

Iodine assimilation by marine diatoms and other phytoplankton in nitrate-replete conditions

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Abstract

Several marine phytoplankton species, primarily diatoms, were examined for the accumulation of iodide (10 species) and iodate (9 species) using radioactive iodine-125 in *f/2* artificial seawater, a nitrate-enriched medium. Iodide accumulation (net uptake) rates were variable, and diatoms exhibited the highest rates. *Emiliania huxleyi* and *Synechococcus* sp. did not accumulate iodide. Accumulation rates ranged from 0 to 1.7 fmol cell⁻¹ d⁻¹. The diatom *Porosira glacialis* accumulated the greatest amount of iodide and was used to determine efflux rates of iodide. Iodide efflux was characterized by two distinct phases of iodide release: an initial rapid release rate of 10 amol cell⁻¹ min⁻¹ from the free space and a subsequent cellular release rate of 0.13 amol cell⁻¹ min⁻¹, which corresponds to a daily cellular release rate of 0.19 fmol cell⁻¹ d⁻¹. Accumulation of iodate ranged from 0 to 19 amol cell⁻¹ d⁻¹, with *P. glacialis* displaying the highest rate. *Emiliania*, *Synechococcus*, and *Chaetoceros* did not show significant iodate accumulation. Iodide is the preferred chemical species of iodine for uptake under nitrate-replete conditions: iodide accumulation rates ranged from 3 to 90 times larger than those for iodate for the species studied. If the iodate accumulated is tightly coupled to its reduction to iodide, the accumulation rates suggest that phytoplankton-mediated iodate reduction is not environmentally significant. If diatoms can reduce iodate to iodide, their overall contribution to surface-water iodide, while living, would be further reduced because of their ability to reassimilate released iodide.

Inorganic iodine exists in disequilibrium in surface seawater as iodide and the thermodynamically favored form, iodate (Wong 1991). The relatively high concentration of iodide in temperate coastal waters and subtropical-tropical waters is thought to be related to biological productivity (Wong 2001; Wong et al. 2002; Chance et al. 2007). Both chemical species are potentially available for uptake by marine phytoplankton. The first measurements of iodide and iodate assimilation used the diatom *Navicula* sp., and it was shown to use both forms, though iodide was the preferred form (Sugawara and Terada 1967). There are no other reports of iodide uptake by phytoplankton. Iodate uptake, however, has been reported for several phytoplankton, and it has been suggested that they may be significant reducers of iodate to iodide (Moisan et al. 1994). Although the role of phytoplankton in iodate reduction has been questioned, especially in colder waters (Truesdale et al. 2003), the facilitated reduction of iodate to iodide by phytoplankton cultures has been demonstrated (Wong et al. 2002; Chance et al. 2007).

Presently, a mechanism by which phytoplankton can reduce iodate to iodide at a rate that is environmentally significant has not been adequately described (Truesdale et al. 2003). Dissimilatory (extracellular) iodate reduction by anaerobic marine bacteria isolated from sediments has been reported (Councell et al. 1997; Amachi et al. 2007). A dissimilatory mechanism for iodate reduction to iodide has not yet been reported for phytoplankton. If phytoplankton have a dissimilatory mechanism, it would probably be different from that of an anaerobe, because phytoplankton are oxygenic photoautotrophs. Thus, a more plausible first step in phytoplankton-mediated iodate reduction would be

its uptake into the cell prior to reduction, possibly by nitrate reductase (Tsunogai and Sase 1969; Hung et al. 2005). Iodide could then be released from the cell by efflux or from cell lysis. In this paper, we report both iodide and iodate accumulation rates for several phytoplankton species in a nitrate-replete culture, using the radioisotope I-125 to directly measure cellular assimilation. This information is needed in assessing the importance of phytoplankton in the biogeochemical cycling of iodine in marine waters.

Methods

Phytoplankton cultures—Marine phytoplankton used for this study were Bacillariophyta: *Chaetoceros neogracile* (CCMP 1317), *Navicula* sp. (CCMP 547, lost to culture and no longer available), *Nitzschia punctata* (UTEX 2041), *Nitzschia* sp. (CCMP 580), *Porosira glacialis* (CCMP 651), *Skeletonema costatum* (UTEX 2308), *Thalassiosira pseudonana* (NEPCC 58); Prymnesiophyta: *Emiliania huxleyi* (CCMP 370), *Phaeocystis* sp. (CCMP 1524); and Cyanophyta: *Synechococcus* sp. (CCMP 1334, axenic). The primary cultures were maintained in *f/2* seawater medium (Guillard 1975) at 18°C under a 12:12 light:dark photoperiod. The light sources used were cool white fluorescent lamps with a photon flux density (PFD) of 20 μmol photons m⁻² s⁻¹. Two cold-water diatoms (*Nitzschia* sp., *P. glacialis*) were incubated under the same conditions, except they were kept at 5°C and the PFD was 10 μmol photons m⁻² s⁻¹ under Gro-Lux lamps. All cultures were unialgal. The antibiotics penicillin and streptomycin were together used prior to the experimental procedure to reduce bacterial contamination in cultures of *S. costatum* and *T. pseudonana* (James 1978). The

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antibiotics were not used during the uptake study. The technique was not successful in creating axenic cultures but only reduced bacterial contamination. *Synechococcus* sp. remained axenic throughout the study.

Artificial seawater—All accumulation experiments were performed using artificial *f/2* seawater with defined iodide and iodate concentrations. The artificial seawater was prepared according to the Marine Biology Laboratory Trace Solution Formula (Cavanaugh 1975), using low-iodide ($0.2 \mu\text{g g}^{-1}$) NaCl and KCl salts (Baker Ultrex®). All the other salts were analytical-grade reagents and did not contribute significantly to the iodide or iodate concentration of the artificial seawater. The salts were dissolved in 100 mL of glass-distilled water. The background iodide concentration of the artificial seawater was 41 nmol L^{-1} as calculated using the iodide contamination listed in the certificates of analysis of the two Ultrex® salts and from iodide contamination listed on the other reagents. Iodate was not a listed contaminant in any of the reagents used. The iodate concentration in the artificial seawater was therefore assumed to be negligible. The final nitrate concentration was $\sim 880 \mu\text{mol L}^{-1}$.

The iodide concentration of the artificial seawater was increased to 100 nmol L^{-1} using a $100 \mu\text{mol L}^{-1}$ analytical-grade NaI solution for the iodide experiments. The *f/2* nutrients were then added to the artificial seawater mixture. The *f/2* nutrient solutions diluted in deionized water to their final *f/2* concentrations were analyzed separately for iodide contamination using the Gran's known-addition method (Gran 1952) in conjunction with an Orion model 960 Autochemistry system fitted with an iodide-specific electrode. The iodide level of the *f/2* was below the limit of detection of the instrument (less than 10 nmol L^{-1}). After adding the *f/2* nutrients, the medium was autoclaved, cooled, and sterile filtered ($0.2\text{-}\mu\text{m}$ pore diameter). The postautoclaving filtration step was added to remove any particulates that might bind the radioactive label. The iodide speciation was not altered by autoclaving (see Results section). The radioactive tracer iodide-125 was added just before inoculation with cells.

For the iodate accumulation experiments, the iodate concentration was increased to 100 nmol L^{-1} by addition of analytical-grade KIO_3 . The iodide concentration was at background (41 nmol L^{-1}). The *f/2* nutrients were added, and the medium was sterile filtered ($0.2\text{-}\mu\text{m}$ pore diameter). The iodate medium was not autoclaved out of concern that autoclaving may alter the speciation of the iodate. The radioactive tracer iodate-125 was added just before inoculation with cells.

