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C. Panzeca, A. Beck, Karine Leblanc, G.T. Taylor, D.A. Hutchins, et al.. Potential cobalt limitation of vitamin B12 synthesis in the North Atlantic Ocean. *Global Biogeochemical Cycles*, 2008, 22 (GB2029), 7 pp. 10.1029/2007GB003124 . hal-00702673

HAL Id: hal-00702673

<https://hal.science/hal-00702673>

Submitted on 22 Nov 2021

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Potential cobalt limitation of vitamin B₁₂ synthesis in the North Atlantic Ocean

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Received 15 October 2007; revised 11 February 2008; accepted 21 February 2008; published 26 June 2008.

[1] While recent studies have confirmed the ecological importance of vitamin B₁₂, it is unclear whether the production of this vitamin could be limited by dissolved Co, a trace metal required for B₁₂ biosynthesis, but found at only subnanomolar concentrations in the open ocean. Herein, we demonstrate that the spatial distribution of dissolved B₁₂ (range: 0.13–5 pmol L⁻¹) in the North Atlantic Ocean follows the abundance of total dissolved Co (range: 15–81 pmol L⁻¹). Similar patterns were observed for bacterial productivity (range: 20–103 pmol ³H leucine L⁻¹ hr⁻¹) and algal biomass (range: 0.4–3.9 μg L⁻¹). In contrast, vitamin B₁ concentrations (range: 0.7–30 pM) were decoupled from both Co and B₁₂ concentrations. Cobalt amendment experiments carried out in low-dissolved Co waters (~20 pmol L⁻¹) enhanced B₁₂ production two-fold over unamended controls. This study provides evidence that B₁₂ synthesis could be limited by the availability of Co in some regions of the world ocean.

Citation: Panzeca, C., A. J. Beck, K. Leblanc, G. T. Taylor, D. A. Hutchins, and S. A. Sañudo-Wilhelmy (2008), Potential cobalt limitation of vitamin B₁₂ synthesis in the North Atlantic Ocean, *Global Biogeochem. Cycles*, 22, GB2029, doi:10.1029/2007GB003124.

1. Introduction

[2] Vitamin B₁₂ availability plays an important role in marine ecosystems as it is required for the synthesis of several vital enzymes, including methylmalonyl-CoA mutase, methionine synthase and type II ribonucleotide reductase [Raux *et al.*, 2000; Martens *et al.*, 2002]. However, actual cellular quotas for vitamin B₁₂ among phytoplankton taxa are largely unknown [Carlucci and Bowes, 1972; Croft *et al.*, 2006; Droop, 2007]. In the few species for which B₁₂ growth kinetics data are available, half-saturation constants, K_s, average 1.4 pmol L⁻¹ (± 2.6 SD [Droop, 2007]), which is within the range of ambient open ocean concentrations of 0.3–2.4 pmol L⁻¹ [Panzeca, 2007]. This suggests that in some areas of the world's oceans some phytoplankton taxa are exposed to subsaturating vitamin B₁₂ concentrations, essentially living under a vitamin-limiting regime. In fact, recent experimental results from both coastal and open ocean environments clearly demonstrate that ambient levels of dissolved B₁₂ were growth-limiting because phytoplankton abundance was enhanced and community structure was altered by B₁₂ amendments [Sañudo-Wilhelmy *et al.*, 2006;

Panzeca *et al.*, 2006; Bertrand *et al.*, 2007; Gobler *et al.*, 2007].

[3] The biogeochemical cycling of Co has been well-studied especially in regards to its function in the enzyme carbonic anhydrase, where it has been shown to substitute for Zn in some phytoplankton species [Price and Morel, 1990; Sunda and Huntsman, 1995; Yee and Morel, 1996]. In addition, de novo synthesis of vitamin B₁₂ also requires Co [Raux *et al.*, 2000; Martens *et al.*, 2002], whose total dissolved concentrations in the open ocean are in the picomolar range (5–105 pmol L⁻¹ [Knauer *et al.*, 1982; Saito and Moffett, 2002]). Moreover, only a small fraction (fM) of the total dissolved Co pool is in the free ionic, or bioavailable form [Ellwood and van den Berg, 2001]. Therefore, vitamin B₁₂ synthesis could potentially be limited by Co availability. However, no field study has evaluated whether Co abundance influences the production and distribution of this important organic cofactor in oceanic surface waters.

