SEE COMMENTARY

Multiple B-vitamin depletion in large areas of the coastal ocean

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B vitamins are some of the most commonly required biochemical cofactors in living systems. Therefore, cellular metabolism of marine vitamin-requiring (auxotrophic) phytoplankton and bacteria would likely be significantly compromised if B vitamins (thiamin B_1, riboflavin B_2, pyridoxine B_6, biotin B_7, and cobalamin B_{12}) were unavailable. However, the factors controlling the synthesis, ambient concentrations, and uptake of these key organic compounds in the marine environment are still not well understood. Here, we report vertical distributions of five B vitamins (and the amino acid methionine) measured simultaneously along a latitudinal gradient through the contrasting oceanographic regimes of the southern California-Baja California coast in the Northeast Pacific margin. Although vitamin concentrations ranged from below the detection limits of our technique to 30 pM for B_2 and B_{12} and to ∼500 pM for B_1, B_6, and B_7, each vitamin showed a different geographical and depth distribution. Vitamin concentrations were independent of each other and of inorganic nutrient levels, enriched primarily in the upper mesopelagic zone (depth of 100–300 m), and associated with water mass origin. Moreover, vitamin levels were below our detection limits (ranging from ≤0.18 pM for B_{12} to ≤0.81 pM for B_1) in extensive areas (100s of kilometers) of the coastal ocean, and thus may exert important constraints on the taxonomic composition of phytoplankton communities, and potentially also on rates of primary production and carbon sequestration.

biogeochemistry | Northeast Pacific Ocean | coenzymes | water circulation | LC-MS

The evolution of organic catalysts (enzymes and coenzymes) that accelerated biochemical reactions by orders of magnitude compared with inorganic catalysis alone was a critical step for the emergence of major cellular processes (1). Well-known coenzyme molecules include the B vitamins, which catalyze many important biochemical reactions in central metabolism (2). Those reactions include rearrangement-reduction of C–C bonds and methyl transfer reactions, synthesis of deoxyribose/fatty acids/carbohydrates/branched-chain amino acids, electron transfer in oxidation-reduction reactions, and CO_2 fixation (2). This means that without an exogenous source of B vitamins, central metabolism in vitamin-requiring (auxotrophic) organisms would be compromised and the cells would not be able to grow, a situation that could have great bearing on many biological processes in the ocean, including the biological carbon pump.

In the marine environment, vitamins have been implicated as important factors regulating the growth and succession of phytoplankton species (3). This inference was based on the fact that many species of eukaryotic phytoplankton require vitamins when they are cultured in the laboratory (3–7). Although it was thought that marine prokaryotes were the major producers of vitamins in the sea (4), genomic data indicate that it is common for both eukaryotic phytoplankton and bacterioplankton to be auxotrophic for at least one B vitamin, mainly because they lack the complete biosynthetic pathways required to produce them (8). Therefore, if some marine bacteria also require vitamins for growth, their high biomass and rate of division (8) suggest that they might be quantitatively the most important consumers of those growth factors in the sea. These genomic and laboratory culture data imply that there are exogenous sources of B vitamins in the marine environment (4, 6–10). Furthermore, depletion and/or enrichment of various B vitamins is governed by the specificity of the predominant phytoplankton species for those vitamins. For example, a bloom of a B_{12}-auxotroph would deplete waters of that vitamin, although potentially enriching them with others (e.g., B_1, B_7). 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.

Despite their ecological relevance, the factors controlling the synthesis, uptake, and excretion of B vitamins in the marine environment are not fully understood. In general, coastal waters contain the highest concentrations of vitamins and open ocean habitats contain the lowest (10). In addition, it appears that waters of intermediate depth (depth of ∼200–500 m) contain higher levels than those above and below that depth (11). The seasonal distributions of vitamins indicate that their concentrations vary from undetectable to a few picomoles per liter (11, 12). However, those studies were mostly limited to vitamin B_{12} (9–16), and it is unclear whether or not those results can be extended to other essential B vitamins. The limited information that exists suggests that this is not the case (16–20). Only a few studies have been comprehensive enough to estimate the importance of vitamins in primary productivity and species succession, and they also have been focused mainly on the role of vitamin B_{12} (19, 20). Although concentrations of dissolved B_{12} and B_1 in some marine systems have been published (20), direct determination and depth profiles of B_7 have never been reported, and vitamins B_2 and B_6 have not been measured in any aquatic system. Therefore, a comprehensive assessment of the role of vitamins in marine ecology has also been difficult.

