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Trace metal transport by marine microorganisms: implications of metal coordination kinetics

ROBERT J. M. HUDSON*† and FRANÇOIS M. M. MOREL*

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Abstract—Marine microorganisms have transport systems capable of accumulating essential trace metals present at low oceanic concentrations—1 pM to 1 nM. In marine phytoplankton, Fe, Mn, Zn and Ni transport has been shown to involve complexation by membrane carriers. By analysing the kinetics of the transport process and accounting for the inherently slow coordination reactions of some of these metals, we predict optimum properties and minimum numbers of sites for the transport systems. Limits to trace metal uptake, and thereby to growth rates, may arise from finite space for these transport sites in the membrane, competition from other metals and the rate of diffusion to the cell. These types of nutrient limitation should exhibit different size dependencies and therefore be important in determining ecosystem structure. The concentrations of inorganically complexed species of nutrient metals remaining in the surface ocean appear to be correlated with predicted rates of metal complexation by trace metal transport sites, suggesting that kinetic lability controls the bioavailability of these metals and their rate of removal from the surface ocean.

INTRODUCTION

ONE of the striking recent discoveries of chemical oceanography is the remarkably low seawater concentration of many elements, some of which are essential to the growth of the biota and are accordingly depleted at the surface like the major nutrients. A number of questions arise from this discovery. How do marine microorganisms acquire necessary trace elements? What are the limits of the uptake process? How low can the concentrations of an element be to support marine life? Conversely, how low can organisms deplete surface seawater concentrations? Our knowledge of the trace element physiology of marine phytoplankton (the organisms that we presume to be responsible for most trace element uptake) and of the uptake process has progressed sufficiently that we can now begin to address these questions. Here we attempt to do this by bringing together recent information on the seawater chemistry of trace elements, on the physiology of phytoplankton, and on the chemical kinetics of coordination reactions. We examine how the rates and selectivity of chemical reactions with transport sites (JACKSON and MORGAN, 1978) and the physics of diffusion and fluid motion in the cell's environs constrain cellular uptake rates of micronutrients (MUNK and RILEY, 1952; PASCHIAK and GAVIS, 1974). This

*Ralph M. Parsons Laboratory, 48-311, Massachusetts Institute of Technology, Cambridge, MA 02139, U.S.A.
†Present address: Institute of Marine Sciences, University of California, Santa Cruz, CA 95064, U.S.A.

discussion from first principles benefits from the hindsight gained in our recent study of iron uptake by algae (HUDSON and MOREL, 1990).

Some of the conclusions we reach might be surprising to physiologists and biochemists: the uptake of micronutrients requires many transport molecules (perhaps more than for more abundant nutrients) working far from saturation. These transport molecules must thus be small and need not be fast. Other conclusions may interest philosophically inclined geochemists and ecologists: for some elements such as Fe or Zn, the ambient concentrations in surface seawater are low enough that diffusion limits their ability to sustain maximal growth rates. In addition, total concentrations of inorganic species of nutrient trace metals in the surface ocean appear to be correlated with their kinetic lability, suggesting that ocean-average uptake rates by phytoplankton are more closely related to the rates of complexation than equilibrium binding of metals with cellular uptake sites.

METAL UPTAKE KINETICS

The classical model for uptake kinetics consists of two steps—reversible binding for the substrate to a transport site or carrier and irreversible transport into the cell (Fig. 1). For a trace metal (M), the transport sites are thought to be ligands (L) associated with the plasma membrane and exposed to the medium at the cell's external surface (WILLIAMS, 1981). Under steady state conditions, the rate of metal uptake via this mechanism follows the well-known Michaelis–Menten hyperbolic rate law (SEGEL, 1976). The maximum uptake rate (ρ_{max}) and the half-saturation constant (K_ρ) are simple functions of the total number of transport ligands ($[L_T]$) and the apparent kinetic constants for the complex formation (k_f) and dissociation (k_d) and metal internalization (k_{in}) reactions.

Most often it is assumed that the reaction between the transport ligand and the dissolved metal approaches equilibrium because the internalization step is slow relative to the formation and dissociation of the transport complex. (Such a condition, in which some fast reactions achieve equilibrium while others proceed relatively slowly, is the definition of pseudoequilibrium.) In this case, the half-saturation constant for transport is simply the inverse of the apparent metal–ligand stability constant ($K'_{ML} = k_f/k_d$) and the transport system may be termed *thermodynamically controlled* (Fig. 1A). Since at thermodynamic equilibrium the extent of metal–ligand binding must be a function of the free metal ion activity or concentration ($[M^{Z+}]$), the uptake rate and half-saturation constant may also be expressed in terms of $[M^{Z+}]$.

Pseudoequilibrium need not be achieved, however, to obtain saturable uptake kinetics. In the opposite limiting case, where internalization is faster than dissociation, a *kinetically controlled* condition results from the steady-state balance between rates of transport complex formation and internalization. The half-saturation constant is then the ratio of the two corresponding rate constants (Fig. 1B). In this case, the rate of uptake depends on the relative reaction rates and concentrations of each species in solution. Since the aquated free ions and inorganic complexes of a metal are generally more kinetically labile than its organic chelates and the relative reactivities of different inorganic complexes are not clearly established (see below), we will assume that uptake rates by kinetically controlled transport systems exhibit a dependence on the total concentration of inorganic species ($[M']$).

The well-documented dependence of some trace metal transport rates on free metal ion activity (SUNDA and HUNTSMAN, 1985; ANDERSON and MOREL, 1982), however, does not

prove that these trace metal transport systems are thermodynamically controlled. Because experimental measurements are typically made by varying chelators and metal concentrations in a constant pH medium, the free metal ion activity is proportional to the concentration of all inorganic complexes in solution, which are in rapid equilibrium with each other. Thus, dependence on free metal ion activity could simply reflect kinetic-control of uptake by one or more inorganic species (JACKSON and MORGAN, 1978). Iron transport in the two marine phytoplankters we have recently examined appears to be nearer kinetic than thermodynamic control (HUDSON and MOREL, 1990).

Since only the Michaelis–Menten parameters, K_ρ and ρ_{max}, and not the individual rate constants are observable under steady-state conditions, discriminating between transport systems that are thermodynamically and kinetically controlled requires examination of transient uptake rates. In principle, transients are observable in pulse-chase experiments and at the start of a short term uptake experiment, although very few such experiments have been performed with marine organisms (HUDSON and MOREL, 1990).

One of the three kinetic parameters of the transport mechanism, k_f, is known to a first

