

# Acquisition and Utilization of Transition Metal Ions by Marine Organisms

Alison Butler

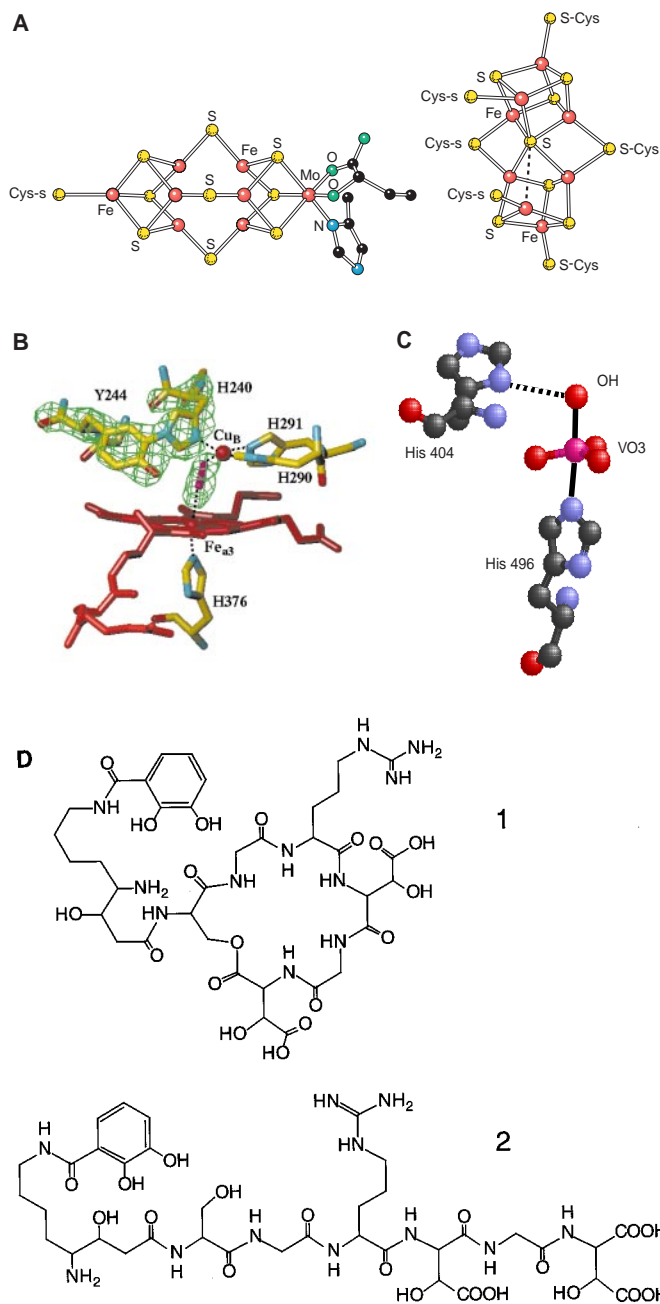
## REVIEW

Recent research has revealed that trace metals, particularly transition metals, play important roles in marine productivity. Most of the work has been on iron, which shows a nutrient-depleted profile in the upper ocean. Marine organisms have a variety of means for acquiring iron and other transition metal ions that differ from those of terrestrial organisms.

