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Forming the primary nitrite maximum: Nitrifiers or phytoplankton?

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Abstract

As intermediary in a number of key biological processes, the dynamics of oceanic NO_2^- concentrations have historically been used as an indicator of the balance between oxidative and reductive pathways in the marine nitrogen cycle. As appreciation of the role of NO_2^- in the marine nitrogen cycle grew through the 1960s and 1970s, and data sets from different ocean basins became available, a common feature was observed in stratified water columns: a peak in NO_2^- concentrations at the base of the euphotic zone, with near zero concentrations both shallower and deeper. These concentrations are significant; they commonly range between 10 and 400 nmol L^{-1} but as high as 4,500 nmol L^{-1}. This peak in NO_2^- concentration is termed the primary nitrite maximum (PNM). Since the 1960s, the mechanisms sustaining the ubiquitous PNM have remained uncertain, with available data supporting either bacterial nitrification or NO_2^- release by phytoplankton. Simple box models have reproduced the PNM feature with nitrification as the source of NO_2^-, whereas others have succeeded solely with phytoplankton. Conclusive identification of the mechanism(s) maintaining the PNM in the world's oceans has yet to be achieved, but the preponderance of data supports phytoplankton excretion, with nitrification likely playing only a supporting role. Furthermore, there are a number of potentially important inconsistencies in the role of nitrification between culture studies and field observations. Biological–physical interactions are likely also important in controlling PNM formation and maintenance.

Nitrite (NO_2^-) is a dynamic component of the marine nitrogen cycle that is produced and consumed by a variety of processes. These processes are both reductive and oxidative and therefore are critical in a proper interpretation of ocean nitrogen cycling. Production of NO_2^- in the aerobic water column can occur via the following pathways: (1) chemoautotrophic oxidation of ammonium (NH_4^+) by ammonium oxidizing microbes, including both bacteria and archaea (Brandhorst 1959; Olson 1981*b*; Francis et al. 2005); (2) light-limited, incomplete assimilatory reduction of nitrate (NO_3^-) by phytoplankton (Vaccaro and Ryther 1960; Kiefer et al. 1976; Collos 1998), bacteria (Wada and Hattori 1971), and potentially archaea; and (3) photolytic reduction of NO_3^- (Zafiriou and True 1979). Rapid attenuation of the high-energy, short wavelengths of light in the upper euphotic zone severely limits the importance of the latter process at the depths of the primary nitrite maximum (PNM), but it may be important during periods of convective mixing in some regions where elevated NO_2^- concentrations are found throughout the euphotic zone (Lipschultz et al. 1996; Al-Qutob et al. 2002). Anaerobic processes, such as dissimilatory reduction of NO_3^- by denitrifying bacteria or the recently discovered anaerobic ammonium oxidation (anammox) pathway (Kuypers et al. 2003), likely make minimal contributions to NO_2^- concentrations (e.g., anaerobic microzones in marine snow particles) in the euphotic zones of open-ocean gyres.

Oxidation of NH_4^+ to NO_2^- provides energy for growth in ammonium-oxidizing organisms. Although very little is currently known about the physiological capacity of ammonium-oxidizing archaea, some Crenarcheota have recently been shown to have the capacity for ammonium oxidation (Francis et al. 2005; Konneke et al. 2005), and Murray et al. (1999) have noted temporal correlation between crenarchaeal abundance and NO_2^- concentrations in the Santa Barbara Channel. This recent discovery raises the central issue of the relative role of ammonium-oxidizing bacteria (AOB) versus other ammonium-oxidizing organisms (AOO) in nitrification and hence the degree to which culture knowledge applies to the ocean. For clarity we make several distinctions in terminology with respect to AOO. In the past, ammonium oxidation was believed to be carried out solely by bacteria and thus the term AOB was used in field studies as well as in culture studies. In this review, we use the term AOB only when referring to culture work (where the organisms are known), and use the term AOO when referring to field data that generally are an unknown mix of bacterial and archaeal nitrifiers.

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During the process of ammonium oxidation, energy is released and CO_2 is fixed via the Calvin-Benson cycle. However, the free energy yield of NH_4^+ oxidation is low, contributing to the slow growth rates of these organisms in culture (Ward 2005). Studies using fluorescent probes based upon cultured representatives have found that AOB, of the β and γ proteobacteria clades, are generally present in very low relative abundance (<1% of total bacterial numbers; Ward 2002) and show little variability in vertical profiles (Ward et al. 1984), supporting earlier observations based upon immunofluorescent labeling (Ward 1982). Despite limited variability in abundance, there is very high genetic diversity (O'Mullan and Ward 2005) with small-scale (tens of meters) variability within vertical profiles often exceeding temporal variability at a single station/depth. Lack of vertical variability in cell densities of β and γ clades of AOB is surprising given data suggesting that the process of NH_4^+ oxidation (and presumably growth rates of AOB) is photoinhibited by irradiances greater than 1–5% of surface sunlight (Hooper and Terry 1974; Olson 1981*b*; Guerrero and Jones 1996*a*). Although high light levels do not appear to be lethal to AOB, they are surely inhibitory (e.g., Horrigan et al. 1981) and one would expect higher AOB abundance at depth, a more favorable environment for both irradiance and concentrations of growth substrates. Newly available data on genetic diversity in AOB (e.g., O'Mullan and Ward 2005) and ammonia-oxidizing archaea (Francis et al. 2005) may help explain this and other conundrums, but this area of research remains very fertile ground for future research.

Release of NO_2^- by marine phytoplankton during reduction of NO_3^- is well documented in both field and laboratory experiments (*see* review by Collos 1998 and references therein). Several proposed physiological mechanisms might explain incomplete reduction of NO_3^- and subsequent release of NO_2^- by phytoplankton. Light limitation of intracellular NO_2^- reduction was originally suggested as the primary reason for release of NO_2^- by phytoplankton at the base of the euphotic zone (Vaccaro and Ryther 1960; Kiefer et al. 1976). This hypothesis was based, in part, upon cultures of the diatom *Thalassiosira pseudonana* grown in batch mode under a single, moderate light intensity (~90 μmol m^{-2} s^{-1}), and significant NO_2^- accumulation was observed while media NO_3^- concentrations were in excess of 75 μmol L^{-1} (Kiefer et al. 1976). In contrast, Olson (1980) has shown that NO_2^- release in steady-state, nutrient-replete (>20 μmol L^{-1} NO_3^-) cultures of the diatom *T. pseudonana* actually increased with increasing irradiance, reaching maximal release rates at ~10% full sunlight. Given the energetic requirements of NO_2^- reduction, it makes "physiological" sense that NO_2^- release could increase with irradiance, but that result would require very poor coupling between intracellular NO_3^- and NO_2^- reduction. One cannot refute, however, the observation that the PNM consistently occurs near the base of the euphotic zone. Regardless of the relation between irradiance and NO_2^- release in phytoplankton, both scenarios require the initial uptake of NO_3^- as a source for nitrite, and NO_3^- concentrations are often undetectable throughout much of the euphotic zone. More recently, Lomas and Glibert (1999, 2000) have proposed that small-scale/short-term increases in the ambient irradiance field in excess of cellular acclimation capabilities may result in NO_2^- release by phytoplankton as a cellular protection mechanism. This hypothesis again requires elevated NO_3^- concentrations, but relates NO_2^- release to the rate of change in the light field to which phytoplankton are exposed, not an absolute irradiance value.

It has also been suggested that iron limitation restricts ferredoxin production, resulting in reduced chloroplastic nitrite reductase activity relative to cytoplasmic nitrate reductase activity and subsequent NO_2^- release into the surrounding medium (Milligan and Harrison 2000). Both light and iron limitation work to restrict the flow of intracellular energy needed to assimilate nitrate. Sciandra and Amara (1994) observed that nitrogen limitation differentially affected nitrate and nitrite reductase enzyme abundance through a different physiological regulation mechanism in a marine dinoflagellate. When nitrogen became limiting, nitrite reductase enzyme abundance (and consequently activity level) was disproportionately reduced relative to nitrate reductase abundance, so that when nitrogen was resupplied, NO_2^- accumulated from excess NO_3^- reduction capacity and was released into the media. These mechanisms of nitrite release by phytoplankton are all consistent with culture data showing that although NO_3^- can accumulate internally to very high internal concentrations, NO_2^- does not (e.g., Dortch et al. 1984), presumably because of toxicity (Painter 1970).

