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**Selecting potential summer phytoplankton eutrophication
indicator species for the northern Baltic Sea**

M.Sc Thesis

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CONTENTS

1. INTRODUCTION	3
1.1. Eutrophication.....	3
1.2. General characteristics of the Baltic Sea and the Gulf of Finland.....	4
1.3. EU Water Framework Directive and aims of the present thesis.....	6
2. MATERIAL AND METHODS.....	8
2.1. Study area	8
2.2. Data collection and analyse	10
2.3. Statistical analyse.....	10
3. RESULTS	13
3.1. Hydrometeorological and hydrophysical conditions	13
3.2. Variation of nutrient concentrations	16
3.3. Dynamics of phytoplankton.....	21
3.4. The relationships between abiotic and biotic variables	26
4. DISCUSSION.....	28
SUMMARY	32
KOKKUVÕTE (Summary in Estonian).....	33
ACKNOWLEDGEMENTS	35
REFERENCES	36

1. INTRODUCTION

1.1. Eutrophication

Planktonic algae significantly contribute to the overall biomass in marine ecosystems and algal blooms at sea are naturally occurring phenomena. However, these mass occurrences of microscopic algae have become more frequent and intense due to the eutrophication of the sea (HELCOM, 2003). Eutrophication is a problem in many marine coastal areas (Vollenweider, 1992; Turner & Rabelais, 1994; Nixon, 1995; Billen & Garnier, 1997; Cloern, 2001; Elmgren, 2001; Paerl et al., 2003), including the Baltic Sea (Larsson et al., 1985; Elmgren, 1989; Rosenberg et al., 1990). Coastal seas are highly diverse and productive ecosystems, which makes them a very valuable resource from a human perspective (Weckström, 2005). However, as more than three-quarters of the world's population live in coastal catchment areas, they are subject to growing pressures (Vitousek et al., 1997; Jickells, 1998; Weckström, 2005). Within only a few decades, numerous previously pristine, oligotrophic estuarine and coastal waters have undergone a remarkably troubling transformation to more mesotrophic and eutrophic conditions (Paerl, 1997).

It is caused by increased nitrogen and phosphorus input from the densely populated and intensively cultivated catchment area and from the atmosphere, resulting in an increase of phytoplankton biomass, primary production and turbidity in the euphotic zone and indirectly in oxygen deficit in deep water layers (Jørgensen et al., 1990; Gray et al., 2002; Wasmund & Uhlig, 2003). A vicious circle can develop where anoxic conditions affect the nutrient cycle in the bottom water and sediments, intensifying eutrophication and accelerating oxygen depletion. This is because in anoxic conditions phosphorus is released from sediments back into the water, while nitrogen accumulates as ammonium because of a lack of denitrification and nitrogen release from sediments. These nutrients are then further utilized by more primary producers, compounding eutrophication through a factor known as internal nutrient load (HELCOM, 2003).

Although the sources and pathways of nutrient inputs to aquatic ecosystems can be estimated with reasonable certainty, it has been difficult to achieve reductions in the different sources (Boesch, 2002; Carstensen et al., 2006). However, some coastal

ecosystems have experienced reductions in inputs of phosphorus and nitrogen primarily through improvement in treatment of wastewater and reductions in point sources, although relatively little progress has been made in reducing diffuse sources of nutrients (Butt & Brown, 2000; Boesch, 2002; Conley et al., 2002; Carstensen et al., 2006).

1.2. General characteristics of the Baltic Sea and the Gulf of Finland

The Baltic Sea is one of the largest brackish water areas on Earth, with a drainage basin that is four times larger than its surface area (Elmgren & Larsson, 2001). The Baltic Sea is extremely vulnerable to anthropogenic disturbance due to its special hydrographical conditions, simple ecosystem structures and poor water exchange with the North Sea (Voipio, 1981). The nutrient load of the Baltic Sea has strongly increased from its natural level (Cederwall & Elmgren, 1990; HELCOM, 1990; Wulff et al., 1994; Kautsky & Kautsky, 2000). It has been estimated an eightfold increase in P and a fourfold increase in N loading during the last century (Weckström, 2005). Increased input of nutrients to the Baltic Sea has changed the biological structure and ecological processes in both coastal and open sea areas (Bonsdorff et al., 2002).

The Gulf of Finland is an eutrophied shallow elongated basin in the northeastern part of the Baltic Sea. The Gulf has a mean depth of 37 m, a length of 400 km, a maximum width of 135 km and a total volume of approximately 1100 km³. The annual freshwater inflow from rivers (dominated by the river Neva) is about 110 km³. The freshwater runoff and the saltwater inflow from the Baltic Proper strongly influence the hydrography and ecology of the Gulf (Kononen et al., 1996; Alenius et al., 1998; Kahru et al., 2000). The nutrient discharges from the catchment area of the Gulf of Finland decreased with 40% during the 1990s. The reductions are especially valid for eastern and southern Gulf of Finland, i.e. in Russian and Estonian waters. The total load to the Gulf of Finland is still 2–3 times larger compared to the rest of the Baltic Sea (Pitkänen et al., 2001).

The overall water circulation in the Gulf of Finland is counter-clockwise, the inflow of the saltier northern Baltic Proper water occurs along the Estonian coast and the outflow of the fresher Gulf water occurs along the Finnish coast (HELCOM, 1996). In summer when the water column is thermally stratified, wind-driven coastal upwelling is an

important mesoscale phenomenon that dramatically changes the euphotic layer temperature and nutrient conditions in the Baltic Sea. The surface-layer water temperature may drop more than ten degrees within hours, while nutrient concentrations increase markedly (Niemi, 1979; Haapala & Alenius, 1994; Alenius et al., 1998). The northern coast of the Gulf of Finland is one of the main wind-driven upwelling areas in the Baltic Sea (Myrberg & Andrejev, 2003). The southern coast is respectively one of the main downwelling areas (Laanemets et al., 2004).

Generally phytoplankton growth and biomass in the Gulf of Finland are nitrogen-limited (Granéli et al., 1990; Kivi et al., 1993; Lignell et al., 2003), except for the filamentous cyanobacteria that fix atmospheric nitrogen. The growth of filamentous cyanobacteria in the Baltic Sea is mainly temperature-, trace element- or phosphorus-limited, phosphorus being the main nutrient setting limits for bloom intensity (Lehtimäki et al., 1997; Stal et al., 1999; Rydin et al., 2002; Lignell et al., 2003).

1.3. EU Water Framework Directive and aims of the present thesis

In 2000 the European Parliament and the Council adopted the EU Water Framework Directive (WFD). The purpose of this Directive is to establish a framework for the protection of inland surface waters, transitional waters, coastal waters and groundwater (European Union, 2000). The WFD provides national and local authorities with a legislative basis for the maintenance and recovery of water quality to achieve good ecological and chemical status for all surface waters and good chemical status for groundwater (Andersen et al., 2004).

Member States of the European Union are expected to develop classification schemes that describe the present ecological status of surface waters based on information on background conditions against which recent anthropogenic disturbance can be assessed, and develop efficient and cost-effective monitoring strategies focusing on biological parameters (ECRU, 2004). The monitoring activities shall in principle cover ecological status and chemical status and monitoring networks should in principle be based on indicators that are indicative of the status of each relevant quality element – biological, hydromorphological or physio-chemical (Andersen et al., 2004).

Biological quality elements are components of the aquatic ecosystem that can be measured using parameters such as species composition or biomass (Vermaat et al., 2005). According to the WFD, phytoplankton is one of the biological quality elements for the classification of ecological status of surface waters (lakes, rivers, coastal and transitional waters). The ecological status in coastal waters is characterised by using phytoplankton taxonomic composition, abundance, biomass and the frequency of phytoplankton blooms. The increase in nutrient input leads directly to an increase in phytoplankton biomass and phytoplankton biomass may serve as an indicator of the trophic state. Therefore it will be important to identify phytoplankton taxa that are the most suitable indicators for assessing the ecological status and eutrophication of coastal waters.

As far as we know there are very few published relationships between the trends in nutrient concentrations and the biomass of total phytoplankton or single taxa. The impact of nutrient enrichment on the phytoplankton community structure in the northern Baltic Sea has been observed by Lagus et al. (2004) and Vuorio et al. (2005). Gasiùnaite

et al. (2005) tried to delineate a set of phytoplankton community indicators in the Baltic Sea that are independent of season and salinity, but indicative of trophic status of different coastal areas.

Aims of the present thesis are:

1. To describe temporal and spatial inter-seasonal variability of the biomass of the phytoplankton and investigate if there exist general biomass pattern for different species.
2. To identify the phytoplankton taxa, which wet weight biomass shows good statistical relationships with enhanced nutrients concentrations and is independent of temperature or salinity and therefore may prove indicative for the assessment of eutrophication.

2. MATERIAL AND METHODS

2.1. Study area

The study was conducted in the Gulf of Finland. The sampling transect was located in the central part of the Gulf between Helsinki and Tallinn. The map of the study area and the locations of sampling points along the transect between Tallinn and Helsinki in the Gulf of Finland are shown in Fig.1.

There are some differences between Finnish and Estonian coastal areas. The shallower Finnish coast characterized by an archipelago zone, which in many places forms a mosaic of islands. There are several shallow bays on both sides of the Helsinki as well as within its limit. The average depth of the inner bays in the Finnish coastal waters is only 1-3 meters, and less than 20 meters in the outer bays. Even the open sea south of the city is shallow, being less than 30 meters deep (Laurila et al., 2004). Estonian shore waters are also quite shallow, but deepen rapidly. A deep trench in the northern part allows deep water from Gulf of Finland to enter Tallinn Bay. Tallinn Bay is 20-45 (80) meters deep. The open part of the bay covers 250 km² and its maximum depth is 90 m (Martin et al., 2003).

There are no major rivers in both regions, only small rivers, such as Pirita (Tallinn area) and Vantaa (Helsinki area), bringing fresh water into the sea (Pesonen et al., 1995; Martin et al., 2003). The water mass in the northern part of the gulf is fresher than in that found along the southern coast, with surface salinities at the Estonian coast 0.5- 1.5 higher than on the Finnish side (Andrejev et al., 2004). Salinity varies in the Finnish side between 0-7 in the bays and 3-8 in the open sea (Finni et al., 2001b). The salinity in the Tallinn Bay varies between 5.5 and 9.5 (Martin et al., 2003).

The Tallinn Bay area is considered to be a “hot spot” because the large urban area of the city of Tallinn and intensive shipping activities of Tallinn harbor influence the nutrient conditions of the coastal waters (Martin et al., 2003). In Helsinki area since 1971, all municipal wastewater discharges have been directed into the open sea via an offshore sea outlet. Since 1994, the wastewater of the capital region has been treated in 2 effective plants (Finni et al., 2001b).