A major limitation in the study of B vitamins in the ocean has been the lack of an analytical technique for accurate concentration measurements in seawater. Although dissolved concentrations of B_1, B_7, and B_{12} have been measured in several marine systems (16–18), those results were obtained indirectly using microbiological assay methods that may not accurately reflect ambient conditions (18). Furthermore, these assays were

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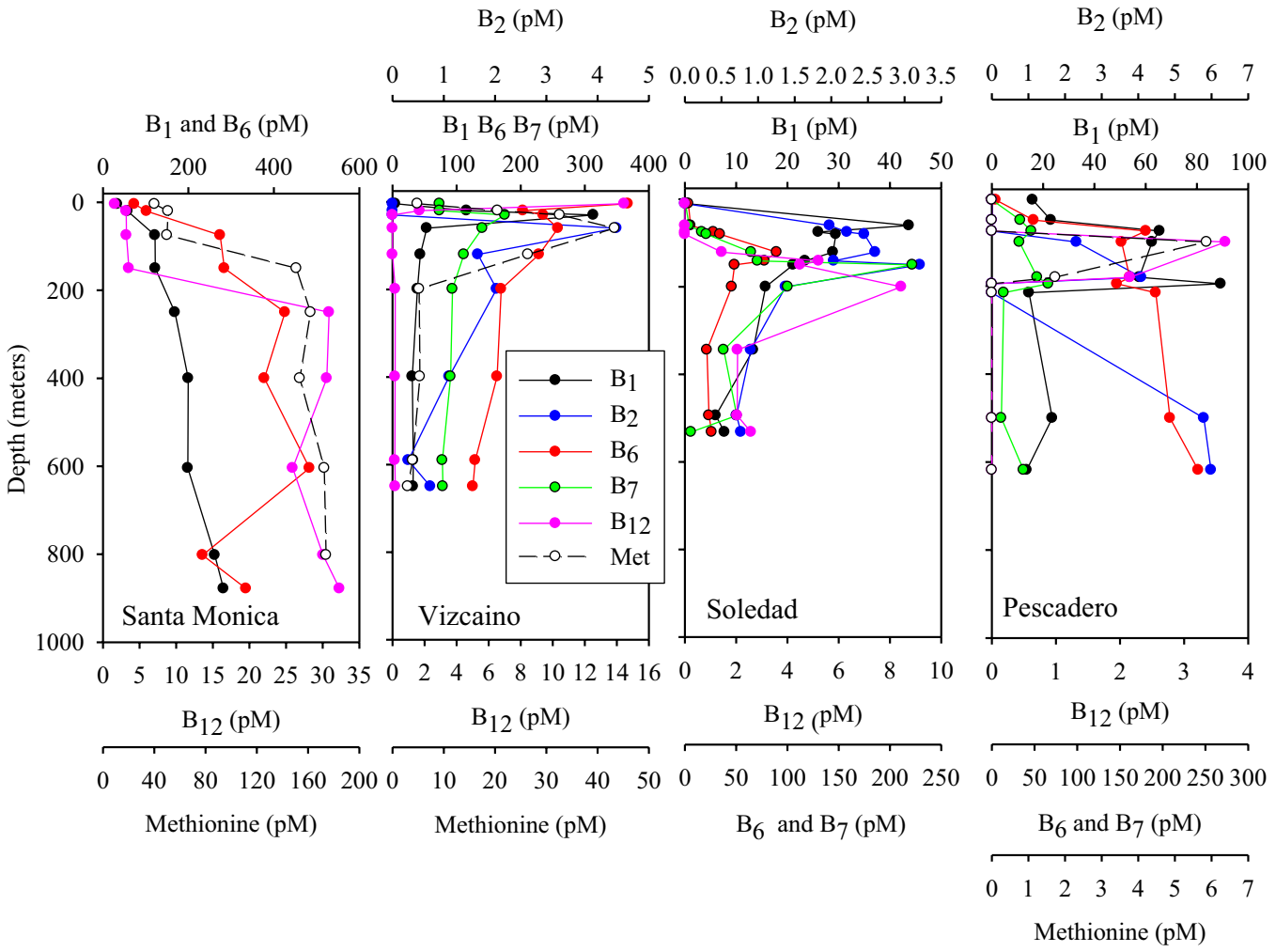


