

Boron and Marine Life: A New Look at an Enigmatic Bioelement

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Abstract On the occasion of the 200th anniversary of the discovery of boron, we review the oceanic biogeochemistry of boron as well as suitable analytical techniques for its determination. This overview includes aspects of biogeochemistry including geochemical stable isotope variations, uptake, transport, storage, nutritional value, toxicity, and distribution within biological materials, providing a framework for discussion of the role of boron in marine organisms, which remains largely enigmatic.

Keywords AI-2 · Algae · Boromycin · Boron · Nutrient · Biogeochemical cycles · Paleo-pH · Stable isotopes · Toxicity · Vibrioferri · Transport

Introduction

Boron was first isolated by Joseph Louis Gay-Lussac, Louis Jacques Thénard, and Humphry Davy in 1808 (Davy 1808; Gay-Lussac and Thenard 1808, respectively). On the

200th anniversary of this event, we would like to reflect on its role in the ocean and in marine life in particular.

While there has been extensive interest in the use of boron as a surrogate of pH in paleoclimate studies in the context of climate change-related questions (vide infra), the high (0.4 mM) concentration in seawater and the depth-independent (non-nutrient-like, Fig. 1) concentration profile of this element have led to boron being neglected as a potentially biologically relevant element in the ocean (Uppstrom 1974). However, recent advances in genomics, trace element detection, biochemistry, and natural products discovery have renewed interest in this previously largely neglected element.

Since the 1920s, boron has been known to be an essential micronutrient for all higher terrestrial plants and many other organisms, but also to be toxic at higher environmental concentrations (Warington 1923; Goldbach et al. 2001 for review). Thus, the beneficial range of boron appears rather narrow. A large volume of literature has focused on the element from the perspective of terrestrial agriculture (reviewed by Tanaka and Fujiwara 2008) with established examples of both boron deprivation (Warington 1923) and boron toxicity (Yau et al. 1995). In consequence, both boron-tolerant and boron-efficient cultivars have been developed. In contrast to the generally boron-poor terrestrial environment, the relatively high concentration of boron in the marine environment suggests that boron deficiency is not likely an issue for marine primary productivity despite the fact that it is also an essential element for marine algae. However, the potential toxicity of boron coupled with its high concentration in the ocean, suggest the need for some sort of homeostatic control mechanisms in marine organisms. The control of boron concentrations is a critical issue in the context of seawater desalination (Öztürk et al. 2008). The remarkable observation that a number of marine natural

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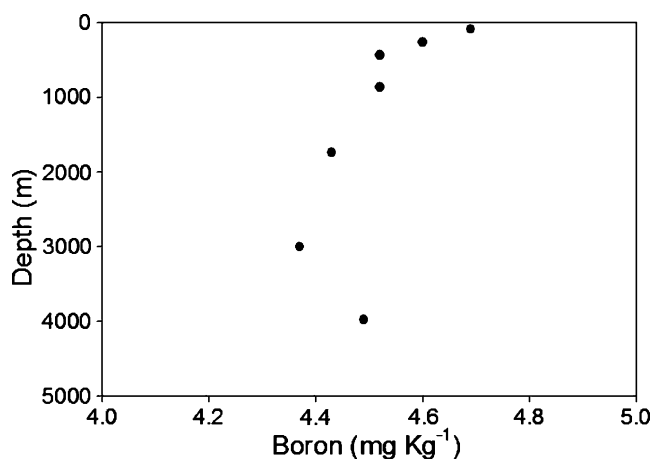
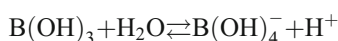


Fig. 1 Representative depth profile of total dissolved boron concentrations at 18° 22' N, 165° 54' W (Uppstrom 1974)

products contain boron suggests that organisms have not only adapted to the presence of high boron concentrations in their environment but have made use of its abundance for a variety of biological uses.

General Introduction to the Chemistry of Boron

Boron typically exists as trigonal planar BX_3 species given its $2s^2 2p^1$ electronic configuration. However, this electron-deficient element often reacts with electron pair donors via its empty p_z orbital to produce formally dative bonded tetrahedral “adducts”. Thus, trigonal planar boric acid is the primary soluble form of boron in natural waters. As a very weak acid, with a pK_a of 9.15, boric acid and the sodium borates exist predominantly as undissociated boric acid $[B(OH)_3]$ in dilute aqueous solution below pH 7. Above pH 10, the tetrahedral metaborate anion $B(OH)_4^-$ becomes the main species in solution. Consequently, since at least the 1940s, $B(OH)_3$ and $B(OH)_4^-$ have been recognized as the primary boron species in seawater (Sverdrup et al. 1942). However, boric acid is not formally a proton donor, but rather reacts with a hydroxyl ion from water to form the $B(OH)_4^-$ adduct and releases a proton in the process via the reaction:

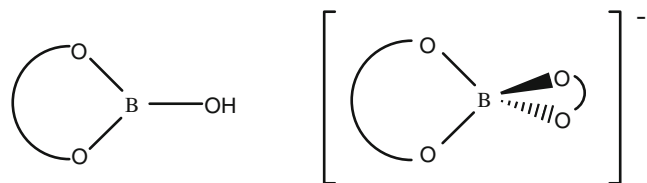


At high boron concentrations (>0.025 M) between pH 6 and 11, highly water soluble polyborate ions such as $B_3O_3(OH)_4^-$, $B_4O_5(OH)_4^{2-}$, and $B_5O_6(OH)_4^-$ form, but such concentrations are almost never observed in the environment and hence these species are of little importance biologically.

In seawater, inorganic boron content generally bears a linear relationship to chloride content. A ratio of 0.00024 g boron/g of total halogen expressed as chloride ion has been

calculated (UNEP 1998) with dissolved species comprised largely of partially dissociated boric acid along with weakly associated ion-pairs of neutral and positively charged borate complexes of sodium, magnesium, and calcium. Noakes and Hood (1961) concluded that organic-bound boron contributes very little to the total boron content of seawater, but that boron associated with organic matter covaried with dissolved oxygen content, with the lowest concentrations found in oxygen minimum zones.

Although the reaction chemistry of boron can be complex with a myriad of theoretically and technologically important known boron hydrides and carboranes, the only biologically relevant reactions of likely importance in the mildly basic aqueous milieu of seawater are those with nitrogen and oxygen donors. The reaction of boric acid/borate with hydroxyl donors such as alcohols, phenols, diols, polyols, or polysaccharides to produce alkoxides or “boroesters” constitute the most important of these reactions. Indeed, the essentiality of boron for plants likely derives from the reaction of borate with carbohydrates that promotes the cross-linking and stabilization of the plant cell wall (see below). Both mono- and diesters are possible and these are most stable when derived from “chelating” dihydroxy species. The monoesters maintain a trigonal planar configuration with no net charge, while the more important diesters form spirocyclic compounds that are anionic and tetrahedral in geometry.



Since water is a product of these “esterifications,” decreasing the activity of the solution (i.e. reaction in a less polar environment) will strongly drive the reaction toward the product. However, even in aqueous solutions, the equilibrium constants of such “esterifications” are often sufficiently large to allow significant product formation (Hunt 2001). While 1,3 diols with six-membered chelate ring structures should be preferred on steric grounds to the more ring-strained 1,2 diols with five-membered chelate ring structures, the latter is favored energetically and is the mode most frequently observed (Rietjens and Steenbergen 2005). Also important is the relative pK_a of the reacting alcohol or phenolic species. Typically, the more acidic the alcohol, the lower the pH that significant complexation occurs. At higher pH, competition with hydroxide ion to form borate becomes important.

Considerable controversy has arisen as to whether the reactive boron species in these reactions is boric acid or

borate. Initially, since such reactions with alcohols occur primarily at alkaline pH, the reactive boron species was assumed to be borate, which predominates at high pH (Fig. 3a). However, recent work has shown unequivocally that this view is incorrect and that the reactive species even in alkaline pH is in fact boric acid (Bishop et al. 2004; Rietjens and Steenbergen 2005).

Analytical Methods

Boron is a surprisingly difficult element to measure at the trace concentrations (i.e., <100 µg/L or <9.2 µM) expected to be important in biological studies. Problems include: (1) a lack of certified reference materials for inter-laboratory comparisons and validation; (2) sample collection, storage, and preparation issues; and (3) analysis concerns including severe “memory effects” and potential losses or contamination due to volatilization and surface adsorption (Downing et al. 1998). Obviously, the presence of boron in common borosilicate glasses precludes their use during sample collection and preparation, but more insidious is the presence of glass components in analytical instrumentation. The preparation of boron-free water for analytical purposes also requires special attention (Kano and Darbouret 1999). If concentrations of interest are relatively high and more sophisticated instrumentation is unavailable, a number of optical and or fluorescence methods are available for the determination of boron (UNEP 1998). However, the analytical methods of choice are inductively coupled plasma atomic emission spectroscopy (ICP-AES) and inductively coupled plasma mass spectrometry (ICP-MS), which are relatively fast, require small sample volumes, are subject to minimal spectral or mass interferences, and applicable to a wide variety of sample types. ICP-AES has detection limits on the order of 0.5 mg/L (0.05 mM), whereas ICP-MS has detection limits on the order of 1 µg/L

(0.09 µM) and the added advantage of determining the stable isotope ratios of $^{10}\text{B}/^{11}\text{B}$ (Lécuyer et al. 2002). Stable isotope ratios can also be measured by ion microprobe or thermoionization mass spectrometry (TIMS, Spivack and Edmond 1986; Chaussidon and Albarède 1992). Such stable isotope ratios can be particularly valuable for biological tracer, biogeochemical cycling, and paleoclimate studies (see below) given the absence of radioactive boron isotopes and ready availability of various isotopically pure ^{10}B -bearing compounds.