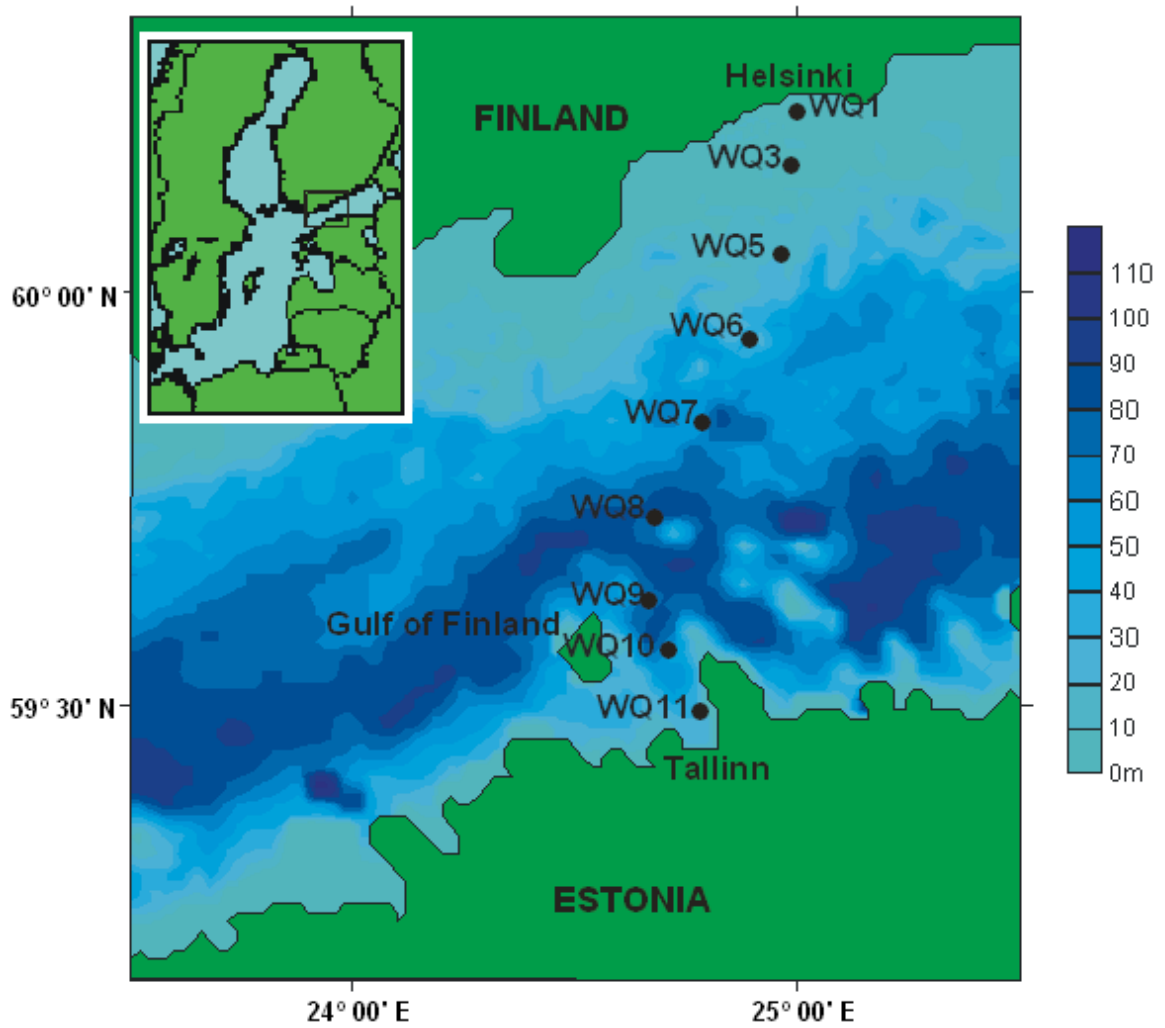


Fig. 1. Map of the study area in the Gulf of Finland, Baltic Sea. Sampling points along the transect between Tallinn and Helsinki (WQ1-WQ11) are indicated.

2.2. Data collection and analyse

Data used in present study were partly collected in the frame of the national monitoring program with an automated equipment onboard passenger ferries *Wasa Queen* (in 1997-1999), *Finnjet* (in 2000- 2003) and *Romantika* (2004) plying between Tallinn and Helsinki in summer period (June to August; weeks 22-35) in years 1997- 2004.

The water was pumped constantly through the sensors from a fixed depth (ca 5 m) onboard of moving ship. The in vivo temperature and salinity were recorded quasi-continuously with spatial resolution of 150 m using an Aanderaa thermosalinograph and Turner Design fluorometer. The measurement location was determined with a GPS navigator. Measurements were repeated every 0.5-3 days in the same sea area depending on the schedule of the ferry.

The water samples for the analysis of phytoplankton species composition were obtained using an automated sampler (ISCO). Nine water samples were taken once a week along transect and kept refrigerated (4 °C) in the dark before the analysis. The water samples were analysed at an on-shore laboratory for nutrient concentrations as well as phytoplankton species composition and biomass. Phytoplankton samples were preserved with acid Lugol solutions and analysed using inverted microscope technique (Utermöhl, 1958). Wet weight biomass of phytoplankton was calculated from cell geometry (HELCOM, 1988) and using cell volume tables (Phyto, Software Kahma Ky. Altogether 1008 phytoplankton samples were analysed.

2.3. Statistical analyse

14 dominant taxa of the Gulf of Finland (Table 1)- that formed mass occurrences in phytoplankton community in summer periods within several studied years- were selected for statistical analysis by using wet weight biomass values. Biomass variables of Oscillatoriales (mainly from genera *Pseudanabaena* and *Planktolyngbya*), *Anabaena* spp., *Aphanizomenon* sp., *Nodularia spumigena* Mertens, *Hemiselmis virescens* Droop, *Plagioselmis prolunga* Butcher, *Prorocentrum minimum* Schiller, *Heterocapsa triquetra* Stein, *Cyclotella choctawhatcheeana* Prasad, *Skeletonema costatum* Greville, *Cylindrotheca closterium* Reimann and J. Lewin, *Eutreptiella gymnastica* Thronsen,

Monoraphidium contortum Komárková-Legnerová and *Pyramimonas* spp. were used. All species except *Hemiselmis virescens* and *Plagioselmis prolunga* were analysed separately.

At first, temporal distribution of wet weight biomass for each selected taxon was investigated to determine time periods with clearly elevated values. Investigated species are steadily presented in summer phytoplankton community, but become massive only in certain periods. Therefore weeks with clearly elevated biomass values were used for further analysis.

Variation in biomass of different species among sampling points and weeks was subsequently tested for significance with an ANOSIM (analysis of similarity) using the software package PRIMER, version 5 developed at the Plymouth Marine Laboratory (Clarke & Warwick, 2001). ANOSIM provides a way to test statistically whether there is a significant difference between two or more groups of sampling units. The R statistic values, generated by ANOSIM, are a relative measure of separation of a priori- defined groups. A zero (0) indicates that there is no difference among groups, while a one (1) indicates that all samples within groups are more similar to one another than any samples from different groups (Clarke & Gorley, 2001).

Weeks with clearly elevated biomass values of phytoplankton were related to total phosphorus (TP), total nitrogen (TN), $\text{NO}_3+\text{NO}_2\text{-N}$ (DIN), $\text{PO}_4\text{-P}$ (DIP), DIN: DIP ratio, temperature and salinity. The relationships between abiotic and biotic variables were tested by correlation, regression and multivariate BIO-ENV analysis. Some earlier studies in the Gulf of Finland have revealed that the time lag between a nutrient pulse and the response of phytoplankton is few days (Rantajärvi et al., 1998). Therefore DIN and DIP were taken with one-week differences compared with biotic variables for statistical analyses. Other abiotic variables used in analysis were with the same spatial and temporal coverage as the phytoplankton biomass.

For multivariate BIO-ENV analyses the software package PRIMER was used (Clarke & Warwick, 2001). This procedure takes into consideration that often more than one environmental variable explains the structure of biological communities. The BIO-ENV routine calculates a measure of agreement between the two similarity matrices, the fixed biotic triangular matrix and each of the possible abiotic triangular matrices. This is done

by rank correlating the matching elements in the two similarity matrices with a standard Spearman rank correlation coefficient (Clarke & Gorley, 2001).

MS EXCEL and software STATISTICA 6.0 (StatSoft, Inc., 2004) were used for figures.

Table 1. The dominant phytoplankton species in the Gulf of Finland that were used for statistical analysis (classification according to Hällfors, 2004)

Division	Class	Order	Species
Cyanophyta	<i>Nostocophyceae</i> (<i>Cyanophyceae</i>)	Oscillatoriales	Mainly from genera <i>Pseudanabaena</i> and <i>Planktolyngbya</i>
		Nostocales	<i>Anabaena</i> spp. <i>Aphanizomenon</i> sp. <i>Nodularia spumigena</i> Mertens
Cryptophyta	<i>Cryptophyceae</i>	Cryptomonadales	<i>Hemiselmis virescens</i> Droop <i>Plagioselmis prolunga</i> Butcher
Dinophyta	<i>Dinophyceae</i>	Prorocentrales	<i>Prorocentrum minimum</i> Schiller
		Peridinales	<i>Heterocapsa triquetra</i> Stein
Chrysophyta	<i>Diatomophyceae</i>	Eupodiscales	<i>Cyclotella</i> <i>chocktawhatcheeana</i> Prasad <i>Skeletonema costatum</i> Greville
		Bacillariales	<i>Cylindrotheca closterium</i> Reimann and J. Lewin
Euglenophyta	<i>Euglenophyceae</i>	Euglenales	<i>Eutreptiella gymnastica</i> Thronsen
Chlorophyta	<i>Chlorophyceae</i>	Chlorococcales	<i>Monoraphidium contortum</i> Komárková-Legnerová
	<i>Prasinophyceae</i>	Chlorodendrales	<i>Pyramimonas</i> spp.

3. RESULTS

3.1. Hydrometeorological and hydrophysical conditions

Usually, the minimums of the water temperature were recorded in the beginning of the summer in weeks 22-26 (minimum 5.2 °C; week 22, year 2002) and maximums in the end of July or in August in weeks 29-34 (maximum 23.2, week 31, year 2003). The mean water temperature only slightly varied (from 15.0 °C to 15.8 °C) at the sampling points of biological measurements in 1997-2004 (Fig.2).

The mean water salinity at the sampling points of biological measurements ranged between 5.1- 5.8 in 1997-2004 and it increased slowly along the study transect from the Finnish coast to the Estonian coast (Fig.3). Although water salinity varied between different weeks, no regular changes in temporal pattern of salinity were observed.

