Response to Prof. Oliver Zafiriou.

Comments from Prof. Oliver Zafiriou are in black while our response in red and changes in manuscript are in blue.

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."

Thank you for your advice, we have amended our manuscript according to your advice.

In artificial seawater samples of our study, NO photoproduction rates from dissolved nitrite showed increasing trends with decreasing pH, increasing temperatures and increasing salinity. This means several factors would affect NO photoproduction rates, thus it is understandable that there were no significant relationships between NO photoproduction rates with pH, water temperature and salinity as well as nitrite concentrations in natural seawater samples from WTNP since the several factors were different between sampling stations. Besides, we also estimated NO concentration in the surface water, the sea-to-air flux, and the photoproduction rates in the mixed layer in our study area.

This is consistent with ref10, which found a strong correlation of R with [NO$_2^-$] at >0.3 μM (no data below that) with Y intercept R= 2 × 10^{-12} very close to the reported R here 2.1 ±1.3 × 10^{-12} (Table 1). The implication is that, despite oceanic [NO$_2^-$] varying ~0.02-0.5 μM (what is [NO$_2^-$] 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 [NO$_2^-$] detection limit is about 0.05 μmol L^{-1}, while $^{1}/_{2}$ of the detection limit (0.025 round-off to 0.02) was used as the concentration of the sampling stations below the detection limit.

"The concentrations of dissolved inorganic nitrogen (DIN = nitrate, nitrite, and ammonium) from the cruise were analyzed using an automated nutrient analyzer (SKALAR San++ system, SKAlAR, Netherlands) onboard. The detection limits were 0.05 μmol L^{-1} for nitrate, nitrite and ammonium.

When the concentration was below detection limit, $^1/_2$ of the detection limit (0.025 round-off to 0.02) was used."

In the study of Anifowose and Sakugawa (2017), NO_2^- concentration, which varied from ~0.02-0.3 μmol L^{-1}, showed linear correlation with R_{NO} (1.4-9.2 $\times 10^{-12}$ mol L^{-1} s^{-1}, R^2=0.9537) in the surface seawater from the Seto Inland Sea in 2013 and 2014, so the average rate 2.1 ±1.3 $\times 10^{-12}$ mol L^{-1} s^{-1} in our study (under simulator) was inside 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, [NO_2^-], and possibly also [O_2] and [NH_4^+] (as NH_3), 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.

If we take the missing 30% of f_{NO} as the experimental error, then in our study, using the J_{NO} in the artificial seawater, the average $\%f_{NO}$ value in natural water was calculated to be 52% (–30%), indicating that there are other unknown nitrogenous compounds. For example, NO_2^- can be produced from NO_3^- photolysis ($NO_3^- \xrightarrow{hv} NO_2^- + \frac{1}{2}O_2$) or other organic matters which could further lead to NO production (Kieber et al., 1999; Goldstein and Rabani, 2007; Minero et al., 2007; Benedict et al., 2017). Thus, unidentified marine biogeochemical factors might account for the 48% (+30%) of the NO production while un-assessed method variables might account for 30% of the NO production.

"In our study, the average $\%f_{NO}$ value in natural water was 52% (-30%), this indicated that there are about 48% (+30%) other unknown nitrogenous compounds, for example, NO_2^- produced from NO_3^- photolysis (R7) or from other organic matter which could further lead to NO production (Benedict et al., 2017; Goldstein and Rabani, 2007; Kieber et al., 1999; Minero et al., 2007)."

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 O_2 (Ref 9, fig1) was never shown, $NO + O_2$ kinetics follow $[NO]^2[O_2]$ – slow at low [NO]. DAF-2T likely can form with or without O_2 (see them, affect DAF-2T yields (only 1-18%, an 18× variation! (ref 9)), so that matrix effect evaluation requires assessing these “YD factors” in the matrix at hand.