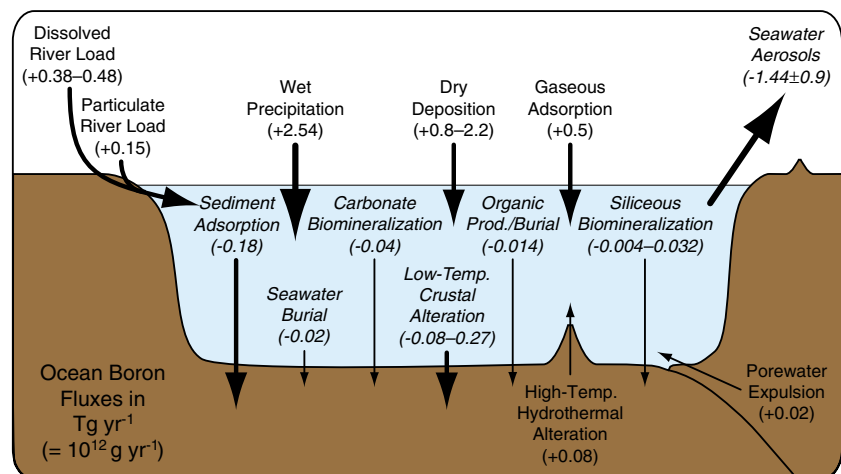
Given sufficient materials and boron concentrations, ^{11}B boron nuclear magnetic resonance (NMR) is a method that provides structural information as well as quantitative determination. This technique has been used extensively in laboratory studies to provide unparalleled information (Chuda et al. 1997); unfortunately, natural materials frequently do not contain sufficiently high concentrations.

Marine and Geochemistry of Boron

Biogeochemical Cycle from an Ocean Perspective

Like most elements, boron is cycled through the atmosphere, hydrosphere, lithosphere, and biosphere by a variety of processes. Park and Schlesinger (2002) provide a quantitative literature review of this global biogeochemical cycle; here we focus on fluxes into and out of the ocean (Fig. 2), the largest surficial boron reservoir at $\sim 6.2 \times 10^6$ Tg (Harriss 1969). Oceanic boron fluxes are dominated by exchanges with the atmosphere; with outflux largely through seawater aerosol production and influx through direct precipitation, direct dry deposition, and gaseous absorption. The hydrosphere is the next largest flux to the ocean through riverine delivery of boron from natural (i.e., atmospherically delivered and weathering produced) and anthropogenic (e.g., soap, fertilizer, fire retardant) sources,

Fig. 2 Boron influxes and outfluxes for the global ocean in Tg year⁻¹ (based on estimates from Park and Schlesinger 2002)



with the latter having approximately doubled from prehistoric times through active mining and widespread societal applications.

Though small compared to the above, fluxes among the lithosphere and ocean are arguably the most complex: lithosphere-to-ocean fluxes include hydrothermal venting at divergent plate boundaries (i.e., oceanic ridges where new crust is produced) and sedimentary porewater expulsion at convergent plate boundaries (i.e., oceanic trenches where crust and overlying sediments are subducted). Ocean-to-lithosphere fluxes include adsorption onto sediments (particularly clay minerals), burial of seawater (i.e., porewater), and seawater-driven alteration of oceanic crust at low temperatures (i.e., away from divergent plate boundaries). Finally, the biosphere transfers dissolved boron into organic matter, which is largely oxidized in the water column with little burial, as well as calcitic and siliceous skeletons that contribute to marine sediments.

Based on the above processes, the estimated boron influx to the global ocean is 4.47–5.97 Tg year⁻¹ and the boron outflux from the global ocean as 0.86–2.88 Tg year⁻¹. Park and Schlesinger (2002) attribute this major imbalance to a relatively poorly constrained atmospheric budget (cf. recent work by Rose-Koga et al. 2006; Xiao et al. 2007); exclusion of this atmospheric component produces a relatively balanced boron budget for the ocean (i.e., influx of 0.63–0.73 versus outflux of 0.32–0.87). In addition to elemental fluxes, boron stable isotopic variations (i.e., $\delta^{11}\text{B}$; see next section) within and among these reservoirs provide additional constraints on the ocean boron budget, though these largely ignore the atmosphere given their geoscience focus (e.g. Seyfried et al. 1984; Spivack et al. 1987; You et al. 1993; Wunder et al. 2005).

