

Nitrite dynamics in the open ocean — clues from seasonal and diurnal variations

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Supplement 1. Determination of ammonium

Special care was taken in the analysis of ammonium due to its extremely low concentrations and high sensitivity to contamination and potential post-sampling transformations. Water for ammonium analysis was sampled very carefully from the Niskin spigot into a 5 ml syringe while exercising great care to avoid any atmospheric contamination. Samples (4 ml) were taken in triplicates (March to November 2000) or quadruplicates (December 2000 onwards) and dispensed into 14 ml polyethylene vials. Both syringe and vials were rinsed 3 times with sample water before filling.

Ammonium was determined using a modified fluorometric method (Protocol A of Holmes et al. 1999). The method uses a stable working reagent, orthophthaldialdehyde (OPA) that forms a fluorescent complex with ammonium. Corrections for background fluorescence (BF) were determined by measuring the fluorescence on samples immediately after the addition of OPA. Typical BF readings were 2 to 3 fluorometer arbitrary units, which were 2 to 3% of the fluorometer response to an ammonium concentration of 0.1 $\mu\text{mol l}^{-1}$. This value was subtracted from the fluorescence readings of the samples. Matrix effect (ME) for samples (collected between March and November 2000) was minimized by using standard calibration curves constructed with nutrient deplete, filtered surface seawater sampled during maximum stratification (August) in the Gulf of Aqaba (GOA). A more rigorous approach to correct for ME (adopted from November 2000 onwards) applied ammonium standard additions of increasing concentration to each individual sample.

During each cast, seawater samples were split into four 4 ml sub-samples, 3 of which were spiked with different ammonium concentrations (up to maximum final concentration of about 0.2 $\mu\text{mol l}^{-1}$) and one was left un-spiked. The spike solution added to replicates of each sample was of 0, 0.1, 0.2, and 0.3 ml of a 2.5 $\mu\text{mol l}^{-1}$ ammonium stock solution. OPA (1 ml) was added into all sub-samples. The samples were incubated for 3 h and measured with a Hoefer (DyNA QuantTM 200) fluorometer on-board ship. Ammonium concentrations were calculated with the equation:

$$K \frac{(C_s V_s + C_n \times n \times V_n + C_{\text{OPA}} V_{\text{OPA}})}{V_t} = F_n \quad (\text{S1})$$

where K ($\mu\text{mol l}^{-1}$)⁻¹ is a constant, C_s is the ammonium concentration in the sample ($\mu\text{mol l}^{-1}$); V_s is the volume of the sample (4 ml), C_n is the ammonium concentration of the spike solution ($\mu\text{mol l}^{-1}$), V_n is the volume of the spike (0.1 ml); V_t is the total volume (sample + spike + OPA = 5 + 0.1n), n

is an integer that varies from 0 to 3 and represents the serial number of the spike added, and F_n is the fluorometer reading in arbitrary units. The concentration of the ammonium in the OPA, C_{OPA} , was found to be practically zero; therefore Eq. (S1) reduces to:

$$F_n(5 + 0.1n) = (4KC_s) + n(0.1KC_n) \quad (S2)$$

The ammonium concentration for each sample was calculated from the slope and intercept by linear regression analysis in which $F_n(5 + 0.1n)$ is the dependent variable, n is the independent variable, $4KC_s$ is the offset and $0.1KC_n$ is the slope. The constant K is calculated from the slope by:

$$K = \frac{\text{slope}}{0.1C_n} \quad (S3)$$

Ammonium concentration, C_s , is calculated by substituting Eq. (S3) in the offset:

$$C_s = \frac{\text{offset}}{4K} \quad (S4)$$

The analytical error and the overall error in ammonium analysis were determined by Eq. (S5):

$$\text{Deviation (nmol l}^{-1}\text{)} = 1000 \times \left| \left(\frac{[F_n(5 + 0.1n)] - \text{offset}}{\frac{\text{slope}}{C_n}} - (n \times C_n) \right) \left(\frac{0.1n}{5 + 0.1n} \right) \right| \quad (S5)$$

This formula calculates the deviation between C_n added and C_n calculated from Eqs. (S2 to S4). The average deviation for all ammonium analyses was $\pm 0.0032 \mu\text{mol l}^{-1}$.

