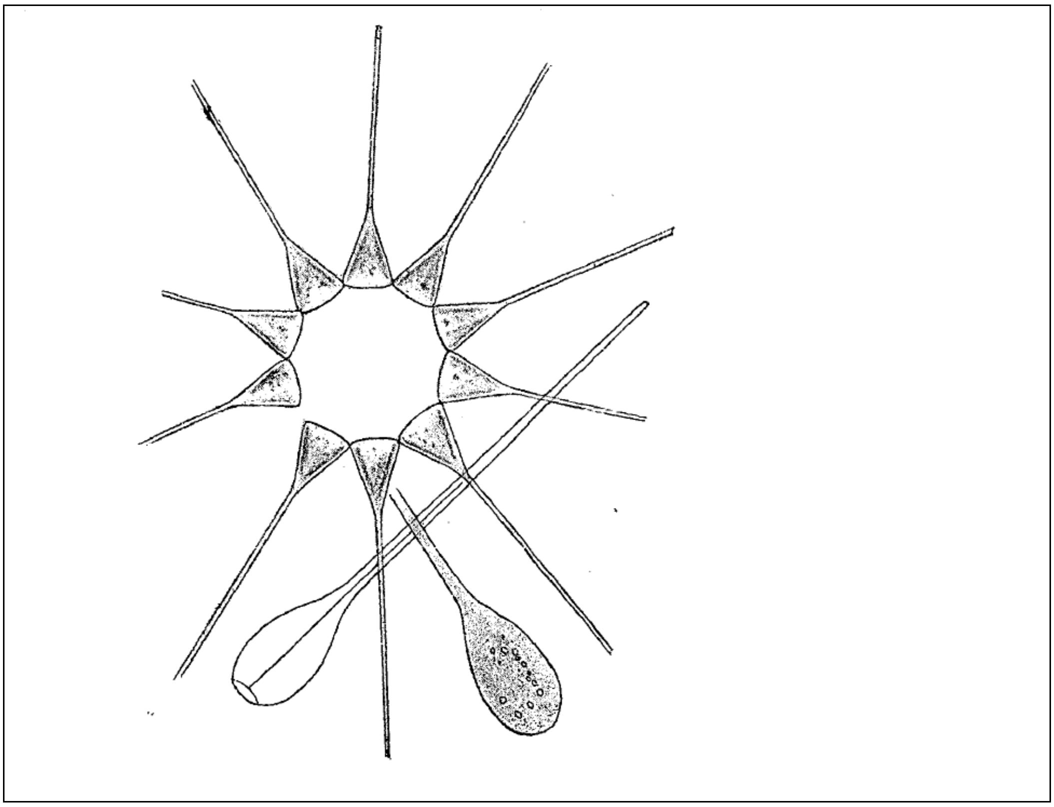

PHYOPLANKTON IN COASTAL WATERS – EVALUATION OF THE LENGTH OF THE SEASONAL ASSESSMENT PERIOD

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PHYOPLANKTON IN COASTAL WATERS – EVALUATION OF THE LENGTH OF THE SEASONAL ASSESSMENT PERIOD

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

This report is a deliverable of one of the scientific sub-projects of WATERS focusing on phytoplankton in coastal waters. The report presents an evaluation of the suitability of different seasonal periods for the assessment of chlorophyll and phytoplankton biovolume. The results provide a basis for final recommendations on assessment period for phytoplankton indicators.

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

Contents

Summary

The period for phytoplankton status assessment is currently June-August for all coastal Swedish waters. It may however be inappropriate to use the same period for all coastal Swedish waters because of the large latitudinal climate gradient. We evaluated the most suitable assessment period in different Swedish coastal areas; the Gulf of Bothnia, the Baltic proper and the west coast. For the Gulf of Bothnia, we suggest to change the assessment period to July-August or July-September. This will reduce the variance of the phytoplankton biovolume, by ensuring that remains of the spring bloom in June are excluded. This will not cause any changes in the classification boundaries. For the Baltic Proper, we suggest to change the assessment period to July-August. The chlorophyll EQR in June often deviates systematically from the July-August period. The use of the period July-August would not increase uncertainty, even if fewer samples are included if a monthly sampling is maintained in some areas. Correction factors for the differences between the June-August and July-August periods are suggested and can be used as guidelines for new classification boundaries. For the Skagerrak and Kattegat areas a prolonged assessment period (May-September) is suggested as the differences between months are small. Presently, monthly sampling is generally performed, and an expanded period would generate more data points in the assessment. The report also evaluates the effects of a monthly sampling interval on assessment periods that include the spring bloom and also the effect of a 90th percentile indicator on such data sets. In order to include the spring bloom in the assessment period, sampling much more frequently than monthly is required. With a monthly sampling there is a risk of biased assessment due to changes in the timing of the spring bloom.

Svensk sammanfattning

Perioden som används för statusklassning av växtplankton i svenska kustvatten är för närvarande juni till augusti. På grund av klimatgradienten längs den långa kuststräckan kan det dock vara olämpligt med samma period för hela landet. I denna rapport utvärderas lämplig period utifrån klorofyll- och biovolymsdata. För Bottniska viken föreslås att perioden ändras till juli-augusti, eller möjligen juli-september, för att undvika att rester från vårblomningen kommer med i sommarmedelvärdet. Vi bedömer att detta inte påverkar nuvarande klassgränser. För Egentliga Östersjön föreslås också perioden juli-augusti, då juni ofta avviker från juli-augusti. Även om färre prover medräknas i medelvärdet vid eventuellt fortsatt månatlig provtagning bedömer vi att detta inte påverkar osäkerheten i statusklassningen. Skillnader mellan perioderna juni-augusti och juli-augusti har beräknats och kan användas som riktlinjer för ändrade klassgränser. För Skagerrak och Kattegatt föreslås en länge provtagningsperiod (maj-september) eftersom skillnaderna mellan månaderna förefaller små. Rapporten utvärderar också effekten av att inkludera perioden med vårblomning i statusbedömningen vid provtagning en gång per månad. Resultatet visar att månatlig provtagning då inte räcker på grund av det ofta snabba och tidsförskjutna vårblomningsförloppet. Med månatliga data från våren är risken stor att få en missvisande bild.

1. Introduction

1.1 Current Swedish assessment system for phytoplankton

The current assessment system for coastal phytoplankton is based on chlorophyll *a* concentrations and phytoplankton biovolume (Naturvårdsverket 2007, HVMFS 2013:19). When biovolume data are missing, the classification is based on chlorophyll only. A reference value has been defined for each type area (west coast and Gulf of Bothnia) or is determined for each sample according to measured salinity (The Baltic Proper) (Table 1.1). The latter procedure includes the calculation of an adjusted reference value for total nitrogen according to the salinity and the type area's assigned background nitrogen concentration for rivers and the open sea (linear physical mixing model). The adjusted nitrogen reference value is used in an empirically based nitrogen-chlorophyll relationship to calculate the corresponding chlorophyll reference value. The assessment period used in Sweden is presently June-August. The summer period June-August was chosen because of its relative stability and because it is normally an intensively sampled period (Larsson et al. 2005). The recommendation is that at least 3 measurements per year are made and that the classification should be based on data from at least three years during the latest 6-year period.

TABLE 1.1. Chlorophyll reference values and class boundaries for Swedish type areas (HVMFS 2013:19). For type areas 8, 12, 13 and 24 values are corrected according to measured salinity. Low salinity means that these reference values are adjusted to a higher value.

Type area	Rv (µg/l)	HG	GM	MO	OD
West coast					
1n	1.3	0.76	0.62	0.35	0.19
1s	1.6	0.76	0.57	0.35	0.2
2	1.9	0.79	0.53	0.34	0.23
3	1.1	0.79	0.63	0.31	0.18
25	1.8	0.86	0.67	0.44	0.28
4	1.0	0.83	0.67	0.33	0.17
5	1.0	0.83	0.67	0.33	0.17
6	0.9	0.82	0.59	0.37	0.18
Baltic Proper					
7	1.2	0.8	0.67	0.35	0.15
8	1.2	0.8	0.67	0.35	0.15
9	1.2	0.8	0.67	0.35	0.15
10	1.2	0.8	0.67	0.35	0.15
11	1.2	0.8	0.67	0.35	0.15

12	1.2	0.8	0.67	0.35	0.15
13	1.2	0.8	0.67	0.35	0.15
14	1.2	0.8	0.67	0.35	0.15
15	1.2	0.8	0.67	0.35	0.15
24	1.2	0.8	0.67	0.35	0.15
Bothnian Sea					
16	1.4	0.78	0.61	0.33	0.14
17	1.2	0.8	0.6	0.32	0.14
18	1.4	0.78	0.61	0.33	0.14
19	1.2	0.8	0.6	0.32	0.14
Bothnian Bay					
20	1.3	0.72	0.57	0.28	0.12
21	1.1	0.73	0.55	0.3	0.13
22	1.2	0.67	0.52	0.28	0.12
23	1.1	0.73	0.55	0.3	0.13

1.2 Assessment periods in other countries

The assessment systems for coastal phytoplankton in most EU countries around the Baltic Sea and North Sea include only chlorophyll and total phytoplankton biovolume, with a few exceptions (Höglander et al. 2013).

Assessment periods differ between countries in the Baltic Sea and North-East Atlantic geographical intercalibration groups (Baltic GIG and NEA GIG). Within the Baltic GIG, periods range from May-September (Denmark, Germany), June-September (Estonia, Latvia, Lithuania, Poland), July-September (Finland) and June-August (Sweden). Within the NEA GIG the whole productive period is used (February-October or March-September), although Denmark uses May-September for the Kattegat area.

1.3 The uncertainty framework

This report deals with the choice of optimal assessment period, which requires that the relevant sources of uncertainty are evaluated (Lindegarth et al. 2012).

The goal of a successful Water Framework Directive (WFD) monitoring program is to correctly assess the present status and the temporal trends, i.e. if the status (in terms of EQR[1]) tend to improve or not. Since assessments are prone to various errors, the WFD requires that the degree of uncertainty of an assessment is specified. Two basic aspects of uncertainty that relate to status classification are defined by the WFD: precision and confidence (Lindegarth et al. 2012). The precision is the uncertainty of the estimated parameter, usually the mean. The confidence of an assessment is dependent on how the mean and its precision relates to a certain classification boundary.

[1] Ecological Quality Ratios: the reference value divided by the measured value

In principle, the sources of uncertainty that affects precision can be separated into different components: temporal (including inter-annual, seasonal, diurnal, irregular variability), spatial (including large-scale gradients and small-scale variability), and measurement errors (Lindegarth et al. 2012). Partly, some of these factors contribute "fixed" variation, which can potentially be compensated for, e.g. consistent seasonal regularity. This is opposed to "random" variation, which can be compensated for only by increasing the number of samples. The sources of uncertainty can also interact; e.g. large-scale spatial gradients can differ between years.

Thus, the confidence of a status assessment will depend on 1. The given natural temporal and spatial sources of uncertainty; 2. The measurement errors; 3. The sampling frequency and the temporal and spatial distribution of the sampling occasions; and 4. How the resulting EQR-value and its confidence limits relate to the classification boundaries.

