Institutionen för Miljöanalys

Nitrogen as a limiting factor for phytoplankton biomass in nutrient poor lakes

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by

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Abstract

Both nitrogen and phosphorus may limit the algal biomass in lakes. Phosphorus is generally considered to be the most limiting factor in fresh waters, and therefore addition of that nutrient leads to enhanced algal production; eutrophication. In recent years, the role of nitrogen as a eutrophicating factor has come into focus, and some models have been published describing the combined effect of phosphorus and nitrogen on algal biomass. These published equations were applied on data for 103 Swedish reference lakes from the years 1996-97 to see their validity.
To further evaluate the eutrophicating effect of nitrogen in nutrient poor lakes, new models were made based on the Swedish data. Multiple linear regression using epilimnic data for the whole vegetation period (May—September) revealed the best equation as:

$\log(\text{Chl-a}) = -1.243 + 0.710\log(\text{TP}) + 0.515\log(\text{TN}) + 0.251\log(\text{Abs\_F})$

$$r^2 = 0.64, \; n = 143, \; p < 0.01$$

The results indicate that algal biomass (Chl-a) in nutrient poor lakes depend not only on total phosphorus concentrations (TP), but also on total nitrogen concentrations (TN) and water colour (Abs_F). The new models were validated on independent data from 1998.

Introduction

Predictions and modelling play an important role in limnology (Peters 1986, Jörgensen 1995). For global and local environmental questions it is essential to have reliable models that predict the outcome of certain treatments, for instance concerning lake restoration projects.

Lake eutrophication refer to enrichment in nutrients, notably nitrogen and phosphorus, and therefore enhanced production of algal and higher plant biomass. In 1840, von Liebig suggested that one limiting factor is responsible for the yield (in this case algal growth) at one occasion. This limiting factor might for instance be a nutrient, light, temperature, pH or grazing by zooplankton (fig. 1). But the relationship is more complex than that. Due to different optimal conditions for different algal species, more than a single factor will be limiting for the algal community growth at any time even though only one factor limits each individual species.
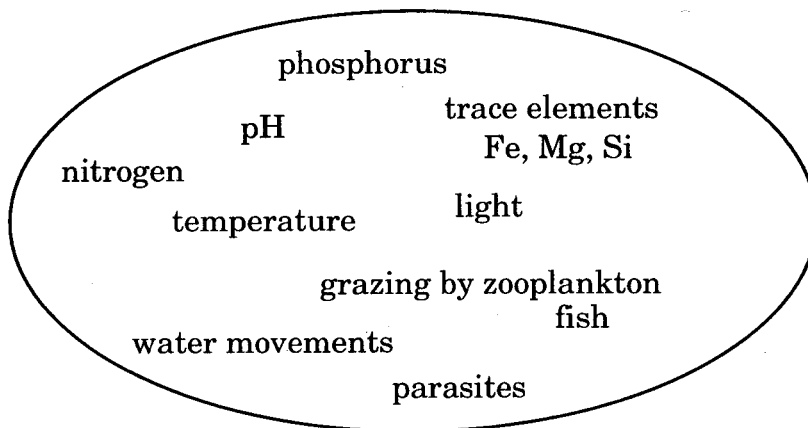
phosphorus

pH   trace elements
             Fe, Mg, Si

nitrogen

temperature   light

grazing by zooplankton
                    fish
water movements

parasites

Fig. 1. Possible factors influencing the algal biomass in nutrient poor lakes.

Totally over 60 chlorophyll:nutrient relationships are published before 1989 (Prarie et al. 1989). They cover both Northern latitudes and subtrophic lakes and have a large variation in the data used.

Many authors (Sakamoto (1966), Dillon and Rigler (1974), and OECD (1982) show a strong correlation between total phosphorus (TP) concentration and phytoplankton biomass (measured as chlorophyll a, Chl-a). Although the models are generalizations, this implies that phosphorus is the major element controlling algal biomass and that a lower phosphorus concentration will result in a reduced phytoplankton biomass. The simplified overall view is that P is limiting in freshwater while N is limiting on land and in the oceans (Cloot and Roos 1996). However, Smith (1982), Prairie et al. (1989), and Seip (1994) also included nitrogen as a significant factor regulating the algal biomass in freshwaters.

A review made by Elser et al. in 1990 of whole-lake and laboratory experiments on the limitation by phosphorus and/or nitrogen to phytoplankton growth shows a need for a balanced view of which nutrient is most limiting for phytoplankton biomass. It is commonly observed that the most pronounced phytoplankton responses to enrichment in whole-

lake experiments occur when both N and P are added together. Only if the stimulation of nitrogen or phosphorus alone equals the combined effect of them is it certain that only one nutrient was limiting. In the laboratory bioassay studies they found considerable deficiencies in the work made by the various investigators. In spite of that, combined N+P enrichment enhanced algal production much more than did addition of N or P singly. On average, the algal response did not differ for P vs. N enrichment.

The following question is adressed in this report since increased nitrogen deposition has been observed in Sweden (Kindbom et al. 1994):

Does nitrogen together with phosphorus act as a eutrophicating factor in Swedish nutrient poor lakes (oligo-, meso-, and dystrophic (brown due to humic acids)?

One way to answer the question was to develop an equation that describe the relation between algal biomass, measured as chlorophyll a (Chl-a) concentration, and the concentrations of nitrogen- and phosphorus compounds.

The aim was to build a model that use the concentration of biologically avaliable nutrients in a lake before the productive season starts in spring to predict the phytoplankton biomass (Chl-a) at the end of the growth season when almost all nutrients are incorporated in the organisms. Late summer (August) is a period with a relative stable and large phytoplankton standing crop.

An alternative was to develop a model from the mean growth season (May—September) concentrations of nutrients and chlorophyll.

Important to consider is that a model is simply an approximation of the real system. The modelling procedure according to Jörgensen 1995 is:
1. Definition of the problem (space, time and subsystems).
2. Selection of the right model complexity.
3. Conceptualize the model (for instance with a diagram of the variables).
4. Formulation of mathematical equations.
5. Verification (is the model stable in the long term? Does the model react as expected?).
6. Sensitivity analysis (most sensitive components of the model).
7. Calibration (to improve the parameter estimation).
8. Validation (model test on independent dataset).

The relevant steps were followed in this work.

