A time series of incubation experiments to examine the production and loss of CH$_3$I in surface seawater

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Abstract In order to investigate production pathways of methyl iodide and controls on emissions from the surface ocean, a set of repeated in vitro incubation experiments were performed over an annual cycle in the context of a time series of in situ measurements in Kiel Fjord (54.3$^\circ$N, 10.1$^\circ$E). The incubation experiments revealed a diurnal variation of methyl iodide in samples exposed to natural light, with maxima during day time and losses during night hours. The amplitude of the daily accumulation varied seasonally and was not affected by filtration (0.2 μm), consistent with a photochemical pathway for CH$_3$I production. The methyl iodide loss rate at nighttime correlates with the concentration accumulated during daytime suggesting a first-order loss mechanism ($R^2 = 0.29$, $p < 0.01$). Daily (24 h) net production ($P_{net}$) was similar in magnitude between in vitro and in situ mass balances. However, the estimated gross production ($P_{gross}$) of methyl iodide ranged from $-0.07$ to 2.24 pmol L$^{-1}$ d$^{-1}$ and was up to 5 times higher in summer than $P_{net}$ calculated from the in situ study. The large excess of $P_{gross}$ over $P_{net}$ in summer revealed by the incubation experiments is a consequence of large losses of CH$_3$I by as-yet uncharacterized processes (e.g., biological degradation or chemical pathways other than Cl$^-$ substitution).

1. Introduction

Methyl iodide is a major carrier of gas phase iodine from the ocean into the atmosphere. In the atmosphere, CH$_3$I participates in several catalytic cycles important for air quality and climate [Lovelock and Maggs, 1973], many investigations have been performed to identify its sources. In the ocean, several different potential sources of CH$_3$I have been proposed. Macroalgae were shown to produce CH$_3$I in coastal regions [Lovelock, 1975a, 1975b; Manley et al., 1992; Laturnus et al., 1995; Nightingale et al., 1995]. For the ocean as a whole, phytoplankton have been hypothesized to be more important than macroalgae in terms of global biological CH$_3$I production [Moore and Tokarczyk, 1993; Manley and delaCuesta, 1997; Shaw et al., 2003; Smythe-Wright et al., 2006; Hughes et al., 2011]; however, estimates of such biological sources vary and are controversial. Smythe-Wright et al. [2006] estimated global biological production rates of $5.3 \times 10^{11}$ g yr$^{-1}$ with Prochlorococcus as a primary source based on culture experiments and observations in the Atlantic and Indian oceans. However, Brownell et al. [2010] calculated a global CH$_3$I production rate of only $8.5 \times 10^{10}$ g yr$^{-1}$ (equivalent to 0.6 Mmol yr$^{-1}$) from Prochlorococcus, which is 1000-fold lower than estimated by Smythe-Wright et al. [2006]. Hughes et al. [2011] attempted to reconcile these estimates by suggesting that the rates of CH$_3$I production by Prochlorococcus in culture varies with cell physiological status. Other biological production sources in the ocean have been reported. Amachi et al. [2001] reported experiments suggesting that oceanic bacteria (e.g., Alteromonas macleodii IAM) are capable of producing CH$_3$I under environmental concentrations of I$^-$ (0.1 μM). Klick and Abrahamsson [1992] argued for the possibility of planktonic CH$_3$I production based on observation of diatom-rich seawater samples with higher CH$_3$I concentrations. A set of similar investigations has been conducted which also made the case for CH$_3$I production by diatoms (>10 μm) [Moore et al., 1996; Manley and delaCuesta, 1997; Xie et al., 1999; Toda and Itoh, 2011].