The dominant biological pathways of NO_2^- consumption in the lower portion of the euphotic zone are believed to be phytoplankton uptake and oxidation to NO_3^- by nitrite oxidizing bacteria (NOB). It is worth noting the recent discovery of the gene for nitrite reduction, *nir*K, in AOB (Casciotti and Ward 2001), which raises the possibility of a role for AOB as both source and sink for nitrite. Phytoplankton uptake of NO_2^- has long been recognized (*see* review by Collos 1998), although it is not commonly measured. Nitrite assimilation is energetically expensive, requiring the reducing potential of four electrons for every molecule of NO_2^- reduced. Moreover, the reduction of NO_3^- and NO_2^- is segregated within algal cells in the cytoplasm and the chloroplast, respectively, requiring still further energy for the active transport of NO_2^- across the chloroplast membrane. This energy requirement would suggest that NO_2^- uptake (and reduction) would be highest in the upper euphotic zone and then decrease as irradiance diminishes with depth. Furthermore, the difference in energetic needs for NO_3^- and NO_2^- reduction suggests that NO_3^- uptake may continue deeper in the water column than NO_2^- uptake, a key aspect of light-dependent phytoplankton NO_2^- release models. Indeed, there is poor intracellular coordination of NO_3^- and NO_2^- reduction reactions, and light limitation (or iron and nitrogen limitation for that matter) of NO_2^- reduction doesn't necessarily result in the cessation of NO_3^- uptake and reduction. The physiological reason for this poor coupling is unclear as it seems to "waste" energy with no net gain in nitrogen metabolism—that is if we assume a static physical environment.

Nitrite oxidation, currently believed to be restricted to bacteria, is an autotrophic process like NH_4^+ oxidation wherein energy for growth is derived from the oxidation step. Available data suggest that NOB abundances are low and do not vary substantially over the water column (Ward et al. 1984). This lack of variability might indicate resource limitation, although attempts to define a rate response to increased substrate (and therefore energy) availability in field experiments with AOO and NOB have yielded inconsistent results. Several studies have shown that NH_4^+ oxidation rates in unamended samples may (Hashimoto et al. 1983; Ward et al. 1984) or may not (Ward and Kilpatrick 1990) be linearly related to ambient NH_4^+ concentrations. Direct amendment studies reach similar conclusions, with both positive responses (Hattori and Wada 1971; Wada and Hattori 1971) and the lack of a response (Olson 1981*b*; Ward 1987; Ward and Kilpatrick 1990). Clarification of the mechanisms controlling the abundance of nitrifiers, especially if NH_4^+ concentration is not the primary control variable, should be a high research priority.

Given the variety of pathways that might produce or consume NO_2^- within the euphotic zone, it is critical to recognize that only through an imbalance among the various production and consumption processes that net NO_2^- accumulation occurs and the PNM is formed. The second central requirement, and one that has been largely overlooked, is that the net NO_2^- production rate must exceed the rate of physical dispersion (e.g., diffusion, mixing) for the PNM to form. Rates of physical dispersion can therefore be used to constrain necessary rates of net NO_2^- production for comparison with rates of nitrification and phytoplankton NO_2^- release.

Proposed models of PNM formation and maintenance

Photoinhibition of nitrifying bacteria—The most frequently cited model of PNM formation is differential photoinhibition of AOO and NOB as proposed by Olson (1981*b*). In comprehensive culture and field experiments, Olson (1980; 1981*a,b*) directly studied most of the biological processes involved in NO_2^- production and consumption in the Southern California Bight. He observed that at high light and with available NO_3^-, phytoplankton actively released NO_2^-, and this release decreased with depth (Olson 1980). He proposed that as one progressed deeper with depth in the euphotic zone, NO_2^- uptake decreased more rapidly than NO_3^- uptake, resulting in a short vertical distance over which NO_3^- uptake continued but NO_2^- uptake had ceased—resulting in net NO_2^- release to the environment. Nitrite oxidation by NOB exceeded or equaled NO_2^- release, so that despite the inclusion of phytoplankton in the model, the real focal point of net NO_2^- production at the PNM was nitrification. In further experiments, Olson (1981*a*) incubated natural microbial assemblages over a range of manipulated irradiances and measured NH_4^+ and NO_2^- oxidation rates. He observed, as did earlier workers (Hooper and Terry 1974), that although both oxidation rates decreased with irradiance, NO_2^- oxidation decreased more. Olson (1981*a*) hypothesized that the same differential photoinhibition mechanism would occur in the ocean so that at the base of the euphotic zone (~1% photosynthetically active radiation [PAR]), ammonium oxidation would dominate and therefore account for the observed PNM throughout the world ocean. A similar study on ocean surface-film nitrifying bacteria and archaea (Horrigan et al. 1981) demonstrated that light (~10% of surface irradiance) was not lethal to natural AOO when presented in an 8 : 16 light : dark (LD) cycle, but it did retard their activity. Furthermore, although a 16 : 8 LD cycle completely inhibited AOO activity, it recovered after several days in the dark. On the other hand, NOB activity, at any light cycle other than complete darkness, was not only completely inhibited but also did not recover. The studies of Olson and Horrigan are consistent with the observations of Wada and Hattori (1971) where NH_4^+ oxidation rates exceeded NO_2^- oxidation rates, leading to net accumulation of NO_2^- at the rate of 0.1 nmol L^{-1} h^{-1}. It is worth noting that this net imbalance was very small relative to observed NH_4^+ and NO_2^- oxidation rates of tens of nanomoles per liter per hour.

Given the slow growth rates of AOB and the occasionally observed increase in NH_4^+ oxidation rate with increased concentrations, observations of a peak in NH_4^+ concentrations slightly shallower than the PNM (e.g., Collos and Slawyk 1983; Woodward and Rees 2001) takes on additional importance. High-quality, low-level NH_4^+ measurements are difficult and data sets, limited. Collos and Slawyk (1983) observed two "patterns" in NH_4^+ and NO_2^- depth distributions. When the PNM was in the upper portion of the euphotic zone, NH_4^+ concentrations were undetectable, in contrast to a deep PNM at the base of the euphotic zone where NH_4^+ concentrations were elevated (>100 nmol L^{-1}). Woodward and Rees (2001) observed only a single pattern, a PNM at ~1% PAR and a distinct NH_4^+ maximum ~10 m shallower. This relation between NH_4^+ and NO_2^- is not always present, however (e.g., Lipschultz 2001). As for NO_2^-, patterns in NH_4^+ concentrations are controlled by complex interactions between biological and physical processes, and therefore some variability should be expected. Possible explanations for this subsurface peak in NH_4^+ concentration are a zone of focused microbial particulate organic nitrogen remineralization, a zone of intense zooplankton grazing, or perhaps high-light–induced NH_4^+ release by phytoplankton (Brzezinski 1988; Lomas et al. 2000). The latter process could couple the photoinhibition and phytoplankton release models still further, complicating the view of the marine NO_2^- cycle and supporting the need for further research.

Guerrero and Jones (1996*a,b*), in direct contrast to the findings of Olson (1981*b*), observed that AOB were more sensitive to photoinhibition than NOB. Furthermore, they explicitly suggested that their experiments using cultures of *Nitrobacter* and *Nitrococcus* (NO_2^- oxidizers) and *Nitrosomonas* (NH_4^+ oxidizer) were free of artifacts such as overly high cell densities and unrealistic experimental light doses that had affected some earlier results (cf. Hooper and Terry 1974; Olson 1981*b*; Guerrero and Jones 1996*b*). Taken at face value, the light inhibition experiments of Guerrero and

Jones (1996*b*) would suggest that nitrification does not contribute to the PNM, as the production of NO_2^- would be more inhibited than its consumption.

A second study by Guerrero and Jones (1996*a*) studied dark recovery of AOB and NOB from photoinhibition and observed that the AOB species tested recovered quickly (~50% activity regained within 5 h), whereas NOB generally recovered very slowly or not at all, findings generally consistent with those of Horrigan et al. (1981). Guerrero and Jones put forth the hypothesis that it is the differential *recovery from photoinhibition* and not photoinhibition directly that links pathways of nitrification to the formation of the PNM. A necessary correlate of this hypothesis is that nitrification within the euphotic zone only contributes to net NO_2^- production at night or other times when the depth of photoinhibition is shallower than the extent of vertical mixing, thereby allowing the AOB to recover in darkness. Olson (1981*b*) did not consider the possibility of dark recovery in his model, perhaps because the differential inhibition of nitrification in the light was sufficient to lead to net NO_2^- accumulation. The data of Horrigan et al. (1981) and Guerrero and Jones (1996*b*) show that a finite amount of time is needed for cultured AOB to recover before initiation of NH_4^+ oxidation. Regardless, assuming sufficient time for AOB to recover, nitrification could still contribute to PNM formation, just by a different mechanism of action than originally proposed by Olson (1981*b*).

