae Watch System, operated by SMHI since 2002. At present mainly surface scums of cyanobacteria are detected. Observation period is 1 June to 31 August.

Available methods

Water sampling metods

To estimate phytoplankton composition (biodiversity), abundance and biomass water samples are needed. Remote sensing techniques and measurements of e.g. chlorophyll fluorescence only give proxy parameters for phytoplankton biomass. In situ imaging flow cytometry hold great promise to give species information but have not yet been evaluated in Swedish waters. There is also one *in situ* system based on molecular biology available commercially (the Environmental Sample processor) that gives information on selected species. This, remote sensing and flow cytometry are discussed further below.

Tube sampling

Tube sampling is the standard HELCOM method for collecting water samples for phytoplankton analyses. Samples for chlorophyll a should be collected from the tube and also from distinct depths. The water collected in the tube is mixed and sub samples are preserved using Lugol's solution and formaldehyde. The reason to use a 10 m long tube is to minimize the risk of missing thin layers of phytoplankton in the upper part of the sea. HELCOM COMBINE also allows for pooling of samples from 1, 2.5, 5, 7.5 and 10 m depth. Tubes may be cumbersome to use in winter because of stiffness at low temperatures, and in rough weather.

Sampling using Niskin or GoFlo bottles

To use the standard water sampling bottles that are used for collecting nutrient samples etc. is convenient for collecting phytoplankton samples. Most often the sampling bottles are mounted on a rosette frame together with sensors for e.g. chlorophyll fluorescence. This makes it possible to close sampling bottles in or near chlorophyll fluorescence maxima of phytoplankton. In this way e.g. samples of harmful algae that accumulate in thin layers may be collected.

Sampling using FerryBox-systems

Information on FerryBox-systems is found in Karlson et al. (2010). A map of FerryBox systems in the Baltic Sea is presented in Fig. 4. Many FerryBox-systems include water sampling devices that can collect e.g. 24 1 L water samples. The water sampling bottles are pre-filled with preservative. When the ship reaches e.g. a certain latitude a water sample is collected and the phytoplankton are preserved directly.

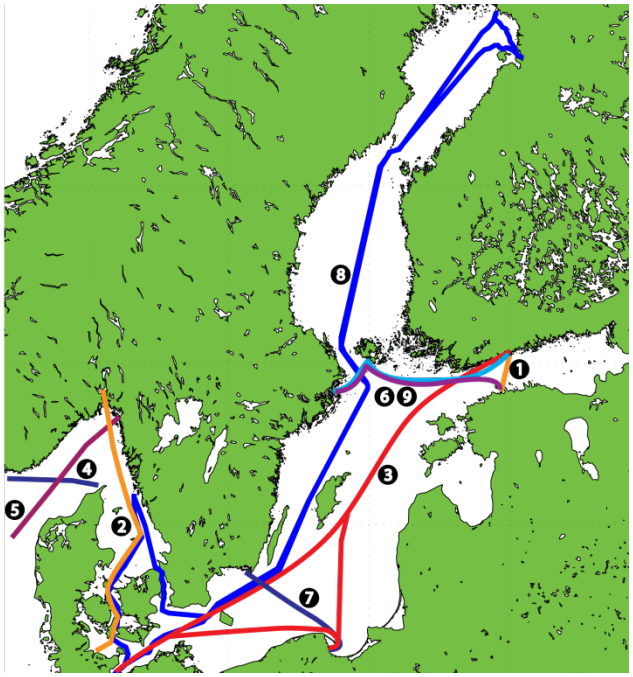

Fig. 4. FerryBox –systems in the Baltic Sea area. Route 8 is the ship TransPaper operated by SMHI. Route 7 is no longer in operation

Automated water samplers on buoys

In the United Kingdom CEFAS operates a system of oceanographic buoys. Some of these are fitted with automated water sampling devices that collect up to 50 samples in plastic bags prefilled with preservative. Triggering of sampling is made by a timer. A similar system is found in Chesapeake bay in the USA.

About net sampling and the Continuous Plankton Recorder

A phytoplankton net may be used to collect samples. Often a vertical net tow 20-0 m is made. Mesh size is mostly 10 or 20 µm. Unfortunately this method does not collect phytoplankton in a quantitative way. Sampled volume is unknown, small species pass through the net and fragile species break. However it is useful to collect rare species and to get an overview of the larger, robust, phytoplankton.

The Continuous Plankton Recorder (CPR) operated by the Sir Alister Hardy Foundation for Ocean Science (SAHFOS, www.sahfos.org) is a device that is towed behind merchant vessels. Plankton are collected on a silk mesh and preserved in formalin. Many zooplankton and some large, robust phytoplankton are collected. The data is only semi quantitative. Long time series of CPR-data has proven very valuable to identify changes in plankton communities. Samples from a CPR-route ending in Gothenburg are available at SAFHOS but the samples from Skagen-Gothenburg have not been analysed.

Abundance and biodiversity of phytoplankton

To determine the abundance of phytoplankton cells are counted in the microscope or using automated cell counters, e.g. Flow Cytometers. The unit for abundance is usually cells per Litre or cells per mL. To determine biodiversity or species composition organisms should be identified to the species level. Microscopy is the classical method and includes both light microscopy, fluorescence microscopy and electron microscopy. The latter is necessary if the biodiversity of smaller cells is needed. Molecular methods give information on the diversity at the genetic level. Most often the information is not directly comparably to cell counts. Microscopy and molecular methods for quantitative phytoplankton analyses are described in a UNESCO – IOC Handbook edited by Karlson et al. (2010).

Light microscopy

The most common method for quantitative analysis of phytoplankton is the Utermöhl method originally described in the 1930's but since then further developed and also described in the handbook. Organisms are concentrated through sedimentation. An inverted microscope with high quality optics is necessary to carry out the analyses. Qualified training in phytoplankton identification, taxonomy and systematics is very important to ensure high quality results. Also recurring inter calibrations/ring tests among persons carrying out analyses are important for consistent results. A disadvantage with the method is that organisms smaller than about 5-10 µm cannot be identified, at least no to the species level. Another problem is that a relatively small volume (10-20 mL) is most often analysed. This means that rare species may be overlooked.

Fig. 5 An inverted microscope suitable for analysing phytoplankton using the Utermöhl method. Photo by Bengt Karlson.

Fluorescence microscopy

Sample for fluorescence microscopy are most often concentrated through filtering. Fluorescence microscopy is used either with unstained samples that have autofluorescence, e.g. from photosynthetic pigments, or with stained samples. Common stains are fluorescent RNA-probes, calcofluor for thecate dinoflagellates and DAPI for cell nuclei. Fluorescence can be used with inverted or regular microscopes. A combination of staining with calcofluor and light microscopy with the Utermöhl method is presented by Edler and Elbrächter in the handbook mentioned above. Analysis of autotrophic picoplankton is often

carried out using fluorescence microscopy. The autofluorescence from phycobilins in cyanobacteria of *Synechococcus* type makes analysis simple. The sample is concentrated by filtering. A protocol for analysing autotrophic picoplankton in the Gulf of Bothnia is available from the University of Umeå, Norrby laboratory.

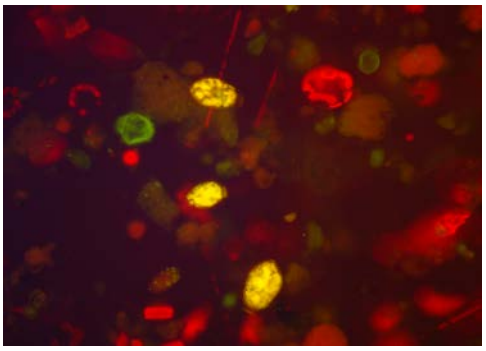

Figure 6. Fluorescence of phytoplankton as seen in the fluorescence microscope. Photo by Bengt Karlson.

