An itemized response (blue words) to the reviewers' comments and suggestions


Anonymous Referee #1

This paper describes a new method for NO in water samples and reports studies of NO in coastal waters. This seems to be the first such report in coastal waters and one of the few in any marine environment.

Since NO is undoubtedly a difficult analyte to measure, and since there is little work on it, larger-than-usual uncertainties in results are tolerable; however, at some point very uncertain data lose usefulness.

Although this work appears to be carefully done and well described in many aspects, there are major ambiguities and concerns about this method, including major uncertainties as to how reported [NO] values relate to [NO] in situ. Both aspects need clarification in order to render the MS acceptable

Method – chemistry: (1) The equation (line141-144) is incomplete and disagrees with that reported: It is unbalanced, since NO has an odd number or electrons whereas the products do not.

Line141-144: The reaction diagram is cited from Miles et al. (1995). The relatively nonfluorescent DAN reacts rapidly with the NO-derived N-nitrosating agent to yield its highly fluorescent product NAT. The mistake has been corrected in the revised manuscript, as indicated below.

2,3-Diaminonaphthalene (DAN)

2,3-Naphthotriazole (NAT)
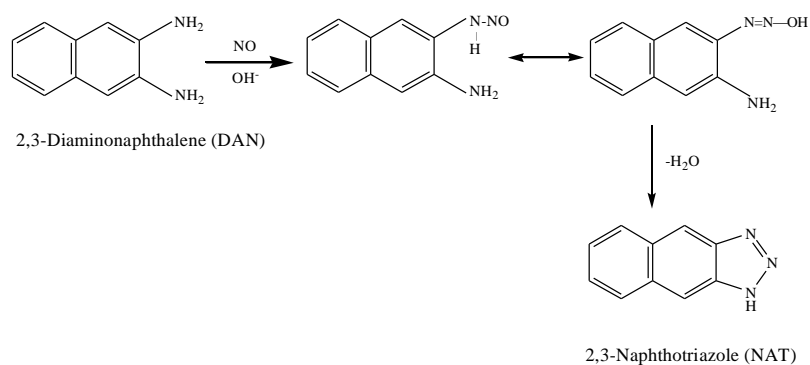
Also, (2) O2 is involved in the DAN->NAT reaction: Biol. Pharm. Bull 21(12) 1247-1250 (1998) states": : : The reaction of NO and O2 with 2,3 diaminonaphthalene (DAN) produced a fluorescent triazole." but in this study the only O2 present is the variable amount in the sample. Less-soluble O2 is stripped out faster than is NO, so as NO reaches the DAN solution, the pO2 varies over time, potentially altering the (NO->NAT) yield.

Yes, the reaction of NO and O_2 with 2,3 diaminonaphthalene (DAN) produced a fluorescent triazole. However, the mechanism of this fluorescence has not yet been established in detail, although the fluorescence increased dose-dependently by NO addition (Nakatsubo et al., 1998). In seawater samples, the concentration of NO (10^{-4} M order of magnitude) was far higher than that of NO (10^{-10} M order of magnitude). Both of them were stripped out and reached the DAN solution finally, thus the NO in samples could almost quantitatively

transform into NAT.

(3) The equation (line 232) is incorrect.
Line 232: The equation has been modified, as indicated below.

RC（%）= NO (sw) / NO (DAN)× 100%.

Where NO (DAN) stands for the NO directly injected to the DAN solution and NO (sw) stands for the NO measured from the sample in degassing column according to the method described above.

(4) NO reacts with O2 in both gas phase and solution; rate = k[NO]ˆ2[O2] (ks are known). No evaluation of the roles of these reactions potentially consuming NO is given in the time between sampling and analysis (30 minute stripping period).
The rate law obtained from the oxidation of NO is

$$-d[NO]/dt=4k[NO]^2[O_2]$$

with $k=2*10^6M^{-2}s^{-1}$. The reaction of NO with O_2 could consume NO in the stripping period indeed. However, we have evaluated the NO recovery coefficient of our purge-and-trap system as described in (3). The resulting NO recovery coefficients ranged from 80.2% to 90.0%, with an average of 83.8%. Furthermore, three replicates of in-situ seawater were measured using our system and method, the aqueous NO solution did not change within one hour, which was also demonstrated by Lutterbeck and Bange (2015).