The overall error was determined on samples taken well below the photic zone and far from bottom sediments (all ammonium is oxidized), assuming that ammonium concentration in these samples is ~ 0 . The standard deviation for ammonium concentrations in deep water samples collected in the same cast was $\pm 0.009 \mu\text{mol l}^{-1}$, which is assigned as the overall error because it includes sampling and handling errors also. The detection limit of this modified ammonium analysis as determined on deionized water ($>18.3 \text{ M}\Omega$) was found to be $\sim 0.0035 \mu\text{mol l}^{-1}$.

Supplement 2. Differential photorecovery as a mechanism for primary nitrite maximum (PNM) formation (Guerrero & Jones 1996b)

The vertical distance between the base of the shallow mixed layer (shallower than 25 m) and the PNM is ~ 80 m (Fig. 1d,i in the main paper). It is very unlikely that over such distance within the stratified water column of the Gulf of Aqaba (GOA), nitrite would accumulate at the PNM by differential recovery of ammonium versus nitrite oxidizers (Guerrero & Jones 1996b). Differential recovery leads to nitrite accumulation when ammonium and nitrite oxidizers that were exposed to UV radiation at the surface mixed layer are transported to the base of the photic zone. The ammonium oxidizers recover in deep dark water (the dark recovery zone) at a certain rate while the nitrite oxidizers are irretrievably harmed. This differential recovery leads to nitrite accumulation and hence may serve as an indirect mechanism for the PNM formation. In the GOA, the long distance and the density gradient within the thermocline prevent the ammonium and nitrite oxidizers, which were exposed to UV at the mixed layer, of reaching the depth of the PNM fast enough to show significant differential recovery.