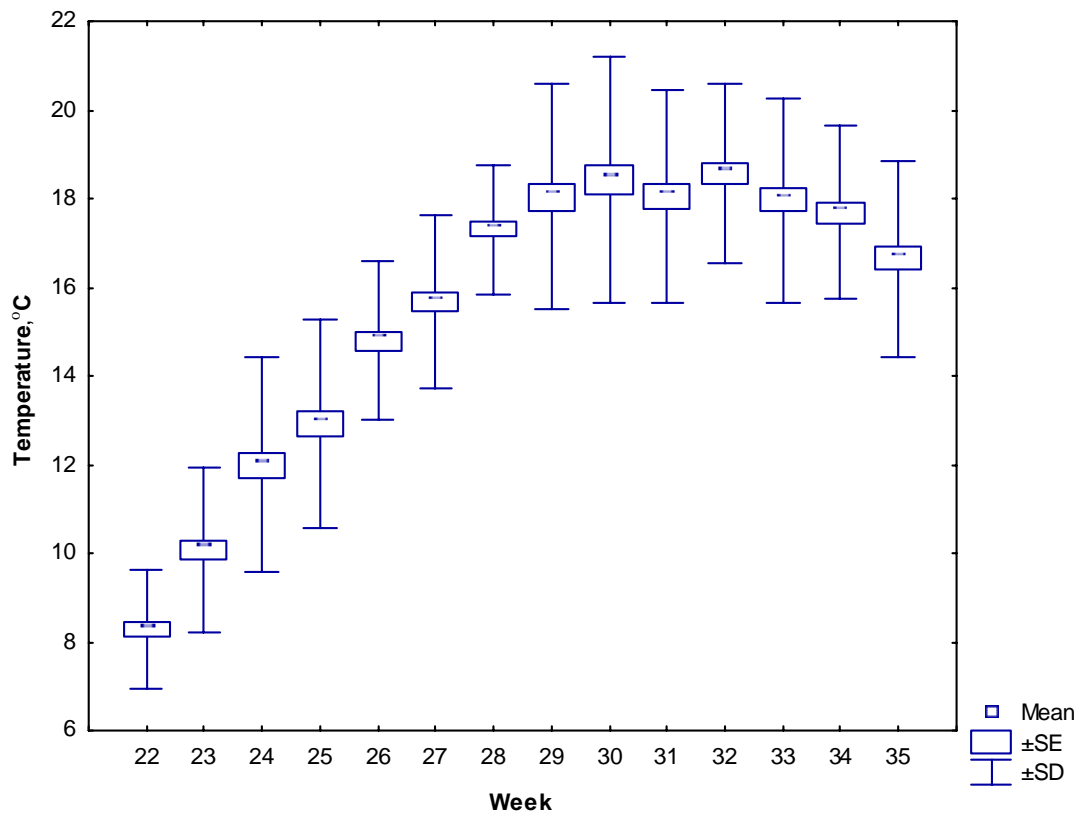
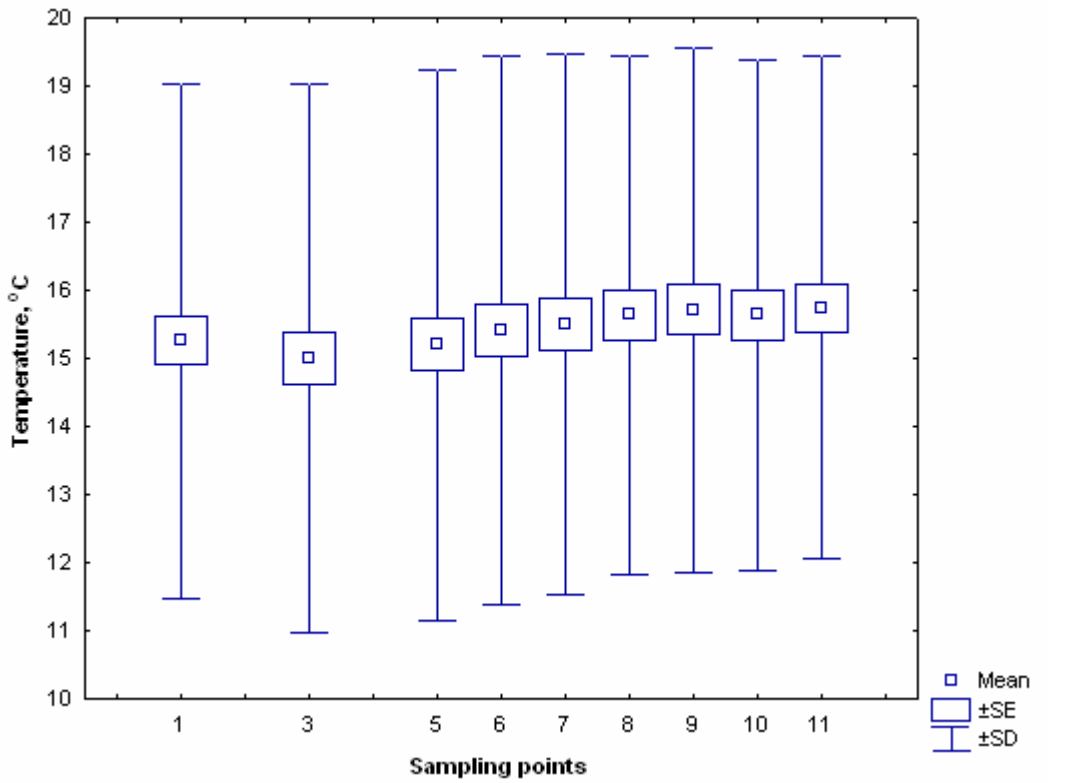


Fig. 2. Mean water temperature, standard error (SE) and standard deviation (SD) at 5 m depth at the sampling points WQ1-WQ11 (upper figure) and across the Gulf of Finland in weeks 22-35 (lower figure) in years 1997-2004.

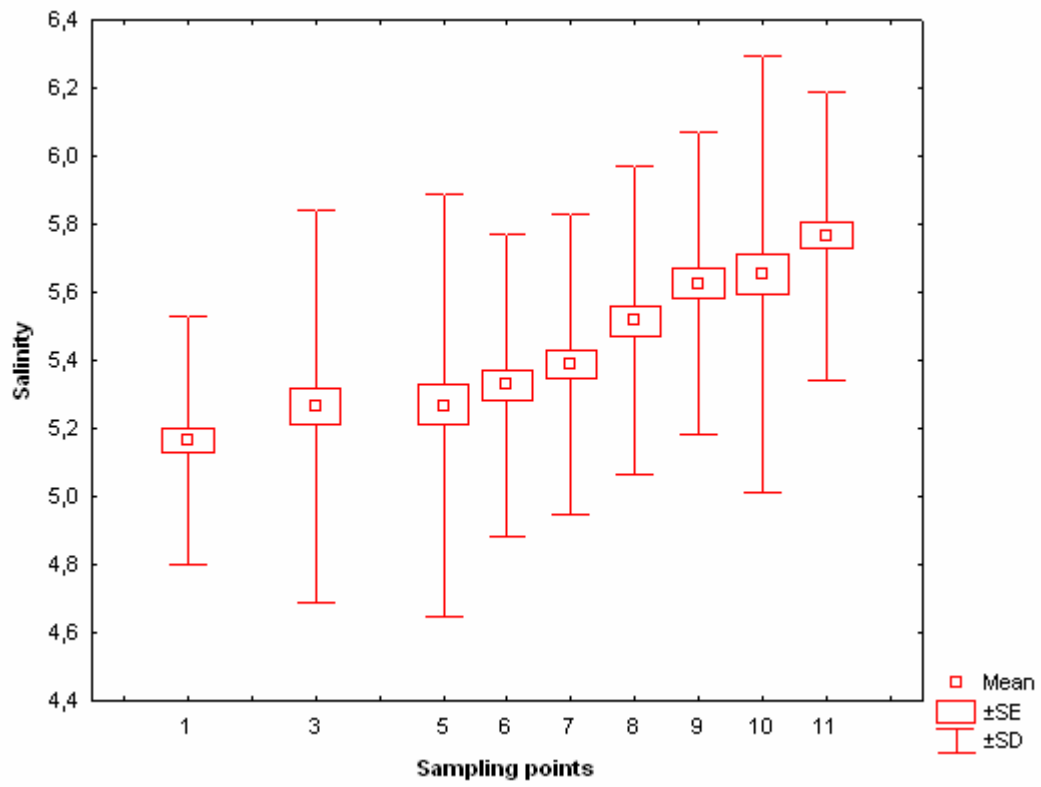


Fig. 3. Mean water salinity, standard error (SE) and standard deviation (SD) at 5 m depth at the sampling points in years 1997-2004.

3.2. Variation of nutrient concentrations

The concentrations of DIN and DIP remained low during the whole period of the study (Fig. 4 and 5). The measured concentrations ranged between 0.006 to 1.00 $\mu\text{mol l}^{-1}$ of DIP and 0.008 to 23.57 $\mu\text{mol l}^{-1}$ of DIN and were mostly close to the detection limits. The largest values of DIN and DIP were measured near to the Finnish coast at the sampling points of biological measurements WQ1 and WQ3. Also somewhat larger values were measured at the sampling points WQ10 and WQ11. The concentrations of DIN and DIP at the sampling points in 1997-2004 are shown in figure 4. Usually, the concentrations of DIP decreased in the first part and increased in the second part of the summer in 1997-2004. That kind of regular changes was not observed in the concentrations of DIN (Fig.5). The DIN: DIP ratio remained mainly below the Redfield molar ratio (N: P = 16: 1) and was very low (average value was 2.28) during the study period.

The mean concentrations of TP stayed between 0.69- 1.17 $\mu\text{mol l}^{-1}$ and TN between 24.68- 33.64 $\mu\text{mol l}^{-1}$ at the sampling points during the investigation period in 1997-2004 (Fig. 6). The mean concentrations of TP and TN were markedly larger at the sampling points WQ1 (1.17 $\mu\text{mol l}^{-1}$ TP; 33.64 $\mu\text{mol l}^{-1}$ TN) and WQ3 (0.89 $\mu\text{mol l}^{-1}$ TP; 27.28 $\mu\text{mol l}^{-1}$ TN) and slightly larger at the sampling points WQ10 (0.74 $\mu\text{mol l}^{-1}$ TP; 26.10 $\mu\text{mol l}^{-1}$ TN) and WQ11 (0.77 $\mu\text{mol l}^{-1}$ TP; 26.52 $\mu\text{mol l}^{-1}$ TN). The mean concentrations of TP, measured across the Gulf of Finland in different weeks in 1997-2004, varied between weeks and were its highest level in weeks 22-23. The mean concentrations of TN were somewhat larger in weeks 28-32 (Fig.7).