Biom mineralization and Boron Isotopes as a Paleo-pH Proxy

Modern seawater averages ~39.5‰ in its $\delta^{11}\text{B}$ as expressed in standard delta notation measured relative to NBS SRM-957 (IUPAC 1998). Ambient pH controls the speciation of this dissolved boron among largely $\text{B}(\text{OH})_3$ and $\text{B}(\text{OH})_4^-$ (Fig. 2a; Dickson 1990; DOE 1994) as well as the $\delta^{11}\text{B}$ of these species through coordination-based differences in vibrational energy, with trigonal $\text{B}(\text{OH})_3$ enriched in ^{11}B relative to tetrahedral $\text{B}(\text{OH})_4^-$ (Fig. 2b; Zeebe 2005). Theoretic estimates of thermodynamic equilibrium fractionation factors (α) for this $\text{B}(\text{OH})_3$ – $\text{B}(\text{OH})_4^-$ system range between 1.0176 to 1.030 at 300 K (i.e., enrichment factors (ϵ) of 18–30‰ for $\text{B}(\text{OH})_4^-$ at 25°C for typical seawater; Fig. 3b, gray curves), with some recent theoretical estimates of 1.026–1.030 (Oi et al. 2000; Liu and Tossel 2005; Zeebe 2005) that are comparable to recent abiotic experimental values of 1.0285 and 1.0272 (Byrne et al. 2006; Klochko et al. 2006, respectively).

Based on these boron–pH relationships, paleoceanographers have aggressively sought to understand the thermodynamics and kinetics of boron elemental and isotopic systematics in order to infer past ocean pH from the $\delta^{11}\text{B}$ of lattice-bound $\text{B}(\text{OH})_4^-$ in the carbonate skeletons of marine organisms (e.g. Hemming and Hanson 1992; Spivack et al. 1993; Sanyal et al. 1995; Pearson and Palmer 2000; Hönisch and Hemming 2005; Hemming and Hönisch 2007; Zeebe et al. 2009). The combination of such a “paleo-pH” proxy with another concentration-based parameter of the marine carbonate system, such as $[\text{CO}_3^{2-}]$, ΣCO_2 , or total alkalinity, provides critical constraints on the history of carbonate equilibria, which in turn modulates paleoatmo-

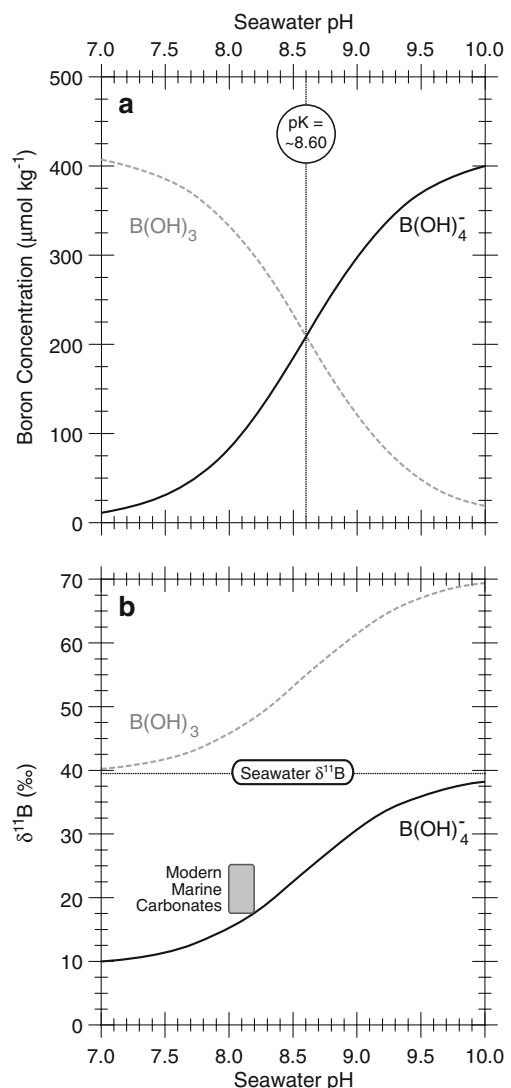


Fig. 3 Elemental and isotopic principles underlying the $\delta^{11}\text{B}$ paleo-pH proxy. **a** The concentration of dissolved boron species as a function of pH at $T=25^\circ\text{C}$, $S=35$, and total boron concentration = $416 \mu\text{mol kg}^{-1}$ ($\sim 405 \mu\text{M}$) (DOE 1994) with estimated pK value for boron (Dickson 1990). **b** Predicted $\delta^{11}\text{B}$ of $\text{B}(\text{OH})_3$ and $\text{B}(\text{OH})_4^-$ as a function of seawater pH assuming a fractionation factor of 1.030 (Zeebe et al. 2008)

spheric $p\text{CO}_2$, a major parameter in global climate history and dynamics. Given uncertainties in fractionation factors and potential effects of biomineralization, many researchers have pursued a naturalistic approach of culturing planktonic foraminifera and scleractinian corals over a range of pH values to produce species-specific $\delta^{11}\text{B}$ –pH calibrations (Fig. 4; various symbols and curves). These calibrations show offset but congruent relationships and are also more congruent with fractionation factors that are smaller (i.e., 1.018 and 1.020) than those predicted by most recent theoretical and experimental work.