Radioactive stock preparation—Iodide-125, as NaI, was supplied carrier free in basic solution in 74-MBq (2 mCi) aliquots (Amersham). Radiochemical purity was documented as 99%. Primary stocks were diluted in sterile glass-distilled water before use and added to the artificial seawater to a specific activity of $18.5 \text{ kBq nmol}^{-1}$ ($0.5 \mu\text{Ci nmol}^{-1}$) and a concentration of 1.85 kBq mL^{-1} . The concentration of iodide-125 was $0.024 \text{ nmol L}^{-1}$ compared to 100 nmol L^{-1} iodide in the artificial seawater.

Iodate-125 is not commercially available. Therefore, it was produced from iodide-125 via acidification and permanganate oxidation, followed by chromatographic purification (de la Cuesta 1996). The radiochemical purity of the iodate-125 was determined to be greater than 93% using thin-layer chromatography and image analysis (de la Cuesta 1996). No iodide-125 was detected on the chromatogram. The specific activity of the iodate-125 stock was $1.59 \text{ MBq nmol}^{-1}$ ($43 \mu\text{Ci nmol}^{-1}$; the same as the starting iodide concentration). The iodate-125 was then added to the artificial seawater medium to a specific activity of 37 kBq nmol^{-1} ($1.0 \mu\text{Ci nmol}^{-1}$) and a radioactivity of 3.7 kBq mL^{-1} . The concentration of iodate-125 was $0.047 \text{ nmol L}^{-1}$ compared to 100 nmol L^{-1} iodate in the artificial seawater.

Phytoplankton and bacteria enumeration—Phytoplankton densities were obtained using an electronic particle counter or light microscopy-hemocytometer (for chain diatoms, diatoms embedded in mucilage, and the large diatom *P. glacialis*). *Synechococcus* sp. cells were enumerated with fluorescence microscopy (excitation at 490 nm, BP490 filter; Olympus BHT, BH2-RFL) relying on the fluorescence of the phycobiliproteins.

Biomass densities of *Phaeocystis* sp. cultures (Prymnesiophyta) were based on chlorophyll *a* (Chl *a*) due to the large (up to 5-mm diameter) mucilaginous colonies formed by the alga. The pigments were extracted in 90% acetone and analyzed spectrophotometrically (Parsons et al. 1984). Chl *a* from *Phaeocystis* sp. was subsequently normalized to cell number from samples taken from cultures grown under the same temperature and light regime as in the accumulation experiments. The samples for enumeration were prepared by disrupting the colonies by shaking. Mean cell numbers were correlated to mean Chl *a* samples. Acridine orange epifluorescence microscopy (Hobie et al. 1977) was used to enumerate any bacteria that were present in the cultures at the beginning and end of the 5-d experiment.

Accumulation experiments—Phytoplankton cells were grown in ^{125}I -labeled artificial medium for up to 5 d. *Nitzschia* sp. (CCMP 580) was not used in the iodate accumulation experiments because the culture died and could not be replaced before the iodate-125 decayed. Light and temperature regimes were the same as for the primary cultures. Parallel cultures were grown in nonradioactive (unlabeled) artificial medium for phytoplankton and bacterial enumeration. Approximately 1×10^6 cells were inoculated per 4 mL of artificial seawater medium. Sterile, 5-mL, capped, clear polystyrene culture tubes were used as culture vessels. Phytoplankton cells, from a primary culture in exponential growth, were added to 3.8 mL of medium in each tube: 5 tubes of labeled medium for accumulation (1 tube per day except where noted otherwise), 15 tubes (3 tubes per day) of labeled medium with no cells for filter background determinations, 5 tubes of unlabeled medium for phytoplankton cell counts (1 tube per day), and 2 tubes for bacterial enumeration ($t = 0$ and 5 d). All tubes were placed on an orbital shaker at 80 revolutions per minute (rpm) to reduce cell-boundary-layer resistance. To deter-

mine the precision of the accumulation experiments, replicate iodide-125 incubations were performed ($n = 3$) for *P. glacialis* and *Phaeocystis* sp., and replicate iodate-125 incubations were performed ($n = 3$) for *P. glacialis*, *Phaeocystis* sp., and *S. costatum*.

Every 24 h, the I-125 incubation medium in an incubation tube was vacuum filtered onto a 25-mm nitrocellulose filter (0.45- μm pore diameter). The samples were washed five times with 3 mL of 0.45- μm -filtered seawater supplemented with either 1 $\mu\text{mol L}^{-1}$ iodide or iodate to facilitate the exchange of absorbed radiolabel adhering to the filter and cell surface with nonlabeled anion. This number of seawater rinses was required to remove loosely bound radiolabel from control filters (see Wash experiment section). Filters with cells were then placed in a plastic culture tube for counting in a gamma counter for 10 min. Phytoplankton and bacteria from the nonradioactive tubes were also sampled and enumerated as described previously. Additional iodide and iodate accumulation experiments were performed on *P. glacialis* that lasted 5 h because initial experiments showed rapid accumulation during the first 24 h.

The amount of radiolabel accumulated in the cells was the amount that remained after the wash (which removed radiolabel from the cells and filter surface). Some radiolabel, however, remained associated with the filter after numerous washes. The mean filter background counts (controls) were subtracted from the initial determined counts from the cells on the filter to give the actual amount of label accumulated in the cells. In order to characterize the theoretical confines of the incubation experiments, we chose to define the theoretical limit of detection (TLOD) as three times the standard deviation of the mean counts from the filter wash controls (Long and Winefordner 1983) divided by the mean cell number for the 24-h period preceding the cell harvest day. We consider this TLOD to be a conservative estimate of the sensitivity of the experiments. All accumulation points, even those below the TLOD, were used to determine accumulation rates by linear regression analysis, except where noted.

Wash experiments—The number of washes needed to remove extracellular free-space label and that adsorbed to the filter was determined by collecting the filtrate of five 3-mL washes of cells and filter in separate filter flasks. The cells were previously incubated in labeled medium for 5 d. The filtrate was counted in the gamma counter to determine at which wash step the label was reduced to background. The rinse profile for the iodate filter blank was similar to that of iodide filter blank. Approximately 0.5% of the radiolabel continued to adhere to the filter after this washing procedure. The background radioactivity was subtracted from the cellular accumulation for each day to obtain net cellular accumulation.

Iodide efflux by *P. glacialis*—Efflux experiments with *P. glacialis* using iodide-125 in artificial f/2 seawater medium (total iodide = 100 nmol L^{-1}) were performed. The cells were incubated for 3 h in 4-mL clear plastic tubes at 5°C with a PFD of 10 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$. After incubation,

Table 1. Iodide accumulation rates of marine phytoplankton determined over a 5-d period.

	Iodide accumulation (amol cell ⁻¹ d ⁻¹) ($p < 0.05$)	r^2
Bacillariophyta		
<i>Chaetoceros neogracile</i>	2.1	0.97
<i>Navicula</i> sp.	1.1	0.90
<i>Nitzschia punctata</i> *	0.44	0.93
<i>Nitzschia</i> sp.	10	0.96
<i>Porosira glacialis</i> †	56	0.74
5-h experiment	1700	0.97
<i>Skeletonema costatum</i> ‡	0.15	0.74
<i>Thalassiosira pseudonana</i>	0.78	0.81
Prymnesiophyta		
<i>Emiliania huxleyi</i>	0	—
<i>Phaeocystis</i> sp.†	0.42 (2.6)*	0.43
Cyanophyta		
<i>Synechococcus</i> sp.	0	—

* One or more data points below limit of detection.

† Data based on replicates of $n=3$.