Systematic spatial variability, gradients, can to some extent be handled by careful location of stations to get a representative value for the water body. If gradients within a water body are variable, several stations may be needed. Small-scale, random variations (patchiness) can be handled by sample replication. However, in most pelagic monitoring programs the random small-scale variability is not separated from temporal variability, which is usually considered more important. Measurement errors are minimized by good quality control of sampling and analysis. One important part here is intercalibrations between laboratories (analytical methods) and persons (taxonomic skills).

1.4 Typical seasonal pattern of temperate phytoplankton

There are often distinct seasonal variations in phytoplankton abundance and biomass. In spring, a phytoplankton spring bloom quickly develops as light conditions improve. This is often followed by a clear water phase, after which nutrient inputs to the surface layer often stimulate somewhat higher biomass in late summer. As the stratification is broken in autumn, an autumn bloom may develop if light is still sufficient. Generally, the summer period is usually relatively stable compared to the more dynamic spring and autumn periods. To describe the spring bloom properly, high-frequency sampling is used in the national monitoring, e.g. at stations Släggö (Skagerrak coast, Gullmar fjord) and at Askö station B1 (coast of the N. Baltic proper). Although the summer period is generally more stable, it can also be affected by blooms. Large cyanobacterial blooms in outer coastal areas of the Baltic proper can confer large spatial and temporal variability of chlorophyll and phytoplankton biomass. Temporarily, strong upwelling events can drastically reduce phytoplankton biomass when surface water is replaced by colder deep-water. Such upwelling events bring nutrients to the surface layer and may thus initiate a bloom, temporally or spatially separated from the time and place of the up-welling event. The development of phytoplankton blooms is often strongly influenced by the weather systems passing Scandinavia. This results in a temporal variability on the scale of several days to a few weeks.

1.5 Factors affecting the choice of seasonal assessment period

It is clearly fruitful to separate systematic (or "fixed") seasonal variability components, such as difference in means between months, from random within-month variability. Systematic variability can potentially be accounted for, at least if all months are sampled with high enough intensity to describe such systematic differences properly. On the contrary, seasonal patterns of random variability, as revealed by differences in within-month variances between months, may suggest that excluding the more variable period (a certain month) is a good option.

The choice is also affected by the possible sampling effort and how it can be distributed in time. In the ideal case, the sampling effort can be adapted to the estimated components of uncertainty and to the differences that are necessary to detect. In reality, the sampling effort is also defined by existing monitoring, by some re-arrangements of existing monitoring, or by a new monitoring program, with its economical and logistical limitations. Thus, a decision on the assessment period must deal with all these factors.

Furthermore, the link between anthropogenic pressure and the response of the plankton is important. Earlier work (e.g. Larsson et al. 2005) shows that there is a generally a strong link between nutrient and chlorophyll concentrations in summer. This report does not evaluate this effect further, assuming that there is a sufficiently strong link between pressure and response for the summer months, currently included in the assessment, compared to spring and autumn months.

In principle, a very short assessment period, i.e. a month or less, with several sampling occasions may decrease temporal variability, but this may be at the cost of losing representativeness (even for the relatively stable summer period), since all sampling occasions may be affected by the same short-term events, such as blooms, upwelling or other weather-related phenomena. A very intense sampling programme may also be logistically difficult to perform. Only one sampling per year, usually in August, has been used in campaigns to get a good spatial coverage with many sampled water bodies (e.g. Bothnian Bay in 2011). If repeated yearly, such sampling may provide a reasonable basis for status assessment in areas with relatively small dynamic excursions in phytoplankton biomass, although within- and between-year variation cannot be separated. This type of monitoring is used in much of lake monitoring (Lindegarth et al. 2013). Some coastal sampling campaigns have focused on the summer period only, as have some coastal monitoring programs focused at status assessment and status follow-up. Such spatially broad sampling programs usually complement temporally more intense monitoring programmes that are restricted to some of the monitoring stations.

Many coastal monitoring programs are traditionally performed with monthly sampling. Given that programs are not modified, an advantage of having a longer seasonal assessment period is that more data from such monitoring are used in the assessment. In this case, extending the period and including a somewhat more variable month, may be advantageous if the increased variance is compensated for by the increased number of measurements included, decreasing the uncertainty of the estimate of the mean (in terms of standard error or confidence interval).

When the seasonal assessment period was evaluated during the development of the current assessment system, the summer period June-August was chosen because of its relative stability and because it is normally the most intensively sampled period. The evaluations of the assessment period (Larsson et al. 2005), using data from the high-frequency stations B1 (outer coastal) and BY31 (off-shore) in the Baltic Proper, showed a lower chlorophyll concentration in June relative to July-August, but similar (B1) or higher (BY31) variance in June. The Secchi depth was larger in June, and had higher variance (B1). It was concluded that to minimize variation, the period July-August should be considered during revision of the assessment system, but that the gain was unclear, especially since no inner coastal stations were evaluated (Larsson et al. 2005).

1.6 Aim of the study

This study evaluates alternative time periods relative to the current assessment period June-August for the phytoplankton parameters total biovolume and chlorophyll *a*. In particular, we wanted to evaluate whether sampling in the Gulf of Bothnia in June would be problematic, since the spring bloom there occurs later than in other areas which has been suggested to occasionally influence the intended summer assessment. Likewise, we wanted to evaluate the influence of samples from June in the Baltic Proper. For the Kattegat and Skagerrak areas we specifically wanted to evaluate sampling also in the March-October and May-September, the latter used by authorities in Denmark in the Kattegat.

It should be noted that this evaluation of the length of the assessment period focuses on the current phytoplankton indicators chlorophyll and biovolume. Since the WATERS project also aims to develop and evaluate species-based indicators, the results of this report need to be complemented when species-based indicators have been fully developed.

Since many monitoring programs adopt a monthly sampling interval, irrespective of differences between months concerning within-month phytoplankton variability, we also specifically explored the effect of a monthly sampling interval on the outcomes of various seasonal assessment periods. The main focus was on the pros and cons of including the usually more variable spring months in an assessment, given that a monthly sampling frequency is adopted. The analysis was based on high-frequency monitoring data from two stations. This analysis is particularly relevant since it is not possible to separate between the within-month and the between-year variation for a certain month from datasets with monthly data only.

2. Methods

2.1 Used data

For the Gulf of Bothnia we used data from the national monitoring in the northern Bothnian Sea (coastal stations B3 and B7, 1995-2012) and C3 (off-shore), and A13 (off-shore) (hose-integrated data 0-10 meters). This was complemented by data from regional programs (~0.5 m depth for chlorophyll). Typically, the monitoring is once per month yearly.

For the Baltic Proper we used data from the high-frequency national (B1) and Himmerfjärden monitoring programs, which have weekly sampling in spring (April- mid-May) and biweekly sampling in summer (June-September). Only hose-integrated chlorophyll samples (Himmerfjärden 0-14 m, B1 0-20 m) were available for all stations and all months and therefore of primary focus in this study. High-frequency phytoplankton biovolume data was available for stations B1 and H4 only. We also used data (chlorophyll 0-5 m depth integrated measurement) from the Stockholm Vatten control program which has monthly sampling interval. Since sampling is often done in the beginning or the end of a month, we interpolated the Stockholm Vatten-data to form more representative monthly values. For certain months, chlorophyll and biovolume EQR-values were available or could be calculated. For B1 and Himmerfjärden, chlorophyll data for EQR-calculation were from 0 m or 0-14 m data that were transformed by an empirical relationship to correspond to 0 m-data.

For the Kattegat and the Skagerrak a total of nine stations were selected to represent most of the coastline (depth 0-10 meters, sampled by hose for biovolume and by hose [national stations] or integrated discrete depths [regional stations] for chlorophyll). The stations included two high-frequency stations in the national monitoring program with biweekly sampling during the whole year (Släggö and N14 Falkenberg). Seven other stations with monthly sampling from the Water control programs of Bohuskusten and Halland were also included in the analyses. The stations were chosen both to represent most of the coastline but also depending on available biovolume measurements. Biovolume measurements for a longer time period are only available from two coastal stations in the national program and two stations in the Water control program of Halland in Kattegat. N14 Falkenberg is located just outside the border of the WFD but was included due to lack of stations with long-term biovolume data. The selected period was the last 6 years with available data (2007-2012) for both clorophyll and phytoplankton biovolume.

2.2 Evaluation of monthly means and variance

Difference between months in terms of medians and within-month variability were evaluated primarily by station-wise graphic plots. Plots show medians, 25-75% range and non-outlier range (Statistica 10) for all the data available from a selected several-year period.

2.3 Evaluation of seasonal means and variance

Yearly seasonal means were formed for selected seasonal periods. Coefficients of variation were calculated as standard deviation of yearly means divided by yearly mean values. Confidence intervals (95% level) were calculated from the yearly seasonal means and its standard error using the t-distribution and the number (n) of included years. Thus, this approach excludes systematic variation between months but includes between-year variation of the different periods, i.e. a month that has large between-year variability will increase the variance of periods when it is included. However, this approach compares the periods using the evenly distributed data available (i.e. current monitoring), and means that longer seasonal periods include more months but also more data points. Thus it is important to note here that this approach does not compare periods with a similar sampling effort allocated to it (e.g. not 5 samplings allocated in June to August compared to 5 sampling occasions in May-Sep, rather it compares 3 sampling occasions Jun-Aug versus 5 occasions May-Sep). If the same sampling effort would be allocated, the results would be more correlated to monthly variance as calculated according to section 2.2.

For the Bothnian Sea, Bothnian Bay and the Baltic proper, the current assessment period June-August was compared to the alternative periods July-August and July-September, i.e. excluding June. For the Kattegat and Skagerrak we also tested the period May-September, currently used by authorities in Denmark in the Kattegat, and the full growth period March-October, used by authorities in countries in the NEA GIG.

2.4 Evaluation of the effects of monthly sampling interval for uncertainty of seasonal means

The two stations in the north-western Baltic Proper, B1 and H4, which are sampled at high frequency over the entire growing season for phytoplankton, were used to simulate the effect of monthly sampling on the uncertainty of different seasonal means. A database with daily values of chlorophyll concentrations was created from the linearly interpolated weekly (spring) or biweekly (summer) data (Figure 3.20). This dataset was used to simulate monthly sampling over the season March to October by retrieving data for a specific day each month (e.g. 5 Mar, 5 Apr, 5 May etc. or e.g. 14 Mar, 14 Apr, 14 May etc.). Since part of the within-month variability is due to a seasonal trend rather than random, this approach should yield more realistic results than sampling from an estimated statistical distribution each month. The "true" within-month variability was estimated from the simulated daily values.

3. Results

3.1 Differences between months

3.1.1 Gulf of Bothnia

Most coastal control programs in the Gulf of Bothnia have limited data available for July. However, since we expected that June should be the most problematic month, i.e. with deviating chlorophyll and biovolume concentrations and variance, we included also monitoring stations that enabled comparison of June with August and September. Only one of the studied stations in the Bothnian Bay (Figures 3.1-3.2) appeared to have higher chlorophyll concentrations and variance in June, but this station (P300) was represented by only a few measurements.

Also stations in the Bothnian Sea (Figures 3.3 and 3.4) generally showed similar chlorophyll concentration and variance in June and August, or higher in August (several K-stations in the north-eastern Hälsingland coastal area, Figure 3.4).

However, on the contrary, phytoplankton biovolume concentrations and variance were clearly higher in June compared to later months at several stations (Figure 3.5).

