Production of methyl halides by *Prochlorococcus* and *Synechococcus*

D. K. Brownell,[1] R. M. Moore,[1] and J. J. Cullen[1]

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[1] The ocean is the dominant source of atmospheric methyl iodide (CH_3I). The mechanisms of CH_3I production in the marine environment are poorly understood. A previous laboratory and field study suggested *Prochlorococcus marinus*, a ubiquitous marine cyanobacterium, is a globally significant biological producer of CH_3I. In this study, CH_3I concentrations were measured in cultures of *P. marinus* (high-light-adapted MED4 and low-light-adapted CCMP 1427) and the marine cyanobacterium *Synechococcus*. Cell-normalized production rates from *P. marinus* cultures (MED4) averaged 1.6 (±0.9) molecules of CH_3I cell^{-1} d^{-1}; these rates were 1000-fold lower than production rates reported for the previous study, which reported comparable concentrations of CH_3I in cultures but cell concentrations about 1000-fold lower than measured in this study. Extrapolating CH_3I production rates from the current study yields a global production rate of 0.6 Mmol yr^{-1} which accounts for 0.03% of the estimated global CH_3I production, suggesting *P. marinus* is not a globally significant source of CH_3I.

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1. Introduction

[2] The oceans are a major source of methyl iodide (CH_3I) to the atmosphere and also play a significant role in the natural cycles of methyl chloride (CH_3Cl) and methyl bromide (CH_3Br). All of these compounds are important as sources of halogens to the atmosphere and, in particular, the stratosphere where the released halogens are active in the catalysis of ozone loss [*Solomon et al.*, 1994]. For each compound, marine sources have been identified, but it has not been possible to match marine production rates with losses from the ocean, namely, efflux to the atmosphere, chemical loss (for CH_3Br and CH_3I), and biological uptake. While there has been some success in the quantifying of methyl halide production by macrophytes in coastal waters [*Manley and Dastoor*, 1987; *Nightingale et al.*, 1995; *Giese et al.*, 1999], it is the vastly greater areas of the pelagic oceans that appear to account for most of the production of methyl halides. Until recently, reported production by a number of laboratory-cultured phytoplankton [*Manley and de la Cuesta*, 1997; *Itoh et al.*, 1997; *Scarratt and Moore*, 1999] was too small to account for observed oceanic concentrations of CH_3I. This was apparently changed by *Smythe-Wright et al.*'s [2006] data indicating that the widespread photosynthetic bacterium, *Prochlorococcus marinus* (MED4), had produced CH_3I in laboratory cultures at rates that would be of major significance to its oceanic budget. The potential significance of that study, as well as the need to improve the quantification of CH_3I production, led us to repeat experiments with *P. marinus* and to extend them to another common photosynthetic bacterium, *Synechococcus*. Although the experiments were directed primarily at better understanding marine sources of CH_3I, the experiments did provide a useful but less complete data set on the production of CH_3Cl and CH_3Br by the same organisms.

2. Methods

[3] Unialgal high-light-adapted CCMP 1986 (clone MED4), low-light-adapted CCMP 1427 *Prochlorococcus marinus*, and *Synechococcus* sp. CCMP 2370 (clone WH 8102) strains were obtained from the Provasoli-Guillard National Center for Culture of Marine Phytoplankton (Maine). Stock cultures were incubated under 40 W Vita-Lite full-spectrum fluorescent bulbs using a 12:12 light:dark cycle at 60–70 μmol photons m^{-2} s^{-1} of photosynthetically active radiation (PAR). Desired light levels (20 μmol photons m^{-2} s^{-1}) for low-light-adapted *P. marinus* were achieved by the use of neutral density filters (Mylar). PAR (μmol photons m^{-2} s^{-1}) was measured with a Biospherical Instruments' Quantum Scalar Laboratory irradiance sensor (model QSL-2100) under dry operation. *P. marinus* cultures and *Synechococcus* sp. cultures were held at 20°C ± 1°C and 21°C ± 1°C, respectively. The media recipes, PRO99 and SN from the Provasoli-Guillard National Center for Culture of Marine Phytoplankton, based on the publications of *Moore et al.* [2002] and *Waterbury et al.* [1986], were made from aged coastal seawater. The phosphate concentrations in the media were modified to obtain different nutrient treatments (replete [P] = 220 μM and low phosphate (low P) [P] = 5 μM). All nutrient stock solutions were sterilized by

[1]Department of Oceanography, Dalhousie University, Halifax, Nova Scotia, Canada.