Method optimization: Table 1's large variations in reaction efficiency clearly establish that near-optimal conditions need to be better defined. At 300 ml/min/45 min (13.5 L gas) the NAT yield is 21%, while at 400 ml/min/30 min (12 L gas), the yield is 69%. Thus, a 12.5% decrease in purge gas volume results in a 328% increase in DAN yield! This huge sensitivity demands better characterization of yield-controlling factors. Also, how can the efficiency also drop at longer times - is the DAN/NAT solution unstable?
This huge sensitivity was related to the status of DAN solution. Under the impact of N_2 gas flow, DAN formed many small bubbles. When the flow reached a certain volume, the trapping liquid was almost bubble - like. The specific surface area was greater when the bubbles were smaller and more; the contact area of NO and DAN was larger; and the reaction was more fully. However, when the flow became even larger, greater bubbles formed, the specific surface area decreased; the reaction yield of the reaction of DAN with NO reduced. The flow rate of 400 mL/min and purging time of 30 min was identified as the optimal experiment condition through the experiments of different flow rate and purging time.
Our experiments showed that the DAN solution was stable in 12 h and the NAT solution did not change within 4 h.

Calculations: Figure 4 lacks critical points at $[NO_2^-]$=0. The bottom 3 curves are roughly the same, _850 units _7%. Is this a "method blank"? Is any blank subtracted? For lab and at-sea measurements, the equation relating fluorescence units to [NO] should be given, along with any blank term(s) used.
According to the reviewer's suggestion, we have added the blank and used [NO] to replace

fluorescence units in Figure 4. The bottom 3 curves represent the variations of NO concentrations in different concentrations of nitrite solutions in the dark or under UV-B radiation of 1h. The blank was subtracted.
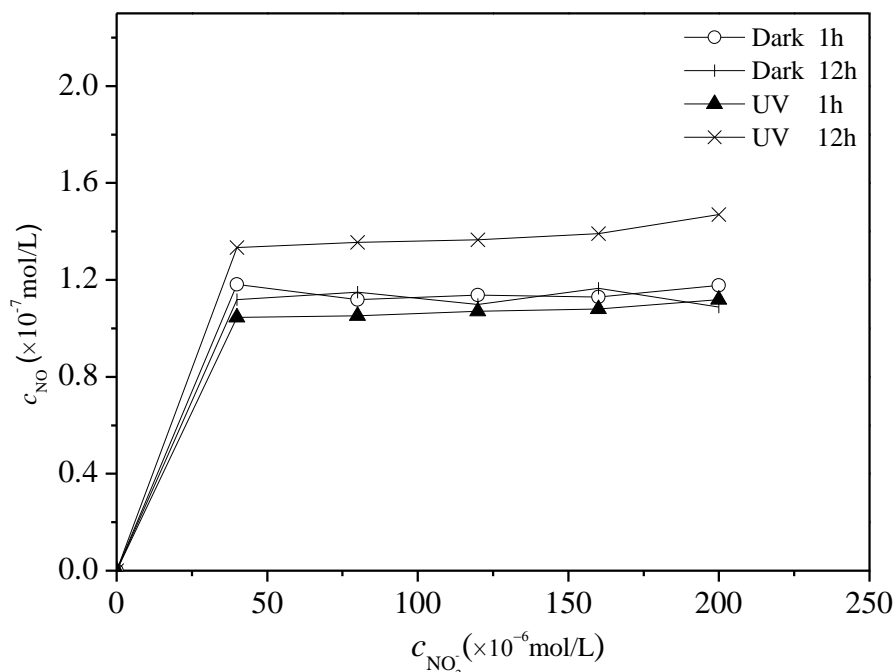

Fig. 4 The variations of NO concentrations in seawater with different concentrations of nitrite in the dark or under UV-B radiation

Here these high NO_2^- concentrations were design to demonstrate no effect caused by NO_2^- on the detection method, thus low concentrations of NO_2^- also has no effect. On the other hand, the fluorescence intensity could not be detected with low concentrations of NO_2^-.

