Bromoperoxidase and iodoperoxidase enzymes and production of halogenated methanes in marine diatom cultures

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Abstract Halogenated methanes produced in the oceans are important as carriers of chlorine, bromine, and iodine into the atmosphere. There they play roles in the regulation of ozone in the stratosphere and perhaps in the Arctic troposphere at polar sunrise. While the mechanisms for the production of some polyhalogenated compounds by marine macrophytes have previously been substantially elucidated, the same has not been true in the case of marine phytoplankton. We describe laboratory experiments on the production of various brominated and iodinated compounds in cultures of marine diatoms, obtained from the Provasoli-Guillard Center for Culture of Marine Phytoplankton collections (Bigelow Laboratory for Ocean Sciences, Maine, USA; CCMP). Species examined included *Nitzschia* sp. (CCMP 580), *Nitzschia arctica*, *Porosira glacialis*, and two *Navicula* sp. (CCMP 545 and 546). A suite of brominated compounds, notably bromoform and dibromomethane, is produced by the *Nitzschia* and *Porosira* species. *Nitzschia* sp.(CCMP 580) was grown in sufficient quantities to allow the identification of a bromoperoxidase enzyme, which is assumed to be responsible not only for the $CHBr_3$ and CH_2Br_2 production but also for CH_2I_2 which was measured in those cultures. Chloroiodomethane was produced, either directly by the algae or by a photochemical reaction of CH_2I_2. One *Navicula* species (CCMP 545), found to produce CH_2I_2 and CH_2ClI, was shown to possess an iodoperoxidase. Bromoform and dibromomethane were not detected in cultures of this species. Other compounds produced in certain of these non axenic cultures included methyl and ethyl iodide, and bromoiodomethane.

Introduction

Halogenated methanes produced in the oceans are important as carriers of chlorine, bromine, and iodine into the atmosphere. Those with longer atmospheric lifetimes such as methyl chloride and bromide are able to carry the halogens into the stratosphere where they are involved in the natural regulation of ozone concentrations [*Molina and Rowland*, 1974; *Wofsy et al.*, 1975; *Solomon et al.*, 1994]. Those with shorter lifetimes can, in some cases, affect tropospheric chemistry, for example, by acting as a source of bromine atoms [*Barrie et al.*, 1988], or, in the case of the iodine compounds, play an important role in the transfer of iodine from the oceans to the terrestrial environment. When intense convection occurs in the troposphere, even the short-lived compounds can be transported to the stratosphere [e.g. *Kritz et al.*, 1993]; furthermore, low concentrations of iodine could exert a potentially significant role in the lower stratosphere on account of its predominant speciation as the free radicals, I and IO [*Solomon et al.*, 1994].

It has long been known that marine macrophytes (seaweeds) produce a wide range of halogenated compounds, some of them volatile [*Fenical*, 1981; *Gschwend et al.* 1985;

Nightingale et al., 1995]. Only rather recently has clear evidence been provided for the production of volatile halogenated organic compounds by some species of marine microalgae (phytoplankton) [*Sturges et al.*, 1992; *Tokarczyk and Moore*, 1994; *Tait and Moore*, 1995]. The significance of phytoplankton as producers is that they have an ocean-wide distribution, unlike macrophytes which are largely confined to the coastal zone. All of the diatoms chosen for this study are Arctic diatoms and were chosen primarily due to the strong correlation observed in the Arctic between tropospheric ozone loss and organic bromine in the troposphere [*Barrie et al.*, 1988].

In this paper we describe experiments which demonstrate the production, in unialgal laboratory cultures, of a range of halogenated methane compounds, notably bromoform ($CHBr_3$), dibromomethane (CH_2Br_2), dibromochloromethane ($CHBr_2Cl$), diiodomethane (CH_2I_2), and chloroiodomethane (CH_2ClI). Gas Chromatograph Mass Spectrometer, GC-MS, measurement of headspace gases from some plankton cultures did provide information on a number of other compounds, including methyl chloride (CH_3Cl), methyl bromide (CH_3Br), and bromoiodomethane (CH_2BrI), which were not covered by our normal technique of analyzing liquid samples by GC with electron capture detection. We also show that in the diatom species investigated, haloperoxidases are present and are probably involved in the biosynthesis of certain of these compounds.

Methods

Non axenic cultures of the following diatoms were obtained from CCMP: *Nitzschia* sp. (CCMP 580), *Nitzschia arctica* (CCMP 1116), *Nitzschia seriata, Porosira glacialis* (CCMP 651), *Navicula* sp. (CCMP 545), and *Navicula* sp. (CCMP 546).

The experiments used Pyrex glass vessels (600 mL) which were filled, leaving a headspace of approximately 100 mL, with f/2 enriched Sargasso Sea water [*Guillard*, 1972], autoclaved, and cleared of volatiles by sparging with ultra pure air enriched with CO_2. They were inoculated with small aliquots (appoximately 2 mL) of monoalgal cultures in a sterile hood using a glass syringe and wide-bore needle. The inocula were at mid-logarithmic growth stage. The concentration of CO_2 in the gas mixture was approximately 900 parts per million (ppm) checked at the beginning of the experiment with the use of an infrared CO_2 analyzer. The pH of the cultures was monitored during the course of the experiments, it started at about 7, and at no time did it exceed 8.5.

After inoculation, algae and medium were purged with a low flow of the air/CO_2 mixture for a further 24 hours. Time zero samples were then withdrawn for cell counts and halocarbon analyses, and the culture vials were sealed using stainless steel (Swagelok) fittings. All species, except when irradiance conditions are specified to be different, were cultured for GC analysis in a laboratory incubator at 6°C. Continuous cool white light at 12 μmol quanta m^{-2} s^{-1} was used as deemed appropriate for high latitude algae. In one experiment, *Porosira glacialis* and *Nitzschia* sp. were cultured for GC-MS analysis in 40 μmol quanta m^{-2} s^{-1} of continuous cool white light. In addition to the algal cultures, a sample of medium with no algal inoculation was kept as a control in an identical flask and treated in the same manner as the algae.