‡ Initial rate based on 1 day of accumulation.

the cells were gently filtered onto a nitrocellulose membrane (25-mm diameter, 0.45- μm pore diameter). The cells were then rinsed (100 nmol L^{-1} iodide artificial f/2 seawater at 5°C) five times in 3-mL increments. The rinse medium was left on the filter for 30 s between each rinse. The filtrate was collected using a new filter flask after each rinse. After rinsing, efflux was determined by placing 4 mL of 100 nmol L^{-1} artificial seawater in the filter tower atop the cells. The filter apparatus was placed in the incubator (5°C, 10 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$) for 10 min and shaken (orbital shaker 50 rpm). The medium was then filtered and counted. The procedure was repeated at 10-min intervals for 2 h, after which the filter and cells were counted to determine the amount of iodide-125 remaining in the cells after efflux. Identical control experiments were performed on the nitrocellulose filters alone to determine the amount of label adsorbed to the filter. Net efflux was calculated from the filter-subtracted values.

Results

Iodide accumulation experiments—A large variation in rates of iodide accumulation occurred among the species (Table 1). Phytoplankton growth rates (Table 2) and selected growth curves (Figs. 1, 2) were included to characterize growth during the incubation period. Similar iodide-125 wash kinetics (data not shown) were observed for diatom species independent of whether the species exhibited no detectable accumulation, low accumulation, or high accumulation. The first two washes removed most of the radiolabel, suggesting that surface adsorption (cell and filter) rather than cell leakage was the dominant mechanism.

A synopsis of growth and iodide accumulation by the various species is outlined as follows: *S. costatum* (Fig. 1A) showed linear growth after day 2; iodide accumulation was

Table 2. Growth rates and bacterial contamination of iodide accumulation cultures.

	Phytoplankton growth rate (doublings d ⁻¹)	Bacteria-to-phytoplankton ratio		Bacteria number‡ (×10 ⁶ cells) day 5
		day 0	day 5	
Bacillariophyta				
<i>Chaetoceros neogracile</i>	0.78	6	0.9	1.9
<i>Navicula</i> sp.	0.35	10	17	13
<i>Nitzschia punctata</i>	0.54	2	3	5.1
<i>Nitzschia</i> sp.	0.25	6	4	1.8
<i>Porosira glacialis</i> *:†	—	37	—	—
<i>Porosira glacialis</i> †	-0.083	107	123	20
<i>Skeletonema costatum</i>	0.49	2	0.7	2.0
<i>Thalassiosira pseudonana</i>	0.66	6	2	3.0
Prymnesiophyta				
<i>Emiliania huxleyi</i>	0.66	55	27	91
<i>Phaeocystis</i> sp.†	0.21	12	13	22
Cyanophyta				
<i>Synechococcus</i> sp.	0.0025	0.1	0.2	11

* 5 h of accumulation, therefore no growth data.

† Replicate experiment $n=3$.

‡ Total cell number per 4 mL of culture.

linear and just above detection limit. *Nitzschia* sp. (Fig. 1B) showed lag growth to day 4, followed by robust growth; iodide accumulation was linear throughout. *Phaeocystis* sp. (Fig. 1C) showed lag growth for 1 possibly 3 days, followed by robust growth (the possibility exists that cells in the tube sampled on day 3 failed and were not representative of the general growth pattern); iodide accumulation was immediate and ceased after 1 d. *P. glacialis* (5 d; Fig. 2A) showed decline in cell numbers until day 4, followed by an increase; iodide accumulation was linear throughout. All *P. glacialis* cells appeared viable (i.e., pigmented), and we observed no empty frustules.

The diatoms *C. neogracile*, *Navicula* sp., and *T. pseudonana* exhibited relatively high iodide accumulation rates (Table 1), all points were above the TLOD (not shown), and they showed good growth (Table 2). *N. punctata*, like *S. costatum*, exhibited accumulation rates that were near the TLOD, with one or more points below the TLOD. Phytoplankton that exhibited no detectable iodide accumulation were *E. huxleyi* and *Synechococcus* sp.; the filter control values were equal to or greater than the cell plus filter values.

To test the precision of the accumulation experiments, replicates ($n = 3$) were performed using the diatom *P. glacialis* (Fig. 2) and the prymnesiophyte *Phaeocystis* sp. (Fig. 1C). The mean cellular accumulation values and standard deviations at each time interval for both organisms are shown. The amount of iodide-125 associated with the cells plus filters (not subtracting the filter controls) was significantly greater than that associated with the filter controls only, indicating cellular accumulation.

P. glacialis exhibited an iodide accumulation rate that was over five times that of any of the other species examined based on the 5-d accumulation experiments (Table 1). However, the amount of iodide accumulated in the cell leveled off after the first day (Fig. 2A), suggesting that the initial iodide accumulation rate was not main-

tained. To define the kinetics of the accumulation of the initial rapid phase, accumulation rates were determined every hour for a 5-h period. Accumulation to this point was linear, significant, and very rapid (1.7 fmol cell⁻¹ d⁻¹; Fig. 2B); cell numbers remained constant.

Because it had the highest iodide accumulation rate, *P. glacialis* was used to verify the fact that autoclaving of the artificial seawater medium did not significantly affect the iodide concentration of the medium. The accumulation averages of the two treatments (autoclaving vs. nonautoclaving) were not statistically different (t -test, $df = 2$, $n = 3$; for each treatment, $p > 0.05$).

Bacterial numbers during iodide uptake—In order to assess the role of bacteria in iodide accumulation, bacteria-to-phytoplankton ratios (B:P ratios) for day 0 and 5 were calculated for all species (Table 2). Although bacterial numbers increased in all cultures, where the B:P ratios increased, they did so slightly. Relatively high bacterial contamination (>20 bacteria per phytoplankton cell) occurred with *E. huxleyi* and *P. glacialis*; the former showed no iodide accumulation, and the latter showed the greatest iodide accumulation.

Efflux of iodide by *P. glacialis*—Two distinct phases of iodide release were observed, an initial release rate (from free space) of 10.0 amol cell⁻¹ min⁻¹ and a subsequent release rate (cellular efflux) of 0.13 amol cell⁻¹ min⁻¹, which correspond to a daily cellular release rate of 1.9×10^2 amol cell⁻¹ d⁻¹ (Fig. 3). *P. glacialis* accumulated 47 ± 13 amol cell⁻¹ or 32% of gross iodide uptake (accumulation = gross uptake - amount released from free space and cell). The iodide accumulation rate by *P. glacialis* was approximately 9× greater than the cellular efflux.

Iodate accumulation experiments—Net cellular accumulation of iodate was determined for several species of marine