Supplement 3. Supplemental figures

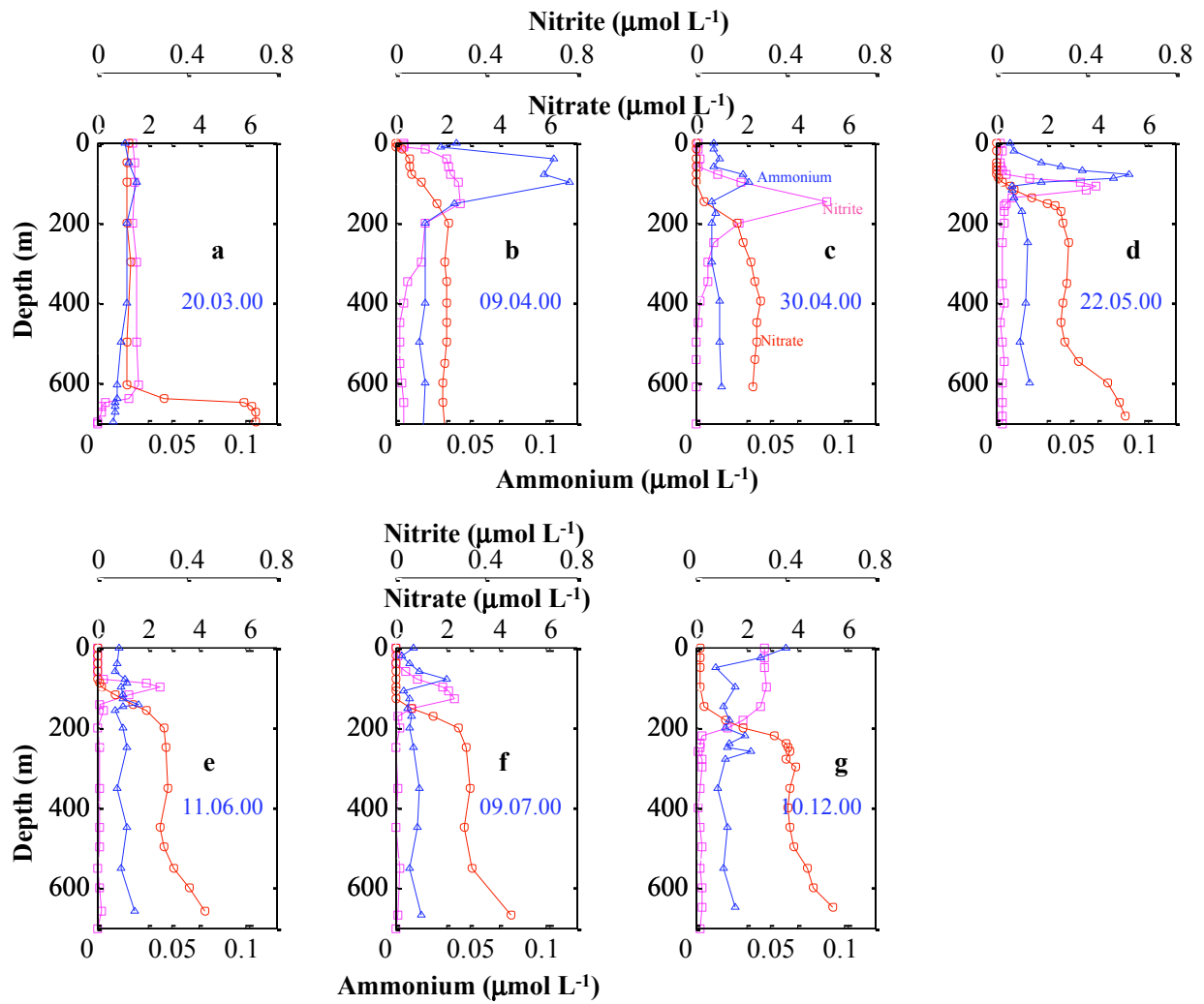


Fig. S1. Nitrate, nitrite and ammonium profiles at Stn A during 2000. Blue numbers indicate sampling date (dd.mm.yy)