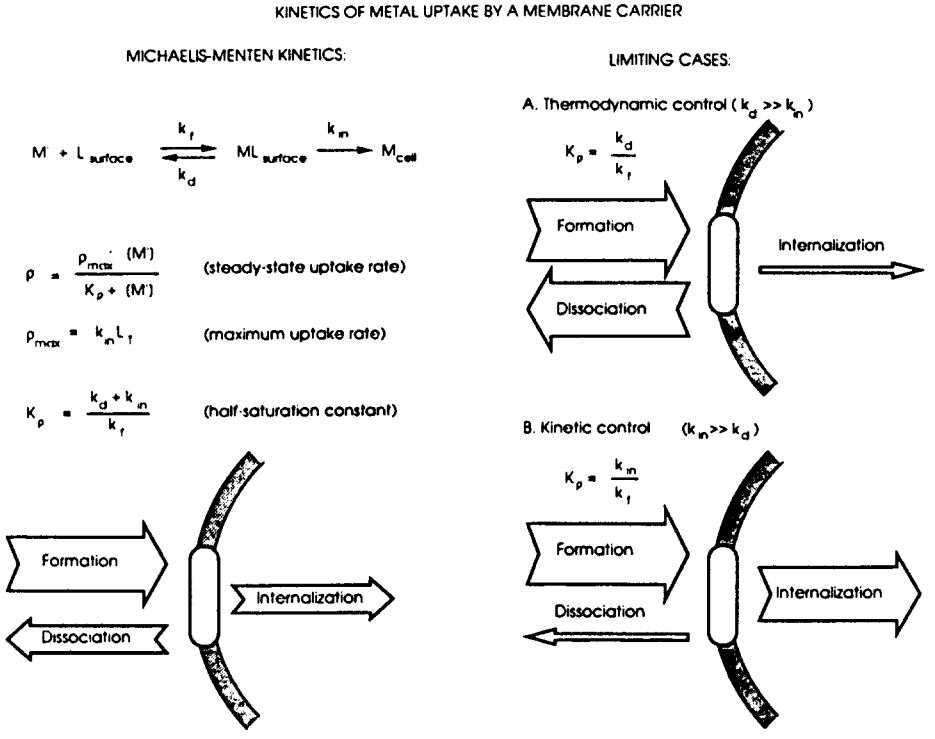

Fig. 1. Kinetics of metal uptake by a membrane carrier. Michaelis–Menten parameters for steady transport (K_ρ and ρ_{max}) depend on the rate constants for metal-transport ligand ($ML_{surface}$) complex formation (k_f), dissociation (k_d) and internalization (k_{in}) and on the total number of transport ligands ($[L_T]$). Arrow widths indicate the relative rates of each reaction for undersaturated transport systems with the same number of total ligands. Since each system is assumed to have the same k_f, the rate of complex formation is the same in all cases. Two limiting cases for the relative rates of intracellular uptake vs dissociation of the complex are: (A) thermodynamic control, where complex dissociation is much faster than internalization and (B) kinetic control, where the rate of internalization is much greater than dissociation.

approximation *a priori*. The formation rate constant of a metal coordination complex is inherently limited by the rate of dehydration of the inorganic metal species after formation of an outer-sphere complex, or ion-pair, according to the Eigen–Wilkins mechanism (BURGESS, 1988):

$$M(H_2O)_6^{Z+} + L \overset{K_{os}}{\rightleftharpoons} M(H_2O)_6^{Z+} \cdots L \overset{k_{-w}}{\rightarrow} ML(H_2O)_5. \quad (1)$$

The complex formation rate constant is the product of the outer-sphere complex stability constant (K_{os}) and the water loss rate (k_{-w}):

$$k_f = K_{os} \cdot k_{-w}. \quad (2)$$

The outer-sphere complex stability constant, which reflects the charges of the metal and the ligand, lies in the range 0.3–10 in seawater for cations of charge 0 to +2 paired with ligands of charge 0 to −2 (Table 1). We will adopt a value of 1 M^{-1} for K_{os}. Water loss rates range from 10^4 to 10^9 s^{-1} for the metals considered here, yielding second order rate constants substantially lower than the diffusion-limited values of 10^9–10^{10} M^{-1} s^{-1} (BASOLO and PEARSON, 1967).

Complexation by inorganic ligands, such as hydroxide or chloride, can accelerate water loss and subsequent ligand exchange kinetics as long as the inorganic ligand binds the metal less strongly than the incoming ligand, a condition which is likely true in seawater except for sulfide. Catalysed rates of water loss have been measured for some complexes of

Table 1. *Rate constants, oceanic concentrations and physiological parameters for trace metal transport in* T. weissflogii

Metal	k_f* (M^{-1} s^{-1})	Oceanic concentrations†			Metal quotas‡	Calculated transport parameters§	
		$[M_T]$	$[M']$	$p[M'^+]$	$Q_{0.9}$ (amol $cell^{-1}$)	$[L']_{min}$ (amol $cell^{-1}$)	k_m^{lim} (s^{-1})
Mn	3×10^7	1	1	9.2	80	0.04	3×10^{-2}
Zn	4×10^7	0.2	0.004	11.7	20	5	2×10^{-4}
Ni	1×10^5	2	2	9.0	20	2	2×10^{-4}
Fe	2×10^6	0.06	0.06	20.2	80	10	1×10^{-4}
Co	4×10^6	0.02	0.02	10.9			
Cd	4×10^8	0.002	0.0006	13.7			
Cu	1×10^9	0.6	0.002	12.7			

* K_{os} estimated from ion pair stability constants in seawater assuming $Z_L = -1$ (MILLERO and SCHREIBER, 1982). k_{-w} are values for M^{2+} (MARGERUM *et al.*, 1978; BURGESS, 1988) except Ni and Co (speciation-weighted averages for M^{2+} and MCl^+) and Fe (observed k_f for Fe(III)-deferriferrioxamine B reaction in seawater at pH 8 (HUDSON *et al.*, 1992)).

†Dissolved concentrations in mixed layer of central North Pacific Ocean: Mn, Ni, Co, Cd (BRULAND, 1983), Cu (COALE and BRULAND, 1988), Zn (BRULAND, 1989) and Fe (MARTIN *et al.*, 1989). Measured organic complexation: Cu (COALE and BRULAND, 1988), Zn (BRULAND, 1989) and Cd (K. W. BRULAND, 1992). Inorganic speciation from TURNER *et al.* (1981).

‡Cellular metal quotas in *T. weissflogii* required to grow at ~90% of μ_{max} (Fe and Ni are intracellular metal only). Based on 750 fl cell with 12 pmol C $cell^{-1}$ (N. M. PRICE, personal communication). Fe and Mn (HARRISON and MOREL, 1986), Zn (SUNDA and HUNTSMAN, 1992), Ni (PRICE and MOREL, 1991).

§Calculated as in Table 2 assuming $\mu_{max} = 2.1$ div day^{-1}.

interest in seawater, but the data are incomplete. Chloride complexation of Ni and Co accelerates water loss by factors of 10–20 (MARGERUM *et al.*, 1978). Hydroxide complexation of Fe increases water loss rates from the $Fe(OH)^{2+}$ ion 1000-fold over the Fe^{3+} ion (GRANT and JORDAN, 1981) and greater increases have been predicted, although not measured, for the predominant species in seawater. The constant used here for iron, $2 \times 10^6\ M^{-1}\ s^{-1}$, was actually measured for a microbial iron chelator in seawater (HUDSON *et al.*, 1992) and is close to measured values in marine phytoplankton, 0.9×10^6 and $1.3 \times 10^6\ M^{-1}\ s^{-1}$ (HUDSON and MOREL, 1990). The zinc constant, $4 \times 10^7\ M^{-1}\ s^{-1}$, matches reasonably well the value of $>7 \times 10^6\ M^{-1}\ s^{-1}$ obtained by BRULAND (1989) for the high affinity zinc-binding ligand found in seawater.

Although the maximum complexation rate is generally limited by water loss from the metal species, observed rates do depend on the nature of the ligand. If the ligand is initially bound to an alkaline earth metal or if deprotonation of the ligand is required, complex formation can be slowed dramatically (MARGERUM, 1963; HERING and MOREL, 1988). In chelate formation, steric factors may cause closure of the chelate ring to become rate limiting (BURGESS, 1988). Thus, the values calculated from water loss rates represent approximate upper limits to complexation rates obtainable in seawater.