Metalloproteins comprise a third to a half of all known proteins. Metals function in catalysis, play structural roles, and activate biochemical processes. Many essential life processes, including photosynthesis, respiration, and nitrogen fixation, involve multi-electron transformations. The essential steps in all of these processes are catalyzed by metalloenzymes (Fig. 1, A and B). These enzymes contain iron and other transition metal ions that can exist in multiple oxidation states. Other essential life processes, such as proteolysis and the equilibration of carbon dioxide and bicarbonate ion are hydrolytic transformations that are also catalyzed by metalloenzymes. Usually these metalloenzymes contain active-site transition metal ions that do not undergo oxidation state changes [for example, Zn(II)] but which function as Lewis acid–type catalysts. The essential transition metal ions for terrestrial organisms include vanadium to zinc of the first-row transition metal series and molybdenum in the second-row series. Iron is the most abundant transition metal ion in most terrestrial organisms. Iron levels are also high in most lakes, estuaries, streams, and rivers, whereas levels of other transition metals vary widely. In contrast, the transition metal composition of the open ocean differs dramatically from that of terrestrial environments (Fig. 2) (1, 2). Recent advances in sampling and analytical techniques have permitted the metal ion composition and speciation (oxidation state and degree and type of ligation) of the ocean to be defined (1). Molybdenum is the most abundant transition metal in surface seawater at 100 nM, followed by vanadium at 20 to 35 nM (3). By contrast iron levels in surface seawater are extremely low, 0.02 to 1 nM (4–7). These metals show a nutrient-like distribution profile in that the elements are depleted in surface waters, where most primary production occurs. Despite its relative scarcity, iron is essential to marine organisms, and iron levels represent one of the key limitations in marine ecosystems. In this article, I cover some of the mechanisms by which marine organisms acquire iron and use other essential metal ions, compared to their better understood terrestrial counterparts.

We know relatively little about marine bioinorganic chemistry, but recent studies are beginning to unravel some of the mysteries. Marine microorganisms acquire iron through novel siderophores (8, 9) and new siderophore-mediated and other processes (10, 11). Phytoplankton substitute flavodoxin for ferredoxin in times of severe iron stress, replacing the iron-sulfur cluster with an organic cofactor (12, 13). The carbonic anhydrases (CA) of diatoms, which have no homology to other known CA, are particularly apt to substitute Co for Zn in the active site and some even contain Cd (14). Vanadium enzymes catalyze peroxidative halogenation reactions, which in most terrestrial organisms is accomplished by Fe(III)-heme enzymes (15), and tung-

sten is required for catalysis of oxidoreductases in hyperthermophilic archaea (16). Given the unique metal ion composition and speciation of ocean waters, it seems likely that many other bioinorganic processes that are specific to oceanic organisms remain to be discovered.



**Fig. 1.** Metal sites of (A) nitrogenase (*Azotobacter vinlandii*) [left: Cofactor (46); right: P-cluster (47); cluster drawings adapted from (49)]; (B) bovine heart cytochrome c oxidase (48); (C) vanadium chloroperoxidase (*Curvularia inaequalis*) (33); and (D) alterobactins A (1) and B (2) (8).