Electron microscopy

Electron microscopy is necessary to identify many small phytoplankton organisms to the species level. Examples include the coccolithophorids and many other haptophytes, e.g. *Prymnesium polylepis*. This species was earlier known as *Chrysochromulina polylepis*. A bloom in 1988 had devastating effects on the marine ecosystem in the Kattegat and the Skagerrak. Many other phytoplankton are small and not identified using light microscopy. Electron microscopy is costly but necessary to increase the knowledge on the phytoplankton community structure. It is also needed when invasive species arrive and during harmful algal blooms of new species. It is suggested that Scanning Electron Microscopy and Transmission Electron Microscopy is included in the long term monitoring in one location in the Baltic Sea and in one location in the Skagerrak.

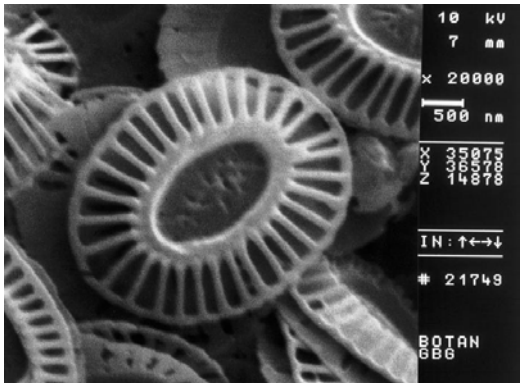

Figure 7. Coccoliths from *Emiliania huxleyi*. Scanning electron microscopy, photo by Bengt Karlson. Source: www.nordicmicroalgae.org

Flow Cytometry

Flow cytometers are particle counters that were originally developed for counting and differentiating blood cells. Most models use one or a few lasers for creating light that is used for exciting fluorescent particles. Also scattering properties of particles are used to differentiate cells. Early models used in the

late 1980´s filled half a cargo container since the lasers were large at that time. Today small bench top units are available. For phytoplankton research they were first mainly used for pico- and nanoplankton and the fluorescent and scattering properties of the algae were used to differentiate the algae to a very rough group level. Later imaging flow cytometers were developed, now available as in situ instruments (Sosik and Olsen 2007 and Olsen and Sosik, 2007). Fluorescence of chlorophyll is commonly used to trigger a camera and every particle with fluorescence, i.e. phytoplankton, is documented in a digital image. Automated image analysis is used to identify the organisms and to measure size etc. This is not perfect but works well today. Software must be trained by experts on local phytoplankton. Supervision by a phytoplankton specialist is of necessity. When new species are observed the software has to be trained for these. There are currently at least three imaging flow cytometers available commercially that are useful for phytoplankton analyses (Fig. 8). They are available both as desktop instruments and as *in situ* instruments deployable e.g. on oceanographic buoys.

- The FlowCam
  http://www.fluidimaging.com/products-particle-vision-pv-series.htm
- The CytoSense
  http://www.cytobuoy.com/
- The FlowCytoBot
  http://www.mclanelabs.com/master_page/product-type/samplers/imaging-flowcytobot

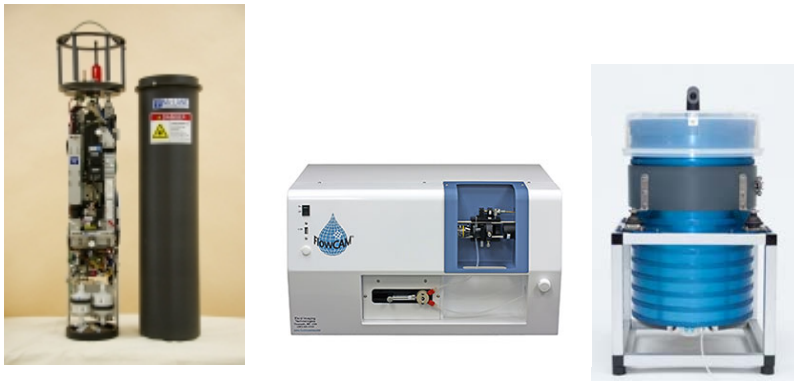

Fig. 8. Imaging flow cytometers from left to right: The FlowCytoBot from McLane, the FlowCam from FluidImaging and the CytoSense from CytoBuoy. Sources of images: FlowCam: FlowCytoBot: http://www.mclanelabs.com (7 October 2015), http://www.fluidimaging.com (7 October 2015), and Cytobuoy: http://www.cytobuoy.com (7 October 2015).

Zooscan

Another automated instrument for counting, sizing and identifying plankton is the Zooscan (Gorsky et al 2010). This is aimed at organisms larger than approximately 0.2 mm up to several centimetres i.e. zooplankton and larger phyto- and micro-zooplankton. The system may be suitable e.g. for colonies of cyanobacteria but microscopy will still be needed. Samples are analysed in the laboratory. The throughput of zooplankton samples is claimed to be 100x higher than for microscopy analyses. The image analysis software needs training by a zooplankton specialist knowledgeable of the local zooplankton to be analysed. Supervision by a zooplankton specialist is of necessity.

Figure 9. The Zooscan. Source http://www.hydroptic.com (7 October 2015).

Laboratoire Océanographie de Villefranche sur Mer

http://www.obs-vlfr.fr/LOV/ZooPart/ZooScan/

Molecular Biological Methods

Molecular methods in phytoplankton have evolved rapidly the last decades. Only some of the methods have recently been implemented in monitoring programs. The methods can roughly be divided into three categories:

1. Molecular probes targeting single species, analysis by fluorescence microscopy
    a. example: fluorescent probes targeting *Alexandrium* sp. (a producer of Paralytic Shellfish Toxins) are used or have been tested in monitoring for harmful algae in Scotland and in New Zealand.
2. Molecular probes aimed at identifying multiple species
    a. Example. In the EU project MIDTAL probes for selected harmful algae were developed. A protocol for preparing and analysing the harmful algae in a mix of phytoplankton was developed. Samples are concentrated through filtering or centrifuging.
3. Analyses of genes from the whole plankton community. Lowered cost for sequencing has made it possible to sequence genes from many samples. Genes specific for selected species or genera may be detected. The samples analysed may be concentrated through filtering. Filtered samples include a mix of pelagic bacteria. phytoplankton and microzooplankton. A method used in the Baltic Sea for pelagic bacteria (Andersson et al. 2010) is currently being developed further to include phytoplankton. An example from the fresh water literature is Eiler et al. 2013.

In the publication Microscopic and Molecular Methods for Quantitative Phytoplankton Analysis (Karlson et al. 2010) several different molecular methods for analysing phytoplankton are described. The handbook is one result of a workshop held in 2005. Another result is the comparison of results obtained using microscopical and molecular methods (Godhe et al 2007). In 2005 the molecular methods did not work that well in general but some

methods were really useful, e.g. species specific probes used with fluorescence microscopy. Since then progress has been substantial, especially regarding the reduction of cost for sequencing. Today it cannot be considered expensive to sequence plankton samples. The cost is mainly in interpreting the data. Molecular methods should at present only be considered as a complement to quantitative optical methods for phytoplankton analysis. It would be a good idea to store samples collected now for further analysis in the future when methodology has developed further.

