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FEATURE ARTICLE

Transformation of iodate to iodide in marine phytoplankton driven by cell senescence

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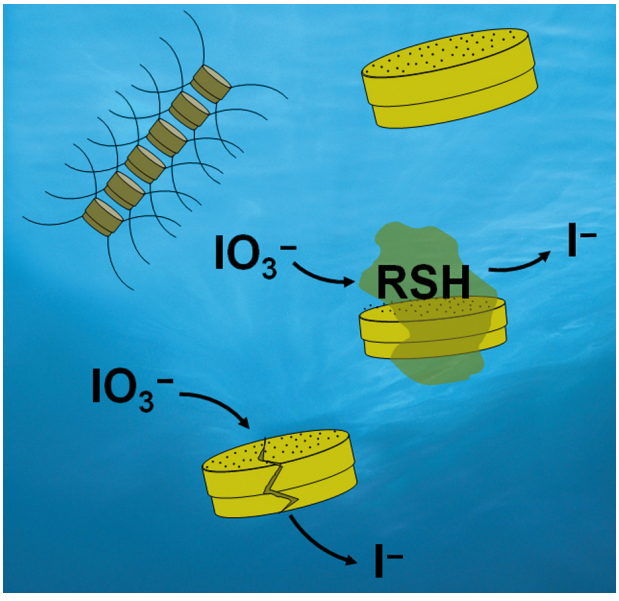
ABSTRACT: Previous studies have suggested that phytoplankton play an important role in the biogeochemical cycling of iodine, due to the appearance of iodide in the euphotic zone. Changes in the speciation of iodine over the course of the growth cycle were examined in culture media for a variety of phytoplankton taxa (diatoms, dinoflagellates and prymnesiophytes). All species tested showed the apparent ability to reduce iodate to iodide, though production rates varied considerably among species (0.01 to 0.26 nmol l^{-1} μg^{-1} chl *a* d^{-1}), with *Eucampia antarctica* the least and *Pseudo-nitzschia turgiduloides* the most efficient iodide producers. Production was found to be species specific and was not related to biomass (indicated by e.g. cell size, cell volume, or chl *a* content). In all species, except for the mixotrophic dinoflagellate *Scrippsiella trochoidea*, iodide production commenced in the stationary growth phase and peaked in the senescent phase of the algae, indicating that iodide production is connected to cell senescence. This suggests that iodate reduction results from increased cell permeability, which we hypothesize is due to subsequent reactions of iodate with reduced sulphur species exuded from the cell. A shift from senescence back to the exponential growth phase resulted in a decline in iodide and indicated that phytoplankton-mediated oxidation of iodide to iodate was likely to be occurring. Iodide production could not be observed in healthy cells kept in the dark for short periods. Bacterial processes appeared to play only a minor role in the reduction of iodate to iodide.

KEY WORDS: Iodine speciation · Iodide · Iodate · Antarctic diatoms · Nitrate reductase · Glutathione · Sulphur species · Cell senescence



Intracellular material (RSH-thiols) leaking from phytoplankton cells converts iodate to iodide. RSH: organic sulphur species

Diagram: Peter Croot & Linn Hoffmann

INTRODUCTION

Iodine has recently been identified as a potential key element involved in climate change, as iodide reacts rapidly with O_3, and is believed to be a major sink for atmospheric O_3 at the sea surface (Garland et al. 1980). This reaction forms HOI and I_2, and has also been suggested as a source of organic halogens (RI) (Martino et al. 2009). RI released from the sea to the atmosphere will undergo photolysis and oxidation in the atmosphere to form IO (Saiz-Lopez et al. 2007, Schonhardt

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et al. 2008), which is the major source of new particles in the atmosphere (von Glasow 2005), with the potential to influence cloud properties and hence the climate (O'Dowd & de Leeuw 2007). In coastal regions, I_2 can also directly be released into the atmosphere by kelp and seaweed and reacts here to equally form IO. Thus, information on the cycling of iodine species in the ocean is important for assessing the impact of iodine in tropospheric ozone chemistry and for climate dynamics. While biology clearly plays a role in the marine iodine cycle, it is still not clear what the link between biological processes and the speciation of iodine in seawater is.

Iodine exists principally in open seawater as the inorganic redox forms iodate (IO_3^-) and iodide (I^-), with a total concentration of 400 to 500 nmol l^{-1} in most oceanic regions. While iodate predominates in the deep ocean (Tsunogai & Sase 1969, Elderfield & Truesdale 1980, Farrenkopf et al. 1997), significant amounts of iodide are found in surface and near-bottom layers (Kennedy & Elderfield 1987, Wong 1991, Luther et al. 1995). The interconversion of the redox couple iodate–iodide within the euphotic zone, together with the biophilic nature of iodine, has given rise to the idea that iodine speciation is linked to primary productivity (Sugawara & Terada 1967, Tsunogai & Henmi 1971, Elderfield & Truesdale 1980, Moisan et al. 1994, Campos et al. 1996, Truesdale et al. 2000). The evidence for a link between iodate reduction and primary productivity came from field observations that suggested surface iodate decreases towards the productive equatorial regions (Tsunogai & Henmi 1971, Jickells et al. 1988, Campos et al. 1996, Truesdale et al. 2000). Additional evidence was found in distinct correlations between iodate and macronutrient concentrations at several stations (Elderfield & Truesdale 1980, Truesdale 1994, Campos et al. 1999). However, a clear link between primary productivity and the reduction of iodate has yet to be shown, and other explanations for the global distribution of iodate have been put forward, e.g. vertical mixing (Truesdale et al. 2000) and regional differences in phytoplankton community (Campos et al. 1999). Although abiotic mechanisms have been proposed for the reduction of iodate and the consequent formation of iodide (Spokes & Liss 1996), the presence of iodide in surface waters is typically ascribed to biological activity (Campos et al. 1996).

Laboratory experiments on the influence of phytoplankton on iodine speciation have led to varying results. In the first such work of this type, Sugawara & Terada (1967) examined iodide and iodate assimilation by the marine diatom *Navicula* sp. using radiotracers. In their work this diatom preferentially assimilated iodide over iodate and appeared to accomplish the conversion of iodide to iodate and vice versa. A recent radiotracer study into the accumulation rates of iodide and iodate by a number of phytoplankton batch culture experiments confirmed this preference for iodide over iodate in the uptake by phytoplankton, but also showed large differences between species (de la Cuesta & Manley 2009). Other laboratory experiments with phytoplankton cultures have shown significant iodate uptake rates (Moisan et al. 1994). The conversion of iodate to iodide has been shown in batch culture at ambient iodate concentrations (Wong et al. 2002, Chance et al. 2007). Much of this work has been carried out to test the hypothesis put forward by Tsunogai & Sase (1969) that nitrate reductase can reduce iodate to iodide in the ocean when nitrate is limiting. This hypothesis grew out of the earlier finding by Egami & Sato (1947) that nitrate reductase is capable of reducing iodate under physiological conditions. Contrastingly, a number of studies found no relationship between iodine and biological activity or only observed iodide increases in phytoplankton cultures at iodate concentrations 10-fold greater than those naturally found (Truesdale 1978b, Butler et al. 1981, Waite & Truesdale 2003). A detailed study (Waite & Truesdale 2003) into the nitrate reductase hypothesis in which cells were grown on ammonia and the enzyme was deactivated by replacing molybdenum with tungsten in the growth media showed that iodate reduction was relatively insensitive to the function of nitrate reductase.