Published equations have a wide range in the data used, from ultra-oligotrophic to hyper-eutrophic lakes. The difference in concentration ranges for the data in this work and those used by one of the published models (Smith 1982) is shown in fig. 2. It is evident that the narrow ranges used here will affect the results of the modelling. A general classification of lakes according to theire nutrient status is shown in table 1.
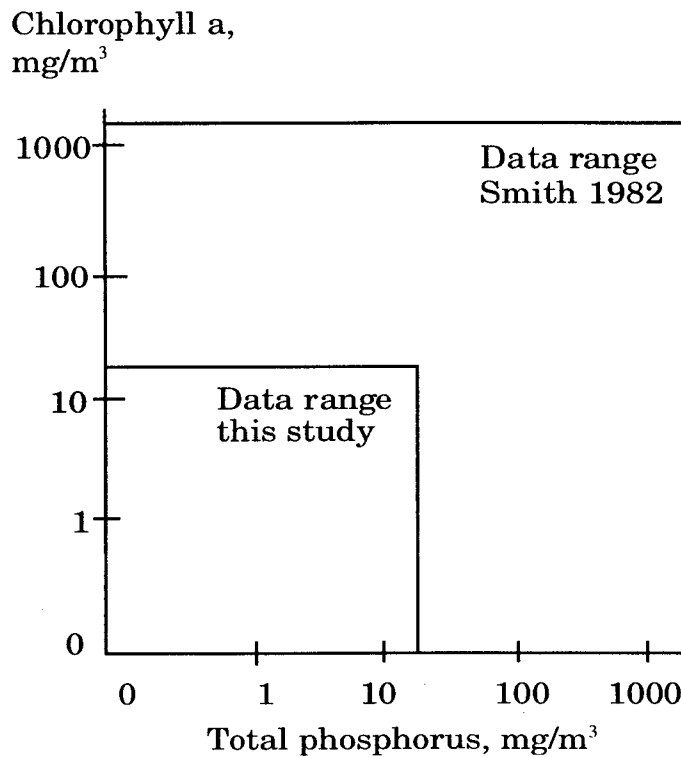
Chlorophyll a, mg/m³

Fig. 2. Data range of phosphorus (TP <26 mg/m³) and chlorophyll (<37mg/m³) in this study compared to the data range (TP <4000 mg/m³) in the published nutrient-chlorophyll relationship by Smith (1982). Observe the logarithmic scale.

Table 1. Classification of lake trophic status (OECD 1982).

	Oligotrophic lake - low production	Mesotrophic	Eutrophic lake -high production
Total phosphorus (TP) mg/m³	< 10	10-35	> 35-100
Total nitrogen (TN) mg/m³	< 500	500-1500	> 1500-2000

The theory of the total phosphorus—chlorophyll (TP—Chl-a) relationship assumes that all TP (or a large and constant fraction) is recycled and therefore biologically avaliable. TP consist of soluble phosphate (PO_4), dissolved organic phosphorus compounds, and particulate phosphorus (in living cells, organic detritus, and adsorbed on abiotic particulate surfaces) (Cloot and Roos 1996). Of all the phosphorus compounds present in a lake, phytoplankton can directly utilize only soluble phosphate (PO_4) for growth.

The amount of biologically avaliable phosphate can therefore determine the photosynthesis rate. In contrast, the phytoplankton standing crop is proportional to the total phosphorus (TP) concentration (soluble + particulate), since most of it is assumed to be included in the plankton.

When PO_4 supplies are low, phytoplankton excrete extracellular enzymes called alkaline phosphatases, which function by splitting off PO_4 originally bound to an organic molecule, like a humic acid or in excretion products from zooplankton (Horne and Goldman 1994).

No such enzymes are excreted for nitrogen cleavage from organic nitrogen compounds. Generally nitrogen is released slower from dead material than phosphorus (Golterman 1975). Part of the total nitrogen (TN) is also tied up in humic acids and therefore decomposed very slow, if at all (Horne and Goldman 1994). Plants utilize nitrogen mostly in the form of ammonium (NH_4^+) and nitrate (NO_3^-), and in most oligotrophic lakes recycling is the main source of nitrogen during summer. Since the recycling is not complete, in a model, inorganic nitrogen (Inorg-N = $NH_4^+ + NO_3^- + NO_2^-$) instead of total nitrogen (particulate + inorganic) might give a more reliable value of the nitrogen avaliable for growth.

The internal algal nitrogen:phosphorus ratio (TN:TP) is around 7 by weight, but differ greatly between species (Heckey and Kilham, 1988). Usually a comparison between the nutrient ratio in the organism and the ratio in water gives an acceptable estimate of the most limiting substance. In general, if the ratio in water of TN:TP is > 10 by weight, phosphorus is considered to limit phytoplankton growth (nitrogen is present in access), and if N:P < 10, nitrogen limits growth. Partly based on this generalization, criteria for critical load (critical deposition) of nitrogen as a eutrophicating factor is made (Kämäri et al. 1992). In lakes with a high N:P ratio, nitrogen is considered not to limit the algal biomass and additional nitrogen deposition is not inducing any further eutrophication.

In this report, water chemistry mesurements from the so-called Swedish reference lakes were used. The whole projekt was initiated in 1983 by the Swedish Environmental Protection Agency (SEPA). The aim was to investigate long term changes, for instance concerning acidification. The lakes are distributed all over the country and are selected to be unaffected or little affected by lokal human activity. They vary greatly in size, mean depth, and altitude (Wilander 1997). Since 1996 the measurements include not only waterchemisty but also chlorophyll a concentrations (algal biomass), and this paper is the first to use these chlorophyll data for calculations.

Materials and methods

Data

Measurements from 103 Swedish reference lakes for the years 1996-97 were used. These belongs either to a set of 20 lakes sampled about 8 times per year or those sampled 3-4 times per year (Persson 1996, Wilander 1997).

All analyses were made at the accredited laboratory at the Department of Environmental Assessment in Uppsala, Sweden. Total phosphorus (TP), total nitrogen (TN), ammonium (NH_4^+), nitrate (NO_3^-), nitrite (NO_2^-), chlorophyll a (Chl-a) concentrations and absorbance of filtered water (water colour, Abs_F) were measured using standardized methods (Wilander 1997).

The data was divided in four groups: spring, late summer, growth season, respectivly annual mean values. The criteria for each group are given below. In order to describe the upper boundaries for each dataset the maximum values and 90 percentile are presented (90 % of the values were less than or equal to this value).

Spring data set

Criteria: Epilimnic samples with low temperature and low chlorophyll a concentrations to insure that samples were taken around spring overturn before the start of, or in the beginning of the productive season. Secondly, low total phosphorus concentrations to ensure nutrient poor conditions. Upper limits for the selected samples are equal to the maximum values for sampling depth, temperature, total phosphorus and chlorophyll a (table 2).

Where possible, mean values of total phosphorus (TP), total nitrogen (TN), and inorganic nitrogen (Inorg-N= NH_4^++ NO_3^-+ NO_2^-) during February—May for each lake and year were calculated. For 77% of the values (140/182) only one spring measurement each year was avaliable.

Table 2. Spring (February—May) data set. Selected samples have these properties (nutrients and chlorophyll in mg/m^3). Number of observations, n=182.

	Maximum	90 percentile
Sampling depth, m	5.0	2.8
Temperature, °C	6.2	3.8
Chlorophyll a (Chl-a)	3.0	2.4
Total phosphorus (TP)	26	15
Total nitrogen (TN)	1633	810
Inorganic nitrogen (Inorg-N)	986	246

Summer data set

Criteria: Upper limits for the selected samples are equal to the maximum values for sampling depth, chlorophyll a, and total phosphorus (table 3). Mean values of chlorophyll a (Chl-a) concentrations and mean absorbance (Abs_F) during July—September were calculated. Absorbance was included as an indicator of light conditions (water colour). For 76% (138/182) of the values only one summer sample from each year was avaliable.

Table 3. Summer (July—September) data set. Selected samples have these qualities. n=182. Nutrient and chlorophyll a concentrations were measured in mg/m^3.