Biomass of phytoplankton

The biomass of phytoplankton must not be confused with the abundance, production or the productivity. The unit of phytoplankton biomass is often the biovolume (e.g. mL L^{-1}) or the weight of the organisms per volume of water (e.g. mg L^{-1}). Also the carbon content per volume of water is used e.g. mg C L^{-1}). Sometimes chlorophyll a is used as a proxy for the biomass of phytoplankton, more on that below.

Biomass based on microscopy

Microscopy is in many ways the best way to estimate phytoplankton biomass. In the standard analysis using the inverted microscope cell dimensions are measured. A standardised method has been developed by microscopists active in the HELCOM-Phytoplankton Expert Group (Olenina et al 2006). A list of geometrical shapes and size classes of phytoplankton species is updated every year by the group. The updated list is available at http://www.ices.dk/marine-data/vocabularies/Documents/PEG_BVOL.zip . The web site www.nordicmicroalgae.org provide images and other information on the microalgae. A European wide standard with recommendations for calculating phytoplankton biovolumes is in development by CEN. Biovolumes are converted to carbon based on many measurements of the carbon content of different phytoplankton species (Menden-Deuer and Meunier 2000). An example of results from biomass estimates based microscopy is presented in Fig. 10. The organisms > 2 µm were analysed using the Utermöhl method (inverted microscope) and the organisms <2 µm, i.e. the autotrophic picoplankton, were analysed using fluorescence microscopy. The example is from August 2011 and autotrophic picoplankton dominated at most stations except for in the Bothnian bay.

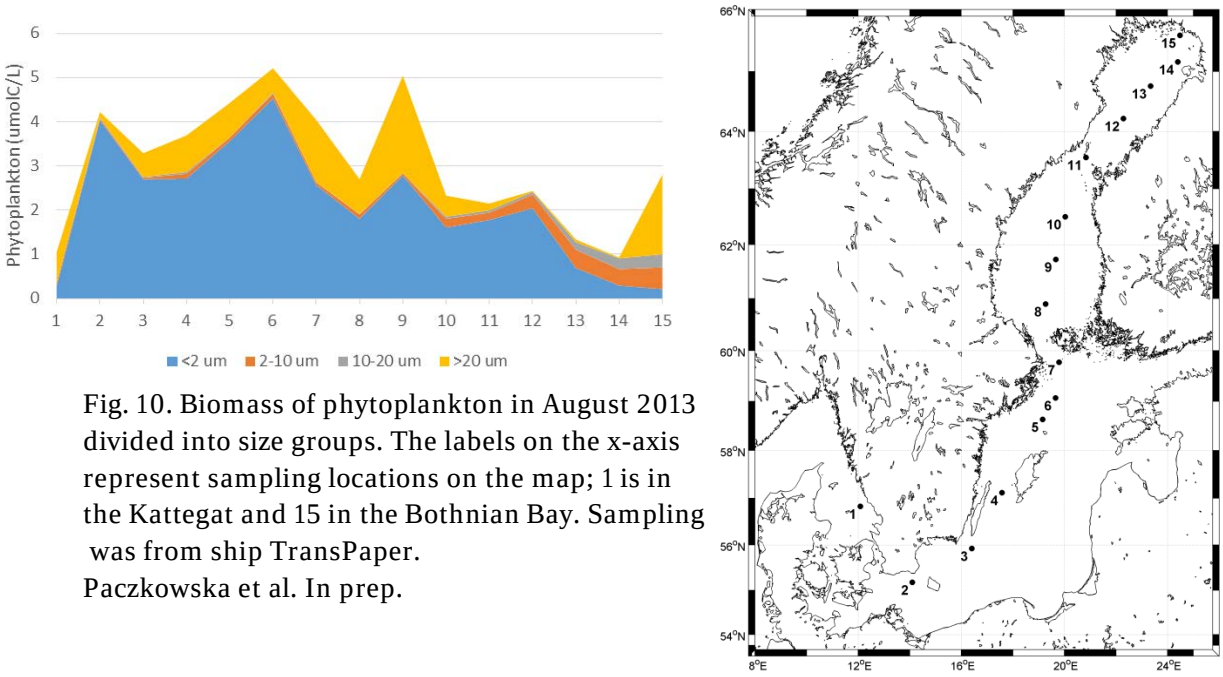

Fig. 10. Biomass of phytoplankton in August 2013 divided into size groups. The labels on the x-axis represent sampling locations on the map; 1 is in the Kattegat and 15 in the Bothnian Bay. Sampling was from ship TransPaper.
Paczkowska et al. In prep.

Chlorophyll a and other photosynthetic pigments

Chlorophyll a per volume of water is used as a proxy for phytoplankton biomass. Since chl. a. content is not a constant fraction of phytoplankton biomass this proxy must be used with caution. Light history and nutrient conditions and also other factors may influence the chl.a. content of microalgae. Chlorophyll a is mostly estimated using water sampling and subsequent filtering and extraction of the pigment which is measured using a spectrophotometer or a laboratory fluorometer. A more exact method is High Performance Liquid Chromatography (HPLC) which separates the different photosynthetic pigments before they are quantified. HPLC is by many considered the new standard for chl. a analysis. HPLC also gives information on pigments such as Chl. b, Chl. C_1 , C_2, C_3 and on carotenoids. Some of these pigments are specific for certain phytoplankton groups, e.g. peridinin for most dinoflagellates. Thus HPLC analysis gives what is called chemotaxonomic information on the phytoplankton community.

In vivo and in situ chlorophyll fluorescence

The chlorophyll in live phytoplankton produces red fluorescence when exposed to light, e.g. sunlight or the blue excitation light in *in vivo* fluorometers. *In vivo* fluorometers mounted on CTD's and other *in situ* instruments are often called *in situ* fluorometers. These may also be mounted on oceanographic buoys or in FerryBox systems on ships of opportunity, e.g. ferries. The fluorescence of chlorophyll is related to the concentration of chlorophyll which is a proxy for phytoplankton biomass. Unfortunately chl. fluorescence is influenced by the composition of phytoplankton and of the light history of the organisms. The night time to day time chl. fluorescence of the same phytoplankton community may vary with a factor of 2-3. In Fig. 11 data on hourly measurements of chlorophyll fluorescence at approximately 2 m depth in the Kattegat are presented. Note the low day-time values and the high night time values. It is likely that the same phytoplankton community was present day and night. Night time data are most consistent. Thus it is recommended to use night time chl. fluorescence only for near surface sensors. Another example of data from an in situ fluorometer mounted on an oceanographic buoy in the Koster fjord in the Skagerrak is presented in Fig. 12. Reference data is from the water sampling for the Water Quality Association of the Bohus Coast (BVVF).

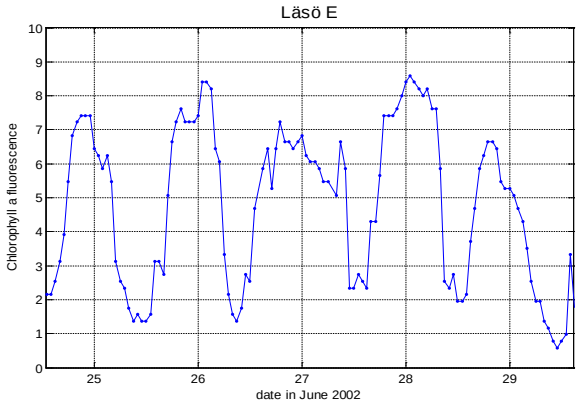

Fig. 11. Variability of in *vivo* chlorophyll fluorescence measured at approximately 2 m depth using the SMHI oceanographic buoy Läsö E. in the Kattegat in 2002. Night time to day time ratio is about 2-3.

