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Hughes, Claire orcid.org/0000-0002-9512-8052, Barton, Eleanor, Hepach, Helmke et al. (7 more authors) (2021) Oxidation of iodide to iodate by cultures of marine ammonia-oxidising bacteria. MARINE CHEMISTRY. 104000. ISSN: 0304-4203

<https://doi.org/10.1002/essoar.10503313.2>

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Final published manuscript at <https://doi.org/10.1016/j.marchem.2021.104000>

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Oxidation of iodide to iodate by cultures of marine ammonia-oxidising bacteria

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24 **Highlights:**

- 25 • Oxidation of iodide to iodate by marine nitrifying bacteria demonstrated for first time
- 26 • Laboratory cultures of ammonium oxidising bacteria produced iodate from iodide substrate
- 27 • Nitrification used to parameterise iodide sink in global marine iodine cycling model
- 28 • Changes in nitrification may increase sea surface iodide, impacting atmospheric chemistry

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30

31

32

33 Abstract

34 Reaction with iodide (I⁻) at the sea surface is an important sink for atmospheric ozone, and causes
35 sea-air emission of reactive iodine which in turn drives further ozone destruction. To incorporate this
36 process into chemical transport models, improved understanding of the factors controlling marine
37 iodine speciation, and especially sea-surface iodide concentrations, is needed. The oxidation of I⁻ to
38 iodate (IO₃⁻) is the main sink for oceanic I⁻, but the mechanism for this remains unknown. We
39 demonstrate for the first time that marine nitrifying bacteria mediate I⁻ oxidation to IO₃⁻. A significant
40 increase in IO₃⁻ concentrations compared to media-only controls was observed in cultures of the
41 ammonia-oxidising bacteria *Nitrosomonas* sp. (Nm51) and *Nitrosococcus oceani* (Nc10) supplied
42 with 9-10 mM I⁻, indicating I⁻ oxidation to IO₃⁻. Cell-normalised production rates were 15.69 (±4.71)
43 fmol IO₃⁻ cell⁻¹ d⁻¹ for *Nitrosomonas* sp., and 11.96 (±6.96) fmol IO₃⁻ cell⁻¹ d⁻¹ for *Nitrosococcus oceani*,
44 and molar ratios of iodate-to-nitrite production were 9.2±4.1 and 1.88±0.91 respectively. Preliminary
45 experiments on nitrite-oxidising bacteria showed no evidence of I⁻ to IO₃⁻ oxidation. If the link
46 between ammonia and I⁻ oxidation observed here is representative, our ocean iodine cycling model
47 predicts that future changes in marine nitrification could alter global sea surface I⁻ fields with
48 potential implications for atmospheric chemistry and air quality.

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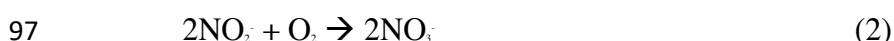
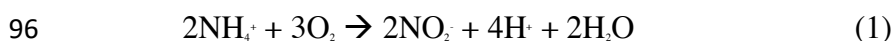
51

52 **Introduction**

53 Iodine plays an important role in catalytic ozone destruction and new particle formation in the
54 troposphere, thereby impacting the oxidative capacity of the atmosphere (Sherwen *et al.*, 2016) and
55 the Earth's radiation balance (O'Dowd *et al.*, 2002). Sea-to-air iodine transfer is known to be the
56 main source of iodine to the atmosphere (Carpenter, 2003; Sherwen *et al.*, 2016). Reactive inorganic
57 iodine (I_2 , HOI) emissions resulting from the reaction of gas-phase ozone with sea surface iodide (I^-)
58 is now thought to be the dominant mechanism mediating sea-air iodine emissions (Carpenter *et al.*,
59 2013). The strength of the surface reactive iodine flux is related to sea surface I^- concentrations
60 (Carpenter *et al.*, 2013) so knowledge of ocean I^- distributions is required in order to estimate the
61 significance of this process. Furthermore, a detailed understanding of the processes controlling
62 inorganic iodine speciation is needed to allow us to develop predictive capacity regarding sea surface
63 I_2 , ozone-deposition rates and sea-air emission of reactive iodine.

64 Total inorganic iodine is found at 400-500 nM in seawater and predominantly exists as iodate (IO_3^-)
65 and I^- (Chance *et al.*, 2014) with inter-conversion between these two species alongside physical
66 mixing being the main causes of spatial and temporal variability in sea surface I^- . Iodate is the
67 thermodynamically stable form and the dominant form in the deep ocean. The existence of relatively
68 higher levels of I^- in the euphotic zone (reviewed by Chance *et al.*, 2014) has led to the suggestion
69 that IO_3^- reduction to I^- is linked to primary productivity. This theory has been supported by
70 observations of I^- production in cultures of a wide range of marine phytoplankton (e.g. Chance *et al.*,
71 2007; Bluhm *et al.*, 2010; Hepach *et al.*, 2020) and some field studies (Chance *et al.*, 2010). The
72 mechanism of biogenic iodate reduction to iodide is not yet known, but may be related to senescence
73 processes (Bluhm *et al.*, 2010; Hepach *et al.*, 2020; Carrano *et al.*, 2020). Reduction of IO_3^- to I^- by
74 phytoplankton nitrate reductase enzymes (Hung *et al.*, 2005), or macroalgal cell surface reductases
75 (Carrano *et al.*, 2020), has also been suggested but neither has been confirmed as a significant route
76 of conversion.

