EUROPEAN COMMISSION
DIRECTORATE-GENERAL
Joint Research Centre

Institute for Environment and Sustainability
Inland and Marine Waters Unit
I-21020 Ispra (VA), Italy

INLAND & MARINE WATERS

Monitoring strategies for phytoplankton in the Baltic Sea coastal waters

Heiskanen, A-S., Carstensen, J., Gasiūnaitė, Z., Henriksen, P., Jaanus, A., Kauppila, P., Lysiak-Pastuszak, E., Sagert, S.

CHARM

2005

EUR 21583 EN

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EUR 21583 EN

Printed in Italy

List of Authors

Anna-Stiina Heiskanen
European Commission
Joint Research Centre
Institute for Environment and
Sustainability, TP 290
I-21020 Ispra (VA), Italy
email: anna-stiina.heiskanen@jrc.it

Jacob Carstensen[1]
Department of marine ecology
National Environmental Research Inst.
Frederiksborgvej 399
P.O.Box 358
DK-4000 Roskilde, Denmark
email: JAC@DMU.dk

Zita R. Gasiūnaitė
Coastal Research and Planning Institute,
Klaipeda University
H. Manto 84,
LT 92294 Klaipeda, Lithuania
e-mail: zita@corpi.ku.lt

Peter Henriksen
Department of marine ecology
National Environmental Research Inst.
Frederiksborgvej 399
P.O.Box 358
DK-4000 Roskilde, Denmark
email: pet@dmu.dk

Andres Jaanus
Estonian Marine Institute
University of Tartu
Mäealuse 10a
12618 Tallinn, Estonia
e-mail: andres@sea.ee

Pirkko Kauppila
Finnish Environment Institute
P.O.Box 140,
FIN-00251 Helsinki, Finland
email: pirkko.kauppila@ymparisto.fi

Elzbieta Lysiak-Pastuszak
Institute of Meteorology and Water
Management Maritime Branch ul.
Waszyngotna 42
81-342 Gdynia, Poland
e-mail: Elzbieta.Lysiak-Pastuszak@imgw.pl

Ingrida Purina
Institute of Aquatic Ecology
University of Latvia
8 Daugavgrivas str.,
LV-1048 Riga, Latvia
e-mail: ingrida@hydro.edu.lv

Sigrid Sagert
University of Rostock,
Institute for Aquatic Ecology,
Albert-Einstein-Str. 23,
D-18051 Rostock, Germany
e-mail: sigrid.sagert@biologie.uni-rostock.de

[1] Current address: *European Commission, Joint Research Centre, Institute for Environment and Sustainability, TP 280, I-21020 Ispra (VA), Italy, email: jacob.carstensen@jrc.it*

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Summary

Phytoplankton monitoring in the Baltic Sea is to a large extent harmonised through the HELCOM COMBINE protocol. This ensures that the methods of sampling and analysis are quite similar and that data should be relatively comparable. There are differences in the spatial and temporal coverage of samples taken within the different monitoring programs. Moreover, within the national monitoring programs there can be large variations in the number samples taken at different stations, between years and during the year. Most monitoring stations are sampled more frequently during summer. Although the chlorophyll a and species-specific phytoplankton biomass has been measured routinely and by standard methods since the early 1970s, most national monitoring programs have had a reasonable monitoring effort after about 1990 only. New methods for collecting data, such as ships-of-opportunity and remote sensing, provide additional information to the traditional shipboard sampling and other new emerging technologies may provide alternative means for monitoring phytoplankton.

We investigated the variation in phytoplankton biomass on the basis of the CHARM phytoplankton database and proposed a statistical method to improve the precision of biomass indicators. The precision of the annual phytoplankton biomass can be greatly improved by taking the seasonal variation into account, but describing the correlation structure in data contributes to improved precision as well. This latter method attempts to separate variations in phytoplankton biomass into systematic and random variations, thereby obtaining more correct estimates of the residual variance. Consequently, the number of observations required to obtain a given precision could almost be reduced by 50%, simply by interpreting data from another perspective. Nevertheless, variations in the phytoplankton biomass are still substantial and it may not be realistic to expect precisions below 30% from biweekly to monthly sampling. However, it is possible that improved modelling of the variations by including covariables may reduce the residual variance even further, improve the precision and thereby reduce the monitoring requirements, but this will require more detailed analysis that are outside the scope of the present work.

Sampling several monitoring stations will increase the number of observations used to characterise given water bodies and consequently improve the precision. However, if monitoring stations are located too close to each other there is a risk of information redundancy. Our analysis of spatial correlation from the Gulf of Finland and the Curonian Lagoon suggests that distances between stations should not be less than 5 km for more enclosed areas such as bays, lagoons, and estuaries, and approximately above 15 km for open waters. Distances above 10 km for coastal areas may prove reasonable.

Monitoring within the Water Framework Directive (WFD) aims at classification on an Ecological Quality Ration (EQR) scale, although classification based on uncertain information has not yet been operationally considered in the Common Implementation Strategy (CIS). Classification of phytoplankton biomass on an EQR scale will most likely require a precision less than 10% to obtain confidence intervals within a single classification level. Otherwise, it will be difficult to obtain a distinctive univocal classification. The concept of uncertainty for classifications needs to be stressed and forwarded to the working groups under CIS.

More work will still be needed to identify robust indicators for the structural changes of the phytoplankton community due to nutrient loading (and eventually also other) pressures. While such phytoplankton classification metrics are still under development, some phytoplankton parameters could be suitable to be used in the identification of the areas in risk of failing the environmental objectives (Article 5 of the WFD). However, it is important to conduct a similar analysis of variability and precision for the indicators of other biological quality elements for prioritisation of the monitoring efforts.

1. Introduction

The Water Framework Directive (WFD, 2000/60/EC) creates a new legislative framework to manage, use, protect, and restore surface and ground water resources within the river basins (or catchment areas) and in the transitional (lagoons and estuaries) and coastal waters in the European Union (EU). The WFD aims to achieve sustainable management of water resources, to reach good ecological quality and prevent further deterioration of surface- and ground waters, and to ensure sustainable functioning of aquatic ecosystems (and dependent wetlands and terrestrial systems).

The WFD stipulates that the ecological status of the surface water is defined as "… *an expression of the quality of the structure and functioning of aquatic ecosystems associated with surface waters, classified in accordance with Annex V*." (WFD, Article 2: 21). This implies that classification systems for the ecological status should evaluate how the structure of the biological communities and the overall ecosystem functioning are altered in response to anthropogenic pressures (e.g. nutrient loading, exposure to toxic and hazardous substances, physical habitat alterations, etc.). The WFD states following "… [ecological quality classification] *shall be represented by lower of the values for biological and physico-chemical monitoring results for the relevant quality elements*…" (Annex V, 1.4.2). Furthermore it is required that the ecological quality of water bodies should be classified into five quality classes (high, good, moderate, poor, and bad) using Ecological Quality Ratio (EQR), defined as the ratio between reference and observed values of the relevant biological quality elements. WFD, Annex V, lists the following phytoplankton quality elements, to be monitored and used in the WFD compliant assessment of the coastal and transitional waters:

- Phytoplankton composition and abundance of phytoplankton taxa
- Average phytoplankton biomass and water transparency
- Frequency and intensity of phytoplankton blooms

According to the WFD (Annex V), declining ecological quality of coastal and transitional waters is characterised by slight ('good status') or moderate ('moderate status') disturbance in the composition of phytoplankton abundance and taxa, slight or moderate changes in the biomass compared to the high status, and slight or moderate increase in the frequency and duration of phytoplankton blooms.

The phytoplankton community is widely considered the first biological community to respond to eutrophication pressures and is the most direct indicator of all the biological quality elements. Most phytoplankton species respond positively and predictable to nutrient enrichment in all European coastal areas (Olsen et al. 2001).

In the CHARM phytoplankton group, we wanted to investigate whether the present monitoring data from coastal areas around the Baltic could be used for WFD compliant assessment of the coastal waters, allowing establishment of the reference conditions and classification scales.

Also we wanted to explore possibilities if the taxonomic phytoplankton data could be used to develop ecological quality indicators that would have low natural variability and could be sensitive to ecosystem changes due to anthropogenic pressures, particularly with respect of eutrophication. Finally our aim was to suggest approaches for monitoring of phytoplankton parameters based on the analysis of the applicability of the current monitoring data.

The WFD CIS Guidance Document no. 7 on Monitoring provides general advice on the interpretation of the legal texts on monitoring requirements. However, this guidance does not provide concrete examples how to deal with problems of deciding the monitoring network, number of stations, frequency and seasonal duration of sampling, and which parameters to monitor and which metrics to use or taxonomic resolution to choose. Therefore it is useful to illustrate by means of practical examples how these factors impact the confidence and precision of the classifications, when phytoplankton quality element is used in the assessment. Since the microscopy analyses are very time consuming and require specific expertise on taxonomic identification of phytoplankton species, it is useful to illustrate what level of taxonomy resolution would be required to have the same precision as if more simple integrative parameters, such as chl *a* would be used.

For this we made an overview of the approaches in monitoring strategies in the current phytoplankton monitoring programs in the Baltic Sea. The overview is largely based on the phytoplankton data combined from the national coastal monitoring databases of Denmark, Germany, Poland, Lithuania, Latvia, Estonia, and Finland as well as from the national HELCOM databases into the CHARM phytoplankton database. The

Alg@line ship-of-opportunity data from the Gulf of Finland was collected and provided by the Estonian Marine Institute and the Finnish Institute of Marine Research as parties of the Alg@line consortium.

The data in the CHARM phytoplankton database was analysed to obtain information on the magnitudes of variation in phytoplankton biomass observations, and how this would affect the precision of ecological classification. We also determined the number of samples required to obtain a given precision.

2. State of monitoring systems

The national monitoring programs within the Baltic Sea have to a large extent been coordinated within the HELCOM COMBINE program. The conduct of the measurements consequently follows the HELCOM guidelines and data are generally comparable across the different countries and areas. There are, however, differences in the national monitoring programs beyond the requirements of HELCOM, and these differences are outlined below.

2.1 Phytoplankton monitoring in Denmark

Phytoplankton is monitored as part of the Danish national and regional monitoring programmes. Chlorophyll *a* (chl *a*) concentration is used as an indirect measure of total phytoplankton biomass in most areas. Concurrent with hydrochemical measurements, chl *a* concentrations have been measured by spectrophotometry since the late 1970s.

In addition, but at a smaller number of stations, primary production is measured by ^{14}C incorporation and phytoplankton is characterised and quantified (as carbon biomass) from microscopy. Primary production is measured as carbon fixation over 2 hours in incubations in artificial light at *in situ* temperature. Dark uptake is subtracted from the uptake in light and the relationship between carbon uptake and light is established from 12 measurements. Area production is calculated from data for surface light, light attenuation in the water column, chlorophyll concentration in the samples and the distribution of chlorophyll with depth as measured from a fluorescence profile. The result is given in mg C m^{-2} d^{-1}. Measurements of primary production were initiated in the late 1970s.

Water samples for microscopy are integrated samples representing the top 10m of the water column. Samples are collected using an integrating hose or as discrete samples from several depths mixed prior to analysis. In shallow estuaries < 10 m deep, samples are integrated samples from the surface down to 0.5 m above the bottom. Individual species are enumerated in an inverted microscope (Utermöhl method) and approx. 10 individuals from each taxon are measured for calculation of biovolume and conversion to carbon biomass. Phytoplankton counts and biomass calculations were initiated at a few

open water stations in 1979 and included in the monitoring of a larger number of coastal stations and estuaries in the mid 1980s.

In the present monitoring programme (2004-2009) Chl *a* is measured 1-47 times per year at 122 stations. Primary production and phytoplankton composition/biomass is measured 4-26 times per year at 15 stations.