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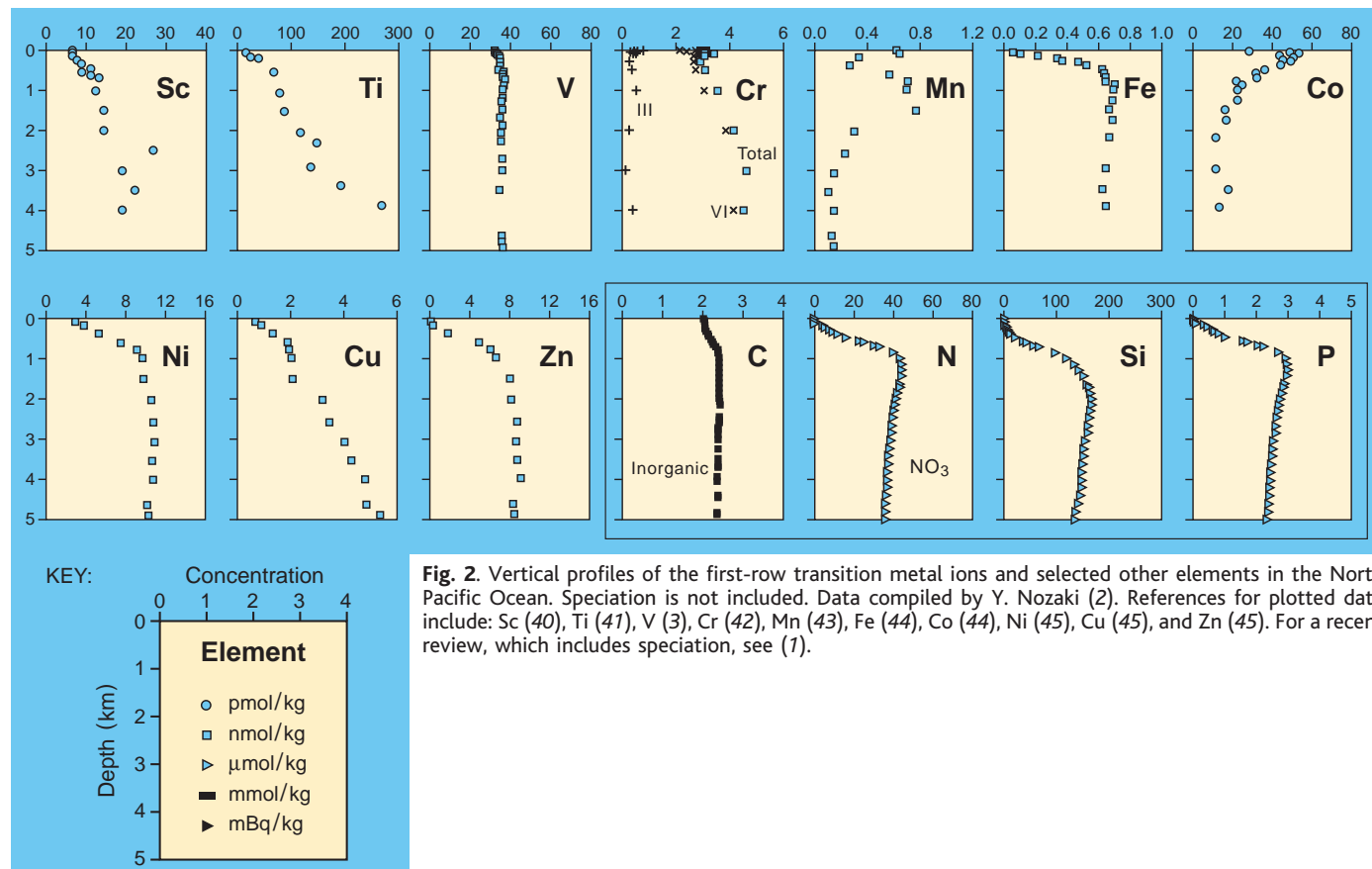
Iron is arguably the most prevalent metal ion among the different classes of terrestrial metalloproteins (Fig. 1, A and B). Its importance is magnified by its insolubility under the neutral pH conditions of biological systems. At pH 7, only  $10^{-18}$  M aqueous  $\text{Fe}^{3+}$  is present in solution; yet, bacteria typically require micromolar levels of total iron for growth (17). Microorganisms have evolved an elaborate mechanism to acquire iron, which is precisely regulated and exquisitely selective (18). Under aerobic conditions, bacteria produce siderophores to solubilize and sequester iron (III). Siderophores are low molecular weight compounds that coordinate Fe(III) with a high affinity (18). They are produced under conditions of iron demand in conjunction with their outer membrane receptor proteins. Under conditions of excess available Fe(III), the biosyntheses of siderophores and outer membrane receptor proteins are repressed.

Iron is also arguably the most important transition metal ion in the ocean, precisely because of its relatively low abundance. Surprisingly, a consensus on the solubility of ferric species in the ocean has not been reached, although the speciation of Fe(III) in surface waters is beginning to be understood (10). Most (>99%) ferric ion is complexed by organic ligands, having conditional stability constants in seawater on the order of  $10^{19}$  to  $10^{22} \text{ M}^{-1}$  (19–21). A major challenge will be to elucidate the nature of these ligands or ligand classes. The low levels of iron have been shown to limit primary production of phytoplankton in vast regions of the world's oceans, including the subarctic Pacific, the equatorial Pacific, and the Southern Ocean (22, 23). These ocean waters are characterized by high nitrate and low chlorophyll (HNLC) levels; they are replete in major nutrients but depressed in rates of primary production, and most importantly, are deficient in iron (approximately 20 to 50 pM). Two separate iron addition experiments carried out by the IronEx I and II studies demonstrated that primary production increased when iron was added at a level of 1 to 2 nM. A surprising result of the IronEx II study was

that the concentration of the organic ligand (that is, 0.5 nM at ambient levels) increased dramatically within a day or two of the mesoscale injection of iron (to 2 nM) (24). This rapid increase raises the question whether these Fe(III) chelating ligands are siderophores or derived from siderophores. Little is known about siderophore production by eukaryotic phytoplankton, although it has been reported (25). Siderophore production by bacteria, phytoplankton or other marine microorganisms, could be an adaptive response of these ambient iron-deficient populations (24).