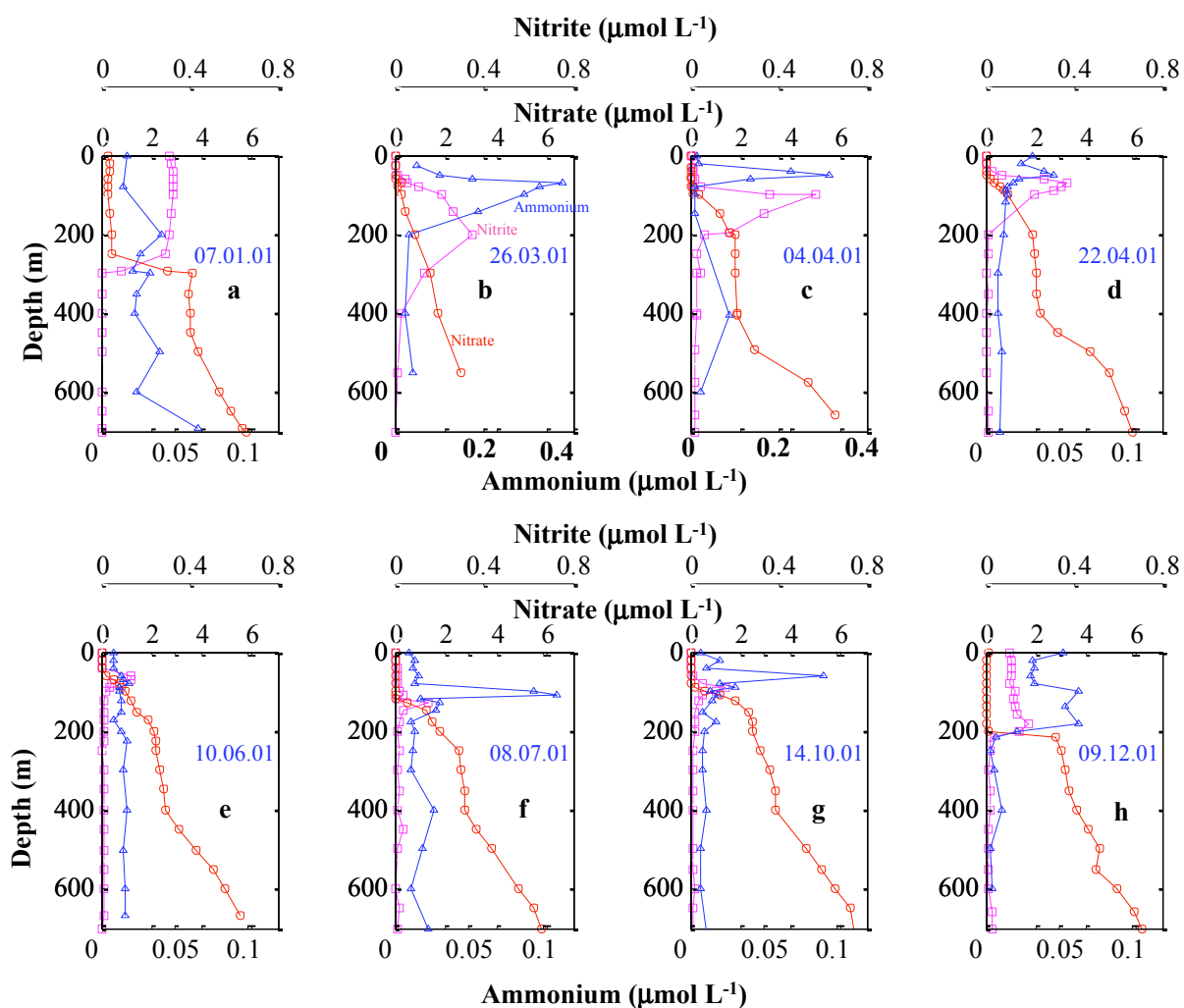


Fig. S2. Nitrate, nitrite and ammonium profiles at Stn A during 2001. Blue numbers indicate sampling date (dd.mm.yy). (b,c) Note the different scale of ammonium during the onset of stratification

