On the issue of limiting nutrient and predictions of cyanobacteria in aquatic systems

Lars Håkanson*, Andreas C. Bryhn and Julia K. Hytteborn, Dept. of Earth Sciences, Uppsala Univ., Villav. 16, 752 36 Uppsala, Sweden

*Corresponding author: Lars.Hakanson@geo.uu.se

Abstract

This study aims at bridging the gap between freshwater and marine eutrophication studies by presenting (1) a cross-system analysis of the relationship between chlorophyll and the total nitrogen (TN) to total phosphorus (TP) ratio (2) a general model to predict concentrations of cyanobacteria from data on TP, the TN/TP-ratio, salinity and temperature, and (3) a general trophic level classification for aquatic systems based on chlorophyll classes (for oligo-, meso-, eu- and hypertrophic systems). The data compiled in this study concerns more than 500 lakes and coastal areas covering a very wide domain in terms of nutrient concentrations and salinity. There was no simple relationship between the TN/TP-ratio and empirical chlorophyll concentrations or concentrations of cyanobacteria. Variations in TP rather than TN generally seem to be more important to predict variations among systems in chlorophyll-a and cyanobacteria. Different “bioavailable” forms of the nutrients (DIN, DIP, phosphate, nitrate, etc.) have been shown to have very high coefficients of variation (CV), which means that many samples are needed to obtain reliable empirical data which are necessary in models aiming for high predictive power and practical usefulness.

Key words: Chlorophyll, cyanobacteria, phosphorus, nitrogen, salinity, temperature, lakes, marine systems, empirical data, models

Originally published in **Science of the Total Environment 2007, issue 379, pages 89-108.**

<http://dx.doi.org/10.1016/j.scitotenv.2007.03.009>

© Elsevier Publishers. **Note that there may be minor differences between this version and the published version. In particular, BG is sometimes used as an abbreviation of bluegreens, while CB is instead used as an abbreviation for cyanobacteria in the printed version.**

Introduction, background and aim

Eutrophication is a common problem to both freshwater and marine ecosystems. Important objectives in the aquatic sciences are therefore to find general patterns and relationships that explain how nutrients affect algal growth in various ecosystems and to quantify the extent to which ecosystems differ in their response to nutrient loads and concentrations. Photolithoautotrophic bacteria (traditionally called bluegreen algae; hereafter referred to as cyanobacteria, CB), play two key roles in eutrophication contexts: they can form extensive nuisance blooms that may be toxic (Smith, 2003), and many cyanobacterial species can fix large amounts of dissolved gaseous nitrogen of atmospheric origin (Rahm et al., 2000; Tönno, 2004).

This study aims at highlighting important issues related to the variability and representativity of data, discussing predictive models for chlorophyll concentrations and investigating how chlorophyll is related to total nitrogen (TN), total phosphorus (TP) and salinity, predicting the biomass of cyanobacteria, and finally, at introducing a general scheme to classify aquatic systems into the classical categories oligotrophic, mesotrophic, eutrophic and hypertrophic. This scheme will be based on algorithms to predict chlorophyll from TP, TN and salinity, and on other results from this work regarding cyanobacteria. The paper takes an ecosystem perspective. Society and science are more interested in the conditions in lakes and coastal areas rather than at sampling stations. The focus is set on lakes and coastal areas and not on rivers and open marine sites.

Fig. 1 illustrates key questions addressed in this work. It shows the main processes regulating fluxes (atmospheric input, river inflow) of nutrients (nitrogen and phosphorus) to a given aquatic system, internal fluxes (sedimentation, resuspension, diffusion, denitrification and burial) including the very important relationship between the amount of the nutrient in dissolved (bioavailable) form and the amount in particulate form (the only part that can settle out due to gravity). The average composition of algae is given by the Redfield ratio (7.2 by mass; see Redfield et al., 1963) and 16:1 (by atoms). So, by definition, all algae need both nitrogen and phosphorus and one focus of this work concerns the factors limiting the primary production as expressed by chlorophyll-a concentrations in the water.

Fig. 1. Overview of important transport processes and mechanisms related to the concept of “limiting” nutrient.

A crucial point raised in fig. 1 concerns the equilibria between nutrients in dissolved and particulate phases, the time scales of these interactions and what is actually meant by “limiting” nutrient. At short time scales (seconds to days), it is evident that the causal agent regulating/limiting biouptake and primary production is the concentration of the nutrient in bioavailable forms (as illustrated in fig. 2A). This also implies that the bioavailable forms are very variable (see the coming section on data and methods) and it is known from, e.g., Lake Balaton (Istvanovics and Somlyódy, 2001) that in highly productive systems there may be difficulties to actually measure nutrients in dissolved forms, especially phosphorus, since these forms are picked up so rapidly by the algae. This also explains why there are no practically useful predictive models for chlorophyll based on dissolved forms, but rather on total-N (TN) or total-P (TP; see tables 1 and 2). So, at longer time scales (weeks to years), and for all practical purposes in water management, one must recognize the difference between what is actually, causally the “limiting” agent in primary production at short time scales, and what is the form of the nutrient limiting predictions of chlorophyll (or cyanobacteria) at the ecosystem scale for longer periods of time. This paper is focused on the latter aspects, and the time scale is set to the growing season (mainly months June, July and August). Most of the data emanate from the temperate zone of the Northern Hemisphere.

Table 1. Regressions illustrating the key role of total phosphorus (TP) in predicting different variables expressing primary production in lakes. Sec_{MV} = mean annual Secchi depth (in m), D_m = mean lake depth (in m), r² = coefficient of determination, n = number of lakes used in the regression, ww = wet weight.

	Equation	Range for x	r ²	n	Units	Reference
A. Targets in this work						
Chlorophyll (summer mean)	=0.28 · TP ^{0.96}	2.5-100	0.77	77	µg/l	OECD (1982)
Cyanobacteria	=43 · TP ^{0.98}	8-1300	0.71	29	mg ww/m ³	Smith (1985)
B. Related variables						
Chlorophyll (summer max.)	=0.64 · TP ^{1.05}	2.5-100	0.81	50	µg/l	OECD (1982)
Max. prim. prod. (TP>10)	=20 · TP-71	7-200	0.95	38	mg C/m ³ ·d	Peters (1986)
Max. prim. prod. (TP<10)	=0.85 · TP ^{1.4}				mg C/m ³ ·d	Peters (1986)
Mean prim. prod. (TP>10)	=10 · TP-79	7-200	0.94	38	mg C/m ³ ·d	Peters (1986)
Mean prim. prod. (TP<10)	=0.85 · TP ^{1.4}				mg C/m ³ ·d	Peters (1986)
Phytoplankton	=30 · TP ^{1.4}	3-80	0.88	27	mg ww/m ³	Peters (1986)
Macrophyte cover	= 0.50 · (Sec _{MV} /D _m)			229	%	Vorobev (1977)
Nanoplankton	=17 · TP ^{1.3}	3-80	0.93	23	mg ww/m ³	Peters (1986)
Net plankton	=8.6 · TP ^{1.7}	3-80	0.82	23	mg ww/m ³	Peters (1986)

Table 2. Compilation of empirical regression models to calculate chlorophyll (Chl in µg/l) from total-P (TP in µg/l) in lakes.

Chl=0.64 · TP ^{1.05}	summer max; from OECD, 1982
Chl=0.28 · TP ^{0.96}	summer average; from OECD, 1982
Chl=0.073 · TP ^{1.451}	Dillon and Rigler, 1974
Chl=0.56 · TP ^{0.87}	world-wide lakes; from Nürnberg, 1996
Chl=0.54 · TP ^{0.80}	N. Am. lakes; from Nürnberg, 1996
Chl=0.36 · TP-0.50	soft water lakes; Håkanson et al., 2005
Chl=0.5 · TP	weekly averages; Håkanson and Boulion, 2002

The question about “limiting” nutrient is certainly central in aquatic ecology and has been treated in numerous papers and textbooks (e.g., Dillon and Rigler, 1974; Smith, 1979; Riley and Prepas, 1985; Howarth, 1988; Hecky and Kilham, 1988; Evans et al., 1996; Wetzel, 2001). Empirical models to predict various expressions of primary production (chlorophyll, cyanobacteria, macrophyte cover, nanoplankton, etc.) are exemplified in table 1 and lake models to predict chlorophyll-a concentrations (Chl in µg/l) are given in table 2. Even though some of the primary production variables in table 1 are processes and others are state variables, they are often used as proxies of one another because of their strong correlations with TP. For this work, data have been collected from lakes, brackish coastal areas, estuaries and marine coastal areas (see next section and table 3) in order to provide a holistic and comprehensive data base to derive new models of the kind illustrated in table 2.

The salinity is of paramount importance to the prediction of chlorophyll (see table 4). The salinity influences the aggregation of suspended particles (Håkanson, 2006), which is of particular interest in understanding variations in water clarity, which in turn regulate the depth of the photic zone and hence also primary production. The saltier the water, the greater the flocculation of suspended particles. Salinity is also suggested to influence the outbreak of blooms of cyanobacteria (CB). An increased salinity together with N-limitation and increased

light seems to control factors of N₂-fixing cyanobacteria in estuaries, according to Moisander et al. (2002).

Fig. 2. A. Variations in chlorophyll-a concentrations, phosphate and nitrate in the Baltic Sea (using data from the a at the Gotland depth between 1993 to 2003; data base from SMHI, Sweden).
 B. The relationship between chlorophyll and total-N (TN) using data from 22 Baltic coastal areas (data from Wallin et al., 1992).
 C. The relationship between chlorophyll and TN using monthly median values from Ringkobing Fjord for the period 1980-2004 (data from Håkanson et al., 2006).
 D. The relationship between chlorophyll and total-P (TP) using data from the Bothnian Bay, the Bothnian Sea and the Oslo Fjord (data from Maguus Karlsson, unpublished).