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Table 1. Summary of Experiments

Experiment	Cyanobacterium Strain	Number of Replicates per Nutrient Treatment	$^{13}CH_3I$ Injection	Biomass Measurements Obtained
1	Low-light-adapted *P. marinus* (CCMP 1427)	2	No	Yes
1	High-light-adapted *P. marinus* (MED4)	2	No	Yes
2	High-light-adapted *P. marinus* (MED4)	3	Yes	Yes
3	*Synechococcus* sp.	3	Yes	No

filtration and added aseptically to the aged coastal seawater. The media were sterilized by filtration through a 0.2 μm filter (Whatman POLYCAP 75 AS) into an autoclaved container.

[4] Batch culture experiments were used, and the concentrations of methyl iodide (CH_3I), methyl bromide (CH_3Br), and methyl chloride (CH_3Cl) were measured over time. Cultures were incubated in borosilicate glass vessels (1.4 L) containing filtered seawater medium using stock culture growth conditions. A T fitting at the top of the vessel allowed a long syringe needle to be inserted into the culture to withdraw a sample while contaminant-free air plus CO_2 (4000 ppm) was being supplied at a low flow rate through the sidearm of the T fitting to prevent influx of laboratory air. The sampling port and sidearm were sealed with Swagelok fittings while not in use. Replicate cultures (two or three) were grown, as well as phytoplankton-free controls. In the second and third set of three experiments, $^{13}CH_3I$ was added (10–20 p*M*) to the medium to monitor any CH_3I loss through processes other than chemical degradation. The culture vessels were sampled at inoculation (time of 0 days) and, once growth was observed, every 2–5 days for approximately 30 days. Samples were analyzed for biomass and methyl halides. In the *P. marinus* cultures, divinyl chlorophyll *a* (chl a_2) was measured using fluorescence by the method of *Welschmeyer* [1994] (using chlorophyll *a* as a standard) and cell concentration by flow cytometry. Duplicate 5 mL volumes of culture were filtered through two GF/F filters with their fibres laid perpendicular to each other. The filters were then placed in 10 mL of 90% acetone (vol/vol) for 24 h in the dark at −15°C.

[5] Methyl halides were extracted from a 37.2 mL volume transferred from a sample loop containing a glass pipette into a purge vessel held at 40°C. The sample was purged with a helium stream (40 mL min^{-1} for 12 min), which then passed through a cold condenser at 2°C and a magnesium perchlorate tube to remove water vapor. CO_2 was removed by an Ascarite II trap, and volatile compounds were trapped in a stainless steel tubular trap (30 cm long, 0.5 mm ID) held at −150°C over liquid nitrogen. Methyl halides were released into a helium carrier gas stream (4 mL min^{-1}) by heating the trap to 45°C and carried to a pair of DB-624 columns (ID 0.53 mm, film thickness 3 μm, J&W Scientific), comprising a precolumn (30 m) and a main column (70 m), used in a series, followed by a Finnigan TraceMS quadrupole mass spectrometer for detection. Compounds eluting after CH_3I were discarded by backflushing the first column. Selected ion monitoring (SIM) acquisition mode was used to monitor *m/z* 50, 94 and 96, 142, 143 (for CH_3Cl, CH_3Br, CH_3I, and $^{13}CH_3I$) and 53 and 55, 97 and 99, and 145 (for deuterated internal standards, CD_3Cl, CD_3Br, and CD_3I). The internal standards were injected upstream of the cryotrap with every sample and were used to correct for any drift in the detector sensitivity. Detection limits calculated as 3 times the standard deviation of the blank were 1.36, 0.05, and 0.26 pM for CH_3Cl, CH_3Br, and CH_3I, respectively. A blank measurement was made by running the system without injecting any water sample. Calibration was achieved by injection into the purge gas stream with Hamilton gas tight syringes of 100 and 200 μL volumes of a gravimetrically produced gas standard containing 1961.2 ppb CH_3Cl, 98.9 ppb CH_3Br, and 98.7 ppb CH_3I diluted in nitrogen and contained in an Aculife-treated aluminum cylinder (Scott Specialty Gases, Plumsteadville, Pennsylvania). Atmospheric temperature and pressure were recorded for each standard injected and used later to correct the volume of the syringes to standard temperature and pressure (0°C, 1013 mbar). This allowed calculation of the methyl halide concentration within the liquid phase of the culture vessels. The amount in the headspace immediately prior to the removal of a liquid sample was then calculated assuming equilibrium between the two phases and using the Henry's law constants from *Moore et al.* [1995], *Moore* [2000], and *De Bruyn and Saltzman* [1997]. The methyl halide production within the flask between two sampling times is given by the difference in total content in the flask (i.e., combined liquid phase and headspace) corrected for the loss (gain in the case of CH_3Cl) due to nucleophilic substitution reactions, calculated using the rate constants of *Elliott and Rowland* [1993], as well as the loss due to dilution of the headspace during sample removal. These production rates were then divided by the average biomass measured for the two time points to obtain biomass-normalized methyl halide production rates.