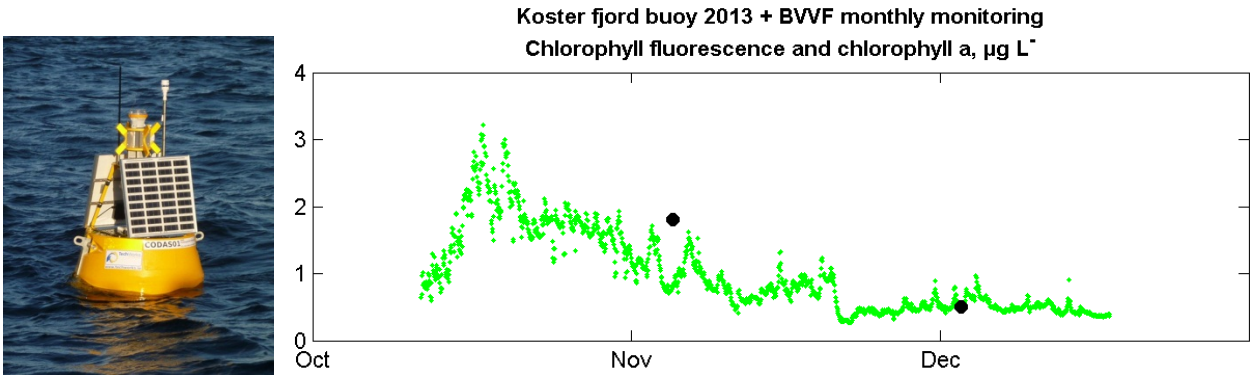

Figure 12. *In vivo* chlorophyll fluorescence in the Kosterfjord in 2013 measured at approximately 1 m depth. Black dots represent water samples for chl. a. analysed in the laboratory as part of the regional monitoring program (BVVF).

Light attenuation

John Cullen, working in Canada and others has used measurements from passive optical instruments, e.g. light attenuation at selected wavelengths, as a proxy for chlorophyll. There are articles in the IOC-UNESCO volume on Real-time coastal observing systems etc. (Babin et al. 2008) and several other publications (e.g. Cullen et. al 1997). In short light attenuation at 490 nm is a robust measurement of chlorophyll a that can be used in instrumented oceanographic buoys and also during CTD-casts. There is no photo quenching effect. An extensive study was carried out in the five years Lunenburg Bay Project in Canada. Much more information is available in the UNESCO volume.

Flow Cytometry

Regular flow cytometry does not give information on the size or biomass of phytoplankton directly. However, by analysing beads with a known size information on scattering properties may be used to infer information on size of phytoplankton. Imaging Flow Cytometry does provide size of organisms and also the area of the organism in the images. These measurements can be converted to biomass of individual organisms.

Molecular biological methods

Most molecular methods do not give direct information on the biomass of phytoplankton. Some give information related to abundance, i.e. cell numbers. Molecular methods are in general based on the content of DNA or RNA of the organisms. Since the number of RNA copies in a cell may vary substantially DNA based methods may have greater potential for giving information on biomass.

Remote sensing

Remote sensing of ocean colour gives the opportunity to cover large sea areas during cloud free conditions. Information on chlorophyll a, a proxy for phytoplankton biomass, surface scums of cyanobacteria and on coccolithophorids may be the most important products related to phytoplankton. At present there are few suitable satellites available, mainly the NASA satellites Aqua and Terra with the MODIS sensor and the NPP with the VIIRS sensor. EnviSAT with the MERIS sensor is not available since May 2012. In 2014 or 2015 the launch of the ESA Sentinel-3 satellite is planned. This means that high quality satellite data suitable for work with algal blooms is likely to become available in 2015. A second Sentinel satellite with ocean colour sensor is also planned. Problems with satellite remote sensing of phytoplankton include cloud cover, influence from humic substances and non-phytoplankton particles. In shallow area the sea floor may influence the data. Only the uppermost part of the water column is observed. A rule of thumb is one Secchi depth. In Figs. 13-15 examples of satellite data on phytoplankton is shown. Remote sensing of phytoplankton in the Baltic Sea area has been reviewed recently by Kratzer et al (2011). The private company Brockmann Geomatics Sweden AB provides a web site www.vattenkvalitet.se with some satellite based information. It is claimed that status class (Water Framework Directive) of the water bodies can be determined using MERIS satellite data. Since information on phytoplankton abundance and species composition is lacking this is not possible. However, satellite based measurements of ocean colour do give useful information that complement water sampling and subsequent analysis of phytoplankton and chlorophyll.

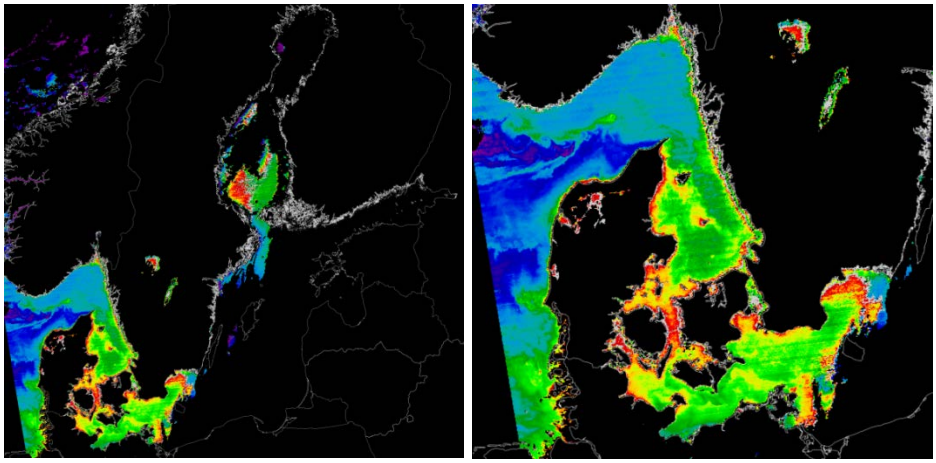

Fig. 13. The phytoplankton spring bloom was observed using the MODIS sensor on 1 April 2013. The cloud free conditions were unusual. Colour indicate chlorophyll concentrations. The bloom in the Bothnian Sea is probably an artefact. Source NOAA-MODIS processed by SMHI.

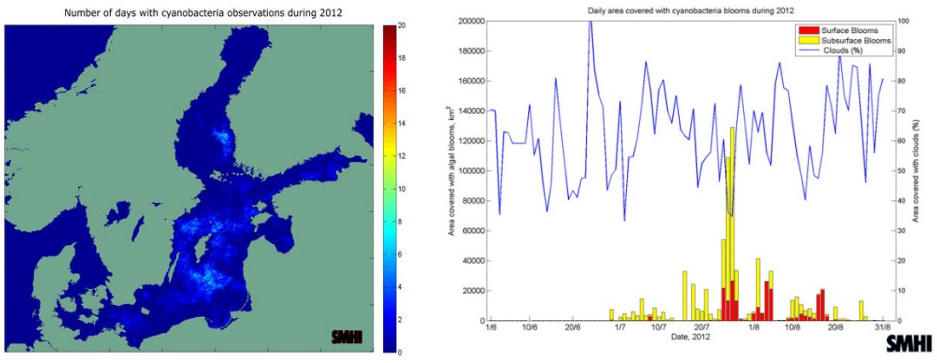

Fig. 14. Left. Surface accumulations of cyanobacteria observed using satellite remote sensing. The scale indicates number of days with observations of surface scums in 2012 between 1 June and 31 August. Right: Graph shows area with cloud cover and area with cyanobacteria observations. Source Baltic Algae Watch System at SMHI and Öberg and Hansson 2012.

