A New General Approach to Quantify Nitrogen Fixation Exemplified for the Baltic Proper

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Abstract: This work uses empirical data from the HELCOM database and a new empirically-based model to predict the concentration of cyanobacteria in the Baltic Proper. The aim has been to estimate nitrogen fixation. The inherent variabilities/patchiness in the variables regulating nitrogen fixation are great. This means that different approaches may provide complementary information so that several relatively uncertain estimates may together provide less uncertainty in the estimate for nitrogen fixation in a given system. We show that there is marked variability in nitrogen fixation among different years (a factor of 20 between the year 2001 with the smallest value and 2005 with the highest value of about 900 kt/yr of N-fixation). The mean value for the period from 1997 to 2005 was 190 kt/yr. TN/TP based on median monthly data has been higher than the Redfield ratio of 7.2 since 1994. 6.5% of all individual data (n = 3001) from the surface-water layer (44 m) in the Baltic Proper for samples with temperatures higher than 15°C (when risks of getting cyanobacteria blooms are favoured) have TN/TP lower than 7.2. The mean TN/TP is 20 for surface-water sites with temperatures higher than 15°C, indicating that the average trophic conditions in the Baltic Proper are likely more limited by phosphorus than nitrogen. Nitrogen fixation is an important contributor to the nitrogen concentration and we give overall budgets for nitrogen and phosphorus in the Baltic Proper, including nutrient data from land uplift, which is the most important contributor for nutrients and often neglected in discussions about sources of nutrients to the Baltic Sea.

INTRODUCTION

Quantifying nitrogen fixation is essential for estimating the nitrogen balance in marine waters and understanding the causes of marine eutrophication. Since nitrogen fixation is triggered by high temperatures, low non-gaseous nitrogen concentrations and high phosphorus concentrations in the water, this process may determine which nutrient regulates primary production in the long run, and thus which nutrient should be abated in order to remediate marine eutrophication [1, 2].

Fig. (**1**) illustrates the main processes regulating external fluxes (atmospheric input and 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) and particulate form (the only part that can settle out due to gravity) forms. The focus of this work is on nitrogen fixation.

Many papers discuss nitrogen fixation in the Baltic Sea [3-7]. Table **1** gives two empirical models for lakes as a background for this work. The first is the well-known OECD-model [8] predicting mean summer chlorophyll-a concentrations from total phosphorus concentrations (TP). This model yielded a coefficient of determination (r^2) of 0.77

when tested for lakes from a wide trophic level domain (characteristic TP-concentrations from 2.5 µg/l, i.e., oligotrophic, to 100 µg/l, i.e., hypertrophic). The second empirical model in Table **1** concerns the main topic of this work, a predictive model for cyanobacteria based on readily accessible x-variables, such as the TP-concentration in this model (from [9]), which gave an r^2 of 0.71 when tested for 29 systems covering a very wide limnological domain (TP from 8 to 1300 µg/l). Smith's empirical model for cyanobacteria applies to lakes.

The aim of this paper is to use a new empirically-based model from [10] to predict cyanobacteria and nitrogen fixation in the Baltic Proper. This model will be outlined in the next section.

Table **2** provides background for this work. This table summarizes transport processes to, within and from the Baltic Proper. The value (bolded) for nitrogen fixation is 130 000 tons per year. This value indicates an order-of-magnitude figure for fixation in the Baltic Proper. The value is, however, very uncertain. References [11] and [12] gave values of 100 kt/yr, [13] gave 130 kt/yr for the Baltic Proper and the Gulf of Finland, [14] estimated values from 18.2 to 186 kt/yr for the Baltic Proper and the Mecklenburg Bay and [4] noted a ranged of 30 to 260 kt/yr for the period 1992 to 1997. Yearly estimates of nitrogen fixation from the Baltic Proper are given in Table **3** (according to [5]). These values were calculated as increases in TN minus atmospheric deposition plus particulate N sedimentation at the Landsort Deep, and extrapolated to other basins according to local increases in TN. The summer increase in TN strongly coincided with blooms of N-fixing cyanobacteria in 1997 and 1998 and

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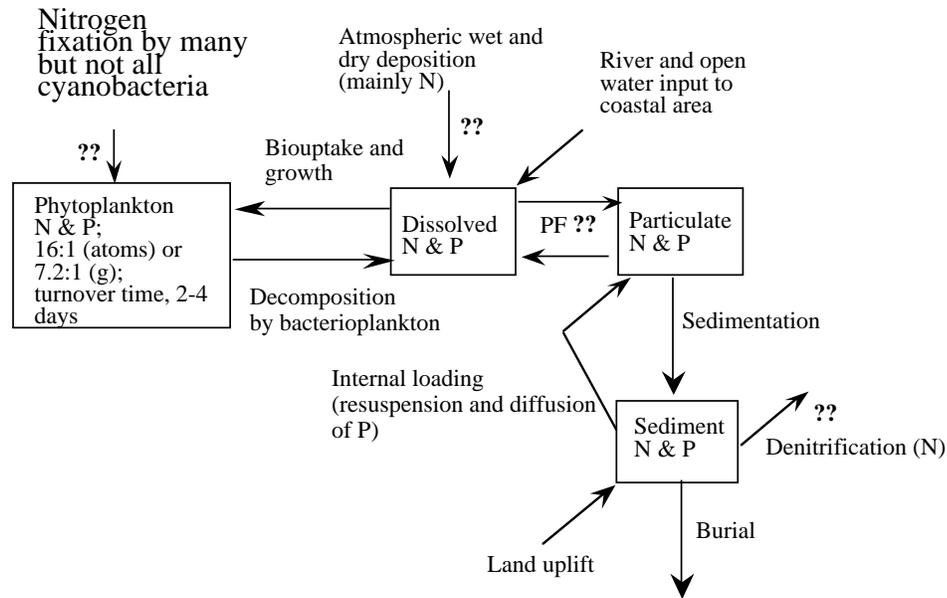
Fig. (1). Overview of important transport processes and mechanisms related to the concept of "limiting" nutrient. PF is the particulate fraction.

these values are about 60% of those calculated by [6]. [7] estimated the N-fixation rate in the Baltic Proper to about a factor of 2 times the values in Table **3** (434 - 792 kt/yr), while earlier estimates have generally been much lower and have not included night measurements, the complete growing season, or the important contribution from phytoplankton smaller than 10μm.

Hence, all approaches to quantify the annual fluxes of nutrients in Fig. (**1**) are more or less uncertain. To quantify nitrogen fixation should be one of the most difficult and uncertain because the cyanobacteria show such very high coefficients of variation and this will be discussed in a following section.

Table 1. **Regressions Illustrating the Key Role of Total Phosphorus (TP) in Predicting Chlorophyll-a (Chl, Summer Values) and the Biomass of Cyanobacteria (CB). r^2 = Coefficient of Determination, n = Number of Lakes Used in the Regression**

	Eq.	Range for TP	r^2	n	Units	Reference
Chl	$0.28 \cdot TP^{0.96}$	2.5-100	0.77	77	μg/l	[8]
CB	$43 \cdot TP^{0.98}$	8-1300	0.71	29	μg/l	[9]

It is important to identify all major sources of nutrient inputs not just to the Baltic Proper but to all polluted water systems and to quantify all major fluxes because this will determine the expectation one would have on different, often costly, measures to reduce nutrient emissions and improve ecosystem conditions. If the total inflow of nitrogen to the Baltic Proper from countries/processes is 1 800 000 tons per year (Table **2**), and if Swedish anthropogenic emissions are just 2.6% of all the nitrogen transport to the Baltic Proper, this will determine the expectations that one would have on such reductions, and also provide a possibility to compare

the cost-effectiveness of alternative approaches to reduce nitrogen fluxes to the system.