Although one could easily conclude, depending on one's predilection, that there are artifacts present in either culture studies or field measurements, as well as disconnects between species we can culture and those that dominate in nature (e.g., Archaea), the fact remains that only a handful of studies have examined the hypothesis that differential photoinhibition or recovery supports PNM formation, and they reach different conclusions. These data also contradict more recent work suggesting that complete nitrification occurs within the euphotic zone during daylight (e.g., Ward 1985; Raimbault et al. 1999; Lipschultz 2001). Moreover, as mentioned earlier, it is the decoupling of these two half reactions that leads to NO_2^- accumulation, and therefore it is critical to understand the environmentally controlled metabolic responses of both AOO and NOB. It would seem that the activity of nitrifiers is a complex interaction between light dose (both intensity and duration) and timescales of dark recovery, and clearly begs for further study to reconcile this component of our PNM conceptual model.

NO_2^- release by phytoplankton—Rakestraw (1936) and Vaccaro and Ryther (1960) are among the first references to the importance of phytoplankton as a local source of NO_2^- in the PNM, but it wasn't until the model proposed by Kiefer et al. (1976) that the role of phytoplankton was formalized. They studied the PNM in the North Pacific, including distributions of phytoplankton biomass, irradiance, nutrient concentrations, physiological growth rates, and NO_3^- uptake rates. Coupled with laboratory data on NO_2^- release by the diatom *T. pseudonana*, Kiefer et al. (1976) created a box model incorporating all of the measured biological fluxes as well as the eddy diffusive fluxes of NO_2^- away from the PNM and of NO_3^- into the lower euphotic zone. They concluded that phytoplankton release of NO_2^- alone was sufficient to form and maintain the PNM against vertical dispersion. In this model, the central control on NO_2^- release was light limitation for the reduction of NO_2^- to NH_4^+ in phytoplankton, but to sustain it, sufficiently high NO_3^- concentrations needed to be available to support meaningful NO_3^- uptake rates. Consequently, a necessary correlate of this model is that the nitracline and sufficient phytoplankton biomass need to be in proximity to the PNM. Interestingly, in their model, nitrification was neither required nor considered for the formation or maintenance of the PNM.

Seven years later, French et al. (1983) were motivated to revisit the role of phytoplankton excretion in the PNM after observing strong diel variability of NO_2^- in the PNM of the Gulf of Mexico. By using a quasi-Lagrangian approach, lateral advection could be eliminated to a first approximation as a source of the increasing daytime NO_2^- concentrations, and physical dispersion could therefore be restricted to the vertical domain. PNM concentrations were at a minimum, ~200 nmol L^{-1}, during the night and rapidly increased to ~600 nmol L^{-1} before the end of the subsequent photoperiod. Using a similar box model to that of Kiefer et al. (1976), they estimated rates of biological NO_2^- production and eddy diffusive losses. They concluded that NO_2^- production by phytoplankton during the day exceeded diffusive and any other loss processes so that the PNM "grew in" over the course of the day. This net production of ~400 nmol L^{-1} NO_2^- is even more impressive given that eddy diffusion was maximal (equivalent to ~50 nmol L^{-1} h^{-1}) during the day but negligible at night. They observed a slight shoaling of the PNM (from ~80 m to ~65 m) during the transition from day to night that they attributed to diel shoaling of the 1% light depth as the sun sets and therefore the depth at which there is net phytoplankton NO_2^- release. During the night, when phytoplankton ceased releasing NO_2^- (presumably due to a reduction/cessation of NO_3^- uptake), NO_2^- concentrations decreased primarily because of net NO_2^- oxidation by NOB (~50 nmol L^{-1} h^{-1}) with a relatively minor contribution (<5 nmol L^{-1} h^{-1}) from eddy diffusion. If diffusion were a dominant term leading to the decrease in PNM concentration (from 600 nmol L^{-1} to 200 nmol L^{-1} in 6 h), then one might expect to see increases in NO_2^- concentrations above and below the PNM. This peak broadening didn't happen: indeed, NO_2^- concentrations decreased significantly at nearly all depths, not just the PNM. These data suggest that NO_2^- oxidation, at least in this system, can exceed NH_4^+ oxidation, leading to *consumption* of the PNM by nitrifiers, and therefore a very limited contribution of NH_4^+ oxidation to daily PNM formation. This small data set was the first to illustrate the temporally dynamic nature of the processes contributing to the formation and dissipation of the PNM. However, if their conclusion is correct that there is significant dark NO_2^- oxidation, it appears contradictory to the work of Horrigan et al. (1981) and Guerrero and Jones (1996*a*) where NOB did not recover from light exposure. This

disparity between "manipulative" experiments and field observations suggests a possible disconnect in understanding of nitrifier metabolism and their role in the PNM. Further study is warranted, paying particular attention to match experimental to natural conditions where these data will be interpreted, and perhaps more importantly addressing the substantial impact of biological diversity on observed biogeochemical processes.

Both of these studies (Kiefer et al. 1976; French et al. 1983) rely on a model of light limitation of net phytoplankton NO_2^- reduction to explain the production of the PNM. It is inconsistent with the data of Olson (1980) that show NO_2^- release increasing with light up to 10% of incident irradiance. In Olson's model (1981*b*), NO_2^- uptake exceeds release in the upper euphotic zone so there is no accumulation, but he also suggests that NO_3^- uptake continues at near zero light with no net release of NO_2^- to the media (*see* his Fig. 5). Is NO_2^- accumulating internally within the diatom, or has he found an irradiance where NO_2^- uptake equals release and lower irradiances would show further net NO_3^- release? There also remain questions on variability in the depth (and therefore the light and nutrient concentrations that the resident phytoplankton are exposed to) of the PNM. At least two studies show that on the timescale of hours, the PNM can shoal or deepen by tens of meters (French et al. 1983; Dore and Karl 1996*b*). There is also the possibility of a disconnect between the species used in culture experiments examining NO_2^- release (primarily diatoms and flagellates) and the cyanobacteria and picoeukaryotes that are dominant at the PNM in the ocean. As with the understanding of nitrifiers, understanding of the mechanisms linking phytoplankton to the PNM is incomplete.

The PNM in the Sargasso Sea

How applicable are these various models for the formation of the PNM in the Sargasso Sea? During the highly stratified summer months, the northwestern Sargasso Sea (Zafiriou et al. 1992; Lipschultz et al. 1996), just like many other ocean regions (Kiefer et al. 1976; Dore and Karl 1996*a*; Al-Qutob et al. 2002), shows a distinct PNM near the base of the euphotic zone that is surprisingly high (mean 58 nmol L^{-1}; range 0–200 nmol L^{-1}) in concentration, given the very oligotrophic nature of the system (Fig. 1). There is a striking vertical coherence between the depths of the deep chlorophyll maximum (DCM), the PNM, and the nitracline (Fig. 1) as initially predicted by Kiefer et al. (1976). Using the range of NO_2^- release rates (as a percentage of NO_3^- uptake rates) given in Collos (1998) as a starting point and scaling to the phytoplankton biomass levels found in the Sargasso Sea, estimates of NO_2^- release by phytoplankton could range from 12 to 190 nmol L^{-1} d^{-1}. Given the generally accepted notion that NO_2^- release may result from light limitation, it is logical to conclude that phytoplankton at the PNM likely lack the energy to take the NO_2^- back up after release. Therefore given the concentrations of NO_2^- in the Sargasso Sea, PNM turnover times from phytoplankton release alone could range from a few hours to several days. Such

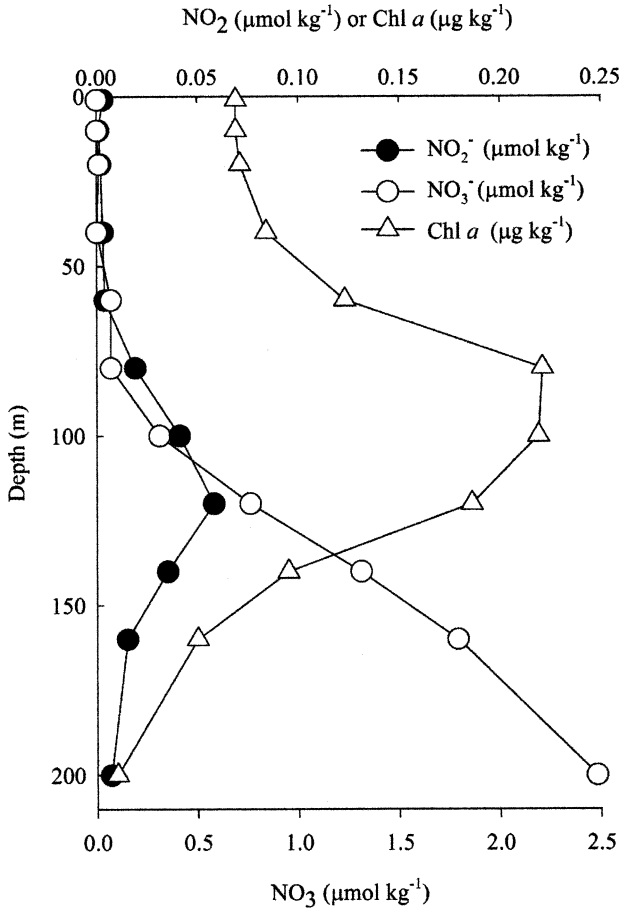

Fig. 1. Average profiles of NO_2^- and NO_3^- concentrations (μmol kg^{-1}) and phytoplankton biomass (chlorophyll *a* [Chl *a*]; μg kg^{-1}) during the stratified June to October period at BATS. Each profile is the average of ~85 cruise profiles. Note NO_3^- concentrations are divided by 10 and Chl *a* values are presented as μg kg^{-1} for scaling purposes only.

a short timescale would likely allow for the PNM to be maintained against vertical diffusion in the Sargasso Sea (e.g., Dusenberry 1999; Planas et al. 1999) and allow it to be rapidly re-formed after short-lived (~days) physical perturbations.