Phosphorus is often recognized as the most crucial limiting nutrient for lake primary production in most but not all lakes (see tables 1 and 2; Schindler, 1977, 1978; Bierman, 1980; Chapra, 1980; Boynton et al., 1982; Wetzel, 2001; Persson and Jansson, 1988; Boers et al., 1993). Nitrogen is regarded as a key nutrient in some marine areas (Redfield, 1958; Ryther and Dunstan, 1971; Nixon and Pilson, 1983; Howarth and Cole, 1985; Howarth, 1988; Hecky and Kilham, 1988; Ambio, 1990; Nixon, 1990; Livingston, 2001). However, in a

cross-systems study, Guildford and Hecky (2000) found that TP was much more strongly correlated to chlorophyll than TN was, although in a similar study, Smith (2006) noticed a strong correlation between TN and chlorophyll and concluded that both nutrients should be abated in eutrophicated waters.

Table 3. Compilation of main references (A) and data (B) used in this work for the general, comprehensive data base. Note that all data used in this data base represent median surface water values for the growing season.

base. Note that all data used in this data base represent median surface water values for the growing season.					
A. Area	Country	Salinity range	Main references		
Chesapeake Bay	U.S.A.	<2-35	http://www.chesapeakebay.net/data/wq_query1.cfm?db=CBP_WQDB		
Italian coast	Italy	15-40	http://dataservice.eea.europa.eu/dataservice/metadetails.asp?id=836		
Crimean lakes	Ukraine	25-275	Golubkov et al. (2006)		
Baltic Sea	Sweden	2.4-5.5	Magnus Karlsson, ÅF, pers. comm.		
Bothnian Bay	Sweden	0.3-1.5	http://dataservice.eea.europa.eu/dataservice/metadetails.asp?id=836		
West coast	Sweden, Norway	6.4-27	http://dataservice.eea.europa.eu/dataservice/metadetails.asp?id=836		
Swedish lakes	Sweden	0	http://info1.ma.slu.se/db.html		
Baltic coast	Sweden, Finland	5-7.5	Wallin et al. (1992); Håkanson (2006)		
Lakes, shelf, marine	Several	0-36	Guildford and Hecky (2002)		
Ringkøbing Fjord	Denmark	3.9-14.5	Petersen et al. (2006); Håkanson et al. (2006)		
B.		Total-N (µg/l)	Total-P (µg/l)	Chlorophyll (µg/l)	Salinity (‰)
Mean (MV)		533	40.5	7.9	15.9
Median (M50)		461	32.1	5.2	12.5
Minimum		1	<1	0.12	0
Maximum		2110	1090	60.3	275
Standard deviation (SD)		320	61.4	9.27	14.4
Coeff. of variation among systems (CV)		0.60	1.52	1.17	0.91
Number of data (n)		658	582	709	714

Table 4. Predicting median summer values of chlorophyll-a concentrations in lakes and marine coastal areas from TP, TN and salinity (from a data base compiled by Håkanson and Eklund, Uppsala University).

A. Predicting chlorophyll from TP and salinity; n = 542; r² = 0.71;

$$\text{Chl} = Y_{\text{sal}} \cdot \text{TP}$$

(TP range: <1 to 1090 µg/l; salinity range: 0 to 275)

Chl = Chlorophyll-a concentration in µg/l

TP = TP-concentration in µg/l

$Y_{\text{sal}} = Y_4$ [dimensionless moderator for the influence of salinity on chlorophyll]

$Y_1 = \text{if Salinity} < 2.5\text{‰ then } (0.20 - 0.1 \cdot (\text{Salinity}/2.5 - 1)) \text{ else } (0.20 + 0.02 \cdot (\text{Salinity}/2.5 - 1))$

$Y_2 = \text{if Salinity} < 12.5\text{‰ then } Y_1 \text{ else } (0.28 - 0.1 \cdot (\text{Salinity}/12.5 - 1))$

$Y_3 = \text{if Salinity} > 40 \text{ then } (0.06 - 0.1 \cdot (\text{Salinity}/40 - 1)) \text{ else } Y_2$

$Y_4 = \text{if } Y_3 < 0.012 \text{ then } 0.012 \text{ else } Y_3$

B. Predicting chlorophyll from TN and salinity; n = 623; r² = 0.68

Salinity < 10: $(\text{Chl}/\text{TN})^{0.5} = -0.014 \cdot \text{abs}(\text{salinity} - 10)^{0.5} + 0.145$

Salinity > 10: $y = (\text{Chl}/\text{TN})^{0.5} = -0.0122 \cdot \text{abs}(\text{salinity} - 10)^{0.5} + 0.145$

(TN range: 1 to 2110 µg/l; salinity range: 0-36)

TN = TN-concentration in µg/l

A target regression related to this study is the first model, the OECD-model for average summer conditions (OECD, 1982) in table 1. This empirical model is based on data from 77 lakes and the TP-concentrations range from 2.5 to 100 µg/l. An interesting aspect concerns the r²-value (r² = the coefficient of determination), which is 0.77. That is, 77% of the variation

among these 77 lakes in mean/median summer chlorophyll values can be statistically explained by variations in TP. The slope is 0.28 and the exponent close to 1 (the exponent is 0.96 for the summer averages and 1.05 for the summer maximum values). In this study, data from many systems have been used, also including information on TN, temperature and salinity.

There are four highlighted spots with question marks in fig. 1, indicating that it is very difficult to quantify them in a general manner. Three of them are denitrification, atmospheric wet and dry deposition, and the particulate fraction, which is necessary for quantifying nutrient sedimentation. The fourth one, atmospheric nitrogen fixation is also very important in contexts of mass-balance calculations for nitrogen (Rahm et al., 2000). Without empirically well-tested algorithms to quantify atmospheric and internal nitrogen fixation, crucial questions related to the effectiveness of remedial measures to reduce nutrient discharges to aquatic systems cannot be properly evaluated. It also means that it is generally very difficult to understand, model and predict changes in measured TN-concentrations in the water phase, since such changes in concentrations are always mechanistically governed by mass-balances, i.e., the quantification of the most important transport processes regulating the given concentrations. Smith (1990) presented two models for predicting nitrogen fixation from TP; one model for temperate lakes ($r^2 = 0.54$) and another one for estuaries ($r^2 = 0.88$). Our present aim is to expand this approach, together with the model for cyanobacteria in table 1, and predict the biomass of cyanobacteria with one model which should be valid for a wide salinity range. A second step in future work will be to predict nitrogen fixation in a similar manner, taking into account that fixation rates at many sites have been substantially revised upwards during the last decade due to better measurement techniques and better understanding of the process (Capone, 2001; Wasmund et al., 2005).

Table 1 gives a regression based on 29 data between TP and cyanobacteria which yields an r^2 of 0.71. The TP-concentrations in this regression vary between 8 and 1300 $\mu\text{g/l}$. A question for this paper is: Is it possible to derive a more general model for cyanobacteria that would also be valid in a wide salinity range?

As stressed, the relative abundance of cyanobacteria compared to other algal groups is closely related to the TN/TP ratio. Cyanobacteria have been found to dominate lake primary production at $\text{TN/TP} \leq 29/1$ (by weight) and are much less abundant at higher ratios, while

nitrogen-fixing cyanobacteria tend to dominate at $TN/TP \leq 22/1$ (Havens et al., 2003). An alternative view is based on observations that TP is a better predictor of the absolute biomass of CB than TN and TN/TP (Smith, 1985, 2003; Downing et al., 2001). According to this view, TN/TP should only be used for identifying the primary growth-limiting resource, and not for predicting CB. This controversy will be addressed at the end of this paper.

Fig. 2 illustrates some of the problems addressed in this work. Using data from the Baltic Sea, fig. 2A gives a situation where the chlorophyll-a concentrations show a typical seasonal “twin peak” pattern with a pronounced peak in April. The higher the primary production, the more bioavailable nitrogen (nitrate, ammonium, etc.) and phosphorus (phosphate) are being used by the algae (the spring bloom is mainly diatoms) and eventually the nitrate concentration drops to almost zero and the primary production decreases. The main limiting nutrient for the spring peak observed in fig. 2A is nitrate. Fig. 2B illustrates a regression between chlorophyll and TN based on empirical data from 22 Baltic coastal areas (median values for the summer period; data from Wallin et al., 1992). One can note a very high r^2 (0.89). So, 89% of the variation in median chlorophyll-a values among these 22 coastal areas may be statistically (but not necessarily causally) related to the measured variations in TN. Does this prove that nitrogen is the “limiting” nutrient? Certainly not, as will be demonstrated in this work. Fig. 2C shows the relationship between chlorophyll-a and TN in Ringkøbing Fjord, Denmark, a lagoon with a salinity in the range between 5 and 15 (data from Petersen et al., 2006; Håkanson et al., 2006). In this lagoon, there is a very poor correlation between TN and chlorophyll ($r^2 = 0.04$) and later on the correlation between chlorophyll and different forms of nutrients in this lagoon will be studied. The point here is that this is only one coastal area, and the regression in fig. 2B is based on data from “only” 22 coastal areas. In this work, the aim is to put results like these in a much wider context and data from about 500 lakes and coastal areas will be introduced. Chlorophyll-a will be studied in relation to total-P (TP) concentrations and fig. 2D illustrates that there are many coastal areas (data in this regression come from Magnus Karlsson, unpublished) where there exist just as powerful relationships between TP and chlorophyll (Chl) as between TN and Chl in fig. 2B. In the following methodological section, questions concerning the scatter around regression lines like these will be addressed, and in particular with respect to how the scatter depend on the variability and uncertainty in the empirical data.

