

The Role of B Vitamins in Marine Biogeochemistry

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Abstract

The soluble B vitamins (B₁, B₇, and B₁₂) have long been recognized as playing a central metabolic role in marine phytoplankton and bacteria; however, the importance of these organic external metabolites in marine ecology has been largely disregarded, as most research has focused on inorganic nutrients and trace metals. Using recently available genomic data combined with culture-based surveys of vitamin auxotrophy (i.e., vitamin requirements), we show that this auxotrophy is widespread in the marine environment and occurs in both autotrophs and heterotrophs residing in oligotrophic and eutrophic environments. Our analysis shows that vitamins originate from the activities of some bacteria and algae and that taxonomic changes observed in marine phytoplankton communities could be the result of their specific vitamin requirements and/or vitamin availability. Dissolved vitamin concentration measurements show that large areas of the world ocean are devoid of B vitamins, suggesting that vitamin limitation could be important for the efficiency of carbon and nitrogen fixation in those regions.

We are now pretty sure that the plankton communities influence each other—that there are what we may call group-symbioses on the great scale so that the kind of plankton which we may expect to be present in a certain sea-area must depend, to some extent, on the kind of plankton that was previously present.

—Johnstone, Scott & Chadwick (1924)

INTRODUCTION

Both oceanic food webs and climate dynamics are influenced by phytoplankton activity, as photosynthetic carbon fixation affects atmospheric and oceanic CO₂ concentrations (Dugdale & Goering 1967, Falkowski et al. 1998). Therefore, in the past several decades, a large amount of research has focused on the role of iron and other mineral nutrients in regulating primary production in the ocean (Boyd et al. 2000). These efforts have led to major breakthroughs in our understanding of the fundamental roles of those inorganic microconstituents in the carbon, nitrogen, and sulfur cycles. However, laboratory experiments with phytoplankton have suggested that diversity and associated biological processes observed in the ocean might not be the result simply of the availability of a small set of trace elements; some biologically active organic compounds may also be essential exogenous growth factors for many phytoplankton species (Provasoli 1963).

Decades ago, Carlucci, Droop, Guillard, Provasoli, and Swift, among others, stated that phytoplankton dynamics in the ocean are strongly influenced by both the availability of and phytoplankton requirements for different B vitamins (Carlucci & Silbernagel 1969, Droop 1957a, Haines & Guillard 1974, Provasoli & Carlucci 1974, Swift 1980). Those initial studies concentrated on vitamins B₁ (thiamin), B₇ (biotin), and B₁₂ (cobalamin), mainly because laboratory studies showed that many marine algae require these vitamins (Provasoli & Carlucci 1974). Since then, the idea that B-vitamin auxotrophy (i.e., B-vitamin requirements) is common in many phytoplankton and bacterial taxa has become widely accepted (Croft et al. 2006, Giovannoni et al. 2005). Fittingly, recent picomolar B₁₂ amendments in both coastal and open-ocean environments have confirmed the ecological importance of organic metabolite availability in various marine systems and suggested that ambient levels of some vitamins, such as B₁₂, may be insufficient to support maximum productivity (Bertrand et al. 2007, Koch et al. 2011, Panzeca et al. 2006, Sañudo-Wilhelmy et al. 2006).

Here, we highlight some of the studies conducted on B vitamins in marine systems. Although recent results have indicated that vitamins other than B₁, B₇, and B₁₂ can be essential for some chemoheterotrophic marine microbes (Carini et al. 2013, Giovannoni et al. 2005), we focus on these three vitamins because they are required, alone or in combination, by most marine autotrophic phytoplankton and bacteria. We also discuss the biochemical importance of B vitamins, the complex interdependencies associated with external metabolites (ectocrine relationships) and vitamin auxotrophy in the marine microbial community, and the oceanography of B vitamins.

B VITAMINS AS BIOLOGICALLY ACTIVE COMPOUNDS IN THE OCEAN

Historically, major efforts have been dedicated to elucidating the mechanisms that trigger phytoplankton blooms and lead to temporal and spatial phytoplankton species successions in the ocean (Leblanc et al. 2009, Mahadevan et al. 2012). Although those studies have shown that a combination of ambient physical and chemical conditions could favor the predominance of certain algae at specific times and/or locations, they have also shown that, with the exception of iron requirements (Boyd et al. 2000), nutritional requirements are similar across all algae (Redfield 1958). Therefore, phytoplankton blooms and species successions in the ocean, which are usually dominated by

one or a few phytoplankton species, cannot be explained entirely by physical parameters and the availability of mineral macro- and micronutrients. For example, changes in environmental factors cannot easily explain a temporal succession of phytoplankton species of the same genera (e.g., diatoms), but differing vitamin requirements of the species can (Guillard 1968).

These types of observations led several researchers in the 1950s, 1960s, and early 1970s to hypothesize that much of the dynamics observed in phytoplankton successions could be the result of species-specific requirements for some growth factors, such as the varied requirements for essential B vitamins (Carlucci & Silbernagel 1969, Droop 1957b, Haines & Guillard 1974, Provasoli 1963, Provasoli & Carlucci 1974). Their experiments established that several marine phytoplankton species release or excrete extracellular B vitamins (Carlucci & Bowes 1970a) and that high concentrations of dissolved vitamins are associated with high phytoplankton biomass (Carlucci 1970). Mixed-culture experiments also demonstrated the nutritional relationship between vitamin producers and auxotrophs (Carlucci & Bowes 1970b). Therefore, as suggested by the statement of Johnstone et al. (1924) that serves as the epigraph of this article, through vitamin cycles (e.g., rates of production, excretion, and uptake), some organisms influence the growth of other organisms by producing and releasing necessary organic growth factors. Depletion and enrichment of various B vitamins in the ocean are thus governed by the requirements of the predominant phytoplankton species. For example, a bloom of a B₁₂ auxotroph would deplete waters of that vitamin, although it would also potentially enrich them with others (e.g., B₁ and B₇). Species-specific B-vitamin requirements would then influence the next algal bloom by determining which vitamins are available, favoring the species whose vitamin specificity matches that availability (Provasoli 1963).

This nonpredatory interrelationship among different organisms, mediated by ectocrines that favor or hinder the growth of other members of the community, was first postulated by Lucas (1947). As initially stated by Johnston (1955), “the production and persistence of metabolites in a body of sea water imposed a ‘biological history’ on the water and provided a basis for ecological ‘succession’.” However, an evolutionary understanding of the release of those external metabolites is still missing. Lewis (1986) hypothesized that the excretion of ectocrines is consistent with his allelochemical-signal hypothesis, in which the release of organic metabolites is significant only to the receptor organisms and is neutral to the producers. This hypothesis seems to be consistent with some laboratory and field observations showing that the release of organic compounds is related to the diel cell death–cell division cycles of picophytoplankton (Llabrés et al. 2011). However, even though it is well known that environmental stressors such as light intensity can influence phytoplankton cell cycles (Berges & Falkowski 1998), no study has evaluated how these stressors can affect the availability and excretion of vitamins. Similarly, it is unknown whether ambient vitamin concentrations are related to production by living phytoplankton or to the contribution of the damaged, nonliving cells that can comprise an important fraction of oceanic phytoplankton communities (Veldhuis et al. 2001). Additionally, the roles of viral infections (Wilhelm & Suttle 1999) and senescence (Mykkestad 2000) in vitamin production are currently unconstrained.

It is well established that phytoplankton and bacteria produce a great variety of extracellular substances of varied chemical structures (Ittekkot et al. 1981). It is also recognized that the availability of those substances often plays an important role in phytoplankton growth and physiology as well as in ecosystem dynamics—what could be called a group symbiosis on the large scale. However, despite those compelling arguments, evaluating the role of vitamins in marine systems has been difficult. Their low ambient dissolved concentration has limited vitamin measurements to a few locations in the world ocean (Carlucci 1970, Carlucci & Cuhel 1977, Menzel & Spaeth 1962, Panzeca et al. 2008, Sañudo-Wilhelmy et al. 2012). A full understanding of the biogeochemical

role of vitamins in the ocean requires detailed knowledge of the actual vitamin requirements of representative phytoplankton species as well as those of other bacterial microbes.

CHEMICAL STRUCTURE AND BIOCHEMISTRY OF B VITAMINS

B vitamins are small organic molecules other than proteins, fats, sugars, and mineral nutrients that are required in both primary and secondary metabolism in all domains of life. Funk (1912), who isolated vitamin B₁ (thiamin), coined the term “vitamine” because the isolated compound, wrongly thought to be an amine, is vital for life. The *e* was later dropped because many vitamins are not amines. Vitamins are classified by their solubility; B vitamins are water soluble owing to the presence of several hydroxyl groups in their molecular structures. As additional B vitamins were isolated and identified, they were given sequential subscripts, although this numbering now has gaps because many of the compounds originally thought to be vitamins were later shown to be other types of chemical compounds. Vitamins are among the most commonly required growth factors, as all B vitamins provide cofactors for enzymatic reactions (coenzymes) of intermediary metabolism and are involved in many important metabolic pathways (Madigan & Martinko 2005).

Vitamin B₁ is formed from the joining of two independently synthesized moieties. The compounds 4-amino-5-hydroxymethylpyrimidine diphosphate (HMP-PP) and 4-methyl-5-(2-phosphoethyl)-thiazole (THZ-P) are condensed to generate thiamin monophosphate (TMP). Many bacterial genomes encode a kinase that then phosphorylates TMP to generate the active form of the vitamin, thiamin pyrophosphate (TDP) (Begley et al. 1999, Rodionov et al. 2002). Because of TDP's important role in numerous anabolic and catabolic reactions in the central metabolisms of nearly all organisms, some have suggested that this vitamin is quite ancient in origin (Frank et al. 2007). As a coenzyme, TDP aids in the catalysis of a suite of reactions, primarily by breaking and forming C-N, C-H, C-S, C-O, and C-C bonds (Frank et al. 2007). Of these, the C-C bond is the most important in the marine photic zone because of the irreplaceable role of the TDP-requiring enzyme transketolase in the Calvin-Benson CO₂ fixation cycle. Breslow (1958) is credited with first proposing the mechanism by which TDP breaks and forms these bonds via a “zwitterionic” intermediate, where the negative charge is localized on carbon C2 (between the S and N atoms in the THZ moiety) (**Figure 1a**). The formation of this carbanion equivalent leads to many fates—protonation (as in pyruvate decarboxylase), oxidation (as in pyruvate oxidase and pyruvate:ferredoxin oxidoreductase), or combination with other molecules (as in pyruvate dehydrogenase and transketolase) (Kluger & Tittmann 2008)—which allows TDP enzymes to make a wide array of products in many metabolic pathways.

