

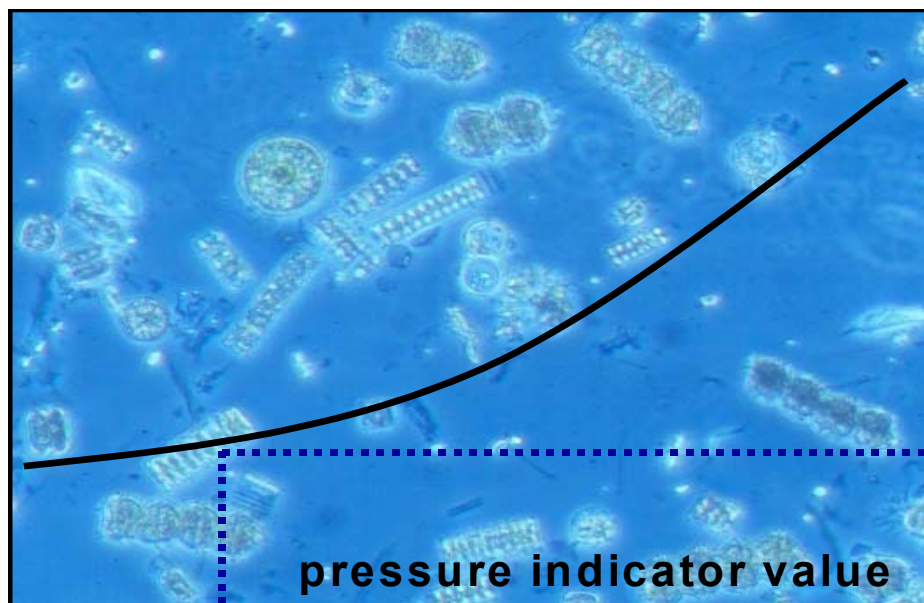


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Developing reference conditions for phytoplankton in the Baltic coastal waters



Part II: Examples of reference conditions developed for the Baltic Sea

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List of Contents

<i>Executive Summary</i>	1
1. Introduction	3
2. Reference conditions for biomass	5
2.1 <i>Historical values of nutrients and phytoplankton biomass</i>	5
2.2 <i>Empirical chlorophyll-secchi depth relationships</i>	7
2.3 <i>Application of transparency for reconstruction of historical phytoplankton conditions</i>	9
2.4 <i>Empirical chlorophyll-nutrient level relationships (annual means)</i>	11
2.5 <i>Empirical chlorophyll-nutrient level relationships (spring bloom)</i>	13
2.6 <i>Paleoecological methods</i>	18
2.7 <i>A simulation study of phytoplankton in the Baltic Sea one century ago</i>	20
3. Reference conditions for phytoplankton composition	22
3.1 <i>Spring bloom composition related to nutrient levels</i>	22
4. Reference conditions for bloom frequency	28
4.1 <i>Empirical bloom frequency-nutrient loading relationships</i>	28
5. Reference conditions for species diversity	30
6. References	33
Acknowledgement	35

Executive Summary

For the implementation of the Water Framework Directive reference conditions have to be established for the various quality element. In this report we outline a number of approaches that can be pursued for establishing reference conditions for phytoplankton at a regional level and discuss the problems associated with their application. This report does not exhaust the topic of establishing reference conditions in the different coastal types of the Baltic Sea, while it should be digested as a source for inspiration when analyses are carried out at a regional level.

Phytoplankton has been quantitatively analysed in the Baltic Sea since the 1970s with increasing frequency of sampling over the last decade. Prior to this only few qualitative studies have been carried out, providing insufficient material to estimate conditions from before the intensification of eutrophication in the Baltic Sea to be considered as reference conditions.

In the Gulf of Bothnia, the outer coastal areas have not been substantially impacted by anthropogenic nutrients inputs. Thus the distribution of phytoplankton data from these sites may therefore represent reference conditions. However, adopting this approach for all Finnish coastal waters, summer chlorophyll a reference conditions vary between 1.2 to 3.4 $\mu\text{g l}^{-1}$. Paleoecological studies from the Laajalahti bay (which is a shallow bay in the south coast of Finland) suggest a reference value for annual summer chlorophyll a of 10 $\mu\text{g l}^{-1}$. However, this value may not be representative for other shallow bays in southern Finland. In the Kattegat our investigations suggest reference values of approximately 2.1 $\mu\text{g l}^{-1}$ for the mesohaline coastal waters, although this is only a rough estimate that needs to be supported by other studies.

Using relationships between secchi depths and phytoplankton biomass is a feasible approach to establish reference conditions, given that there are sufficiently long-time series of historical secchi depth measurements and that other light attenuating substances would have had trends comparable to that of phytoplankton. In Finnish coastal waters this approach suggests reference conditions from 1.6 to 2.0 $\mu\text{g l}^{-1}$, i.e. a narrower span compared to the values established from reference sites. Consistent relationships between chlorophyll and secchi depths are found basin-wide in the Baltic Sea, and this approach is an option for establishing reference conditions provided that the underlying assumptions are carefully examined and their implications evaluated.

Relationships between nutrient levels and phytoplankton biomass have been established for annual means, whereas it is difficult to link the spring bloom biomass to the winter nutrient level due to the strong spatial and temporal variability of the spring bloom intensity and the inadequate amount of data to verify such variability in most monitoring programs. It should be stressed that these relationships are associated with a degree of uncertainty, and that the predicted reference conditions from these relations have a similar degree of uncertainty.

The spring blooms in the Baltic Sea are mainly composed of diatoms. Several diatom species tend to increasingly dominate the total biomass with increasing nutrient levels, particularly with nitrogen. Species frequently observed in the southern Baltic Sea, the Gulf of Riga and Gulf of Finland, such as *Skeletonema sp.*, *Thalassiosira sp.* and *Chaetoceros sp.* all showed increased dominance of the spring biomass with higher DIN levels (winter nutrient concentrations). Therefore, we propose that the reference conditions for these spring bloom indicator species could be estimated using reference conditions for nitrogen. Again, it should be acknowledged that such reference conditions are inherently uncertain due to the considerable variation in data.

The frequency of summer phytoplankton blooms was linked to the external nitrogen input to the coastal Kattegat. Using this relationship, with an adjustment for spatial variation, we suggest that in the reference conditions 3 to 5% of all summer observations would be blooms. This is, of course, a rough estimate and more detailed analyses on a regional level are needed to confirm these values.

Phytoplankton species abundance is a difficult indicator to assess from monitoring data, as the number of species recognised in a sample highly depends on the taxonomical skills of the person analysing the sample. Moreover, the taxonomy of phytoplankton is constantly developing and the awareness of new types of species is increasing. These factors will impact the use and reliability of species abundance and diversity in the classification of coastal waters. At least, robust and unbiased indicators of the structural changes of phytoplankton communities need to be developed before phytoplankton species composition can be applied for classification of coastal waters of the Baltic Sea.

1. Introduction

The classification of water bodies in the implementation of the Water Framework Directive (WFD) relies on the establishment of reference conditions for various qualitative elements. Four approaches have been outlined in the WFD for estimating such reference conditions: (i) identification of non-impacted sites with similar typology, (ii) predictive modelling including mechanistic modelling and hind-casting methods, (iii) historical data or paleo-reconstruction methods, and (iv) expert judgement. This report describes the status for estimating reference conditions for phytoplankton in the EU-project CHARM².

In a previous report (Heiskanen et al., in press /a) it was concluded that the entire Baltic Sea has experienced changes in nutrient levels making it virtually impossible to find non-impacted sites representative of reference conditions (option i). However, Samuelsson et al. (2004) have considered stations in the Swedish part of the Gulf of Bothnia to be relatively non-impacted and therefore used recent data for deriving reference conditions. These values were only applicable for the Gulf of Bothnia and could not be transferred to other Swedish coastal sites. Quantitative methods for monitoring phytoplankton in the Baltic Sea were first introduced in the 1970s and the earlier qualitative studies are difficult to encompass in a WFD monitoring framework. Paleoecological studies have been employed as part of the EU-project MOLTEN³ to estimate past levels for phytoplankton and nutrients. However, the sediments sampled at several sites were not suitable for this technique. These considerations have rendered modelling approaches and expert judgements as the most generally applicable methods for deriving reference conditions.

Phytoplankton is a key component of most coastal ecosystems, while phytoplankton modelling is not an easy task since it involves many other different components that contribute to the production and loss of biomass (Figure 1). Moreover, the phytoplankton community is comprised of many different species, for convenience often merged into functional groups that have different strategies in a complex mosaic of

² Characterisation of the Baltic Sea Ecosystem: Dynamics and Function of Coastal Types (2001-2004); <http://charm.dmu.dk>

³ Monitoring long-term trends in eutrophication and nutrients in the coastal zone: Creation of guidelines for the evaluation of background conditions, anthropogenic influence and recovery (2001-2004) <http://craticula.ncl.ac.uk:8000/Molten/jsp/index.jsp>

mechanisms, some of these, perhaps, still unknown. To complicate matters even further phytoplankton biomass and composition are also governed by the physical transport mechanisms that can be extremely dynamic in the coastal area. The variation in phytoplankton biomass and composition is therefore inherently enormous, and the conduct of the measurements further adds to this variation in present monitoring data.

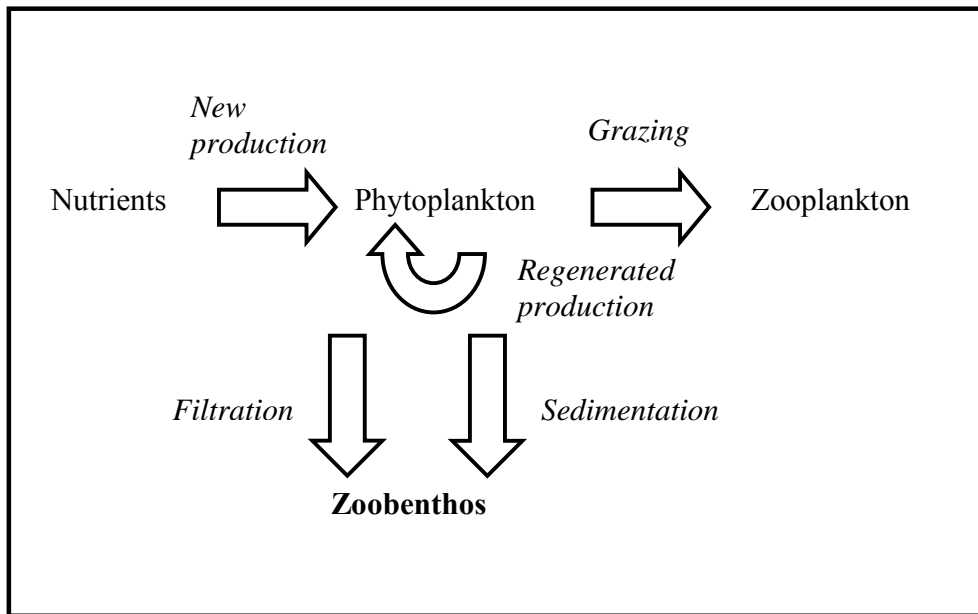


Figure 1: Conceptual model showing the production and loss processes (in italics). Only the major pathways of phytoplankton are shown and the physical processes are not included.

A comprehensive phytoplankton database has been compiled within the framework of the CHARM project covering a large part of coastal waters of the Baltic Sea. The database contains bio-volumes at species level with additional taxonomical, morphological, functional and size group distribution for the different species recorded. In addition, hydrophysical and –chemical measurements from the same samples have been collected from the contributors and combined with the phytoplankton data. The CHARM phytoplankton database includes data from 1970 to 2001, however, with the largest amount of data sampled within the last two decades. Data were contributed from all countries around the Baltic Sea except for Sweden and Russia.