[6] Precision was measured at the end of each experiment, except for the last one, where sufficient medium was not available. The coefficients of variation (%) for the two experiments culturing *P. marinus* are ±1.0 and ±3.4 for CH_3I, ±1.5 and ±2.5 for CH_3Br, and ±1.5 and ±4.3 for CH_3Cl (n = 4). Accuracy of the measurements was estimated using the concentrations of the standard, known to be about 5%.

3. Results

[7] Three experiments were conducted in an effort to quantify the rate of methyl halide production from *Prochlorococcus marinus* and *Synechococcus* sp. cultures. High-light- (CCMP 1986, also known as MED4) and low-light-adapted (CCMP 1427) strains of *P. marinus* were cultured in the first experiment, high-light-adapted *P. marinus* was cultured in the second, and *Synechococcus* sp. (CCMP 2370) in the third (Table 1). The experiments using the high-light-adapted strain of *P. marinus* (MED4) showed similar results among replicates and good reproducibility between the two experiments.

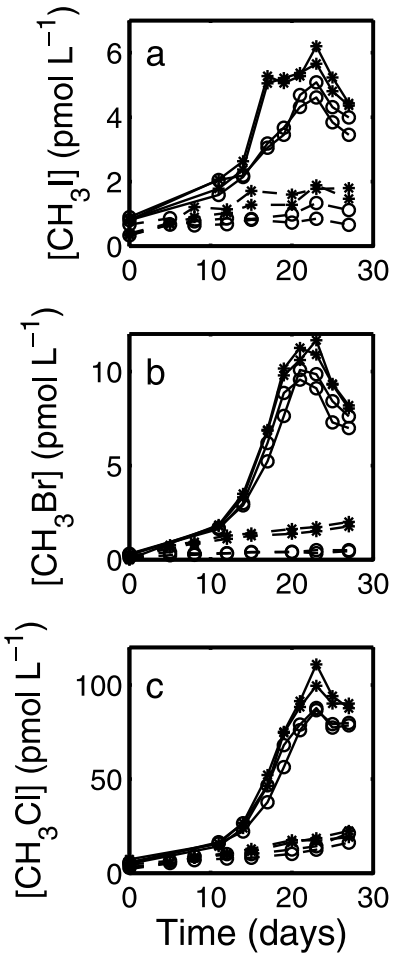

Figure 1. Liquid phase concentration of (a) CH_3I, (b) CH_3Br, and (c) CH_3Cl measured from high-light-adapted strain of *P. marinus* (MED4) cultures grown under low-P (asterisks) and replete (circles) nutrient conditions (experiment 1). Controls are shown as dashed lines.

[8] The difference between the two experiments on the high-light-adapted *P. marinus* was that in the second experiment, $^{13}CH_3I$ was added to provide information on loss rates of CH_3I to show whether there were losses in addition to chemical reaction with Cl^- and the effect of withdrawing samples. In each experiment, *P. marinus* was grown under two nutrient treatments (low P and replete). The biomass-normalized rates of production of methyl halides were similar in both experiments, but a higher background level of CH_3I existed in those to which $^{13}CH_3I$ had been added, apparently due to the presence of $^{12}CH_3I$ in the added $^{13}CH_3I$. For this reason, the production of methyl halides will be illustrated for experiment 1 (Figure 1), but the rates of production of methyl halides normalized to biomass are provided for both experiments (Table 2). The ordinates in Figure 1 are the measured concentrations of methyl halides in the liquid phase. The growth of the cultures was quantified by measuring the chlorophyll a_2 (μg L^{-1}) and cell concentrations (cell mL^{-1}); these quantities are presented in Figure 2. Average rates of production of CH_3I (together with

Table 2. Average Biomass-Normalized Production Rates of Methyl Halides Measured in *P. marinus* Cultures Under Low P and Replete Conditions[a]