A source of CH$_3$I via photochemical production in surface seawater has been suggested frequently as an alternative to biological production. Moore and Zafiriou [1994] presented experimental evidence for a photochemical production pathway based on short-term (maximum 2 h) incubations. Longer-term (>24 h) field incubation experiments with open ocean waters conducted by Richter and Wallace [2004] and
Wang et al. [2009] were also interpreted to support photochemical production. The former study included experiments with poisoned and filtered samples. A photochemical mechanism was inferred by Happell and Wallace [1996] from their geographical correlation analysis between measured CH$_3$I concentrations and a number of environmental factors. Shi et al. [2014] presented a temporal correlation analysis of seasonal concentration variations in a nearshore environment that was also consistent with photochemical production of CH$_3$I but which could not rule out a biological source when temporal variability of the net production rate was considered. Archer et al. [2007] presented a temporal correlation analysis in shelf waters that noted the similarity of the seasonal trends of CH$_3$I concentration and light, consistent with a photochemical production mechanism. However, based on their observations of the variations of [CH$_3$I] in the water column they argued for a biological production pathway. Ziska et al. [2013] noted higher CH$_3$I concentrations in subtropical gyres, which they considered consistent with photochemical production. Bell et al. [2002] simulated oceanic and atmospheric concentrations of CH$_3$I assuming a photochemical CH$_3$I source dependent on only light and DOC. Stemmner et al. [2014] also modeled the CH$_3$I production globally and suggested that a photochemical process is the best way to explain the global surface concentration variations. However, the relative importance of biological and photochemical production of CH$_3$I is still debated, perhaps because biological and photochemical processes are likely to be coupled in that a source of organic carbon (as well as light) is required for photochemical production of CH$_3$I. We note that the highest CH$_3$I production rates in the above-mentioned culture experiments did not explicitly rule out the possibility of abiotic, photochemical production taking place within the cultures.

The main sink of methyl iodide from the surface ocean has generally been considered to be its loss to the atmosphere. Estimates of this global sea-to-air flux vary from 1.9 \times 10^{11} to 1.3 \times 10^{12} g yr$^{-1}$ [Liss and Slater, 1974; Rasmussen et al., 1982; Singh et al., 1983; Reifenhauser and Heumann, 1992; Moore and Groszko, 1999; Bell et al., 2002; Richter and Wallace, 2004; Ziska et al., 2013]. A second major sink for methyl iodide in seawater is nucleophilic substitution by chloride and bromide, as suggested first by Zafiriou [1975]. Elliott and Rowland [1993] calculated the temperature dependent chemical loss rate of methyl iodide, with the rate constant for the dominant reaction with chloride at 22°C being 1.0 \times 10^{-6} L mol$^{-1}$ s$^{-1}$. Bell et al. [2002] used these rates to calculate a global in situ sink of CH$_3$I via reaction with Cl$^{-1}$ of 2.6 \times 10^{11} g yr$^{-1}$. A third possible sink for CH$_3$I is biological degradation. Bell et al. [2002] hypothesized that an additional biological loss should exist based on their model calculations. Moore [2006] estimated a “nonchemical loss” (defined as in situ losses due to processes other than reaction with chloride) from incubation experiments with open ocean surface waters. The loss rates ranged from <1 to 15% d$^{-1}$ assuming a pseudo first-order loss process. In this study, we will refer to this “nonchemical loss” as “nonchloride loss” to allow for the possibility of additional uncharacterized chemical loss processes as well as biological (e.g., microbial) removal processes.

Another potential sink of CH$_3$I from surface seawater is lateral and, especially, vertical mixing with water containing lower concentrations of CH$_3$I. However, even in open ocean waters where CH$_3$I concentrations decrease below the euphotic zone, the downward mixing loss has been shown to be negligible in comparison with the sea-to-air flux and chemical loss [e.g., Richter and Wallace, 2004].

In this work, a set of repeated long-term incubation (in vitro) experiments of ~2.5 days duration were conducted over 1 year using samples of seawater collected from a nearshore, brackish environment, in order to investigate sources and sinks of methyl iodide. Simultaneously, and at the same location, the seasonal cycle and mass balance of CH$_3$I concentrations were investigated [Shi et al., 2014]. In this paper, production and loss rates of CH$_3$I are estimated based on the results of the in vitro incubation experiments, and used to calculate the daily mass balance of CH$_3$I over an annual cycle, which is compared with results from the in situ study.

