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Do top-down and bottom-up controls interact to exclude nitrogen-fixing cyanobacteria from the plankton of estuaries? An exploration with a simulation model

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Abstract. Explaining the nearly ubiquitous absence of nitrogen fixation by planktonic organisms in strongly nitrogen-limited estuaries presents a major challenge to aquatic ecologists. In freshwater lakes of moderate productivity, nitrogen limitation is seldom maintained for long since heterocystic, nitrogen-fixing cyanobacteria bloom, fix nitrogen, and alleviate the nitrogen limitation. In marked contrast to lakes, this behavior occurs in only a few estuaries worldwide. Primary production is limited by nitrogen in most temperate estuaries, yet no measurable planktonic nitrogen fixation occurs. In this paper, we present the hypothesis that the absence of planktonic nitrogen fixers from most estuaries is due to an interaction of bottom-up and top-down controls. The availability of Mo, a trace metal required for nitrogen fixation, is lower in estuaries than in freshwater lakes. This is not an absolute physiological constraint against the occurrence of nitrogen-fixing organisms, but the lower Mo availability may slow the growth rate of these organisms. The slower growth rate makes nitrogen-fixing cyanobacteria in estuaries more sensitive to mortality from grazing by zooplankton and benthic organisms.

We use a simple, mechanistically based simulation model to explore this hypothesis. The model correctly predicts the timing of the formation of heterocystic, cyanobacterial blooms in freshwater lakes and the magnitude of the rate of nitrogen fixation. The model also correctly predicts that high zooplankton biomasses in freshwaters can partially suppress blooms of nitrogen-fixing cyanobacteria, even in strongly nitrogen-limited lakes. Further, the model indicates that a relatively small and environmentally realistic decrease in Mo availability, such as that which may occur in seawater compared to freshwaters due to sulfate inhibition of Mo assimilation, can suppress blooms of heterocystic cyanobacteria and prevent planktonic nitrogen fixation. For example, the model predicts that at a zooplankton biomass of 0.2 mg l^{-1}, cyanobacteria will bloom and fix nitrogen in lakes but not in estuaries of full-strength seawater salinity because of the lower Mo availability. Thus, the model provides strong support for our hypothesis that bottom-up and top-down controls may interact to cause the absence of planktonic nitrogen fixation in most estuaries. The model also provides a basis for further exploration of this hypothesis in individual estuarine systems and correctly predicts that planktonic nitrogen fixation can occur in low salinity estuaries, such as the Baltic Sea, where Mo availability is greater than in higher salinity estuaries.

Introduction

Net primary production in many of the earth's ecosystems, both aquatic and terrestrial, is limited by nitrogen. A variety of mechanisms such as denitrification and mobility of nitrate can contribute to nitrogen limitation, yet the widespread prevalence of such limitation is somewhat surprising given the ability of many types of bacteria to fix atmospheric N_2 and thereby alleviate shortages of nitrogen (Vitousek & Howarth 1991; Vitousek & Field, this volume). To understand how nitrogen limitation can persist over time, one must be able to understand why nitrogen fixation is sometimes unable to make up the deficit of nitrogen relative to phosphorus and other elements.

Estuaries and coastal seas of the temperate zone comprise one type of ecosystem in which net primary production is often limited by nitrogen (Nixon & Pilson 1983; D'Elia et al. 1986; Howarth 1988; NRC 1993) and in which rates of nitrogen fixation tend to be very low (Horne 1977; Fogg 1987; Howarth et al. 1988a). This behavior is in marked contrast to many temperate lakes, where P limitation of production is common (Schindler 1977; NRC 1993) and where nitrogen fixation rates are often high (Horne & Goldman 1972; Horne 1977; Flett et al. 1980; Howarth et al. 1988a). In fact, nitrogen fixation in lakes is one mechanism which leads to phosphorus limitation of production; generally in mesotrophic and eutrophic temperate lakes, when the N:P ratio is low, heterocystic species of cyanobacteria bloom in the plankton and fix nitrogen. This can alleviate the shortage of nitrogen and result in long term limitation of production by P (Schindler 1977; Flett et al. 1980; Howarth et al. 1988b). However, heterocystic cyanobacteria are rare or absent from the plankton of most estuaries and coastal seas even when these ecosystems are strongly nitrogen limited, and rates of nitrogen fixation in the plankton of most estuaries are immeasurably low (Horne 1977; Doremus 1982; Fogg 1987; Howarth et al. 1988a; Paerl 1990; NRC 1993). Two major exceptions are the Baltic Sea (Lindahl & Wallstrom 1985; Wallstrom 1988, 1991; Wallstrom et al. 1992; Niemisto et al. 1989; Moisander et al. 1996; Lehtimaki et al. 1997) and the Harvey-Peel Inlet in southwestern Australia (Huber 1986; Lukatelich & McComb 1986).

Ecologists have long been intrigued by the contrast between abundant nitrogen fixation by planktonic cyanobacteria in lakes and the relative absence of this process in most estuaries and coastal seas. The dichotomy between lakes and estuaries is so striking that one is compelled to search for a single factor to explain the difference, and the literature contains numerous hypotheses where one factor such as turbulence or a low availability of Mo (an element required for nitrogen fixation) is invoked to explain the relative lack of nitrogen fixation in coastal ecosystems compared to lakes (Doremus 1982; Paerl 1985; Howarth & Cole 1985; Fogg 1987; Valiela 1991). Nonetheless, no one explanation has gained widespread acceptance, and evidence inconsistent with or weakening of many of the single-control hypotheses has accumulated (Marino et al. 1990; Cole et al. 1993; Howarth et al. 1993, 1995).

Two trace metals, molybdenum (Mo) and iron (Fe), are both required for nitrogen fixation, and both are likely to be less available in seawater than in freshwaters (Howarth et al. 1988b; Marino et al. 1990). For Mo, the low availability is the result of competitive inhibition of molybdate uptake by sulfate. Even though dissolved Mo is fairly abundant in seawater compared to other trace metals (Howarth et al. 1988b), the thermodynamically stable form of Mo in oxic waters is molybdate, an anion which is stereochemically similar to sulfate. Thus, sulfate can inhibit Mo uptake by cyanobacteria and other phytoplankton (Howarth & Cole 1985; Cole et al. 1993). Inhibition of Mo assimilation by sulfate has also been seen in physiological studies with a variety of other organisms (Stout & Meagher 1948; Elliot & Mortenson 1975; Huising & Matrone 1975; Cardin & Mason 1976). In 1985, we suggested that the availability of Mo in estuaries may be sufficiently low as to exclude nitrogen-fixing cyanobacteria (Howarth & Cole 1985). However, our subsequent work has shown that sulfate only partially inhibits Mo uptake at the sulfate and Mo concentrations which characterize seawater (Marino et al. 1990; Cole et al. 1993). This suggests that while Mo availability may contribute to the relative absence of heterocystic cyanobacteria in the plankton of estuaries, it is unlikely to be the sole cause (Marino et al. 1990). The role of Fe in regulating nitrogen-fixation by planktonic cyanobacteria in estuaries has received much less study than has Mo (Howarth et al. 1988b; Marino et al. 1990; Vitousek & Howarth 1991), although the role of Fe in limiting the growth of oceanic phytoplankton has received much recent study (see for example, Martin et al. 1994), and Fe availability has been suggested as a regulator of nitrogen fixation in oceanic waters (Michaels et al. 1996; Falkowski 1997). We suspect that as with Mo, a relatively low availability of Fe in estuaries compared to lakes (Howarth et al. 1988b; Marino et al. 1990) may slow the growth rate of cyanobacteria but is unlikely to act as an absolute constraint.

We have come to believe that the relative lack of nitrogen fixation by plankton in estuaries compared to lakes is not the result of a single factor, but rather is caused by an interaction of a bottom-up control by availabilities of essential trace metals and a top-down control by grazing. In this paper, we present the results of a simulation model which explores this interaction.

The hypothesis

Our hypothesis is that low availabilities of Mo and/or Fe in estuaries lead to slow growth rates of planktonic, nitrogen-fixing cyanobacteria. This leaves the cyanobacteria vulnerable to consumption by generalized grazing animals, leading to very low numbers and thus low rates of nitrogen fixation. Our hypothesis does not state that herbivores in estuaries specifically seek out filamentous, heterocystic cyanobacteria as food (which would be a poor strategy, given the virtual absence of such cyanobacteria). Rather, we suggest that estuarine ecosystems contain generalized grazers which are capable of feeding on fine cyanobacterial filaments and are likely to do so incidentally to their feeding on other particles when filaments are present.

