Iodate production in cultures of marine ammonia-oxidising bacteria: implications for future inorganic iodine distributions in the oceans

Claire Hughes[1,1], Eleanor Barton[1,1], Helmke Hepach[1,1], Rosie Chance[1,1], Matt Pickering[1,1], Karen Hogg[1,1], Andreas Pommerening-Röser[2,2], Martin Robert Wadley[3,3], David P. Stevens[3,3], and Timothy D. Jickells[3,3]

[1]University of York
[2]University of Hamburg
[3]University of East Anglia

November 30, 2022

Abstract

Reaction with iodide (I) at the sea surface is an important sink for atmospheric ozone, and causes sea-air emission of reactive iodine which in turn drives further ozone destruction. To incorporate this process into chemical transport models, improved understanding of the factors controlling marine iodine speciation, and especially sea-surface iodide concentrations, is needed. The oxidation of I to iodate (IO) is the main sink for oceanic I, but the mechanism for this remains unknown. We demonstrate for the first time that marine nitrifying bacteria mediate I oxidation to IO. A significant increase in IO concentrations compared to media-only controls was observed in cultures of the ammonia-oxidising bacteria sp(Nm51) and (Nc10) supplied with 9-10 mM I, indicating I oxidation to IO. Cell-normalised production rates were 15.69 (±4.71) fmol IO cell d for sp., and 11.96 (±6.96) fmol IO cell d for , and molar ratios of iodate-to-nitrite production were 9.2±4.1 and 1.88±0.91 respectively Preliminary experiments on nitrite-oxidising bacteria showed no evidence of ItoIO oxidation. If the link between ammonia and I oxidation observed here is representative, our ocean iodine cycling model predicts that decreases in marine nitrification under ocean acidification could lead to significantly higher sea surface I. A global sensitivity analysis suggests a 0.13 nM increase in sea surface I concentrations per percentage decrease in nitrification rate. In turn, this could result in increased O deposition to the sea surface and sea-air iodine emissions, with implications for atmospheric chemistry and air quality.

Hosted file

`essoar.10503313.2.docx` available at https://authorea.com/users/561300/articles/608854-iodate-production-in-cultures-of-marine-ammonia-oxidising-bacteria-implications-for-future-inorganic-iodine-distributions-in-the-oceans

Posted on 30 Nov 2022 — CC-BY-NC 4 — https://doi.org/10.1002/essoar.10503313/v2 — This a preprint and has not been peer reviewed. Data may be preliminary.

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

			[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		
Bacterial strain	Day	Analyte	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Nitrosomonas sp.	0	Nitrite	0.0	0.0	0.0	0	0	0	18145	26130	21010	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	318	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	6182	8490															
	6		19	19	-58	18112	8764	12893															
	8		35	35	45	16403	24243	15702															
Nitrosococcus oceani	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	81	15000	28036	11970															
	12		25	28	46	19369	7185	5519															

Posted on 30 Nov 2022 — CC-BY-NC 4 — https://doi.org/10.1002/essoar.10503313/v2 — This a preprint and has not been peer reviewed. Data may be preliminary.

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

Claire Hughes[1*], Eleanor Barton[1*], Helmke Hepach[1], Rosie Chance[2], Matt Pickering[1], Karen Hogg[3], Andreas Pommerening-Röser[4], Martin R. Wadley[5], David P. Stevens[5] and Tim D. Jickells[6]

[1] Department of Environment and Geography, University of York, Wentworth Way, Heslington, York, YO10 5NG, UK

[2] Wolfson Atmospheric Chemistry Laboratory, Department of Chemistry, University of York, Heslington, York, YO10 5DD, UK

[3] Bioscience Technology Facility, Department of Biology, University of York, Wentworth Way, York, YO10 5DD, UK

[4] University of Hamburg, Mikrobiologie & Biotechnologie, Ohnhorststr. 18, D-22609 Hamburg, Germany

[5] School of Mathematics, University of East Anglia, Norwich Research Park, Norwich, NR4 7TJ, UK

[6] School of Environmental Sciences, University of East Anglia, Norwich Research Park, Norwich, NR4 7TJ, UK

*authors contributed equally to the manuscript

Corresponding author: Rosie Chance (rosie.chance@york.ac.uk)

Highlights:

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

Abstract

Reaction with iodide (I^-) at the sea surface is an important sink for atmospheric ozone, and causes sea-air emission of reactive iodine which in turn drives further ozone destruction. To incorporate this process into chemical transport models, improved understanding of the factors controlling marine iodine speciation, and especially sea-surface iodide concentrations, is needed. The oxidation of I^- to iodate (IO_3^-) is the main sink for oceanic I^-, but the mechanism for this remains unknown. We demonstrate for the first time that marine nitrifying bacteria mediate I^- oxidation to IO_3^-. A significant increase in IO_3^- concentrations compared to media-only controls was observed in cultures of the ammonia-oxidising bacteria *Nitrosomonas* sp. (Nm51) and *Nitrosoccocus oceani* (Nc10) supplied with 9-10 mM I^-, indicating I^- oxidation to IO_3^-. Cell-normalised production rates were 15.69 (±4.71) fmol IO_3^- $cell^{-1}$ d^{-1} for *Nitrosomonas* sp., and 11.96 (±6.96) fmol IO_3^- $cell^{-1}$ d^{-1} for *Nitrosococcus oceani*, and molar ratios of iodate-to-nitrite production were 9.2±4.1 and 1.88±0.91 respectively. Preliminary experiments on nitrite-oxidising bacteria showed no evidence of I^- to IO_3^- oxidation. If the link between ammonia and I^- oxidation observed here is representative, our ocean iodine cycling model predicts that future changes in marine nitrification could alter global sea surface I^- fields with potential implications for atmospheric chemistry and air quality.