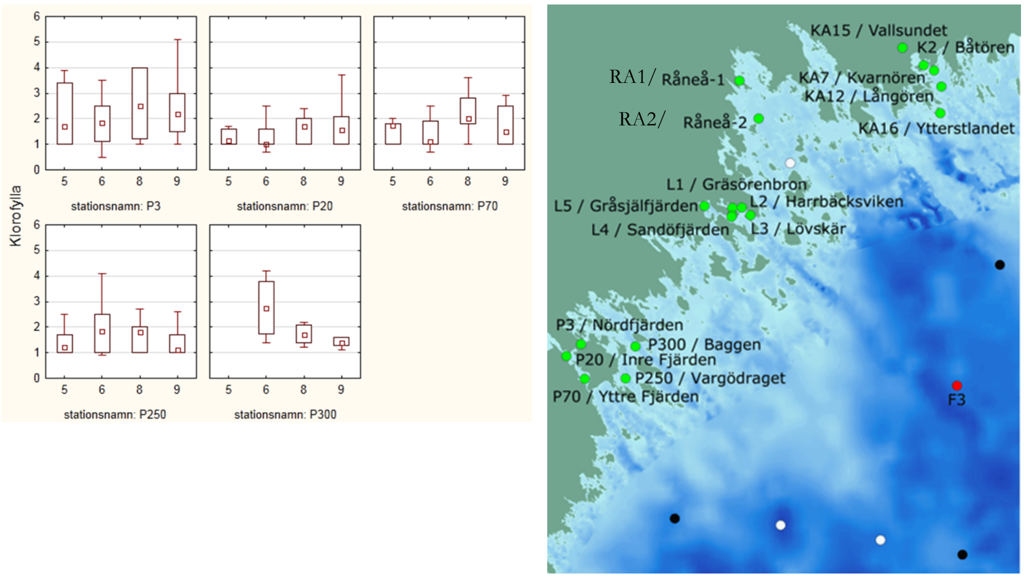


FIGURE. 3.1. Monthly chlorophyll (µg L^{-1}) for time period 1994-2010, for stations in the Bothnian Bay. For stations P20, P250, and P 70, n= 26/18 for June/Aug. respectively. For station P3 n=22/7, station P300 n=4/5. The data in June were not confined to the latter part of the month, but distributed over the full month. The figure shows median, 25-75% range (box) and non-outlier range (error bars). Map from www.smhi.se.

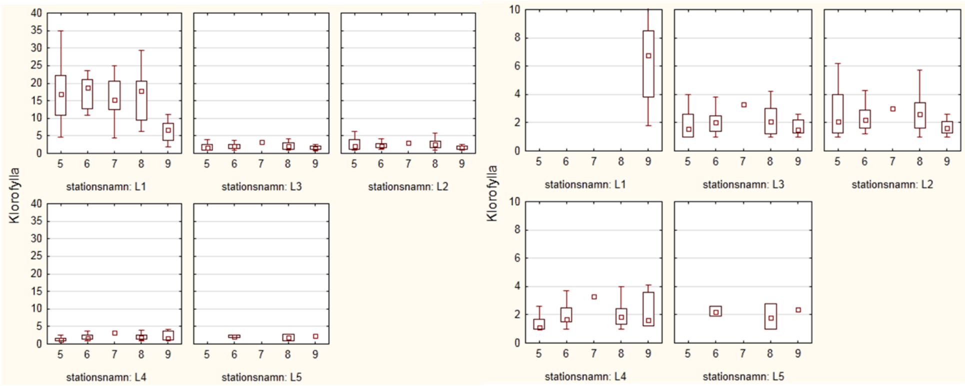


FIGURE 3.2. Monthly chlorophyll, May (5) to September (9) (µg L^{-1}) for time period 1993-2010. Stations in Bothnian Bay. Different y-scale on panels to the right. For stations L1-L5, n= 12/14, 31/26, 31/23, 22/16 and 3/3 for June/August respectively. July and September are represented by fewer measurements, except for station L1. The figure shows median, 25-75% range (box) and non-outlier range (error bars). For map, see fig. 3.1.

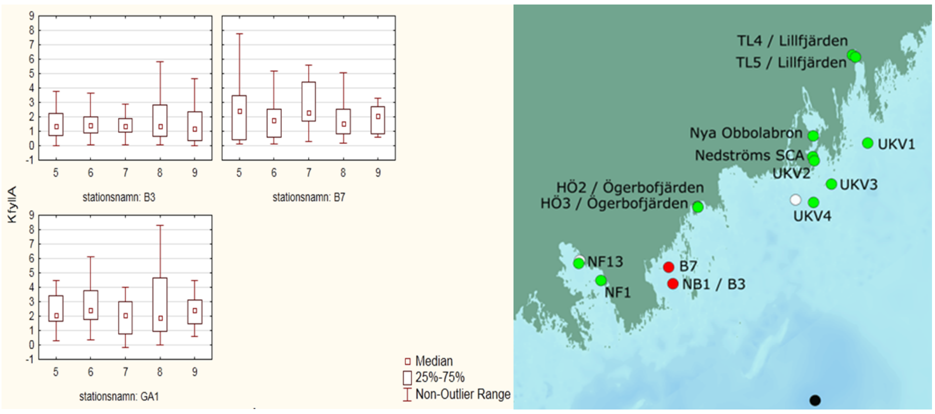


FIGURE 3.3. Monthly chlorophyll May (5) to September (9) (µg L^{-1}) for time period 1989-2012 (B3), 2000-2012 (B7) and 1997-2012 (GA1). Stations in northern Bothnian Sea. For stations B3, B7 and GA, n=42/39/29/42/35, 20/16/9/18/9, 17/23/11/16/10 for May-September respectively.

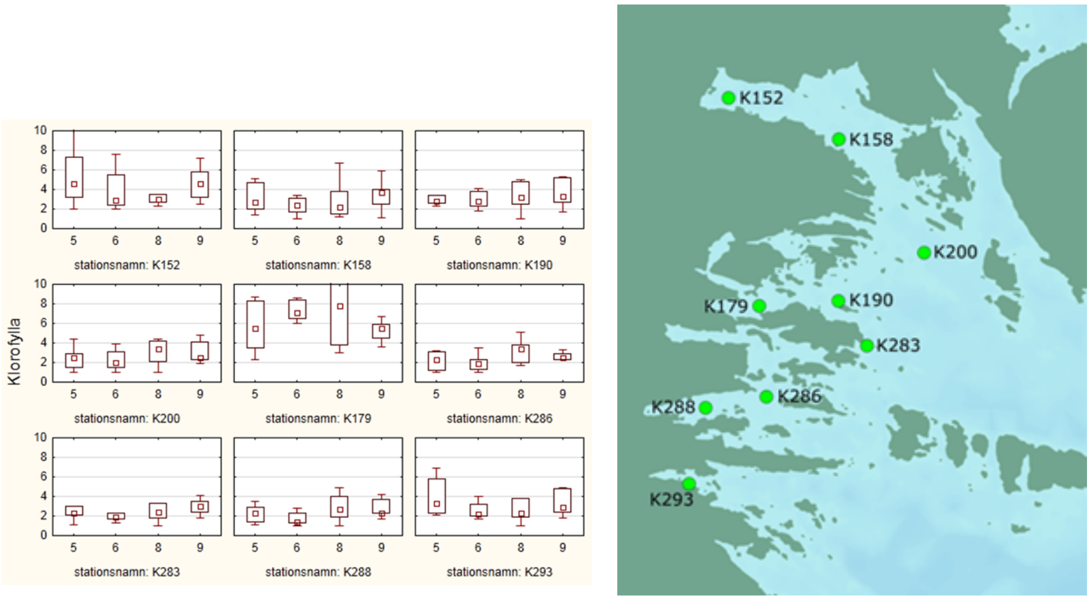


FIGURE 3.4. Monthly chlorophyll May (5) to September (9) (µg L^{-1}) for time period 2002-2011. Stations in central Bothnian Sea (NÖ Hälsinglands VVF). n=7/10/10/10 per station for May-September, respectively. Most data in June are from the first part of the month. The figure shows median, 25-75% range (box) and non-outlier range (error bars).

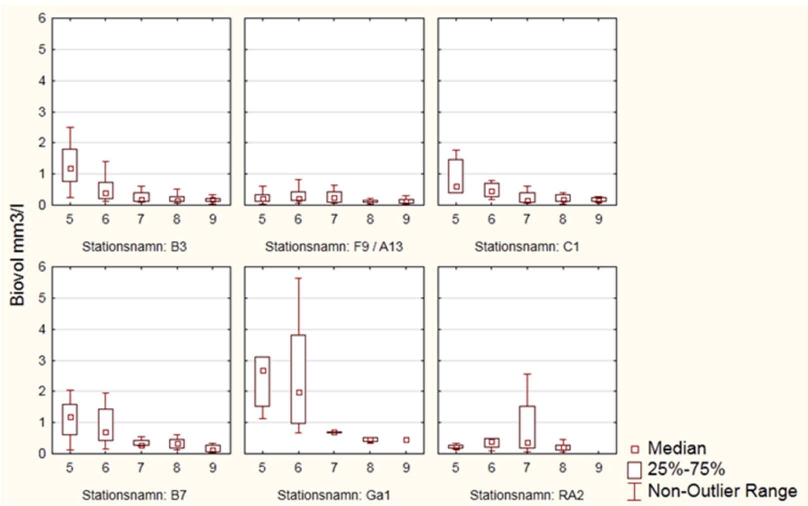


FIGURE 3.5. Monthly biovolume May (5) to September (9) (mm^3 L^{-1}) for time periods 1995-2012 (B3, F9/A13), 2001-2012 (B7), 1995-2000 (C1), 2008-12 (Ga1, RA2). Stations are located in both Bothnian Bay (RA2, F9/A13) and Bothnian Sea (B3, B7, Ga1, C1). n=23/22/13/20/17, 21/23/13/21/16, 7/9/7/9/8, 11/11/4/9/4, 5/8/5/6/1 and 578/4/6/0 for stations B3, F9/A13, C1, B7, Ga1 and RA2 for May-September, respectively. The figure shows median, 25-75% range (box) and non-outlier range (error bars).

3.1.2 Baltic Proper

Comparison of data for May-September, for inner stations in the Himmerfjärden area (H7 and H6 in Figures 3.6), shows a generally higher chlorophyll concentration in May and, to a lesser extent, June, compared to July-August. Lower chlorophyll concentrations in May and June were observed for intermediate and outer coastal stations (e.g. H3, H2 and B1, Figure 3.6). This was also reflected in higher chlorophyll EQR values for June at the intermediate and outer stations (Figure 3.6).