[4] In order to test our hypothesis that ambient vitamin B₁₂ distributions are influenced by Co availability, we measured dissolved B₁₂ concurrently with Co concentrations in the water column and in phytoplankton in surface waters of the North Atlantic Ocean. In addition, shipboard Co amendment experiments were conducted using phytoplankton communities collected in two contrasting oceanographic regimes to evaluate whether the production of vitamin B₁₂ is limited by this trace element. One experiment took place on the Iceland Shelf, a neritic environment where dissolved Co levels were expected to be relatively high, and the other in the open waters of the North Atlantic, where

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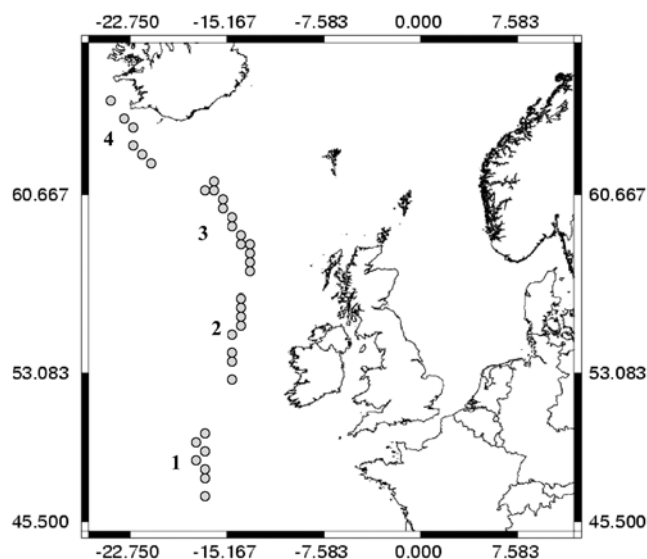


Figure 1. Map of sampling transect in the North Atlantic (start 47.10° N, 20.07° W; end 66.34° N, 24.58° W). Circles represent sampling locations (region 1, n = 8; region 2, n = 8; region 3, n = 15; region 4, n = 10). Numbers represent sampling regions 1–4.

dissolved Co levels were presumed to be very low. Furthermore, in order to validate the importance of Co for B₁₂ synthesis we also measured ambient concentrations and production of vitamin B₁, which does not require Co for its biosynthesis.

2. Materials and Methods

2.1. Sample Collection and Analysis

[5] Samples were collected on the R/V Seward Johnson as part of the North Atlantic Spring Bloom (NASB) 2005 project from 6 June 2005 to 11 July 2005 along four geographical transects from the Azores to Iceland (Figure 1). The North Atlantic bloom is one of the most dramatic and predictable biological events in the world ocean [Esaias *et al.*, 1986]. The obvious implications of this regional phytoplankton bloom for ocean carbon and nutrient cycling led to its selection as the first study site for the Joint Global Ocean Flux Study (JGOFS), during the North Atlantic Bloom Experiment (NABE) in 1989–1990 [Ducklow and Harris, 1993; Harrison *et al.*, 1993]. Integrated primary production during the bloom can reach levels of 50–150 mmol C m² d⁻¹ [Lochte *et al.*, 1993; Chipman *et al.*, 1993]. Investigators on the NASB cruise determined how biological community structure correlates with regional biogeochemistry (Leblanc *et al.*, manuscript in preparation, 2008), as well as possible future climate change-driven influences on the bloom [Feng *et al.* [2008]; J. M. Rose *et al.*, The effects of increased pCO₂ and temperature on the North Atlantic Spring Bloom: II, Microzooplankton dynamics, submitted to *Limnology and Oceanography*, 2008].