78 Oxidation of I⁻ back to IO₃⁻ is the dominant sink for I⁻, but is a relatively slow reaction with rate
79 estimates ranging from ~4 to 670 nM yr⁻¹ (Chance *et al.*, 2014; Hardisty *et al.*, 2020). The rates and
80 processes involved in I⁻ to IO₃⁻ oxidation are associated with large uncertainty (Truesdale *et al.*, 2001;
81 Amachi *et al.*, 2008), and the mechanisms involved remain undefined. This uncertainty has been
82 suggested to be one of the factors hindering the development of mathematical models of iodine
83 transformations in the global oceans (Truesdale *et al.*, 2001). Abiotic oxidation of I⁻ back to IO₃⁻ in
84 the ocean (e.g. by oxygen, hydroxyl radicals, hydrogen peroxide and ozone) is thought to occur so
85 slowly as to be insignificant (e.g. Wong, 1991), and so I⁻ oxidation to IO₃⁻ is also thought to be
86 associated with marine microbiological activity. I⁻ oxidation to I₂ has been observed in bacterial
87 isolates obtained from a range of environments including seawater aquaria (Gozlan *et al.*, 1968),
88 natural gas brines (Iino *et al.*, 2016) and seawater/marine mud (Fuse *et al.*, 2003). Additionally,
89 based on field observations, a number of studies (Truesdale *et al.*, 2001; Žic *et al.*, 2013) have
90 proposed that I⁻ oxidation to IO₃⁻ is linked to nitrification in marine systems. Nitrification is the two-
91 stage biological transformation of ammonia (NH₃) to nitrate (NO₃⁻) (Equations 1 and 2; Koops &
92 Pommerening-Röser, 2001) mediated by chemoautotrophic ammonia-oxidising bacteria (AOB), and
93 nitrite-oxidising bacteria (NOB). Previously thought to only occur outside of the euphotic zone,
94 nitrification is now known to occur throughout the oceanic water-column (reviewed by Yool *et al.*,
95 2007).



98 A link between I⁻ oxidation/ IO₃⁻ production and nitrification is yet to be confirmed but, if established,
99 would suggest that I⁻ oxidation to IO₃⁻ is widespread throughout the world's oceans (Yool *et al.*,
100 2007).

101

102 The primary aim of this study was to establish whether I oxidation to IO_3^- is associated with marine
103 nitrification. Our objectives were to determine if IO_3^- production occurs in cultures of marine
104 ammonia- and nitrite-oxidising bacteria supplied with I, determine the relative rates of IO_3^-
105 production and nitrification and explore the possible implications of the findings.

106

107 **Methods**

108 ***Cultures***

109 Stock bacterial cultures were taken from the existing culture collections of the authors. Two marine
110 AOB cultures, *Nitrosomonas* sp. Nm51 (C-15) and *Nitrosococcus oceani* Nc10 (C-107, ATCC
111 19707) were investigated for IO_3^- production in the presence of I as the only iodine source. These
112 strains were originally isolated from seawater in the south Pacific and the north Atlantic respectively
113 (Watson and Mandel, 1971). Cultures were grown in the dark in a water bath at 25 °C in autoclaved
114 ESAW artificial seawater mixture (Berges *et al.*, 2001) made up using distilled water. The ESAW
115 media was supplemented with 7-8 mM ammonium chloride and potassium phosphate. We also
116 conducted preliminary tests on three active marine NOB: *Nitrospira marina* Nb-295 (isolated from
117 Gulf of Maine, Watson *et al.*, 1986); *Nitrospina gracilis* 3/211 (isolated from the south Atlantic,
118 Watson and Waterbury, 1971); *Nitrococcus mobilis* Nb-231 (ATCC 25380, isolated from Galapagos
119 seawater, Watson and Waterbury, 1971). However we saw no evidence of IO_3^- production in any of
120 the NOB cultures studied and these results are not discussed further. Handling of cultures was done
121 at all times in a biosafety cabinet using sterile equipment.

122

123 ***Experimental Set Up***

124 For the AOB experiments triplicate cultures were incubated alongside triplicate media-only controls
125 for periods of 8-12 days. The experiments were kept as short as possible to avoid significant changes
126 in pH in the bulk media which would impact inorganic iodine speciation. Hence experiments were
127 only run until an increase in nitrite across two time-points was observed. Samples were taken at
128 regular intervals of between 1 to 6 days for pH measurement, cell counts and determination of NO_2^- ,
129 IO_3^- , I^- and $\text{NH}_4^+/\text{NH}_3$ concentrations. In all cases, I^- (Aristar) was added to be at similar concentrations
130 with the NH_4^+ required in the growth media. The levels of I^- are much higher than those encountered
131 in the oceans (global ocean median=77 nM I^- [interquartile range 28-140 nM], Chance *et al.*, 2014)
132 but were chosen to be similar to the levels of NH_4^+ . This is because in the marine environment
133 nitrifiers would be exposed to similar ratio of NH_4^+ and I^- . For example, Rees *et al.* (2006) show that
134 $\text{NH}_4^+/\text{NH}_3$ occurs at concentrations ranging from 60-300 nM in the Atlantic between 60°N to 50°S.

135

136 ***pH***

137 A spectrophotometric method using a Lambda 25 UV/Vis spectrophotometer (Perkin-Elmer) and m-
138 cresol purple dye (Dickson *et al.*, 2007) with measurements at 730, 578 and 434 nm was used to
139 determine pH in the cultures and media-only controls. Salinity, needed for the pH calculation, was
140 calculated from conductivity measured using a calibrated Hanna Instruments hand-held probe.

141

142 ***Cell counts***

143 Immediately after sampling, 4 mL of the culture was fixed with 15 μL of 50% glutaraldehyde (Alfa
144 Aesar), flash frozen in liquid nitrogen and placed in a -80 °C freezer for later determination of cell
145 density. Cell counts were made using a Beckman Coulter Cytoflex S flow cytometer (flow rate of 10
146 $\mu\text{L min}^{-1}$) within 2 months of collection. DAPI (Sigma; 2 $\mu\text{g mL}^{-1}$) stained samples were excited by a

laser at 405 nm and the emitted fluorescence detected using an avalanche photodiode detector with a reflective band pass filter 450/45. The flow cytometer thresholds were set using the 405 nm laser side scatter and the DAPI fluorescence signals.