Fig. 1. Depth-profiles of dissolved B-vitamin and methionine concentrations measured at different locations (Santa Monica, Vizcaino, Soledad, and Pescadero) along the California-Baja California margin. The fluorescence maximum was detected at 50 m in Santa Monica, at 20 m in Vizcaino, and at a broad maximum between 50 and 75 m in Soledad and Pescadero. B-vitamin depth distributions were spatially variable and independent of each other, suggesting that Redfield-type stoichiometric relationships (37) cannot be used to predict the actual concentrations of these different organic factors.

conducted in 0.45–0.8 μm filtered seawater that may have included B-vitamin–producing bacteria in the filtrate (11, 18). Herein, we have developed a method to measure five dissolved B vitamins (B_1, B_2, B_6, B_7, and B_{12}) and methionine, a key amino acid that is often made by a B_{12}-dependent enzyme (4) directly in seawater, simultaneously using a modification of the method developed by Okbamichael and Sañudo-Wilhelmy (21) (*SI Appendix*). Furthermore, we used this technique to establish spatial and depth distributions of all those B vitamins along the southern California-Baja California coast in the Northeast Pacific margin (*SI Appendix*, Fig. S1 and Table S1). This margin is characterized by contrasting oceanographic conditions, attributable to the convergence of different water masses of subarctic, central North Pacific, and equatorial origin, which are likely to contain different phytoplankton and bacterial assemblages (22–24). We hypothesize that these water masses possess different growth factors, because different bacterioplankton and phytoplankton species seem to require and/or synthesize different vitamins (*SI Appendix*, Table S2).

Results and Discussion

Vertical distributions of dissolved B vitamins and methionine showed large spatial variability along the California-Baja California margin, ranging from undetectable to 30 pM for B_2 and B_{12} and from undetectable to ~500 pM for B_1, B_6, and B_7 (Fig. 1 and *SI Appendix*, Table S1). Fig. 1 compares the vertical profiles for all B vitamins and methionine measured in four distinct oceanographic regimes sampled along our transect: the northernmost station (Santa Monica; Fig. 1*A*) with hydrographic conditions typical of a subarctic domain (*SI Appendix*, Fig. S2); near 28° N latitude (Vizcaino; Fig. 1*B*), a latitudinal boundary that causes well-known biological discontinuities between northern and southern waters off Baja California (25); the southernmost station along our transect (Soledad; Fig. 1*C*), with waters of subtropical origin (*SI Appendix*, Fig. S2); and a station influenced by the Gulf of California (Pescadero; Fig. 1*D*). In contrast to the typical pattern observed for inorganic nutrients in the world ocean (surface minimum, a middepth maximum, and a gradual decrease with depth; *SI Appendix*, Fig. S3), the vertical distributions of B vitamins and methionine measured in this study were site-specific and independent of each other (Fig. 1). To understand the factors controlling the vertical distributions of B vitamins observed in our study, future studies will need to combine vitamin measurements with microbial community and metabolism information, as well as temporal stability and turnover times of each vitamin from each of the sampling sites.