Available estimates of concurrently measured NH_4^+ and NO_2^- oxidation in the ocean suggest that these processes may be reasonable well coupled, perhaps favoring rates of NO_2^- oxidation (Ward 2002). If we use estimates of net NO_2^- production (e.g., Wada and Hattori 1971; Dore and Karl 1996*b*), the timescale of PNM turnover by nitrification would only increase. Therefore it is unlikely that nitrification would contribute significantly to the formation of the PNM in the Sargasso Sea. On the basis of these estimations, we believe that in the Sargasso Sea, the PNM is produced primarily through phytoplankton NO_2^- excretion.

Coherence of the nitracline, PNM, and the DCM

The coherence of the depths of the PNM, nitracline, and DCM have been presented for a range of ocean regions;

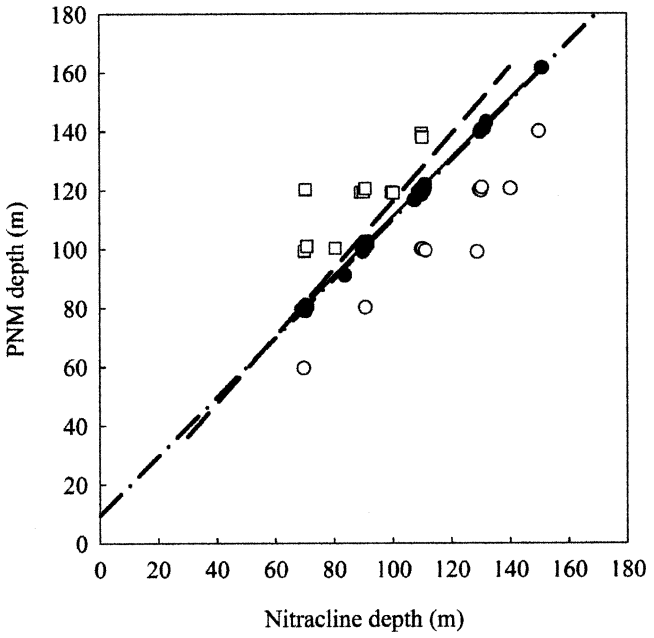

Fig. 2. Relation between nitracline depth (defined as half the distance between the first depth where $NO_3^- > 100$ nmol L^{-1} and the sampling depth above) and PNM depth (defined as the depth of highest NO_2^- concentration). Symbols are data from BATS. Closed symbols are from stations with ~10 m offset between the PNM and nitracline depths, with the solid regression line representing the best fit to only these data. Open symbols represent data where the PNM is deeper (squares) and shallower (circles) than the 10-m relation. The heavy dashed line is reproduced from Dore and Karl (1996), and the heavy dash-dot line is reproduced from Herbland and Voituriez (1979).

indeed it was part of the rationale that first led early investigators to hypothesize that phytoplankton were a controlling influence on the PNM. The Sargasso Sea is no different, further supporting our contention that phytoplankton are predominantly important in PNM formation and maintenance (Fig. 1). More recently, Adornato et al. (2005) have used high vertical-resolution profiles (<1 m sampling interval) to show an *exact* coherence of the PNM and the DCM along a transect into the North Pacific Subtropical Gyre. Dore and Karl (1996*b*), defining the PNM as the depth of maximum NO_2^- concentration, described an extremely tight relation between the PNM and the nitracline, which they defined as half the distance between the first sampling depth where $NO_3^- > 100$ nmol L^{-1} and the sampling depth immediately above. The 10-m offset between the depths of the PNM and the nitracline is partially due to the vertical sampling resolution of 20 m, which precludes a closer vertical definition of the relation. As Dore and Karl did not always sample NO_2^- and NO_3^- concentrations on the same cast, the nearly perfect relation between PNM and nitracline depths that they observed implies that the timescale of a biological response to any physical perturbation is likely shorter than the timescale of that physical perturbation. Although the bulk of the data collected in the Sargasso Sea follows this 10-m relation, ~15% of our data fall above and ~15% below this relation despite a similar absolute range in nitracline depths between the Pacific and the Atlantic (Fig. 2). For the Sargasso Sea data, this is not a potential artifact of internal waves (cf. Dore and Karl 1996*b*), as NO_2^- and NO_3^- profiles were always collected from the same cast on each cruise, but rather suggest that there may be different physical processes (or different timescales for these processes) at work in the Atlantic than in the Pacific subtropical gyres, leading to different profiles of NO_2^- concentration. We hypothesize that these disparities from the 10-m relation are indeed the result of a physical perturbation and subsequent biological response that differs between the Pacific and Atlantic Oceans and is driven by short-lived (hours to days) changes in the physical environment that affect the spatial relation between the nitracline, DCM, and the 1% light depth. This hypothesis is based upon the common explanation for the spatial relation between the DCM and the nitracline that phytoplankton serve as a nitrate "filter" (Cullen and Eppley 1981) and can quantitatively remove NO_3^- (in a static system) down to approximately the 1% PAR level. If we are correct in believing that formation of the PNM is driven primarily by the balance between phytoplankton uptake and release of NO_2^- (following NO_3^- reduction), and that the irradiance at which this occurs is ~1% PAR, then any physical processes that move the nitracline and DCM away from 1% PAR depth (Sargasso Sea long-term average 91 ± 13 m; Siegel et al. 2001), or that alter the depth of 1% PAR relative to the nitracline and DCM, could lead to this observed decoupling (i.e., separation distances >10 m) of the PNM and nitracline depths.

First we focus on scenarios explaining data where the PNM is deeper (20–50 m) than the nitracline. We envision two conditions (Fig. 3), one where there is an oscillation between periods (i.e., several days) of high- and low-incident irradiance, and one where there is vertical displacement of the nitracline relative to the 1% PAR depth by processes such as an internal wave or possibly the passage of an eddy. Note that these physical perturbations would have to have a timescale at least as short as that of the biological response timescale for these patterns of decoupled PNM and nitracline to emerge. In the first scenario (Fig. 3A–C), a shift from high to low irradiance due to consecutive cloudy days, for example, would result in a shallower 1% PAR depth. Given the absolute light requirement of NO_3^- and NO_2^- uptake (and NO_2^- release), we would expect, over time, the depths of the nitracline to shoal as phytoplankton in the depths between the former and the current 1% PAR depths can no longer take up NO_3^-. If sufficient time passes at the new 1% PAR depth without further physical disturbance, a new PNM would form at the new 1% PAR/nitracline interface. During this time, there would be a "relic" PNM at the original nitracline depth that would no longer be actively maintained and so would simply diffuse away or be consumed by NOB. This physical perturbation/biological response model would be consistent with the NO_2^- profiles observed by Dore and Karl (1996b) in the North Pacific that show an "upper" and a "lower" PNM, although they attributed the lower PNM to nitrification. After a transition back from low to high irradiance, one can easily envision that the