The average composition of algae is given by the Redfield ratio 7.2, if the calculation is done in g (see [15] and [16]) and 16:1, if the calculation is based on the number of atoms.

Another critical uncertainty 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 [17]. This also implies that the bioavailable forms are quickly regenerated so their supply is poorly described by their concentrations at any given sampling site [18]. This also explains why there are no practically useful predictive models for chlorophyll based on dissolved forms (DIN, DIP, phosphate, nitrate, etc.), but rather on TN or TP (see Table **1** or [19, 20]). At longer time scales (weeks to years), and for all practical purposes in water management, one must recognize the difference between what is 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 uses an empirical model for total cyanobacteria, which concerns the latter aspects, the ecosystem scale (the entire Baltic Proper) using monthly data.

The problem to understand and predict TN-concentrations in marine systems is accentuated by the fact that there are no (to the best of our knowledge) practically useful models to quantify the particulate N-fraction in saltwater systems (but such approaches are available for phosphorus in lakes and brackish systems, see [21]. In mass-balance modelling, it is imperative to have a reliable algorithm for particulate nitrogen, since the particulate fraction (PF) is the only fraction that by definition can settle out due to gravity.

Table 2.   **Background Budget on the Transport of Nitrogen and Phosphorus to and from the Baltic Proper (t/yr); from [48]. The Data from the Swedish Environmental Protection Agency (SNV [49]) Concern Mean Values for the period 1982 and 1989; Data from HELCOM [43] is for 2000**

	Total-N			Total-P		
	SNV	HELCOM	H&L*	SNV	HELCOM	H&L*
A. From countries/rivers						
Sweden	44 300	46 636		1780	1219	
Baltic states	72 600	145 697		1890	5408	
Finland	-	35 981		-	1874	
Russia	-	90 229		-	5863	
Poland	109 900	191 521		19 100	12 698	
Germany	20 000	20 602		2750	512	
Denmark	51 000	27 664		7860	1193	
Sum inflow from countries:	297 800	558 046		33 380	28 767	
Order-of-magnitude value:	≈ 500 000			≈ 30 000		
B. From processes and water inflow from adjacent basins						
Precipitation	289 900	192 400		3420	-	
Nitrogen fixation	**130 000**	**-**	**-**	**-**		
Land uplift			480 000			160 000
Inflow from Kattegat			120 000			14 000
Inflow from Bothnian Sea			340 000			14 000
Sum from processes:			1 261 000 – 1 359 000			191 420
Order-of-magnitude value:			≈ 1 300 000			≈ 190 000
Total inflow (A+B):			≈ 1 800 000			220 000
C. Water outflows to adjacent basins						
To the Bothnian Sea			340 000			24 000
To Kattegat			260 000			18 000
Total outflow:			≈ 600 000			40 000
D. Other terms						
Burial in sediments		(3·180 000)* = 540 000			(220 000 – 40 000) = 180 000	
Denitrification		(1 800 000 - 600 000 – 540 000) = 660 000				

* The nitrogen concentration is set to be 3 times higher than the phosphorus concentration in these sediments. H&L = [48]. The role of land uplift and the task of finding the most cost-efficient remedial strategy for the Baltic Sea are discussed in more detail in [55].

From previous modelling work [22], one can conclude that it is also very difficult to quantify denitrification (Fig. **1**). Denitrification depends on sediment red-ox conditions, i.e., on sedimentation of degradable organic matter and the oxygen concentration in the deep-water zone, but also on the frequency of resuspension events, on the presence of mucus-binding bacteria, and on zoobenthos and bioturbation. Given this complexity, it is easy to understand why empirically well-tested algorithms to quantify denitrification on a monthly basis do not exist to the best of our knowledge. The atmospheric wet and dry deposition of nitrogen may (as indicated in Fig. **1**) be very large (in the same order as the tributary inflow) and patchy [23], which means that for large coastal areas and smaller systems distant from measurement stations, the uncertainty in the magnitude of atmospheric wet and dry N-deposition is also generally very large.

Table 3.   **Yearly N-Fixation (kt/yr) in the Baltic Proper Based on Data from [5]**

Year	Minimum	Maximum
1994	290	350
1995	360	420
1996	180	250
1997	340	430
1998	180	330

The question about "limiting" nutrient is central in aquatic ecology and has been treated in numerous papers [19, 20, 23-26]. The salinity is of paramount importance to the predictions of chlorophyll and cyanobacteria. The salinity influences the aggregation of suspended particles [21], of particular interest in understanding variations in water clarity, which in turn regulate the depth of the photic zone and hence primary production. The saltier the water, the greater the flocculation of suspended particles. An increased salinity together with N-limitation and increased light seem to control factors of N-fixing cyanobacteria in estuaries, according to [27].

The 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 TN/TP $\leq$ 29 (by weight) and are much less abundant at higher ratios, while nitrogen-fixing cyanobacteria tend to dominate at TN/TP $\leq$ 22 [28]. [9] found TP to be a better predictor of the biomass of cyanobacteria (CB) than TN and TN/TP.

THE MODEL

This approach does not concern cyanobacteria produced in the benthic zone. In the derivation of the model [10], data from many databases were used, and Table **4** gives a compilation of information used as well as data used for the Baltic Proper (from the HELCOM data base). Fig. (**2A**) gives the (log-log) regression between chlorophyll-a concentrations and total cyanobacteria (CB = median values for the growing season) using lake data. One can note a highly significant and mechanistically understandable strong positive co-

Table 4. Compilation of Main References (A), Data and Statistics (B) for the General, Comprehensive Data Base. Note that All Data Used in this Data Base Represent Median Surface Water Values for the Growing Season, and (C) Data from HELCOM (http://www.ices.dk/ocean/asp/helcom/helcom.asp?Mode=1) Used in this Work for Water Temperature, Salinity, Total-N and Total-P from the Entire Baltic Proper Between 1990 and 2005 and Chlorophyll-a Data from 1974 to 2005

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	[51]
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	[20, 52]
Lakes, shelf, marine	Several	0-36	[25, 50]
Ringkobing Fjord	Denmark	3.9-14.5	[53, 54]

B.	Total-N (µg/l)	Total-P (µg/l)	Chlorophyll (µg/l)	Salinity psu
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

C.	Temp (°C)	Salinity	Total-N (µg/l)	Total-P (µg/l)	Chl-a (µg/l)
Min	-0.71	1.29	7.0	0.3	0.1
Max	24.02	24.89	2493	780.6	71.6
Mean (MV)	6.44	8.41	298.1	44.83	2.11
Median (M50)	4.96	7.40	291.4	25.71	1.70
Standard dev. (SD)	4.27	2.33	78.39	41.93	2.23
Coeff. of var. (CV)	0.66	0.28	0.26	0.94	1.06
Number of data (n)	43125	43502	41690	44152	14384