Nitrite concentration

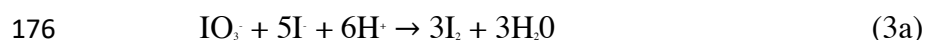
NO₂⁻ was measured in 0.45 µm (Millex) filtered samples using a spectrophotometric method (Lambda 25 UV/Vis spectrophotometer, Perkin-Elmer) developed by Norwitz & Keliher (1984). The method involves diazotizing nitrite with sulfanilamide (Fisher, analytical reagent grade) and coupling with N-1-naphthylethylenediamine dihydrochloride (Fisher, analytical reagent grade) to form a coloured azo dye which is measured spectrophotometrically at 540 nm. The method was calibrated using NaNO₂ standards (Fisher, analytical reagent grade) prepared in the ESAW-based media.

Iodate Concentration

IO₃⁻ concentrations were measured in 0.45 µm (Millex) filtered samples using a manual version of the spectrophotometric (Lambda 25 UV/Vis spectrophotometer) method detailed in Truesdale & Spencer, 1974 and Jickells *et al.*, 1988. Absorbance was measured at 350 nm. Strictly, this method determines all oxidised (0 to +5 oxidation state) forms of inorganic iodine, but in seawater derived media this is predominantly IO₃⁻, and so will be referred to as IO₃⁻ iodate hereafter. The method was calibrated using potassium iodate (Aristar) standard solutions made up in ESAW.

Some validation and modification to the method was required due to the nature of our experimental set-up. Chapman & Liss (1977) show that NO₂⁻ can interfere with spectrophotometric IO₃⁻ measurements (using sulfamic acid) at ambient seawater concentrations with a 15% error. Clearly significant interference would be an issue for our experiments where NO₂⁻ was being produced so we ran tests. We found that the presence of NO₂⁻ up to 10 µM had negligible impact on IO₃⁻ measurements

171 (between 0.1-50 μM). We did however identify that the high starting concentration of I ($\sim 10\ \mu\text{M}$) in
172 the culture media was problematic. The iodate analysis method comprises two steps: the first
173 involves an initial absorbance reading after the addition of sulfamic acid; the second involves the
174 addition of excess I. Under acidic conditions I reacts with IO_3^- to form I_2 (equation 3a) which reacts
175 with excess I to form the coloured ion I_3^- (equation 3b) that can be measured spectrophotometrically.



178 The difference between the first and second absorbance readings is then used to calibrate the method.
179 In the case of our experiments the media already contained excess I so the formation of I_2 and I_3^- was
180 initiated as soon as the acid was added in the first step. Hence we calibrated the method based on a
181 single absorbance reading obtained after acid and then additional I was added. Calibrations and
182 standard checks revealed this approach did not have any impact on the quality of the data.

183

184 ***Ammonium Concentration***

185 NH_4^+ concentrations were measured in 0.45 μm (Millex) filtered samples with a Seal Analytical
186 Autoanalyser 3 according to method G-109-93 rev. 10 (Seal Analytical) using sodium salicylate,
187 dichloro-isocyanuric acid and citrate buffer. The method was calibrated using standards ranging from
188 0-2 mg/L prepared from dilutions of a 1000 mg/L ammonium standard solution (Merck).

189

190 ***Iodide Concentration***

191 I concentrations were determined using a Dionex ICS-2000 ion chromatograph equipped with an
192 EGC III KOH elugen cartridge, AG18 (2 x 50 mm) guard column, AS18 (2 x 250 mm) analytical
193 column, ASRS 300 (2 mm) suppressor, DS6 heated conductivity cell and AS40 autosampler.

194 Samples were diluted 100-fold with 18 MΩ deionised water for analysis and 5 μL was injected onto
195 the ion chromatograph. Aqueous potassium hydroxide was used as the eluent at a flow rate of 0.25
196 mL min⁻¹ with a gradient program starting from an initial concentration of 2 mM hydroxide (hold 1
197 min) to 20 mM at 18 min then to 41 mM at 19 min (hold 2 min) before returning to 2 mM. The I⁻
198 retention time was 19 min. The instrument was calibrated with matrix-matched standards ranging
199 from 0-100 nM (I⁻), prepared from dilutions of a 1000 mg/L iodide standard solution (Fisher
200 Scientific) with 18 MΩ deionised water and containing a final concentration of 1% ESAW.

201

202 ***Data Analysis***

203 As in Guerrero and Jones (1996), the NH₄⁺ oxidation rate is defined here as the rate of increase in
204 NO₂⁻. Similarly, we define the rate of I oxidation as the rate of increase in IO₃⁻. This is appropriate as
205 no other iodine species were supplied to the cultures and conversion between I⁻ and IO₃⁻ is known to
206 be the main cause of variability in inorganic iodine speciation (Bluhm *et al.*, 2010; Chance *et al.*,
207 2014). Average NO₂⁻ and IO₃⁻ production rates were calculated for each replicate culture using
208 Equation 4.

$$209 \quad \text{Production Rate (nM day}^{-1}\text{)} = \frac{(C_{\text{end}} - C_0)}{t} \quad (4)$$

210 where C₀ and C_{end} are the NO₂⁻ or IO₃⁻ concentrations observed at the start and end of the experiment
211 and t is the experimental duration in days. Cell-normalised rates were calculated by dividing these
212 rates by the final cell density observed in each AOB culture and are hence likely to be minimum
213 values.