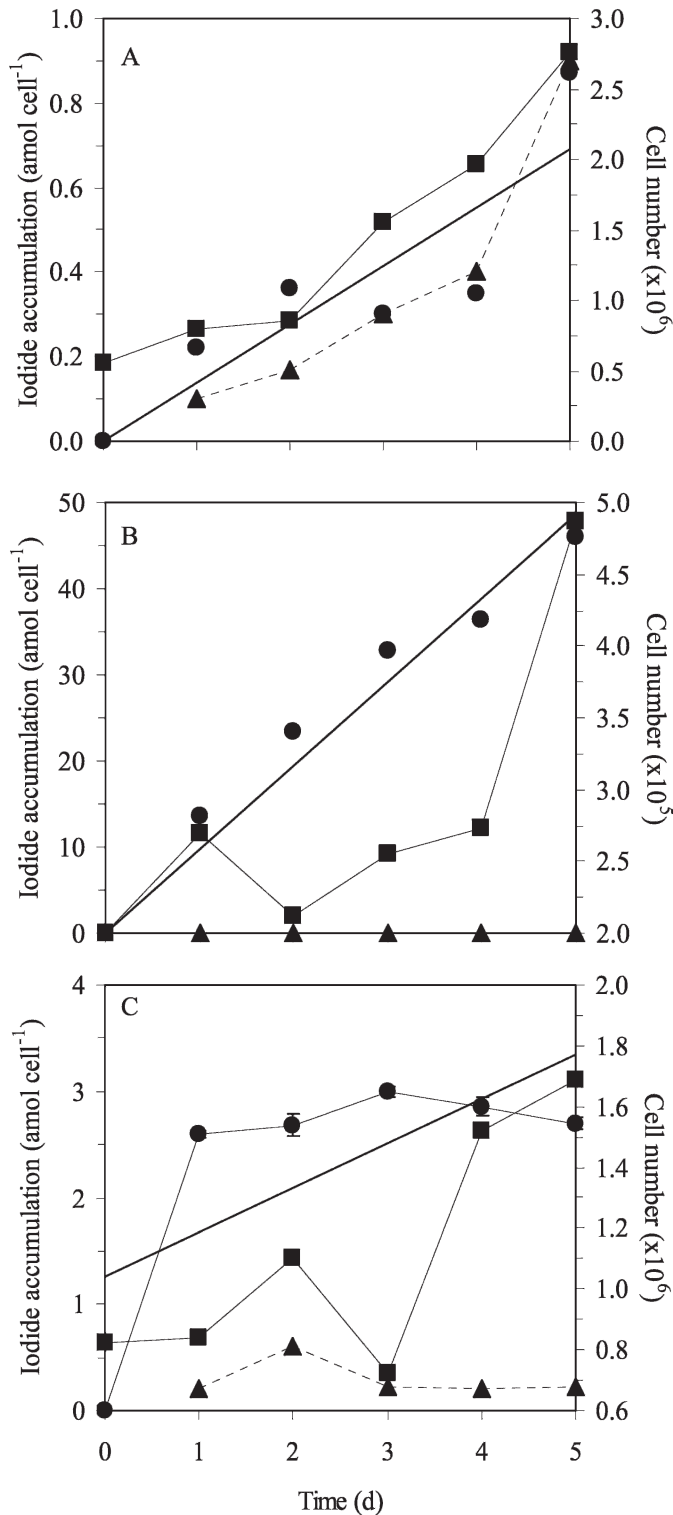


Fig. 1. Iodide accumulation by (A) *Skeletonema costatum* ($r^2 = 0.74$ at $p < 0.05$, with y -intercept at 0), (B) *Nitzschia* sp. ($r^2 = 0.96$ at $p < 0.05$, with y -intercept at 0), and (C) *Phaeocystis* sp. ($n = 3$, ± 1 SD; $r^2 = 0.42$ at $p < 0.05$). Circles = iodide accumulation, triangles = detection limit, squares = phytoplankton cell number.

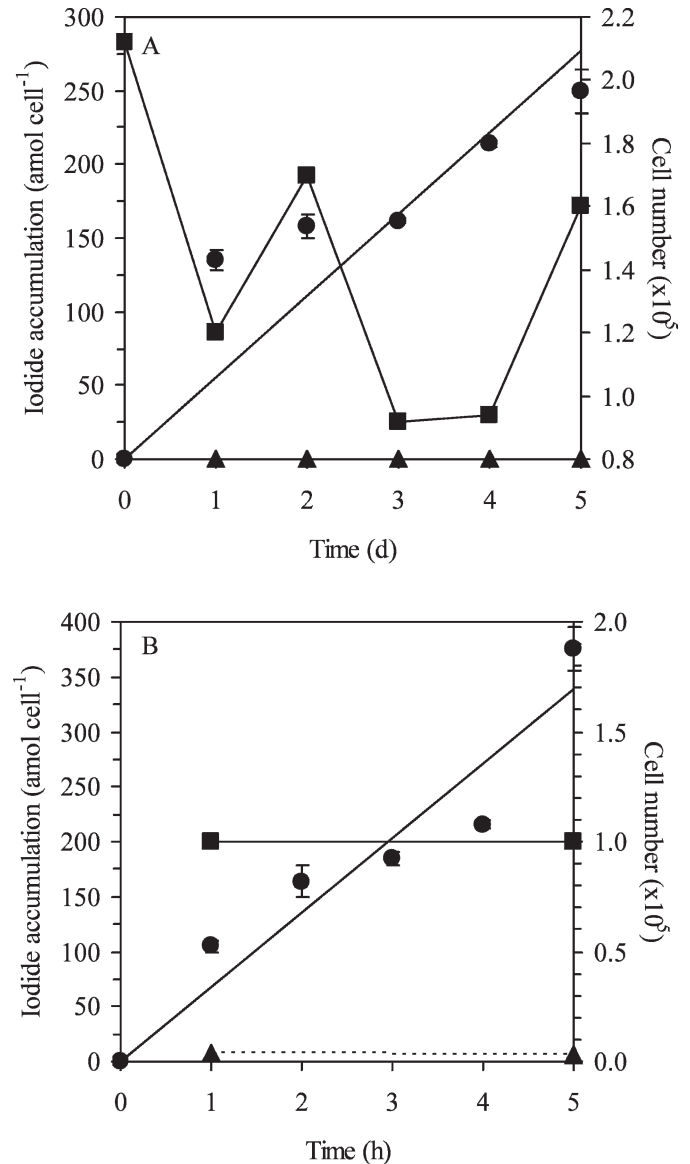


Fig. 2. Iodide accumulation by *Porosira glacialis* ($n = 3$, ± 1 SD). Circles = iodide accumulation, triangles = detection limit, squares = phytoplankton cell number. (A) 5-d experiment ($r^2 = 0.74$ at $p < 0.05$, with y -intercept at 0), (B) 5-h experiment ($r^2 = 0.97$ at $p < 0.05$, with y -intercept at 0). Dashed line indicates limit of detection based on filter background at 1 h ($n = 3$ rinsed filters); cell number was determined at hour 1 and assumed to be constant over 5 h.

phytoplankton (Table 3). All species exhibited a similar wash profile to that of the filter control (not shown). As with iodide, the wash data suggested that much of the label was associated with cell and filter surface adsorption.

The precision of the accumulation procedure was determined using replicates ($n = 3$) for several of the species used (Table 3). *P. glacialis* accumulated iodate over the entire period (Fig. 4), and the amount of label present (cell + filter) was significantly greater than the filter controls. The iodate accumulation rate for *P. glacialis* was greater than all the other phytoplankton. The accumulation data points for days 1 and 2 for *S. costatum*

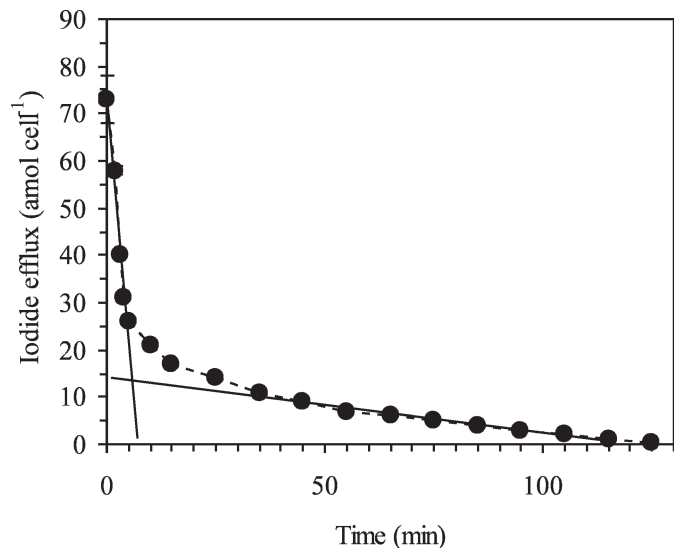


Fig. 3. Iodide efflux by *Porosira glacialis* ($n = 3$, ± 1 SD). Free-space efflux was calculated by linear regression analysis of points 1 through 5 ($10.04 \text{ amol cell}^{-1} \text{ min}^{-1}$; $r^2 = 0.97$). Cellular efflux was calculated by linear regression analysis of points 9 through 18 ($0.13 \text{ amol cell}^{-1} \text{ min}^{-1}$; $r^2 = 0.94$).

and *Phaeocystis* sp. (Fig. 5) were based on cell + filter counts, which were not significantly different from the filter controls.

