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2 **Yellow Sea off Qingdao**

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35

36 **Abstract** We developed a new method for the determination of dissolved nitric oxide (NO) in discrete seawater
37 samples based on a combination of a purge-and-trap set-up and fluorometric detection of NO.
38 2,3-diaminonaphthalene (DAN) reacts with NO in seawater to form the highly fluorescent 2,3-naphthotriazole
39 (NAT). The fluorescence intensity was linear for NO concentrations in the range from 0.14 nmol L⁻¹ to 19 nmol
40 L⁻¹. We determined a detection limit of 0.068 nmol L⁻¹, an average recovery coefficient of 83.8% (80.2-90.0%),
41 and a relative standard deviation of ±7.2%. With our method we determined for the first time the temporal and
42 spatial distributions of NO surface concentrations in coastal waters of the Yellow Sea off Qingdao and in Jiaozhou
43 Bay during a cruise in November 2009. The concentrations of NO varied from below the detection limit to 0.50
44 nmol L⁻¹ with an average of 0.26 ± 0.14 nmol L⁻¹. NO surface concentrations were generally enhanced
45 significantly during daytime implying that NO formation processes such as NO₂⁻ photolysis are much higher
46 during daytime than chemical NO consumption which, in turn, lead to a significant decrease of NO
47 concentrations during nighttime. In general, NO surface concentrations and measured NO production rates were
48 higher compared to previously reported measurements. This might be caused by the high NO₂⁻ surface
49 concentrations encountered during the cruise. Moreover, additional measurements of NO production rates
50 implied that the occurrence of particles and a temperature increase can enhance NO production rates. With the

51 method introduced here we have a reliable and comparably easy to use method at hand to measure oceanic NO
52 surface concentrations which can be used to decipher both its temporal and spatial distributions as well as its
53 biogeochemical pathways in the oceans.

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

56

57 **1 Introduction**

58 As a reactive atmospheric trace gas, nitric oxide (NO) plays important roles in tropospheric
59 chemistry: It is a key player in the formation of acid rain and ozone (Williams et al., 1992; Lee et al.,
60 1997; Mazzeo, et al., 2005). NO is an intermediate of both the terrestrial and marine nitrogen cycle
61 (Ward and Zafiriou, 1988; Williams et al., 1992; Canfield et al., 2010; Chen et al., 2010; Thamdrup,
62 2012; Voss et al., 2013). It has a variety of sources in seawater, including nitrite photolysis and various
63 microbial processes such as denitrification, anammox and dissimilatory nitrate reduction to ammonia
64 (Law, 2001; Schreiber et al., 2012; Martens-Habbena et al., 2015). Because of its chemical reactivity,
65 NO usually does not accumulate in large amounts in seawater and the ocean as a source of atmospheric
66 NO is, therefore, negligible in a global context (Zehr and Ward, 2002; Bange, 2008). Moreover, NO
67 was found to have significant effects on the growth of marine algae (Zhang et al., 2005; Liu et al., 2004;
68 2005; 2006; 2014). To this end, the determination of the spatial and temporal distributions of NO in the
69 ocean as well as deciphering its oceanic production processes and their major influencing factors are
70 essential to improve our understanding of the biogeochemical cycling NO in the ocean.

71 Because of its low concentrations in seawater caused by its fast diffusion and high chemical
72 reactivity, measurements of NO in seawater are very difficult. Therefore, there are only a few methods
73 available to determine NO (Hetrick and Schoenfish, 2009), see Tab. 1. The electrochemical method
74 using sensors in seawater medium achieved a detection limit of 42 nmol L⁻¹ (Xing et al., 2005; Zhang
75 et al., 2005). Olasehinde et al. (2009) developed a method for the determination of photochemically

76 generated NO in natural waters adopting 4,5-diaminofluorescein as a probe compound and a
77 measurement of reversed-phase high performance liquid chromatography (HPLC) with fluorescence
78 detector. The NO concentrations and signal intensities exhibited a good linearity correlation over the
79 range of 0.025-10 nmol L⁻¹ triazolofluorescein. Zafiriou and McFarland (1980) determined the NO
80 concentration of seawater by using a flow system to equilibrate the seawater samples with a gas stream
81 coupled to a chemiluminescence detector. They report an analytical precision of ±3% and an accuracy
82 of ±20%. More recently, Lutterbeck and Bange (2015) developed an improved method of a
83 chemiluminescence NO analyser connected to a stripping unit, and the limit of detection was 0.25
84 nmol L⁻¹ using a 20 mL seawater sample volume. Until now only these two chemiluminescence
85 methods were applied successfully to determine NO concentrations in seawater samples. The
86 N-nitrosation of 2,3-diaminonaphthalene (DAN) results in the highly fluorescent 2,3-naphthotriazole
87 (NAT), which could be used to detect NO concentrations as low as 10 nmol L⁻¹ (Miles et al., 1995). We
88 adopted this method for seawater medium instead of NaOH medium and the calibration curve
89 exhibited linearity over the concentration range of 1.4 - 1400 nmol L⁻¹ NO (Liu et al., 2009). However,
90 this assay cannot be used to detect trace levels of NO in seawater samples directly.

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

95

96 2 Materials and methods

97 2.1 Instrumental set-up

98 The analytical system consists of a degassing column to purge NO from seawater samples, a
99 reaction chamber where NO reacts to form a fluorescent compound (Fig. 1), and a fluorescence
100 spectrophotometer (F-4500, Hitachi Co., Japan). The 800 mL degassing column has a sodium silicate

101 bonded sand core at the bottom to disperse the nitrogen (N₂) purge gas stream. There are four ports on
102 the column: (1) a gas port at the bottom of the degassing column where the high purity N₂ purge gas
103 (99.999%, Qingdao Heli Industry Gas Center, China) or a NO standard gas mixture (5.4 ppmv, NO/N₂)
104 (Beijing Sida Standard Substance Co., China) are introduced, (2) a drain port as outlet for water
105 samples, (3) an inlet port where water samples are pushed into the degassing column with N₂, and (4) an
106 outlet port on the top of the degassing column connected with the reaction chamber.