214

215

216 **Results**

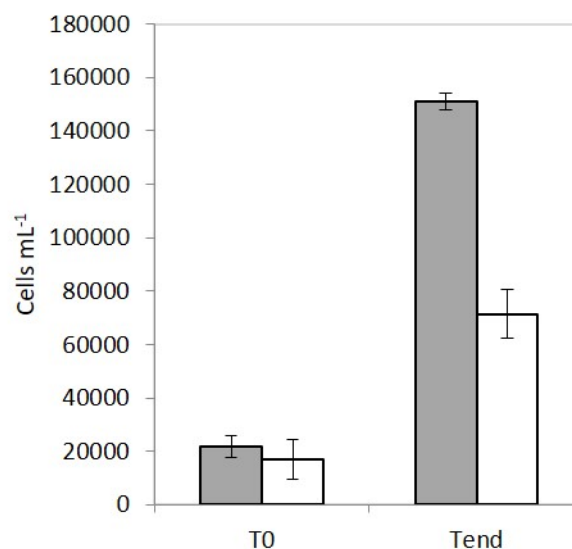
217 **Cell counts and pH**

218 Increases in cell density were observed in all replicates of *Nitrosomonas* sp. and *Nitrosococcus*
219 *oceanii* between the start and end of the experiment indicating growth (Figure 1). Average initial cell
220 density in the *Nitrosomonas* sp. cultures was 21,767 ($\pm 4,046$) cells mL⁻¹ and this increased to 150,983
221 ($\pm 7,585$) cells mL⁻¹ by the end of the experiment (8 days). For *Nitrosococcus oceanii* start and end (12
222 days) cell densities were 16,947 ($\pm 3,098$) and 71,430 ($\pm 9,062$) cells mL⁻¹, respectively. Average pH
223 levels in the culture experiments calculated from measurements at each time point (data not shown)
224 were 7.69 (± 0.07) for *Nitrosomonas* sp. and 7.41 (± 0.12) for *Nitrosococcus* sp. These pH levels are
225 consistent with those found in the media-only controls (7.64 ± 0.07 for *Nitrosomonas* sp; 7.64 ± 0.15
226 for *Nitrosococcus oceanii*).

227

228

229



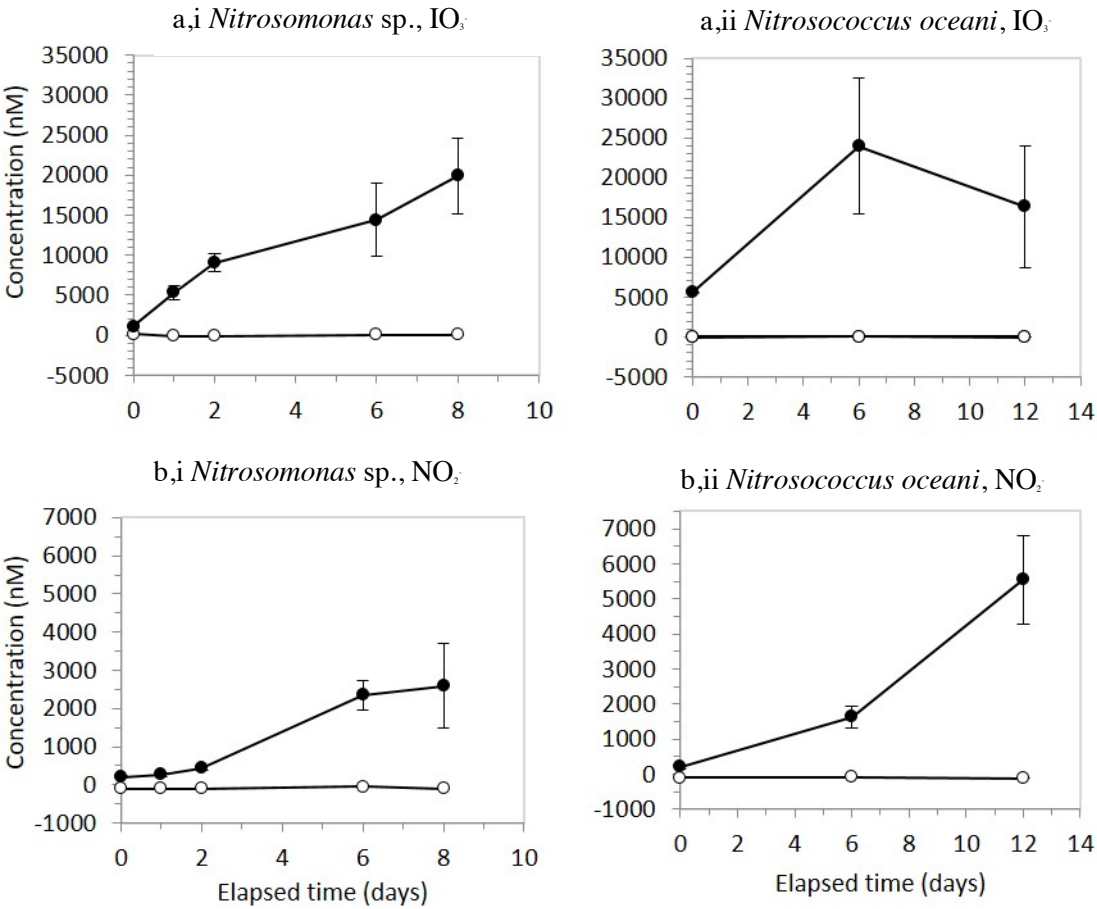
230

231 **Figure 1.** Average cell number in the *Nitrosomonas* sp. (grey bars) and *Nitrosococcus oceanii* (white
232 bars) cultures used in this study at the start (T₀) and end (T_{end}; 8 days for *Nitrosomonas* sp. and 12
233 days for *Nitrosococcus oceanii*) of each experiment. Error bars are standard deviations from three
234 replicate cultures.

