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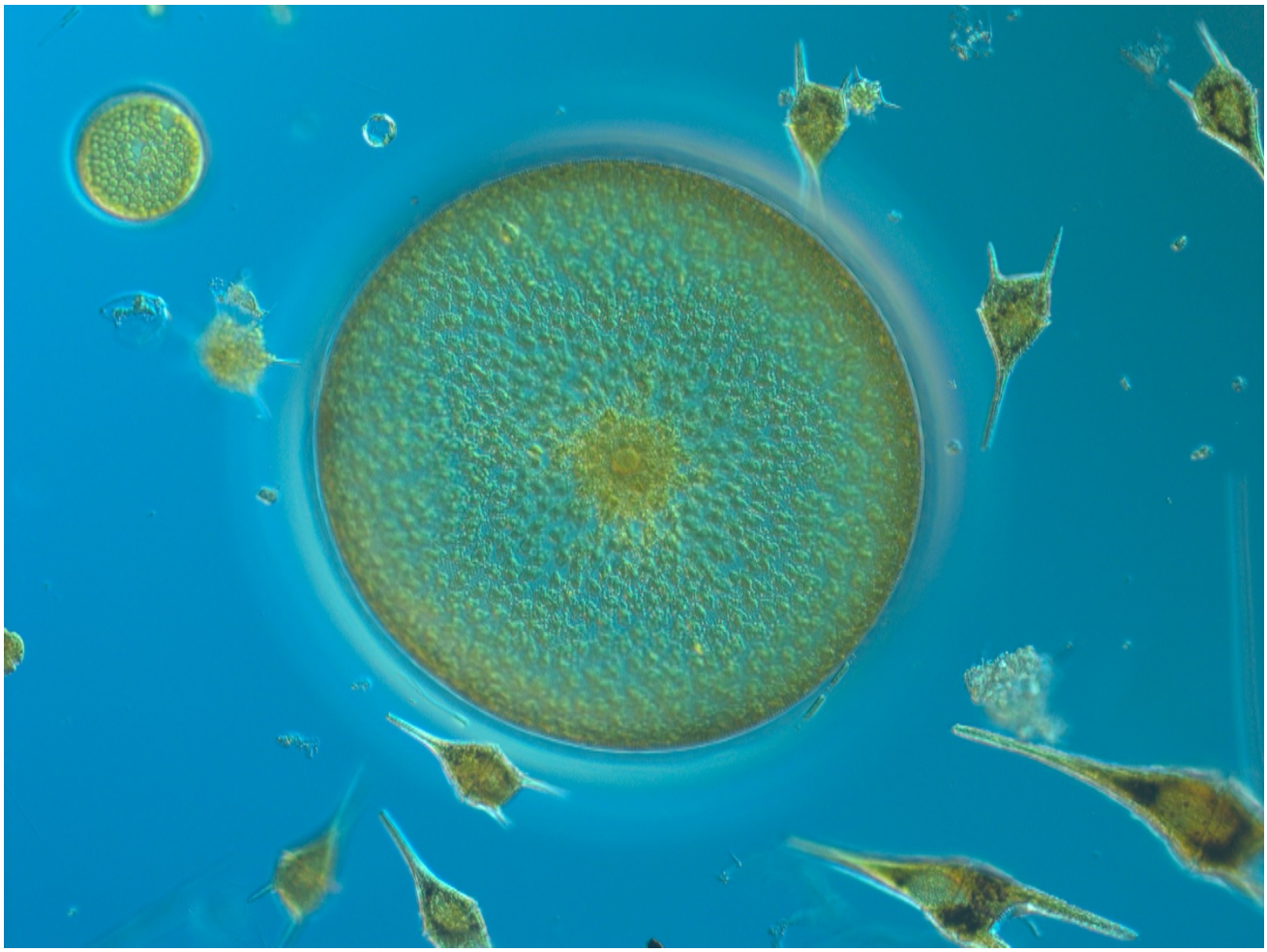
Variability and Trends of Phytoplankton in the Baltic Sea and Kattegat-Skagerrak



Swedish Agency for Marine and Water Management report 2015:33

Variability and Trends of Phytoplankton in the Baltic Sea and Kattegat-Skagerrak

Bengt Karlson, Patrik Strömberg and Ann-Turi Skjevik

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

Swedish Agency for Marine and Water Management
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Deputy Director General Ingmar Berglund is legally responsible for the publication of this report.
Cover illustration: Phytoplankton from the Skagerrak: In the middle the diatom *Coscinodiscus concinnus* surrounded by several dinoflagellates from the genus *Ceratium*. Photo: Malin Mohlin.
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Sammanfattning och slutsatser

Rapporten syftar till att beskriva resultat från analys av tidsserier från marina miljöövervakningsprogram i Sverige med avseende på växtplankton. En övergripande fråga är om den svenska miljöövervakningen kan fånga upp förändringar med nuvarande provtagningsfrekvens. De tre huvudsyftena är att: (I) analysera statistisk styrka för tidsserier av växtplanktonbiomassa, (II) analysera hur statistiskt lika stationer är med avseende på artsammansättning och (III) analysera tidsmässig variabilitet vad gäller artsammansättning. Resultaten kan ge en fingervisning om hur långa mätserier som krävs beroende på vilken fråga som skall besvaras, d.v.s. vilken storlek på förändring över tid som behöver detekteras. Resultaten ger även inblick i hur stor variabiliteten i växtplanktons biodiversitet är mellan stationerna, eller enklare utryckt hur lika stationer är.

För att kvantifiera hur mycket växtplankton som finns kan olika parametrar undersökas, exempelvis klorofyll och biovolym. Klorofyll är specificerat i direktiv för att beskriva miljöstatus i havet som en indikator för eutrofiering. Klorofyllprovtagning sker med huvudsakligen två olika metoder i haven runt Sverige. Prover tas dels med slang, normalt 0-10 m, och dels med vattenhämtare från fasta djup. När medelvärden från provtagning vid fasta djup jämfördes med data från slangprovtagning kunde ingen skillnad påvisas. En slutsats är att klorofylldata baserad på slangprovtagning kan användas tillsammans med medelvärdesbildade data från fasta djup. För att undersöka om klorofyll *a* fungerar som en så kallad proxy (~ersättare) för växtplanktonbiomassa jämfördes data på total biovolym av växtplankton med mängden klorofyll *a* i ett datamaterial från 1983 till 2014. En stor del av data kommer från 2010 och senare. I det undersökta datamaterialet finns en signifikant, men svag, korrelation mellan växtplanktonbiomassa, mätt som biovolym, och klorofyll *a*. ($n= 3119$, $p < 0.01$, $R^2 = 0.439$).

Resultat gällande statistisk styrka på tidsserier av total biovolym av växtplankton visar att det i medeltal tar 23 år att upptäcka en förändring på 1% ($p<0.05$, power = 80%). En förändring på 10% upptäcks på 7 år och en förändring på 40% på 5 år. Resultat gällande statistisk styrka på tidsserier av klorofyll *a* visar att det i medeltal tar 33 år att upptäcka en förändring på 1%. En förändring på 10% upptäcks på 14 år och en förändring på 40% på 7 år. Det är värt att notera att dessa siffror är baserade på verkliga data från miljöövervakningen och den variabilitet som finns i datamaterialet. När data delades upp i olika geografiska områden enligt den så kallade typindelningen av kustvatten visade det sig att datamängden i de olika typerna varierar stort. Det innebär att det i vissa områden i stort sett saknas dataunderlag för att göra en analys av statistisk styrka. En annan slutsats är att det finns en betydligt mindre mängd data gällande växtplanktons biodiverstitet och biomassa baserat på cellräkning och cellvolymsbestämningar jämfört med mängden data på klorofyll *a*.

Data delades även in havsområdesvis för att visa vilken statistisk säkerhet datamaterialet ger om provtagningar fortsätter med samma frekvens som hittills. För att upptäcka en förändring på 5% i klorofyll med en statistisk säkerhet på 80% krävs det i medeltal:

Kattegatt-Skagerrak: 16 års data
Öresund och södra Egentliga Östersjön: 11 års data
Egentliga Östersjön: 7-> 50 års data
Bottenhavet: 19-41 års data
Bottenviken: 13-35 års data

För att undersöka skillnader i biodiversitet användas data från de så kallade kampanjårs-studierna som genomfördes under perioden 2010 - 2012. Då genomfördes provtagning av växtplankton på betydligt fler stationer än i de normala övervakningsprogrammen. Resultat av klusteranalys (Euclidian distance) på artsammansättning visar att en provtagningsfrekvens på en gång i veckan fångar den naturliga variationen i biodiversitet. Provtagning en gång i månaden fångar inte den naturliga variabiliteten i biodiversitet. När det gäller rumslig upplösning så har stationer inom samma vattenmassa, t.ex. södra Kattegatt, ungefär samma artsammansättning. När det gäller stationer nära kusten, finns betydande skillnader i biodiversitet mellan prover insamlade i närliggande vikar och fjärdar/fjordar.

Summary and conclusions

The aim of the report is to describe results from analyses of time series of phytoplankton data from the Swedish marine monitoring programs. One issue is if it is possible to describe environmental change with the current sampling frequency. The three main aims are: (I) to investigate the statistical strength of time series of phytoplankton biomass, (II) to investigate the variability of species composition between stations and (III) to investigate the temporal variability regarding species composition. Results indicate how long time series are needed to detect change at a certain level. Also the variability in biodiversity is shown with some examples.