Introduction

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

Total inorganic iodine is found at 400-500 nM in seawater and predominantly exists as iodate (IO_3^-) and I^- (Chance *et al*., 2014) with inter-conversion between these two species alongside physical mixing being the main causes of spatial and temporal variability in sea surface I^-. Iodate is the thermodynamically stable form and the dominant form in the deep ocean. The existence of relatively higher levels of I^- in the euphotic zone (reviewed by Chance *et al*., 2014) has led to the suggestion that IO_3^- reduction to I^- is linked to primary productivity. This theory has been supported by observations of I^- production in cultures of a wide range of marine phytoplankton (e.g. Chance *et al*., 2007; Bluhm *et al*., 2010) and some field studies (Chance *et al*., 2010). Proposed mechanisms for IO_3^- reduction to I^- by marine phytoplankton include nitrate reductase enzymes (Hung *et al*., 2005) and reactions of iodate with reduced sulphur species exuded from cells during senescence (Bluhm et al., 2010), but neither has yet been confirmed as the dominant route of conversion. I^- oxidation to IO_3^- is also known to occur with rate estimates ranging from ~4 to 670 nM yr^{-1} (reviewed in Chance *et al*., 2014). Abiotic oxidation of I^- back to IO_3^- in the ocean (e.g. by oxygen, hydroxyl radicals, hydrogen

peroxide and ozone) is thought to occur so slowly as to be insignificant (e.g. Wong, 1991), and so I^- oxidation to IO_3^- is also thought to be associated with marine microbiological activity. The rates and processes involved in I^- to IO_3^- oxidation are associated with large uncertainty (Truesdale *et al*., 2001; Amachi *et al*., 2008), and the mechanisms involved remain undefined. This uncertainty has been suggested to be one of the factors hindering the development of mathematical models of iodine transformations in the global oceans (Truesdale *et al*., 2001).

I^- oxidation to I_2 has been observed in bacterial isolates obtained from a range of environments including seawater aquaria (Gozlan *et al*., 1968), natural gas brines (Iino *et al*., 2016) and seawater/marine mud (Fuse *et al*., 2003). Additionally, based on field observations, a number of studies (Truesdale *et al*., 2001; Žic *et al*., 2013) have proposed that I^- oxidation to IO_3^- is linked to nitrification in marine systems. Nitrification is the two-stage biological transformation of ammonia (NH_3) to nitrate (NO_3^-) (Equations 1 and 2; Koops & Pommerening-Röser, 2001) mediated by chemoautotrophic ammonia-oxidising bacteria (AOB), and nitrite-oxidising bacteria (NOB). Previously thought to only occur outside of the euphotic zone, nitrification is now known to occur throughout the oceanic water-column (reviewed by Yool *et al*., 2007).

$$2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 4H^+ + 2H_2O \quad (1)$$

$$2NO_2^- + O_2 \rightarrow 2NO_3^- \quad (2)$$

A link between I^- oxidation/ IO_3^- production and nitrification is yet to be confirmed but, if established, would suggest that I^- oxidation to IO_3^- is widespread throughout the world's oceans (Yool *et al*., 2007).

The primary aim of this study was to establish whether I^- oxidation to IO_3^- is associated with marine nitrification. Our objectives were to determine if IO_3^- production occurs in cultures of marine

ammonia- and nitrite-oxidising bacteria supplied with I^-, determine the relative rates of IO_3^- production and nitrification and explore the possible implications of the findings.

Methods

Cultures

Two AOB cultures (*Nitrosomonas* sp. [Nm51] and *Nitrosococcus oceani* [Nc10]) were investigated for IO_3^- production in the presence of I^- as the only iodine source. Cultures were grown in the dark in a water bath at 25 °C in autoclaved ESAW artificial seawater mixture (Berges *et al*., 2001) made up using distilled water. The ESAW media was supplemented with 7-8 mM ammonium chloride and potassium phosphate. We also conducted preliminary tests on three active marine NOB (*Nitrospira marina* [295], *Nitrospina gracilis* [3/211], *Nitrococcus mobilis* [231]) but saw no evidence of IO_3^- production in any of the cultures studied. These results are not discussed further. Handling of cultures was done at all times in a biosafety cabinet using sterile equipment.

Experimental Set Up

For the AOB experiments triplicate cultures were incubated alongside triplicate media-only controls for periods of 8-12 days. The experiments were kept as short as possible to avoid significant changes in pH in the bulk media which would impact inorganic iodine speciation. Hence experiments were only run until an increase in nitrite across two time-points was observed. Samples were taken at regular intervals of between 1 to 6 days for pH measurement, cell counts and determination of NO_2^-, IO_3^-, I^- and NH_4^+/NH_3 concentrations. In all cases, I^- (Aristar) was added to be at similar concentrations with the NH_4^+ required in the growth media. The levels of I^- are much higher than those encountered in the oceans (global ocean median=77 nM I^- [interquartile range 28-140 nM], Chance *et al*., 2014) but were chosen to be similar to the levels of NH_4^+. This is because in the marine environment

123 nitrifiers would be exposed to similar ratio of NH_4^+ and I^-. For example, Rees *et al*. (2006) show that
124 NH_4^+/NH_3 occurs at concentrations ranging from 60-300 nM in the Atlantic between 60°N to 50°S.

125

126 ***pH***

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

131

132 ***Cell counts***

133 Immediately after sampling, 4 mL of the culture was fixed with 15 μL of 50% glutaraldehyde (Alfa
134 Aesar), flash frozen in liquid nitrogen and placed in a -80 °C freezer for later determination of cell
135 density. Cell counts were made using a Beckman Coulter Cytoflex S flow cytometer (flow rate of 10
136 μL min^{-1}) within 2 months of collection. DAPI (Sigma; 2 μg mL^{-1}) stained samples were excited by a
137 laser at 405 nm and the emitted fluorescence detected using an avalanche photodiode detector with a
138 reflective band pass filter 450/45. The flow cytometer thresholds were set using the 405 nm laser
139 side scatter and the DAPI fluorescence signals.

140

141 ***Nitrite concentration***

142 NO_2^- was measured in 0.45 μm (Millex) filtered samples using a spectrophotometric method
143 (Lambda 25 UV/Vis spectrophotometer, Perkin-Elmer) developed by Norwitz & Keliher (1984). The
144 method involves diazotizing nitrite with sulfanilamide (Fisher, analytical reagent grade) and coupling
145 with N-1-naphthylethylenediamine dihydrochloride (Fisher, analytical reagent grade) to form a

146 coloured azo dye which is measured spectrophotometrically at 540 nm. The method was calibrated
147 using $NaNO_2$ standards (Fisher, analytical reagent grade) prepared in the ESAW-based media.