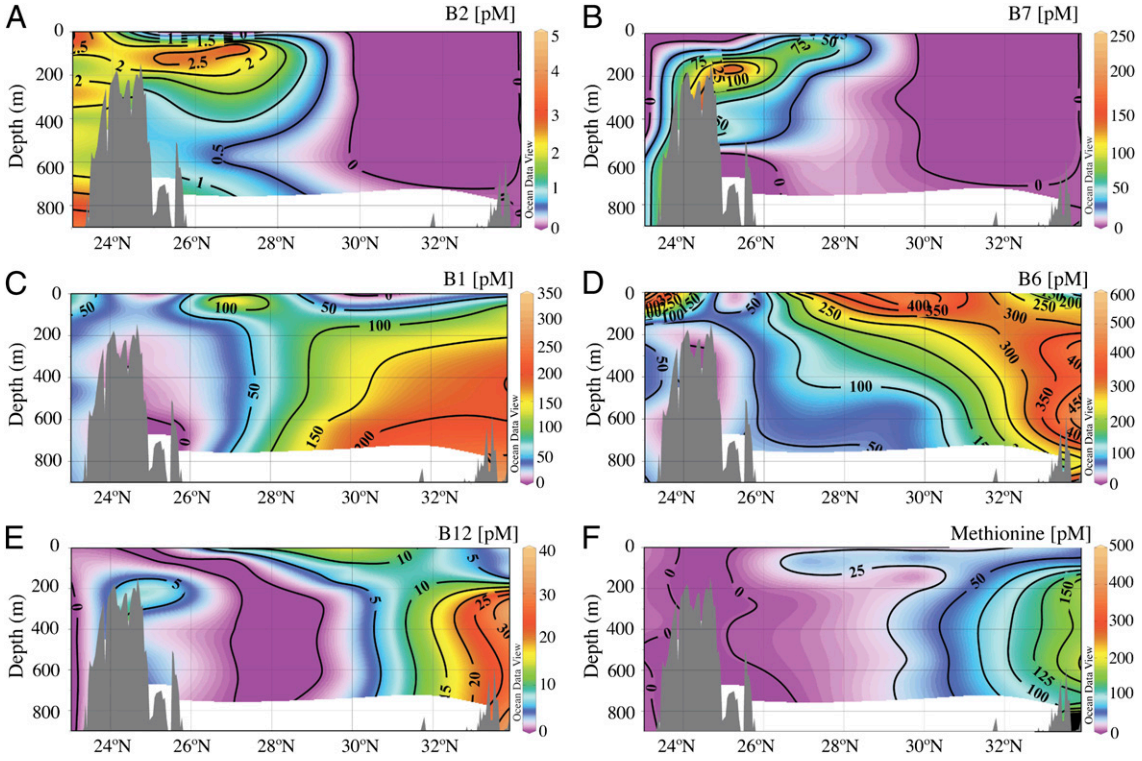


Fig. 2. Latitudinal distribution of dissolved B-vitamin and methionine concentrations measured along the California-Baja California margin in the autumn of 2009. These distributions show that B vitamins and methionine were undetectable in large areas of the coastal ocean, potentially influencing phytoplankton dynamics in those regions. The locations of the sampling stations and depths are shown in Fig. 3*B*. This figure was generated using Ocean Data View (odv.awi.de).

Despite those limitations, the B-vitamin maxima observed in the upper mesopelagic zone at some locations (Fig. 1) suggest that the microbial plankton groups found below the photic zone do not only contribute substantially to heterotrophic metabolism and nutrient cycling in the ocean (26) but that heterotrophic bacterioplankton may be net producers (either by remineralization of detritus and/or from excretion during growth or release attributable to cell lysis) of the growth factors required by eukaryotic auxotrophic phytoplankton found in the photic zone. However, our analysis of available genomic data and other published data indicates that heterotrophic bacteria are not the only de novo B-vitamin synthesizers (27, 28). The ubiquitous marine cyanobacteria, *Prochlorococcus* and *Synechococcus*, are also vitamin producers that make most, if not all, of their vitamins de novo (29) (*SI Appendix*, Table S2); thus, more research on the sources and sinks of the vitamins measured herein remains to be done.

Distributions of dissolved B vitamins (and methionine) showed large spatial variability along the California-Baja California margin (Fig. 2). Although we detected some vitamin B_6 in surface waters south of 28° N, the zonal distributions revealed the following trends: (*i*) the boundary conditions at 28° N separate waters lacking some B vitamins from those that contain them, and (*ii*) vitamins had different potential sources: those transported into the region from biological producers in tropical-subtropical waters (B_2 and B_7) vs. those from subarctic regions (B_1, B_6, B_{12}, and methionine; Fig. 2). Comparing those sectional latitudinal distributions with the main water masses along the margin (Fig. 3 *A* and *B*), we can attribute the large values of B_2 and B_7 (and B_6 in surface waters south of 24° N) to input of southern waters, composed of subtropical surface water and equatorial subsurface water (*SI Appendix*, Fig. S2) and transported into the region by the California undercurrent (Fig. 3*B*). Concentrations of vitamins B_2 and B_7 were undetectable in waters north of 28° N within the Southern California Bight eddy. In contrast, we can ascribe the source of vitamins B_1, B_6, and B_{12} and methionine to the equator-ward transport of northern water (subarctic water) composed of California current (CC) and North Pacific intermediate water (Fig. 3 and *SI Appendix*, Fig. S2). The presence of these waters seems to influence the distribution of those dissolved constituents observed between 100 and 800 m. Vitamins B_1, B_6, B_{12}, and methionine were nearly undetectable south of 28° N. The zonal distributions also showed a subsurface B_1 maximum at ~100 m at 28° N associated with deeper northern waters being brought to the surface by upwelling (Fig. 3*B*). The geographical patterns observed during our study (Fig. 2) are consistent with the presence of two independent eddies north and south of 28° N latitude during our sampling (Fig. 4), as well as an offshore flow at that latitude responsible for the north-to-south discontinuity in vitamin distributions. Although we did not establish microbial diversity during our cruise, the coupling between dissolved B-vitamin distributions and water mass origin is consistent with recent results showing that biogeographical patterns in microbial distributions are strongly influenced by ocean hydrodynamics (30). Future studies combining B-vitamin measurements with prokaryotic diversity will be needed to address that relationship.