	Biomass-Normalized Production Rates					
	Molecules per Cell per Day			Femtomoles per Microgram chl a_2 per Day		
	Average	1 SD	Maximum	Average	1 SD	Maximum
Experiment 1, n = 8 (Days 11–21)						
CH_3I						
Low [P]	1.7	1.5	4.2	2.4	2.1	5.2
Replete	1.3	0.5	2.1	2.1	0.8	3.4
CH_3Br						
Low [P]	4.1	1.3	5.6	6.7	2.3	9.8
Replete	4.5	1.4	5.9	6.2	2.4	10.8
CH_3Cl						
Low [P]	31.2	8.5	40.4	42.7	13.1	64.0
Replete	25.1	6.5	32.1	38.0	10.0	52.5
Experiment 2, n = 12 (Days 13–21)						
CH_3I						
Low [P]	1.6	0.9	3.2	3.3	1.8	6.1
Replete	1.6	0.5	2.4	2.7	1.0	4.8
CH_3Br						
Low [P]	15.9	14.4	43.1	13.1	2.3	15.6
Replete	7.8	2.3	12.5	12.9	2.9	20.3
CH_3Cl						
Low [P]	74.8	19.5	96.6	149.6	38.5	206.6
Replete	85.4	32.7	169.0	139.5	35.0	191.0

[a]Low P, initial [P] = 5 μM; replete, initial [P] = 220 μM. Two experiments are presented here. The *n* is the number of time steps used to calculate the mean and standard deviation. Experiment 1 used four time steps from two replicate flasks; experiment 2 used four time steps from three replicate flasks.

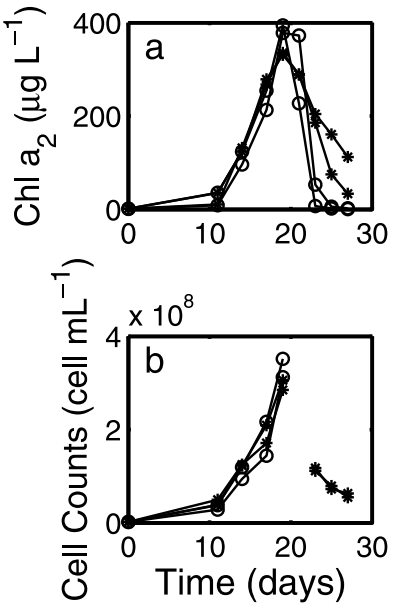

Figure 2. (a) Chl a_2 and (b) cell abundances measured from cultures in experiment 1 versus time. Circles and asterisks indicate cultures grown under replete and low-P nutrient conditions, respectively.

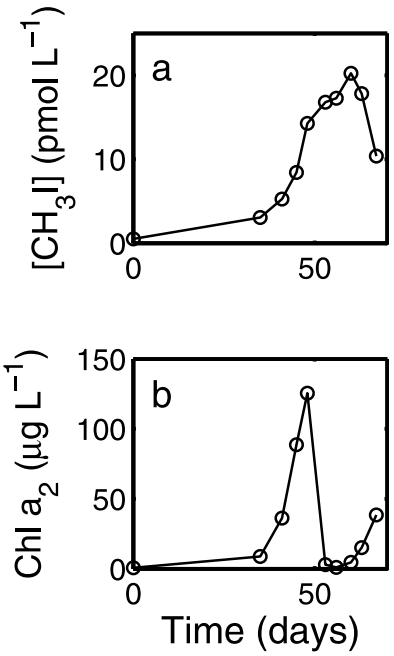

Figure 3. The concentration of (a) CH_3I and (b) chlorophyll a_2 versus time in a low-light-adapted *P. marinus* strain culture (experiment 1).

CH_3Br and CH_3Cl) have been calculated from the data in Figure 1 and normalized to the average chlorophyll a_2 and cell concentration at each time step during the exponential curve of the culture growth; means, standard deviations, and maxima are presented in Table 2.

[9] During experiment 1, only one of four cultures of the low-light-adapted strain of *P. marinus* grew successfully. For comparison with the high-light-adapted strain, the result of this single low-light-adapted culture is shown in Figure 3. While it is apparent that the chl a_2-normalized production rate of CH_3I was higher (20 ± 4 fmol μg chl a_2^{-1} d^{-1}) than in the high-light cultures (Table 2), the results will not be discussed further on account of the absence of replicates.