Compared to other phytoplankton, heterocystic cyanobacteria species that typically dominate nitrogen fixation in lakes may be particularly vulnerable to grazing (Schaffner et al. 1994; Epp 1995). Nitrogen fixation in these cyanobacteria occurs only in specialized, nonphotosynthetic cells called heterocysts (Bothe 1982; Gallon 1992). The energy needs for nitrogen fixation in the heterocyst are supported by other photosynthetic cells, and generally, many photosynthetic cells are required in a filament to build and support nitrogen fixation in one heterocyst (Turpin et al. 1985; Rowell & Kerby 1991). Thus, considerable filament growth must occur before any nitrogen fixation can occur. It is likely that if a filament is fully or partially grazed before enough photosynthetic cells are produced, the cyanobacteria will not produce heterocysts or fix nitrogen.

The growth rate of the cyanobacteria is an important parameter in our overall hypothesis. In freshwater studies, zooplankton have been found to feed on relatively fine and short cyanobacterial filaments, while larger and longer filaments are less susceptible to grazing (Schaffner et al. 1994; Epp 1995). Thus, fast-growing cyanobacteria are of a vulnerable size for only a short period of time until they grow sufficiently to become larger filaments. However, if low Mo and/or Fe availabilities in estuaries slow the growth rate of cyanobacteria, filaments will remain small and more vulnerable to grazing mortality for a longer period of time. Hence, the biological availabilities of Mo and Fe are not absolute constraints on growth, but may be factors which lower the growth rate of cyanobacteria in estuaries relative to lakes and thereby increase the vulnerability of the cyanobacteria to grazing.

Calanoid copepods (particularly *Acartia* species), ctenophores, and a variety of bivalves (mussels, oysters) are examples of filter feeding animals common in estuaries which may feed on cyanobacterial filaments. In a series of mesocosm experiments using seawater of salinity greater than 30‰, we found that planktonic, nitrogen-fixing cyanobacteria (*Anabaena* sp.) grew and fixed nitrogen when zooplankton populations were kept low (by adding zooplanktivorous fish) and benthic filter feeders were absent. However, the nitrogen-fixing cyanobacteria were suppressed by the addition of the blue mussel (*Mytilis edulis*) or higher densities of zooplankton in the mesocosms (Chan et al., manuscript in preparation). In short-term grazing experiments, we further demonstrated that *Acartia* sp. were able to feed upon cyanobacterial filaments from the mesocosms (Chan et al., manuscript in preparation), and consumption of filamentous cyanobacteria by *Mytilis edulis* has been reported by others (Falconer et a. 1992). Nitrogen-fixing, heterocystic cyanobacteria have not been reported in the plankton of any estuary in North America (Howarth et al. 1988a; Howarth & Marino 1990), including Narragansett Bay, Rhode Island (Karentz and Smayda 1998) which was the source of water for our mesocosm experiment. In fact, at salinities greater than 12‰, planktonic heterocystic cyanobacteria have been reported only for two estuaries: one in southwestern Australia (Huber 1986; Lukatelich & McComb 1986) and one in Tasmania (Jones et al. 1994). Our experiment thus provides strong evidence that a top-down control is likely to be important in excluding these nitrogen fixers from the plankton of estuaries. This influence and the interaction with the growth rate of the nitrogen-fixing cyanobacteria as influence by the availability of the trace metal Mo is explored in the model presented here.

Model structure

We constructed our model using STELLA ("research" version 4.0). The structure of the model is illustrated graphically in Figure 1. We have kept the structure quite simple so as to ease interpretation of model behavior. While we have used realistic parameters, we have specifically chosen not to duplicate much of the complexity of nature. For instance, while it is well known that increasing concentrations of inorganic nitrogen can suppress nitrogen fixation (Howarth et al. 1988b) and that the build up of inorganic nitrogen from nitrogen fixation over a growing season can contribute to the decline of nitrogen-fixing cyanobacteria (Wallstrom 1991), our model has no such feedbacks. The model is designed only to study the factors involved with the initiation of a bloom of nitrogen-fixing cyanobacteria, and not the decline of these blooms. This is justified since the purpose of the model is to examine why nitrogen-fixing blooms do not generally occur in estuaries and not to predict the extent or duration of such blooms once they form. The

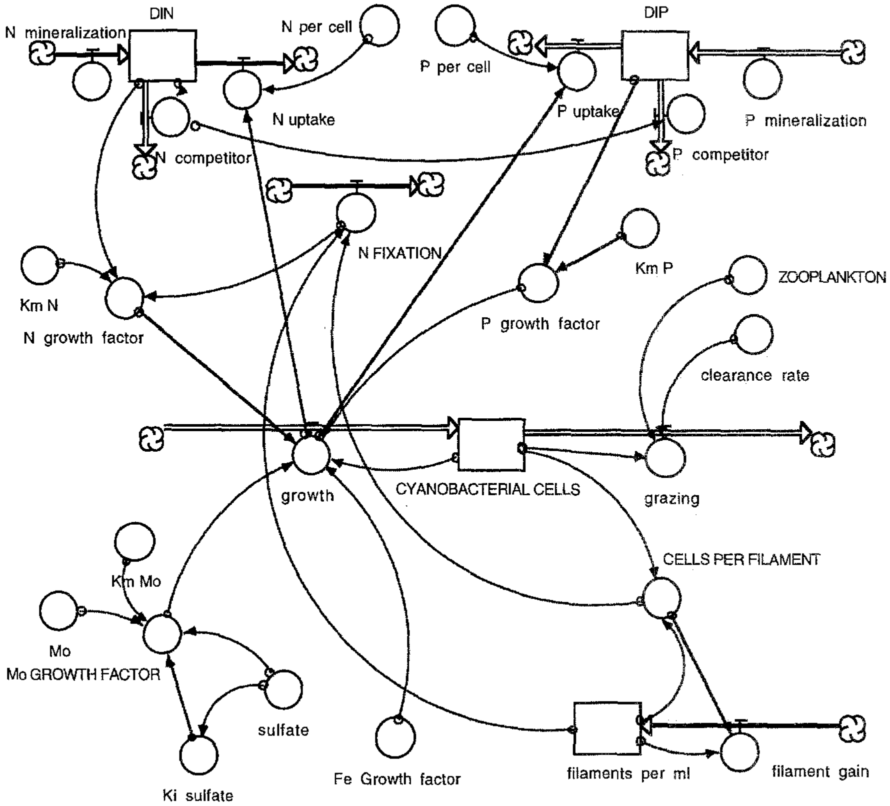

Figure 1. STELLA graphical representation of the simulation model. See text for equations underlying model structure.

initial conditions for the model are intended to be representative of temperate lakes immediately after the start of summer stratification. In the presentation below, we show the model as a series of differential equations. In practice, the STELLA software solves these as a series of difference equations using a time step of 0.1 hour.

The abundance of cyanobacterial cells (C) is calculated from their rate of growth (GROW) and their mortality from grazing by zooplankton (GRAZE).

$$dC/dt = (GROW - GRAZE) * C. \tag{1}$$

The initial condition is taken as 240 cyanobacterial cells per ml, a very low abundance. Our hypothesis is that grazing by both zooplankton and benthic filter feeders may be important in regulating blooms of heterocystic cyanobacteria. However, for the sake of simplicity, in the model we explicitly consider only grazing by crustacean zooplankton; grazing by benthic filter feeders or by other zooplankton (such as ctenophores) would have a similar effect. In the model, grazing is a function of zooplankton biomass and the rate of feeding by zooplankton (described as a clearance rate of volume of water filtered per zooplankton biomass per time).

$$\mathrm{GRAZE} = \mathrm{Z} * \mathrm{CLEAR}, \tag{2}$$

where Z is the total biomass of crustacean zooplankton and CLEAR is the clearance rate. For any given model run, the zooplankton biomass is held constant. This is of course unrealistic, since zooplankton biomass varies over time in real ecosystems, and will be related both to the overall rate of primary production and to top-down controls from fish and other predators. However, holding zooplankton biomass constant makes comparisons among systems easier. For standard model runs, we set zooplankton biomass at 0.2 mg l^{-1}, a value frequently encountered in mesotrophic and eutrophic lakes and estuaries (Durbin & Durbin 1981; Pace 1986; Varmo et al. 1989; Tackx et al. 1990; Pace et al. 1992; White & Roman 1992; Uitto 1996; Canfield & Jones 1996; Elmgren & Larsson 1997). For clearance rate, we use a value of 5 ml hr^{-1} per mg of zooplankton. Studies for zooplankton feeding on cyanobacterial filaments have reported a range of clearance rates from 0.2 to over 200 ml hr^{-1} per mg of zooplankton, but the majority of these reported rates are greater than 5 ml hr^{-1} per mg of zooplankton (Holm et al. 1983; Haney 1987; Burns & Xu 1990; Schaffner et al. 1994; Burns & Hegarty 1994; Fulton 1988; James & Forsyth 1990; Sellner et al. 1994; Hanson et al. 1998). Thus, our chosen rate is relatively conservative. For any given model run the parameter GRAZE is a constant.

