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Determination of dissolved nitric oxide in coastal waters of the Yellow Sea off Qingdao

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Abstract We developed a new method for the determination of dissolved nitric oxide (NO) in discrete seawater samples based on a combination of a purge-and-trap set-up and fluorometric detection of NO. 2,3-diaminonaphthalene (DAN) reacts with NO in seawater to form the highly fluorescent 2,3-naphthotriazole (NAT). The fluorescence intensity was linear for NO concentrations in the range from 0.14 nmol L^{-1} to 19 nmol L^{-1}. We determined a detection limit of 0.068 nmol L^{-1}, an average recovery coefficient of 83.8% (80.2-90.0%), and a relative standard deviation of ±7.2%. 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. The concentrations of NO varied from below the detection limit to 0.50 nmol L^{-1} with an average of 0.26 ± 0.14 nmol L^{-1}. NO surface concentrations were generally enhanced significantly during daytime implying that NO formation processes such as NO_2^- photolysis are much higher

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during daytime than chemical NO consumption which, in turn, lead to a significant decrease of NO concentrations during nighttime. In general, NO surface concentrations and measured NO production rates were higher compared to previously reported measurements. This might be caused by the high NO_2^- surface concentrations encountered during the cruise. Moreover, additional measurements of NO production rates implied that the occurrence of particles and a temperature increase can enhance NO production rates. With the method introduced here we have a reliable and comparably easy to use method at hand to measure oceanic NO surface concentrations which can be used to decipher both its temporal and spatial distributions as well as its biogeochemical pathways in the oceans.

Keywords: Nitric oxide (NO), determination method, coastal waters of the Yellow Sea, distribution, production rate

1 Introduction

As a reactive atmospheric trace gas, nitric oxide (NO) plays important roles in tropospheric chemistry: It is a key player in the formation of acid rain and ozone (Williams et al., 1992; Lee et al., 1997; Mazzeo, et al., 2005). NO is an intermediate of both the terrestrial and marine nitrogen cycle (Ward and Zafiriou, 1988; Williams et al., 1992; Canfield et al., 2010; Chen et al., 2010; Thamdrup, 2012; Voss et al., 2013). It has a variety of sources in seawater, including nitrite photolysis and various microbial processes such as denitrification, anammox and dissimilatory nitrate reduction to ammonia (Law, 2001; Schreiber et al., 2012; Martens-Habbena et al., 2015). Because of its chemical reactivity, NO usually does not accumulate in large amounts in seawater and the ocean as a source of atmospheric NO is, therefore, negligible in a global context (Zehr and Ward, 2002; Bange, 2008). Moreover, NO was found to have significant effects on the growth of marine algae (Zhang et al., 2005; Liu et al., 2004;

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2005; 2006; 2014). To this end, the determination of the spatial and temporal distributions of NO in the ocean as well as deciphering its oceanic production processes and their major influencing factors are essential to improve our understanding of the biogeochemical cycling NO in the ocean.

Because of its low concentrations in seawater caused by its fast diffusion and high chemical reactivity, measurements of NO in seawater are very difficult. Therefore, there are only a few methods available to determine NO (Hetrick and Schoenfisch, 2009), see Tab. 1. The electrochemical method using sensors in seawater medium achieved a detection limit of 42 nmol L^{-1} (Xing et al., 2005; Zhang et al., 2005). Olasehinde et al. (2009) developed a method for the determination of photochemically generated NO in natural waters adopting 4,5-diaminofluorescein as a probe compound and a measurement of reversed-phase high performance liquid chromatography (HPLC) with fluorescence detector. The NO concentrations and signal intensities exhibited a good linearity correlation over the range of 0.025-10 nmol L^{-1} triazolofluorescein. Zafiriou and McFarland (1980) determined the NO concentration of seawater by using a flow system to equilibrate the seawater samples with a gas stream coupled to a chemiluminescence detector. They report an analytical precision of ±3% and an accuracy of ±20%. More recently, Lutterbeck and Bange (2015) developed an improved method of a chemiluminescence NO analyser connected to a stripping unit, and the limit of detection was 0.25 nmol L^{-1} using a 20 mL seawater sample volume. Until now only these two chemiluminescence methods were applied successfully to determine NO concentrations in seawater samples. The N-nitrosation of 2,3-diaminonaphthalene (DAN) results in the highly fluorescent 2,3-naphthotriazole (NAT) which could be used to detect NO concentrations as low as 10 nmol L^{-1} (Miles et al., 1995). We adopted this method for seawater medium instead of NaOH medium and the calibration curve exhibited linearity over the concentration range of 1.4 - 1400 nmol L^{-1} NO (Liu et al., 2009). However,

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this assay cannot be used to detect trace levels of NO in seawater samples directly.

In this paper, we describe a modified spectrofluorometric method using a purge-and-trap technique which can be used to quantify NO in seawater samples. This method was applied in a first field study on the distribution and production rates of dissolved NO in coastal waters of the Yellow Sea off Qingdao and Jiaozhou Bay.

2 Materials and methods

2.1 Instrumental set-up

The analytical system consists of a degassing column to purge NO from seawater samples, a reaction chamber where NO reacts to form a fluorescent compound (Fig. 1), and a fluorescence spectrophotometer (F-4500, Hitachi Co., Japan). The 800 mL degassing column has a sodium silicate bonded sand core at the bottom to disperse the nitrogen (N_2) purge gas stream. There are four ports on the column: (1) a gas port at the bottom of the degassing column where the high purity N_2 purge gas (99.999%, Qingdao Heli Industry Gas Center, China) or a NO standard gas mixture (5.4 ppmv, NO/N_2) (Beijing Sida Standard Substance Co., China) are introduced, (2) a drain port as outlet for water samples, (3) an inlet port where water samples are pushed into the degassing column with N_2, and (4) an outlet port on the top of the degassing column connected with the reaction chamber.