[10] In experiment 2, which differed from 1 only in the addition of $^{13}CH_3I$, production rates of CH_3I normalized to biomass were similar to those measured in the first experiment, but production rates of CH_3Br and CH_3Cl were higher by a factor of 3 and 4, respectively. We have no explanation for these differences.

[11] Experiment 3 showed that *Synechococcus* sp. does produce methyl halides (Figure 4). Cell and chlorophyll *a* (chl *a*) concentrations were measured (Figure 5), but much of the biomass adhered to the walls of the culture vessel resulting in severe underestimates of biomass. Maximum concentrations of CH_3I and CH_3Cl measured in the *Synechococcus* cultures were 2 to 3 times higher than those measured in the *P. marinus* cultures, while CH_3Br concentrations were similar in both experiments. Methyl halide production rates normalized to biomass were not calculated because of the lack of accurate biomass measurements.

[12] To determine whether there is significant biological loss, $^{13}CH_3I$ was added to controls and cultures in experiments 2 and 3. In each flask a decline in concentration is expected as a result of chemical loss (reaction with Cl^-) and dilution as liquid is withdrawn and replaced with contaminant-free air. These losses have been calculated with allowance being made for the distribution of the tracer between liquid and gas phases and assuming that only the former is subject to chemical loss. The predicted rate constants for the exponential decline in concentration are 0.034 and 0.036 d^{-1} for the culture flasks and control flasks, respectively, the difference being attributable to the smaller volume of the control flasks (1260 mL versus 1450 mL). It was found that the measured rate of decline of the tracer in the controls (0.037 d^{-1}) closely matched the predicted value, while in the culture flasks the decline was faster than predicted (0.049 d^{-1} versus 0.034 d^{-1}) (Figure 6). This value is for the second half of the growth period when the decline was somewhat steeper. It is assumed that biological processes were responsible for the additional loss rate of 0.015 d^{-1}. Such a loss is small

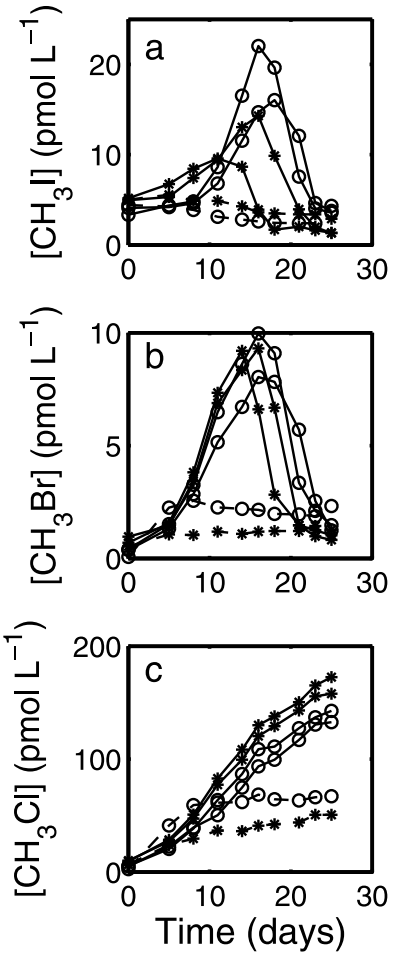

Figure 4. The concentrations of (a) CH_3I, (b) CH_3Br, and (c) CH_3Cl measured in the liquid phase in *Synechococcus* cultures grown under low-P (asterisks) and replete (circles) nutrient conditions. Controls are shown as dashed lines.

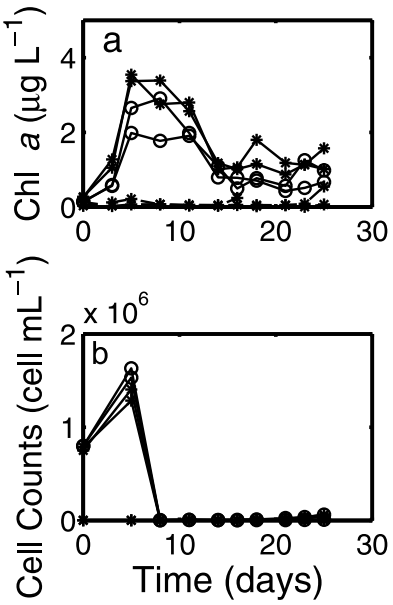

Figure 5. (a) Chl *a* and (b) cell concentrations measured over time in *Synechococcus* cultures. Circles and asterisks indicate cultures grown under replete and low-P nutrient conditions, respectively. Values are underestimated because cells adhered to the walls of the culture vessels. Controls are shown as dashed lines.

enough, compared with net rates of production, to have no influence on the conclusions drawn from this study.