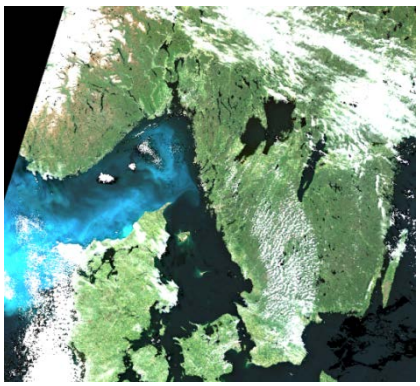

Fig. 15. Image of coccolithophorid bloom in the North Sea and the Skagerrak, 5 June, 2010. Source NOAA-MODIS processed by SMHI.

Frequency of algal blooms

The frequency of algal blooms is a parameter of interest. But before monitoring of this is discussed algal blooms must be defined. In this context only high biomass blooms are being considered. One possibility is to use sudden changes in biomass as criteria. Examples of high biomass blooms:

- The spring bloom
- Summer cyanobacteria bloom in the Baltic Sea
- Blooms related to upwelling events
- Blooms caused by direct anthropogenic activities, e.g. supply of high nutrient concentrations through eutrophication

To measure frequency of blooms a high frequency sampling programmes is needed. A combination of satellite remote sensing, automated in situ instruments and FerryBox-systems may be the best option. FerryBox systems and the emerging network of oceanographic buoys could be used for measuring chl. fluorescence to detect sudden changes in phytoplankton biomass. An example is found in Fig. 18. Reference sampling every two weeks or every month would be needed as well.

Harmful algal blooms

Harmful algal blooms are recurrent phenomena in the seas surrounding Sweden. Most are natural but some be caused by anthropogenic activities. New types of harmful algae may become common in Swedish waters due to climate change and because of transport of invasive species to our waters. It should be noted that harmful algae do not need to have high biomass to be harmful. One example is the biotoxin algae that cause diarrhetic shellfish toxins that accumulate in bivalves. Harmful algal blooms are of different types. The main ones are:

- Blooms of biotoxin producing algae. The toxins may accumulate e.g. in shellfish.
  examples, blooms of *Dinophysis* causing Diarrhetic Shellfish Poisoning, *Alxandrium* spp. causing paralytic Shellfish Poisoning
- Blooms of fish killing algae
  examples, *Prymnesium polylepis* bloom in 1988, blooms of *Pseudochattonella* sp.from 1998 and onwards.
- Blooms of toxic cyanobacteria
  example, bloom of *Nodularia spumigena*
- Nuisance blooms, e.g. surface accumulations of non-toxic cyanobacteria that cause concern for tourists and others
  example, bloom of *Aphanizomenon*

Monitoring of harmful algae should be part of the standard monitoring of phytoplankton. Resources for extra sampling and analyses of phytoplankton during extreme events should be available. It is important to have highly qualified persons available to identify organisms during novel types of blooms. In areas where bivalves are harvested special monitoring is required. If fish farming becomes an industry in Sweden monitoring of fish killing algae may be required.

Discussion

The directives – only part of the story

The MSFD and the WFD provide incitements for monitoring of phytoplankton but there are other good reasons. Climate change is one. Changes in pH, temperature, stratification, riverine input etc. are bound to influence phytoplankton communities. To understand changes long term datasets are necessary. Fisheries, aquaculture and the use of marine ecosystem services are becoming more important. These are all affected by phytoplankton production, biomass and composition. Harmful algae blooms may be of concern as well.

The value of long time series

Long time series of phytoplankton are becoming very valuable. Good examples include the Helgoland ROADS time series that started in 1962. Sampling has been carried out essentially all weekdays since then. In Norway a three day a week sampling programme at Flödevigen in the Southern part of the country is also very valuable. Scotland has the Stonehaven time series with sampling every week and England the time series at station L4 near Plymouth with weekly phytoplankton sampling. The sampling series at stations BATS, near Bermuda, and at station ALOHA, near Hawaii, may be most well-known for pH data showing ocean acidification but the data on phytoplankton and photosynthetic pigments are also important. In Sweden we have longer time series of phytoplankton in the sea than most countries. We should keep on sampling and analysing and treat this long term scientific research with respect. Parameters should be added and frequency increased but do not touch the basic measurements. One addition that is suggested is to store samples for future analyses. Methods unknown to us today may be used to analyse these samples at a later date.

Food webs

The methods used in the existing national marine monitoring program in Sweden where essentially decided in the 1970's. HELCOM monitoring of phytoplankton started around 1978 in Sweden. Since then a lot of new knowledge about the plankton food web has been gained and new methods have been developed. A major change is the incorporation of the pico- and the nanoplankton in the food web. Autotrophic picoplankton is at present missing from the monitoring of phytoplankton except for in the Gulf of Bothnia. The biomass often makes up a large part of the phytoplankton biomass, e.g. up to 80% in the Baltic Proper in August 2011 (Fig. 10 and Agneta Andersson, pers. comm.) and they dominate in abundance (cell numbers) of phytoplankton in summer in the Skagerrak (Kuylenstierna and Karlson 1994) . In oligotrophic conditions they dominate phytoplankton production. Mesocosm experiments have shown that picoplanktonic cyanobacteria may be affected by ocean acidification (Schultz et al 2012).

The new knowledge is reflected in that new methods that incorporate pico- and nanoplankton and microzooplankton is included in monitoring in some locations. Unfortunately this has not happened in the Swedish Marine Monitoring Programme or in the HELCOM and OSPAR monitoring programmes yet. Since a revision of the Swedish programme is planned to be implemented in 2015 now is the time to make changes.

Fluorescence microscopy or Flow Cytometry should be used for analysing autotrophic picoplankton in preserved water samples. Microzooplankton can be analysed together with the larger phytoplankton but a larger volume need to be concentrated than today since microzooplankton abundances are lower than abundances for phytoplankton. To overcome the problem with small volume analysed it is suggested that a volume of 1 L should be concentrated either through sedimentation or through filtering to provide a concentrated sample for analyses of rare phytoplankton species and of micro zooplankton.

About temporal and spatial resolution

The sampling in monitoring programmes needs to be representative of the sea areas to be monitored. Phytoplankton distribution in the sea is patchy both vertically and horizontally and also in time, examples are presented in Figs. 16-18. This is an effect of physical and biological processes and it makes monitoring a challenge. The use of several methods is a necessity. It is also necessary to realise that a full picture of the distribution of phytoplankton is not achievable. But to understand effects of eutrophication, climate change, etc. a resolution high enough to detect changes is necessary. In this report essentially no data is presented but experience and other publications indicate that monthly sampling of phytoplankton in the seas surrounding Sweden is too low to detect such changes. Thus a minimal frequency of two weeks at sentinel sites (sentinel ~vaktpost) is suggested with weekly sampling during blooms. High frequency sampling using automated techniques should be used and also evaluated further.