To quantify the biomass of phytoplankton different parameters may be investigated, e.g. chlorophyll content and the biovolume of phytoplankton. Chlorophyll *a* is designated in directives for describing the environmental status of the seas as an indicator for eutrophication. Sampling for chlorophyll is usually made using two different methods in the seas surrounding Sweden. Samples are collected using a hose, normally from 0-10 m depth, or by sampling at discrete depths, e.g. 1, 5 and 10 m. No difference was observed when comparing data from the hose sampling with depth-averaged data from the discrete depths. Thus the data from hose-sampling can be used together with the data based on sampling at discrete depths. To investigate if chlorophyll *a* works as a proxy for phytoplankton biomass data on total biovolume of phytoplankton, based on cell counts and cell volume estimates, was compared to chlorophyll *a* data. The data set include data from 1983 to 2014. A large part of the data emanates from 2010 and later. In the investigated data set there is a significant, but weak, correlation between chlorophyll *a* and total biovolume ($n= 3119$, $p < 0.01$, $R^2 = 0.439$).

Results about the statistical strength (power) of the time series of total bio-volume of phytoplankton indicate that it on average takes 23 years to detect a change of 1% ($p<0.01$, power = 80%). A change of 10% is detected after 7 years and a change of 40% is detected in 5 years. Results regarding the statistical strength of time series of chlorophyll *a* show that it on average takes 33 years to detect a change of 1%. A change of 10% is detected after 14 years and a change of 40% in 7 years. Please note that these figures are based on data from monitoring programs that include the variability observed. When the data set was divided into geographical areas called the type areas it was evident that the amount of available data in the different type areas varies a lot. In some areas there is not enough data to carry out an analysis of the statistical strength. Another conclusion is that there is substantially less data on phytoplankton biodiversity and biomass based on cell counts and cell volume estimates compared to the amount of data on chlorophyll *a*.

Data was also split according to sea basins to show the statistical strength in the data if sampling frequency continues as up to now. To detect a change of

5% of chlorophyll a with the power of 80% the number of years needed if the present sampling frequency continues is on average:

- The Kattegat-Skagerrak: 16 years
- The Sound and the Southern Baltic Proper: 11 years
- The Baltic Proper: 7->50 years
- The Bothnian Sea: 19-41 years
- The Bothnian Bay: 13-35 years

To investigate differences in biodiversity, data from intense sampling campaigns made in the period 2010-2012 was used. The sampling was made at a much larger number of locations compared to the normal monitoring program. Results of cluster analysis (Euclidian distance) on the species composition show that weekly sampling describes the natural variability in phytoplankton biodiversity well while sampling once a month does not resolve the natural variability in biodiversity. When investigating the spatial variability, i.e. the differences in species composition between stations, results indicate that samples from the same water mass, e.g. the southern Kattegat, are similar. The differences in closely located bays and fjords are large in regard to plankton biodiversity.

Introduction

Aim

The aim of this report is to evaluate if the existing Swedish coastal marine phytoplankton monitoring programs are suitable to fulfil the needs of the EU Water Framework Directive (EU 2000). The results are also relevant for the EU Marine Strategy Framework Directive (EU 2008, 2010). The Swedish Agency for Marine and Water Management gave SMHI specific questions to address. Accordingly the report covers only a few aspects of the many problems involved in phytoplankton monitoring. The mission from the Swedish Agency for Marine and Water Management, SWAM, was to investigate Swedish phyto-plankton data. The objectives were; I to investigate the power of the data to detect given change in time per station and II to analyse how "similar" the stations where based on species composition. To answer these questions data where quality controlled and prepared for analysis. Then power- and cluster analyses were carried out. Similarities were identified by using the Euclidian distances. The distances were then visualized as tree diagrams.

Overview of requirements from directives etc.

The Swedish environmental objectives (miljömålen)

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

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

Of great importance are also:

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

The EU Water Framework Directive (WFD)

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

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

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

The EU Marine Strategy Framework Directive (MSFD)

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

Descriptor 1:

Biological diversity is maintained. The quality and occurrence of habitats and the distribution and abundance of species are in line with prevailing physiographic, geographic and climate conditions.

Descriptor 2:

Non-indigenous species introduced by human activities are at levels that do not adversely alter the ecosystem.

Descriptor 4:

All elements of the marine food webs, to the extent that they are known, occur at normal abundance and diversity and levels capable of ensuring the long-term abundance of the species and the retention of their full reproductive capacity.

4.3. Abundance/distribution of key trophic groups/species

— Abundance trends of functionally important selected groups/species (4.3.1).

4.3.1— groups with fast turnover rates (e.g. phytoplankton, zooplankton, jellyfish, bivalve molluscs, short-living pelagic fish) that will respond quickly to ecosystem change and are useful as early warning indicators,

Descriptor 5:

Human-induced eutrophication is minimised, especially adverse effects thereof, such as losses in biodiversity, ecosystem degradation, harmful algal blooms and oxygen deficiency in bottom waters.

Overview of ongoing phyto-plankton monitoring programmes

Introduction to the monitoring programmes

The national marine environmental monitoring programme in Sweden performs monthly monitoring of several parameters including phytoplankton and chlorophyll. In a few locations the sampling is more frequent. The main focus of the national program is the off shore areas but a limited number of coastal sampling locations are also included. In addition to the national program several regional programs are in operation. Some of these include phytoplankton monitoring while some only include chlorophyll as a parameter.

National Marine Monitoring Programme

Phytoplankton is sampled approximately monthly at the stations indicated with red dots (see map in Fig. 1). At the high frequency stations B3/B7, B1, BY31, Släggö and Anholt E sampling is made approximately 24 times a year. A 10 m long tube is used for sampling, except at stations B1 and BY31 where the sampling carried out by the Stockholm University is made using a 20 m tube. The HELCOM COMBINE manual recommends 10 m. Acid Lugol's solution is used for preservation in general. Alkaline Lugol's is used for coccolithophorids in the Kattegat-Skagerrak during part of the year. Samples for autotrophic picoplankton are at present only collected in the Gulf of Bothnia and analysed by the Umeå University.

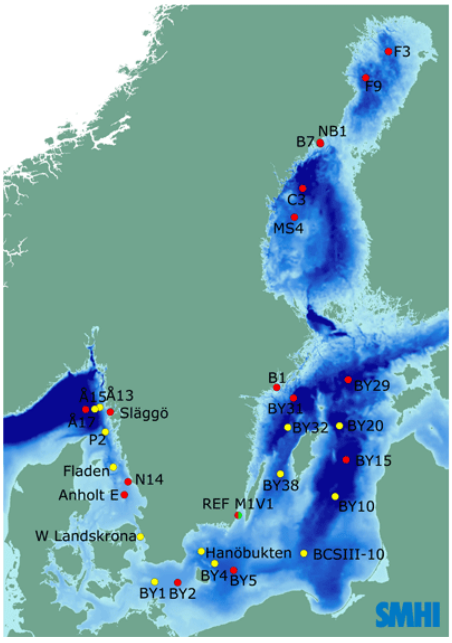


Fig 1. Map shows sampling locations in the National Marine Monitoring Program 2015. Stations with red marks are funded by SWAM while those labelled with yellow marks are funded directly through SMHI.

● National Monitoring Program funded by the Swedish Agency for Marine and Water Management (carried out by SMHI, Umeå university, and Stockholm university))

● Stations in the sampling program funded by SMHI.

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

Swedish National Food Administration - monitoring for microalgae producing biotoxins

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

Regional monitoring programmes

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

Table 1. Regional monitoring programmes listed at the National data host for marine biological and oceanographic data in January 2014. There are also monitoring programs that have not delivered data to the national data host, e.g. Svealands vattenvårdsförbund. These are not listed. Please note that only some of the regional monitoring programmes include phytoplankton monitoring. Source: http://www.smhi.se/klimatdata/oceanografi/Havsmiljodata

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

Overview of phytoplankton sampling in 2014

A map of phytoplankton sample data reported to the national data host for marine biological data, i.e. the Swedish National Oceanographic Data Centre at SMHI is shown in Fig. 2. Only locations where biomass of phytoplankton have been determined based on cell volume estimates are included. The map includes data from both national and regional monitoring programs. In some cases the crosses represent more than one sampling event during a month. Source: http://sharkweb.smhi.se accessed on 15 November 2015. Missing from the maps are data from e.g. Öresund water quality association sampling events (no biovolume data), National monitoring program for biotoxin producing algae administered by the National Food Administration (no biovolume data). A data set from the Svealands Vattenvårdsförbund is missing since the data has not been delivered to the national data host.

