

Rejoinder to: Influence of river discharge in the tropical and subtropical North Atlantic Ocean

We would like to start this response to the comment of Cotrim da Cunha et al. (2007, 2009) by thanking them for the time and effort they spent analyzing our published manuscript (Tovar-Sanchez et al. 2006). Although Cotrim da Cunha et al. recognized the validity of our new comprehensive data set that includes the first simultaneous measurements of metals in aerosols and in field populations of *Trichodesmium*, they argue with the interpretation of some of our results. Our responses to the comment of Cotrim da Cunha et al. are the following:

Northeastern subtropical Atlantic Ocean circulation, riverine freshwater input, and riverine and dust Fe inputs—Cotrim da Cunha et al. indicated that regional circulation patterns in the subtropical Atlantic Ocean preclude any

freshwater transport to the locations sampled in the COCA cruise. They based that argument on a schematic representation of ocean circulation in the tropical and subtropical Atlantic, as well as basinwide diagrams of the annual average surface salinity, river runoff, and iron deposition (figs. 1 and 2 in the Cotrim da Cunha et al. commentary). Such diagrams are, however, idealized representations of far more complex realities (Maximenko et al. 2008). The annual mean values for salinity, river flow, and iron fluxes used in these diagrams (fig. 2 in the Cotrim da Cunha et al. commentary) conceal short-term and small-scale spatial variability, such as that observed during the COCA cruise of May–June 2003 reported in Tovar-Sanchez et al. (2006). Cotrim da Cunha et al. also tried to substantiate their argument about the lack of freshwater influence in the

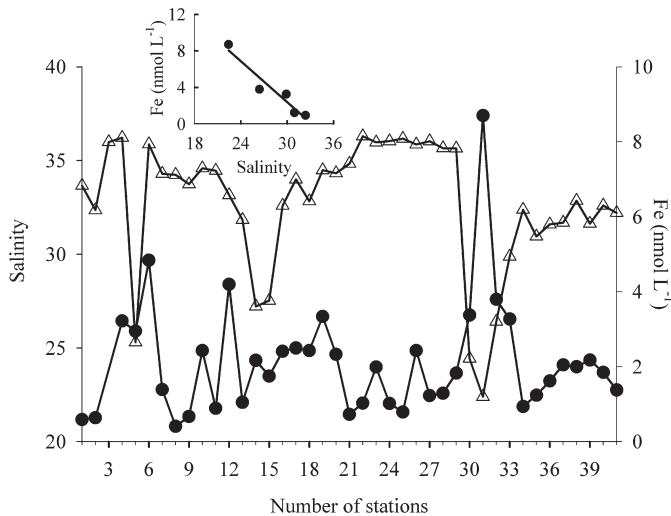


Fig. 1. Surface salinities (triangles) and dissolved iron (circles) measured during the MP08 cruise in the western Atlantic Ocean. The mixing line in the inset represents the conservative mixing between dissolved Fe and salinity at six stations sampled during a 24-h period within Amazon plume.

eastern subtropical Atlantic Ocean by reanalyzing our salinity data reported in web appendix I of Tovar-Sanchez et al. (2006). However, they only reported the average salinity for the whole region (36.59), and pointed out that this salinity is not representative of an “area under riverine freshwater influence.” Unfortunately, they overlooked the fact that there was a difference of 1.32 between our offshore stations (salinity = 37.30) and our most coastal station (salinity = 35.98), supporting our interpretation that some of the COCA stations could have been freshwater influenced. Our results regarding the influence of African rivers on the metal composition measured in *Trichodesmium* are consistent with previous studies by the authors of the commentary, showing that 36% of the regional primary production of the subtropical and tropical Atlantic Ocean is sustained by nutrient and iron inputs from African rivers (Cotrim da Cunha et al. 2005). The fact that the samples in the eastern Atlantic were collected when river discharge from African rivers was expected to be low, and the area should be dominated by dust deposition, was acknowledged in the methods section by Tovar-Sanchez et al. (2006). In fact, consistent with that hypothesis, the metal composition of most of the *Trichodesmium* samples collected in the COCA cruise clustered with the Saharan aerosols (fig. 3 in Tovar-Sanchez et al. 2006). However, we observed some deviations that we attributed to the influence of the Niger River or its tributaries. Although our results are based on a tremendous sampling and analytical effort to evaluate the importance of the most probable sources of metals to the region (South American and African rivers as well as Saharan aerosols), Cotrim da Cunha et al. did not provide any alternative hypothesis or any empirical data to support their statements that “it is improbable that *Trichodesmium* metal composition at COCA stations was influenced by riverine inputs at any season of the year.” The fact that we found relatively low-

salinity waters in areas sampled during the COCA cruise contradicts that strong assertion.