[6] The study area was divided into four provinces based on water temperature, salinity and known oceanic circulation patterns. Subtropical waters influenced region 1 (average temperature and salinity ± standard deviations: 14.5°C ± 0.73, 35.4 ± 0.10 ‰) while region 2, located over the Rockhall Hatton Plateau, was influenced by waters of the North Atlantic Current (12.0 ± 0.81°C, 35.3 ± 0.03 ‰). Region 3 was located in the deep Iceland Basin (11 ± 0.43°C, 35.2 ± 0.03 ‰) and region 4 (9.9 ± 0.79°C, 35.0 ± 0.22 ‰) comprises the shallow areas of the Iceland Shelf (Figure 1). Phytoplankton communities varied in each region, with region 1 phytoplankton typical of a central gyre community (cyanobacteria and nanoflagellates). A mixture of coccolithophores, diatoms and dinoflagellates dominated waters in region 2, while coccolithophores dominated phytoplankton community composition in the north (regions 3 and 4, Leblanc *et al.*, manuscript in preparation, 2008).

[7] Surface water samples were collected and analyzed for inorganic nutrients (PO₄³⁻, NO₃⁻), total chlorophyll *a*, bacterial productivity, dissolved vitamins (B₁₂, and B₁) and Co in different pools (total dissolved, labile dissolved and intracellular phytoplankton). Bacterial productivity, inorganic nutrients (PO₄³⁻, NO₃⁻) and total chlorophyll *a* samples were collected from a Niskin Bottle Rosette Sampler. Inorganic nutrients (PO₄³⁻, NO₃⁻) and total chlorophyll *a* were quantified by standard protocols [Parsons *et al.*, 1984] while bacterial productivity was measured by ³H leucine incorporation after Kirchman [1993]. Additional surface water samples (total, labile, intracellular Co; B₁, B₁₂) were pumped directly on-board using a weighted tow-fish and acid-washed Teflon[®] tubing. Samples for total and labile dissolved Co analyses were filtered through 0.22 μm polypropylene capsule filters and collected in 1-L acid-washed LDPE bottles. Samples for intracellular Co were collected on 0.4 μm acid-washed polycarbonate filters and quantified using the oxalate wash method described by Tovar-Sanchez *et al.* [2003]. These values were normalized to P as the above method also quantifies intracellular P [Sañudo-Wilhelmy *et al.*, 2004]. Dissolved vitamin (B₁ and B₁₂) samples were collected in the same way as the dissolved Co, but in 4-L collapsible cubitainers. Filtered samples for total dissolved and labile Co measurements were preconcentrated using established protocols [Bruland *et al.*, 1979; Beck and Sañudo-Wilhelmy, 2007] and quantified by High Resolution Inductively Coupled Plasma Mass Spectrometry (Thermo Finnegan Element 2). The portion of the total dissolved pool that was extracted onto preconditioned Chelex-100 resin at a flow rate of 10 ml/min was operationally defined as the kinetically labile Co species [Beck and Sañudo-Wilhelmy, 2007]. For vitamin analyses, 4 L of seawater were solid-phase extracted on board and analyzed by Reversed Phase High Performance Liquid Chromatography according to the method of Okbamihael and Sañudo-Wilhelmy [2004, 2005]. Methodological relative standard deviation values for replicated analyses were below 10% for inorganic nutrients and dissolved B-vitamins, while recoveries of dissolved Co were well-within 10% of certified reference materials.

2.2. Co Addition Experiments

[8] Two Co amendment experiments were conducted to quantify B₁₂ and B₁ production; one in region 2 typical of open ocean conditions, and another in region 4 using water from the Iceland Shelf. Inorganic nutrient concentrations at the onset of amendment experiments in region 2 were 4.3 $\mu\text{mol L}^{-1}$ NO₃⁻ and 0.25 $\mu\text{mol L}^{-1}$ PO₄³⁻, whereas in region 4 inorganic nutrient concentrations were 6.8 $\mu\text{mol L}^{-1}$ NO₃⁻ and 0.45 $\mu\text{mol L}^{-1}$ PO₄³⁻. Similarly, ambient total dissolved and labile dissolved Co concentrations were 17 pmol L⁻¹ and 7.8 pmol L⁻¹, respectively, in region 2 and about two times higher in region 4 (32 pmol L⁻¹ total dissolved, 12.7 pmol L⁻¹ labile dissolved). Ambient dissolved B₁₂ concentrations were 0.67 and 1.9 pmol L⁻¹, and B₁ levels were 2.6 and 3.7 pmol L⁻¹ in regions 2 and 4, respectively.