Vitamin B₇ (biotin) is a soluble coenzyme that was first identified in the 1930s based on its importance to microbial growth (Wood & Barden 1977) and its role in egg-white toxicity in animals (György et al. 1941). Ultimately, it was determined that the avidin protein found in egg whites tightly and irreversibly binds B₇ and thus causes a B₇ deficiency (Waldrop et al. 2012); the characteristics of this binding have been extensively utilized in biotechnology (Cronan & Reed 2000).

Structurally, B₇ comprises two rings (an ureido and a tetrahydrothiophene) and a valeric side chain (Waldrop et al. 2012) (**Figure 1b**). In the coenzymatic form, it is covalently attached, aids in the transfer of CO₂, and is required for three basic classes of enzymes: class I, carboxylases (e.g., pyruvate carboxylase, which performs the first step in gluconeogenesis); class II, decarboxylases [e.g., methylmalonyl-coenzyme A (CoA) decarboxylase, which performs the last step in *Micrococcus* lactate fermentation]; and class III, transcarboxylases (e.g., transcarboxylase, which is important in fermentation to propionate in the propionibacteria) (Samols et al. 1988, Waldrop et al. 2012, Wood & Barden 1977). Most identified B₇-requiring enzymes are in class I and thus are involved in CO₂ fixation (Waldrop et al. 2012), including in the recently discovered autotrophic role of

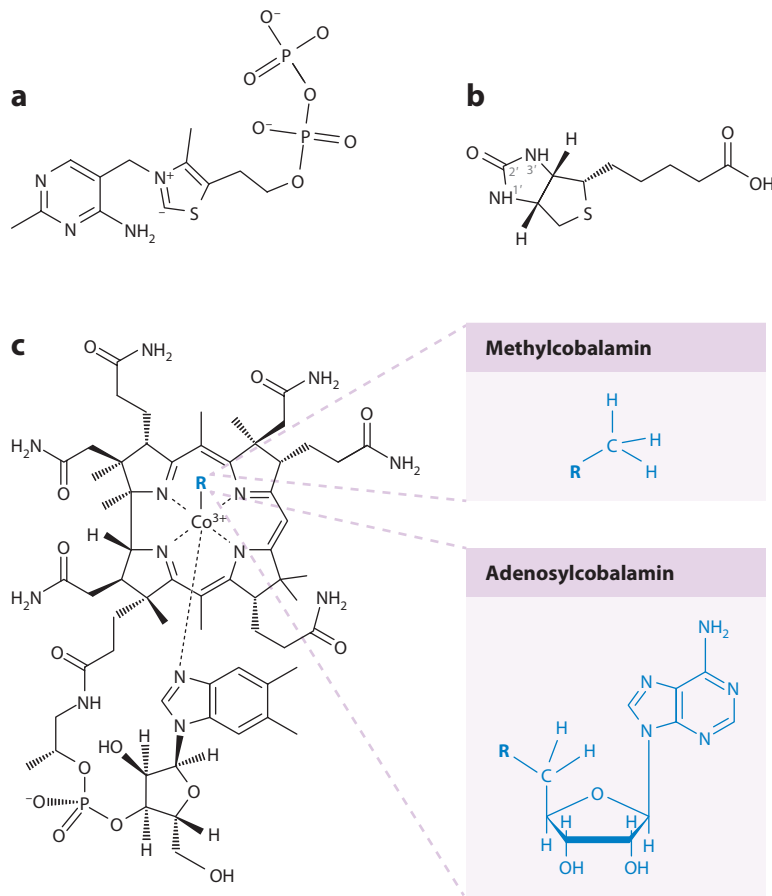


Figure 1

Structures of B vitamins. (a) Vitamin B₁ [thiamin pyrophosphate (TDP)] structure as bound by TDP-requiring enzymes and described by Frank et al. (2007). Note the formation of the carbanion on the C2 [between the S and N atoms on the 4-methyl-(β-hydroxyethyl) thiazole moiety]. (b) Vitamin B₇ (biotin) structure, which comprises two rings (an ureido and a tetrahydrothiophene) and a valeric side chain. The latter is covalently attached in B₇-requiring enzymes. (c) Vitamin B₁₂ (cobalamin) structure, which comprises a corrin ring that coordinates an atom of cobalt. The two major forms are adenosylcobalamin and methylcobalamin, which have R groups of 5'-deoxy-5'-adenosyl and methyl ligands, respectively (*insets*).

biotin-dependent acetyl-CoA carboxylase by an archaeal ammonia oxidizer (Walker et al. 2010). Interestingly, all B₇-requiring enzymes appear to be of common descent (Samols et al. 1988). Catalysis by these enzymes can be broken into two half reactions: (a) the binding of B₇ with CO₂ at the 1' N atom in the carboxylase active site, and (b) the transfer of the bound carboxylate to an acceptor molecule residing in the carboxyltransferase active site (Waldrop et al. 2012). Although the biochemical role of B₇ in CO₂ transfer has been accepted for decades (Wood & Barden 1977), recent protein structural determinations have shed new light on the subject, leading some authors to argue that the knowledge gained from the study of B₇-dependent enzymes could provide a possible catalytic mechanism for future atmospheric CO₂ amelioration (Waldrop et al. 2012). Thus, the mechanisms mediated by this cofactor are still vibrant and important areas of research.

Vitamin B₁₂ (cobalamin), also known as “nature’s most beautiful cofactor” (Stubbe 1994), is large, structurally complex, and rich in nitrogen (Bettendorff & Wins 2009, Rébeillé et al. 2007) (**Figure 1c**). It has two important active forms: adenosylcobalamin and methylcobalamin, which have R groups of 5'-deoxy-5'-adenosyl and methyl ligands, respectively (Gruber et al. 2011) (**Figure 1c**). Enzymes that use these forms can be broken up into three basic classes: isomerases (or mutases), methyl transferases, and reductive dehalogenases (Banerjee & Ragsdale 2003). The most ubiquitous B₁₂-dependent enzymes identified in marine taxa are methylcobalamin-dependent methionine synthase and adenosylcobalamin-dependent methylmalonyl-CoA mutase, which play important roles in amino acid synthesis and carbon resupply to the TCA cycle and central metabolism, respectively (Dowling et al. 2012, Matthews et al. 2003). Interestingly, the forms of B₁₂ associated with these two enzymes go through different intermediates to generate their products: In methionine synthase, the Co-C bond in methylcobalamin is heterolytically cleaved to generate methionine, whereas in methylmalonyl-CoA mutase (and other adenosylcobalamin enzymes), the Co-C bonds are homolytically cleaved, transiently generating a radical in the presence of a substrate (Banerjee & Ragsdale 2003, Marsh & Meléndez 2012, Randaccio et al. 2010). Although not all B₁₂-dependent enzymes use a radical intermediate, the way that these restrictive enzymes generate and control radicals serves as a paradigm for understanding these important and reactive species (Marsh & Meléndez 2012).

VITAMIN AUXOTROPHY IN THE OCEAN

Laboratory culture studies have shown that many marine photosynthetic eukaryotic phytoplankton need vitamins B₁, B₇, and B₁₂, either alone or in combination; of these three, B₁₂ is the most commonly required, followed by B₁ and B₇. Provasoli & Carlucci (1974), Croft et al. (2006), and Tang et al. (2010) provided lists of vitamin requirements based on cultures of single species of marine algae from different phytoplankton groups, and the subject of phytoplankton auxotrophy has been and will continue to be reviewed often as work continues. B-vitamin auxotrophy seems to be widespread; of the more than 300 phytoplankton species studied so far, more than half require B₁₂, almost a quarter require B₁, and a smaller fraction (8%) require B₇ (**Figure 2**). This list will almost certainly continue to grow as new organisms are cultured. Furthermore, the general trend of auxotrophy in marine eukaryotes obtained in growth culture experiments seems to be consistent with the information generated by whole-genome sequencing projects (**Table 1**).

Based on laboratory and genomic results, it is generally accepted that eukaryotic phytoplankton cannot synthesize vitamin B₁₂ de novo (Bertrand et al. 2012, Carlucci & Bowes 1970b, Haines & Guillard 1974, Helliwell et al. 2011), although all of the organisms surveyed have the B₁₂-dependent methionine synthase enzyme. Only 3 out of 10 species also have the B₁₂-independent methionine synthase enzyme (**Table 1**), suggesting that some algae have adapted to overcome B₁₂ limitation in the environment by using alternative enzymes, whereas others, such as the diatom *Thalassiosira pseudonana* and the coccolithophore *Emiliania huxleyi*, have an absolute B₁₂ requirement and depend on an exogenous source of this growth factor (**Table 1**). All eukaryotic phytoplankton species whose genomes have been sequenced have a de novo pathway for B₇ synthesis, suggesting that these species have high cellular quotas for this vitamin. In contrast, of the sequenced eukaryotes, only stramenopiles contain both the *thiC* and *thiG* genes necessary for synthesis of the HMP and THZ moieties of B₁, respectively, suggesting that all other eukaryotic phytoplankton strains acquire this vitamin (or at least one of the moieties) from the environment (**Table 1**). Overall, the genomic results presented in **Table 1** are consistent with laboratory cultures showing that some eukaryotic phytoplankton synthesize some vitamins, whereas others

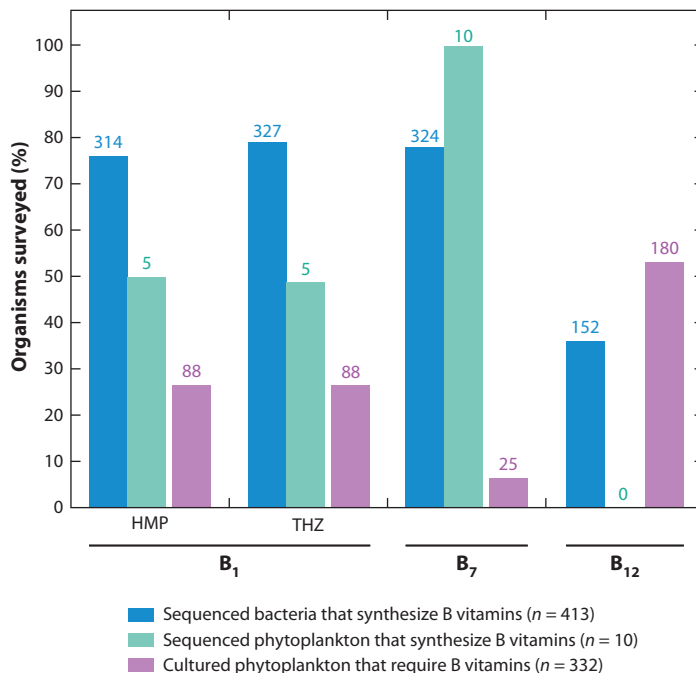


Figure 2

Percentages of marine species that synthesize (based on whole-genome sequencing) or require (based on culture experiments) B vitamins. The sequencing analyses are described in more detail in **Tables 1** and **2**. The numbers of B-vitamin-requiring species are from culture data compiled by Tang et al. (2010). Abbreviations: HMP, 4-amino-5-hydroxymethylpyrimidine; THZ, 4-methyl-(β -hydroxyethyl) thiazole.

do not. Furthermore, the findings that some algae excrete substantial quantities of vitamins add the algae to the roster of vitamin sources in the ocean.