y = 0.83x + 0.35; r² = 0.86; n = 76 lakes

y = 5.85x - 4.01; r² = 0.76; n = 86[if CB >0]
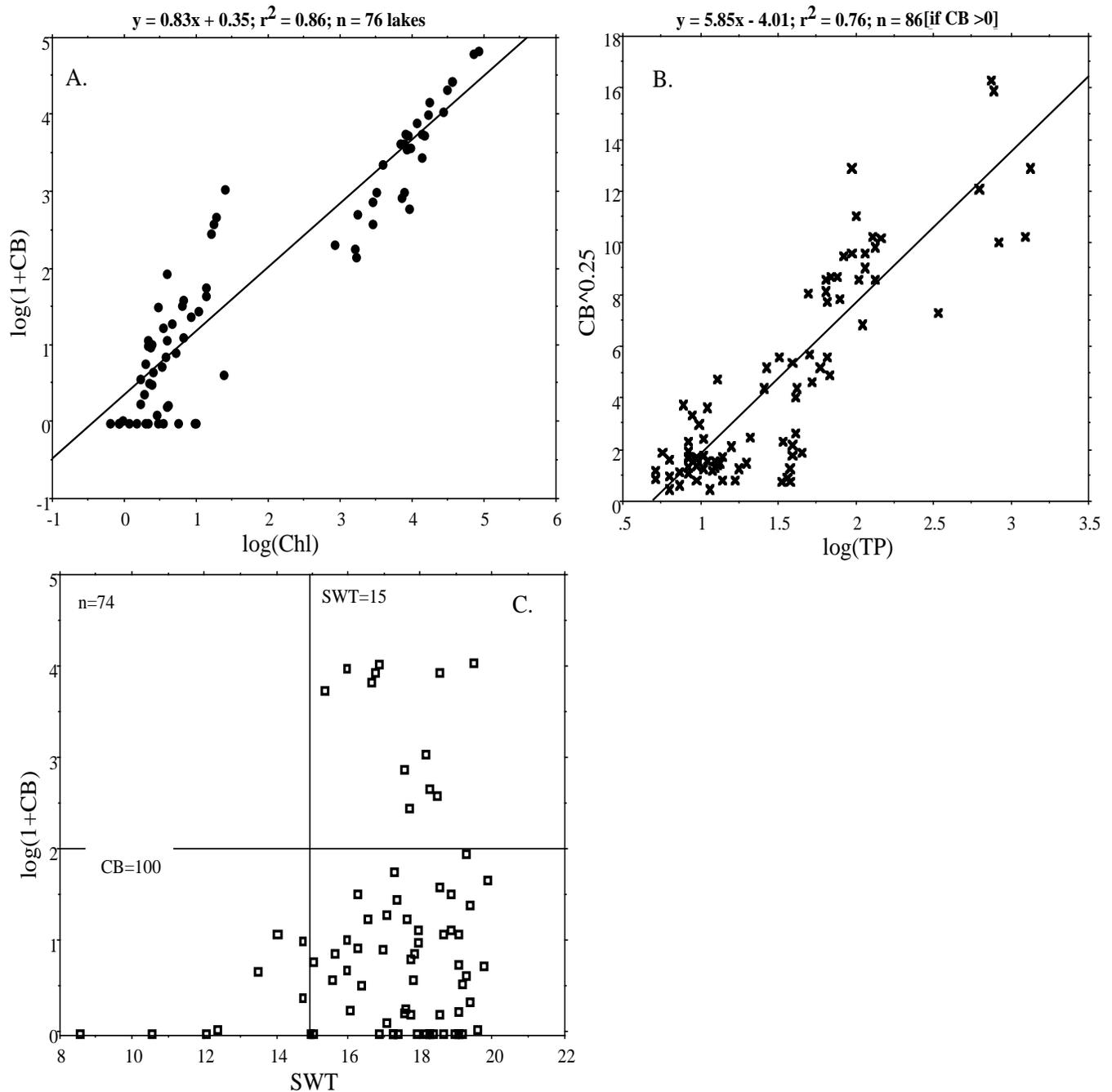
Fig. (2). (**A**) Regression between log(1+CB) and log(Chl) based on data from 76 lakes. The figure also gives the coefficients of determination (r²) and the regression lines. Cyanobacteria (CB) in μg ww/l (ww = wet weight), chlorophyll-a concentrations (Chl) in μg/l. (**B**) Regression between CB (transformed into CB^{0.25}; CB in μg ww/l) and log(TP) (TP in μg/l) using median values for the growing season from 86 systems with CB-values higher than zero. (**C**) Scatter plot between cyanobacteria (transformed into log(1+CB); CB in μg ww/l) versus surface water temperatures (SWT in °C) based on data from 74 systems. From [10].

variation between these two measures of primary production in lake water. This relationship might have looked different had it been based on daily, weekly or monthly values and it is probable that this co-variation also exists for many marine systems. The following calculations first show maximum values for nitrogen fixation.

In a following section, we will also discuss the fraction of N-fixing species of the total biomass of cyanobacteria in the Baltic Proper.

CB Versus TP

The basic regression is given in Fig. (**2B**) (r² = 0.76; n = 86). This regression includes data from more systems than the equation given by [9] and it also yields a higher r²-value (0.76 as compared to 0.71).

CB Versus Temperature

In the literature, temperatures between 15 and 17 °C have been reported as the minimum for cyanobacteria blooms in

Model for cyanobacteria
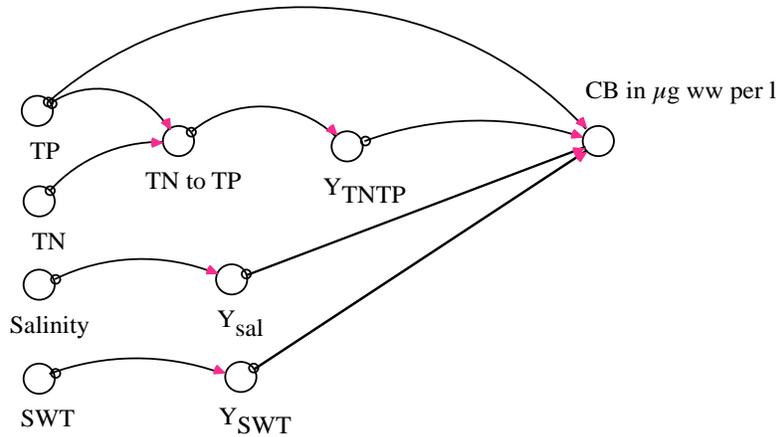
$$CB = ((5.85 \cdot \log(TP) - 4.01)^4) \cdot Y_{TNTP} \cdot Y_{SWT} \cdot Y_{sal}$$

CB in μg ww/l
Salinity in psu
SWT= Surface water temperature in °C
Total-N (TN) in μg/l
Total-P (TP) in μg/l

Y_{TNTP} = if TN/TP < 15 then (1-3·(TN/TP/15-1)) else 1
Y_{SWT} = if SWT ≥ 15 then (0.86+0.63·((SWT/15)^1.5-1)) else (1+1·((SWT/15)^3-1))
Y_{sal} = if salinity <10 then (2.1+1.1·((salinity/10)^2-1)) else (2.1-115·((salinity/10)^0.01-1))

Model domain: 4 < TP < 1300; 165 < TN < 6830; 0 < salinity < 40; 8 < SWT < 25

Fig. (3). Outline of the model to predict median summer values of cyanobacteria in lakes and coastal areas (from [10]).

freshwater systems and in the Baltic Sea [7, 29, 30]. Laboratory experiments on cyanobacteria also support this conclusion [31-33]. There are also reports that cyanobacteria have a requirement of temperatures of about 20-21 ºC to form blooms. Those reports are from a freshwater lake in Canada [34], the North Pacific Ocean [35] and an estuary in Australia [36]. The optimal growth temperatures in laboratory experiments are usually around 25 ºC for many species [31, 33, 37], but these experiments often use species from temperate areas. With higher temperatures, growth rate usually starts to decrease. In field data from the Baltic Sea [3], this decrease in growth rate is not shown because there are few occasions with temperatures higher than 20 ºC.

Fig. (**2C**) 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 CB exceeding 100 µg/l (median values for the growing season) have temperatures higher than 15 °C. Fig. (**3**) gives a compilation of the model. The dimensionless moderator for temperature influences on CB (Y_{SWT}) is given by:

If SWT ≥ 15 °C, then

Y_{SWT} = (15/17.5)·(1+0.63·((SWT/15)$^{1.5}$-1)) else

Y_{SWT} = (1+1·((SWT/15)^3-1))   (1)

This means that if SWT = 15 °C, Y_{SWT} = 0.86; if SWT = 17.5, Y_{SWT} = 1; if SWT = 25, Y_{SWT} = 1.48, 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.