In addition to phytoplankton, heterotrophic marine bacteria have been shown to be limited by the low levels of iron in the ocean (26, 27). Heterotrophic bacteria compete successfully with phytoplankton for iron. Thus it is of tremendous interest to elucidate the molecular mechanisms that are used by oceanic bacteria and phytoplankton to acquire iron. Many oceanic bacteria have been shown to produce siderophores, although few structures have yet been determined (28–30). We have identified aerobactin, a known terrestrial siderophore from an oceanic *Vibrio* species (30). We have also isolated and determined the structures of alterobactins A (1) (Fig. 1) and B (2) from *Alteromonas luteoviolacea*, found in oligotrophic and coastal seawater; alterobactin A has a very high thermodynamic stability constant for ferric ion (8). The conditional stability constant of the Fe(III) alterobactin A complex at pH 8 is at least equal to or greater than the ferric complex of the oceanic ligands. Thus alterobactin A can clearly function well in the ocean. While we are beginning to elucidate the siderophore-mediated uptake of some oceanic bacteria, even less is known about the molecular mechanisms phytoplankton use to sequester iron. Phytoplankton may use cell-surface reductases to obtain iron from chelated complexes, including possibly marine siderophores from other microorganisms (10, 11).

Can parallels be drawn from the behavior of iron to other metal ions? On the one hand, iron is unique in the neutral pH conditions of biological



**Fig. 2.** Vertical profiles of the first-row transition metal ions and selected other elements in the North Pacific Ocean. Speciation is not included. Data compiled by Y. Nozaki (2). References for plotted data include: Sc (40), Ti (41), V (3), Cr (42), Mn (43), Fe (44), Co (44), Ni (45), Cu (45), and Zn (45). For a recent review, which includes speciation, see (1).

systems because of its poor solubility. The siderophore-mediated system for Fe(III) uptake used by microorganisms is essential because it solubilizes ferric hydroxide, which would otherwise be unavailable (50). But a second advantage of the siderophore system is specificity. If no other organism can recognize a particular microorganism's ferric siderophore, then the iron is reserved for that species, resulting in productive chemical warfare. On the other hand, because of the much greater solubility of other first-row transition metal ions, which are largely present in the divalent oxidation state, specific metal ligands are not required to increase the bioavailability of these metals. The consensus in terrestrial systems is that microorganisms can acquire these metal ions through other uptake pathways.

In seawater, many of the first-row transition metal ions are partially or fully complexed by, as yet, undefined organic ligands (1). The initial evidence points to metal-specific organic ligands, distinct from the organic ligand or ligand classes complexing Fe(III) in the ocean (31). Thus marine microorganisms living in surface seawater may have evolved specific uptake systems for metals other than iron, as an effective competition strategy to enhance acquisition of these dilute metal ions. But it is also possible that the organic complexing ligands may mitigate the toxicity of the uncomplexed, aquatic species (for example, Cu). At this point we know little about the nature of the ligands, other than the conditional stability constant of the metal-ligand complexes.