So, an important aim of this work is to try to add a new dimension to the debate on “limiting” nutrient by using a very comprehensive data set collected by us from the literature and different websites. Conditions in a very wide salinity range will be discussed (which is not often done) and the main task is to try to find general patterns in variations among areas in chlorophyll-a concentrations and in concentrations of cyanobacteria. Are such variations mainly governed by variations in phosphorus and/or nitrogen? How important are variations in salinity and temperature? Is it useful to account for different forms of the nutrients?

Data and statistical considerations

Four main dataset have been used in this study, (1) the general comprehensive data base, (2) the data base from Ringkobing Fjord, (3) the data base for Swedish lakes, (4) a database from River Danube, Central Europe. Table 3A gives a compilation of information related to the general, comprehensive data base and table 3B gives a compilation of the variables in this data base, i.e., chlorophyll, TN, TP and salinity (cyanobacteria are not included in this data base). One can note that this work has used a very comprehensive dataset, probably the most comprehensive ever to address the problems of how TN, TP and salinity affect the variability in chlorophyll concentrations among aquatic systems. The models to predict chlorophyll from TN, TP and salinity in table 4 emanate from this data base. The idea with this work is to use this data base and focus on the “limiting” nutrient question. The salinity in these systems ranges from zero to 275‰ in hypersaline Crimean lakes; the median salinity is 12.5. The range in nutrients is from oligotrophic systems (TP<1 µg/l) to hypertrophic systems (TP>1000 µg/l).

The second main data base used in this work comes from Ringkobing Fjord, Denmark (see Petersen et al., 2006; Håkanson et al., 2006) and it includes data on cyanobacteria as well as chlorophyll and nutrients. This lagoon is connected to the open sea (North Sea) through a sluice, so in many ways this system may also be regarded as a lake close to the sea. The area of the lagoon is about 300 km², the mean depth 1.9 m and the maximum depth 5.1 m. This large and shallow lagoon is dominated by resuspension processes.

The third data base also concerns CB and chlorophyll-a concentrations, but in lakes. Data covers four lakes studied by Smith (1985) and another 58 Swedish lakes. The latter data are freely available at www.ma.slu.se. Lake-typical medians from the growing season (June-August) have been calculated and analysed. These lakes represented a wide limnological

domain with median summer TP between 4 and 1000 µg/l, median summer TN between 165 and 4000 µg/l and median summer pH between 4.6 and 8.4.

The fourth dataset covers median monthly data on chlorophyll and cyanobacteria from the River Danube (using data from Håkanson et al., 2003) and the idea is to compare CVs for chlorophyll with CVs for cyanobacteria. Since data for such a comparison for coastal areas are not accessible to us, these data from River Danube has been used instead. The basic factors regulating the two CV-values (i.e., nutrients, temperature, light, salinity, predation, analytical uncertainties related to sampling) should be similar in most aquatic systems, except that it is known that the actual CV-values are higher in rivers than in lakes or coastal areas because of the variations in water discharge (see Håkanson, 2006).

In compiling the data bases used in this work, only systems where there are at least 3 samples available for the growing season were generally accepted. This means that the median values are very uncertain for some of the areas, and quite reliable for many of them. This uncertainty may be expressed by the coefficient of variation ($CV = SD/MV$; SD = standard deviation; MV = the mean value) and table 5 gives a compilation of characteristic CV-values for many of the variables discussed in this work. It is evident that a significant part of the scatter around the regression lines will depend on the fact that many of the data used in the regressions are uncertain.

Table 5. Coefficients of within-system variation (CV) for variables from (A) brackish coastal areas in the Baltic Sea collected during the growing season and (C) from Ringkøbing Fjord.

A. Brackish coastal areas (data from Wallin et al., 1992; Nordvarg, 2001; Håkanson, 2006)				
	Monthly			
Secchi depth	0.19			
Chlorophyll-a	0.25			
Salinity	0.05			
Total-N	0.13			
Inorganic-N	0.31			
Total-P	0.16			
Inorganic-P	0.28			
B. Ringkøbing Fjord (data from Pedersen et al., 1995; Petersen et al., 2006)				
	Daily	Monthly	Yearly	All
SPM	0.20	0.38	0.70	0.81
Secchi depth	0.11	0.20	0.42	0.68
Chlorophyll-a	0.18	0.30	0.56	1.00
Total-N	0.07	0.12	0.24	0.51
Total-P	0.15	0.27	0.62	0.70
Salinity	0.08	0.08	0.24	0.33
Water temperature	0.03	0.10	0.53	0.56

The sampling formula (eq. 1 from Håkanson, 1984) is derived from the basic definitions of the mean value, the standard deviation and the Student's t value, which expresses how many samples are required (n) in order to establish a lake mean value with a specified certainty:

$$n = (t \cdot CV/L)^2 + 1 \quad (1)$$

where t = Student's t, which specifies the probability level of the estimated mean (strictly, this approach is only valid for variables from normal frequency distributions), and CV = coefficient of variation within a given ecosystem. L is the level of error accepted in the mean value. Since one often determines the mean value with 95% certainty (p = 0.05), the t-value is often 1.96. Since most water variables discussed in this work (see fig. 3, which also illustrates the sampling formula) have CV's between 0.1 to 2, one can calculate the error in a typical estimate. If n = 5 and CV = 0.33, then L is about 33%. This calculation has profound implications for the quality of our knowledge of aquatic systems. It indicates that for most water variables, existing empirical estimates are only rough measures of the area-typical mean or median value.

Given the high CV-values for many water variables, one can ask: Is it possible to reduce the CV so that more reliable empirical mean/median values can be obtained? It is a standard practice to approximate the CV for a mean value according to eq. 2 (see Håkanson et al., 2003), but then one must also presuppose that the mean value comes from a random sample from a normal frequency distribution. Many water variables often have seasonal or long-term temporal trends or patterns and when this is the case, one cannot use this approach to reduce the CV without due reservations and generally take more sample than suggested by eq. 2 to reach the requested reliability in the mean value (for example that the error, L, is smaller than 20% of the mean):

$$CV_{MV} \approx CV_{ind}/\sqrt{n} \quad (2)$$

where CV_{MV} is the CV for the mean and CV_{ind} is the CV for the individual data; n is the number of data used to determine the mean value.

Fig. 3. Graphical illustration of the sampling formula (Håkanson, 1984) and a compilation of characteristic CV-values for coastal areas based on monthly samples for the variables discussed in this work.

From a statistical point of view, an equation has been derived (Håkanson, 1999) which gives the highest r^2 -value that one in practice can hope to achieve as a function of (1) the number of samples (n_i) for each y_i -value in a regression, (2) the number of data points in the regression (n), (3) the standard deviations related to all individual data points, (4) the standard deviation of all points in the regression and (5) the range of the y -variable. The derivation of eq. 3 is based on an algorithm for the 95% confidence interval (CI) for individual y for independent validations (N) (from Håkanson and Peters, 1995), giving CI as a function of range in y , n and r^2 , and the sampling formula (eq. 2), giving the error L as a function of CV and n . If CI is set equal to L and if n and N are set to 10, and if the minimum value in the range ($y_{\max} - y_{\min}$) is assumed to be small in comparison to $y_{\max}$, and if $y_{\max}$ is set to 1 (which is valid for relative values), then:

$$r_r^2 = 1 - 0.66 \cdot CV^2 \quad (3)$$

It should be noted that the CV-value in eq. 3 should be the characteristic within-system variability (sometimes abbreviated as CV_y).

This work uses simple statistical methods described by Håkanson and Peters (1995). This means regression analysis including transformations of x- and y-variables to obtain as normal frequency distributions as possible (the mean value for each variable should be as close to the median as possible).

Variability in water variables

Table 6 gives CV-values for twelve variables in 58 Swedish lakes. Cyanobacteria have significantly higher CV-values than other variables and are therefore much more difficult to predict with a high certainty. It is also important to note that dissolved inorganic nutrient fractions have considerably higher CV-values than TN and TP. Thus, dissolved inorganic nutrient fractions are, in relation to TN and TP, not only very poorly correlated with chlorophyll and Secchi depth (Pienitz et al., 1997), but given their high inherent uncertainties, it is much more costly to determine reliable mean/median values which can be used in ecosystem models, whose scientific quality and usefulness is given by their predictive power.

Table 6. CV-values for twelve water variables based on data from 58 Swedish lakes. Median refers to the CV-value in the median lake, while Min and Max denote CV-values in the lakes with the lowest and highest variability, respectively. Values describe the summer period and are typically based on 66 measurements and in no case less than 6.

Variable	Median	Min	Max
Cyanobacteria	1.76	0.64	3.91
Ammonium	0.74	0.42	3.91
Nitrate + nitrite	0.73	0.09	2.78
Total algal biomass	0.68	0.34	1.83
Phosphate	0.58	0.24	1.14
Chlorophyll	0.43	0.25	1.29
Total-P	0.37	0.23	0.78
Total-N	0.24	0.04	0.72
Secchi depth	0.21	0.11	0.51
Temperature	0.14	0.10	0.37
Calcium	0.11	0.03	0.83
pH	0.04	0.01	0.09

From table 7, one can note that there is a certain seasonal pattern in the CV-values from River Danube with highest values in October and November and lowest values in December and January, that the CVs for the cyanobacteria are generally higher than the CVs for chlorophyll and that, on average, the CV for the cyanobacteria is a factor of 1.3 higher. This is the key information stressed by this example. So, one should expect that the r^2 -value that one can hope to achieve would be lower for cyanobacteria than for chlorophyll and this is also the information conveyed in table 1 ($r^2 = 0.71$ and 0.77 , respectively, for lakes) and in table 6 (CV = 1.76 for CB and 0.43 for Chl in lakes).