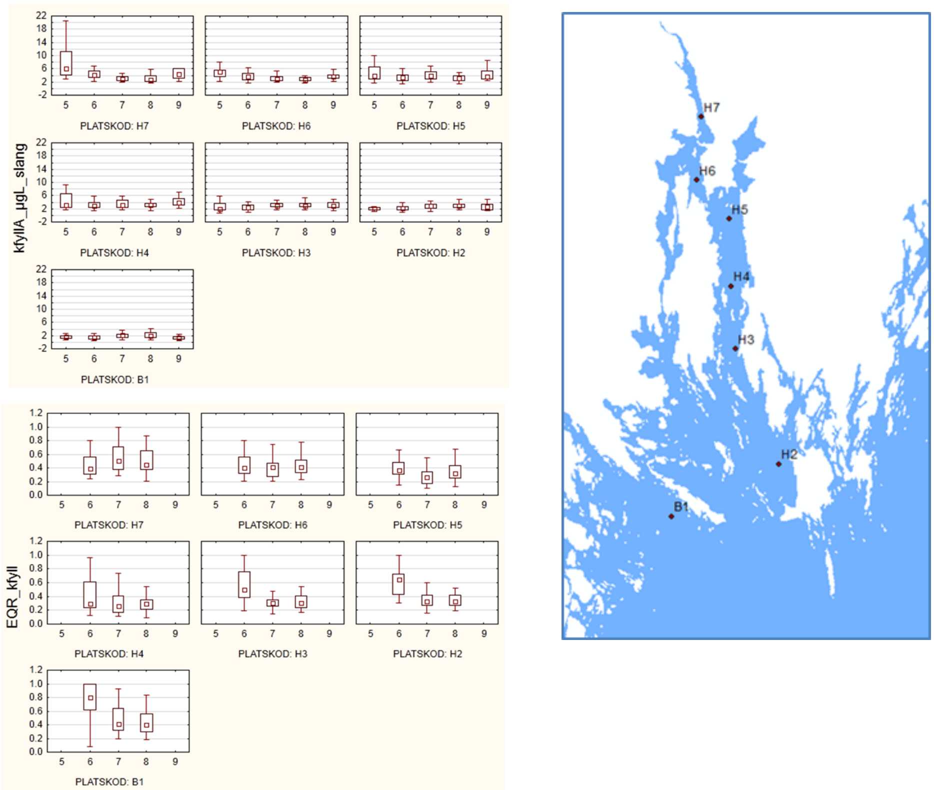


FIGURE 3.6. Monthly chlorophyll May (5) to September (9) (µg L^{-1}) and EQR for time period 1999-2012 in Himmerfjärden area. The figure shows median, 25-75% range (box) and non-outlier range (error bars). Monthly n=27-34, except station H7 where n=11-15.

A high biovolume was also found for station H4 in May, but not in June or at the outer station B1 (Figure 3.7).

In the central Stockholm archipelago, relatively high chlorophyll concentrations were evident for most stations in May, and also for the inner stations in June (Figure 3.8). For the intermediate stations the concentrations were rather similar between June-August, and at the outer stations concentrations in June were lower than in July-August.

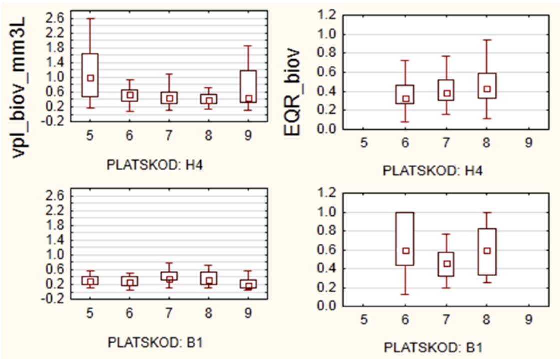


FIGURE 3.7. Monthly biovolume May (5) to September (9) (mm^3 L^{-1}) and EQR for time period 1999-2012 in Himmerfjärden area. The figure shows median, 25-75% range (box) and non-outlier range (error bars).

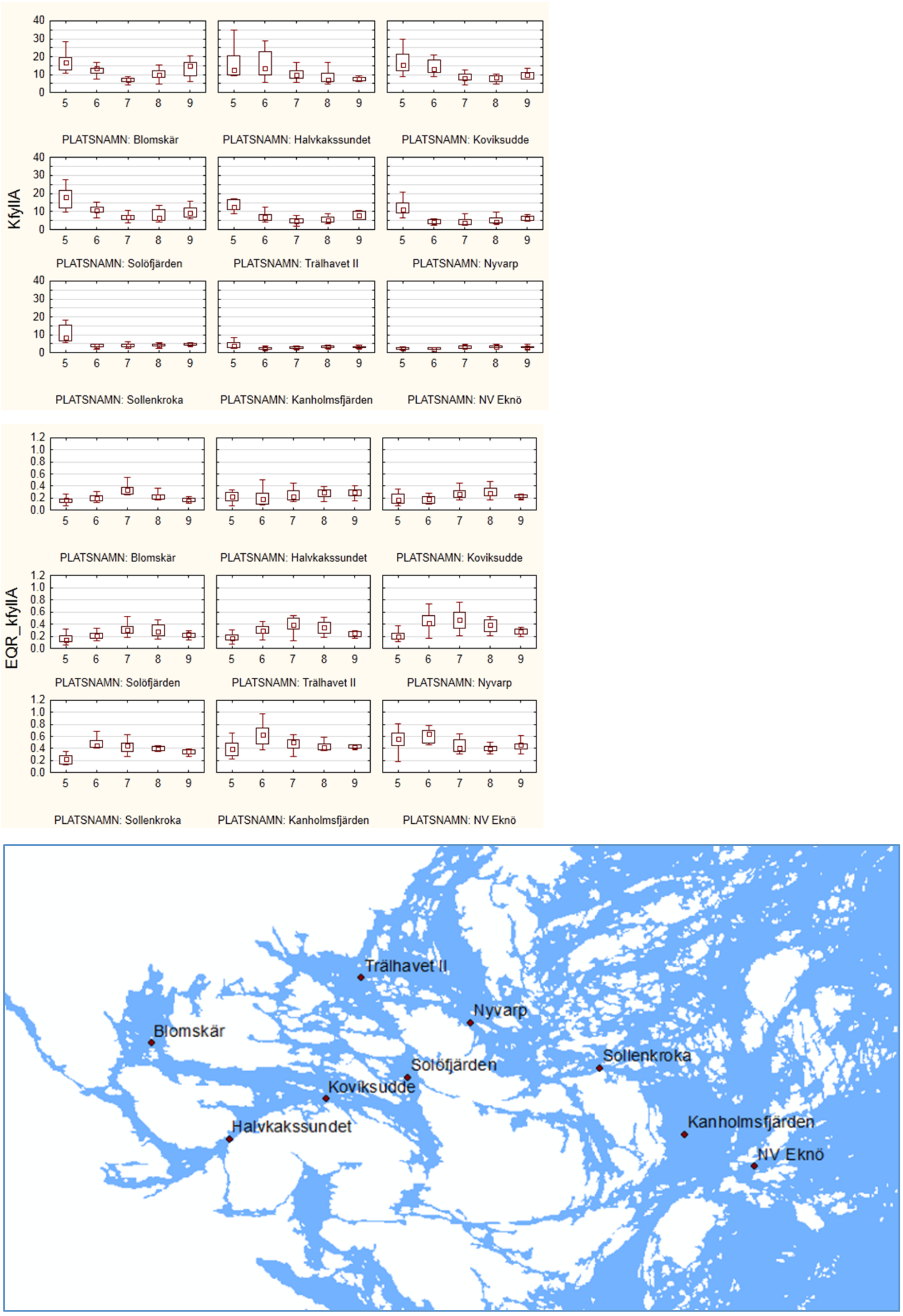


FIGURE 3.8. Monthly chlorophyll May (5) to September (9) (μgL^{-1}) and EQR for time period 1999-2012 in central Stockholm archipelago area. The figure shows median, 25-75% range (box) and non-outlier range (error bars).

3.1.3 Kattegat and Skagerrak

Comparison of months for each parameter for the whole year reveals that the spring bloom can linger into April depending on location of the station. Variability in April is high at stations in the Koster Fjord and in the fjord system around the Island of Orust (Koljö Fjord) and in the Byfjord. Large differences between years in April are most evident in fjord systems where water exchange is low, such as Koljö Fjord and Byfjord. However, the spring bloom mainly occurs in February or in March on the Swedish west coast. The spring blooms often develop first in the Kattegat and then progressively later northwards. This is an effect of stronger stratification in the southern part. Spring bloom in the enclosed fjords usually starts last.

July, being part of the existing evaluation period, shows great variances between years for biovolume, but not for chlorophyll *a* (Figures 3.9-3.10). Looking at correlations between chlorophyll *a* and biovolume clearly indicate that there is a discrepancy between both parameters especially during the summer period (Figure 3.11). Overall, the months between May and September seem to have the least variance for most parameters.

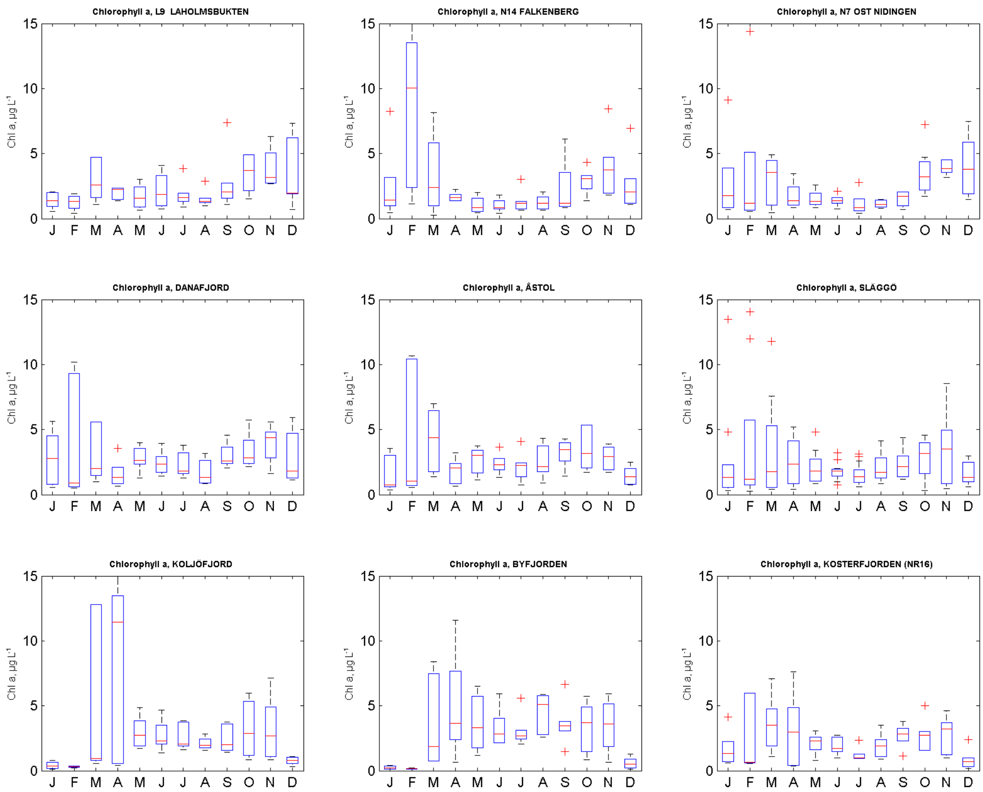


FIGURE 3.9. Monthly chlorophyll (µg L^{-1}) for January to December (2007-2012). The central mark is the median, the edges of the box are the 25th and 75th percentiles, the whiskers extend

to the most extreme data points not considered outliers, and outliers are plotted individually. For map of stations, see fig. 3.11.