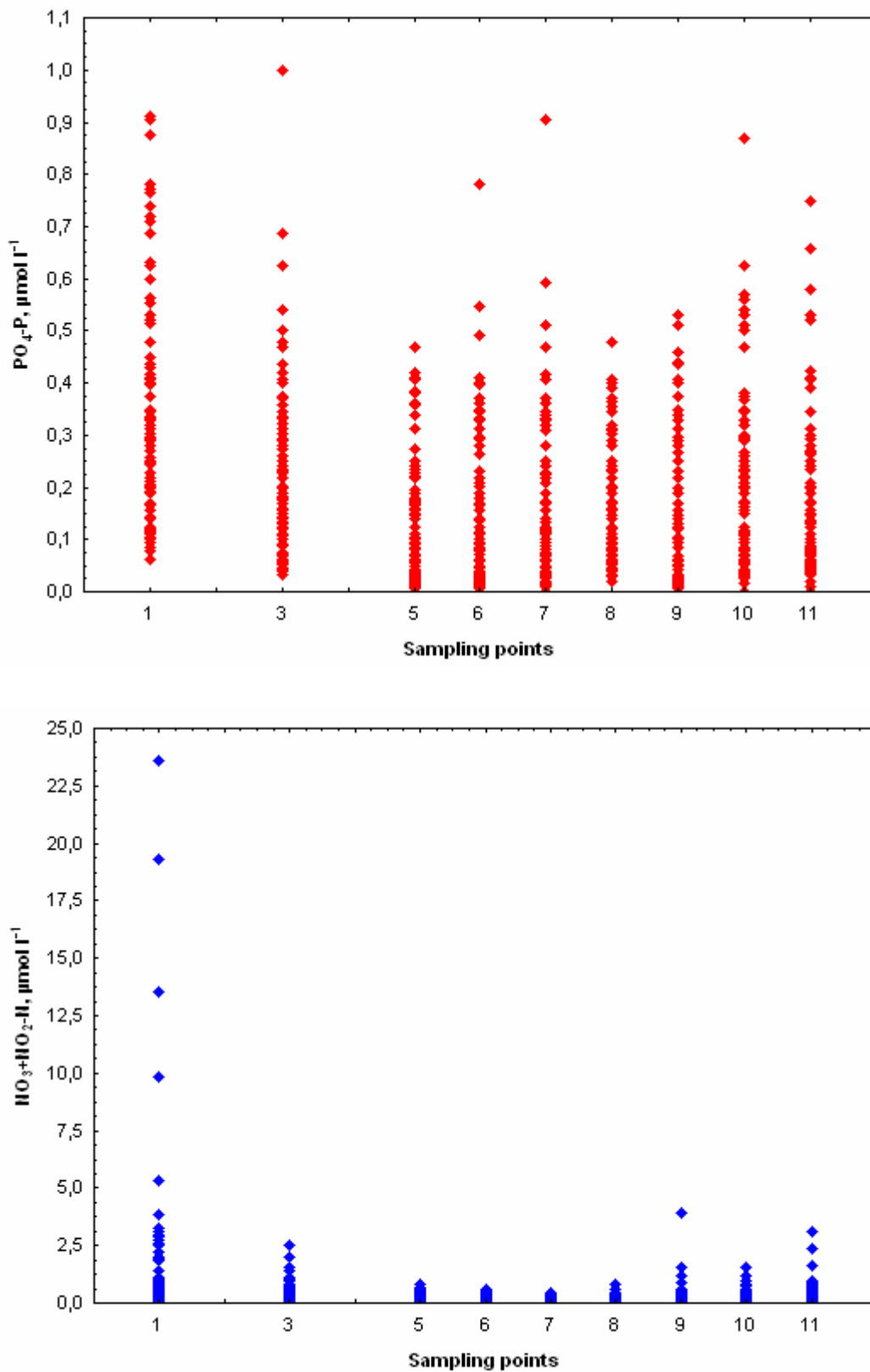


Fig. 4. The concentrations ($\mu\text{mol l}^{-1}$) of DIP ($\text{PO}_4\text{-P}$; upper figure) and DIN ($\text{NO}_3+\text{NO}_2\text{-N}$; lower figure) measured during the investigation period at the sampling points WQ1-WQ11 in years 1997-2004.

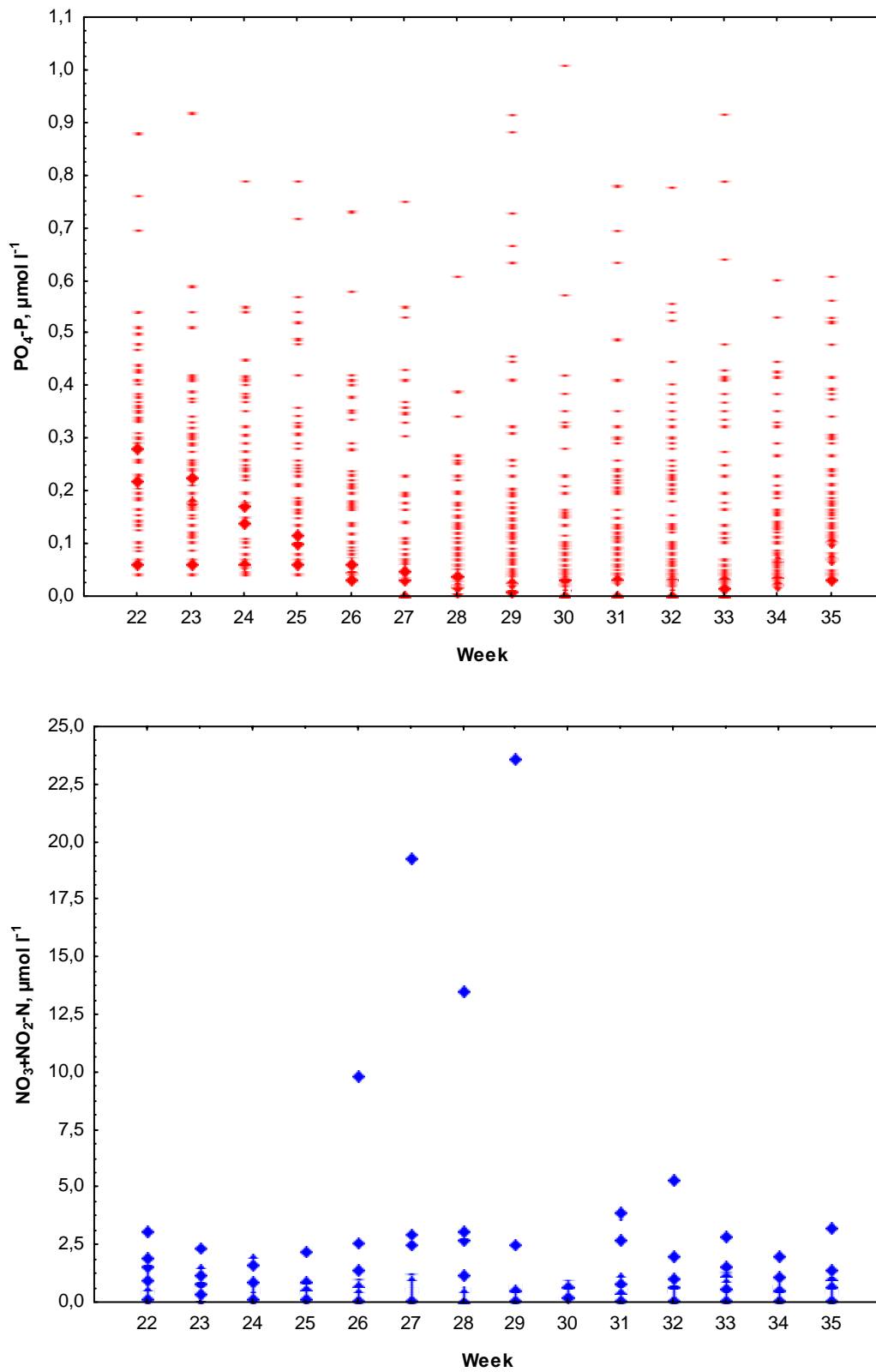


Fig. 5. The concentrations ($\mu\text{mol l}^{-1}$) of DIP ($\text{PO}_4\text{-P}$; upper figure) and DIN ($\text{NO}_3+\text{NO}_2\text{-N}$; lower figure) measured across the Gulf of Finland in weeks 22-35 in years 1997-2004.

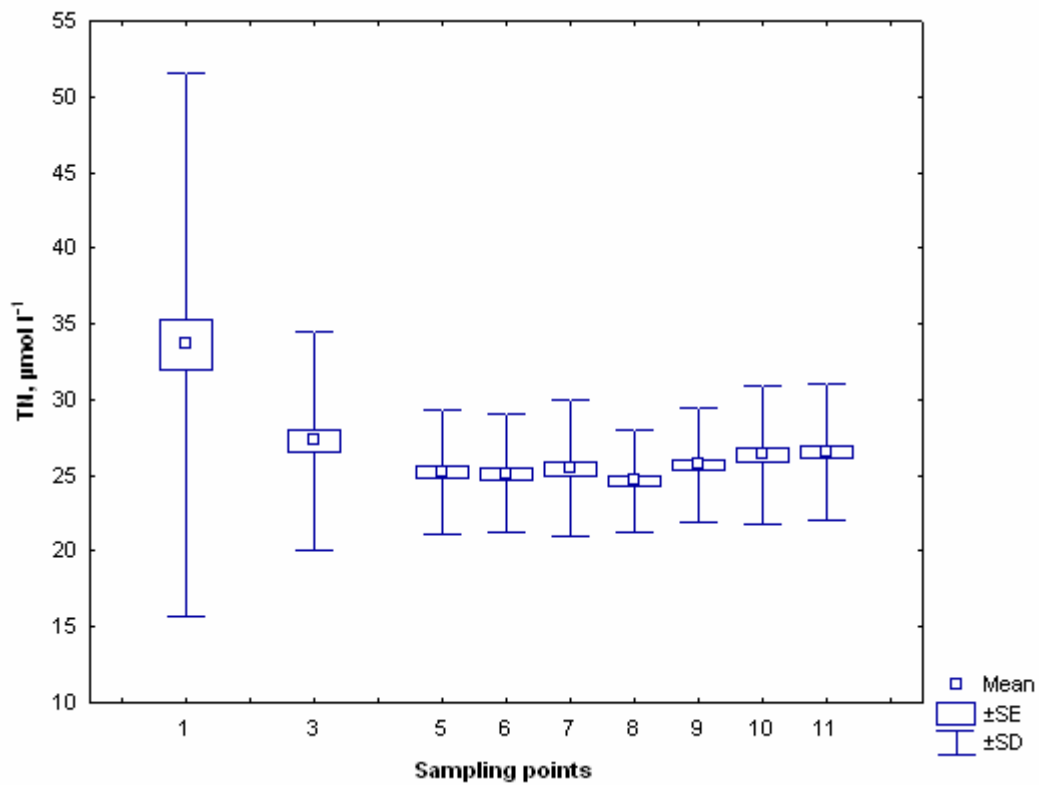
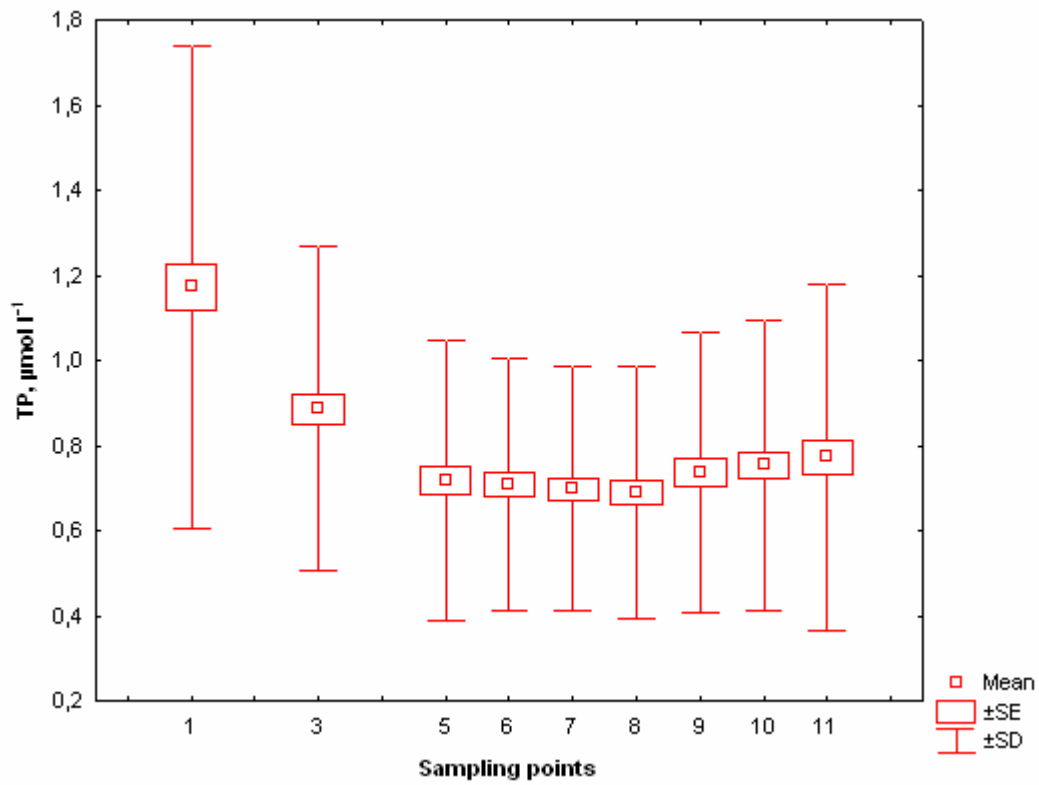