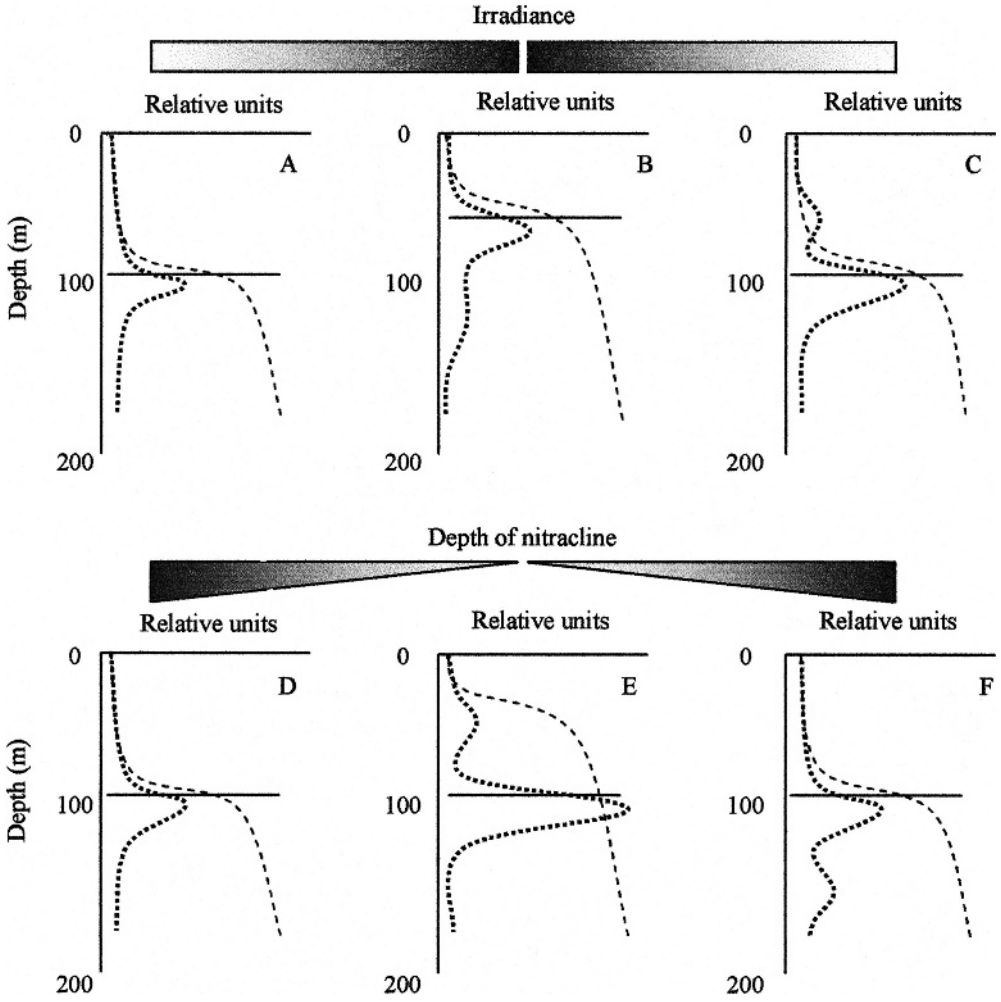

Fig. 3. Conceptual models of relative changes in the depth of 1% PAR (mean 93 m based on Siegel et al. 1997; solid line), nitracline (dashed line), and the primary nitrite maximum (dotted line) under the scenarios described in the text. Panels A–C represent a transition from periods of high to lower incident PAR (A→B) and back again (B→C). Panels D–F represent the vertical uplifting of the nitracline (due to an internal wave or eddy heaving; D→E) and subsequent relaxation (E→F). These models are described further in the text.

surface PNM would rapidly be consumed by phytoplankton as there is now sufficient light to reduce NO_2^- to NH_4^+, and a new PNM would quickly form at the new 1% PAR/nitracline interface where NO_3^- concentrations are high and light is limiting. The nitracline would slowly deepen because of increased NO_3^- uptake until reaching the new 1% PAR depth. Thus, for some time period we would expect the new PNM to be significantly deeper than the nitracline, because of the 1% PAR depth deepening faster than the nitracline is eroded. Moreover, the elevated NO_3^- concentrations at the new 1% PAR depth will likely lead to higher NO_2^- release rates and hence higher NO_2^- concentrations than typically observed. We suspect the sum of these processes might result in characteristic "shapes" of the vertical NO_2^- profile. For example, while the nitracline is shoaling following a shallower 1% PAR depth, one would expect the shallow slope of the PNM to be very steep, but a "tail" below the peak in the PNM. On the other hand, as the nitracline deepens following a deepening of the 1% PAR depth, both the shallower and deeper slopes would be very steep, with perhaps a relic PNM observed (Fig. 3A–C).

In the second scenario (Fig. 3D–F), internal waves or perhaps eddy heaving would move the nitracline and PNM closer to the sea surface, but doesn't change the depth of 1% PAR (as light-attenuating particles are just being redistributed throughout the water column and not increasing in abundance as yet). Because of the lack of change in the 1% PAR depth, the NO_3^- concentration is now greater at this depth (an important second condition for the formation of a PNM) and we would expect that a much higher concentration PNM would result because of the higher rate of NO_3^- uptake (supported by the higher NO_3^- concentrations), and continued light-limited NO_2^- release. No shallow PNM would form because of high irradiances; indeed both the nitracline and the original PNM that were uplifted would rapidly erode back to the 1% PAR depth if the isopycnal remained shallow for

a sufficiently long period of time. The PNM, again for a finite period of time, would be >10 m deeper than the nitracline. As the isopycnal relaxes with the passing of the internal wave or eddy, the PNM would deepen and disappear (because of consumption or diffusion) with another new PNM reforming at the 1% PAR depth. One might expect the vertical NO_2^- profiles in this scenario to oscillate between a larger PNM below a smaller NO_2^- "peak" and a larger PNM above a smaller NO_2^- peak, depending upon which phase of the physical disturbance is acting on the system at that time. Under scenarios such as these, we hypothesize that one can observe a wide range of depth differences between PNM and the nitracline. However, although we don't know the response timescales for changes in the depth of the DCM, nitracline, and PNM, we do know that the response timescale of the PNM must be shorter than that of the DCM and nitracline for us to catch the system in one of these transition states.

Switching attention to the oceanographic condition where the PNM is shallower than the nitracline (~15% of the Sargasso Sea data set), it initially appears a bit more difficult to explain. An interesting observation on the relation shown in Fig. 2 is that when the PNM is shallower than the nitracline, it is more closely coupled (9 of 11 instances, PNM is only ~10 m shallower than the nitracline) than the opposite case. In contrast, when the PNM is deeper than the nitracline, it is 20–50 m (11 of 11 instances) deeper. This closer coupling on the shallower side of the nitracline is consistent with the higher light levels and the greater likelihood for NO_2^- uptake. Overall, the NO_2^- concentrations are very low (10–50 nmol L^{-1}) and it is easy to overinterpret this slight offset; however, this could be a "significant" feature and there may be physiological explanations. Although likely just a coincidence, it is interesting that there are the same number of occurrences higher and lower over the 15 years of the Sargasso Sea data record where NO_2^- concentrations have been determined separately.

There are several scenarios, all linked to the DCM, that could explain the shallower PNM. The DCM, on average, exhibits a peak on the shallow side (~10 m) of the PNM and the nitracline (Fig. 1), and therefore could contribute to this small NO_2^- pulse above the nitracline. One explanation for this is a lack of coordination between intracellular NO_3^- reduction steps. Cultures of the marine flagellates *Dunaliella tertiolecta* and *Monochrysis lutheri*, grown to steady state on NO_3^-, when supplemented with additional NO_3^- displayed significant NO_3^- uptake (in the dark) but released back into the media as NO_2^- 26% and 100%, respectively, of that uptake (Laws and Wong 1978). The diatom *Thalassiosira allenii* released NO_2^- at only 5% of the dark NO_3^- uptake rate, but the absolute NO_2^- release rates were the same among all three species. This poor coordination between intracellular NO_3^- and NO_2^- reduction pathways is also seen in the dinoflagellates *Scrippsiella trochoidea*, *Alexandrium minutum*, and *Heterosigma cartarae* (Flynn and Flynn 1998; Clark and Flynn 2002), but not in the diatom *Thalassiosira weissflogii* (Clark et al. 2002). It is reasonable to hypothesize that NO_2^- shallower than the nitracline could arise from "sloppy" phytoplankton in the DCM exposed to periodic low-level NO_3^- injections or very short-lived light/dark transitions at sunrise (e.g., Anderson and Roels 1981) or sunset (French et al. 1983) when the 1% PAR depth is rapidly deepening or shoaling. The physiological mechanism for this could be the transient, light-induced uncoupling of NO_3^- and NO_2^- reduction (Lomas et al. 2000) whereby NO_3^- is reduced and NO_2^- released to the media to dissipate excess absorbed light energy.