The NO standard gas cylinder is linked to the degassing column via a gas-tight syringe (Shanghai Anting Injector Co., China). The N_2 gas cylinder is connected to the degassing column via a deoxygen tube (Agilent Technologies, USA) to remove traces of O_2 and a glass rotameter to monitor the gas flow (0.1-1 L min^{-1}, Jiangyin, China). These two gas streams enter the degassing column via the port at the bottom of the flask, controlled by a three-port valve. The tubing used is made of polytetrafluoretyhylene

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(PTFE, 1/8-inch tubing outer diameter [o.d.]). Moreover, an Ultraviolet-Visible spectrophotometer (UV-2550, Shimadzu Co., Japan) and an Automatic Analytical Balance (Beijing Sartorius Co., China) were used in this work.

The degassing column, reaction chamber and the syringe were degreased with organic solvents and rinsed several times with methanol and distilled water in order to minimize potential contamination and adsorption effects. The degassing column was cleaned initially with detergent, rinsed with water, acetone, methanol, and distilled water, and then treated for 30 min with 10% (v/v) HCl in an ultrasonic bath, followed by rinsing with distilled water. Subsequently, those parts of the set-up which comes into contact with the sample solutions were rinsed with methanol, water, HCl solution, and dilute NaOH solution. No significant difference was found from the test of the set up loaded with a water sample and without a water sample (dry run).

2.2 Preparation of DAN and NO solutions

A 2,3-diaminonaphthalene (DAN, ≥ 95%, GC, Sigma-Aldrich Chemical Co., USA) stock solution was prepared fresh with a concentration of 10 mmol L^{-1} in dimethylformamide (Sigma-Aldrich Chemical Co., USA) and kept in the dark at -21 ℃ until used. DAN solutions of 40 μmol L^{-1} were prepared from the stock solution in Milli-Q water, 10 mmol L^{-1} NaOH aqueous solution and filtered natural seawater, respectively. (Natural seawater was sampled from the coastal waters off Qingdao and was filtered through a 0.45 μm acetate cellulose membrane (Millipore, USA). The DAN solutions were purged with N_2 gas for 30 min to remove oxygen (O_2), then stored on ice and transferred to a refrigerator at 4 ℃ before use.

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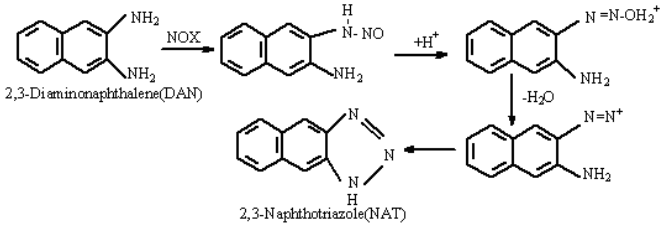
132 An aliquot of 10 mL Milli-Q water was bubbled with N_2 gas at a flow of 10 mL min^{-1} for 1h to
133 remove O_2 after 10 min of ultrasonic degassing. The solution was then bubbled with high purity NO
134 gas (99.9%, Dalian Date Gas Ltd, China) for 30 min. The concentration of the saturated NO stock
135 solution was 1.4 mmol L^{-1}, which should be used within 3h (Lantoine et al., 1995). A series of diluted
136 NO solutions were prepared in N_2-purged water from the NO stock solution using a syringe (Xing et al.,
137 2005).
138
139 **2.3 Fluorometric detection of NO**
140 DAN reacts with NO_x (= NO + NO_2) in alkaline medium and forms the highly fluorescent
141 2,3-naphthotriazole (NAT):



142
143 Based on this reaction a fluorometric method was originally developed for the detection of NO in
144 oxygenated media (Misko et al., 1993; Miles et al., 1995) and has been adapted to detect NO in seawater
145 medium instead of aqueous NaOH medium. The wavelength for NAT excitement is 383 nm and the NAT
146 emission is monitored at a wavelength of 410 nm (Liu et al., 2009).
147
148 **2.4 The influence of nitrite in seawater on the reaction of DAN and NO**
149 NO can be formed from nitrite (NO_2^-) in seawater (Zafiriou and McFarland, 1981). Therefore, we
150 tested a potential interference of dissolved NO_2^- by adding different concentrations of NO_2^- to

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seawater samples. The tests were conducted in the dark or with ultraviolet B (UV-B) radiation (HR 1×18 w, Xinghui Electric Instrument Factory, China). The final concentrations of NO_2^- in the seawater samples were set to 40, 80, 120, 160, and 200 μmol L^{-1}, respectively, and the reaction time was 1 h or 12 h.

2.5 Sampling and analysis

Sampling was conducted aboard the R/V '*Dong Fang Hong 2*' on a cruise to the coastal waters off Qingdao and Jiaozhou Bay from 4 to 6 November 2009. The locations of sampling stations are shown in Fig. 2. The surface seawater samples were collected from 1 m depth at 11 stations using 8 L Niskin bottles mounted on a Seabird CTD Rosette (Sea-Bird Electronics, Inc., USA). A time-course observation of 24 h was carried out at station 10 near the mouth of Jiaozhou Bay.