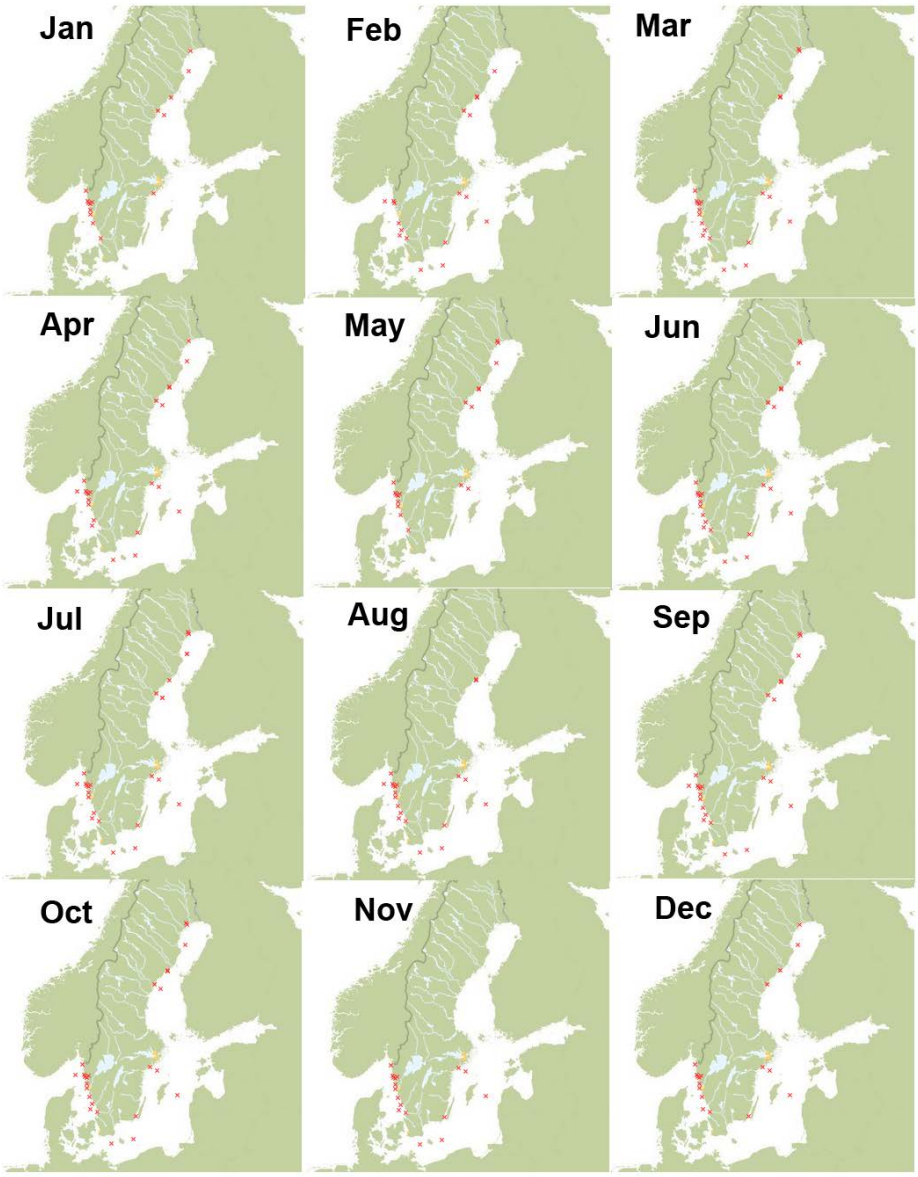


Fig. 2. Sampling locations for phytoplankton in 2014. Maps include samples from the national marine monitoring program and most regional monitoring programmes and any short term sampling. Please note that any phytoplankton sampling that does not include analysis of biomass based on cell volumes is excluded. Source: www.shark.smhi.se accessed on 16 November 2015.

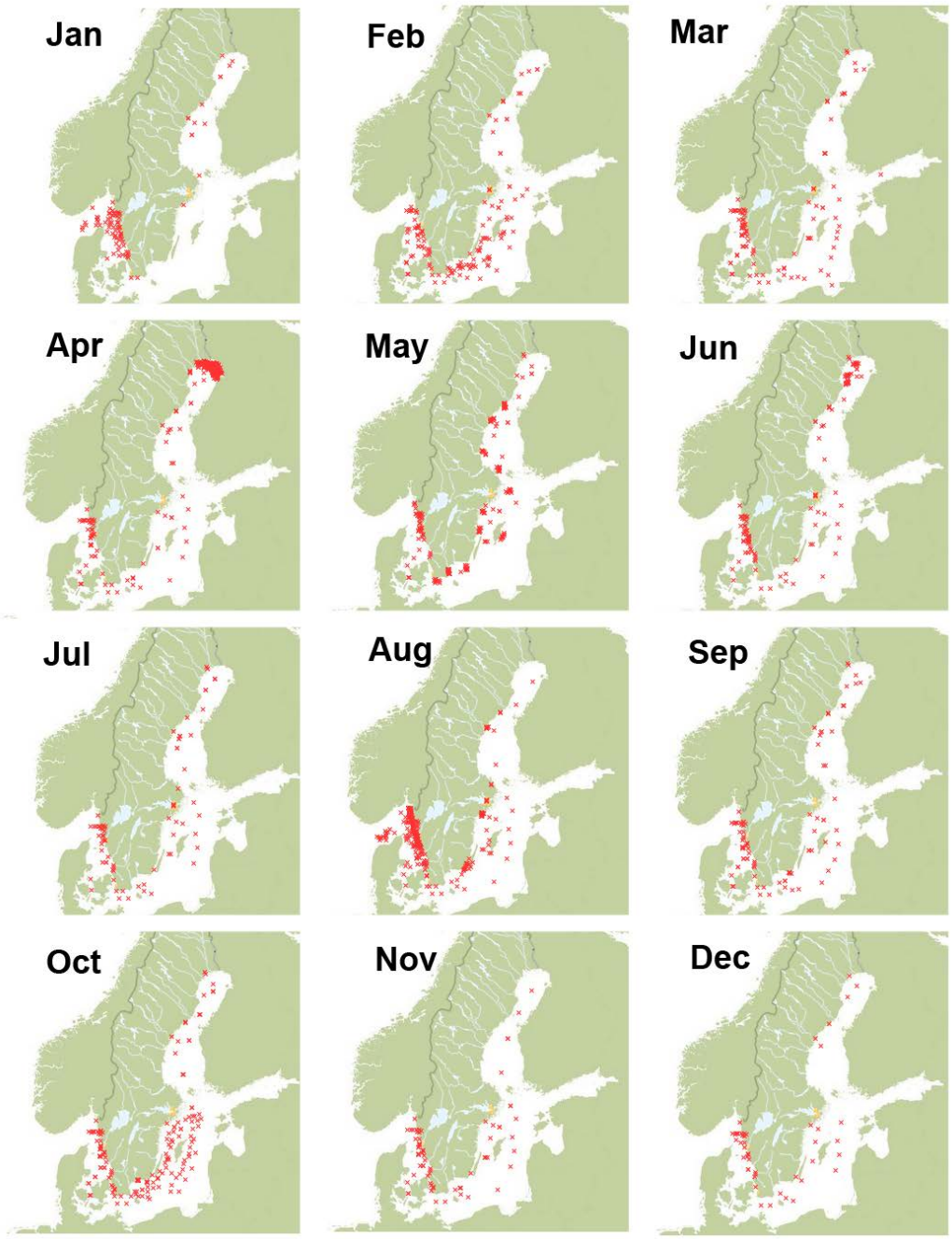


Fig. 3. Sampling locations for chlorophyll *a* in 2014. Maps include samples from the national marine monitoring program, most regional monitoring programmes and any short term project sampling. Source: www.sharkweb.smhi.se accessed on 16 December 2015.

Material and methods

Data

All data was retrieved from the Swedish Oceanographic Data Centre at SMHI. Phytoplankton data and data on chlorophyll *a* were downloaded from http://www.sharkweb.smhi.se. The data is the results of national marine monitoring programs and regional programs (see acknowledgements). The data used is freely available at the national data host for marine biology and oceanography in Sweden. More data exist but have not been reported to the national data host and was unavailable for the work presented.

Water sampling was carried out in national and regional monitoring programmes. In Fig. 2 a map showing the sampling locations for phytoplankton in 2014 is found. Fig. 3 illustrates sampling of chlorophyll *a* 2014. In years 2010, 2011 and part of 2012 the numbers of stations sampled were greatly increased in certain areas since special phytoplankton sampling campaigns were carried out. A report for the Svealand coast was published by Höglander et al. (2011). The data from the sampling campaigns in the Kattegat-Skagerrak in 2011-2012 and in the Gulf of Bothnia 2010-2011 are previously unpublished.

Tube sampling is the standard HELCOM method for collecting water samples for phytoplankton analyses. The depth interval is 0-10 m. At stations B1 (near Askö, Archipelago of Stockholm) and BY31 the depth interval was 0-20 m. The water collected in the tube was mixed and sub samples preserved using Lugol's. The reason to use a 10 m long tube is to minimize the risk of missing thin layers of phytoplankton in the upper part of the sea. Samples for chlorophyll *a* were mainly collected from distinct depths or by using the tube.

Biodiversity, abundance and biomass of phytoplankton

Samples were analysed according to HELCOM (2015) and Olenina et al (2006) using the Utermöhl method. Organisms > 2 µm were identified to the most detailed level possible. The 2015 list of the HELCOM-Phytoplankton Expert Group was used to standardize names. The list also includes geometrical shapes and size classes of phytoplankton species for biovolume calculations and is updated every year by the group. The updated list is available at http://www.ices.dk/marine-data/vocabularies/Documents/PEG_BVOL.zip. Several species observed in the Skagerrak are not part of the HELCOM-PEG list as the area belongs to the North Sea. For these species a separate amendment to the HELCOM-PEG list was compiled by the SMHI oceanographic unit in Gothenburg. Biovolumes are converted to carbon based on measurements of the carbon content of different phytoplankton species (Menden-Deuer and Meunier 2000).

Chlorophyll *a*

The methods followed recommendations by HELCOM (2015). Water samples were concentrated by filtration onto Whatman GF/F filters. Photosynthetic pigments were extracted using ethanol. The concentration of chlorophyll *a* was measured using a laboratory fluorometer or a spectrophotometer.

Data processing

All Swedish coastal stations where phytoplankton has been sampled and biovolume have been calculated have been used for this report.