Experiments examining changes in iodine speciation have been predominantly performed with temperate, tropical and, to a lesser extent, cold water species, with Antarctic species having been totally neglected. Surface iodide concentrations are low in the Southern Ocean, although with some surface maxima, which are possibly due to biological activity (Campos et al. 1999, Truesdale et al. 2000, K. Bluhm et al. unpubl.). During an Antarctic mesocosm experiment carried out by Truesdale et al. (2003), no changes in iodine speciation were observed. The authors suggested that the large, chain-forming diatom *Thalassiosira antarctica*, which dominated the mesocosm blooms, is unable to perform the reduction of iodate to iodide. As phytoplankton blooms in polar regions typically exist over more than 30 d (Boyd 2004), the 25 d duration of their mesocosm experiment apparently did not catch the end of the bloom. The importance of this lies in the observation from an earlier field study conducted over a seasonal cycle in the Mediterranean by Tian et al. (1996), which indicated that iodate reduction was related to regenerated production and not primary production directly. Thus, the mesocosm work of Truesdale et al. (2003) may not have run long enough to observe the critical phase of senescence. As most of all the earlier culture studies were focused on examining the purported link between iodate reduction and nitrate re-

ductase, this suggested to us that the differences and contradictions between these earlier experiments may have been related to the duration of the experiments and to the phase of culture growth of the cells during the experiments.

In the present study, we tested whether the different growth phases exhibited different iodate reduction rates. We examined the iodate reduction over long-term culture experiments in which the cells pass through the 5 characteristic phases of growth in cultures (Fogg & Thake 1987): (1) lag phase, (2) exponential phase, (3) phase of declining relative growth rate, (4) stationary phase, and (5) senescent or declining phase. This work was performed in the laboratory under nitrate replete conditions with 6 different species of phytoplankton (4 Antarctic diatoms, 1 coccolithophore and 1 dinoflagellate), representing coastal and oceanic species from cold to temperate waters.

MATERIALS AND METHODS

Phytoplankton cultures. Three Antarctic diatom strains, *Fragilariopsis kerguelensis, Chaetoceros debilis* and *Pseudo-nitzschia turgiduloides*, were isolated from the Southern Ocean during the iron fertilization experiment EIFEX in February/March 2004 by P. Assmy (AWI-Bremerhaven). A fourth Antarctic diatom *Eucampia antarctica* (CCMP 1452) and the tropical strain of the coccolithophore *Emiliania huxleyi* (CCMP 371) were obtained from the Provasoli-Guillard Centre for the Culture of Marine Phytoplankton, Bigelow Laboratory, USA. The dinoflagellate *Scrippsiella trochoidea* was isolated from the southern North Sea in 2001 by U. Tillman (AWI-Bremerhaven). All species were non-axenic, and their characteristics are listed in Table 1. *E. huxleyi*, although isolated from the tropical ocean, was cultured under temperate conditions and is referred to here as a temperate species.

Experimental setup. Several experiments were carried out to determine the iodide production mechanism and are listed in Table 2. In general, all species examined were grown in seawater collected from their natural habitat, with nutrients at *f*/2 concentrations, according to the method of Guillard and Ryther (Guillard & Ryther 1962, Guillard 1975) The final phosphate and silicate concentrations in the medium were 36 and 106 µmol l^{-1}, respectively. Additionally, all diatom species were supplied double the usual silicate concentrations (212 µmol l^{-1}) to ensure that silicate is not the limiting nutrient in the culture. Initially, 5 µmol l^{-1} of iodate were added to the culture medium.

Table 1. Species characteristics of the 2 temperate strains and 4 cold water diatoms used in the experiments and their natural distribution. Average values are quoted; n > 30. Coccol.: coccolithophorid; dino.: dinoflagellate; cosmopol.: cosmopolitan

Phytoplankton species	Algal group	Size (µm^2)	Volume (µm^3)	Carbon (pg cell^{-1})	Area volume^{-1}	Distribution
Temperate species						
Emiliania huxleyi	Coccol.	47	31	5	1.49	Cosmopol. oceanic, not polar regions
Scrippsiella trochoidea	Dino.	1335	4045	581	0.33	Cosmopol. neritic/estuarine, not polar regions
Cold water species						
Chaetoceros debilis	Diatom	90	63	8	1.42	Cosmopol., mainly cooler waters
Pseudo-nitzschia turgiduloides	Diatom	1168	1388	102	0.84	Southern cold water
Fragilariopsis kerguelensis	Diatom	1697	4677	200	0.36	Southern cold water
Eucampia antarctica	Diatom	2711	6741	276	0.40	Southern cold water

Table 2. Overview of experiments. L:D: light:dark cycle; PFD: photon flux density

Experiments	Species	Temp. (°C)	L:D (h)	PFD (µmol quanta m^{-2} s^{-1})	Iodate added (µM)	Nitrate added (µM)	Nitrate depletion
Experiment 1							
(a) Iodide production	Temperate	18	12:12	50/100	5	88	No
	Cold water	4	16:8	50/100	5	88	No
(b) Iodide production	*Chaetoceros debilis*	4	16:8	50	1	44	Yes
Experiment 2							
Metabolites and bacteria	*Pseudo-nitzschia turgiduloides*	4	16:8	50	5	88	No
Experiment 3							
Dark production	*Pseudo-nitzschia turgiduloides*	4	0:24	–	5	88	No

All cultures used were monoclonal, and culture handling was done under a laminar flow hood to prevent any outside contamination. The sample bottles and lids were sterilized via autoclaving, and sterile-filtered (Sartobran 300 capsules with a filter combination of 0.45 and 0.2 µm) growth media was used. In Expt 1a the nitrate concentration was lowered to *f*/20, with a final concentration of ~88 µmol l^{-1}. Duplicates of each culture and phytoplankton species were run in parallel. One was exposed to a photosynthetically active photon flux density (PFD) of 50 µmol quanta m^{-2} s^{-1} and the other to 100 µmol quanta m^{-2} s^{-1}. Aliquots were sampled regularly for a total of 55 d or until cells reached mortality. Growth conditions for each experiment are listed in Table 2.

Expt 1b was carried out with a *Chaetoceros debilis* culture at lower initial iodate and nitrate concentrations of approximately 1 and 44 µmol l^{-1}, respectively. After cells used up all nitrate and the photosynthetic efficiency (F_v/F_m; description see below) reached a value below 0.3, a second nitrate addition with a concentration of *f*/20 (~88 µmol l^{-1}) was performed on Day 24. Sampling frequency was the same as in Expt 1a. Cell-free controls (no phytoplankton cells added) using the same filtered seawater media were run in parallel to each experiment (1a and 1b) under the same lighting and temperature conditions.

An extra control was obtained for Expt 2 by filtering a senescent *Pseudo-nitzschia turgiduloides* culture (this species was selected based on its performance in the first set of experiments)—initially grown in 88 µmol l^{-1} nitrate and 5 µmol l^{-1} iodate enriched seawater—over a 5 µm mesh to remove phytoplankton cells but to retain all dissolved organic matter and bacteria in the water. This was done to see whether metabolites or bacteria have an effect on the conversion of iodate to iodide or if it is exclusively done by the phytoplankton. The filtrate was not re-supplied with nutrients or iodate, as sufficient concentrations still prevailed and sampling was done over a period of 30 d.

Expt 3 was conducted to ascertain whether the conversion of iodate to iodide is light dependent and connected to photosynthesis. Two sets of cultures of *Pseudo-nitzschia turgiduloides* and *Chaetoceros debilis* were placed in the dark and sampled over a period of 31 d.

Measured parameters and analytical methods. Nutrient samples were filtered over cellulose acetate filters (pore size 2 µm), and the filtrate was stored frozen (–20°C) until analysis. Measurements were performed using standard methods for macronutrient analysis after Grasshoff et al. (1999). Samples for chlorophyll *a* (chl *a*) measurements were filtered on glass fibre filters (GF/F-Whatman) and immediately stored at –20°C. The frozen filters were placed in polypropylene vials together with 11 ml of 90% acetone and glass beads (2 and 4 mm). Thereafter, the closed vials were placed in a cell mill for at least 5 min until the filters were completely homogenized. The vials were then centrifuged at –5°C (10 min at 4160 × *g*, and the supernatant was measured fluorometrically with a Turner fluorometer according to the method of Welschmeyer (1994).