To evaluate vitamin auxotrophy in prokaryotes, we determined the presence or absence of the vitamin synthesis pathways of more than 400 species of marine bacteria, with a special focus on the microbial taxa most commonly found in marine environments: Cyanobacteria, Alpha- and Gammaproteobacteria, and Bacteroidetes. Because these whole-genome-sequenced representatives are cultured, their physiologies may differ from those of in situ uncultured communities. Nevertheless, they are good model organisms to predict the ecological functions of abundant marine microbial taxa (e.g., whether they are vitamin synthesizers or consumers). Overall, 76%, 78%, and 37% of the marine bacteria had de novo pathways for synthesizing B₁, B₇, and B₁₂, respectively (**Figure 2**). These data imply that many marine bacteria require vitamins for growth and therefore must compete with other marine organisms for those vitamins. As the division rates of bacteria can be faster than those of phytoplankton, bacteria may be the most important consumers of B vitamins in the ocean, as Koch et al. (2012) recently demonstrated empirically in some marine environments.

The members of Alphaproteobacteria make up an extremely diverse class, ranging from obligate oligotrophic bacteria in the SAR11 cluster to those that grow on high organic matter concentrations like *Rhodobacterales*. Bacteria in the SAR11 cluster are the most abundant taxon in the ocean, accounting for more than 60% of the sequences in the Global Ocean Sampling project (Rusch et al. 2007). All sequenced SAR11 genomes lack de novo pathways for synthesizing vitamins B₁, B₇ and B₁₂ (**Table 2**). It is important to point out, though, that the entire vitamin B₁ biosynthetic

Table 1 Presence of B-vitamin synthesis genes and pathways in whole-genome-sequenced algae, based on data from the Integrated Microbial Genomes database (<http://img.jgi.doe.gov>)

Phylogenetic group		Gene or pathway present ^a					
Group	Strain	B ₁ HMP moiety (<i>tbiC</i>)	B ₁ THZ moiety (<i>tbiG</i>)	B ₇ (<i>bioB</i>)	B ₁₂ path-way	B ₁₂ -ind. (<i>metE</i>)	B ₁₂ -dep. (<i>metH</i>)
Chlorophyta (green algae)	<i>Chlorella</i> sp. NC64A	✓		✓		✓	✓
	<i>Micromonas pusilla</i> CCMP1545			✓			✓
	<i>Micromonas pusilla</i> RCC299			✓			✓
	<i>Ostreococcus lucimarinus</i> CCE9901			✓			✓
	<i>Ostreococcus tauri</i> OTH95			✓			✓
Stramenopiles/heterokonts (diatoms, pelagophytes, Phaeophyceae)	<i>Thalassiosira pseudonana</i> CCMP1335	✓	✓	✓			✓
	<i>Phaeodactylum tricorutum</i> CCAP1055/1	✓	✓	✓		✓	✓
	<i>Aureococcus anophagefferens</i> CCMP1984 ^b	✓	✓	✓			✓
	<i>Ectocarpus siliculosus</i> Ec32	✓	✓	✓		✓	✓
Haptophyceae (coccolithophores)	<i>Emiliania huxleyi</i> CCMP1516		✓	✓			✓

^aB₁ synthesis was determined using the *tbiC* and *tbiG* genes for the production of the 4-amino-5-hydroxymethylpyrimidine (HMP) and 4-methyl-(β-hydroxyethyl) thiazole (THZ) moieties, respectively; B₇ synthesis was determined using the *bioB* biotin synthase gene. The B₁₂ synthesis pathway was considered present when a genome contained more than 75% of the B₁₂ de novo synthesis clusters of orthologous groups (COGs) (COG0007, COG0310, COG0368, COG1010, COG1270, COG1429, COG1492, COG1797, COG1903, COG2073, COG2082, COG2087, COG2099, COG2109, COG2241, COG2242, and COG2243). In addition to B₁₂ synthesis genes, both B₁₂-independent (B₁₂-ind.) and B₁₂-dependent (B₁₂-dep.) methionine synthesis genes were included.

^bHarmful algal bloom species.

pathway is not absent. They encode for proteins required to produce the B₁ THZ moiety (*tbiG*) (Table 2), suggesting that the acquisition of an exogenous HMP moiety alone could be sufficient to synthesize vitamin B₁. This hypothesis is consistent with laboratory results carried out decades ago and reported by Droop (1962) that showed that some B₁ auxotrophs could satisfy their requirement for that vitamin through the uptake of one of the moieties alone. Another common feature of SAR11 bacteria is that none of the analyzed strains appear to require B₁₂, owing to the absence of enzymes requiring the vitamin.

Although detectable in most seawater samples, *Rhodobacterales* organisms usually predominate in marine ecosystems during and after algal blooms (Allers et al. 2007, Buchan et al. 2005), and they are represented by a relatively large number of cultured bacteria. They have been identified as potential algal symbionts that provide the phytoplankton with B₁₂ in exchange for dissolved organic carbon (Wagner-Dobler et al. 2010). It is noteworthy that 86% of *Rhodobacterales* have

Table 2 Distribution of B-vitamin synthesis genes and pathways in whole-genome-sequenced bacterioplankton species, based on data from the Integrated Microbial Genomes database (<http://img.jgi.doe.gov>)

Phylogenetic group ^a				Gene or pathway present (%) ^b			
Phylum	Class	Order	Genus	B ₁ HMP moiety (<i>tbiC</i>)	B ₁ THZ moiety (<i>tbiG</i>)	B ₇ (<i>bioB</i>)	B ₁₂ pathway
Cyanobacteria	—	Chroococcales ^c	<i>Acaryochloris</i> (2)	100	100	100	100
			<i>Crocospaera</i> (1)	100	100	100	100
			<i>Cyanobacterium</i> (1)	0	100	0	100
			<i>Cyanobium</i> (1)	100	100	100	100
			<i>Cyanothece</i> (3)	100	100	100	100
			<i>Synechococcus</i> (13)	100	100	96	96
		Prochlorales ^d	<i>Prochlorococcus</i> (18)	100	100	100	100
		Nostocales (2)	—	100	100	100	100
Oscillatoriales (3)	—	100	100	100	100		
Proteobacteria	Alphaproteobacteria	SAR11 cluster (11) ^e	—	0	100	0	0
		Caulobacteriales (3)	—	100	100	0	0
		Magnetococcales (1)	—	100	100	100	100
		Parvularculales (1)	—	100	100	0	0
		Rhizobiales (15)	—	93	93	60	87
		Rhodobacterales (50)	—	50	72	44	86
		Sphingomonadales (7)	—	100	100	100	0
	Gammaproteobacteria	Alteromonadales (49)	—	94	89	100	6
		Chromatiales (8)	—	100	100	100	37
		Oceanospirillales (13)	—	100	100	100	46
		Pseudomonadales (6)	—	83	66	100	0
		Thiotrichales (9)	—	89	89	89	11
		Legionales	<i>Reinekea</i> (1)	100	100	100	0
		Vibrionales (33)	—	97	100	94	3
Xanthomonadales (1)	—	100	100	100	0		
Bacteroidetes	Cytophagia (10)	—	—	60	60	70	0
	Flavobacteriia (25)	—	—	36	24	56	0
	Sphingobacteriia (5)	—	—	60	60	80	0

^aNumbers in parentheses are the number of genomes analyzed for each group. Shading highlights the groups that dominate in most oceanic waters according to Rusch et al. (2007). Dashes are used in cases where the particular subgroup is not relevant.

^bB₁ synthesis was determined using the *tbiC* and *tbiG* genes for the production of the 4-amino-5-hydroxymethylpyrimidine (HMP) and 4-methyl-(β-hydroxyethyl) thiazole (THZ) moieties, respectively; B₇ synthesis was determined using the *bioB* biotin synthase gene. The B₁₂ synthesis pathway was considered present when a genome contained more than 75% of the B₁₂ de novo synthesis clusters of orthologous genes (COGs) (COG0007, COG0310, COG0368, COG1010, COG1270, COG1429, COG1492, COG1797, COG1903, COG2073, COG2082, COG2087, COG2099, COG2109, COG2241, COG2242, COG2243).

^cChroococcales (including *Synechococcus*) represented 4.2% of the reads from the Global Ocean Sampling expedition (Rusch et al. 2007).

^dProchlorales (*Prochlorococcus*) represented 14.5% of the reads from the Global Ocean Sampling expedition.

^eThe SAR11 cluster represented 63.8% of the reads from the Global Ocean Sampling expedition.

a de novo pathway for B₁₂ synthesis (**Table 2**). At the same time, a relatively high percentage of *Rhodobacterales* lack the B₁ and B₇ pathways, suggesting that their symbiotic relationship with phytoplankton could involve these bacteria depending on algal vitamins, as some phytoplankton can synthesize B₁ and B₇ de novo (**Tables 1 and 2**).

Gammaproteobacteria is also an extremely diverse group in the marine environment, representing both readily cultured bacteria, like the genus *Vibrio*, and the strictly oligotrophic bacteria of the SAR86 clade, which are among the most abundant uncultivated members of microbial assemblages in surface waters (Dupont et al. 2011, Rusch et al. 2007). As previously observed in other oligotrophic bacteria, like SAR11, members of the SAR86 clade lack complete biosynthetic pathways for vitamins B₇ and B₁₂, and some lack the B₁ pathway as well (Dupont et al. 2011). However, in contrast to SAR86, most easily cultured Gammaproteobacteria species have an opportunistic lifestyle, which allows them to efficiently assimilate pulses of organic matter and consequently to reach very high concentrations over short periods of time (Gilbert et al. 2011). Although Gammaproteobacteria is a highly diverse class, almost all the cultured organisms have the B₁ and B₇ biosynthetic pathways (**Table 2**). Nevertheless, with the exception of the genera *Halomonas*, *Marinomonas*, and *Neptuniibacter*, marine Gammaproteobacteria species lack the genes for B₁₂ synthesis (**Table 2**).