[9] Phytoplankton community composition determined microscopically at both stations was dominated by *Emiliana huxleyi* and other coccolithophores (Leblanc et al., manuscript in preparation, 2008). Unfiltered seawater for the amendment experiments was collected in 4-L clear polycarbonate bottles in the same manner as surface water Co samples. The experiment consisted of 6 bottles; 2 initials, 2 controls, and 2 Co amended (2.0 nmol L⁻¹ in region 2 and 0.2 nmol L⁻¹ in region 4). In addition, 2 bottles were also amended with B₁₂ at the same locations (100 pmol L⁻¹ in both regions) to determine the potential effect of B₁₂ on algal biomass. All experimental samples were handled in a class-100 trace metal clean van, and were then placed in a deckboard incubator at ambient temperature and ~30 % of surface light intensity for 3 d. Subsamples were collected for dissolved B₁₂, B₁ and algal biomass on day 1 and again at the end of the experiment (3 d later) and analyzed as described above.

2.3. Statistical Analysis

[10] To validate differences in measured total, labile dissolved Co, and dissolved B₁₂ between study regions a One-Way Analysis of Variance (ANOVA) was performed on the mean of total measurements within each region.

3. Results and Discussion

3.1. Spatial Trends in Dissolved Constituents

[11] Total dissolved surface concentrations of vitamin B₁₂ ranged from 0.1–2.5 pmol L⁻¹ along the sampling transects (Figure 2a). Generally, mean concentrations increased from south to north (mean: region 1, 0.7 pmol L⁻¹; region 2, 0.5 pmol L⁻¹; region 3, 0.7 pmol L⁻¹; region 4, 1.4 pmol L⁻¹). Mean total dissolved B₁₂ concentrations were not significantly different in regions 1–3 (one-way ANOVA, $p = 0.3$), while mean B₁₂ concentrations nearly doubled from region 1 to region 4 (one-way ANOVA; $p = 0.001$). The range of B₁₂ measured in this study was comparable to concentrations measured in the Southern Ocean (0.3–2.4 pmol L⁻¹ [Panzeca, 2007]), and up to two orders of magnitude lower than those measured in temperate coastal embayments (5–87 pmol L⁻¹ [Sañudo-Wilhelmy et al., 2006; Gobler et al., 2007]).

[12] Vitamin B₁₂ distributions were similar to spatial patterns of bacterial productivity, as both parameters show a general increase from region 1 to region 4 (Figures 2a and 2b). Bacterial productivity ranged from 20–103 pmol ³H leucine L⁻¹ hr⁻¹, and was relatively uniform in regions 1 and 2 with mean values of 31 and 33 pmol ³H leucine L⁻¹ hr⁻¹, respectively (Figure 2b). Similar to total dissolved B₁₂ distributions, bacterial productivity doubled from region 1 (31 pmol ³H leucine L⁻¹ hr⁻¹) to region 4 (67 pmol ³H leucine L⁻¹ hr⁻¹). These analogous distributions are not unexpected since bacteria are the primary producers of this organic growth factor [Raux et al., 2000; Martens et al., 2002].

[13] The same spatial trend was also observed for algal biomass (as total chlorophyll *a*). Mean chlorophyll *a* concentrations were 0.75, 1.3, 2.6, and 3.2 $\mu\text{g L}^{-1}$ in regions 1–4, respectively (Figure 2c). These results are contrary to what has been observed in coastal systems where algal biomass showed an inverse correlation with dissolved vitamin B₁₂ [Sañudo-Wilhelmy et al., 2006]. Concentrations of inorganic nutrients PO₄³⁻ and NO₃⁻ increased from regions 1–3 (mean PO₄³⁻, NO₃⁻ ($\mu\text{mol L}^{-1}$): region 1, 0.14, 2.5; region 2, 0.18, 3.3; region 3, 0.3, 4.3) similar to the above parameters, but decreased in region 4 (mean PO₄³⁻, NO₃⁻ ($\mu\text{mol L}^{-1}$): 0.24, 2.8). It is unclear whether those distributions reflect differences in inorganic nutrient inputs within the different sampling regions due to vertical advection and terrestrial runoff, or increased algal uptake in region 4.