The photosynthetic efficiency (F_v/F_m) (Suggett et al. 2003, Rottgers 2007) of the cells was assessed with a Phyto-PAM phytoplankton analyzer (WALZ). In the present work samples of phytoplankton culture were dark adapted for 30 min before measurement. Optimal values of F_v/F_m lie around 0.4 to 0.6 for phytoplankton cultures, lower values indicate cells under stress from nutrient or iron limitation (Maxwell & Johnson 2000).

For cell enumeration all cultures except *Emiliania huxleyi* were preserved with LUGOL's solution at a final concentration of 4%. *E. huxleyi* was preserved with 0.2 µm prefiltered formaldehyde at a final concentration of 1%. All samples were stored at 4°C in the dark for subsequent counting. Cells were enumerated using inverted light microscopy (Axiovert 135, Zeiss) according to Utermöhl (1958). Only viable cells that were still auto-fluorescing were counted. The cell size of the different species and groups was determined, and their biovolume was calculated from equivalent geometrical shapes (Hillebrand et al. 1999). The cell volume was then converted to cellular carbon content through carbon conversion equations using a carbon to volume relationship recommended by Menden-Deuer & Lessard (2000).

Bacterial abundances were determined by flow cytometry according to Gasol & Del Giorgio (2000). Samples were fixed with 0.2 µm prefiltered formaldehyde (2% final concentration) in 5 ml cryovials, deep-frozen in liquid nitrogen after a 30 min dark incubation, and stored at –80°C. Before analysis, the thawed samples were stained with SYBR Green 1 (Molecular Probes, final concentration 5 µM, diluted in dimethyl sulfoxide [DMSO]) for 15 min in the dark. Samples were run through a FACScalibur flow cytometer (Becton & Dickinson). Bacterial biomass was calculated from abundance data using a conversion factor of 20 fg C $cell^{-1}$ (Lee & Fuhrman 1987).

Iodine speciation. Samples were filtered over cellulose acetate filters (pore size 2 µm) and, if not measured immediately, stored frozen (–20°C) until analysis. Iodide was determined by cathodic stripping square wave voltammetry according to the method of Luther et al. (1988), modified by Campos (1997), with a detection limit of from 0.1 to 0.2 nmol l^{-1} and a precision of better than 5%. Iodate was determined spectrophotometrically by its conversion to the I_3^- ion with

sulphamic acid, to remove interference by nitrite and potassium iodide, after the method of Truesdale (1978a). Samples were measured in a 5 cm cuvette with a 'Unicam' spectrophotometer (UV 300, Thermo-Forma) at a wavelength of 350 nm. The detection limit of the method is ~20 nmol l^{-1}. Samples were measured in triplicate for both iodide and iodate, and standard deviations were gained from the triplicates measured.

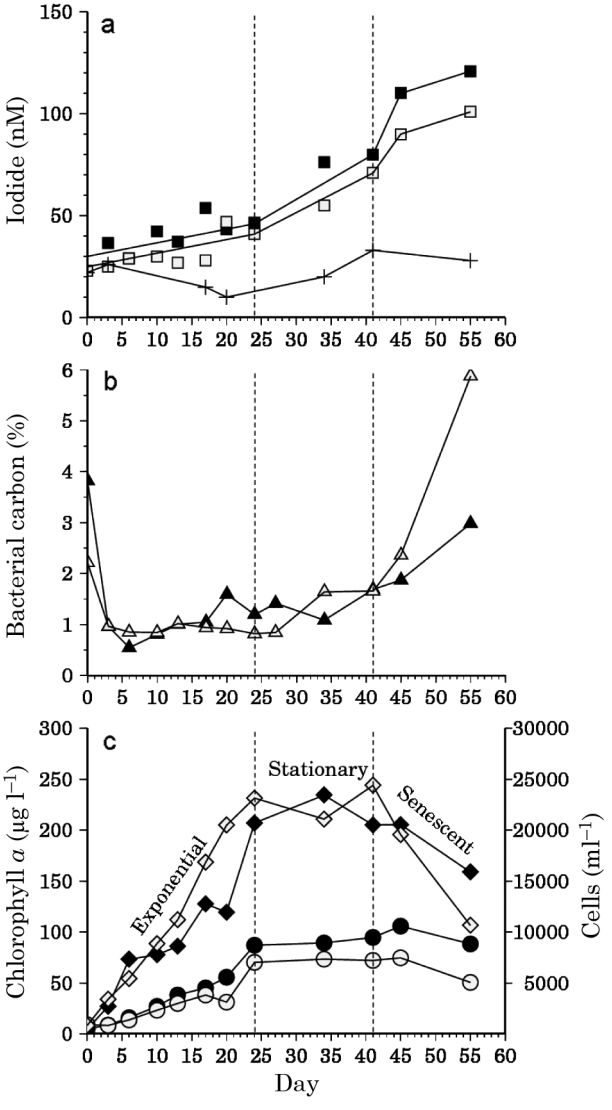

Fig. 1. *Fragilariopsis kerguelensis*. (a) Iodide production, (b) bacterial carbon and (c) chl *a* and cell numbers. Bacterial carbon is given as percentage of the total carbon present in the culture flask. Dashed lines: position of the different growth phases; solid symbols: samples grown at 50 µmol quanta m^{-s} s^{-1}; open symbols: samples grown at 100 µmol quanta m^{-s} s^{-1}; squares: iodide production; crosses: control without algal cells; triangles: bacterial carbon; diamonds: cell numbers; circles: chlorophyll *a*. Note that the scales for the *y*-axes change for each species. Error bars are smaller than the size of the symbols

RESULTS

Chl *a*, growth and bacterial carbon

The changes in iodide production, bacterial carbon, chl *a* concentration and cell numbers for all species tested are shown in Figs. 1 to 4. As expected for all species, less chl *a* per cell was observed in samples grown under the higher light intensity of 100 µmol quanta m^{-2} s^{-1}. The growth phases were identified by visual inspection of the cell count data. The exact time when cells entered a particular growth phase varied between species. The exponential phase, as indicated by a rapid increase in both chl *a* and cell numbers until a maximum value is reached, lay within the first 8 to 18 d for most of the species except *Eucampia antarctica* and *Fragilariopsis kerguelensis*. Both of these species are slow growing due to heavy silicification and a great