CB Versus Salinity

In hypertrophic lakes, the biomass of cyanobacteria can be very high with concentrations of about 100 mg/l [9]. [38, 39] found no data on N-fixing planktonic species in estuaries and coastal seas, except for the Baltic Sea and the Peel-Harvey estuary, Australia. Also results from [40] support this general absence of N-fixing cyanobacteria in estuaries. There are more than 10 nitrogen fixing cyanobacteria species in the Baltic Proper [6]. The number of species and the nitrogen fixation rates have been considerably revised upwards during recent years [7]. A field study in the Baltic Sea [3] indicates that in this brackish environment species of cyanobacteria have, interestingly, the highest biomass at salinities of 7 – 8 psu and that the blooms in Kattegat and Belt Sea are more frequent if the salinity is below 11.5 psu. Results from a laboratory experiment with cyanobacteria from the Baltic Sea support the highest growth rates at salinities between 5 and 10 psu [33]. According to [41], blooms of cyanobacteria in marine environments may not be as common as in freshwater systems. In marine systems, there are just a few dominant genera. In a field study in the Pacific Ocean [35], there was no correlation between the salinity and the abundance of cyanobacteria and no cyanobacteria were found in the cooler, less saline waters. In this model (Eq. 2), the influences of the

salinity on cyanobacteria are motivated by empirical data from many systems [10]:

If the salinity < 10 psu, then

$Y_{sal} = (2.1+1.1 \cdot ((salinity/10)^2 - 1))$, else

$Y_{sal} = (2.1-115 \cdot ((salinity/10)^{0.01} - 1))$ (2)

This means that at a salinity of 10 psu, Y_{sal} is 2.1 and CB a likely factors of 2.1 higher than in freshwater systems.

CB Versus TN/TP

Fig. (**4A**) shows a scatter plot between log(CB) and TN/TP. High CB-values only appear in systems with relatively low TN/TP. The regression between log(CB) and TN/TP attains a maximum value (r^2 = 0.73; log(CB)=0.142·TN/TP+5.47; n = 61) if only data from systems with TN/TP smaller than 40 are used. The upper curve (circles) in Fig. (**4B**) gives the r^2-values when only systems with TN/TP smaller than 10, 15, 20,….. 100, respectively, were used in the regressions. The lower curve in Fig. (**4B**) gives similar results when only systems with higher TN/TP were used. Note that there is no statistically significant (p<0.01) relationship between log(CB) and TN/TP if TN/TP is higher than 15.

The basic regression between CB and TP given by Eq. 1 is complemented with a dimensionless moderator related to TN/TP, Y_{TNTP}, defined by:

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

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 without any correction, but for systems with TN/TP < 15, Eq. 3 is used. If, e.g., TN/TP = 7.2, then $Y_{TNTP} = 2.56$, and the CB-value a factor of 2.56 higher than the value suggested by the basic regression.

The general model for cyanobacteria may give rather uncertain predictions for systems with high TN/TP and low temperatures. However, during such conditions, the N-fixation should be small. Predicting conditions with high CB is evidently more important in calculations of N-fixation. Due to the fact that all methods to estimate N-fixation in entire systems at longer time scales (such as month or years) are very uncertain, there is good reason to regard the approach presented here as complementary to other approaches.

Measured N-fixation tends to follow a similar pattern as the prevalence of cyanobacteria [6, 38, 39]. Analyses using gene sequencing techniques have suggested that more organisms than we currently know may fix nitrogen in both lakes and marine systems [42].

DATA AND DATA UNCERTAINTY

To estimate the N-fixation in the Baltic Proper, we have used one main dataset, the HELCOM data compiled in Table **4C** [43]. All calculations have used median monthly values on TN, TP, salinity and temperature since these are the obligatory driving variables in the model.

The uncertainty in the model variables 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 for 58 lakes. One should note from Table **5** that cyanobacteria should be expected to have significantly higher CVs than most other variables and are therefore more difficult to predict with a high certainty. It is also important to note that dissolved inorganic nutrient fractions have considerably higher CVs than TN and TP. Thus, dissolved inorganic nutrient fractions are, in relation to TN and TP, not only poorly correlated with chlorophyll and Secchi depth [44], but given their high inherent uncertainties, it is much more costly to determine reliable mean/median values that can be used in ecosystem models, whose scientific quality and usefulness is given by their predictive power.
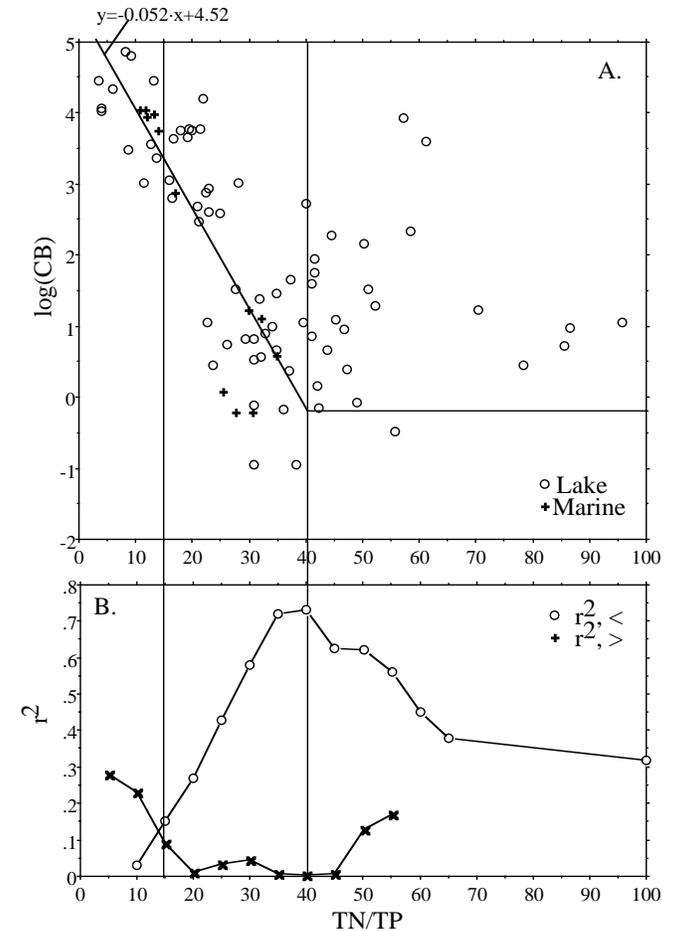
Fig. (4). (**A**) Scatter plot between log(CB) and TN/TP. The regression line is based on data from systems where TN/TP < 15. (**B**) Compilation of r^2-values between log(CB) and TN/TP using data from systems when successively smaller (<) and higher (>) TN/TP have been omitted. The maximum r^2 is obtained for systems with TN/TP < 40; very low r^2-values are obtained if the regressions are done for systems with TN/TP > 15 (from [10]).

The basic factors regulating CVs for chlorophyll and cyanobacteria (i.e., nutrients, temperature, light, salinity, predation, analytical uncertainties related to sampling) should be similar in most aquatic systems. [45] showed that for the River Danube, the CV for the cyanobacteria was, on average, a factor of 1.3 higher than for chlorophyll.

This means that one should expect that the r^2-value 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).

Table 5. Coefficients of Variation (CV) for Twelve Water Variables Based on Data from 58 Swedish Lakes (from [10]). The Values Describe Conditions During the Growing Season 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

RESULTS

Calculating Maximum Nitrogen Fixation in the Baltic Proper

Table **6** exemplifies the calculation routine using data for 2004. The table gives the median monthly values for surface-water temperatures (i.e., for samples taken above the theoretical wave base of 44 m in the Baltic Proper; see [46]), salinity, TN, TP and TN/TP, as well as the dimensionless moderators for salinity (Y_{sal}), surface-water temperature (Y_{SWT}) and TN/TP (Y_{TNTP}). The calculated monthly concentrations of total cyanobacteria (CB in µg/l) are given as well as the calculated maximum potential N-fixation. The table also shows the dimensional adjustments used in the calculation. From Table **6**, one can note:

- The maximum potential N-fixation (in 2004) in the Baltic Proper was about 255 kt, with highest values in July, August and September.