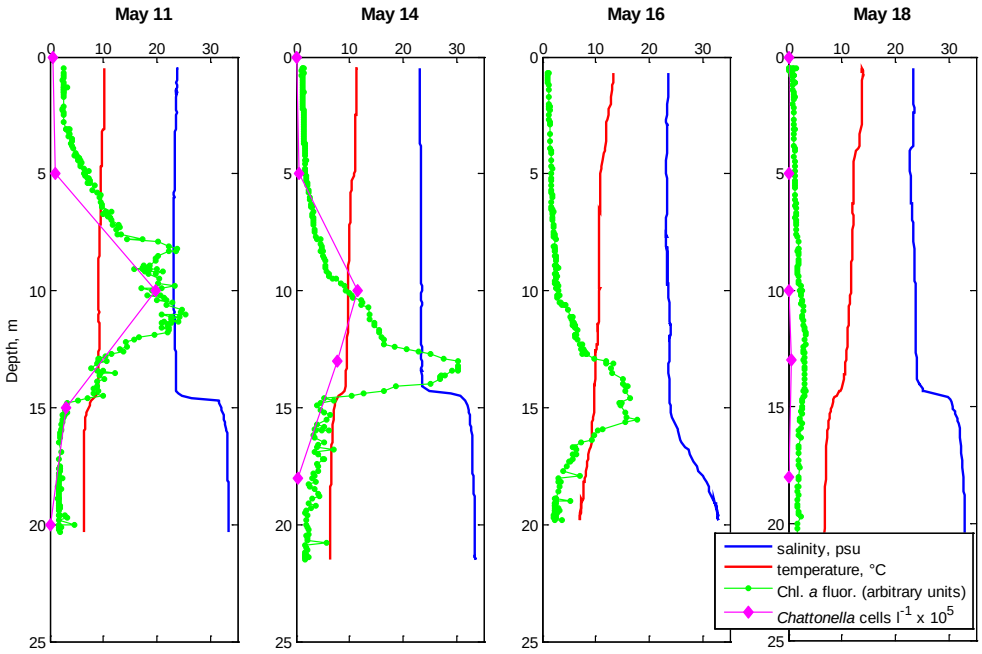

Fig 16. Data from sampling at Valö near Gothenburg during the 1998 bloom of *Pseudochattonella* first identified as *Chattonella*.

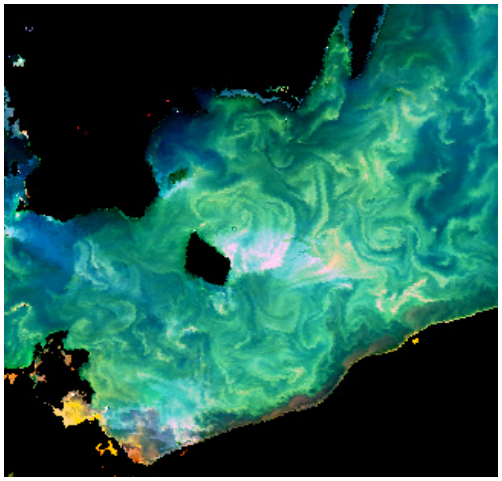

Fig 17. A satellite image of surface accumulations of cyanobacteria in the Southern Baltic Sea illustrates patchy horizontal distribution. NASA/MODIS, July 2013. Image processed by SMHI.

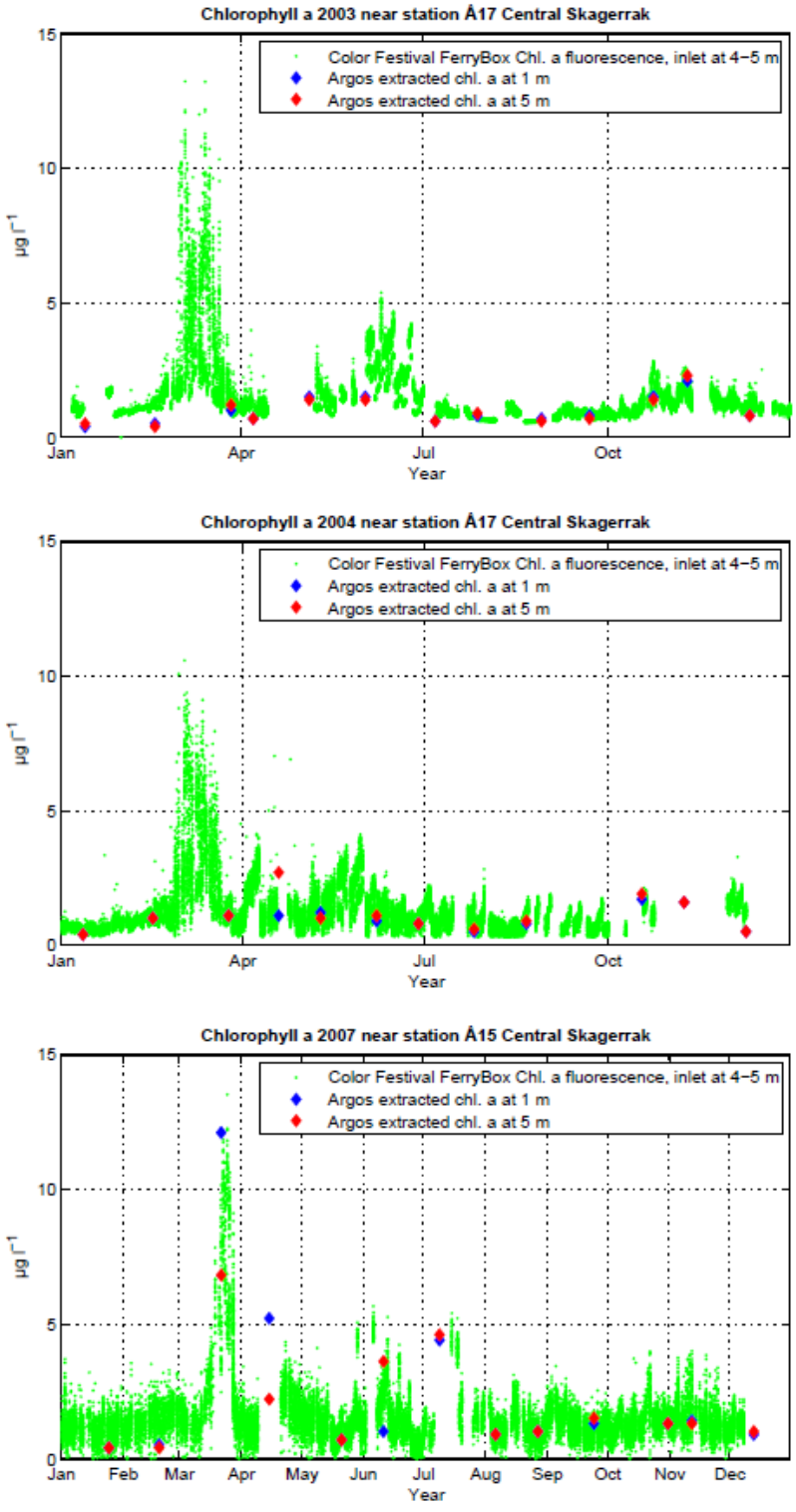

Fig 18. Algal blooms detected using chlorophyll fluorescence measurements in a FerryBox system in the Skagerrak compared to monthly sampling of chlorophyll a from R/V Argos. In year 2003 and 2004 the spring bloom was missed completely on the standard monitoring but detected using the Ferrybox system. In 2007 also the standard monitoring observed the spring bloom. Note other high biomass blooms missed by the standard monitoring programme. From Karlson et al 2010.

Summary of methods and costs

In tables 1 and 2 a summary of the benefits and disadvantages of the methods mentioned in the report is found. The tables also include comments about costs.

