B Vitamins as Regulators of Phytoplankton Dynamics

Without an adequate supply of dissolved vitamins, many species of phytoplankton do not grow. Additions of inorganic nutrients like phosphorus and nitrogen, and trace metals like iron, are not alone adequate to sustain life—a practical lesson learned quickly by experimental biologists when they try to keep eukaryotic phytoplankton cultures alive in their labs. The reason is that coenzymes such as B vitamins are also required for many metabolic pathways. For example, vitamin B_1 serves as a cofactor for a large number of enzymatic systems, including the pyruvate dehydrogenase complex required for the metabolism of carbohydrates (glycolysis) and amino acid synthesis [*Vandamme*, 1989]. Vitamin B_{12} is used primarily to assist two enzymes: methionine synthase, which is involved in DNA synthesis, and methylmalonyl CoA mutase, which is required for inorganic carbon assimilation [*Lindemans and Abels*, 1985].

Recognition of the importance of external metabolites such as B vitamins for phytoplankton growth is not new. The pioneering research of Alberto F. Carlucci, Michael R. Droop, and Luigi Provasoli carried out in the 1950s, 1960s, and 1970s clearly established that exogenous B vitamins are essential for growth of numerous phytoplankton species, notably taxa with exoskeletons, such as diatoms and coccolithophores with high sinking-velocities. What is surprising is that the oceanographic community has largely ignored the importance of extracellular vitamins for the past 30 years, as most recent research efforts have been focused on studying how mineral nutrients influence phytoplankton blooms.

Laboratory data generated decades ago [e.g., *Provasoli and Carlucci*, 1974] strongly suggest that a large percentage of all phytoplankton species in culture collections are B_{12} auxotrophs (which require an exogenous source of B_{12}). In addition, the recent study of *Croft et al.* [2005] provides evidence suggesting an algal-bacterial symbiosis in which algal exudates provide substrate for bacterial metabolism in exchange for vitamin B_{12} produced by bacteria.

These results are not unexpected since prokaryotes are believed to be the major producers of vitamins [e.g., *Provasoli,* 1963; *Provasoli and Carlucci,* 1974]. Thus, synergistic interactions between phytoplankton and bacteria may be the most important mechanism governing biogeochemical cycling of vitamins in the marine environment.

Despite numerous laboratory culture studies [e.g., *Provasoli and Carlucci,* 1974; *Croft et al.,* 2005], there is very little field evidence supporting the hypothesis that vitamin availability directly influences phytoplankton growth at the community level. The field verification of the vitamin requirement is critical because although the recent genomic analysis of *Croft et al.* [2005] shows that phytoplankton require vitamin B_{12}, this requirement could be extremely low and inconsequential for phytoplankton blooms and marine food dynamics. However, the limited field data that exist for temperate coastal waters suggest that indeed exogenous dissolved vitamin B_{12} preferentially stimulates growth of large phytoplankton [*Sañudo-Wilhelmy et al.,* 2006]. If such field data is correct, then B-vitamin availability may influence phytoplankton species composition.

In contrast to tiny picophytoplankton and nanophytoplankton, larger phytoplankton taxa typically have larger predators that form rapidly sinking fecal pellets, which increase the downward export of organic matter [*Longhurst and Harrison,* 1989]. If findings reported by *Sañudo-Wilhelmy et al.* [2006] are operative in offshore waters, then B vitamin availability may exert control indirectly over proportions of surface productivity exported to deeper waters, by influencing which phytoplankton taxa dominate. In other words, the efficiency of the biological pump and the carbon sequestration potential of the ocean may be controlled by B vitamin cycling, as well as by macronutrient and iron availability.

B-Vitamins and Phytoplankton in the Southern Ocean

Understanding how the combination of B vitamins and iron contributes to limiting surface primary productivity is of particular importance in high-nitrate/low-chlorophyll (HNLC) regions such as the Southern Ocean, where macronutrients are abundant. Such regions thus can provide a possible sink for atmospheric carbon. Recent field experiments carried out during the 2005 Complex Pelagic Interactions in the Southern Ocean (ICEPOS) campaign suggest that phytoplankton growth in the Southern Ocean indeed is colimited by B vitamins and iron (Figure 1). Similar to previous reports [e.g., *de Baar et al.,* 1999], these results show that experimental amendments of iron alone to surface water samples increased net accumulation of phytoplankton biomass twofold over control treatments after 12 days ($p < 0.05$, t test; Figure 1).

