

This paper's major ocean-relevant finding is that "NO photoproduction from the natural seawater samples from the WTNP did not show any correlations with pH, water temperature and salinity as well as dissolved nitrite concentrations." This is consistent with ref10, which found a strong correlation of R with [NO₂⁻] at >0.3uM (no data below that) with Y intercept R= 2 x 10⁻¹² – very close to the reported R here, 2.1 ±1.3 x 10⁻¹² (Table 1) . The implication is that, despite oceanic [NO₂⁻] varying ~0.02-0.5 uM (what is [NO₂⁻] detection limit?) in this study, the major source(s) of NO are unknown, consistent with R10's correlation and suggesting that the method unfortunately may have been applied in regions where R is outside the DAF-2 method's range of validity.

The method used is "DAF-2" method for NO (ref 9), previously used in seawater (ref 10, in a major journal). Thus it is not surprising that the authors utilized DAF-2. However, this review argues that the DAF-2 results are highly questionable because its response factor may vary in uncharacterized ways under varying conditions, such as T, spectral quality and intensity of light, amount and nature of CDOM that yields ROS and other radicals, [NO₂⁻], and possibly also [O₂] and [NH₄⁺] (as [NH₃]), and redox-active trace metals. Thus the central issue is: To what extent the RNO values found (and lack of correlation) are due to unidentified marine biogeochemical factors vs. un-assessed method variables? The authors need to clarify these aspects in detail.

Danger: the DAF-2 method is assumed to involve a complex series of reactions (below), terminating in DAF-2 □ DAF-2T. Yet the postulated central role of O₂ (Ref 9, fig1) was never shown, NO + O₂ kinetics follow [NO]²[O₂] - slow at low [NO]. DAF-2T likely can form with or without O₂ (see below). Obviously, inaccurately assessed additional DAF-2T sources, and reactions competing with them, affect DAF-2T yields (only 1-18%, an 18x variation! (ref 9)), so that matrix effect evaluation requires assessing these "YD factors" in the matrix at hand.

- Method chemistry #1 (from ref 9): "However, DAFs do not react directly with NO but rather with the oxidized form of NO. In fact, it has been proposed that the reaction mechanism of DAF with NO involves N₂O₃ according to the following scheme: NO + O₂ □ 2NO₂ (2) 2NO₂ + 2NO □ 2N₂O₃ (3)" Thus the simplest case involves truly pure water + light + nitrite +DAF-2. In the presence or absence of O₂, the dominant reaction of OHdot, which has not been considered, is OHdot + NO₂⁻ □ NO₂,that N₂O₃ can form in the absence of O₂; the presence of O₂ adds a second pathway forming DAF- 2T. Furthermore, can other oxidants convert NO to NO⁺, which may be able react with DAF-2 to form DAF-2T.

- Method chemistry #2 also, (ref 9) “Since ...OH was generated along with NO upon NO₂- was a possibility that the degradation of DAF-2 could be a result of the reaction of ·OH with DAF-2. To study this, we carried out a 30 min irradiation of 0.2 μM DAF-2 with 100μM H₂O₂ in Milli-Q water and analyzed DAF-2 before and during the illumination period, at suitable intervals. The signal intensities of DAF-2 were constant during the illumination period (Figure 5), suggesting that the degradation of DAF-2 under these conditions could not be attributed to the reaction of DAF-2 with OH radicals. “ and “the mean value (±standard deviation) of YD 0.042 ±0.003 was used in all calculations of RNO.” How was YD measured in a way relevant to seawater? Ref 9 never showed that a significant amount of OHdot was formed by the irradiation of HOOH; also, another reaction, OHdot +HOOH → HOH + HOOdot; → HOOdot → O₂- + H+, might compete with OHdot + DAF-2 destruction.

Thus even in the simplest “pure water” matrix, the DAF-2 method calibration is inadequate. But in this paper we do not care about “pure water,” except insofar as it can validate the method. In seawater, OHdot also forms other inorganic radicals (Br₂·, CO₃·) that have major effects on the NO₂- + hv → pathways. These reactions presumably make YD factors from pure water irrelevant, yet ref 9 used a pure-water value. There seem to be no determinations of YD in this paper.