COHERENCE OF TRANSPORT SYSTEM PARAMETERS, CELLULAR REQUIREMENTS AND ENVIRONMENTAL AVAILABILITY

In order to explore the implications of chemical kinetics for trace metal transport in marine microorganisms, we will consider a specific marine diatom, *Thalassiosira weissflogii*, and a single environment, the surface waters of the open ocean. The open ocean was chosen because its trace metal concentrations are probably the lowest to which phytoplankton must adapt and because it is homogeneous relative to coastal waters. Although plunging the coastal diatom, *T. weissflogii*, into the open ocean may not be an ecologically successful venture, it is a useful *gedanken* experiment since our knowledge of its trace metal requirements permits us to explore the issues of trace metal limitation in marine organisms. The limited data available for oceanic species show that they have somewhat lower trace metal requirements than *T. weissflogii*. Thus, we present the following discussion realizing that the numerical values are only approximate.

Slow coordination kinetics mandate numerous surface ligands

At steady-state, the transport complex formation rate must at least equal the uptake rate (Fig. 1 and Table 2). Since the complexation rate is proportional to the inorganic metal concentration ([M']) and the total number of *free* transport ligands ([L']), a low [M'] necessitates a high [L'] to achieve an uptake rate sufficient for the cellular requirement. Consider iron(III), for example, which has a k_f of $2 \times 10^6\ M^{-1}\ s^{-1}$ and a surface seawater concentration of 0.06 nM. The diatom *T. weissflogii* utilizing nitrate as a N source, in order to obtain the cellular iron quota ($Q_{0.9}$) of 8×10^{-17} mol Fe $cell^{-1}$ (Fe:C = 7×10^{-6}) that sustains a growth rate 90 per cent of its maximum ($\mu_{max} = 0.06\ h^{-1}$), requires at least 1×10^{-17} mol uncomplexed transport ligand per cell ($[L']_{min}$). (Note: all following discussion of cellular requirements refers to the steady uptake rate needed to grow at $\mu_{0.9} = 0.9 \cdot \mu_{max}$.) Perhaps fortuitously, this value closely approximates observations of transportable

Table 2. Dependence of transport system properties on complexation kinetics, cellular requirements and oceanic concentrations of trace metals

(A) Minimum free transport ligand number ($[L']_{min}$):
Constraint: complex formation rate > cellular requirement

$$k_f \cdot [L'] \cdot [M'] > \mu_{0.9} \cdot Q_{0.9}.$$

Result:

$$[L']_{min} = \frac{\mu_{0.9} \cdot Q_{0.9}}{k_f \cdot [M']}$$

(B) Minimum internalization rate constant allowing minimal total ligand number (k_{in}^{lim}):
Constraint: transport system undersaturated and kinetically-controlled.

$$k_d + k_{in} > k_f \cdot [M']; \qquad k_{in} > k_d.$$

Result:

$$k_{in}^{lim} = k_f \cdot [M'].$$

(C) Minimum internalization constant allowing full utilization of complexed metal (k_{in}^{μ}):
Constraint: low ratio of metal in transport complex to intracellular requirement

$$\frac{[ML]}{Q} = \frac{\mu}{k_{in}} < 1 \qquad \text{for all } \mu$$

Result:

$$k_{in}^{\mu} > \mu_{max}.$$

surface iron in *T. weissflogii* when grown under iron stress (HUDSON and MOREL, 1990) and in fact is an extraordinarily high concentration of membrane carriers, as discussed later.

The requirement for large numbers of transport ligands is most acute in the surface ocean, where total concentrations of nutrient metals are in the range of 0.1–2 nM and organic complexation can reduce the concentration of labile species even further, as shown for other metals in Table 1. For several metals—Ni, Fe and Zn— the combination of slow reaction kinetics and low metal concentrations requires that the cellular concentration of free transport ligands be comparable to the cellular concentration of the metal. Note that this conclusion is independent of the cellular metal requirement.

Undersaturation and kinetic control minimizes the total number of transport ligands

The above calculations of the minimum number of free ligands are based on the necessity for the transport complex formation rate to be at least as fast as the intracellular uptake rate. The total number of transport ligands required (free and complexed) depends on both the extent of kinetic vs thermodynamic control and the degree of saturation of the transport system. If the rate of complex dissociation exceeds the rate of metal internalization (thermodynamic control) then the rate of binding must be much faster than uptake and the free ligands more numerous than in the kinetically controlled case (Fig. 1). Attaining the minimum *total* number of ligands also requires that the transport system remain undersaturated, because complexation kinetics fix the number of *free* ligands

needed to sustain growth and saturation of sites only increases the total number by adding *complexed* ligands. Undersaturation of transport systems at the lowest metal concentrations permitting maximum growth rates, the conditions where the most ligands would be required, has in fact been observed for Fe (HUDSON and MOREL, 1990) and Mn (SUNDA and HUNTSMAN, 1986).

The advantages of undersaturation and kinetic control can be seen quantitatively for ligands of different binding strengths in Fig. 2. Again consider the uptake of a metal at its ambient inorganic concentration, e.g. 0.06 nM for Fe. We define a strong ligand as one that is more than half-saturated at ambient [M′], i.e. $K'_{ML} > [M']^{-1}$ or $K'_{FeL} > 2 \times 10^{10}$ M^{-1}, and a fast internalization rate as one that causes undersaturation under kinetic control of transport, i.e. $k_{in} > k_f[M']$ or $k_{in} > 2 \times 10^6 M^{-1} s^{-1} \cdot 6 \times 10^{-11} M = 1 \times 10^{-4} s^{-1}$ for Fe. For a ligand of given binding strength (K'_{ML}), the total number of ligands ($[L_T]$) necessary to acquire the metal at a given rate decreases with increasing internalization rate constant up to the point where transport is both undersaturated—$(k_{in} + k_d)/k_f > [M']$—*and* kinetically controlled—$k_{in} > k_d$. We refer to such transport systems as *kinetically limited*. Kinetically limited transport can be achieved at lower values of k_{in} for stronger ligands since they dissociate more slowly, i.e. have a lower k_d. However, the use of ligands stronger than necessary for ambient metal concentrations to saturate the ligand, i.e. $K'_{ML} \cdot [M'] > 1$, does not permit further decreases in k_{in} since undersaturation must be maintained. Thus, reaction kinetics and ambient concentrations together define the minimum value of the internalization rate constant ($k_{in}^{lim} = k_f \cdot [M']$; Table 2) possible for transport systems that approach the minimum total number of ligands ($[L_T] \approx [L']_{min}$). By this reasoning alone, neither high ligand affinity for the metal nor thermodynamic control is necessary to transport metals at low environmental concentrations.

Competition from analogous metals limits the degree of kinetic control

Thus far we have considered optimal properties of metal transport systems without regard to the presence of competing metals in the organisms' environment. Many metals are similar enough chemically that it is difficult for ligands to bind one to the exclusion of others. The metal internalization step is also of limited selectivity, as demonstrated by experimental observations of the intracellular accumulation of nonessential metals (REUTER and MOREL, 1981; HARRISON and MOREL, 1983). Thus, metals that are chemically similar to the micronutrient create an additional constraint for transport systems—selectivity.

Classical competitive inhibition of nutrient uptake arises from analogous substrates binding to transport sites, thereby decreasing the number of sites available to transport the nutrient (Table 3A). In such a case, the organism must compensate either by increasing the total number of ligands to maintain [L′] or by evolving a transport system with an inhibition constant (K_X) that is normally undersaturated with respect to the nonessential metal (X).

Nonessential metals need not saturate the transport system to have an effect. Intracellular inhibition of cellular functions can occur if the uptake of nonessential metal relative to the essential one is excessive. Applying our model of metal transport simultaneously to a case where two metals are transported by a single transport system suggests that the ratio of the rates of essential and nonessential transport ($S_{M:X}$) should differ under kinetic and thermodynamic control (Table 3B). In fact, KRASNE and EISENMANN (1976) have demon-

strated the influence of both kinetic and equilibrium factors on the selectivity of alkali metal cation transport.