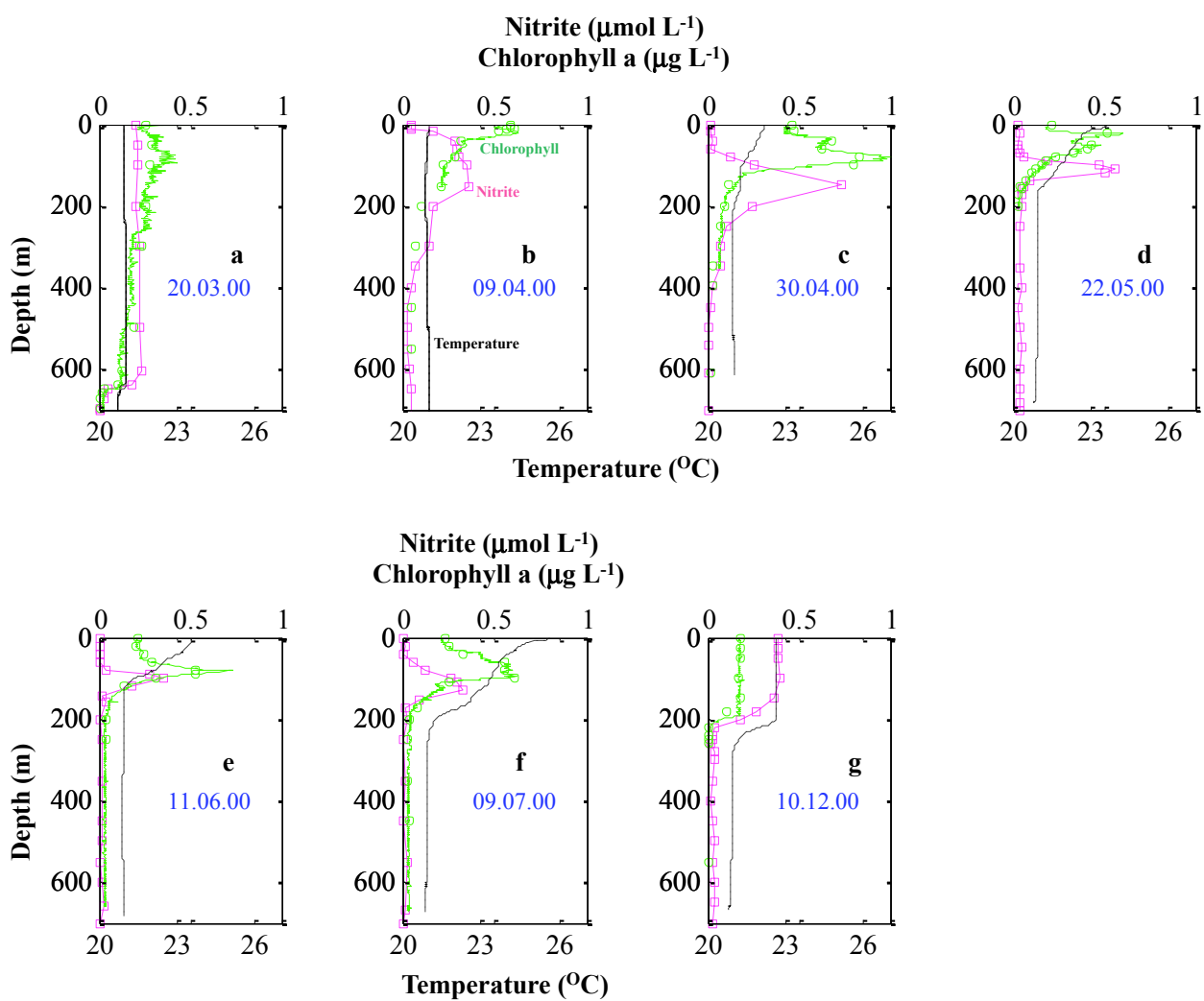


Fig. S3. Temperature, chl *a* and nitrite profiles at Stn A during 2000. Blue numbers indicate sampling date (dd.mm.yy)