107 The NO standard gas cylinder is linked to the degassing column via a gas-tight syringe (Shanghai
108 Anting Injector Co., China). The N₂ gas cylinder is connected to the degassing column via a deoxygen
109 tube (Agilent Technologies, USA) to remove traces of O₂ and a glass rotameter to monitor the gas flow
110 (0.1-1 L min⁻¹, Jiangyin, China). These two gas streams enter the degassing column via the port at the
111 bottom of the flask, controlled by a three-port valve. The tubing used is made of polytetrafluorethylyene
112 (PTFE, 1/8-inch tubing outer diameter [o.d.]). Moreover, an Ultraviolet-Visible spectrophotometer
113 (UV-2550, Shimadzu Co., Japan) and an Automatic Analytical Balance (Beijing Sartorius Co., China)
114 were used in this work.

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

123

124 **2.2 Preparation of DAN and NO solutions**

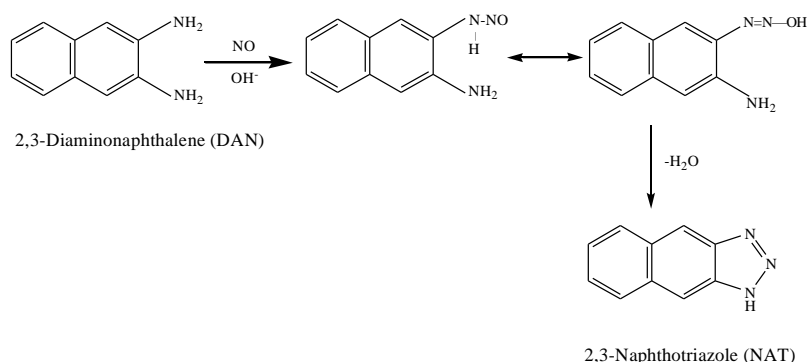
125 A 2,3-diaminonaphthalene (DAN, $\geq 95\%$, GC, Sigma-Aldrich Chemical Co., USA) stock solution
126 was prepared fresh with a concentration of 10 mmol L^{-1} in dimethylformamide (Sigma-Aldrich Chemical
127 Co., USA) and kept in the dark at $-21 \text{ }^\circ\text{C}$ until used. DAN solutions of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

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 gas
134 (99.9% , Dalian Date Gas Ltd, China) for 30 min. The concentration of the saturated NO stock solution
135 was 1.4 mmol L^{-1} , which could be used within 3h (Lantoine et al., 1995). A series of diluted NO
136 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 ($= \text{NO} + \text{NO}_2$) in alkaline medium, thus forms the highly fluorescent
141 2,3-naphthotriazole (NAT) as follows:



142

143 The reaction of NO and O_2 with 2,3 diaminonaphthalene (DAN) produced a fluorescent triazole.
144 Although the mechanism of this fluorescence has not yet been established in detail, the fluorescence was
145 reported to increase dose-dependently by NO addition (Nakatsubo et al., 1998). In seawater samples, the

146 concentration of O₂ (10⁻⁴ M order of magnitude) was far higher than that of NO (10⁻¹⁰ M order of
147 magnitude). Both of them were stripped out and reached the DAN solution finally, thus, the NO in
148 samples could almost quantitatively transform into NAT. Based on this reaction, a fluorometric method
149 was originally developed for the detection of NO in oxygenated media (Misko et al., 1993; Miles et al.,
150 1995) and has been adapted to detect NO in seawater medium instead of aqueous NaOH medium. Our
151 experiments showed that the DAN solution was stable in 12 h and the NAT solution did not change
152 within 4 h. The wavelength for NAT excitement is 383 nm and the NAT emission is monitored at a
153 wavelength of 410 nm (Liu et al., 2009).

154

155 **2.4 The influence of nitrite in seawater on the reaction of DAN and NO**

156 NO can be formed from nitrite (NO₂⁻) in seawater (Zafiriou and McFarland, 1981). Therefore, we
157 tested a potential interference of dissolved NO₂⁻ by adding different concentrations of NO₂⁻ to seawater
158 samples. The tests were conducted in the dark or with ultraviolet B (UV-B) radiation (HR 1×18 w,
159 Xinghui Electric Instrument Factory, China). The final concentrations of NO₂⁻ in the seawater samples
160 were set to 40, 80, 120, 160, and 200 μmol L⁻¹, respectively, and the reaction time was 1 h or 12 h.

161

162 **2.5 Sampling and analysis**

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

168 A 500 mL Wheaton glass serum bottle was rinsed with the seawater three times before it was filled
169 with seawater quickly through a siphon. When the overflowed sample reached the half volume of the
170 bottle, the siphon was withdrawn rapidly and 0.5 mL saturated HgCl₂ solution was added to stop

171 biological activities and the bottle was sealed quickly. All glass bottles were covered with aluminum
172 foil to prevent NO_2^- photolysis during sampling.

173 Because NO reacts with O_2 both in the gas phase and in aqueous solution, we purged our set-up
174 for 1h with N_2 gas and sealed it before the measurements. In a first step, a certain amount of standard
175 NO gas was transferred to the reaction chamber via the degassing column by injecting it from a gas
176 tight syringe into the N_2 carrier gas stream. In the reaction chamber NO reacts with the DAN solution.
177 After the measurement of the NO gas standard, a 500 mL seawater sample was injected into the
178 degassing column and purged with N_2 gas and immediately transferred into the reaction chamber where
179 it reacts with 10 mL DAN solution. The gas flow is controlled to ensure that the reaction of NO with
180 DAN solution was completed. Finally, the fluorescence intensity of the resulting NAT solution was
181 measured with the F-4500 fluorescence spectrophotometer.