Cyanobacterial growth rate is a function of the intrinsic, maximum possible rate and several multipliers which lower the rate of growth based on availabilities of phosphorus, nitrogen, and molybdenum (referred to as "growth factors" in Figure 1).

$$\mathrm{GROW} = 0.03 * \mathrm{P}_{\mathrm{mult}} * \mathrm{N}_{\mathrm{mult}} * \mathrm{Mo}_{\mathrm{mult}}. \tag{3}$$

The maximum growth rate of 0.03 hr^{-1} is reasonable for freshwater cyanobacteria (Gibson & Smith 1982) and is about twice the rate observed for planktonic cyanobacteria isolated from the Baltic Sea (Wallstrom 1991). The phosphorus-availability multiplier ($\mathrm{P}_{\mathrm{mult}}$) is simply a Michaelis-Menten function of dissolved inorganic phosphorus (DIP).

$$\mathrm{P}_{\mathrm{mult}} = \mathrm{DIP}/(\mathrm{DIP} + \mathrm{Km}_{\mathrm{P}}), \tag{4}$$

where Km_{P} is the half-saturation constant for DIP uptake. We assume a value of 2 μM for Km_{P}, a value typical for both freshwater and Baltic Sea nitrogen-fixing cyanobacteria (Nalewajko & Lean 1978; Wallstrom 1991). We handle the nitrogen-availability multiplier (N_{mult}) in an analogous manner for the case when no nitrogen fixation is occurring. However, when nitrogen fixation is occurring, we assume that this fully meets the nitrogen needs for growth and so nitrogen imposes no constraint on growth. This gives nitrogen-fixing cyanobacteria a competitive advantage when DIN concentrations are low.

$$\text{If N fixation} > 0,\ N_{mult} = 1, \tag{5}$$

$$\text{If N fixation} = 0,\ N_{mult} = \text{DIN} / (\text{DIN} + Km_N), \tag{6}$$

where Km_N is the half-saturation constant for the uptake of dissolved inorganic nitrogen (DIN). We assume a value of 20 μm for Km_N, a reasonably low value for filamentous cyanobacteria (Zevenboom & Mur 1978). For the molybdenum-availability multiplier (Mo_{mult}), we use an extended Michaelis-Menten type equation which also includes the inhibitory effect of sulfate (Cole et al. 1993):

$$Mo_{mult} = 4.2 * Mo/[Mo + Km_{Mo} * (1 + \text{sulfate}/Ki)], \tag{7}$$

where Km_{Mo} is the half-saturation "constant" for uptake of molybdate (in nM), Ki is the inhibition "constant" for the effect of sulfate on Mo uptake (in mM), Mo is the ambient molybdenum concentration in nM, sulfate is the ambient sulfate concentration in mM, and 4.2 is a scaling factor so that Mo_{mult} equals 1 for average freshwater concentrations of Mo and sulfate (5 nM and 0.11 mM, respectively; Marino et al. 1990). Cole et al. (1993) demonstrated that Km_{Mo} and Ki are not truly constants but rather vary in response to the concentrations of Mo and sulfate in natural waters. In waters where sulfate is high, the phytoplankton community responds to some extent so that per mole of sulfate, the sulfate is somewhat less inhibitory (larger Ki) than in systems where the sulfate concentration is lower. We estimate Ki as a function of sulfate concentration (in mM), following Cole et al. (1993):

$$Ki = 12 * \text{sulfate}/(0.3 + \text{sulfate}). \tag{8}$$

Thus, Ki is constant at 12 mM for ecosystems with high sulfate concentrations (greater than 10 to 15 mM). At progressively lower sulfate concentrations, Ki decreases (inhibition is proportionately greater per mole of sulfate, although the overall inhibition is still less since the molar concentration of sulfate is less).

From Mo assimilation data from several ecosystems as well as several cultures of cyanobacteria (benthic as well as planktonic), Cole et al. (1993) concluded that Km_{Mo} could be predicted as a function of the ambient Mo concentration (in nM) according to the following equation:

$$\log Km_{Mo} = 0.415 * \log Mo + 0.68. \tag{9}$$

However, this equation in combination with Equation 7 and Equation 8 suggests that Mo is actually more available in seawater (Mo = 110 nM; sulfate = 28 mM; Marino et al. 1990) than in average freshwaters, perhaps by more than 40%. This is strongly at variance with the observation that Mo is equally partitioned between particulate and dissolved phases in some freshwaters but is present overwhelmingly as the dissolved form in seawater; that is, independent evidence strongly shows that the biological availability of Mo is much less in seawater than in freshwater (Howarth et al. 1988b) since the ratio of dissolved to particulate metals is a good indicator of their biological availability in natural waters (Morel & Hudson 1985).

Therefore, we have further considered the analysis of Cole et al. (1993). Their analysis included data from cultures where the Mo concentration from the original source ecosystem is poorly known. Further, cyanobacteria may have evolved in the culture conditions; most commonly used culture media have concentrations of Mo and other trace metals which are much higher than seen in natural waters. During many generations of growth in Mo-rich media, the cyanobacteria may have increased their half-saturation values for Mo uptake. Gibson and Smith (1982) note that nutrient uptake systems in cyanobacteria both evolve and adapt physiologically to the ambient concentration of substrates. Using the data of Cole et al. (1993) for natural systems but deleting their culture data from the analysis yields the following equation to predict $\mathrm{Km_{Mo}}$ as a function of Mo concentration:

$$\log \mathrm{Km_{Mo}} = 0.95 * \log\mathrm{Mo} + 0.53. \tag{10}$$

Note that within the variation of the data of Cole et al. (1993), Equations 9 and 10 are not statistically distinguishable. Note also that Equation 10 has less scatter ($r^2 = 0.74$) than does Equation 9 ($r^2 = 0.43$). While using Equation 9 with Equations 7 and 8 suggests that Mo is more available in seawater than in average freshwaters, using Equation 10 in place of Equation 9 predicts that Mo availability is 58% less in seawater than in average freshwaters. This is more in line with other available data, such as the partitioning of dissolved and particulate Mo (Howarth et al. 1988b). Equation 10 also makes greater physiological sense: it predicts that $\mathrm{Km_{Mo}}$ values for any given system (average freshwater and seawater) are consistently somewhat greater than the Mo concentrations (by some 3-fold), whereas Equation 9 (the original equation from Cole et al. 1993) similarly predicts a $\mathrm{Km_{Mo}}$ value for freshwaters that is somewhat higher than the Mo concentration (by some 2-fold) but a $\mathrm{Km_{Mo}}$ value for seawater that is considerably lower than the Mo concentration (by 3-fold). It makes greater physiological sense that the half-saturation constant be more consistently related to the Mo concentration. We have therefore used Equation 10 to estimate $\mathrm{Km_{Mo}}$ values in our simulation model.