Twice each week the cultures were sampled for algal cell concentrations and halocarbons. The culture vessel was first gently swirled to mix the contents, then the air/CO_2 line was connected and the vessel slightly pressurized to prevent contamination by laboratory air during sampling. A sample of approximately 2 mL was removed for cell counting using a glass syringe and a 20-cm-wide bore steel needle. Cell counts were done on 10-μL samples using a Fuchs-Rosenthal grid within 6 hours of collection. Duplicates were counted, and if they were not within 10% of each other, quadruplicates were counted. After the cells had settled in the culture vessel a further 8 mL was withdrawn for Gas Chromatograph- Electron Capture Detector, GC-ECD, analysis, and the vessel was resealed.

Unfiltered samples (5 mL) were injected into a glass purge vessel of a Tekmar LSC 2000 purge and trap system and purged with helium (40 mL min^{-1}) for 5 min at 40° C. Halocarbons were trapped on a Tenax trap at room temperature and water vapor was removed by flushing the trap with the flow of helium for a further 2 minutes. Next, the trap was preheated to 180°C at which point the He carrier gas stream was diverted through the trap at 7 mL min^{-1} and desorption effected at 185°C for 2 min. The desorbed gases passed through the Tekmar Moisture Control Module held at 5°C for further removal of traces of water vapor and then passed into a 30m DB-624 column in Varian 3400 GC. The column was held at 35°C for 5 min and programmed to 110°C at 5°C min^{-1}.

Chromatograms were collected on computer and later reintegrated with the Varian Star Integrator software.

Samples of laboratory purified distilled water were analyzed between successive algal samples in order to eliminate the possibility of cross contamination and at the beginning and the end of a daily series. Standards were prepared by serial dilution of the pure compounds; in the first step the dilution of the compounds into methanol was checked gravimetrically. Concentrated standards were stable for a few months. A working standard was prepared daily by dilution of a methanol solution with the laboratory purified distilled water, and small aliquots (2-8 μL) of this were injected through a septum port into the purge vessel containing prepurged medium. Detection limits and precisions of the analyses (based on measurements of standards) were CHBr$_3$, 0.11 pM, precision, 4%; CH$_2$Br$_2$, 0.04 pM, precision, 2.2%; CH$_2$ClI, 0.02 pM, precision, 0.8%.

In one experiment gas samples were also collected for GC-MS analysis. A 20 cm 1/4-inch steel tube, internally electropolished and fitted at each end with a stainless steel bellows valve (Nupro), was used to contain each gas sample. This was first warmed to about 80°C while flushed with high-purity air and then cooled in a chamber over liquid nitrogen to a temperature of -150°C. A stream of air at approximately 50 mL min^{-1} was passed via a frit through the culture vessel, through a condenser at 0°C, and then through a drying trap filled with magnesium perchlorate. This stream then flowed through the cold trap until 1 L of gas had been sampled, at which time the Nupro valves were closed and the exits capped with Swagelok fittings as a further precaution against leakage. The tubes were stored in a deep freeze (approximately -20°C) for several weeks before being analyzed. Further details of the GC-MS measurement procedures are given by *Moore et al.* [1994].

To examine species for the presence of haloperoxidases, large cultures of *Nitzschia* sp. (CCMP 580) and *Navicula* sp. (CCMP 545) were grown. Algal material was filtered, stored deep frozen at -70°C, and transported on dry ice before analysis.

To extract and purify the enzymes, 3 g (wet weight) of *Nitzschia* sp. (CCMP 580) was homogenized in 20 mL 0.1 M Tris/HCl (pH 8.3) and centrifuged for 30 min at 12,000 x g. The supernatant containing bromoperoxidase activity was applied to a 2-mL column of DEAE-Sephacel (Pharmacia). The column was first extensively rinsed with 0.1M Tris/HCl (pH 8.3). The enzyme was eluted from the column with 1.0 M NaCl in 0.1M Tris/HCl (pH 8.3), and fractions of 0.5 mL were collected and tested for brominating activity [*Manthey and Hager*, 1981].

Brominating activity was determined spectrophotometrically by measuring the bromination of monochlorodimedone (MCD) (ϵ = 20.2 mM^{-1} cm^{-1} at 290 nm) in a medium with 0.05 mM H$_2$O$_2$, 0.1 M KBr, 50 mM MCD, and either 0.1 M potassium citrate (pH 4-7) or 0.1 M phosphate (pH 7-8). For inactivation/reactivation experiments the enzyme was first dialyzed against 0.1 M citrate (pH 8.3) with 1 mM ethylenediamine-tetraacetic acid (EDTA) and then against 0.1 M Tris/HCl (pH 8.3). For a vanadium-containing enzyme this treatment will result in the loss of enzymatic activity which can be restored by the addition of vanadate (VO_4^{3-}) [*Wever and Kustin*, 1990].

For the *Navicula* sp. (CCMP 545), 5 g of the algae was homogenized and partially purified on a DEAE Sephacel

column as above. The supernatant was tested for brominating activity as above and also tested for iodoperoxidase activity as measured by the triiodide formation [Alexander, 1962]. The assay mixture was 10 mM in KI, 0.1 nM in H_2O_2, and 100 mM in potassium phosphate (pH 6.7). Addition of the enzyme started the reaction which resulted in an increase in absorbance at 350 nm due to the formation of I_3^-.

Results and Discussion

The findings of these experiments will be presented starting with the products of the Nitzschia sp. (CCMP 580), Nitzschia arctica, and Porosira glacialis cultures which were characterized by brominated dihalomethanes and trihalomethanes. Next, the results of the Navicula cultures will be discussed. It will subsequently be shown how the product patterns may be related to the enzyme systems of the respective algae. One of the species, Nitzschia seriata, was not seen to produce any of the polyhalogenated halocarbons examined here. Table 1 summarizes which diatoms were observed to produce which compounds. However, one must keep in mind that the absence of the production of a specific compound in our laboratory experiments does not exclude that production under other conditions.