Method	Routine method or in development	Temporal resolution	Spatial resolution	Information about biodiversity and invasive species	Comments about costs etc.
Water sampling from research vessels and microscope analyses (and Flow Cytometry or Molecular methods)	Routine	Low	Low	High	Ship time is expensive
Water sampling using FerryBox systems and microscope analyses (and Flow Cytometry or Molecular methods)	Routine	Medium	Medium	High	No cost for ship time, only surface water sampling
Water sampling using buoys and microscope analyses (and Flow Cytometry or Molecular methods)	Routine in the United Kingdom. Automated sampling devices also used in Chesapeake Bay	Medium	Low	High	Sampling device is about 170 000 SEK. A network of buoys is needed.
Water sampling from any platform and subsequent analysis of extracted chlorophyll a	Routine	Low-Medium depending of platform	Low-Medium depending of platform	Low (essentially no infomation)	Low cost for analysis. Samples need to be filtered and frozen within 24 hours after sampling.
Water sampling from any platform and subsequent HPLC-analysis of extracted pigments	Routine	Low-Medium depending of platform	Low-Medium depending of platform	Low-Medium	Medium cost for analysis but a HPLC-instrument is needed, ~300 000 to 500 000 SEK. Samples need to be filtered and frozen within 24 hours after sampling.
Satellite remote sensing	Routine but algorithms are not wel developed for the Baltic Sea	Medium - High Observations only during cloud free condtions	High horizontally, low vertically	Low (essentially no infomation)	Data from ESA and NASA are free. To produce useful products for Sweden substantial addtitonal resources are necessary. A system of validation with in situ sampling is necessary.
Chlorophyll fluorescence sensors on buoys	Routine, e.g. the SmartBuoy network in the UK	High	Low	Low (essentially no infomation)	A network of buoys is needed. Regular service and reference sampling is necessary.
Chlorophyll fluorescence sensors in FerryBox systems	Routine	High	High horizontally, low vertically	Low (essentially no infomation)	No cost for ship time. Regular service and reference sampling is necessary.
Light attenuation at specific wavelengths on buoys	Mainly used in Canada (the author is not well aqcuainted with the situation elsewhere)	High	Low	Low (essentially no infomation)	Sensors at two depths or more are needed. Regular service and reference sampling is necessary.

Table 1. A summary of the benefits and disadvantages of some of the sampling and analyses methods mentioned in the report. The table also include comments about costs.

Method	Routine method or in development	Information about biodiversity and invasive species	Suitablility for Harmful Algal Bloom observations	Comments about costs etc.
Light microscopy, the Utermöhl method	Routine	High	High	Cost for instrument ~300 000 to 500 000 SEK. Time consuming method, analysis time ~4 hours. A skilled microscopists with expert knowledge of the phytoplankton community is neccesary. A relatively small volume is analysed in the present routine method.
Electron microscopy, SEM and TEM	Method is well developed but only used at high quality monitoring sites	Very high, needed to identify small species, e.g. coccolithophorids, Chrysoschromulina, Prymnesium etc.	Needed mainly to identify small or new species	Time consuming method and high cost of instruments. Expert knowledge on phytoplankton taxonomy is needed. The availablity of Scanning Electron and Transmission Electron Microscopes is limited but in Sweden several universities and hospitals have instruments. Cost for instrument > 1 000 000 SEK.
Fluorescence microscopy of autotrophic picoplankton	Routine	Low	Low	Quick method, only limited knowledge of phytoplankton taxonomy is needed. Cost for instrument ~300 000
Flow cytometry for analysis of autotrophic picoplankton	Routine	Low	Low	Very quick method if many samples are analysed at a time, only limited knowledge of phytoplankton taxonomy is needed. A standard flow cytometer is needed, cost ~300 000 to 600 000 SEK.
Imaging Flow Cytometry	Probably only used routinely in the Netherlands but being evaluated e.g. in USA, France and in the UK.	Medium to high	High	Analysis time ~20 minutes. To run analyses only minor knowledge of phytoplankton is needed but a person with expert knowledge of the local phytoplankton community is neccesary for quality control. Cost for instrument ~700 000 to 1000 000 SEK
In Situ Imaging Flow Cytometry	Not yet used routinely but long term deployments have been made in the USA	Medium to high	High	Analysis time ~20 minutes. Analyses are made automatically but a person with expert knowledge of the local phytoplankton community is neccesary for quality control. Cost for instrument is probably ~700 000. A buoy is also needed.
The Continuous Plankton Recorder - microscopy	Routine	Low for phytoplankton, high for zooplankton	Low	Analyses is by microscopy and time consuming. The method is only semiquantitative. A disadvantage is that many small or fragile species are missed. An advantage is that very long time series exist in some areas. Cost for instrument, unknown, probably rented from SAHFOS.
Zooscan	Routine, e.g. in France	Low for phytoplankton, high for zooplankton	Low	The method is included in the table mainly because it may be suitable for larger phytoplankton, e.g. colonies of cyanobacteria. It seems to be very useful for analysing high numbers of samples of larger zooplankton. Cost of instrument is ~400 000 SEK
Molecular methods, probes for single cells	Routine in a few places, e.g. Scotland and New zealand	Low	High, for selected species	Probes for selected species from the local phytoplankton community must be available. Basic instrumentation for molecular biological work is needed to use these methods. Cost for instruments is unknown to the author.
Molecular methods, multiple probes for multiple species	In development	Low	High, for selected species	Probes for selected species from the local phytoplankton community must be available. The results are not fully comparable to cell counts and identification using microscopy. The only method/device known to the author is the device produced in the EU Project MIDTAL that handles approximately 20-30 species. Sample processing include many prepative steps but several samples can be handled simultaneously. A laboratory equipped for molecular biological work is needed as well as a special device. Cost is unknown.
Molecular methods - analyses of genes from the whole plankton community	In development	Low	Not known	It is possible to process many samples at a low cost for sequencing,. The exact cost is unknown to the author. The results are not fully comparable to cell counts and identification using microscopy. The results should be considered semi-quantitative and include information on bacteria, phytoplankton and microzooplankton. Cost for sequencing has plummeted. Interpreting the data is time consuming.

Table 2. A summary of the benefits and disadvantages of the analyses methods mentioned in the report. The tables also include comments about costs.

Conclusions and recommendations

General conclusions

The Swedish Environmental Objectives, EU Marine Strategy Framework Directive and the EU Water Framework Directive as well as the HELCOM and OSPAR conventions calls for monitoring of phytoplankton both at the species level and at the community level. Also invasive species and harmful algae should be part of observing programs. Climate change effects on the phytoplankton community should also be considered. The current national monitoring program of phytoplankton does not resolve natural variability in time and space at a level of detail needed to fulfil the directives and conventions.

1. The Swedish national marine monitoring programme for phytoplankton should be consistent in all basins in the seas surrounding Sweden.
2. At least one off shore and one coastal station in each basin should be considered high priority sentinel sites, i.e. long time series stations for observing changes related to eutrophication, climate change etc.
3. The whole phytoplankton community should be monitored including autotrophic picoplankton.
4. Long time series using traditional sampling methods and microscopy should be maintained and complemented with data from imaging flow cytometry and molecular biological methods.
5. In the Skagerrak and the Kattegat the calcium carbonate containing group coccolithophorids should be monitored specifically since these organisms are likely to be affected by ocean acidification early.
6. Measurements of chlorophyll a as a proxy for phytoplankton biomass should be continued. HPLC analyses of photosynthetic pigments should be considered at the high priority sentinel sites.
7. Automated water sampling using devices on buoys and ships of opportunity (FerryBox systems) should be used to increase sampling frequency.
8. Automated measurements of night time chlorophyll fluorescence on oceanographic buoys should be used to obtain data on the frequency of high biomass blooms.
9. In the Baltic Sea measurements of phycocyanin fluorescence can be used to observe cyanobacteria blooms.
10. Remote sensing of ocean colour should be used to complement the in water data to obtains better spatial coverage. The remote sensing data should be used with caution since cloud cover, problems with interference of non-plankton particles, humic substances in the water and effects of the sea floor in shallow water cause methodological problems. Remote sensing data give essentially no information on species composition but provide information on chlorophyll, a proxy for total phytoplankton biomass, information on surface accumulations of cyanobacteria and on blooms of coccolithophorids.
11. The Continuous Plankton Recorder should be used where long time series of CPR-data already exist.