The model makes no attempt to mass balance either nitrogen or phosphorus, as to do so would require detailed knowledge of the rates of recycling from cyanobacteria and other phytoplankton as they are consumed. The model allows for a continuous input of nitrogen and phosphorus from the combined sources of mineralization within the ecosystem and external loading, termed "N mineralization" and "P mineralization" in the model and called N_{min} and P_{min} in Equations 11 and 12, below. P mineralization was set at 0.006 μM hr^{-1}, a value based on the DIP needed to support a somewhat eutrophic rate of primary productivity for a shallow freshwater lake (assuming a C:P ratio by moles of 105:1, a depth of 2 m, and a rate of primary production of 0.36 g m^{-2} day^{-1}; Grobbelaar & House 1995). N mineralization was set at a rate of 0.04 μM hr^{-1}, a rate which reflects an N:P molar ratio of 6.7 during recycling due to denitrification. Initial conditions for DIN and DIP are 10 and 1 μM, respectively. Thus, the model starts with the assumption of a nitrogen-limited water column (and therefore one favorable to nitrogen fixation), and the mineralization inputs tend to maintain this nitrogen limitation with a continuously low N:P ratio compared to the Redfield ratio. The model contains two sinks for these nutrients: assimilation by the cyanobacterial cells (called "N uptake" and "P uptake" in Figure 1), and assimilation by the rest of the phytoplankton community (termed "N competitor" and "P competitor" in Figure 1). Thus, the equations for DIN and DIP concentration are given by

$$\mathrm{dDIN/dt} = N_{min} - N_{up} - N_{com}, \tag{11}$$

$$\mathrm{dDIP/dt} = P_{min} - P_{up} - P_{com}, \tag{12}$$

where N_{up} and P_{up} represent nitrogen and phosphorus assimilation by the cyanobacterial cells and N_{com} and P_{com} represent nitrogen and phosphorus assimilation by the rest of the phytoplankton community. Rates of uptake of phosphorus by the cyanobacteria are expressed simply as their rate of growth multiplied by an assumed amount of phosphorus per cell (P_{cell}, assumed to be 0.22×10^{-12} moles P per cell):

$$P_{up} = C * \mathrm{GROW} * P_{cell} * (1,000\mathrm{ml}\ \mathrm{l}^{-1}). \tag{13}$$

A similar approach is taken for nitrogen uptake by the cyanobacteria when they are not fixing nitrogen. However, when the cyanobacteria are fixing nitrogen, this is assumed to be sufficient to meet all of their nitrogen needs, and so no assimilation of DIN occurs.

$$\text{If N fixation} > 0,\ N_{up} = 0. \tag{14}$$

$$\text{If N fixation} = 0,\ N_{up} = C * GROW * N_{cell} * (1{,}000\ \text{ml}\ l^{-1}). \tag{15}$$

The nitrogen content per cell, N_{cell}, is assumed to be 3.3×10^{-12} moles N, so the N:P ratio in the cyanobacteria is 15:1 by moles.

The rest of the phytoplankton community assimilates nitrogen as a function of the DIN concentration according to a Michaelis-Menten equation:

$$N_{com} = 0.08 * [DIN/(DIN + 10)]. \tag{16}$$

Note that the half-saturation constant for the competitor community, 10 μM, is lower than that used for the nitrogen-availability multiplier for the growth of the cyanobacteria (20 μM; Equation 6), so that the model assumes the competitors use DIN more efficiently than the cyanobacterial cells. Equation 16 indicates that when DIN concentrations are well above the half-saturation constant of 10 μM, nitrogen assimilation will be 0.08 μM hr^{-1}; this is twice the rate of nitrogen mineralization and input assumed in the model (see N_{min}, above) and would be typical of the nitrogen assimilation rate seen in a somewhat eutrophic lake (given assumptions similar to those used to parameterize P_{min}). Phosphorus assimilation by the competitor phytoplankton community is scaled to nitrogen assimilation by assuming a molar ratio of N:P of 15:1:

$$P_{com} = N_{com}/15. \tag{17}$$

Thus, nutrient assimilation by the competitor community will help maintain a low N:P ratio in the water, and maintain nitrogen limitation.

In the model, nitrogen fixation is a function of the length and number of cyanobacterial filaments. For the baseline standard run, we assume that a filament must have 19 photosynthetic cells in order to support the energy needs of producing and supporting a heterocyst (the site of nitrogen fixation). Filaments grow through the addition of cyanobacterial cells, with new cyanobacterial cells being assumed to be evenly distributed among all filaments. Thus,

$$C_{fil} = C/F, \tag{18}$$

where C_{fil} is the number of cyanobacterial cells per filament and F is the number of filaments per ml of water. As filaments grow to be longer than 50 cells, the model assumes that they break in half to form twice as many filaments, all half as long. The initial condition for the number of filaments is 80 filaments per ml (leading to 3 cells per filament since the initial condition for C is 240 per ml). Any filament having 19 or more cells per filament will

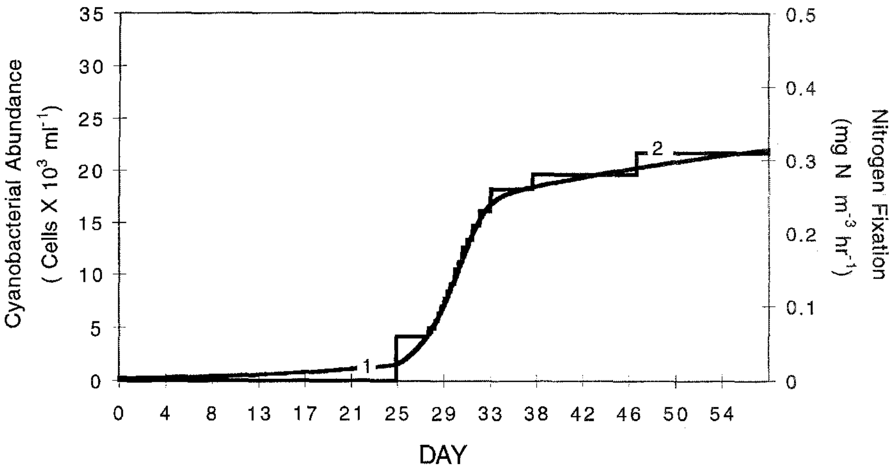

Figure 2. Model results for number of cyanobacterial cells over time (line labeled "1") and for rates of nitrogen fixation (line labeled "2") over time under "standard" model run for average freshwater conditions.

support one heterocyst, while shorter filaments will support no heterocysts. A reasonable value for nitrogen fixation rates per heterocyst, based on a literature survey of rates in several lakes as well as the Baltic Sea, is 7×10^{-13} g N hr^{-1} per heterocyst (Howarth et al. 1993). Thus,

$$\text{If } C_{\text{fil}} \geq 19, \text{ N fixation} = F * 0.0007, \tag{19}$$

$$\text{If } C_{\text{fil}} < 19, \text{ N fixation} = 0. \tag{20}$$

The units of nitrogen fixation so estimated are ng N ml^{-1} hr^{-1}, or equivalently mg N m^{-3} hr^{-1}.

Model results and discussion

A standard run of the model for average freshwater conditions is given in Figure 2 (zooplankton biomass = 0.2 mg l^{-1}; sulfate = 0.11 mM; Mo = 5 nM). After some 25 to 30 days, the cyanobacterial cells start to increase in number exponentially, as does the rate of nitrogen fixation. Although this may seem like a long time for bloom initiation, it is in fact quite characteristic of the time necessary for cyanobacterial blooms to form in temperate lakes after the start of summer stratification (which is taken as the initial condition for the model). As the bloom progresses in the model run, the number of cyanobacterial cells starts to level off as phosphorus concentrations become controlling, and rates of nitrogen fixation plateau at approximately 0.3 mg N m^{-3} hr^{-1}. This rate is typical of those often found in eutrophic freshwater lakes (Howarth et al. 1988a). Thus, despite its simplicity, the model accurately predicts the general behavior of cyanobacterial bloom formation and planktonic nitrogen fixation in eutrophic lakes. This strongly suggests that the model incorporates the major mechanisms controlling the initiation of nitrogen fixation by planktonic cyanobacteria in lakes.

The reader is reminded that the model is not intended to include the mechanisms which would allow prediction of the duration and decline of blooms, and so the cyanobacterial numbers and rates of nitrogen fixation would remain at their high levels for as long as the model ran. In nature, blooms would decline due to the gradual accumulation of nitrogen from nitrogen fixation, from the deepening of the water column at the time of fall overturn, or from other such causes.