2. Reference conditions for biomass

The earliest quantitative monitoring data on phytoplankton biomass (microscopic identification and enumeration, and chlorophyll) date back to around 1970, but the frequency and spatial extent of these data are rather limited. Due to the large variation in phytoplankton biomass and that eutrophication of the Baltic Sea started even earlier, these data cannot be used for determining reference conditions. Instead variations in the phytoplankton biomass may be related to other supporting elements for which reference conditions may exist from other studies. Here we shall investigate such empirical relations from the CHARM database in addition to the paleoecological results obtained through MOLTEN for Laajalahti estuary.

2.1 Historical values of nutrients and phytoplankton biomass

Historical time series data have been used to establish reference conditions for nutrients and phytoplankton biomass (chlorophyll a) in Swedish coastal waters (Sahlsten and Hansson 2004, Samuelsson et al. 2004). Using a similar approach, long-term monitoring data were evaluated in Finnish coastal waters to estimate its applicability and to compare the results between the common types of Sweden and Finland.

The data on total N, total P and chlorophyll a originated from the national monitoring of the Finnish Environment Administration. The monitoring comprised altogether 130 sampling stations and 2540 samples covering at longest the time period of 1962-2004. In most of the stations the monitoring was started in the 1970s. Heavily loaded innermost coastal areas were not included in this data set. The time series data were considered to assess average concentrations levels and trends in each coastal type. Frequency distributions were used by assuming that 10% deviation might be acceptable for reference conditions (see Andersen et al. 2004, Sahlsten and Hansson 2004).

Trends of nutrients and chlorophyll a varied along Finnish coastal waters; partly this was due to changes in loading and partly due to natural variation. The trends were usually less clear in the Gulf of Bothnia compared with the other sea areas (Pitkänen et al. 2001, Kauppila et al. 2004). In the open and coastal Gulf of Finland as well as in the Archipelago Sea, inorganic N increased in the 1970s and 1980s. In many areas, especially

in the Gulf of Bothnia, changes in concentrations could be linked to strong variations in river water flows. For example variation of summertime nutrients and chlorophyll a in the coastal area off Haukipudas were clearly affected by water flows from the River Kiiminkijoki, a forested basin in the NE Bothnian Bay receiving small amounts of municipal loading compared to many other river basins. Lack of major changes in the long-term trends implies that some outer coastal areas in the Gulf of Bothnia might be close to their natural background conditions.

The "background" concentrations of wintertime TN and TP, estimated from the frequency distribution data, ranged from 237 $\mu\text{g TN l}^{-1}$ and 12 $\mu\text{g TP l}^{-1}$ in the Bothnian Sea outer coastal type to 410 $\mu\text{g TN l}^{-1}$ and 27 $\mu\text{g TP l}^{-1}$ in the Gulf of Finland inner coastal type (Table 1). Summertime chlorophyll a was smallest (1.2 $\mu\text{g l}^{-1}$) in the Bothnian Bay outer coastal type and largest (3.4 $\mu\text{g l}^{-1}$) in the Gulf of Finland inner coastal type.

The concentrations of TN and TP were higher in the Finnish side of the Gulf of Bothnia compared to values in the Swedish side of the coast. This may partly be due to the fact that only open water stations in the Swedish side of the Gulf of Bothnia were used to estimate reference conditions for the outer coastal types. Sampling periods in Sweden and Finland also differed to some extent from each other (Table 1). Additionally, methodological differences in chemical analyses between and within the countries during the whole monitoring period must be taken into account. Contrary to nutrients, however, summertime chlorophyll a concentrations in the Finnish outer coastal types of the Gulf of Bothnia and in the intercalibration types of middle Archipelago / Åland Sea / Stockholm Archipelago were quite similar to the values given in the Swedish coast (Table 1).

Comparing the "background" values of this study with the trends of nutrients and chlorophyll a showed that taking 10% deviation from the frequency distribution data may give values close to conditions in less-rainy years rather than a reliable estimate for reference conditions. Natural variation may in many cases be greater than this approach can give. Furthermore, percentage of deviation from the lowest values may not be same for different sea areas. The background values for the Finnish coastal waters presented in this report should be taken as tentative. Detailed analyses based on more specific information e.g. on the locations of sampling sites in each coastal types are required

(digital maps on Finnish coastal types are still under work) Furthermore, national consensus in the results are also needed.

Table 1: Reference concentrations of TN, TP ($\mu\text{g l}^{-1}$) and phytoplankton biomass as chlorophyll *a* ($\mu\text{g l}^{-1}$) in the Finnish coastal types (see Figure 3 below). The values are 10% deviations from the lowest concentrations, calculated from the frequency distribution data in the 1962/1970-2000s. The values in brackets are 10% deviation concentrations of the Swedish data originating mainly from 1980-2003; individual samplings measured in 1950-1970 (Sahlsten and Hansson 2004, Samuelsson et al. 2004). Note that the latitudes of the Finnish and Swedish coastal types in different sea areas are not accurately the same, especially in the Bothnian Bay and Quark. The borderlines of Finnish coastal types are still tentative and will be changed.

Coastal types	Code FIN	Code SWE	Winter Jan.-Mar (Nov-Feb)		Summer Jul-Sep (Jul-Aug)		Chl <i>a</i>
			TN	TP	TN	TP	
Bothnian Bay							
- inner type	J	22	349 (168)	12 (5.9)	240 (99)	8 (2.5)	1.8 (2)
- outer type	K	23	285 (238)	6.7 (3.7)	240 (210)	6 (3.7)	1.2 (1.1)
Quark							
- inner type	H	20	350 (238)	8.5 (8.4)	258 (196)	11 (5.6)	1.6 (1.3)
- outer type	I	21	288 (238)	7 (4.0)	223 (210)	7 (4.3)	1.4 (.)
Bothnian Sea							
- inner type	F	16,18	290 (154-210)	14 (1.9-9.9)	230 (154)	12 (5-9)	1.5 (2)
- outer type	G	17,19	237 (196)	12 (7.7)	192 (154-168)	8.5 (5.9-7.1)	1.3 (1.4)
WE archipelago							
- inner type	C		340	20	295	15	3.0
- middle type	D	13	285	18	270	12.5	1.9 (2)
Gulf of Finland							
- inner type	A		410	19	308	19.3	3.4
- outer type	B		398	27	295	15	3.0

2.2 Empirical chlorophyll-secchi depth relationships

Secchi depth is one of the earliest standard monitoring measurements carried out within the Baltic Sea. It has been widely used on research cruises since the beginning of the 1900s, however, due to the limited number of cruises carried out in the beginning of the

century the data from this period is still rather limited. Sanden and Håkansson (1996) have described the trends in secchi depths since the 1930s for the open part of the Baltic Sea. Records from shallow coastal areas should be analysed with caution if water depths are in the range or lower than the secchi depths.

Light is attenuated by water and phytoplankton biomass as well as by many other substances, both dissolved and particulate. The relationship between secchi depths and chlorophyll is often modelled by means of a simple empirical relation

$$\frac{1}{SD} = kw + kchl \cdot Chl$$

where kw is a site-specific attenuation coefficient for that particular type of water and $kchl$ is the attenuation coefficient for chlorophyll. First, it should be stressed that kw is site-specific and that estimated values of kw may not implicitly be applied to other water bodies. Second, estimating such relationships for a specific site using recent data and extrapolation this relationship to historical data for secchi depths implicitly takes the assumption that all other light attenuating substances have not changed over the same period of time or at least that they have changed correspondingly to the trends of chlorophyll. In coastal areas the attenuation of light from suspended solids can be quite substantial. For most coastal ecosystems it is reasonable to assume that the resuspension of material from the sediments due to physical forcing does not reflect any time trend, but that the particulate organic loading has increased similarly to chlorophyll. It is obvious that variations in the other light attenuating coefficients will cause scatter in the chlorophyll to secchi depths plots.

Investigating 58 stations from the CHARM database with a reasonable amount of data, we found that 47 stations had a significant relationship between the log-transforms of chlorophyll and secchi depths ranging from -0.59 to -2.71 for the slope with a tendency for coastal and estuarine stations to have a stronger decrease in chlorophyll with increasing secchi depths. The empirical relations found in Sanden and Håkansson (1996) and for German data in Heiskanen et al. (in press) had slopes within the range given above. The analysis from the CHARM database further shows that there is a strong seasonal pattern in the relationship suggesting that secchi depth data should be normalised for differences in solar radiation, both in terms of seasonal variation and the

time of the day for conducting the secchi measurement. However, there is insufficient data in the CHARM database to carry out this normalisation.

Reference conditions for chlorophyll can be established by means of reference conditions for secchi depths and the empirical chlorophyll-secchi depth relationship, provided that the trends of other light attenuating substances can be assumed to have similar trends to chlorophyll.

2.3 Application of transparency for reconstruction of historical phytoplankton conditions

In the northern Baltic Sea, observations of secchi depth were started by the Finnish Institute of Marine Research (FIMR) during the cruises of "NAUTILUS" in the summer of 1914 (Granqvist (1921)). After the interruption of the cruises by the First and Second World Wars, they continued again in the late 1960s. Observation stations are mainly located in the open sea, and partly in outer coastal waters of the northern Baltic Sea. Comparison between the old and present monitoring values are weakened by methodological differences: secchi depth before the second world were observed by the disc of the diameter of 0.6 m, whereas the diameters of the disc are today 0.2-0.3 m. Correction factor has been given by Launiainen et al. (1989).

In the northern Baltic Sea, secchi depth in the early 1990s was on average 9.5 m, deviating from the mean by ca. 2.5 m (Launiainen et al. 1989). The values in these outer and open sea areas were on average 2.5 to 3 m smaller than at present. Old values of secchi depths were available at 16 stations in the outer coastal waters of Finland in 1914 and 1921. The smallest values (2.9 m) were recorded in the Gulf of Finland and the greatest (10-11 m) in the outer Archipelago Sea and the Bothnian Bay.

Linear regression analyses were used to consider relationships between secchi depth and phytoplankton biomass (as chlorophyll a and biovolume) in summer conditions (July to September). The coastal monitoring data on secchi depth and chlorophyll a originated from 130 national coastal stations stored in the database of the Finnish Environment Administration (see chapter 3). The mean annual secchi depth in this summertime data set was 3.4 m, ranging from 0.3 m to 9.8 m. The differences between the main coastal areas were small. The CHARM database includes information on both

secchi depth and phytoplankton biovolume at 60 Finnish coastal stations. However, in this data set the range of secchi depth was too small (maximum 7.1 m) for reliable reconstructions of phytoplankton biovolume by the highest secchi depth values of the historical data.

In the Finnish coastal waters, the relationship between secchi depth and summertime chlorophyll a was weak compared to the study by Sanden and Håkansson (1996) in the southern Baltic Sea. This was due to considerable influence of river waters, which are strongly colored by humic and clay substances. Chlorophyll a explained 35% of the variation of secchi depth in the Gulf of Finland and the Bothnian Sea, whereas in the Bothnian Bay and Archipelago Sea the coefficients of determination were greater ($R^2 = 0.40$). Combining chlorophyll a with $PO_4\text{-P}$ and the total depth of the sampling site improved the coefficient of determination ($R^2 = \text{ca. } 0.5$). Thus, the greater the depth and the smaller the concentrations of chlorophyll a and $PO_4\text{-P}$, the greater is the secchi depth. In Finnish estuaries TP alone accounted 53% of the variation in secchi depth (Kauppila 2004). This implied that most of the TP was bound to algae, but extinction of particle scattering also had an effect on the optical properties of the seawater.