Based on the above relationships, Zeebe et al. (2009) note that (1) regardless of the true fractionation factor for the seawater $\text{B}(\text{OH})_3$ – $\text{B}(\text{OH})_4$ system, $\text{B}(\text{OH})_4$ must experience additional isotopic fractionation during biomineralization, otherwise these species-specific data would produce a single curve that would be the $\delta^{11}\text{B}$ –pH relationship for $\text{B}(\text{OH})_4$ and (2) the $\delta^{11}\text{B}$ of $\text{B}(\text{OH})_4$ cannot be robustly deduced from the $\delta^{11}\text{B}$ of carbonates and vice versa. In addition, Zeebe (2005) stress that neither these uncertainties in fractionation factors nor these observed differences among species-specific calibrations preclude the parallel development and application of species-specific paleo-pH proxies.

An important question for robust application of this paleo-pH proxy is that the $\delta^{11}\text{B}$ of the oceanic reservoir is constant or independently constrained through time. First-order limitations are based largely on estimated oceanic boron residence times, which range widely from ~ 3 to ~ 20 million years (Spivack and Edmond 1987; Park and

Schlesinger 2002, respectively). Geochemical studies support relatively stable oceanic $\delta^{11}\text{B}$ for the last ~ 10 million years, but geochemical modeling suggests that seawater $\delta^{11}\text{B}$ has varied over a range of 6–10‰ over the past 60 to 120 million years, which could account for observed $\delta^{11}\text{B}$ variations in many paleo-pH studies (Lemarchand et al. 2000; Simon et al. 2006). The need to constrain oceanic variation in $\delta^{11}\text{B}$ remains an important issue for application of the paleo-pH proxy in deeper time as independent geologic evidence supports significant secular variations in ocean pH (e.g. Royer et al. 2001).

Boron as a Nutrient of Marine Algae

While a number of studies report that boron is essential for the growth and development of marine algae, the specific role(s) of the element remain unclear. Boron was recognized as an essential nutrient for terrestrial plants since the 1920s (Warington 1923), whereas symptoms of boron toxicity have been reported for soil concentrations >1 ppm (e.g. Yau et al. 1995).

Pioneering studies in the 1940s and 1950s reported that boron is essential for different groups of algae and cyanobacteria. For example, in the marine red algae *Bangia* and *Porphyra*, boron at seawater concentrations is essential for sustained growth (Henkel 1952). The freshwater green alga *Chlorella* has a similar boron requirement for growth (McIlrath and Skok 1958), whereas boron deficiency in the cyanobacterium *Nostoc* leads to chlorosis (Eyster 1952). Consequently, boron has been recognized as an important constituent of algal culture media (Spector 1956). Lewin demonstrated the requirement of boron for the growth of marine pennate and centric diatoms (Lewin 1965; Lewin 1966a; Lewin 1966b; Lewin and Chen 1976), with cell division being much reduced at boron concentrations less than 0.5 mg L^{-1} ($\sim 0.05 \text{ mM}$; i.e. $\sim 10\%$ natural seawater concentration) and ceasing completely at lower concentrations (Lewin 1966a). Under boron-deficient conditions, the content of phenolic compounds in the diatom *Cylindrotheca fusiformis* is more than doubled, while the majority of other major cell constituents remain unchanged (Lewin and Chen 1976). Shortly after these diatom-based studies, the essential role of boron for the development of *Fucus edentatus* Pyl. (Phaeophyceae) was also recognized (McLachlan 1977): omission of boron from the culture medium resulted in moribund embryos, but normal development could be restored by adding 1–4 μM boron. Gametogenesis in brown algal gametophytes (of the kelps *Laminaria japonica* and *Laminaria angustata* and of the sulfuric acid-producing *Desmarestia ligulata*) is strongly affected by the availability of boron and iron which show partially antagonistic effects, with high boron concentra-