The model is quite sensitive to changes in zooplankton biomass. Compared to the standard run for freshwater conditions, increasing the zooplankton biomass from 0.2 mg l^{-1} to 0.4 mg l^{-1} and 0.6 mg l^{-1} slows the initiation of cyanobacterial blooms (Figure 3(a)) and planktonic nitrogen fixation (Figure 3(b)) by some 8 and 30 days, respectively. This is consistent with the observation that these levels of higher zooplankton biomass can sometimes suppress planktonic nitrogen fixation in freshwater ecosystems, even when these systems are strongly nitrogen limited with low DIN concentrations and low DIN:DIP ratios (Lynch & Shapiro 1981; Schaffner et al. 1994; Epp 1995; Hairston et al., manuscript in preparation). In an experiment with replicate freshwater ponds under strongly nitrogen-limited conditions, Schaffner et al. (1994) found virtually no planktonic, heterocystic cyanobacteria in ponds with high zooplankton densities, while nitrogen-fixing heterocystic cyanobacteria were abundant in ponds with lower zooplankton densities. The product of the biomass of zooplankton in the ponds and the empirically determined clearance rate of zooplankton on cyanobacteria in those same ponds (Schaffner et al. 1994; N. Hairston, pers. comm.) indicates that average grazing rates of 2.3 ml l^{-1} hr^{-1} (high zooplankton ponds) suppressed cyanobacterial blooms, while average grazing rates of 0.6 ml l^{-1} hr^{-1} (low zooplankton ponds) allowed abundant populations of nitrogen-fixing cyanobacteria. In our simulation model runs, the 0.2 mg l^{-1} biomass levels corresponds to a grazing rate of 1 ml l^{-1} hr^{-1}, while the 0.6 mg l^{-1} biomass corresponds to a grazing rate of 3 ml l^{-1} hr^{-1}. Thus, the model is sensitive to changes in zooplankton grazing pressure at the same levels as were observed experimentally to control the development of heterocystic cyanobacterial blooms in the plankton of freshwater ponds.

The effects of increased grazing pressure on cyanobacterial blooms reflect both direct increases in cell mortality and the suppression of heterocyst devel-

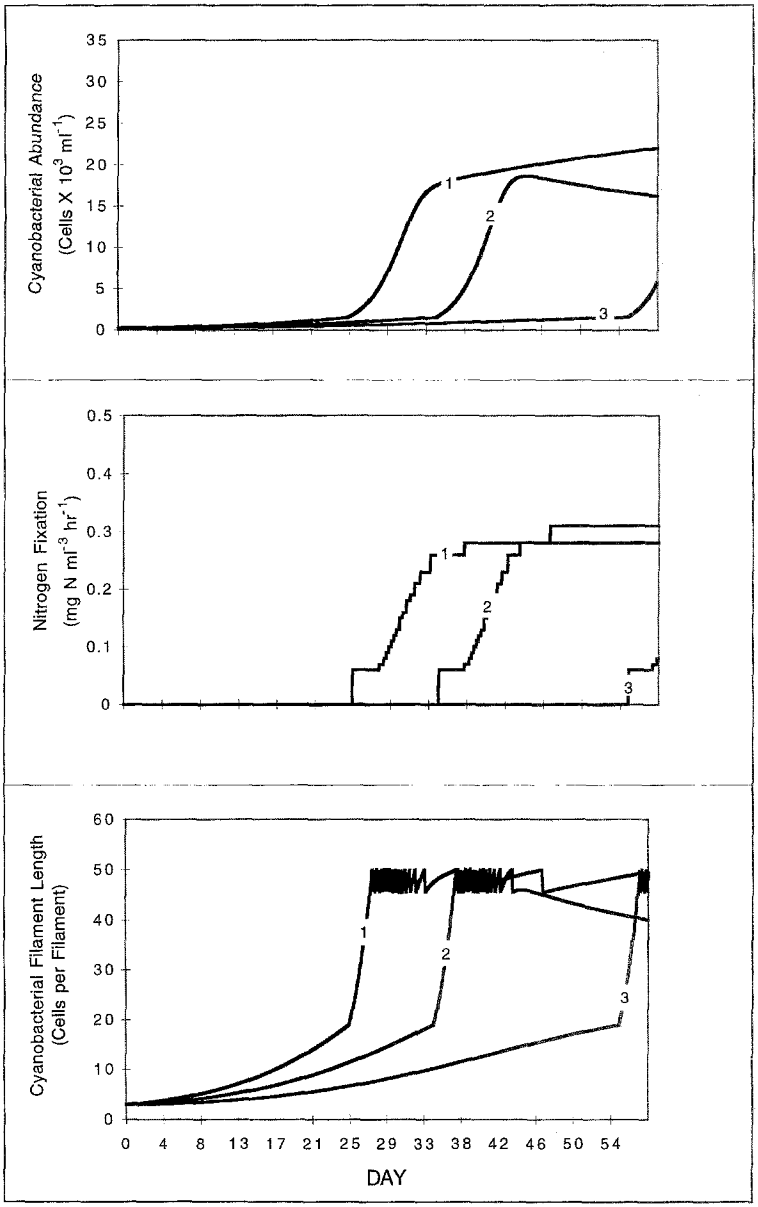

Figure 3. Sensitivity analysis for increasing zooplankton biomass while keeping other parameters as in the standard freshwater model run shown in Figure 2. The response to cyanobacterial cell numbers (3a, top), nitrogen fixation rates (3b, middle), and number of cyanobacterial cells per filament (3c, bottom) is shown as zooplankton biomass is increased from 0.2 mg l^{-1} (line labeled "1"; standard run conditions) to 0.4 mg l^{-1} (line labeled "2") and to 0.6 mg l^{-1} (line labeled "3").

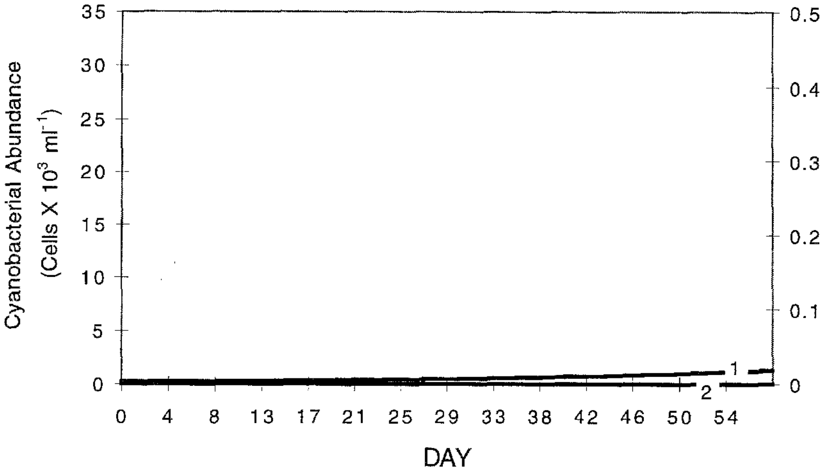

Figure 4. Model results for seawater concentrations of sulfate and Mo, showing numbers of cyanobacterial cells (line labeled "1") and rates of nitrogen fixation (line labeled "2") over time. Except for sulfate and Mo concentrations, all model parameters are the same as in the standard freshwater model run shown in Figure 2. This figure is scaled to the results shown in Figure 2.

opment associated with reduced mean filament length. In the model, nitrogen fixation occurs only when filaments have 19 or more photosynthetic cells to support the energy needs of a heterocyst. Increasing zooplankton biomass from 0.2 mg $l^{-1}$to 0.4 mg l^{-1} slows the rate of filament growth, and further increases in zooplankton biomass to 0.6 mg l lead to persistence of short filaments (Figure 3(c)). Once filaments become long enough to support the energy need of building a heterocyst and fixing nitrogen, nitrogen fixation commences and exponential growth rapidly accelerates.

In addition to being sensitive to grazing pressure, the model is quite sensitive to changes in Mo availability as well (Figure 4). Holding the zooplankton biomass constant at 0.2 mg l^{-1} but increasing the Mo and sulfate concentrations from average freshwater values (5 nM and 0.11 mM, respectively) to average seawater values (110 nM and 28 mM respectively; Marino et al. 1993) results in virtually no increase in cyanobacterial cell numbers and no nitrogen fixation over a time period of 2 months (compare Figure 4 with Figure 2, the standard freshwater model run). This result is based solely on slowing the growth rate of cyanobacterial cells due to a lower Mo availability in seawater than in freshwaters. For average freshwater values of Mo and sulfate, the Mo-availability multiplier (Mo_{mult}) is 1.0, but for seawater values of Mo and sulfate, the value of Mo_{mult} predicted from Equations 7, 8, and 10 is 0.42. Thus, the model estimates that the cyanobacterial growth rate is 58% lower in seawater than in average freshwaters. As discussed above, there is