Reference chlorophyll a concentrations were reconstructed by using the historical secchi depth values in 1914 and 1921 given by Granqvist (1921) and Granqvist and Jurwa (1922), after correction them according to Launiainen et al. (1989) to enable comparison between the old and newer values. Reconstructed chlorophyll a concentrations varied slightly in Finnish coastal waters compared to variation in historical secchi depth values. Chlorophyll a was highest in the Gulf of Finland ($1.9\text{-}2.0 \mu\text{g chl l}^{-1}$) and lowest in the Bothnian Bay and the middle and outer Archipelago Sea ($1.6\text{-}1.7 \mu\text{g chl l}^{-1}$). In the Quark, chlorophyll a concentrations were $1.7\text{-}1.8 \mu\text{g l}^{-1}$. In the Gulf of Bothnia, reconstructed chlorophyll a concentrations were higher than gained by using 10% deviation of the frequency distribution data (Table 1). By contrast, in the Archipelago Sea and in the Gulf of Finland the reconstructed chlorophyll a values were smaller and probably closer to reference values compared with the concentrations, estimated from the frequency distribution data (Table 1).

2.4 Empirical chlorophyll-nutrient level relationships (annual means)

Establishing empirical chlorophyll-nutrient level relationship is more complicated than the secchi depth-chlorophyll relationships, because nutrients and phytoplankton biomass interact in a highly dynamic and, to some extent, inversely manner, since phytoplankton consume inorganic nutrients while growing (Figure 1). Before establishing empirical relationships between phytoplankton biomass and nutrient levels, the dynamical data should be aggregated into mean levels.

Phytoplankton and abiotic data, including nutrient levels, were separated into 38 distinct water bodies (see Carstensen et al. 2004), some of these characterised by more than one monitoring stations. The mean annual phytoplankton biomass, chlorophyll, DIN, DIP and DSi levels, denoted by X in the following, were calculated by means of a general linear model for the log-transform of these variables:

$$\text{Log}(X) = \text{water body} + \text{station}(\text{water body}) + \text{year} + \text{month}$$

where *water body* described the mean proportion for the 38 water bodies (Table 2), *station (water body)* described the variation between monitoring stations within the water body, *year* described the interannual variation common to all water bodies and *month* described differences between months of sampling. Mean levels of the transformed observations for the 38 water bodies were calculated as marginal means from this model, i.e. producing mean values that were not biased by skewed sampling in time or space. This implied that the mean values for water bodies were represented by the mean level of all monitoring stations within the water body. These mean values were subsequently transformed back to the original scale by the exponential function to derive geometric mean values.

There was a good correlation between phytoplankton biomass levels and nutrient levels (Figure 2) with the relationship for dissolved inorganic nitrogen being the most significant for both chlorophyll and phytoplankton biomass. The relationships were

Table 2: Typologies for the water bodies investigated and their grouping used in the analyses later. The grouping is compared to the typologies definition for salinity and station depths obtained from Carstensen et al. (2004) and retention typology obtained from Schernewski and Wielgat (2004). Western Baltic Sea was meso- and polyhaline and main Baltic Sea mainly oligo- and mesohaline. Estuaries were generally shallow and coastal types deep, although this was not consistent.

No.	Water body	Location	Type	Typology		
				Salinity	Depth	Retention
1	Bothnian Bay Finnish coast	Main Bal.	Coastal	oligo	deep	<30 d
2	Bothnian Sea Finnish coast	Main Bal.	Coastal	oligo	deep	<30 d
3	Inner archipelago	Main Bal.	Coastal	oligo	deep	<30 d
4	Tvärminne coast	Main Bal.	Coastal	meso	deep	<30 d
5	Coast east of Helsinki	Main Bal.	Coastal	oligo	shallow	<30 d
6	Huovari	Main Bal.	Coastal	oligo	deep	<30 d
7	Narva Bay	Main Bal.	Estuary	oligo	deep	<30 d
8	Gulf of Finland	Main Bal.	Coastal	oligo	deep	<30 d
9	Tallinn Bay	Main Bal.	Coastal	oligo	shallow	<30 d
10	Pärnu Bay	Main Bal.	Estuary	oligo	shallow	<30 d
11	Gulf of Riga coastal	Main Bal.	Coastal	oligo	shallow	<30 d
12	Gulf of Riga open-part	Main Bal.	Coastal	oligo	deep	<30 d
13	Curonian Lagoon	Main Bal.	Estuary	oligo	shallow	>30 d
14	Lithuanian coast	Main Bal.	Coastal	meso	deep	<30 d
15	Bight of Gdansk coastal	Main Bal.	Coastal	meso	shallow	<30 d
16	Bight of Gdansk open-part	Main Bal.	Coastal	meso	deep	<30 d
17	Coast off Swinoujscie	Main Bal.	Coastal	meso	deep	<30 d
18	Oderhaff	Main Bal.	Estuary	oligo	shallow	>30 d
19	Greifswalder Bodden	Main Bal.	Coastal	meso	shallow	<30 d
20	Prohner Wiek/Bodden	Main Bal.	Coastal	meso	shallow	<30 d
21	East of Rügen	Main Bal.	Coastal	meso	deep	<30 d
22	West of Rügen	Main Bal.	Coastal	meso	shallow	<30 d
23	Der Grabow	Main Bal.	Estuary	oligo	shallow	<30 d
24	Warnow estuary	West Bal.	Estuary	meso	shallow	<30 d
25	Warnemünde coast	West Bal.	Coastal	meso	deep	<30 d
26	Mecklenburg Bight	West Bal.	Coastal	meso	deep	<30 d
27	Western Baltic open-part	West Bal.	Coastal	meso	deep	<30 d
28	South Little Belt	West Bal.	Coastal	meso	deep	<30 d
29	Great Belt	West Bal.	Coastal	meso	deep	<30 d
30	The Sound	West Bal.	Coastal	meso	deep	<30 d
31	Kolding Fjord	West Bal.	Estuary	poly	shallow	<30 d
32	Vejle Fjord	West Bal.	Estuary	poly	shallow	<30 d
33	North Little Belt	West Bal.	Coastal	meso	deep	<30 d
34	Horsens Fjord	West Bal.	Estuary	poly	shallow	<30 d
35	Århus Bight	West Bal.	Coastal	poly	deep	<30 d
36	Mariager Fjord	West Bal.	Estuary	meso	deep	>30 d
37	Coastal Kattegat	West Bal.	Coastal	poly	shallow	<30 d
38	Skive Fjord	West Bal.	Estuary	poly	shallow	<30 d

generally better for chlorophyll than for phytoplankton biomass, indicating that chlorophyll may provide a more robust measure of total phytoplankton biomass than biomass found from microscopy samples.

There were also 3 specific water bodies with high phytoplankton biomass that may have been considered outliers in the regressions. These water bodies, Nordvorpommern Lagoon, Oderhaff and Curonian Lagoon, are all characterised by long retention time (>30 days) according to Schernewski and Wielgat (2004) and consequently large turnover rates. Thus, the relationships could be improved by incorporating information on retention times either as covariate or in a stratified analysis, although there are few water bodies in the CHARM database with long retention times.

These relations could potentially be used for establishing reference conditions for phytoplankton biomass, if reference conditions for nutrient levels are known. It should, however, be stressed that the considerable scatter in the regressions and the use of log-log scale may result in low precision for the reference conditions. Furthermore, benthic grazers may affect phytoplankton at shallow water stations by lowering the biomass relative to the expected level from the nutrient concentrations.

2.5 Empirical chlorophyll-nutrient level relationships (spring bloom)

An alternative to consider the annual mean levels of phytoplankton biomass versus annual mean nutrient levels is to correlate the spring bloom maximum concentration to the winter nutrient level. From the data in the CHARM database an algorithm has been developed to identify potential observations of the spring bloom. Using the HELCOM region-specific definition for the spring period, the maximum phytoplankton concentration was found. Provided that there were at least 3 observations within the spring period for a given station at a given year and that the biomass of the maximum observation was at least twice the average of the other observations, this particular observation was considered representative for the spring bloom. For this spring bloom observation the dominating species and its functional group were identified. This resulted

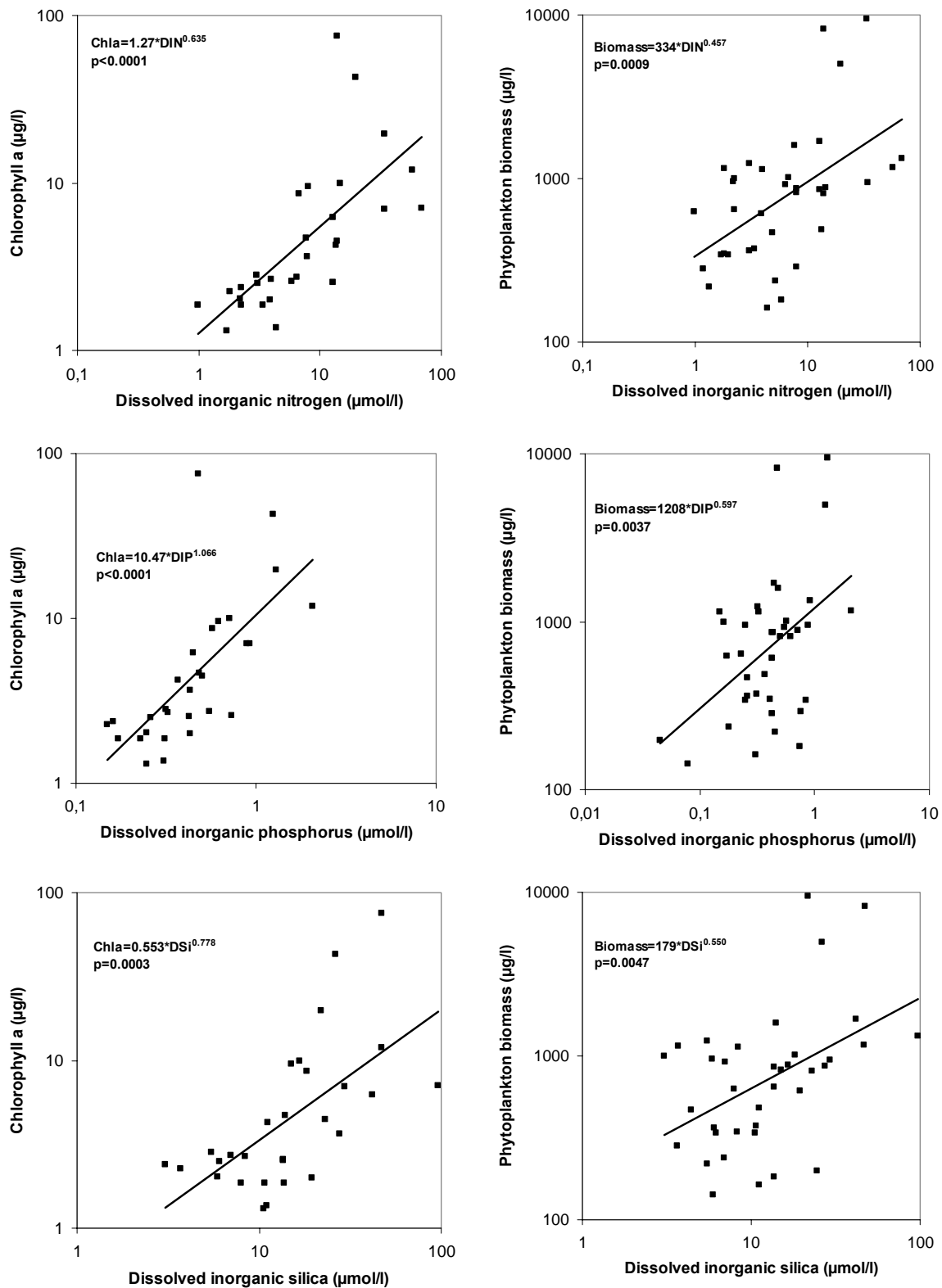


Figure 2: Annual mean for chlorophyll (left panel) and phytoplankton biomass (right panel) versus annual mean nutrient levels for 38 water bodies within the Baltic Sea.

in 387 identified spring bloom observations. For each of these observations the average nutrient level in the winter period prior to the spring bloom was calculated.