In our study, the external standard method was used with a series of NO standard as follows: an aliquot of 10 mL Milli-Q water was bubbled with N_2 gas at a flow of 10 mL min^{-1} for 2 h to remove O_2 after 10 min of ultrasonic and heat degassing. The solution was then bubbled with high-purity NO gas (99.9 %, Dalian Date Gas Ltd., China) for 30 min. The concentration of the saturated NO stock solution was 1.4 mmol L^{-1}, which could be used within 3 h (Lantoine et al., 1995). A secondary standard of NO solution was also prepared in N_2-purged water from the NO stock solution (Xing et al., 2005; iu et al., 2017). The series samples were trapped by DAF-2 by injecting series of NO standard solution into DAF-2 solution (1.4 μmol L^{-1} in artificial seawater) using different (micro)syringes. Then the measured product (DAF-2T) peak area was plotted against NO concentration, and the standard curve was $y = 0.101\ x$ (x: μmol L^{-1}, y: nmol L^{-1}); the intercept was removed because in our irradiation experiment, the peak area of the control samples (wrapped in aluminum foil) was subtracted from all the samples. Thus, our detection method was somewhat a little different from Ref 9 although the reaction between NO and DAF-2 in our study was the same as Ref 9.

• 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 N_2O_3 according to the following scheme: $NO + O_2 \rightarrow 2NO_2$ (2) $2NO_2 + 2NO \rightarrow 2N_2O_3$ (3)” Thus the simplest case involves truly pure water + light + nitrite +DAF-2. In the presence or absence of O_2, the dominant reaction of •OH, which has not been considered, is $•OH + NO_2^- \rightarrow NO_2$, that N_2O_3 can form in the absence of O_2; the presence of O_2 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.

In the Supporting Information to accompany the manuscript of ref #10, Olasehinde et al (2010) studied the effect of the addition of benzene which served as •OH scavenger, and the results showed

(Supporting Information of ref #10, page 5 line 4) “no appreciable difference between the fluorescence intensity of DAF-2T formed in the presence and absence of benzene, suggesting the negligible effect of •OH radicals on the nitric oxide generated in equation S1. Further, it has been shown that 2 μM DAF-2 was sufficient to effectively scavenge all NO• formed from the irradiation of 10 μM NO_2^- in Milli-Q water in the presence of other *in situ* generated radicals (*5*).” Thus we think that the influence of •OH, whether existed in the water samples or photolyzed from NO_2^-, could be neglected.

Method chemistry #2 also, (ref 9) “Since …•OH was generated along with NO upon NO_2^- 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 100 μM H_2O_2 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 *R*NO.” How was *Y*D measured in a way relevant to seawater? Ref 9 never showed that a significant amount of OH• was formed by the irradiation of HOOH; also, another reaction, $OH\bullet + HOOH \rightarrow HOH + HOO\bullet$; $HOO\bullet \rightarrow O_2^- + H^+$, might compete with OH• + DAF-2 destruction. Thus even in the simplest “pure water” matrix, the DAF-2 method calibration is in adequate. But in this paper we do not care about “pure water,” except insofar as it can validate the method. In seawater, OH• also forms other inorganic radicals (Br_2^-, CO_3^-) that have major effects on the $NO_2^- + h\nu \rightarrow$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.

It is agreed that *Y*D factors in Milli-Q water is different from those in seawater medium. As mentioned above, the external standard method was used in our study. The *Y*D value of Ref 9 was not used in our study, and we think *Y*D was similar in our artificial seawater standards to that in our seawater samples. Although *Y*D was lower (only 1-18% with an 18× variation), the studies by Olasehinde et al (2009; 2010), Anifowose et al (2015) and Anifowose and Sakugawa (2017) showed good results and provide a new method to evaluate NO concentration and its production and consumption in the seawater.

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.

Thank you for your advice, we would improve our sampling method with a small boat in the future if the condition permits or we would take photolysis samples from the mixed layer.

The possibility that some NO forms from NH_4^+ (NH_3) via photochemical reactions is ignored. The reported [NH_4^+] 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 NHx 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 NHx 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, $CO_3^- + NH_3 \rightarrow NH_2\bullet$; $NH_2\bullet + O_2 \rightarrow NH_2OO\bullet$, $NH_2OO\bullet \rightarrow NO + H_2O$.