	Maximum	90 percentile
Sampling depth, m	2.0	1.0
Temperature, °C	24.6	22.9
Chlorophyll a (Chl-a)	37	14
Total phosphorus (TP)	33	16
Total nitrogen (TN)	1342	689
Absorbance (Abs_F)	0.80	0.21
pH	8.11	7.31

Growth season data set

Criteria: Epilimnic samples with low TP, TN and Chl-a concentrations to ensure nutrient poor conditions. The maximum values of sampling depth, chlorophyll a and total phosphorus sets the upper limits for the selected samples.
Growth season (May—September) averages of Chl-a, TP, TN, Inorg-N, and Abs_F were calculated for each lake (table 4).
At least two measurements from each year and lake were avaliable. For 29 % of the samples (42/143) more than two measurements make up the average value.

Table 4. Growth season (May—September) data set. Selected samples have these properties (nutrients and chlorophyll in mg/m^3). n=143.

	Maximum	90 percentile
Sampling depth, m	2	1.3
Chlorophyll a (Chl-a)	23	8.1
Total phosphorus (TP)	25	15
Total nitrogen (TN)	968	661
Inorganic nitrogen (Inorg-N)	532	100
Absorbance (Abs_F)	0.68	0.22

Annual data set

Criteria: epilimnic samples from nutrient poor lakes sampled at least six times per year. Maximum values of sampling depth, Chl-a and TP set the upper limit of the selected samples.
Yearly average values were calculated (table 5).

Table 5. Annual data set. Selected samples have these properties. n=23. Nutrients and chlorophyll a concentrations measured in mg/m³.

	Maximum	90 percentile
Sampling depth, m	2.8	2.8
Chlorophyll a (Chl-a)	17	11
Total phosphorus (TP)	22	14
Total nitrogen (TN)	897	734
Absorbance (Abs_F)	0.47	0.38

Statistics

Multiple linear regression analysis using logarithmically transformed mean values of TP, TN, Inorg-N, Abs_F and Chl-a were performed. Data was transformed to base 10 logarithms to meet the requirement of the statistical analysis (stabilized variance).
Goodness-of-fit was determined as r^2, the proportion of the total variance associated with the regression (Fowler and Cohen 1992).
Lack-of-fit was examined by plotting the residuals of the relationship (observed values minus predicted values) against other parameters like temperature and pH.
The statistical program JMP was used (JMP 1995).

Results

The results of the work is presented in three parts, one with evaluations of published chlorophyll:nutrient relationships, one with new models derived from Swedish data, and finally validation of the new models.

Evaluation of published chlorophyll:nutrient relationships

Several published chlorophyll:nutrient relationships are based on phosphorus exclusively, but some include both nitrogen and phosphorus as significant factors. The focus in this study was on the latter. Seven published models for Northern latitude lakes were evaluated, and three types of relationships based on spring, summer, respectivly annual nutrient concentrations were found. The various equations were, according to their restrictions, applied on data from the Swedish reference lakes. The non restricted Swedish data range was TP 2,5-26 mg/m^3, TN 130-1633 mg/m^3, TN:TP 17-188 (by weight). The TP range in the Swedish lakes was very narrow compared to data used by the authors of the published models.

Mean spring nutrients—mean summer chlorophyll relationships

The aim with these relationships is to predict the algal biomass in a lake during late summer just by collecting a water sample during spring overturn. Two published equations were tested (table 6).

Table 6. Published nutrient—chlorophyll relationships for spring mean nutrients to summer mean chlorophyll-a concentrations (mg/m^3). The concentration and TN:TP ranges refer to the conditions used by the authors of the model. The equations tested on Swedish data under the same restrictions gave r^2 Swedish data.

Model	Equation Log(Chl-a)=	TP range	TN:TP ratio	r^2/n	r^2/n Swedish data
Dillon and Rigler (1974)	1.449log(TP)-1.136	3-180	-	0.95/46	0.34/182
Prairie et al. (1989)	0.517log(TP)+0.838log (TN) -2.213	3-5000	5-75	0.81/133	0.37/133

r^2=correlation coefficient.
n=number of observations.

In the model by Dillon and Rigler (1974) only phosphorus (TP 3-180 mg/m^3) is used to predict algal biomass. Prairie et al. (1989) use both P and N with a TN:TP interval of 5-75.

With data from the Swedish reference lakes the published equations gave $r^2=0.34$ respectivly 0.37. This means that only 34% resp 37% of the variation in algal biomass (chlorophyll-a concentration) was explained by variation in nutrient concentration (TP and TN) when using these equations on Swedish data. A shortage was that the Swedish data set often was based on only one sample in spring and one in summer.

Lack-of-fit was examined by plotting the residuals (observed values in the Swedish lakes minus predicted values by the published equations) against other parameters like summer absorbance (Abs_F), total nitrogen, pH value, or temperature (fig. 3, 4). A residual value greater than zero means that the observed Chl-a concentration in a lake was higher than the Chl-a concentration predicted from the published models.
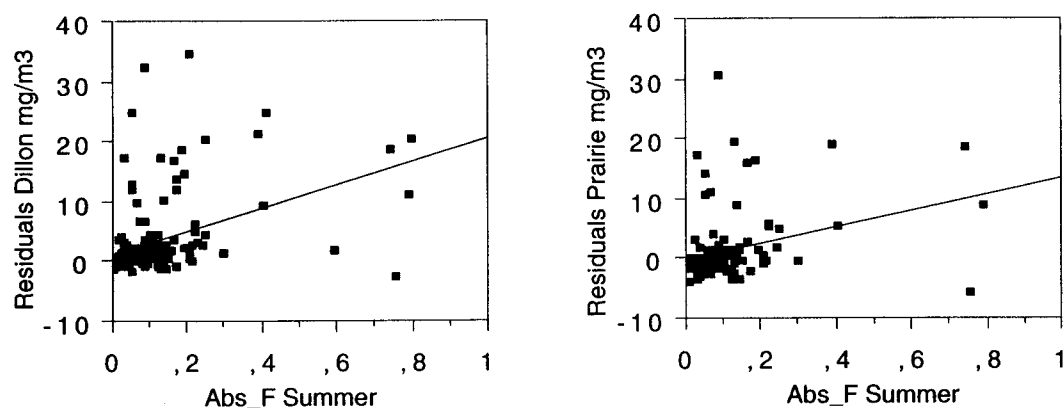

fig 3. Residuals (observed values from the Swedish reference lakes minus predicted values by the equation of Dillon and Rigler (1974) respectivly Prairie et al. 1989) plotted against summer water colour (Abs_F).