The growth curves of the algae are shown in Figure 1a, 1b, and 1c, showing algal growth curves for the two experiments with Nitzschia sp. and Porosira glacialis that differed in the light intensity used, as well as a third experiment with Nitzschia arctica. Production of $CHBr_3$ and CH_2Br_2 is shown in Figure 1d -1i.

Dihalomethane and Trihalomethane Production

In the case of one of the Nitzschia species, N. seriata, no halocarbon production was observed (not shown). For the other two species of Nitzschia and for Porosira glacialis, $CHBr_3$ was the predominant halocarbon product followed by CH_2Br_2 (Figure 1). $CHBr_2Cl$, not shown, was measured at typically 20 times lower concentrations than $CHBr_3$, with maximum concentrations of approximately 90 pM at higher light levels and approximately 30 pM at the lower irradiance. For all of these compounds, concentrations started at control levels, increased during the growth of the culture, and then maintained almost constant high levels or in some instances decreased. Two reasons may be offered for declines in concentration. There is some loss of volatile compounds during sampling when the headspace was briefly flushed with high purity air and removal of larger amounts of material in the set of cultures used for GC-MS analysis (identified in Figure 1 as the experiment with higher irradiance levels). Such losses would have to be combined with relatively low production rates at this stage of the culture.

As well as producing dibromomethane and tribromomethanes, the Nitzschia arctica, Nitzschia sp. (CCMP 580) and Porosira glacialis cultures showed the presence of CH_2ClI at levels up to 1000 pM (Figure 2). The Nitzschia sp. culture also showed clear evidence for production of CH_2I_2, with concentrations of up to 3500 pM being measured (Figure 2). There are several possibilities for the source of the CH_2ClI. One is that it is produced directly by the organism; a second is that it is produced by reaction of

Table 1. Diatom Species and the Presence or Absence of Significant Halocarbons Examined and Enzymes Examined

Nitzschia sp. (CCMP 580)	Nitzschia arctica	Nitzschia seriata	Porosira glacialis	Navicula sp. (CCMP 545)	Navicula sp. (CCMP 546)	
yes	yes	--	yes	--	--	CH_3Br
yes	yes	--	yes	--	--	CH_2Br_2
yes	yes	--	--	yes	--	CH_2I_2
yes	yes	--	yes	yes	--	CH_2ClI
yes	--	--	yes	--	--	CH_3I
yes	--	--	yes	--	--	C_2H_5I
yes	--	--	yes	--	--	CH_2BrI
b	nt	nt	nt	i	nt	enzyme

yes means significant quantities of the compound or enzyme were produced and differed substantially from the control treatment.; - means either insignificant quantities were produced or it was not possible to say that the quantities were substantially different from the control treatment. This does not mean that these organisms are unable to produce this compound or enzyme; b means that a bromoperoxidase enzyme was found; .i means that an iodoperoxidase enzyme was found; nt means that these organisms were not tested for this compound or enzyme. CCMP is the Provasoli-Guillard Center for Culture of Marine Phytoplankton Collections.
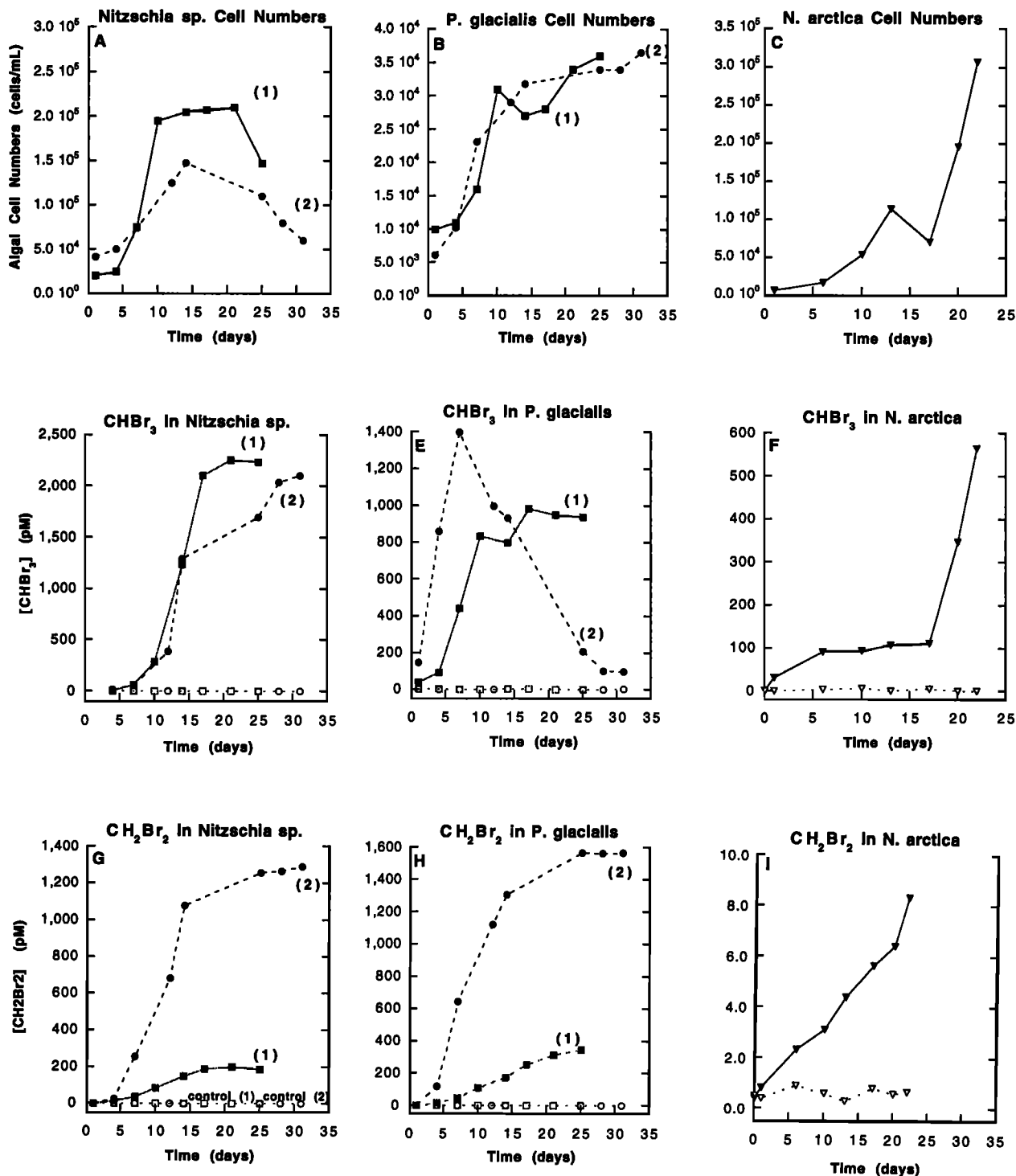
Figure 1. Growth curves of *Nitzschia* sp. (CCMP 580), *Porosira glacialis*, and *Nitzschia arctica*, and production of CHBr$_3$ and CH$_2$Br$_2$ in those cultures. Solid lines with solid squares are from the low light experiment 1, dashed lines with solid circles are from the higher light experiment 2, and solid lines with solid inverted triangles are from the *N. arctica* culture. The open symbols are the controls in their respective experiments. (a) Growth curves of *Nitzschia* sp.; (b) Growth curves of *P. glacialis*; (c) Growth of *N. arctica*; (d) production of CHBr$_3$ in the *Nitzschia* sp. culture; (e) production of CHBr$_3$ in the *P. glacialis.* culture; (f) production of CHBr$_3$ in the *N. arctica* culture.;(g) production of CH$_2$Br$_2$ in the *Nitzschia* sp. culture.; (h) production of CH$_2$Br$_2$ in the *P. glacialis.* culture.; (i) production of CH$_2$Br$_2$ in the *N. arctica* culture.