A 500 mL Wheaton glass serum bottle was rinsed with the seawater three times before it was filled with seawater quickly through a siphon. When the overflowed sample reached the half volume of the bottle, the siphon was withdrawn rapidly and 0.5 mL saturated $HgCl_2$ solution was added to stop biological activities and the bottle was sealed quickly. All glass bottles were covered with aluminum foil to prevent NO_2^- photolysis during sampling.

Because NO reacts with O_2 both in the gas phase and in aqueous solution we purged our set-up for 1h with N_2 gas and sealed it before the measurements. In a first step, a certain amount of standard NO gas was transferred to the reaction chamber via the degassing column by injecting it from a gas tight syringe into the N_2 carrier gas stream. In the reaction chamber NO reacts with the DAN solution. After the measurement of the NO gas standard, a 500 mL seawater sample was injected into the degassing column and purged with N_2 gas and immediately transferred into the reaction chamber where

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it reacts with 10 mL DAN solution. The gas flow is controlled to ensure that the reaction of NO with DAN solution was completed. Finally, the fluorescence intensity of the resulting NAT solution was measured with the F-4500 fluorescence spectrophotometer.

In order to prevent NO photochemical generation, the entire glass parts were wrapped with aluminum foil. The purge-and-trap procedure was conducted at room temperature of 20 ℃.

2.6 O_2, nutrients, DOC and chlorophyll *a* measurements

Dissolved O_2 (DO) concentrations were determined according to the Winkler method. The concentrations of dissolved nitrate, nitrite and ammonia were measured by using an AutoAnalyzer 3 (SEAL Analytical, USA). The detection limits of the method were 0.003, 0.015 and 0.040 μmol L^{-1} for nitrate, nitrite and ammonia, with the precision less than 1%. The intensity of sunlight was monitored by the use of a TES-1322A actionometer (Taishi Co. Taiwan). Dissolved organic carbon (DOC) was determined by a high-temperature combustion method using a Shimadzu TOC-5000 Analyzer with an Al–Pt catalyst (Shimadzu Co., Japan). The precision of the DOC measurements was less than 2%. Concentrations of chlorophyll a were measured with a bbe Cuvette Fluorometer (bbe-Moldaenke GmbH, Kiel, Germany).

2.7 NO production rates

Experiments for NO production by NO_2^- photolysis were conducted at station 10 as follows: Aliquots of 10 mL untreated seawater samples from 0.2 m depth or Millipore membrane (0.45 μm) filtered samples were distributed into three 14 mL glass vials. The initial concentrations of NO_2^- and DOC in seawater were 0.75 μmol L^{-1} and 439 μmol L^{-1} C, respectively. Then 200 μL of 20% NaN_3

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solutions (instead of saturated $HgCl_2$ solution to avoid contamination by the photosensitive Hg) and 20 μL of 1 mmol L^{-1} DAN solutions were added. The vials were sealed with rubber septa and aluminum crimp tops, and were exposed to sunlight on the deck at ambient temperatures (17 ℃) or at 13 ± 2 ℃ in a water bath supplied with the ambient seawater. For "dark" controls vials were wrapped in aluminum foil. The intensity of sunlight ranged from 67565 lux to 71500 lux (average: 69430 lux). After irradiation by sunlight for 30 min, the NO concentrations were measured with the method described above. The NO photolysis production rates were computed as the increase of the NO concentrations during the incubation time.

We also measured NO production rates in natural seawater at station 10. Three transparent polyethylene buckets (3.5 L) were filled with the surface seawater from 0.2 m depth. The buckets were exposed to sunlight in the water bath on deck. The experiment began at 8:30h (local time) and the NO production rates and chlorophyll *a* concentrations were concurrently measured in 2 h intervals. An aliquot of 10 mL sample was collected from each bucket using a glass syringe, distributed and sealed in a 14 mL glass vial, and then incubated under the same conditions as the bucket samples. Three vials per sample were used in the experiments. After 30 min of incubation, solutions of 20 μL DAN (1 mmol L^{-1}) were injected into the vials, respectively. Concentrations of NO were detected and NO production rates were calculated.

3 Results and Discussion

3.1 Method evaluation

Both the purge time and flow of the purge gas (N_2) significantly influence the yield of the NO + DAN reaction and thus, the overall purge efficiency (see Tab. 2). The optimal (i.e. maximum) reaction

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yield was 85% after 30 min of purging at a flow of 400 mL min^{-1}.

The set-up was tested for internal NO production or loss by comparing the fluorescence intensity from NO-free gas or NO calibration gas passing through the degassing column with the fluorescence intensity from the same gas bypassing the degassing column. This procedure was repeated with both a dry degassing column and a moistening degassing column (by a minimum amount of filtered seawater). Neither NO production nor NO loss by adsorption was observed in the set-up in all test runs.

Seawater samples from coastal waters off Qingdao were analyzed in the lab up to of 7 times and gave a relative standard deviation of ±7.2%. The detection limit of our method was determined to be 0.068 nmol L^{-1} (S/N = 3), which is lower than most of the reported detection limits for NO measurements in seawater (see Tab. 1)

The NO recovery coefficient of our purge-and-trap system was estimated by the addition of the same volume of a NO standard solution to (i) 500 mL NO-free seawater in the degassing column and (ii) to 10 mL DAN solution (with a DAN concentration of 40 μmol L^{-1}) in the reaction chamber. The recovery coefficient (RC) of NO was calculated according to:

$$RC（\%）= NO\ (DAN) / NO\ (sw) \times 100\%.$$

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. The resulting NO recovery coefficients ranged from 80.2% to 90.0%, with an average of 83.8%.