Fig. 6. Mean concentrations ($\mu\text{mol l}^{-1}$), standard error (SE) and standard deviation (SD) of total phosphorus (TP; upper figure) and total nitrogen (TN; lower figure) measured during the investigation period at the sampling points WQ1-WQ11 in years 1997-2004.

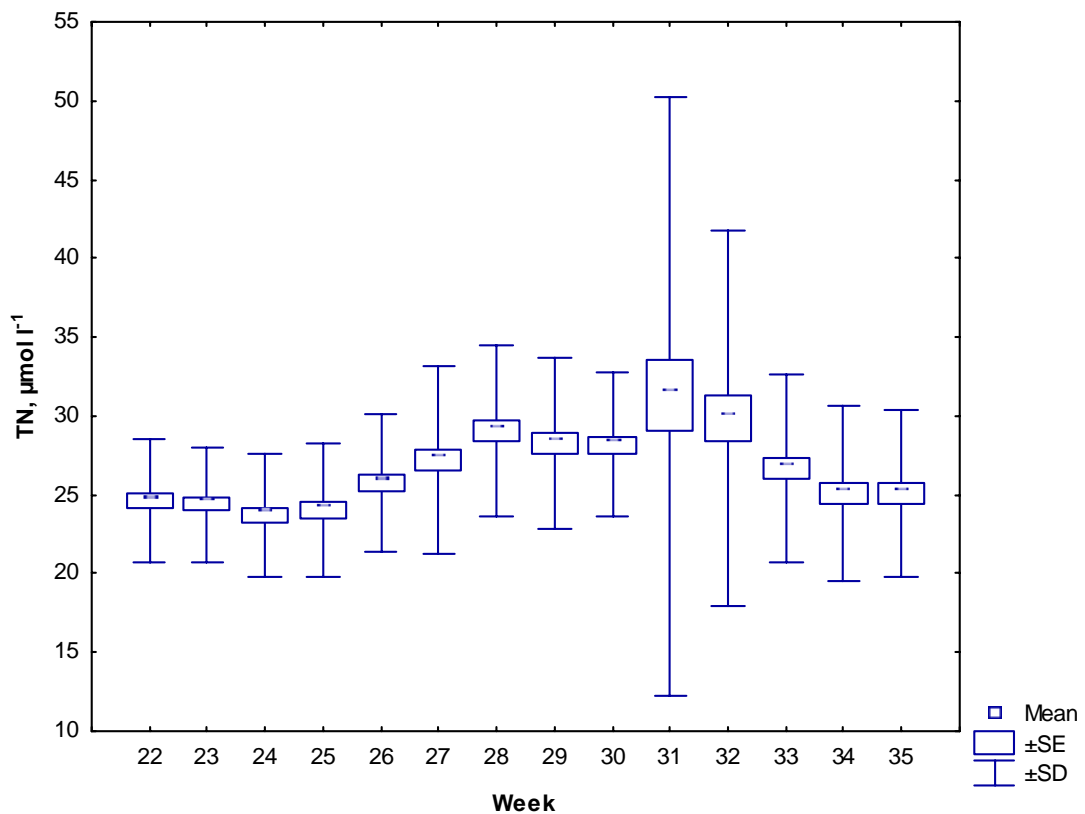
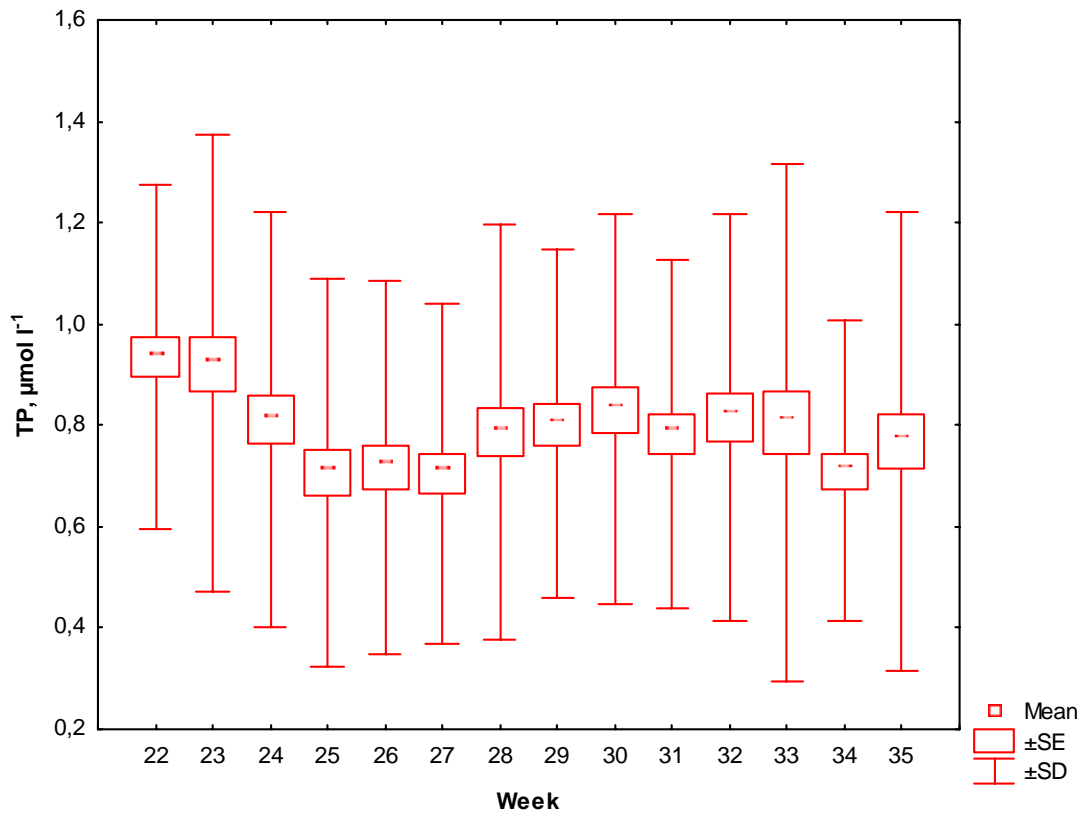


Fig. 7. Mean concentrations ($\mu\text{mol l}^{-1}$), standard error (SE) and standard deviation (SD) of total phosphorus (TP; upper figure) and total nitrogen (TN; lower figure) measured across the Gulf of Finland in weeks 22-35 in years 1997-2004.

3.3. Dynamics of phytoplankton

The short duration of the study (years 1997-2004) preclude any assessment of long-term changes in biomass of phytoplankton species. 8-year monitoring data still provide important information on assessment of inter-seasonal variability of the biomass of the phytoplankton. The inter-seasonal biomass of phytoplankton varied between different years. Still the general biomass pattern during the investigation period can be distinguished for each species (Fig. 8).

The maximum biomass values of *Skeletonema costatum* and *Monoraphidium contortum* were observed mainly in May. The biomass of *Aphanizomenon* sp. was larger in June-July, *Anabaena* spp., *Nodularia spumigena*, *Eutreptiella gymnastica* and *Pyramimonas* spp. in July. Oscillatoriales, *Hemiselmis virescens*, *Plagioselmis prolunga* and *Heterocapsa triquetra* reached their biomass peak generally in July-August. *Cyclotella choctawhatcheeana* and *Cylindrotheca closterium* showed the highest biomass in the August. The dinophyte *Prorocentrum minimum* appeared among dominant species only in the end of the August (mainly in years 1999 and 2003).

ANOSIM showed that weeks with clearly elevated biomass values were more similar to one another than any other weeks i.e. weeks with larger biomass differ significantly from other weeks. Weeks with clearly elevated biomass values and ANOSIM statistics R are shown in table 2.