2.2 Phytoplankton monitoring in Finland

In Finland's coastal waters, the monitoring of phytoplankton chlorophyll a is carried out by many organisations. The total combined network is ca. 1000 sampling stations (Figure 1) covering the entire extent of the Finland's coastal waters (Kauppila et al. 2004). The monitoring in the open sea is performed by the Finnish Institute of Marine Research (FIMR), but only a few of the stations are located inside the Finnish coastal types characterised according to the WFD. The samples are mostly taken twice a year, but some representative stations are visited for sampling more frequently.

Finnish Environment Administration (FEA) is carrying out the national monitoring of coastal water quality since 1979 covering ca. 100 sampling stations (Fig. 1). Thirteen of these stations are sampled intensively - 16-20 times per year - whereas at the others the hydrography and other water chemistry (including chlorophyll a) are screened twice a year. Phytoplankton biomass and species composition are analysed at five intensive stations in the open water period. These stations represent the coastal waters of the main sea areas around Finland.

Data on phytoplankton biomass (as chlorophyll a and biovolume) and species composition have also been gathered during the cruises of the research vessel "Muikku", which has visited several monitoring stations in the coastal Gulf of Finland and the Archipelago Sea in the summers of the late 1990s and early 2000s. Realisation of these cruises, carried out in the cooperation with the Finnish Environment Institute (SYKE) and the Regional Environmental Centers (RECs), depends on outside funding.

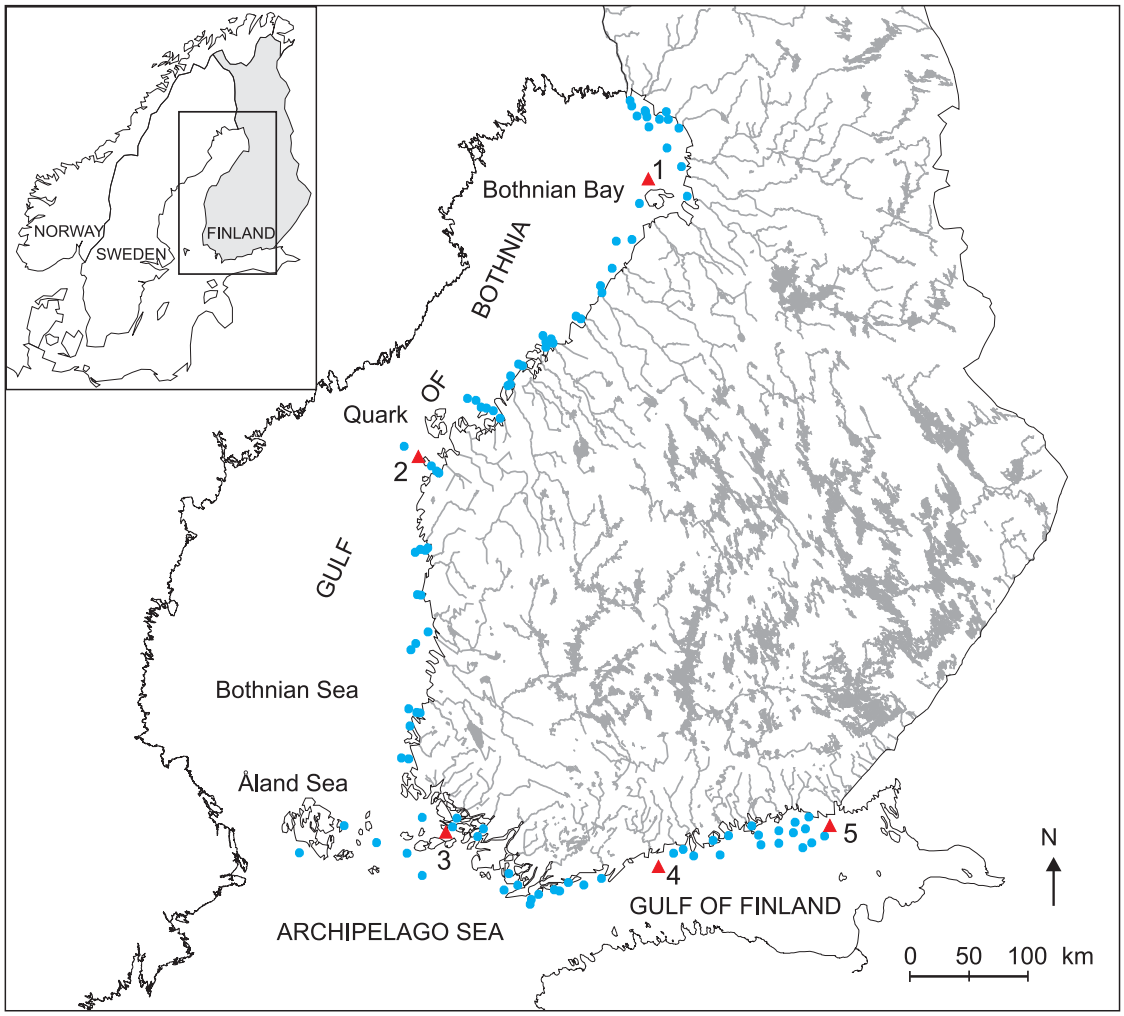

Figure 1: Locations of the national monitoring stations of the Finnish Environment Administration. Intensive monitoring stations including in this report are Hailuoto (1), Bergö (2), Seili (3), Länsi-Tonttu (4) and Huovari (5).

The network of local monitoring programs covers most of the sampling stations. The obligation of polluters to carry out local monitoring is based on the Water Act, and the programmes are approved by the Regional Environment Centers of the FEA. Variables in the programmes depend both on the qualities and amounts of loading, and the characteristics of recipient waters. Data on phytoplankton biomass (biovolume) and species composition are seldom included into the local monitoring programmes. Samples of chlorophyll a as well as hydrography and other chemical variables are usually taken 2 to 6 times per year.

The use of satellite remote sensing in the project of SYKE enables efficient monitoring of spatial water quality variation in Finnish inland and coastal waters (Härmä et al. 2001, Koponen et al. 2002). Best results are obtained by combining remote sensing with the results of traditional monitoring, which is based on water sampling at fixed stations. The development of the interpretation algorithms also requires detailed measurement of optical properties of water. The most important determinations include the absorbtion coefficient (400 and 750 nm) in filtered water and suspended solids both of which are taken from the depth of 1 m. Samples are measured in each of the 13 intensive coastal stations from April to August. The aim is to produce remote sensing based water quality maps for coastal waters over large areas.

LANDSAT ETM and Aqua MODIS images have been used in the estimation of turbidity, concentration of total suspended solids, surface accumulation of algal blooms and Secchi disk for selected areas, e.g. Helsinki sea area. Chlorophyll a and humic substance algorithms have been developed using AISA airborne spectrometer and portable spectrometer data.

Alg@line has provided 10 years of innovative plankton monitoring and research and information service in the Baltic Sea (Rantajärvi 2003). The unattended measurements and sampling on ferries and cargo ships make up the main bulk of collected data. Today there are several 'ship-of-opportunity' regularly crossing different areas of the Baltic, of which routes also cross the coastal waters of Finland. The monitoring is carried out in coordination by the FIMR. In Finland, RECs are taken part in this monitoring.

The national monitoring program, carried out both in the open sea by the FIMR and in the coastal waters by the FEA, is part of the international Baltic Monitoring Programmes of the Helsinki Commission (HELCOM), which has been operating since 1979. In the beginning of 1998, the monitoring programmes of HELCOM were revised and the COMBINE Programme was set up by officially putting together the monitoring programmes of the coastal waters (CMP) and open sea (BMP). The monitoring results are reported annually to the HELCOM database, which is maintained by the International Council of the Exploration of the Sea (ICES). The state of the Baltic Sea is mainly reported by HELCOM in periodic assessments. Additionally, Finland is committed to

deliver water quality data from several open and coastal water stations to the Eurowaternet - network of the European Environment Agency (EEA) - to be used for indicator reports. These reports are important for the implementation of the European water policy.

In Finland, phytoplankton chlorophyll a is measured from composite samples (surface to twice the Secchi depth) and analysed according to Lorenzen (1967). In the 1980s, the samples were usually extracted with acetone, but since the early 1990s with ethanol (ethyl alcohol). Samples of phytoplankton (surface to twice the Secchi depth) are taken with a Ruttner-sampler and preserved with acid Lugol's solution. Cells are counted with a Zeiss IM35 inverted microscopy using the technique of Utermöhl (1958). Cell numbers are converted to biomass (ww) using the volumes of the phytoplankton database of the Finnish Environment Administration, most of which have been calculated according to Edler (1979).

2.3 Phytoplankton monitoring in Estonia

Regular phytoplankton monitoring in Estonian coastal waters started in 1993. Intensive monitoring has been focused on three hot spot areas, including 3 stations in each (Tallinn, Narva and Pärnu bays). Phytoplankton samples have been collected monthly (in March and from September to November) or fortnightly (from April to August). The overall number of phytoplankton samples is 100-120 per year. Reductions in the sampling programme are mainly due to ice-cover in early spring and weather conditions (strong winds), as nowadays only small vessels are used. The latter is the reason of less frequent data coverage for offshore/reference stations as compared to the coastal stations. 1-2 times a year (usually in early spring and in the end of May), all Estonian monitoring stations (36) are monitored, including chlorophyll a and phytoplankton analysis. Those so-called seasonal cruises may give information on the onset and fading of spring bloom in different sub-basins in a longer time scale.

In 1997, Estonian Marine Institute joined the operational monitoring system onboard merchant ships (Alg@line). Phytoplankton is an essential part of the unattended monitoring with high-frequent (weekly) sampling during the vegetation period from April to November. EMI is responsible for 9 Alg@line stations located in the central Gulf of

Finland between Tallinn and Helsinki. Depending on the system order, the number of operational phytoplankton samples on that transect is 200-225 a year. Since 2000, operational monitoring is a part of the Estonian national monitoring programme.

All monitoring data are stored in the Access-database administrated by the Estonian Marine Institute. Alg@line data have been also sent to the data administrator at the Finnish Institute of Marine Research. A new GUI-based based database for the Alg@line ship-of-opportunity data administrated by FIMR is under development.

The annual reports of the Estonian coastal water monitoring are available from the web-site http://www.seiremonitor.ee/alam/05/index.php (in Estonian, with English summary).

The ordinary monitoring samples have been collected monthly to fortnightly by pooling of water from 3 discrete sampling depths (1, 5 and 10m). The samples collected automatically from the merchant ships represent probably the most productive layer (~5 m) and the sampling was conducted with an interval of one week during the vegetation period from April-November. Analysis procedure follows the guidelines of HELCOM COMBINE (http://www.helcom.fi/Monas/CombineManual2/PartC/CFrame.htm). Chlorophyll *a* has been measured spectrophotometrically using ethanol as solvent. Until 1999, acetone was used to extract chlorophyll *a*. To correct earlier measurements, these two solvents were used in parallel during 1999-2002. Ethanol proved to be more effective, giving 9.5 % more yield in average and 9-12.4 % depending on the dominating algal group. The smallest difference was found during dinoflagellate dominance and the biggest when cyanobacteria prevailed (unpublished data).

Samples for microscopic determination of phytoplankton species and for biomass calculations have been taken simultaneously with the water for nutrient and chlorophyll a analyses. Samples have been treated according to HELCOM COMBINE manual. Since 2003, the counting procedure has been performed using PhytoWin counting programme (Software Kahma Ky). The Alg@line phytoplankton data collected from the Tallinn-Helsinki transect in 1997-2002 was also transferred into PhytoWin. By the identification of phytoplankton taxa the checklists of the Baltic Sea phytoplankton species have been used (Edler et al., 1984; Hällfors, 2004). To improve the quality of the phytoplankton counting method and the comparability of the results between different laboratories, a

standardized species list with fixed size-classes and biovolumes has been compiled by the HELCOM phytoplankton expert group (Olenina et al., 2005). The present list is recommended to be used for calculation of phytoplankton biomass in routine monitoring of Baltic Sea phytoplankton and is aimed to become an integral component of PhytoWin. It will be updated as new information is obtained.