In order to check the linearity of our method, a solution of 10 mL 40 μmol L^{-1} DAN was injected into the reaction chamber and purged with N_2 gas at a rate of 10 mL min^{-1} for 5 min prior to the actual measurements. A series of NO-free seawater samples placed in the degassing column were spiked with

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different volumes of the NO standard gas (mixing ratio 5.4 ppmv NO/N_2) and analyzed according to the procedure described above. The resulting fluorescence intensity was linear with the NO concentrations in the range from 0.14 to 19.0 nmol L^{-1} ($y = 7.4286x + 0.6188$, $R = 0.9976$, $P < 0.0001$) (Fig. 3).

The results of the samples spiked with varying concentrations of dissolved NO_2^- are given in Fig. 4. In general, samples with the same NO_2^- concentration showed higher fluorescence when UV-irradiated or kept in dark for 12 h compared to samples under short term (i.e. 1 h) UV irradiation or kept in dark. This points a significant NO production under UV irradiation ($n=5$, $F=76.13$, $p=2.32\times10^{-5}$) and (albeit weaker) NO dark production from NO_2^-. Higher NO_2^- concentrations resulted in a slight increase of fluorescence when irradiated. Therefore we conclude that the measurements of NO should be done in the dark as soon as possible after sampling when high NO_2^- concentrations occur.

To assess the influence of the interferences of dissolved organic matter, trace metals, nutrients, and other substances in seawater, the NO/fluorescence intensity relationship should be determined when the method is applied in different oceanic regions.

With our method we are able to detect > 0.068 nmol L^{-1} NO in discrete seawater samples with a volume of 500 mL. With a larger degassing column, even lower concentrations of NO might be determined.

A U-shaped tube and cold bath (i.e. a water trap) was initially placed between the degassing column and the recation chamber in order to eliminate small amounts of water carried by the N_2 gas stream. However, we found that the fluorescence intensities did not show significant differences when the water trap was removed.

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261 3.2 Distribution of dissolved NO in coastal waters of Qingdao

262 Fig. 5 shows the NO concentrations of surface seawater in coastal waters off Qingdao (stations
263 S01-S09) and in the Jiaozhou Bay (stations S10 and S11). The concentrations of NO ranged from
264 below the detection limit (stat. 02 and 03) up to 0.50 ± 0.01 nmol L^{-1} (stat. S08), with an overall mean
265 of 0.26 ± 0.14 nmol L^{-1}. It is noteworthy that the higher NO concentrations seem to be related to the
266 time point of sampling (given in local time): Samples of stations 2 and 3 were collected at night time,
267 22:30h and 00:50h, respectively, while samples for stations 5, 6, 7 and 8 were collected during the
268 day time (08:58h - 15:38h). (Stations S09 and S10 have been measured in Jiaozhou Bay and, thus,
269 their NO concentrations are directly not comparable with the stations off Qingdao). Our results are
270 generally consistent with the findings in the aquatic ecosystem of Daya Bay in China (Zhang et al.,
271 2006) and the nitrite-rich surface waters of the central equatorial Pacific Ocean (Zafiriou et al., 1980),
272 indicating that sunlight could be a main factor affecting NO formation in seawater. The
273 concentrations of NO in coastal surface waters off Qingdao were found to be an order of magnitude
274 higher than those in surface waters during day time in the central equatorial Pacific Ocean (0.05 nmol
275 L^{-1}) (Zafiriou et al., 1980; Zafiriou and McFarland, 1981). This difference is probably related to the
276 concentrations of NO_2^- in seawater. Zafiriou et al. (1980) proposed that sunlight photolyzes NO_2^- in
277 surface water by the following reaction:

278 $$NO_2^- + H_2O \xrightarrow{hv} NO + \cdot OH + OH^-.$$

279 According to the reaction above, high concentrations of NO_2^- together with strong solar irradiation
280 could cause enhanced concentrations of NO in seawater. The sunlight intensity of the central
281 equatorial Pacific is generally higher than that of coastal waters of Qingdao (located at 36°05'N);
282 however, the coastal waters off Qingdao at the time of our measurements exhibited an average NO_2^-

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concentration of 0.49±0.25 μmol L^{-1}, which was much higher than that observed concentration in the central equatorial Pacific Ocean (~0.1 μmol L^{-1}).

The diurnal variation of NO concentrations and other parameters in surface seawater are shown in Fig. 6. Concentrations of NO presented a significant diurnal variation within 24 h. The peak value appeared at 15:00h (local time) with a concentration of 0.81 nmol L^{-1}. After that the concentration of NO decreased with time gradually until a minimum value occurred at 03:00. Obviously, the concentration of dissolved NO at this station was influenced by the in-situ sunlight intensity. However, the maximum NO concentration appeared not at 12:00h but at 15:00h, which suggesting that there were other influencing factors besides sunlight irradiation.

3.3 NO production rates in coastal waters

The results of the NO irradiation experiments are given in Fig. 7. The production rate of NO through seawater irradiation was 1.52×10^{-12} mol L^{-1} s^{-1} which is slightly higher than that NO production rate of the 0.45 μm Millipore filtered samples (1.46×10^{-12} mol L^{-1} s^{-1}). The difference may indicate that particles in seawater could increase the NO production rate. The non-filtered samples incubated in the water bath had a lower NO production rate (1.44×10^{-12} mol L^{-1} s^{-1}) compared to the other non-filtered treatment, which could be ascribed to the difference of the temperature. The ambient temperature and water bath were 17 ℃ and 13 ℃, respectively, thus the higher temperature may resulted in a higher photolysis rate. The photochemical production rates of NO in Qingdao coastal waters during the daytime were generally higher than that reported from the central equatorial Pacific Ocean (0.4-1.2×10^{-12} mol L^{-1} s^{-1}) (Zafiriou and McFarland, 1981).