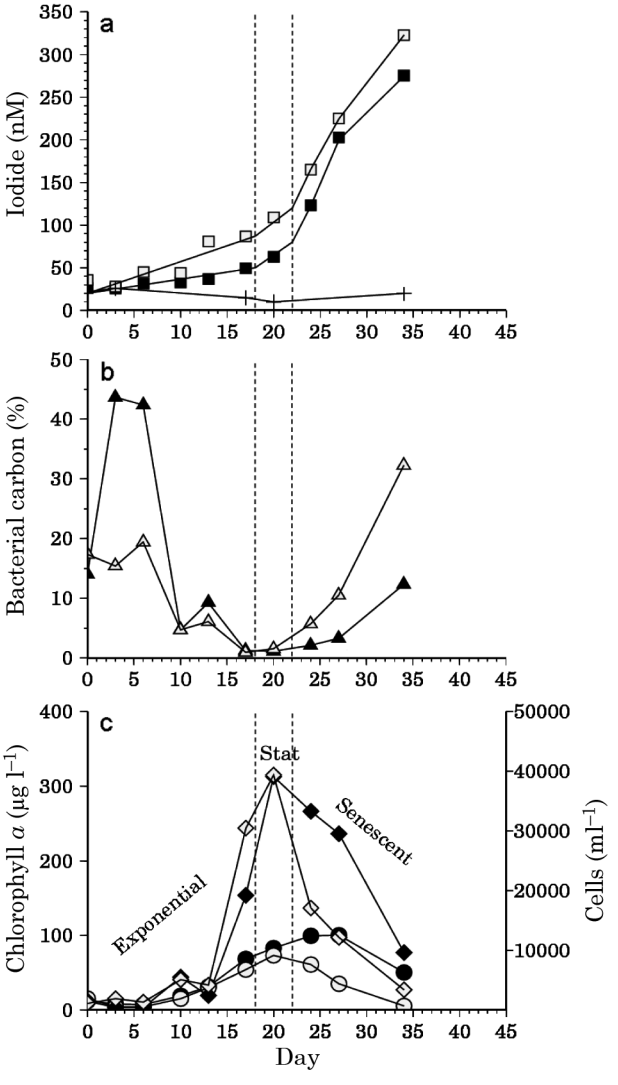

Fig. 2. *Pseudo-nitzschia turgiduloides*. See Fig.1 for description

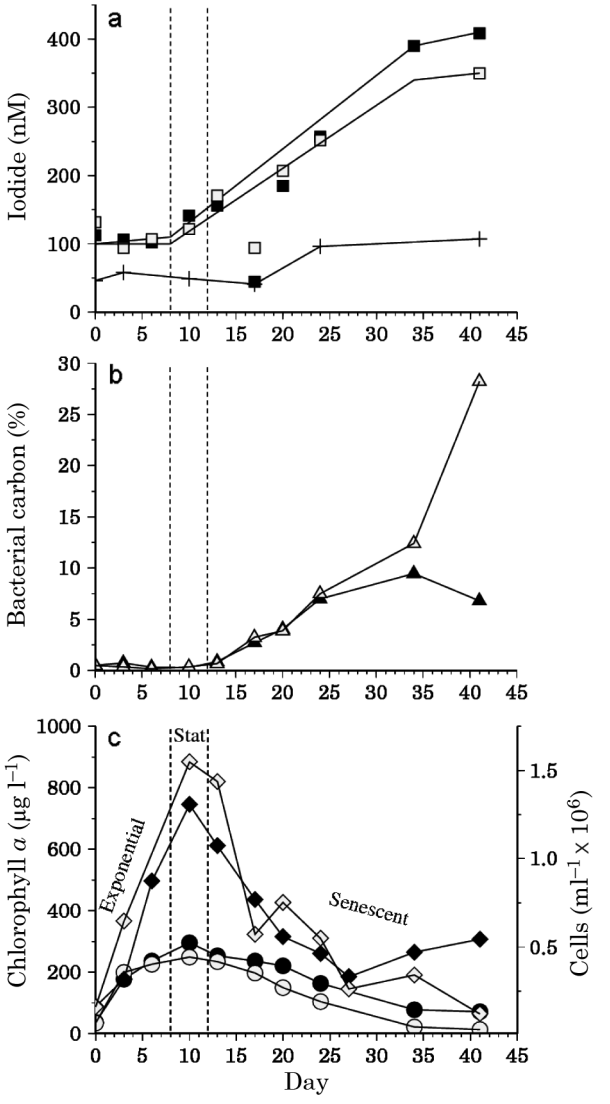

Fig. 3. *Emiliania huxleyi*. See Fig.1 for description

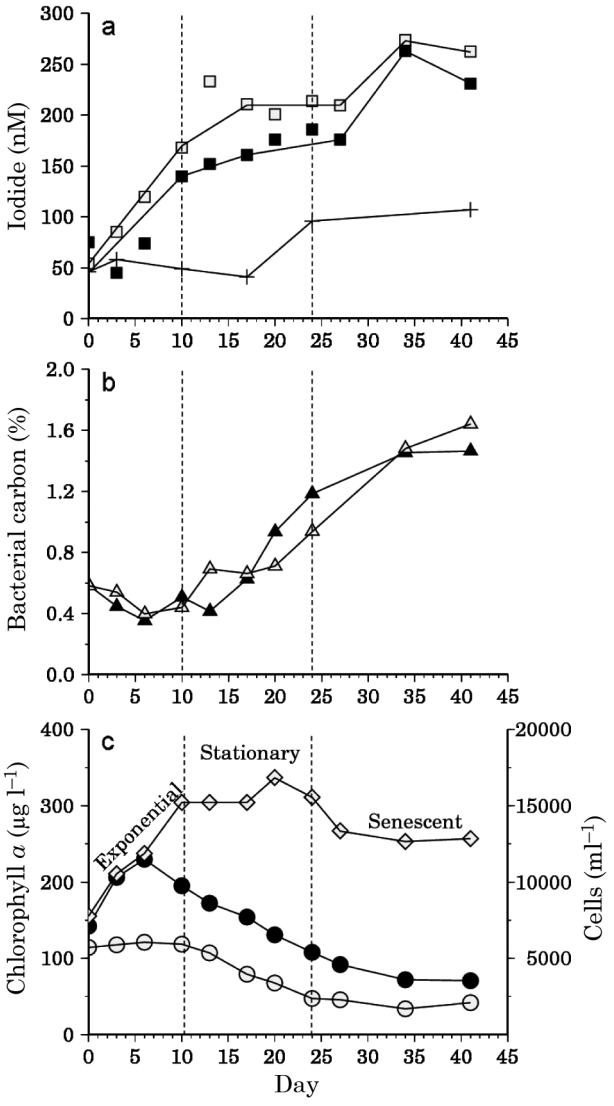

Fig. 4. *Scrippsiella trochoidea*. See Fig.1 for description

cell size (Table 1), and their exponential phase continued until Day 24 (Fig. 1c, only shown for *Fragilariopsis kerguelensis*).

In most species, except *Emiliania huxleyi* and *Pseudo-nitzschia turgiduloides*, the stationary growth phase was between 4 and 17 d long (Figs. 1c & 4c), whereas in *E. huxleyi* and *P. turgiduloides* a distinct stationary phase could not be observed (Figs. 2c & 3c). Their cell numbers increased exponentially and went straight into senescence after reaching a maximum. This might be due to the timing of sampling, so that we missed the stationary phase, or just their fast-growing behaviour, so that the culture never really entered a stationary phase and went straight from exponential growth to senescence (Figs. 2c & 3c). Throughout the experiment most of the cultures did not run into nutrient limitation. Nitrate, silicate and phosphate concentrations were still high when cells reached a senescent growth phase, with >30 µmol l^{-1} for nitrate, >60 µmol l^{-1} for silicate and >23 µmol l^{-1} for phosphate. The only 2 exceptions were *Scrippsiella trochoidea*, which used up all the nitrate present within 10 d, and *Fragilariopsis kerguelensis*, which used up all the available silicate and phosphate before cells showed a rapid decline in cell numbers (data not shown).

Elevated iodate levels have sometimes been found to affect phytoplankton growth (Sugawara & Terada 1967, Zheng et al. 2005) and sometimes without effect (Waite & Truesdale 2003, Chance et al. 2007). The behaviour and growth of the phytoplankton was not affected by the added iodate. Measured chl *a* and cell densities appeared similar to untreated samples, and species showed normal growth rates of µ = 0.11 to 0.31 cell doublings d^{-1} (Timmermans et al. 2004) through-

out the experiments, suggesting that the added iodate neither enhanced nor inhibited cell growth.