[14] Similar to the spatial distributions of total dissolved B₁₂, concentrations of total dissolved Co were also not significantly different within regions 1–3 (one-way ANOVA; $P = 0.6$), but were significantly higher (one-way ANOVA; $p = 0.002$) in region 4 (mean: region 1, 19 pmol L⁻¹; region 2, 25 pmol L⁻¹; region 3, 26 pmol L⁻¹; region 4, 45 pmol L⁻¹; Figure 2d). These concentrations are in agreement with those reported previously for Atlantic (5–87 pmol L⁻¹ [Saito and Moffett, 2002]) and Pacific waters (30–105 pmol L⁻¹ [Knauer et al., 1982]). Spatial trends in labile dissolved Co concentrations were also similar to the gradients observed for the total dissolved Co and B₁₂ pools (Figure 2e), and increased from region 1 to region 4 (mean concentrations of 4 pmol L⁻¹, 5 pmol L⁻¹, 5 pmol L⁻¹, and 16 pmol L⁻¹ from region 1 to 4 respectively; Figure 2e). This kinetically labile, and potentially more bioavailable Co fraction [Campbell, 1995] accounted for about 20–40% of the total dissolved Co pool.

[15] B-vitamins are chemically labile organic molecules with half-lives in seawater of <24 h [Gold et al., 1966; Carlucci et al., 1969], suggesting that elevated concentrations must generally be associated with local production. Thus, similar spatial trends in B₁₂, total dissolved, and labile dissolved Co pools (Figure 2) suggest that B₁₂ synthesis could be responsive to the availability of this trace element. Increasing Co concentrations from south (region 1) to north (region 4) may be partially driven by inputs from coastal margins (from reduced sediments and/or freshwater sources [Knauer et al., 1982; Sañudo-Wilhelmy and Flegal, 1996]). Consistent with a coastal source, the highest levels of Co (and B₁₂) were measured in the low-salinity (35.0 ± 0.06 ‰) waters of the continental shelf in region 4. In contrast, the