2.4 Phytoplankton monitoring in Latvia

The phytoplankton monitoring in the Gulf of Riga and Latvian coast of the Baltic Sea started already in 1976. Marine monitoring is performed by the Centre of Marine Monitoring (Institute of Aquatic Ecology, University of Latvia). From 1976 till 1991 phytoplankton was sampled in 45 stations (30 in the Gulf of Riga and 15 in the open Baltic Sea). Sampling frequency was 3-4 times per year. Samples were collected from 0m, 10m, 20m depth. Phytoplankton analyses were performed separately for each depth and average values were calculated mathematically. Since 1991 phytoplankton samples were collected in the Gulf of Riga in 11 stations (7-8 times per year) and 2 stations (20-22 times per year). In the open part of the Baltic Sea phytoplankton was collected in 4 stations 3 times per year and in 6 stations 5 times per year only chlorophyll a was sampled. Integrated samples from 0-10m were used for phytoplankton analysis.

Samples for microscopic determination of phytoplankton species and for biomass calculations have been taken simultaneously with the water for nutrient and chlorophyll a analyses. Before 1991 samples for determination of phytoplankton were fixed with formaldehyde, but later with Lugol solution. Samples have been treated according to HELCOM COMBINE manual. By the identification of phytoplankton taxa the checklists of the Baltic Sea phytoplankton species have been used (Edler et al., 1984; Hällfors, 2004). To improve the quality of the phytoplankton counting method and the comparability of the results between different laboratories, HELCOM phytoplankton expert group has compiled a standardized species list with fixed size-classes and phytoplankton biovolumes.

Data are also reported to HELCOM/ICES database and to EEA. They are used in producing HELCOM assessments and thematic reports, and in corresponding reports produced by EEA. Every year Environment Agency of Latvia publishes comprehensive

environment report where one chapter is dedicated to marine issues. Report is in Latvian, however lately it is translated also to English (www.vdc.lv).

2.5 Phytoplankton monitoring in Lithuania

The phytoplankton monitoring in the Curonian lagoon started already in 1981, and in the Lithuanian coastal zone of the Baltic Sea since 1984. Nowadays monitoring is performed by the Centre of Marine Research (Ministry of the Environment of the Republic of Lithuania). In the Curonian lagoon, phytoplankton is sampled at 10 stations, 3 (May, August, November- 5 stations), 5 (May-September- 1 station) or 12 (each month- 4 stations) times per year from surface layer.

In the Baltic sea phytoplankton is sampled at 17 stations, 2 (seasons not determined- 1 station), 3 (spring, summer, autumn-11 stations) or 4 (spring, summer, autumn, winter- 5 stations) times per year. Integrated samples from 0, 2.5, 5, 7.5 and 10 depths are further analysed according to Utermohl method. Chlorophyll a and abiotic parameters are analysed simultaneously.

There are still no changes in the phytoplankton monitoring strategy, related to WFD. The proposal to increase sampling frequency (to 1 time per month) in three stations in the Baltic Sea and in one station in the Curonian lagoon (station 14) is now under consideration. More information on the Lithuanian monitoring program can be found in Stankevicius (1998) and at the homepage of the Centre of Marine Research: http://www1.omnitel.net/juriniai_tyrimai/index.htm

2.8 Phytoplankton monitoring in Poland

The station network of the Polish monitoring program is coordinated with HELCOM COMBINE. Phytoplankton samples are collected at the following stations (see Figure 2):

- in the coastal lagoons: KW, ZP6, 11
- in the coastal zone and the bays: ZN2, P110, Sw3, Dz6 (this station is not marked in the chart, it is situated close to the mouth of the river Dziwna in the vicinity of the station B15), MR (a new station, not marked in the chart, situated between stations

B15 and K6), K6, DR (new station, between K6 and P16), P16, LP (new station, between P16 and L7), L7,

- in the off-shore region: P1, P140.

Sampling is done 5 times per year, typically in the months March/April, June, August, September, and November. Sampling is conducted according to the COMBINE manual (www.helcom.fi). The phytoplankton indicators used in the assessments are: species composition, abundance and biomass. The methodology employed in the monitoring program is according to the COMBINE manual.

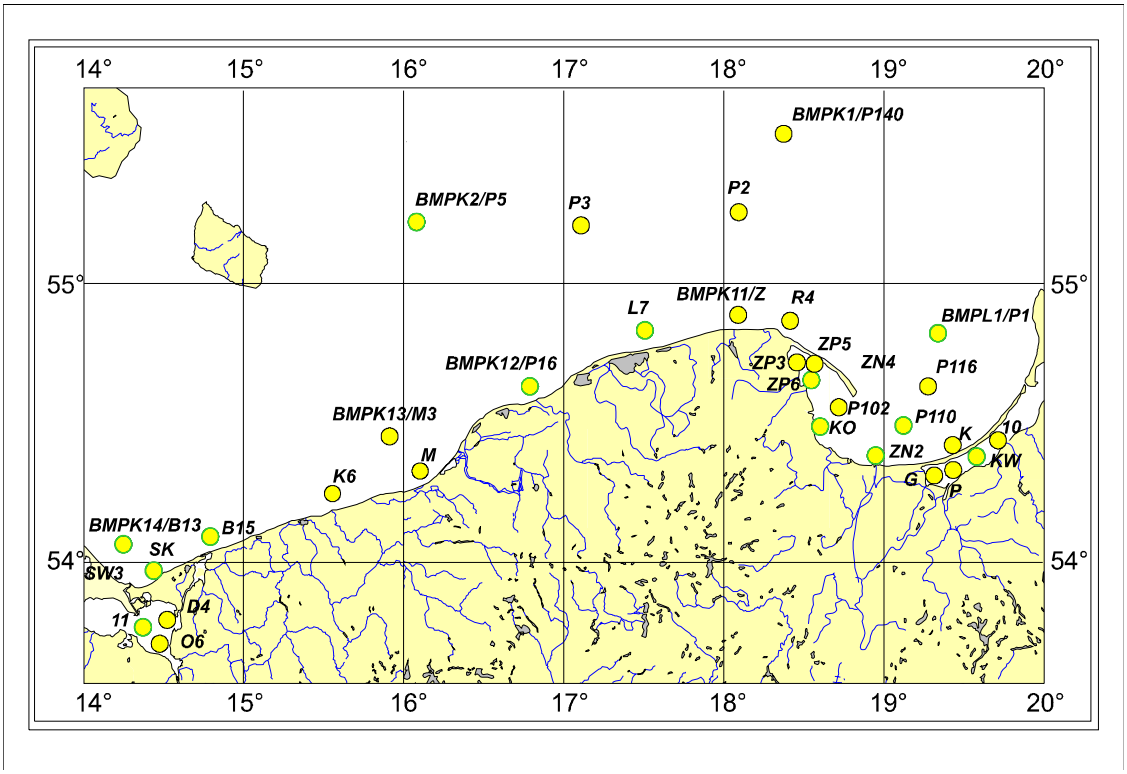

Figure 2: Overview of the Polish monitoring network for phytoplankton.

More specific information can be found in HELCOM (2002) and the annual reports from the Polish monitoring program (Warunki srodowiskowe polskiej strefy poludniowego Baltyku w 2000 (Environmental conditions in the Polish zone of the southern Baltic Sea in 2000), annual bulletin of the Maritime Branch of the Institute of Meteorology and Water Management in Gdynia, published since 1987, (in Polish)).

2.9 Phytoplankton monitoring in Germany

The current monitoring program is designed primarily for the HELCOM-assessment. National monitoring strategies, which fulfil the requirements of the WFD, will be developed in the next month under supervision of the responsible national authorities. Currently, the available sampling sites are not sufficient to deliver the data basis necessary for a biological evaluation of the water quality.

Table 1: The German monitoring program for phytoplankton. Samples were also analysed for abiotic variables (salinity, temperature, nutrients, etc.) Method according to HELCOM COMBINE.

institute name	station code/ geographic region	geographic position		phytoplankton parameter	frequency per year
		North	East		
IOW	BMPJ1, Gotland Deep	57°19,20'	20°03,00'	species composition; abundance; biomass; chl *a*	5
	BMPK1, South Gotland Sea	55°33,30'	18°24,00'		
	BMPK2, Bornholm Deep	55°15,00'	15°59,00'		
	BMPK5, Arkona Basin	54°55,50'	13°30,00'		
	BMPK8, Darss Sill	54°43,40'	12°47,00'		
	BMPM1, Kadet Trench	54°28,00'	12°13,00'		
	BMPM2, Mecklenburg Bight	54°18,90'	11°33,00'		
	OB, Oder Bank	54°05,00'	14°09,60'		
LANU	225059, Kiel Fjord	54°27,55'	10°14,70'	species com-position and abundance of main taxa; chl *a*	9-15
	225003, Flensburg Fjord	54°50,10'	9°49,60'		
	225019, innere Flensburg Fjord	54°50,40'	9°29,07'		
	BMPN3, Kiel Bight	54°36,00'	10°27,00'		
LUNG	GB19, Greifswalder Bay	54°12,40'	13°34,00'	species composition; abundance; biomass; dominant species; potential toxic species; chl *a*	10-20
	KHM, Sczecin Lagoon	53°49,50'	14°06,00'		
	O5, Mecklenburg Bight Warnemünde	54°13,90'	12°04,00'		
	O9, Darss Sill Hiddensee	54°37,40'	13°01,70'		
	O11, Arkona Sea Sassnitz	54°32,10'	13°46,20'		
	O22, Lübeck Bight	54°06,60'	11°10,50'		
	OB4, Pomeranian Bight Ahlbeck	54°00,40'	14°14,00'		
	WB3, Lübeck Bight Walfisch	53°57,00	11°24,50'		

The current monitoring program (so-called BLMP-program) is administered by the Bundesamt für Seeschifffahrt und Hydrographie (BSH; http://www.bsh.de/en/Marine%20data/Observations/BLMP%20monitoring%20programme/index.jsp) with the following participating institutions (Table 1):

- IOW- Baltic Sea Research Institute Warnemünde (abbreviated IOW for Institut für Ostseeforschung Warnemuende)
- LUNG – State office of environment, nature conservation and geology of Mecklenburg- Western Pomerania (abbreviated LUNG for Landesamt für Umwelt, Naturschutz und Geologie)
- LANU - State office of nature and environment of Schleswig-Holstein (abbreviated LANU for Landesamt für Natur und Umwelt)

The monitoring stations are distributed along the entire German Baltic Sea coastline (Figure 3).

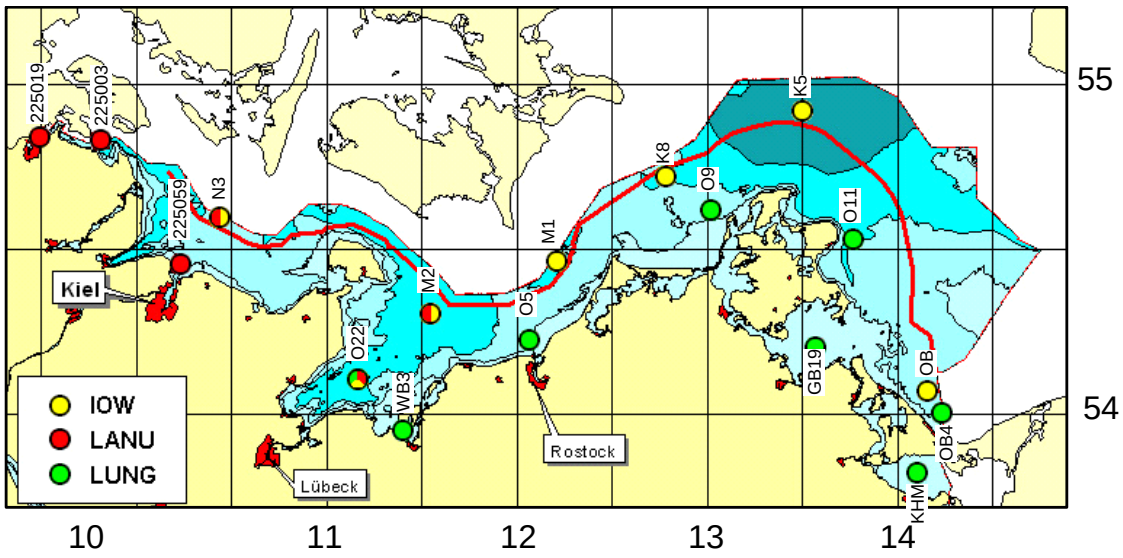

Figure 3: Position of the German monitoring stations for phytoplankton.