148

149 ***Iodate Concentration***

150 IO_3^- concentrations were measured in 0.45 μm (Millex) filtered samples using a manual version of the
151 spectrophotometric (Lambda 25 UV/Vis spectrophotometer) method detailed in Truesdale &
152 Spencer, 1974 and Jickells *et al.*, 1988. Absorbance was measured at 350 nm. Strictly, this method
153 determines all oxidised (0 to +5 oxidation state) forms of inorganic iodine, but in seawater derived
154 media this is predominantly IO_3^-, and so will be referred to as IO_3^- iodate hereafter. The method was
155 calibrated using potassium iodate (Aristar) standard solutions made up in ESAW.

156 Some validation and modification to the method was required due to the nature of our experimental
157 set-up. Chapman & Liss (1977) show that NO_2^- can interfere with spectrophotometric IO_3^-
158 measurements (using sulfamic acid) at ambient seawater concentrations with a 15% error. Clearly
159 significant interference would be an issue for our experiments where NO_2^- was being produced so we
160 ran tests. We found that the presence of NO_2^- up to 10 μM had negligible impact on IO_3^- measurements
161 (between 0.1-50 μM). We did however identify that the high starting concentration of I^- (~10 μM) in
162 the culture media was problematic. The iodate analysis method comprises two steps: the first
163 involves an initial absorbance reading after the addition of sulfamic acid; the second involves the
164 addition of excess I^-. Under acidic conditions I^- reacts with IO_3^- to form I_2 (equation 3a) which reacts
165 with excess I^- to form the coloured ion I_3^- (equation 3b) that can be measured spectrophotometrically.

166 $$IO_3^- + 5I^- + 6H^+ \rightarrow 3I_2 + 3H_20 \qquad (3a)$$

167 $$I_2 + I^- \rightarrow I_3^- \qquad (3b)$$

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

173

174 ***Ammonium Concentration***

175 NH_4^+ concentrations were measured in 0.45 μm (Millex) filtered samples with a Seal Analytical
176 Autoanalyser 3 according to method G-109-93 rev. 10 (Seal Analytical) using sodium salicylate,
177 dichloro-isocyanuric acid and citrate buffer. The method was calibrated using standards ranging from
178 0-2 mg/L prepared from dilutions of a 1000 mg/L ammonium standard solution (Merck).

179

180 ***Iodide Concentration***

181 I^- concentrations were determined using a Dionex ICS-2000 ion chromatograph equipped with an
182 EGC III KOH elugen cartridge, AG18 (2 x 50 mm) guard column, AS18 (2 x 250 mm) analytical
183 column, ASRS 300 (2 mm) suppressor, DS6 heated conductivity cell and AS40 autosampler.
184 Samples were diluted 100-fold with 18 MΩ deionised water for analysis and 5 μL was injected onto
185 the ion chromatograph. Aqueous potassium hydroxide was used as the eluent at a flow rate of 0.25
186 mL min^{-1} with a gradient program starting from an initial concentration of 2 mM hydroxide (hold 1
187 min) to 20 mM at 18 min then to 41 mM at 19 min (hold 2 min) before returning to 2 mM. The I^-
188 retention time was 19 min. The instrument was calibrated with matrix-matched standards ranging
189 from 0-100 nM (I^-), prepared from dilutions of a 1000 mg/L iodide standard solution (Fisher
190 Scientific) with 18 MΩ deionised water and containing a final concentration of 1% ESAW.

191

192 ***Data Analysis***

193 As in Guerrero and Jones (1996), the NH_4^+ oxidation rate is defined here as the rate of increase in
194 NO_2^-. Similarly, we define the rate of I^- oxidation as the rate of increase in IO_3^-. This is appropriate as
195 no other iodine species were supplied to the cultures and conversion between I^- and IO_3^- is known to
196 be the main cause of variability in inorganic iodine speciation (Bluhm *et al*., 2010; Chance *et al*.,
197 2014). Average NO_2^- and IO_3^- production rates were calculated for each replicate culture using
198 Equation 4.

199 $$\text{Production Rate (nM day}^{-1}) = \frac{(C_{end} - C_0)}{t} \qquad (4)$$

200 where C_0 and C_{end} are the NO_2^- or IO_3^- concentrations observed at the start and end of the experiment
201 and t is the experimental duration in days. Cell-normalised rates were calculated by dividing these
202 rates by the final cell density observed in each AOB culture and are hence likely to be minimum
203 values.

204

205

206 **Results**

207 ***Cell counts and pH***

208 Increases in cell density were observed in all replicates of *Nitrosomonas* sp. and *Nitrosococcus*
209 *oceani* between the start and end of the experiment indicating growth (Figure 1). Average initial cell
210 density in the *Nitrosomonas* sp. cultures was 21,767 (±4,046) cells mL^{-1} and this increased to 150,983
211 (±7,585) cells mL^{-1} by the end of the experiment (8 days). For *Nitrosococcus oceani* start and end (12
212 days) cell densities were 16,947 (± 3,098) and 71,430 (±9,062) cells mL^{-1}, respectively. Average pH
213 levels in the culture experiments calculated from measurements at each time point (data not shown)
214 were 7.69 (±0.07) for *Nitrosomonas* sp. and 7.41 (±0.12) for *Nitrosococcus* sp. These pH levels are

consistent with those found in the media-only controls (7.64±0.07 for *Nitrosomonas* sp; 7.64±0.15 for *Nitrosococcus oceani*).

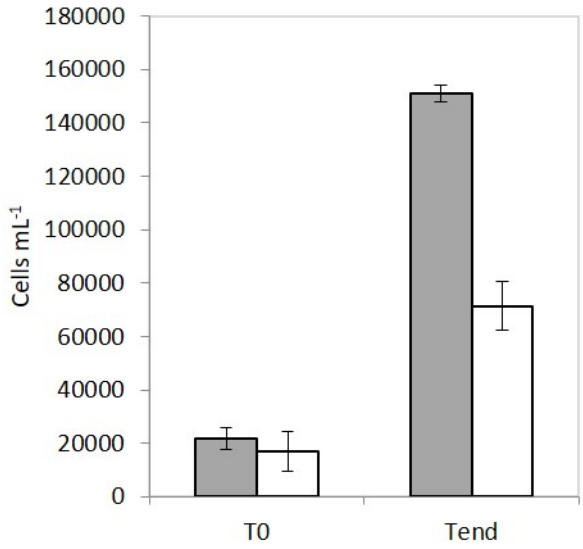


Figure 1. Average cell number in the *Nitrosomonas* sp. (grey bars) and *Nitrosococcus oceani* (white bars) cultures used in this study at the start (T_0) and end (T_{end}; 8 days for *Nitrosomonas* sp. and 12 days for *Nitrosococcus oceani*) of each experiment. Error bars are standard deviations from three replicate cultures.