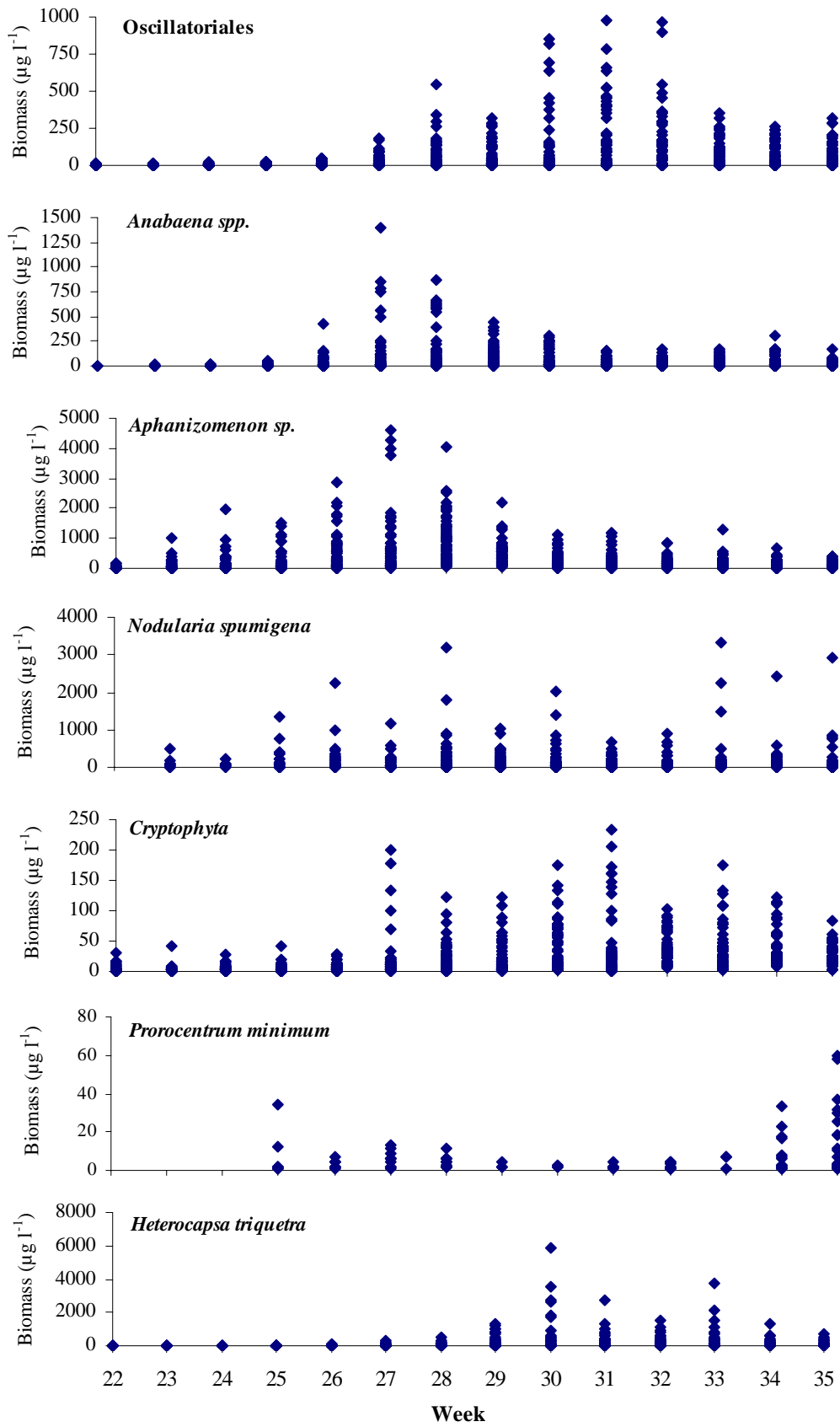


Fig.8. The biomass of dominant phytoplankton species measured across the Gulf of Finland in weeks 22-35 in 1997-2004.

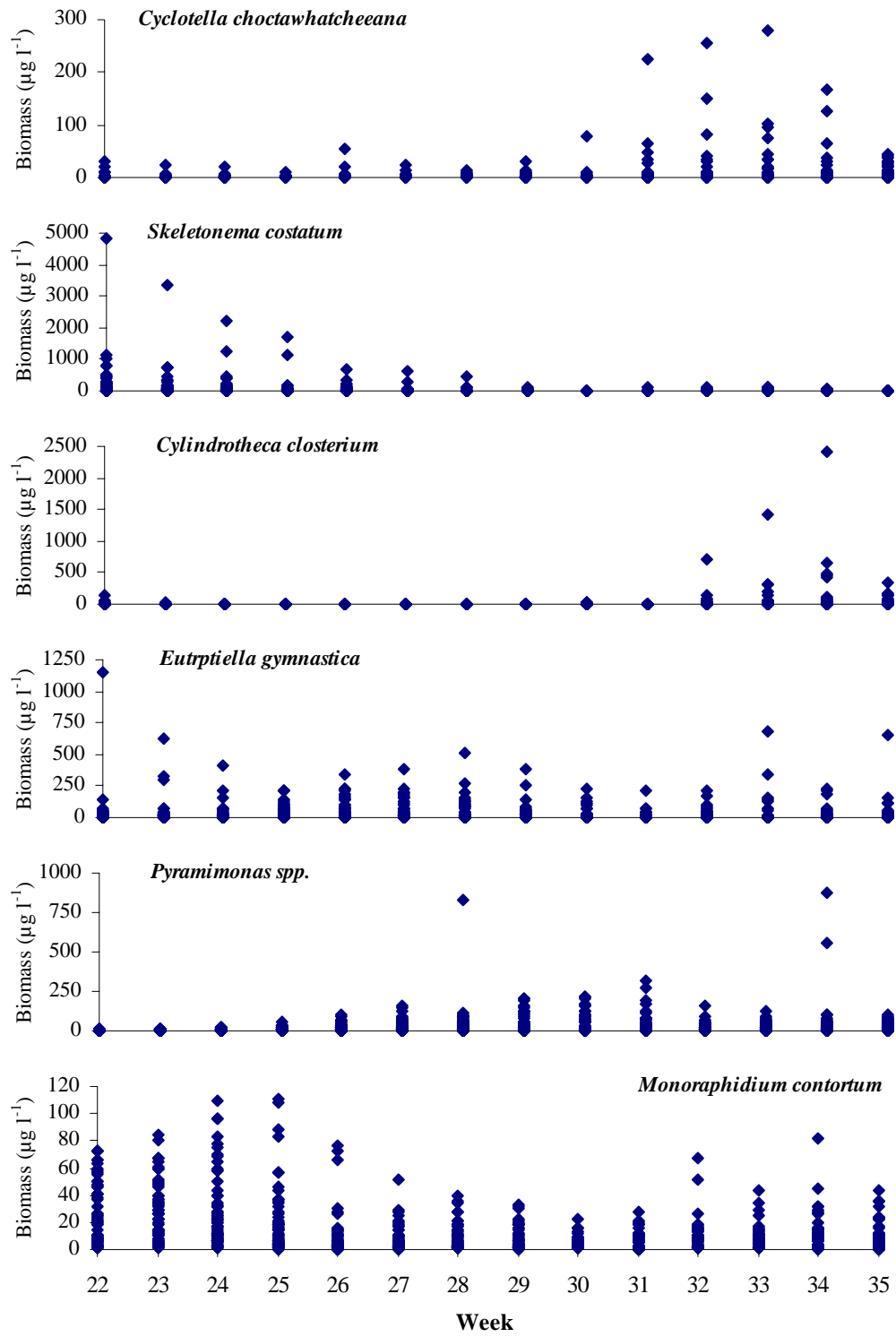


Fig. 8. *Continued*

Table 2. Weeks with larger biomass values in 1997-2004, ANOSIM statistic R and significance level of statistic R

Species	Selected weeks	ANOSIM statistic R	Significance level
Oscillatoriales	28-35	0,183	0,001
<i>Anabaena</i> spp.	27-30	0,139	0,001
<i>Aphanizomenon</i> sp.	25-31	0,232	0,001
<i>Nodularia spumigena</i>	27-31	0,199	0,001
<i>Hemiselmis virescens</i> <i>Plagioselmis prolunga</i>	27-34	0,307	0,001
<i>Prorocentrum minimum</i>	34-35	0,234	0,001
<i>Heterocapsa triquetra</i>	28-35	0,293	0,001
<i>Cyclotella choctawhatcheeana</i>	31-35	0,162	0,001
<i>Skeletonema costatum</i>	22-26	0,387	0,001
<i>Cylindrotheca closterium</i>	32-35	0,302	0,001
<i>Eutreptiella gymnastica</i>	25-28	0,065	0,04
<i>Pyramimonas</i> spp.	27-35	0,215	0,001
<i>Monoraphidium contortum</i>	22-26	0,097	0,001

The biomasses of *Heterocapsa triquetra*, *Cyclotella choctawhatcheeana*, *Skeletonema costatum*, *Cylindrotheca closterium* and *Eutreptiella gymnastica* were similarly to nutrient concentrations usually larger at the sampling points situated near the coast (mainly in Finnish coastal waters) than in central Gulf of Finland (Fig. 9.). Still ANOSIM-test showed only slight statistical differences between biomass values in stations situated near the coast and in central Gulf of Finland: *H. triquetra* (ANOSIM R=0,044; p=0,01), *C. choctawhatcheeana* (ANOSIM R=0,100; p=0,02), *S. costatum* (ANOSIM R=0,043; p=0,01), *C. closterium* (ANOSIM R=0,052; p=0,02) and *E. gymnastica* (ANOSIM R=0,081; p=0,001).

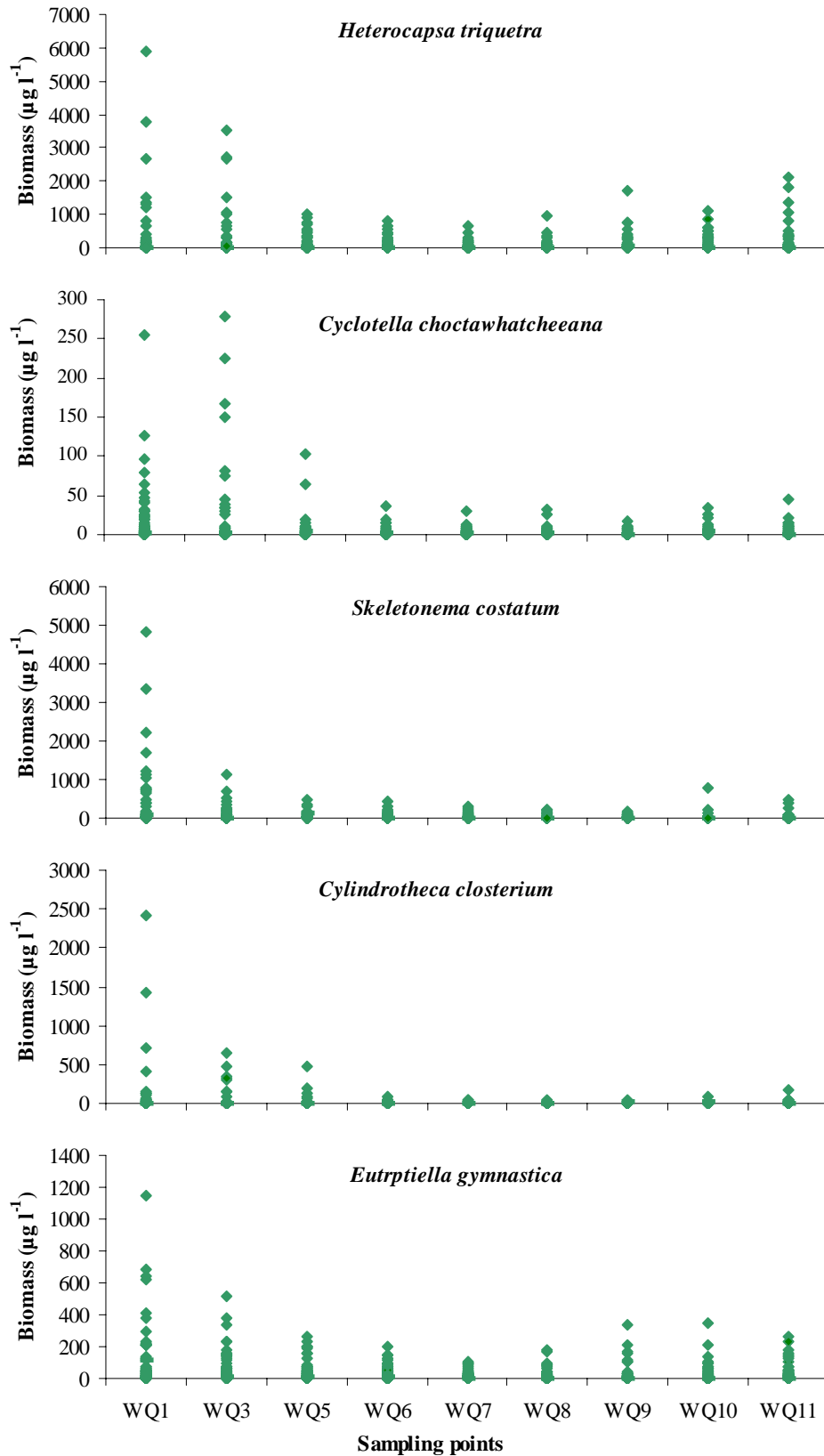


Fig. 9. The biomass of *H. triquetra*, *C. choctawhatcheana*, *S. costatum*, *C. closterium* and *E. gymnastica* measured during the investigation period along the Helsinki-Tallinn ferry route at the sampling points (WQ1-WQ11) in years 1997-2004.