What had not been shown before was that the addition of combined vitamins B_1 and B_{12} also stimulated growth, yielding a significant 1.5-fold increase in abundance relative to control incubations ($p < 0.05$). Combined enrichment of B vitamins and iron yielded a synergistic threefold increase in biomass, suggesting potential colimitation by iron and B vitamins.

Bacterial production measurements in the same incubation experiments reveal no evidence of vitamin limitation (Figure 1). While the addition of iron alone or the combination of added iron and B vitamins increased bacterial production twofold over control treatments, the addition of vitamins alone had no effect on bacterial production. The iron alone and iron and B vitamin combined treatments yielded indistinguishable production rates, suggesting that increased bacterial production was caused solely by iron addition. These results are expected because prokaryotes, in contrast to eukaryotic phytoplankton, appear able to synthesize B vitamins [e.g., *Provasoli and Carlucci,* 1974; *Croft et al.,* 2005]. The observed iron stimulation of bacterial production in the Southern Ocean observed in the ICEPOS cruise is consistent with previous studies [e.g., *Hutchins et al.,* 2001]. However, it is unclear whether iron availability influences vitamin B-synthesis by bacteria.

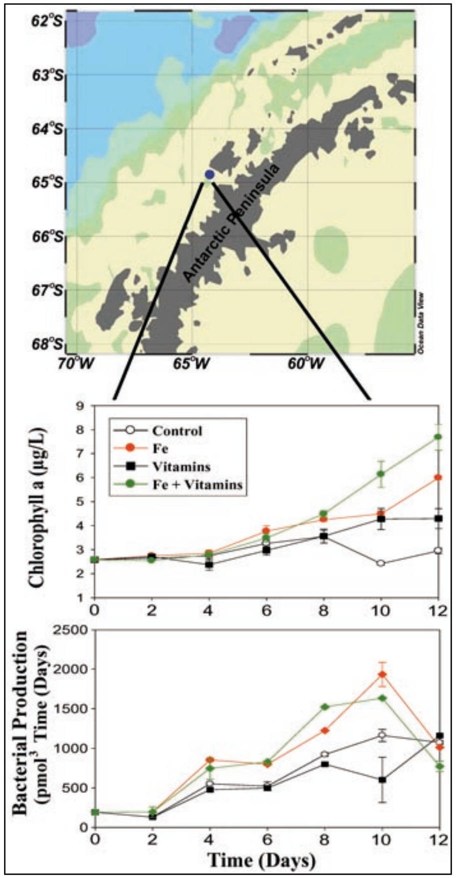

Fig. 1. Effect of vitamin (B_1 and B_{12}) and iron amendments on net production of total phytoplankton biomass (as chlorophyll a*) and bacterial production (as ^{3}H-leucine incorporation). Experiments were conducted from 8 to 20 February 2005 at one location (64.87ºS, 64.18ºW) in the Antarctic Peninsula sector of the Southern Ocean. Levels of inorganic nutrients were considered not limiting as they were in the micromolar range (1.5 µM PO_4, 19.41 µM NO_3). The decline in bacterial production observed after 10 days is attributed to mortality. Error bars represent standard deviation of duplicate incubations.*

By C. Panzeca, A. Tovar-Sanchez, S. Agustí, I. Reche, C. M. Duarte, G. T. Taylor, and S. A. Sañudo-Wilhelmy

B Vitamins cont. on page 596

cont. from page 593

Implications for the Biological Pump

Because of the importance of the Southern Ocean in influencing atmospheric carbon dioxide levels and Earth's climate [e.g., *Sarmiento and Orr,* 1991], a fuller understanding of mechanisms controlling carbon sequestration via phytoplankton production and sedimentation in that region is vital. While major iron fertilization studies performed in the Southern Ocean clearly established the importance of this trace element on phytoplankton growth in that region [e.g., *de Baar et al.,* 2005, and references therein], the results presented in Figure 1 show, for the first time, the importance of B vitamins in a HNLC region.

These results are not unique to the Southern Ocean, as vitamin B_{12} additions also increased growth of large phytoplankton taxa in temperate coastal waters [*Sañudo-Wilhelmy et al.,* 2006], suggesting that vitamin limitation also might affect coastal environments where inorganic nutrients and bioactive trace metals are abundant.