A synopsis of growth and iodate accumulation by the various species is outlined as follows: *P. glacialis* (5 d; Fig. 4A) showed no growth and strong linear iodate accumulation over the entire period. *S. costatum* (Fig. 5A) showed little growth and no iodate accumulation until day 3, with points below detection limit prior to day 3. *Phaeocystis* sp. (Fig. 5B) showed 1-d lag growth followed by steady growth, and iodate accumulation was steady up to day 4, with points below detection limit prior to day 3. The iodate accumulation rates for *Navicula* sp. and *N. punctata* were based on linear regressions that included data above the TLOD except for day 1; all showed growth throughout the experiment. *T. pseudonana* displayed little growth but had an iodate accumulation pattern similar to *Navicula* sp. and *N. punctata*. *E. huxleyi*, *C. neogracile*, and *Synechococcus* sp. showed no measurable uptake, while only *E. huxleyi* displayed no growth (Tables 3, 4).

Bacterial numbers during iodate uptake—Bacteria-to-phytoplankton ratios were generally higher than in the iodide cultures (Tables 4, 2, respectively), presumably because the medium was not autoclaved (see Methods). *E. huxleyi*, *S. costatum*, and *P. glacialis* had the highest B:P ratios, yet *E. huxleyi* showed no iodate accumulation, and *P. glacialis* had the highest value.

Discussion

Experimental considerations—Experiments measuring ion uptake by algal or plant cells using radioisotopes must distinguish between uptake by various cellular compartments (Walker and Pitman 1976). In plants and green algae,

Table 3. Iodate accumulation rates of marine phytoplankton determined over a 5-d period.

	Iodate accumulation ($\text{amol cell}^{-1} \text{ d}^{-1}$)	r^2 ($p < 0.05$)	I ⁻ : IO ₃ ⁻ accumulation ratio
Bacillariophyta			
<i>Chaetoceros neogracile</i>	0	—	Undefined
<i>Navicula</i> sp.*	0.14	0.62	7.8
<i>Nitzschia punctata</i> *.†	0.046	0.84	9.6
<i>Porosira glacialis</i> ‡	5.08	0.98	11.6
5-h experiment	19	0.48	89.5
<i>Skeletonema costatum</i> *.‡	0.048	0.82	3.1
<i>Thalassiosira pseudonana</i> *.‡	0.031	0.68	25.2
Prymnesiophyta			
<i>Emiliania huxleyi</i>	0	—	Undefined
<i>Phaeocystis</i> sp.*.‡	0.072	0.89	36.1
Cyanophyta			
<i>Synechococcus</i> sp.	0	—	Undefined

* One or more data points below limit of detection.

† Rates and r^2 values based on the omission of day 2 data.

‡ $n = 3$.

these compartments are the apoplast (“free space”), cytoplasm, and tonoplast (large vacuole). Most algae lack a tonoplast. Cellular uptake measures the amount of labeled ion that passes through the cell membrane into the cell. Apoplastic uptake includes that fraction of the labeled ion that enters the water associated with the cell covering (“water free space”), which has slight diffusion resistance, and that which is ionically bound to the extracellular material (significant diffusion resistance; referred to as the Donnan free space; Walker and Pitman 1976). Many phytoplankton have a polysaccharide cell wall, and some produce extracellular polysaccharide mucilage. *Phaeocystis* and *Synechococcus* have a polysaccharide-based cell covering. *Phaeocystis* can also produce large amounts of mucilage. Whereas diatoms do not have a polysaccharide cell covering, their siliceous cell covering is enclosed by an organic layer. Many diatoms, such as *Navicula* sp. and *Nitzschia punctata*, can also produce large amounts of mucilage. Polysaccharides are polyanionic at seawater pH and bind few anions. Algal cells also have extracellular proteins and glycoproteins that are attached to the cell-membrane surface, and that are constituents of cell walls and mucilage. The polypeptides of these compounds have some positive-charged groups; however, anion uptake into Donnan free space is usually small (Walker and Pitman 1976).

The various cellular compartments can be identified from both high-resolution uptake and efflux kinetic experiments. Apoplastic uptake of the labeled ions is rapid (min), and when determining uptake rates over short periods of time, when there is no significant cell growth, it can be easily distinguished from the slower, more constant cellular uptake. Likewise, ion efflux from the apoplast is much more rapid than from the cell. Iodide-125 efflux from *P. glacialis* clearly showed only two phases (Fig. 3),

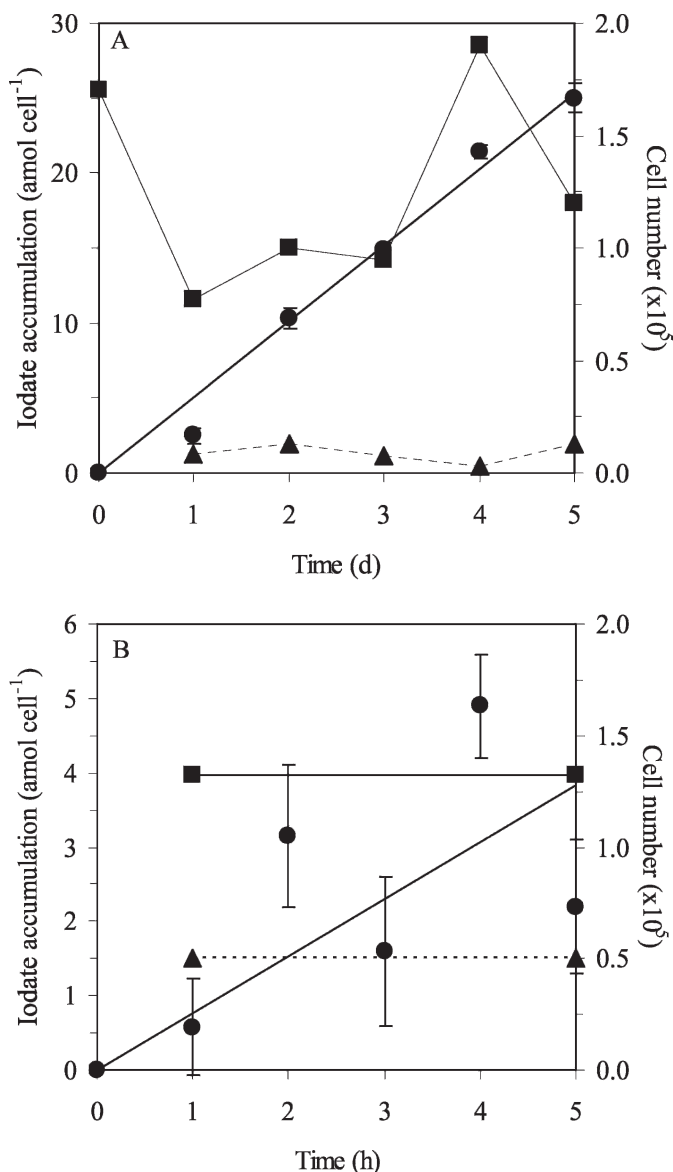


Fig. 4. Iodate accumulation by *Porosira glacialis* ($n = 3$, ± 1 SD). Circles = iodate accumulation, triangles = detection limit, squares = phytoplankton cell number. (A) 5-d experiment ($r^2 = 0.98$, $p < 0.05$ with y -intercept at 0), (B) 5-h experiment ($r^2 = 0.48$ at $p < 0.05$ with y -intercept at 0). Dashed line indicates limit of detection based on filter background at 1 h ($n = 3$ rinsed filters); cell number was determined at hour 1 and assumed to be constant over 5 h.

indicating a two-compartment system for this diatom; apoplast and cell.