2.10 Algaline ships-of-opportunity

The Alg@line project was generated in 1993 to improve the coverage of extending pelagic monitoring in the Baltic Sea (Rantajärvi, 2003). The project, coordinated by FIMR, is carried out in joint cooperation of several research institutes and shipping

companies. It offers an extensive and inexpensive automated sampling method on board merchant ships. This 'Ships-of-opportunity' (SOOP) approach, unattended measurements and sampling on ferries and cargo ships form the basis of the Alg@line data collection.

Alg@line has its main emphases on the high frequency monitoring of phytoplankton and zooplankton in the Baltic Sea. It provides early warning system for harmful algal blooms, and by taking into account spatial and temporal dimensions it gives more adequate information on plankton communities and dynamics than traditional monitoring. In addition, the continuously measured hydrographical parameters on board SOOP give high frequency information of the water masses. This is important as the hydrographical processes, such as upwelling, which strongly regulate the plankton patterns. Alg@line data are used to validate ecological and hydrodynamic models and as reference data for optical remote sensing measurements. The indicator reports and environmental assessment provide follow-up tools for the basis of administrative decision-making.

New innovative approaches are under development to expand the use of Alg@line monitoring data. New sensors are to be installed onboard in cargo ships. At present the SOOP recordings *in vivo* fluorescence of chlorophyll *a* provides a relative measure of phytoplankton biomass. This is due to the fact that the ratio of *in vivo* fluorescence to chlorophyll *a* is dependent to phytoplankton species composition and physiological status of cells. The recording of in vivo fluorescence of chlorophyll a is not the best measure for cyanobacteria blooms. Phycobilin pigments of cyanobacteria could be used instead. There is a plan that a pilot project would record *in vivo* fluorescence of phycocyanin on board SOOP. This could offer a better tool to detect intensity and coverage of cyanobacteria blooms in the Baltic Sea.

New steps taken with optical remote sensing will also be connected to Alg@line monitoring in near future. Season specific algorithms for MODIS will be developed to estimate other phytoplankton pigments than chlorophyll *a*, such as phycobilins. The Alg@line ship borne monitoring provides reference data for the calibrations of the new satellite images.

3. Data availability and variation

An assessment of the recommendations for phytoplankton monitoring strategies essentially must take its starting point in analysing the existing monitoring programs. In this section we shall provide an overview of the data compiled within the CHARM database and produce some key statistics to describe the present state of phytoplankton monitoring in the Baltic Sea. These results will subsequently be used for determining appropriate number of samples (sample sizes) in the next section.

3.1. Overview of the CHARM database

Within the CHARM project phytoplankton data from the national, HELCOM, and Alg@line databases of seven countries (Denmark, Germany, Poland, Lithuania, Latvia, Estonia, and Finland) have been collected and stored in a database. The database contains bio-volumes at species level with additional taxonomical, morphological, functional and size group distribution for the different species recorded. In addition, hydrophysical and –chemical measurements from the same samples as well as, to some extent, wind observations have been combined with the phytoplankton data.

In the following we shall consider one sample as a one visit at a monitoring site, although there may be taken samples at several depths to characterise the profile. The idea is to demonstrate the monitoring effort in terms of ship-time and to a lesser degree the time associated with analysing the samples. Although the time used for species identification and enumeration of a phytoplankton sample can be costly, the most expensive part of a monitoring program is generally the ship-time used for travelling between monitoring stations, particularly for the open water stations.

There were generally few samples taken in the 1970s and 1980s compared to the 1990s (Figure 4). The highest number of samples (n=1071) was collected in 1997. The national monitoring programs has apparently had their up-and-downs, most visible for the German and Finnish monitoring programs in the mid 1980s, and the Latvian monitoring program in the early 1990s. The introduction of the Alg@line sampling in 1997 increased the number of samples associated with Estonia by factors of 5-6. Similarly, the introduction of regional phytoplankton monitoring in the late 1980s in Denmark resulted

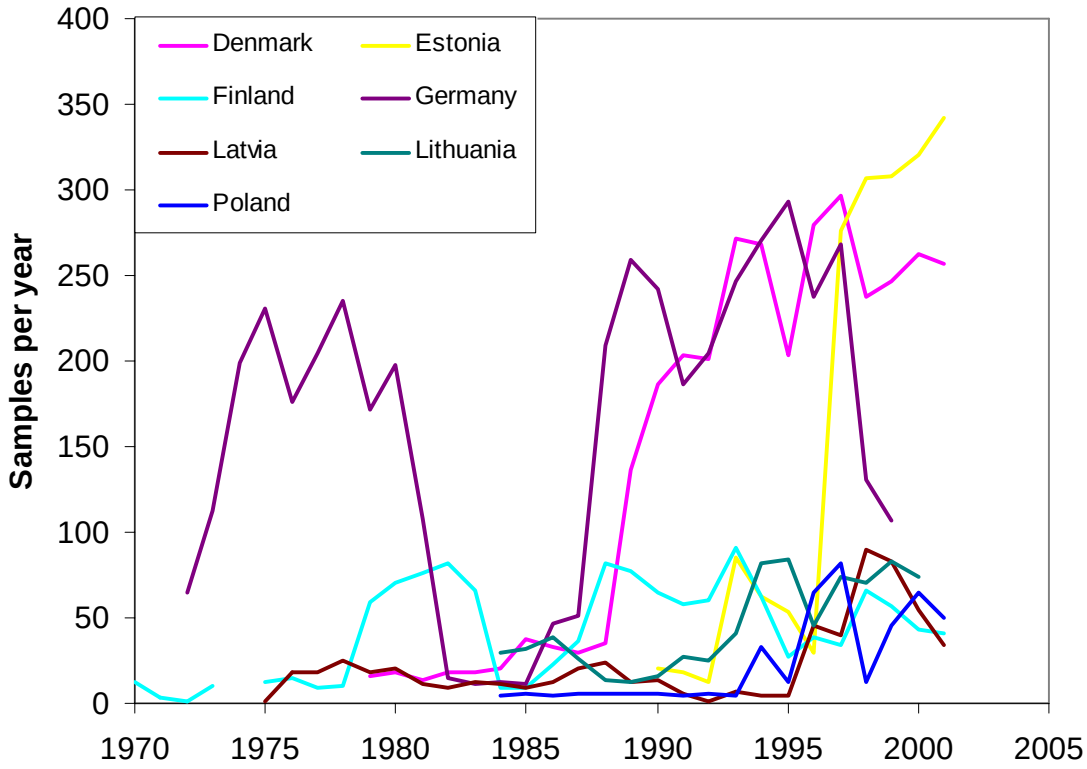

Figure 4: The number of samples per year for the different countries providing data. Note that Algaline data are shown under Estonia.

in increases in the number of samples by factors of 5-10. The data spanned from 1970 to 2001.

The monitoring data also has a bias towards more samples taken during summer than winter (Figure 5). The use of specific month for monitoring was particularly pronounced in the Finnish, Estonian, Latvian and Lithuanian monitoring programs (May and August). There were 2 to 4 times as many data in the summer period as during winter. It can also be seen that the Estonian monitoring program does not have any sampling in January or February, and in the Polish data there were only one sample taken in January and December. The Finnish monitoring data are also relatively scarce from December throughout March. This strong seasonal bias in the number of samples taken are due to problems with ice cover and bad weather during winter, and the fact that the phytoplankton biomass is generally low in the winter months and therefore not considered as information-rich as samples taken during the summer period. Such

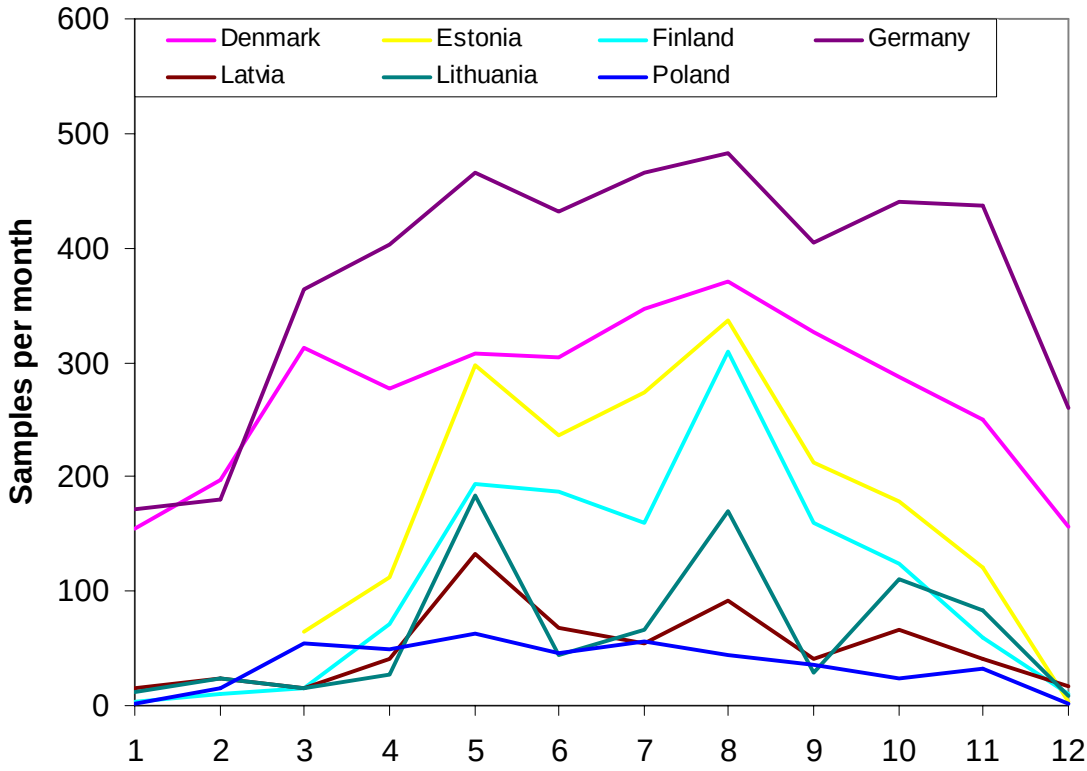

Figure 5: The number of samples per month for the different countries providing data. Note that Algaline data are shown under Estonia.

skew ness of data have to be taken into account when comparing data across different years, months and countries.

There are similarly large differences in the number of samples taken at the different stations and the number of stations each country provided (Figure 6). Denmark provided only 13 stations ranging from 122 to 398 samples per station as opposed to Poland that had 85 stations where only 7 had more than 10 samples (maximum of 95 samples at the station with the most data) and 10 stations only had 1 sample. Germany provided data from many stations (n=56), most stations had more than 30 samples taken. The seven Estonian stations with the most data were all from the Alg@line project. There were 46 stations that had more than 100 samples taken in total, most of these from Germany and Denmark. It should be acknowledged, however, that the total number of samples may have been taken over several years and therefore does not provide a direct indication of the monitoring frequency.