3.4. The relationships between abiotic and biotic variables

The relationships between abiotic and biotic variables analysed by linear correlation, regression and multivariate BIO-ENV analysis are shown in table 3. The biomass of *Aphanizomenon* sp., *Anabaena* spp., *Nodularia spumigena* and Cryptomonadales did not give any significant positive correlation ($p > 0.001$) with nutrients. *N. spumigena*, Oscillatoriales and Cryptomonadales gave positive correlation ($p < 0.001$) with temperature. Oscillatoriales also gave positive results with TP ($p < 0.001$) and in addition BIO-ENV analyses that linked the biomass data to all studied abiotic variables showed correlation with TN and DIN: DIP ratio. BIO-ENV analyses showed that the nutrient variables that best grouped with the biomass variables of *Anabaena* spp. and *Aphanizomenon* sp. were DIP and DIN. *N. spumigena* grouped best with TP, DIN and temperature and Cryptomonadales with TP and TN.

The biomass of the dinophyte *Prorocentrum minimum* did not show any correlation with abiotic variables ($p > 0.001$). Still, multivariate BIO-ENV analyses gave the best match with TP and DIN. Correlation analysis showed positive correlation ($p < 0.001$) between another dinophyte *Heterocapsa triquetra* and nutrients DIP, TP and DIN and negative correlation with temperature. BIO-ENV analyses showed best link with salinity, DIN and DIN: DIP ratio.

The diatoms *Cyclotella choctawhatcheeana*, *Cylindrotheca closterium* and *Skeletonema costatum* showed strong relationship with nutrients. The biomass of *C. choctawhatcheeana* gave good positive correlation with TP, TN and DIN ($p < 0.001$), *C. closterium* with TP, DIP ($p < 0.001$) and *S. costatum* with TN, DIN and DIN:DIP ratio ($p < 0.001$). In addition *C. choctawhatcheeana* correlated negatively with salinity ($p < 0.001$). BIO-ENV analyses showed that the abiotic variables that best grouped with *C. choctawhatcheeana* were salinity, TP and TN. *C. closterium* grouped with TP and TN and *S. costatum* with temperature, TP and TN.

The biomass of the euglenophyte *Eutreptiella gymnastica* did not show any correlation with abiotic variables ($p > 0.001$). Still, multivariate BIO-ENV analyses showed positive correlation with TP, DIN and salinity.

The biomass of *Pyramimonas* spp. showed positive correlation and *Monoraphidium contortum* negative with TN ($p < 0.001$). *M. contortum* correlated negatively with salinity

and temperature and *Pyramimonas* spp. with salinity ($p < 0.001$). Multivariate BIO-ENV analyses grouped *Pyramimonas* spp. with temperature, salinity and TN and *M. contortum* with temperature, salinity and TP.

Table 3. Coefficients of determination (r^2), correlation (r) and Spearman correlation (BIO-ENV) between phytoplankton biomass and abiotic variables. Values with $p < 0.001$ are marked with bold; n= number of samples

		Temperature	Salinity	TP	PO ₄ -P	TN	NO ₃ +NO ₂ -N	DIN:DIP
<i>Oscillatoriales</i>	r	0,24	-0,17	0,38	0,02	0,11	-0,01	-0,04
Weeks 28-35	r^2	0,06	0,03	0,15	0,00	0,01	0,00	0,00
n=575	BIO-ENV	0,013	0,013	0,069	0,000	0,057	0,037	0,050
<i>Anabaena</i> spp.	r	-0,06	-0,21	0,01	-0,15	0,02	-0,08	-0,05
Weeks 27-30	r^2	0,00	0,04	0,00	0,02	0,00	0,01	0,00
n=288	BIO-ENV	0,031	0,136	0,025	0,080	0,039	0,110	0,055
<i>Aphanizomenon</i> sp.	r	0,03	-0,11	-0,08	-0,21	0,03	-0,07	-0,04
Weeks 25-31	r^2	0,00	0,01	0,01	0,04	0,00	0,01	0,00
n=501	BIO-ENV	0,088	-0,010	0,048	0,138	-0,006	0,062	0,065
<i>Nodularia spumigena</i>	r	0,23	-0,05	-0,22	-0,19	0,05	-0,07	-0,05
Weeks 27-31	r^2	0,05	0,00	0,05	0,04	0,00	0,00	0,00
n=359	BIO-ENV	0,087	0,025	0,063	0,090	0,026	0,066	0,073
<i>Hemiselmis virescens</i>	r	0,21	-0,05	-0,21	-0,22	0,09	-0,04	0,06
<i>Plagioselmis prolonga</i>	r^2	0,04	0,00	0,04	0,05	0,01	0,00	0,00
Weeks 27-34	BIO-ENV	-0,019	0,024	0,039	0,009	0,054	0,006	-0,018
n=575								
<i>Prorocentrum minimum</i>	r	-0,04	-0,05	0,24	-0,08	0,00	-0,16	-0,10
Weeks 34-35	r^2	0,00	0,00	0,06	0,01	0,00	0,03	0,01
n=144	BIO-ENV	0,010	-0,073	0,075	0,002	-0,082	0,061	-0,016
<i>Heterocapsa triquetra</i>	r	-0,19	0,00	0,23	0,23	0,03	0,26	0,03
Weeks 28-35	r^2	0,04	0,00	0,05	0,05	0,00	0,07	0,00
n=575	BIO-ENV	0,020	0,088	0,026	0,028	0,039	0,071	0,108
<i>Cyclotella choctawhatcheeana</i>	r	0,10	-0,34	0,34	0,16	0,19	0,18	-0,03
Weeks 31-35	r^2	0,01	0,11	0,12	0,03	0,04	0,03	0,00
n=360	BIO-ENV	-0,015	0,073	0,162	0,090	0,064	0,050	-0,022
<i>Skeletonema costatum</i>	r	-0,12	-0,16	0,14	0,03	0,31	0,31	0,22
Weeks 22-26	r^2	0,01	0,02	0,02	0,00	0,10	0,10	0,05
n=288	BIO-ENV	0,105	0,020	0,099	0,052	0,111	0,028	0,043
<i>Cylindrotheca closterium</i>	r	0,06	-0,12	0,44	0,21	0,04	-0,01	-0,06
Weeks 32-35	r^2	0,00	0,01	0,20	0,05	0,00	0,00	0,00
n=288	BIO-ENV	-0,004	0,080	0,203	0,013	-0,026	0,084	0,021
<i>Eutreptiella gymnastica</i>	r	0,04	0,01	0,01	0,01	0,00	0,00	0,00
Weeks 25-28	r^2	0,19	-0,10	-0,10	-0,10	0,07	-0,03	-0,05
n=288	BIO-ENV	-0,010	0,025	0,061	0,018	-0,001	0,039	0,003
<i>Pyramimonas</i> spp.	r	0,21	-0,19	-0,02	-0,13	0,21	0,00	0,07
Weeks 27-35	r^2	0,04	0,04	0,00	0,02	0,04	0,00	0,01
n=597	BIO-ENV	0,062	0,090	0,014	0,052	0,075	0,021	0,012
<i>Monoraphidium contortum</i>	r	-0,20	-0,32	0,02	0,04	-0,16	-0,04	-0,01
Weeks 22-26	r^2	0,04	0,10	0,00	0,00	0,03	0,00	0,00
n=288	BIO-ENV	0,040	0,108	0,126	0,017	0,098	-0,047	-0,021

4. DISCUSSION

Solar radiation and nutrient availability are the dominant physical factors controlling phytoplankton production in the sea. The most common use of the term eutrophication is related to inputs of mineral nutrients, primarily nitrogen and phosphorus, to coastal waters. The Gulf of Finland is the sub-basin of the Baltic Sea that is most seriously affected by the effects and consequences of eutrophication. Analyzing long-term physical, chemical and biological data (1980–2002), Lundberg et al. (2005) brought out clear differences between coastal and offshore areas. During the 1990s the nutrient discharges from the catchment area of the Gulf of Finland decreased with 40% and the reductions are especially clear for the southern, i. e. Estonian coastal waters (Pitkänen et al., 2001). Thus, the differences in phytoplankton dominance on both coastal open sea gradient and between Finnish and Estonian coastal stations might be expected. Some species were distinguished (*Heterocapsa triquetra*, *Skeletonema costatum*, *Cyclotella choctawhatcheeana*, *Cylindrotheca closterium* and *Eutreptiella gymnastica*) that had usually larger biomass at the stations close to the coast, mainly in Finnish coastal waters.

Generally phytoplankton biomass is nitrogen-limited in the Gulf of Finland (Granéli et al., 1990; Kivi et al., 1993; Lignell et al., 2003), an exception being the filamentous cyanobacteria, which are able to fix gaseous nitrogen. Among the selected cyanobacterial taxa only non-N₂-fixing Oscillatoriales gave positive relationship with TP. Oscillatoriales showed also good positive correlation with temperature and negative correlation with salinity indicating its freshwater origin and preference for warm water. Based on recent plankton data and concerning the relationship between plankton and trophic level, Finni et al. (2001b) attribute the dominance of oscillatorean cyanobacteria to considerably eutrophic or hypereutrophic conditions. On the other hand, Nixdorf et al. (2003) also state that the dominance of Oscillatoriales in lakes is a result of the anthropogenic induced eutrophication process, but analyzing phytoplankton data from shallow hypertrophic lakes they concluded that turbid mixed layers with highly light deficient conditions seem to be the key factors favoring the dominance of some oscillatorean genera like *Pseudanabaena*. Some earlier investigations also suggest that Oscillatoriales are able to outcompete N₂-fixing cyanobacteria under declining photosynthetically active radiation (references therein).