Table 7. Statistical river data for (A) chlorophyll ($\mu\text{g/l}$) and (B) cyanobacteria ($\mu\text{g/l}$) based on samples from 1995 to 1999 from the Regensburg station (the Danube). n = number of data, MV = monthly mean value, SD = standard deviation, CV = coefficient of variations (from Håkanson et al., 2003).

A. Chl	Jan	Feb	Mar	April	May	June	July	Aug	Sep	Oct	Nov	Dec
n	17	18	25	34	54	58	76	58	46	46	18	9
MV	2.62	9.58	9.06	26.69	33.57	21.88	21.81	19.65	6.67	6.63	3.84	3.72
SD	1.33	7.57	7.10	13.99	17.64	20.16	14.03	15.72	6.74	9.79	3.67	2.39
CV_{Chl}	0.51	0.79	0.78	0.52	0.53	0.92	0.64	0.80	1.01	1.48	0.95	0.64
B. Cyanobacteria												
n	17	18	25	31	44	45	50	43	32	40	17	8
MV	0.06	0.20	0.25	0.83	1.26	0.78	0.32	0.41	0.36	0.29	0.12	0.14
SD	0.04	0.19	0.21	0.61	0.91	0.71	0.26	0.35	0.55	0.55	0.11	0.09
CV_{CB}	0.71	0.93	0.86	0.73	0.72	0.91	0.81	0.86	1.52	1.90	0.92	0.66
CV_{CB}/CV_{Chl}	1.39	1.33	1.10	1.40	1.36	0.99	1.27	1.08	1.50	1.28	0.97	1.03
MV	1.27											

Equations 2 and 3 applied on cyanobacteria in lakes lead to the conclusion that the characteristic CV for individual data (CV_{ind}) on bluegreens is 1.76, a very high value. The minimum number of samples used to determine mean and median values for cyanobacteria for the growing season in this work is n = 6. This means that the CV for the mean (CV_{MV}) have been reduced from 1.76 to about $1.76/\sqrt{6} = 0.72$ and the highest reference r_r^2 to 0.66. For most of the lakes, n is larger than 6 and a typical value is n = 10, which means that one cannot expect to achieve a predictive model for cyanobacteria from our dataset for lakes which is higher than $r_r^2 = 0.80$. However, for most of the brackish systems and marine systems, n is smaller than 6. So, all things considered, one cannot expect to achieve and r^2 -value higher than 0.75 to 0.80 using this dataset. The only way to derive a better predictive model for cyanobacteria would be to base that model on more reliable mean or median values, and such

values can only be obtained if significantly more samples are taken in regular monitoring programs than is generally done today.

Nutrients, chlorophyll and cyanobacteria in Ringkøbing Fjord

According to the basic theory related to the Redfield ratio, one would have expected that there would have been higher concentrations of cyanobacteria during periods when the Redfield ratio is lower than 7.2, since this would have favored algae which can take up and utilize atmospheric nitrogen (Sellner, 1997; Gruber and Sarmiento, 1997). Fig. 4 shows that there is no clear relationship between the TN/TP-ratio (curve 1 gives measured annual median values since there are no monthly or seasonal data available to us on cyanobacteria from the growing season in this lagoon) and empirical chlorophyll concentrations (curve 3, annual values) or concentrations of cyanobacteria (curve 4, annual values). The TN/TP-ratio is never lower than 7.2 (curve 2 gives the Redfield ratio). In this lagoon (but probably not more generally, see later), there were no cyanobacteria when the TN/TP-ratio was higher than 15.

Fig. 4. Variation in the TN/TP-ratio (curve 1), empirical median annual data in chlorophyll-a concentrations (curve 3) and empirical annual data on the concentration of cyanobacteria in Ringkøbing Fjord (curve 4) in the years from January 1989 to January 2005. The figure also gives the Redfield ratio (TN/TP = 7.2). Ratios are dimensionless and units of the other variables are stated in the figure.

Table 8 gives results for many regressions between chlorophyll (as y-variable) and potential x-variables (TP, TN and different forms of the nutrients) using data from Ringkøbing Fjord. One can note that there are major differences among the x-variables in how they correlate to chlorophyll, that the bioavailable forms correlate poorly and this is also the case (as stressed in fig. 2C) for TN in this lagoon. From this, one can conclude that in Ringkøbing Fjord,

quantitative statistical analyses based on empirical data indicate that phosphorus may regulate primary production all years in the entire trophic gradient from very eutrophic conditions during the period 1980 to 2004. However, this does not contradict observations conveying that short-term nitrogen limitation occurs in Ringkobing Fjord, similar to observations from many other natural waters, such as the brackish Chesapeake Bay (Fisher et al., 1992).

Table 8. Relationships between different nutrients, different forms of nutrients and nutrient ratios versus chlorophyll-a in Ringkobing Fjord. Six different averaging methods have been used on median log values: (1) annual values of all data, (2) all data adjusted to give equal weight to each of the four seasons, (3) spring values, (4) summer values, (5) autumn values and (6) winter values. All correlations are based on log-values. Data are from the period 1986-2004, although some series that only include TP, NH_x and Chl also cover 1985 (except for winter values). Modified from Håkanson et al. (2006)

	All data	Season adjusted	Spring	Summer	Autumn	Winter
r ² (TP vs Chl)	0.96	0.94	0.86	0.92	0.93	0.56
	p	p	p	p	p	p
r ² (OrtP vs Chl)	0.23	0.32	0.03	0.31	0.57	0.30
	n	n	n	p	m	m
r ² (TN vs Chl)	0.33	0.24	0.06	0.76	0.58	0.13
	p	p	n	p	p	n
r ² (NO _x vs Chl)	0.50	0.06	0.00	0.45	0.27	0.00
	m	n	n	m	m	n
r ² (NH _x vs Chl)	0.42	0.59	0.55	0.23	0.52	0.35
	m	m	m	m	m	m
r ² (DIN vs Chl)	0.56	0.09	0.00	0.21	0.33	0.02
	m	n	n	n	m	n
r ² (TN:TP vs Chl)	0.81	0.77	0.73	0.73	0.63	0.46
	m	m	m	m	m	m
r ² (DIN:OrtP vs Chl)	0.45	0.00	0.01	0.55	0.15	0.33
	p	n	p	p	p	p

Significance at the p<0.05 level; p = positive, m = negative, n = not significant

r² = coefficient of determination, TP = total phosphorus, TN = total nitrogen, OrtP = orthophosphate, Chl = chlorophyll, NO_x = nitrate + nitrite, NH_x = ammonium + ammonia, DIN = dissolved inorganic nitrogen = NO_x + NH_x. Due to changes in methods data for Chl, for OrtP and for TN, NO_x, DIN are more reliable from October 1984, from January 1986 and from May 1986, respectively.

Nutrients and chlorophyll from the comprehensive data base

Results from the comprehensive data base are given first in fig. 5, which shows chlorophyll data on the y-axis and the TN/TP-ratio on the x-axis. This scatter plot involves data from 495 aquatic systems in a wide salinity range. There is a significant negative correlations coefficient ($r = -0.26$; $p < 0.0001$) and one can note that if the Redfield ratio would be the criteria for nutrient limitation, then practically all systems have TN/TP-ratios higher than 7.2 and there would be very few nitrogen limited systems. However, as stressed in fig. 4, cyanobacteria do exist also when the TN/TP-ratio is significantly higher than 7.2 taken as mean/median values for the growing season. The Redfield ratio as a demarcation line for

nutrient limitation has also been challenged by several authors (e. g., Guildford and Hecky, 2000).

Fig. 5. Scatter plot between chlorophyll-a concentrations and the TN/TP-ratio based on data from 495 systems.

Fig. 6 gives results when the TN/TP-ratio is plotted against salinity (the transformation is $\log(1+\text{salinity})$), which gives a normal frequency distribution for the data on the x-axis). The figure also gives the regression line and basic statistics. One can see that the salinity influences the TN/TP-ratio significantly – the higher the salinity, the lower the TN/TP-ratio. This may have several reasons, e.g., that waters with low salinity are being influenced by relatively P-rich freshwater inputs. The Redfield ratio of 7.2 for the TN/TP-ratio has been marked in fig. 6 and most systems have TN/TP-ratios higher than that.

Chlorophyll versus cyanobacteria from the lake data base

Fig. 7 gives the (log-log) regression between chlorophyll-a concentration and cyanobacteria (CB = median values for the growing season). One can note a highly significant and mechanistically understandable strong positive co-variation between these two measures of

primary production in lake water. Evidently, this relationship may look different had it been based on daily, weekly or monthly values.

Fig. 6. Scatter plot between the TN/TP-ratio for the growing season and the salinity (transformed into $\log(1+\text{salinity})$) based on data from 494 systems. The figure also gives the regression line and basic statistics.

It has also been tested if it was possible to use the lake dataset to predict the biomass of nitrogen-fixing cyanobacteria (CB_{fix}) but that turned out to be more difficult than predicting CB, in part because there were much fewer empirical data available. There was a division line at $TN/TP = 24$, below which CB_{fix} increased sharply. The least uncertain way to predict CB_{fix} was to calculate the median CB_{fix}/CB -ratio from the five lakes in Smith (1985), which gave $CB_{\text{fix}} = 0.33 \cdot CB$. A regression of empirical data against modelled data gave an r^2 -value of 0.36 ($n = 29$), although this value increased to 0.84 when two data points from one of the lakes was excluded. Possibly, these data points may emanate from summers with unusual weather conditions.