- This corresponds very well to the overall values given in Table **3**.

  The calculated lowest and highest values for the maximum potential annual N-fixation in the Baltic Proper for the period 1997 to 2005 are given in Table **7**. One can note:

- The variation among the years is very high: the smallest value is 45 kt, the highest 908 kt.

- The average annual value is 191 kt.

Both [5] and [6] measured higher rates in 1997 than in 1998, but our results show higher rates in 1998 (58 and 71 kt, respectively). However, the order-of-magnitude is about the same in our measurements for these two years, and the

uncertainty in the calculated values is higher than the difference between these two years.

Fig. (**5**) illustrates the modelled monthly concentrations of cyanobacteria from 1996 to 2005. One can see clear seasonal patterns and that the modelled values are very high for 2005, which also had a very high and much discussed algal bloom.

Using the model, one can also clarify the factors contributing to the calculated values. Fig. (**6**) illustrates predictions if the four x-variables (i.e., the monthly median values of temperature, salinity, TN and TP) were reduced by 25%. A reduction in TN would increase the predicted biomass of total cyanobacteria considerably, while reductions in salinity, temperature and TP would lower the predicted values; the clearest response would be from reductions in temperature. According to our results, the high CB-values in 2005 may be attributed to relatively high TP-concentrations and high temperatures in the summer and fall.

TN/TP and Cyanobacteria

If TN/TP is lower than 7.2, phytoplankton species that can take up dissolved nitrogen of atmospheric origin will be favoured. Empirical data in Fig. (**4**) show that there is a threshold limit for the ratio not at 7.2 but rather at 15. Fig. (**7**) gives a scatter plot of all available data on TN/TP (n = 24048) from the surface-water layer in the Baltic Proper from 1990 to 2005; Fig. (**8**) shows variations in median monthly TN/TP in relation to the Redfield ratio of 7.2 and the threshold ratio of 15. From these two figures, one can note that there are no major changes in the general temporal trend. There is also a very large scatter in the data and clear seasonal patterns.

Table **8** gives a compilation of TN/TP statistics for the Baltic Proper, divided into four categories: (1) all data from the surface-water layer, (2) all data for situations with temperatures higher than 15 °C, (3) all data with Temp > 15 °C and from water depths < 20 m, and (4) all data with Temp > 15 °C, water depths < 20 m and TP > 10 µg/l. This table also gives information on the percentage of the data with TN/TP smaller than 7.2 and smaller than 15. The main conclusion from this table is that less than 7% of the values are smaller than 7.2 and that between 30 and 50% of the TN/TP-ratios are smaller than 15. Fig. (**9**) gives a frequency distribution for the data at sites with temperatures higher than 15°C.

The key information in Figs. (**7-9**) and Table **8** is that the conditions in the surface-water layer of the Baltic Proper often favour cyanobacteria. However, in more than 70% of the situations when the water temperature is higher than 15 °C and the risks of blooms of cyanobacteria are highest, the system is not N-limited but P-limited (Fig. **9**). To minimize the overall primary phytoplankton production in the Baltic Proper (eutrophication), one should mainly reduce the discharges of phosphorus to the systems. To achieve a goal of less eutrophication in the Baltic Proper, N-reductions would be more or less meaningless, which is well in line with results presented by [1]. In 30% of the situations, when TN/TP is lower than 15, reductions in N would favour nitrogen fixing cyanobacteria, which should be avoided.

Fig. (**10**) gives a regression between median monthly concentrations of chlorophyll-a and modelled concentrations

Table 6.   **Calculation of Monthly and Annual Maximum Potential Nitrogen Fixation in the Baltic Proper Using Data from 2004**

Month	Temp	Salinity	TN	TP	TN/TP	Y_{sal}	Y_{SWT}	Y_{TNTP}	CB	N-Fix
2004	°C	psu	µg/l	µg/l					µg ww/l	t/Month
1	3.8	7.1	276	22.9	12.0	1.56	0.02	1.59	10	2363
2	2.4	7.2	266	25.7	10.4	1.57	0.00	1.93	4	937
3	2.0	7.1	275	29.4	9.3	1.55	0.00	2.13	4	870
4	3.5	6.9	261	25.4	10.3	1.52	0.01	1.95	12	2917
5	6.2	7.1	254	21.1	12.0	1.55	0.07	1.59	34	8171
6	10.0	7.1	259	18.9	13.7	1.55	0.29	1.26	82	19748
7	12.5	7.1	265	20.0	13.3	1.55	0.58	1.35	202	48451
8	16.5	7.0	270	19.5	13.9	1.54	0.96	1.23	284	68315
9	13.4	7.0	269	19.2	14.0	1.54	0.71	1.20	197	47328
10	9.7	7.0	265	20.8	12.8	1.53	0.27	1.44	110	26359
11	8.5	6.9	261	19.8	13.1	1.53	0.18	1.37	62	14899
12	6.0	7.1	265	25.7	10.3	1.56	0.06	1.94	61	14619
								Max. annual N-fixation (t)		254977

$CB \cdot 8.7 \cdot 10^{12} \cdot 10^{-9} \cdot 0.2 \cdot (0.42/4.19) \cdot 0.147 \cdot (1/3.2) \cdot 30$.
$CB \cdot V$ (m³)·(µg to t)·(ww to dw)·(dw to C)·(C to N)·(1/d)·(d to month).

of total cyanobacteria. The co-variation is significant ($p = 0.0008$), but the scatter around the regression line is considerable ($r^2 = 0.11$). Two reasons for this low r^2-value is that the range in the monthly chlorophyll data is relatively small in the Baltic Proper (from 0.2 to 4 µg/l), and that the CV for the empirical chlorophyll data is high.

We have also investigated if it would be possible to test these model predictions of cyanobacteria against empirical data on cyanobacteria. The basic idea was to use information accessible from SMHI (see SMHI website), but that turned out to be very difficult for several reasons. The satellite images only give qualitative (not quantitative) information related to non specified algal blooms in the uppermost water layer at defined hours and the results depend very much on the cloudiness. Our model predictions concern the inventory of total cyanobacteria in the entire surface-water layer (0 to 44 m) in the entire Baltic Proper on a monthly basis, and to transform the information conveyed by the satellite images has been beyond the possibilities of this work.

The Fraction of N-Fixing Cyanobacteria

All calculations so far are based on total concentrations of cyanobacteria (CB_{tot}) using the model in Fig. (**3**). The fraction of N-fixing cyanobacteria (CB_{fix}) is not identical to, but lower than, CB_{tot}. We have estimated the ratio CB_{fix}/CB_{tot} using data from the Baltic Proper. The data comes from two monitoring stations, one outside the Askö research station (B1) and one at the Landsort deep (BY31). We have accessed the data at, http://www2.ecology.su.se/dbbm/JS.html.

Cyanobacteria from the order Nostocales can fix nitrogen in heterocysts. Nitrogen fixation is rather energy consuming, with each heterocyst requires 12 to 20 photosynthetic cells to provide the necessary energy supply [47].

Table 7.   **Compilation of Calculated Annual Maximum Potential Nitrogen Fixation in the Baltic Proper Between 1997 and 2005. Note that the Results from 2005 are Based on Interpolated Data for the Missing Months**

Year	N-Fix (t/yr, max.)
1997	58303
1998	70960
1999	60443
2000	116916
2001	45181
2002	131898
2003	73642
2004	254977
2005	908087
Min	45181
Max	908087
Mean	191156
Median	73642
SD	276448
CV	1.45
n	9

Recent studies indicate that other primary producers (non-heterocystous cyanobacteria) smaller than 10 µm may contribute considerably to nitrogen of the total N-fixation in

the Baltic Proper may be fixation [6]; measurements indicated that up to 43% done by these organisms, while not contributing much to total biomass.