The cyanobacterial surface accumulations first occurred in the Baltic Sea in the 19th century (Finni et al., 2001a) and these are natural phenomena (Kahru et al., 1994; Finni et al., 2001a; Bianchi et al., 2000). In our study the biomass of these species (*Anabaena* spp., *Aphanizomenon* sp. and *Nodularia spumigena*) showed positive relationship neither with dissolved nutrients nor total nitrogen or phosphorus. Therefore only the blooming of blue-green algae may not, by itself, be a sign of high trophic conditions, suggested also by Gasiùnaite et al., 2005.

Cryptophytes *Hemiselmis virescens* and *Plagioselmis prolunga* gave the strongest positive relationship with temperature and based on the current results it seems that they are not indicative for the assessment of eutrophication level.

Many of the common dinoflagellates may potentially be mixotrophic species, partly independent of ambient nutrient conditions (Jacobson & Andersen, 1994; Gasiùnaite et al., 2005). Abundance of the Baltic Sea *Prorocentrum minimum* has been generally not related to salinity or temperature (Hajdu et al., 2004). It could be a dominant species at both high and low salinity (from 4.8 to 15), and its temperature range is broad (from 2.7 to 26.4 °C). Also, any relationships between *P. minimum* and salinity or temperature were found in present study. However, dense populations of *P. minimum* usually occur from July to October at temperatures above 10 °C and the study period (June to August) may be too limited for final conclusions. Still, multivariate BIO-ENV analyses showed positive relationship between dinophyte *P. minimum* and TP, DIN. A positive correlation between the successes of *P. minimum* in the Baltic Sea and high concentrations of TP and TN has previously observed by Hajdu et al. (2004) and Pertola et al. (2004). However, the mass occurrence of *P. minimum* in the northern Baltic areas is probably related to intrusion of water masses from southern basins under favourable meteorological and hydrodynamical conditions.

The dinophyte *Heterocapsa triquetra* showed strong relationship with TP, DIP and DIN. This species is often causing red-tides (discoloured water) and is probably favoured by frequent mixing with more continuous supply of nutrients, for example by a stimulating effect of water movements caused by ferries (Lindholm & Nummelin, 1999). The same authors relate the development of red tide to high phosphorus level and low TN:TP ratio with earlier indications of success of *H. triquetra* in quite eutrophic conditions (e. g. Niemi & Hällfors, 1974). However, Olli & Seppälä (2001) demonstrated apparent vertical movements of *H. triquetra* (diurnal surface and

nocturnal deep aggregations) in a mesocosm experiment. This leads to the speculations that some motile and migratory active species are greatly independent on the availability of nutrient supply in the mixed surface layers.

Diatoms generally prevail during the first phase of the succession (spring bloom) in temperate and boreal coastal areas and they are generally adapted to more turbulent environments characterized by high nutrient concentrations (Margalef, 1978). Some diatom species are still a significant part of summer and autumn phytoplankton communities as well (Baltic Sea incl.) and may form secondary biomass peaks. Biomass of the diatoms *Cyclotella choctawhatcheana*, *Cylindrotheca closterium* and *Skeletonema costatum* were well related to the nutrients and similarly to nutrient concentrations, the highest values of biomasses were observed at the sampling points situated near the coast (WQ1 and WQ3). *C. choctawhatcheana* and *C. closterium* showed strongest relationship with TP and *S. costatum* with TN and DIN. On the contrary to our study a nutrient enrichment experiment conducted by Lagus et al. (2004) revealed that *S. costatum* is P-limited. In despite that difference seems that *C. choctawhatcheana*, *C. closterium* and *S. costatum* may be reliable indicators in terms of eutrophication. The biomass of diatoms didn't correlate with temperature or salinity with an exception of the biomass of the diatom *C. choctawhatcheana* that correlated negatively with salinity. This may set some limits in using the species as water quality indicator in larger, more than basin-wide scales or requires salinity adjustments when giving numerical values for certain quality classes.

The biomass of the euglenophyte *Eutreptiella gymnastica* was positively related with TP and DIN. Olli et al. (1996) characterize this species as having a particular combination of environmental adaptations like high growth potential in environments of elevated nutrient levels and flexible behavior (vertical migrations, cyst formation). In our material, *E. gymnastica* reached bloom-like concentrations almost throughout the investigation period and showed rather irregular shape of spatio-temporal distribution. It is true that the absolute maximum biomass values were recorded in coastal sites, but the clearly opportunistic character of such short-term peaks does not allow relating *E. gymnastica* blooms to the background biogenic level unambiguously.

The biomass of prasinophyte *Pyramimonas* spp. was positively related with TN and temperature and negatively with salinity. More information about *Pyramimonas* spp. will be needed for making any conclusions.

The chlorophyte *Monoraphidium contortum* has been observed by Kononen (1988) and Kuosa (1988) to be abundant at relatively low salinities (from 3 to 4), high phosphorus concentrations and low N:P ratios. Multivariate BIO-ENV analyses showed that the abiotic variables that best grouped with the biomass of *M. contortum* were TP, salinity and temperature. However correlation analyses gave strong negative correlation with salinity and temperature and any correlation with enhanced nutrients.

SUMMARY

The increase in nutrient input leads directly to an increase in phytoplankton biomass and phytoplankton biomass may serve as an indicator of the trophic state. The main aim of the present thesis was identify the phytoplankton taxa, which wet weight biomass shows good statistical relationship with the concentrations of the enhanced nutrients and is independent of temperature or salinity and therefore may prove indicative for the assessment of eutrophication. It was possible to distinguish the general temporal inter-seasonal biomass pattern for each species during the investigation period. In addition the biomasses of *Heterocapsa triquetra*, *Cyclotella choctawhatcheeana*, *Skeletonema costatum*, *Cylindrotheca closterium* and *Eutreptiella gymnastica* were similarly to nutrients usually larger at the sampling points situated near the coast than in the central Gulf of Finland.

The relationships between abiotic and biotic variables were analysed by linear correlation, regression and multivariate BIO-ENV analysis. *Anabaena* spp., *Aphanizomenon* sp. and *Nodularia spumigena* showed no positive correlations with nutrients. Also based on the current study seems that Cryptophytes, *Monoraphidium contortum* and *Pyramimonas* spp. that correlated with temperature or salinity are not indicative for the assessment of eutrophication level. The author suggests that some species are opportunistic and the short-term changes in their biomass are not related to the eutrophication levels, for example *E. gymnastica*, *H. triquetra*. Among 14 selected taxa only three species (the diatoms *S. costatum*, *C. choctawhatcheeana* and *C. closterium*) were found that responded regularly to enhanced nutrient concentrations being larger in coastal areas. Thus, these species are suggested as potential and reliable indicators in terms of eutrophication. Also the biomass of diatoms didn't correlate with temperature or salinity with an exception of the biomass of the diatom *C. choctawhatcheeana* that correlated negatively with salinity. This may set some limits in using the species as water quality indicator in larger, more than basin-wide scales or requires salinity adjustments when giving numerical values for certain quality classes. In addition, the gradient of the nutrients used in present work might have been too restrictive and for further work more data is needed from sea areas with different trophic and hydrological conditions.

KOKKUVÕTE (Summary in Estonian)

Potentsiaalsed eutrofeerumise indikaatorliigid Läänemere põhjaosa suvises fütoplanktonis

Kaire Toming

Veetaimestiku, sh fütoplanktoni, liigilist koosseisu, arvukust ja biomassi loetakse pinnavee bioloogilisteks kvaliteedinäitajateks vastavalt Euroopa Liidu Veepoliitika Raamdirektiivile. Toitainete juurdevool põhjustab fütoplanktoni biomassi suurenemise ning sellest tulenevalt on fütoplanktoni biomass heaks indikaatoriks vee kvaliteedi hindamisel. Magistritöö peamine eesmärk on leida fütoplanktoni liigid, mille biomasside ja suurenenud toitainete sisalduste vahel esineb statistiliselt usaldusväärne seos ning mis on samal ajal sõltumatud vee soolsusest ja temperatuurist.

Leidmaks, milline fütoplanktoni liik võib osutada indikatiivseks veekeskkonnas sisalduvate toitainete suhtes, analüüsiti 14 Soome lahe suvise fütoplanktoni dominantliigi biomasside sesoonseid mustreid ning veekeskkonna hüdrograafilisi ja keemilisi parameetreid. Kasutatud andmed on kogutud Soome lahe keskosast Tallinn-Helsingi vahel sõitvate spetsiaalse automaatse proovivõtmise aparatuuriga varustatud reisilaevade abil suveperioodil (nädalad 22-35) aastatel 1997-2004. Iga liigi biomassi jaoks joonistus välja üldine temale omane ajaline muster. Sarnaselt toitainetele olid *Heterocapsa triquetra*, *Cyclotella choctawhatcheeana*, *Skeletonema costatum*'i ja *Cylindrotheca closterium*'i biomassid suuremad rannalähedastes jaamades, võrreldes Soome lahe keskosaga.

Biootiliste ja abiootiliste tegurite vahelise seose uurimiseks kasutati regressioon- ja korrelatsioonanalüüsi ning mitmemõõtmelist BIO-ENV analüüsi. *Anabaena* spp., *Aphanizomenon* sp. ja *Nodularia spumigena* biomasside ja toitainete sisalduse vahel positiivset usaldusväärset seost ei esinenud. Samuti võib käesolevale tööle tuginedes öelda, et temperatuuri või soolsusega tugevalt korreleerunud *Monoraphidium contortum*, *Pyramimonas* spp. ja krüptofüüdid ei ole sobilikud troofsustaseme hindamiseks. Lisaks on töös vaadeldud liikidest mõned (nt *Eutreptiella gymnastica*, *Heterocapsa triquetra*) pigem oportunistid lühiajaliste muutuste suhtes ning töö autor ei seostaks neid konkreetse mereala troofsustasemega. 14 dominantliigi hulgast reageerisid suurenenud toitainete sisaldusele korrapäraselt vaid kolme liigi biomassid (ränivetikad

Skeletonema costatum, *Cyclotella choctawhatcheeana* ja *Cylindrotheca closterium*). Lisaks olid nimetatud liikide biomassid suuremad rannalähedastes jaamades ning joonistus välja ka biomasside üldine ajaline muster. Seega võiksid nimetatud liigid olla usaldusväärsed indikaatorid vee troofsustaseme hindamisel. Samuti puudus üldjuhul korrelatsioon ränivetikate ning soolsuse ja temperatuuri vahel. Erandiks oli soolsusega negatiivselt korreleerunud *C. choctawhatcheeana*, mis võib seada teatud piirid nimetatud liigi kasutamisel vee kvaliteedi indikaatorina.

Kokkuvõtteks tuleb lisada, et töös kasutatud toitainete gradient võis olla liiga kitsapiiriline ning sellest tulenevalt oleks edasistesse uurimustesse kasulik kaasata erineva troofsuse ja hüdroloogiliste tingimustega merealaid.

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