Bacterial densities are expressed in bacterial carbon (µg C l^{-1}) and are considered in relation to the phytoplankton biomass also expressed in micrograms of carbon per litre. Bacterial numbers in the batch cultures varied between phytoplankton species, but usually were the minor component. Numbers peaked at the end of the experiment and within the senescent growth phase of the phytoplankton (Figs. 1b to 4b).

Production of iodide

Iodide production was only observed in the presence of the algae, with no production in the cell-free controls (Figs. 1a to 4a). In Expt 1, the production of iodide was observed in all species examined. The average iodide production rates per day (k) were estimated as the slope of a linear regression analysis, over the entire duration of the experiment, and the results are listed in Table 3. The correlation coefficients R^2 were always between 0.77 and 0.96. The amount of total iodide produced over the length of the experiment varied within species from 78 to 302 nmol l^{-1}, and k ranged from 1.25 to a maximum of 7.97 nmol l^{-1} d^{-1} (Table 3). *Emiliania huxleyi* and *Pseudo-nitzschia turgiduloides* had the highest k with the greatest amount of total iodide produced relative to the other species.

The rate k was additionally normalised to the so-called 'time-averaged chl *a*'; this was done with the trapezoidal method. This method is used to approximate the area under a curve (chl *a* vs. time curve in this case) by circumscribing n number of trapezoids under this curve. The area of the trapezoids is then summed. This method is used to gain average chl *a* values for the whole length of the experiment for each species. The same method was used to normalise k to cell densities and is named 'time-averaged cell density' here. According to this method *Pseudo-nitzschia turgiduloides* was by far the most efficient producer on a chl *a* basis, with a maximum production rate of 0.26 nmol l^{-1} $µg^{-1}$ chl *a* d^{-1}. All other species revealed lower rates between 0.01 and 0.06 nmol l^{-1} $µg^{-1}$ chl *a* d^{-1} (Table 3). Production of iodide could be observed in cultures with higher iodate concentrations than naturally found in seawater (5 µmol l^{-1}), but also at concentrations close to natural (1 µmol l^{-1}; Fig. 5). In Expt 1b iodide production was observed in the *Chaetoceros* species tested. The k rates were comparable to those in Expt 1a, where we added 5 times more iodate.

Table 3. Characteristics of the iodide production rates in cultures of marine phytoplankton. The iodide production rate in Expt 1b was only calculated over the time period when nitrate was depleted. Iodate: Initial iodate concentrations added. Iodide: total iodide produced over the course of the experiment. Avg. chl *a*: time averaged chl *a* gives an average chl *a* value for each species. Iodide production rates: rates represent the slope of a linear regression analysis of iodide concentration versus time. k/chl *a*: is the rate k normalised to time-averaged chl *a*. Error estimates are at the 95% confidence interval. R^2: correlation coefficient

Phytoplankton species	Iodate (µM)	Iodide (nM)	Chl *a*/cell (pg $cell^{-1}$)	Avg. chl *a* (µg l^{-1})	Iodide production rates: Rate (k) (nM d^{-1})	k/chl *a* (pM $µg^{-1}$ d^{-1})	k/cell × 10^{-7} (aM $cell^{-1}$ d^{-1})	R^2
Expt 1a								
Temperate species								
Emiliania huxleyi								
50 µmol m^{-2} s^{-1}	5	302	0.29	170	6.8 ± 1.6	40 ± 9	11 ± 2	0.94
100 µmol m^{-2} s^{-1}	5	256	0.19	129	7.2 ± 3.6	55 ± 27	9 ± 5	0.77
Scrippsiella trochoidea								
50 µmol m^{-2} s^{-1}	5	186	9.6	122	3.4 ± 1.2	28 ± 9	265 ± 89	0.89
100 µmol m^{-2} s^{-1}	5	208	6.55	67	3.4 ± 1.7	50 ± 25	296 ± 149	0.78
Cold water species								
Chaetoceros debilis								
50 µmol m^{-2} s^{-1}	5	166	0.47	111	4.2 ± 0.8	38 ± 7	16 ± 3	0.95
100 µmol m^{-2} s^{-1}	5	142	0.29	61	3.8 ± 1.1	63 ± 18	14 ± 4	0.87
Pseudo-nitzschia turgiduloides								
50 µmol m^{-2} s^{-1}	5	249	4.15	53	7.7 ± 2.9	146 ± 54	493 ± 182	0.81
100 µmol m^{-2} s^{-1}	5	295	3.03	31	8.0. ± 2.2	255 ± 71	643 ± 179	0.87
Fragilariopsis kerguelensis								
50 µmol m^{-2} s^{-1}	5	78	4.08	68	1.3 ± 0.3	18 ± 4	80 ± 17	0.92
100 µmol m^{-2} s^{-1}	5	98	291	51	1.5 ± 0.3	30 ± 6	93 ± 19	0.92
Eucampia antarctica								
50 µmol m^{-2} s^{-1}	5	122	43.29	160	2.0 ± 0.8	12 ± 5	500 ± 207	0.80
100 µmol m^{-2} s^{-1}	5	152	37.07	111	2.8 ± 0.4	25 ± 4	853 ± 124	0.96
Expt 1b								
Chaetoceros debilis	1	101 ± 23	–	63 ± 1.2	3.5 ± 0.4	56 ± 5	–	0.71

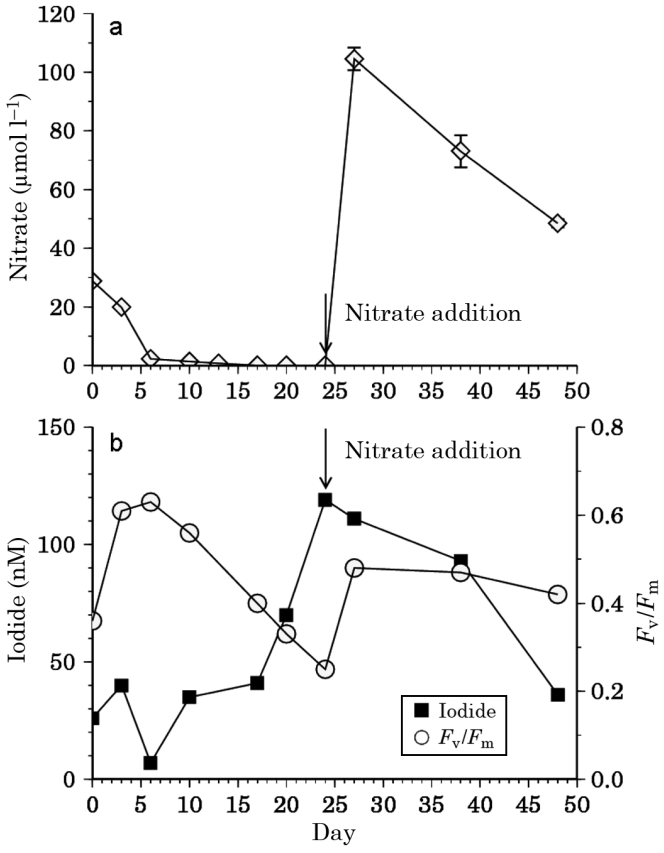

Fig. 5. *Chaetoceros debilis.* (a) Nitrate, (b) iodide and photosynthetic efficiency (F_v/F_m) in *C. debilis* during Expt 1b. Error bars are smaller than the size of the symbols

Iodide production vs. growth phase

All species, except *Scrippsiella trochoidea*, showed the same behaviour when relating the production of iodide to the state of their growth phase (Figs. 1 to 4, Table 4). During the exponential growth phase no or negligible amounts of iodide were produced; once cells reached the stationary phase, iodide increased rapidly and peaked in the senescent phase. In contrast *S. trochoidea*, a mixotrophic dinoflagellate, showed an increase in iodide accompanying their exponential phase (Fig. 4).