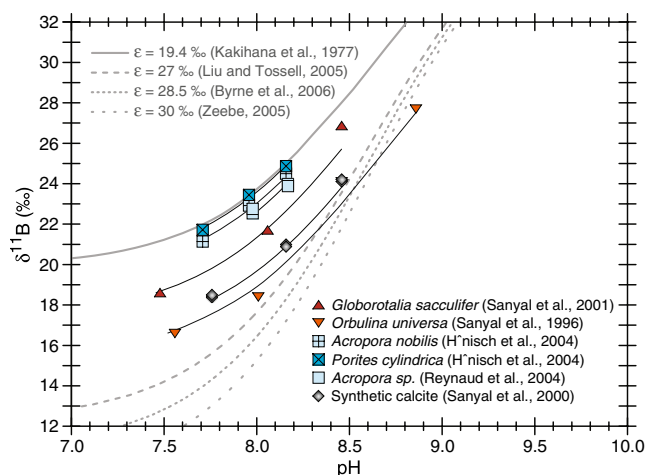


Fig. 4 Comparison of theoretical and empirical relationships between pH and $\delta^{11}\text{B}$. Gray curves represent various theoretical fractionation factors (α) that produce various enrichment factors (ϵ). Symbols and associated black curves represent species-specific culturing data for calcitic planktonic foraminifera (i.e., *Globorotalia sacculifer*, *Orbulina universa*), aragonitic scleractinian corals (i.e., *Acropora*, *Porites*), and synthetic calcite. Error bars (± 1 s) omitted for clarity; pH uncertainty typically ± 0.1 U and temperature uncertainty typically better than $\pm 0.75^\circ\text{C}$ (modified from Hemming and Hönisch 2007 and Zeebe et al. 2008)

tions acting inhibitory which can be alleviated by Fe^{3+} supplements (Motomura and Sakai 1984). The partially antagonistic interplay between iron and boron is particularly striking in light of the more recent findings that Fe^{3+} and borate compete for binding to microbial siderophores (Amin et al. 2007; Harris et al. 2007), even though no functional link between the two effects has been established yet.

Little is known about the boron content of marine algae and more research is definitely needed regarding this aspect. Öy (as quoted by Black and Mitchell 1952) published some figures for minor elements in the brown algae *Ascophyllum nodosum*, *Laminaria* sp., *Fucus serratus* and *Fucus vesiculosus*, with boron concentrations typically in the range of 100 ppm. Since this concentration is higher than that of the seawater, this implies that there must be some sort of active uptake and homeostatic control mechanism in place in these organisms.

The role of boron in higher plants including boron uptake and molecular aspects of boron transporters has been reviewed recently (Tanaka and Fujiwara 2008), with a major function being the cross-linking of pectins for the maintenance of cell wall integrity (Kobayashi et al. 1996). In general, borate reacts most strongly with sugars that have cis-diols on a furanoid ring as a result of both steric and electronic forces. Therefore, compounds bearing cis-diols on a furanose ring form far stronger complexes with borate than those on a pyranoid ring such as the more common alpha-D-glucose. This affinity is reflected in the differing equilibrium constants ($\sim 10^3$ for glucose vs. $\sim 10^7$ for ribose) (Hunt 2001). In fact, only two natural sugars have the strongly boron-binding cis-diol functionality on a furanose ring, ribose and apiose. It was found that the essentiality of boron to higher plants stems from its ability to crosslink cell wall carbohydrates. Therefore, it is not surprising that the first isolated B-polysaccharide was identified as a complex of the peptic polysaccharide rhamnogalacturonan II (RG-II) (Matoh and Kobayashi 2001). RG-II is notable in that it has the richest known diversity of sugars and linkages. Monomeric RG-II has four side chains, two of which contain apiose. The apiosyl residues are engaged in B-binding and borate crosslinks between apiofuranosyl

residues of two 2-O-methyl-D-xylose sidechains resulting in a dimeric complex between two molecules of monomeric RG-II and one borate. The presence of boron crosslinks in marine plants was initially described for the sulfated polysaccharide of the green seaweed *Ulva lactuca* (Haug 1976) and has been confirmed for red, green, and brown macroalgae by ^{11}B NMR analysis (Chuda et al. 1997). Even though it is tempting to speculate that boron may play a key role for the cell wall integrity of marine algae, substantial further research clearly is needed for a better understanding of the biological significance of the boron requirement by marine algae.

Boron Transport and Regulation

A broad systematic understanding of boron uptake mechanisms is lacking for animals, though uptake is known to occur across mucous membranes of the gastrointestinal and respiratory systems. In contrast, higher plants are fairly well studied with regards to boron uptake (Tanaka and Fujiwara 2008). Boric acid is an uncharged and undissociated molecule over much of the physiological pH range and is therefore considered to be the species taken up (Raven 1980). Boron uptake was largely assumed to occur through passive diffusion, but recent studies over the last decade have shown that uptake occurs as an active process mediated by active BOR transporters or facilitated by nodulin-like intrinsic proteins (NIPs). BOR1, a member of the SLC4 anion-exchanger superfamily (Frommer and Von Wiren 2002) which also includes bicarbonate transporters, was identified as the first transport protein involved in active xylem loading in the higher plant model *Arabidopsis* (Takano et al. 2002). Shortly afterwards, the same group found a member of the major intrinsic proteins, namely NIP5;1, to be essential for boron uptake and plant development under boron limitation (Takano et al. 2006).