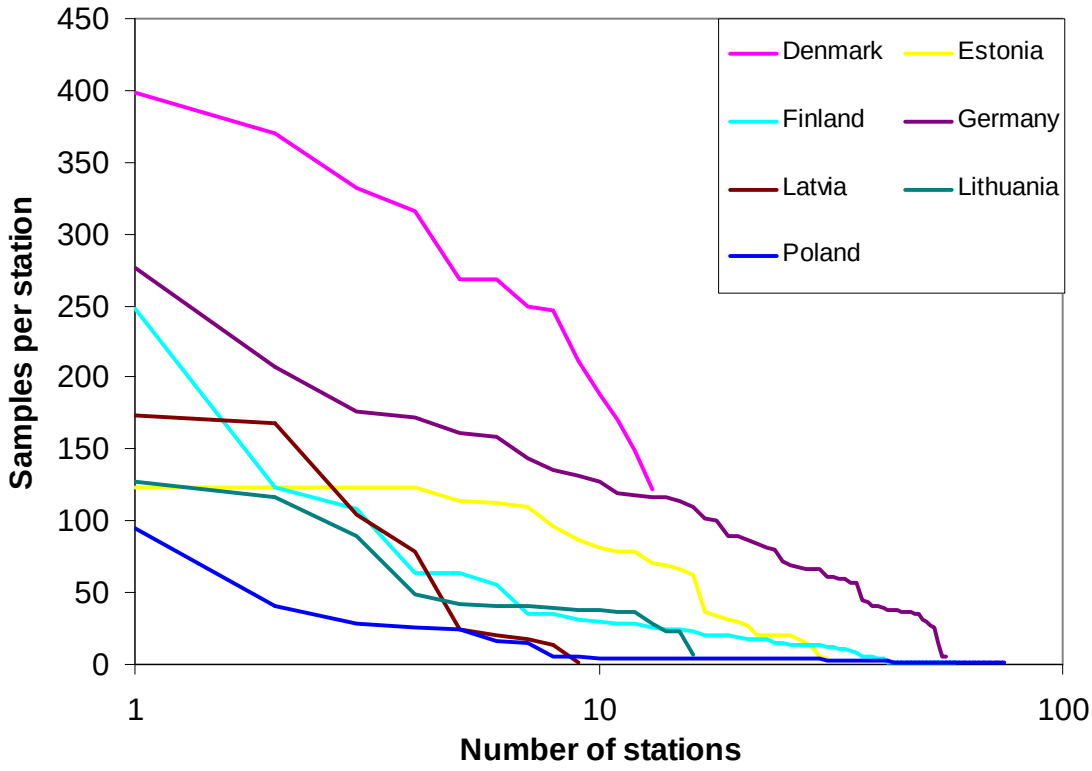

Figure 6: The number of samples per station for the different countries providing data. Stations have been ordered according to the number of samples taken. For better illustration of the differences the X-scale is logarithmic.

3.2 Frequency of monitoring

All the national monitoring programs appear to have adopted a strategy of intensive sampling at selected stations and more elaborate monitoring at other stations. This is clearly seen in the monitoring frequencies that reflect variations by at least factor two in the monitoring frequencies (Figure 7). The most intensively monitored stations have almost weekly to biweekly samples, except for the Latvian, Lithuanian and Polish monitoring programs where the typical frequency is less (about monthly) for the most intensively sampled stations. The 10 most intensive sampled Estonian stations were all from the Alg@line project. Otherwise the Estonian monitoring frequencies were comparable to the two other Baltic States and Poland. However, it should be stressed that the monitoring frequency was generally lower in the other years than those depicted in Figure 7.

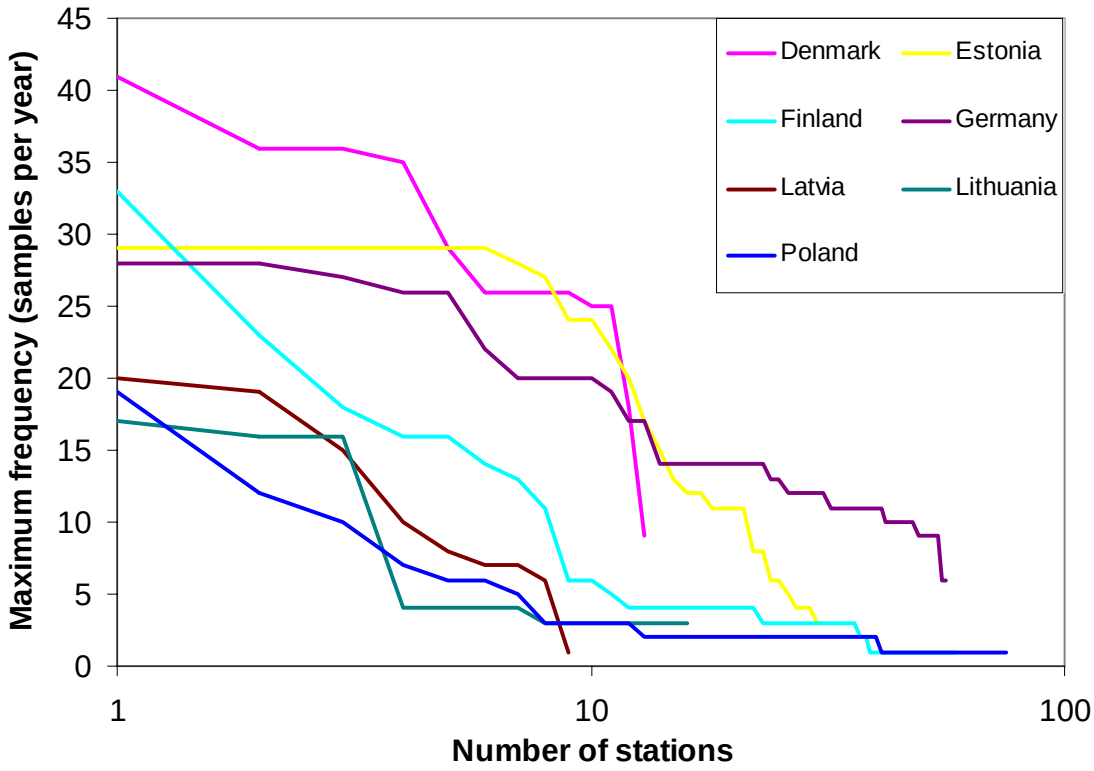

Figure 7: The maximum number of samples in a given year per station for the different countries providing data. Stations have been ordered according to their maximum sampling frequency. For better illustration of the differences the X-scale is logarithmic.

Phytoplankton monitoring is generally conducted in the summer period (Figure 5), but the sampling frequency is also more intense in the summer months (Figure 8). Considering the most intensively sampled year at a given station in each of the national monitoring programs there are large variations in the time period between two consecutive samples: Danish station D-5503 varied between 5 and 28 days (for 1997), Estonian station E-WQ10 varied between 5 and 24 days (for 1998), Finnish station F-Kyvy-8 varied between 1 and 21 days (for 1993), German station G-GOAP8 varied between 5 and 34 days (for 1975), Latvian station LA-119 varied between 9 and 57 days (for 1998), Lithuanian station Lt-Cl-12 varied between 13 and 35 days (for 1997), and Polish station P-ORU varied between 6 and 42 days (for 1996). For instance the Finnish station F-Kyvy-8 was monitored approximately every 3 to 4 days during the spring period

and about every week in July-September, whereas there were no samples in January-February and October-December. The times of sampling were definitely not uniformly distributed over the seasons, hence complicating the application of classical time series analysis methods.

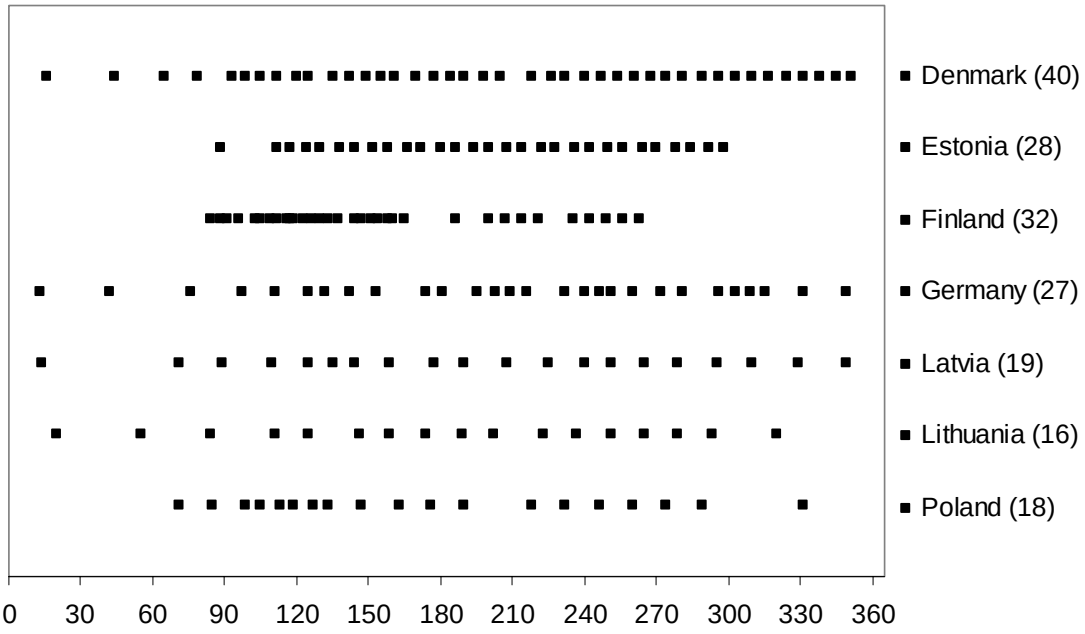

Figure 8: Time of sampling during the year at the most intensively monitored stations for each country. The numbers of samples taken during the most intensively sampled year are given in parentheses.

3.3 Temporal variations

Phytoplankton data mostly exhibit a strong seasonal variation and year-to-year variations that affect both the magnitude and appearance of the seasonal cycle. Estimating the seasonal cycle for the most intensively sampled station from each country by employing a fourth order harmonic to the log-transform of the biomass confirmed this (Figure 9). Some stations had a very pronounced spring bloom (e.g. F-Kyvy-8, LA-119, and E-WQ10), typically located in open-waters, whereas other more coastal and estuarine stations (D-5503, G-GOAP8, Lt-Cl-12, and P-ORU) had a relatively high biomass throughout most of the productive season. The mean biomass for the seven stations considered varied by more than by factor of 20, with station E-WQ10 in the open-part of

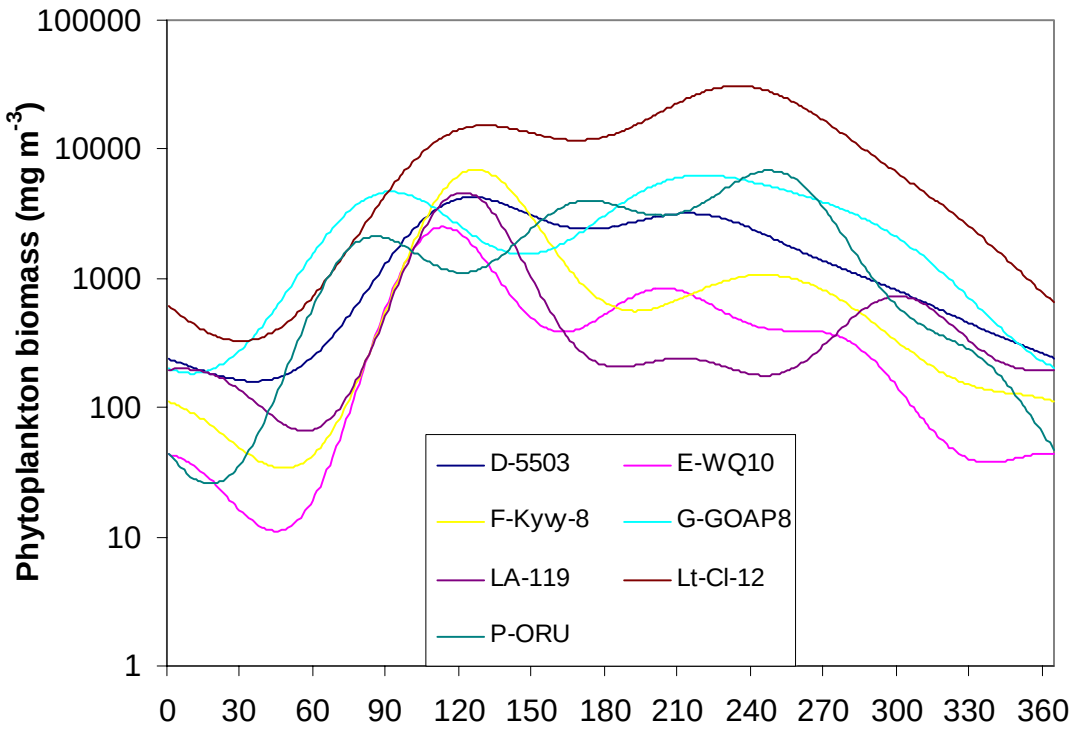

Figure 9: Seasonal cycle of phytoplankton biomass estimated by a fourth order harmonic for the most intensively monitored stations for each country. Note the logarithmic scale on the secondary axis. The first part of the station name indicate the national monitoring program (D=Denmark, E=Estonia, F=Finland, G=Germany, LA=Latvia, Lt=Lithuania, P=Poland).

the Gulf of Finland having the lowest, and Lt-Cl-12 in the Curonian Lagoon having the highest biomass.