Our results show that selected B vitamins and methionine are undetectable in large areas (100s of kilometers) of the coastal ocean (Fig. 2). The undetectable ambient B-vitamin concentrations in our study are not likely attributable to phytoplankton uptake, because the levels of chlorophyll *a* at our locations were low and not typical of phytoplankton blooms, although we cannot rule out scavenging by heterotrophic bacteria (*SI Appendix*, Fig. S1). Phytoplankton growth rate, abundance, and diversity, as well as biological processes, such as carbon fixation, could be limited by the scarcity of essential organic growth factors in large regions of the world ocean. The latest taxonomic survey (5) indicated that about 92% of rhodophytes, 91% of dinophytes, and

60% of haptophytes and heterokontophytes tested require an exogenous source of vitamin B_{12}. Another large percentage of phytoplankton species requires B_1 and B_7 (5) and the requirements for vitamins B_2 and B_6 have not yet been investigated. However, eukaryotic phytoplankton species are not the only organisms that depend on the B vitamins synthesized by other organisms. For example, genome annotation suggests that *Candidatus* Pelagibacter ubique (HTCC1062), a member of the abundant and ubiquitous SAR11 clade of heterotrophic marine bacteria, lacks biosynthetic pathways for vitamins B_1, B_5, B_7, B_{12} and organosulfur compounds, such as methionine (8, 31).

Oceanic food webs as well as climate dynamics are influenced by phytoplankton and bacterioplankton activity, because photosynthetic carbon fixation and heterotrophic respiration affect atmospheric and oceanic carbon dioxide concentrations (32). B-vitamin auxotrophy is widespread among microbial planktonic taxa, including large, fast-sinking phytoplankton species that influence carbon sequestration via the biological pump, as well as bacterioplankton that sustain elemental cycles. Our field results show that because large areas in the ocean do not contain sufficient B vitamins, the efficiency of the biological pump may be controlled not only by the availability of mineral nutrients, such as Fe, N, and P, but by vitamin accessibility. Although recent field studies have confirmed the ecological importance of B_{12}, because phytoplankton growth was enhanced by picomolar B_{12} amendments in both coastal and open ocean environments (12, 14, 15, 19, 20), no field study has yet addressed the relevance of the other B vitamins measured in the present study. Because some bacterial groups seem to be major producers of the organic cofactors required by eukaryotic phytoplankton, B vitamins illustrate the complexity of the prokaryote/eukaryote codependency via ectocrine relationships in the marine environment. Our results show that B-vitamin distributions are linked to ocean circulation, which, in turn, might have an impact on the ecological geography of the sea (33). Furthermore, it appears that concentrations of B vitamins are maximal in the upper mesopelagic zone. Hence, climate-driven changes in water-column stratification (34) and ocean circulation (35) could reduce vitamin input from the mesopelagic zone to the surface ocean and cause changes in bacterioplankton biogeography, respectively. This climate shift might disrupt ecosystem function via important vitamin-dependent biological processes, such as primary production and associated carbon export in the ocean.