Phytoplankton data was extracted from SHARKweb and thoroughly analysed to discover any errors. The errors that have been found have been corrected in the database in cooperation with the data providers. The corrections made have also been noted in a data specific change log file added with the new import to the database. Phytoplankton data was downloaded from SHARKweb from the whole time series available, 1983-2013. The data set was imported to the Plankton toolbox (Karlson et al. 2015) where the following steps were made:
Sample with minimum depths > 0 were excluded.
Sample with maximum depths other than 10 and 20 meters were excluded.
All heterotrophic organisms were excluded.
Data were aggregated to biota to get total biovolume per sampling event.
Consequently, total phytoplankton biovolume per station, from 0-10 or 0-20 meters were achieved.
For the analysis of similarities between stations (question 1d), additional biovolume data have been exported from the sample campaign period 2010 – April 2012, during which many extra stations were sampled during different lengths of time and various numbers of stations.
In the Bothnian Bay and Bothnian Sea, the campaign included July and August 2011 and 39 stations. In the Northern Baltic proper, the campaign included July and August 2010 and 23 stations sampled twice. In the Kattegat and Skagerrak areas, sampling was performed 12 times at 5 different stations giving a total of 60 samples.
The campaign data as well as the ordinary data 2010 - April 2012 were exported from SHARKweb. The data set was imported to the Plankton Toolbox where the following steps were made:

- Sample minimum depths > 10 meters were excluded, all other depths were kept.
- All heterotrophic organisms were excluded.
- Data were aggregated to the scientific level identified by the microscopist.

Software for statistical analysis

Analysis of large datasets, as in this study, is best performed using programs where scripts can be written. The open source software R was used since it is free and used in countless studies including statistical research. Except for the obvious advantage of free easily available tools, we would like to also stress that

this eases the process of reproducing scientific studies and must hence be encouraged.
In this study standard functions, supplied with the R base installation, and some additional packages were used; for model II regression lmodel2 (http://cran.r-project.org/web/packages/lmodel2/vignettes/mod2user.pdf). We also developed methods for power analysis in cooperation with Prof. Jacob Carstenssen (DTU). These will be described in details in a separate section. All our R-code may be supplied if requested.

Power analysis

The task was to investigate annual constant trends of different magnitudes, spanning from 1%, 5%, ...%, i.e. the annual change or slope equals 0.01, 0.05, ... Power analysis where then used to investigate if enough data (number of observations) were available to obtain enough certainty, i.e. probability, that the observed time series trends (1%, 5%...) were "real" statistically. In the analysis the probability to detect the trends was to be reported as statistical power β.
P-value indicates the risk of detecting a trend that is not true. Often a level of 5% or 1% are chosen, we use the 5% (0.05) level. Statistical power β is the likelihood of detecting a trend (or effect) where one is, in fact actually, present. β is often chosen to be 80% which is what we chose. Generally speaking, β is likely to be higher as the number of observations increase and/or as variability of the data decreases.
As in most statistical analyses, also in the field of time series analysis there are an infinite number of methods. In this study the task was to analyse constant annual changes, or trends, i.e. changes in annual means. We wanted to report useful numbers and the choice was made to report the number of years required to obtain β of (minimum) 80% based on the uncertainty of the data (residual standard error from linear regression and number of samples per year) on the current station and the slope (annual change). Hence we adopted the following programming logic (J. Carstensen *pers.com*.):

Power analysis script, step by step, for each station in the data:

- Initial check so a minimum of 3 years containing a minimum of 3 samples and depth must be 0-10 meters, except for two stations; B1 and BY31 that were excluded from the depth criteria.
- Calculate annual means
- Calculate the residual standard error, RSE, from one initial linear regression of year vs. values. RSE indicate how much of the fitted values deviate from the true values (in statistics we talk about the true distribution out in nature and the estimate we can get from sampling, i.e. there is a true mean of the distribution and the estimate that we normally refer to as the mean, same in regression studies, these are a model of the true relationship).
- Create random time-series from 5 to 50 years and estimate β for changes of 1%, 5%, 10%, 20% and 40% annual change (slopes 0.01, 0.05, ...). This is done by using the (mean) number of observations per

year, RSE + a stochastic normal distribution (noise, using function rnorm in R). R-code, 'linear model' shown in the textbox.
- For every level of change, run 1000 randomisations
- Fit a linear regression of y and years (ranging from 1 to 5 minimum or 1 to 50 years as maximum)
- Estimate how many of these regressions are significant, p-value at least 0.05
- The quota of number of significant regressions of the 1000 (n_sig_pvalues/1000) are significant multiplied by 100 will give the β%.
- For every time series 5 to 50 years save β. Stop if β=80%. There is no point in analysing longer time series if β=80% was obtained after, say 8 years.
- Report the number of years required to reach β=80%. If it takes longer than 50 years return 'no solution found'

In essence a large RSE and small number of samples per year results in a longer theoretical time period required to obtain β of 80%. It is also easier to detect a large annual change (slope) than a smaller.

The following **four quantities** have an intimate relationship:

1. sample size
2. effect size
3. significance level = P(Type I error) = probability of finding an effect that is not there
4. power = 1 - P(Type II error) = probability of finding an effect that is there

Given any three, we can determine the fourth.

Statistical relationship of chlorophyll and biovolume

A model-II regression was chosen to analyse the relationship between chlorophyll and biovolume, since the aim was to analyse two parameters that aren't controlled by the investigator, they are both subject to natural variation, i.e. "random". Legendre & Legendre (1998) describes the logic behind model-II regressions. In short the idea is to estimate a confidence interval for the slope and intercept and then fit the line. This method is described in the literature as being less prone to overestimate the slope.
Both chlorophyll and biovolume are log-normally distributed and were hence log-transformed prior to being regressed. The model II regression was run in R using the lmodel2 function (with 1000 permutations) from the lmodel2 package.

Similarity in species composition

Another aim of the study was to investigate how similar stations are to one another based on the occurrence of species. Subset of data with a large number of locations sampled during a short time was used to determine spatial variability. The temporal variability was investigated by using data from stations sampled with a high frequency in year 2011. First the data was aggregated from the level of size classes within a taxon to the species level. Then a Euclidian distance matrix was calculated. This distance matrix was then visualized as a tree diagram, showing the relationships. We also used cluster analysis to identify clusters in the tree (distance matrix). In essence the Euclidean distance matrix was calculated then a (ward.d) clustering method was used to identify clusters. The distance matrix was visualised in tree plots.

Results and discussion

Tube sampling or sampling at discrete depths

Phytoplankton often occurs in thin layers in the sea. This is a potential problem for sampling because it would make a large difference if a high biomass thin layer is sampled or missed. To minimize the problem tube or hose sampling is often used. In this way a sample from e.g. 0-10 m depth is collected and mixed before sub samples are collected for analysis. This was compared with data based on samples collected at discrete depths, e.g. 0, 5 and 10 m. The data from discrete depths was averaged mathematically, with a trapezoid method, to the same depth interval as the corresponding tube samples collected at the same time.

To investigate whether there is a statistical difference between chlorophyll sampled with hose versus bottle sampling at discrete depths data from sampling events where both methods was used. The results are presented in Fig. 4 and the statistical test in a text box below. There is no difference. Two-three outliers are evident, but generally the chlorophyll data follows the expected log-normally distributed pattern. This means it is safe to continue with statistical analysis and to draw conclusions from it.

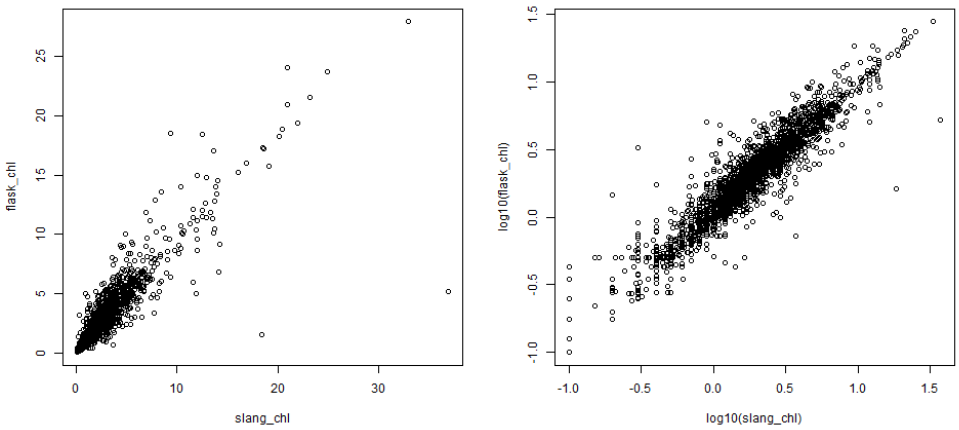


Fig. 4. The $\log_{10}$ relationship between chlorophyll *a* data collected using tube samples (x-axis “slang”) and chlorophyll *a* data from discrete depths (y-axis, “flask”). The data from discrete depths was averaged to represent the same depth interval as the tube data.

Statistical test for comparing chlorophyll *a* data from tube samples with data from discrete depths

Results from t-test to investigate the difference of integrated bottle measurements and hose chlorophyll.