Where there is significant cell growth, cellular uptake can be measured only by removing the ions from the free space. The washing protocol was used to remove labeled iodide and iodate from the free space prior to radioisotope counting. Examination of the wash data for the various species (not shown) and detailed efflux kinetics (determined for *P. glacialis* only; Fig. 3) showed successful removal. The values reported are, therefore, net cellular uptake, which equals gross cellular uptake minus any cellular efflux

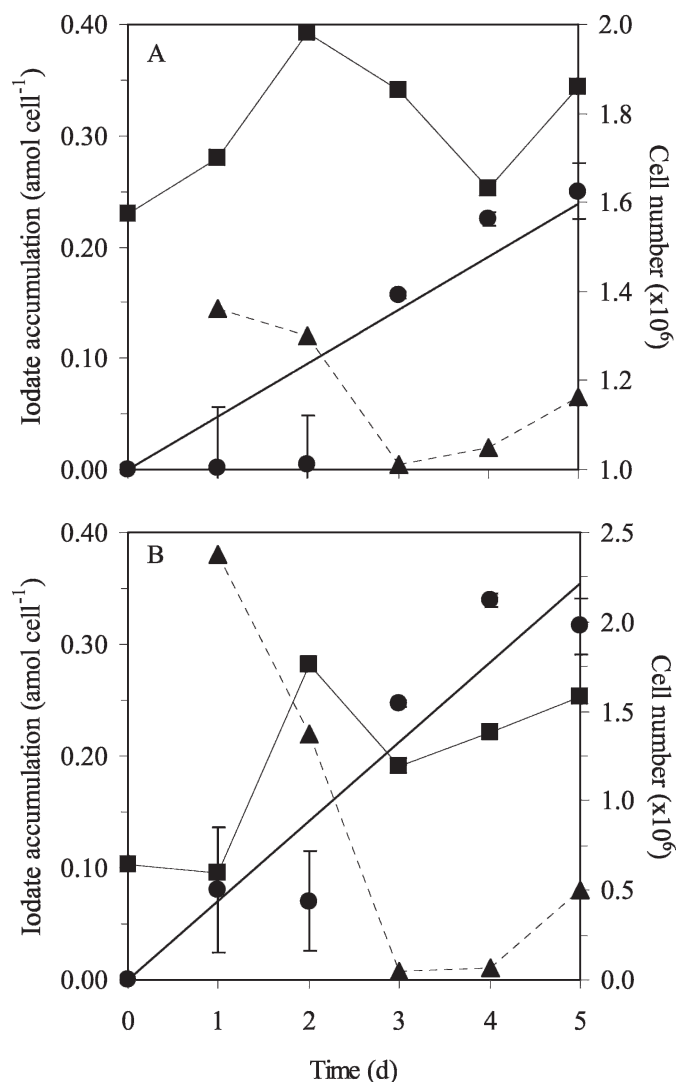


Fig. 5. Iodate accumulation by (A) *Skeletonema costatum* ($r^2 = 0.82$ at $p < 0.05$ with y -intercept at 0) and (B) *Phaeocystis* sp. ($r^2 = 0.89$ at $p < 0.05$ with y -intercept at 0). Circles = iodate accumulation, triangles = detection limit, squares = phytoplankton cell number ($n = 3$, ± 1 SD).

(loss of label from inside the cell to its surroundings). Where iodide and iodate accumulation remained linear over the course of these experiments, any interference due to free-space uptake (or another compartment) was most likely minimal.

Cellular efflux rates are generally much slower than cellular uptake rates, depending on the ion and physiological state. The calculated cellular efflux rate of iodide for *P. glacialis* was $0.13 \text{ amol iodide cell}^{-1} \text{ min}^{-1}$ (Fig. 3). This value was $\sim 2\%$ of the net cellular uptake rates reported (using the conservative rates of $56 \text{ amol iodide cell}^{-1} \text{ min}^{-1}$). The rates of iodide and iodate efflux were presumed to be small for all phytoplankton measured.

The filters used to isolate phytoplankton and hold them during the wash retained label that could not be removed by washing. Although identical in composition, there was

Table 4. Growth rates and bacterial contamination of iodate accumulation cultures.

	Phytoplankton growth rate (doublings d ⁻¹)	Bacteria-to-phytoplankton ratio		Bacteria number† (×10 ⁶ cells)
		day 0	day 5	day 5
Bacillariophyta				
<i>Chaetoceros neogracile</i>	0.33	46	20	5.7
<i>Navicula</i> sp.	0.33	11	35	17
<i>Nitzschia punctata</i>	0.196	3	16	45
<i>Porosira glacialis</i> *	-0.096	29	161	19
<i>Skeletonema costatum</i> *	0.051	0.9	2	3.0
<i>Thalassiosira pseudonana</i> *	0.035	0	0	0
Prymnesiophyta				
<i>Emiliana huxleyi</i>	-0.146	51	51	87
<i>Phaeocystis</i> sp.*	0.268	5	7	10
Cyanophyta				
<i>Synechococcus</i> sp.	0.07	0	0	0

* Replicate experiment $n=3$.

† Total cell number per 4 mL of incubation culture.

high variability in label retention among the filters. Label retention by the filters may have prevented the detection of significant iodide uptake for *E. huxleyi* and *Synechococcus*, and iodate uptake for *C. neogracile*, *E. huxleyi*, and *Synechococcus*.

In our experiments, iodide (at 100 nmol L⁻¹) assimilation was measured in the absence of iodate. Iodate (at 100 nmol L⁻¹) assimilation was measured in the presence of unavoidable trace contamination of iodide (41 nmol L⁻¹). We do not think that the presence of iodide affected the rate of iodate uptake because they most likely utilize different uptake mechanisms because of differences in size, shape, and charge separation. To our knowledge, however, there is no evidence supporting or refuting this contention.

Although it has long been known that iodide, and not iodate, is rapidly accumulated by seaweeds, it has been shown that iodide uptake by brown seaweeds actually requires a haloperoxidase-facilitated oxidation to hypoiodous acid (HOI), which enters the cell (Küpper et al. 1998). The uptake of this neutral molecule circumvents the need for proton-anion co-transport, and it probably uses a facilitated-diffusion mechanism (Küpper et al. 1998).

Bacterial considerations—Most phytoplankton cultures are available only with their associated bacteria. It is difficult to produce and maintain axenic cultures, and phytoplankton may require the associated bacteria for normal growth. It is, therefore, always a challenge to separate phytoplankton responses from those of the surrounding bacteria.

Although bacteria were present in most of the cultures, no correlation was observed between bacterial numbers and iodide or iodate accumulation. *E. huxleyi* had a high level of bacterial contamination on day 5 (2.3×10^7 cells mL⁻¹) but exhibited no detectable rate of iodide uptake, whereas mean bacterial levels on day 5 for those species that had high iodide accumulation rates (*C. neogracile*, *Nitzschia* sp., and *P. glacialis*) were much less (3.6×10^6 cells mL⁻¹). If bacteria had made a significant contribution

to the uptake of iodide, an enhanced rate of iodide accumulation should have been associated with *E. huxleyi*, which had the highest levels of bacterial contamination. *S. costatum* and *T. pseudonana* were treated with antibiotics and had minimal bacteria contamination (Tables 2, 4) yet moderate iodide uptake. These two species also had significant iodate uptake, while *C. neogracile* and *E. huxleyi*, with moderate bacterial levels, had no detectable iodate uptake. The bacteria-to-phytoplankton ratios found in culture were generally lower than those found in natural waters (Vaquer et al. 1989). We conclude that we were measuring uptake by phytoplankton and not bacteria.

Iodide accumulation and efflux—Iodide accumulation (net uptake) was found to vary greatly between phytoplankton species, and two species showed no detectable accumulation (Table 1). Positive growth rates were demonstrated by all species that exhibited low and high iodide accumulation, with the exception of *P. glacialis*, which showed an apparent decline in cell numbers (Table 2). As a group, the diatoms exhibited the highest rates of iodide accumulation. Any loss of iodide-125 due to its incorporation and loss as volatile organo-iodine (e.g., CH₃I or CH₂I₂) would not be counted as assimilated iodide by the procedure used. Production rates of CH₃I by many of the species used in this study were several orders of magnitude less than the iodide accumulation rates (Manley and de la Cuesta 1997). However, iodide accumulation may have been underestimated due to the loss of label as CH₂I₂ because production rates of 0.2–5 fmol cell⁻¹ d⁻¹ have been recorded (Moore et al. 1996).