The yearly means for the stations considered also reflected substantial interannual variation by station-specific factors ranging from 2 to 16 between the lowest and highest concentration years (Figure 10). Investigating the correlations between stations for the annual means resulted in two significant values; however, this corresponded to the expected amount of null-hypothesis rejections from multiple testing (type I error) given that there is no correlation. It should be stressed, though, that the number of overlapping years between the investigated stations was rather low.

The standard errors of the means varied from 13% up to 200% of the mean value depending mainly on the number of observations the mean was calculated from. The residual variance (Table 2) was largest in the estuaries (D-5503 and Lt-Cl-12) and

smallest at the more open-water stations (F-Kyvy-8 and E-WQ10). The seasonal cycle model combined with yearly means for the interannual variation explained between 49% (at D-5503) and 76% (at F-Kyvy-8) of the total variation in the log-transformed biomasses.

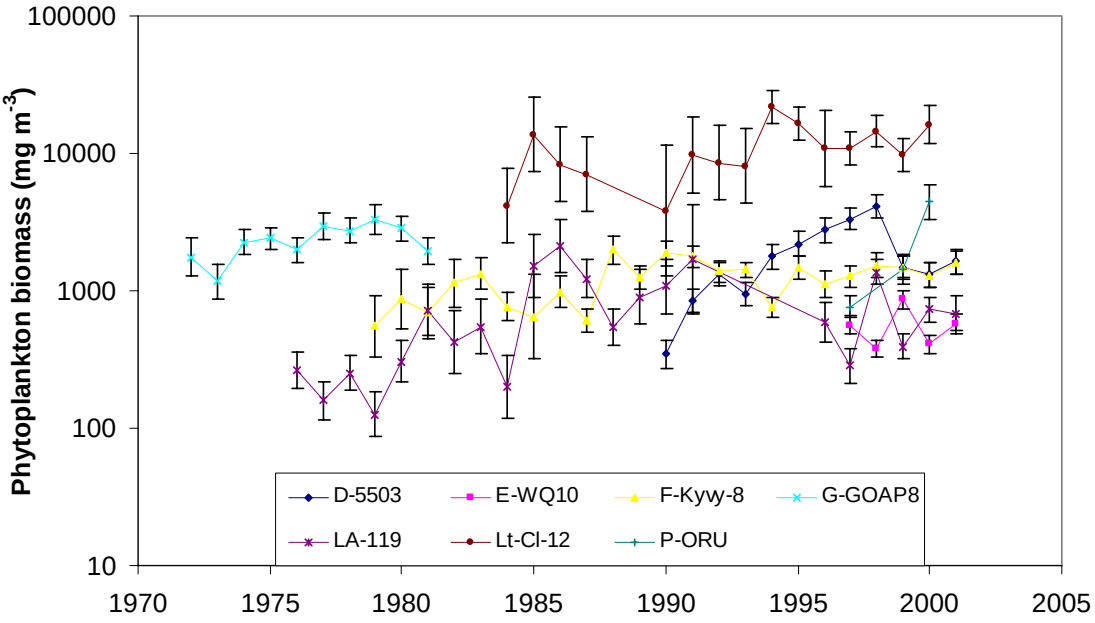

Figure 10: Interannual variation in mean phytoplankton biomass at the most intensively monitored stations for each country. The seasonal variation in data was extracted by means of the seasonal cycles in Figure 9. Error bars mark the standard errors of the means.

The covariance structure of the residuals from the model was investigated to determine any potential autocorrelation in the time series that was not described by the station-specific fixed seasonal cycle. The autocorrelation was described by means of an exponential function, where the correlation between observations decayed with the number of days (d_{ij}) between the observations ($\sigma^2[\exp(-dij/\theta)]$). Furthermore, a variance component ($\sigma^2{}_m$) describing the uncorrelated error of the measurement itself was also included in the covariance structure. The covariance structure was estimated on the log-transformed phytoplankton biomasses that were assumed normal distributed.

Table 2: Statistics from fitting a seasonal model combined with interannual variation for the most intensively monitored station for each country.

Station	R^2	Residual variance	Overall mean	Year		Seasonal cycle	
				df	*p*	*df*	*p*
D-5503	0.49	1.33	7.37	11	<0.0001	8	<0.0001
E-WQ10	0.58	0.54	6.27	4	0.0011	8	<0.0001
F-Kyvy-8	0.76	0.49	7.13	22	0.0008	8	<0.0001
G-GOAP8	0.53	0.86	7.76	9	0.2458	8	<0.0001
LA-119	0.73	0.82	6.27	21	<0.0001	8	<0.0001
Lt-Cl-12	0.58	1.15	9.44	14	0.5706	8	<0.0001
P-ORU	0.71	0.66	7.25	2	0.0002	8	<0.0001

The covariance structure was well determined with most parameters significant at 5% significance levels for stations D-5503, E-WQ10, F-Kyvy-8, and LA-119, whereas the parameters were less well determined for station Lt-Cl-12 (Table 3). The covariance structure could not be determined for G-GOAP8 and P-ORU. The measurement variance was generally larger than the variance component for the autocorrelation, up to 4 times larger, suggesting that a large portion of the total variance derives from the conduct and analysis of the sample, i.e. reflecting the variance in the phytoplankton biomass (log-transformed), if several samples were taken at the same location and at the same time. For phytoplankton biomass observations on the original scale these values correspond to variations between 58% for E-WQ10 and 174% for D-5503. The deviations from the fixed seasonal cycle, modelled by means of an autoregressive correlation structure, were typically correlated more than 50% for 1-2 weeks. This component, although formulated as a stochastic model, can be interpreted as systematic, non-random variations in the mean phytoplankton biomass that we are not able to model through a fixed component.

Table 3: Estimation of the covariance structure for the most intensively monitored stations for each country. The covariance structure could not be estimated for G-GOAP8 and P-ORU, most likely due to infrequent sampling relative to the time constants in the covariance structure.

Station	Measurement var. σ^2_m		Correlation var. σ^2		Decay parameter θ	
	Estimate	*p*	Estimate	*p*	Estimate	*p*
D-5503	1.0137	<0.0001	0.3307	0.0053	22.40	0.0009
E-WQ10	0.2101	0.0396	0.2804	0.0056	13.03	0.0782
F-Kyvy-8	0.3227	0.0001	0.1101	0.0748	8.76	0.0013
LA-119	0.5119	0.0345	0.1691	0.2660	13.06	0.0240
Lt-Cl-12	0.7623	0.2468	0.1829	0.4336	12.39	0.1851

Eutrophication assessments are often based on the calculation of mean values, i.e. annual mean or summer means of phytoplankton. In terms of deriving unbiased values for these means, simple averages fulfil this requirement provided that the monitoring data are approximately equidistantly distributed over the considered period. Moreover, the standard error of the mean is calculated by standard deviation divided by the squareroot of *n-1* (*n* is the number of observations that the mean is based on). The assumption of a constant mean value is not true, probably not even for the summer period (see Figure 9). This implies that seasonal variation and systematic variation modelled by the autoregressive model above are misinterpreted as completely random variation. Consequently, the standard deviation is a gross overestimate of the random variation, which has important implications for the number of observations required to obtain a given precision (see below). Neglecting the seasonal variation by averaging over the entire year resulted in residual variances 2-3 times larger than those in Table 2.

The residual variance decreasing and R^2 increased when including the seasonal cycle and the autocorrelation structure in addition to standard averaging of summer values (Table 4). However, due to the reduction in data (summer observations only) and the truncation of the time series at start and end of the summer period the autocorrelation structure could only be determined for a single station.

Table 4: Coefficients of determination and residual variance for summer phytoplankton biomass (May-September) for 1) averaging only,2) including a seasonal cycle and 3) including autocorrelation. Only E-WQ10 had sufficient data to estimate the autocorrelation structure.

Station	w/o seasonal cycle		w. seasonal cycle		w. autocorrelation	
	R^2	Res. Var.	R^2	Res. Var.	R^2	Res. Var.
D-5503	0.20	0.915	0.28	0.855	-	-
E-WQ10	0.19	0.616	0.43	0.477	0.77	0.256
F-Kyvy-8	0.12	1.273	0.72	0.425	-	-
G-GOAP8	0.14	1.116	0.39	0.876	-	-
LA-119	0.29	2.675	0.82	0.769	-	-
Lt-Cl-12	0.22	0.638	0.52	0.463	-	-
P-ORU	0.25	0.975	0.64	0.801	-	-

3.4 Spatial variations

Designing a monitoring network it is also important to consider the potential spatial correlation. Obviously, there is no point in positioning two monitoring stations next to each other, but how close can they be located without producing redundant information? We investigated the spatial correlation structure for two separate areas: the Alga@line transect in the Gulf of Finland and the Curonian Lagoon. Before investigation the spatial correlation a spatial trend common to all data was estimated and subtracted from the data. For the Alga@line data the spatial trend showed increasing phytoplankton biomass from Tallinn towards Helsinki (from SSW to NNE), whereas there was a decreasing trend from North to South in the Curonian Lagoon corresponding to the axis of the estuary and the location of monitoring stations.

Estimating spatial correlation structure (exponentially decreasing correlation with distance) for the residuals subjected to the different monitoring cruises revealed for the Alga@line data a variance of 0.1656 for the measurement error and microscale variation, whereas the systematic spatial correlation variance was of the same magnitude (0.1815). The estimated distance coefficient (θ=21.29 km) showed that the spatial correlation was 0.5 within a range of 15 km and 0.1 within a range of 50 km. Thus, locating monitoring stations closer than 15 km in an open-water ecosystem such as the Gulf of Finland may

result in some degree of data redundancy. The Alga@line stations were typically about 6 to 12 km apart, but most cruises would only sample a limited number of the stations.

In the Curonian Lagoon it was not possible to estimate a spatial correlation structure after the spatial trend was removed. This may be due to a combination of scarcity in the data or that the distance between monitoring stations is larger than the range of spatial correlation. In the latter case spatial correlation ranges would be less than the typical 5 to 10 km between stations in the monitoring program. It should be recognised that many of the cruises did not sample all stations and therefore there may be relatively few observations with short inter-station distances. However, it seems plausible that correlation scales could be less than 5 km in lagoons such as the Curonian Lagoon when compared to a scale of approximately 15 km in the open-waters and considering the often highly dynamic and changing environment of estuaries.

These considerations lead to suggest that distances between monitoring stations should be around 5 km or more in enclosed areas such as bays, lagoons, and estuaries, around 10 km or more in coastal areas and at least 15 km in open waters in order to avoid redundancy in the monitoring data. These results are rough estimates that may be applied more as a rule-of-thumb rather than a categorical design criterion.

4. Sample size determination

The mean level of an indicator is usually estimated by averaging over the observations. If the seasonal variation is not accounted for the uncertainty of the estimate will be too high, however, in order to estimate the seasonal variation there should be a reasonable amount of data available. Data requirements are even higher (particularly high frequency data), if an autocorrelation structure is also to be estimated. In this section we shall describe the basic methods for determining the number of samples required (sample sizes) in order to have a given precision with a given confidence, and we shall employ these methods to indicators for annual and summer phytoplankton biomass. We shall refer to sample size by the statistical definition as the number of observations to be sampled.