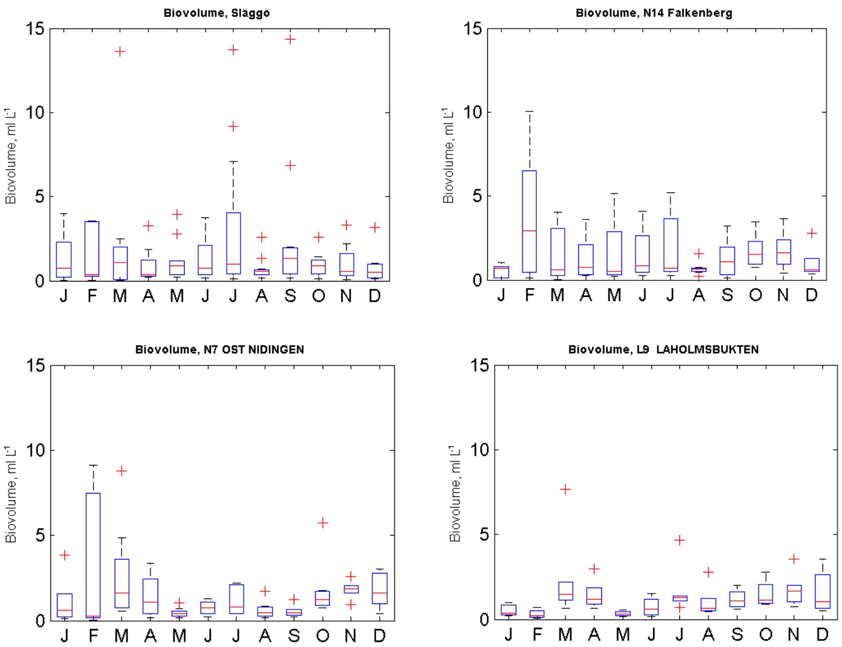


FIGURE 3.10. Monthly biovolume (mm^3 L^{-1}) for time period 2007-2012. On each box, the central mark is the median, the edges of the box are the 25th and 75th percentiles, the whiskers extend to the most extreme data points not considered outliers, and outliers are plotted individually.

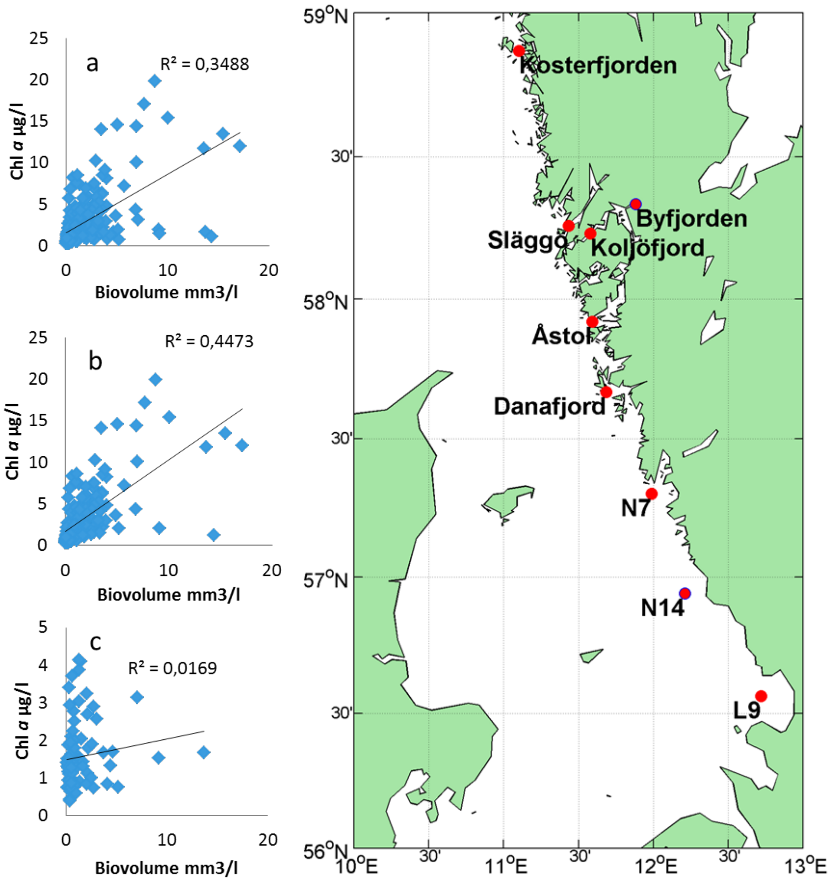


FIGURE 3.11. Correlation between measured chlorophyll *a* and biovolume from samples between 2007-2012 including a) the whole year b) the whole year except summer and c) summer. Map shows stations included in the analyses.

3.2 Differences between seasonal periods

3.2.1 Gulf of Bothnia

The generally small differences in chlorophyll between months resulted in small differences also between compared seasonal periods (Figure 3.12). At station A13 in the Bothnian Bay the biovolume was 32% lower, and the between-year coefficient of variation (CV) 16% lower, in July-September than June-August. At this station also chlorophyll was somewhat lower in July-September than June-August (mean 16% lower, CV 31% lower). At station B3 in Örefjärden in the Bothnian Sea the biovolume was 22% lower in July-September, but CV similar to June-August. Chlorophyll was somewhat higher in July-September. At station C3 in the Bothnian Sea biovolume was also lower in July-September (21%), but with similar CV, and with no difference for chlorophyll. Since

September is similar to July and August we get similar results if we exclude September and look at Jul-Aug only (not shown in Figure 3.13).

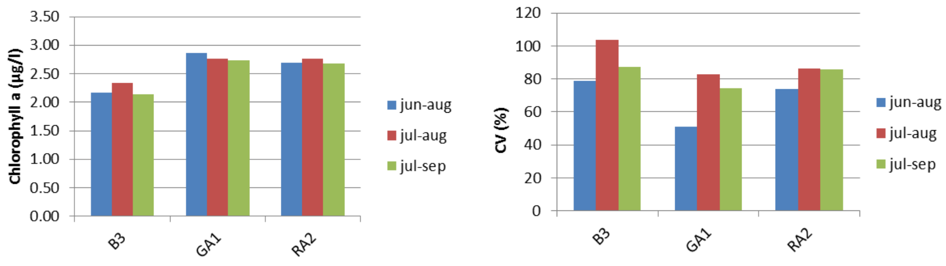


FIGURE 3.12. Mean chlorophyll concentrations (left panel) for the period 1989-2012 (station B3),1997-2012 (stations GA1 and RA2). Between-year variation of seasonal means as CV (right panel).

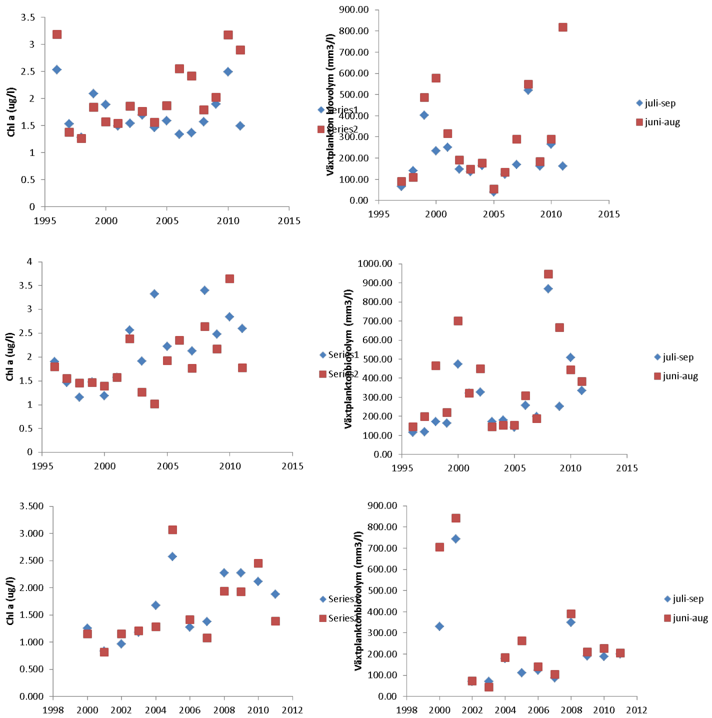


FIGURE 3.13. Yearly phytoplankton chlorophyll (left panels) and biovolume (mm3/m3) (right-panels) and for the seasonal periods Jun-Aug (red squares) and Jul-Sep (blue diamonds). Data are for station A13 in the Bothnian Bay (upper panels), B3 in the Bothnian Sea (middle panels), and C3 in the Bothnian Sea (lower panels).

3.2.2 Baltic Proper

Comparing the overlapping time periods June-August and July-August, differences in mean concentrations of chlorophyll are relatively small. The pattern with higher chlorophyll in June compared to July and August at inner stations, and lower chlorophyll in June at outer stations is reflected in the mean values for the time periods but differences are small (Figures 3.14 and 3.16). Generally, there were small differences in variation between the periods June-August and July-August, but with a tendency for higher variation for the June-August period. The inclusion of data for September increases the concentration and variance of chlorophyll at station H7 (Figure 3.14) and biovolume at station H4 (Figure 3.15).

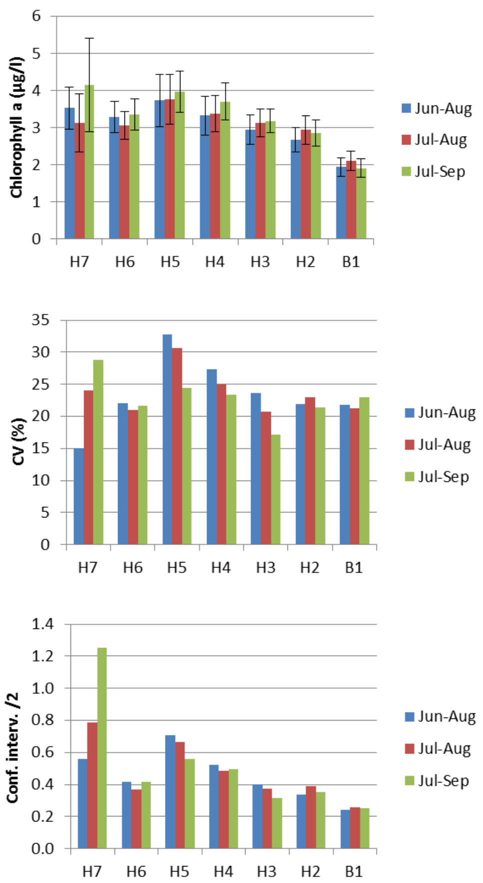


Figure 3.14. Mean chlorophyll concentrations (1999-2012, Himmerfjärden stations H2-H7 and the national station B1), coefficient of variation (CV = SD/mean*100), and 95% confidence interval of the mean, shown as error bars in top panels and as confidence deviation from mean (confidence interval/2) in lower panels. The means and the variation are based on yearly means (N= 14), i.e. the variation is between-year variation.