Cellular accumulation of iodide-125 by several diatom species (*N. punctata*, *Navicula* sp., and *T. pseudonana* kinetic data not shown for these species) began to level off after day 3, and accumulation rates for these species were based on accumulation to day 3 (Table 1). This decline in the accumulation rate may have been due to several factors. If the efflux rates were significant, as the specific activity of

iodide within the cell approached the specific activity of the medium, the rate of elimination of radioactive iodine from the cells would begin to diminish the apparent rate of uptake of radioactive iodide into the cell (Moisan et al. 1994). However, the iodide efflux rate determined for *P. glacialis* was much lower than iodide uptake, and this could be true for the other phytoplankton. If the cells became extremely leaky under physiologically stressful incubation conditions, or died during the course of the experiment, labeled iodide would be released back into the solution; however, there were no noticeable lysed cells. Significant release of I-125 iodocarbons after day 3 could also have caused such a leveling off.

Iodide accumulation by *P. glacialis* over a 5-h period was 30× that of the accumulation rate determined over a 5-d period, perhaps as a result of cell stress over the 5-d uptake experiment.

The iodide accumulation curve for *Phaeocystis* sp. (Fig. 1C) showed a sharp increase within the first day and then a plateau. Little or no growth appears to have occurred until after day 3. Much of the iodide may have been adsorbed to the large mucilaginous matrix that surrounds the cells of *Phaeocystis* colonies to such a degree that washing of the cells did not remove all of the adsorbed iodide. This is supported by the finding that the other prymnesiophyte, *E. huxleyi*, which does not produce this matrix, exhibited no iodide accumulation (Table 1). The number of cells in this mucilage can increase without a proportional increase in the amount of mucilage, although there tends to be a log/log relationship between colonial volume and cell number per colony (Rousseau et al. 1990). If the *Phaeocystis* cells did indeed grow (i.e., if growth data for day 3 are erroneous), the leveling off may be due to efflux rates being equal to cell uptake. *Synechococcus* sp. exhibited no iodide accumulation, even though it, like *E. huxleyi*, exhibited positive growth rates (Table 2).

Iodate accumulation—Except for *Phaeocystis* and *Synechococcus*, the growth rates for the phytoplankton used in the iodate accumulation experiment were significantly lower than the growth rates for the same algae used in the iodide accumulation experiment. Although iodate toxicity has been reported for the diatom *Navicula* sp., this occurred at 2 $\mu\text{mol L}^{-1}$ iodate (Sugawara and Terada 1967). Iodate concentrations of 250 $\mu\text{mol L}^{-1}$ had no effect on growth of phytoplankton cultures (Waite and Truesdale 2003). The iodate concentration of 100 nmol L^{-1} used in our experiments was near the typical iodate concentration (125 nmol L^{-1}) in temperate coastal surface seawater (Truesdale 1978). If iodine is a required nutrient for phytoplankton, as it is for many macroalgae (Kuwabara and North 1980), and if its main biologically available form is iodide, as has been shown for macroalgae (Küpper et al. 1998), it is possible that the phytoplankton incubated in iodate experienced iodine-limited growth because of the low iodide concentration present (41 nmol L^{-1}).

The prymnesiophyte *Phaeocystis* sp. and the diatoms *Navicula* sp., *N. punctata*, *P. glacialis*, *S. costatum*, and *T. pseudonana* were the only species of those tested that accumulated iodate (Table 3). With the exception of *P.*

glacialis (Fig. 4A), the accumulation rates for these species included data points for days 1 and 2 that were below the theoretical limit of detection. If there was a true 2-d delay in iodate accumulation, then these rates are underestimated. The apparent delay in iodate accumulation could be attributable to the possible biological (bacterial) or chemical reduction of iodate to iodide, and the subsequent rapid uptake of radioactive iodide. However, as seen for iodide uptake, the rate of iodate uptake in each culture was not correlated to the number of bacteria present, which strongly suggests they had no discernible effect on iodate uptake. The cyanobacteria *Synechococcus* sp. did not show detectable iodate uptake, which is consistent with the findings of Waite and Truesdale (2003) but contrary to the findings of Wong et al. (2002).

Tsunogai and Sase (1969) demonstrated that nitrate-reducing bacteria have the capacity to reduce iodate to iodide via nitrate reductase (NR). More recently, NR activity from crude extracts of the diatom *S. costatum* and natural phytoplankton assemblages has shown iodate reductase activity (Hung et al. 2005). In contrast, iodate reduction by the prymnesiophyte *Isochrysis galbana* was not associated with NR activity (Waite and Truesdale 2003). Whether or not iodate is reduced by most phytoplankton NR remains unresolved. Iodate uptake and its assimilation (reduction to iodide) may be two separate processes. Iodate may share the same membrane carrier protein as one of the nutrient anions such as nitrate, phosphate, or molybdate. If so, the relative concentrations of these anions would affect iodate uptake (i.e., competitive inhibition). Higher rates of iodate uptake may occur in nitrate-depleted media. The culture medium used in our experiments was extremely rich in nitrate ($\sim 900 \mu\text{mol L}^{-1}$; with no ammonium) compared to iodate (100 nmol L^{-1}), which should have ensured the presence of NR in phytoplankton, while at the same time allowing nitrate to outcompete iodate for uptake and for reduction by NR.

There have been several studies measuring, either directly or indirectly, the uptake of iodate by phytoplankton. The present study and these other studies have used different protocols and expressed iodate assimilation differently. Moisan et al. (1994) reported iodate uptake rates by diatoms ranging from 0.48 to 5.8 $\text{nmol } \mu\text{g Chl } a^{-1} \text{ d}^{-1}$. Using Chl *a* values determined from nonexperimental but comparable cultures (data not shown), our rates translate to 1×10^{-4} to $2 \times 10^{-3} \text{ nmol } \mu\text{g Chl } a^{-1} \text{ d}^{-1}$. The rates reported by Moisan et al. (1994) were higher by factors of ~ 700 for *T. pseudonana* and 5000 for *S. costatum*. They detected iodate uptake in *E. huxleyi*, while we did not.

Although their study also measured iodate uptake using iodate-125, there were several differences in methodology from ours, which could have influenced the rates obtained: (1) A total iodate concentration of $\sim 200 \text{ nmol L}^{-1}$ was used, which is twice that of ours; (2) uptake was measured in f/20 seawater media, while we used f/2 artificial seawater; and (3) filter retention and cellular surface adsorption of label were determined independent of the experimental samples, whereas we removed label associated with each for all experimental samples prior to counting (five 3-mL washes).

The use of a higher iodate concentration would have, at best, made their measurements of iodate uptake twice that of our determinations, assuming first-order uptake kinetics. The effect of using a media concentrated in nitrate, phosphate, silicate, micronutrients, trace metals, and vitamins ($f/2$ vs. $f/20$) on iodate uptake is unknown. One possibility is that iodate uptake might be affected if it shares the same membrane carrier protein as one of the nutrient anions. If one assumes that one of these anions competitively inhibits iodate uptake in a proportional manner, then their rates of iodate uptake would be $10\times$ greater than our determination.