Bacteroidetes is an important group of bacterioplankton, accounting for as much as half of the bacterial community at some locations (Cottrell & Kirchman 2000). Consistent with being particularly efficient at degrading complex organic matter (e.g., algal exudates and lysis products), Bacteroidetes species have been associated with natural occurring algal blooms (DeLong et al. 1993, Pinhassi et al. 1999, Riemann et al. 2000). All currently sequenced Bacteroidetes isolates are predicted to be B₁₂ auxotrophs (**Table 2**); only 24% of Flavobacteria species can synthesize B₁, and 56% can synthesize vitamin B₇. These results suggest that Bacteroidetes species could be especially limited by the availability of exogenous sources of the three vitamins, and because these bacteria commonly grow attached to particles, this lifestyle could potentially allow them to obtain vitamins from vitamin-producing organisms in macroaggregates.

Cyanobacteria species are among the most abundant photosynthetic organisms in the ocean (Chisholm et al. 1988, Johnson 2006, Rusch et al. 2007). Although not found in polar environments, they account for a large fraction of the microbial community in temperate and subtropical waters (Campbell et al. 1997, Partensky et al. 1999). Different Cyanobacteria taxa thrive in different environments. For example, *Prochlorococcus* occurs mainly in temperate oligotrophic waters, where it can account for 21–43% of the photosynthetic biomass, whereas *Synechococcus* can bloom during higher-nutrient pulses (Partensky et al. 1999). Despite these apparent physiological differences, nearly all Cyanobacteria species possess the de novo pathways for synthesizing B₁, B₇, and B₁₂ (**Table 2**), suggesting that these organisms have high vitamin quotas and might be some of the major of B-vitamin producers in most of the ocean. Nevertheless, some Cyanobacteria species, such as the uncultured UCYN-A diazotrophs, lack the B₁ and B₇ biosynthetic pathways, and *Synechococcus* sp. PCC7002 lacks the B₁₂ biosynthetic pathway (**Table 2**). B₁ is an essential cofactor for the function of a variety of enzymes, including pyruvate:ferredoxin oxidoreductase, which is crucial for electron transfer to nitrogenase (Brostedt & Nordlund 1991). This metabolic requirement for exogenously supplied B₁ presumably impacts the quantity and timing of nitrogen fixation by UCYN-A diazotrophs, which seem to be ubiquitous in large areas of the ocean (Moisander et al. 2010).

It is notable that some marine bacteria, such as the SAR11 isolate “*Candidatus* Pelagibacter ubique,” reach concentrations approaching 10⁵ cells/mL despite an almost complete inability to make B vitamins (Carini et al. 2013, Morris et al. 2002). In Pelagibacter and other auxotrophic bacteria (**Table 2**), the genes have likely been deleted because the vitamins whose synthesis they

enable are available in the organisms' natural environment. In support of the supposition that the dissolved organic matter in the microbial loop is a source of food as well as growth factors, recent work has partially explained the absence of B₁₂ synthesis genes in "*Ca. P. ubique*," showing that instead of synthesizing methionine, these bacteria acquire it (or other reduced sulfur compounds) from the environment (Tripp et al. 2008). Furthermore, although others have shown that heterotrophic bacteria can be B-vitamin producers in the ocean (Croft et al. 2005, Provasoli 1963, Provasoli & Carlucci 1974), organic matter from dead phytoplankton is needed to stimulate growth of these bacteria and their subsequent vitamin production (Haines & Guillard 1974). This suggests that in well-stratified open-ocean waters away from land sources and coastal upwelling areas, direct phytoplankton-bacteria interactions may be important for supplying B vitamins to the ecosystem (Croft et al. 2005, Kazamia et al. 2012). However, as shown in **Table 2**, heterotrophic bacteria are not the only B-vitamin producers in the ocean. The picoplankton *Prochlorococcus* and *Synechococcus*, which can reach abundances of 10⁵–10⁶ cells/mL (Johnson 2006, Waterbury et al. 1986), are also predicted to be capable of de novo B-vitamin synthesis and, fitting with this prediction, can grow without supplemental vitamins (Bonnet et al. 2010).

It is important to note that the early studies in the 1950s and 1960s on the function of vitamins in the ocean were carried out before the discovery of marine *Prochlorococcus* and *Synechococcus*, thereby underscoring the significance of studying the roles of these organisms in B-vitamin synthesis. The oceanic diazotrophs *Crocospaera* and *Trichodesmium* are also vitamin synthesizers (**Table 2**), but with the important difference that their ability to produce B vitamins is independent of available nitrogen, a characteristic that is likely important in the nitrogen-limited oligotrophic oceans. Taken together, these data imply that the oceanic cyanobacteria are not competing for most exogenous B vitamins with other bacteria and eukaryotes, but instead are obligate producers.

Thus, our search of available genomic data indicates that some heterotrophic bacteria and the ubiquitous marine cyanobacteria are vitamin producers that make most if not all of their B vitamins de novo, whereas other microbes are nearly complete consumers that are predicted to acquire most of their B vitamins from their environment (**Tables 1 and 2**). However, the notion that vitamins are solely produced by prokaryotes is incorrect, and both vitamin-producing and vitamin-consuming bacteria and algae coexist in the ocean. This nutritional interdependency among different bacteria and eukaryotic phytoplankton suggests a chemical symbiosis in which some vitamin producers are themselves dependent on other vitamins produced by other organisms. The exchange of these growth factors among bacteria and phytoplankton supports Lucas's (1947) hypothesis that ectocrine substances mediate community integration in marine systems.

UNDERSTANDING THE BIOCHEMISTRY OF MARINE AUXOTROPHY

To begin to understand the biochemical reasons for vitamin auxotrophy in marine bacteria and eukaryotic plankton, we inspected the available genomes for vitamin-requiring enzymes. **Table 3** presents these enzymes and the reactions they catalyze.

Compared with vitamins B₇ and B₁₂, vitamin B₁ has the greatest range of required enzymes (**Figure 3a**, **Table 3**). Of the 30 B₁ (TDP)-requiring enzymes, 21 were encoded in at least one of the marine genomes surveyed (**Figure 3a**). In general, B₁-requiring enzymes are involved in both catabolic and anabolic reactions in central metabolism (**Figure 4**). To simplify our analysis, we defined two groups of B₁-requiring enzymes: core and specialized. The core enzymes were present in all taxonomic groups surveyed (**Figure 3a**) and play a critical role in central metabolism (**Figure 4**); the specialized enzymes were present in only some taxa and were present in lower abundance within each taxon (**Figure 3a**). The five core B₁-requiring enzymes are pyruvate dehydrogenase (which catalyzes the first committed step of the TCA cycle by converting pyruvate to acetyl-CoA),

Table 3 B-vitamin-requiring enzymes as defined from the ExPASy database (<http://www.expasy.org>), along with their EC numbers and the reactions they catalyze

Enzyme	EC number	Reaction catalyzed
Vitamin B₁ (thiamin pyrophosphate)-requiring enzymes		
Acetolactate synthase	2.2.1.6	2 pyruvate ↔ 2-acetolactate + CO ₂
Benzoylformate decarboxylase	4.1.1.7	Benzoylformate ↔ benzaldehyde + CO ₂
1-Deoxy-D-xylulose-5-phosphate synthase	2.2.1.7	Pyruvate + D-glyceraldehyde 3-phosphate ↔ 1-deoxy-D-xylulose 5-phosphate + CO ₂
Fructose-6-phosphate phosphoketolase	4.1.2.22	D-Fructose 6-phosphate + phosphate ↔ acetyl phosphate + D-erythrose 4-phosphate + H ₂ O
5-Guanidino-2-oxopentanoate decarboxylase	4.1.1.75	5-Guanidino-2-oxo-pentanoate ↔ 4-guanidinobutanal + CO ₂
Indolepyruvate decarboxylase	4.1.1.74	3-(Indol-3-yl)pyruvate ↔ 2-(indol-3-yl)acetaldehyde + CO ₂
Indolepyruvate ferredoxin oxidoreductase	1.2.7.8	(Indol-3-yl)pyruvate + CoA + 2 oxidized ferredoxin ↔ S-2-(indol-3-yl)acetyl-CoA + CO ₂ + 2 reduced ferredoxin + H ⁺
α-Ketoglutarate dehydrogenase	1.2.4.2	2-Oxoglutarate + (dihydrolipoyllysine-residue succinyltransferase) lipoyllysine ↔ (dihydrolipoyllysine-residue succinyltransferase) S-succinyl-dihydrolipoyllysine + CO ₂
3-Methyl-2-oxobutanoate dehydrogenase (2-methylpropanoyl-transferring)	1.2.4.4	3-Methyl-2-oxobutanoate + [dihydrolipoyllysine-residue (2-methylpropanoyl)transferase] lipoyllysine ↔ [dihydrolipoyllysine-residue (2-methylpropanoyl)transferase] S-(2-methylpropanoyl)-dihydrolipoyllysine + CO ₂
Oxalyl-CoA decarboxylase	4.1.1.8	Oxalyl-CoA ↔ formyl-CoA + CO ₂
2-Oxoglutarate synthase	1.2.7.3	2-Oxoglutarate + CoA + 2 oxidized ferredoxin ↔ succinyl-CoA + CO ₂ + 2 reduced ferredoxin + 2 H ⁺
Phosphoketolase	4.1.2.9	D-Xylulose 5-phosphate + phosphate ↔ acetyl phosphate + D-glyceraldehyde 3-phosphate + H ₂ O
Phosphonopyruvate decarboxylase	4.1.1.82	3-Phosphonopyruvate ↔ 2-phosphonoacetaldehyde + CO ₂
Pyruvate decarboxylase	4.1.1.1	A 2-oxo acid ↔ an aldehyde + CO ₂
Pyruvate dehydrogenase (acetyl-transferring)	1.2.4.1	Pyruvate + CoA + NAD ↔ acetyl-CoA + CO ₂ + NADH + H ⁺
Pyruvate oxidase	1.2.3.3	Pyruvate + phosphate + O ₂ ↔ acetyl phosphate + CO ₂ + H ₂ O ₂
Pyruvate synthase	1.2.7.1	Pyruvate + CoA + 2 oxidized ferredoxin ↔ acetyl-CoA + CO ₂ + 2 reduced ferredoxin + 2 H ⁺
Sulfoacetaldehyde acetyltransferase	2.3.3.15	2-Sulfoacetaldehyde + phosphate ↔ acetyl phosphate + sulfite
Sulfopyruvate decarboxylase	4.1.1.79	3-Sulfopyruvate ↔ 2-sulfoacetaldehyde + CO ₂
Tartronate-semialdehyde synthase	4.1.1.47	2 glyoxylate ↔ tartronate semialdehyde + CO ₂
Transketolase	2.2.1.1	Sedoheptulose 7-phosphate + D-glyceraldehyde 3-phosphate ↔ D-ribose 5-phosphate + D-xylulose 5-phosphate
Vitamin B₇ (biotin)-requiring enzymes		
Acetyl-CoA carboxylase	6.4.1.2	ATP + acetyl-CoA + HCO ₃ ⁻ ↔ ADP + phosphate + malonyl-CoA
Geranoyl-CoA carboxylase	6.4.1.5	ATP + geranoyl-CoA + HCO ₃ ⁻ ↔ ADP + phosphate + 3-(4-methylpent-3-en-1-yl)pent-2-enediyl-CoA
Methylcrotonoyl-CoA carboxylase	6.4.1.4	ATP + 3-methylcrotonoyl-CoA + HCO ₃ ⁻ ↔ ADP + phosphate + 3-methylglutaconyl-CoA