235

236 **Iodine and nitrogen speciation**

237 Figure 2 shows that significant increases in the concentrations of IO_3^- (compared to media-only
 238 controls) were observed alongside NO_2^- production in both AOB cultures studied. In *Nitrosomonas*
 239 sp. (Figure 2ai and 2bi) there was a steady increase in IO_3^- concentrations throughout the experiment
 240 reaching a maximum of 19,921 ($\pm 4,754$) nM by the end of the experiment (day 8). In contrast NO_2^-
 241 concentrations reached a maximum of 2,360 (± 386) nM by day 6 and remained at around that level
 242 until the end of the experiment. In *Nitrosococcus oceanus* (Figure 2aai and 2bai) IO_3^- concentrations
 243 increased rapidly during the initial stages of the experiment reaching 23,943 ($\pm 8,568$) nM by day 6.
 244 IO_3^- concentrations at the end of the experiment (day 12) were 16,365 ($\pm 7,603$) nM. NO_2^-
 245 concentrations increased gradually throughout the experiment reaching 5,547 ($\pm 1,251$) nM by day
 246 12. There was larger variability in IO_3^- concentrations between replicates for *Nitrosococcus oceanus*
 247 but despite this a clear increase in all replicates was observed.



248

Figure 2. Changes in iodate (a) and nitrite (b) concentrations in cultures (closed symbols) and media-only controls (open symbols) for two cultures of ammonia-oxidising bacteria: i) *Nitrosomonas* sp.; and, ii) *Nitrosococcus oceanii* supplied with 9-10 mM iodide and 7-8 mM NH_4^+ . Error bars show the standard deviation of three replicate cultures.

Average production rates of IO_3^- and NO_2^- are presented in Table 1. In *Nitrosomonas* sp. average rates (\pm standard deviation) were 2,348 (\pm 593) nM IO_3^- day⁻¹ and 298 (\pm 141) nM NO_2^- day⁻¹. In *Nitrosococcus oceanii* averages rates were 897 (\pm 640) nM IO_3^- day⁻¹ and 445 (\pm 99) nM NO_2^- day⁻¹. Minimum cell-normalised rates (based on the final cell density observed in each culture) were 15.69 (\pm 4.71) fmol IO_3^- cell⁻¹ day⁻¹ and 1.96 (\pm 0.88) fmol NO_2^- cell⁻¹ day⁻¹ for *Nitrosomonas* sp., and 11.96 (\pm 6.96) fmol IO_3^- cell⁻¹ day⁻¹ and 6.19 (\pm 0.56) fmol NO_2^- cell⁻¹ day⁻¹ for *Nitrosococcus oceanii*. Molar ratios of iodate-to-nitrite production were 9.2 \pm 4.0 for *Nitrosomonas* sp. and 1.88 \pm 0.91 for *Nitrosococcus oceanii*.

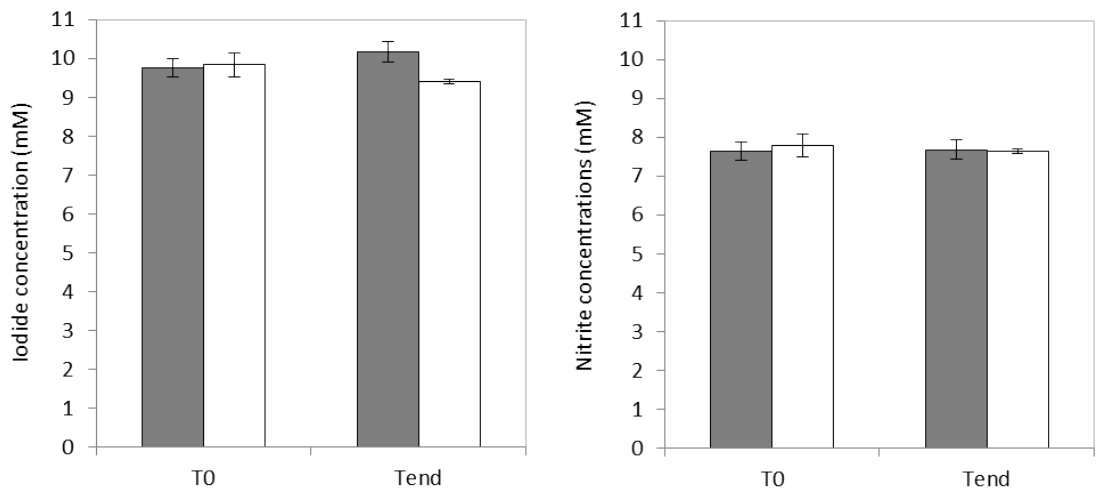
Table 1. Nitrite and iodate production rates (\pm standard deviations) observed in cultures of the ammonia-oxidising bacteria *Nitrosomonas* sp. and *Nitrosococcus oceanii*. Cell-normalised values are a minimum as they are calculated using maximum cell densities.

Culture	Nitrite		Iodate	
	nM day ⁻¹	fmol cell ⁻¹ day ⁻¹	nM day ⁻¹	fmol cell ⁻¹ day ⁻¹
<i>Nitrosomonas</i> sp.	298 (\pm 141)	1.96 (\pm 0.88)	2,348 (\pm 593)	15.69 (\pm 4.71)
<i>Nitrosococcus oceanii</i>	445 (\pm 99)	6.19 (\pm 0.56)	897 (\pm 640)	11.96 (\pm 6.96)

Figure 3 shows that, within error, a decline in I or NH_4^+ concentrations was not observed during either of the AOB experiments. Average starting I or NH_4^+ concentrations in *Nitrosomonas* sp. were 9.8 (\pm 0.2) mM and 7.6 (\pm 0.1) mM respectively. At the end of the experiment these values were 10.2 (\pm 0.3) mM I and 7.7 (\pm 0.1) mM NH_4^+ . For *Nitrosococcus oceanii* the start and end concentrations were 9.8 (\pm 0.3) and 9.4 (\pm 0.1) mM for I and 7.8 (\pm 0.1) and 7.7 (\pm 0.1) mM for NH_4^+ . This result was expected as the average standard deviations associated with the observed concentrations of I or NH_4^+ (i.e. 0.1 to 0.3 mM) are at least an order of magnitude higher than the maximum levels of IO_3^- and

276 NO₂⁻ observed in the culture experiments, i.e. very little of the initial stock of NO₂⁻ or NH₄⁺ was
 277 oxidised during the experiments.