Homologs, clustering in the same clade with BOR1, the yeast protein YNL275w (BOR1p) and the BTR1 protein of humans, are found in the genomes of the diatoms *Thalassiosira pseudonana*, *Phaeodactylum tricorutum*, and the brown seaweed *Ectocarpus siliculosus* (Fig. 5). This

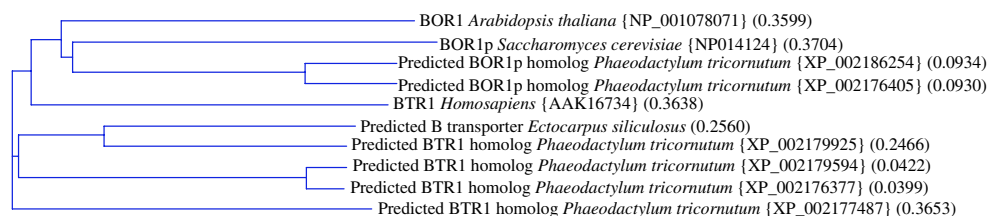


Fig. 5 Phylogenetic analysis of putative bicarbonate/borate transporters sequences. Tree generation was done using VectorNTI (Invitrogen) based on three known borate transporters (BOR1, BOR1p, and BTR1).

Nucleotide database accession numbers are provided in *brackets* and distances are in *parentheses*

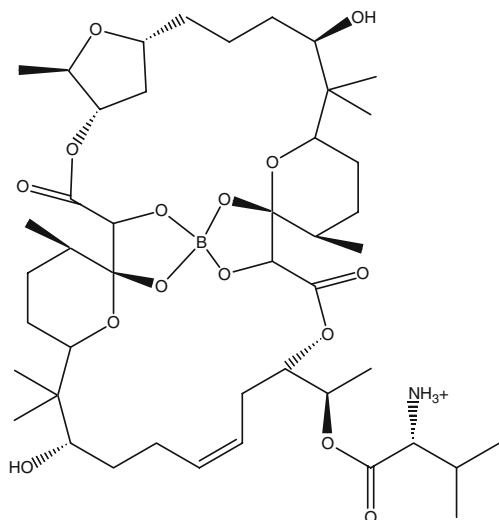


Fig. 6 The streptomycete-derived antibiotic boromycin

observation suggests that boron transport and regulation is widespread among marine plants.

However, because of the similarities between the purported borate transporters and their bicarbonate analogs, detailed biochemical studies will be needed to confirm the physiological function/substrates of these gene products.

Boron-containing Natural Products from Marine Prokaryotes

Considering the abundance of boron in the Earth's crust and ocean, it is surprising how few boron-containing natural products are known—however, it is similarly striking that

only one of them is of terrestrial origin with all others coming from marine microorganisms. All boron natural products known to date are prokaryotic metabolites. An obvious, intriguing question for further research is whether eukaryotes use boron in a similar manner in their metabolism and whether they produce any boron-containing natural products.

The first identified boron-containing natural product was boromycin (Fig. 6; Hütter et al. 1967; Dunitz et al. 1971; Marsh et al. 1974), which was isolated from a culture of *Streptomyces antibioticus* ETH 28 829 that originated from a terrestrial soil sample from the Ivory Coast. The second boron natural product, the macrodiolide antibiotic aplasmomycin, was found in the related, but marine *Streptomyces griseus* (Okami et al. 1976). The biosynthesis of this unusual compound has been elucidated (Chen et al. 1979).

The next high-profile natural product was the quorum sensing molecule autoinducer-2 (AI-2), which was first isolated from the bioluminescent marine bacterium *Vibrio harveyi* (Chen et al. 2002). In fact, its production by a range of other bacteria is remarkable and AI-2 has been postulated to function in inter-species communication (Miller and Bassler 2001). However, recently it was discovered that the active form of AI-2 found in terrestrial bacteria such as *Salmonella typhimurium* does not contain boron but rather it is simply the furanose sugar itself. This finding was confirmed by the crystal structures of the two molecules bound to their respective receptors, which shows unequivocally that boron is bound to the AI-2 of marine bacteria but not to the AI-2 of terrestrial bacteria (Miller et al. 2004, Fig. 7). This is a powerful example of how marine organisms have had to adapt to the high concentrations of boron in their environment or vice versa.