Western tropical Atlantic Ocean and the Amazon River plume—Cotrim da Cunha et al. claimed that the metal variability observed in the *Trichodesmium* colonies collected off the coast of South America was inconsistent with changes in the Amazon River discharge (reflected in the salinity measurements), as Tovar-Sanchez et al. (2006) stated. We agree with the suggestion of Cotrim da Cunha et al. that we probably oversimplified our interpretation of the timing of river influence, as we could not find actual river discharge data for our sampling periods during the preparation of the manuscript. However, we believe that using average salinity, without taking into consideration the variability of the whole data set, as Cotrim da Cunha et al. did, could itself be misleading. For example, comparing the average salinity reported by Cotrim da Cunha et al. during the MP08 cruise (34.35) and the MP03 cruise (31.55) suggests that the influence of the Amazon is higher during MP03, thereby contradicting our initial finding. However, a plot of dissolved Fe and salinity for MP08 (Fig. 1; A. Tovar-Sanchez and S. A. Sañudo-Wilhelmy unpubl.) shows a very strong inverse relationship, substantiating the influence of the river during that cruise, despite a higher average salinity. We were surprised that Cotrim da Cunha et al. used the quote “because Amazon plume waters cannot be identified by geographical characteristics. . .” from Cooley and Yager (2006) as part of their critique. The fact is that that sentence in the manuscript of Cooley and Yager is an assumption made in their methods section, not a final result or conclusion of their research. In addition, the work of Cooley and Yager (2006) on dissolved inorganic carbon and alkalinity in the Amazon plume is not applicable, as far as we know, to identify sources of trace metals to any marine system.

Riverine Fe: Al and Mn: Al as proxies for tracing metal sources to Trichodesmium sp. in the Atlantic Ocean—Cotrim da Cunha et al. also criticized our use of riverine metal concentrations (and ratios) as proxies of metal sources to the subtropical Atlantic Ocean. To substantiate that statement, Cotrim da Cunha et al. cited several inappropriate references on metal cycling in the Columbia River and the Hebrides shelf edge area that do not describe the estuarine behavior of trace metals from samples collected in the Amazon or Niger rivers. This suggests to us that they faced the same problem that we faced in writing the manuscript: the behavior of metals in the estuarine region of those rivers is virtually unknown. However, there are plenty of references showing that the behavior observed in one estuary cannot be extrapolated to another estuarine system, as implied in the Cotrim da Cunha et al. comment. For example, copper distributions measured in the Changjiang River showed conservative mixing behavior (Edmond et al. 1985), whereas the same metal in the Rhine Estuary showed a nonconservative removal (Duinker and Nolting 1977). As previously stated by Dagg et al. (2004), the chemical composition of large rivers, such as the Amazon, cannot be understood by

scaling up the processes occurring in small rivers, as implied by Cotrim da Cunha et al. The limited research that exists regarding metal behavior in the Amazon River suggests that dissolved metals (e.g., Cu, Ni, Cd) behave conservatively within the plume (Boyle et al. 1982). Consistent with those results, our iron distribution in MP08 (Fig. 1) shows conservative behavior of this element within the Amazon River plume. If that is the case, the metal ratio of two elements whose concentrations are only affected by mixing should be conserved in any section of the plume. It is unclear to us how Cotrim da Cunha et al. can use the work of Maring and Duce (1987) on dissolution experiments of aluminum from atmospheric aerosols collected in the tropical North Pacific (using seawater and artificial rainwater) to claim that “about 50% of the gross dissolved riverine flux of this element is retained in the estuarine zone.” To further substantiate the trapping efficiency in the Amazon, Cotrim da Cunha et al. cited the Amazon River water–seawater mixing experiments reported by Bergquist and Boyle (2006), showing that almost 90% of the dissolved iron flocculated by salinity 18. In drowned river valley estuaries where river flow is generally small compared with the volume of the tidal prism (Dyer 1973), salinities in that range are found several tens of kilometers inland (Flegal et al. 1991; Sañudo-Wilhelmy and Gill 1999; Dagg et al. 2004). However, during the MP cruises, salinities near 20 were measured in relatively deep waters more than 150 km off shore (Fig. 1), suggesting that a large fraction of the flocculated metals is dispersed over the subtropical–tropical Atlantic, and therefore can influence the metal composition of *Trichodesmium* colonies. Although river plumes are affected by many complex factors that are not fully understood, as recently pointed out by Dagg et al. (2004), we used the only riverine metal data available to identify potential sources of metals to the subtropical Atlantic. We believe that our empirical results were coherent and consistent with our initial hypothesis on the impact of rivers on the metal composition of *Trichodesmium*. We believe that further field studies (especially off the coast of Africa), instead of arguments on the basis of published studies that are not representative of our area of study, will be needed to unambiguously determine if our interpretation was correct or not.