4. Discussion

[13] *Smythe-Wright et al.* [2006] provided the only report of biological production of CH_3I in the ocean, occurring at rates that would account for a major contribution to the ocean-to-atmosphere flux that has been estimated from measured surface concentrations. They reported a production rate of 2.25 pmol (μg fresh weight)$^{-1}$ d^{-1}, which would rank this species as the most prolific source of CH_3I in the ocean, exceeding the maximum production rate by macroalgae (normalized to biomass) by 3 orders of magnitude. Were that so, *P. marinus* would arguably be the prime source of oceanic CH_3I, at least in midlatitudes. However, the results presented here are in marked contrast with those of *Smythe-Wright et al.* [2006]. Our mean production rate of 1.6 molecules cell^{-1} d^{-1} may be combined with a mean cell diameter of 0.7 μm to yield a production rate of 0.016 pmol (μL of cell volume)$^{-1}$ d^{-1}, equal to 0.014 fmol (μg fresh weight)$^{-1}$ d^{-1} (assuming a cell specific gravity of 1.15). This represents a difference of a factor of 1.6×10^5 between the two studies, even though the maximum concentrations of CH_3I reported by *Smythe-Wright et al.* [2006] were only a factor of 4 higher than ours. The primary reason for the large difference between the studies lies in the reported cell abundances. Our cultures had maximum abundances 3×10^8 cells mL^{-1}, generally consistent with published results [*Bertilsson et al.*, 2003], whereas *Smythe-Wright et al.* [2006] reported a maximum of 5×10^5 cells mL^{-1}. The latter concentration scarcely exceeds the range that may be measured in ocean waters [*Partensky et al.*, 1999] and would impart no perceptible color to a culture. Much smaller contributions to the differences between the studies may be attributed to different assumptions of the cell diameter, which strongly affect the conversion factors between cell abundance and fresh weight. Both studies report the maximum rate of production of CH_3I, which occurs during a short period during the exponential growth phase of the cultures. Applying such a rate to natural populations may exaggerate the potential for methyl halide production.

[14] In this study, we have provided evidence that *P. marinus* produces CH_3I at a much lower rate than reported by *Smythe-Wright et al.* [2006]. Their study does itself contain evidence for an inconsistency between its laboratory and field studies. One may compare the rate of production estimated from their laboratory study of *P. marinus*, which is given as 2.25 pmol CH_3I (μg fresh weight of *P. marinus*)$^{-1}$ d^{-1}, with the production rate that is needed to support CH_3I concentrations reported (along with *Prochlorococcus* abundances) for the Atlantic and Indian oceans. For example, approximate values for the CH_3I concentration and *Prochlorococcus* abundance in the southern part of the CHAOS cruise track around 25°S are 30 p*M* and 6 μg *P. marinus* carbon L^{-1}. A conversion factor of 226 fg C μm^{-3} [*Bertilsson et al.*, 2003] and a cell density of 1.15 were used to calculate

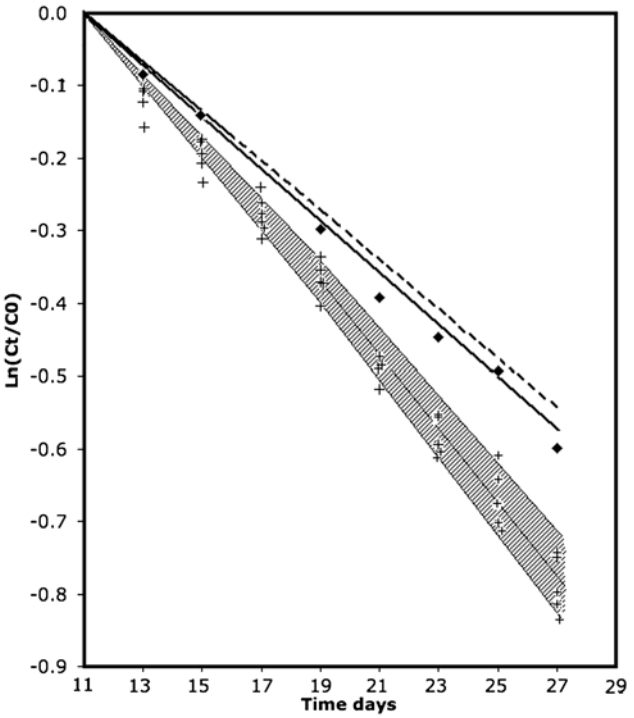

Figure 6. The ln (C_t/C_o) for $^{13}CH_3I$ plotted against time in *P. marinus* cultures (experiment 2). The shaded area indicates the decline of $^{13}CH_3I$ measured in the culture vessels. The solid line represents the predicted decline for the control (chemical and physical processes alone), and the diamonds are the measured control. The dashed line is the predicted decline for the culture samples.