Welch Two Sample **t-test**
data: log10(slang_chl) and log10(flask_chl)
t = -3.9716, df = 4270.975, **p-value = 7.257e-05**
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
-0.06372060 -0.02160217
sample estimates:
mean of x mean of y
0.2390058 0.2816672

Probability (power) of detecting trends

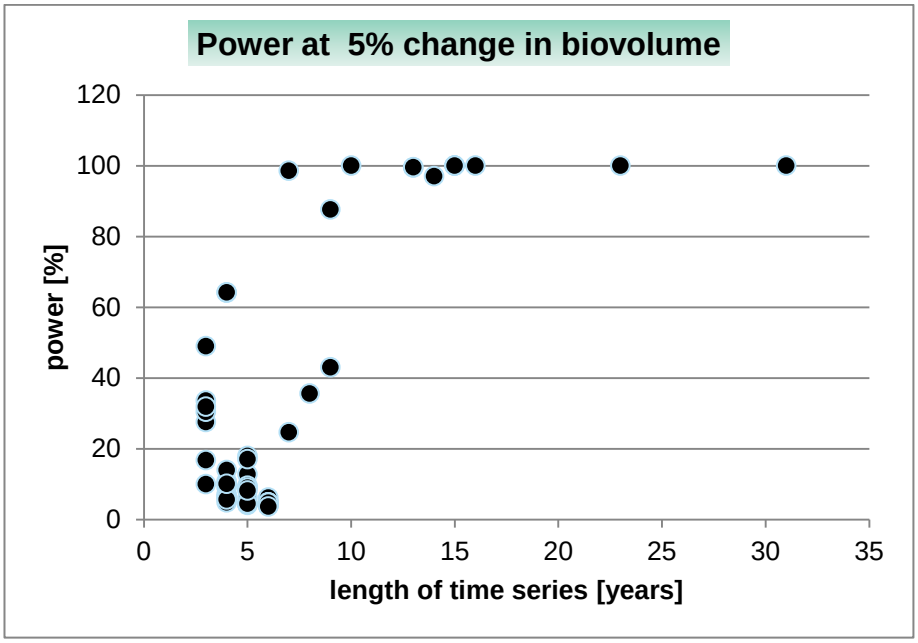
A problem for marine monitoring is to sample at a frequency high enough to detect environmental change. High short term variability will make it difficult to identify long term trends. This is a potential problem for detecting trends based on phytoplankton biomass since the phytoplankton respond quickly to environmental change, both short term changes and long term changes.

Chlorophyll

40 stations where analysed. On average, to detect an annual change with power of 80% and p-value 0.05, it takes for: 1% 33 years, 5% 19 years, 10% 14 years, 20% 10 years and 40% 7 years. The minimum modelled time series where 5 years.

With increasing length of time series, generally the power to detect a trend is increasing. If the trend is stronger (in this case for example 20%) fewer years are required to detect the trend. To illustrate the effect of length of time series on power the 5% effect size are used as an example, where length of time series (“x”) is plotted against power (“y”). The results show that about 15 years of measurements are required to detect a 5% change in chlorophyll (Fig. 5B).

A



B

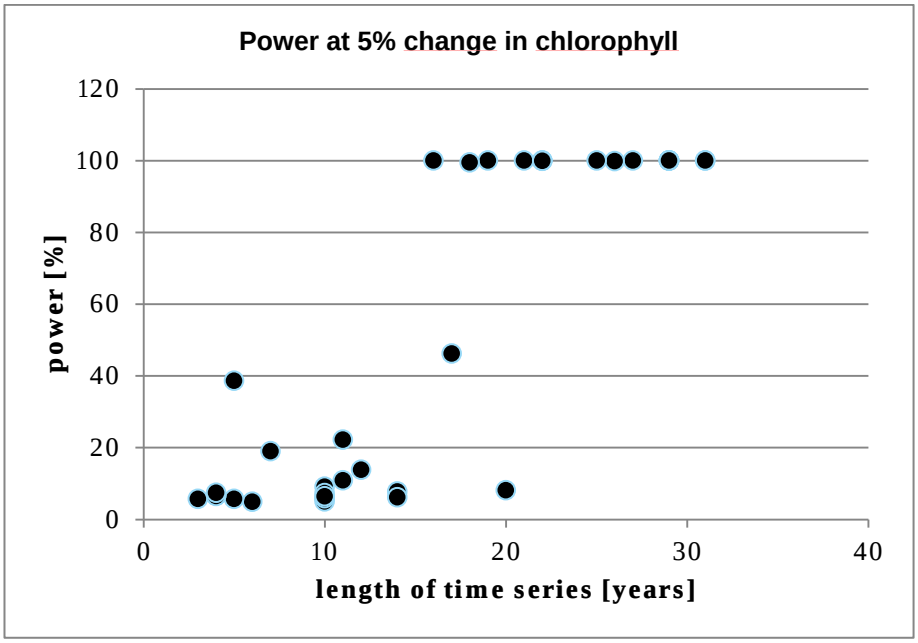


Fig. 5. With increasing length of time series, generally the power to detect a trend is increasing. If the trend is stronger fewer years are required to detect the trend. To illustrate the effect of length of time series on power the 5% effect size are used as an example, where the length of the time series is plotted vs. power. The results show that about 13 years of measurements are required to detect a 5% change in biovolume (plate A). The same plot for chlorophyll (plate B) shows that approximately 15 years is required for a 5% change. The 'outlier' at low power after 20 years is due to very high variability at that station.

For the examples shown below at Släggö there were 14 years of data. Based on the number of observations per year, 25, and the residual standard error of 0.85, it takes 31 years to detect an annual change in in chlorophyll of 1%, with a power of 80%. The existing time series duration of 14 years has a power of 12% to detect the 1% change and if Släggö is sampled for 6 years, power is 7% to detect the 1% annual change. This means power is very low in all cases, indicating the more data is necessary from this station. All results are show in appendix.

Table 1. Example of results from the station Släggö. n yrs = length of time series. n obs/yr = mean number of observations, mean = mean biovolume, st.ddev. = standard deviation, res.st.dev. = residual std of initial regression. % change = % annual change, yrs to 80% B = number of years required to obtain a power 80% for the given d. B initial = what is the power initially to detect the given d. B after 6 yrs = power after six years. The analysis is restricted to a maximum number of years of 50 and minimum of 5.

Släggö					
yrs	**n yrs**	**n obs / yr**	**mean**	**st.dev.**	**res.st.dev.**
2000-2013	14	25	1.36	0.85	0.88
		% change	**yrs to 80% B**	**B initial**	**B after 6 yrs**
		1	31	12	7
		5	12	97	15
		10	8	100	45
		20	6	100	95
		40	5	100	100
L9 Laholmsbukten					
yrs	**n yrs**	**n obs / yr**	**mean**	**st.dev.**	**res.st.dev.**
2005-2013	14	11	1.21	0.35	0.35
		% change	**yrs to 80% B**	**B initial**	**B after 6 yrs**
		1	23	9	5
		5	9	88	34
		10	6	100	85
		20	5	100	100
		40	5	100	100

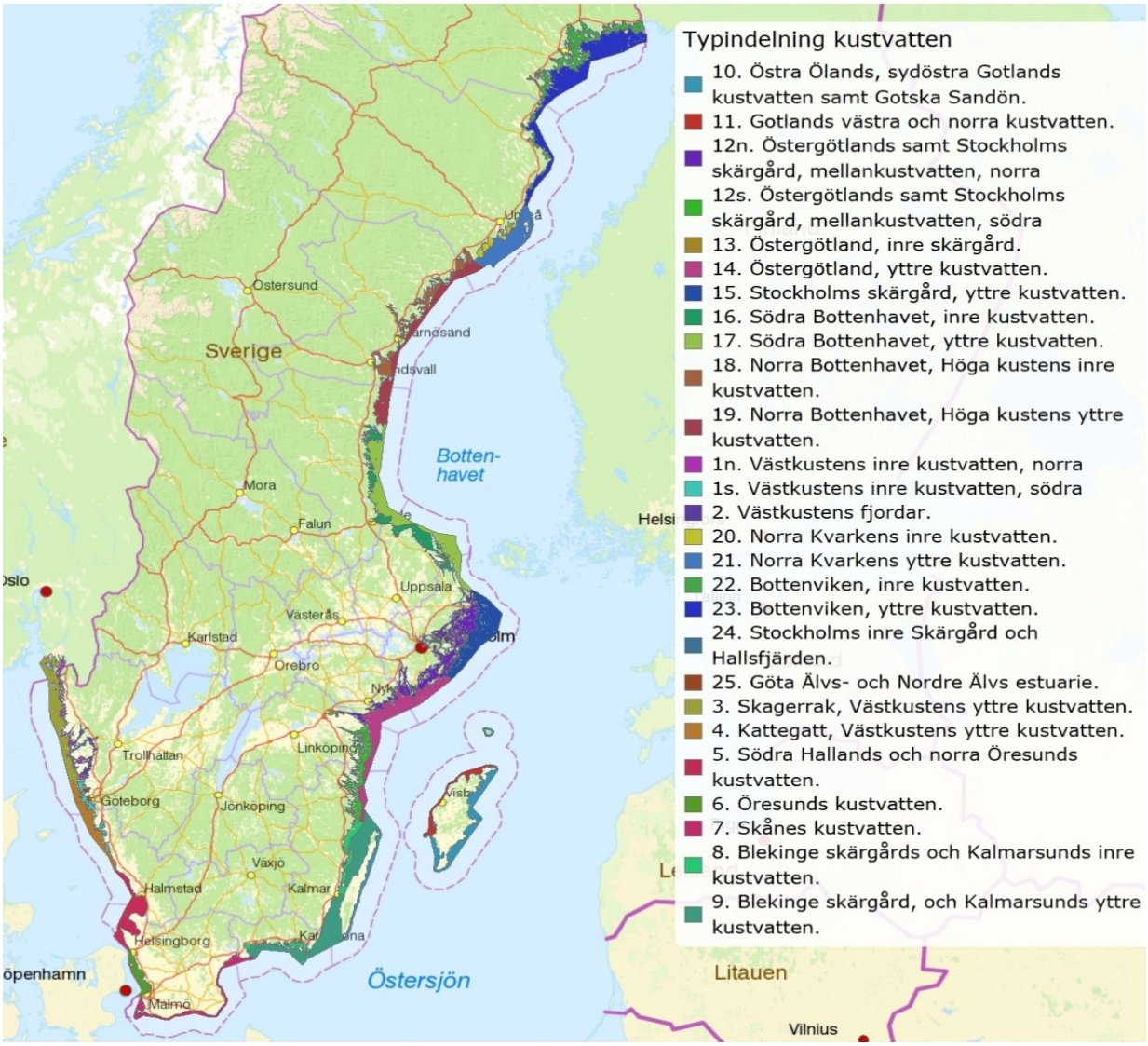
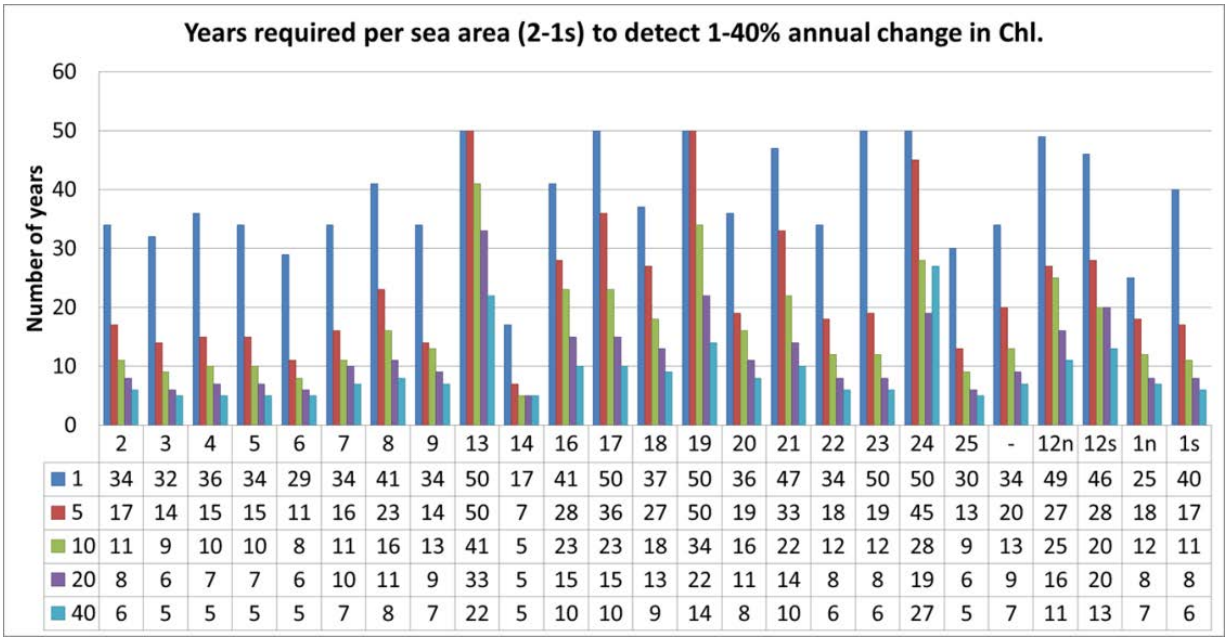