Iodine and nitrogen speciation

Figure 2 shows that significant increases in the concentrations of IO_3^- (compared to media-only controls) were observed alongside NO_2^- production in both AOB cultures studied. In *Nitrosomonas* sp. (Figure 2ai and 2bi) there was a steady increase in IO_3^- concentrations throughout the experiment reaching a maximum of 19,921 (±4,754) nM by the end of the experiment (day 8). In contrast NO_2^- concentrations reached a maximum of 2,360 (±386) nM by day 6 and remained at around that level until the end of the experiment. In *Nitrosococcus oceani* (Figure 2aii and 2bii) IO_3^- concentrations increased rapidly during the initial stages of the experiment reaching 23, 943 (±8,568) nM by day 6. IO_3^- concentrations at the end of the experiment (day 12) were 16,365 (±7,603) nM. NO_2^-

concentrations increased gradually throughout the experiment reaching 5,547 (±1,251) nM by day 12. There was larger variability in IO_3^- concentrations between replicates for *Nitrosococcus oceani* but despite this a clear increase in all replicates was observed.

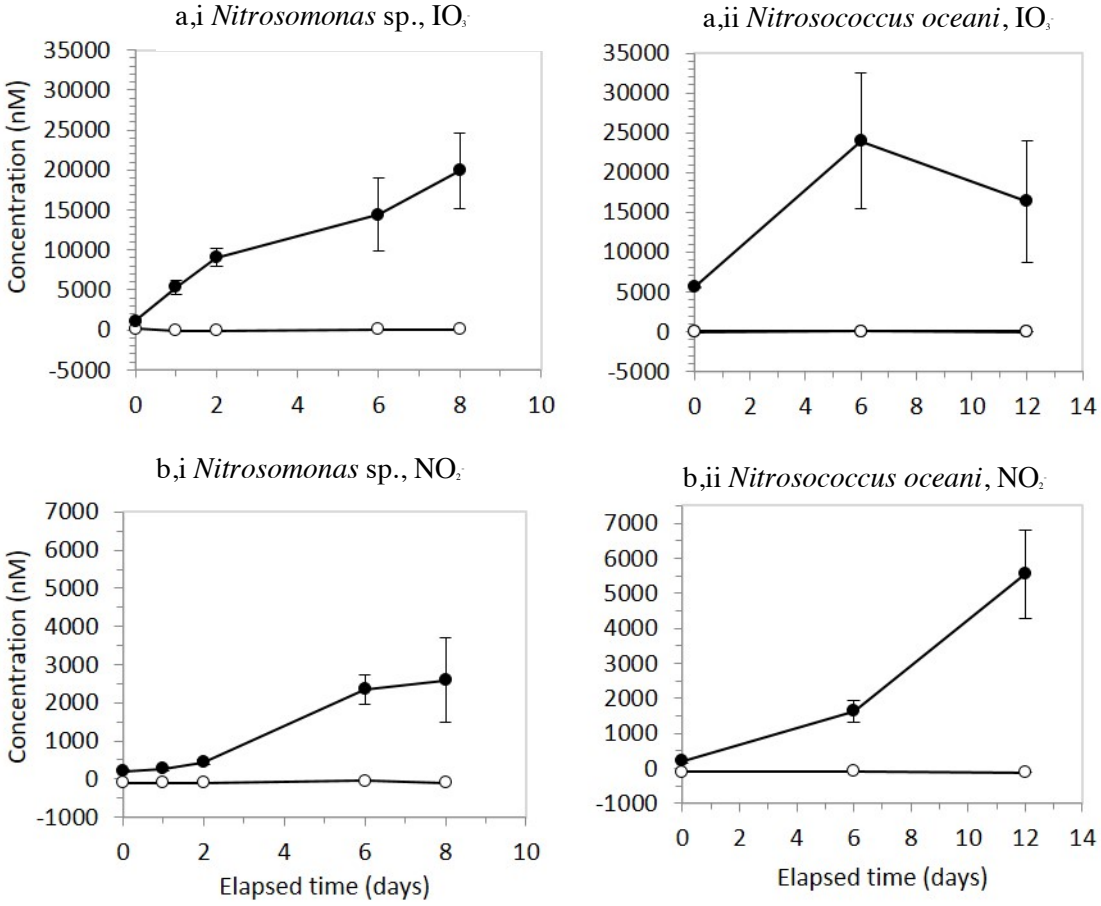


Figure 2. Changes in iodate (a) and nitrite (b) concentrations in cultures (closed symbols) and media-only controls (open symbols) for two cultures of ammonia-oxidising bacteria: i) *Nitrosomonas* sp.; and, ii) *Nitrosococcus oceani* supplied with 9-10 mM iodide and 7-8 mM NH_4^+. Error bars show the 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 (±standard deviation) were 2,348 (±593) nM IO_3^- day^{-1} and 298 (±141) nM NO_2^- day^{-1}. In *Nitrosococcus oceani* averages rates were 897 (±640) nM IO_3^- day^{-1} and 445 (±99) nM NO_2^- day^{-1}. Minimum cell-normalised rates (based on the final cell density observed in each culture) were 15.69 (±4.71) fmol IO_3^- $cell^{-1}$ day^{-1} and 1.96 (±0.88) fmol NO_2^- $cell^{-1}$ day^{-1} for *Nitrosomonas* sp., and 11.96

(±6.96) fmol IO_3^- $cell^{-1}$ day^{-1} and 6.19 (±0.56) fmol NO_2^- $cell^{-1}$ day^{-1} for *Nitrosococcus oceani*. Molar ratios of iodate-to-nitrite production were 9.2±4.0 for *Nitrosomonas* sp. and 1.88±0.91 for *Nitrosococcus oceani*.