Table 3. Chl *a*-Normalized CH_3Cl and CH_3Br Production Rates From Literature and This Study[a]

Species	Biomass Normalized Production Rates (pmol (μg chl *a*) $^{-1}$ d^{-1})		Reference
	CH_3Cl	CH_3Br	
Phaeodactylum tricornutum	0.35	0.01	*Scarratt and Moore* [1996]
Thalassiosira weissflogii	0.05	0.01	*Scarratt and Moore* [1996]
Chaetoceros calcitrans	0.01	0.002	*Scarratt and Moore* [1998]
Emiliania huxleyi	0.25	0.04	*Scarratt and Moore* [1998]
Prorocentrum sp.	0.1	0.02	*Scarratt and Moore* [1998]
Phaeodactylum tricornutum	0.2	0.04	*Scarratt and Moore* [1998]
Synechococcus sp.	0.03	0.002	*Scarratt and Moore* [1998]
Phaeocystis sp.	1.0	0.11	*Scarratt and Moore* [1998]
Guillardia theta	NA	0.027	*Sæmundsdóttir and Matrai* [1998][b]
Hemiselmis rufescens	NA	0.059	*Sæmundsdóttir and Matrai* [1998][b]
Chaetoceros diversum	NA	0.153	*Sæmundsdóttir and Matrai* [1998][b]
Amphidinium carterae		0.069	*Sæmundsdóttir and Matrai* [1998][b]
Prorocentrum micans	NA	0.101	*Sæmundsdóttir and Matrai* [1998][b]
Pycnococcus provasolii	NA	0.015	*Sæmundsdóttir and Matrai* [1998][b]
Phaeocystis sp.	NA	0.480	*Sæmundsdóttir and Matrai* [1998][b]
P. marinus (Experiment 1)	0.04	0.01	This study
P. marinus (Experiment 2)	0.13	0.01	This study

[a]NA, not available.
[b]Experiments in this study did not measure the release of CH_3Cl.

the *P. marinus* abundance in units of fresh weight per liter: 30.6 μg L^{-1}. With a production rate of 2.25 pmol CH_3I (μg fresh weight)$^{-1}$ d^{-1}, this could yield 69 pmol CH_3I L^{-1} d^{-1}. The mixed layer depth was 50 m or more so that the areal production rate was equal to or greater than 3440 nmol m^{-2} d^{-1} which, on average, should be balanced by losses. The maximum temperature for this section was 21.5°C, so the maximum chemical loss rate of CH_3I would be 0.04 d^{-1} or 140 nmol m^{-2} d^{-1}. With a concentration of 30 pM and an estimated exchange velocity of 5 m d^{-1}, the loss to the atmosphere would be 150 nmol m^{-2} d^{-1}. This means that there would be a production surplus or unaccounted losses of 3150 nmol CH_3I m^{-2} d^{-1} (or 63 pmol CH_3I L^{-1} d^{-1}). Thus, the *Smythe-Wright et al.* [2006] laboratory estimate of CH_3I production by *P. marinus* is greatly in excess of what is apparently required to support the reported surface water concentration, even if *P. marinus* were the sole source. By contrast, our own *P. marinus* production rate, lower by a factor of about 1.6×10^5, if used in the same example, would yield a production rate of only 0.0004 pmol CH_3I L^{-1} d^{-1}, which would be inadequate to support a concentration of 0.1 p*M*, much less 30 p*M*, against chemical loss, even in the absence of any flux to the atmosphere.