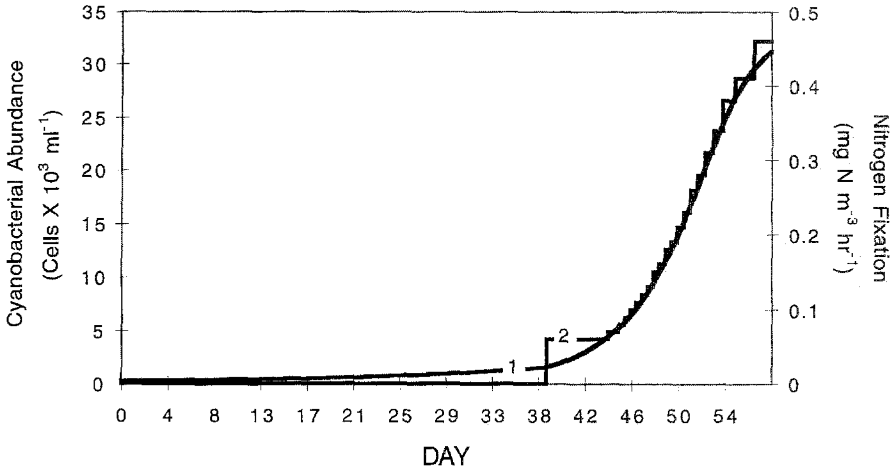

Figure 5. Model results for seawater concentrations of sulfate and Mo, as in Figure 4, except zooplankton biomass is reduced to zero from the standard model run conditions of 0.2 mg l^{-1}.

substantial error associated with estimating the extent to which sulfate inhibits Mo assimilation, so the actual change in growth rate may be more or less than we estimate. Nonetheless, the model clearly shows that relatively small decreases in cyanobacterial growth rate can have a profound effect on bloom initiation and on rates of nitrogen fixation. As we hypothesized, the ecological effect of zooplankton grazing can magnify the influence of a relatively small change in growth rate, so that the physiological effect of lower trace metal availability can result in the exclusion of heterocystic nitrogen fixers from the plankton. If Fe availability is less in estuaries than in lakes (as discussed further below), this too could have a similar effect.

The virtually complete suppression of nitrogen fixation over a two-month period in the seawater model run is not solely a result of the lower Mo availability, but rather results from the interaction of grazing and the slower growth rate caused by low Mo availability. Thus, when the model is run with seawater concentrations of sulfate and Mo but with the zooplankton biomass set at zero, a cyanobacterial bloom does eventually occur (Figure 5). The bloom occurs some 2 weeks later than in the standard freshwater run, but numbers of cyanobacterial cells and rates of nitrogen fixation are comparable to those in the standard freshwater model run, or perhaps even somewhat higher (Figure 5).

The Baltic Sea is one of the very few estuaries in the world where planktonic heterocystic cyanobacteria occur and fix nitrogen at reasonable rates. Our model correctly predicts this (Figure 6). The Baltic Sea is of relatively low salinity, with salinities ranging from near zero in the northern

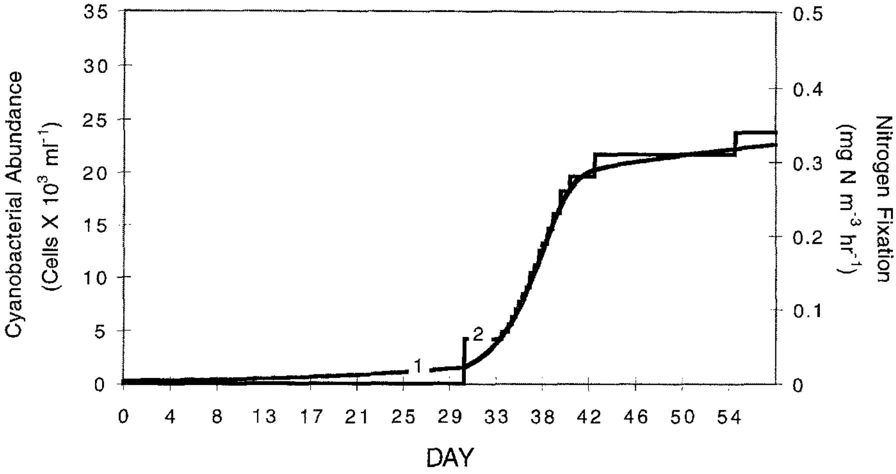

Figure 6. Model results for Baltic Sea concentrations of sulfate and Mo. All other parameters are as in the standard freshwater model run conditions as shown in Figure 2.

Gulf of Bothnia to 8 ‰ in the southern Baltic. The highest rates of nitrogen fixation are generally found in regions where the salinity is 6 ‰ or less (Lindahl & Wallstrom 1985; Wallstrom 1988, 1991; Wallstrom et al. 1992; Niemisto et al. 1989; Moisander et al. 1996; Lehtimaki et al. 1997). Since Mo and sulfate behave conservatively as a function of salinity in most oceanic waters (Howarth et al. 1988b) including the Baltic Sea (Howarth & Marino, unpublished data), we can estimate that at a salinity of 6 ‰, the sulfate concentration would be 4.8 mM and the Mo concentration would be 18 nM. Using Equations 8 and 10 to estimate the sulfate inhibition constant and Mo half saturation constant at these ambient concentrations, Equation 7 predicts a value for Mo_{mult} of 0.78. That is, the predicted rate of cyanobacterial growth is 22% less than for average freshwaters but is 86% faster than in full-salinity seawater (Howarth & Marino 1998). Figure 7 shows in general how we predict Mo availability will vary in estuaries as a function of salinity, expressed relative to average freshwater availability where the Mo_{mult} is taken as 1. Keeping the zooplankton biomass at 0.2 mg l^{-1} (the value used for the standard, freshwater run of the model, and a value typical for the coastal waters of the Baltic Sea; Varmo et al. 1989; Uitto 1996; Elmgren & Larsson 1997), our simulation model predicts that the initiation of a cyanobacterial bloom would be delayed by approximately 6 days in the Baltic Sea compared to average freshwaters, and that rates of nitrogen fixation would be virtually the same as in an average freshwater ecosystem after this short delay (compare Figure 6 with Figure 2).

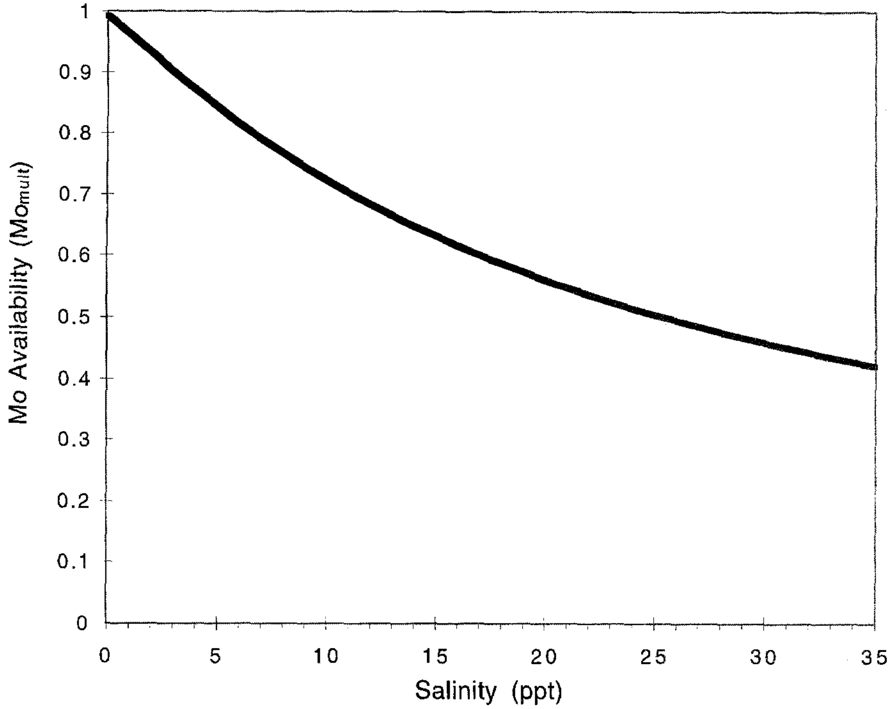

Figure 7. Estimated values of Mo availability in estuaries (expressed as the Mo$_{mult}$ which is taken as a value of 1.0 for average freshwater values of Mo and sulfate) as a function of salinity; based on Equations 7, 8, and 10. A simple mixing model is used to estimate sulfate and Mo concentrations at any given salinity based on their concentrations in seawater and in average freshwaters. Mo availability decreases in a non-linear fashion as salinity increases from freshwater to full-strength seawater.