The question does arise, however, about the applicability of these prior culture studies to the ocean gyres when marine cyanobacteria and as yet largely uncharacterized picoeukaryotes dominate the autotrophic biomass (Chisholm et al. 1988; Partensky et al. 1999; Worden et al. 2004). A further complication, *Prochlorococcus* has been shown to have high-light– and low-light–adapted ecotypes ideally suited to the different environmental regimes (e.g., Moore et al. 1995; Moore and Chisholm 1999). *Prochlorococcus* has its numerical abundance, 10–40% of the autotrophic biomass, in the Sargasso Sea at ~100 m, spatially coincident with the PNM, the nitracline, the average 1% PAR depth, and the zone of potential iron limitation (Durand et al. 2001; Siegel et al. 2001; Sedwick et al. 2005; Fig. 1). Most significant to the formation of the PNM, the low-light ecotype isolated from the Sargasso Sea, strain SS120, cannot grow on NO_3^- but can grow on NO_2^- (Moore et al. 2002), an observation that has been validated by genomic analysis showing that other low-light strains lack the genes coding for nitrate reductase (Dufresne et al. 2003; Rocap et al. 2003). However, at least late in the summer, SS120 is a very small fraction (~0.5%) of the total *Prochlorococcus* population (Zinser et al. 2006) and conclusions based upon this single strain may be biased. Preliminary studies on natural *Prochlorococcus* populations, using a combined flow cytometry–stable isotope tracer technique (Lipschultz 1995), contradict these culture conclusions and have shown that both NO_3^- and NO_2^- are assimilated and oxidized nitrogen uptake rates are a significant fraction, ~15%, of total measured nitrogen uptake (NO_3^-, NO_2^-, NH_4^+, and urea; Casey et al. unpubl. data). It is intriguing to speculate on the role of this taxonomic group in nitrogen cycling in the Sargasso Sea; this remains a very active area of research.

According to our models, when the PNM is significantly deeper than the nitracline, the NO_2^- peak is expected to be broader and higher in concentration than when the PNM is shallower than the nitracline. In the latter case, one might expect the PNM concentrations to be very low because of enhanced uptake at the slightly higher irradiance levels. A comparison of the vertical profiles of NO_2^- under these two conditions is in part supportive of these expectations (Fig. 4). There is no difference in the average depth-dependent shape of the NO_2^- profile between the two conditions, with both showing a maximum at 100–120 m and similar depth ranges where concentrations are elevated. However, NO_2^- concentrations are significantly ($p < 0.05$) greater at 100–120 m when the PNM is deeper than the nitracline.

All of these physical disturbance scenarios and the emergence of "decoupled" PNM and nitracline depths

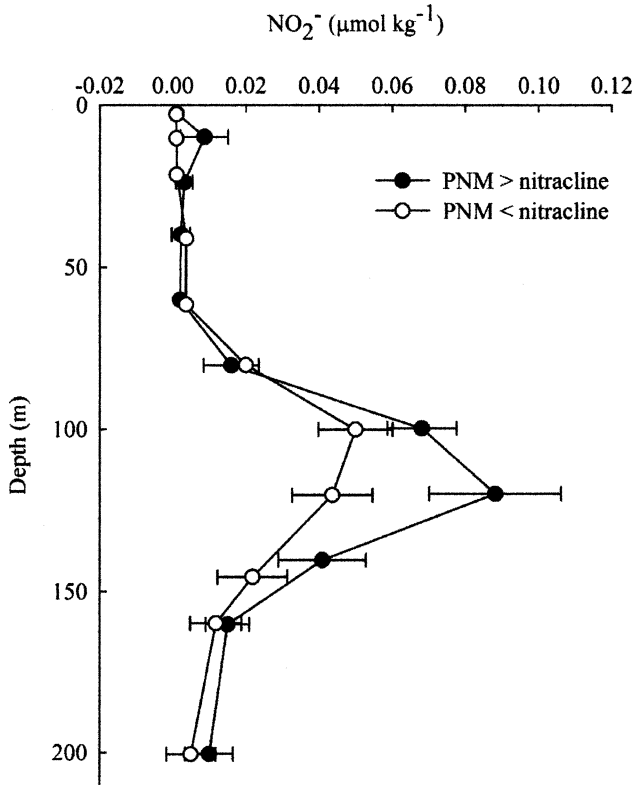

Fig. 4. Average NO_2^- profiles for those stations at BATS where the PNM was more than 10 m deeper than the nitracline and those stations where the PNM was shallower than the nitracline.

require that the timescale of changes in the physical environment at the Bermuda Atlantic Time-series Study (BATS) are shorter than that of biological responses (and apparently shorter than those operating in the Pacific), such that biological processes are playing "catch-up" to these physical perturbations. Also, as time-dependent changes, one would expect to see a wide range of possible NO_2^- profiles as the PNM "grows in" and is "eroded away". In the North Pacific Subtropical Gyre, the kinetic energy spectra shows the dominant mode to be Rossby waves with a period of ~90 d (Sakamoto et al. 2004), much longer than the timescale of biological response given that phytoplankton growth rates in this region can approach one division per day (Landry et al. 2003). In the North Atlantic Ocean, the kinetic energy spectra is dominated by mesoscale eddies (Smith et al. 2000), but the turnover timescales of these features, and therefore the timescale on which isopycnals would shoal, is not well constrained. Consequently the relative importance of internal waves and eddies in the Atlantic, with regard to decoupling the PNM and the nitracline, can not be evaluated at this time, but should be a focus of future research as it affects other aspects of physical/biological interactions in the ocean.

Winter mixing

The Sargasso Sea also provides an interesting example where NO_2^- concentrations display large seasonal oscillations in both concentrations and the shape of euphotic zone profiles. Frequently during the winter season, NO_2^- concentrations within the mixed layer, and often throughout significant portions of the mixed layer, are greatly elevated relative to summer concentrations (Fig. 5). It is important to note that even in the face of this active mixing there remains a distinct PNM at ~100 m. The duration of these periods of elevated NO_2^- concentrations is poorly defined given the biweekly sampling interval during this period, but they likely persist for 4–6 weeks (Lipschultz et al. 1996). These elevated concentrations represent a nearly 10- to 100-fold increase in NO_2^- inventories over the summer period, with NO_2^- concentrations often approaching those of nitrate. Interestingly, since 1996, maximum euphotic zone NO_2^- concentrations have been reduced while remaining elevated well below the euphotic zone. One possible explanation is that the lower NO_3^- concentrations in the euphotic zone, likely driven by reduced winter mixing depths during this period (Hansell and Carlson 2001; Lomas and Bates 2004), may lead to a lower capacity for NO_2^- release.

If we extend the internal wave model (Fig. 3D–F) to an extreme, i.e., the period of wintertime convective mixing when organisms experience rapid and relatively continuous depth and irradiance changes in the presence of elevated NO_3^- concentrations, we suggest that this model can explain the observed patterns in NO_2^- concentrations at this time of year as well. For all the reasons discussed above for the summer period, such as photoinhibition of AOO and NOB activity and the observation that seasonal chlorophyll distributions are intricately tied to seasonal patterns in NO_2^- concentrations (Figs. 3, 5), we are even more confident that the winter season elevated NO_2^- concentrations in the Sargasso Sea (or other regions where NO_2^- is present throughout the euphotic zone) are the result of phytoplankton release processes. Indeed this is likely an extreme case of the variable irradiance-NO_2^- release model of Lomas and Glibert (1999, 2000). During periods of active convective mixing in the Sargasso Sea, diel oscillations in mixed-layer depth can exceed 100 m, depths greater than the euphotic zone depth (Johnson unpubl. data), and therefore cells experience rapid and wide-ranging changes in absolute irradiance, with resulting stress on the cellular energy machinery of marine phytoplankton.

Under very similar physical conditions in the Red Sea, Al-Qutob et al. (2002) also observed elevated NO_2^- concentrations through the entire euphotic zone. In fact, NO_2^- constituted up to 80% of the total oxidized nitrogen present in the Red Sea. On the basis of bioassay-type experiments and nutrient time courses, they also concluded that phytoplankton release was the source of the elevated NO_2^- concentrations, although they did not propose a specific mechanism for NO_2^- release. Although infrequently published, this pattern of elevated of NO_2^- concentrations through the upper ocean (<100 m) is not uncommon. The global WOCE database (http://www.ewoce.org/data/) contains many examples of elevated NO_2^- concentrations through the mixed layer (Fig. 6). Within this data set nearly every pattern of NO_2^- profiles can be found, and there is widespread geographical