Aerosol Fe: Al and Mn: Al as proxies for tracing metal sources to Trichodesmium sp. in the Atlantic Ocean—Cotrim da Cunha et al. also disagreed with the use of metal:aluminum ratios as tracers of Saharan aerosols in our study, and concluded that “phytoplankton metal concentrations are unlikely to reflect the metal ratios from bulk aerosols.” The fact is that field-collected *Trichodesmium* colonies trap Saharan dust particles (Rueter et al. 1992). Therefore it is not surprising that metal ratios in those colonies may reflect that source, as presented by Tovar-Sanchez et al. (2006). The use of Fe, Al, and Mn as tracers of Saharan dust is substantiated in the scientific literature (Statham and Burton 1986; Kremling and Streu 1993; Measures and Vink 2000). Our use of metal ratios to identify metal sources is not new; it has previously been applied to identify African dust transported over the

tropical Atlantic (Schneider 1985; Chiapello et al. 1997), as well as the source of particles collected in deep North Atlantic subtropical waters (Kremling and Streu 1993). Despite the solubility concerns highlighted in the Cotrim da Cunha et al. commentary, the Sahara signal in the metal ratios was still evident in samples collected at 1020-m and 4120-m depths by Kremling and Streu (1993), suggesting to us that those solubility arguments may not apply to our *Trichodesmium* samples collected in surface waters.

Normalization of metal ratios to Al—Cotrim da Cunha et al. also questioned the use of aluminum as a normalizing element in our manuscript, because this element is mostly found in lithogenic rather than biogenic materials. They cited the work of Frew et al. (2006), in which particulate aluminum was used to estimate the amount of biogenic particulate iron. We are very aware of the use of aluminum to differentiate between lithogenic and biogenic materials, as we developed some of the chemical protocols to differentiate those two pools (Tovar-Sanchez et al. 2003) and carried out some of the metal analyses reported by Frew et al. (2006). However, the objective of the Frew et al. (2006) manuscript was to address iron bioavailability, which requires differentiating intra- and extracellular metal pools. In contrast, the objective of Tovar-Sanchez et al. (2006) was to differentiate between dust deposition and riverine sources of metals, using metals with a strong particle affinity such as aluminum, iron, and to a lesser extent manganese, all of which are expected to be found mostly in the extracellular pool. For that reason, we reported total (surface-scavenged and intracellular combined) metal concentrations measured in the colonies, as clearly described in the methods section of Tovar-Sanchez et al. (2006). It is not appropriate for Cotrim da Cunha et al. to pose the question “why *Trichodesmium* would bioaccumulate aluminum to a higher *internal* concentration than iron, which is a required element” or make statements about the “cellular *internalization* of iron” or “passive versus active *internalization* mechanisms” because Tovar-Sanchez et al. (2006) did not measure, report, or discuss intracellular metal concentrations. We also do not believe that Cotrim da Cunha et al. can claim that the results reported by Duarte et al. (2006) on how pulse additions of aerosol materials affect new production in the COCA cruises contradict our results on the effect of tropical rivers on metal composition of *Trichodesmium*. The field incubation results reported by Duarte et al. (2006), carried out at two locations far from the coast (320 km and 610 km off shore) where freshwater sources are unlikely to be relevant, showed only a biological response to the addition of aerosols (metal composition of phytoplankton was not measured in those experiments). In any case, if Cotrim da Cunha et al. believe that the results reported by Duarte et al. (2006) are important for their argument, they should have noticed that Duarte et al. (2006) also reported that cyanobacteria did not show any growth response to the aerosol inputs.