Previous experiments about NO_2^- photolysis were also carried out in the laboratory (Li et al.,

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2011): The production of NO was observed after 3 h illumination of 10-100 μmol L^{-1} NO_2^- in Milli-Q water. There was an increasing trend of NO concentrations with the NO_2^- concentrations. For natural seawater, it was observed to have an increasing trend of NO concentration with the illumination time (Li et al., 2011). The process of sunlight photolysis of NO_2^- in surface water was demonstrated, which was consistent with the results of Zafiriou et al. (1980) and Olasehinde et al. (2009).

The on-deck incubation experiments for the production rates of NO in Qingdao coastal waters, together with chlorophyll *a* concentrations and sunlight intensities, are shown in Fig. 8. The production rates of NO exhibited a clear variation during the course of the day with a maximum value appearing at 14:30h (local time). The maximum value of 2.52×10^{-12} mol L^{-1} s^{-1} was about seven-fold higher than the minimum value at 08:30h. The production rates of NO kept an increasing trend from 08:30h to 14:30h. The mean production rate in Qingdao coastal waters was 1.51×10^{-12} mol L^{-1} s^{-1} during the day. The variation of the production rates of NO did not follow the trends in chlorophyll *a* concentrations and solar radiation. Therefore, the production pattern of NO in marine environments deserves further research.

4 Summary

For the determination of NO concentrations in discrete seawater samples we developed a new method by combining a purge-and-trap set-up with fluorometric detection of NO. The method showed a linear fluorescence intensity for NO concentrations ranging from 0.14 nmol L^{-1} to 19 nmol L^{-1}. The detection limit is 0.068 nmol L^{-1} (S/N =3), the average recovery coefficient is 83.8% (80.2～90.0%), and the relative standard deviation is ±7.2%. Our method was applied to measure concentrations of NO in surface layer of the coastal waters off Qingdao and Jiaozhou Bay. NO concentrations varied

327 from below the detection limit to 0.50 nmol L^{-1}, with an average of 0.26 ± 0.14 nmol L^{-1}. The
328 concentrations of NO in coastal waters off Qingdao were an order of magnitude higher than those in
329 surface waters of the central equatorial Pacific. NO surface concentrations were generally enhanced
330 significantly during daytime implying that NO formation processes such as NO_2^- photolysis are much
331 higher during daytime than chemical NO consumption which, in turn leads to the observed significant
332 decrease of the NO concentrations during nighttime. The measurements of NO production rates
333 showed that the occurence of particles and an increase in temperature can enhance NO production.

334 We conclude that our method can be applied to measure (i) NO concentrations in the ocean
335 surface, (ii) NO production and consumption pathways in oceanic waters and (ii) NO production rates
336 in culture experiments.

337

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421 Figure Captions

422

423 Fig. 1 The purge-and-trap system for the determination of dissolved nitric oxide in seawater

424 **Fig. 2** Loations of the sampling stations in the coastal waters off Qingdao and Jiaozhou Bay

425 **Fig. 3** Relationship between nitric oxide concentrations and fluorescence intensities

426 **Fig. 4** The fluorescence variations of NAT in seawater with different concentrations of nitrite in the

427 dark or under UV-B radiation

428 **Fig. 5** The concentrations of NO in the surface water off Qingdao and Jiaozhou Bay

429 **Fig. 6** The diurnal variations of NO concentrations and related parameters in the surface seawater at

430 station 10

431 **Fig. 7** The production rates of NO by seawater irradiation under natural light after different treatments

432 **Fig. 8** The variations of NO production rates, chlorophyll *a* concentrations and sunlight intensities in the

433 incubation experiments with Qingdao coastal waters

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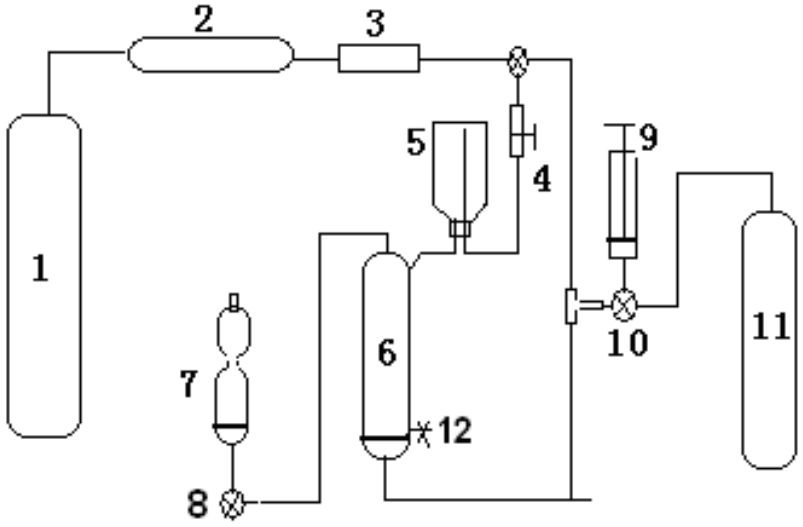

436

437 Fig. 1 The purge-and-trap system for the determination of dissolved nitric oxide in seawater

438 (1. N_2 gas; 2. Deoxygenation tube; 3. Glass rotameter; 4. 2-port valve; 5. Sample vial; 6. Degassing

439 column; 7. Reaction chamber; 8 and 10. 3-port valves; 9. Gas-tight syringe; 11. NO standard gas; 12.