As an hypothetical example, we consider the case of a transport ligand whose relative affinities for divalent metals parallel those of ethylenediamine, which increase in the sequence Mn < Cd < Zn < Co < Ni < Cu. Given the metals' speciation in seawater and assuming equal internalization rates, we can compute their relative transport rates (Fig. 3). Under thermodynamic control, such a transport system in seawater would take up Ni, which because of its high binding affinity and a high proportion of the free ionic species is taken up the fastest, $10^{7.7}$ times faster than Cd, which is taken up the slowest.

If the internalization rates of the same transport system were increased to the kinetically controlled limit, the uptake rate would depend only on the forward rate constant, the number of ligands and the concentrations of the metals. If the internalization rates for a series of metals were high enough (i.e. the same and very high for all or simply greater than the individual dissociation rates) then they all would be transported under kinetically controlled conditions. The selectivity of the transport system would then be "passive" and correspond to the relative reaction rates of the metals (S_{kinetic}). According to the these calculations, this "passive" selectivity would be Mn $\gg$ Cu $\gg$ Cd > Ni > Zn > Co in seawater (Fig. 3) and be independent of the metals' affinities for the transport ligand.

The conditions under which the selectivity of a transport system may be improved relative to the passive selectivity observed under pure kinetic control are illustrated in Fig. 4. As long as transport of both metals is kinetically controlled (the quadrant of the surface pointing forward from the plane of the paper), the relative rates of M and X uptake are independent of the nature of the ligand, which defines k_d for M and X, and the relative internalization rates. Thermodynamic control of micronutrient transport alone actually decreases transport selectivity since nutrient uptake must decrease relative to complexation. Improvements in the selectivity only occur in the portion of the surface where the nonessential metal is thermodynamically controlled to a greater degree than the micronutrient, i.e. $k_{in}^{X}/k_d^{X} < k_{in}^{M}/k_d^{M}$. Thus, an ideal transport system would be kinetically controlled with respect to the micronutrient to minimize the ligands required, but thermodynamically controlled with respect to any nonessential metals to maximize its selectivity.

However, such an ideal system may be difficult to realize. Since internalization involves a dissociation step, k_{in} and k_d are likely to be correlated, e.g. k_{in}/k_d approximately constant for different metals. One solution may involve a two-step transport process in which k_{in} is modulated for essential and nonessential metals. For a given differentiation of internalization kinetics, larger differences between the equilibrium affinities of a ligand for two metals permit larger improvements in selectivity. Thus, selectivity requirements may force transport systems toward higher affinity ligands and internalization rates nearer thermodynamic control.

Slow internalization kinetics of the metal–ligand complex

The internalization rate constants required to attain kinetically limited transport of metals in open ocean waters are remarkably small; that is, metal turnover is slow, compared to other membrane transport processes (cf. Tables 1 and 4). Returning to our previous example of iron uptake in *T. weissflogii*, at a minimum of 1×10^{-17} mol cell^{-1} of ligand and a steady uptake rate of 4×10^{-18} mol cell^{-1} h^{-1}, a given transport ligand is transporting at most one Fe atom every 2 h on average. Since the steady-state uptake rate

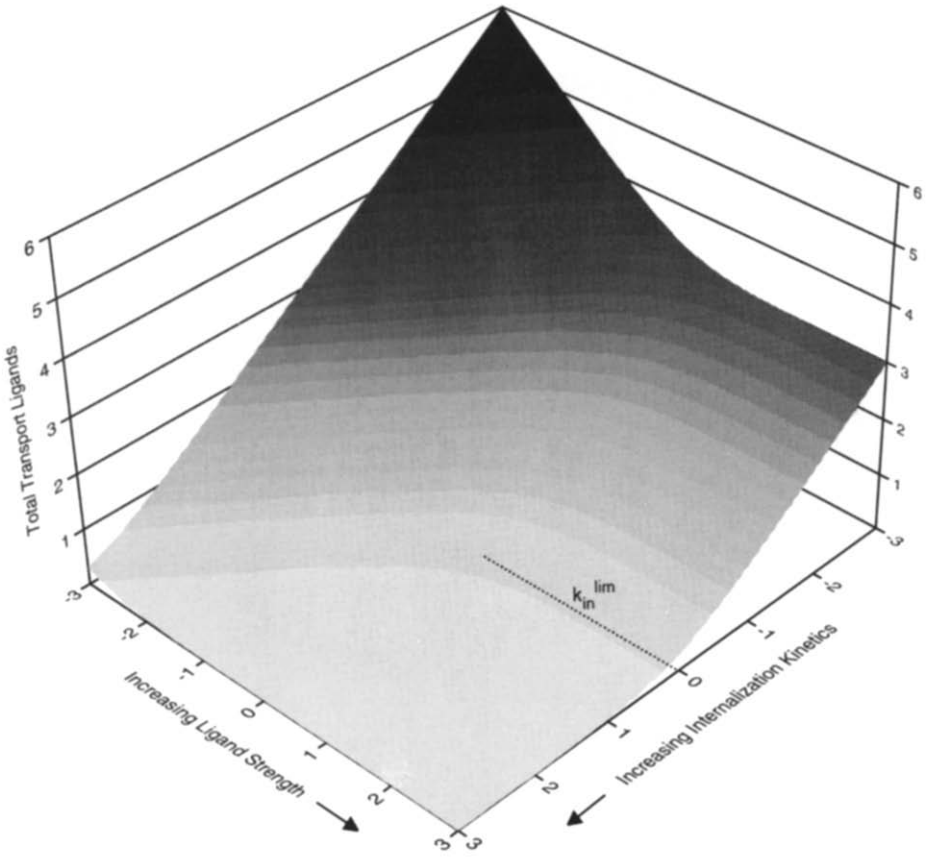

Fig. 2. Dependence of the total number of metal transport ligands ($[L_T]/[L']_{min}$) on the binding strength of the transport ligand ($K'_{ML} \cdot [M']$) and the internalization rate constant ($k_{in}/k_t \cdot [M']$). All axes are logarithmic. k_t, $[M']$ and $[L']_{min}$ are held constant.