The only other estuaries in the world where heterocystic, nitrogen-fixing cyanobacteria have been reported among the plankton in waters of salinities greater than a few ‰ are in southwestern Australia and in Tasmania. Compared to most estuaries in North America or Europe, these estuaries undergo extreme salinity variations seasonally, with salinities varying from near zero to well over 30 ‰ (Hearn & Lukatelich 1990). Blooms of planktonic, filamentous cyanobacteria begin in these estuaries during the low-salinity events (Huber 1986; Lukatelich & McComb 1986; Jones et al. 1994). This is broadly consistent with our hypothesis: the low salinity events would increase cyanobacterial growth rates by increasing the availability of Mo and Fe, and might also decrease mortality from grazing by zooplankton and benthic animals if the animal species present at high salinities were lost by the sudden intrusion of low-salinity water.

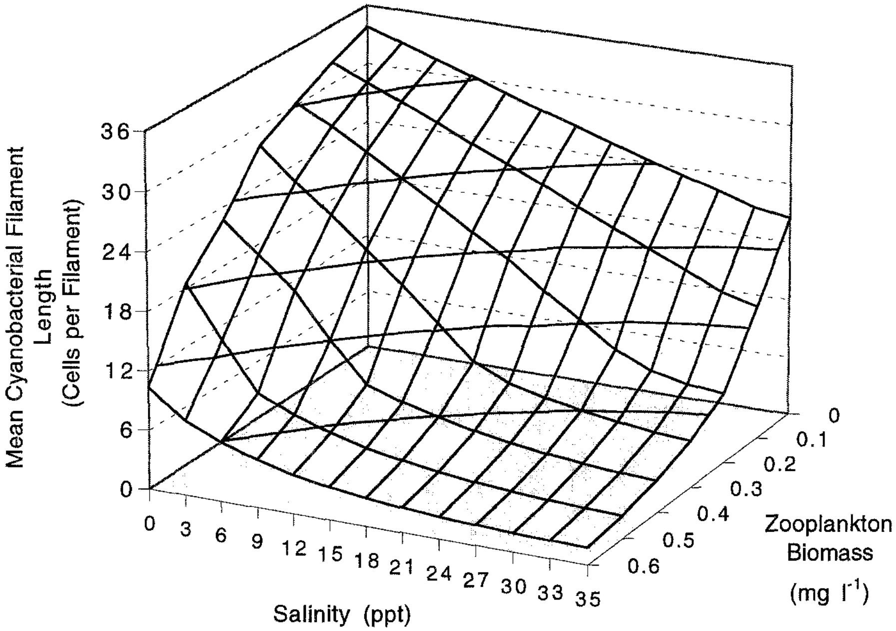

Figure 8. Model results illustrating responses of mean cyanobacterial filament length to variations in salinity and zooplankton biomass. Mean number of cells per filament (averaged over the course of a bloom) are depicted for individual model runs representing 91 combinations of salinity and zooplankton biomass. Salinity gradient ranges from "standard" freshwater conditions to full salinity seawater, while zooplankton biomass is set at 7 densities between 0.0 mg l^{-1} to 0.6 mg l^{-1}. Mo availability and growth factor are estimated based on Equations 7, 8 and 10.

Part of the explanation for the sensitivity of the model to both cyanobacterial growth rates and to grazing lies in the importance of having a sufficient number of photosynthetic cells in a cyanobacterial filament to support nitrogen fixation in the nonphotosynthetic heterocyst. The cyanobacteria have no competitive advantage over other phytoplankton until they begin to fix nitrogen, and they may in fact often be relatively poor competitors for assimilating DIN (as our model assumes). However, the cyanobacteria cannot fix nitrogen until they grow sufficiently long filaments, and this is difficult to do under significant grazing pressure (Figure 8). As salinity increases, the accompanying decrease in the availability of Mo accentuates the effects of zooplankton grazing on mean filament length (averaged over the entire time span of the model). Increasing zooplankton biomass from 0 to 0.2 mg ml^{-1} results in a rapid decline in mean cyanobacterial filament length from 20 to 7 cells per filament (Figure 8). For cyanobacteria growing in freshwater conditions, mean filament length declines only from 32 to 30 cells per filament over the same zooplankton biomass gradient (Figure 8).

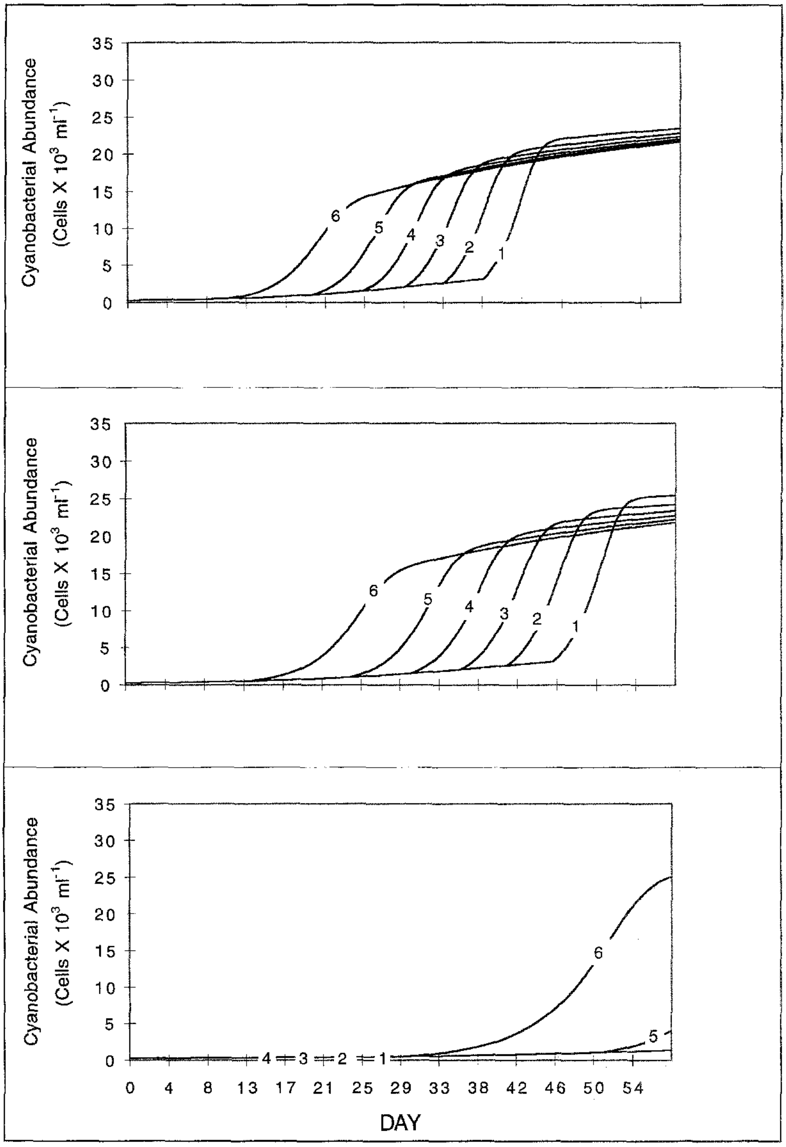

Figure 9. Sensitivity analysis showing model results for number of cyanobacterial cells over time as the number of photosynthetic cells assumed necessary to support the energetic needs of a heterocyst is altered. Top panel (9a) is for standard freshwater conditions, middle panel (9b) is for Baltic Sea concentrations of sulfate and Mo, and bottom panel (9c) is for seawater concentrations of sulfate and Mo. Other parameters are as in the standard model run shown in Figure 2. Line labeled "1" represents 38.5 cells per heterocyst, that labeled "2" represents 32 cells per heterocyst, that labeled "3" represents 25.5 cells per heterocyst, that labeled "4" represents 19 cells per heterocyst (the standard model run condition), that labeled "5" represents 12.5 cells per heterocyst, and that labeled "6" represents 6 cells per heterocyst. Values as low as 6 are outside of the range usually seen in nature.