440 Drain)

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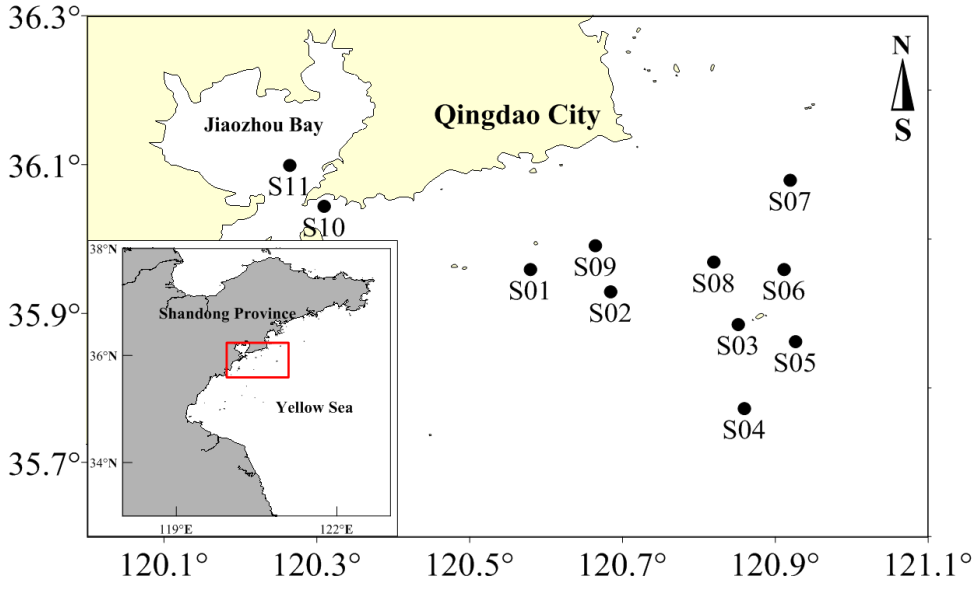

442

443 **Fig. 2** Location of the sampling stations in the coastal waters off Qingdao and Jiaozhou Bay

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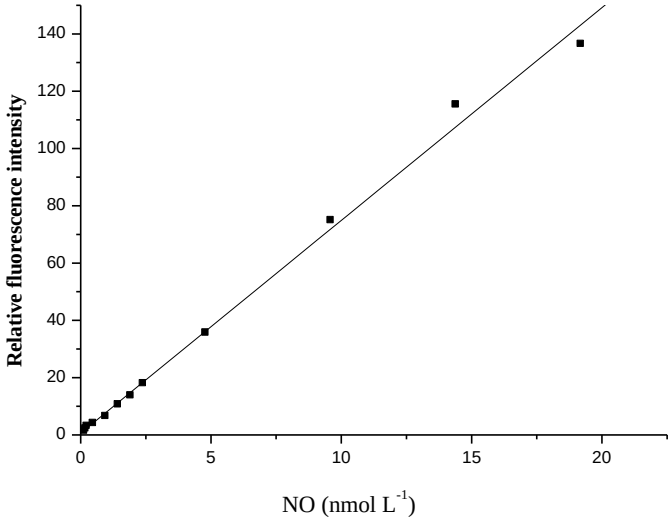

445

446 **Fig. 3** Relationship between nitric oxide concentrations and fluorescence intensities

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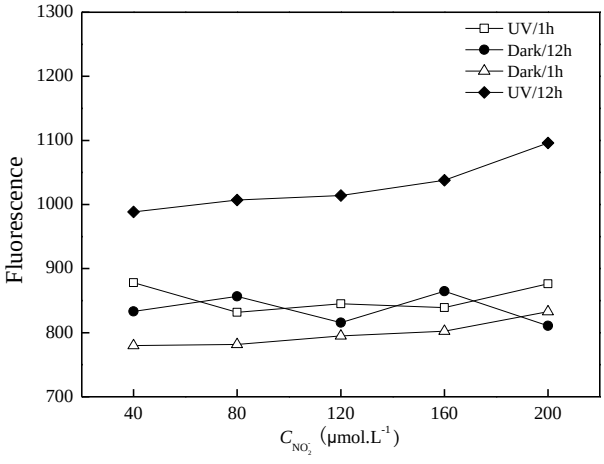

448

449 **Fig. 4** The fluorescence variations of NAT in seawater with different concentrations of nitrite in the