Fig. 6. The coastal sea areas of Sweden have been divided into different types. The map illustrates the types listed in the key to signs. The information on type areas is found in VISS - Vatteninformationssystem Sverige http://www.viss.lansstyrelsen.se/.



	2	3	4	5	6	7	8	9	13	14	16	17	18	19	20	21	22	23	24	25	-	12n	12s	1n	1s
1	34	32	36	34	29	34	41	34	50	17	41	50	37	50	36	47	34	50	50	30	34	49	46	25	40
5	17	14	15	15	11	16	23	14	50	7	28	36	27	50	19	33	18	19	45	13	20	27	28	18	17
10	11	9	10	10	8	11	16	13	41	5	23	23	18	34	16	22	12	12	28	9	13	25	20	12	11
20	8	6	7	7	6	10	11	9	33	5	15	15	13	22	11	14	8	8	19	6	9	16	20	8	8
40	6	5	5	5	5	7	8	7	22	5	10	10	9	14	8	10	6	6	27	5	7	11	13	7	6

Fig. 7. The possibility to detect trend in areas listed in Fig. 6, x-axis; area names y-axis; number of years required to detect the trend (required to obtain B=80%), colour of bars; %change. Stacked bars. Numbers indicate the number of years required to detect a change of 1%,5%, 10% and 40%. The analysis is restricted to a maximum number of years of 50 (so it may be more than 50 in those cases its exactly 50 years) and minimum of 5 (hence may be shorter than 5 years, then reported as 5).

For the **different areas**, as defined in figure 6 times to reach 80% power for changes in:

- Region 1-to 5 (**Skagerrak-Kattegat**): 1% 38 years, 5% 16 years, 10% 10 years, 20% 7 years, 40% 5 years.
- Region 6 – 9 (**the Sound – S.Baltic**): 1% 28 years, 5% 11 years, 10% 7 years, 20% 5 years, 40% 5 years.
- Region 10 – 15 (**Baltic proper**): 1% 15-no solution (i.e. + 50 years), 5% 7- no solution (i.e. + 50 years) years, 10% 16-38 years, 20% 5-25 years, 40% 5-16 years.
- 16 – 19 (**Bothnian sea**) : 1% no solution (i.e. + 50 years), 5% 19-41 years, 10% 13-26 years, 20% 9-17 years, 40% 6-14 years.
- 20 – 25 (**Bothnian bay**) : 1% no solution (i.e. + 50 years), 5% 13-35 years, 10% 9-22 years, 20% 6-15 years, 40% 5-10 years.

In general it seems here that a status change in chlorophyll is more easily detected in the Sound and south Baltic area.

Biovolume

56 stations where analysed. On average, to detect an annual change with power of 80% and p-value 0.05, it takes for: 1% 23 years, 5% 9 years, 10% 7 years, 20% 5 years and 40% also 5 years. The minimum modelled time series where 5 years. For 19 stations the power analysis algorithm could not be solved (i.e.

takes more than 50 years), this was due to the fact there were too few observations (only two/year on average).

With increasing length of time series, generally the power to detect a trend is increasing. If the trend is stronger (in this case for example 20%) fewer years are required to detect the trend. To illustrate the effect of length of time series on power the 5% effect size is used as an example, where length of time series is plotted against power. The results show that about 13 years of measurements are required to detect a 5% change in biovolume (figure 2b).

Relationship biovolume vs. chlorophyll

The results from the model II regression of chlorophyll, x, and biovolume, y, show that the relationship for this particular dataset is $\log_{10}(y) = 2.076\log_{10}(x) - 0.877$ with $r^2=0.4$, n=3119, p-value < 0.01.

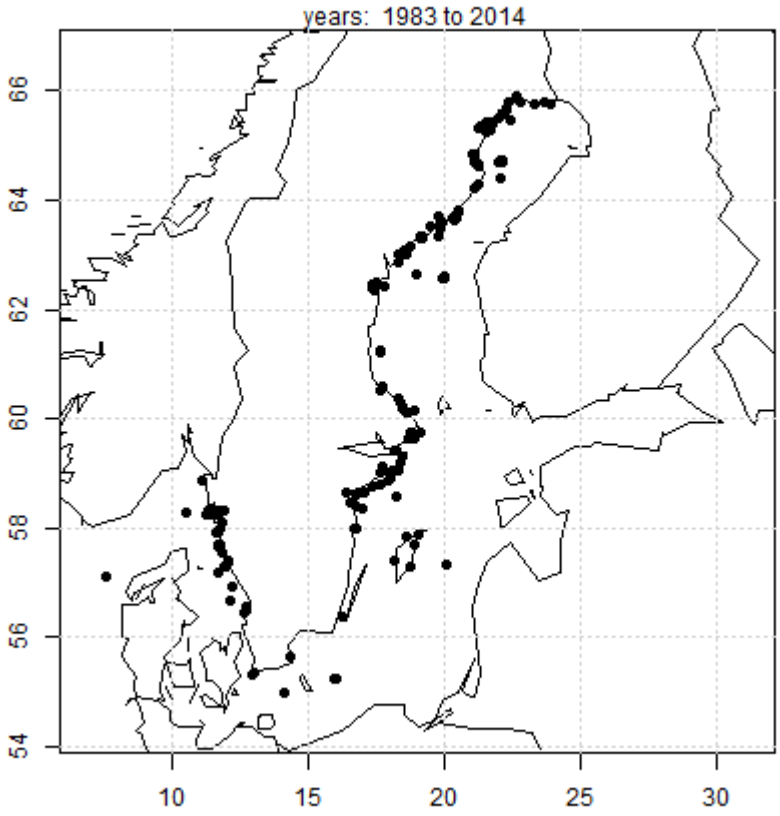


Fig. 8. The map illustrates sampling locations for the data used in Fig. 9. It is locations where phytoplankton was analysed using microscopy and cell volumes were measured resulting in data on total biovolume of phytoplankton. In the same samples chlorophyll *a* was analysed. The majority of the data emanates from national monitoring programmes and the phytoplankton sampling campaigns in years 2010-2012.

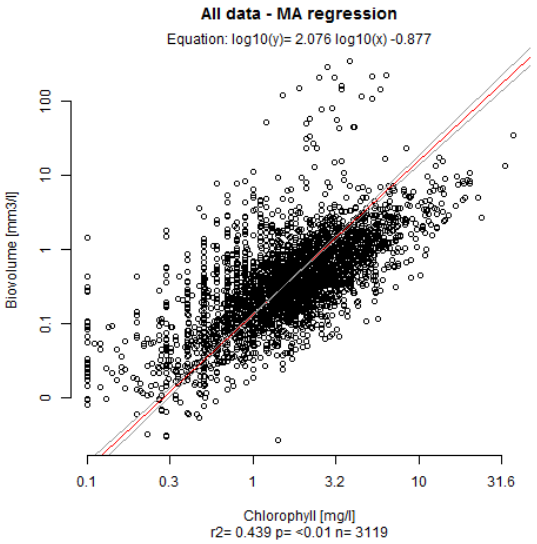


Fig. 9. Left frame: The relationship between total $\log_{10}$ biovolume of phytoplankton and $\log_{10}$ chlorophyll *a*. A model 2 regression was used.