Influence of nitrate on iodide production

In Expt 1b the lower initial nitrate concentrations of ~44 µmol l^{-1} used in this setup caused a total nitrate consumption in the first 6 d (Fig. 5a). Over the following 18 d, cells showed a corresponding decline in F_v/F_m, whilst iodide concentrations increased with an iodide production rate of 0.06 nmol l^{-1} μg^{-1} chl *a* d^{-1}. The re-supply of nitrate on Day 24 led to a recovery in the F_v/F_m but a decline in iodide (Fig. 5b).

Depletion of iodate

Iodate depletion was only observed when iodide concentrations typically exceeded 60 nmol l^{-1}, and this was due to the combination of high iodate concentrations and the precision of the iodate method (5%). With the initial iodate concentration of 5 µmol l^{-1}, the error on the iodate measurement is 250 nmol l^{-1}. Although, in some species (*Emiliania huxleyi* and *Pseudo-nitzschia turgiduloides*), a corresponding drawdown in iodate concentrations was observable (Fig. 6). Overall, however, a total mass balance was obtained for iodate and iodide throughout the experiments within experimental error, suggesting organic or particulate iodine species were <20 nmol l^{-1} throughout the experiment. Thus, for the remainder of the manuscript, we concentrate solely on the iodide results.

Bacterial influences and dark incubation

Batch cultures were non-axenic, and the bacterial influences on iodide production needed to be observed. This was examined in Expt 2, where an algal-cell-free filtrate control, containing bacteria and phytoplankton exudates of a senescent *Pseudo-nitzschia turgiduloides* culture, showed no notable iodide production (Fig. 7). Additionally, the iodide concentrations in the dark incubation in Expt 3 did not change over the course of the experiment. Cells showed healthy F_v/F_m values of from 0.4 to 0.5 and were sufficiently supplied with macro- and micronutrients, but did not show an increase in chl *a* or cell numbers due to the lack of light.

Table 4. Duration and growth phase in which iodide production took place in each phytoplankton species

Phytoplankton species	Duration (d)	Phase		
		Exponential	Stationary	Senescent
Temperate species				
Emiliania huxleyi	8–41	No	Yes	Yes
Scrippsiella trochoidea	3–41	Yes	No	Yes
Antarctic diatoms				
Chaetoceros debilis	10–41	No	Yes	Yes
Pseudo-nitzschia turgiduloides	18–34	No	Yes	Yes
Fragilariopsis kerguelensis	24–55	No	Yes	Yes
Eucampia antarctica	24–55	No	Yes	Yes

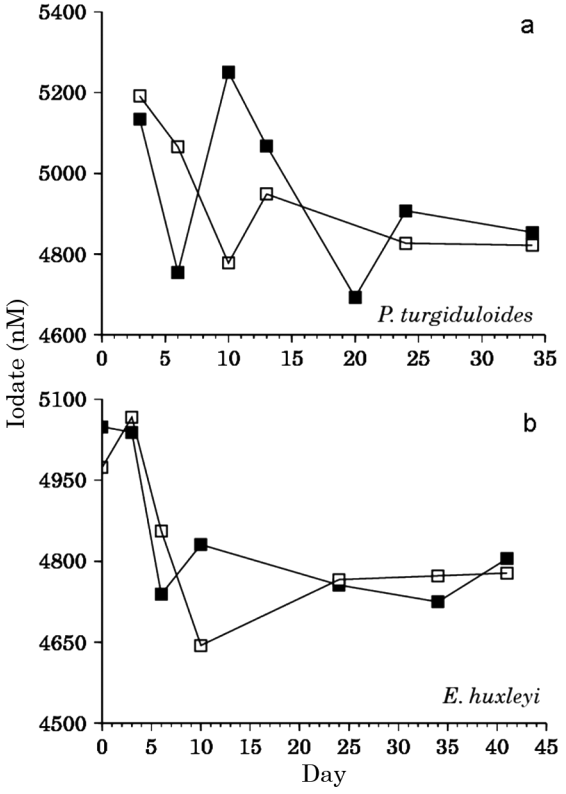

Fig. 6. Iodate depletion in (a) *Pseudo-nitzschia turgiduloides* and (b) *Emiliania huxleyi* over the course of the experiment. (■) samples grown at 50 µmol quanta m^{-s} s^{-1}; (□) samples grown at 100 µmol quanta m^{-s} s^{-1}. Error bars are smaller than the size of the symbols. Note that the scales for the *x*- and *y*-axes are different for each species

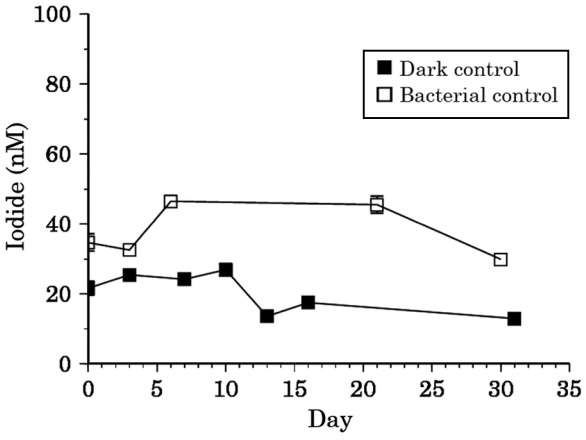

Fig. 7. *Pseudo-nitzschia turgiduloides*. Bacterial control and dark control of a *P. turgiduloides* culture. Bacterial control: 5 µm filtrate of a senescent *P. turgiduloides* culture. Dark control: exponentially growing *P. turgiduloides* culture placed in the dark. See Table 2, Expts 2 and 3

DISCUSSION

Iodide production rates (*k*) per day

Iodide production by phototrophs

All species tested produced significant amounts of iodide through the conversion of iodate. Average production rates per day were in agreement with those reported in previous studies (Wong et al. 2002, Chance et al. 2007) and are shown in Table 3. In all cultures, except *Scrippsiella trochoidea*, the iodide production began in the stationary growth phase (Figs. 1 to 4). *Pseudo-nitzschia turgiduloides* showed the highest iodide production of all species tested. During RV 'Polarstern' cruise ANTXXIV-3 in March 2008, we found elevated iodide values in the neritic Weddell Sea Zone compared to samples taken further north along the Zero Meridian (K. Bluhm et al. unpubl.). *P. turgiduloides* has been found to exist predominantly in this region of the southern Polar Ocean (Almandoz et al. 2008).

Iodide production by mixotrophs

Scrippsiella trochoidea is a mixotrophic dinoflagellate and able to perform phagotrophy. Phagotrophy is usually the primary nutritional mode in mixotrophic dinoflagellates, as they are only able to reach maximum growth rates phagotrophically (Raven 1997). These species have a high nutrient demand, especially when grown under autotrophic conditions, which was reflected in our experiments by total nitrate consumption within 10 d. Consequently, for mixotrophs, photosynthetic carbon fixation has been interpreted as a survival strategy when food densities are low (Andersson et al. 1989, Sanders et al. 1990). Mixotrophs utilise digestive enzymes to consume organic matter, and it has been demonstrated that this process can be used to assimilate colloidal iron (Maranger et al. 1998). A low pH environment is required to dissolve colloidal iron, and under such conditions the redox equilibrium between iodide and iodate is shifted towards iodide once the pH is below 5.7 (Sillen 1961). We then suggest that mixotrophs may passively reduce iodate to iodide via the ingestion of seawater when feeding. Interestingly, this would furthermore suggest that protozoans, which have been shown to dissolve iron colloids (Barbeau et al. 1996), may also contribute to iodate reduction in the ocean.

Is the iodide from iodate reduction?