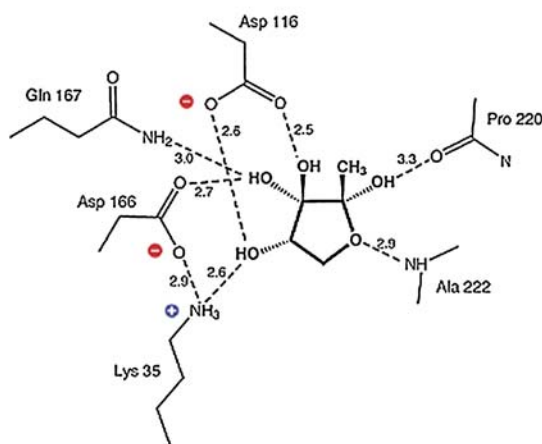
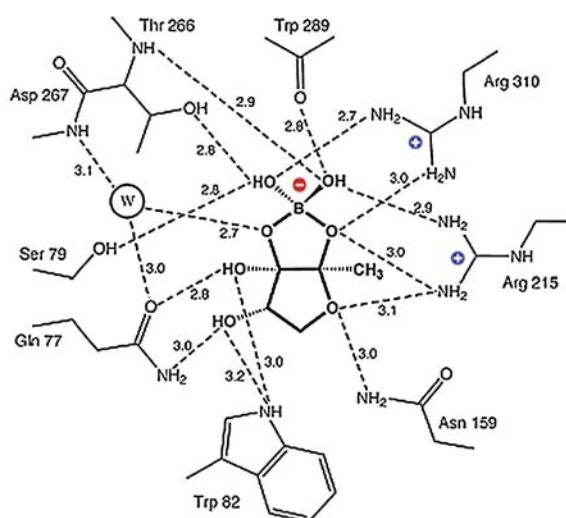


Fig. 7 A comparison of the binding of the AI-2 signal molecule, 2-methyl-2,3,3,4-tetrahydroxytetrahydrofuran (R-THMF) to the binding site in its receptor, LsrB, from *S. typhimurium* (left) with that in the



corresponding LuxP receptor from *V. harveyi* (right). Note the presence of boron only in the latter

Most recently, we became interested in boron-binding siderophores (Fe^{3+} chelators) of marine bacteria. The first such case was a surprise finding of a compound similar to the known siderophore vibrioferrin produced in parallel to this chelator by cultures of a *Marinobacter* sp. (strain DG 893) (Amin et al. 2007). Borate occurring either naturally in seawater or in the borosilicate glass of the used flasks competed with Fe^{3+} for binding to these ligands. A more systematic investigation subsequently revealed that a number of siderophores, such as rhizoferrin and petrobactin, share this hitherto unrecognized property with vibrioferrin, with the relevant functionality being the availability of a vicinal dianionic oxygen containing binding group (i.e., citrate or catecholate) in the siderophore backbone (Harris et al. 2007). In contrast, hydroxamate siderophores, such as aerobactin, which lack this functional group, do not bind boron (Harris et al. 2007). The biological significance of this feature remains at the moment unknown, but considering the abundance of boron in the ocean and the importance of siderophores to marine microbial trace element acquisition it is likely to be very relevant. One attractive hypothesis is that while siderophores are known to have as their primary function the binding and transport of iron from the environment into microbial cells, increasing evidence suggests that they may also play another significant role as signaling or "quorum sensing" molecules. The observed affinity of certain siderophores for borate allows for significant concentrations of B-siderophores to exist even at oceanic pH. These concentrations, although small, could well be sufficient for them to function as signaling molecules as with AI-2. Binding of the tetrahedral boron to these siderophores will also result in a conformation that would be different from either the free siderophore or its iron complex, allowing a distinction to be made between its iron uptake and any cell signaling roles. This area is ripe for further experimentation.

Conclusions and Outlook

Findings in several disciplines suggest that boron, an abundant element in the ocean, is involved in a range of aspects of marine life and it is considered an essential trace element. While all boron-containing and boron-binding low-molecular weight metabolites are of prokaryotic origin, boron also plays an important role in stabilizing plant and algal cell walls by cross-linking carbohydrates. The molecular biology of boron transporters in different branches of the tree of life is beginning to emerge, but the knowledge remains far from phylogenetically representative at the current time. Studies in biological oceanography investigating the correlation of boron with phytoplankton abundance (in particular, whether boron in seawater is drawn

down in algal blooms) would appear very timely. Also, the potential occurrence of low-molecular weight, boron-containing compounds in eukaryotes and much of the other remaining physiological roles of this element remain largely enigmatic at this stage and require significant, further research effort.

We would like to close by highlighting a number of biotechnology-related perspectives. The central role of boron in quorum sensing of marine bacteria opens perspectives for the control of bacterial biofilms both on man-made surfaces in the sea and in a wider context. The involvement of boron in cell wall stability has potential implications for the resistance of plants and algae to infection, for the technical extractability of cell contents and for the texture of edible seaweeds. In the latter context, it is unknown whether the boron content of seaweeds and seafood is beneficial or problematic from a nutritional standpoint and definitely requires further study.

Overall, one can conclude that the current state of knowledge has opened some intriguing perspectives for further fundamental and applied research about the role of this enigmatic bioelement.

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