Temporal variability in species composition

To investigate the temporal variability in phytoplankton, composition data from year 2011 from the high frequent sampling locations around Sweden was used. For the Northern Bothnian Sea the data from stations B3 and B7, which are located close to each other, was pooled.

Sea area	**Station name(s)**	**Number of samples collected in 2011**
Skagerrak	Släggö (mouth of the Gullmar Fjord)	21
Kattegat	Anholt E (open sea)	19
The Northern Baltic Proper	B1 (near Askö, southern archipelago of Stockholm)	24
The Bothnian Bay-Northern Quark	B3 and B7 (Öre estuary)	20

The results from cluster analysis based on biovolume data at the species level is shown in Figs. 10-12. A general conclusion after studying the cladograms is that there is substantial short term variability. If the short term variability was fully resolved it would be reasonable to find adjacent dates on the same branches in the cladograms. Here follows some examples when this was observed. In the Kattegat (Anholt E) two samples from March are on adjacent branches in the cladogram. Both samples are from the diatom spring bloom. Also during other times of year adjacent dates often cluster pairwise. The

sampling at Anholt E is most often carried out pairwise with just a few days between. This is due to logistics when two sampling events are made during the same cruise with a research vessel. In the Northern Baltic Proper four samples collected during the period 28 March to 18 April form a clade. This is likely to represent spring bloom sampling. Here the weekly sampling resolved the bloom well. Also samples four collected in July and August at B1 are found in the same clade indicating a relatively high similarity. In the Northern Quark (B3 and B7) samples collected from late April to early July cluster together. In general samples collected at adjacent dates seldom show a high similarity. This is interpreted as an indication that the natural temporal variability in phytoplankton composition is not fully resolved using the present sampling frequency.

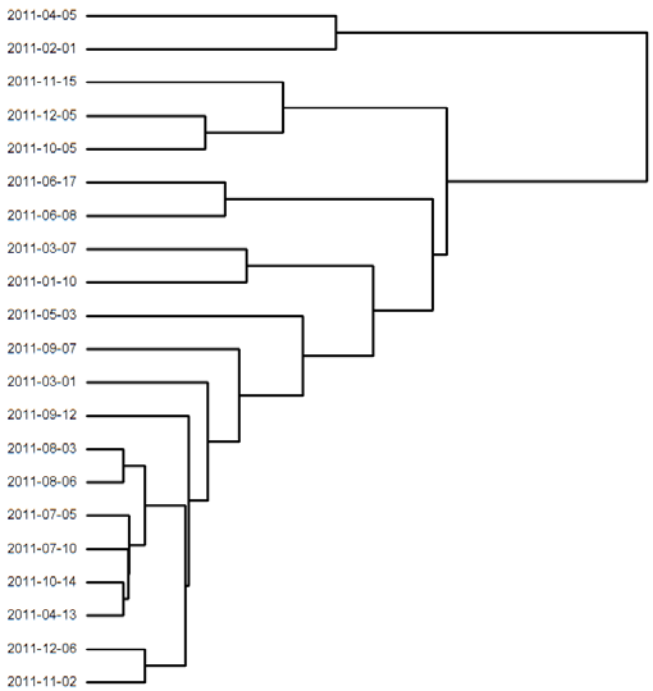


Fig. 10. Similarity in species composition at station Släggö in the Skagerrak in year 2011. The result from cluster analysis is based on biovolume data at the species level. The horizontal line length represents the relative Euclidian distance.

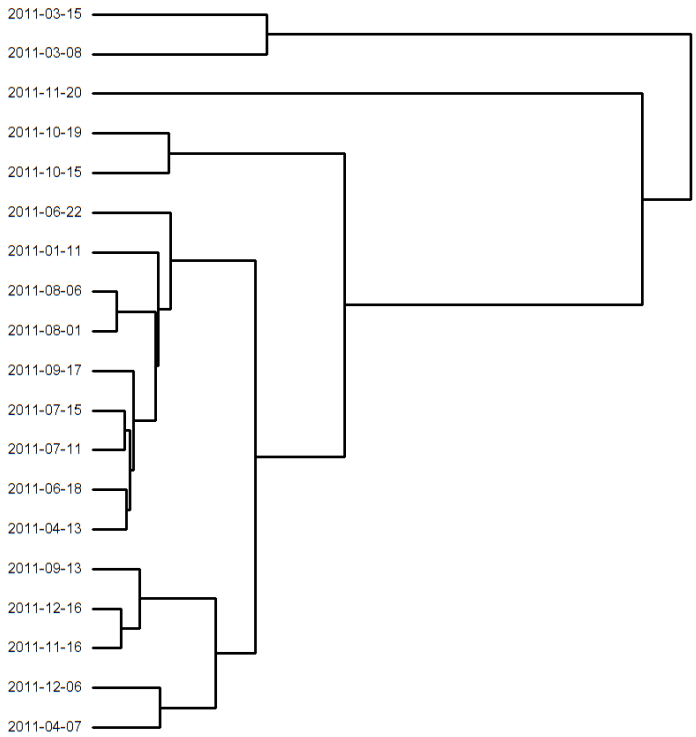


Fig. 11. Similarity in species composition at station Anholt E in the Kattegat in year 2011. The result from cluster analysis is based on biovolume data at the species level. The horizontal line length represents the relative Euclidian distance.

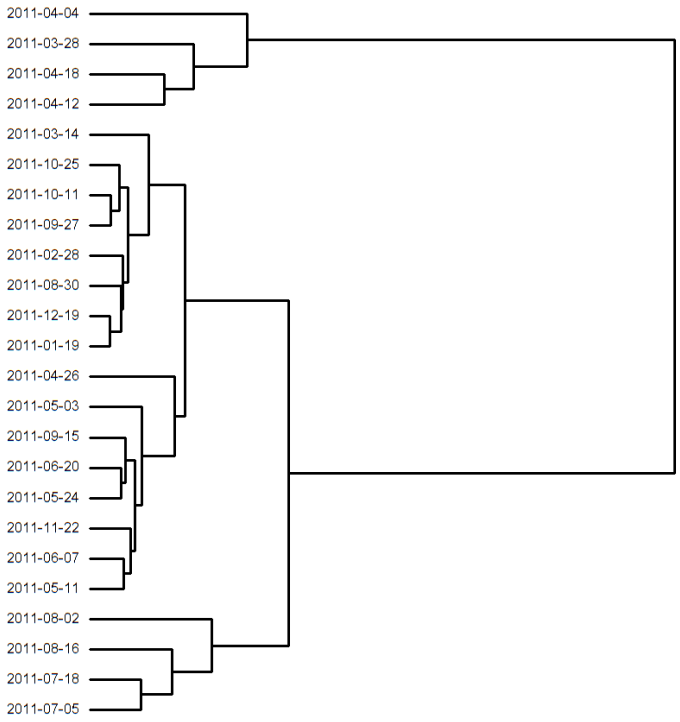


Fig. 12. Similarity in species composition at station B1 in the Northern Baltic Proper in year 2011. The result from cluster analysis is based on biovolume data at the species level. The horizontal line length represents the relative Euclidian distance.

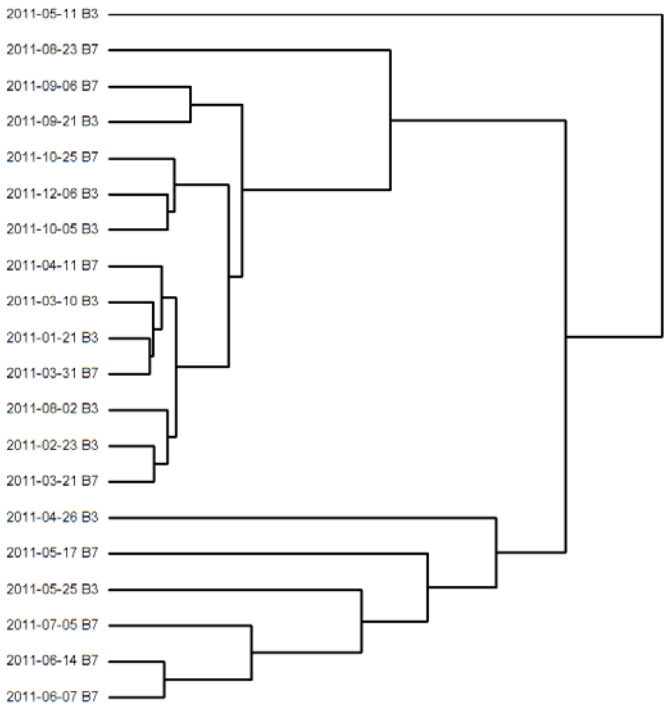


Fig. 13. Similarity in species composition at stations B3 and B7 in the Northern Quark in year 2011. The result from cluster analysis is based on biovolume data at the species level. The horizontal line length represents the relative Euclidian distance.