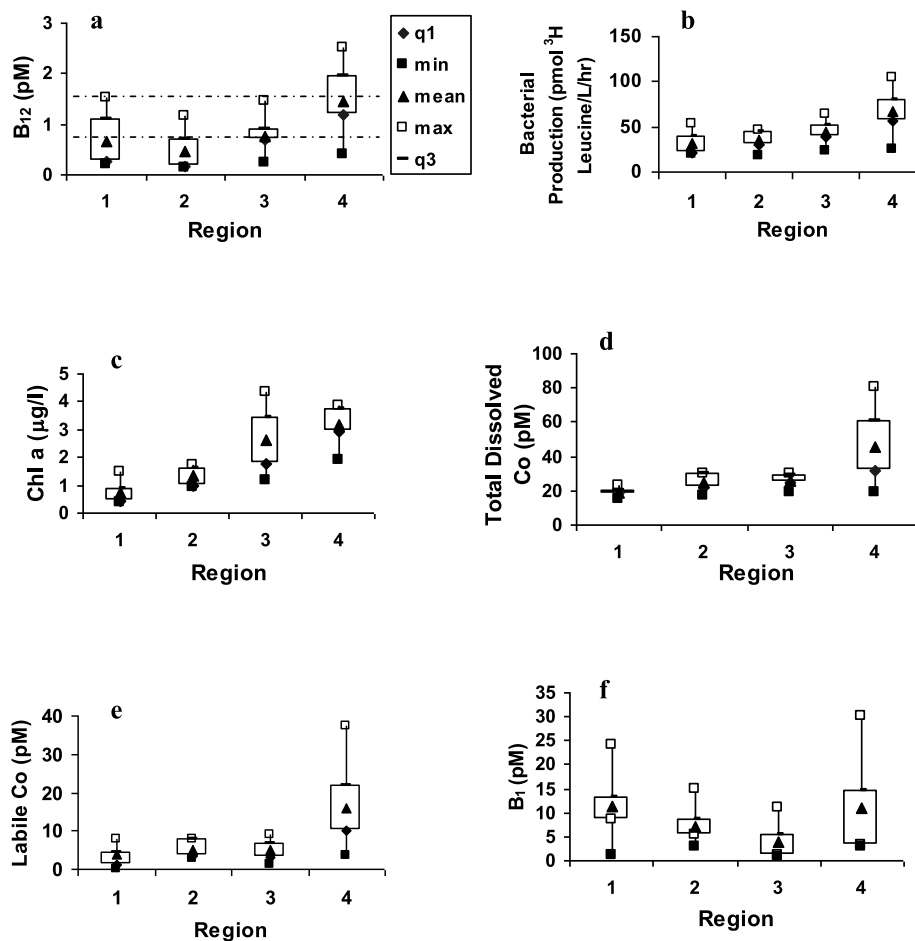


Figure 2. Box and whisker plots of (a) total dissolved B₁₂, horizontal lines represent K_s values reported by *Droop* [2007] (see text); (b) bacterial productivity; (c) total Chl; (d) total dissolved Co; (e) labile dissolved Co; and (f) total dissolved B₁. Quartile 1 (q1) represents the 25th percentile and Quartile 3 (q3) represents the 75th percentile.

lowest ambient levels of both Co and B₁₂ were detected farther south (region 1; $35.4 \pm 0.02\%$) in an area dominated by subtropical gyre waters distant from any coastal source.

[16] The south to north gradient of dissolved B₁ was not as prominent as that observed for B₁₂ and Co. The range of total dissolved B₁ concentrations was highest in region 4 (range; 2.8–30 pmol L⁻¹), although the mean B₁ concentrations were highest in region 1 (11 pmol L⁻¹; Figure 2f). The lowest B₁ levels were measured in regions 2 and 3 (mean: 6.8, 4.0 pmol L⁻¹, respectively). Overall, B₁ concentrations were five to ten times higher than ambient B₁₂ levels, and the two B-vitamin concentrations were not correlated. This decoupling between ambient B₁ and B₁₂ distributions could reflect different production and consumption rates, or different cellular requirements of these two vitamins among phytoplankton and bacterial communities. This is consistent with the survey reported by *Croft et al.* [2006] which showed that of the phytoplankton species

surveyed 10% had a known B₁ requirement versus 50% which required an exogenous supply of B₁₂.

3.2. Importance of Cobalt for Phytoplankton Dynamics and B₁₂ Synthesis in the North Atlantic

[17] To evaluate the potential for Co and vitamin B₁₂ limitation of phytoplankton growth in the North Atlantic, we compared the water column and intracellular phytoplankton stoichiometric ratios measured during our study with those reported in the literature (Figure 3). Labile dissolved Co: total dissolved P ratios (range, mmol Co: mol PO₄³⁻: region 1, 0.001–0.2; region 2, 0.01–0.04; region 3, 0.01–0.03; region 4, 0.004–0.4) were on average 1–2 orders of magnitude lower than intracellular Co:P ratios measured during this study in the field-collected phytoplankton (range, 0.12–6.2 mmol Co: mol PO₄³⁻, Figure 3). Lowest mean intracellular Co:P (0.12 mmol Co: mol PO₄³⁻) was found in region 1 and the highest mean ratio (2 mmol Co: mol PO₄³⁻) in region 4 (Figure 3b). These stoichiometric