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

184

185 **2.6 O_2 , nutrients, DOC and chlorophyll *a* measurements**

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

195

196 2.7 NO production rates

197 Experiments for NO production by NO_2^- photolysis were conducted at station 10 as follows:
198 Aliquots of 10 mL untreated seawater samples from 0.2 m depth or Millipore membrane (0.45 μm)
199 filtered samples were distributed into three 14 mL glass vials. The initial concentrations of NO_2^- and
200 DOC in seawater were 0.75 $\mu\text{mol L}^{-1}$ and 439 $\mu\text{mol L}^{-1}$ C, respectively. Then 200 μL of 20% NaN_3
201 solutions (instead of saturated HgCl_2 solution to avoid contamination by the photosensitive Hg) and 20
202 μL of 1 mmol L^{-1} DAN solutions were added. The vials were sealed with rubber septa and aluminum
203 crimp tops, and were exposed to sunlight on the deck at ambient temperatures (17 $^\circ\text{C}$) or at 13 ± 2 $^\circ\text{C}$
204 in a water bath supplied with the ambient seawater. For “dark” controls vials were wrapped in
205 aluminum foil. The intensity of sunlight ranged from 67565 lux to 71500 lux (average: 69430 lux).
206 After irradiation by sunlight for 30 min, the NO concentrations were measured with the method
207 described above. The NO photolysis production rates were computed as the increase of the NO
208 concentrations during the incubation time.

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

218

219 3 Results and Discussion

220 3.1 Method evaluation

221 Both the purge time and flow of the purge gas (N₂) significantly influence the yield of the NO +
222 DAN reaction and thus, the overall purge efficiency (see Tab. 2). The optimal (i.e. maximum) reaction
223 yield was 85% after 30 min of purging at a flow of 400 mL min⁻¹. The error of these measurements was
224 in the range of 8-25%. They were triplicates. Now the scheme looks a bit rough. We will increase the
225 measurements of the purge flow rates between 350 and 450 mL per minute, especially around 400
226 mL/min. It is possible to obtain even better results with fine tuning.

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

232 Seawater samples from coastal waters off Qingdao were analyzed in the lab up to of 7 times and
233 gave a relative standard deviation of ±7.2%. The detection limit of our method was determined to be
234 0.068 nmol L⁻¹ (S/N = 3), which is lower than most of the reported detection limits for NO
235 measurements in seawater (see Tab. 1)

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

240
$$RC(\%) = NO(\text{sw}) / NO(\text{DAN}) \times 100\%.$$

241 Where *NO*(DAN) stands for the NO directly injected to the DAN solution and *NO*(sw) stands for the
242 NO measured from the sample in degassing column according to the method described above. The rate
243 law obtained from the oxidation of NO is

244
$$-d[\text{NO}]/dt = 4k[\text{NO}]^2[\text{O}_2]$$

245 with $k = 2 \times 10^6 \text{ M}^{-2} \text{ s}^{-1}$. The reaction of NO with O₂ could consume NO in the stripping period. The NO

246 recovery coefficients of the purge-and-trap system were evaluated and ranged from 80.2% to 90.0%,
247 with an average of 83.8%. Furthermore, three replicates of in-situ seawater were measured using our
248 system and method, the aqueous NO solution did not change within one hour, which was also
249 demonstrated by Lutterbeck and Bange (2015).

250 In order to check the linearity of our method, a solution of 10 mL 40 $\mu\text{mol L}^{-1}$ DAN was injected
251 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
252 measurements. A series of NO-free seawater samples placed in the degassing column were spiked with
253 different volumes of the NO standard gas (mixing ratio 5.4 ppmv NO/ N_2) and analyzed according to
254 the procedure described above. The resulting fluorescence intensity was linear with the NO
255 concentrations in the range from 0.14 to 19.0 nmol L^{-1} ($y = 7.4286x + 0.6188$, $R = 0.9976$, $P < 0.0001$)
256 (Fig. 3).

257 The results of the samples spiked with varying concentrations of dissolved NO_2^- are given in Fig.
258 4. The blank had no signal when measured with the fluorescence spectrophotometer after UV-B radiation
259 or in the dark. That is, the NO concentrations were zero. Nitrite did not cause significant problems with
260 natural samples during the measurement process. In general, samples with the same NO_2^- concentration
261 showed higher fluorescence when UV-irradiated or kept in dark for 12 h compared to samples under
262 short term (i.e. 1 h) UV irradiation or kept in dark. This points a significant NO production under UV
263 irradiation ($n=5$, $F=76.13$, $p=2.32 \times 10^{-5}$) and (albeit weaker) NO dark production from NO_2^- . Higher NO_2^-
264 concentrations resulted in a slight increase of fluorescence when irradiated. Therefore we conclude that
265 the measurements of NO should be done in the dark as soon as possible after sampling when high NO_2^-
266 concentrations occur. Here these high NO_2^- concentrations were design to demonstrate no obvious effect
267 caused by NO_2^- on the detection method, thus, low concentrations of NO_2^- also do not affect this method.
268 On the other hand, the fluorescence intensity could not be detected with low concentrations of NO_2^- .

269 To assess the influence of the interferences of dissolved organic matter, trace metals, nutrients, and
270 other substances in seawater, the NO/fluorescence intensity relationship should be determined when

271 the method is applied in different oceanic regions.

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

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

279 NO is a conceptually important intermediate in N-cycle biogeochemistry, product of ocean
280 photochemistry, and putative inter-cellular signal. Unfortunately, our knowledge about the oceanic NO
281 distribution and the major pathways of NO is very poor. There are only a few published NO
282 concentration measurements available because a reliable and easy to use method to determine dissolved
283 NO at in-situ concentrations in seawater samples is missing. We try to find a solid method both
284 convenient for many labs and sensitive enough, which seems to have promise.