Oceanography: seawater samples were from 1 meter, using a CTD, greatly increasing the chances that some samples are contaminated by the ship. 1-m samples for measurements that may be sensitive to trace contaminants (such as RNO) are best obtained using a small boat away from the ship, or taken in the mixed layer from a few meters below the ship’s hull depth.

The possibility that some NO forms from NH₄⁺ (NH₃) via photochemical reactions is ignored. The reported [NH₄⁺] seem high (~0.2-1.2 μM) and do not vary spatially as expected (<https://agupubs.onlinelibrary.wiley.com/doi/full/10.1029/2007GB003039>): “Generally speaking, seawater NH_x concentrations are lower in regions of low productivity; nutrient-limited communities being more efficient at utilizing recycled nitrogen and thus maintaining a lower ambient concentration. Thus high latitudes tend to have substantially greater NH_x concentrations than low latitudes in the open ocean, with high-productivity coastal and shelf seas tending to have highest concentrations, irrespective of latitude [Johnson, 2004].” . Were NH₄⁺ data influenced by ship’s sewage-related effluents (vapor or liquids)? NH₄⁺ in seawater forms nitrite and nitrate via singlet oxygen reactions that may involve NO intermediates, also, CO₃⁻ + NH₃ → NH₂dot; NH₂dot + O₂ → NH₂OOdot, NH₂OOdot → NO + H₂O.

The otherwise useful table 1 needs a “Method” column, and it should be noted that the method of Zafiriou and McFarland almost certainly does not remove NO fast enough to give a total NO formation rate (as the DAF-2 method is intended to do), so is not directly comparable.

Since almost all oceanic mixed-layer NO data are now from the DAF-2 method (Table 1), it would be useful for this Discussion to clearly establish the limits of its applicability. END. This paper's major ocean-relevant finding is that "NO photoproduction from the natural seawater samples from the WTNP did not show any correlations with pH, water temperature and salinity as well as dissolved nitrite concentrations." This is consistent with ref10, which found a strong correlation of R with [NO₂⁻] at >0.3uM (no data below that) with Y intercept R= 2 x 10⁻¹² – very close to the reported R here 2.1 ±1.3 x 10⁻¹² (Table 1) . The implication is that, despite oceanic [NO₂⁻] varying ~0.02-0.5 uM (what is [NO₂⁻] detection limit?) in this study the major source(s) of NO are unknown, consistent with R10's correlation and suggesting that the method has, unfortunately, been applied in regions where R is outside its range of validity.

The method used is "DAF-2" method for NO (ref 9), previously used in seawater (ref 10, in a major journal). Thus it is not surprising that the authors utilized DAF-2. However, this review argues that the DAF-2 results are highly questionable because its response factor may vary in uncharacterized ways under varying conditions, such as T, spectral quality and intensity of light, amount and nature of CDOM that yields ROS and other radicals, [NO₂⁻], and possibly also [O₂] and [NH₄⁺] (as [NH₃]), and redox-active trace metals. Thus the central issue is: To what extent the R_{NO} values found (and lack of correlation) are due to unidentified marine biogeochemical factors vs. un-assessed method variables? The authors need to clarify these aspects in detail.

Danger: the DAF-2 method is assumed to involve a complex series of reactions (below), terminating in DAF-2 → DAF-2T. Yet the postulated central role of O₂ (Ref 9, fig1) was never shown, NO + O₂ kinetics follow [NO]²[O₂] - slow at low [NO]. DAF-2T likely can form with or without O₂ (see below). Obviously, inaccurately assessed additional DAF-2T sources, and reactions competing with them, affect DAF-2T yields (only 1-18%, an 18x variation! (ref 9)), so that matrix effect evaluation requires assessing these “Y_D factors” in the matrix at hand.