Nutrients and cyanobacteria from all utilized data bases

Fig. 8A gives the regression between CB (transformed as $CB^{0.25}$) and TN (log-transformed data) for 86 systems with $CB > 0$. One can note a highly significant co-variation ($r^2 = 0.63$) but that the scatter around the regression line is high. The scatter is even higher for the actual

(non-transformed) data. Fig. 8B gives the regression between TN and TP (log-log) and for these 103 systems the co-variation is high ($r^2 = 0.83$). Smith (2006) noted a weaker but still

Fig. 7. Regression between $\log(1+CB)$ and $\log(Chl)$ based on data from 76 lakes. The figure also gives the coefficients of determination (r^2) and the regression lines. Cyanobacteria (CB) in mg ww/l, chlorophyll-a concentrations (Chl) in $\mu\text{g/l}$.

highly significant correlation ($r^2 = 0.55$) in a cross-systems analysis. This means that generally the two nutrients have common sources and that they are transported to the systems from the same tributaries and the same major point source emissions and redistributed within the systems by the same general transport fluxes (sedimentation, resuspension, mixing, burial, etc. see Håkanson, 2006). Both nutrients are also incorporated in the phytoplankton (as stressed, the average chemical composition of the which is $C_{106}N_{16}P$; see Redfield et al., 1963). Fig. 8C gives the regression between the TN/TP-ratio (log) and $\log(TP)$. The co-variation is strong but partly spurious (see Håkanson, 1999) since TP is included in the factors

on both axes. From these results, an outline of a model to predict characteristics summer values for cyanobacteria in a wider salinity range will be given.

Fig. 8. Regressions between (A) $CB^{0.25}$ and $\log(TN)$, (B) $\log(TN)$ versus $\log(TP)$ and (C) $\log(TN/TP)$ versus $\log(TP)$. The figure also gives the number of systems used in the regressions (n), the coefficients of determination (r^2) and the regression lines. CB in μg ww/l, TN and TP in $\mu g/l$.

An outline of a general empirically-based model for cyanobacteria in water

It should be stressed that the following model derivation in many ways may be regarded as a working hypothesis since it has been beyond the scope of this work to test the model more thoroughly with independent data. This approach does not concern cyanobacteria produced in the benthic zone and it does not differentiate between cyanobacteria fixing atmospheric nitrogen and non-fixing species. To expand this modeling in such directions, one would need a more comprehensive set of reliable data (that meet the criteria given by the sampling formula). Today, such data are not available to us.

The model has been derived in the following steps.

1. CB versus TP

The basic regression is given in fig. 9.

$$CB^{0.25} = 5.85 \cdot \log(TP) - 4.01 \quad (4)$$

($r^2 = 0.76$; $n = 86$)

Fig. 9. The basic regression between CB (transformed into $CB^{0.25}$; CB in mg ww/l) and $\log(TP)$ (TP in $\mu\text{g/l}$) using median values for the growing season from 86 systems with CB-values higher than zero.

It should be stressed that this regression includes data from many more systems than the equation from Smith (1985) given in table 1. It also gives a higher r^2 -value (0.76 as compared to 0.71) so it is more general and provides better predictive power. As already discussed,

given the inherent uncertainties in the empirical CB-data, one should not expect to derive predictive models for cyanobacteria that give higher r^2 -values than 0.75 to 0.80 for the actual data and eq. 4 gives an r^2 of 0.76 for the transformed data (and 0.49 for the actual data).

2. CB versus TN/TP-ratio

Fig. 10A shows a scatter plot between $\log(\text{CB})$ and the TN/TP-ratio. One can note that the scatter is high but also that some interesting general conclusions can be made:

- High CB-values only appear in systems with relatively low TN/TP-ratios.
- The regression between $\log(\text{CB})$ and TN/TP attains a maximum value ($r^2 = 0.73$; $\log(\text{CB})=0.142 \cdot \text{TN/TP}+5.47$; $n = 61$) if one only use data from systems with TN/TP-ratios smaller than 40. The upper curve (circles) in fig. 10B gives the r^2 -values when only systems with TN/TP-ratios smaller than 10, 15, 20,..... 100 were used in the regressions. The lower curve in fig. 10B gives similar results when only system with higher TN/TP-ratios were used and then one can note that there is no statistically significant ($p<0.01$) relationship between $\log(\text{CB})$ and TN/TP if TN/TP is higher than 15.
- So, two critical TN/TP-ratios, 15 and 40, have been identified. This information will be used in the following where the basic regression between CB and TP given by eq. 4 will be complemented with (multiplied by) a dimensionless moderator (see Håkanson, 1999, for discussions on modeling using dimensionless moderators) Y_{TNTP} defined as:

$$\text{If TN/TP} < 15 \text{ then } Y_{\text{TNTP}} = (1-3 \cdot ((\text{TN/TP})/15-1)) \text{ else } Y_{\text{TNTP}} = 1 \quad (5)$$

This means that for systems with TN/TP (based on median values for the growing season) higher than 15, one can use the basic regression (eq. 4) without any correction for the TN/TP-ratio (i.e., eq. 4 multiplied by 1), but for systems with lower TN/TP-ratios, eq. 5 will provide a correction factor. If, e.g., $\text{TN/TP} = 7.2$, then $Y_{\text{TNTP}} = 2.56$, and the CB-value a factor of 2.56 higher than the value suggested by eq. 4. The use of the TN/TP ratio as a determinant of the biomass of cyanobacteria has been questioned by some authors (see Smith, 2003 for a review). Eq. 5 for Y_{TNTP} is supported by the data in fig. 10 and future data and testing may clarify the general validity of this approach.

Fig. 10. CB (in mg ww/l) and the TN/TP-ratio. A. Scatter plot between $\log(\text{CB})$ and the TN/TP-ratio. The given regression line is based on data from systems where $\text{TN/TP} < 15$.

B. Compilation of r^2 -values between $\log(\text{CB})$ and TN/TP using data from systems when successively smaller ($<$) and higher ($>$) TN/TP-ratios have been omitted. The maximum r^2 is obtained for systems with smaller TN/TP-ratios than 40; very low r^2 -values are obtained if the regressions are done for systems with TN/TP-ratios higher than 15.

3. CB versus temperature

In the literature, temperatures between 15 and 17 °C have been reported as the minimum for cyanobacteria blooms in freshwater systems and in the Baltic Sea (Reynolds and Walsby, 1975; Edler, 1979; Wasmund, 1997). Laboratory experiments on cyanobacteria also support this conclusion (Konopka and Brock, 1978; Lehtimäki et al., 1994, 1997) since many species of cyanobacteria have a slow growth rate below 15 °C. To complicate matters, there are also reports that cyanobacteria have a requirement of temperatures of about 20-21 °C to form blooms and become dominant in a system. Those reports are from a freshwater lake in Canada (McQueen and Lean, 1987), from the North Pacific Ocean (Marumo and Asaoka, 1974) and from an estuary in Australia (Lukatelich and McComb, 1986).

The optimal growth temperatures are species specific for cyanobacteria, but around 25 °C for many species from temperate areas according to laboratory experiments (Konopka and Brock, 1978; Robarts and Zohardy, 1987; Lehtimäki et al., 1997), but these experiments often use species from temperate areas. With higher temperatures, the growth rate usually starts to decrease (Konopka and Brock, 1978). In field data from the Baltic Sea (Wasmund, 1997), this decrease in growth rate is not shown because there are few occasions with temperatures higher than 20 °C. Tilman and Kiesling (1984) studied the cyanobacteria response to temperature in Lake Superior and concluded that they were disfavoured at temperatures under 17 °C and dominating at temperature over 24 °C. Data from the Pacific Ocean area (Marumo and Asaoka, 1974; Lukatelich and McComb, 1986) indicate that the cyanobacteria in this area may have another response to temperature. Evidently, it would be interesting to study if cyanobacteria react to temperature changes differently in tropical areas and saline environments as compared to fresh and brackish waters in the temperate zone.

Fig. 11 gives data on the relationship between CB ($\log(1-CB)$) and surface water temperatures (SWT in °C) from 74 systems. One can note that all systems with higher CB-values than 100 (median values for the growing season) have temperatures higher than 15 °C.

As already stressed (fig. 7), there exist a close co-variation between chlorophyll-a and CB-concentrations in lakes. Since no more data relating CB to temperature have been accessible to us than those shown in fig. 11, the data base from Ringkobing Fjord has been used to investigate the role of surface water temperatures (SWT) versus chlorophyll, or rather versus the ratio between chlorophyll (Chl) and TP (see fig. 12). Note that the aim has been to try to

minimize the possible influences of salinity by taking data only in the salinity range between 5 and 10. The regression in fig. 12 is based on daily median values ($n = 299$) and there is a highly significant relationship ($p < 0.0001$) between Chl/TP and SWT (but the scatter around the regression is also evident and the r^2 -value is 0.21).

Fig. 11. Scatter plot between cyanobacteria (transformed into $\log(1+CB)$; CB in $\mu\text{g ww/l}$) versus surface water temperatures (SWT in $^{\circ}\text{C}$) based on data from 74 systems.