Tracing multiple sources for natural samples—Cotrim da Cunha et al. also believed that we did not properly apply

the metal-ratio mixing diagram to identify sources of trace metals to the *Trichodesmium* colonies, as some of the samples did not conform to the two end-member mixing diagram shown in fig. 3 of Tovar-Sanchez et al. (2006). As samples were collected in both the western and eastern Atlantic, it was of course expected that a two end-member diagram could not fully explain the metal composition of *Trichodesmium* along the entire transect. For example, although most of the metal ratios in samples collected in the western Atlantic conformed to the mixing of two sources (the Amazon River and Saharan aerosols), that was not the case with some of the COCA samples (fig. 3 in Tovar-Sanchez et al. 2006). Similar deviations from conservative mixing behavior are common in areas with more than two sources (Sañudo-Wilhelmy and Flegal 1994). In fact, deviations from the Amazon–Sahara mixing line prompted our search for another additional potential source in the eastern Atlantic (e.g., the Niger River) to explain the results measured in some of the COCA samples. Cotrim da Cunha et al. were correct that in our analysis we did not include other potential sources such as shelf pore waters, sediments, or upwelling. This omission was for two reasons: first, our most coastal stations were located in several hundreds of meters of water depth, at 100 km and 140 km off shore in the eastern and western Atlantic respectively, suggesting that those coastal inputs may be insignificant compared with the Amazon (which transports 20% of the total freshwater discharged into the world's ocean; Dagg et al. 2004; Wetz et al. 2006) and air plumes of African dust (which can mobilize about 5×10^7 metric tons of iron per year; Sañudo-Wilhelmy and Flegal 2003). Second, we do not know the metal composition of any of those potential sources and therefore they cannot be included in our analysis. We hope that future research by Cotrim da Cunha et al. and others will generate the empirical data needed to test their hypothesis regarding the potential relevance of those sources. Cotrim da Cunha et al. correctly pointed out a mistake in the units reported in the web appendix, as we reported aluminum levels in *Trichodesmium* as nmol colony⁻¹ instead of pmol colony⁻¹.

Regulation of internal metal concentration by Trichodesmium sp.—Cotrim da Cunha et al. claimed that metals in *Trichodesmium* cannot be used to identify their potential sources because their internalization is regulated, as suggested by the abundance and diversity of genes encoding metal uptake. However, the abundance of those genes is simply indicative of a metal requirement for a metabolic function, not a quantitative indication of how much metal is required for that function. As recently shown for Co and vitamin B₁₂ synthesis, the internal requirement may be extremely low (Panzecca et al. 2008), such that metal ratios measured in the total metal pool of phytoplankton may not be affected. Because Tovar-Sanchez et al. (2006) did not measure the intracellular metal pool in the *Trichodesmium*, we do not have any experimental evidence of how metal internalization might affect our results. However, our field results (Tovar-Sanchez et al. 2006) suggest that the impact of this process may not be very relevant in the Atlantic,

where dissolved metal levels are among the highest (Statham and Burton 1986; Measures and Vink 2000; Sañudo-Wilhelmy et al. 2001) and probably in excess relative to demands. In such high metal concentration environments, we would expect that phytoplankton metal requirements are easily met, and metal levels in phytoplankton reflect high metal levels in “luxury” internal storage pools and the surface scavenged pool. This last process is consistent with the chemical reactivity of the metals used to identify sources by Tovar-Sanchez et al. (2006). It is a long-standing paradigm that phytoplankton metal uptake rates and cellular content are a function of both biochemical demand and ambient concentrations (Sunda and Huntsman 1995). Because of processes like surface scavenging and luxury uptake, phytoplankton total trace metal content is substantially more variable than major elemental ratios, and there is no absolute “Redfield ratio” for most trace elements. Our paper simply examined the possibility that external metal inputs may be one important factor influencing the elemental composition of Atlantic *Trichodesmium* colonies; of course biological requirements are another important factor.

In summary, we believe that the conclusions in Tovar-Sanchez et al. (2006) are well founded, on the basis of empirical data obtained in three major oceanographic cruises. In this rejoinder, we have considered how the results presented by Tovar-Sanchez et al. (2006) can accommodate the criticisms of Cotrim da Cunha et al. This exercise has allowed further scrutiny of those results along lines not considered in the initial publication, providing evidence of their robustness. In the process, we have identified several key questions that require further research and thank Cotrim da Cunha et al. for contributing to this exercise.