CH$_2$I$_2$ with chloride in the presence of light [*Class and Ballschmiter*, 1987; *Moore and Tokarczyk*, 1993]. This is consistent with the decline in CH$_2$I$_2$ and concurrent increase in CH$_2$ClI after the period of active algal growth (Figure 2a

and 2c). A combination of these processes might be occurring. It should be noted that the decline in CH$_2$I$_2$ is larger than the observed increase in CH$_2$ClI; this may be due to subsequent reaction of CH$_2$ClI photochemically and by nucleophilic
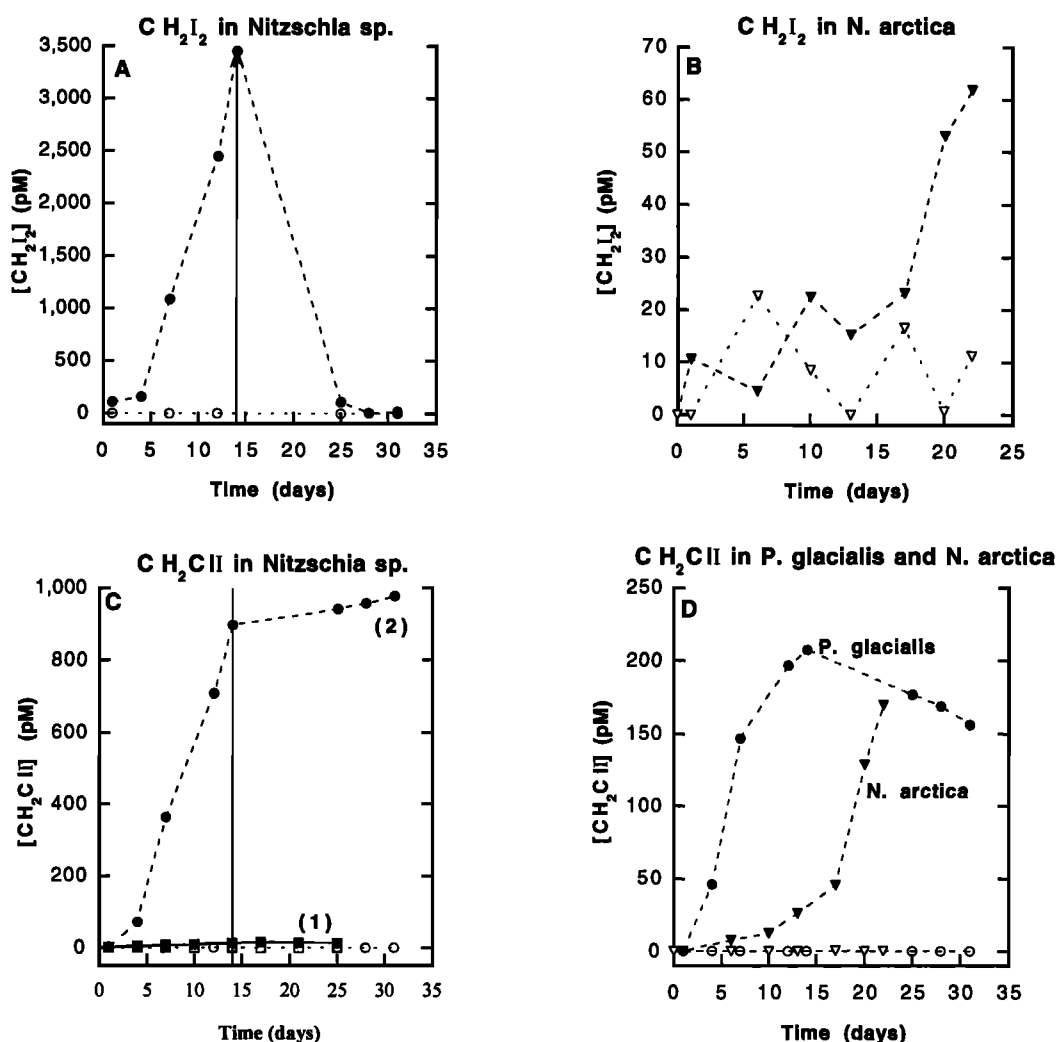
Figure 2. CH$_2$I$_2$ production by *Nitzschia* sp. (CCMP 580) and *Nitzschia arctica* and CH$_2$CII production in cultures of *Nitzschia* sp., *N. arctica*, and *Porosira glacialis*. Solid line with solid squares is from the low light experiment 1, dashed lines with solid circles are from the higher light experiment 2, and dashed lines with solid inverted triangles are from the *N. arctica* culture. The open symbols are the controls in their respective experiments. The vertical lines in Figures 2a and 2c show when cell growth stopped, CH$_2$I$_2$ production stopped, and CH$_2$CII production continued. (a) CH$_2$I$_2$ production in the *Nitzschia* sp. culture.; (b) CH$_2$I$_2$ production in the *N. arctica* culture.; (c) CH$_2$CII production in cultures of *Nitzschia* sp.; (d) CH$_2$CII production in cultures of *N. arctica* and *P. glacialis*.