From the results in fig. 12, it is hypothesized that the temperature may influence CB in the same manner as Chl. This would imply that if the median surface water temperature for the growing season is higher than 15 $^{\circ}\text{C}$, one would expect a higher production of cyanobacteria. The basic regression (eq. 4) includes data from systems with a median surface water temperature of 17.5 $^{\circ}\text{C}$. This means that the dimensionless moderator for temperature influences on CB (Y_{SWT}) may be given by:

$$\text{If } SWT > 15 \text{ }^{\circ}\text{C}, \text{ then } Y_{\text{SWT}} = (0.86 + 0.63 \cdot ((SWT/15)^{1.5} - 1))$$

$$\text{else } Y_{\text{SWT}} = (1 + 1 \cdot ((\text{SWT}/15)^3 - 1)) \quad (6)$$

This means that if $\text{SWT} = 15^\circ\text{C}$, then $Y_{\text{SWT}} = 0.86$; if $\text{SWT} = 17.5$, $Y_{\text{SWT}} = 1$; if $\text{SWT} = 25$, $Y_{\text{SWT}} = 1.48$; $\text{SWT} = 10^\circ\text{C}$, $Y_{\text{SWT}} = 0.30$, etc.

So, when the temperature is 25°C , the risks of getting high concentrations of cyanobacteria a factor of 1.48 higher than at 17.5°C , if all else is constant, using this approach.

Fig. 12. Illustration of water temperature (surface water temperatures, SWT , in $^\circ\text{C}$; the x-axis gives $\sqrt{\text{SWT}}$) influences on the ratio between chlorophyll and TP [$\log(\text{Chl}/\text{TP})$] using daily median values from Ringkøbing Fjord in the salinity range from 5 to 10‰.

4. CB versus salinity

In many freshwater lakes, the biomass of cyanobacteria can be very high (Reynolds, 1987). In brackish systems, the situation is probably slightly different. Howarth et al. (1988a) found no data on N_2 -fixing planktonic species in estuaries and coastal seas, except for the Baltic Sea and Pell-Harvey estuary, Australia. Also results from Marino et al. (2006) support this general lack of N_2 -fixing cyanobacteria in estuaries. Common freshwater cyanobacteria species may be unable to deal with a higher concentration of Na^+ , since their saline tolerance is low and they have an inefficient efflux system for Na^+ (Thomas et al., 1988). This is an indication that

there may be limited amounts of cyanobacteria in brackish waters. That conclusion is not, however, supported by other studies. There are a few species of cyanobacteria tolerant to brackish water (Lehtimäki et al., 1997; Wasmund, 1997; Moisander et al., 2002 and Mazur-Marzec et al., 2005). A field study from the Baltic Sea (Wasmund, 1997) indicates that in this brackish environment species of cyanobacteria have, interestingly, the highest biomass at 7 – 8‰ and that the blooms in Kattegat and Belt Sea are more frequent if the salinity is below 11.5‰. A laboratory experiment with cyanobacteria from the Baltic Sea supports the results that the highest growth rate was at salinities in the range between 5 and 10‰ (Lehtimäki et al., 1997).

Water blooms of cyanobacteria in marine environments may not be as common as in freshwater systems but according to Sellner (1997) they can be the dominating factor in carbon and nutrient fluxes in some saline systems. In marine systems, there are just a few dominant genera. In a field study in the Pacific Ocean (Marumo and Asaoka, 1974), there was no correlation between the salinity and the cyanobacteria abundance and no cyanobacteria were found in the cooler, less saline subarctic waters. Those marine cyanobacteria species are mainly found in high-saline conditions. In the data discussed by Marumo and Asaoka (1974), the salinity was around 32 - 36‰.

Fig. 13 gives the relationship between the Chl/TN-ratio and salinity based on these data from 621 systems in the salinity range from 0 to 36. In this modeling, it has been assumed that CB would be related to salinity in the same manner as chlorophyll. This means that the salinity moderator may be derived from the information given by the two regressions in fig. 13 for salinities higher and lower than the threshold value of 10‰. The maximum expected CB-values should hence be found if the median salinity for the growing season is 10; lower values should generally be found in systems with lower and higher salinities.

The two regressions in fig. 13 have been transformed into an algorithm expressing how changes in salinity are likely to affect the predictions of cyanobacteria in relation to the basic regression (eq. 4).

$$\begin{aligned} \text{If the salinity} < 10\text{‰, then } Y_{\text{sal}} &= (2.1 + 1.1 \cdot ((\text{salinity}/10)^2 - 1)) \\ \text{else } Y_{\text{sal}} &= (2.1 - 115 \cdot ((\text{salinity}/10)^{0.01} - 1)) \end{aligned} \quad (7)$$

This means that at a salinity of 10, Y_{sal} is 2.1 and CB a likely factors of 2.1 higher than in freshwater systems; if the salinity is 5, Y_{sal} is 1.28; if the salinity is 20, Y_{sal} is 1.3; and if the salinity is 36, Y_{sal} is 0.62.

Fig. 13. Scatter plot of all available data relating the ratio Chl/TN to salinity. The figure also gives two regressions for salinities either below or higher than the threshold value of 10.

The CB-model is summarized in fig. 14.

The general model for cyanobacteria presented here may give rather uncertain predictions for systems with high TN/TP-ratios and low temperatures. However, during such conditions, cyanobacteria generally play a small role for the N_2 -fixation and as a nuisance to people and animals. Predicting CB when it is most prevalent is evidently more essential in contexts of water management, and our model serves this purpose quite well given the restrictions related to the inherent uncertainties in the available empirical data. Due to the lack of alternatives, there is also good reason to use the approach presented here until more data have been

collected and stronger predictive models developed. Measured N₂-fixation tends to follow a similar pattern as the prevalence of cyanobacteria (Howarth et al., 1988; Tönno, 2004). Also, analyses using modern gene sequencing techniques have suggested that more organisms than is currently known may fix nitrogen in both lakes and marine systems (Zehr et al., 2003). This motivates the need for predicting general bioproduction patterns for cyanobacteria and other groups of organisms in waters with low TN/TP-ratios, rather than solely focussing on certain species. Evidently, it would be very interesting to have access to many more data on the relationship between cyanobacteria and salinity.

$$BG = ((5.85 \cdot \log(TP) - 4.01)^4) \cdot Y_{TNTP} \cdot Y_{SWT} \cdot Y_{sal}$$

BG in µg ww/l

Salinity in ‰

SWT = Surface water temperature in °C

Total-N (TN) in µg/l

Total-P (TP) in µg/l

$$Y_{TNTP} = \text{if } TN/TP < 15 \text{ then } (1 - 3 \cdot (TN/TP/15 - 1)) \text{ else } 1$$

$$Y_{SWT} = \text{if } SWT > 15 \text{ then } (0.86 + 0.63 \cdot ((SWT/15)^{1.5} - 1)) \text{ else } 1$$

$$Y_{sal} = \text{if salinity} < 10 \text{ then } (2.1 + 1.1 \cdot ((\text{salinity}/10)^2 - 1)) \text{ else } (2.1 - 115 \cdot ((\text{salinity}/10)^{0.01} - 1))$$

Model domain: $4 < TP < 1300$; $165 < TN < 6830$; $0 < \text{salinity} < 40$; $8 < SWT < 25$

Fig. 14. Outline of the model to predict median summer values of cyanobacteria in lakes and coastal areas.

Practical use of results in water management

A fundamental question in water management is: If there is a change in an abiotic state variable, like TP, TN, salinity and/or temperature, will there also be changes in important bioindicators, variables of ecosystem effects and/or changes in ecosystem function and structure? It is possible to quantify and predict such changes? Classification systems like the one presented in this section may not in themselves provide answers to such questions, but they can provide a scientific framework for such analyses.

There is a classical way of categorizing lakes into different trophic levels by oligo-, meso-, eu- and hypertrophic classes. This basic system from Naumann (1931) has been modified and improved on many occasions. In this work, the system put forward by Håkanson et al. (2006) will be used. It differentiates the trophic categories by chlorophyll-a concentrations in steps of 2, 6, 20 µg/l using median values for the growing season. The model presented here for cyanobacteria will be used and cyanobacteria will be added to the classification system, as Willén (2000) has previously done for lakes. This means that:

- The same classes of chlorophyll are used for all systems independently of salinity; chlorophyll is a standard measure of primary production and this is what the trophic categories should reflect. There are many systems like this (see Håkanson and Boulion, 2002 for a compilation) using not chlorophyll but nutrient concentrations as key criteria, but this system uses strict classes of chlorophyll-a concentrations based on data from the growing season.
- The classification is shown in table 10. The freshwater system is defined as having salinities in the range between 0 and 5; the brackish systems are those with salinities between 5 and 20 and the marine or saltwater systems (including saline lakes) have salinities higher than 20. In table 10, values using a salinity of 2.5 have been used for the freshwater systems, a salinity of 12.5 for the brackish systems and a salinity of 36 for the marine/saltwater systems, since a salinity of 36 is common in many marine coastal areas around the world.
- The values for the cyanobacteria in the different trophic classes have been calculated by setting $TN=15 \cdot TP$, which is typical for many systems where cyanobacteria may be present.

It should be stressed that this table is certainly not meant as a final statement concerning a general system to classify aquatic areas into trophic categories. The model for cyanobacteria introduced in this work should be critically tested and such tests may change the model, which in turn would require modifications in the information summarized in table 10. But

while waiting for such results, table 10 is one more step on the road toward a general classification system of trophic categories for aquatic systems. It should also be clear that the concentrations of cyanobacteria may be very high at individual sites high up in the water column after warm periods with no winds since many cyanobacteria can float. During such events, the media tend to be alert to produce alarm reports concerning “toxic algal blooms” and this often have consequences for the tourist industry since tourists tend to avoid coastal areas where these harmful blooms appear. The model presented in this work cannot predict such single events and conditions at specific sites but it is meant to provide a scientific basis for interpretations of such algal blooms.

Table 9. Compilation of equations to predict Secchi depth in lakes and coastal areas from TP-concentrations and salinity.