(Continued)

Table 3 (Continued)

Enzyme	EC number	Reaction catalyzed
Methylmalonyl-CoA decarboxylase	4.1.1.41	(S)-methylmalonyl-CoA \leftrightarrow propanoyl-CoA + CO ₂
Oxaloacetate decarboxylase	4.1.1.3	Oxaloacetate \leftrightarrow pyruvate + CO ₂
Propionyl-CoA carboxylase	6.4.1.3	ATP + propanoyl-CoA + HCO ₃ ⁻ \leftrightarrow ADP + phosphate + (S)-methylmalonyl-CoA
Pyruvate carboxylase	6.4.1.1	ATP + pyruvate + HCO ₃ ⁻ \leftrightarrow ADP + phosphate + oxaloacetate
Urea carboxylase	6.3.4.6	ATP + urea + HCO ₃ ⁻ \leftrightarrow ADP + phosphate + urea-1-carboxylate
Vitamin B₁₂ (cobalamin)-requiring enzymes		
Ribonucleotide reductase	1.17.4.2	2'-Deoxyribonucleoside triphosphate + thioredoxin disulfide + H ₂ O \leftrightarrow ribonucleoside triphosphate + thioredoxin
Methionine synthase	2.1.1.13	5-Methyltetrahydrofolate + L-homocysteine \leftrightarrow tetrahydrofolate + L-methionine
Methylaspartate ammonia-lyase	4.3.1.2	L-Threo-3-methylaspartate \leftrightarrow mesaconate + NH ₃
Ethanolamine ammonia-lyase	4.3.1.7	Ethanolamine \leftrightarrow acetaldehyde + NH ₃
Methylaspartate mutase	5.4.99.1	L-Threo-3-methylaspartate \leftrightarrow L-glutamate
Methylmalonyl-CoA mutase	5.4.99.2	(R)-methylmalonyl-CoA \leftrightarrow succinyl-CoA

acetolactate synthase (which plays a role in branch-chain amino acid synthesis), 1-deoxy-D-xylulose-5-phosphate synthase (which plays a role in terpenoid and B₁ biosynthesis), transketolase (which plays a role in the Calvin-Benson cycle and the pentose phosphate pathway), and α -ketoglutarate dehydrogenase (which plays a role in the TCA cycle) (Berg et al. 2007). The 16 specialized enzymes consist primarily of pathways for alternative carbon (noncarbohydrate) metabolism in both aerobic and anaerobic environments. These specialized enzymes likely allow marine microbes to be generalists and thrive in both copio- and oligotrophic environments (Lauro et al. 2009, Moran et al. 2004).

Each taxonomic group has a distinct complement of B₁-dependent enzymes (**Figure 3a**). The Cyanobacteria group had the fewest B₁-requiring enzymes (seven) of the groups surveyed, which fits with the highly conserved photoautotrophic metabolism of these organisms (Waterbury 2006). The exception is UCYN-A, which is a symbiotic photoheterotroph (Thompson et al. 2012, Tripp et al. 2010). UCYN-A is the only Cyanobacteria member that we surveyed (and arguably the only one in existence) that does not conform to the conserved metabolism of the rest of the phylum, and as such it has a slightly different enzyme complement, lacking acetolactate synthase and thus generating an auxotrophy for valine, isoleucine, and leucine (Tripp et al. 2010).

Alphaproteobacteria and Gammaproteobacteria taxa contained the most B₁-requiring enzymes (18 and 17, respectively) (**Figure 3a**). The taxa that we surveyed consisted of a highly metabolically diverse group of heterotrophic bacteria, and as such they require the ability to utilize a large variety of carbon sources. The metabolic diversity within the Proteobacteria groups is further shown by the abundance of the core B₁-requiring enzymes, which were present in more than 90% of the Proteobacteria genomes. Comparatively, the specialized B₁-requiring enzymes showed great diversity in the Proteobacteria phylum (**Figure 3a**). This enzymatic diversity allows this group to exploit a wide variety of niches in the ocean.

The B₁-requiring enzymes in Bacteroidetes were much less diverse compared with those of Proteobacteria (**Figure 3a**). Bacteroidetes species are well known for their abundance in the ocean and their ability to metabolize the high-molecular-mass portion of dissolved organic matter (including cellulose and chitin) (Abell & Bowman 2005, Kirchman 2002). Our findings, paired with

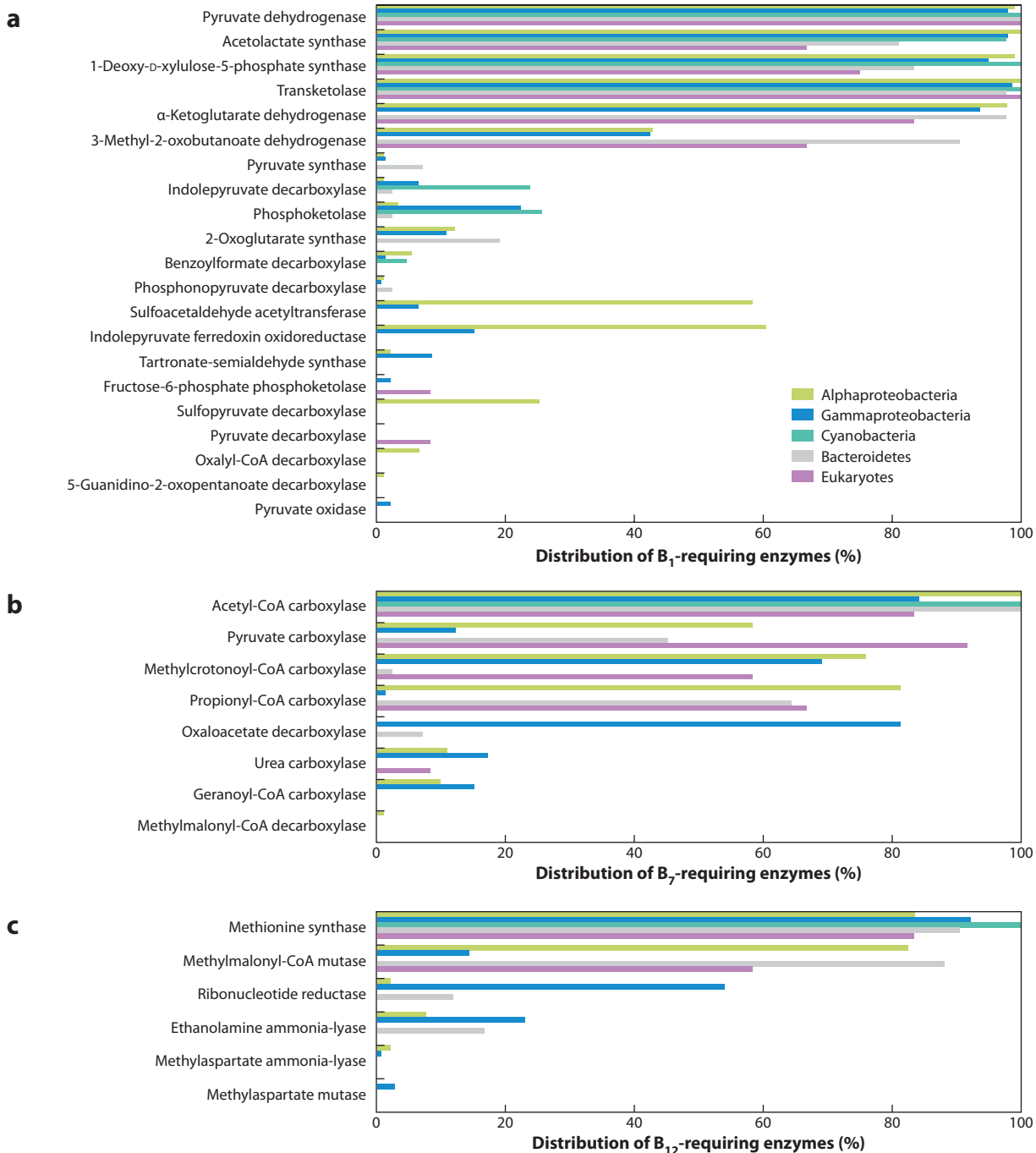


Figure 3

Distribution of (a) B₁-, (b) B₇-, and (c) B₁₂-requiring enzymes in the most abundant marine taxa. Genomes were searched for EC numbers of vitamin-requiring enzymes in the Integrated Microbial Genomes database (<http://img.jgi.doe.gov>). The microbial genomes of interest were then queried in this database by searching the available metadata for the taxa and marine habitat.