Two issues were raised during the reviewing process of this manuscript: what was the iodine source of the observed iodide in our experiments, and what possible alternatives are there to iodate reduction? We do not

believe iodine could stem from any source other than iodate for the following reasons. (1) The concentration of organic iodine in the air is not significant to provide a source of iodide to seawater via air–sea gas exchange (the equilibrium concentrations would be in the range of 0.1 nM) (Martino et al. 2009). (2) The idea that the cells themselves are a source of iodine to the experiment is valid, but this source is also insignificant to explain the observed changes. Using the canonical I:C mole ratio of Wong et al. (1976) of ~1×10^{-4} and the estimated initial C content of the added phytoplankton biomass at the start of each experiment (range: 90 to 5500 µg C l^{-1}), we derive a mean iodine addition of 3.1 ± 2.3 nM for the phototrophs and 41 ± 4 nM for the mixotroph described in the previous section. Thus, in most of the experiments, the cell biomass would have provided insignificant iodine to have caused the observed iodide signal. Only in the case of the mixotroph is it possible that a significant detectable amount of iodine was added to the culture in the form of particulate iodine, but that this is still less than the observed iodide signal. Thus, we conclude that in the absence of a significant additional known source of iodine in the experiment, the observed iodide signal can only have come from reduction of the iodate in solution.

Iodide production rate (*k*) per cell

Interestingly, *Fragilariopsis kerguelensis* produced considerably less iodide per cell (0.86×10^{-7} nmol l^{-1} $cell^{-1}$) compared to its remote relative *Pseudo-nitzschia turgiduloides* (5.37×10^{-7} nmol l^{-1} $cell^{-1}$), although the latter species is 1.5 times smaller with 3.5 times less cell volume (Table 1). Our initial hypothesis was that, if it was purely a metabolic process by the cells, then the production of iodide per cell should be proportional to some indicator of biomass (cell volume) or cell surface area. However, in the present study, we did not observe any significant relationship between cell volume, or cell surface area, and the amount of iodide produced. We conclude from this that iodide production is species specific and related to some specific physiological process or processes that differ between the phytoplankton examined here.

Iodide production rate (*k*) based on chl *a*

The simple iodide production rates per day (*k*) were equal in both light regimes in basically all species, but *k* normalised to chl *a* was always higher at higher light intensities based on the decrease in chl *a* per cell (Figs. 1 to 4, Table 3). Due to a greater light availability, cells may photo-adapt, leading to chloroplasts with less chl *a*, which, however, produce the same amount of iodide per day compared to chloroplasts from cells grown under lower light. Highest rates per chl *a* were observed in *Pseudo-nitzschia turgiduloides* (0.26 nmol l^{-1} $µg^{-1}$ chl *a* d^{-1}), and this species was a clear example of the processes outlined above (Table 3). *Emiliania huxleyi* produced iodide with a maximum rate of 0.06 nmol l^{-1} $µg^{-1}$ chl *a* $d^{—1}$, which is comparable with the findings of Wong et al. (2002), but 4 times lower than those observed by Chance et al. (2007) during their studies. This might be due to divergent calculations of the average chl *a* concentrations. In our study we used the trapezoidal method, as did Wong et al. (2002), and obtained comparable values. Chance et al. (2007) normalised their values to the simple average chl *a* concentration over the selected period. They observed an iodide production in *E. huxleyi* only in the exponential growth phase of the algae and no production in the stationary phase, with their cells apparently not reaching the senescent phase. This is in contrast to our results for the same species, although a different strain cultured under similar conditions (nutrients and light). However, we suggest that part of the difference may be due to their use of a coulter counter for cell enumeration, which does not distinguish between live and dead cells, unlike epi-fluorescence microscopy. Thus, in their data, the senescence phase does not appear, as cells are still counted even though they may be lacking chloroplasts.

Why was nitrate not totally consumed?

Some of our cultures apparently did not run into macronutrient limitation (Expt 1a), and, thus, we must look for an alternative reason for the passage into the senescent phase. Physiological death and subsequent lysis of phytoplankton cells have been shown to result from a number of factors apart from nutrient limitation:

(1) Light limitation due to high cell densities in the batch cultures. Nitrate reduction in phytoplankton strongly depends on light energy and will be hampered under these conditions (Berges & Falkowski 1998).

(2) Changes in alkalinity and pH. Uptake of inorganic carbon by phytoplankton during photosynthesis may increase pH and cells may become carbon limited as CO_2 concentrations decrease under these conditions (Hansen 2002).

(3) Viral lysis. Cells get infected by specific viruses, which induce a loss in the viability of the algal cell and, hence, automortality (Suttle 1992, Bratbak et al. 1998, Nagasaki et al. 2004, Bettarel et al. 2005).

(4) Apoptosis. Autocatalysed cell death, which can be induced by pathogen exposure or through the produc-

tion of superoxide radicals (oxidative stress) (Antunes et al. 2001, Segovia et al. 2003).

All possibilities mentioned result in cell death followed by a rapid release of cellular constituents to the surrounding medium, which serves as a food supply for bacteria. Consequently, bacterial numbers increase rapidly in this growth phase of the phytoplankton. We suggest here that viral lysis was most likely the reason for cell senescence and cell lysis in our batch cultures, as light limitation, as seen in the dark control experiments, apparently had little effect on cell mortality. Interestingly, light is apparently required for viral replication in some marine phytoplankton, and this may be the reason why the dark control cells remained intact (Baudoux & Brussaard 2008). Diatoms are relatively insensitive to light deprivation, which is concordant with previous observations that non-spore-forming species such as *Thalassiosira weissflogii* survive several weeks in good condition (Peters & Thomas 1996). We did not measure pH or alkalinity in our cultures, so that we cannot totally rule out that cells might also have become carbon limited, although, with the added nitrate concentrations of 88 µmol l^{-1}, cells would have rather become nitrate limited.

Iodate reduction by nitrate reductase

The enzyme nitrate reductase (NR) has been postulated to perform the reduction of iodate to iodide in phytoplankton and bacteria when nitrate is limiting in the ocean (Tsunogai & Sase 1969). Our work does not support that view, as our cultures from the first experiment did not run into nitrate limitation although we still observed iodide production. Instead, we believe the reduction of iodate is connected to cell viability and senescence. This also supports the findings of Waite & Truesdale (2003), who still found iodide production even when NR was deactivated and cells were grown on ammonia instead of nitrogen.

Iodide oxidation to iodate by diatoms

We did, however, see an interesting effect of nitrate with *Chaetoceros debilis* (Fig. 5) when iodide production was initiated during cell senescence caused by nitrate limitation. However, upon re-supply of nitrate, iodide production stopped and the concentration declined while cells resumed exponential growth. Thus, an iodide oxidation mechanism must have been active during this time that oxidised 80 nmol l^{-1} iodide over 25 d. Diatoms have been observed previously to oxidise iodide to iodate (Sugawara & Terada 1967), and other phytoplankton have been shown to possess iodoperoxidases (Murphy et al. 2000, Hill & Manley 2009), though these enzymes are normally not capable of oxidising iodide to iodate. A review of the literature on this subject reveals only one reference to a chloroperoxidase in the fungus *Caldariomyces fumago* that can oxidise iodide to iodate (Thomas & Hager 1968). Iodoperoxidases are also present in marine bacteria (Gozlan & Margalith 1973, 1974, Amachi et al. 2005), though none of these studies found oxidation through to iodate. Another possibility would be that iodide had been incorporated into the phytoplankton cells, or lost as I_2.