Spatial variability in species composition

To investigate spatial variability in species composition the data from enlarged sampling campaigns in 2010 – 2011 was used. It should be noted that the sampling campaigns had slightly different arrangements. In the Skagerrak-Kattegat the sampling was made monthly during a 12 month period. When putting the campaign data together with the data from regular sampling programmes this resulted in 19 different stations sampled one July 2011. Along the Svealand coast the sampling campaign was made in July and August 2010. For the period 15 July to 2 August 2010 this resulted in a data set of 21 sampling locations when combined with the data from station B1. In the Gulf of Bothnia the samples for the campaign were collected in July and August 2011. A dataset of 42 stations from August 2011 was used for the cluster analysis. In the Kattegat-Skagerrak the samples no. 9, 18 and 19 from the Skälderviken Bay, and the Laholms Bay form a separate cluster. Also samples from stations 7,8 and 15, representing Åstol, Danafjord and the Kosterfjord form a cluster. All of these are from the outer part of the archipelagos likely representing a some-what homogenous community in the Baltic current moving northward along the coast. Along the Svealand coast sample 15 from the inner part of the Archipelago of Stockholm is very different from the other samples. In the Gulf of Bothnia it is difficult to find geographical patterns in the similarities of stations based on species composition.

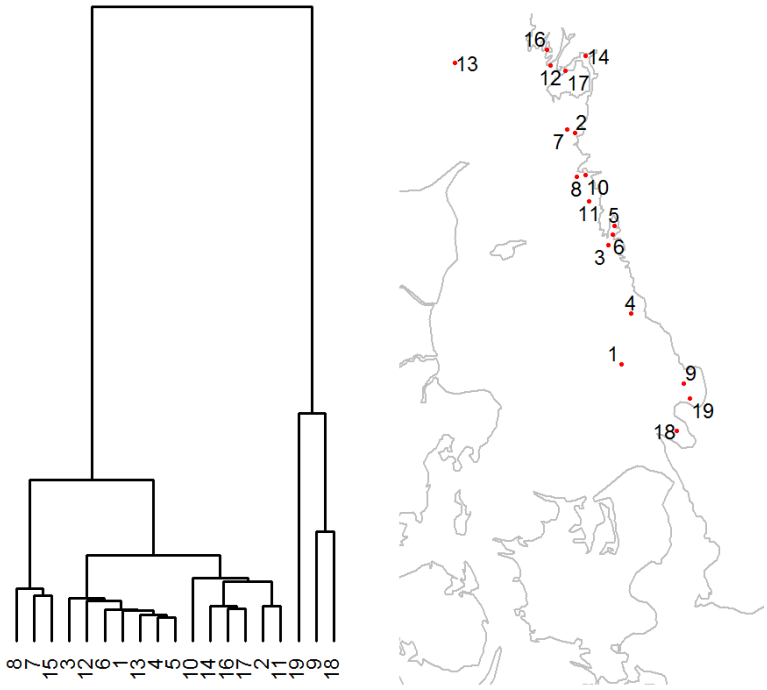


Fig. 14. Similarity in species composition in July 2011 in the Kattegat-Skagerrak. The result from cluster analysis is based on biovolume data at the species level. The horizontal line length represents the relative Euclidian distance. Station 15, the Kosterfjord, near the border between Sweden and Norway, is not included on the map.

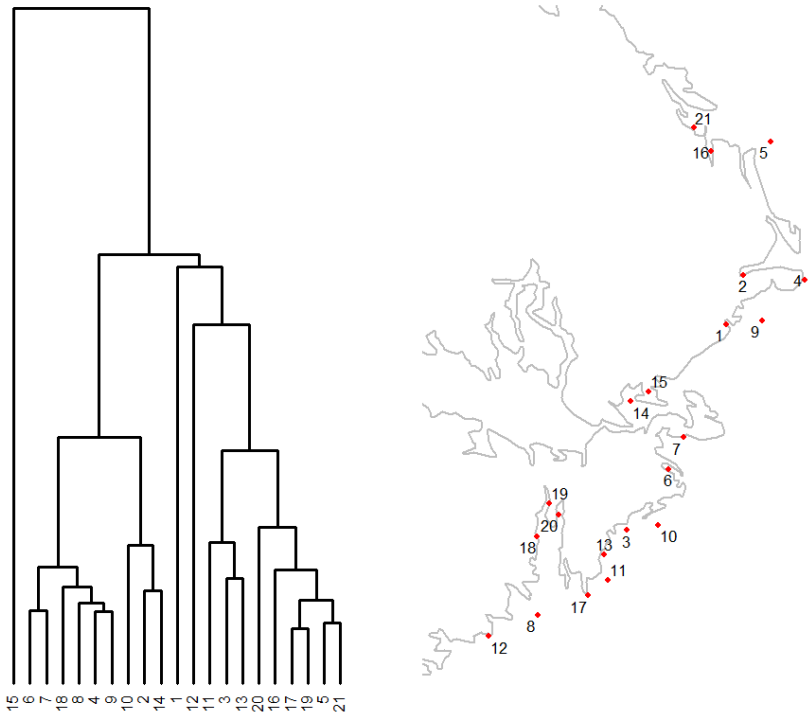


Fig. 15. Similarity in species composition in July 2011 along the Svealand coast. The result from cluster analysis is based on biovolume data at the species level. The horizontal line length represents the relative Euclidian distance.

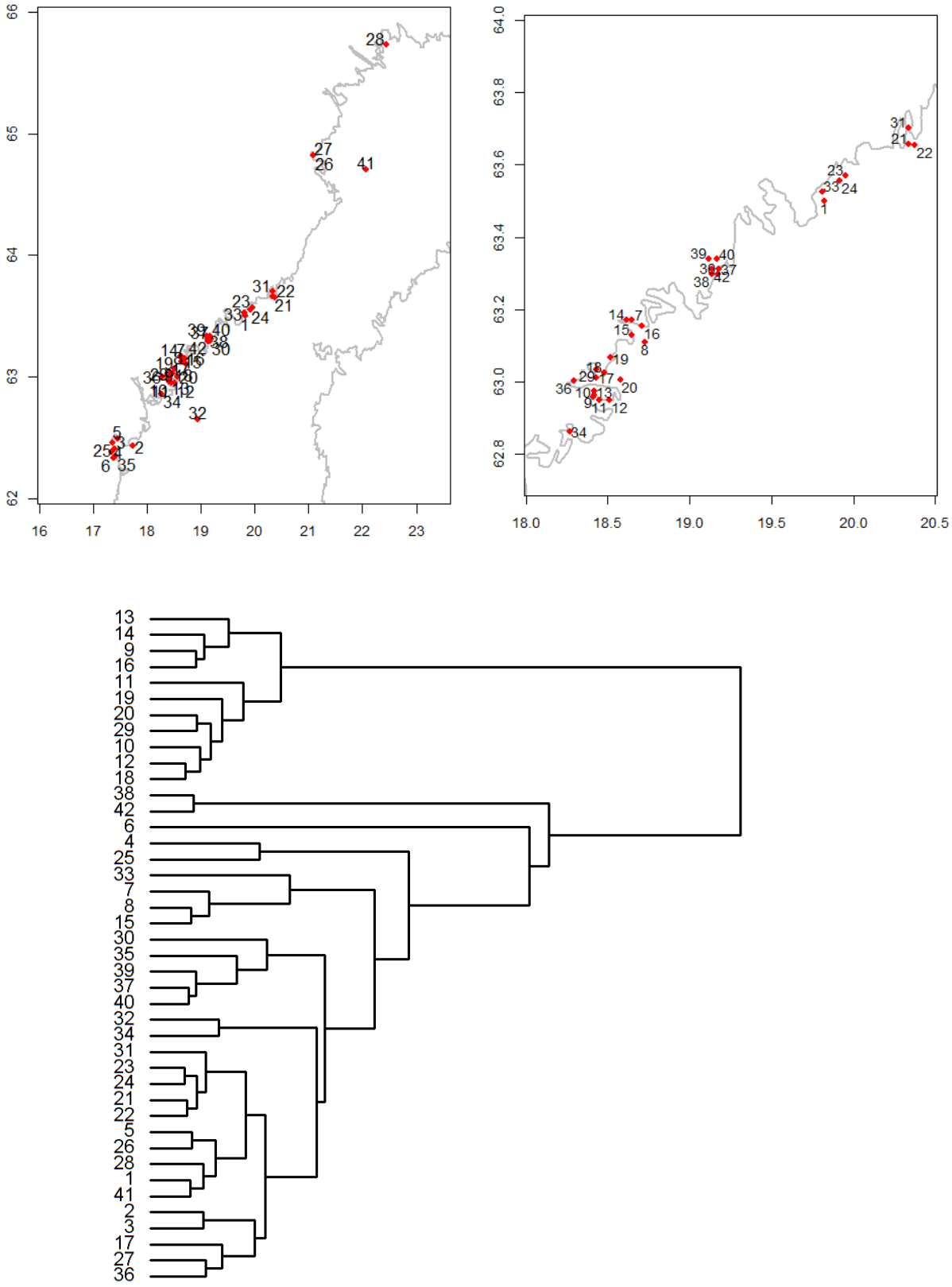


Fig. 15. Similarity in species composition in August 2011 in the Gulf of Bothnia. The result from cluster analysis is based on biovolume data at the species level. The horizontal line length represents the relative Euclidian distance.

Acknowledgements

The data from the Swedish National Marine Monitoring Programme was produced by SMHI, Oceanographic unit in Gothenburg, the University of Stockholm, Department of Ecology, Environment and Plant Sciences and the University of Umeå, Umeå Marine Sciences Centre. Also data from Bohusläns Vattenvårdsförbund and Hallands kustkontrollprogram was used. The study would not have been possible without access to the data and the data providers are thus acknowledged. Jacob Carstensen, Aarhus University, gave advice regarding the statistical analysis (power analysis) but is not responsible for the analysis or conclusions made.

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