In contrast to the deficit of iron in surface seawater, vanadium is abundant, and the bioinorganic chemistry of vanadium is diverse. Most marine tunicates acquire vanadium in large quantities, but the functional significance of the sequestered vanadium has eluded investigators since its discovery in seawater near the turn of the century (32). Vanadate is also important in halide metabolism in marine algae. Vanadium haloperoxidases, found in virtually all classes of marine algae, catalyze halogenation reactions and are thought to be responsible for the vast array of halogenated marine natural products (14). The x-ray structure of vanadium chloroperoxidase from *Curvularia inaequalis*, a fungus with ultimate marine origins which is similar to the marine vanadium bromoperoxidase, reveals a remarkably simple active site (Fig. 1C) (33). The protein complexes hydrogen vanadate ion,  $\text{HVO}_4^{2-}$ , with one histidine ligand. Yet vanadate ion itself, which is the second most abundant transition metal ion in seawater, does not catalyze peroxidative halogenation under ambient seawater conditions (for example, pH 8;  $\sim 1\text{--}10\ \mu\text{M}\ \text{H}_2\text{O}_2$ ;  $0.5\ \text{M}\ \text{Cl}^-$ ,  $\sim 1\text{--}10\ \text{mM}\ \text{Br}^-$ , and  $\sim 1\text{--}10\ \mu\text{M}\ \text{I}^-$ ). On a molecular level we are beginning to understand the mechanism of halide oxidation and the peroxidative halogenation process (14, 33). On a global level, algal blooms produce massive quantities of volatile chlorinated and brominated hydrocarbons (34), which may lead to depletion of the Arctic ozone layer (35). However, on a functional level, the significance of these halogenated compounds is not entirely understood. In contrast to marine organisms, relatively few halogenated compounds are known in terrestrial organisms which usually have Fe(III)-heme halogenating enzymes [for example, chloroperoxidase (*Caldariomyces fumago*), eosinophil peroxidase, lactoperoxidase, myeloperoxidase, thyroid peroxidase, and so forth].

Seeking consequences of low iron levels in much of the world's oceans clearly inspired the exploration of the biogeochemistry of this element on a global scale and also initiated investigations into its bioinorganic chemistry at a molecular level. Insofar as it controls oceanic primary production, Fe must have a direct effect on the global C cycle (39) and may have played a role in its variations over geologic times (38). Iron availability must also affect the N cycle because it is involved in the catalysis of all nitrogen redox transformations. In particular, the reduction and assimilation of nitrate by phytoplankton and the fixation of  $\text{N}_2$  by cyanobacteria (which involves nitrogenase, Fig. 1A) may both be limited by low Fe (36, 51). The low oceanic concentration of Zn (and substituted metals Co and Cd) may have a

direct effect on the C cycle by limiting the ability of primary producers to acquire and fix inorganic C (37). The mechanistic investigations of the marine vanadium haloperoxidase, the enzyme containing vanadium has influenced global considerations of the natural halogen cycle. Many details of the underlying bioinorganic or bioorganic (that is, not involving metal ions) processes remain unknown. Most of what we know about global element cycles comes from studies of terrestrial organisms, where metalloenzymes are involved in most steps of these cycles. As the examples of iron and vanadium discussed above have shown, the bioinorganic mechanisms in marine organisms often differ from the terrestrial ones, and this influences global element cycles in new ways. Detailed studies into the bioinorganic chemistry of other transition metal ions, together with increased knowledge of the concentration and speciation of these ions in the oceans, are required to obtain a complete picture of global element cycles comprising both terrestrial and oceanic components.

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52. I thank K. W. Bruland, M. G. Haygood, G. W. Luther, III, and F. M. M. Morel for valuable discussions and M. T. Simpson for assistance with graphics. Supported by grants from the National Science Foundation (CHE 9529374), the National Institutes of Health (GM38130) and the AP Sloan Foundation. Partial support for my work on marine haloperoxidases and siderophores is also sponsored by NOAA, U.S. Department of Commerce under grant number NA66RGO447, project number R/MP-76 through the California Sea Grant College System and in part by the California State Resources Agency.

# Climate-Ocean Variability and Ecosystem Response in the Northeast Pacific

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## REVIEW

The role of climatic variation in regulating marine populations and communities is not well understood. To improve our knowledge, the sign, amplitude, and frequency of climatic and biotic variations should be compared as a necessary first step. It is shown that there have been large interannual and interdecadal sea-surface temperature changes off the West Coast of North America during the past 80 years. Interannual anomalies appear and disappear rather suddenly and synchronously along the entire coastline. The frequency of warm events has increased since 1977. Although extensive, serial, biological observations are often incomplete, it is clear that climate-ocean variations have disturbed and changed our coastal ecosystems.