The role of bacteria

Many phytoplankton cultures are often only available with their associated bacteria; diatoms in particular require the associated bacteria for normal growth (Fukami et al. 1997, Croft et al. 2005, Grossart & Simon 2007). Therefore, it is difficult to separate phytoplankton responses from those of the surrounding bacteria. Bacteria were present in all cultures to varying amounts; highest numbers were observed in the cultures of *Pseudo-nitzschia turgiduloides* and *Emiliania huxleyi* towards the end of the experiment (>30% of total carbon biomass; Figs. 2b & 3b). The increase in bacteria goes hand in hand with the increase in iodide, and we cannot totally rule out that bacteria also reduce a certain amount of iodate to iodide as they are capable of doing so (Tsunogai & Sase 1969, Amachi et al. 2007). In all other cultures, bacteria were minor constituents and can be neglected (Figs. 1b to 4b). These results are corroborated by the results of the bacteria control experiment (Expt 2) in which no significant changes in iodide were observed (Fig. 7).

Influence of light

No influence of light on the iodide production in the deployed range (50 and 100 µmol quanta m^{-2} s^{-1}) was observed in Expt 1a, as samples showed similar *k* rates under both light regimes (Table 3). However, cells that were kept in the dark for several days did not show any iodide production (Fig. 7), indicating that light did have some effect. However, as both *Pseudo-nitzschia turgiduloides* and *Chaetoceros debilis* went into a resting phase during which they no longer took up nutrients, iodide production was probably not related to photosynthesis and the highest iodide production occurred when C fixation was minimal. Cells held in the dark were still viable with F_v/F_m values never dropping below 0.4, which makes us believe that no cell lysis took place.

The role of cell permeability

From our experiments it is apparent that part of the high iodide production rates in the later growth phases are related to cell senescence. When cells become extremely permeable under stressful conditions, such as nutrient limitation or viral infection, metabolites will be released back into the surrounding media and can react with the components of that media, including iodate. The release of cellular material can be hastened during this time by direct cell lysis mediated either by viruses (Nagasaki et al. 2004, Bettarel et al. 2005) or apotosis (Antunes et al. 2001, Segovia et al. 2003). In the present study, we cannot determine which process caused the cells to lyse, but evidence that this process was occurring can be found in the decrease in cells containing intact chloroplasts, as observed by microscopy, and the subsequent increase in bacterial numbers, presumably from the release of labile dissolved organic matter (DOM). The release of cellular metabolites, especially reduced sulphur compounds such as sulphide and glutathione (GSH), which are present in high concentrations inside phytoplankton cells (Matrai & Keller 1994) and are able to reduce iodate to iodide under typical seawater conditions (Hird & Yates 1961, Jiazhong & Whitfield 1986), seems to be the most likely cause for the increase in iodide at this time. Our finding is consistent with phytoplankton culture experiments investigating sulphide production (Walsh et al. 1994), during which increases in the dissolved free-sulphide concentration were observed during the senescence phase. Additionally, field work on sulphide in the ocean suggests that metal complexation is important (Cutter et al. 1999) in reducing the extent of the reaction between sulphide and iodate; thus, in phytoplankton cultures in which the metal speciation is dominated by EDTA complexes, we can infer that reduction rates of iodate would be maximal.

Interestingly, our data (Figs. 3 & 5b) indicate that iodate loss, presumably due to uptake by phytoplankton, preceded iodide production by several days. This suggests 2 possibilities: firstly, that the source of the iodide is from the release of iodide accumulated in the cells prior to cell lysis, or, secondly, that there was a conversion of iodate into another form of iodine (iodo-organics) that was unreactive to our analytical methods and which slowly converted to iodide. Iodo-organics are clearly produced during phytoplankton growth (Brownell et al. 2010), but they have not been found in nanomolar concentrations and, typically, are easily photolysed even by phytosynthetically active radiative light (Martino et al. 2006). The uptake of iodate into phytoplankton cells has been studied recently by de la Cuesta & Manley (2009), and based on their work we estimated that the cellular uptake of iodate may explain the initial drawdown of iodate. This would also imply that iodate is not reduced prior to uptake by the cell, as we see no evidence for increased iodide concentrations at this time. Once in the cell, however, iodate is most likely reduced to iodide by glutathione or other reduced organic species as indicated above.

Our findings apparently contradict the earlier work of Wong et al. (2002), who found no clear relationship between iodide production and the growth phase of the culture. Indeed, they ruled out cell lysis as a possible cause, though this may have been because they did not measure cell numbers or any proxy of cell permeability. In the present work, we can examine the potential of cell lysis by determining the rate of iodide increase as a function of cell mortality, for time points at which both chl *a* and cell numbers are decreasing, as a function of the estimated C content per cell, here used as a proxy for S content (Fig. 8). Our results suggest that, for a first approximation, there is a relationship between C content and iodide production. Species-specific variations in the S:C ratios (Matrai & Keller 1994) may be responsible for the variations in this relationship, though, unfortunately, there are no data available for most of the species we examined here.

Ecological relevance

In the open ocean, the presence of iodide in the euphotic zone and its oxidation back to iodate in deeper waters are linked to biological activity controlling the cycling of micronutrients like iodine. The data obtained during the present study suggest that the production of iodide is a process connected to cell

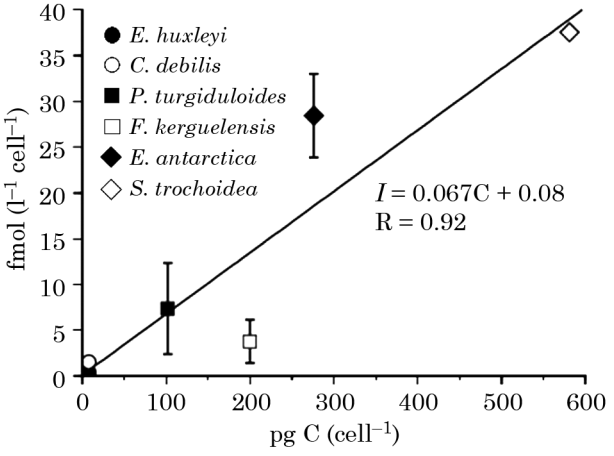

Fig. 8. Calculated iodide production (I) (±SE) per dead cell (calculated as the difference in cell numbers between time points) as a function of the estimated C content per cell. Only data showing both a decrease in chl *a* and cell numbers after the maximum values were reached are used. For full taxonomic names see Table 1

senescence and cell permeability, and we speculate that it may be linked to internal sulphur species. If, for example, 10^5 diatom cells l^{-1}—these organisms being present in a range from 10^4 to 10^6 cells l^{-1} in the Southern Ocean (Kopczynska et al. 1986, 1998, Olguin et al. 2006)—produced iodide at a rate we measured for our Antarctic species (average rate: 3.2×10^{-7} nmol l^{-1} $cell^{-1}$ d^{-1}), their total iodide contribution would be 0.0316 nmol l^{-1} d^{-1}.

Conclusions

The influence of phytoplankton on the biogeochemical cycle of iodine was investigated in a set of experiments carried out with a variety of phytoplankton taxa. From those results we derive the following conclusions:

In batch cultures of marine phytoplankton the reduction of iodate to iodide is observed, but principally in the late phases of cell growth. The production of iodide is only observable in the stationary and/or senescent growth phase of the algae, the only exception among the mixotrophic species being *Scrippsiella trochoidea*. In this species iodide production commenced at the very beginning of the experiment.

Cells were grown under nitrate-replete conditions, and it is clear that the reduction of iodate to iodide is not related to nitrate availability, but more to the viability of the cells. Iodine is assimilated by phytoplankton cells preferentially as iodide (de la Cuesta & Manley 2009), but it still remains unclear whether phytoplankton has an essential metabolic need for iodine. Further investigations are needed to define the role of iodine for phytoplankton needs. The importance of light or nutrient limitation and the resulting senescence in phytoplankton should be observed further in terms of iodide production and the link to S species. A second step would be to examine the behaviour of natural assemblages instead of monoclonal batch cultures.

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