reaction with Cl⁻. Dichloromethane, an expected product, was not measured. Diiodomethane might also react in seawater, yielding products in addition to CH$_2$CII.

It is in the production of the two iodinated compounds, CH$_2$CII and CH$_2$I$_2$, that similarity is seen in the halocarbon production by the group of organisms discussed so far and by *Navicula* sp. (CCMP 545). This species produced both of these iodinated compounds (Figure 3) but did not yield brominated compounds such as bromoform. A second *Navicula* species (CCMP 546) produced neither compound, a result which cannot be attributed simply to differences in the abundance of the algal cells since the CH$_2$I$_2$ normalized to cell concentration of the *Navicula* sp. (CCMP 545) was more than triple that of the *Navicula* sp. (CCMP 546) culture (Figure 3).

Haloperoxidases

Two phytoplankton species, *Nitzschia* sp. (CCMP 580) and *Navicula* sp. (CCMP 545), representing the

organobromine- and organoiodine-producing groups of diatoms, respectively, were grown in bulk so as to provide sufficient material for determining whether haloperoxidase enzymes were present. These enzymes, which catalyze the destruction of hydrogen peroxide, are widespread in nature and have been shown to be present in various species of macrophytes [e.g., *Wever et al.*, 1991]. Bromoperoxidases catalyze iodination and bromination reactions and form polyhalogenated (iodinated or brominated) products. There is substantial evidence that the presence of brominated methanes such as CHBr$_3$ and CH$_2$Br$_2$ found in coastal areas is due to the activity of peroxidases present in brown, red, and green macroalgae (for reviews see *Wever*, [1993] and *Wever et al.*, [1993]).

It should be noted that these peroxidases differ significantly in their specificity toward the halides they are able to oxidize. The iodoperoxidases are able to oxidize only iodide [*De Boer et al.*, 1986], whereas the bromoperoxidases
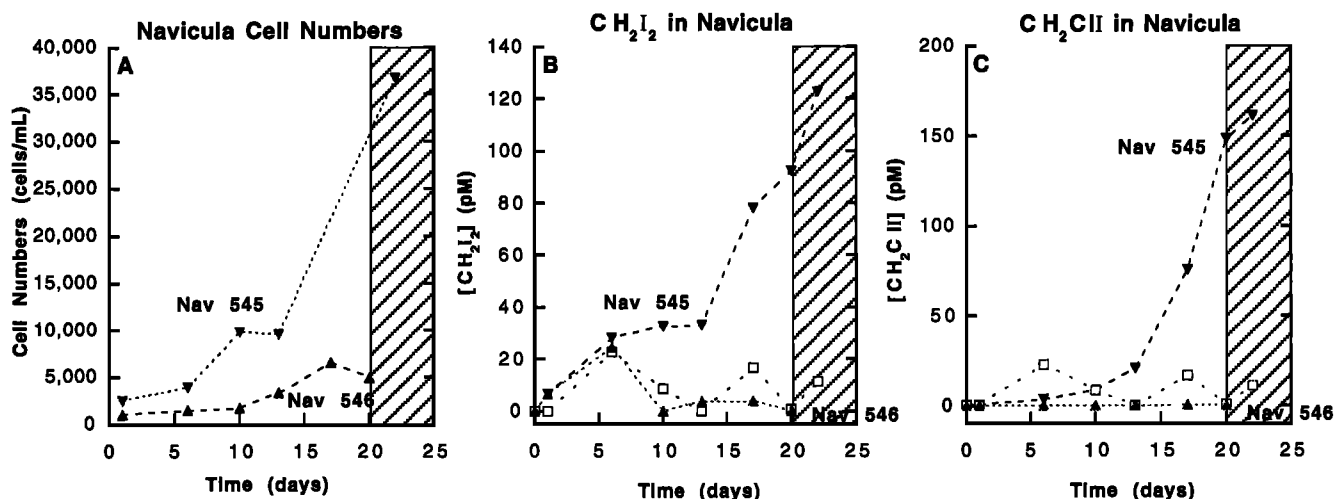
Figure 3. Growth curves of two *Navicula* sp. (CCMP 545+ 546) and production of CH_2I_2 and CH_2ClI in those cultures. dashed lines with solid inverted triangles are for the *Navicula* sp. (CCMP 545) (Nav 545); dashed lines with solid triangles are for the *Navicula* sp. (CCMP 546) (Nav 546); dashed lines with open squares are for the control treatment. The shaded portions cover the time that Nav 545 was kept in the dark. (a) *Navicula* growth curves; (b) production of CH_2I_2 in *Navicula* cultures; (c) production of CH_2ClI in *Navicula* cultures.

are able to oxidize both iodide and bromide. It has been reported, however, that a bromoperoxidase is also able to oxidize chloride, albeit at a very slow rate [Soedjak and Butler, 1990]. These enzymes generally will oxidize first iodide, followed by bromide then chloride, dependent on the specific activity and K_m for the halide, as well as their pH optimum.