1. Predicting SPM (mg/l) from TP (µg/l); from Håkanson, 2006):

$$\text{SPM} = 0.0235 \cdot C_{\text{TP}}^{1.56}$$

This equation is based on data from 51 lakes and coastal areas and gives an r^2 of 0.895.

2. Predicting Secchi depth (Sec in m) from SPM (mg/l) and salinity (sal in ‰) (from Håkanson, 2006):

$$\text{Sec} = 10^{-(10^{-(0.15 \cdot \log(1+\text{sal})+0.3)-1})+0.5) \cdot (\log(\text{SPM})+0.3)/2+(10^{-(0.15 \cdot \log(1+\text{sal})+0.3)-1})}$$

Note that if Secchi depth is calculated to be higher than 200 m in very low-productive systems, there is a boundary rule which never permits the value to be higher than 200 m.

Table 10. Characteristic features in (A) freshwater systems, (B) brackish coastal systems and (C) marine coastal systems of different trophic levels (see also OECD, 1982; Håkanson and Jansson, 1983; Wallin et al., 1992; Håkanson and Boulion, 2002; Håkanson et al., 2006). All data represent characteristic (median) values for the growing season for the surface water layer.

A. Fresh water systems, salinity < 5‰					
Trophic level	Secchi* (m)	Chl-a (µg/l)	Total-N (µg/l)	Total-P (µg/l)	Cyanobacteria** (µg ww/l)
Oligotrophic	>5	<2	<60	<8	<2.2
Mesotrophic	3-5	2-6	60-180	8-25	2.2-250
Eutrophic	1-3	6-20	180-430	25-60	250-1400
Hypertrophic	<1	>20	>430	>60	>1400
B. Brackish systems, salinity 5-20‰					
Oligotrophic	>8	<2	<70	<10	<9.5
Mesotrophic	4.5-8	2-6	70-220	10-30	9.5-380
Eutrophic	1.5-4.5	6-20	220-650	30-90	380-2500
Hypertrophic	<1.5	>20	>650	>90	>2500
C. Marine systems, salinity >20‰					
Oligotrophic	>11	<2	<110	<15	<55
Mesotrophic	6-11	2-6	110-290	15-40	55-680
Eutrophic	2-6	6-20	290-940	40-130	680-4040
Hypertrophic	<2	>20	>940	>130	>4040

Relationships between chlorophyll, TP, TN and salinity calculated from Håkanson (2006).

* Secchi depth calculated from Håkanson (2006), see table 9.

** Concentration of cyanobacteria (CB) calculated from our model when TP/TP is set to 15, surface water temperature to 17.5 °C and the salinity to 2.5, 12.5 and 36, respectively for fresh water, brackish and marine systems.

Concluding comments

In the introduction, it was asked: Are variations in chlorophyll-a concentrations and in cyanobacteria mainly governed by variations in phosphorus and/or nitrogen? How important are variations in salinity and temperature? Is it useful to account for different forms of the nutrients? Our study shows:

- Variations in total phosphorus generally seem to be more important than variations in total nitrogen to predict variations among systems in chlorophyll-a and cyanobacteria if median characteristics values for the surface water from the growing season are being used.
- Variations in salinity and water temperature are very important in predicting chlorophyll-a and cyanobacteria and regression models for such predictions have been presented.
- At the ecosystem scale, it is generally pointless to base predictions of chlorophyll-a and cyanobacteria on different “bioavailable” forms of the nutrients (DIN, DIP, phosphate, nitrate, etc.) because such forms do not predict cyanobacteria and chlorophyll well and they have been shown to have very high CV-values, which means that many samples are needed to obtain reliable empirical data which are necessary in models aiming for high predictive power and practical usefulness.
- Data have been collected from many data bases. It is evident that many, maybe most, regular monitoring programs collect too few data of the target water variables discussed in this work. If too few samples are taken and if the CV-values are high (which is generally the case; see tables 5, 6 and 7), then the mean and/or median values requested to characterize the system and to detect critical changes and thresholds in the system will be very uncertain. This may imply that the fundamental objective of and reason for the monitoring cannot be achieved.
- It should be stressed that these results clearly demonstrate that it generally would be wise (1) to take more samples than is often done in monitoring programs and (2) to be cost-efficient and focus the efforts on fewer water variables. The focus should be set on total-P and total-N rather than on dissolved nutrients on the basic rules given by the sampling formula (related to CV and the number of samples needed to determine the mean value for the system with an error smaller than 20% of the mean), and on key variables for water clarity (Secchi depth), primary production (chlorophyll-a concentrations) and cyanobacteria (a crude measure of toxic algae) since these variables can be predicted rather well today, since they are included in many regular monitoring programs and since they can be understood by a general public which (at the end of the day pay for most/many of our monitoring programs).
- In the future, it would be very interesting to try to learn more about the relationships between total chlorophyll and chlorophyll-a, between total cyanobacteria and nitrogen

fixating cyanobacteria, and to have access much more and more reliable data on how temperature and salinity influence variations within and among systems in the target variables for water management.

Acknowledgements

This work has been carried out within the framework of an EU-project (no., 003933-2), Thresholds, coordinated by Prof. Carlos M. Duarte, CSIC-Univ. Illes Balears, Spain. A special thanks to Jens Würgler Hansen for support concerning data regarding Ringkobing Fjord.

Literature references

Ambio. Special issue. Marine eutrophication. Ambio 1990; 19: 102-176.

Bierman VJ Jr. A comparison of models developed for phosphorus management in the Great Lakes. In: Loehr C, Martin CS, Rast W, editors. Phosphorus management strategies for lakes. Ann Arbor Science Publishers, Ann Arbor, 1980, pp. 235-255.

Boers PCM, Cappenberg TE, van Raaphorst W, editors. Proceeding of the Third International Workshop on Phosphorus in Sediments. Hydrobiologia, 1993; issue 253, 376 p.

Boynton WR, Kemp WM, Keefe CW. A comparative analysis of nutrients and other factors influencing estuarine phytoplankton production. In: Kennedy VS, editor. Estuarine comparisons. Academic Press, London, 1982, pp. 69-90.

Capone DG. Marine nitrogen fixation: what's the fuss? Curr Opin Microbiol 2001; 4: 341-348.

Chapra SC. Application of the phosphorus loading concept to the Great Lakes. In: Loehr C, Martin CS, Rast W, editors. Phosphorus management strategies for lakes. Ann Arbor Science Publishers, Ann Arbor, 1980, pp. 135-152.

Dillon PJ, Rigler FH. The phosphorus-chlorophyll relationship in lakes. Limnol Oceanogr 1974; 19: 767-773.

Downing JA, Watson SB, McCauley E. Predicting Cyanobacteria dominance in lakes. *Can J Fish Aquat Sci* 2001; 58: 1905-1908.

Edler L. Phytoplankton succession in the Baltic Sea. *Acta Bot Fennica* 1979; 110: 75-78

Evans MS, Arts MT, Robarts RD. Algal productivity, algal biomass, and zooplankton biomass in a phosphorus-rich, saline lake: deviations from regression model predictions. *Can J Fish Aquat Sci* 1996; 53:1048-1060.

Fisher TR, Peele ER, Ammerman JW, Harding LW. Nutrient limitation of phytoplankton in Chesapeake Bay. *Mar Ecol Prog Ser* 1992; 82:51-63.

Golubkov MS, Golubkov SM, Gubelit YI, Litvinchuk LF. Primary production and composition of phytoplankton assemblages in hypersaline lakes of Crimea peninsula. *Proc Zool Inst Russ Acad Sci* 2006 (in press).

Gruber N, Sarmiento JL. Global patterns of marine nitrogen fixation and denitrification. *Global Biogeochem Cycles* 1997; 11: 235-266.

Guildford SJ, Hecky RE. Total nitrogen, total phosphorus, and nutrient limitation in lakes and oceans: Is there a common relationship? *Limnol Oceanogr* 2000; 45: 1213-1223.

Håkanson L. Sediment sampling in different aquatic environments: statistical aspects. *Wat Res Res* 1984; 20: 41-46.

Håkanson L. Water pollution - methods and criteria to rank, model and remediate chemical threats to aquatic ecosystems. Backhuys Publishers, Leiden, 1999, 299 p.

Håkanson L. Suspended particulate matter in lakes, rivers and marine systems. The Blackburn Press, New Jersey, 2006, 331 p.

Håkanson L, Boulion V. The Lake Foodweb - modelling predation and abiotic/biotic interactions. Backhuys Publishers, Leiden, 2002, 344 p.

Håkanson L, Malmaeus JM, Bodemar U, Gerhardt V. Coefficients of variation for chlorophyll, green algae, diatoms, cryptophytes and blue-greens in rivers as a basis for predictive modelling and aquatic management. *Ecol Mod* 2003; 169: 179-196.

Håkanson L, Blenckner T, Bryhn AC, Hellström SS. The influence of calcium on the chlorophyll-phosphorus relationship and lake Secchi depths. *Hydrobiologia* 2005; 537: 111-123.

Håkanson L, Bryhn AC, Eklund JM. Modelling phosphorus and suspended particulate matter in Ringkobing Fjord to understand regime shifts. *J Mar Sys* 2006 (in press).

Håkanson L, Jansson M. Principles of lake Sedimentology. Springer, Berlin, 1983, 316 p.

Håkanson L, Peters RH. Predictive limnology. Methods for predictive modelling. SPB Academic Publishing, Amsterdam, 1995, 464 p.

Havens KE, James RT, East TL, Smith VH. N:P ratios, light limitation, and cyanobacterial dominance in a subtropical lake impacted by non-point source nutrient pollution. *Environ Poll* 2003; 122: 379-390.

Hecky RE, Kilham P. Nutrient limitation of phytoplankton in freshwater and marine environments: A review of recent evidence on the effects of enrichment. *Limnol Oceanogr* 1988; 33: 796-822.