285

286 3.2 Distribution of dissolved NO in coastal waters of Qingdao

287 Fig. 5 shows the NO concentrations of surface seawater in coastal waters off Qingdao (stations
288 S01-S09) and in the Jiaozhou Bay (stations S10 and S11). The concentrations of NO ranged from
289 below the detection limit (stat. 02 and 03) up to $0.50 \pm 0.01 \text{ nmol L}^{-1}$ (stat. S08), with an overall mean
290 of $0.26 \pm 0.14 \text{ nmol L}^{-1}$. It is noteworthy that the higher NO concentrations seem to be related to the
291 time point of sampling (given in local time): Samples of stations 2 and 3 were collected at night time,
292 22:30h and 00:50h, respectively, while samples for stations 5, 6, 7 and 8 were collected during the day
293 time (08:58h - 15:38h). (Stations S09 and S10 have been measured in Jiaozhou Bay and, thus, their
294 NO concentrations are directly not comparable with the stations off Qingdao). Our results are
295 generally consistent with the findings in the aquatic ecosystem of Daya Bay in China (Zhang et al.,

296 2006) and the nitrite-rich surface waters of the central equatorial Pacific Ocean (Zafiriou et al., 1980),
297 indicating that sunlight could be a main factor affecting NO formation in seawater. The concentrations
298 of NO in coastal surface waters off Qingdao were found to be an order of magnitude higher than those
299 in surface waters during day time in the central equatorial Pacific Ocean (0.05 nmol L^{-1}) (Zafiriou et
300 al., 1980; Zafiriou and McFarland, 1981). This difference is probably related to the concentrations of
301 NO_2^- in seawater. Zafiriou et al. (1980) proposed that sunlight photolyzes NO_2^- in surface water by the
302 following reaction:



304 According to the reaction above, high concentrations of NO_2^- together with strong solar irradiation
305 could cause enhanced concentrations of NO in seawater. The sunlight intensity of the central
306 equatorial Pacific is generally higher than that of coastal waters of Qingdao (located at $36^\circ 05' \text{N}$);
307 however, the coastal waters off Qingdao at the time of our measurements exhibited an average NO_2^-
308 concentration of $0.49 \pm 0.25 \text{ } \mu\text{mol L}^{-1}$, which was much higher than that observed concentration in the
309 central equatorial Pacific Ocean ($\sim 0.1 \text{ } \mu\text{mol L}^{-1}$).

310 NO is a short-lived intermediate of various microbial processes of the nitrogen cycle, which is
311 involved in denitrification (Kampschreur et al., 2007), anammox (Kartal et al., 2011) and archaea
312 ammonia-oxidizing (Martens-Habbena et al., 2015) processes. Zafiriou and McFarland (1981) analyzed
313 NO in seawater samples at the sea surface of the central equatorial Pacific by stripping NO into an air
314 and N_2 stream by passing it through the chemiluminescence - type detector. Thus, the NO
315 concentrations were underestimated to some extent because seawater samples were suboxic or anoxic.
316 However, time-dependent losses from microbial processes were minimized. Lutterbeck and Bange
317 (2015) improved the method above to determine dissolved NO in discrete seawater samples of the
318 eastern tropical South Pacific Ocean. The contamination by O_2 diffusion into the continue samples
319 could be further minimized. This work was also designed to detect dissolved NO in discrete seawater

320 samples with a combination of a purge-and-trap set-up and fluorometric NO analyzer. The HgCl₂
321 solution was added to stop biological activities during the stripping. However, the disposal of these
322 Hg-contaminated solutions is a tough proposition. To improve the method the purge-and-trap set-up
323 could be modified and the stripping time could be reduced, then the addition of HgCl₂ solution may be
324 removed in the future.

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

332

333 3.3 NO production rates in coastal waters

334 The results of the NO irradiation experiments are given in Fig. 7. The production rate of NO
335 through seawater irradiation was $1.52 \times 10^{-12} \text{ mol L}^{-1} \text{ s}^{-1}$ which is slightly higher than that NO production
336 rate of the 0.45 μm Millipore filtered samples ($1.46 \times 10^{-12} \text{ mol L}^{-1} \text{ s}^{-1}$). The difference may indicate that
337 particles in seawater could increase the NO production rate. The non-filtered samples incubated in the
338 water bath had a lower NO production rate ($1.44 \times 10^{-12} \text{ mol L}^{-1} \text{ s}^{-1}$) compared to the other non-filtered
339 treatment, which could be ascribed to the difference of the temperature. The ambient temperature and
340 water bath were 17 °C and 13 °C, respectively, thus the higher temperature may resulted in a higher
341 photolysis rate. The photochemical production rates of NO in Qingdao coastal waters during the
342 daytime were generally higher than that reported from the central equatorial Pacific Ocean
343 ($0.4\text{-}1.2 \times 10^{-12} \text{ mol L}^{-1} \text{ s}^{-1}$) (Zafiriou and McFarland, 1981).

344 Previous experiments about NO₂⁻ photolysis were also carried out in our laboratory (Li et al.,

2011): The production of NO was observed after 3 h illumination of 10-100 $\mu\text{mol L}^{-1}$ 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 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.

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 $2.52 \times 10^{-12} \text{ mol L}^{-1} \text{ s}^{-1}$ 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 $1.51 \times 10^{-12} \text{ mol L}^{-1} \text{ s}^{-1}$ 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.

367

368 4 Summary

369 For the determination of NO concentrations in discrete seawater samples we developed a new

370 method by combining a purge-and-trap set-up with fluorometric detection of NO. The method showed
371 a linear fluorescence intensity for NO concentrations ranging from 0.14 nmol L⁻¹ to 19 nmol L⁻¹. The
372 detection limit is 0.068 nmol L⁻¹ (S/N =3), the average recovery coefficient is 83.8% (80.2~90.0%),
373 and the relative standard deviation is ±7.2%. Our method was applied to measure concentrations of
374 NO in surface layer of the coastal waters off Qingdao and Jiaozhou Bay. NO concentrations varied
375 from below the detection limit to 0.50 nmol L⁻¹, with an average of 0.26 ± 0.14 nmol L⁻¹. The
376 concentrations of NO in coastal waters off Qingdao were an order of magnitude higher than those in
377 surface waters of the central equatorial Pacific. NO surface concentrations were generally enhanced
378 significantly during daytime implying that NO formation processes such as NO₂⁻ photolysis are much
379 higher during daytime than chemical NO consumption which, in turn leads to the observed significant
380 decrease of the NO concentrations during nighttime. The measurements of NO production rates
381 showed that the occurrence of particles and an increase in temperature can enhance NO production.