278



279

280 **Figure 3.** Start and end concentrations of a) iodide and b) ammonia in cultures of *Nitrosomonas* sp.
 281 (grey bars) and *Nitrosococcus oceani* (white bars). Error bars show the standard deviation of three
 282 replicate cultures.

283

284

285

286

287 Discussion

288 Iodate production by ammonia-oxidising bacteria

289 Our results confirm that IO₃⁻ production occurs in cultures of the ammonia-oxidising bacteria

290 *Nitrosomonas* sp. and *Nitrosococcus oceani* supplied with I⁻, but not in cultures of nitrite oxidising

291 bacteria. Coincident increases in NO₂⁻ (Figure 2) show that both cultures were actively oxidising

292 ammonia throughout the experiments at rates of 1.96±0.088 fmol NO₂⁻ cell⁻¹ day⁻¹ for *Nitrosomonas*

293 sp. and 6.19±0.56 fmol NO₂⁻ cell⁻¹ day⁻¹ for *Nitrosococcus oceani*. Whilst these cell-normalised

294 oxidation rates are of the same order as those reported in the literature (e.g. 6-20 fmol NO₂⁻ cell⁻¹ day⁻¹;

295 Ward *et al.*, 1987; 1989) they are at the lower end. This is consistent with the approach taken here to

296 calculate the rates by normalising to the final (highest) cell densities. It is also worth noting that the

297 cultures were at an early stage of growth and had relatively low cell densities during the experiment.
298 This was done to avoid significant changes in pH in the bulk media which would impact inorganic
299 iodine speciation (*Section 3.2*). The observation of an increase in IO_3^- concentrations alongside active
300 biological ammonia oxidation supports previous studies (e.g. Truesdale *et al.*, 2001; Zic *et al.*, 2013)
301 which have shown that high aqueous concentrations of IO_3^- are found in regions of enhanced
302 nitrification, and provides the first direct confirmation of a biological basis for at least one
303 mechanism of iodide oxidation

304

305 Whilst we did not set out to establish the mechanism for I^- to IO_3^- oxidation by marine nitrifiers, some
306 speculations can be made. As I^- oxidation to IO_3^- requires the transfer of six electrons, it may occur in
307 a series of one- or two- electron transfer steps. Initially, I^- may be oxidised to molecular iodine ($\text{I}^- \rightarrow$
308 I_2), a reaction which is thermodynamically unfavourable at the pH of seawater (Luther *et al.*, 1995).
309 Further oxidation to IO_3^- by disproportionation ($\text{I}_2 \rightarrow \text{HOI} \rightarrow \text{IO}_3^-$) can occur spontaneously, but in
310 seawater is subject to competition with reduction of I_2 by organic matter (Truesdale & Moore, 1992;
311 Truesdale *et al.*, 1995). It is not known whether the ammonia-oxidisers mediate just the first stage of
312 I^- oxidation, with the observed IO_3^- production due to subsequent spontaneous reactions in the culture
313 media, or if they are involved in driving the complete conversion of I^- to IO_3^- . However, bacteria
314 which just oxidise I^- to I_2 have been isolated from seawater aquaria (Gozlan, 1968), I-rich natural gas
315 brine waters (Amachi *et al.*, 2005) and marine environmental samples (Fuse *et al.*, 2003; Amachi *et*
316 *al.*, 2005).

317

318 The observed IO_3^- production is either linked to the nitrification process itself or associated with other
319 metabolic activities of the AOB studied. Truesdale *et al.* (2001) has proposed that I^- oxidation to IO_3^-
320 would be energetically advantageous for chemoautotrophic AOB. In that case the key enzymes used
321 to obtain energy during the oxidation of NH_4^+ to NO_2^- (ammonia monooxygenase [AMO] and

hydroxylamine oxidoreductase [HAO]) could also have the potential to use I as a substrate. The observed IO₃⁻-to-NO₂⁻ molar production rates (9.2±4.0 for *Nitrosomonas* sp. and 2.3±1.1 for *Nitrosococcus oceanus*) are intriguing. If AMO/HAO are involved, this suggests that the enzymes have higher affinities for I than NH₄⁺/NH₂OH given the similar concentrations of I and NH₄⁺ used in the experiments. Other enzymes that have been implicated in I oxidation include the chloroperoxidases (Thomas & Hager, 1968) but we do not know if they occur in AOB. The exact metabolic pathway driving the observed IO₃⁻ production and its controls (i.e. substrate concentrations, light intensity) will need to be determined in future work. To establish if such further experimentation is warranted we need to explore whether the link between nitrification and I oxidation is likely to be an important part of inorganic iodine cycling in seawater.

Implications for inorganic iodine speciation in the oceans

Our culture studies suggest that the molar rate of I oxidation (IO₃⁻ production) is ~2-9 times higher than that for ammonia oxidation (nitrification). Note that although ammonium and iodide concentrations were much higher in the experimental media than in the oceans, the concentration ratio of these species was comparable to that found naturally. Ammonia oxidation rates in seawater range from below detection to 10⁻³ nM day⁻¹ (Table 2). Literature estimates of the rate of I oxidation in the marine environment range from ~4 to 670 nM year⁻¹ or 0.01 to 1.84 nM day⁻¹ (reviewed in Chance *et al.*, 2014). If the oxidation molar ratios observed in this study (~2-9) are representative, predicted rates of I oxidation are in-line (i.e. 2-9 times higher) with the lower end of observed ammonia oxidation rates (Table 2).

Table 2. Ammonia-oxidation rates measured in a range of ocean regions.