The difference in iodate uptake rates between this study and that by Moisan et al. (1994) may also be a result of methods used to distinguish true cellular uptake from impassive cell surface adsorption and filter retention of the iodate-125. Contrary to their results, we found a dramatic retention of radioactivity on the filters through which labeled culture medium was passed. They, however, used polycarbonate filters that had larger pore diameter (1 or 2 μm), whereas 0.45- μm pore-diameter nitrocellulose filters were used in this study. Also, they did not wash each of their samples (cells plus filter) as was done in our study, but they instead used values based on separate determinations of filter retention (after first removing abiotic particulates $<0.2 \mu\text{m}$) and cellular surface adsorption, the latter using glutaraldehyde-killed cells. Glutaraldehyde dramatically changes the surface properties of cells; it binds to and cross links proteins and thus changes the quality and quantity of available ionic sites. Treatment can also cause cell shrinkage. Glutaraldehyde-fixed cells will have different adsorption characteristics than untreated cells. Their estimate of cell surface adsorption of iodate may have been dramatically underestimated.

There have been studies measuring iodate depletion by phytoplankton with the purpose of demonstrating phytoplankton-mediated iodate reduction to iodide (Wong et al. 2002; Chance et al. 2007). These studies did not utilize iodate-125, and instead measured iodate and iodide directly in the medium; both also used $f/20$ media. Wong et al. (2002) used three iodate concentrations (ambient [360 nmol L^{-1}], $5 \mu\text{mol L}^{-1}$, and $10 \mu\text{mol L}^{-1}$) and determined iodate depletion rates (at 360 nmol L^{-1}) ranging from 0.02 (*E. huxleyi*) to 0.8 (*Amphidinium carterae*) $\text{nmol } \mu\text{g Chl } a^{-1} \text{ d}^{-1}$. If one assumes a proportional effect based on iodate concentration ($3.6\times$), and assumes that nitrate competitively inhibits iodate uptake in a proportional manner ($10\times$), our rates are very similar to that study (i.e., for *S. costatum*: their value 0.21, our value $0.4 \text{ nmol } \mu\text{g Chl } a^{-1} \text{ d}^{-1}$).

Another study of phytoplankton depletion of iodate was performed in $f/20$ media containing either 300 nmol L^{-1} or $10 \mu\text{mol L}^{-1}$ iodate (Chance et al. 2007). Cultures exposed to the lower iodate concentration depleted iodate at a rate between 2 (*E. huxleyi*) and 150 (*Nitzschia* sp.) $\text{amol cell}^{-1} \text{ d}^{-1}$. Again, if one assumes a proportional effect based on iodate concentration ($3\times$) and one assumes that nitrate competitively inhibits iodate uptake in a proportional manner ($10\times$), our values would be very similar. Chance et al. (2007) also reported that *Nitzschia* sp. (CCMP 580) cultures demonstrated a rise and fall in iodide levels during

iodate depletion and ascribed it to iodide assimilation. In our study, this species took up iodide at a high rate, as did the other cold-water species *P. glacialis* (Table 1). They also reported that *T. pseudomana* did not show significant iodide production from iodate; we measured a small rate of iodate uptake, 4% of the measured uptake of iodide. For those cases where cultures depleted more iodate from the medium than iodide produced in the medium, it is possible that released iodide was rapidly reassimilated.

All of the studies to date demonstrate that *E. huxleyi* assimilates (or depletes) iodate at a low rate compared to other species, and that diatoms tend to have much higher rates compared to other species. Our experiments do not have the ability to determine if iodate reduction occurred, and to date, studies have yet to show that phytoplankton reduction of iodate to iodide is ecologically significant. Although a variety of phytoplankton species, many of which have a global distribution, were examined in these studies (including ours), the number of different species examined was small.

Ecological relevance—Clearly iodide was the preferred species of iodine for uptake by marine phytoplankton in this study. For the species that took up iodate, the rates were much lower than the rates of iodide accumulation (Table 3). The lowest ratio of iodate to iodide is ~ 1 in very productive waters, whereas in more oligotrophic conditions, the ratio can be ~ 40 (Wong 1991). Therefore, based on the uptake rates determined from our experiments, in productive waters, iodide uptake would be favored by phytoplankton. In more oligotrophic conditions, iodate uptake could dominate. These results demonstrate that, as a group, diatoms have a proclivity to accumulate iodide and iodate, and the former is preferred. This is consistent with the finding that diatoms concentrate iodine over ambient seawater iodine levels and contain much higher cell iodide concentrations than other phytoplankton groups (Vinogradov 1953). Because diatoms readily accumulate iodide, they can affect the distribution of iodine radionuclides released from nuclear power plants and other sources into the oceans. After the Chernobyl nuclear power plant disaster, relatively high levels of ^{131}I were detected in marine diatoms (Snoeijs and Notter 1993).

Diatoms appear to have an active iodine metabolism. They can assimilate both iodate (Moisan et al. 1994; Wong et al. 2002; Chance et al. 2007; this study) and iodide (this study), and they release mono- and polyiodomethanes (Moore et al. 1996; Manley and de la Cuesta 1997). It has recently been demonstrated that diatoms, especially those from cold waters, have the ability to generate and release HOI formed from the oxidation of iodide by a putative extracellular bromoperoxidase (Hill and Manley 2009). The HOI so released can react with dissolved organic carbon (DOC) to form diiodomethane (Carpenter et al. 2005). *P. glacialis* is unusual in that it has the highest rates of iodide and iodate uptake, and HOI release ($271 \text{ fmol cell}^{-1} \text{ h}^{-1}$; Hill and Manley 2009). It would be interesting to determine its ability to reduce iodate to iodide.

One remaining question, however, is if diatoms, and phytoplankton in general, can significantly affect inorganic

iodine speciation in seawater (Truesdale et al. 2003; Waite and Truesdale 2003). Culture experiments have shown iodate reduction to iodide by phytoplankton (Wong et al. 2002; Chance et al. 2007). Another study has shown the production of iodide by certain phytoplankton species (*S. costatum*, *I. galbana*), but only at iodate concentrations not naturally found (Waite and Truesdale 2003). These results are, however, consistent with ours in demonstrating an uptake of iodate. The low rates of iodate uptake that we measured are more compatible with the results of Waite and Truesdale (2003). Our results do not show the assimilatory fate of iodate taken up.

Iodate reductase (IR) has not been identified from marine phytoplankton. IR activity has been shown to be associated with NR activity in enzyme extracts of *S. costatum* (Hung et al. 2005). However, *I. galbana*, cultured to deactivate NR, still displayed iodate reduction to iodide, although only at unnaturally high iodate concentrations (Waite and Truesdale 2003). The apparent contradiction in results may point to a taxon-specific (or species-specific) response; the NR of certain groups may have IR activity, while others may not. Waite and Truesdale (2003) suggested that iodate may serve as a photosynthetic electron acceptor that is employed when other electron acceptors are reduced, producing iodide; this remains to be demonstrated. The reduction of iodate to iodide by an extracellular dissimilatory mechanism similar to that described for an anaerobic marine bacterium (Amachi et al. 2007) has not been demonstrated for phytoplankton, nor would it be anticipated, because phytoplankton are strict aerobes, and they are photosynthetic. The methods used in our study would not have detected a dissimilatory mechanism.

Estimations of annual global accumulation of iodide by phytoplankton, using the cell-normalized rates of iodide accumulation (Table 1), converting cell number to grams dry weight (Parsons et al. 1961; g dry wt cell⁻¹: 2.3×10^{-10} for *Nitzschia* sp., *N. punctata*, *Navicula* sp., *S. costatum*, *T. pseudonana*; 6.7×10^{-8} for *P. glacialis*; 3.4×10^{-11} for *Phaeocystis* sp.), and using 1.3×10^{15} g dry wt for the global phytoplankton standing crop (Whittle 1977), range from 2.4×10^{12} g yr⁻¹ to 2.9×10^{14} g yr⁻¹, with a mean of 6.6×10^{13} g yr⁻¹. Estimates of global iodide accumulation by phytoplankton are much greater than those estimated for macroalgae (10^{11} g yr⁻¹; Manley 1984).

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