450 dark or under UV-B radiation

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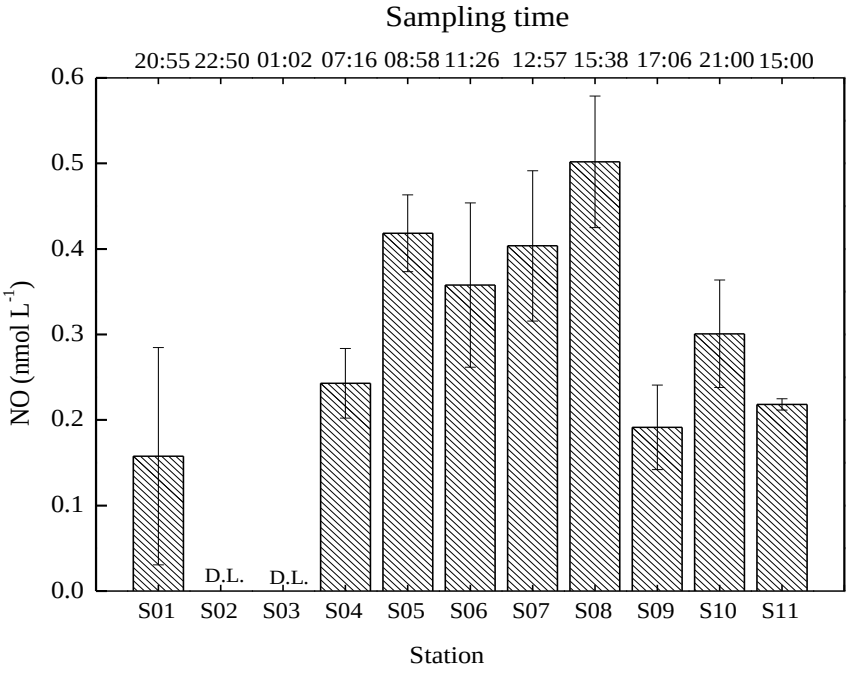

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453

454 **Fig.5** The concentrations of NO in the surface waters off Qingdao (stations S01-S09) and Jiaozhou

455 Bay (stations S10 and S11)

456 D.L. stands for concentration below the detection limit.

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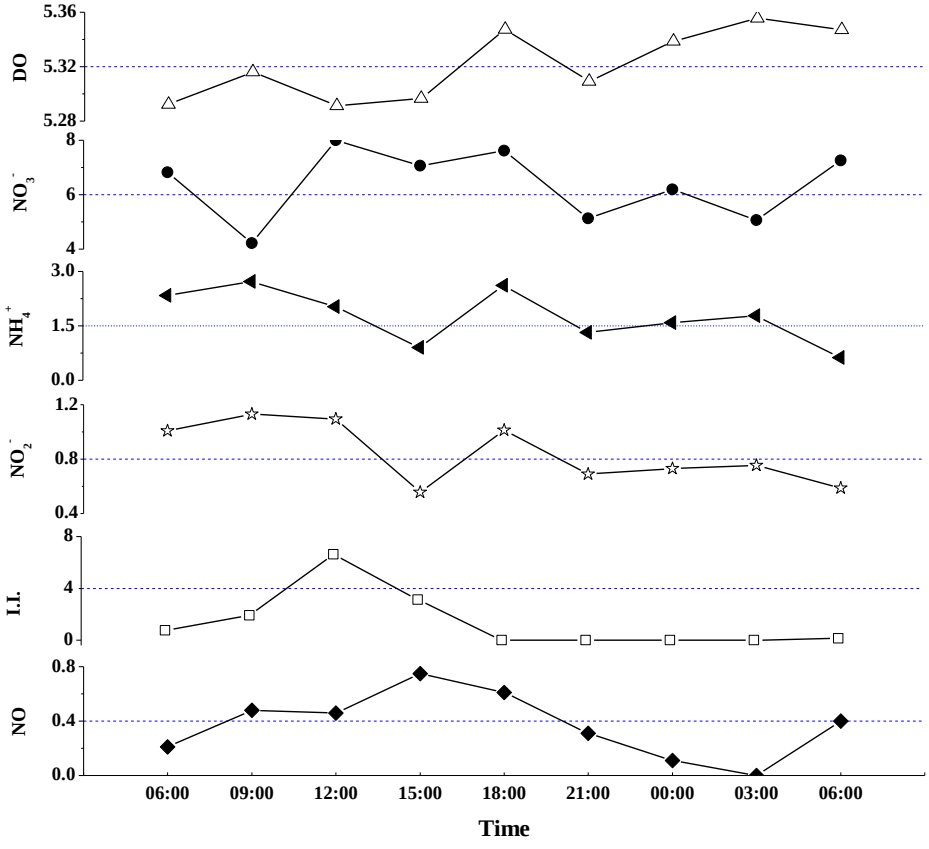

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459

460 **Fig. 6** The diurnal variations of NO concentrations and related parameters in the surface seawater at

461 station 10 (Units: DO (mL L^{-1}), NO_3^-, NO_2^-, NH_4^+ (μmol L^{-1}), I.I.-illumination intensity ($\times 10^4$ lux), NO

462 (nmol L^{-1}))

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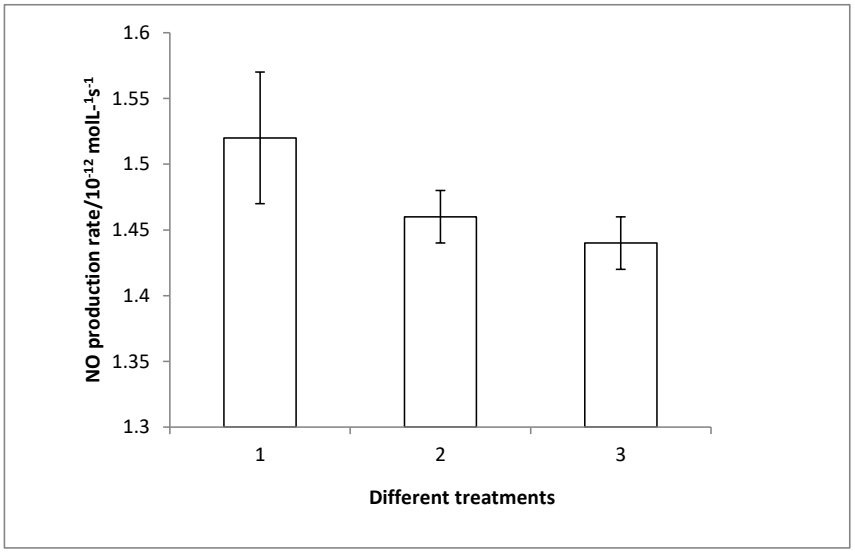