For cyanobacterial filaments in natural systems, the number of photosynthetic cells associated with each heterocyst can vary widely from 12 to over 50 cells (Ogawa & Carr 1969; Horne & Goldman 1972; Horne et al. 1972; Kling et al. 1994; Gronlund et al. 1996). In the standard model run, we assume that 19 cells are needed to support the energetic needs of a heterocyst. In model runs where this number is increased (to 25.5 and to 32) or decreased (to 6 and to 12.5), the timing of bloom initiation is altered for conditions otherwise representative of the standard baseline freshwater run, but the eventual size of the bloom remains unchanged (Figure 9(a)). When we run this sensitivity analysis for Baltic Sea conditions of sulfate and Mo (and the standard zooplankton biomass value of 0.2 mg l^{-1}), the result is similar with blooms occurring approximately 6 days later than in the freshwater runs (Figure 9(b)). However, for seawater values of sulfate and Mo, the model shows no response except at the lowest number of cells per heterocyst (Figure 9(c)). Cyanobacterial cells begin to bloom in the seawater run within the 2-month time frame of the model only when we assume that 6 photosynthetic cells are sufficient to support a heterocyst. Such a low ratio of photosynthetic cells to heterocysts has not been observed in natural waters, probably because more cells are needed to support the energetic needs of heterocysts. Thus, while the model results show some sensitivity to the assumed number of cells needed to support a heterocyst, the overall comparison of freshwater and seawater results appears quite robust.

Presumably, grazing would place less of a constraint on the growth of unicellular, nonfilamentous cyanobacteria capable of nitrogen fixation than on heterocystic species. However, in lakes and in the few estuaries where planktonic nitrogen fixation has been observed, the organisms responsible for fixation are filamentous cyanobacteria and not unicellular organisms (Horne & Goldman 1972; Horne 1977; Flett et al. 1980; Howarth et al. 1988 a, b; Lindahl & Wallstrom 1985; Lukatelich & McComb 1986; Howarth & Marino 1998). Picoplankton-sized unicellular cyanobacteria (*Synechococcus* sp.) and heterotrophic bacteria can fix nitrogen, but in laboratory studies often require anoxic conditions to do so and have never been observed to fix nitrogen in oxic water columns, perhaps because of an inability to protect the nitrogenase enzyme from poisoning by oxygen (Gallon & Stal 1992; Bergman et al. 1997; Howarth & Marino 1998). The major nitrogen-fixing organism in oligotrophic oceanic waters, *Trichodesmium*, is colonial but nonheterocystic, with nitrogen fixation and photosynthesis occurring in the same cells (Carpenter & Capone 1992). We would therefore hypothesize that it may be less sensitive to the effects of grazing than are heterocystic cyanobacteria, such as those that are dominant nitrogen-fixers in lakes. This may in fact be part of the reason that this organism thrives in so much of the oligotrophic tropical and subtropical ocean water of the world; it may be constrained in growth rate by low availabilities of Mo and Fe, but a slow growth rate is tolerable without the need to produce long filaments before commencing nitrogen fixation. However, *Trichodesmium* is found in oligotrophic ocean waters, and has not been observed in the mesotrophic or eutrophic waters more characteristic of estuaries. The reasons for this remain unknown, although perhaps this species simply cannot compete well in higher nutrient environments (Howarth & Marino 1998).

Future model refinements

We have deliberately kept this version of our model simple so that interpretation is relatively straight forward. We have also only attempted to model the factors which regulate the initiation of cyanobacterial blooms and the maximum rates of nitrogen fixation, and not the factors which determine the length of bloom of the timing of bloom collapses. Even within this context, though, we see several ways in which the model could be refined in the future to further explore the controls on bloom initiation.

One refinement would be to include the inhibitory effect of DIN on heterocyst production and nitrogen fixation (Howarth et al. 1988b). The availability of some DIN is essential to support growth of cyanobacterial filaments before they become large enough to support a heterocyst and fix nitrogen, and the model currently includes this dynamic. However, DIN concentrations as low as 0.14 to 1.6 μM have been suggested to suppress heterocyst formation in a eutrophic lake (Horne et al. 1979), presumably because the cyanobacteria used the available DIN rather than pay the higher energetic cost of fixing nitrogen.

Another refinement would be to explicitly include the effect of benthic filter-feeding animals on cyanobacteria and on nitrogen fixation. From our mesocosm experiments (Marino et al., manuscript in preparation; Chan et al., manuscript in preparation), we know that benthic estuarine filter feeders such as blue mussels can feed on planktonic cyanobacteria, and their role as grazers may be as great as or greater than that of zooplankton in preventing the initiation of cyanobacterial blooms in estuaries. Freshwater filter feeders such as zebra mussels can also feed on planktonic cyanobacteria and may be important in regulating nitrogen fixation in some lakes.

A refinement to the zooplankton-grazing portion of the model would be to model the clearance rate at which zooplankton graze as a function of the abundance of cyanobacterial filaments. Several studies have found that at increasing densities of filaments, the clearance rate slows down, and some studies have even found a cessation of feeding at moderate to high densities of cyanobacterial filaments (Holm et al. 1983; Gliwicz & Lampert 1990; Schaffner et al. 1994). Our simulation model currently uses a constant clearance rate. Modifying the model so as to reduce clearance rates at higher filament densities would magnify the difference between the standard freshwater run (Figure 2) and the run with seawater concentrations of sulfate and Mo (Figure 4).

In our current model, zooplankton grazing removes cyanobacteria cells equally from all filaments, thereby shortening filaments (Equation 2). This is consistent with the behavior observed by Schaffner et al. (1994) for feeding by cladocerans on cyanobacteria. Our preliminary experiments with estuarine copepods (*Acartia* sp.) also suggest that they may feed on cyanobacterial filaments in this manner (Chan et al., manuscript in preparation). However, Schaffner et al. (1994) observed that freshwater copepods feed on cyanobacterial filaments by snipping pieces out of the middle, thereby not only eating some cells but creating much shorter filaments (and a greater number of filaments). We suspect that this type of feeding behavior would have an even greater influence on suppressing cyanobacterial blooms because of the large reduction in the number of photosynthetic cells in a filament; fewer cells are available to support the energetic requirements of the nonphotosynthetic heterocyst. The model could be used to explore the quantitative effect on nitrogen fixation of these contrasting feeding styles.

The model is parameterized to represent a mesotrophic or eutrophic ecosystem, and it contains no structural connection between zooplankton biomass and nutrient availability. In reality, ecosystems with greater nutrient availability have higher rates of primary productivity and support larger zooplankton populations (Pace 1986; Leibold et al. 1997). Such a linkage could be added to the model, perhaps simply relating zooplankton biomass to nutrient availability using regressions from comparative analyses. With such a change, the model could better explore the factors regulating nitrogen fixation in oligotrophic environments, including fixation by *Trichodesmium* in oceanic environments.

This version of our model does not consider the effect of Fe availability on cyanobacterial growth rate since we are unaware of adequate quantitative data on the relative availability of Fe in estuaries in comparison to lakes and on the effects of Fe availability on heterocystic cyanobacterial growth. Although Figure 1 suggests that there is an Fe-availability multiplier in the model, we have simply set this factor at 1. However, concentrations of both total Fe and dissolved Fe tend to be lower in estuaries than in lakes, so it is quite likely that the biological availability of Fe is also lower (Howarth et al. 1988b; Marino et al. 1990). Given the high Fe requirement of nitrogen-fixers and the low solubility of Fe in seawater, it seems quite plausible that

Fe may limit N-fixing activity in estuarine and marine waters (Rueter 1982; Howarth et al. 1988b; Brand 1991; Vitousek & Howarth 1991; Michaels et al. 1996; Falkowski 1997). Thus, our model probably underestimates the extent to which cyanobacterial growth rates in estuaries would be lower than in lakes.

Conclusions

We hypothesize that a low availability of Mo in estuaries compared to lakes does not act as an absolute physiological constraint against the occurrence of nitrogen-fixing cyanobacteria, but that low Mo availability may slow the growth rate of the cyanobacteria and thereby make them more sensitive to mortality from generalized grazing by zooplankton and benthic organisms. Our simple, mechanistically based simulation model indicates that this hypothesis is quite reasonable. Relatively small and environmentally realistic changes in either zooplankton biomass or Mo availability can result in the earlier or later initiation of cyanobacterial blooms, development of heterocysts, and subsequent nitrogen fixation. The model correctly predicts that increasing zooplankton biomass from 0.2 mg l^{-1} to 0.6 mg l^{-1} in freshwater ecosystems can suppress blooms of nitrogen-fixing cyanobacteria. The model also predicts that at a zooplankton biomass of 0.2 mg l^{-1}, cyanobacteria will bloom and fix nitrogen in lakes but not in estuaries of full-strength seawater salinity. Further, the model correctly predicts that planktonic nitrogen fixation can occur in low salinity estuaries, such as the Baltic Sea, and the model is consistent with the finding of nitrogen fixation in two estuaries in southwestern Australia and Tasmania as these experience drastic seasonal reductions in salinity.

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