These observations were partitioned into four distinct groups depending on whether data originated from an estuary or a coastal area and if the station was located in the western Baltic (west of the Drogden and Darss sills) with strong advective currents and higher salinities or in the more stable brackish waters of the remaining Baltic Sea. Although the geographical extents of the different subgroups were quite different, the resulting numbers of observations in each group were more similar. This grouping of observations partly reflected the typologies within the Baltic Sea, however in a simplified manner (Table 2).

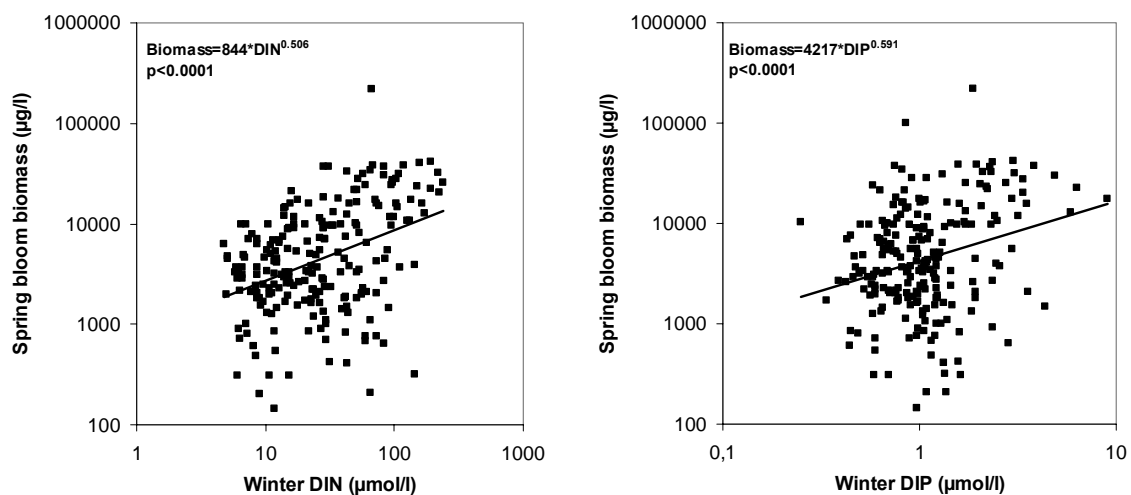
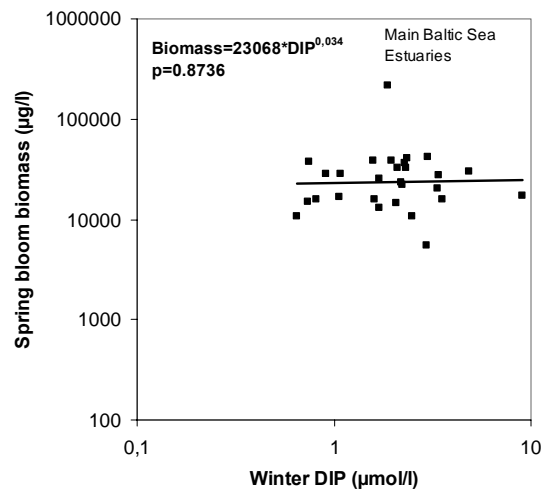
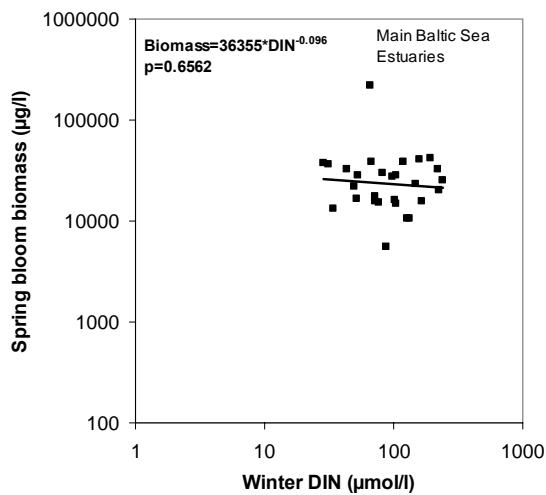
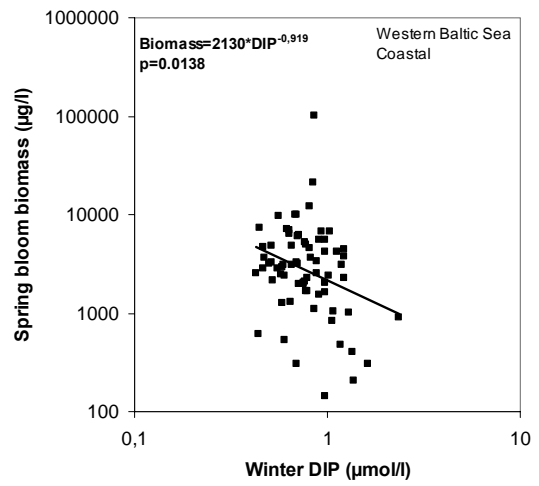
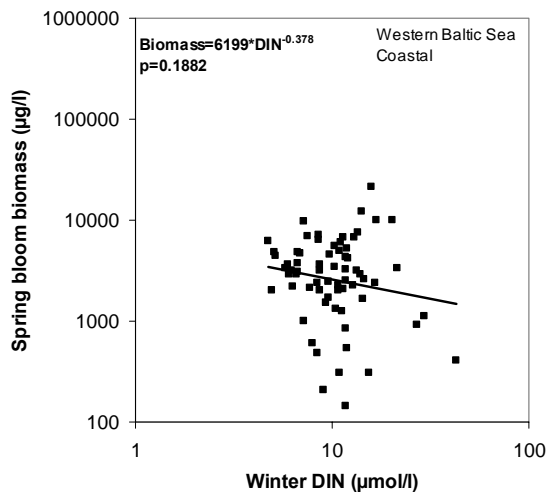
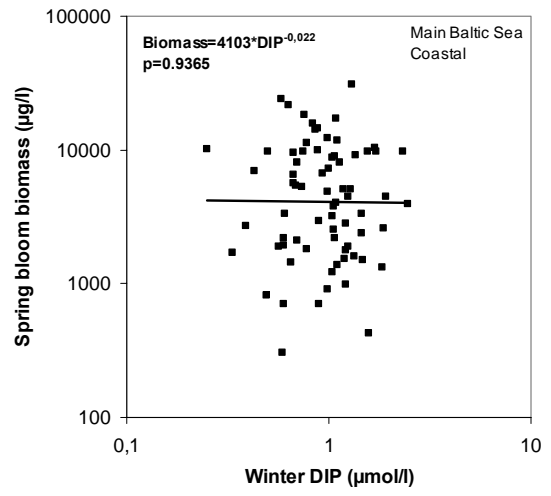
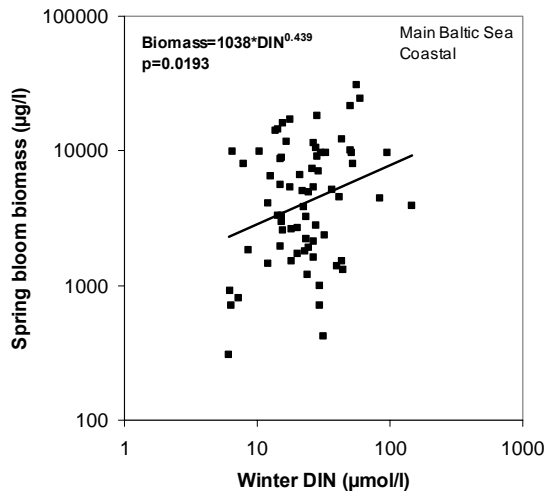


Figure 3: The maximum spring biomass defined according to criteria for spring bloom versus winter nutrient levels.

If all observations were considered concurrent there was a significant correlation between the phytoplankton biomass of the identified spring bloom and the mean DIN and DIP levels during winter (Figure 3). It should, however, be acknowledged that data for these relationships were quite scattered and the significance was achieved by the large number of observations. For all data the best relationship was obtained using winter DIN levels. However, if we investigate the same relationship for the 4 different groups separately, the relationship is not at all clear (Figure 4).



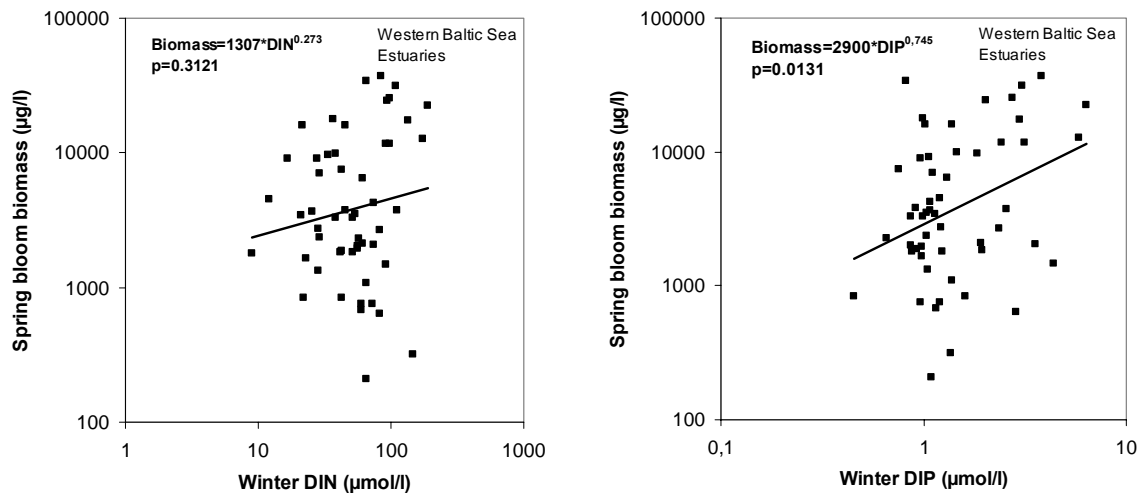


Figure 4: The spring bloom biomass versus winter nutrient levels for 4 different groups of stations.

Only 3 out of the 8 relationships were significant, and one of these significant relationships actually showed a negative correlation in contrast to the expected. It should also be stressed that the significant relationships were still characterised by considerable scatter and that the p-value was not very low considering the many observations used in the regressions. The four groups of observations actually appear to form separate groups of observations and consequently, the significant relationships when using all data could merely be interpreted as differences between the 4 groups.

The spring bloom is considered to be a transient, highly dynamic event that can be difficult to capture in a monitoring program. Furthermore, the spring can develop as one or several intense and short-lived blooms, or it can develop over several weeks gradually transforming the inorganic nutrients into biomass without attaining its maximum potential due to simultaneous losses. Thus, although there should be a conceptual link between the winter nutrient level and the magnitude of the spring bloom, in practice, the identification of this relationship is difficult to obtain from monitoring data. It is therefore unlikely that the spring bloom biomass can provide a reliable indicator for classification and that reference conditions can be established for this indicator.