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

	Nitrite		Iodate	
Culture	**nM day^{-1}**	**fmol $cell^{-1}$ day^{-1}**	**nM day^{-1}**	**fmol $cell^{-1}$ day^{-1}**
Nitrosomonas sp.	298 (±141)	1.96 (±0.88)	2,348 (±593)	15.69 (±4.71)
Nitrosococcus oceani	445 (±99)	6.19 (±0.56)	897 (±640)	11.96 (±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 (±0.2) mM and 7.6 (±0.1) mM respectively. At the end of the experiment these values were 10.2 (±0.3) mM I^- and 7.7 (±0.1) mM NH_4^+. For *Nitrosococcus oceani* the start and end concentrations were 9.8 (±0.3) and 9.4 (±0.1) mM for I^- and 7.8 (±0.1) and 7.7 (±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 NO_2^- observed in the culture experiments, i.e. very little of the initial stock of NO_2^- or NH_4^+ was oxidised during the experiments.

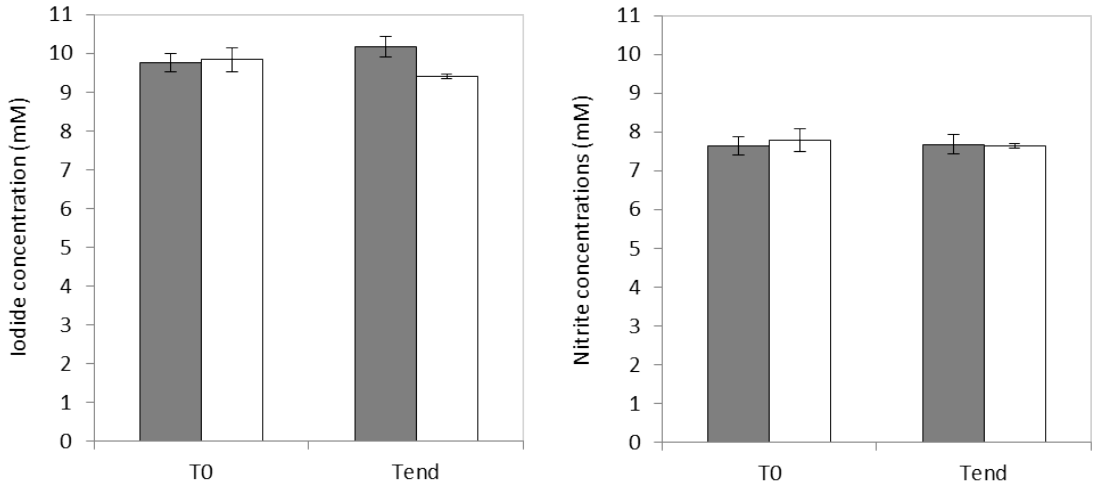


Figure 3. Start and end concentrations of a) iodide and b) ammonia in cultures of *Nitrosomonas* sp. (grey bars) and *Nitrosococcus oceani* (white bars). Error bars show the standard deviation of three replicate cultures.

Discussion

Iodate production by ammonia-oxidising bacteria

Our results confirm that IO_3^- production occurs in cultures of the ammonia-oxidising bacteria *Nitrosomonas* sp. and *Nitrosococcus oceani* supplied with I^-, but not in cultures of nitrite oxidising bacteria. Coincident increases in NO_2^- (Figure 2) show that both cultures were actively oxidising ammonia throughout the experiments at rates of 1.96±0.0.88 fmol NO_2^- $cell^{-1}$ day^{-1} for *Nitrosomonas* sp. and 6.19±0.56 fmol NO_2^- $cell^{-1}$ day^{-1} for *Nitrosococcus oceani*. Whilst these cell-normalised oxidation rates are of the same order as those reported in the literature (e.g. 6-20 fmol NO_2^- $cell^{-1}$ day^{-1}; Ward *et al*., 1987; 1989) they are at the lower end. This is consistent with the approach taken here to calculate the rates by normalising to the final (highest) cell densities. It is also worth noting that the cultures were at an early stage of growth and had relatively low cell densities during the experiment. This was done to avoid significant changes in pH in the bulk media which would impact inorganic iodine speciation (*Section 3.2*). The observation of an increase in IO_3^- concentrations alongside active

290 biological ammonia oxidation supports previous studies (e.g. Truesdale *et al*., 2001; Zic *et al*., 2013)
291 which have shown that high aqueous concentrations of IO_3^- are found in regions of enhanced
292 nitrification, and provides the first direct confirmation of a biological basis for at least one
293 mechanism of iodide oxidation
294
295 Whilst we did not set out to establish the mechanism for I^- to IO_3^- oxidation by marine nitrifiers, some
296 speculations can be made. As I^- oxidation to IO_3^- requires the transfer of six electrons, it may occur in
297 a series of one- or two- electron transfer steps. Initially, I^- may be oxidised to molecular iodine ($I^- \rightarrow$
298 I_2), a reaction which is thermodynamically unfavourable at the pH of seawater (Luther *et al*., 1995).
299 Further oxidation to IO_3^- by disproportionation ($I_2 \rightarrow HOI \rightarrow IO_3^-$) can occur spontaneously, but in
300 seawater is subject to competition with reduction of I_2 by organic matter (Truesdale & Moore, 1992;
301 Truesdale *et al*., 1995). It is not known whether the ammonia-oxidisers mediate just the first stage of
302 I^- oxidation, with the observed IO_3^- production due to subsequent spontaneous reactions in the culture
303 media, or if they are involved in driving the complete conversion of I^- to IO_3^-. However, bacteria
304 which just oxidise I^- to I_2 have been isolated from seawater aquaria (Gozlan, 1968), I-rich natural gas
305 brine waters (Amachi *et al*., 2005) and marine environmental samples (Fuse *et al*., 2003; Amachi *et*
306 *al*., 2005).
307
308 The observed IO_3^- production is either linked to the nitrification process itself or associated with other
309 metabolic activities of the AOB studied. Truesdale *et al*. (2001) has proposed that I^- oxidation to IO_3^-
310 would be energetically advantageous for chemoautotrophic AOB. In that case the key enzymes used
311 to obtain energy during the oxidation of NH_4^+ to NO_2^- (ammonia monooxygenase [AMO] and
312 hydroxylamine oxidoreductase [HAO]) could also have the potential to use I^- as a substrate. The
313 observed IO_3^--to-NO_2^- molar production rates (9.2±4.0 for *Nitrosomonas* sp. and 2.3±1.1 for
314 *Nitrosococcus oceani*) are intriguing. If AMO/HAO are involved, this suggests that the enzymes

have higher affinities for I^- than NH_4^+/NH_2OH given the similar concentrations of I^- and NH_4^+ 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 IO_3^- 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 (IO_3^- 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 10^2 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.