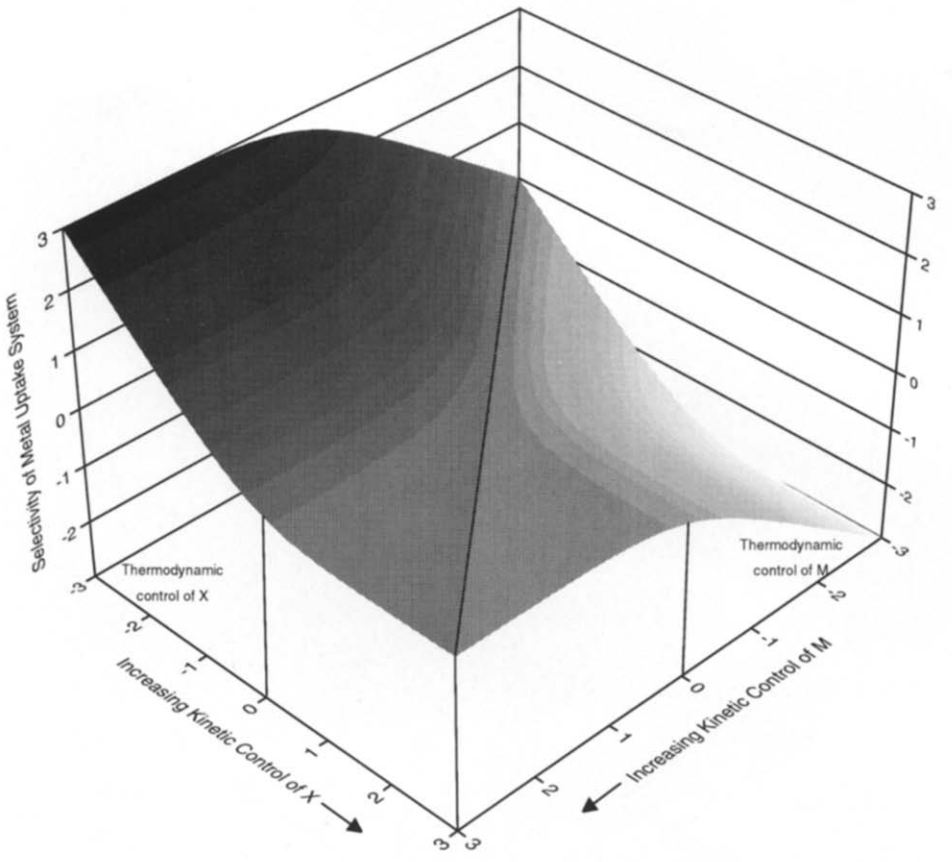

Fig. 4. Dependence of transport selectivity ($S_{M,X}/S_{kinetic}$) on the degree of kinetic control of essential (k_m^M/k_d^M) and nonessential (k_m^X/k_d^X) metals' uptake. All axes are logarithmic. Calculated as in Table 3.

Table 3. Metal competition and selectivity of uptake

(A) Competitive inhibition of micronutrient M uptake of by nonessential metal X.

$$\rho_M = \frac{\rho_{max}^M \cdot [M']}{K_\rho \cdot (1 + [X']/K_X) + [M']}$$

where

$$K_X = \frac{k_d^X + k_{in}^X}{k_f^X}.$$

(B) Relative rates when both M and X are transported via the same mechanism ($S_{M:X}$).

$$S_{M:X} = \frac{\rho_M}{\rho_X} = \frac{k_f^M \cdot [M'] \cdot k_{in}^M \cdot (k_d^X + k_{in}^X)}{k_f^X \cdot [X'] \cdot k_{in}^X \cdot (k_d^M + k_{in}^M)}.$$

(C) Relative uptake rates when transport of both M and X is kinetically-controlled ($S_{kinetic}$)

$$S_{kinetic} = \frac{\rho_M}{\rho_X} = \frac{k_f^M \cdot [M']}{k_f^X \cdot [X']}.$$

is roughly 2% of the measured saturated uptake rate, about 2% of the transport ligands must be bound to iron, but turn over 40-times faster, i.e. $1/k_{in}$ should be *ca* 3 min. The turnover time observed in pulse-chase experiments (HUDSON and MOREL, 1990), 5 min, differs in proportion to the total number of ligands actually observed, 1.7×10^{-17} mol cell^{-1}.

Transport systems for other metals should also exhibit slow internalization kinetics, as the calculated k_{in}^{lim} suggest (Table 1). The corresponding turnover times ($1/k_{in}^{lim}$) vary

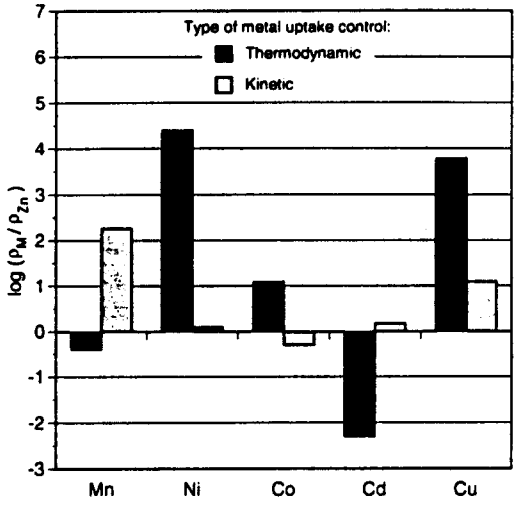

Fig. 3. Selectivity of divalent metal uptake under kinetic and thermodynamic control. Uptake rates of each metal relative to zinc are shown for an hypothetical transport system with ethylenediamine-like ligands and equal internalization rate constants for each metal (see Table 3B–C). Stability constants for ethylenediamine from MOREL (1983); concentrations and k_f given in Table 1.

Table 4. Numbers and turnover times of typical transport molecules

Molecule (type of transport)	MW (kDal)	Radius (nm)	Maximum number* (mol cell^{-1})	Turnover time (s)
Phospholipid (lipid flip-flop)	0.9	0.4	1.3×10^{-15}	$> 10^5$
Valinomycin (K^+ ionophore)	1.1	0.6	3×10^{-16}	10^{-4}
Bacteriorhodopsin (active H^+ channel)	26	1.7	4×10^{-17}	n.a.
S.R. Ca ATPase (active Ca^{2+} carrier)	102	2.3	2×10^{-17}	5×10^{-3}
Mitochondrial ATPase (active H^+ pump)	460	5	4×10^{-18}	n.a.

Data from STRYER (1981), METZLER (1977), JAIN and WAGNER (1980) and CRAM (1980).

n.a. no applicable.

*Numbers of molecules calculated for a spherical organism of radius equal to *T. weissflogii*—5.6 μm. Phospholipids assumed to make up entire membrane; other molecules assumed to occupy one-half of membrane area.

between 30 s and 3 h. As noted above for iron, the actual internalization rate constant should be significantly faster than the minimum value (k_{in}^{lim}) to avoid saturating the system. Measured half-saturation constants for Mn (SUNDA and HUNTSMAN, 1986) and maximum formation rate constants (Table 1) can be used to estimate $1/k_{in}$ values of > 20 s and > 1 s, respectively. These turnover times are still much greater than the time needed to traverse the plasmamembrane, typically 10^{-3} s for ionophores or active transport enzymes (Table 4).

Slow internalization kinetics may therefore reflect slow kinetics of release from the transport ligand, either via intracellular dissociation or ligand exchange. Since dissociation kinetics depend on complex stability (MARGERUM, 1963), internalization kinetics may ultimately reflect the thermodynamic stability of the complex. In the case of iron bound to siderophores, intracellular release is so slow as to require reduction of the metal or degradation of the ligand in some cases (RAYMOND and CARRANO, 1979). In addition, increasing the internalization rate constant beyond the point where transport becomes kinetically limited results in no decrease in ligands required (Fig. 2) or improvement in selectivity (Fig. 4), so the incentive to develop a faster release mechanism is nil.

A lower limit for physiologically reasonable internalization rate constants that is independent of the requirement to remain undersaturated arises from the organism's need to utilize the metal intracellularly after binding to the transport ligand (Table 2C). Slow internalization relative to the organism's maximum growth rate (μ_{max}) would result in larger quantities of metal bound to the transport ligand than in use inside the cell. Consequently, a constraint applicable to transport systems for all metals whose supply potentially limits cell growth would be $k_{in} \gg \mu_{max}$. This constraint also defines a minimum half-saturation constant attainable for metal transport ($K_\rho^{min} = \mu_{max}/k_f$). For example, at a growth rate of 1 div day^{-1} and a forward rate constant of 4×10^7 M^{-1} s^{-1} for zinc, the lowest internalization rate is $\sim 10^{-5}$ s^{-1} and the corresponding half-saturation constant, 4×10^{-12} M, is very near oceanic inorganic zinc concentrations.

LIMITS TO ATTAINABLE RATES OF NUTRIENT UPTAKE AND GROWTH

To this point, we have focused on the required characteristics of trace metal transport systems capable of satisfying physiological requirements. While phytoplankton do regulate their transport systems in response to nutrient deficiencies, there are, of course, limits to the range of nutrient concentrations at which maximal growth rates can be maintained. For trace metals, these limits may be consequences of the biophysics of the cell membrane, the rate of physical transport processes outside the cell or the ability of transport systems to selectively accumulate a metal when competing analogs are present.