2.6 Paleocological methods

Paleocological reconstruction has been used to establishing reference conditions in the inner coastal areas of the Baltic Sea (e.g Clarke et al. 2003) by the MOLTEN project. For example in Laajalahti this method gave relative good results (Kauppila et al. 2004, Weckström et al. 2004). The pollution history of Laajalahti outside of Helsinki has been traced by paleocological methods as described by Weckström et al. (2004), Vaalgamaa (2004), Kauppila et al. (2004a) and Clarke et al. (2003), and reference conditions have been established in Kauppila et al. (2004 a). At present the bay receives only little external loading, but it is strongly affected by releases of phosphorus from the sediments (Rekolainen 1982, Kauppila et al. 2004 a).

Considering the applicability of reference values of Laajalahti in a broader context, it seems obvious that natural variability – both temporal and spatial – must be separated order to establish reference conditions for other sites. The reference values obtained for Laajalahti are site-specific, and do not reflect natural variability across the whole inner coastal type of the Gulf of Finland. However, nutrient levels in the small embayments of the Gulf of Finland were generally lower than the reference values in Laajalahti (Table 3, Kauppila et al. 2004 b). The results support the findings of Weckström et al. (2002), suggesting that many inner coastal areas in the northern Baltic Sea, with a relatively small human pressure, might still be in good ecological state.

Table 3: Locations (coordinates according to the WGS84 system) and the total depth (Zmax) of studied sampling sites and the mean annual concentrations of TN, TP and chlorophyll a in the innermost coastal areas of the Gulf of Finland in 1990-2003 (Kauppila et al., 2004 b).

Station	lat	long	Zmax (m)	TN $\mu\text{g l}^{-1}$	TP $\mu\text{g l}^{-1}$	Chl $\mu\text{g l}^{-1}$
Laajalahti 87	6005.4	2421.0	3	760	50	22
Kyrkfjärden 171	6005.4	2420.3	3	517	41	8.2
Pikkalanlahti 21	6002.2	2400.3	5.2	463	43	9.2
Pikkalanlahti 198	6011.7	2450.9	4	487	41	11.6
Båtviken 16	6004.1	2418.9	9	446	42	8.2
Fiskarviken 17	6004.5	2419.3	2.6	432	44	8.6

Thus, it seems that the reference conditions established for nitrogen and chlorophyll a (annual levels $600 \mu\text{g TN l}^{-1}$ and $10 \mu\text{g Chl l}^{-1}$) in Laajalahti, might preliminary be used for classification in the inner types of the southern Finnish coasts (see Figure 5).

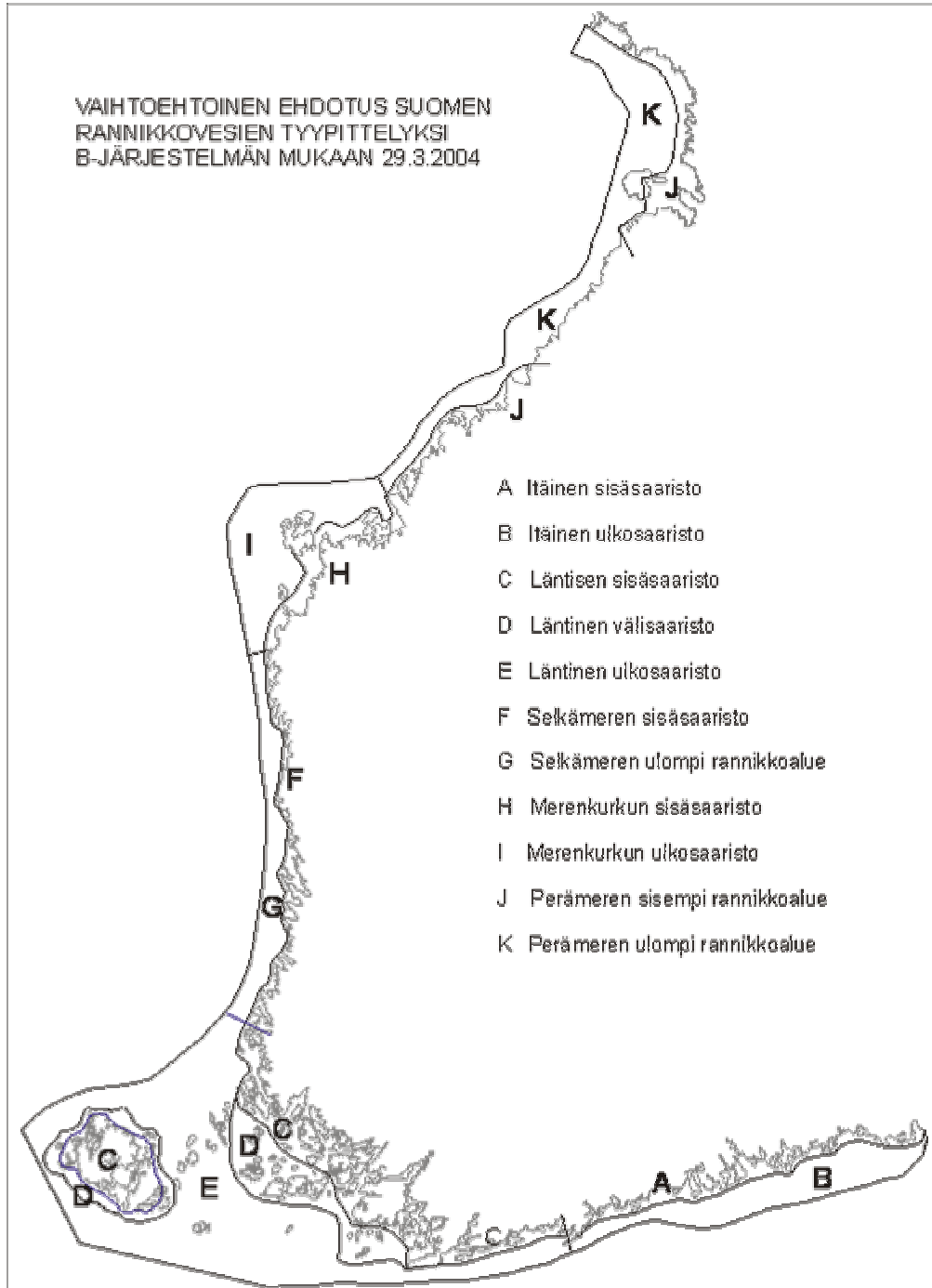


Figure 5: Finnish coastal typology.

2.7 A simulation study of phytoplankton in the Baltic Sea one century ago

A 3-D circulation model with a biogeochemical module (ERGOM) was applied for the simulation of trophic conditions in the Baltic Sea a century ago (Schernewski & Neumann, in press). One aim was to provide reference or background data for nitrogen, phosphorus and chlorophyll, which is required for the implementation of the European Water Framework Directive (WFD). It was assumed that the situation a century ago served this purpose well. Model inputs for this long-term simulation study were the regionally differentiated riverine and atmospheric nutrient loads to the Baltic Sea, which were compiled and calculated for a situation 100 years ago on the basis of various literature sources. For the mixed surface layer of the open Baltic Sea, we suggested maximum winter concentrations for dissolved phosphorus (dissolved inorganic nitrogen) of 0.23 – 0.35 (2.7 - 3.7) mmol/m³. Maximum chlorophyll a concentrations were between 1.8 -2.4 mg/m³. The concentrations of all parameters for different coastal waters varied widely, depending on exposure to nutrient sources. Our simulated nutrient concentrations for the situation a century ago are close to early measured data (1950 - 1960) and suggest that this data is suitable as reference data, as well.

In an extension of this simulation, we estimated the historical concentrations of three phytoplankton groups (diatoms, flagellates and cyanobacteria). The results are still not fully analysed and require detailed evaluation. The model suggests roughly similar historical diatom concentrations compared to today in the open Baltic Sea (Figure 6). However, near the coast the diatom concentrations were much lower a century ago. Even higher concentrations were obtained for the Kattegat. However, currently the results are not reliable enough to derive reference conditions for the three phytoplankton group considered.

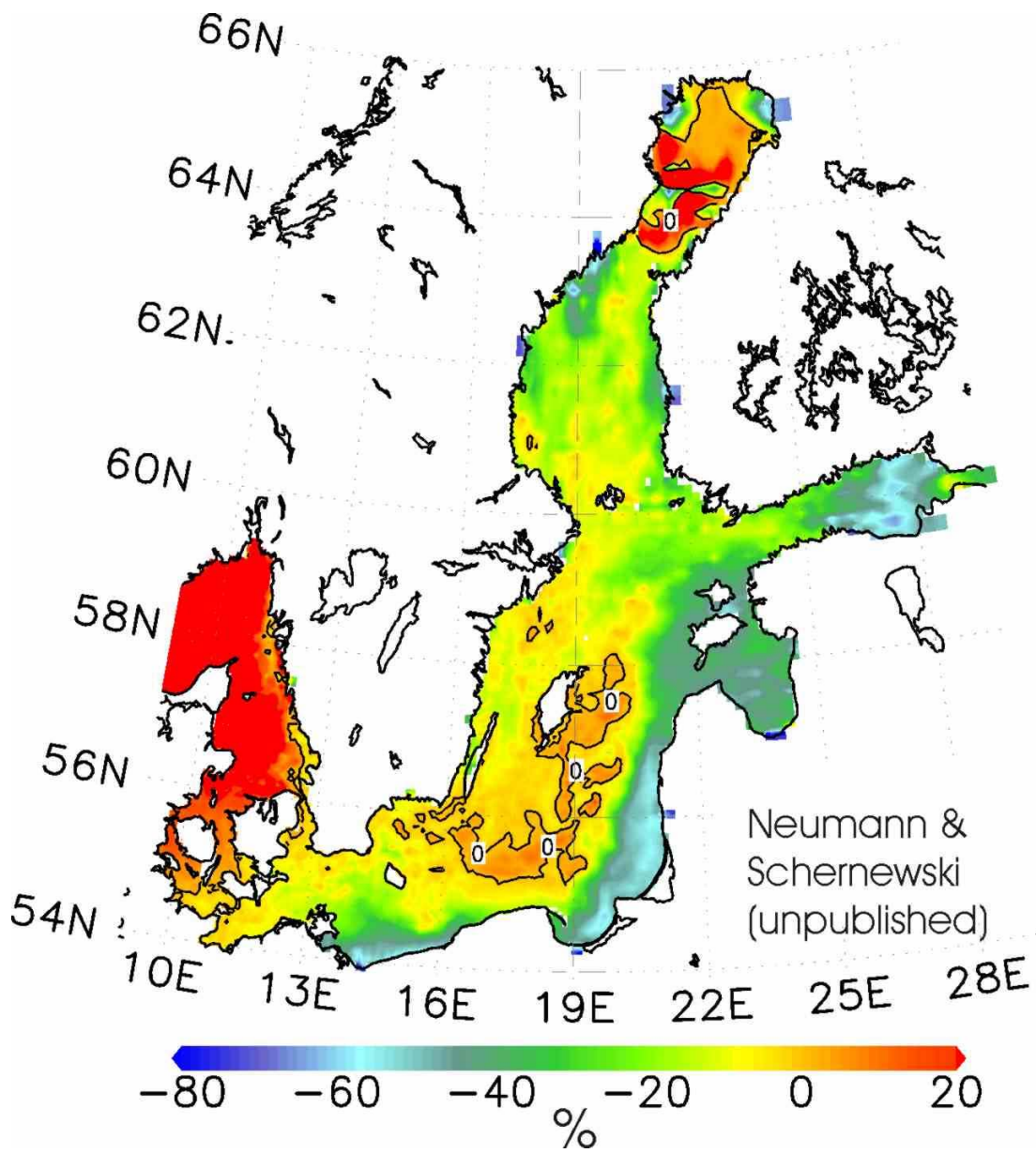


Figure 6: The relative difference between spring diatom blooms nowadays compared to the situation a century ago.