Antonio Tovar-Sanchez¹
Carlos M. Duarte

IMEDEA (CSIC-UIB)
C/Miguel Marqués 21
07190 Esporles (Mallorca), Spain

Adam B. Kustka

Institute of Marine and Coastal Sciences
Rutgers, The State University of New Jersey
71 Dudley Road
New Brunswick, New Jersey 08901

Susana Agustí

IMEDEA (CSIC-UIB)
C/Miguel Marqués 21
07190 Esporles (Mallorca), Spain

Jordi Dachs

Department of Environmental Chemistry (IIQAB-CSIC)
Jordi Girona 18-26
08034, Barcelona, Spain

¹ Corresponding author (antonio.tovar@uib.es).

David A. Hutchins
Douglas G. Capone
Sergio A. Sañudo-Wilhelmy

Department of Biological Sciences
Department of Earth Sciences
University of Southern California
Los Angeles, California 90089

References

- BERGQUIST, B. A., AND E. A. BOYLE. 2006. Iron isotopes in the Amazon River system: Weathering and transport signatures. *Earth Planet. Sci. Lett.* **248**: 54–68.
- BOYLE, E. A., S. S. HUESTED, AND B. GRAND. 1982. The chemical mass-balance of the Amazon plume; II, copper, nickel and cadmium. *Deep Sea Res.* **29**: 1355–1364.
- CHIAPELLO, I., G. BERGAMETATIN, B. CHATENET, P. BOUSQUET, F. DULAC, AND E. SANTOS SOARES. 1997. Origins of African dust transported over the northeastern tropical Atlantic. *J. Geophys. Res.* **102**: 97JD00259, doi: 10.1029/97JD00259.
- COOLEY, S. R., AND P. L. YAGER. 2006. Physical and biological contributions to the western tropical North Atlantic Ocean carbon sink formed by the Amazon River plume. *J. Geophys. Res. Oceans* **111**: C08018, doi:10.1029/2005JC002954.
- COTRIM DA CUNHA, L., E. T. BUITENHUIS, C. LE QUÉRÉ, X. GIRAUD, AND W. LUDWIG. 2007. Potential impact of changes in river nutrient supply on global ocean biogeochemistry. *Global Biogeochemical Cycles* **21**: GB4007, doi:10.1029/2006GB002718.
- , P. CROOT, AND J. LAROCHE. 2009. Influence of river discharge in the tropical and subtropical North Atlantic Ocean. *Limnol. Oceanogr.* **54**: 644–648.
- DAGG, M., R. BENNERB, S. LOHRENTZ, AND D. LAWRENCE. 2004. Transformation of dissolved and particulate materials on continental shelves influenced by large rivers: Plume processes. *Cont. Shelf Res.* **24**: 833–858.
- DUARTE, C. M., J. DACHS, M. LLABRES, P. ALONSO-LAITA, J. M. GASOL, A. TOVAR-SANCHEZ, S. SAÑUDO-WILHEMY, AND S. AGUSTI. 2006. Aerosol inputs enhance new production in the subtropical northeast Atlantic. *J. Geophys. Res. Biogeosci.* **111**: G04006, doi:10.1029/2005JG000140.
- DUINKER, J. C., AND R. F. NOLTING. 1977. Dissolved and particulate trace metals in the Rhine Estuary and the Southern Bight. *Mar. Poll. Bull.* **8**: 65–71.
- DYER, K. R. 1973. *Estuaries: A physical introduction*. John Wiley and Sons.
- EDMOND, J. M., A. SPIVACK, B. C. GRANT, M.-H. HU, Z. CHEN, S. CHEN, AND X. ZENG. 1985. Chemical dynamics of the Changjiang estuary. *Cont. Shelf Res.* **4**: 17–36.
- FLEGAL, A. R., G. J. SMITH, G. A. GILL, S. SAÑUDO-WILHEMY, AND L. C. D. ANDERSON. 1991. Dissolved trace element cycles in the San Francisco Bay estuary. *Mar. Chem.* **36**: 329–363.
- FREW, R. D., D. A. HUTCHINS, S. NODDER, S. SAÑUDO-WILHEMY, A. TOVAR-SANCHEZ, K. LEBLANC, C. E. HARE, AND P. W. BOYD. 2006. Particulate iron dynamics during FeCycle in subantarctic waters southeast of New Zealand. *Global Biogeochem. Cycles* **20**: GB1S93, doi:10.1029/2005GB002558.