Biophysical limits on the transport system

Without more detailed knowledge of the molecular mechanics of metal transport in phytoplankton than is available at present, it is only possible to suggest approximate biophysical limits on transport system capabilities. The likelihood that transport ligands are attached to or embedded in the cell membrane creates a question of crowding due to the large number of ligands required (Table 1). The maximum number of potential transport molecules that can fit (occupy one-half of the membrane area) in the plasma membrane of our model organism, *T. weissflogii*, range from 4×10^{-16} to 4×10^{-18} mol cell^{-1} for ionophores and active transport ion pumps, respectively (Table 4). Realistic limits are likely to be somewhat lower than these since membrane space is required for other essential processes. Our calculations (Table 1) suggest that while Mn transport systems could employ a protein comparable in size to the mitochondrial ATPase, the use of such large proteins for Fe, Zn and Ni transport would crowd the membrane. Although it would seem that minor nutrient transport must necessarily take up a minor portion of the membrane, paradoxically it may not be so. An additional disadvantage to the use of large proteins for trace metal transport, particularly for metals such as Fe, Ni and Zn that require a large number of ligands relative to their quota, is that their production could consume a large fraction of the cellular supply of nitrogen and fixed carbon.

Diffusion limitation

So far we have considered that uptake rates can be increased as long as the cell can synthesize and "install" more surface ligands. There is, of course, a physical limit to this due to the development of a diffusive boundary layer around the cell. Were the cell to be a perfect sink, the metal concentration at the cell surface ($[M']_{surface}$) would be depleted to zero and an infinite number of surface ligands would be required.

What fraction of the maximum diffusion flux can a cell then afford to take up? For a spherical, non-motile cell, the number of ligands required to transport the metal at any fraction of the maximum diffusion rate increases disproportionately in the range of 60–80% of the diffusion limit (Fig. 5). In this range, for the same bulk metal concentration 2.5–5 times as many ligands are required to obtain the same flux as in the absence of the diffusive boundary layer, i.e. if the medium were well-mixed up to the cell surface. Further increases are extremely inefficient, as indicated by the large increase above the number of ligands required in the absence of the diffusion boundary layer. This upper limit is close to the maximum of about 50% of the diffusion-limited flux observed in a survey of

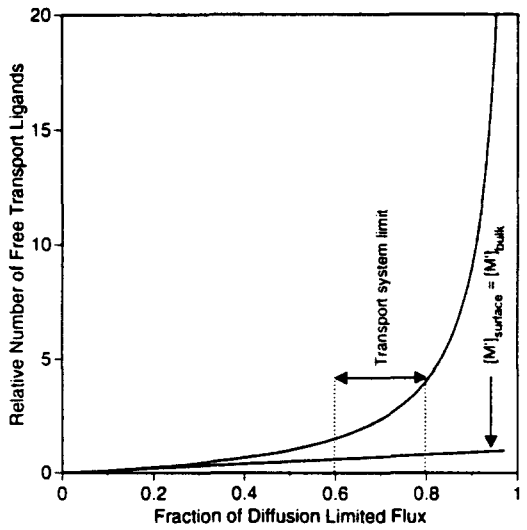

Fig. 5. Relative number of transport ligands ($[L'] \cdot k_f/k_D$) required to sustain uptake at a given fraction of the purely diffusion limited rate ($\alpha_{diffusion}$). The number of ligands required to obtain the same uptake rate if the medium were well-mixed up to the cell surface (dashed line) is shown for reference. See Table 5.

phosphate transport kinetics of phosphorus-limited phytoplankton (J. M. BOWEN and K. D. STOLZENBACH, personal communication).

For an organism to obtain a given fraction ($\alpha_{diffusion}$) of the maximum diffusive flux, the number of free ligands required under kinetically limited transport depends only on the cell diameter and the complex formation rate constant (Table 5). For example, to obtain two-thirds of the maximum diffusion flux of Fe in the oceans, a cell such as *T. weissflogii* with a radius of 5.6 μm would need 6×10^{-17} mol ligands cell^{-1}. For this organism, iron uptake rates up to 20% of the diffusion-limited flux and 1.7×10^{-17} mol ligands cell^{-1} have been observed under iron stress (HUDSON and MOREL, 1990). These findings suggest that the organism increases its transport system to the point where diffusion limitation begins to be felt.

The limits posed by membrane biophysics and by solute diffusion to our model organism's ability to acquire Ni, Fe, Zn and Mn for growth can be seen in Fig. 6. The minimum number of free transport ligands ($[L']_{min}$) required to sustain near-maximal growth rates is inversely proportional to the ambient inorganic metal concentration ($[M']$), as shown by the lines. At the same $[M']$, $[L']_{min}$ for Ni is high relative to other metals due to its slow kinetics; the values for Fe are also high due to a combination of slow kinetics and high quota. $[L']_{min}$ values at oceanic inorganic metal concentration are indicated by the dot on each line. Mn requires a small enough number of ligands, 0.04×10^{-18} mol cell^{-1}, that a large, ion-pump protein could be employed to transport this metal even under thermodynamic control. Zn and Ni each require enough ligands, $2\text{–}5 \times 10^{-18}$ mol cell^{-1}, that large proteins may cause crowding even under kinetic control. Fe uptake, which requires 10×10^{-18} mol cell^{-1}, by moderate- to large-sized proteins would require more of the membrane space than is available.

Diffusion limitation of growth occurs at the metal concentration and corresponding

Table 5. Diffusion limitation of uptake and growth

(A) Minimum free ligands required to obtain 2/3 diffusion limited uptake rate ($[L']_{diffusion}$):
Constraint: complexation rate at surface = 2/3 diffusion limited flux

$$k_f \cdot [L'] \cdot [M']_{surface} = k_D \cdot ([M']_{bulk} - [M']_{surface}),$$

where $k_D = 4 \pi R D$ for a spherical, non-motile cell, D = diffusion coefficient, R = radius of cell, $[M']_{surface} = [M']_{bulk}/3$.
Result:

$$[L']_{diffusion} = \frac{2 \cdot k_D}{k_f}.$$

(B) Ambient concentration allowing 90% of maximal growth rate at 2/3 diffusion limited uptake rate ($[M']_{diffusion}$):
Constraint: cellular requirement = 2/3 diffusion limited flux.

$$\mu_{0.9} \cdot Q_{0.9} = \frac{2}{3} \cdot k_D \cdot [M']_{bulk}$$

Result:

$$[M']_{diffusion} = \frac{3 \cdot \mu_{0.9} \cdot Q_{0.9}}{2 \cdot k_D}.$$

(C) Fraction of diffusion-limited flux obtainable at fixed ρ_{max} and K_ρ for undersaturated conditions ($\alpha_{diffusion}$).
Constraint: uptake rate = rate of physical transport to cell

$$\frac{\rho_{max} \cdot [M']_{surface}}{K_\rho} = k_D \cdot [M']_{bulk} \cdot \alpha_{diffusion}.$$

Result:

$$\alpha_{diffusion} = 1/(1 + k_D \cdot K_\rho/\rho_{max}).$$

Note: $k_D \cdot K_\rho/\rho_{max}$ = "P" of PASCHIAK and GAVIS (1974).

number of ligands indicated on each line by a triangle. The metal concentration at which diffusion limits growth ($[M']_{diffusion}$) decreases from Fe and Mn to Ni and Zn in proportion to their cellular quotas. Interestingly, oceanic Fe and Zn concentrations occur at values where diffusion limitation is of concern. If the parameters on which this analysis is based are correct, phytoplankton in the ocean must have a significantly lower Zn requirement than measured for coastal species in the laboratory. It is thus satisfying to note that indeed oceanic species have lower Zn requirements than neritic species (SUNDA and HUNTSMAN, 1992). In addition, Cd has been shown to partially replace zinc under zinc-limited conditions (PRICE and MOREL, 1990). The slowness of Ni coordination kinetics implies that *T. weissflogii* cells could only attain diffusion limited uptake rates if nearly the entire membrane area were to be carriers the size of phospholipids.