This current study suggests that the availability of B vitamins may be as relevant as iron is to nutrient limitation in the Southern Ocean. B vitamins are required for growth of all unicellular eukaryotes, photosynthetic or not. Therefore, the efficiency of the biological carbon pump also could be controlled by vitamin availability. However, exogenous vitamins may be much scarcer than iron in seawater. In fact, iron is one of the most abundant elements in the Earth's crust, and its availability in the ocean is controlled by large-scale geochemical processes. In stark contrast, the only source of B vitamins is presumably the biosynthetic activity of prokaryotes [e.g., *Provasoli,* 1963; *Provasoli and Carlucci,* 1974; *Croft et al.,* 2005], whose activities are relatively low in the cold Southern Ocean.

A complete understanding of mechanisms controlling marine primary production has been lacking because the organic regulation of phytoplankton fertility largely has been ignored. The prevailing paradigm for many decades has focused solely on iron and inorganic nutrients as determinants of phytoplankton dynamics. The field results presented here necessitate further exploration into the importance of vitamins as growth factors for phytoplankton in the world ocean.

Acknowledgments

This research was supported by the U.S. National Science Foundation (OCE-0351999) and the Spanish National Programme of Polar Research. This research was carried out on the Spanish Research Vessel *Hesperides*.

References

Croft, M. T., A. D. Lawrence, E. Raux-Deery, M. J. Warren, and A. G. Smith (2005), Algae acquire vitamin B-12 through a symbiotic relationship with bacteria, Nature, 438, 90–93.

de Baar, H. J. W., J. T. M de Jong, R. F. Nolting, K. R. Timmermans, M. A. van Leeuwe, U. Bathmann, M. R. van der Loeff, and J. Sildam (1999), Low dissolved Fe and the absence of diatom blooms in remote Pacific waters of the Southern Ocean, *Mar. Chem., 66,* 1–34.

de Baar, H. J. W., et al. (2005), Synthesis of iron fertilization experiments: From the Iron Age in the Age of Enlightenment, *J. Geophys. Res., 110,* C09S16, doi:10.1029/2004JC002601.

Hutchins, D. A., B. J. Cambell, M. T. Cotrell, and S. Takeda (2001), Response of marine bacterial community to iron additions in three iron-limited regimes, *Limnol. Oceanogr., 46,* 1535–1545.

Lindemans, J. and Abels, J. (1985) Vitamin B_{12} and related Corrinoids. In *Modern Chromatographic Analysis of the Vitamins*, Dekker: New York, 499-539.

Longhurst, A. R., and W. G. Harrison (1989), The biological pump—Profiles of plankton production and consumption in the upper ocean, *Prog. Oceanogr., 22,* 123.

Provasoli, L. (1963), Organic regulation of phytoplankton fertility, in *The Sea,* vol. 2, edited by M. N. Hill, pp. 165–219, Wiley-Interscience, New York.

Provasoli, L., and A. F. Carlucci (1974), in *Algal Physiology and Biochemistry,* edited by W. D. P. Stewart, p. 741, Blackwell, Malden, Mass.

Sañudo-Wilhelmy, S. A., C. J. Gobler, M. Okbamichael, and G. T. Taylor (2006), Regulation of phytoplankton dynamics by vitamin B_{12}, *Geophy. Res. Lett., 33,* L04604, doi:10.1029/2005GL025046.

Sarmiento, J. L., and J. C. Orr (1991), Three-dimensional simulations of the impact of Southern Ocean nutrient depletion on atmospheric CO_2 and ocean chemistry, *Limnol. Oceanogr., 36,* 1928–1950.

Vandamme, E. J. (1989), Vitamins and Related Compounds Via Micro-Organisms: A Biotechnological View, In *Biotechnology of Vitamins, Pigments, and Growth Factors,* Elsevier, New York. 1-11.

Author Information

Caterina Panzeca, Marine Sciences Research Center, Stony Brook University, Stony Brook, N.Y., E-mail: cpanzeca@ic.sunysb.edu; Antonio Tovar-Sanchez and Susana Agustí, Mediterranean Institute for Advanced Studies (IMEDEA), Spanish Council for Scientific Research (CSIC)-Universitat de les Illes Balears, Esporles, Islas Baleares, Spain; Isabel Reche, Departamento de Ecologia, Universidad de Granada, Granada, Spain; Carlos M. Duarte, IMEDEA, CSIC-Universitat de les Illes Balears; Gordon T. Taylor, Marine Sciences Research Center, Stony Brook University; and Sergio A. Sañudo-Wilhelmy, Marine Environmental Biology, University of Southern California, Los Angeles.