Environmental [NO] data:
(1) In prior marine NO work by others, the NO source has been assigned to microbial processes that were assumed to continue, perhaps at perturbed rates, even during the stripping step, minimizing any losses, or to nitrite photolysis occurring while samples were stripped. Compared to this work, time-dependent losses were previously minimized (most microbial samples were also suboxic or anoxic). Nonetheless, likely all reported data underestimate [NO] to some extent. In contrast, here the minimum time seems to be (?) minutes in the dark, plus 15 minutes on average in the degasser. Thus comparing these data with literature values without qualification/explanation is unjustified.
According to the reviewer's suggestion, we have tried to discuss the deviation of these detection methods.
NO is a short-lived intermediate of various microbial processes of the nitrogen cycle, which is involved in denitrification (Kampschreur et al., 2007), anammox (Kartal et al., 2011) and archaea ammonia-oxidizing (Martens-Habbena et al., 2015) processes. Zafiriou and McFarland (1981) analyzed NO in seawater samples at the sea surface of the central

equatorial Pacific by stripping NO into an air and N_2 stream by passing it through the same chemiluminescence - type detector. Thus, the NO concentrations were underestimated to some extent because seawater samples were suboxic or anoxic. However, time-dependent losses from microbial processes were minimized. Lutterbeck and Bange (2015) improved the method above to determine dissolved NO in discrete seawater samples of the eastern tropical South Pacific Ocean. The contamination by O_2 diffusion into the continue samples could be further minimized. This work was also designed to detect dissolved NO in discrete seawater samples with a combination of a purge-and-trap set-up and fluorometric NO analyzer. The $HgCl_2$ solution was added to stop biological activities during the stripping. However, the disposal of these Hg-contaminated solutions is a tough proposition.

To improve the method, the purge-and-trap set-up could be modified and the stripping time could be reduced, then the addition of $HgCl_2$ solution may be removed.

(2) In irradiated/sunlit waters, light may also induce NO losses by forming NO-reactive radicals from CDOM. NO loss has been used to estimate rates of CDOM + hv -> radicals {Marine Chem., 30, 45–71 (1990); J. Geophys. Res. 96(C3), 4939–4945 (1991)} and Olasehinde et al. stated, "Thus, our findings indicate that the reaction of NO with photochemically generated free radicals might be a major pathway for NO loss in natural waters." Given high [DOC] in these waters, likely also rich in CDOM (it IS the "Yellow Sea"), it seems likely that NO consumption occurred. Was onlu a small residual [NO] detected?

The production and consumption of NO occur synchronously when sunlight photolyze natural seawater. The photolysis of NO_2^- is to mainly produce NO and OH. On the other hand, the loss of NO happens by forming NO-reactive radicals from CDOM (Zafiriou et al., 1990; Zafiriou and Dister, 1991; Olasehinde et al., 2009).The concentration of NO after exposure to sunlight is a balancing of this production against consumption by radical recombination. The study area has high concentrations of DOC and is rich in CDOM (Liu et al., 2010; Yang et al., 2011), thus, the authentic NO resulted from NO_2^- photolysis was underestimated. The photochemical production rates of NO were only a total value of production and consumption in this study.

(3) What can be reliably said about the reported data's implications for [NO] in-situ, if one ignores the absolute values of [NO] as uncertain and assumes only a roughly constant reaction efficiency? The patterns of Figures 5, 6 seem consistent with a positive light-dependence of the source/sink balance.

NO is a conceptually important intermediate in N-cycle biogeochemistry, product of ocean photochemistry, and putative inter-cellular signal. Unfortunately, our knowledge about the oceanic NO distribution and the major pathways of NO is very poor. There are only a few published NO concentration measurements available because a reliable and easy to use method to determine dissolved NO at in-situ concentrations in seawater samples is missing. We try to find a solid method both convenient for many labs and sensitive enough, which seems to have promise (one anonymous reviewer). With our method we determined for the first time the temporal and spatial distributions of NO surface concentrations in coastal waters of the Yellow Sea off Qingdao and in Jiaozhou Bay during a cruise in November 2009. Our

results implied the presence of NO formation processes such as NO_2^- photolysis, which was closely related to light intensity and nitrite concentration, and that the occurrence of particles and a temperature increase can enhance NO production rates.

The following references are added.

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