Table 8.　Compilation of Statistical Information TN/TP in the Surface-Water Layer of the Baltic Proper Using Data from 1990 to 2005

	All	Temp >15 °C	Depth <20 m	TP >10 µg/l
Min.	1.05	1.74	1.74	1.74
Max.	171	76.9	76.9	59.4
Mean	16.5	20.1	20.4	18.7
Median	15.2	19.5	19.8	18.8
SD	8.1	9.2	9.2	7.4
CV	0.49	0.46	0.45	0.40
n	24048	3001	2880	2577
% < 7.2	7.0	6.5	6.0	6.6
% < 15	49.1	28.8	27.5	30.7
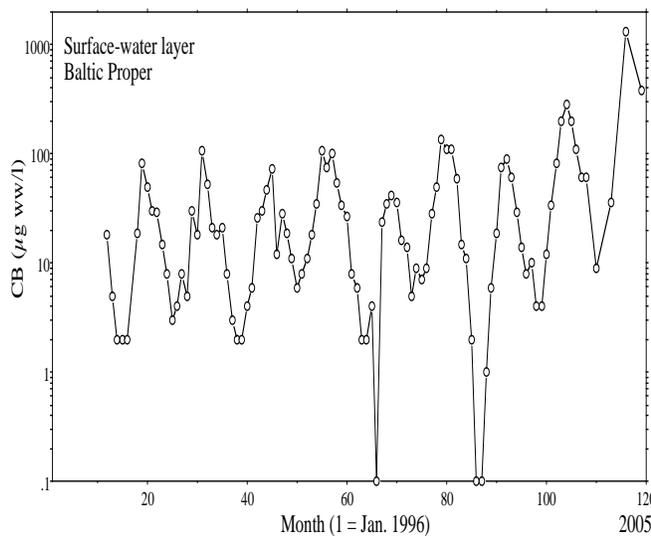
Fig. (5). Modelled concentrations of total cyanobacteria (µg ww/l; ww = wet weight) in the surface-water layer of the Baltic Proper 1996 to 2005.

Because heterocyst-forming species of cyanobacteria do not always fix nitrogen and because small non-heterocystous cyanobacteria may contribute to N-fixation, it is complicated to find a reliable value of CB_{fix}/CB_{tot}. Further complicating matters include the high inherent CV for cyanobacteria and the fact that reliable data for the entire surface-water layer for longer periods of time from the Baltic Proper (or from most systems) are scarce. In this estimation of CB_{fix}/CB_{tot}, only the heterocyst-forming species have been considered. An average ratio of 0.85 was calculated from all 580 values (see Table **9**).

The mean and median values when the water temperature is higher than 15°C (the threshold temperature for cyanobacteria) are between 0.84 and 0.97, respectively. The actual N-fixation may, however, be close to the modelled maximum

values since N-fixation from "small organisms" is not included in this ratio.
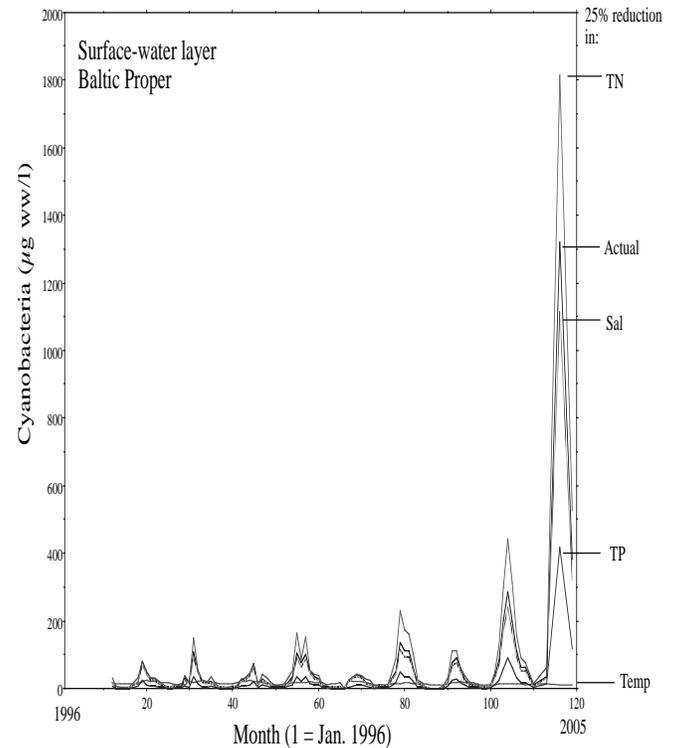
Fig. (6). Simulations to illustrate how 25% reductions in TN, TP, water temperature and salinity (Sal) would likely influence the concentrations of cyanobacteria in the surface-water layer of the Baltic Proper using data from 1996 to 2005. The curve marked "Actual" is based on empirical data from the given period.

[10] calculated CB_{fix}/CB_{tot} using lake data from [9], deriving a value of 0.33, which is considerably lower than these results for the Baltic Proper.
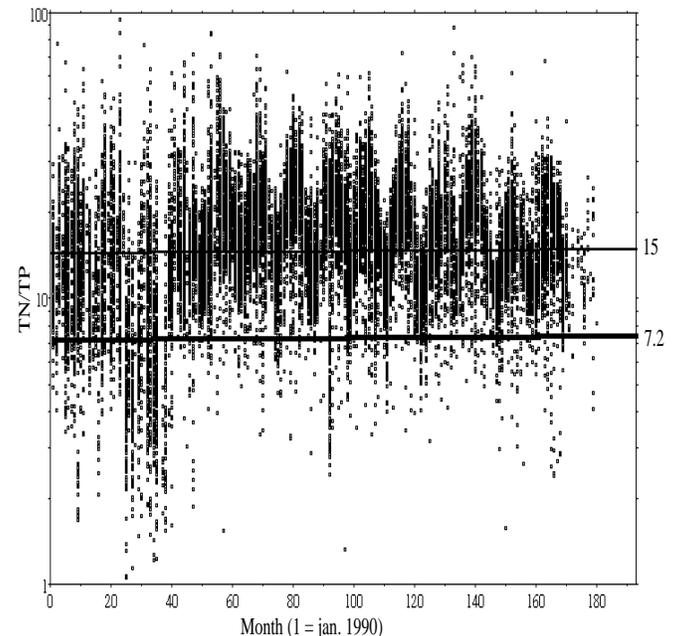
Fig. (7). TN/TP from all (n=24048) surface-water samples in the Baltic Proper from 1990 to 2005.
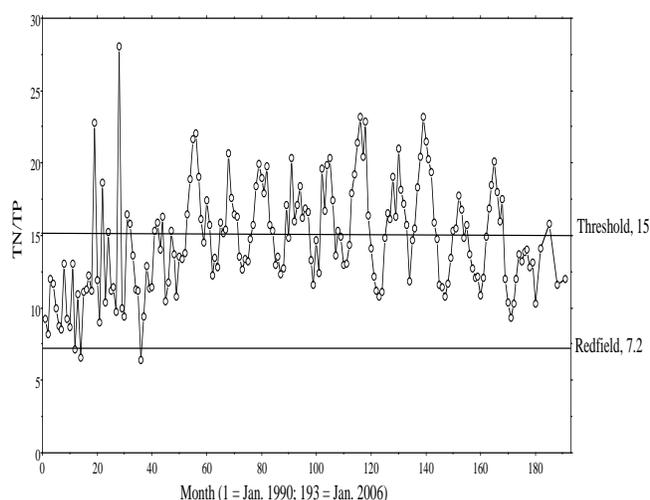
Fig. (8). Variations in median monthly TN/TP in the surface-water layer of the Baltic Proper from 1990 to 2005 in relation to the Redfield ratio of 7.2 and the threshold ratio of 15 (see text).