Howarth RW. Nutrient limitation of net primary production in marine ecosystems. *Ann Rev Ecol* 1988; 19: 89-110.

Howarth RW, Cole JJ. Molybdenum availability, nitrogen limitation, and phytoplankton growth in natural waters. *Science* 1985; 229: 653-655.

Howarth RW, Marino R, Lane J, Cole JJ. Nitrogen fixation in freshwater, estuarine, and marine ecosystems. 1. Rates and importance. *Limnol Oceanogr* 1988a; 33: 669-687.

Howarth RW, Marino R, Cole JJ. Nitrogen fixation in freshwater, estuarine, and marine ecosystems: 2. Biogeochemical controls. *Limnol Oceanogr* 1988b; 33: 688-701.

Istvanovics V, Somlyódy L. Factors influencing lake recovery from eutrophication – the case of Basin 1 of Lake Balaton. *Wat Res* 2001; 35: 729-735.

Konopka A, Brock TD. Effect of temperature on blue-green algae (Cyanobacteria) in Lake Mendota. *Appl Environ Microbiol* 1978; 36: 572 – 576.

Lehtimäki J, Sivonen K, Luukainen R, Niemelä SI. The effects of incubation time, temperature, light, salinity, and phosphorus on growth and hepatotoxin production by *Nodularia* strains. *Arch Hydrobiol* 1994; 130: 269 – 282.

Lehtimäki J, Moisander P, Sivonen K, Kononen K. Growth, nitrogen fixation, and nodularin production by two Baltic Sea cyanobacteria. *Appl Environ Microbiol* 1997; 63: 1647-1656.

Livingston RJ. Eutrophication processes in coastal systems. CRC Press, Boca Raton, 2001, 327 p.

Lukatelich RJ, McComb AJ. Nutrient levels and the development of diatoms and blue-green algal blooms in a shallow Australian estuary. *J Plankton Res* 1986; 8: 597-618.

Marino R, Chan F, Howarth RW, Pace ML, Likens GE. Ecological constraints on planktonic nitrogen fixation in saline estuaries. I. Nitrogen and trophical controls. *Mar Ecol Prog Ser* 2006; 309: 25 – 39.

Marumo R, Asaoka O. Distribution of pelagic blue-green algae in the Northern Pacific Ocean. *J Oceanogr Soc Japan* 1974; 30: 77-85.

Mazur-Marzec H, Zeglinska L, Plinski M. The effect of salinity on the growth, toxin production, and morphology of *Nodularia spumigena* isolated from the Gulf of Gdansk, southern Baltic Sea. *J Appl Phycol* 2005; 17: 171-179.

McQueen DJ, Lean DRS. Influence of water temperature and nitrogen to phosphorus ratios on the dominance of blue-green algae in Lake St. George, Ontario. *Can J Fish Aquat Sci* 1987; 44: 598-604.

Moisander PH, McClinton E, Paerl HW. Salinity effects on growth, photosynthetic parameters, and nitrogenase activity in estuarine planktonic cyanobacteria, *Microb Ecol* 2002; 43: 432-442.

Naumann E. *Limnologische Terminologie. Handb. Biol. Arbeitsmethod., Abt. IX, Teil 8*, Urban und Schwartzberg, Berlin, 1931, 776 p.

Nixon SW. Marine eutrophication: a growing international problem. *Ambio* 1990; 3:101.

Nixon SW Pilson MEQ. Nitrogen in estuarine and coastal marine ecosystems. In: Carpenter, EJ, Capone DG, editors. *Nitrogen in the marine environment*. Academic Press, New York, 1983, pp. 565-648.

Nordvang L. Predictive models and eutrophication effects of fish farms. PhD Thesis, Uppsala University, Sweden, 2001.

Nürnberg GK. Trophic state of clear and colored, soft- and hardwater lakes with special consideration of nutrients, anoxia, phytoplankton and fish. *J Lake Reserv Manag* 1996; 12:432-447.

OECD. Eutrophication of waters. Monitoring, assessment and control. OECD, Paris, 1982, 154 p.

Pedersen OB, Christiansen C, Laursen MB. Wind-induced long term increase and short term fluctuations of shallow water suspended matter and nutrient concentrations, Ringkøbing Fjord, Denmark. *Ophelia* 1995; 41: 273-287.

Persson G, Jansson M. Phosphorus in freshwater ecosystems. *Hydrobiologia* 1988; Vol. 170, 340 p.

- Peters RH. The role of prediction in limnology. *Limnol Oceanogr* 1986; 31: 1143-1159.
- Petersen JK, Hansen JW, Laursen MB, Conley D, 2006. Regime shift in a marine coastal ecosystem. Manuscript, National Environmental Research Institute & County of Ringkjøbing.
- Pienitz R, Smol JP, Lean DRS. Physical and chemical limnology of 59 lakes located between the southern Yukon and the Tuktoyaktuk Peninsula, Northwest Territories (Canada). *Can J Fish Aquat Sci* 1997; 54: 330-346.
- Rahm L, Jönsson A, Wulff F. Nitrogen fixation in the Baltic proper: an empirical study. *J Mar Sys* 2000; 25: 239-248.
- Redfield AC. The biological control of chemical factors in the environment. *Am Sci* 1958; 46: 205-222.
- Redfield AC, Ketchum BH, Richards FA. The influence of organisms on the composition of sea-water. In: Hill N, editor. *The Sea 2*. Interscience, New York, 1963, pp. 26-77.
- Reynolds CS. Cyanobacteria water-blooms. *Adv Bot Res* 1987; 13: 67-143.
- Reynolds CS, Walsby AE. Water-blooms. *Biol Rev* 1975; 50: 437-481.
- Riley ET, Prepas EE. Comparison of the phosphorus-chlorophyll relationships in mixed and stratified lakes. *Can J Fish Aquat Sci* 1985; 42: 831-835.
- Roberts RD, Zohary T. Temperature effects on photosynthetic capacity, respiration, and growth rates of bloom-forming cyanobacteria. *NZJ Mar Freshw Res* 1987; 21: 391 – 399.
- Ryther JH, Dunstan WM. Nitrogen, phosphorus, and eutrophication in the coastal marine environment. *Science* 1971; 171: 1008-1013.
- Schindler DW. Evolution of phosphorus limitation in lakes. *Science* 1977; 195: 260-262.

Schindler DW. Factors regulating phytoplankton production and standing crop in the world's freshwaters. *Limnol Oceanogr* 1978; 23: 478-486.

Sellner KG. Physiology, ecology and toxic properties of marine cyanobacteria blooms. *Limnol Oceanogr* 1997; 42: 1089-1104.

Smith VH. Nutrient dependence of primary productivity in lakes. *Limnol Oceanogr* 1979 24:1051-1064.

Smith VH. Predictive models for the biomass of blue-green algae in lakes. *Wat Resour Bull* 1985, 21: 433-439.

Smith VH. Nitrogen, Phosphorus, and Nitrogen Fixation in Lacustrine and Estuarine Ecosystems. *Limnol Oceanogr* 1990; 35: 1852-1859.

Smith VH. Eutrophication of Freshwater and Coastal Marine Ecosystems: A Global Problem. *Environ Sci Pollut Res Int* 2003; 10:126-139.

Smith VH. Responses of estuarine and coastal marine phytoplankton to nitrogen and phosphorus enrichment. *Limnol Oceanogr* 2006; 51: 377-384.

Thomas J, Apte SK, Reddy BR. Sodium metabolism in cyanobacteria nitrogen fixation and salt tolerance. In: Bothe H, Bruijn FJ, Newton WE, editors. *Nitrogen Fixation: Hundred Years After*. Stuttgart, New York, 1988, pp. 195-201.

Tilman D, Kiesling RL. Freshwater algal ecology: taxonomic trade-offs in temperature dependence of nutrient competitive abilities. In: Klug MJ, Reddy CA, editors. *Current perspectives in microbial ecology. proceedings of the third International Symposium on Microbial Ecology*. American Society of Microbiology, Washington, D.C., 1984, pp. 314-319.

Tõnno I. The impact of nitrogen and phosphorus concentration and N/P ratio on cyanobacterial dominance and N₂ fixation in some Estonian lakes. PhD thesis, Tartu University Press, Tartu, 2004, 106 p.

Vorobev GA. Landscape types of lake overgrowing. Natural conditions and resources of North European part of the Soviet Union. Vologda, 1977, pp. 48-60 (in Russian).

Wallin M, Håkanson L, Persson J. Load models for nutrients in coastal areas, especially from fish farms (in Swedish with English summary). Nordiska ministerrådet, 1992:502, Copenhagen, 1992, 207 p.

Wasmund N. Occurrence of cyanobacterial blooms in the Baltic Sea in relation to environmental conditions. *Int Rev Gesamt Hydrobiol* 1997; 82: 169 – 184.

Wasmund N, Nausch G, Schneider B, Nagel K, Voss M. Comparison of nitrogen fixation rates determined with different methods: a study in the Baltic Proper. *Mar Ecol Prog Ser* 2005; 297: 23-31.

Wetzel RG. *Limnology*. Academic Press, London, 2001, 1006 p.

Willén E. Phytoplankton in water quality assessment - An indicator concept. In: Heinonen P, Giuliano Z, Van der Beken A, editors. *Hydrological and Limnological Aspects of Lake Monitoring*. Wiley and Sons, Ltd, West Sussex, England, 2000, pp 57-80.

Zehr JP, Jenkins BD, Short SM, Steward GF. Nitrogenase gene diversity and microbial community structure: a cross-system comparison. *Environ Microbiol* 2003; 5: 539-554.