Analysis of fig 3 showed that the published equations both underestimated and correctly estimated the chlorophyll concentration in lakes with clear waters (low Abs_F). A tendency of an underestimation of Chl-a in brown lakes (high Abs_F) was also obvious. The equation of Dillon and Rigler (1974) also slightly underestimated chlorophyll-a in lakes over the whole TN range (fig 4). It must be noted that the equation by Dillon and Rigler does not include nitrogen. Residuals from the model by Prairie et al. (1989) plotted against summer pH value (fig. 4) showed an underestimation of Chl-a over the whole pH range. Temperature did not improve the strength in any of the relationships.
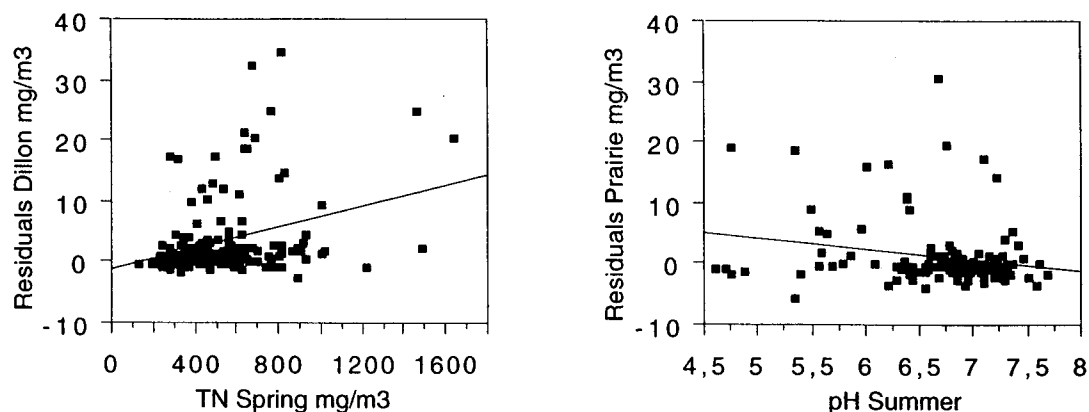
fig 4. Residuals (observed values from the Swedish reference lakes minus predicted values by the equation of Dillon and Rigler (1974) respectivly Prairie et al. (1989) plotted against spring TN respectivly summer pH.

Mean growth season nutrients—mean growth season chlorophyll relationships

These relations are used to analyze the dependence of total phosphorus and total nitrogen concentrations on algal biomass (Chl-a) during the growth season. Four published equations were evaluated (table 7).

Table 7. Published nutrient—chlorophyll relationships for mean growth season (May—September) concentrations of chlorophyll-a (Chl-a), total phosphorus (TP) and total nitrogen (TN) (mg/m^3). The concentration and TN:TP ranges refer to the conditions used by the authors of the model. The equations tested on Swedish data under the same restrictions gave r^2 Swedish data.

Model	Equation Log(Chl-a)=	TP range	TN:TP ratio	r^2/n	r^2/n Swedish data
Smith (1982) A	0.653log(TP) +0.548log(TN)-1.517	2.0-4000	-	0.76/127	0.57/143
Smith (1982) B	0.682log(TP) +0.539log(TN)-1.543	?	>17	0.70/?	0.57/143
Praire et al. (1989)	0.517log(TP) +0.838log(TN)-2.213	3.0-5000	5-75	0.81/133	0.54/129
Seip (1994)	0.467log(TP)•log(TN)-0.667	2-20	-	0.59/117	0.49/136

r^2=correlation coefficient.
n=number of observations.

In all these models, total phosphorus and total nitrogen are used as variables to predict the phytoplankton biomass in lakes. Prairie et al. (1989) did not specify if they used spring or mean growth season nutrient concentrations, therefore the model was evaluated for both.
Overall, lower r^2 values with the dataset for the Swedish reference lakes under the same restrictions indicated that other factors or other proportions of the factors were important for the relationship.

An analysis of the residuals showed that temperature and pH not strongly affected the strength in any of the published relationships (fig 5). Positive residual values mean that the observed Chl-a concentration in a lake was higher than the Chl-a concentration predicted by the model. The model by Seip showed no systematic lack-of-fit at all.
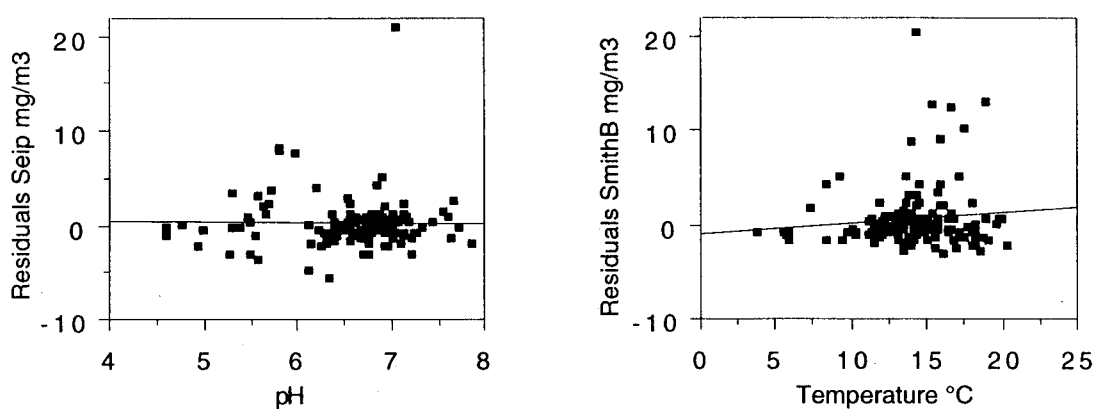

fig 5. Residuals (observed values from the Swedish reference lakes minus predicted values by the equation of Seip (1994) respectivly SmithB (1982) plotted against pH respectivly temperature.

Residuals plotted against water colour (fig 6) showed the same pattern as for the models based on spring nutrient values. An underestimation of Chl-a in clear waters at the same time as a correct estimation in clear waters was obvious.
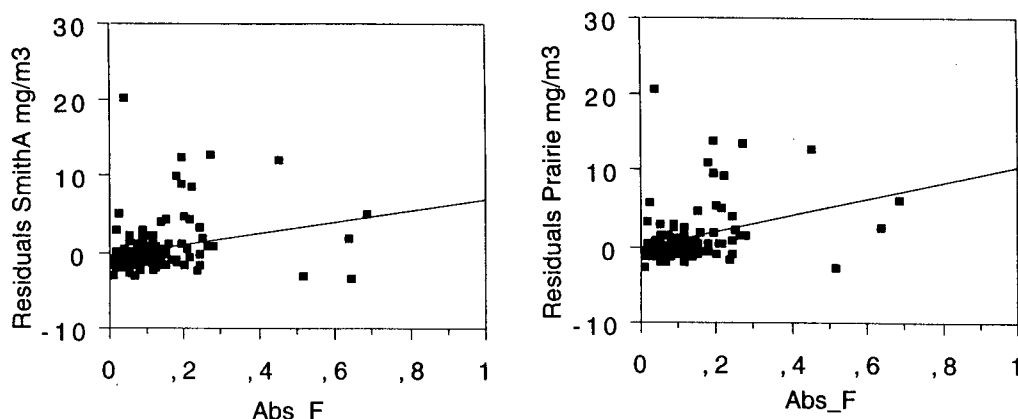
fig 6. Residuals (observed values from the Swedish reference lakes minus predicted values by the equation by SmithA (1982) respectivly Prairie et al. (1989) plotted against water colour (Abs_F).

Mean annual nutrients—chlorophyll relationships

These relations are also used to analyze the influence of phosphorus and/or nitrogen concentrations on algal biomass (Chl-a). One published equation was evaluated (table 8).

Table 8. Published nutrient—chlorophyll relationship for mean annual concentration of chlorophyll-a (Chl-a), and total phosphorus (TP) (mg/m^3). The concentration and TN:TP ranges refer to the conditions used by the authors of the model. The equations tested on Swedish data under the same restrictions gave r^2 Swedish data.