[15] Cultures from the current study were not axenic. It was difficult to measure the heterotrophic bacteria in *P. marinus* cultures using flow cytometry because the heterotrophic bacterial signal overlapped the weakly fluorescent *P. marinus* signal. Previous studies have indicated bacterial production of methyl halides is insignificant. *Manley and de la Cuesta* [1997] dismissed bacterial production of CH_3I because production ceased in the stationary phase of algae growth. They considered that if bacteria were the dominant producers of CH_3I in the cultures, then CH_3I would continue to increase during the stationary phase of algae growth. Numbers of heterotrophic bacteria would continue to increase during stationary phase of algae growth because they are presumably utilizing carbon from dead algal cells [*Manley and de la Cuesta*, 1997]. In the current study, CH_3I production ceased when the cultures of *P. marinus* reached stationary phase, further suggesting production was associated with growth of *P. marinus*. If CH_3I was being produced by bacteria, then this would suggest that *P. marinus* produces CH_3I in even smaller quantities than reported here, further supporting the claim that *P. marinus* is not a significant producer of CH_3I.

[16] Our studies showed maximum concentrations of CH_3I measured from *Synechococcus* sp. cultures were 2 to 3 times higher than those measured in *P. marinus* cultures. *Manley and de la Cuesta* [1997] reported no production of CH_3I in cultures of *Synechococcus* sp. (CCMP 1334).

[17] Biological consumption of CH_3Br and CH_3Cl has been reported in several studies of ocean waters and soils [*King and Saltzman*, 1997; *Tokarczyk et al.*, 2003], but little information exists on parallel processes for CH_3I. Our study showed that biological processes may be responsible for a loss rate of about 0.015 d^{-1}, which is too small to have any significant effect on our estimates of CH_3I production in our cultures.

[18] While no other studies have been made of production of CH_3Cl and CH_3Br in *P. marinus* cultures, we can make a comparison of our current values for *P. marinus* with values for various phytoplankton reported by *Scarratt and Moore* [1996, 1998]. The species and their chl *a*-normalized production rates are listed in Table 3, which also includes calculated chl a_2-normalized production rates from the current study for *P. marinus* cultures. It is apparent that our measured production rates of CH_3Cl and CH_3Br by this species are in the same range as reported for cultures of other species of phytoplankton.

[19] We have estimated global oceanic production rates (G_{CH3X}) of all three methyl halides by *P. marinus* using the average cell-normalized production rates from two experiments, including both nutrient treatments (molecules $cell^{-1}$ d^{-1}

(Table 2)). The typical abundance of *Prochlorococcus* in the ocean was taken as 1.1×10^{11} cells m^{-3} [*Partensky et al.*, 1999], and the average thickness of the layer populated by *Prochlorococcus* was taken as 50 m [*Bouman et al.*, 2006; *Johnson et al.*, 2006]. The area of the ocean in which *P. marinus* is widespread was taken as 30% of the total ocean area [*Partensky et al.*, 1999]. The results for CH_3I, CH_3Br, and CH_3Cl, estimated from maximum production rates calculated from the results of two *P. marinus* experiments, were 0.6, 2.9, and 19 Mmol yr^{-1}, respectively. These quantities may be compared with published estimates of the fluxes of CH_3I and CH_3Cl from ocean to atmosphere [*Moore and Groszko*, 1999; *Moore*, 2000] and with an estimate of total oceanic production of CH_3Br from *Lobert et al.* [1995]. For CH_3I, our *P. marinus* experiments would account for 0.03% of the ocean-to-atmosphere flux estimate from field data by *Moore and Groszko* [1999]. In the case of CH_3Cl, the appropriate comparison is with the flux to the atmosphere from warm waters ($T > 15°C$) of the ocean: *P. marinus* would account for 0.27%. For CH_3Br, the comparison is made with *Lobert et al.*'s [1995] estimate of global marine production: *P. marinus* would account for 0.34%. In each case, the contribution from *P. marinus* is insignificant.

5. Conclusions

[20] While *P. marinus* does produce methyl halides in significant quantities (i.e., above the instrument detection limits) in laboratory cultures, when extrapolated to global scales, the production is sufficient to account for only a small fraction of the estimated oceanic production based on field measurements. The average global production rate of CH_3I estimated from the two *P. marinus* experiments was 0.6 Mmol yr^{-1}, which accounted for 0.03% of the global marine production, strongly suggesting *P. marinus* is not a significant marine source of CH_3I to the atmosphere. The results presented here are in contrast with *Smythe-Wright et al.*'s [2006] study, which reported that *Prochlorococcus* is a major marine source of atmospheric CH_3I.

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D. K. Brownell, J. J. Cullen, and R. M. Moore, Department of Oceanography, Dalhousie University, 1355 Oxford St., Halifax, NS B3H 4J1, Canada. (brownelld@dfo-mpo.gc.ca)