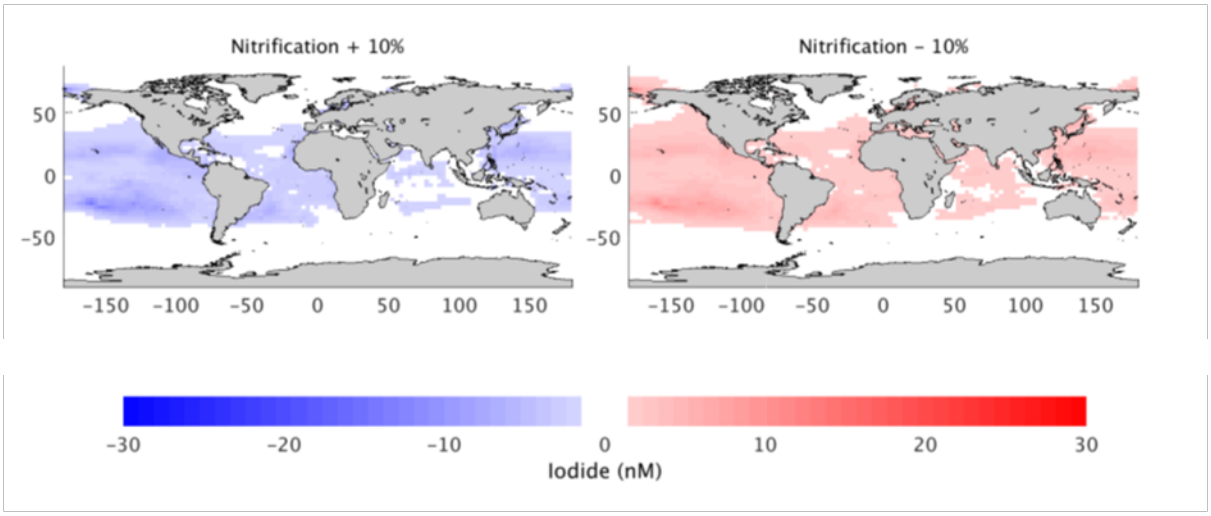
Study	Location	Rate (nM day^{-1})
Newell *et al*. (2011)	Arabian Sea, Indian Ocean	undetected to 21.6
Smith *et al*. (2015)	Northeast Pacific	< 0.01 to 90
Peng *et al*. (2015)	Eastern tropical north Pacific	< 1to 8.6
Newell *et al*. (2013)	Subtropical Atlantic, Sargasso Sea (BATS)	< 2
Lam *et al*. (2007)	Black Sea	7-24
Beman *et al*. (2012)	Gulf of California, eastern tropical north Pacific	0-348

Truesdale *et al*. (2001) derive likely I^- oxidation (or IO_3^- 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 3.89×10^{-4} mol I m^{-2} $year^{-1}$ 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 ≤1,400 cells mL^{-1} in the Black Sea. If we apply a cell density of 1,400 AOB cells mL^{-1} to the average cell-normalised rates of IO_3^- production observed in this study (Table 1) we derive I^- oxidation rates of ~20 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

Environmental factors which are known to be currently undergoing change in the oceans (e.g. oxygen, light, pH, temperature) have all been found to impact rates and patterns of marine nitrification (reviewed by Pajores and Ramos, 2019). Whilst there remains some uncertainty about the future magnitude and, in some cases, sign of the response, some of the expected future changes in

363 marine nitrification are large. For example, whilst some studies have seen no impact on specific
364 marine nitrifiers (e.g. Qin et al., 2014), Beman et al. (2011) suggest that expected rates of
365 acidification could cause a decline in ammonia oxidation by up to 44% within the next few decades.
366 It is hence worth exploring how possible future changes in marine nitrification could impact ocean
367 iodine cycling.

368


369

370 **Figure 4.** Modelled changes in surface I^- concentration (nM) resulting from a) +10%, b) -10%,
371 changes in the rates of nitrification. Negative percent values indicate a decline in the rate of
372 nitrification and *vice-versa*. Negative values on the scale bar indicate a decrease in I^- concentrations
373 and *vice versa*.
374

375 In order to explore the possible impact of future changes in marine nitrification rates on sea surface
376 iodine fields we used the ocean cycling model described in Wadley et al. (2020). Within the model
377 iodide production is driven by primary productivity, and I^- oxidation to IO_3^- linked to nitrification in
378 the mixed layer. Nitrogen fluxes and the spatial distribution of mixed layer ammonia oxidation are
379 derived from a global biogeochemical cycling model (Yool *et al.*, 2007). I^- is oxidised to IO_3^- in
380 association with the ammonia oxidation, with the same I:N:C ratio as associated with iodide
381 production (Truesdale *et al.*, 2001; Long *et al.*, 2015). The model does not use any of the rates
382 derived in the current study as these are based on results from only 2 AOB species cultured at high
383 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 I_2 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 IO_3^- 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 could lead to a change in ozone deposition to the sea surface and sea-air iodine emissions with potentially major implications for atmospheric chemistry and air quality.

Acknowledgements

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

Author contributions

Claire Hughes: Conceptualisation, Methodology, Formal Analysis, Investigation, Writing – Original Draft, Writing – Review & Editing, Visualisation, Supervision, Project Administration, Funding Acquisition. Eleanor Barton: Formal Analysis, Investigation, Writing – Original Draft. Helmke Hepach: Methodology, Validation, Investigation, Resources. Rosie Chance: Conceptualisation, Methodology, Resources, Data Curation, Writing – Review & Editing, Project Administration, Funding Acquisition. Matt Pickering: Methodology, Validation, Resources. Karen Hogg: Methodology, Resources. Andreas Pommerening-Röser: Methodology, Resources. Martin R. Wadley: Conceptualisation, Methodology, Software, Validation, Formal Analysis, Investigation, Writing – Original Draft, Visualisation. David P. Stevens: Conceptualisation, Methodology, Resources, Supervision, Funding Acquisition. Tim D. Jickells: Conceptualisation, Methodology, Supervision, Funding Acquisition.