Model	Equation Log(Chl-a)=	TP range	r^2/n	r^2/n Swedish data
Vollenweider— OECD (1982)	0.96log(TP) -0.553	5.6-1120	0.88/77	0.33/23

r^2=correlation coefficient.
n=number of observations.

Only phosphorus is used to predict the algal biomass. The Swedish dataset was limited to 23 yearly mean observations with each mean valus based on six to eight samples. The correlation coefficient for the OECD relation was 0.88 compared to 0.33 with the Swedish data. The residual analysis showed no systematic lack-of-fit but a tendency of an underestimation of chlorophyll in lakes with brown and nitrogen rich waters.

<u>Prediction of chlorophyll concentrations in Swedish lakes</u>

Two new, different models for the prediction of algal biomass (chlorophyll a concentration) in nutrient poor lakes were developed using mean growth season respectivly spring nutrient concentrations for the Swedish reference lakes as predictors.

New mean growth season nutrients—mean growth season chlorophyll relationships

The studied lakes were all nutrient poor (TP <25, TN <968 mg/m^3) with TN:TP ratios between 17-188 (by weight). They varied from colourless to strongly coloured (Abs_F 0.0-0.7), and from acid to slightly alkaline (pH 4.6-7.9). Multiple linear regression for mean growth season (May–September) values revealed the best model as:

log(Chl-a)=-1.243+0.710log(TP)+0.515log(TN)+0.251log(Abs_F)   (1)

r^2=0.64, p<0.01, n=143

Total phosphorus (TP) and total nitrogen (TN) concentrations together with water colour (Abs_F) gave an r^2=0.64, meaning that these factors explain 64 % of the variance in the observed algal biomass (Chl-a) (fig. 7).
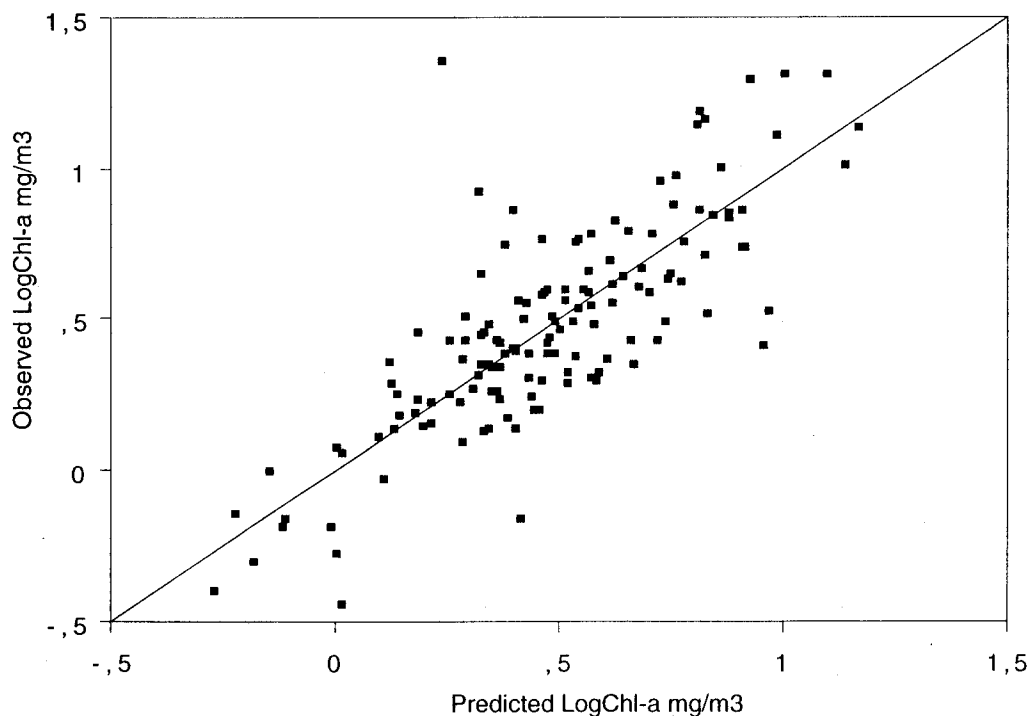

Fig. 7. Chlorophyll-a concentrations in Swedish reference lakes, plotted against Chl-a values predicted from equation 1. r^2=0.64, n=143.

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Inclusion of temperature or pH in the model did not improve the prediction of chlorophyll concentration. Regressions relating the factors in the model alone and together to chlorophyll a are shown in table 9. Total phosphorus respectivly total nitrogen alone as predictors for algal biomass explained 52 respectively 37 % (r^2=0.52, 0.37) of the observed chlorophyll variation. Chl-a as a function of both TN and TP gave r^2=0.57, indicating that nitrogen concentration influence (0.57-0.52) the algal biomass in nutrient poor lakes. Chl-a as a function of watercolour showed a relatively strong correlation (r^2=0.47).

Table 9. Correlation coefficients, r^2, for linear regression of mean growth season (May–September) chlorophyll a versus total phosphorus (TP), total nitrogen (TN), TN:TP ratio (by weight), watercolour (Abs_F) alone and together. n=143. . For exampel, Chl-a=f(TP, TN) expresses chlorophyll as a function of both total phosphorus and total nitrogen concentrations.

	r^2	p
log Chl-a=f(log TP)	0.52	0.001
log Chl-a=f(log TN)	0.37	0.001
log Chl-a=f(log TP+log TN)	0.57	0.001
log Chl-a=f(log TP+log TN:TP)	0.57	0.001
log Chl-a=f(log Abs_F)	0.47	0.001
log Chl-a=f(log TP+log Abs_F)	0.61	0.001
log Chl-a=f(log TP+log TN+log Abs_F)	0.64	0.001

The interdependence among factors that predict algal biomass is shown in table 10. There was a positive but not strong correlation between TP and TN (r^2=0.34). Total phosphorus was however relatively strongly correlated to watercolour (r^2=0.42) indicating that brown waters have higher TP concentrations.

Table 10. Regressions relating mean growth season (May—September) values of total phosphorus (TP) to total nitrogen (TN) and water colour (Abs_F). n=143

	r^2	p
log TP=f(log TN)	0.34	0.001
log TP=f(log Abs_F)	0.42	0.001

New mean spring nutrients—mean summer chlorophyll relationships

Calculations of the relation between nutrient concentration at spring (February—May) and algal biomass during late summer (July—September) yielded the equation:

$$\log(\text{Chl-a})=-0.078+0.605\log(\text{TP})+0.243\log(\text{Inorg-N})+0.393\log(\text{Abs\_F}) \quad (2)$$

$$r^2=0.55, p<0.001, n=182$$

Spring total phosphorus (TP) and spring inorganic nitrogen (Inorg-N) together with summer water colour (Abs_F) gave $r^2=0.55$, meaning that these factors explain 55% of the variance in the observed chlorophyll-a (Chl-a) concentration in the Swedish reference lakes, leaving 45% unexplained. Summer water colour was chosen as an indicator of light conditions during the growth season. Analysis of the residuals did not reveal other factors like temperature and pH as significant for the relationship.
All Swedish lakes were nutrient poor (TP <26, Inorg-N <986 mg/m^3) with TN:TP ratios between 19-162 (by weight) and Inorg-N:TP 3.5-92. They varied from acid to slightly alkaline (pH 4.6-8.1), and from colourless to strongly coloured (Abs_F 0.0-0.8).
Regressions relating chlorophyll a concentrations to different factors in the model are shown in table 11. Total phosphorus, total nitrogen respectivly inorganic nitrogen alone as predictors for algal biomass explained only 34, 23 respectivly 16 % ($r^2=0.34$, 0.23, 0.16) of the chlorophyll variation. Chl-a as a function of water colour showed a relatively strong correlation ($r^2=0.44$). Inorganic nitrogen (Inorg-N), the biologically available form of N, instead of TN in the model slightly improved the chlorophyll a prediction ($r^2=0.55$ respectivly 0.54) (cf. equation 2, and table 11).