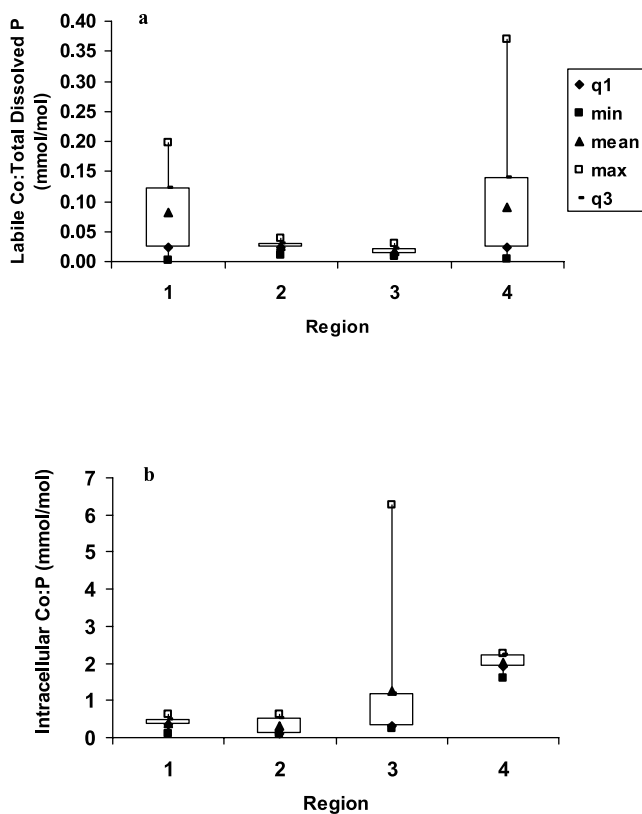


Figure 3. (a) Labile dissolved Co:total dissolved P (mmol:mol), (b) intracellular Co:P (mmol:mol). All ratios reported are normalized to P. Quartile 1 (q1) represents the 25th percentile and Quartile 3 (q3) represents the 75th percentile.

calculations suggest that phytoplankton were not Co-limited during our study. This assumption is consistent with our field amendment experiments which showed that Co additions did not enhance phytoplankton biomass over unamended control incubations (total chl *a*; one-way ANOVA; $P > 0.5$). Furthermore, the intracellular Co:P ratios found in the field-collected phytoplankton populations are in agreement with those reported in cultures of diatoms and coccolithophorids (0.06–20 mmol Co:mol PO_4^{3-} [Sunda and Huntsman, 1995; Ho *et al.*, 2003]).

[18] Although Co availability did not seem to limit phytoplankton growth in the North Atlantic during our study, the availability of this trace metal may exert control over B₁₂ synthesis in areas where ambient Co concentrations are low. Consistent with that hypothesis, Co additions increased B₁₂ concentrations by two-fold over unamended controls in region 2 (Figure 4a), where levels of dissolved Co (and B₁₂) were the lowest (17.1 pmol L⁻¹ Co; 0.67 pmol L⁻¹ B₁₂; Figure 2). In contrast, there was no statistically significant (one-way ANOVA; $p > 0.5$) increase in B₁₂ production over control incubations in waters from region 4 over the Iceland Shelf (Figure 4a) where ambient Co and B₁₂ levels were among the highest (32 pmol L⁻¹ Co; 1.9 pmol L⁻¹ B₁₂; Figure 2). However, since the concentration of Co added in region 4 was ten times lower than the concentration added in

region 2, we cannot totally rule out the fact that ambient Co concentration did not limit B₁₂ synthesis in region 4. Furthermore, B₁ production showed no response to the Co additions, as no difference (one-way ANOVA; $p > 0.5$) was observed over control amendments in either location (Figure 4b). These results were not unexpected given that Co is not required for B₁ synthesis. Although Co concentrations added in our amendments were relatively high (0.2–2 nmol L⁻¹), we believe that our Co additions did not induce a deleterious response as previous Co enrichment studies have shown no negative affect on phytoplankton growth at added Co concentrations ranging from 3 to 8 nmol L⁻¹