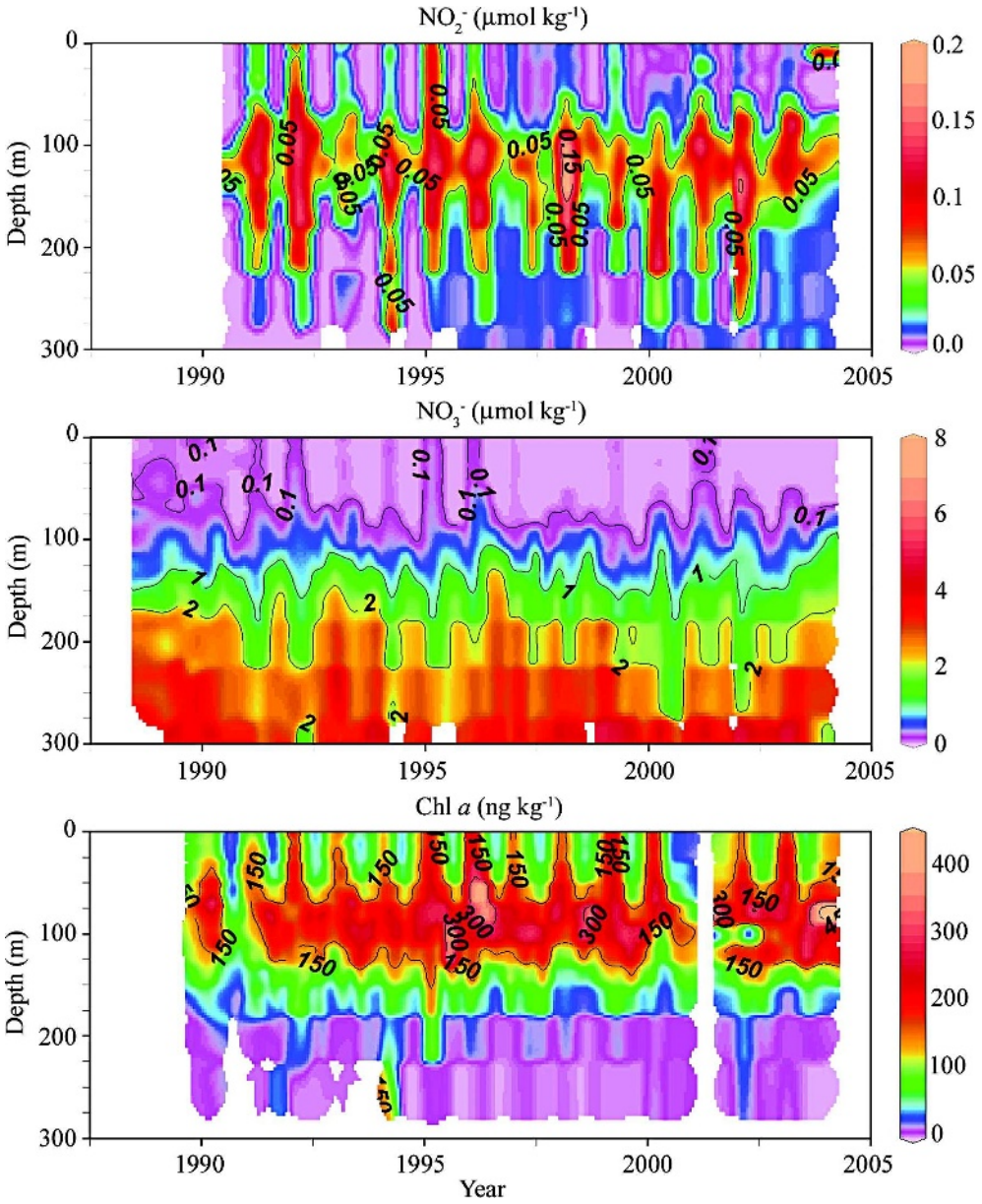

Fig. 5. Time-series contour plot of NO_2^- and NO_3^- concentrations (μmol kg^{-1}) and phytoplankton biomass (Chl *a*; ng kg^{-1}) in the upper 300 m at BATS. Before March 1990, NO_2^- concentrations were not determined separately as part of the BATS core measurements, and therefore not subtracted from the combined NO_3^-/ NO_2^- analysis. From March 1990 to present, the combined NO_3^-/NO_2^- analysis channel is corrected for NO_2^- concentrations.

distribution of profiles showing elevated concentrations throughout the euphotic zone. What separates the Sargasso and Red Seas from the other stations in the WOCE data set (Fig. 6) is the observation that NO_2^- is generally <1–2% of the measured NO_3^- at these other stations. Perhaps there are other important regulatory mechanisms, beyond what we've identified here, that remain to be elucidated when NO_2^- is produced throughout the mixed layer. Pending additional study, it would seem that some of the models we have proposed for the Sargasso Sea (Fig. 3) may have widespread relevance to other regions of the world ocean, further solidifying the importance of physical perturbations (in addition to stratified conditions) as a controlling factor on biological NO_2^- cycling in the global ocean.

Potential iron limitation in the Sargasso Sea and its impact on the PNM

Nitrite reduction by phytoplankton is mediated by the iron-containing cellular reductant ferrodoxin. Therefore iron, as well as light, limitation may exert a control on autotrophic NO_2^- cycling in the Sargasso Sea (e.g., Milligan and Harrison 2000). Specifically, *both* light and iron limitation could decrease rates of autotrophic NO_2^-

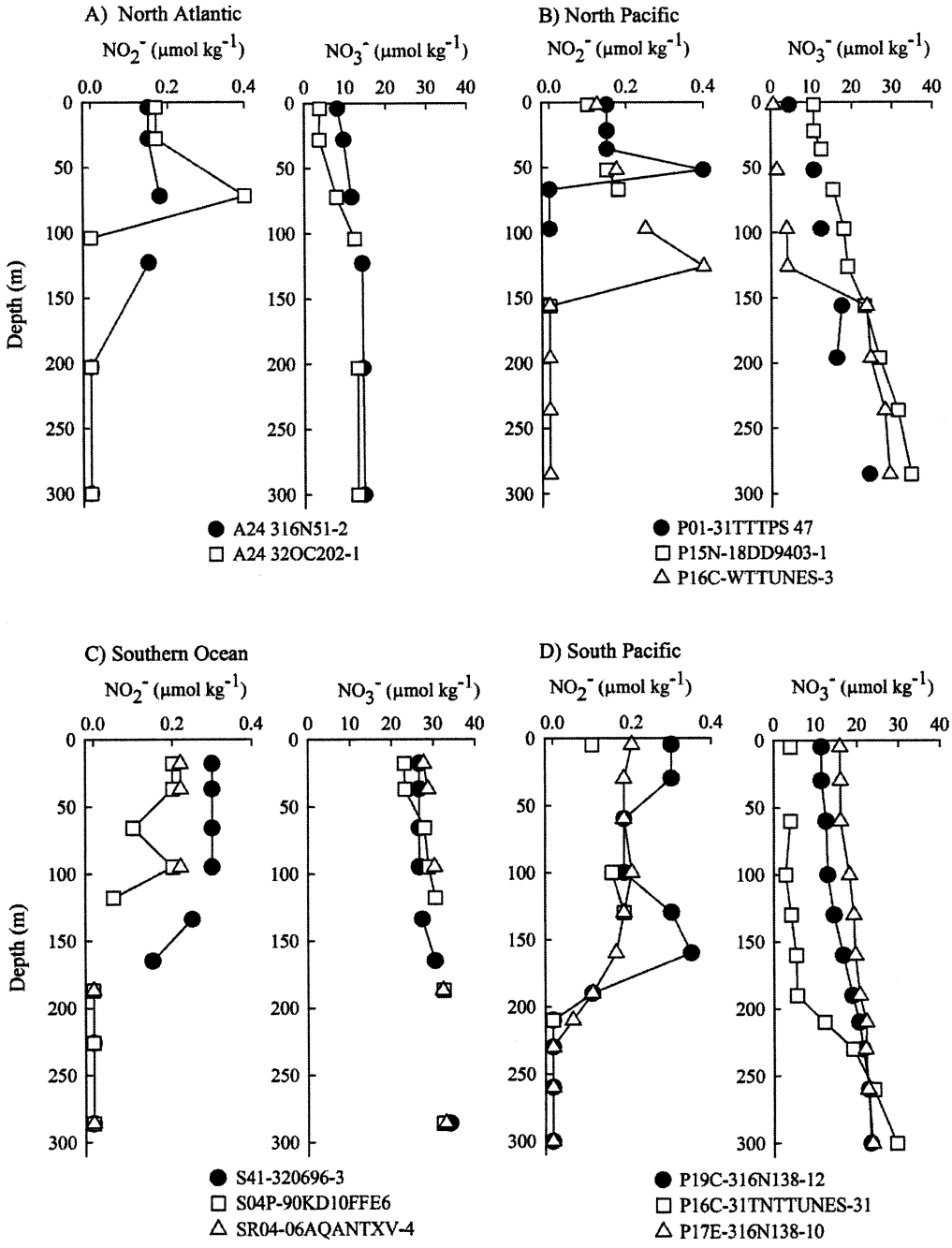

Fig. 6. Examples of NO_2^- and NO_3^- profiles from several ocean basins extracted from the eWOCE dataset (http://www.ewoce.org/data/). The depth axis is restricted to 300 m to match the data for the BATS station.

uptake, leading to the formation of the PNM. Profiles of total iron concentrations in May/July, when the deep PNM has formed after winter mixing, show minima at ~100 m, the depth where the PNM (and approximately the DCM) is found (Sedwick et al. 2005). Under iron-limited conditions in some marine phytoplankton, ferredoxin can be replaced by flavodoxin, a functionally similar molecule that does not contain iron (Laroche et al. 1995, 1996; McKay et al. 1997). Research by Milligan and Harrison (2000) shows that the marine diatom *T. pseudonana*, known to produce flavodoxin, continued to release NO_2^- under iron-limiting, light-saturating conditions, suggesting that the intracellular mechanisms leading to NO_2^- release are more complicated. Perhaps not unexpectedly, *Prochlorococcus* has a unique light-harvesting antenna when grown under iron limitation that increases its competitive ability at the base of the euphotic zone (Bibby et al. 2001, 2003). The interactions between light, iron limitation, and phytoplankton release of NO_2^- have not been directly studied, let alone quantified in conceptual models or in process-oriented field studies. Elucidation of these interactions is important as predictive biological models become structurally more

complex, but we currently lack the necessary data for validation.