Table 11. Regression models relating mean values of late summer (July—September) chlorophyll a (mg/m^3) to spring (February—May) total phosphorus (TP), total nitrogen (TN), inorganic nitrogen (Inorg-N) and summer water colour (Abs_F). n=182

	r^2	p
log Chl-a=f(log TP)	0.34	0.001
log Chl-a=f(log TN)	0.23	0.001
log Chl-a=f(log TP+log TN)	0.40	0.001
log Chl-a=f(log Inorg-N)	0.16	0.01
log Chl-a=f(log TP+log Inorg-N)	0.38	0.001
log Chl-a=f(log Abs_F)	0.44	0.001
log Chl-a=f(log TP+log Abs_F)	0.52	0.001
log Chl-a=f(log TP+log TN+log Abs_F)	0.54	0.001
log Chl-a=f(log TP+log Inorg-N+log Abs_F)	0.55	0.001

<u>*Validation*</u>

The validation of the equations developed (equation 1 and 2) was made using 1998 data for the same reference lakes.

Mean growth season nutrients—mean growth season chlorophyll relationships

To validate the new model (equation 1), data for 64 Swedish reference lakes for the year 1998 were avaliable. Mean growth season values of TP, TN, and Chl-a were calculated and applied on equation 1 under the same restrictions (TP <25, TN <968, Chl-a <23 mg/m^3) as used for the estimation of the constants in the equation. The mean values were based on at least two summer samples (14 % > 2 samples). The correlation coefficient r^2 between the observed Chl-a concentration 1998 and the Chl-a predicted by the model was 0.59, which was acceptable since r^2 for the model was 0.64.

Mean spring nutrients—mean summer chlorophyll relationships

To validate equation 2, values from 98 lakes in the 1998 Swedish data set were avaliable. The restrictions for the model as above were used (spring TP <26, Inorg-N <986, and summer Chl-a <37 mg/m^3). Spring mean TP and Inorg-N combined with summer Abs_F and Chl-a applied on equation 2 gave r^2=0.54 compared with 0.55 for the model.

Discussion

The results indicate that algal biomass in Swedish nutrient poor lakes depend not only on phosphorus concentrations, but also on nitrogen concentrations and on water colour (equation 1). Mean growth season values of TP, TN, Abs_F, and Chl-a explained 64 % (r^2=0.64) of the observed variation in algal biomass. Chl-a as a function of TP exclusively explained only 52% of the variance (table 9). TN included in the model added 5% (0. 57-0.52) to the predictive power. Usually in lakes, total phosphorus to total nitrogen concentrations tend to be highly correlated (Canfield 1983). In this study, a weak correlation between TP and TN was observed (table 10, r^2= 0.34). The addition of correlation with TN in the model might eventually still bee accounted for by the covariance between TN and TP.

Absorbance contributed with an additional 7% (0.64-0.57) to the relationship with TP and TN (table 9). The absorbance values ranged between 0.002—0.7 with 90% of the values less than 0.2 (table 4). Logarithms thus gave negative values, and therefore in the models (equation 1 and 2) absorbance act as a correction factor. For clear waters (low absorbance, log 0.002= -2.7) the predicted chlorophyll concentration was therefore lowered by the model, but in brown waters (log 0.8=-0.1) the

prediction of chlorophyll was only slightly affected by water colour. This influence of absorbance on Chl-a was not expected. The absorbance as a factor in the model was supposed to remove the overestimation of chlorophyll in brown waters where a lot of TN is bound in humic acids and not avaliable for the organisms. Instead this factor had an opposite effect in the equations. One reason might bee that the really brown waters were removed from the test since their TP concentrations were to high and therefore not passed the criteria. TP and watercolour correlated relatively strongly (table 10, $r^2=0.42$), and in some way this affected the model.

Equation 2 was based on nutrient concentrations during spring together with chlorophyll concentration and water colour during summer. The correlation to the observed algal biomass was moderate ($r^2=0.55$). Inorganic nitrogen, the biologically avaliable form of nitrogen, was included in the model as a significant factor. This is discussed in more detail below. Chlorophyll concentration as a function of total phosphorus exclusively (table 11) showed a low correlation ($r^2=0.34$).

Important to consider is that an empirical model is simply a reflection of the dataset it is made up from (Lehman 1986). The model can never adjust for limitations in the data used to build it up. One severe weakness in the material from the Swedish reference lakes was that the measurements often were limited to only 3-4 samples per year. For the last model (equation 2) only 23 % of the lakes had two spring measurements, indicating that the material used for the model was weak. Chlorophyll data during summer showed the same pattern. Summer mean values were possible to calculate only for 24 % of the lakes. The influence of peak or minimum samples was therefore strong (fig. 8 and 9). In the first model (equation 1), all values were based on at least two samples from the growth season, and partly therefore the correlation in the model was better.

Evaluation of published relationships

The reason for the various constants found in the published equations were mainly due to various data sets, mainly with respect to different concentration levels. Somewhat different statistical methods were also used.
When applied on Swedish data, all equations gave low correlation coefficients (table 6, 7, 8). An important reason was the narrow data range (TP < 26 mg/m^3 in the Swedish lakes and the limited number of samples each year.
The model by Smith (1982) was based on data for 127 North American lakes with TP range 2.0–4000 mg/m^3 (table 7), while the model by Seip (1994) was based on 117 lakes from North America and Norway but with a much more narrow TP range (TP 2-20 mg/m^3). A further look at the report by Seip (1994) showed that Chl-a as a function of only TP gave $r^2=0.58$ but 0.59 when TN and TP was used together. The conclusion must be that N did not improve the nutrient:chlorophyll relationship much.
The model by Prairie et al. (1989) based on measurements drawn from the limnological literature were evaluated for both spring and mean growth

season nutrient concentrations since they did not clearly specified their data material (table 6 and 7). Correlation to the Swedish data was better for the mean growth season values.

Analysis of the residuals (observed Chl-a in Swedish lakes minus predicted Chl-a from the models) for the different published equations showed the same pattern for both spring and growth season models. Temperature and pH value did not significantly influence the prediction of Chl-a concentrations in any of the models (fig. 4 and 5). Residuals plotted against water colour (Abs_F) (fig. 3 and 6) showed that the models both correctly estimated and at the same time underestimated the Chl-a concentrations in clear waters. The residuals of the published relationship by Dillon and Rigler (1974) that only use TP as predictor for Chl-a showed that total nitrogen also influence the relationship (fig. 4).