3. Reference conditions for phytoplankton composition

It has been suggested that the phytoplankton composition could be a good descriptor for environmental degradation. Changes in the nutrient ratios, particularly the N:Si ratio, may favour certain groups of species (e.g. diatoms that require silicate for growth). There have been qualitative investigations of the phytoplankton community prior to the intensification of coastal eutrophication in the Baltic Sea, which is considered to have started during the 1950s and 1960s (Richardson 1996). Ecosystem studies relating phytoplankton composition to nutrient availability have been sparse, although changes in nutrient inputs, particularly, decreases in the silicate input to the Baltic Sea have been documented (Conley et al. 2000). Here we will investigate the composition of the spring bloom and relate this to the nutrient levels.

3.1. Spring bloom composition related to nutrient levels

Observations considered as spring blooms were identified from the CHARM database as described in Section 2.5. The spring blooms in the main Baltic Sea coastal waters were mainly dominated by *Achnanthes taeniata*, *Peridiniella catenata*, and *Scrippsiella hangoei*, whereas the dominating species in the western Baltic Sea coastal waters were *Coscinodiscus concinnus* and *Skeletonema costatum*. For the estuarine waters the dominating species in the main Baltic Sea were *Rhodomonas lacustris* as opposed to *Skeletonema costatum* in the western Baltic Sea. In the western Baltic and the coastal waters of the main Baltic Sea the genus *Thalassiosira sp.* also appeared to dominate although there was a great diversity between which specific species within the genus that would form the bloom. Similarly, the genus *Chaetoceros sp.* was quite dominating in the coastal waters throughout the Baltic Sea, whereas only few estuarine spring blooms were dominated by this genus.

A first working hypothesis was that higher nutrient levels would favour opportunistic species such that the spring bloom would be dominated by single species. The dominating species proportion of the total biomass had a slight increasing tendency with winter nutrient levels that was significant for DIN but not for DIP (Figure 7). Employing the same analysis for the four different groups of stations defined in Section

2.5 revealed both increasing and decreasing relationships and none of these were significant.

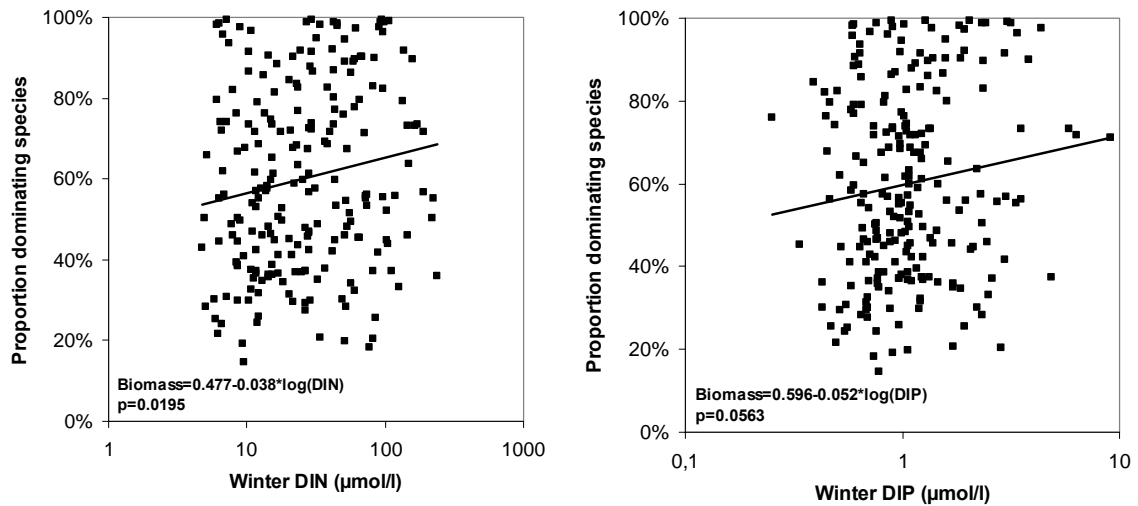
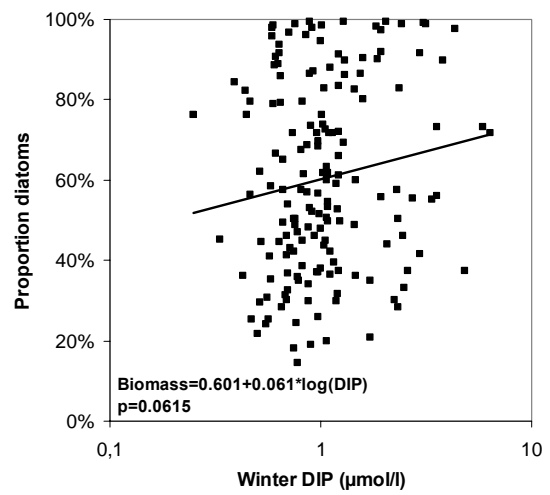
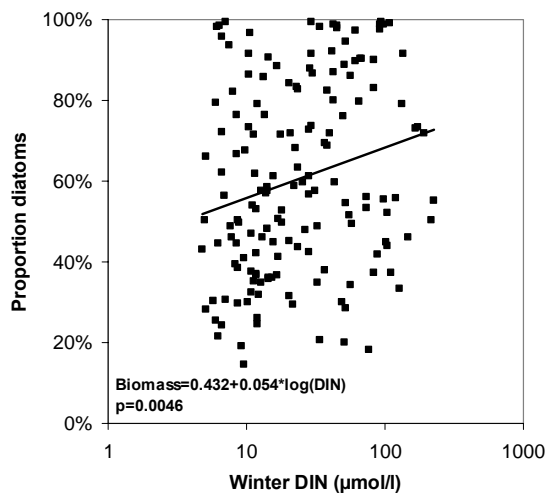
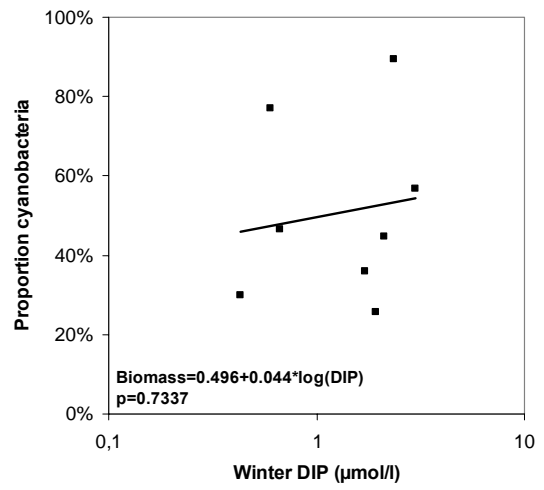
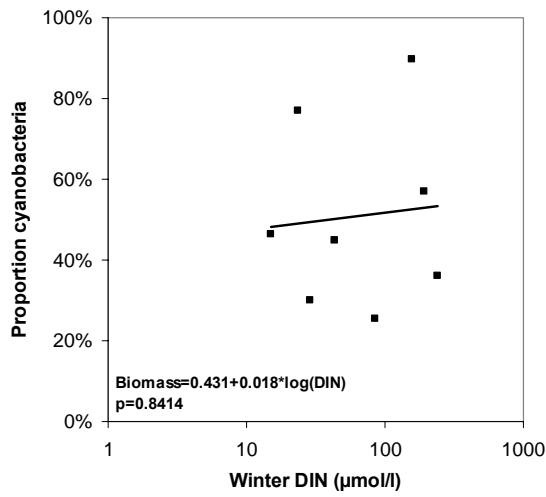
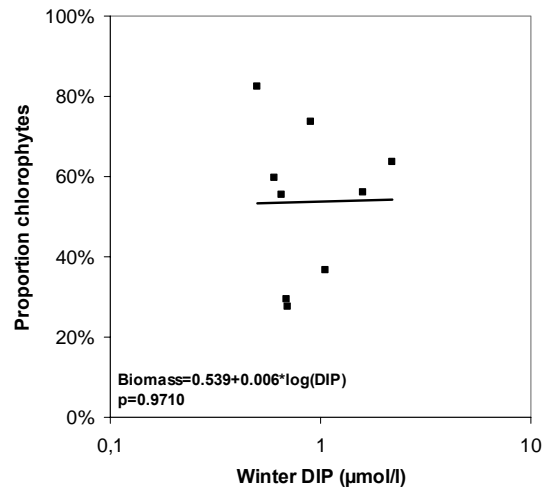
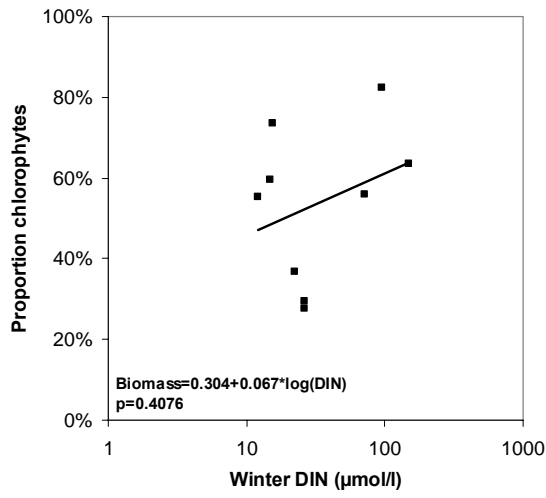


Figure 7: Proportion of the dominating species versus winter nutrient levels for all spring bloom observations.

If we consider the proportion of the dominating species versus the winter nutrient level for the different functional groups, it is apparent that most spring blooms are diatom blooms in the Baltic Sea (Figure 8). Diatoms were also the only functional group that reflected a significant relationship to the winter nutrient level (DIN only), although there is considerably scatter in the plot. Some of this scatter was due to different species dominating the spring bloom and therefore this analysis was detailed down to the genus level for diatoms only. Since the relationship was strongest for DIN, DIP was not considered in the further analyses.