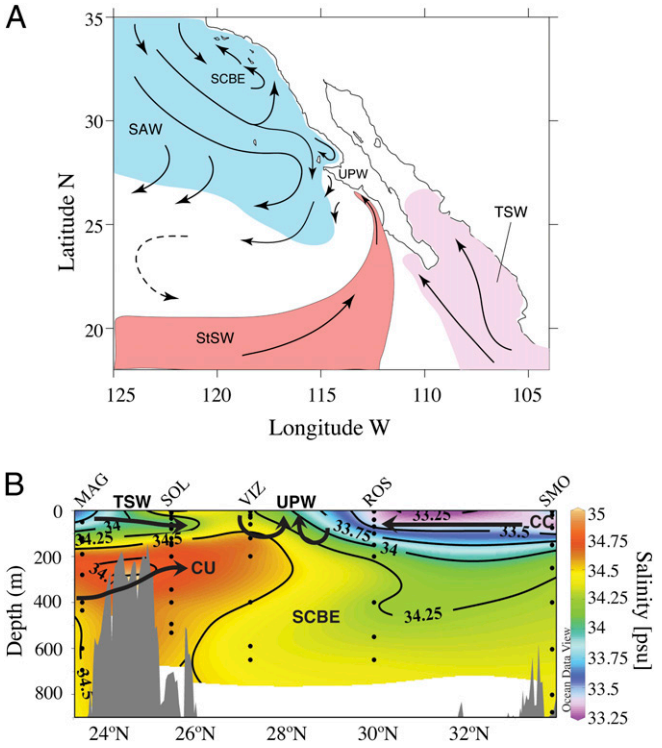


Fig. 3. Water circulation patterns along the California-Baja California margin. (*A*) Diagram illustrates the main water masses typical of our area of study. (*B*) Latitudinal distribution of salinity with water masses observed during our sampling campaign is shown. Water masses: CC, California current; CU, California undercurrent; MAG, Magdalena; ROS, Rosario; SAW, subarctic water; SCBE, Southern California Bight eddy; SMO, Santa Monica; SOL, Soledad; StSW, subtropical surface waters; TSW, tropical surface waters; UPW, upwelling; VIZ, Vizcaino.

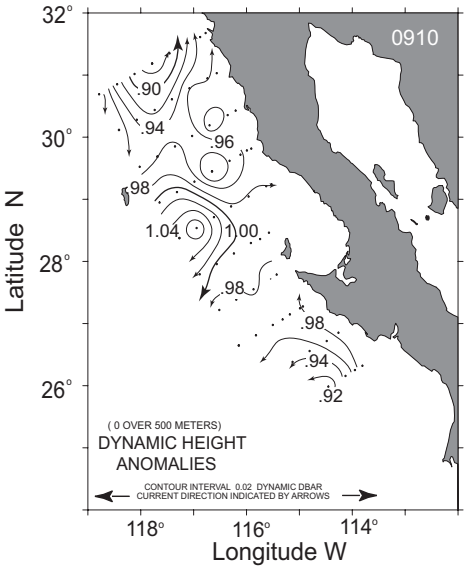


Fig. 4. Dynamic height anomalies (0/500 dbar) calculated along the Baja California Peninsula show the presence of two independent eddies north and south of 28° N latitude during our sampling, as well as an offshore flow at that latitude responsible for the north-to-south discontinuity in vitamin distributions (Fig. 2).

Methods

Water samples for B-vitamin and methionine analyses were collected at multiple depths at six locations (Santa Monica Basin, El Rosario, Vizcaino, Soledad, Magdalena, and Pescadero) along the southern California–Baja California coast in the Northeast Pacific margin (34° N to 23° N latitude) in October 2009 (*SI Appendix*, Fig. S1 and Table S1). Water samples for vitamin and methionine analyses (2-L) were filtered with acid-washed 0.2-μm polypropylene capsule filters, using a low-flow peristaltic pumping system at a rate of about 1 mL/min. Filtered samples were stored frozen in acid-cleaned high-density polyethylene (HDPE) dark bottles until analysis, according to a modification of the technique of Okbamichael and Sañudo-Wilhelmy (21). The technique is fully described in the *SI Appendix*; it consists of a solid-phase extraction onto a C_{18} resin at two different pHs and vitamin quantification using liquid chromatography/tandem MS. The detection limits of the technique were 0.81 pM for B_1, 0.67 pM for B_2, 0.61 pM for B_6, 0.23 pM for B_7, 0.18 pM for B_{12}, and 0.17 pM for methionine. Hydrographic casts were used to calculate the dynamic height anomaly at 0 dbar over the 500-dbar reference level according to Durazo and Baumgartner (36).

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