Inorg-N vs. TN

By using the biologically avaliable form of nitrogen (Inorg-N) instead of TN, the problem with humic acids can be reduced (equation 2). In brown waters a large part of TN is tied up in humic acids and is therefore not accessible for the algae. One difficulty is that the concentration of Inorg-N is very dependent of the season. It fluctuates extremely over the year due to algal production and recycling (Horne and Goldman 1994) (fig 8). During winter, dead organic material in the lake is recycled (mineralized), and since the primary production is low the Inorg-N concentrations rise. Inorganic nitrogen deposited on snow is also brought to the lake during spring. The biologically avaliable nutrients for plants can therefore be found in a lake before the productive season starts in spring. The rest of the year land vegetation takes up most of the deposited nutrients. Measuring the nutrient concentrations during spring circulation (start of the productive season) in each lake is difficult, especially when the onset of the growth season varies between years. With only 3-4 measurements per year, as for the Swedish reference lakes, no precise measurements in the start of the growth season were avaliable.

P vs. N limitation

The general opinion is that phosphorus is the limiting factor in lakes with TN:TP ratios <10 (by weight). According to this definition, all of the studied lakes in this report should be P-limited (TN:TP ratios between 17-188). The striking thing was that nitrogen concentration influence, though not strongly, the prediction of chlorophyll concentration in equation 1.

Elser et al. (1990) found in a thorough literature review of 62 nutrient limitation studies in lakes, that in 82 % of the cases, the largest growth stimulation occured when both P and N were added. This indicates that nutrient poor lakes may be deficient in both P and N (se also fig 8 and 9 with both low TP and Inorg-N concentrations during late summer).

Even small increases in bioavaliable N could therefore induce changes at the community level, as various species have different optimum N:P ratios (Elser et al. 1990).
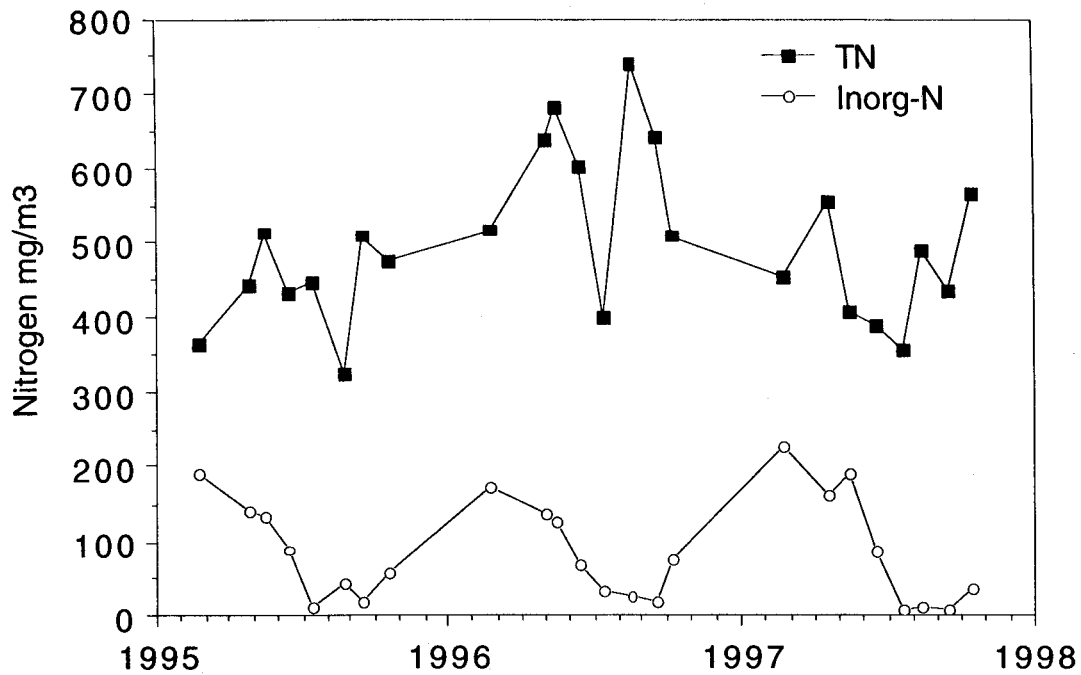
Fig 8. Seasonal variation of total nitrogen (TN) and inorganic nitrogen (Inorg-N, the biological avaliable form of nitrogen) in Lake Fräcksjön, one of the Swedish reference lakes.
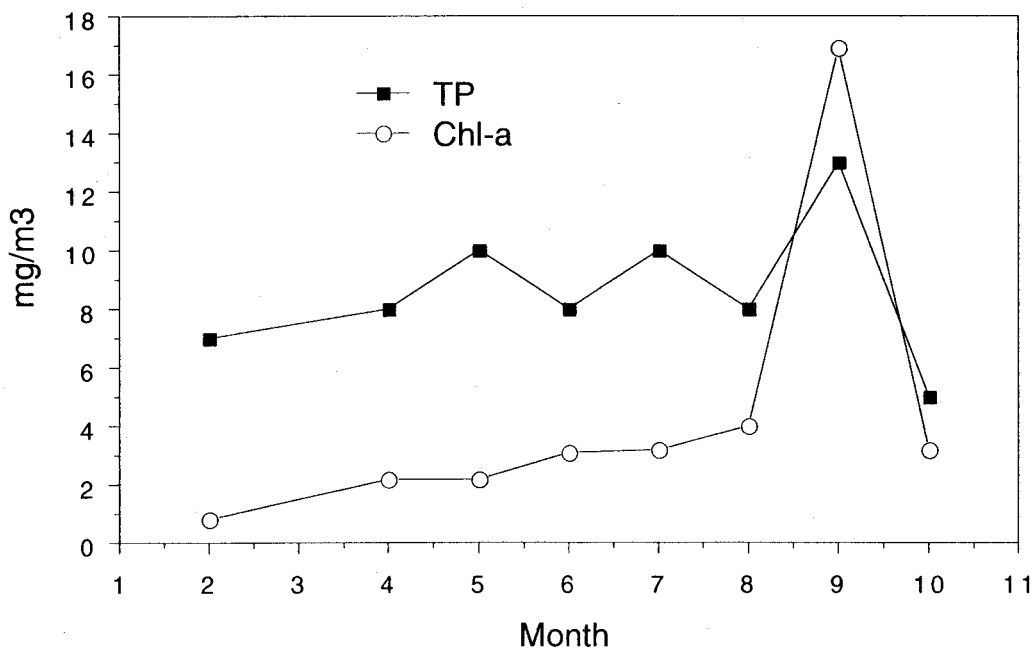
Fig 9. Seasonal variation of total phosphorus (TP) and chlorophyll a (Chl-a) in Lake Fräcksjön, one of the Swedish reference lakes.