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

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

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

444 **References**

445 Amachi, S., 2008. Microbial Contribution to Global Iodine Cycling: Volatilization, Accumulation,
446 Reduction, Oxidation, and Sorption of Iodine. Microbes Environ., 23(4):
447 269.10.1264/jsme2.ME08548

448 Amachi, S., Muramatsu, Y., Akiyama, Y., Miyazaki, K., Yoshiki, S., Hanada, S., Kamagata, Y.,
449 Ban-nai, T., Shinoyama, H. and Fujii, T., 2005. Isolation of iodide-oxidizing bacteria from
450 iodide-rich natural gas brines and seawaters. Microb. Ecol., 49(4): 547

451 Beman, J.M., Chow, C.-E., King, A.L., Feng, Y., Fuhrman, J.A., Andersson, A., Bates, N.R., Popp,
452 B.N. and Hutchins, D.A., 2011. Global declines in oceanic nitrification rates as a
453 consequence of ocean acidification. Proceedings of the National Academy of Sciences,
454 108(1): 208.10.1073/pnas.1011053108

455 Beman, J.M., Popp, B. N. & Alford, S. E., 2012. Quantification of ammonia oxidation rates and
456 ammonia-oxidizing archaea and bacteria at high resolution in the Gulf of California and
457 eastern tropical North Pacific Ocean. Limnology and Oceanography, 57, 712-726

458 Bluhm, K., Croot, P., Wuttig, K. and Lochte, K., 2010. Transformation of iodate to iodide in marine
459 phytoplankton driven by cell senescence. Aquat. Biol., 11(1): 1.10.3354/ab00284

460 Carpenter, L.J., 2003. Iodine in the marine boundary layer. Chem. Rev., 103(12):
461 4953.10.1021/cr0206465

462 Carpenter, L.J., MacDonald, S.M., Shaw, M.D., Kumar, R., Saunders, R.W., Parthipan, R., Wilson,
463 J. and Plane, J.M.C., 2013. Atmospheric iodine levels influenced by sea surface emissions of
464 inorganic iodine. Nature Geoscience, 6(2): 108.10.1038/ngeo1687

465 Chance, R., Baker, A.R., Carpenter, L. and Jickells, T.D., 2014. The distribution of iodide at the sea
466 surface. Environmental Science-Processes & Impacts, 16(8): 1841.10.1039/c4em00139g

467 Chance, R., Malin, G., Jickells, T. and Baker, A.R., 2007. Reduction of iodate to iodide by cold
468 water diatom cultures. Mar. Chem., 105(1-2): 169.10.1016/j.marchem.2006.06.008

469 Chance, R., Weston, K., Baker, A.R., Hughes, C., Malin, G., Carpenter, L., Meredith, M.P., Clarke,
470 A., Jickells, T.D., Mann, P. and Rossetti, H., 2010. Seasonal and interannual variation of
471 dissolved iodine speciation at a coastal Antarctic site. Mar. Chem., 118(3-4):
472 171.10.1016/j.marchem.2009.11.009
473 Chapman, P. and Liss, P.S., 1977. Effect of Nitrite on Spectrophotometric Determination of Iodate in
474 Seawater. Mar. Chem., 5(3): 243
475 Dickson, A. G., Sabine, C. L. & Christian, J. R. (Eds.), 2007. Guide to Best Practices for Ocean CO_2
476 Measurements. PICES Special Publication 3. 191 pp.
477 Fuse, H., Inoue, H., Murakami, K., Takimura, O. and Yamaoka, Y., 2003. Production of free and
478 organic iodine by *Roseovarius* spp. FEMS Microbiol. Lett., 229(2): 189.10.1016/s0378-
479 1097(03)00839-5
480 Gozlan, R.S., 1968. Isolation of Iodine-Producing Bacteria from Aquaria. Antonie Van
481 Leeuwenhoek Journal of Microbiology, 34(2): 226
482 Hung, C.C., Wong, G.T.F. and Dunstan, W.M., 2005. Iodate reduction activity in nitrate reductase
483 extracts from marine phytoplankton. Bull. Mar. Sci., 76(1): 61
484 Iino, T., Ohkuma, M., Kamagata, Y. and Amachi, S., 2016. *Iodidimonas muriae* gen. nov., sp. nov.,
485 an aerobic iodide-oxidizing bacterium isolated from brine of a natural gas and iodine
486 recovery facility, and proposals of *Iodidimonadaceae* fam. nov., *Iodidimonadales* ord. nov.,
487 *Emcibacteraceae* fam. nov. and *Emcibacterales* ord. nov. Int. J. Syst. Evol. Microbiol.,
488 66(12): 5016.https://doi.org/10.1099/ijsem.0.001462
489 Jickells, T.D., Boyd, S.S. and Knap, A.H., 1988. Iodine cycling in the Sargasso Sea and the Bermuda
490 Inshore waters. Mar. Chem., 24(1): 61.10.1016/0304-4203(88)90006-0
491 Koops, H.-P. and Pommerening-Röser, A., 2001. Distribution and ecophysiology of the nitrifying
492 bacteria emphasizing cultured species. FEMS Microbiol. Ecol., 37(1): 1.10.1111/j.1574-
493 6941.2001.tb00847.x

494 Lam, P., Jensen, M.M., Lavik, G., McGinnis, D.F., Müller, B., Schubert, C.J., Amann, R.,
495 Thamdrup, B. and Kuypers, M.M.M., 2007. Linking crenarchaeal and bacterial nitrification
496 to anammox in the Black Sea. Proceedings of the National Academy of Sciences, 104(17):
497 7104.10.1073/pnas.0611081104
498 Li, H.P., Daniel, B., Creeley, D., Grandbois, R., Zhang, S.J., Xu, C., Ho, Y.F., Schwehr, K.A.,
499 Kaplan, D.I., Santschi, P.H., Hansel, C.M. and Yeager, C.M., 2014. Superoxide Production
500 by a Manganese-Oxidizing Bacterium Facilitates Iodide Oxidation. Appl. Environ.
501 Microbiol., 80(9): 2693.10.1128/aem.00400-14
502 Long, A., Dang, A., Xiao, H. and Yu, X., 2015. The Summer Distribution of Dissolved Inorganic
503 Iodine along 18°N in the South China Sea. Journal of Marine Science: Research &
504 Development, 5: 169.10.4172/2155-9910.1000169
505 Newell, S.E., Babbin, A.R., Jayakumar, A. and Ward, B.B., 2011. Ammonia oxidation rates and
506 nitrification in the Arabian Sea. Global Biogeochemical Cycles,
507 25(4).10.1029/2010GB003940
508 Newell, S.E., Fawcett, S.E. and Ward, B.B., 2013. Depth distribution of ammonia oxidation rates and
509 ammonia-oxidizer community composition in the Sargasso Sea. Limnology and
510 Oceanography, 58(4): 1491.10.4319/lo.2013.58.4.1491
511 Norwitz, G. and Keliher, P.N., 1984. Spectrophotometric determination of nitrite with composite
512 reagents containing sulphanilamide, sulphanilic acid or 4-nitroaniline as the diazotisable
513 aromatic amine and N-(1-naphthyl)ethylenediamine as the coupling agent. Analyst, 109(10):
514 1281.10.1039/AN9840901281
515 O'Dowd, C.D., Jimenez, J.L., Bahreini, R., Flagan, R.C., Seinfeld, J.H., Hameri, K., Pirjola, L.,
516 Kulmala, M., Jennings, S.G. and Hoffmann, T., 2002. Marine aerosol formation from
517 biogenic iodine emissions. Nature, 417(6889): 632