4.1. Methods for determining sample sizes

Let y_i denote the i'th observation (i=1,...,*n*) during a given period of time. Assuming the observations to be normal distributed, $N(\mu, \sigma^2)$, the 95% confidence interval for the average of the observations ($\bar{y}$) is

$$\bar{y} \pm t_{n-1,0.975} \cdot \frac{s}{\sqrt{n}}$$

where $t_{n-1,0.975}$ is the 97.5-percentile of the t-distribution with *n*-1 degrees of freedom and s is the estimated standard deviation. Let *d* be the desired precision of the mean with 95% confidence

$$d \geq t_{n-1,0.975} \cdot \frac{s}{\sqrt{n}}$$

which translates into calculating the minimum sample size for obtaining this precision.

(1) $$n \geq \left\{ t_{n-1,0.975} \cdot \frac{s}{d} \right\}^2$$

Note that N also appears on the right-hand side of (1) and therefore *n* should be found iteratively.

In case the observations are independent the standard error of the average is estimated as $\frac{s}{\sqrt{n}}$, where $s = \sqrt{\frac{1}{n-1}(\sum_{i=1}^{n}(y_i - \bar{y})^2)}$. In case the observations are correlated in time (autocorrelated, typically positive) the standard error of the average is generally larger. One of the most simple and commonly used correlation structures for equidistant observations is the autoregressive model of order 1, AR(1), and for this correlation structure the standard error of the average can be estimated as $\frac{s}{\sqrt{n}} \cdot \sqrt{\left\{1+2\left\{\frac{\rho}{1-\rho}\right\}\left\{1-\frac{1}{n}\right\}-2\left\{\frac{\rho}{1-\rho}\right\}^2\left\{1-\rho^{n-1}\right\}/n\right\}}$ where ρ is an estimate for the lag1-correlation. The sample size formula in (1) then becomes

$$(2)\ n \geq \left\{ t_{n-1,0.975} \cdot \frac{s}{d} \sqrt{\left\{1+2\left\{\frac{\rho}{1-\rho}\right\}\left\{1-\frac{1}{n}\right\}-2\left\{\frac{\rho}{1-\rho}\right\}^2\left\{1-\rho^{n-1}\right\}/n\right\}} \right\}^2$$

Again, n also appears on the right-hand side of (2) and must consequently be found iteratively.

The formulas for the sample size, (1) and (2), can also be employed to data that is not normal distributed, provided that *n* is large (> 30) and $t_{n-1,0.975}$ is then replaced by 1.96, the 97.5-percentile of the normal distribution. If the standard error of the distribution is known, and need not be estimated from the observations, then $t_{n-1,0.975}$ is similarly replaced by 1.96.

If the observations have a right-skewed distribution or the absolute uncertainty is scale-dependent of the mean level (i.e. larger observations have a larger absolute uncertainty), it is more convenient to consider the logarithmic transformed observations $x_i = \log_e(y_i)$, where $\log_e$ denotes the natural logarithm. The confidence interval for the log-transformed observations can be calculated as above and back-transformed to the original scale by means of the exponential function. This back-transform of the average and its confidence interval correspond to the geometric average ($\bar{y}_G = \exp(\bar{x})$) and its confidence interval. The upper limit of the confidence interval is $\bar{y}_G \cdot (1+d)$ where *d* is the precision for the geometric average and the minimum samples required to obtain this precision is

(3) $$n \geq \left\{ t_{n-1,0.975} \cdot \frac{s}{\log_e(1+d)} \right\}^2$$

for the case of independent observations. In the case of correlated observations described by an AR(1) correlation structure the sample size is found as

(4) $$n \geq \left\{ t_{n-1,0.975} \cdot \frac{s}{\log_e(1+d)} \sqrt{\left\{ 1 + 2\left\{ \frac{\rho}{1-\rho} \right\}\left\{ 1 - \frac{1}{n} \right\} - 2\left\{ \frac{\rho}{1-\rho} \right\}^2 \left\{ 1 - \rho^{n-1} \right\} / n \right\}} \right\}^2$$

where s is the estimated standard deviation and ρ is an estimate for the log1-correlation of the log-transformed observations. The precision *d* should be entered as a decimal number, e.g. a desired precision of 20% of the geometric average corresponds to *d*=0.20.

In the case that the observations are few and cannot be assumed normal or lognormal distributed the confidence interval can be found by means of bootstrapping (Efron & Tibshirani 1998).

4.2 Sample sizes for annual phytoplankton biomass

In the previous section the standard error of the annual average after employing a seasonal cycle model were calculated (Table 2). These standard errors were all based on more than 30 observations and therefore the t-distribution was approximated by the normal distribution (using the percentile value of 1.96). A precision of 10% is not realistically feasible for phytoplankton biomass by a seasonally adjusted mean value, as this would require more than 100 observations on an annual basis (Table 5). It should be stressed that the numbers in Table 5 do not take the autocorrelation into account that becomes important, if sampling is to be carried out on a weekly basis and maybe also on a biweekly basis.

It is probably more realistic to expect a precision of 40-50% at open water stations and >50% at estuarine and coastal stations. If we include an autocorrelation of ρ=0.5 between weeks and assume that weekly monitoring is carried out (n=52) the precision will be 71% for D-5503, 41% for E-WQ10, 39% for F-Kyvy-8, 54% for G-GOAP8, 53% for LA-119, 65% for Lt-Cl-12 and 46% for P-ORU. Similarly, a biweekly sampling

scheme (n=26) with a correlation of ρ=0.25 between samples would results in precisions of 76% for D-5503, 44% for E-WQ10, 41% for F-Kyvy-8, 58% for G-GOAP8, 56% for LA-119, 69% for Lt-Cl-12 and 49% for P-ORU. Thus, changing the monitoring frequency from weekly to biweekly only has minor increases in the precision of the annual mean, if the autocorrelation is to be interpreted as a completely random process.

Table 5: Number of samples required to obtain a relative precision from d=0.1 to 0.5 in the annual mean phytoplankton biomass, based on a seasonal adjustment. Autocorrelation was not accounted for.

Station	Residual variance	Desired precision of annual mean				
		d=0.1	d=0.2	d=0.3	d=0.4	d=0.5
D-5503	1,3305	563	154	74	45	31
E-WQ10	0,5398	228	62	30	18	13
F-Kyvy-8	0,4883	207	56	27	17	11
G-GOAP8	0,858	363	99	48	29	20
LA-119	0,8162	345	94	46	28	19
Lt-Cl-12	1,1496	486	133	64	39	27
P-ORU	0,6588	279	76	37	22	15

If we, however, consider the autocorrelation to be governed by some underlying mechanistic process and that the "real" source of randomness is described by σ^2_m this has a great implication for the required amount of data (Table 6). It now appears reasonable to have a precision about 50% for estuaries, about 40% for coastal stations and about 30% for open water stations. The number of observations required is proportional to the residual variance and consequently (3), obtaining as precise and unbiased estimates of the random variation is crucial to the sample size determination. For the 5 stations considered the reduction in the number of samples required to obtain a given precision was reduced by 13% to 38% by changing the statistical method of assessment. Improving the description of the seasonal cycle and the correlation structure, and maybe include explanatory variables in the model may further reduce the residual variance and lead to a lesser requirement for the monitoring program.

Table 6: Number of samples required to obtain a relative precision from d=0.1 to 0.5 in the annual mean phytoplankton biomass, based on a seasonal adjustment and autocorrelation model.

Station	Residual variance	Desired precision of annual mean				
		d=0.1	*d*=0.2	*d*=0.3	*d*=0.4	*d*=0.5
D-5503	1,0137	429	117	57	34	24
E-WQ10	0,2101	89	24	12	7	5
F-Kyvy-8	0,3227	136	37	18	11	8
G-GOAP8						
LA-119	0,5119	216	59	29	17	12
Lt-Cl-12	0,7623	322	88	43	26	18
P-ORU						

4.3 Number of samples for summer phytoplankton biomass

Similar to the calculations above for the annual mean phytoplankton biomass, the number of observations required to obtain a given precision were calculated without a seasonal correction (Table 7) and with a seasonal correction (Table 8). Considering that the realistic number of samples within the considered 5 summer months is unlikely to exceed 20 and 10 observations is probably more realistic, the precision to be obtained without accounting for the autocorrelation is around 50%.

It was only possible to estimate a seasonal model including a term for the autocorrelation for E-WQ10 if summer observations were used only. The residual variance of 0.2562 corresponded to an expected precision of 30%, if 14 samples were taken during the summer months. Thus, in this case the monitoring requirements were reduced by almost 50% including the autocorrelation.

It should be noted that the residual variance during the summer period was lower for all stations, except G-GOAP8 and P-ORU, than the residual variance for the annual mean value. However, the realistic number of samples within the summer period is also lower than the number of observations on an annual basis. Assuming that approximately 50% of the annual samples are taken during the summer period, the residual variance of

Table 7: Number of samples required to obtain a relative precision from d=0.1 to 0.5 in the summer (May-September) mean phytoplankton biomass without seasonal adjustment. Autocorrelation was not accounted for.

Station	Residual variance	Desired precision of annual mean				
		d=0.1	*d*=0.2	*d*=0.3	*d*=0.4	*d*=0.5
D-5503	0,9152	387	106	51	31	21
E-WQ10	0,6155	260	71	34	21	14
F-Kyvy-8	1,2729	538	147	71	43	30
G-GOAP8	1,1160	472	129	62	38	26
LA-119	2,6748	1131	309	149	91	63
Lt-Cl-12	0,6376	270	74	36	22	15
P-ORU	0,9753	412	113	54	33	23

the summer means should similarly be 50% lower than the residual variance of the annual means to obtain the same precision in the mean values. However, the variance reduction obtained by considering summer observations only is relatively small and it is therefore recommendable to consider annual mean relative to summer means from the point of obtaining a better precision.

Table 8: Number of samples required to obtain a relative precision from d=0.1 to 0.5 in the summer (May-September) mean phytoplankton biomass with seasonal adjustment. Autocorrelation was not accounted for.

Station	Residual variance	Desired precision of annual mean				
		d=0.1	*d*=0.2	*d*=0.3	*d*=0.4	*d*=0.5
D-5503	0,8547	361	99	48	29	20
E-WQ10	0,477	202	55	27	16	11
F-Kyvy-8	0,4255	180	49	24	14	10
G-GOAP8	0,8762	371	101	49	30	20
LA-119	0,7685	325	89	43	26	18
Lt-Cl-12	0,4625	196	53	26	16	11
P-ORU	0,8008	339	93	45	27	19

5. New emerging technologies for phytoplankton monitoring

Phytoplankton identification and biomass determination by microscopy as well as chlorophyll a measurements have been the standard for phytoplankton monitoring in the Baltic Sea for the last for 3 to 4 decades. Although chlorophyll a is only a proxy measure of the phytoplankton biomass that vary with species composition, season and depth of sampling, it may provide a more robust biomass measure than biomass determined by microscopy but it contains no information on the composition. These constraints with present day methods for phytoplankton monitoring have led investigating alternative techniques, however, many of these are still on an experimental state.

5.1 Pigment analysis

Phytoplankton contain numerous different pigments of which chlorophyll a (chl *a*) is found in all phytoplankton species. For approximately 50 years spectrophotometric analysis of chl *a* has been used as a proxy of phytoplankton biomass. In the 1960s the fluorometric method for measuring chl *a* was introduced. This in vivo analysis of chl *a* has facilitated high-resolution vertical profiling, which has become a regular feature of many monitoring programs. More recently, continuous on-line fluorometric chl *a* measurements have been implemented on a number of ships-of-opportunity (e.g. http://www.fimr.fi/en/itamerikanta/levatiedotus/menetelmat.html) providing a regular spatial coverage of chl *a* measurements previously not possible to obtain. While easily measured and generally providing a good estimate of the biomass of phytoplankton, chl *a* is indicative of only the total phytoplankton biomass with no information on the community structure.