Limits imposed by metal competition

In some cases, limitation of phytoplankton growth rates by nonessential metals has been shown to occur via intracellular or transport competition with nutrient metals. The effect, as observed for Mn–Cu and Zn–Cu interactions (SUNDA *et al.*, 1981; SUNDA and

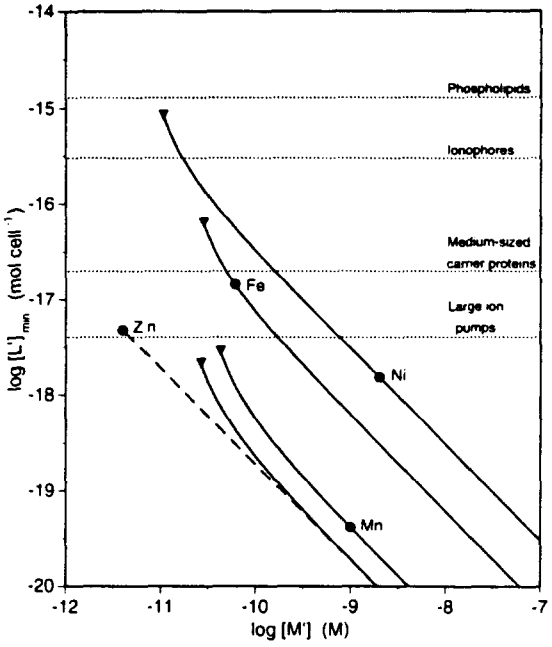

Fig. 6. Dependence of the minimum number of transport ligands ($[L']_{min}$) required to grow at 90% of maximum growth rate on ambient trace nutrient concentration ([M']). The line for each metal terminates at the concentration at which the transport system obtains two-thirds of the diffusion limited uptake rate. Circles (●) indicate oceanic concentrations of inorganic species for each metal. Maximum carrier concentrations in Table 4. Calculations assume that no dissociation of dissolved organic complexes occurs in the boundary layer. $[L']_{min}$ and $[L']_{diffusion}$ calculated as in Tables 2 and 5.

HUNTSMAN, 1983; REUTER and MOREL, 1981) is to cause growth rates to depend on the relative amounts of the competing metals and to decrease before the point at which diffusion limitation of the nutrient begins. This clearly constitutes a third type of nutrient limitation, one which is not absolute, however, in the sense that organisms may evolve to become as selective as they need be. The greater selectivity for Mn relative to Cu in oceanic diatoms over neritic species (SUNDA and HUNTSMAN, 1983, 1986), for example, suggests that organisms can adapt their Mn transport systems or intracellular defenses to the ambient levels of Mn and Cu in their environments. Thus, while this limit may be an important factor in determining the suitability of a given organism for a particular environment, it may not limit all organisms simultaneously and may be most important under transient conditions, such as upwelling events (SUNDA *et al.*, 1981).

ECOLOGICAL AND OCEANOGRAPHIC IMPLICATIONS

Summary of experimental results

Experimental studies of trace metal nutrition and uptake in marine phytoplankters give evidence of two of the types of nutrient limitation discussed above (Table 6). Iron and zinc uptake by phytoplankton appears to be most strongly influenced by diffusion limitation

Table 6. Fe, Mn, Zn *and* Ni *transport in marine diatoms*

Metal (organism)	Observed $[M']_{limit}$* (nM)	Calculated $[M']_{diffusion}$ (nM)	Observed $[M']_{ocean}$ (nM)	$\alpha_{diffusion}$†
Fe (*T. weissflogii*)‡	0.1–0.4	0.03	0.08	0.2
Mn (*T. pseudonana*)§	2–5	0.01	1	0.002
Zn (*T. pseudonana*)‖	0.004–0.01	0.006	0.002	≤ 0.5
Ni (*T. weissflogii*)¶	0.1	0.01	2	≥0.03

n.d. = not determined.

*Metal concentration at which $\mu \approx 0.9\,\mu_{max}$ from growth experiments.

†Defined in Table 5, calculated using measured values of K_ρ and ρ_{max} and $D = 6 \times 10^{-6}$ cm^2 s^{-1} for divalent metals and $D = 9 \times 10^{-6}$ cm^2 s^{-1} for Fe(III) (after Li and Gregory, 1974).

‡Harrison and Morel (1986); Hudson and Morel (1990).

§Sunda and Huntsman (1986).

‖Sunda and Huntsman (1992); Brand *et al.* (1983).

¶Price and Morel (1991).

since the uptake rates reach a significant fraction of the diffusion limited rates and the growth of the diatoms becomes limited at concentrations near the calculated diffusion limit. Manganese provides an example of a type of nutrient limitation where neither transport nor growth rates approach diffusion limited values. Rather, because the interaction of Mn and Cu which controls the growth of this organism through its effect on Mn transport or intracellular utilization (Sunda and Huntsman, 1983), the limitation of growth prior to diffusion limitation is expected.

We have hypothesized that growth limitation by membrane crowding could occur for Ni before the point of diffusion limitation in urea-grown cells (Fig. 6). For *T. weissflogii*, $\alpha_{diffusion}$ and the ratio of $[Ni']_{limit}$ to $[Ni']_{diffusion}$ appear to be intermediate between the competition controlled value for Mn and the diffusion limited values of Fe and Zn (Table 6). From observations of surface-bound Ni, $\sim 5 \times 10^{-18}$ mol cell^{-1} (Price and Morel, 1991), we do not expect that membrane crowding is the limiting factor either, unless large transport proteins are used in Ni uptake. It may be that the organism never experiences Ni levels low enough to reach these limits.

Generalization to oceanic phytoplankton

Generalization of these results to other marine phytoplankton is complicated by the fact that metal quotas are not absolute. For example, substitution by other metals or use of alternate biochemical pathways must occur since oceanic species appear to be capable of growing maximally at zinc and iron concentrations well below those which limit coastal species that take up metals at diffusion limited rates (Brand *et al.*, 1983). Recent studies have shown that an oceanic diatom species requires about 3-fold less iron than *T. weissflogii* (Sunda *et al.*, 1991) and substitution of cadmium and cobalt for zinc has been observed in *T. weissflogii*, providing an example of the utilization of alternative elements when ambient nutrient concentrations are low (Price and Morel, 1990). Cellular metal quotas, however variable, must be limited by biochemical necessity (Raven, 1988) and the widespread occurrence of major nutrient-trace metal correlations for Fe (Martin *et al.*,

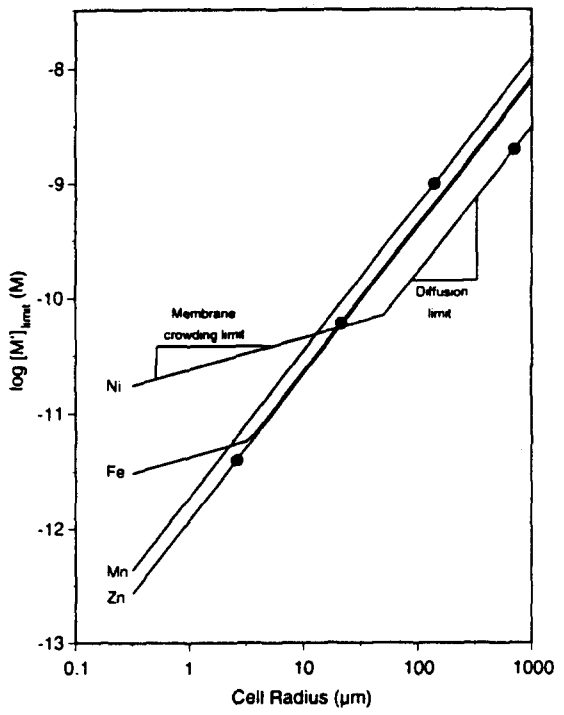

Fig. 7. Size dependence of ambient inorganic metal concentration ($[M']_{limit}$) below which diffusion or membrane area prevents organisms from growing at 90% of maximal growth rate (1.9 div day^{-1}). Calculations are for spherical cells with cell quotas calculated from metal:carbon ratio in *T. weissflogii* (Table 1) and cell carbon-cell volume correlation of STRATHMAN (1967). Diffusion limit calculated as in Table 5B. Membrane crowding limit derived from $[L']_{min}$ (Table 2A), cross-sectional area of Ca-ATPase (Table 4) and allowing up 50% of the membrane area to be occupied by transport sites.