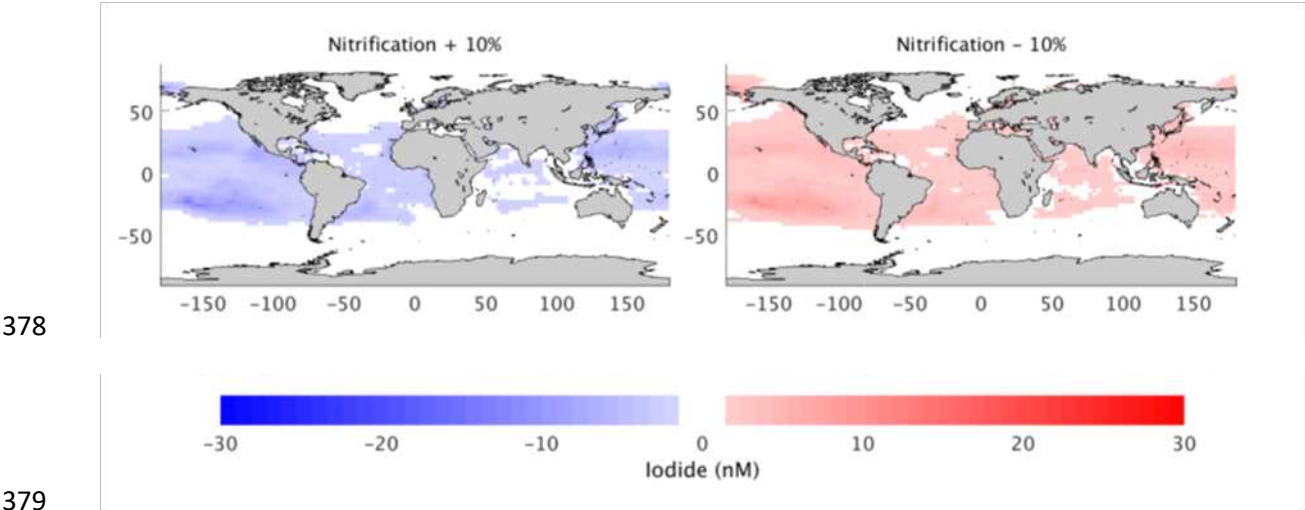
Study	Location	Rate (nM day ⁻¹)
Newell <i>et al.</i> (2011)	Arabian Sea, Indian Ocean	undetected to 21.6
Smith <i>et al.</i> (2015)	Northeast Pacific	< 0.01 to 90
Peng <i>et al.</i> (2015)	Eastern tropical north Pacific	< 1 to 8.6

Newell <i>et al.</i> (2013)	Subtropical Atlantic, Sargasso Sea (BATS)	< 2
Lam <i>et al.</i> (2007)	Black Sea	7-24
Beman <i>et al.</i> (2012)	Gulf of California, eastern tropical north Pacific	0-348

Truesdale *et al.* (2001) derive likely I⁻ oxidation (or IO₃⁻ production) rates for the near surface Black Sea using an iodine budget and this allows us to examine the potential importance of the link between nitrification and I⁻ oxidation on a local scale. They predict a minimum I⁻ oxidation flux of $3.89 \times 10^{-4} \text{ mol I m}^{-2} \text{ year}^{-1}$ which is an average of 0.02 nM day^{-1} at a mixed-layer depth (MLD) of 50 m or 0.11 nM day^{-1} at an MLD of 10 m. Lam *et al.* (2007) report an AOB abundance of $\leq 1,400 \text{ cells mL}^{-1}$ in the Black Sea. If we apply a cell density of $1,400 \text{ AOB cells mL}^{-1}$ to the average cell-normalised rates of IO₃⁻ production observed in this study (Table 1) we derive I⁻ oxidation rates of $\sim 20 \text{ nM d}^{-1}$. This is clearly much higher than the rates suggested in Truesdale *et al.* (2001). This discrepancy could be explained in a number of ways. Firstly, Lam *et al.* (2007) state that net nitrification only takes place within a narrow depth range of the Black Sea water column (i.e. between 71 and 81 m) and, the I⁻ oxidation values derived in Truesdale *et al.* (2001) are minimum values. It is also possible that the AOB studied here have a higher capacity for I⁻ oxidation (per unit ammonia-oxidised) than other ammonia-oxidisers or that our culture conditions (e.g. substrate availability) promoted higher I⁻ oxidation rates than would be observed in marine systems. For example, ammonia-oxidising Archaea (AOA), which can outnumber known bacterial ammonia oxidisers by orders of magnitudes in environments such as the marine water-column (reviewed by Schleper & Nicol, 2010), may have a very different capacity for I⁻ oxidation compared to the AOB studied here. Further studies are needed to establish the relationship between ammonia- and I⁻ oxidation in the marine environment.

Potential implications for future oceanic inorganic iodine distributions

369 Environmental factors which are known to be currently undergoing change in the oceans (e.g.
370 oxygen, light, pH, temperature) have all been found to impact rates and patterns of marine
371 nitrification (reviewed by Pajores and Ramos, 2019). Whilst there remains some uncertainty about
372 the future magnitude and, in some cases, sign of the response, some of the expected future changes in
373 marine nitrification are large. For example, whilst some studies have seen no impact on specific
374 marine nitrifiers (e.g. Qin et al., 2014), Beman et al. (2011) suggest that expected rates of
375 acidification could cause a decline in ammonia oxidation by up to 44% within the next few decades.
376 It is hence worth exploring how possible future changes in marine nitrification could impact ocean
377 iodine cycling.