With the development of modern analytical procedures like high-performance liquid chromatography (HPLC), the use of chemotaxonomical classification of phytoplankton communities from analysis of pigment contents has increased. This method provides a quantitative measure of chl *a* and, in addition, accessory pigments that are more or less unique (’marker pigments’) to specific taxonomic groups (e.g. prasinoxanthin in some prasinophytes and peridinin in most dinoflagellates) and others that are found mainly in one or few groups (e.g. 19’-hexanoyloxyfucoxanthin in

prymnesiophytes and some dinoflagellates, and fucoxanthin in diatoms, chrysophytes, prymnesiophytes, and raphidophytes). The quantification of these pigments provide the basis for calculating the contribution of individual phytoplankton groups to the total amount of chl *a* given sufficient knowledge of the relationship between cellular content of marker pigments and chl *a* in different taxa.

Algorithms for deriving contributions from different phytoplankton groups to total chl *a* have been obtained by multiple regressions or by inverse methods based on individual marker pigments (Gieskes and Kraay, 1983; Letelier et al., 1993; Tester et al., 1995; Kohata et al., 1997). Another, and by now more commonly used, approach has been application of a matrix factorisation program, 'CHEMTAX' (Mackey et al., 1996), using input matrixes of, in principle, all identified and quantified pigments in samples and the corresponding pigment ratios of phytoplankton taxa potentially present. The output from the calculations provides the best fit of contributions from the predefined taxa to the true measured chl *a*.

Characterisation of phytoplankton communities using pigment analysis is cost-efficient and much less time consuming than traditional analysis in the microscope. However, it should be emphasised that the results are not directly comparable to those obtained by the traditional microscopic method. The chemotaxonomical approach provides information at only the class or group level while microscopy provides information about individual species. However, groups of small organisms impossible to identify in the microscope, but containing specific pigments, may be quantified by pigment analysis.

Pigment-based description of phytoplankton composition will be based on calculated contributions from different phytoplankton groups to the total chl *a*. Thus, seasonal or vertical light-induced variations in the ratio of carbon or biovolume to chl *a* will also be reflected in estimates of the biomass of different groups using microscopy and pigment analysis, respectively.

5.2 DNA analysis

DNA techniques cover many different areas and methods, of which some are out-lined briefly below:

- *Effects of contaminants*. Analysis of DNA-strand breaks, formation of DNA-adducts, and expression of mRNA is used as biomarkers for contaminants (Reichert et al. 1999).
- *Community analysis of bacteria and pico-plankton*. Microbial community analysis using DNA-techniques include PCR-based methods such as clone-libraries, finger-printing techniques such as Denaturing-Gradient-Gel-Electrophoresis (DGGE), and microarrays. PCR-based techniques are not fully quantitative unless a specific target organism is of interest, but can have a resolution down to species level. Direct DNA/rRNA techniques, such as In-Situ Fluorescence Hybridisation (FISH), are quantitative but often lacks resolution on species level. DNA/rRNA microarrays are more quantitative than PCR-based arrays.
- *Changes in genetic diversity*. Molecular techniques can be used to determine the relationship between populations of the same species in order to determine whether the intra-species biodiversity has changed.

5.3 Remote sensing

The earth observation satellite data provided by the Sea-viewing Wide Field-of-view Sensor (SeaWiF) can provide a synoptic view of the physical processes and biological compounds in the coastal and marine ecosystems. Such data is potentially very promising to provide an overall synoptic picture of the phytoplankton biomass as well as of the temporal and spatial variability of phytoplankton bloom frequency, provided that the underlying algorithms used in the conversion of the satellite data to chlorophyll a (chl *a*) concentrations are properly calibrated for specific marine areas, and that there is a comprehensive data set of in situ measurements to support the validation of remote sensing products. Optimally remote sensing products and the use of these products in

indicators such as EUTRISK (Druon et al. 2004) could be linked to pressure information (such as nutrient loading) for evaluation of the vulnerability of coastal ecosystems for eutrophication, and to 'a priori' typologies (Schernewski & Wielgat 2004) to enable the development of type specific reference conditions and classification. Although there are intensive on-going activities for development of the regional algorithms for the retrieval of chlorophyll *a*, more research will be needed to allow operational use of remote sensing data for instance for the WFD compliant assessment of coastal and transitional waters.

Recently a project "*Validation of algorithms for chlorophyll a retrieval from satellite data of the Baltic Sea area*", carried out by the EC Joint Research Centre for the HELCOM MONAS was completed (HELCOM, 2004). This project compared four existing regional algorithms for the computation of the chl *a* using the SeaWiFS images from the Baltic Sea. The investigation consisted in comparisons of the in situ chl *a* measurements with those determined with different Baltic Sea algorithms applied to SeaWiFS atmospherically corrected data (Schrimpf and Zibordi 2004).

The atmospheric correction of the ocean colour data from the Baltic Sea appeared to be difficult, mainly due to high solar zenith angles and the relatively high absorption of dissolved organic matter (yellow substance) in the Baltic Sea, which makes it difficult to apply universal chl *a* algorithms in the Baltic. The results of this study indicated that in general the satellite products underestimate chl *a* concentrations in comparison to in situ measurements, and are not completely able to encompass the overall variability of the chl *a* concentrations in situ measurements. However, there was more encouraging comparability when using the Alg@line results (Schrimpf and Zibordi 2004).

The potential applications of remote sensing products for assessment of coastal and marine waters are huge. However, the techniques are not yet ready to allow operational use of such data on a national basis. Based on the project results it was recommended that in order to support the validation of remote sensing products extensive spatial and temporal data sets of marine apparent optical properties for algorithm development would be needed. Such task would require a multi-year activity, involving co-operation of various on-going remote sensing developments and institutes in the Baltic Sea.

6. Monitoring requirements by WFD

The monitoring requirements in the Annex V of the WFD, allow flexibility for the design of the monitoring programs. Three different types of monitoring strategies are described: surveillance, operational, and investigative monitoring, which all have different aims in terms of detection or mapping of the environmental status. Surveillance monitoring is to be carried out as basis for deciding upon the coverage of the operational monitoring, and investigative monitoring functions as check if operational monitoring would be needed for more water bodies than identified on the basis of surveillance monitoring (in case that there is a doubt for risk failing the environmental objectives). Large flexibility is provided in the terms of parameters to be chosen (within the required biological quality elements) and the methods and the sampling strategies to be applied in the monitoring programs. However, it is stated the "Estimates of the level of confidence and precision of the results provided by the monitoring programs shall be given in the [River Basin Management] Plan". Further, WFD states the minimum frequencies of sampling for several quality elements, and if available, international standards for sampling and analysis should be followed. For instance, for phytoplankton parameters sampling is required to be carried at least every 6 month (WFD Annex V, 1.3.4.).

The Guidance on Monitoring under the WFD (Monitoring 2003), that was prepared under the Common Implementation Strategy, outlines a common understanding of all Member States of the interpretations of the Annex V texts, as well as definitions of the terminology and approaches for the monitoring strategies. In the Toolbox of the Best Practices general principles are given for designing and optimization of monitoring programs, on the general requirements for quality assurance and quality control, on the risk, precision and confidence in the assessment, number and location of the monitoring stations, and on the frequency on monitoring. There are no detailed or specific guidance of any of these issues, while it is left for the Member States to decide the details of their monitoring programs.

The guidance foresee some problems in the applicability of phytoplankton as quality element for assessment of coastal and transitional waters. Concerning transitional waters it is stated: "The main difficulties in using phytoplankton as a quality element for transitional waters with pronounced tides are represented by the extremely high natural

spatial and temporal variability of the planktonic communities, which may make phytoplankton monitoring a useless exercise in some transitional waters… ".

Further the guidance underlines the importance of identification of nuisance or potentially toxic species as crucial assessment parameters, although those are is not explicitly required by the WFD. Since the toxicity of the blooms cannot be directly linked to the pressures, this could be used as an indicative parameter. It would be difficult to establish reference conditions or classification scales for bloom toxicity. Qualitative indicators, such as bloom toxicity could be more appropriate for assessment systems like OSPAR comprehensive procedure. Such information could be also used in the determination of the water bodies in risk (or potential problem areas), while those hardly could enable WFD compliant classification at the current stage.

With respect of phytoplankton in coastal waters, the WFD CIS Monitoring guidance states that: "High natural spatial and temporal variability of the planktonic communities requires frequent sampling to ensure meaningful data for classification or detection of events (blooms). Sampling frequency is determined by the variability, and it is recommended a minimum of monthly sampling with optional increased sampling frequency in seasons with main bloom events. Sampling should be performed together with measurements of chemical and physico-chemical parameters. Seasonal sampling is a minimum frequency."

It is obvious that more detailed guidance and examples of determination of the precision and confidence of the monitoring results are needed. The existing high-frequency long-term monitoring data around the Baltic Sea provide excellent database to test the approaches needed for reliable assessment, especially concerning precision and confidence of the classifications based on phytoplankton monitoring results. One of the most important questions is that what level of taxonomic determination would be required for WFD compliant classification. Does the high taxonomic resolution bring added value and more precision in the assessment, or should it be used only in the analysis of the potential occurrence of toxic or harmful blooms for determining the water bodies in risk of failing the environmental objectives.

Three different approaches for interpreting the uncertainty inherent to the calculation of indicators from monitoring data have been outlined in the WFD CIS

Guidance Document no.7 on Monitoring. 1) The benefit-of-doubt approach assumes that the ecological quality is high and that any deterioration in quality has to be shown with sufficient confidence. 2) The face-value does not take the uncertainty into account and just considers the indicator value disregarding how uncertain this estimate may be. 3) The fail-safe approach assumes bad ecological quality and any improvement in the classification has to be shown with sufficient confidence. Whether option 1) or 3) is chosen the precision of an indicator will have large ramifications for the ecological classification and may lead to contrasting results. Although the face-value approach does not explicitly take the confidence of an indicator value into account, the precision should somehow be included in the assessment to avoid potential erroneous conclusions.

The WFD indicator values, such as mean values for phytoplankton biomass, should be standardized by means of dividing the reference condition by the actual mean level to obtain an Ecological Quality Ratio (EQR). The precision of the mean values will also be reflected in the precision of the EQR, although the confidence interval will not be symmetric (Table 9). If a mean with a precision of 10% (d=0.1) corresponds to an EQR of 0.8 then the confidence of the EQR will be [72.7%; 88.9%] and similarly for precisions d=0.2: [66.7%; 100%], d=0.3 [61.5%; 114%], d=0.4 [57.1%; 133%] and d=0.5 [53.3%; 160%]. Considering that the quality classes on average will have 0.2 on the EQR scale, these results suggest that WFD classification with 95% confidence should aim at obtaining a precision of 10% or maybe even lower. Such precisions are not realistically feasible for phytoplankton biomass, and consequently the classification of phytoplankton biomass will not be based on a high level of confidence, unless other means of reducing the residual variance are found.

Table 9: Recalculating precision into EQR scale. Note that the values in the table are relative and not absolute to the calculated EQR.

Precision	d=0.1	d=0.2	d=0.3	d=0.4	d=0.5
EQR lower	-9.1%	-16.7%	-23%	-29%	-33%
EQR upper	+11.1%	+25%	+43%	+67%	+100%

Consequently, the benefit-of-doubt and the fail-safe approaches will most likely lead to different classifications, even if the precision is as low as 10%. In most cases the confidence interval of the mean phytoplankton biomass will include several distinct

classes, and this is a problem that has not yet been seriously considered in the implementation of the WFD.

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Acknowledgement

We gratefully acknowledge the possibility to use the phytoplankton data from the Alg@line project (Finnish Institute of Marine Research) in our analysis. We thank Dr Wolfram Schrimpf for the comments on the chapter 5.3. (remote sensing). This study is supported by the European Commission (CHARM-EVK3-CT-2001-00065).

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