1989), Zn (BRULAND, 1980), Ni (SCLATER *et al.*, 1977) and Cd (BOYLE *et al.*, 1976) suggests a finite range of quotas occur, at least for the phytoplankton which control nutrient removal from the surface of the ocean.

Organism size, well-known as an important factor in the organization of marine ecosystems, provides a convenient basis on which to generalize the effects of the three types of nutrient limitation discussed above. As Fig. 7 suggests, the growth rates of different-sized organisms will not be equally limited by diffusion rates or membrane crowding at a single ambient concentration. With metals whose uptake is limited by competition, organisms of all sizes experience the same challenges of chemical selectivity. Since organism size has profound effects on sinking rates and predation (GOLDMAN, 1988), it is important for modeling pelagic ecosystems to determine which type of nutrient limitation exists.

Assuming that phytoplankton maintain constant metal:carbon ratios and that cellular carbon contents vary as a function of cell volume (STRATHMAN, 1967), we calculate that metal requirements will increase as cell radius (R) to the 2.25 power. Because maximum growth rates are a weak function of cell volume (BANSE, 1982), we have applied a constant growth rate, $\mu_{0.9}$ = 1.9 div day^{-1}, in this analysis. For diffusion-limited uptake, the

maximum rate of supply increases in direct proportion to radius (Table 5), yielding an $R^{1.25}$ relationship of limiting metal concentration to cell radius (Fig. 7). Similarly, for metals whose uptake is limited by membrane space for transport sites, uptake rates vary as radius squared and the growth-limiting trace nutrient concentration should be proportional to $R^{0.25}$. For the slowly reacting metals, Ni and Fe, we expect a transition between limitation of growth by membrane crowding and diffusion to occur for organisms of about 50 and 3 μm, respectively. The high Fe requirements of cyanobacterial species relative to *T. weissflogii* (BRAND, 1992) raises the possibility that membrane crowding may limit Fe uptake in these organisms. Membrane space for Ni carriers should limit its uptake only if Ni is organically-complexed to a significant degree, >95%, in the ocean. For Mn and Zn, membrane crowding should not be a factor.

As a result of the size dependence of diffusion limited uptake rates, a threshold size, indicated for the metals considered here by the points shown in Fig. 7, may exist above which phytoplankton are unable to attain maximal growth rates at ambient concentrations in the ocean. According to our analysis, oceanic Zn concentrations may be low enough that diffusion limits growth rates of cells larger than 4 μm in radius; diffusion of Fe may limit cells of greater than 20 μm radius. Diffusion limitation by Zn and Fe may partly explain why oceanic ecosystems are dominated by pico- and nanoplankton.

Metal concentrations in the surface ocean

For bio-limiting elements in the sea, the composition of plankton and seawater reflect one another (REDFIELD, 1958). We surmise that the existence of stable oceanic trace nutrient profiles reflects the end result of competition among plankton for major and trace nutrients as one or more became limiting. Since the rates of nutrient supply and of removal via settling biogenic particles must balance over the long term, the concentration and bioavailability of dissolved nutrients remaining in the mixed layer must be adequate to sustain the flux of nutrients contained in settling particulate matter.

As the effects of biota on the composition of seawater are most directly felt in the surface layer, we might ask what determines how low plankton are able to reduce trace nutrient concentrations in surface waters of the ocean? As indicated in Table 1, the concentrations of metal as total ($[M_T]$), labile ($[M']$) and free ions ($[M^{Z+}]$) vary over several orders of magnitude despite the fairly narrow range of their concentrations in the deep sea—0.5–12 nM. However, there is an approximately inverse relationship between the measured concentrations ($[M']$) and the coordination kinetics (k_f) of the labile metal species (Fig. 8). The relationship is particularly good for the essential metals, i.e. Zn, Ni, Fe, Co and Cd, with the notable exception of Mn. Even [Cu'] departs from the value expected on the basis of k_f by only a factor of 10 or so. The correlation suggests that the removal of essential trace metals from the surface ocean is controlled by the rates of coordination reactions, perhaps with the metal transport ligands of marine phytoplankton. The deviation of Mn from the behavior of other essential trace metals may result from differences in the nature of its transport systems, as discussed earlier.

The inverse relationship of $[M']$ and k_f may reflect the interdependence of the properties of metal transport systems, the metabolic requirements of phytoplankton and the oceanic concentrations of trace metals. It is noteworthy that the equation developed here to describe this interdependence at the cellular level (Table 2A) approximately fits the observed oceanic data if the *in situ* growth rates of oceanic plankton, about 0.6–0.7

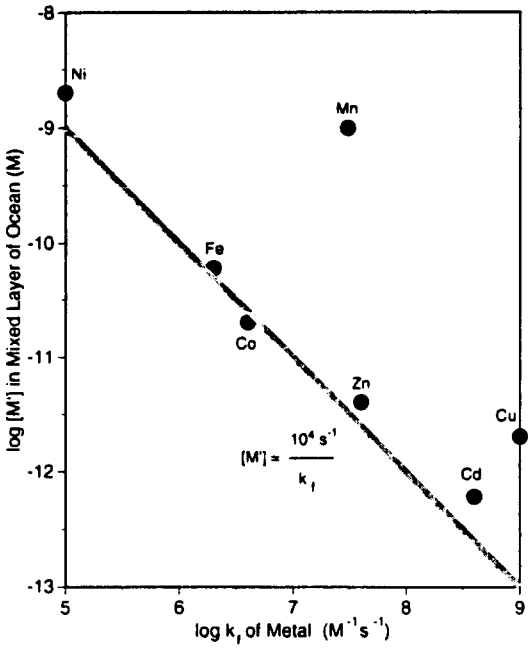

Fig. 8. Correlation of the concentration of inorganic complexes ([M']) and complexation rate constant (k_f) for trace metals in the surface ocean. All data from Table 1. Line is an approximate lower limit fit to data by inspection. A similar graph that does not include Cd complexation was included in MOREL *et al.* (1992).

day^{-1}, are utilized in conjunction with a constraint that $Q_{0.9} \approx 10 \cdot [L']$. Such a constraint, applicable to a significant portion of it not all oceanic phytoplankton, may result from the kinds of requirements for transport system properties considered here. Alternatively, such a relationship might follow from a dependence of the net rate of metal removal from the surface ocean on both the metabolic requirements and the number of transport sites in phytoplankton. Clearly, the generality of the proposed correlation needs to be further investigated. It would also appear to us that greater consideration of the influence of metal complexion kinetics in future studies of the biogeochemistry of trace metals in the surface ocean is in order.

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