The mean compositions of cyanobacteria species or genus at the two monitoring stations in the Baltic Proper in situations with water temperatures higher than 15°C are given in Fig. (**11**). This diagram is included here so that these results from the Baltic Proper may be translated to other systems where different cyanobacteria with other CB_{fix}/CB_{tot}-ratio may prevail. The two nitrogen fixing species *Aphanizomenon* sp. and *Nodularia spumigena* are dominant, with 80% of the biomass. Then follow *Cyandictyon* spp., *Pseudanabaena limnetica*, *Anabaena* spp. and *Anabaena lemmermannii* with a few percent each. The remaining species contribute, on average, less than 1% of the biomass of cyanobacteria.
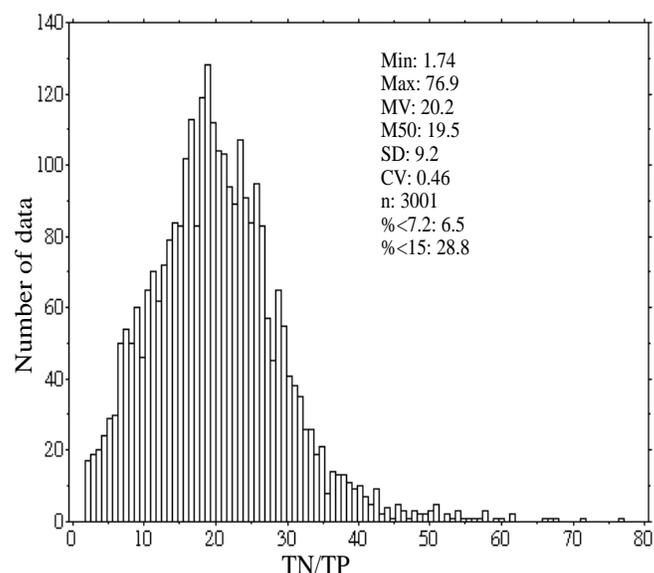
Fig. (9). Frequency distribution for TN/TP for all samples with temperatures higher than 15°C during 1990 to 2005 in the surface-water layer of the Baltic Proper. 6.5% of the data have TN/TP < 7.2; 28.8 % of the data ratios < 15.

CONCLUDING COMMENTS

The empirically-based approach to predict total nitrogen fixation used in this work (from [10]) is general and could be used for other aquatic systems in a wide salinity range. It is

Table 9. The Ratio Between the Volume of Nitrogen Fixation Cyanobacteria to the Total Volume of Cyanobacteria from Two Monitoring Stations in the Baltic Proper, Askö (Data from 1983 to 2005) and the Landsort Deep (Data from 1990 to 2005). MV = Mean Value for All Data; n = Number of Data. Temperature Data (T) from Askö from 1992 to 2004 and Landsort Deep from 1990 to 2004 at 0 to 20 m Depth

Month	Askö			Landsort Deep		
	T(ºC)	n	CB_{fix}/CB_{tot}	T(ºC)	n	CB_{fix}/CB_{tot}
1	1.6	10	0.50	3.0	11	0.79
2	0.8	10	0.68	1.9	8	0.83
3	1.2	11	0.93	1.7	23	0.88
4	2.7	16	0.87	2.7	38	0.90
5	6.5	21	0.94	5.7	28	0.93
6	10.9	38	0.96	9.7	32	0.97
7	13.6	53	0.96	13.5	34	0.87
8	15.3	52	0.87	15.5	34	0.82
9	12.8	37	0.79	13.5	25	0.85
10	9.3	29	0.73	10.3	21	0.69
11	6.4	13	0.91	7.2	14	0.51
12	4.0	10	0.69	5.4	11	0.74
Median for T>15ºC	17.2	30	0.97	16.8	34	0.91
Mean for T>15ºC	17.2	30	0.92	16.6	34	0.84
Mean for all data	7.6	301	0.86	7.8	279	0.84

meant to give seasonal or monthly data for the surface-water layer. When tested using empirical data for the Baltic Proper, there was reasonably good overall correspondence between the estimates using this model and other approaches. For the future, it would be important to have better data on the fraction of nitrogen fixing cyanobacteria not just in the Baltic Proper but more generally, so that a general algorithm could be derived for this important ecosystem characteristic.
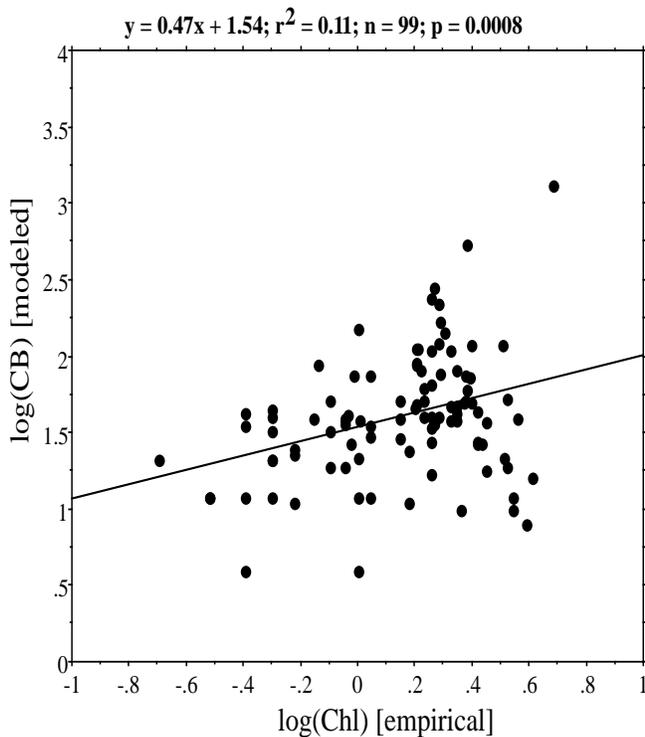
Fig. (10). The relationship between empirical median monthly concentrations of chlorophyll-a (μg/l) and modelled concentrations of cyanobacteria (μg ww/l) using data from 1997 to 2005 for the surface-water layer of the Baltic Proper.
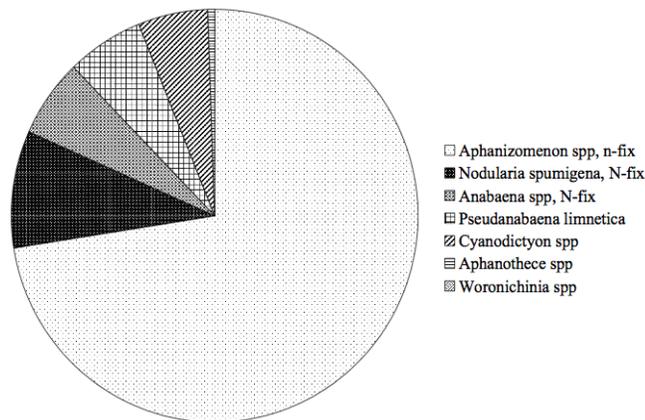
Fig. (11). Average composition of Cyanobacteria at water temperatures higher than 15 °C at Askö (station B1) using data from 30 days sampled between 1992 to 2004 and at the Landsort deep, (station BY31) using data from 34 days collected between 1990 to 2004.

REFERENCES

[1] Savchuk O, Wulff F. Modelling regional and large-scale response of Baltic Sea ecosystems to nutrient load reductions. Hydrobiologia 1999; 393: 35-43.

[2] Tyrrell T. The relative influences of nitrogen and phosphorus on oceanic primary production. Nature 1999; 400: 525-31.

[3] Wasmund N. Occurrence of cyanobacteria blooms in the Baltic Sea in relation to environmental conditions. Int Revue ges Hydrobiol 1997; 82: 169-84.