- KREMLING, K., AND P. STREU. 1993. Saharan dust influenced trace element fluxes in deep North Atlantic subtropical waters. *Deep Sea Res.* **40**: 1155–1168.
- MARING, H. B., AND R. A. DUCE. 1987. The impact of atmospheric aerosols on trace metal chemistry in open ocean surface waters. I. Aluminium. *Earth Planet. Sci. Lett.* **84**: 381–392.
- MAXIMENKO, N. A., O. V. MELNICHENKO, P. P. NILER, AND H. SASAKI. 2008. Stationary mesoscale jet-like features in the ocean. *Geophys. Res. Lett.* **35**: doi:10.1029/2008GL033267.
- MEASURES, C. I., AND S. VINK. 2000. On the use of dissolved aluminum in surface waters to estimate dust deposition to the ocean. *Global Biogeochem. Cycles* **14**: 317–327.
- PANZECA, C., A. J. BECK, K. LEBLANC, G. T. TAYLOR, D. A. HUTCHINS, AND S. A. SAÑUDO-WILHEMY. 2008. Potential cobalt limitation of vitamin B12 synthesis in the North Atlantic Ocean. *Global Biogeochem. Cycles* **22**: GB2029, doi:10.1029/2007GB003124.
- RUETER, J. G., D. A. HUTCHINS, R. W. SMITH, AND N. L. UNSWORTH. 1992. Iron nutrition of *Trichodesmium*, p. 289–306. *In* E. J. Carpenter, D. A. Capone, and J. G. Rueter [eds.], *Marine pelagic Cyanobacteria: Trichodesmium and other diazotrophs*. Kluwer.
- SAÑUDO-WILHEMY, S. A., AND A. R. FLEGAL. 1994. Temporal variations in lead concentrations and isotopic composition in the Southern California Bight. *Geochim. Cosmochim. Acta* **58**: 3315–3320.
- , AND ———. 2003. Potential influence of Saharan dust on the chemical composition of the Southern Ocean. *Geochim. Geophys. Geosyst.* **4**: 1063, doi: 10.1029/2003GC000507.
- , AND G. A. GILL. 1999. Impact of the Clean Water Act on the levels of toxic metals in urban estuaries: The Hudson River Estuary revisited. *Environ. Sci. Technol.* **33**: 3477–3481.
- , AND OTHERS. 2001. Phosphorus limitation of nitrogen fixation by *Trichodesmium* in the central Atlantic Ocean. *Nature* **411**: 66–69.
- SCHNEIDER, B. 1985. Sources of atmospheric trace metals over the subtropical North Atlantic. *J. Geophys. Res.* **90**: 10774–10776.
- STATHAM, P. J., AND J. D. BURTON. 1986. Dissolved manganese in the North Atlantic Ocean, 0°–35°N. *Earth Planet. Sci. Lett.* **79**.
- SUNDA, W. G., AND S. A. HUNTSMAN. 1995. Iron uptake and growth limitation in oceanic and coastal phytoplankton. *Mar. Chem.* **50**: 189–206.
- TOVAR-SANCHEZ, A., S. A. SAÑUDO-WILHEMY, M. GRACIA-VARGAS, R. S. WEAVER, L. C. POPELS, AND D. A. HUTCHINS. 2003. A trace metal clean reagent to remove surface-bound Fe from marine phytoplankton. *Mar. Chem.* **82**: 91–99.
- , AND OTHERS. 2006. Effects of dust deposition and river discharges on trace metal composition of *Trichodesmium* spp. in the tropical and subtropical North Atlantic Ocean. *Limnol. Oceanogr.* **51**: 1755–1761.
- WETZ, M. S., B. HALES, Z. CHASE, P. A. WHEELER, AND M. M. WHITNEY. 2006. Riverine input of macronutrients, iron, and organic matter to the coastal ocean off Oregon, U.S.A., during the winter. *Limnol. Oceanogr.* **51**: 2221–2231.

Edited by: Mary I. Scranton

Received: 1 April 2008

Amended: 7 October 2008

Accepted: 2 October 2008