The biological consequences of climatic variability of the atmosphere and ocean are largely unknown. This is probably because of the mismatch between the scales of important atmospheric and oceanographic processes and the spatial and temporal dimensions of biological research programs (1). However, there is a widespread consensus that marine populations respond to climatic events and that major changes have taken place in the past 20 years in the marine ecosystems of the Pacific (2). Much of the biological, observational evidence is disconnected spatially and often discontinuous temporally, but because the potential consequences of large-scale ecosystem disturbance and disruption are uncertain and possibly detrimental, we must accept less than ideal data in our attempt to understand what is happening. Atmospheric and certain hydrographic properties are much better sampled, especially sea-level pressure (SLP) and sea-surface temperature (SST). By using these two measures, we are learning that the relation between large-scale, low-frequency climatic variability (3) and that of ecosystem and population biology is close.

Temperature variations not only affect an organism's metabolic rates directly but also influence other equally important variables such as sea level and therefore exposure of intertidal organisms, local currents and the movement of planktonic larvae, erosional regimes and therefore substrate structure, photosynthetic light intensity (cloudiness), and water-column stratification and nutrient cycling and therefore production. These environmental variables affect population and community dynamics strongly and, over time, community structure and function. The use of departures of temperature from long-term daily or monthly means (nonseasonal anomalies) can indicate physical

perturbations of the kind that act as ecosystem disturbances.

Disturbance theory is well developed and is central to our understanding of the maintenance of community structure and patterns of diversity (4). But what types of hydrographic perturbations disturb marine ecosystems and what types do not? For example, does a single large, brief warm (cold) event have greater effects than, say, a decadal trend? We cannot answer this question because many of our concepts of the biological consequences of physical perturbations are based on brief, process-oriented studies. Large, low-frequency changes are simply not well detected by short, small-scale studies (1, 5).

There are long-term daily (since 1916) coastal SST time-series measurements over much of the Pacific coast of North America (6) and a shorter (since 1947) oceanic SST series and good, but interrupted, biological measurements of zooplankton, fish catch, and kelp forest communities for two to six decades. Departures from nonseasonal, long-term average SSTs (anomalies) have varied considerably between years and over decades (7). Thus, ecosystem disturbances as indexed by SST changes can be inferred back some 80 years, and their relation to basin-wide patterns of SST and SLP can be studied. Some of these low-frequency coastal temperature anomalies are connected to warm El Niños and cool La Niñas. Particularly strong tropical events during 1957 to 1958 and 1982 to 1983 had noticeable effects on Pacific coast marine populations (8–10). Here, we used anomalies from long-term coastal SSTs to describe environmental perturbations and what is known of the biological consequences. Such knowledge will be necessary for the further development of conceptual models of marine ecosystem dynamics and of fisheries management.

## Physical Changes

*Interannual scales.* Daily SST has been measured for decades at 17 stations along the Pacific coast (6) (Fig. 1). SSTs episodically varied from monthly, nonseasonal means over large areas by up to 3°C (Fig. 2). Large-scale heating and cooling occurred rapidly and apparently synchronously in many instances along the entire 1130 km of coastline. Many of the warm episodes lasted only a couple of months, sometimes less. Remarkable warm events associated with the 1957 to 1958 and 1982 to 1983 tropical El Niños stand out. In both of these cases, the warming off California persisted long after the tropical signal had died out.

Conventional wisdom and a well-established theory of coastally trapped Kelvin waves (11) would lead us to expect that warming episodes should propagate poleward along the West Coast. This signal should be especially pronounced during large equatorial El Niños. Sea-level changes do apparently progress from south to north (12), but there is no consistent evidence in the data (Fig. 2) of south to north movement of warm anomalies. Because monthly averages of SST anomalies might not resolve a south-to-north signal if Kelvin waves pass along the coast in less than 1 month, we plotted daily anomalies from long-term daily means for each station, for segments of our record during which there was a large-scale warm period (the tropical

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