Two pathways have been postulated by which brominated compounds are produced by seaweeds. *Theiler et al.* [1978] demonstrated production of $CHBr_3$ and CH_2Br_2 when a bromoperoxidase isolated from the macrophyte *Bonnemaisonia hamifera* was allowed to react with ketoacids. Bromination of ketoacids will yield a number of unstable intermediates which decay via the haloform reaction to form $CHBr_3$ and CH_2Br_2, and it was proposed that this reaction also occurred in the plant. It follows that when a bromoperoxidase is present in a macrophyte, mixtures of chlorinated, brominated and iodinated compounds will be formed. If, however, an iodoperoxidase is present in a seaweed, mainly iodinated and maybe some brominated compounds will be produced.

Wever et al. [1991] have suggested that some seaweeds produce HOBr directly and that this compound will diffuse into seawater and react with organic materials such as humic and fulvic acids and yield volatile brominated compounds.

Considering the prime role these peroxidases play in the biosynthesis of several volatile halogenated compounds, the presence and nature of these enzymes were investigated for two of the phytoplankton species, *Nitzschia* sp. (CCMP 580) and *Navicula* sp. (CCMP 545). While the algal cultures were not axenic, the enzyme extraction procedure which was used will not break up the bacteria, and they will be spun down during centrifugation. The *Nitzschia* species tested was found to have a bromoperoxidase, and the enzymatic activity of the partially purified enzyme was characterized. When the MCD assay was carried out to determine the brominating activity, it was observed that at 1 mM H_2O_2, the absorbance decrease at

290 nm was markedly nonlinear. At lower H_2O_2 concentrations the rate of bromination of MCD was linear. This observation suggests that the enzyme is inactivated by high H_2O_2 concentrations, a phenomenon seen in heme-containing peroxidases [*Zuurbier et al.*, 1990]. The bromoperoxidase has a distinct pH optimum in the bromination reaction at pH 7.0. This value is similar to that reported for the heme-containing bromoperoxidase from the marine green alga *Penicillus capitatus* [*Manthey and Hager*, 1981].

The effect of Br^- concentration on the rate of enzymatic bromination was also studied and showed that a fairly high concentration of Br^- was required to obtain the maximal rate of the reaction. A K_m for Br^- of about 35 mM was found. For the bromoperoxidase from the green algae *P. capitatus* the affinity for Br^- is also low, in comparison with the K_m for vanadium-containing bromoperoxidases which can range from 0.02 to 12.7 mM [see *Wever et al.*, 1988]. In order to test whether the partially purified bromoperoxidase from the *Nitzschia* contained a heme as a prosthetic group or is a vanadium bromoperoxidase, the enzyme was inactivated at a low pH (see materials and methods), and then vanadate was added at a neutral pH. For a vanadium peroxidase, this treatment will result in total recovery of the brominating activity. However, recovery was not observed. This shows it is not a vanadium bromoperoxidase but is probably a heme-containing peroxidase. The susceptibility of the enzyme to high H_2O_2 concentrations would support this. That the *Nitzschia* contains a bromoperoxidase is in line with our observation that this culture produces a suite of brominated methanes which are similar to those produced by many macrophytes [*Gschwend et al.*, 1985].

Bromoperoxidase activity could not be detected in the *Navicula* species, but the microalga was found to contain an iodoperoxidase (see Methods section). Due to a limited amount of material, the iodoperoxidase could not be characterized further than showing its presence.

This is the first time that either bromoperoxidase or iodoperoxidases have been reported in any marine phytoplankton. In view of the known relationship between the presence of such enzymes in seaweeds and their production of polyhalogenated compounds, it is very likely that the production of $CHBr_3$ and CH_2Br_2 by *Nitzschia* sp. (CCMP 580) and probably by *Nitzschia arctica* and *Porosira glacialis* is due to the action of bromoperoxidases. Since a bromoperoxidase is capable of oxidizing iodide, CH_2I_2 and CH_2BrI could be produced by similar mechanisms. The distinct suite of compounds produced by *Navicula* sp. (CCMP 545) can be attributed to its containing an active iodoperoxidase. The presence of CH_2ClI found in the cultures of all of these organisms could be due either to direct production by the organisms, or to a light catalyzed reaction between CH_2I_2 and Cl^-. This is supported in the experiment with *Navicula* sp. (CCMP 545) (Figure 3). For the last 2 days of the experiment, the control (medium only) and *Navicula* sp. vessels were kept in the dark. During that time, the concentration of CH_2I_2 increased more rapidly, while production of CH_2ClI slowed. We limited this dark period to 2 days to minimize other effects to the cells.

It should be noted that our experiments suggest that phytoplankton belonging to the same genus do not necessarily produce the same halocarbons. Thus neither *Nitzschia seriata* nor *Navicula* sp. (CCMP 546) yielded brominated or iodinated compounds characteristic of haloperoxidase reactions.

Effect of varying light intensity

In a pair of experiments on *Nitzschia* sp. (CCMP 580) and *Porosira glacialis* employing different light levels, it was observed that for CH_2Br_2 and $CHBr_2Cl$ there appeared to be higher concentrations produced in the cultures with a higher level of illumination. This cannot be simply due to differences in the concentrations of cells, since in the case of *Nitzschia* sp.(CCMP 580), the cell densities were actually greater in the low light experiment, while in the case of *P. glacialis* the cell densities were similar in the two experiments. For $CHBr_3$, when the cell abundances are taken into account (Figure 1), it appears that for both species, $CHBr_3$ production was enhanced by high light levels.