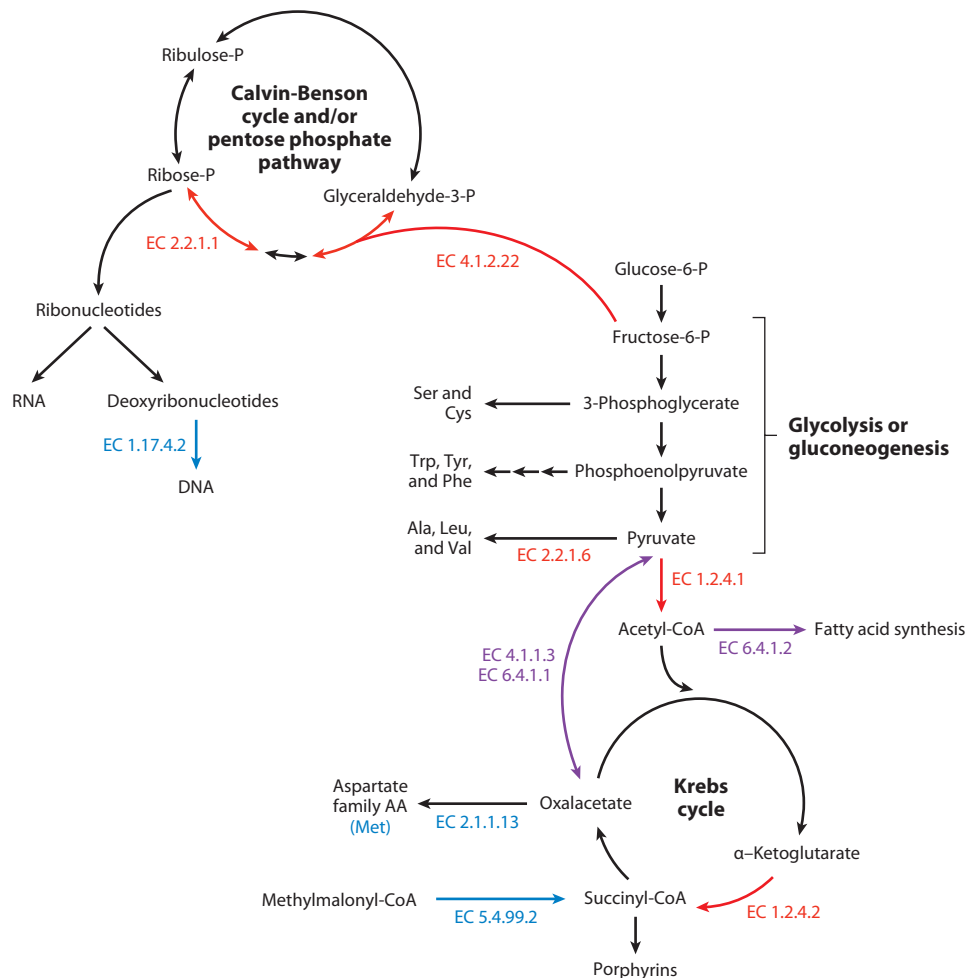


Figure 4

Central metabolic pathways with vitamin-requiring steps highlighted. Red arrows are B₁-dependent steps, purple arrows are B₇-dependent steps, and blue arrows are B₁₂-dependent steps. Specific enzyme names can be found by referring to the EC numbers in **Table 3**.

what is known about the ecology of Bacteroidetes, seem to imply that, as a group, Bacteroidetes is more metabolically similar than Proteobacteria.

There are 12 B₇-dependent enzymes, 8 of which were found in the surveyed marine taxa (**Figure 3b**, **Table 3**). All of these enzymes are predicted to be B₇-dependent carboxylases (Knowles 1989). The most common and widespread B₇-dependent enzyme found was acetyl-CoA carboxylase (**Figure 3b**). This enzyme was present in at least 85% of our taxonomic groups and in 100% of the Cyanobacteria, Alphaproteobacteria, and Bacteroidetes groups. Interestingly, it was also the only B₇-dependent enzyme found in Cyanobacteria. In addition to its recently defined role in archaeal CO₂ fixation, acetyl-CoA carboxylase is essential for fatty acid synthesis in most described microbes; thus, it is crucial for energy storage as well as the production of important cellular components, such as membranes (Jitrapakdee & Wallace 2003).

B₇-dependent pyruvate carboxylase was present in all taxonomic groups surveyed except Cyanobacteria. This is the only other B₇-dependent anabolic enzyme (**Figure 3b**), and it catalyzes the first step in gluconeogenesis by converting pyruvate to oxaloacetate (Jitrapakdee & Wallace 2003) (**Figure 4**). This pathway is common in heterotrophs, as it produces glucose from noncarbohydrate precursors. It is especially important to heterotrophs in the ocean that survive primarily on dissolved organic matter and therefore do not have a stable supply of hexoses (Azam 1998, Azam et al. 1983). Interestingly, the genomically characterized members of the SAR11 clade lack this enzyme (Carini et al. 2013). Cyanobacteria can produce glucose via the Calvin-Benson cycle and therefore do not require gluconeogenesis. B₇-dependent urea carboxylase was present in fewer than 25% of the marine genomes (in Alpha- and Gammaproteobacteria as well as eukaryotes) (**Figure 3b**). This enzyme is part of the urea cycle, which in terrestrial organisms is used primarily to excrete excess nitrogen (Berg et al. 2007). In the nitrogen-limited ocean, however, it is quite possible that these microbes are using this enzyme to metabolize exogenous nitrogen for growth (Dugdale & Goering 1967, McCarthy 1972).

The other B₇-dependent enzymes fall into the broad category of alternative noncarbohydrate metabolism (**Figures 3b and 4**) and are likely used to metabolize lipids and amino acids (Jitrapakdee & Wallace 2003). Owing to the oligotrophic nature of most marine environments, organisms that can utilize a wide range of carbon sources are much better fit for survival. In contrast, cyanobacteria (as photoautotrophs) do not have any of these alternative carbon metabolic pathways. Also in contrast to the general taxonomic themes, the SAR11 clade (which cannot synthesize B₇; Giovannoni et al. 2005) lacks all of the surveyed B₇-requiring enzymes except acetyl-CoA carboxylase. Most Alphaproteobacteria taxa contain more B₇-requiring enzymes than SAR11 does; however, it should be noted that, owing to the abundance of SAR11 in seawater (Giovannoni et al. 2005), if the current genomes are representative, then the majority of these Alphaproteobacteria taxa likely have a B₇-requiring enzymatic profile in situ.

B₁₂ auxotrophy is the most common form of vitamin auxotrophy in marine plankton (Croft et al. 2006) (**Figure 2, Tables 1 and 2**), possibly owing to the vitamin's molecular complexity (Brown 2005, Croft et al. 2006). As such, it is not surprising that only 12 enzymes require B₁₂ as a cofactor, nor that only 6 of them were found in the marine microbes surveyed (**Figure 3c, Table 3**).

In all surveyed taxonomic groups, the gene for B₁₂-dependent methionine synthase (*metH*) was present in at least 80% of the genomes in each group (**Figure 3c**). This indicates that most marine microbes can utilize the more efficient B₁₂-dependent methionine synthesis pathway (*metH*) when B₁₂ is available, as opposed to the less efficient B₁₂-independent pathway (*metE*) (Matthews et al. 2003). Although more than 80% of microbes surveyed can use B₁₂-dependent methionine synthase, none of the surveyed eukaryotes or Bacteroidetes taxa can synthesize B₁₂ (**Figure 2, Tables 1 and 2**), and the Proteobacteria taxa have only a limited ability to do so. This suggests that scavenging of B₁₂ from the dissolved phase must be crucial for these organisms' survival.

Less common B₁₂-requiring enzymes are involved in alternative noncarbohydrate utilization as well as nucleotide synthesis (**Figure 3c, Table 3**). These enzymes were found only in select taxa, which did not include Cyanobacteria (**Figure 3c**). Ribonucleotide reductase, which plays an important role in nucleotide synthesis (Reichard 1997) (**Figure 4**), was present in varying abundances in Proteobacteria and Bacteroidetes. Therefore, the taxa that do not utilize this enzyme must either scavenge reduced ribonucleotides from the water column or utilize an alternative biosynthetic pathway. All other B₁₂-requiring enzymes were present in limited abundance and taxonomic diversity, which may indicate that they have a narrow niche used in alternative carbon source degradation and metabolism.

Within the photic zone, the primary role of B₁₂ appears to be enabling methionine synthesis. Although the abundance and taxonomic diversity of B₁₂-requiring enzymes is low, methionine synthesis is a crucial biochemical process. Furthermore, field experiments have shown that, although B₁₂ is incredibly scarce in the ocean, it can control phytoplankton growth and microbial community structure (Bertrand et al. 2012, Koch et al. 2011). These results validate our genomic surveys of both synthesis and usage of B₁₂ by showing a clear imbalance between the supply of and demand for this vitamin in the environment.

THE OCEANOGRAPHY OF B VITAMINS

Understanding the ecological role of the B vitamins in the marine environment requires knowledge of their dissolved concentrations. Owing to the extremely low concentrations of vitamins in seawater, for more than three decades, ambient concentrations were estimated indirectly using bioassays with both bacteria and algae as test organisms (Carlucci & Silbernagel 1966a,b; Haines & Guillard 1974). However, the pioneers of vitamin studies in marine systems during the 1960s and early 1970s acknowledged that the indirect bioassay technique may not accurately reflect ambient levels of dissolved B vitamins, either because the growth of the test organism is not always truly B-vitamin specific or because incubation periods, ranging from days to weeks, can change vitamin availability and/or the physiological responses of the phytoplankton (Carlucci & Boves 1970a; Carlucci & Silbernagel 1966a,b; Haines & Guillard 1974). However, the similar dissolved B₁₂ concentrations measured using the bioassay technique in the open-ocean waters of different marine systems in the 1950s and 1960s (ranging from undetectable to ~7 pM) along with the levels measured more recently using direct techniques (**Table 4**) suggest that, at least for vitamin B₁₂, the bioassays and the direct quantifications produce similar results. The same applies to B₁₂ measurements in the Antarctic, where bioassay-based measurements of dissolved B₁₂ concentrations (ranging from 0.5 to 2.4 pM; Carlucci & Cuhel 1977, Taylor & Sullivan 2008) were similar to those measured with the direct technique (ranging from 0.5 to 3.5 pM; Panzeca et al. 2009). The similar range of concentrations as well as the similar depth profiles obtained by the two methodologies (**Figure 5a**) in water samples collected in the Pacific Ocean almost 50 years apart suggest that both techniques produce coherent results.

In general, coastal waters have the highest concentrations of dissolved B₁₂, and open-ocean waters have the lowest (**Table 4**). The highest B₁₂ concentrations have been reported in anoxic basins of the Southern California Bight (0–30 pM; Sañudo-Wilhelmy et al. 2012), in shallow Long Inland embayments with restricted water exchange with the adjacent Atlantic Ocean (5–87 pM; Sañudo-Wilhelmy et al. 2006), and in Todos Santos Bay in Baja California, Mexico, during a B₁₂-requiring dinoflagellate (*Lingulodinium polyedrum*) bloom (2–61 pM; Panzeca et al. 2009).