- Method chemistry #1 (from ref 9): ”However, DAFs do not react directly with NO but rather with the oxidized form of NO. In fact, it has been proposed that the reaction mechanism of DAF with NO involves N₂O₃ according to the following scheme: NO + O₂ → 2NO₂ (2) 2NO₂ + 2NO → 2N₂O₃ (3)” Thus the simplest case involves truly pure water + light + nitrite +DAF-2. In the presence or absence of O₂, the dominant reaction of OHdot, which has not been considered, is OHdot + NO₂⁻ → NO₂, that N₂O₃ can form in the absence of O₂; the presence of O₂ adds a second pathway forming DAF- 2T. Furthermore, can other oxidants convert NO to NO⁺, which may be able react with DAF-2 to form DAF-2T.
- Method chemistry #2 also, (ref 9) “Since ...OH was generated along with NO upon NO₂⁻ was a possibility that the degradation of DAF-2 could be a result of the reaction of ·OH with DAF-2. To study this, we carried out a 30 min irradiation of 0.2 μM DAF-2 with 100μM H₂O₂ in Milli-Q water and analyzed DAF-2 before and during the illumination period, at suitable intervals. The signal intensities of DAF-2 were constant during the illumination period (Figure 5), suggesting that the degradation of DAF-2 under these conditions could not be attributed to the reaction of DAF-2 with OH radicals. “ and “the mean value (±standard deviation) of Y_D 0.042 ±0.003 was used in all calculations of RNO.” How was Y_D measured in a way relevant to seawater? Ref 9 never showed that a significant amount of OHdot was formed by the irradiation of HOOH; also, another reaction, OHdot +HOOH → HOH + HOOdot; → HOOdot →O₂⁻ + H⁺, might compete with OHdot + DAF-2 destruction.

Thus even in the simplest “pure water” matrix, the DAF-2 method calibration is inadequate. But in this paper we do not care about “pure water,” except insofar as it can validate the method. In seawater, OHdot also forms other inorganic radicals (Br₂⁻, CO₃⁻) that have major effects on the NO₂⁻ + hv → pathways. These reactions presumably make Y_D factors from pure water irrelevant, yet ref 9 used a pure-water value. There seem to be no determinations of Y_D in this paper.

Oceanography: seawater samples were from 1 meter, using a CTD, greatly increasing the chances that some samples are contaminated by the ship. 1-m samples for measurements that may be sensitive to trace contaminants (such as R_{NO}) are best obtained using a small boat away from the ship, or taken in the mixed layer from a few meters below the ship’s hull depth.

The possibility that some NO forms from NH₄⁺ (NH₃) via photochemical reactions is ignored. The reported [NH₄⁺] seem high (~0.2->1.2 uM) and do not vary spatially as expected (<https://agupubs.onlinelibrary.wiley.com/doi/full/10.1029/2007GB003039>): “Generally speaking, seawater NH_x concentrations are lower in regions of low productivity; nutrient-limited communities being more efficient at utilizing recycled nitrogen and thus maintaining a lower

ambient concentration. Thus high latitudes tend to have substantially greater NH_x concentrations than low latitudes in the open ocean, with high-productivity coastal and shelf seas tending to have highest concentrations, irrespective of latitude [**Johnson, 2004**].” . Were NH_4^+ data influenced by ship’s sewage-related effluents (vapor or liquids)? NH_4^+ in seawater forms nitrite and nitrate via singlet oxygen reactions that may involve NO intermediates, also, $\text{CO}_3^- + \text{NH}_3 \rightarrow \text{NH}_2\text{dot}$; $\text{NH}_2\text{dot} + \text{O}_2 \rightarrow \text{NH}_2\text{OOdot}$, $\text{NH}_2\text{OOdot} \rightarrow \text{NO} + \text{H}_2\text{O}$.

The otherwise useful table 1 needs a “Method” column, and it should be noted that the method of Zafiriou and McFarland almost certainly does not remove NO fast enough to give a total NO formation rate (as the DAF-2 method is intended to do), so is not directly comparable.

Since almost all oceanic mixed-layer NO data are now from the DAF-2 method (Table 1), it would be useful for this Discussion to clearly establish the limits of its applicability. END.