These observations may be explained on the basis that the brominated compounds are products of bromoperoxidase enzymes which utilize H_2O_2 as one substrate. H_2O_2 is commonly associated with metabolism in many types of algae and in other marine microorganisms [Cooper and Lean, 1992]. In the cyanobacteria *Anacystis nidulans*, H_2O_2 has been shown to be produced in greater quantities at higher irradiances, probably through the photosynthetic generation of excess reductant [Patterson and Meyers, 1973]. Similarly, *Wever et al.* [1991] observed that in the brown seaweed *Ascophyllum nodosum*, light had an effect on the production of halogenated products by the intact plant. Increasing the illumination resulted in large increases in the amount of halogenated compounds formed. This phenomenon is probably related to an increase in the generation of H_2O_2 during photosynthesis. That light may also affect the yield and perhaps the nature of halogenated products was also observed by *Collen et al.* [1994]. While different organisms have different patterns of H_2O_2 production, [Stevens et ai., 1973], it is consistent with abiotic photochemistry that

photosynthetic organisms would all tend to have greater H_2O_2 production under increased irradiance [Cooper et al., 1988]. This could account for these observed differences between the experiments at different light intensities.

Methyl Iodide

Methyl iodide production was observed clearly only in the *Nitzschia* sp. (CCMP 580) culture held at higher illumination in which CH_3I concentration reached about 10 times the control levels (Figure 4). One would not expect CH_3I production to be linked to haloperoxidase activity as monohalogenated halocarbons are not formed via the haloform reaction. The absolute values seen in this culture, up to 25 pM, are very low in comparison with field measurements when the high biomass content of the algal culture is taken into account. While batch culture chlorophyll a (chl a) to cell numbers have a large range, to a first order one can make some approximations to compare laboratory data with field measurements. The cell concentrations of up to 150,000 cells m^{-3} may be roughly approximated as 1500 mg chl a m^{-3} [Conover, 1974]. This would give a CH_3I to chlorophyll a ratio of 17 pmol CH_3I mg^{-1} chl a. Concentrations in offshore waters of the North Atlantic reported by *Moore and Tokarczyk* [1993] ranged from less than 0.7 pM to 7 pM, while coastal waters and shallow waters of the Grand Banks had values up to 60 pM. As the chlorophyll a in the offshore waters of the North Atlantic ranged from 0.2 to 1.5 mg chl a m^{-3} [Tait, 1995], the CH_3I to chlorophyll a ratio for those waters would be 470 to 35,000 pmol CH_3I mg^{-1} chl a. Thus, although this is a very rough comparison, the field measurements are 1-3 orders of magnitude higher than the laboratory results. *Porosira glacialis* in the high light experiment did show concentrations of methyl iodide at a few times the control level, but at low light, like the *Nitzschia* sp. (CCMP 580), the values were rarely as high as twice the control levels and therefore were not significant.

Ethyl Iodide

Ethyl iodide was found in the *Nitzschia* sp.(CCMP 580) and *Porosira glacialis* cultures, again with concentrations in the higher light culture being significantly greater than in that with low light (Figure 4). With this compound, levels in the control were below the detection limit. Data from another GC-MS experiment (R.M.Moore, unpublished data, 1993) would suggest that C_2H_5I production is not dependent on the presence of a bromoperoxidase or iodoperoxidase since it was found that the diatom *Odontella mobiliensis* showed no haloperoxidase activity, producing similar concentrations of C_2H_5I but no $CHBr_3$ or CH_2ClI. This is to be expected as C_2H_5I is of the monohalogenated halocarbon group, which would not be produced via the proposed haloperoxidase mechanisms.

Bromoiodomethane

A prominent peak in the ECD traces of *Nitzschia* sp. (CCMP 580) and *Porosira glacialis* cultures was identified by GC-MS as bromoiodomethane. A standard of this compound was then prepared, and the retention time and ECD response under our normal conditions of analysis were determined. In this way it was possible to approximately quantify CH_2BrI in the two cultures (Figure 5).
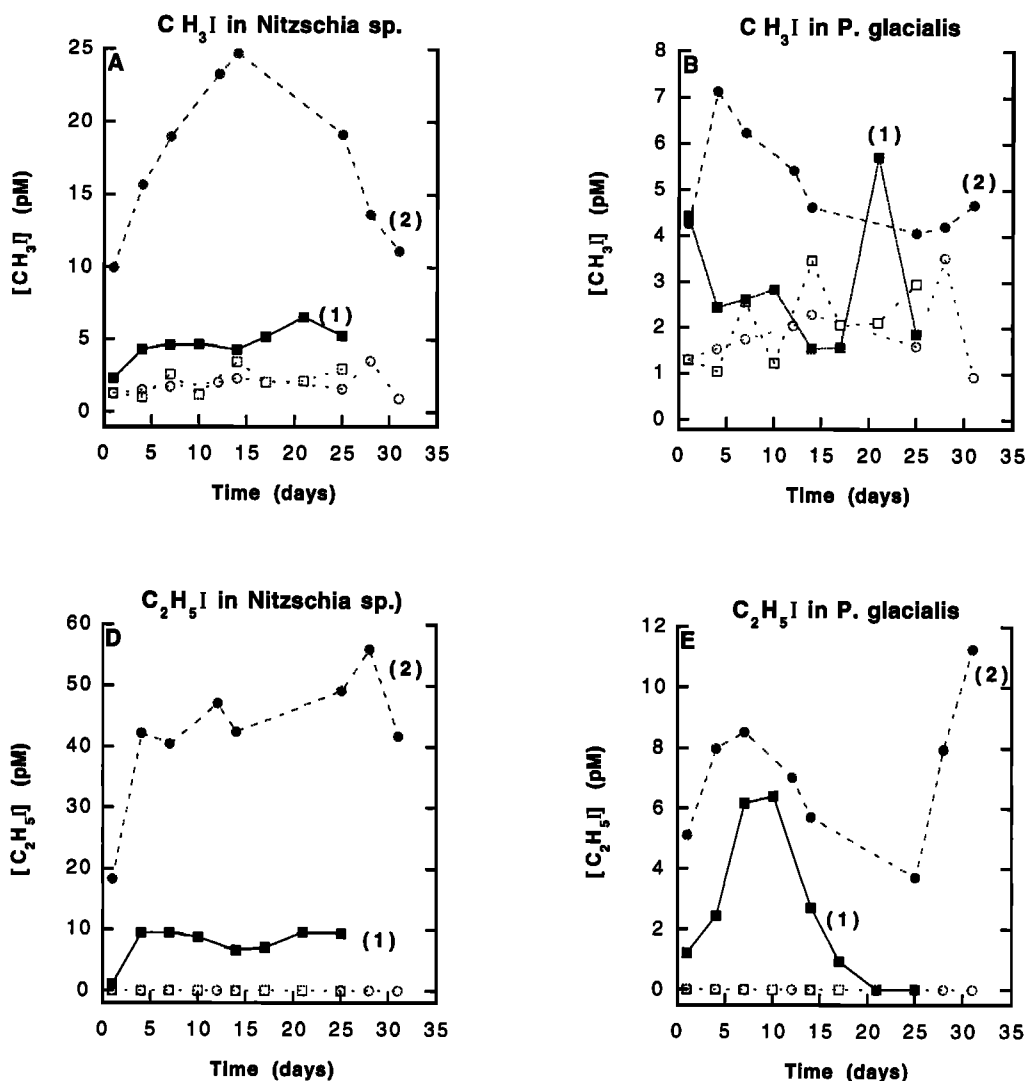
Figure 4. Production of CH₃I and C₂H₅I in *Nitzschia* sp. (CCMP 580) and *Porosira glacialis*. Solid lines with solid squares are from the low light experiment 1, and dashed lines with solid circles are from the higher light experiment 2. The open symbols are the controls in their respective experiments. (a) CH₃I in *Nitzschia* sp.; (b) CH₃I in *P. glacialis*.; (c) C₂H₅I in *Nitzschia* sp.; (d) C₂H₅I in *P. glacialis*.