Vertical distributions of dissolved B₁₂ measured in the Pacific Ocean (off Southern California and Hawaii) also showed similar depth distributions and concentration levels (**Figure 5a**). B₁₂ levels in the Sargasso Sea are approximately an order of magnitude lower than those reported for the Pacific Ocean (<0.3 pM; Menzel & Spaeth 1962). Depth profiles of dissolved B vitamins in the Sargasso Sea based on direct measurements have not been produced, so a comparison with the 1960s bioassay results is not possible. However, depth distributions measured in both oceans seem to be consistent with each other (**Figure 5**). In general, waters of intermediate depth appear to contain higher vitamin levels than those below and above (**Figure 5**). Deep waters of the Pacific Ocean seem to be devoid of B vitamins; increases in concentrations with water depth have been observed only in the low-oxygen basins of the Southern California–Baja California coastal boundary (Carlucci & Silbernagel 1966c, Sañudo-Wilhelmy et al. 2012) (**Figure 5**). The upper

Table 4 Ranges of dissolved vitamin B₁₂ concentrations from different regions of the world ocean

Studied area	Range of B ₁₂ concentrations (pM)	Method	Reference
Oceanic			
Bay of Biscay	0.1–3.7	Bioassay	Daisley & Fisher 1958
Gerlache Strait, Southern Ocean	0.4–4	Direct HPLC detection	Panzeca et al. 2009
Mediterranean Sea	0.5–6.2	Direct HPLC detection	Bonnet et al. 2013
North Atlantic surface waters	0.1–2.5	Direct HPLC detection	Panzeca et al. 2008
Northeast Pacific Ocean	0–2.7	Bioassay	Carlucci & Silbernagel 1966c
Sargasso Sea	0–0.3	Bioassay	Menzel & Spaeth 1962
Southern part of the Indian Ocean	0.1–3.0	Bioassay	Fiala & Oriol 1984
Coastal			
Long Island Sound	5.0–87	Direct HPLC detection	Sañudo-Wilhelmy et al. 2006
San Pedro Basin, California	0.2–1.8	Direct HPLC detection	Panzeca et al. 2009
Southern California and Baja California coast	0–30	Direct LCMS detection	Sañudo-Wilhelmy et al. 2012
Todos Santos Bay, Mexico	2.0–61	Direct HPLC detection	Panzeca et al. 2009
Upwelling site off Mexico	0.4–7	Direct HPLC detection	Panzeca et al. 2009

Abbreviations: HPLC, high-performance liquid chromatography; LCMS, liquid chromatography–mass spectrometry.

water column is the area most involved in biological activity, however, and vitamin levels there likely fluctuate greatly during the year, as observed in some locations off Baja California, Mexico (**Figure 5**). The B-vitamin maxima observed in the upper mesopelagic zone suggest not only that microbial plankton groups below the photic zone contribute substantially to heterotrophic metabolism and nutrient cycling in the ocean, but also that heterotrophic bacterioplankton may be major producers of the growth factors required by eukaryotic auxotrophic phytoplankton in the photic zone.

The few depth profiles available in which vitamins B₁, B₇, and B₁₂ were measured simultaneously indicate that their concentrations and distributions are site specific and independent of one another (Sañudo-Wilhelmy et al. 2012) (**Figure 5**). The varied depth distributions of the different dissolved vitamins contrast with the oceanographically consistent distributions reported for inorganic nutrients and some trace metals (Bruland 1980). Geographic distributions of dissolved B vitamins show large spatial variability in vast areas of the ocean (e.g., the Pacific Ocean and south polar seas), including areas where B vitamins are undetectable (Carlucci & Cuhel 1977, Sañudo-Wilhelmy et al. 2012). In some marine regions, the geographic distributions of dissolved B vitamins are strongly influenced by water hydrodynamics, as these distributions were also coupled to water mass transport (Sañudo-Wilhelmy et al. 2012). The large areas with undetectable levels of dissolved vitamins suggest that in those regions, microbial phytoplankton growth rate, abundance, and diversity could be limited by the scarcity of those organic growth factors.

The causes of the observed vitamin depletions in different areas of the ocean are currently undefined. However, the scarcity of vitamins and the temporal and spatial variability of vitamin concentrations observed in many locations may be due to their nonbiological destruction, as the half-life of some vitamins (such as B₁ and B₁₂) in the alkaline pH of seawater is only a few days, and

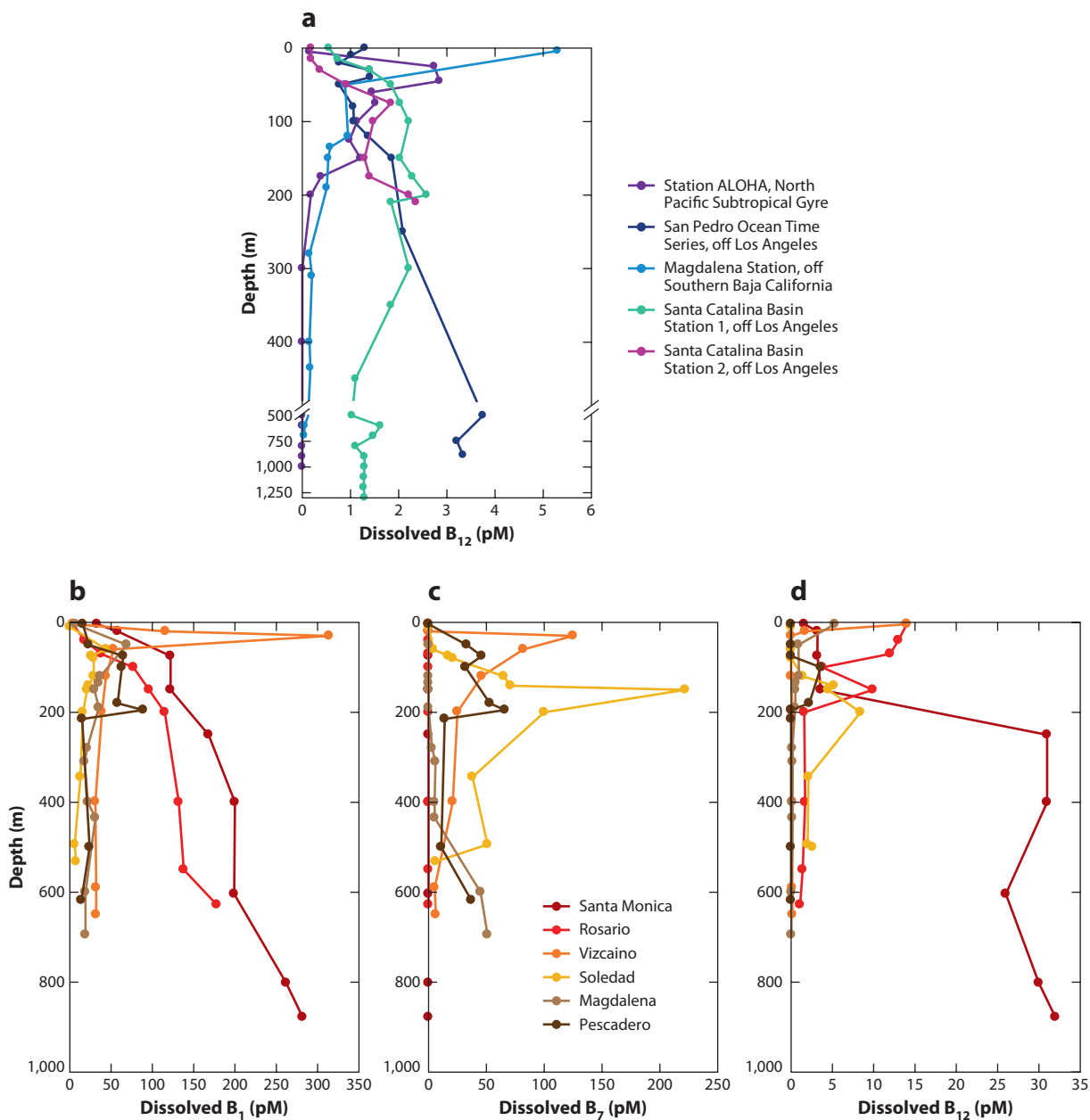


Figure 5

Depth profiles of B vitamins measured in different regions of the Pacific Ocean. (a) Depth profiles of B₁₂ from different regions of the Pacific, measured using either the bioassay method or direct quantification. (b–d) Depth profiles of B₁, B₇, and B₁₂ from different stations off Southern California and Baja California measured using direct quantification (Sañudo-Wilhelmy et al. 2012).

their degradation seems to increase with water temperature and solar radiation (Carlucci et al. 1969, Gold et al. 1966). However, the degradation products of vitamins in seawater remain unidentified, and whether those molecules could satisfy the vitamin requirements of some phytoplankton is unknown.

The role of biological uptake in the depletion of dissolved vitamins in many regions of the ocean has not been properly evaluated, as direct techniques for determining particulate vitamin levels in field populations of phytoplankton have not been optimized. The few studies that have simultaneously measured dissolved and particulate vitamin levels suggest that biological activity could considerably deplete vitamins from the dissolved pool. For example, Carlucci & Bowes (1972) reported that the vitamin content in cultures of phytoplankton was variable and dependent on the vitamin levels in the external medium, with starved cultures containing less than those grown under replete conditions. In the field, Ohwada & Taga (1972) found that particulate B₁ and B₇ concentrations in surface waters of the North Pacific were approximately 1% of the dissolved concentrations, whereas in coastal waters, particulate B₁ and B₇ concentrations were approximately 150% and 50% of the dissolved concentrations, respectively. The rapid turnover rates calculated for B₁₂ with respect to biological assimilation (on the order of a few hours; Koch et al. 2011, Taylor & Sullivan 2008) suggest that water column vitamin depletion due to biological scavenging is feasible in certain environments.

The environmental variables controlling B-vitamin synthesis are still unknown, and the scarcity of dissolved B vitamins observed in some locations could also be the results of a trace metal limitation. Although they are molecules of biogenic origin, the production of some vitamins requires cobalt and iron. Cobalt is the central coordinating ion in the B₁₂ molecule (**Figure 1c**), and the B₁ and B₇ biosynthetic pathways require the trace element iron (Chatterjee et al. 2008, Lin & Cronan 2011). Therefore, the synthesis of these organic growth factors is also linked to seasonal changes and geochemical processes that control the delivery and cycling of trace metals in the ocean.

Panzeca et al. (2009) evaluated the effect of ambient concentrations of dissolved cobalt on vitamin B₁₂ synthesis in the North Atlantic and found that the addition of that trace element enhanced B₁₂ synthesis by almost twofold over unamended controls. Importantly, this effect was observed only in surface waters where the dissolved cobalt concentration was less than 20 pM. Similar data sets are not available to evaluate whether B₁ and B₇ synthesis is iron limited. However, owing to the extremely low ambient concentrations of cobalt and iron in the open ocean (Boyd et al. 2012, Saito & Moffett 2002)—low enough to be considered limiting nutrients in many oligotrophic waters (Boyd et al. 2012)—metal limitation of vitamin synthesis is a real possibility in some regions. Furthermore, the colimitation of phytoplankton growth by iron and B₁₂ has been observed in different regions of the ocean (Bertrand et al. 2007, Koch et al. 2011, Panzeca et al. 2006) and under laboratory conditions (Bertrand et al. 2012), suggesting a strong link between the availability of the organic cofactors and trace metals.