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

385

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- 486

Figure Captions

487

488

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

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

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

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

493 dark or under UV-B radiation

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

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

496 station 10

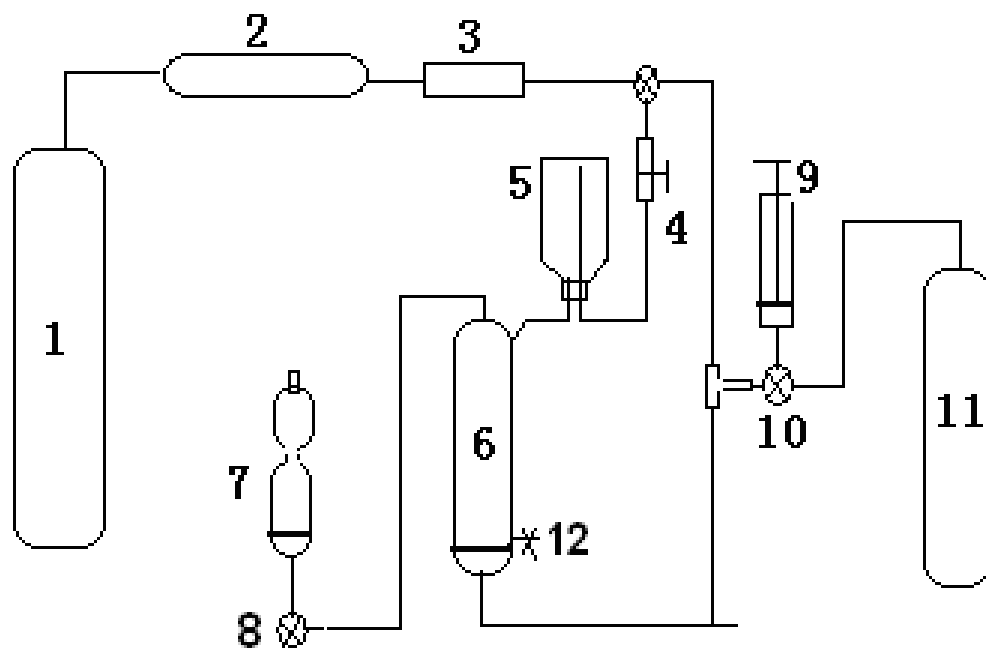
497 **Fig. 7** The production rates of NO by seawater irradiation under natural light after different treatments

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

499 incubation experiments with Qingdao coastal waters

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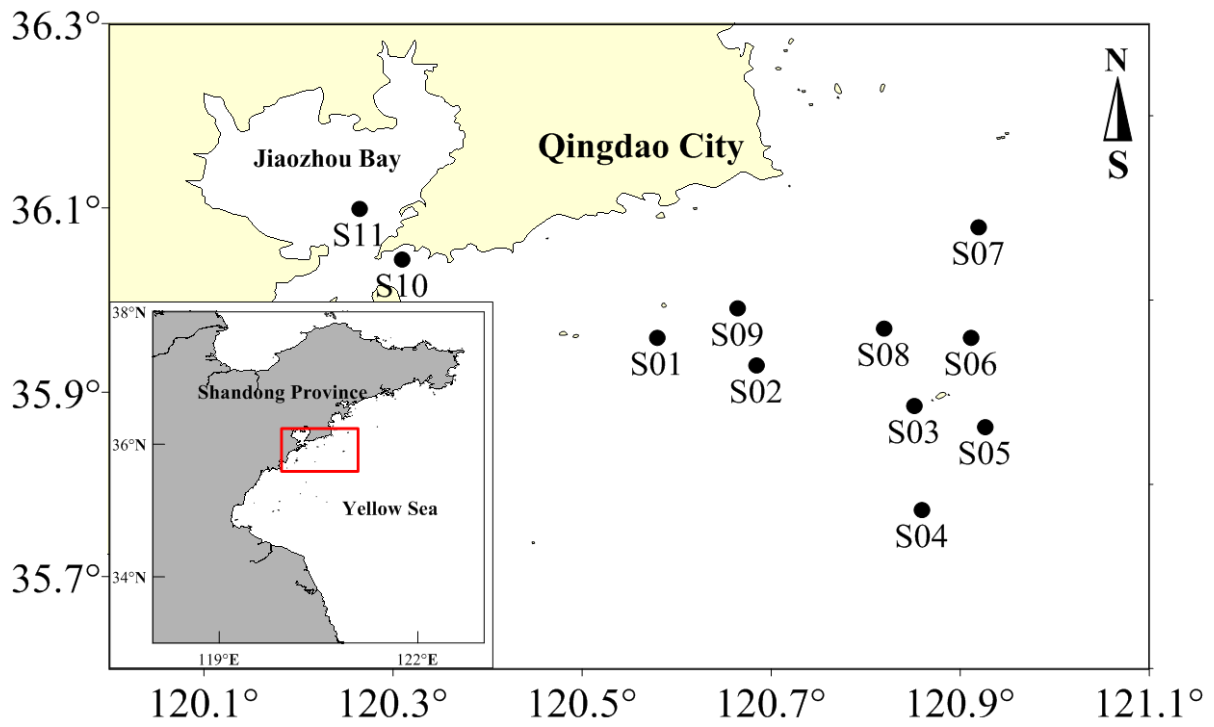
503 Fig. 1 The purge-and-trap system for the determination of dissolved nitric oxide in seawater

504 (1. N₂ gas; 2. Deoxygenation tube; 3. Glass rotameter; 4. 2-port valve; 5. Sample vial; 6. Degassing