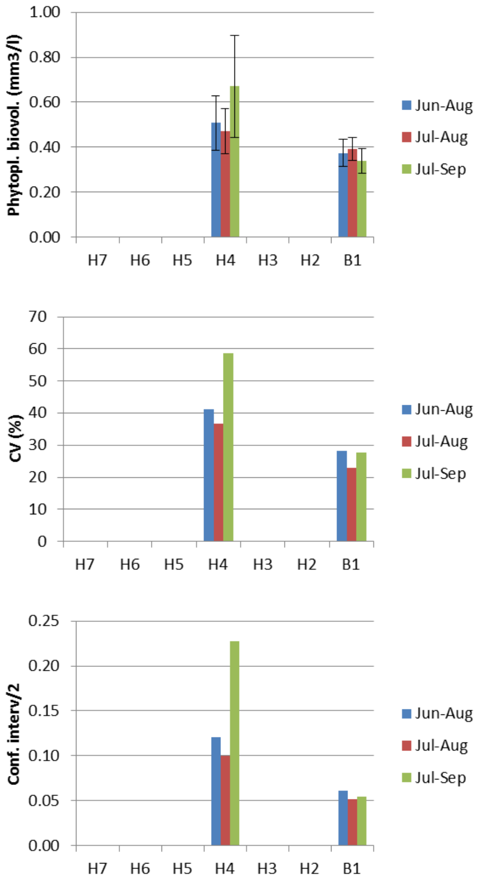


FIGURE 3.15. Phytoplankton biovolume concentrations. Other as in Figure 3.14.

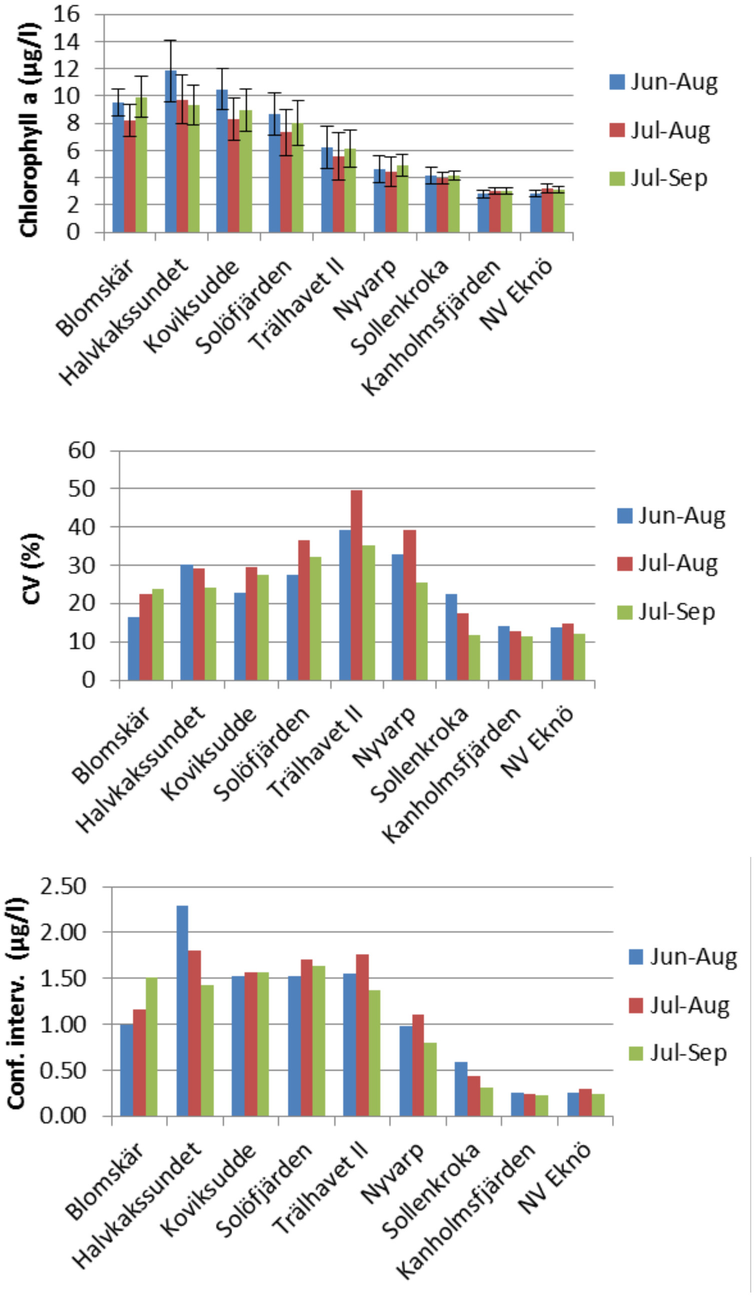


FIGURE 3.16. Chlorophyll concentrations (1999-2010, central Stockholm archipelago), coefficient of variation (CV), and 95% confidence interval, shown as error bars in top left panel and as confidence deviation from mean (confidence interval/2) in lower panel. Based on yearly means of seasonal periods (n=12).

The chlorophyll EQR values for the July-August period were higher for inner stations and lower for outer stations compared to the period June-August (Figure 3.17). The differences for the central Stockholm area indicate that an assessment based on chlorophyll EQR values for July-August data only, should preferably be corrected if it should correspond to the present assessment period June-August. This correction was approximately -10% for inner stations (type area 24), ±0% for intermediate stations (to Sollenkroka, i.e. inner type area 12 west of type 15), and +15% for outer stations (Kanholmsfjärden, i.e. outer 12 west of type 15, and probably type area 15). Data from the Himmerfjärden data indicated that for stations in type 12 (west of type 14), chlorophyll EQR should be corrected by approximately +10% for inner stations, and +20% at outer stations, and in type area 14 by +20%. These differences between the June-August and July-August periods for different areas probably reflect the occurrence of cyanobacteria, which tend to be more abundant in July-August, especially in the outer and southern part of the Stockholm archipelago. Corresponding correction factors for total nitrogen and phosphorus EQR were negligible (<2%), while the correction factor for Secchi depth EQR was +5% for intermediate and outer areas.

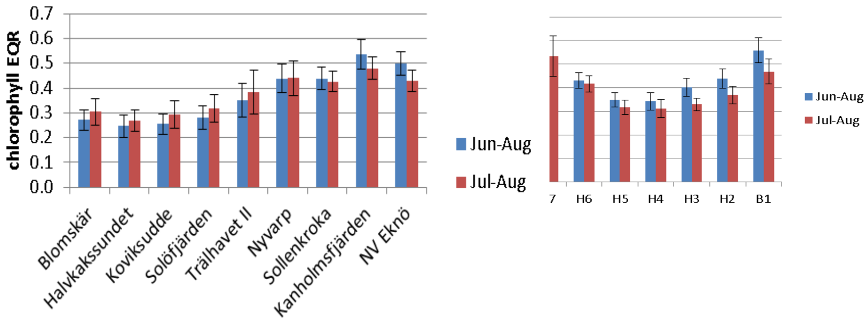


FIGURE 3.17. Chlorophyll EQR 1999-2012 (with 95% confidence interval) for central Stockholm archipelago (left panel) and Himmerfjärden (right panel) areas.

3.2.3 Kattegat and Skagerrak

The stability of the presently used sampling period (June to August) was compared to both shorter and longer periods (Figures 3.18-3.19) for the 6 years 2007-2012. An overall shortening of the existing summer period for chlorophyll *a* by including two months only (June-July or July-August) did not result in an overall better estimation, in terms of CV. As described in the Method section this assumes that the current sampling effort is maintained, both the number of samples and the seasonal distribution (mostly monthly). Longer assessment periods, which include data from more months in the yearly seasonal means, resulted in non-consistent pattern of CV among analysed stations, with approximately similar, lower or higher CV than the current period June-August. As for chlorophyll, biovolume CV was highest for shortest periods, reflecting high outlier values in July. Longer assessments periods than June-August resulted in similar or lower CV depending on station.

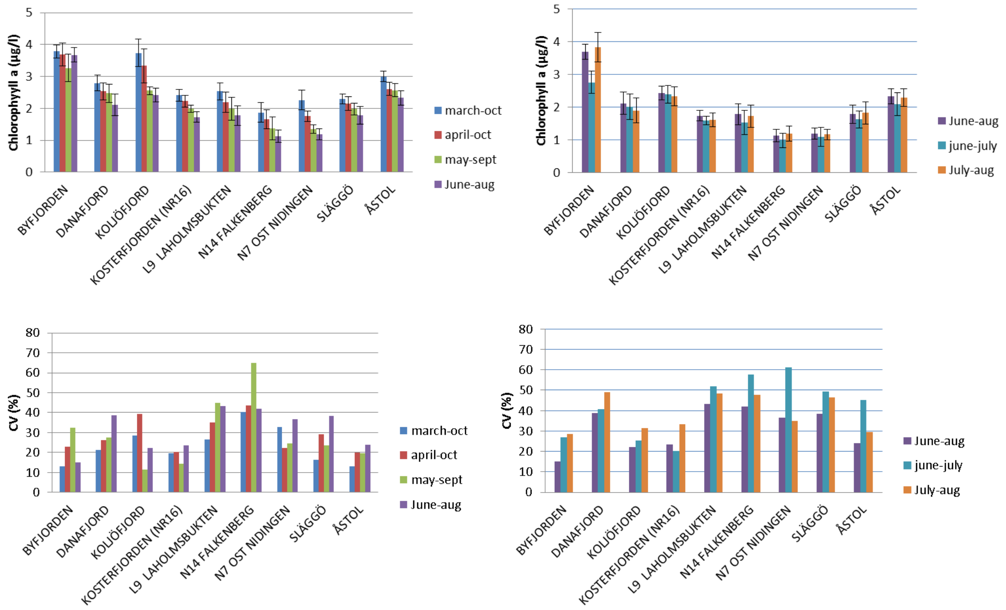


FIGURE 3.18. Mean chlorophyll *a* concentrations (2007-2012 Swedish west coast) and coefficient of variation (% CV) for different seasonal time periods. For comparison, the currently used period (June to August) is included in both graphs. Based on yearly seasonal means formed from monthly interpolated averages. Error bars show standard error (n=6).

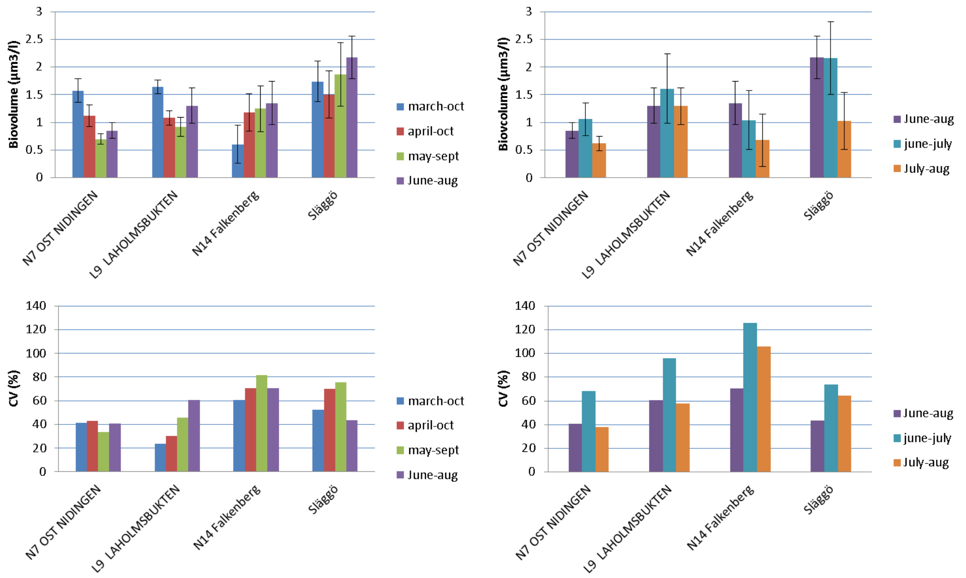


FIGURE 3.19. Mean biovolume concentrations (2007-2012 Swedish west coast) and coefficient of variation (% CV) for different seasonal time periods. The currently used period (June to August) is included in both graphs. Based on monthly interpolated averages. Error bars show standard error (n=6).