Summarized understanding of the PNM and suggestions for future research

The accumulation of NO_2^- at the PNM is driven by an imbalance (temporal or vertical) of production and loss (biological or physical) processes. However, because of inconsistencies between observational data sets, complete understanding of the mechanisms involved in this global ocean feature remains elusive. The data sets that we have presented and discussed above provide fertile ground for generating testable hypotheses.

Notwithstanding the agreement of several literature data sets and data from the Sargasso Sea that NO_2^- cycling in the surface ocean and production of the PNM under stratified conditions appears to be dominated by phytoplankton, many questions remain. A critical question arising from the Sargasso Sea data set, as well as that of Al-Qutob et al. (2002) for the Red Sea, is the interaction between physical forcing and NO_2^- cycling when the water column is not stratified and convective mixing exceeds the depth of the euphotic zone. Can active convective mixing convert phytoplankton from a net sink for NO_2^- in the upper euphotic zone to a net source? If so, does the functional relation between irradiance and NO_2^- release by phytoplankton differ between stratified and well-mixed conditions? Furthermore, despite the elevated NO_2^- concentrations throughout the euphotic zone, there remains a distinct PNM, suggesting that autotrophic NO_2^- release rates can exceed rates of physical dispersion. What are the relevant timescales linking biological responses and physical perturbations? We have proposed several conceptual models driven by "discrete" physical forcing (as opposed to continuous forcing such as winter convective mixing or the continuously stratified summer conditions) that are assumed to be of a timescale shorter than that of biological response—an assumption that needs to not only be reconciled between the Sargasso Sea and the subtropical Pacific, but also seasonally in the Sargasso Sea. These models have been hypothesized to explain variability in the depth-dependent relations between the PNM and the nitracline during stratified summer conditions, but it appears that there are differences between ocean basins in the dominant mode of physical disturbance. Understanding the relations between different physical perturbations, which are acting upon different timescales, and biological responses is an important area for future research if we are to understand the mechanisms of NO_2^- cycling in the ocean.

Understanding the controls on nitrification and its importance to NO_2^- cycling in the euphotic zone and PNM is at least as confused as the understanding of phytoplankton release processes and physical interactions. If nitrification is involved in the formation of the PNM, is the nitrification component controlled by differential photoinhibition or differential recovery from photoinhibition? Why do cultured NOB appear unable to recover from photoinhibition (even after short exposures), but natural NOB populations actively oxidize NO_2^- with apparently little recovery time needed? This conclusion for natural populations is supported by field observations showing that surface NO_2^- and NO_3^- concentrations remain low and constant during the stratified summer in the subtropical gyres, suggesting that nitrification does occur in the surface ocean during daylight at low but measurable rates (Ward 1987; Raimbault et al. 1999; Lipschultz 2001; ~5 nmol L^{-1} d^{-1}). PNM NO_2^- concentrations in the Gulf of Mexico (French et al. 1983) have been shown to increase during the day because of phytoplankton release, yet in the North Pacific (Dore and Karl 1996*a*) NO_2^- concentrations increase at night because of nitrification, suggesting a temporal component (perhaps day/night oscillations in irradiance) as well as a taxonomic component (i.e., phytoplankton vs. bacteria) to understanding the PNM in the ocean. Moreover, there is no a priori reason to believe that all phytoplankton or all nitrifying bacteria display the same physiological characteristics, further complicating our interpretation of the present and future data. Indeed, it is very unlikely that a single mechanism is responsible for the formation of the PNM in all locations, but rather formation of the PNM results from one (or several) of a suite of interrelated mechanisms. Clearly, reconciliation of field and culture observations is needed to fully understand the role of nitrification in forming the PNM. For example, is there an irradiance dose–response relation for nitrification that is not as yet understood? Are we conducting laboratory dose–response experiments on the correct organisms, or are there other nitrifier organisms (or groups of organisms) that are not light inhibited that we should be studying, such as ammonia-oxidizing archaea (Venter et al. 2004; Francis et al. 2005) or nitrite-oxidizing archaea that may well be present in the surface ocean. Nitrite accumulation linked to nitrification would imply an uncoupling of the two oxidation reactions, but available field estimates suggest that NO_2^- oxidation rates are either equal to (Dore and Karl 1996*a*) or greater than NH_4^+ oxidation rates (Ward 2002); so what are the ecosystem controls that allow NO_2^- to accumulate, and what is the role of nitrification?

Answers to these (and likely many other) questions, reconciliation of inconsistencies between culture and field studies, and a more process-oriented field approach to understanding the processes and controls on NO_2^- cycling in the ocean are absolutely essential as global ocean models increasingly include nitrification as a dynamically modeled process (e.g., Oguz et al. 2000; Li and Peng 2002; Denman 2003). One very specific example for the Sargasso Sea is the flexible composition model of Mongin et al. (2003). In this model, nitrification throughout the water column was *required* to keep NH_4^+ concentration fields in line with available data. This model simulation was for the convectively mixed winter period in the Sargasso Sea when the average light dose experienced by nitrifiers as they are mixed through the water column should have exceeded the dose in the PNM during stratified conditions, and therefore should have inhibited nitrification, assuming our view of photoinhibition is correct. Yet the model solutions

required that nitrification produce up to 72% of the euphotic zone nitrate. This case is just one example of where there is a very clear disconnect between available data, understanding of NO_2^- dynamics in the ocean, and how they are modeled.

Future sampling efforts need to consider sampling on the diel timescale and with greater vertical resolution. Future studies would also need to take advantage of new, higher-sensitivity isotope dilution techniques (Lipschultz unpubl. data), new techniques to assess nitrogen uptake by specific taxonomic groups such as flow cytometric sorting (Lipschultz 1995; Casey et al. unpubl. data), as well as new molecular techniques to identify the diversity, number, and activity of marine nitrifying bacteria to distinguish pathways of nitrogen flow in marine ecosystems (Ward 2005). Last, future studies need to consider the possibility of trophic interactions as a control on nitrification rates. Research in coastal regions has clearly shown the importance of both "mutualistic" microbial associations (e.g., Clark and Schmidt 1966; Steinmuller and Brock 1976; Jones and Hood 1980) and trophic cascades (e.g., Verhagen and Laanbroek 1992; Lee and Welander 1994; Lavrentyev et al. 1997). Culture studies with mixed cultures of AOBs and other heterotrophic bacteria are indeed mutualistic, with both NH_4^+ oxidation rates and heterotrophic growth rates increasing when grown together, possibly due to the shuttling of organic metabolites between organisms (e.g., Clark and Schmidt 1967*a,b*; Jones and Hood 1980). Although this exchange is likely to be a minor component in the open ocean because of the overall dilute concentration of particles, it may be important during periods of high particle flux and formation of marine snow. Studies on trophic cascades are more complex, but highlight the importance of grazing pressure on controlling the abundance of nitrifying bacteria and therefore rates of nitrification. To the best of our knowledge, these types of experiments have not been performed in the open ocean and trophic interactions therefore remain potentially important factors in understanding NO_2^- variability in the ocean.

The determination of the "source" of the observed NO_2^- in the ocean is not merely a pragmatic issue; it has large implications for the definition of new production (sensu Dugdale and Goering 1967). One of the major assumptions of the new production paradigm is that euphotic zone nitrification is negligible (i.e., NO_3^- is entrained with deep water, not regenerated within the euphotic zone). We now know this assumption is untrue in stratified surface waters (Ward 1985; Raimbault et al. 1999; Lipschultz 2001), but if we believe our models to be parameterized properly, this assumption may be in question even when NO_3^- is entrained convectively. Nitrite cycling in the ocean is still an area of active research with many unanswered questions.

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