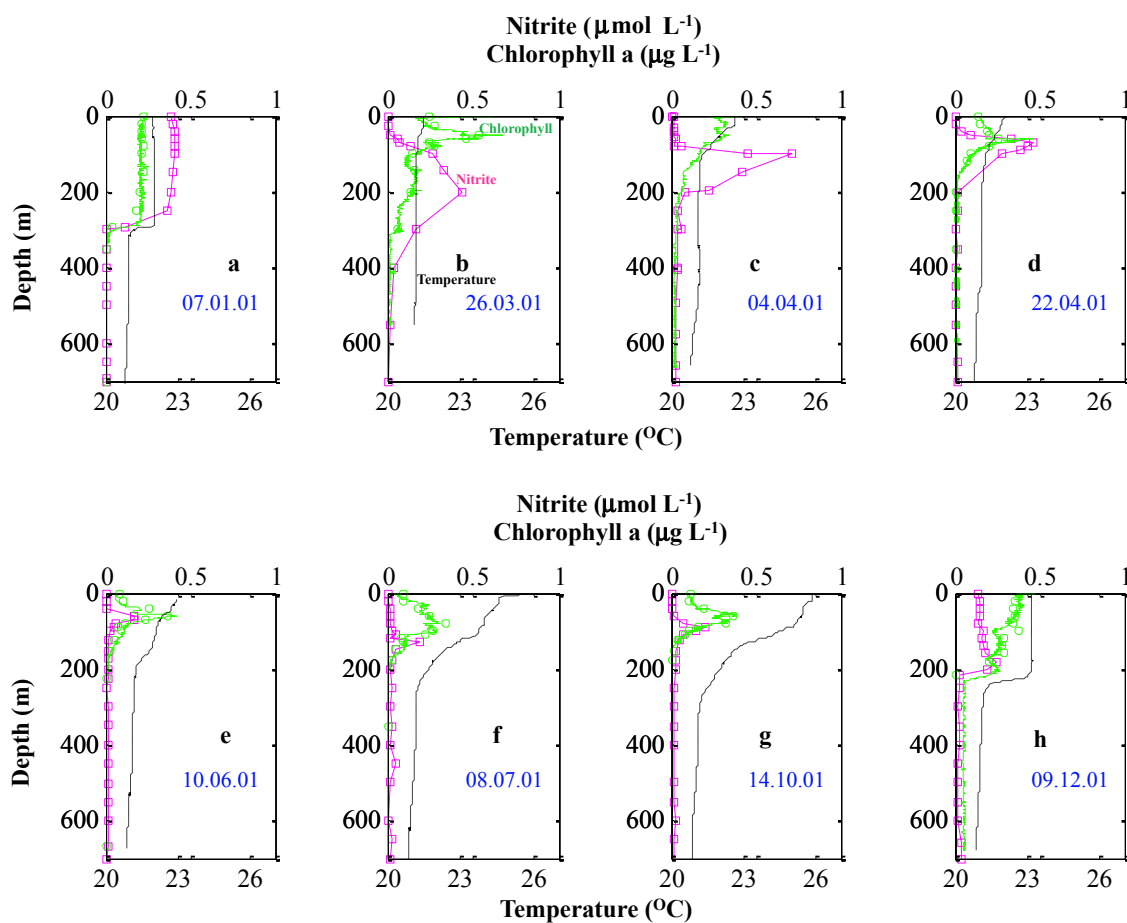


Fig. S4. Temperature, chl *a* and nitrite profiles at Stn A during 2001. Blue numbers indicate sampling date (dd.mm.yy)