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

506 Drain)

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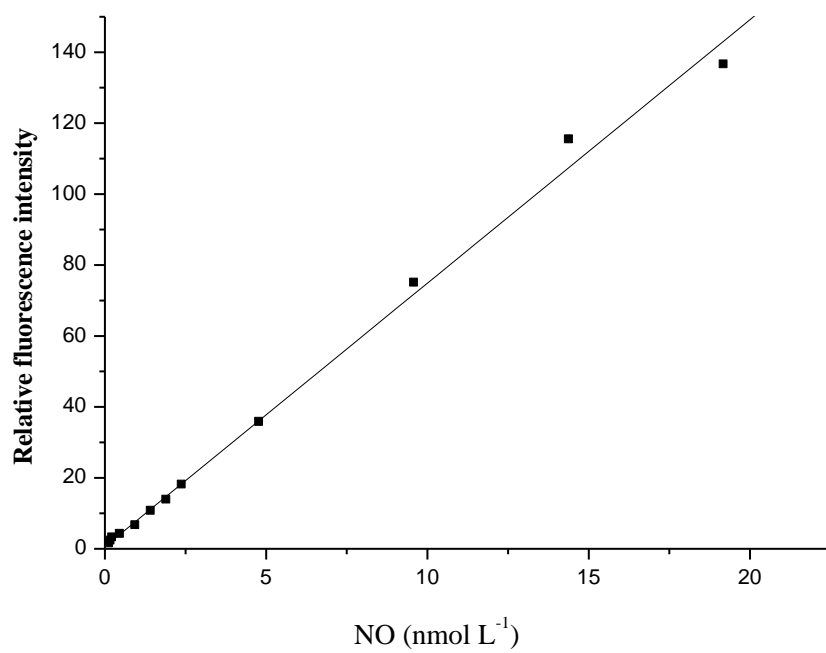


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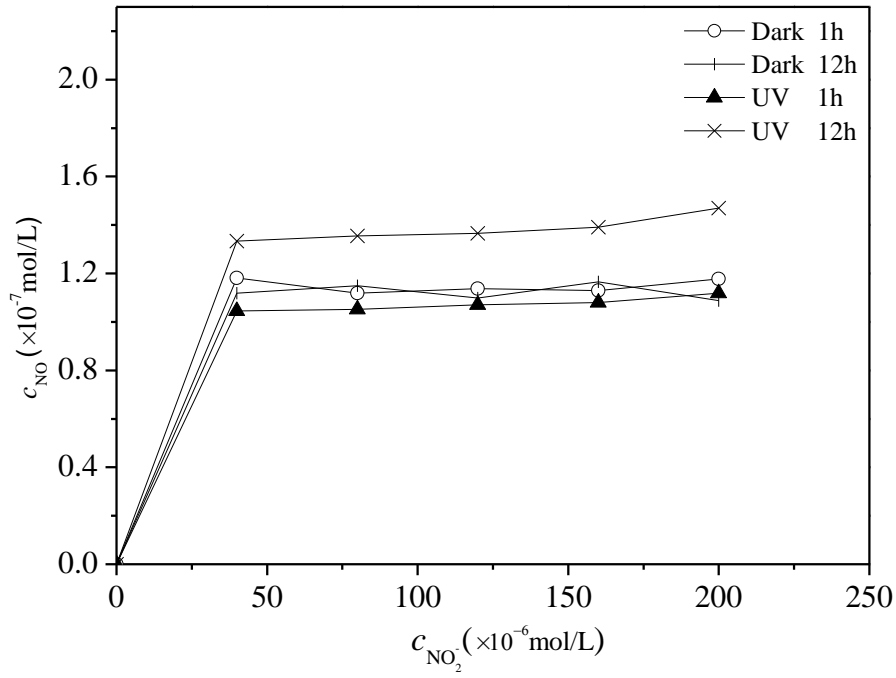
Fig. 2 Location of the sampling stations in the coastal waters off Qingdao and Jiaozhou Bay



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

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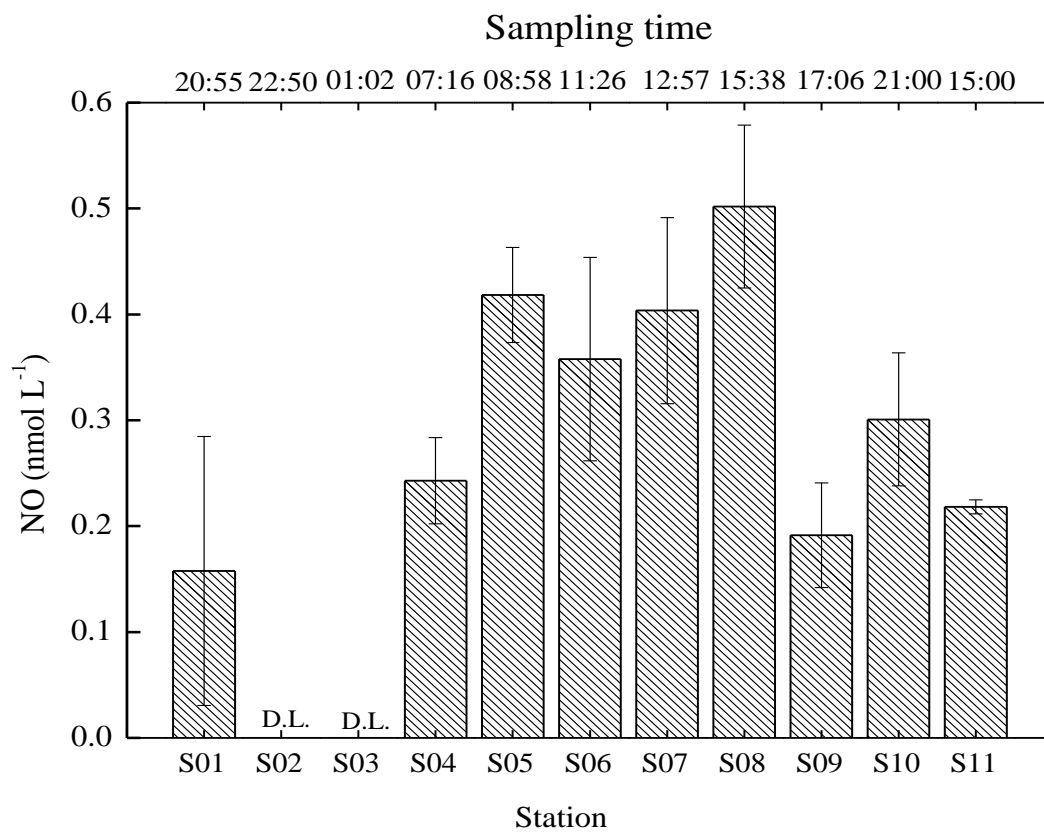