380 **Figure 4.** Modelled changes in surface I concentration (nM) resulting from a) +10%, b) -10%,
381 changes in the rates of nitrification. Negative percent values indicate a decline in the rate of
382 nitrification and *vice-versa*. Negative values on the scale bar indicate a decrease in I concentrations
383 and *vice versa*.
384

385 In order to explore the possible impact of future changes in marine nitrification rates on sea surface
386 iodine fields we used the ocean cycling model described in Wadley et al. (2020). Within the model
387 iodide production is driven by primary productivity, and I oxidation to IO_3^- linked to nitrification in
388 the mixed layer. Nitrogen fluxes and the spatial distribution of mixed layer ammonia oxidation are
389 derived from a global biogeochemical cycling model (Yool *et al.*, 2007). I is oxidised to IO_3^- in

association with the ammonia oxidation, with the same I:N:C ratio as associated with iodide production (Truesdale *et al.*, 2001; Long *et al.*, 2015). The model does not use any of the rates derived in the current study as these are based on results from only 2 AOB species cultured at high substrate concentrations. Model outputs (Figure 4) show that even with small (+/- 10%) changes in ammonia oxidation there is a clear alteration to sea surface I fields. Sea surface I concentrations increase as ammonium oxidation rates decrease and *vice-versa*. For example, the ocean cycling model suggests there could be an average global increase of 0.13 nM I per 1% decrease in nitrification. The outputs suggest that the change in the iodine fields is spatially variable and will increase as the perturbation to ammonia oxidation increases. For example, at the 44% decline in nitrification predicted by Beman *et al.* (2011) the model predicts there will be a 25% increase (+30 nM) in sea surface I in the sub-tropical gyres. Carpenter *et al.* (2013) show that I₂ emissions due to ozone deposition increase near linearly with I concentration. Hence, the predicted changes to sea surface I fields under future ocean acidification could have a major impact on ozone deposition to the sea surface, atmospheric chemistry and resulting sea-air iodine emissions.

5.3. Conclusions

This study has shown that I⁻ oxidation to IO₃⁻ occurs in cultures of ammonia oxidising (nitrifying) bacteria, but not nitrite oxidising bacteria. Our calculations suggest that I oxidation by AOB could be an important control on inorganic iodine speciation in seawater, but to confirm this further study is needed on a wider range of ammonia-oxidisers including ammonia oxidising archaea (AOA). Simulations from our iodine cycling model suggest that changes in nitrification rate, such as those predicted to occur under acidification (Beman *et al.*, 2011), could have an important impact on sea surface I fields. A future change in marine nitrification could alter sea surface I fields. In turn, this

414 could lead to a change in ozone deposition to the sea surface and sea-air iodine emissions with
415 potentially major implications for atmospheric chemistry and air quality.

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419

420 **Acknowledgements**

421 We thank Eva Spieck (University of Hamburg, Germany) for supplying the nitrite oxidising bacteria
422 (NOB) cultures used in our preliminary experiments.

423

424 **Author contributions**

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426 Draft, Writing – Review & Editing, Visualisation, Supervision, Project Administration, Funding
427 Acquisition. Eleanor Barton: Formal Analysis, Investigation, Writing – Original Draft. Helmke
428 Hepach: Methodology, Validation, Investigation, Resources. Rosie Chance: Conceptualisation,
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435 Supervision, Funding Acquisition.

436

437 **Data Statement:** The data presented in this manuscript is available from the British Oceanographic
438 Data Centre (BODC), doi:10.5285/aaa4dab8-7c9d-0cca-e053-6c86abc00b1d

439

440 **Funding:** This work was supported by the Natural Environment Research Council [grant no.
441 NE/N01054X/1]; and the University of York Laidlaw Scholarship [work undertaken by Eleanor
442 Barton].

443

444 **Declaration of interest:** The authors have no financial conflicts of interest with the research in this
445 paper.

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Supplementary Data

Iodate production in cultures of marine ammonia-oxidising bacteria: implications for future inorganic iodine distributions in the oceans
Claire Hughes, Eleanor Barton, Helmke Hepach, Rosie Chance, Matt Pickering, Karen Hogg, Andreas Pommerening-Röser, Martin R. Wadley, David P. Stevens & Tim D. Jickells

Table S1: Concentrations of iodine and nitrogen species, cell counts and pH in bacterial cultures and media-only controls over time

Bacterial strain	Day	Analyte	[Iodate] and [Nitrite], nmol L ⁻¹						Cell count, cells/mL			[Ammonium], mmol L ⁻¹			[Iodide], mmol L ⁻¹			pH					
			Media-only control			Bacteria			Bacteria			Bacteria			Bacteria			Media-only control			Bacteria		
			A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
<i>Nitrosomonas</i> sp.	0	Nitrite:	0.0	0.0	0.0	0	0	0	18140	26130	21030	7.64	7.64	7.61	9.94	9.49	9.84	7.69	7.7	7.71	7.75	7.77	7.78
	1		-0.7	-2.3	-3.2	31	63	71										7.58	7.65	7.64	7.67	7.63	7.7
	2		2.5	2.3	-2.3	257	210	238															
	6		45.1	44.5	37.5	2175	1790	2488															
	8		2.1	-4.6	-7.2	3485	2430	1228	158610	143440	150900	7.75	7.61	7.68	10.4	10.2	9.9	7.54	7.61	7.61	7.62	7.61	7.65
	0	Iodate:	0	0	0	0	0	0															
	1		-98	-98	-212	4047	3529	5022															
	2		-125	-125	-202	8741	6582	8490															
	6		19	19	-58	18112	8764	12893															
	8		35	35	-85	16403	24243	15702															
<i>Nitrosococcus oceanii</i>	0	Nitrite:	0	0	0	0	0	0	19870	17270	13700	7.83	7.72	7.78	9.5	10.0	10.0	7.61	7.60	7.59	7.54	7.50	7.52
	6		30.3	21.3	23.4	1675	1428	1194										7.68	7.69	7.64	7.45	7.44	7.49
	12		-12.9	-12.6	-10	6705	4718	4604	81830	67230	65230	7.69	7.58	7.68	9.5	9.4	9.4	7.66	7.67	7.63	7.21	7.30	7.27
	0	Iodate:	0	0	0	0	0	0															
	6		72	59	61	15000	28036	11970															
	12		25	28	46	19569	7185	5519															