518 Pajores, S. and R. Ramos (2019) Processes and microorganisms involved in the marine nitrogen
519 cycle: knowledge and gaps, Frontiers in Marine Science, 6, 10.3389/fmars.2019.00739
520 Peng, X., Fuchsman, C.A., Jayakumar, A., Oleynik, S., Martens-Habbena, W., Devol, A.H. and
521 Ward, B.B., 2015. Ammonia and nitrite oxidation in the Eastern Tropical North Pacific.
522 Global Biogeochemical Cycles, 29(12): 2034.10.1002/2015GB005278
523 Qin, W., Amin, S.A., Martens-Habbena, W., Walker, C.B., Urakawa, H., Devol, A.H., Ingalls, A.E.,
524 Moffett, J.W., Armbrust, E.V. and Stahl, D.A., 2014. Marine ammonia-oxidizing archaeal
525 isolates display obligate mixotrophy and wide ecotypic variation. Proc. Natl. Acad. Sci. U. S.
526 A., 111(34): 12504.10.1073/pnas.1324115111
527 Schleper, C. and Nicol, G.W., 2010. Ammonia-oxidising archaea--physiology, ecology and
528 evolution. Adv. Microb. Physiol., 57: 1.10.1016/b978-0-12-381045-8.00001-1
529 Schulz, K.G., Barcelos e Ramos, J., Zeebe, R.E. and Riebesell, U., 2009. CO_2 perturbation
530 experiments: similarities and differences between dissolved inorganic carbon and total
531 alkalinity manipulations. Biogeosciences, 6(10): 2145.10.5194/bg-6-2145-2009
532 Sherwen, T., Evans, M.J., Carpenter, L.J., Andrews, S.J., Lidster, R.T., Dix, B., Koenig, T.K.,
533 Sinreich, R., Ortega, I., Volkamer, R., Saiz-Lopez, A., Prados-Roman, C., Mahajan, A.S. and
534 Ordonez, C., 2016. Iodine's impact on tropospheric oxidants: a global model study in GEOS-
535 Chem. Atmospheric Chemistry and Physics, 16(2): 1161.10.5194/acp-16-1161-2016
536 Smith, J.M., Damashek, J., Chavez, F.P. and Francis, C.A., 2016. Factors influencing nitrification
537 rates and the abundance and transcriptional activity of ammonia-oxidizing microorganisms in
538 the dark northeast Pacific Ocean. Limnology and Oceanography, 61(2):
539 596.10.1002/lno.10235
540 Thomas, J.A. and Hager, L.P., 1968. The peroxidation of molecular iodine to iodate by
541 chloroperoxidase. Biochemica Biophysica Research Communications, 32: 770

542 Truesdale, V.W. and Luther, G.W., 1995. Molecular iodine reduction by natural and model organic
543 substances in seawater. Aquatic Geochemistry, 1(1): 89
544 Truesdale, V.W. and Moore, R.M., 1992. Further-Studies on the Chemical-Reduction of Molecular-
545 Iodine Added to Seawater. Mar. Chem., 40(3-4): 199
546 Truesdale, V.W. and Spencer, C.P., 1974. Studies on the determination of inorganic iodine in
547 seawater. Mar. Chem., 2(1): 33.https://doi.org/10.1016/0304-4203(74)90004-8
548 Truesdale, V.W., Watts, S.F. and Rendell, A.R., 2001. On the possibility of iodide oxidation in the
549 near-surface of the Black Sea and its implications to iodine in the general ocean. Deep-Sea
550 Research Part I-Oceanographic Research Papers, 48(11): 2397.10.1016/s0967-
551 0637(01)00021-8
552 Wadley, M.R., Stevens, D.P., Jickells, T.D., Hughes, C., Chance, R., Hepach, H., Tinel, L. and
553 Carpenter, L.J., 2020. A Global Model for Iodine Speciation in the Upper Ocean. Global
554 Biogeochemical Cycles, e2019GB006467.10.1029/2019GB006467
555 Ward, B.B., 1987. Nitrogen transformations in the Southern California Bight. Deep Sea Research
556 Part A. Oceanographic Research Papers, 34(5): 785.https://doi.org/10.1016/0198-
557 0149(87)90037-9
558 Ward, B.B., Glover, H.E. and Lipschultz, F., 1989. Chemoautotrophic activity and nitrification in the
559 oxygen minimum zone off Peru. Deep Sea Research Part A. Oceanographic Research Papers,
560 36(7): 1031.https://doi.org/10.1016/0198-0149(89)90076-9
561 Wong, G.T.F., 1991. The marine geochemistry of iodine. Reviews in Aquatic Sciences, 4(1): 45
562 Yool, A., Martin, A.P., Fernandez, C. and Clark, D.R., 2007. The significance of nitrification for
563 oceanic new production. Nature, 447(7147): 999.10.1038/nature05885
564 Zic, V., Caric, M. and Ciglenecki, I., 2013. The impact of natural water column mixing on iodine and
565 nutrient speciation in a eutrophic anchialine pond (Rogoznica Lake, Croatia). Estuarine
566 Coastal and Shelf Science, 133: 260.10.1016/j.ecss.2013.09.008