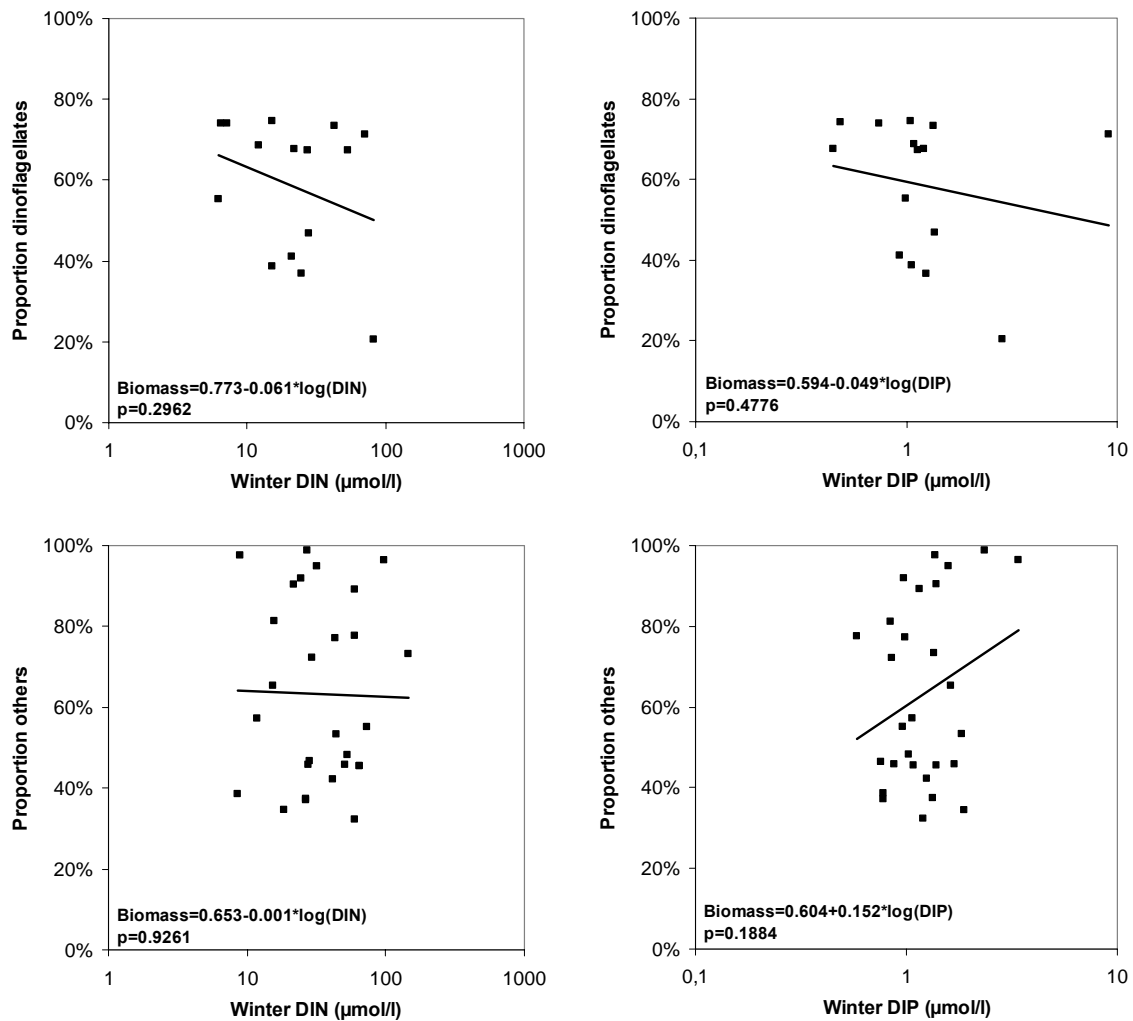


Figure 8: Proportion of the dominating species versus winter nutrient levels partitioned into functional groups.

We only considered genera with at least 10 observations of spring bloom and corresponding DIN levels. Five out of the six genera with sufficient observations showed increasing relationship, although different, of the dominating species with the DIN level (Figure 9). Only *Coscinodiscus sp.* did not show a positive relationship with the DIN level, even if the single outlier observation with the high DIN level was not included (*C. granii* in the Curonian Lagoon). It should also be noted that *Coscinodiscus sp.* generally dominated at lower DIN levels only. Thus, if the spring bloom dominance of *Coscinodiscus sp.* were potentially related to the DIN level then the response would be a threshold mechanism rather than a gradient.

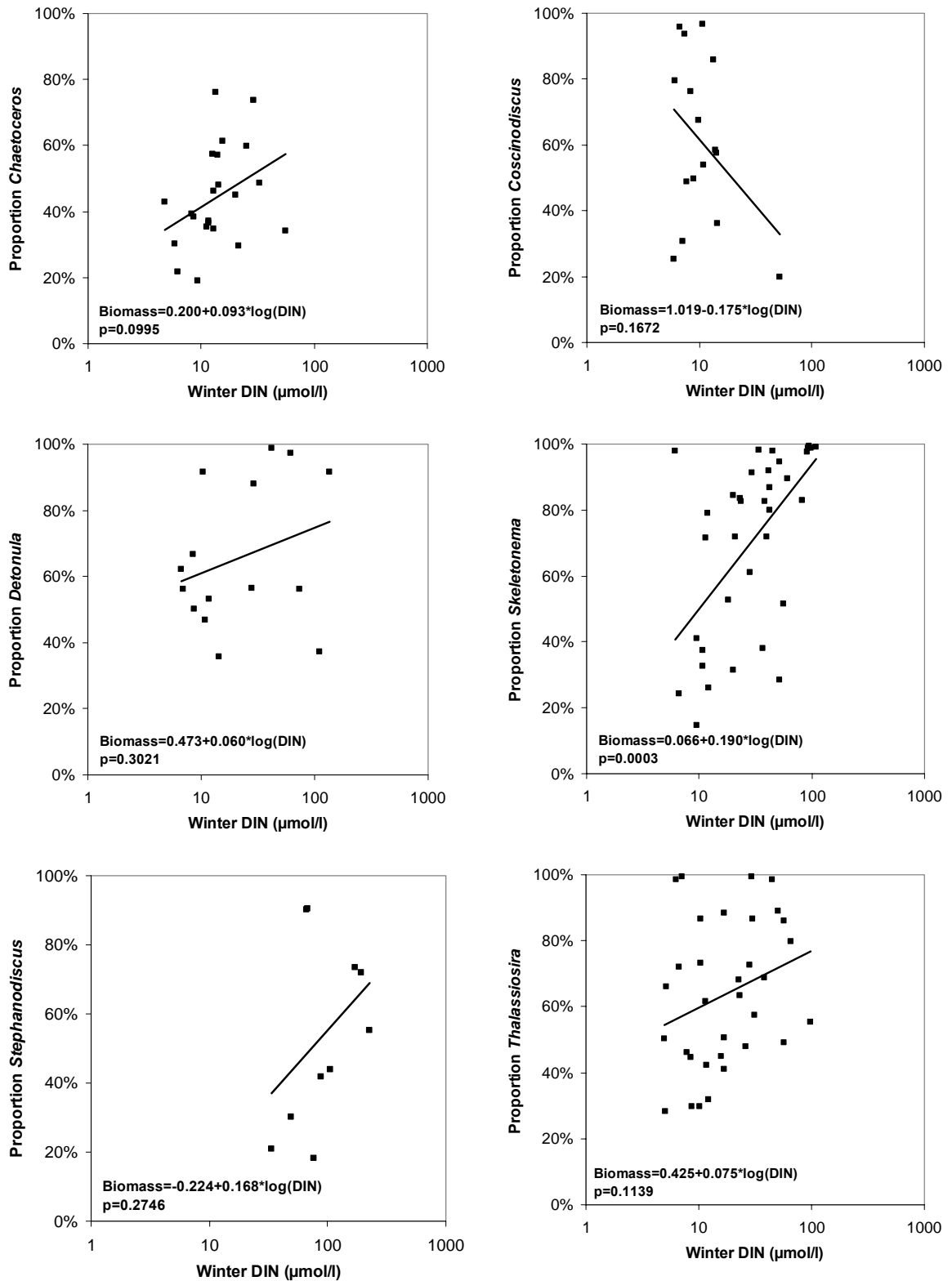


Figure 9: Proportion of the dominating species versus winter DIN level partitioned into the genus for the dominating species.

A highly significant relationship was obtained for *Skeletonema* sp. suggesting that this species becomes more dominant with increasing nitrogen levels, provided that *Skeletonema* sp. is the dominating species. The stations with a *Skeletonema* sp. domination of the spring bloom were mainly located in the Danish and German waters of the southern Baltic Sea.

Thalassiosira sp. appears to be another frequently dominating species that potentially may reflect an increasing dominance with the DIN level. This genus group includes 7 different species with *Thalassiosira baltica* being the most frequent spring species. Removing two of the less frequent species (*T. oceanica* and *T. levanderi*) actually result in a significant relationship with the DIN level ($p=0.0066$). *Thalassiosira* sp. was dominating spring blooms at stations throughout the southern Baltic Sea, the Gulf of Riga and the Gulf of Finland.

Chaetoceros sp. also appeared to have some relationship with the DIN level and is a relatively frequent dominant species of the spring bloom in the southern Baltic Sea as well as in the Gulf of Riga and the Gulf of Finland. The genus includes 8 different species recorded as dominant for the spring bloom in the database. One of these, *C. curvisetus*, was only recorded once in a Danish estuary (Horsens Fjord) and if this observation was removed from the analysis the regression turned out significantly ($p=0.0139$).

The analyses above suggest that some diatom species or genus can be used as indicators of eutrophication, since they become more dominating during the spring bloom with higher nutrient levels. The selection of the specific species to be included in such indicators may need some refinement considering the physiology of the different species. These indicators were applicable to most of the Baltic Sea area except for the Gulf of Bothnia, where there was too little data available to determine key species for the spring bloom and their relation to nutrient levels. The proportion of *Skeletonema* sp., *Thalassiosira* sp. and *Chaetoceros* sp. all showed increasing relationship to the DIN level and therefore reference conditions for these indicators can be established from reference conditions of nitrogen. It should, however, be acknowledged that any classification deriving from these indicators is deemed to be uncertain due to the considerable variation in data.

4. Reference conditions for bloom frequency

It has been suggested that the intensity and frequency of blooms has increased with the nutrient enrichment of coastal waters (Haellegraf 1993). In Carstensen et al. (2004) and Carstensen et al. (in press) it was shown that the most likely cause of summer blooms in the Kattegat is wind-induced entrainment of nutrient-rich bottom water increasing the surface phytoplankton biomass through active growth. From the 1950 to the mid 1990s the pool of inorganic nitrogen in the bottom waters of the Kattegat increased due to the enhanced delivery of nitrogen from terrestrial sources mainly. Although the triggering mechanism underlying these summer blooms are physical, the flux of nitrogen into the surface layer from entrainment will be larger with the increasing nitrogen concentrations in the bottom water. Consequently, for the Kattegat and most likely other parts of the open Baltic Sea the reference condition is a lower frequency of summer blooms than present day level.

4.1. Empirical bloom frequency-nutrient loading relationships

If we employ the regression from Carstensen et al. (2004) and assume that the dry winter of 1996/97 is representative of the nitrogen input reference condition (37,100 tonnes in Sep-May) then the reference conditions for the frequency of summer blooms in the Kattegat can be estimated to be 1.45% (i.e. 1,45% of the summer observations would be fulfil the criteria for phytoplankton bloom). As shown in Carstensen et al. (2004) there are spatial differences in the bloom frequency leading to generally higher levels along the western coastal part of the Kattegat. Thus, reference conditions for the frequency of summer blooms in the coastal parts of the Kattegat may be approximately 2-3 times higher when spatial differences are taken into account.

Using a similar approach for the mean summer chlorophyll concentration in the Kattegat we find a reference condition of $1.41 \mu\text{g l}^{-1}$ as an average for the entire Kattegat. Again, allowing for the spatial differences reported in Carstensen et al. (2004) reference conditions in the coastal areas should approximately be 50% higher.

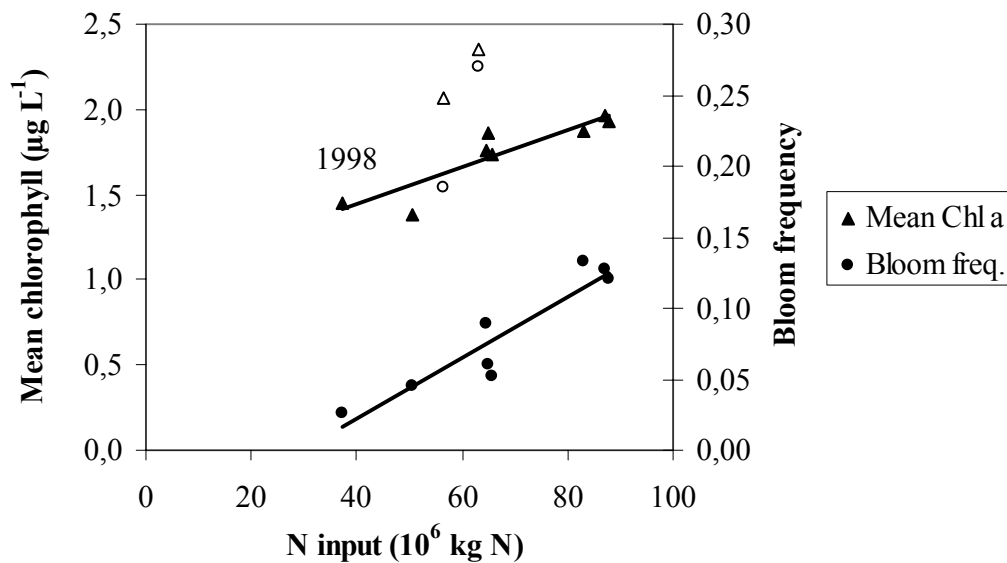


Figure 10: Summer mean chlorophyll concentration and bloom frequency related to nitrogen input to the Kattegat during the 8 previous months (September-April). Nutrient input data include land-based discharges from Denmark and Sweden as well as atmospheric deposition, compiled from the national monitoring programs in the two countries. Regression lines do not include data from 1990 and 1998 (open symbols), which have been marked separately. From Carstensen et al. (2004).