Firstly, NH_4^+ data was not influenced by ship's liquid sewage, because the sewage was released after the samples were collected from CTD. Secondly, about the vapor, we think the samples might not be polluted by NH_3. Because during the cruise to the Yellow Sea and the East China Sea in 2017, the same vessel "Dongfanghong 2" was also used and the same sampling and analytical method were used, while the NH_4^+ were at lower level. However, it seems that in our study, NH_4^+ concentration was higher than Johnson et al. (2008). It might be the typhoon that made deep layer NH_4^+ mixed with surface layer in our study area in winter.

The relevant reactions were added to the manuscript and Laszlo et al. (1998) found that this $CO_3^{\cdot -}$ could also produce by OH. This potential pathway to produce NO was contained in "48% (+30%) other unknown nitrogenous compounds".

“besides, in natural sunlit seawater, photolyzed dissolved nitrate (NO_3^-) could be a potential source of NO through NO_2^- (R 7); during the process of ammonium (NH_4^+/NH_3) oxidation in to NO_2^- and NO_3^-, NO might be an intermedium (Joussotdubien and Kadiri, 1970), or NO could be produced through amino-peroxyl radicals (R 8 to R 11) (Laszlo et al., 1998;Clarke et al., 2008)

$$NO_3^- \overset{h\nu}{\rightarrow} NO_2^- + \frac{1}{2} O_2 \qquad (R\ 1)$$

$$OH^{\cdot} + HCO_3^-/CO_3^{2-} \rightarrow CO_3^{\cdot -} + H_2O/OH^- \qquad (R\ 2)$$

$$OH^{\cdot} + NH_3 \rightarrow NH_2^{\cdot} + H_2O \qquad (R\ 3)$$

$$CO_3^{\cdot -} + NH_3 \rightarrow NH_2^{\cdot} + HCO_3^- \qquad (R\ 4)$$

$$NH_2^{\cdot} + O_2 \rightarrow NH_2O_2^{\cdot} \qquad (R\ 5)$$

$$NH_2O_2^{\cdot} \rightarrow NO^{\cdot} + H_2O \qquad (R\ 6)”$$

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.

The “Method” column was added in revised manuscript.

Regions	*R* (mol L^{-1} s^{-1})	Method	NO (mol L^{-1})	NO_2^- (μmol L^{-1})	Flux (mol m^{-2} s^{-1})	Sampling date	References
Seto Inland Sea, Japan	$8.7–38.8 \times 10^{-12}$	DAF-2	120×10^{-12}	0.5-2	3.55×10^{-12}	Oct 5–9, 2009	Olasehinde et al., 2010
Seto Inland Sea, Japan	$1.4-9.17 \times 10^{-12}$	DAF-2	$3-41 \times 10^{-12}$	~0.02-0.4	0.22×10^{-12}	Sep, 2013 and Jun, 2014	Anifowose and Sakugawa, 2017
Kurose River, Japan	$9.4–300 \times 10^{-12}$	DAF-2	–	-	–	–	Olasehinde et al., 2009
Kurose River (K1 station), Japan	4×10^{-12}	DAF-2	1.6×10^{-12}	0.06	–	Monthly, 2013	Anifowose et al., 2015
Jiaozhou Bay	–	DAN	157×10^{-12}	-	7.2×10^{-12}	Jun, Jul and Aug, 2010	Tian et al., 2016

Jiaozhou Bay and its adjacent waters	–	DAN	$(160 \pm 130)\times10^{-12}$	-	10.9×10^{-12}	Mar 8–9, 2011	Xue et al., 2011
Coastal water off Qingdao	1.52×10^{-12}	DAN	260×10^{-12}	0.75	-	Nov, 2009	Liu et al., 2017
Central equatorial Pacific	$> 10^{-12}$	Chemiluminescence	46×10^{-12}	0.2	2.2×10^{-12}	R/V Knorr 73/7	Zafiriou and Mcfarland., 1981
Northwest Pacific Ocean	$0.5 \pm 0.2\times10^{-12}$	DAF-2	49×10^{-12}	0.06	1.8×10^{-12}	Nov 15, 2015 to Jan 26, 2016	This study

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.