3.3 Effects of monthly sampling interval for uncertainty of seasonal means

Traditionally, many monitoring programs are designed with monthly sampling, in order to describe seasonal variability and to yield seasonal means. This contrasts with programs focused on the status assessment period Jun-Aug, with sampling of the summer period only, but usually with larger spatial coverage, as more stations are usually included (e.g. SKVVF sampling program in the Stockholm region).

Two stations in the north-western Baltic Proper, B1 and H4, which are sampled at high frequency over the entire growing season for phytoplankton, were used to simulate the effect of monthly sampling on the uncertainty of different seasonal means. A database with daily values of chlorophyll concentrations was created from the linearly interpolated weekly (spring) or biweekly (summer) data (Figure 3.20). This dataset was used to simulate different monthly sampling regimes.

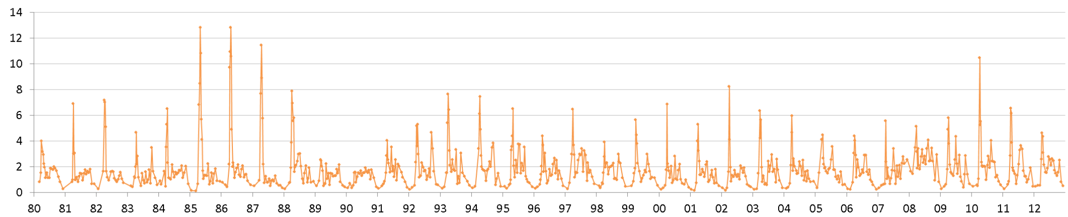


FIGURE 3.20. Chlorophyll concentration 1980-2012 at station B1 in the Baltic Proper, sampled with hose 0-20 m. The sampling interval is weekly in spring and biweekly in summer. The spring bloom peak is usually described by 2-4 successive weekly sampling occasions. The line shows the linearly interpolated data. Station H4 follows a similar seasonal pattern (not shown).

Figure 3.21 shows time-series of the monthly means and standard deviations of all the daily data points interpolated from the dataset of fig 3.20 (station B1). The months March to May are characterized by large within-month variability. This means that for these months the results of monthly sampling in a particular year are very sensitive to the date of the month on which samples are taken. This is because the sampling occasion may precede, be near the peak, or follow the decline of the relatively short-lived spring bloom (fig. 3.20). Figure 3.22 summarizes this data for the entire period for both stations (H4 and B1), showing the monthly means and standard deviations (SD). The coefficient of variation(CV: SD divided by mean) was similar for the two stations, except for May, reflecting the generally later or longer spring bloom peak at station H4. Moreover, figures 3.21 and 3.22 show that the variability in chlorophyll *a* for the summer months June, July and August is relatively low compared to other months.

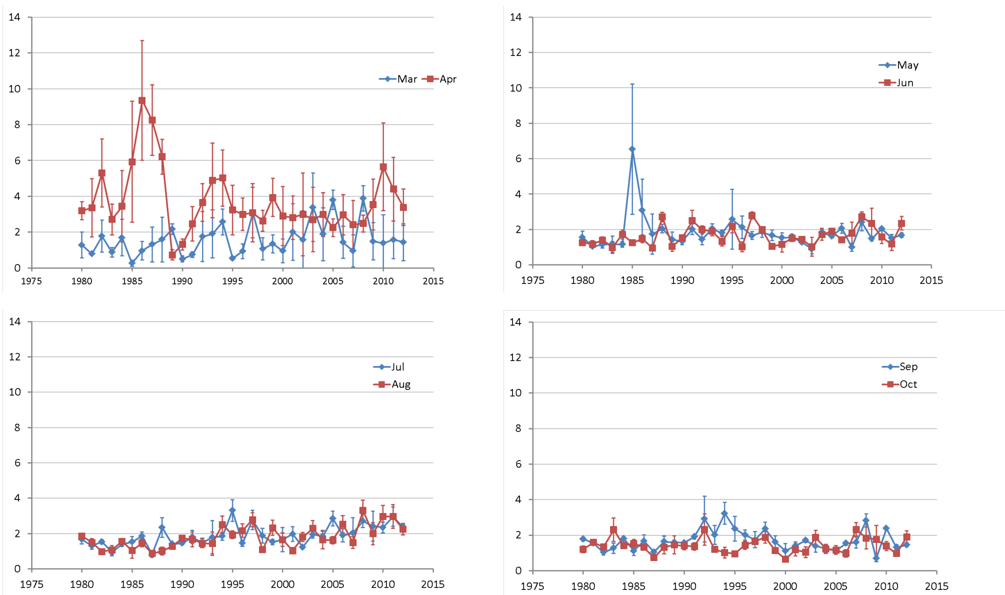


FIGURE 3.21. Monthly mean chlorophyll concentrations (y-axes: µg/l) 1980-2012 at station B1 in the Baltic Proper,calculated from “daily” data, derived from interpolation of weekly to biweekly data. Error bars show standard deviation of “daily” data points. Mar/Apr in upper left panel, May/Jun in upper right panel, Jul/Aug in lower left panel and Sep/Oct in lower right panel.

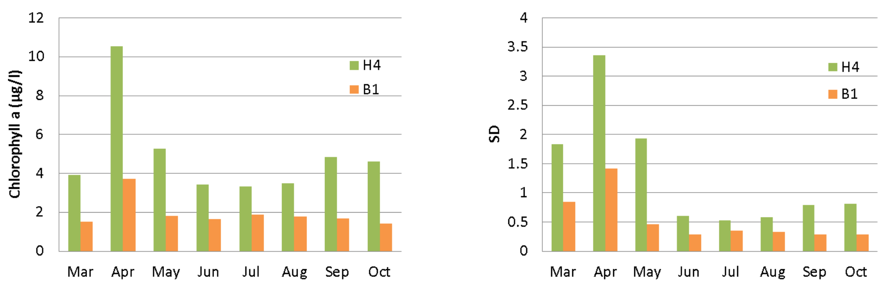


FIGURE 3.22. Monthly mean chlorophyll concentration (left panel) and mean within-month chlorophyll standard-deviation (right panel) for the period 1980-2012 at stations H4 and B1 in the Baltic Proper, calculated from “daily” data, derived from interpolation of weekly to biweekly data.

Clearly, in this case assessments that include monthly data points from the relatively variable months Mar-May will have larger variance relative to assessments including the relatively stable months Jun-Oct only. Nevertheless, it may be argued that for ecological reasons the spring bloom should still be included in the assessment. It may also be argued that the uncertainty introduced is compensated for, simply by adding more data. Although variance is increased, the confidence interval of the mean may decrease, due to an increased number of observations.

To illustrate the theoretical limits for gaining decreased confidence interval with more observations, let us consider a hypothetical data set (A) that is merged with a second data set (B), to increase the number of observations (data set A+B). The combined variance of the pooled data A+B was calculated from the variances of the original data sets. From the variance the confidence interval of the mean was calculated for the pooled data set and compared with the original data sets. In principle, if the variance of data set B is not too high, there may be a smaller confidence interval of the mean for the pooled data set A+B than for the original data set A. Table 3.3.1 shows the maximal variance of the second data set B to get a positive effect (decreased confidence interval) of pooling this data to dataset A (forming A+B). If the SD of B is larger than this maximum relative to A, there is no gain in terms of decreased confidence interval of the mean for A+B compared to the original A. For example, assuming similar mean values for all months that can be included in an assessment and a 33% increase in the number of observations by pooling periods, i.e. an increase from 3 to 4 months included in the assessment, the SD of the additional period should be not more than 50% higher than the SD of the original period to get a positive effect in terms of improved confidence of the mean seasonal value. Thus, there is not always a gain from adding more data unless this data has a variance close to the original data set. In this example the mean value is the same for the merged populations. If the mean value of the added period is higher than the original period, the variance of the added period must be even smaller to get a positive effect. From figure 3.22 it is evident that for stations B1 and H4 the standard deviation (square-root of variance) and the mean are so much higher for the spring months compared to the summer months that there is no gain in confidence for the mean from just including more monthly observations.

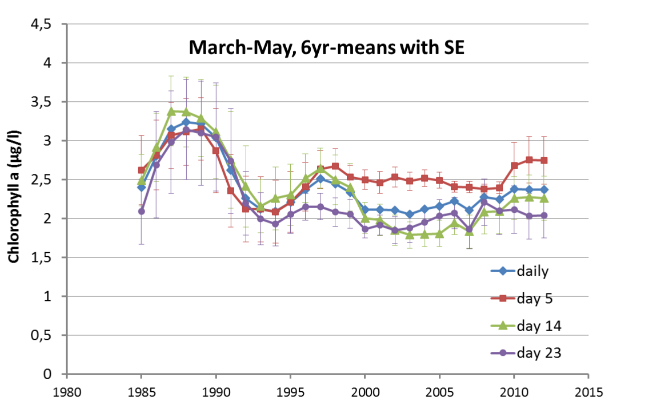
TABLE 3.3.1. The maximum variance (max.Var. B) of an additional period (B) that is merged with an original period (A) with variance s^2, in order to gain decreased confidence interval from the increased number of observations. The pooled variance of the new longer period is also shown (Var. A+B). Example Case 1: When merging a population n A that is 3 times larger than n B, having similar means, the variance of population B must not exceed 1.5 times the variance of population A to yield a gain in terms of decreased confidence interval.

Case	**n A**	**n B**	**Mean A**	**Mean B**	**Var. A**	**max.Var. B**	**Var. A+B**
1	3n	n	x	x	s^2	**1.5** s^2	1.1 s^2
2	3n	2n	x	x	s^2	**1.6** s^2	1.3 s^2
3	3n	3n	x	x	s^2	**1.7** s^2	1.4 s^2

It may be argued that for ecological reasons, the spring bloom should still be included in the assessment, and that a lower confidence is acceptable or that it should be compensated for by sampling in the spring more frequently than monthly. Assuming the spring bloom is included, what would be the consequences of monthly sampling? Most critically: do we get a representative mean?

If a 6 –year assessment cycle is followed and monthly data from 6 years are included in the assessment, one may argue that both within-month and between-year variance is included in the assessment, although we cannot separate these variance components. Thus, although additional uncertainty is introduced by inclusion of monthly data from March-May, 6-year means will, on average, be representative for the seasonal period. However, this assumes within-month variance is random. Unfortunately it is not. As is evident in figure 3.20, there is a distinct yearly spring chlorophyll peak, of much shorter duration than a month. If the timing of the peak would vary randomly within a monthly time frame, monthly sampling would, in principle, yield random values. However, this is not the case. Although the timing of the spring bloom is influenced by random factors, such as weather events, unusually cold winters etc., the timing is largely determined by the general seasonal progression from winter to spring, i.e. the progressively longer days, increasing angle of the sun, which is similar from year to year.

The dataset with daily data obtained from interpolated weekly and biweekly data (station B1) was used to simulate and illustrate the effect of different monthly sampling regimes. This was done simply by distributing sampling to a certain day each month, e.g. either the 5th, 14th, or 23rd, or some other arbitrarily chosen day. From the retrieved monthly data points, seasonal means were computed for spring (March-May), summer (June-August) and the full growth period (March-October). Seasonal means were aggregated into 6-year means (and between-year variability of these), corresponding to the current assessment cycle of the WFD (fig. 3.23). The first period March-May covers the spring bloom (3 sampling occasions), June-August is the present assessment period (3 sampling occasions), and March-October covers the entire growing season (8 sampling occasions).