514

515 **Fig. 4** The variations of NO concentrations in seawater added with different concentrations of nitrite in

516 the dark or under UV-B radiation

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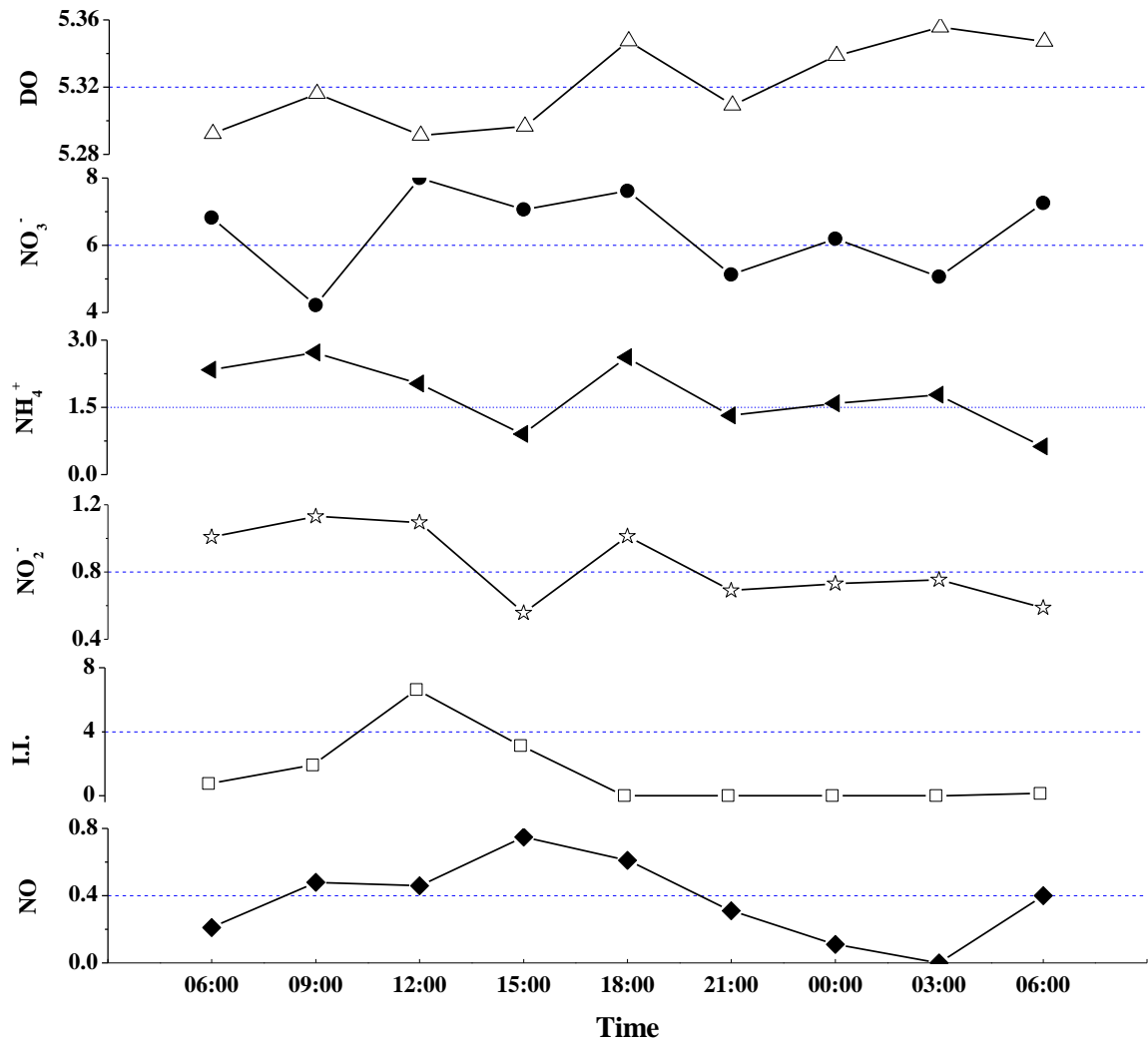
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520 **Fig.5** The concentrations of NO in the surface waters off Qingdao (stations S01-S09) and Jiaozhou Bay

521 (stations S10 and S11)

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

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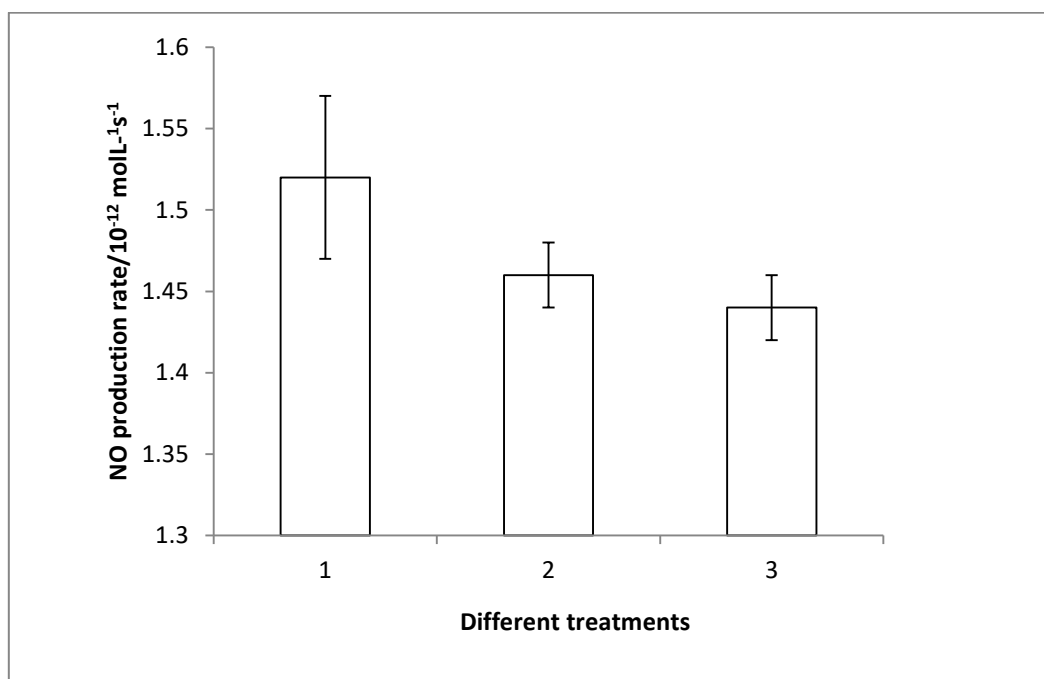