[4] Rahm L, Jönsson A, Wulff F. Nitrogen fixation in the Baltic proper: an empirical study. J Mar Syst 2000; 25: 239-48.

[5] Larsson U, Hajdu S, Walve J, Elmgren R. Baltic Sea nitrogen fixation estimated from the summer increase in the upper mixed layer. Limnol Oceanogr 2001; 46: 811-20.

[6] Wasmund N, Voss M, ad Lochte K. Evidence of nitrogen fixation by non-heterocystous cyanobacteria in the Baltic Sea and recalculation of a budget of nitrogen fixation. Mar Ecol Prog Ser 2001; 214: 1-14.

[7] 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.

[8] OECD. Eutrophication of waters. Monitoring, assessment and control. OECD, Paris 1982; p. 154.

[9] Smith VH. Predictive models for the biomass of blue-green algae in lakes. Water Resour Bull 1985; 21: 433-9.

[10] Håkanson L, Bryhn AC, Hytteborn JA. On the issue of limiting nutrient and predictions of bluegreen algae in aquatic systems. Sci Total Environ 2007; 379: 89-108.

[11] Melvasalo T, Niemi Å, Niemitö L, Rinne I. On the importance of nitrogen fixation in the Baltic Sea ecosystem. Symposium on Ecological Investigations of the Baltic Sea Environment, Riga 16-19 March 1983; pp. 176-89.

[12] Leppänen JM, Niemi Å, Rinne I. Nitrogen fixation of cyanobacteria (blue-green algae) and nitrogen cycle of the Baltic Sea. Symposis 1988; 6: 181-94.

[13] Rönner U. Nitrogen transformations in the Baltic proper: denitrification counteracts eutrophication, Ambio 1985; 14: 134-8.

[14] Hübel H, Hübel M. Blaualgen-Wasserblüten in der Ostsee: Ursachen-Ausmass-Folgen. Dtsch Hydrogr Z (Suppl) 1995; 2:151-8.

[15] Redfield AC, Ketchum BH, Richards FA. In: Hill N, Ed. The influence of organisms on the composition of sea-water. The Sea 2. Interscience, New York 1963; pp. 26-77.

[16] Hassett RP, Cardinale B, Stabler LB, Elser JJ. Ecological stoichiometry of N and P in pelagic ecosystems: comparison of lakes and oceans with emphasis on the zooplankton-phytoplankton interaction. Limnol Oceanogr 1997; 42: 648-62.

[17] Vidal M, Duarte CM, Agusti S. Dissolved organic nitrogen and phosphorus pools and fluxes in the central atlantic ocean. Limnol Oceanogr 1999; 44: 106-15.

[18] Dodds WK. Misuse of inorganic N and soluble reactive P concentrations to indicate nutrient status of surface waters. JNABS 2003; 22: 171-81.

[19] Dillon PJ, Rigler FH. The phosphorus-chlorophyll relationship in lakes. Limnol Oceanogr 1974; 19: 767-73.

[20] Meeuwig JJ, Kauppila P, Pitkänen H. Predicting coastal eutrophication in the Baltic: a limnological approach. Can J Fish Aquat Sci 2000; 57: 844-55.

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

[22] Floderus S. The effect of sediment resuspension on nitrogen cycling in the Kattegat-variability in organic matter transport. UNGI Report 71, Dr. thesis, Uppsala University, Sweden 1989.

[23] Wulff F, Rahm L, Larsson P, Eds. A systems analysis of the Baltic Sea. Springer-Verlag, Berlin, Ecological Studies 2001; Vol. 148: p. 455.

[24] Ryther JH, Dunstan WM. Nitrogen, phosphorus, and eutrophication in the coastal marine environment. Science 1971; 171: 1008-13.

[25] Guildford SJ, Hecky RE. Total nitrogen, total phosphorus, and nutrient limitation in lakes and oceans: Is there a common relationship? Limnol Oceanogr 2000; 45: 1213-23.

[26] Wetzel RG. Limnology. Academic Press, London 2001; p. 1006.

[27] Moisander PH, McClinton E, Paerl HW. Salinity effects on growth, photosynthetic parameters and nitrogenase activity in estuarine planktonic cyanobacteria, Microbila. Ecology 2002; 43: 432-42.

[28] 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 Pollut 2003; 122: 379-90.

[29] Reynolds CS, Walsby AE. Water-blooms. Biol Rev 1975; 50: 437-81.

[30]   Edler Å. Blue-green algal blooms and N:P ratio in the Baltic Sea. Acta Bot Fennica 1979; 110: 57-61.

[31]   Konopka A, Brock TD. Effect of temperature on blue-green algae (Cyanobacteria) in Lake Mendota. Appl Environ Microbiol 1978; 36: 572-6.

[32]   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: 269-82.

[33]   Lehtimäki J, Moisander P, Sivonen K, Kononen K. Growth, nitrogen fixation, and Nodularin production by two Baltic Sea Cyanbacteria. Appl Environ Microbiol 1997; 63: 1647-56.

[34]   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 1987; 44: 598-604.

[35]   Marumo R, Asaoka O. Distribution of pelagic blue-green algae in the Northen Pacific ocean. J Oceanogr Soc Jpn 1974; 30: 77-85.

[36]   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.

[37]   Robarts RD, Zohary T. Temperature effects on phytosynthetic capacity, respiration, and growth rates of bloom-forming cyanobacteria. NZ J Mar Freshwater Res 1987; 21: 391-9.

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

[39]   Howarth RW, Marino R, Lane J, Cole JJ. Nitrogen fixation in freshwater, estuarine, and marine ecosystems. 1. Rates and importance. Limnol Oceanogr 1988; 33: 669-87.

[40]   Marino R, Chan F, Howarth RW, Pace ML, Likens GE. Ecological constraints on planktonic nitrogen fixation in saline estuaries. I. Nutrient and trophic controls. Mar Ecol Prog Ser 2006; 309: 25-39.

[41]   Sellner KG. Physiology, ecology and toxic properties of marine cyanobacteria blooms. Limnol Oceanogr 1997; 42: 1089-104.

[42]   Zehr JP, Jenkins BD, Short SM, Steward GF. Nitrogenase gene diversity and microbial community structure: a cross-system comparison. Environ Microbiol 2003; 5: 539-54.

[43]   HELCOM. Baltic Sea Environment Proceedings No. 100 from the HELCOM website 2000.

[44]   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: 330-46.

[45]   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 Modell 2003; 169: 179-96.

[46]   Håkanson L. Physical geography of the Baltic. The Baltic University. Session 1. Uppsala University 1991.

[47]   Vitousek PM, Cassman K, Cleveland C, *et al.* Towards an ecological understanding of biological nitrogen fixation. Biochemistry 2002; 57/58: 1-45.

[48]   Håkanson L, Lindgren D. On regime shifts and budgets for nutrients in the open Baltic Proper-evaluations based on extensive data between 1974 and 2005. J Coast Res 2008; 24: 246-60.

[49]   SNV. Eutrophication of land, freshwater and the Sea (in Swedish, Eutrofiering av mark, sötvatten och hav). Swedish Environmental Protection Agency, Report 4134, Stockholm 1993; p. 199.

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

[51]   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.

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

[53]   Petersen JK, Hansen JW, Laursen MB, Conley D. Regime shift in a marine coastal ecosystem. Manuscript, National Environmental Research Institute and County of Ringkjøbing 2006.

[54]   Bryhn AC, Håkanson L, Eklund JM. Variabilities and uncertainties in key coastal water variables as a basis for understanding changes and obtaining predictice power in modelling. J Coast Res 2008; in press.

[55]   Håkanson L, Bryhn AC. Eutrophication in the Baltic Sea-present situation, nutrient transport processes, remedial strategies. Springer Verlag, Berlin, Heidelberg 2008; p. 264.