Models

Harris (1994) wrote: "Empirical biomass models work because of the annual averaging of pattern and process and because of some inherent properties of the functioning of pelagic ecosystems".
Or with other words: the cyclic processes in a lake are repeated year after year.
Why linear regression? The model suggests that the response of algal biomass to changing nutrient levels might be linear on a logarithmic scale. That is, a unit increase of P should yield an increment in plant biomass that is independent of the original P concentration in the lake (McCauley et al. 1989). For nutrient poor lakes this is assumed to be the case.
Biologically, regression slopes may be interpreted as a measure of the ability of the phytoplankton to respond to changes in nutrient concentration. Similarly, the corresponding correlation coefficient can be viewed as a measure of the strength of the nutrient–phytoplankton coupling.
According to Harris (1994) a predictive capability must be based upon robust knowledge of ecological structure and function, pattern and process. Even if prediction turn out to be very difficult we need to know what is possible and why. Since empirical limnology contributes little to "understanding", all predictions under conditions outside the limits of the original data sets are accompanied with uncertainty.

Sources of variability

The magnitude of temporal and year-to-year variation can severely limit the power of statistical comparison of nutrient and chlorophyll means (Knowlton 1984). There are a number of factors that help explain the lack of correlation of algal biomass to phosphorus and nitrogen. In this study the low nutrient concentrations and the low sampling intensity were most likely to be the main sources of variability. The nutrient and chlorophyll values used in equation 2 might be peak or lower concentrations, since often only one measurement each spring and summer was avaliable. At least two samples contributed to the mean growth season values, making them more reliable. Analytical difficulties at low concentrations were the second important source of variability. Lakes are also located in different geographical and climatic regions making the light-temperature relation different.

Manipulation test

How do the new models (equation 1 and 2) react on a 10% or on a 100 mg/m^3 increase respectivly decreace in nitrogen concentration? The nitrogen contributions are realistic from precipitation in Sweden (Kindbom et al. 1994), and the reduced values represent an earlier deposition level.

Equation 1 (mean growth season values):

log(Chl-a)=-1.243+0.710log(TP)+0.515log(TN)+0.251log(Abs_F)

A 10 % increase of the observed TN concentrations in the Swedish reference lakes results in a 5.0 % rise in algal biomass (table 12). The corresponding reduction by 10 % of TN results in a decrease in Chl-a concentration by 5.3 %. These values are independent of the original nutrient levels since the changes are in percent. The 100 mg/m^3 changes in nutrient concentrations are in contrast to the 10 % changes dependent on the original nitrogen concentration in each lake. In table 12 a median value of 11.8 % increase respectivly 13.3 % decrease in algal biomass is given. Figure 10 showes that the relative effect of increased or decreased nitrogen concentrations is most pronounced at low Chl-a concentrations since these lakes have low original TN concentrations.

Table 12. Manipulation of total nitrogen (TN) concentrations from the Swedish reference lakes rendering changes in chlorophyll a concentrations when using equation 1.

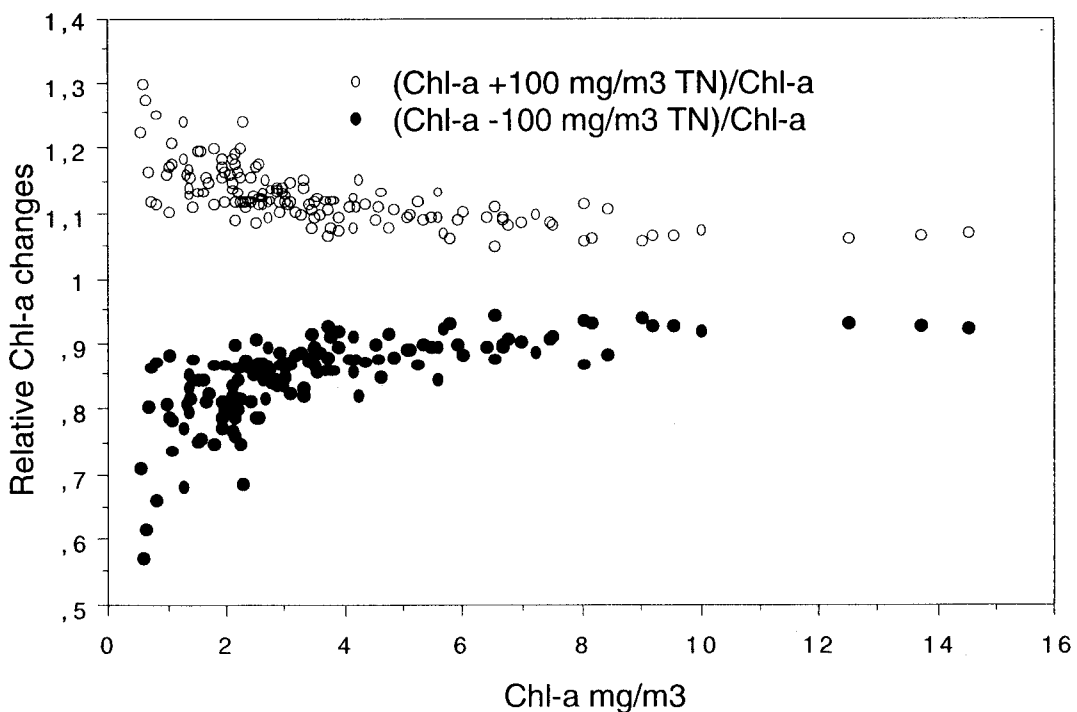| Nutrient status	Chl-a
+ 10 % TN	+ 5.0 %
- 10 % TN	- 5.3 %
+ 100 mg/m3 TN	+ 11.8 % (median value)
- 100 mg/m3 TN	- 13.3 % (median value)

Fig. 10. Calculated chlorophyll a (Chl-a) concentrations in the Swedish reference lakes using equation 1 plotted against the relative Chl-a changes after +100 mg/m^3 respectivly -100 mg/m^3 TN manipulation.

Equation 2 (spring nutrients):

$$\log(\text{Chl-a})=-0.078+0.605\log(\text{TP})+0.243\log(\text{Inorg-N})+0.393\log(\text{Abs\_F})$$

A 100 mg/m^3 increase in the observed Inorg-N concentrations from the Swedish reference lakes results in a change in median Chl-a concentration of 18.6 %. The relative effect of increased inorganic nitrogen concentrations is most pronounced at low Chl-a concentrations since these lakes originally have low Inorg-N concentrations (Inorg-N 26–986 mg/m^3) and addition of 100 mg/m^3 results in a significant change. Up to 45 % increase in Chl-a was observed when the Inorg-N concentration was rised by 100 mg/m^3 (for example a change from 40 to 140 gives a 350 % increase in Inorg-N). Only small changes in Chl-a occured when the Inorg-N concentration was rised or lowered with 10 %. (+2.3 % respectivly -2.5 %.

A first attempt to apply the model by Smith (1982) on data for Swedish lakes was made by Wilander (1995). When an increase of N by 400 mg/m^3 was made to forest lakes the number of oligotrophic lakes diminished from 34 % to 2.2 %. At that time no chlorophyll measurements were performed in the Swedish lakes.

Conclusions

The models developed in the present study show that algal biomass in Swedish nutrient poor lakes depend not only on total phosphorus concentrations, but also on total nitrogen and inorganic nitrogen concentrations. This indicates that nutrient poor lakes may be deficient in both phosphorus and nitogen, a conclusion also drawn by Elser et al. (1990) after a thorough literature review. However, further investigation based on more intense measurements in each lake is needed.

Acknowledgments

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