Recommendations for changes in the current national monitoring program

1. Use caution when making changes to monitoring programs that has long time data series. Maintain existing methodology and complement with new parameters and methods.

Changes in the short term, to be implemented in 2015

1. Methods should be made consistent in all sea basins around Sweden
    a. Autotrophic picoplankton shoud be analyses using fluorescence microscopy or flow cytometry.
    b. Chlorophyll a sampling should be carried out both using tube sampling 0-10 m (or 0-20 m if long time series exist) and at discrete depths (0, 5, 10, 15, 20, 30, 40 and 50 m). At present chl. *a*. is analysed from tube samples only in the Gulf of Bothnia.
2. At least one off shore and one coastal station in each major sea basin should be designated high priority sentinel sites and include sampling of the whole phytoplankton and zooplankton communities. Sampling frequency should be at least every two weeks at these sites during the growth season and weekly during bloom periods. It is likely that a combination of sampling from different platforms is needed to accomplish this frequency.
    a. The major sea basins are:
        i. The Bothnian Bay
        ii. The Northern Quark, the Bothnian Sea and the Åland Sea
        iii. The Northern Baltic Proper
        iv. The Southern Baltic Proper
        v. The Kattegat
        vi. The Skagerrak
3. The present method for analysing larger phytoplankton (nano- and microplankton) should be maintained. Biomass based on cell volumes should always be presented in carbon units.
4. A larger volume of water should be used for analyses of phytoplankton compared to today. This will facilitate higher quality analyses of rare species including early invaders. It will also facilitate analysis of micro-zooplankton in the same samples, a part of the marine food web neglected today.
5. At least three coastal and three off shore stations in each major sea basin should be used for water sampling of chlorophyll a. Analysis of other photosynthetic pigments should be considered.
6. Ships of opportunity with FerryBox systems should be considered part of the national marine monitoring program. Water sampling for phytoplankton analysis and photosynthetic pigments should be carried out and automated measurements of chlorophyll and phycocyanin fluorescence should be made. At present samples and data from the following routes should be included:
    a. Gothenburg-Kemi-Oulu-Husum-Lübeck-Gothenburg (TransPaper, SMHI)
    b. Oslo-Kiel (Color Fantasy, NIVA)
7. Measure light in air and in water during CTD casts during monitoring cruises to calculate the attenuation coefficient at selected wavelengths.

Changes that need to be evaluated before being fully implemented, e.g. in 2018

8. Document phytoplankton samples analysed in the microscope using digital photography at different magnifications. Images should be delivered together with quantitative data to the national data host.
9. Save phytoplankton samples from one location in the Baltic Sea and one in the Skagerrak for future analysis using techniques not available today, e.g. novel molecular techniques. Freeze samples at -80 degrees and store in a sample bank.
10. Implement automated optical imaging techniques in the monitoring of phyto- and zooplankton. This will facilitate analysis of a much larger number of samples than today with only a small increase in cost. However, the new methods will not replace the existing ones fully.
    a. In laboratory Imaging Flow Cytometry of larger phytoplankton and microzooplankton
    b. The Zooscan for large phytoplankton (colonies etc.) and for zooplankton
    c. In situ Imaging Flow Cytometry should be evaluated in the Skagerrak-Kattegat and in the Baltic Sea.
11. Molecular biological methods should be used to complement optical analyses of plankton communities. A goal is that it should be possible to analyse a larger number of samples than at present.
12. The emerging network of coastal oceanographic buoys should be used in phytoplankton monitoring
    a. To evaluate the usefulness of measuring night time chlorophyll fluorescence as a proxy for phytoplankton biomass with the goal of observing frequency of algal blooms.
    b. To evaluate the usefulness of measuring light attenuation (K_d)at selected wavelengths as a proxy for phytoplankton biomass and a proxy for Secchi depth.
    c. Reference sampling should be carried out at least monthly. An evaluation period of 1-3 years is suitable.
    d. Automated water sampling of phytoplankton should be evaluated.
13. Remote sensing of ocean colour should be part of the program. The Sentinel 3 satellite will provide useful data sometime after April 2015. Data should be evaluated for coastal areas and for off shore areas separately. It is likely that data from off shore areas will be of higher quality.
    a. Data from coastal areas, i.e. data from within 1 nautical mile from the coast, split by water types and/or water bodies according to the Water Framework Directive
    b. Data from off shore areas, i.e. data from areas outside 1 nautical mile from the coast. Data can be presented like this: Split data into seven areas:
        i. The Bothnian Bay
        ii. The Northern Quark, the Bothnian Sea and the Åland Sea
        iii. The Gulf of Finland
        iv. The Northern Baltic Proper
        v. The Southern Baltic Proper
        vi. The Kattegat
        vii. The Skagerrak
    c. The main parameters
        i. Chlorophyll a from the most suitable algorithm. A continuous evaluation of quality of results is a necessity.

   ii. Surface accumulations of cyanobacteria
   iii. Coccolithophorid blooms
   iv. Cloud cover
d. Indicators
   i. Length of growing season
   ii. Ratio Number of days with observations: Total number of days (including days with cloud cover)
   iii. Index of integrated yearly phytoplankton biomass
   iv. Index of integrated monthly phytoplankton biomass
   v. Date of peak of spring bloom
   vi. Duration of spring bloom
   vii. Date of peak of cyanobacteria bloom
   viii. Duration and extent of cyanobacteria bloom
   ix. Date of peak of coccolithophorid bloom
   x. Duration and extent of coccolithophorid bloom

14. Data from the Continuous Plankton Recorder should be used where long time series of samples already exist, i.e. in the Kattegat. These samples are stored in formalin but not yet analysed.

Other recommendations

1. An emergency fund with resources for sampling and analyses during harmful algae events should be established. Harmful algal blooms are likely to occur and at present it is difficult to fund the additional sampling needed, analyses of phytoplankton, and specialized techniques for identifying new organism in Swedish waters and for toxin analyses.
2. Use the National Data Host for Marine Biology and Oceanography for hosting all marine phytoplankton data and observations of chlorophyll etc. www.sharkweb.smhi.se
3. The National Food Administration (Livsmedelsverket) governs a phytoplankton monitoring program aimed at observing biotoxin producing microalgae. A cooperation between SwAM and the National Food Administration should be established to cut costs for sampling and analyses and to make high frequency sampling of biotoxin producing algae part of the national sampling programme in areas where bivalves are harvested for human or animal consumption.
4. Use the IOC Harmful Algae Event Database for reporting of harmful algal blooms www.haedat.iode.org
5. Increase cooperation with authorities in Finland, Denmark and Norway for consistent use of methods and for and cost efficient joint sampling programs for phytoplankton.
6. Use HELCOM and OSPAR for developing or establishing joint sampling programs
7. Use the groups HELCOM-PEG (HELCOM Phytoplankton Expert Group) and NOMP (Nordic Marine Phytoplankton Group) for cooperation between phytoplankton specialists and for arranging ring tests etc. Standardisation of species lists should be done through the Swedish Microalgae Committee and the counterparts in Norway (Norwegian Microalgae Committee) and the other countries. Nordic species lists may be maintained at www.nordicmicroalgae.org

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