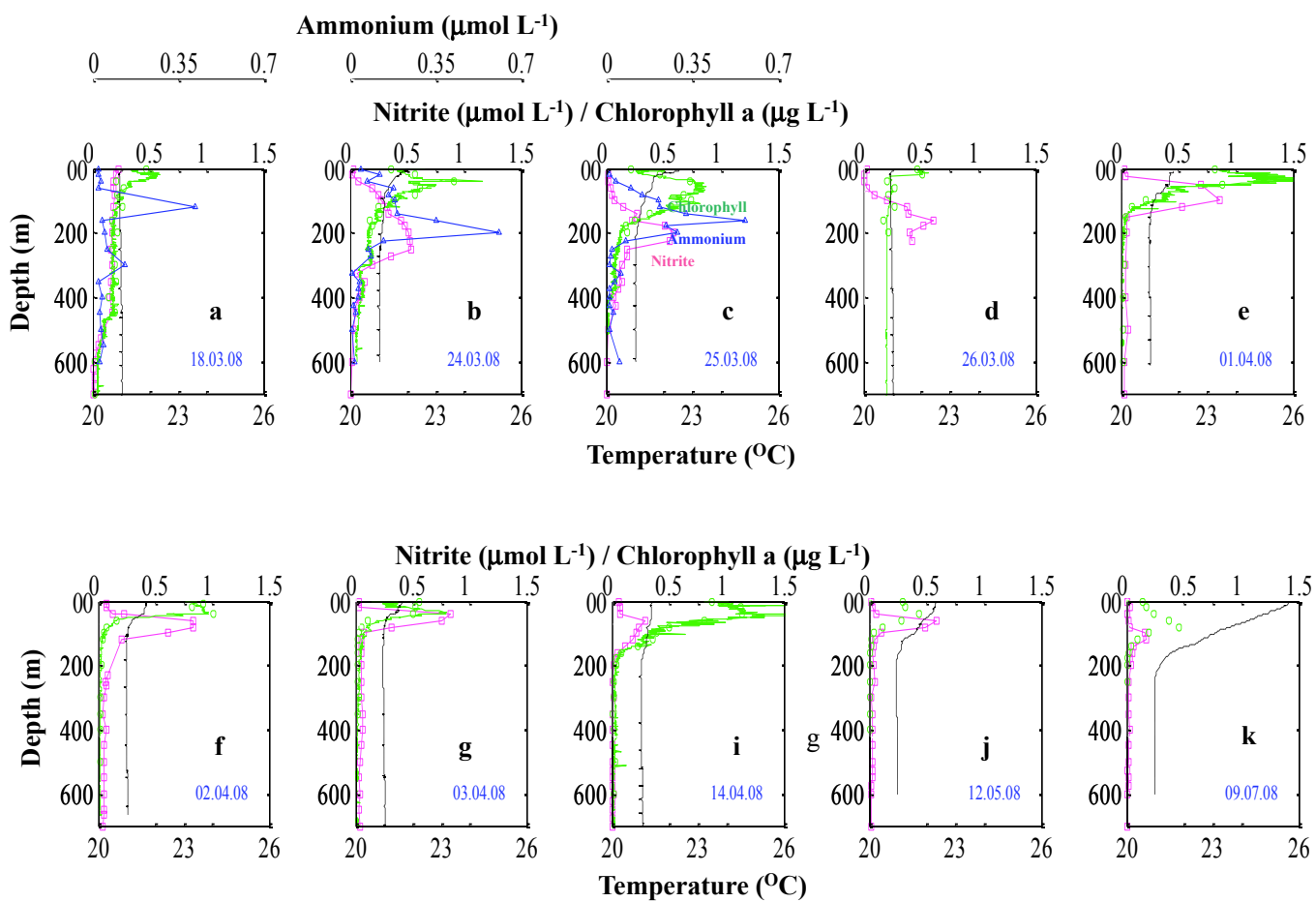


Fig. S5. Temperature, chl *a*, nitrite, and ammonium profiles at Stn A during spring 2008. Blue numbers indicate sampling date (dd.mm.yy)

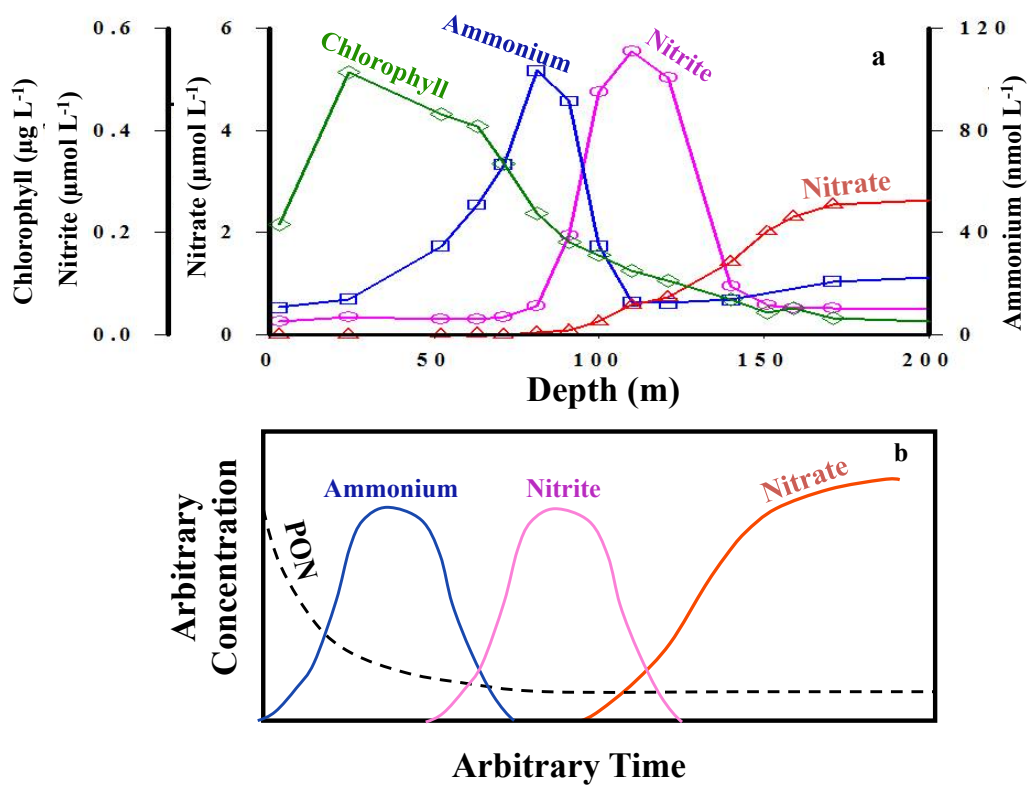


Fig. S6. (a) Representative depth profiles of nitrate, nitrite, ammonium and chl *a* at Stn A (22 May 2000). (b) Schematic illustration of nitrogenous species production and decline in particulate organic nitrogen (PON) from the decomposition of phytoplankton in aerated water stored in the dark (reproduced from Libes 1992)