Seen from Table 1, Olasehinde et al. (2010) and Anifowose and Sakugawa (2017) showed that the detection limits might be about 0.02 μmol L^{-1} of NO_2^- in the Seto Inland Sea, Japan. In our study, although the concentration of NO_2^- ranged from 0.02 to 0.33 μmol L^{-1}, the linear relationship was not found. This might because that other factors like pH, salinity were different between samples collected at different stations.

"According to the photoproduction rates and the relevant NO_2^- in Olasehinde et al. (2010), Anifowose and Sakugawa (2017) (Table 1), the photoproduction rates under lower than 0.02 μmol L^{-1} NO_2^- might not be determined in nearshore waters like the Seto Inland Sea."

The following references are added.

Anifowose, A. J., and Sakugawa, H.: Determination of Daytime Flux of Nitric Oxide Radical (NO·) at an Inland Sea-Atmospheric Boundary in Japan, J Aquat Pollut Toxicol, 1, 1- 6, 2017.

Benedict, K. B., Mcfall, A. S., and Anastasio, C.: Quantum Yield of Nitrite from the Photolysis of Aqueous Nitrate above 300 nm, Environ. Sci. Technol., 51, 4387-4395, 2017.

Clarke, K., Edge, R., Johnson, V., Land, E. J., Navaratnam, S., and Truscott, T. G.: The carbonate radical: its reactivity with oxygen, ammonia, amino acids, and melanins, J Phys Chem A, 112, 10147-10151, 2008.

Goldstein, S., and Rabani, J.: Mechanism of Nitrite Formation by Nitrate Photolysis in Aqueous Solutions: The Role of Peroxynitrite, Nitrogen Dioxide, and Hydroxyl Radical, J. Am. Chem. Soc., 129, 10597, 2007.

Johnson, M. T., Liss, P. S., Bell, T. G., Lesworth, T. J., Baker, A. R., Hind, A. J., Jickells, T. D., Biswas, K. F., Woodward, M. E. S., and Gibb, S. W.: Field observations of the ocean-atmosphere exchange of ammonia: Fundamental importance of temperature as revealed by a comparison of high and low latitudes, Global Biogeochem. Cycles, 22,GB1019-GB1034, 2008.

Joussotdubien, J., and Kadiri, A.: Photosensitized Oxidation of Ammonia by Singlet Oxygen in Aqueous Solution and in Seawater, Nature, 227, 700-701, 1970.

Kieber, R. J., Li, A., and Seaton, P. J.: Production of nitrite from the photodegradation of dissolved organic matter in natural waters, Environ. Sci. Technol., 33, 717-723, 1999.

Lantoine, F., Trévin, S., Bedioui, F., and Devynck, J.: Selective and sensitive electrochemical measurement of nitric oxide in aqueous solution: discussion and new results, J Electroanal Chem, 392, 85-89, 1995.

Laszlo, B., Alfassi, Z. B., Neta, P., and Huie, R. E.: Kinetics and Mechanism of the Reaction of • NH_2 with O_2 in Aqueous Solutions, J Phys Chem A, 102, 8498-8504, 1998.

Minero, C., Chiron, S., Falletti, G., Maurino, V., Pelizzetti, E., Ajassa, R., Carlotti, M. E., and Vione, D.: Photochemincal processes involving nitrite in surface water samples, Aquat. Sci., 69, 71-85, 2007.

Xing, L., Zhang, Z. B., Liu, C. Y., Wu, Z. Z., and Lin, C.: Amperometric Detection of Nitric Oxide with Microsensor in the Medium of Seawater and Its Applications, Sensors, 5, 537-545, 2005.