Evaluating the role of vitamins in marine ecology has been difficult. Only a few studies have been comprehensive enough to estimate the importance of vitamins in primary productivity and species succession. For example, Menzel & Spaeth (1962) reported a moderate diatom bloom in the Sargasso Sea that coincided with the highest B₁₂ levels. Low ambient B₁₂ levels correlated with a shift from diatoms to coccolithophores. Diatoms are auxotrophic for B₁₂, but how the also B₁₂-requiring coccolithophores are able to survive without an exogenous source of B₁₂ is unclear (Helliwell et al. 2011). In an extensive study off La Jolla, California, Carlucci & Bowes (1970a) tried to correlate the concentrations of B₁, B₇, and B₁₂ over a six-month period with phytoplankton composition and succession. However, there was no statistical evidence that primary production

as a whole was limited by any of the vitamins. That said, the positive correlations between high vitamin levels in the water and high standing stocks of phytoplankton led Carlucci (1970) to hypothesize that phytoplankton may secrete vitamins, a supposition that was later proven true.

More recently, Sañudo-Wilhelmy et al. (2006) showed that B₁₂ availability influences the phytoplankton community composition in some coastal embayments, favoring large phytoplankton. Panzeca et al. (2006), Bertrand et al. (2007), and Koch et al. (2011) showed similar results in the Southern Ocean and the Gulf of Alaska: Field-based amendment experiments with B₁₂ enhanced the growth rates of large phytoplankton and shifted the algal communities to diatoms and dinoflagellates. Research on the effects of B₁ and B₇ on phytoplankton growth and diversity have been more limited; however, studies have also shown that B₁ availability could enhance the biomass of large phytoplankton in some coastal environments at certain times of the year (Gobler et al. 2007, Koch et al. 2012).

It is still unclear whether B vitamins are the limiting nutrient according to Liebig's law of the minimum and whether the ambient dissolved vitamin concentrations could be considered limiting at certain times and locations. The fact that field amendments of vitamins increase phytoplankton growth in many regions of the ocean (reviewed in Bertrand & Allen 2012) strongly suggests that phytoplankton communities in large areas are indeed limited by the availability of these organic metabolites. Those results are also consistent with field surveys showing that B vitamins are undetectable in large areas of the ocean (Sañudo-Wilhelmy et al. 2012). However, specific growth rates depend on the cell vitamin quota rather than on the medium concentration directly (Droop 1968). No study has measured intracellular B vitamins directly in phytoplankton, and therefore cell vitamin quotas are not well known. Nevertheless, cell quotas calculated for different species of phytoplankton using growth-rate half-saturation constants ranged from 0.02 to 13 pM for B₁₂, from 6 to 184 pM for B₁, and from 0.06 to 0.28 pM for B₇ (Droop 2007, Tang et al. 2010). The observed phytoplankton vitamin requirements are within the same order of magnitude as the dissolved vitamin levels measured in some marine systems for vitamins B₁₂ and B₁ (0–10 pM for B₁₂ and 0–500 pM for B₁) but are at least an order of magnitude lower than ambient B₇ levels (0–500 pM) (Sañudo-Wilhelmy et al. 2012). This comparison suggests that some limitation by some B vitamins (e.g., B₁₂ and B₁) is plausible, even if these arguments are based on only a relatively small sample set of cultured phytoplankton. Although laboratory data cannot be easily extrapolated to natural situations, where organisms rarely operate under conditions of optimal temperature or nutrition, they do give strong circumstantial evidence and a fair indication of the relative degrees of sensitivity of different organisms to a vitamin, which may help in ecological evaluations. Furthermore, the abundant and widespread distribution in the marine environment of a recently discovered B₁₂ acquisition protein (Bertrand et al. 2012) strongly supports the notion of B-vitamin limitation and the ecological importance of at least B₁₂ in marine systems.

POTENTIAL IMPACT OF B VITAMINS ON THE BIOLOGICAL PUMP

In the sections above, we established that B vitamins are required cofactors that are associated with a large number of biologically important enzymes in varied anabolic and catabolic pathways (Figure 4). Furthermore, most microphytoplanktonic organisms require B vitamins for cell function; some can synthesize them *de novo*, whereas others obtain them from an exogenous source in the environment. Although we are years away from establishing the true ecological role of B vitamins in the marine environment, the present scientific evidence strongly suggests that vitamin

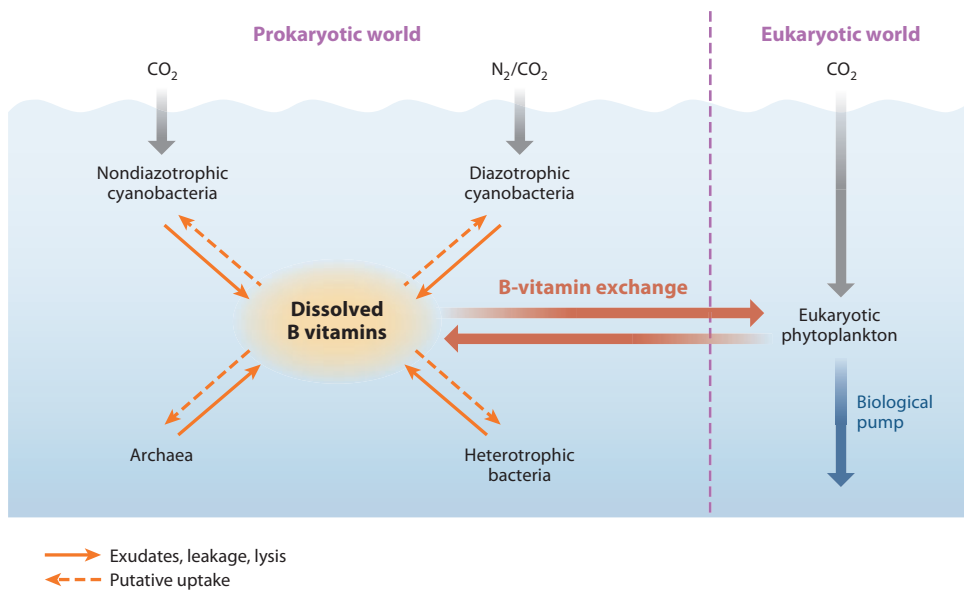


Figure 6

Generalized diagram showing the role of the microbial loop in the cycling of B vitamins in the ocean and the potential role of these organic metabolites in carbon sequestration. The magnitudes of vitamin uptake and release rates, shown as arrows, need to be evaluated.

availability could determine phytoplankton species composition and, in some areas, biomass production (Koch et al. 2011). These ecological responses to the availability or scarcity of some B vitamins could potentially impact the functioning of the system. For example, the major taxonomic and successional groups of phytoplankton are distinct in terms of carbon fluxes. A major part of the particulate matter that sinks to deep waters is made up of large, fast-sinking phytoplankton species such as diatoms, *Phaeocystis*, and coccolithophores. In contrast, flagellates and picoplankton appear to contribute little to the vertical export of particulate organic and inorganic carbon. Therefore, the dominance of one taxon over another owing to their vitamin specificity or vitamin availability could determine the strength of carbon sequestration via the so-called biological pump (Figure 6).

Regardless of whether B vitamins are among the limiting nutrients controlling carbon and nitrogen fixation in the ocean, they are clearly important. The marine vitamin cycle depicted in Figure 6 shows the complex interdependencies of vitamin producers and consumers and how ecosystem functioning (e.g., carbon and nitrogen cycles) is linked to the interspecies exchange of those ectocrines. This exchange is also influenced by the availability of dissolved vitamins in the environment, as organisms that have the biosynthetic pathways could also obtain them from exogenous sources, saving biosynthetic energy (Bonnet et al. 2010). The vitamin traffic among different species, as recently defined by Giovannoni (2012) (Figure 6), could be one reason for some of the connectedness observed in some microbial plankton communities (Fuhrman & Steele 2008). The vitamin cycle also illustrates the importance of synergistic interspecies partnerships to sustaining ecosystem dynamics. In contrast to mineral nutrients, there is no abiotic source of these organic factors, and their availability in the ocean depends solely on the biosynthetic activity of some organisms.

SUMMARY POINTS

1. We are beginning to better understand the varied distributions of dissolved B vitamins in the ocean as well as the widespread nature of vitamin auxotrophy in the microbial community. By searching the genomes of select marine microbial taxa for vitamin synthesis genes and vitamin-requiring enzymes, we have shown that vitamins likely play an important role in global microbially mediated biogeochemical cycles.
2. The dissolved vitamins in the sea originate mainly from the activities of some bacteria as well as some algae. Auxotrophy, or lack of it, does not relate to the metabolic mode of organisms, as both autotrophs and heterotrophs can require some B vitamins. Furthermore, auxotrophy does not strictly correlate with the trophic state of the marine environment, as auxotrophs have been isolated from and metagenomically characterized in varied oceanic regimes, ranging from oligotrophic to eutrophic regions.
3. Changes in the biological properties of waters during an algal bloom (removal of needed vitamins and release of other vitamins) may favor algae that require the vitamin released by the previous bloom (setting up a floral succession). This selective preconditioning of the waters may be one factor in the seasonal succession of algal species. It is also important to appreciate that bacteria are both producers and consumers of vitamins in the marine environment. Therefore, measurements of dissolved vitamins in the water reflect only the balance, not the rate at which release and uptake are occurring.
4. Changes in the phytoplankton community composition in response to species-specific vitamin requirements and vitamin availability could determine the strength of carbon sequestration via the biological pump.

FUTURE ISSUES

1. Any analysis attempting to unravel the role of vitamins in bacterial-phytoplankton blooms and successions will require more-comprehensive studies that consider the variability and specificity of these organisms' vitamin requirements as well as temporal and spatial changes in vitamin availability. Whether variations in vitamin requirements are also related to environmental availability is still unknown. The few data available on ambient vitamin concentrations are mainly from one location and are representative mostly of one sampling. Depth profiles of B vitamins have been measured in only a few locations. The degradation products and different chemical forms of these organic metabolites in seawater remain to be determined.
2. Vitamin producers and consumers need be identified *in situ*, and their contributions to vitamin flux need to be described via manipulative studies. It is important to establish the amount, type, and rate of vitamin production (and degradation) in varied regimes. The interdependence of these consumers and producers and their dependence on different microbial phytoplankton and bacterial species must also be defined.
3. Establishing the true B-vitamin requirements of phytoplankton will require developing a technique that directly measures intracellular vitamin levels. Such a technique will also

be needed to establish potential fluctuations that depend on the cell cycle (or other growth-limiting scenarios) and to help determine whether different requirements arose from distinct evolutionary pressures.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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