The bloom frequency approach in Carstensen et al. (2004) requires considerable data to obtain a well-defined distribution for summer chlorophyll. If such data is available from other areas and the bloom underlying mechanisms are similar to that of the open Kattegat then this approach can be applied as well. In the case of shallow waters with benthic grazing blooms may develop during periods with stratification and sufficient nutrients, however, the distribution of chlorophyll will be different and algorithms for identification of bloom observations need to be developed.

5. Reference conditions for species diversity

In the water framework directive the species abundance of phytoplankton is listed as one of the biological elements. However, robust indicators for phytoplankton abundance have not been developed yet. The problem of defining abundance from phytoplankton samples is that the identification of species highly depends on the taxonomical skills of the person analysing the sample. In many of the early samples species were only identified by their genus and not the specific species. Moreover, the taxonomy has developed and probably will continue to develop increasing the general knowledge of the people analysing the samples. These findings will, at least for the use of historical data, inevitably lead to systematic differences when comparing the number of species recorded. In the CHARM database the average number of species recorded in the samples has increased for almost all countries (Figure 11) in addition to the differences between countries. Thus, species abundance defined as the number of species per sample is not a robust indicator that can be used for classification in the water framework directive.

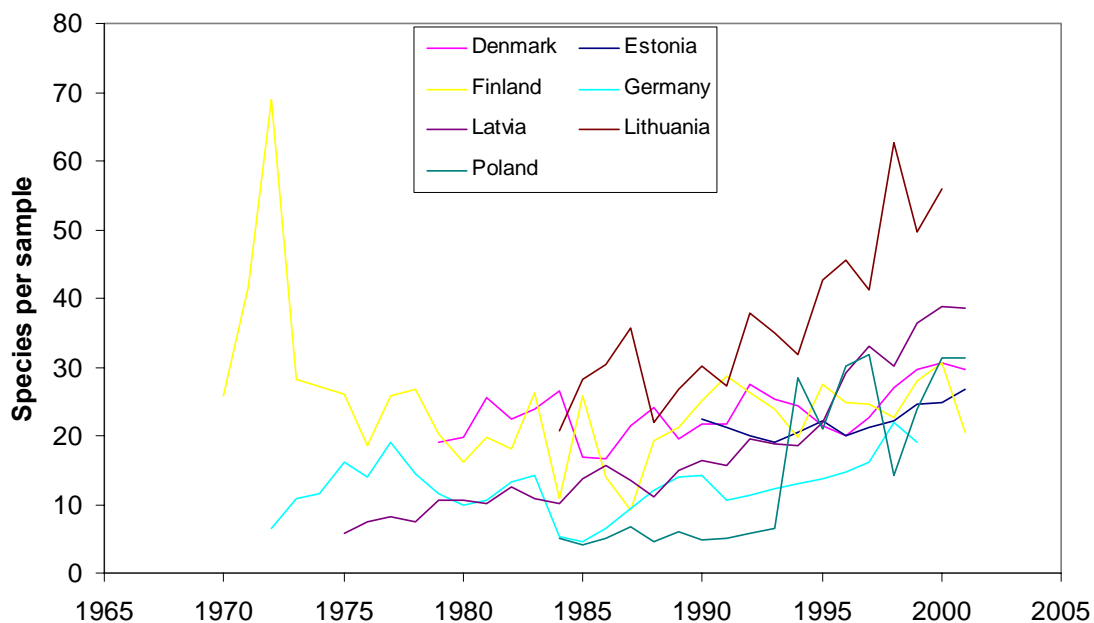


Figure 11: The average number of species recorded in phytoplankton samples for different countries in the CHARM database. The high species abundance in 1972 was from a single sample.

In addition, we also attempted to estimate the long-term changes in phytoplankton diversity by calculating values for some commonly used diversity indices. Diversity indices combine the information related to species abundance and species richness into a single number that can be used to assess the state of the community (Washington 1984). For the purpose of checking the long-term changes in species diversity, we applied Shannon's (Shannon and Weaver 1949), Margalef's (Margalef 1958), and Menhinick's (Menhinick 1964) indices for the available phytoplankton data from German and Polish coastal waters (Gromisz et al., in prep).

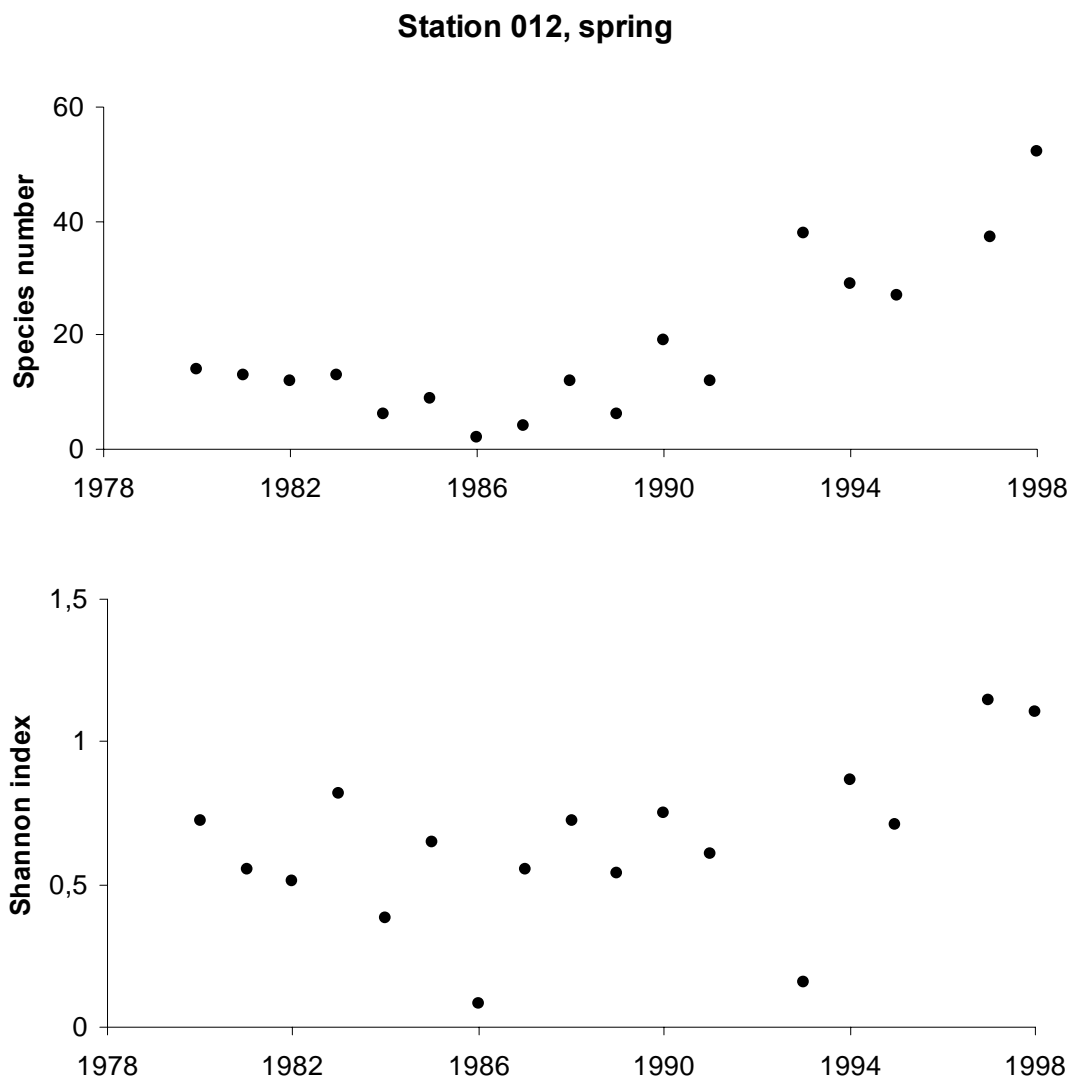


Figure 12: Long -term changes in species number and Shannon index values from 1980 to 1998 in spring at the station O12 in the Mecklenburg Bight, Germany.

A significant long-term increase in the Shannon's index, related to an increase in species number, was noted from 1980 to 1998 at German stations both in spring and summer (Fig. 11), and in the values of the Shannon's, Margalef's and Menhinnick's indices in the Polish stations between 1994 and 2001 (Fig. 12). However, this probably resulted from the improved taxonomic knowledge of the phytoplankton counters and taxonomical revision (splitting of some species into different genera or species).

In the Polish data from the station in the Gdańsk Deep, the phytoplankton biomass was generally determined only for five dominant species. Thus only the McNaughton's index, which deals with just two dominant species, could be calculated for these samples. A clear decrease in McNaughton's values (including the 1994-2001 period) was observed in spring and summer (Fig. 12).

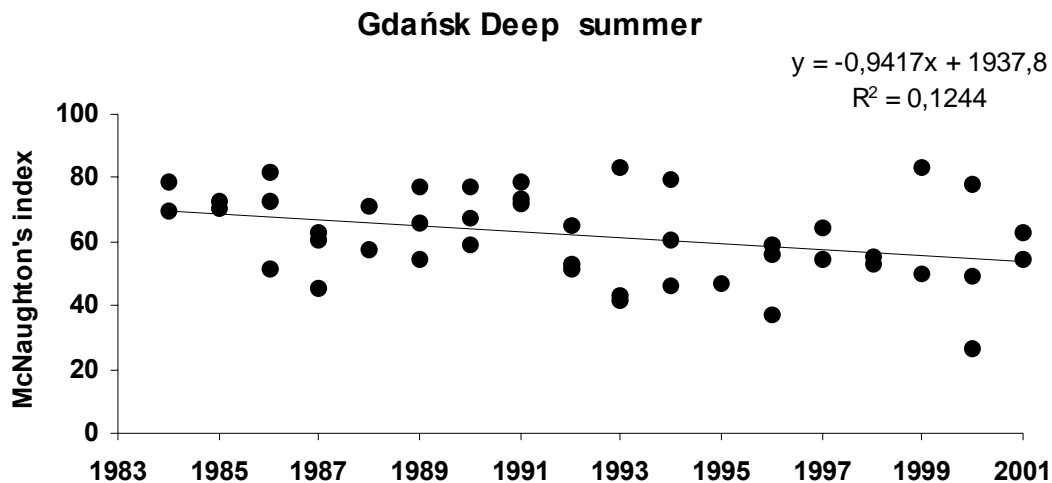


Figure 13: Long-term changes in the values of the McNaughton's index in the Gdańsk Deep (spring and summer).

While the Shannon's index and species number indicate that the results may have been due to methodological bias (i.e. more species identified), this is less evident to the McNaughton's index, which indicates the proportion of the biomass of the two most dominant species of the total biomass of phytoplankton. The results for the Gdansk Deep suggest a trend since early 1980's. Here as well the variability seems to be too high to allow any conclusions to be drawn. However, the applicability of McNaughton's index for detecting changes in the patterns of dominance in the phytoplankton communities could be explored further.

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