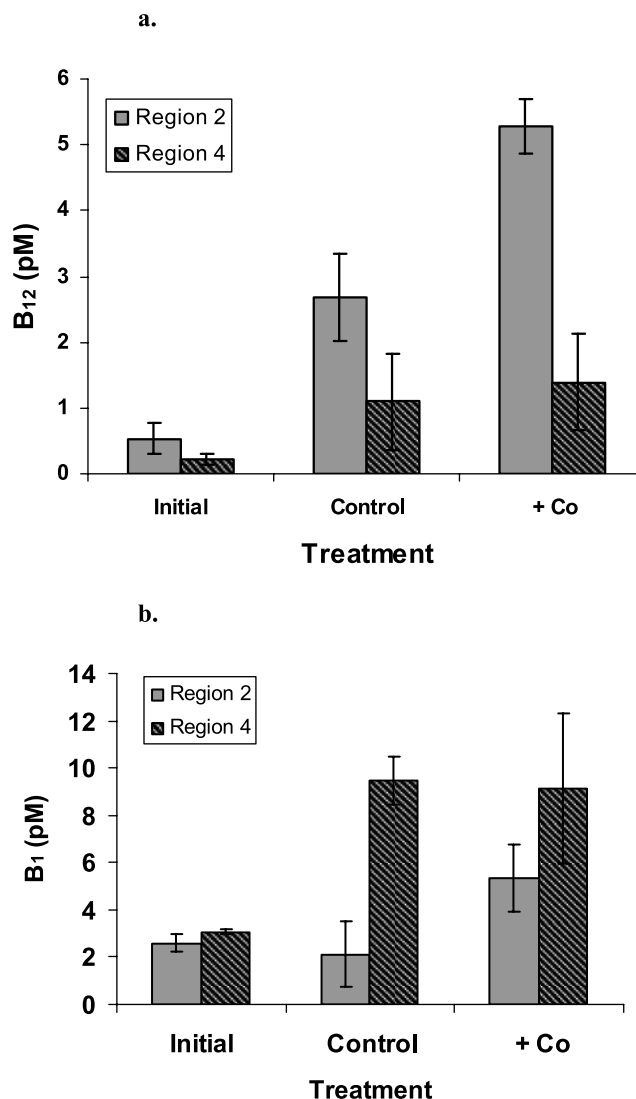


Figure 4. (a) B₁₂ production in Co additions. B₁₂ concentrations increased over control incubations after 3 d in experiments conducted in region 2 only. (b) B₁ concentration in Co amendment experiments. Based on the different oceanographic regimes (see methods), it was assumed that ambient Co concentrations were higher in region 4 than in region 2. Therefore, concentrations of Co added were 0.2 nmol L⁻¹ and 2.0 nmol L⁻¹, respectively.

[Granéli and Risinger, 1994; Mitrovic et al., 2004]. Furthermore, inorganic nutrient (NO₃⁻ and PO₄³⁻) concentrations in the experimental bottles were depleted by 40–60% at the end of the incubations, suggesting that there was active biological growth during the experiment in region 2. Future studies will need to establish the minimal Co levels required for B₁₂ synthesis.

[19] It is unclear which species were responsible for the production of vitamin B₁₂ in our experiments. However, the production of vitamin B₁₂ could reflect the preferential stimulation of cyanobacteria, which possess the genes for B₁₂ synthesis (www.jgi.doe.gov), and are also known to have a higher Co requirement than eukaryotic phytoplankton [Sunda and Huntsman, 1995; Saito et al., 2002]. An alternative hypothesis is that coccolithophorids were responsible for B₁₂ production as originally proposed by Carlucci and Bowes [1972], as these organisms also have a specific requirement for Co [Sunda and Huntsman, 1995]. Although coccolithophorids were abundant in ambient waters from which our field incubations were conducted, their genomic sequence is not yet available to verify whether or not this organism can indeed produce B₁₂. It is also unclear whether or not vitamin B₁₂ was released by healthy cells during our experiments (Figure 4a). However, excess large corrinoid ring molecules, much like those forming vitamin B₁₂'s backbone, are known to be secreted to the surrounding environment by prokaryotes when the intracellular levels are high [e.g., Harris et al., 1993].

[20] While Co has a well-accepted function in carbonic anhydrase in phytoplankton [Price and Morel, 1990; Sunda and Huntsman, 1995; Yee and Morel, 1996], our results suggest that this trace metal may also influence the cycling of vitamin B₁₂ in oceanic waters. Given the increased growth response of phytoplankton to B₁₂ additions in an array of marine systems [e.g., Sañudo-Wilhelmy et al., 2006; Panzeca et al., 2006; Bertrand et al., 2007; Gobler et al., 2007], it could be argued that limitation of B₁₂ synthesis by Co could indirectly influence phytoplankton growth and species composition in some areas of the world ocean.

[21] **Acknowledgments.** This research was supported by the U.S. National Science Foundation (OCE-0351999; OCE-0423418). The authors would like to thank the captain and crew of the RV Seward Johnson for assistance with sample collection. Additional contributions to the manuscript were made by Steven W. Wilhelm for chlorophyll data, Matthew Cottrell and Vanessa Michelou for bacterial productivity measurements, and Mine Berg for inorganic nutrient data.

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