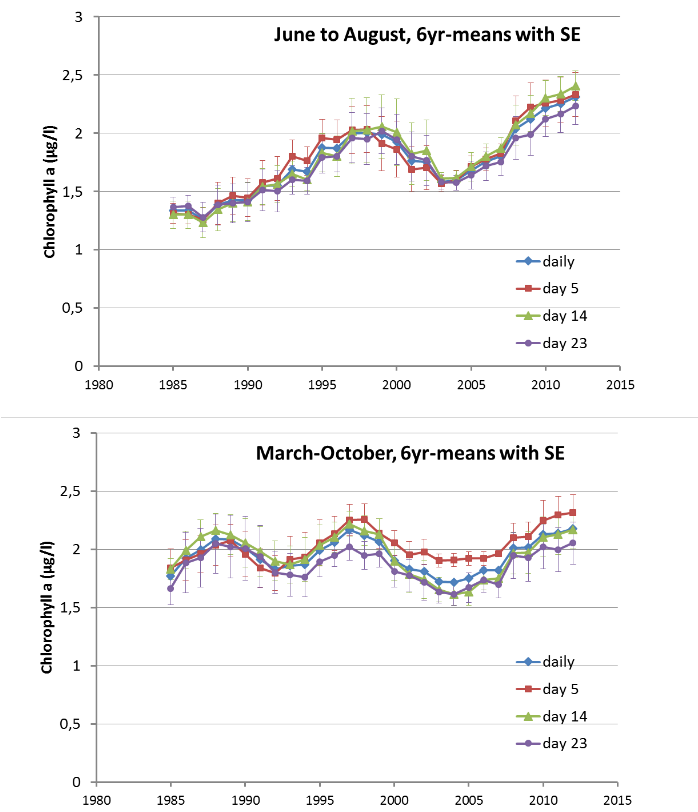


FIGURE 3.23. Running 6-year means for spring (March-May) and summer (June-August) chlorophyll a data, calculated from the full "daily" data set (obtained from interpolated weekly and biweekly data), or monthly data sampled a certain date from the daily data (i.e. the 5th, 14th or 23rd each month). Error bars show standard error for the 6 year-sample included. The 6-year running mean values are shown for the last year of the period (e.g. 1985 for the period 1980-85).

The results (fig 3.23) show that the spring period was characterized by large inter-annual variability in the 1980´s to early 1990´s and that there was relatively small inter-annual variability from the late 1990´s and onwards. However, in this later period the results were very sensitive to the arbitrary choice of sampling date. Sampling the 5th each month resulted in consistently high 6-year mean values. Sampling the 14th or 23rd resulted in relatively low 6-year means. The difference between the estimates is as large as 40%. The reason for this result is that from the late 1990's the timing of the spring peak was consistently near the turn of March to April, i.e. close to the 5th. Fig. 3.24 shows the corresponding retrieved data points of the simulation for the years 2003-2012, illustrating the generally high chlorophyll values the 5th of April, and the generally lower values retrieved if sampling the 14th or 23rd.

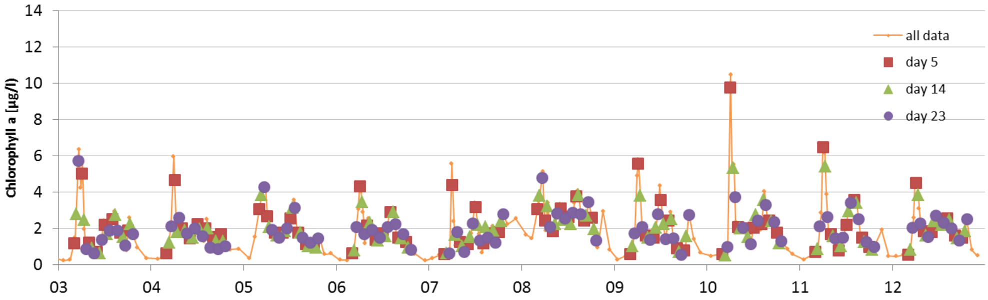


FIGURE 3.24. Chlorophyll data at station B1 2003-2012. Clorophyll values are shown for three different arbitrarily chosen monthly sampling dates, used in fig. 3.23. The figure illustrates the problem that the spring bloom may be sampled near the peak or missed completely with monthly sampling.

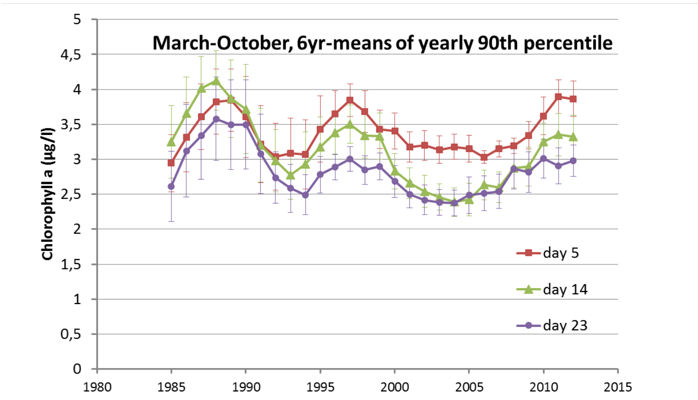


FIGURE 3.25. Running 6-year means for yearly 90th percentile of seasonal period March-May (station B1, Baltic Proper). Results are shown for three different arbitrarily chosen monthly sampling dates, simulating monthly sampling from the interpolated data set. The 6-year running mean values are shown for the last year of the period (e.g. 1985 for the period 1980-85).

In Figure 3.25 we show an analysis of the effect of using a yearly 90th percentile of the data for the period Mar-Oct, if monthly sampled. The 90th percentile was tested since it is used within the NEA-GIG. The results show that the 90th percentile will in principle be an analysis of the spring bloom (c.f. fig. 3.24 and 3.23), but with even larger sensitivity to arbitrary monthly sampling date.

4. Discussion and conclusions

Our results show that in the Gulf of Bothnia phytoplankton biovolume data have larger variance and higher mean concentration in June compared to July-September, indicating a spill-over effect from the spring bloom. Rather surprisingly, this problem was not evident for chlorophyll. Possibly, a low chlorophyll content of the dominating species in the end of the spring bloom contributes to this pattern. The biovolume data suggest that the sampling period for type areas of the Gulf of Bothnia should preferably exclude June, i.e. include July-August only, or possibly be extended to July-September. Including September does not appear to affect the seasonal means of chlorophyll and biovolume and would have the advantage of the inclusion of more data in the assessment. Omitting June would increase the need for sampling in July, which presently is not sampled so well. These changes should not confer any changes in the classification boundaries since they are defined to characterize the summer situation.

For the Baltic Proper, our results indicate some systematic differences of chlorophyll concentrations between June and later months, but that these differ between inner stations (higher in June), intermediate stations (similar between months) and outer stations (lower in June). Accordingly, there were contrasting differences when comparing the June-August and July-August periods for different areas. This pattern probably reflects the effects of continued nutrient inputs to inner areas after the spring bloom, and that cyanobacteria tend to be relatively abundant in July-August in the outer and southern parts of the Stockholm archipelago. Moreover, the uncertainty was not reduced by the inclusion of June, despite more sampling occasions included in the seasonal mean. The July-August period rather showed a lower variance for some stations. This suggests the period July-August is preferable as assessment period compared to the existing period June-August. An advantage of a shorter assessment period is that it facilitates monitoring programs with a greater spatial coverage. The data for the Baltic Proper indicate that September occasionally may be affected by autumn blooms and is therefore less suitable to include in the assessment period. Compared to the present assessment period June-August, sampling only in July-August tends to overestimate EQR (status) in inner coastal areas in the Baltic Proper and tends to underestimate EQR in outer areas. Unless classification boundaries are changed, chlorophyll EQR values based on July-August data only, should preferably be corrected by -10 to +20%, depending on area (see results section). Corresponding correction factors for total nitrogen and phosphorus EQR were negligible, while Secchi depth EQR differences motivate a minor correction for intermediate and outer areas. The calculated factors can be guidelines for changed classification boundaries.

For the Kattegat and the Skagerrak, our results indicate that a shortened period may result in more uncertain assessment. Since sampling frequency at most coastal stations is once a month, a shortened period would result in fewer data points for yearly evaluation. Another possibility is to make the evaluation period longer. The spring bloom peak is often as early as February (see fig. 3.9-10). Thus, assessment periods starting in March or April will include the spring bloom only some years, contributing uncertainty to the assessment. With the unclear improvement from using an assessment period including the spring bloom, and the principal objections of including the spring bloom if there is a monthly monitoring only, the recommendation is to exclude the spring bloom and thereby not start earlier than May. For chlorophyll, there were similar mean concentrations and variances for the different months in the period May-September. The extended period May-September was similar to the currently used summer period (June-August). Increasing the length of the evaluation period would ensure more data points for many assessments. Given the relatively stable assessment period surveys with more limited temporal coverage should also be possible to perform, which is an advantage if a greater spatial coverage is needed. Another advantage is that an assessment period extending from May to September would facilitate comparisons with neighboring waters, as authorities in both Denmark and Germany use this period. Only small corrections of the reference values and class boundaries are expected, but need further evaluation. Furthermore, the summer months show a discrepancy between chlorophyll *a* and biovolume, especially during July. This discrepancy might be less evident if additional months are included. Another possible solution to harmonize chlorophyll a and biovolume would be to transfer biovolume to carbon content. This would however need further analyses and would impose a larger revision.

The analysis of the uncertainty of a monthly sampling interval shows that single measurements in spring are very uncertain and of little value, i.e. sampling programmes with resources to measure only 1-5 times per year should restrict sampling to the suggested status assessment period (summer). Monthly measurements in spring are very sensitive to the arbitrary choice of sampling date. If a reasonably good representation of the spring bloom should be achieved, monthly monitoring is not sufficient.

Moreover, the analysis shows that the 90th percentile of the data, as has been used within the NEA GIG (North-East Atlantic, including Skagerrak and Kattegat), is a very uncertain indicator based on monthly data, since the spring bloom value will be decisive of the indicator.

Acknowledgements

Much of the data used comes from the Swedish National Marine Monitoring Program, today funded by the Swedish Agency for Marine and Water Management and earlier by the Swedish Environmental Protection Agency. Additional data from the west coast comes from the Water Quality Association of the Bohus Coast and the County of Halland Coastal Monitoring Program. Additional data for the Baltic Proper comes from Stockholm Vatten AB and the Himmerfjärden control and research program. In the Gulf of Bothnia additional data were used from SRK programs in County of Norrbottens and from NÖ Hälsinglands VVF.

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PHYTOPLANKTON IN COASTAL WATERS – EVALUATION OF THE LENGTH OF THE SEASONAL ASSESSMENT PERIOD

The period for phytoplankton status assessment is currently June-August for all coastal Swedish waters. It may however be inappropriate to use the same period for all coastal Swedish waters because of the large latitudinal climate gradient. We evaluated the most suitable assessment period in different Swedish coastal areas; the Gulf of Bothnia, the Baltic proper and the west coast.

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