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525

526 **Fig. 6** The diurnal variations of NO concentrations and related parameters in the surface seawater at
 527 station 10 (Units: DO (mL L⁻¹), NO₃⁻, NO₂⁻, NH₄⁺ (μmol L⁻¹), I.I.-illumination intensity (×10⁴ lux), NO
 528 (nmol L⁻¹))

529



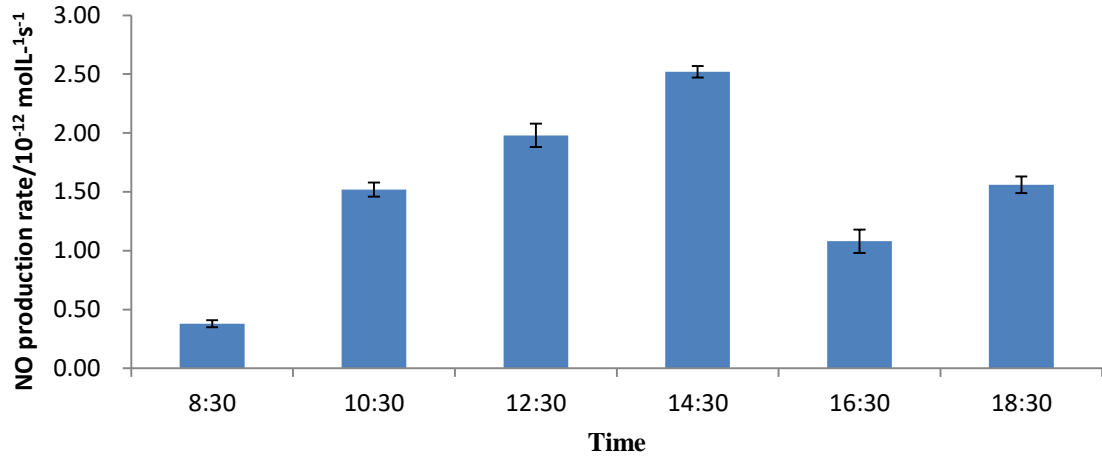
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531 **Fig. 7** The production rates of NO by seawater irradiation under natural light after different treatments

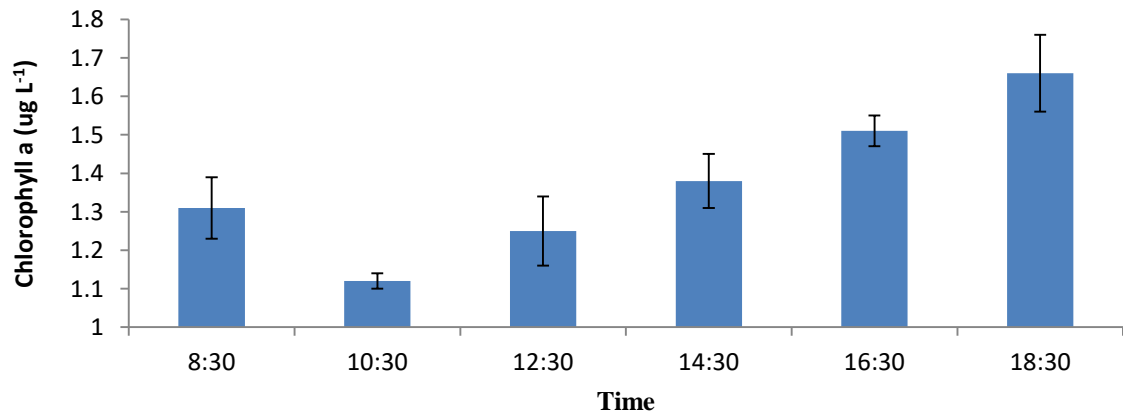
532 (1. Incubated on deck at ambient temperature, 2. 0.45 μm Millipore filtered at ambient temperature, 3.

533 Incubated in water bath supplied with surface seawater)

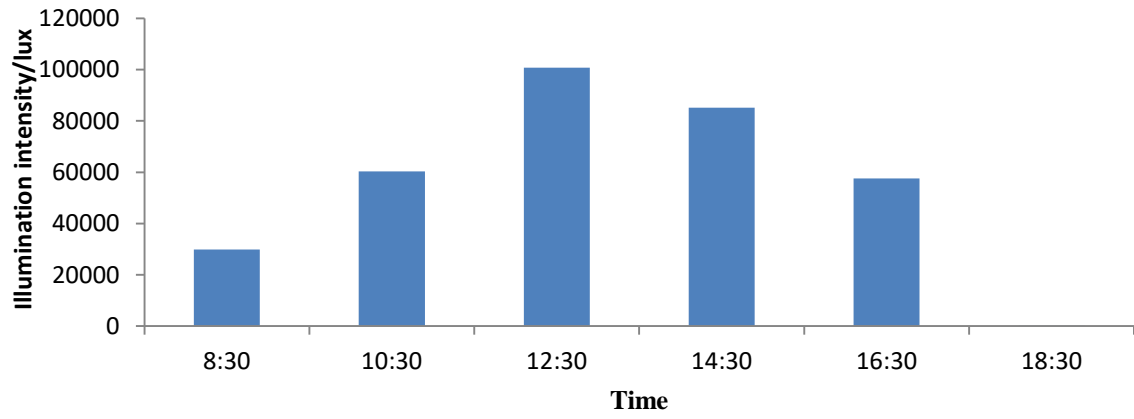
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538 **Fig. 8** The variations of NO production rates, chlorophyll *a* concentrations and sunlight intensity in the
 539 incubation experiments with Qingdao coastal waters

540

541 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

542

543

544

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

545