Fig. 7 The production rates of NO by seawater irradiation under natural light after different treatments (1. Incubated on deck at ambient temperature, 2. 0.45 μm Millipore filtered at ambient temperature, 3. Incubated in water bath supplied with surface seawater)

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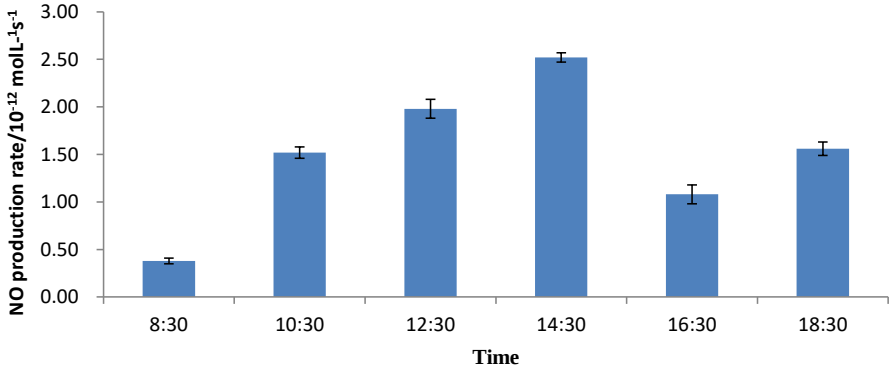

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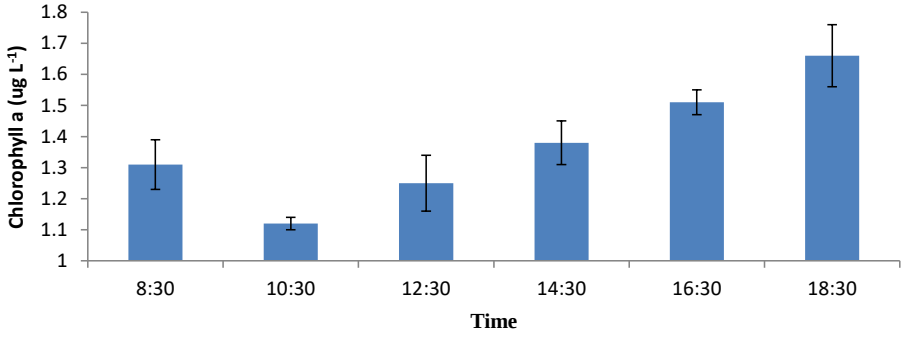

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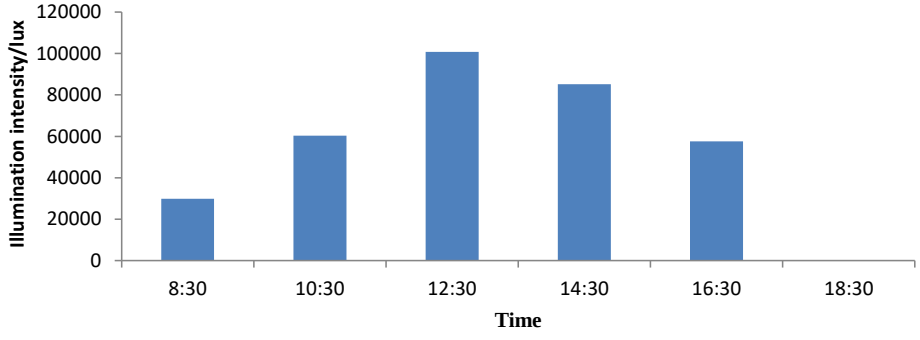

471

472 **Fig. 8** The variations of NO production rates, chlorophyll *a* concentrations and sunlight intensity in the

473 incubation experiments with Qingdao coastal waters

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475 Table 1 The methods for NO detection in seawater

Method	Linearity range (nmol L^{-1})	Detection limit (nmol L^{-1})	Analytical precision	Reference
Microelectrode	140–9900	140	0.24%	Zhang et al. (2003)
Microelectrode	1.4–1400	4.2×10^{-10}	6.30‰	Xing et al. (2005)
Microelectrode	0.4 –4000	30	-	Schreiber et al.(2008)
Fluorescence	1.4–1400	1.4	1.63%	Liu et al. (2009)
HPLC with fluorescence	0.025–10	0.025	3-5%	Olasehinde et al. (2009)
Purge-and-trap with chemiluminescence	-	0.0015	3%	Zafirou and McFarland (1980)
Purge-and-trap with chemiluminescence	-	0.25	3-25%	Bange and Lutterbeck (2015)
Purge-and-trap with fluorescence	0.14–19	0.068	7.2%	This study

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478 **Table 2** Reaction yields of the reaction of DAN with NO (in %)

Purge flow rate /mL min^{-1}	Purge time /min			
	15	30	45	60
200	—	—	—	—
300	—	—	21	34
400	56	85	69	69
500	—	—	22	26
600	—	—	31	33

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