It seems likely that its production mechanism could be similar to that of CH_2Br_2. We have not been able to establish retrospectively whether the compound is present in GC-ECD chromatograms of field samples such as in situ incubations of ice algae in the Beaufort Sea [Moore *et al.*, 1995] or Resolute Bay, North West Territories, Canada [*Moore et al.*, 1993]. By analogy with CH_2I_2, CH_2BrI may be expected to react with Cl⁻ in seawater with the process catalyzed by light. This would tend to lead to a relatively short lifetime for the compound in seawater in comparison with CH_2Br_2 and $CHBr_3$.

Methyl Halides

The GC-MS experiments also showed that methyl chloride and bromide were produced in all the cultures that were measured. It follows that the mechanism for their production is independent of that for polyhalogenated compounds such as $CHBr_3$. It should also be noted that mechanisms proposed for the operation of haloperoxidases [e.g., *Beissner et al.*, 1981] do not yield methyl halides (CH_3X). The reader is referred to

Tait and Moore [1995] for a discussion of methyl chloride production in phytoplankton cultures.

Conclusions

The compounds $CHBr_3$ and CH_2Br_2 have been shown to be produced by the marine diatoms, *Nitzschia* sp. (CCMP 580), *Nitzschia arctica*, and *Porosira glacialis* grown in laboratory cultures. Testing one of these species, *Nitzschia* sp. (CCMP 580), revealed that it possessed a bromoperoxidase enzyme to which we attribute the organism's brominating ability. The same species produced the iodinated compounds, CH_2I_2 and CH_2ClI. In contrast, one species of *Navicula* (CCMP 545) produced the same two iodinated compounds but no detectable $CHBr_3$ or CH_2Br_2; this organism was found to contain an iodoperoxidase enzyme.

While the existence of haloperoxidases in macrophytes is well established and can account for their production of polyhalogenated methanes, this is the first time that similar
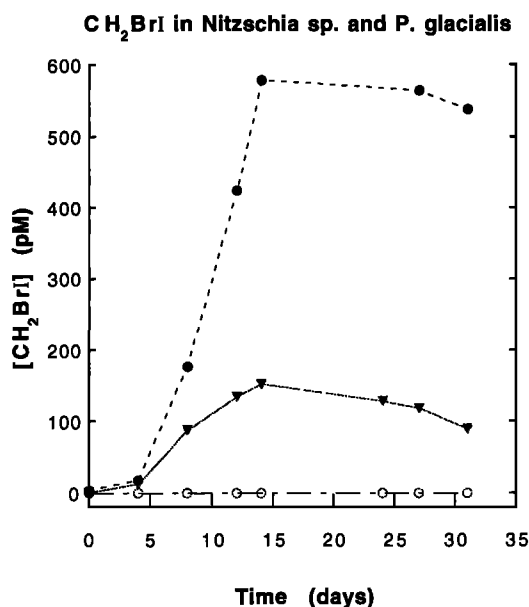
CH₂BrI in Nitzschia sp. and P. glacialis

Figure 5. CH₂BrI production by *Nitzschia* sp. (CCMP 580) and *Porosira glacialis*. The dashed line with solid circles is production in the *Nitzschia* culture and the solid line with the inverted triangles is production in the *P. glacialis* culture. The dashed line with open circles is the medium-only control.

enzymes have been shown to exist in any marine phytoplankton. High light levels promoted production of CHBr₃ and CH₂Br₂ consistent with involvement of photosynthetically produced H₂O₂ and haloperoxidase activity.

Bromoiodomethane, not hitherto reported as a product of phytoplankton, was measured in the *Nitzschia* sp. (CCMP 580) and *Porosira glacialis* cultures. Its production is consistent with the operation of a bromoperoxidase in these organisms.

Methyl iodide was measured at significant levels only in a culture of *Nitzschia* sp. (CCMP 580), and its formation does not show any link with the presence of haloperoxidase activity. Ethyl iodide was seen at significant levels in *Nitzschia* sp. (CCMP 580) and *Porosira glacialis* cultures and was found at similar levels in the culture of the diatom, *Odontella mobiliensis*, that showed no evidence for polyhalogenated compounds (e.g., CHBr₃).

GC-MS measurements which confirmed the identity of the polyhalogenated species also showed that methyl chloride and bromide, though not quantified, were present in every culture that was examined. It appears that the production of all the monohalogenated compounds occurs by mechanisms different from those for polyhalogenated compounds and independent of haloperoxidases.

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