2. Methods

2.1. Incubation Experiments

Incubation experiments with natural seawater (salinity: 13–16) were conducted from August 2009 to November 2010 in the Kiel Fjord. Seawater was sampled directly from the surface (0.5 m) of the Fjord from a pier in front of GEOMAR’s west shore building (54.3° N, 10.1° E). The experiments involved long-term incubations (57 h) of natural seawater samples at natural light levels, as well as in the dark (12 light samples and 8 dark samples per experiment). In this study three treatments of seawater samples were used: natural
seawater samples or “original” untreated samples (O), the “filtered and prepurged samples” (F), and the “nonfiltered and prepurged samples” (NF).

The “filtered and prepurged samples” (F) comprised natural seawater (7 L) which had been filtered stepwise through 20, 5, and finally 0.2 μm membrane filters under 5 bars pressure. After filtration, most of the plankton had been removed, although the samples were not axenic and small bacteria (e.g., <0.2 μm) could have remained. The filtered seawater was bubbled (prepurged) for 5 h with synthetic air (35–45 mL min⁻¹) to reduce the initial concentration of volatile iodinated organic compounds (20–30% of original methyl iodide remained in the seawater). Use of synthetic air assured that dissolved oxygen remained high in the sample (e.g., 150 μmol L⁻¹ in original seawater compared to 138 μmol L⁻¹ after the 5 h purge). The “nonfiltered but prepurged samples” (NF) represented unfiltered natural seawater that had been bubbled for 5 h with synthetic air at the start of experiment.

After these sample treatments, 20 subsamples (each 300 mL) were placed in sealed quartz bottles. Twelve of the subsamples were exposed to the natural light dark cycle, while eight subsamples were kept in the dark. The quartz-flasks were incubated (upside down) at 0.5 m depth under the sea surface in front of GEOMAR’s west shore building in order to keep the experimental conditions as close to natural temperature and light (“sunlit samples”) as possible. The “dark samples” were kept in a closed box filled with water of the same temperature. Samples were taken for analysis every 5 h for the light treatments and every 12 h for the dark treatments, corresponding to approximate measurement times of 08:00, 12:00, 17:00, and 22:00 every day. Every sample was analyzed in triplicate, the mean value of each sample (standard deviation <15%) was used in this work.

2.2. Analysis of Halocarbons

A purge and trap (dynamic headspace) method was used for the analysis. The volatile organic compounds (e.g., CH₃I) were released from the seawater samples into the gaseous phase by a continuous 30 mL min⁻¹ gas flow of helium for 40 min at a purge temperature of 50–60°C. The purged gas stream was dried by Nafion dryers (50 cm length) and the volatiles were trapped in a glass tube (1/4” ID) filled with a 25 mm length of glass beads above liquid nitrogen (N₂) at −100°C. The volatiles were released at high temperature (100°C) and trapped again on a deactivated glass capillary (50 cm length, 0.53 mm ID) in liquid nitrogen. The analytes were then injected into the GC at room temperature. For all measurements a FISONS 8000 gas chromatograph (GC) was used, equipped with a RTX-VGC column (60 m long; coated: 1.4 μm, column diameter: 0.25 mm) and electron capture detector (ECD) with helium carrier gas. The temperature program of the GC oven was 25°C for 6 min, increased to 150°C at 6°C/min, and held at 150°C for 1 min.

Calibrations were performed by injecting microliter volumes of liquid standards via a septum port, into the purge vessel containing prepurged tap water and analyzed in the same way as seawater samples. Three stock standard solutions were prepared in 10 mL pentane. The final standards for calibration were then prepared by serial dilutions of the stock solutions into methanol. All chemicals were purchased from Merck, and all stock solutions were stored at −20°C. Every working standard was measured three times. The standard deviation of triplicate measurements ranged from 13% to 23%. The detection limit of the method was 0.06 pmol L⁻¹.

Meteorological data (collected every 8 min), including solar radiation (SSR) and sea surface temperature (SST), were provided by the Maritime Meteorology research unit at GEOMAR (http://www.geomar.de/en/research/fb1/fb1-me/). The annual cycle of SSR and SST are presented in a companion paper [Shi et al., 2014].

3. Results

3.1. Diurnal Variability

The variations of CH₃I concentrations during a 57 h incubation experiment conducted in July 2010 are plotted in Figure 1. The results show a diurnal variation of methyl iodide under irradiation in both nonfiltered (NF) and filtered (F) samples compared with samples that were kept in the dark. The “sunlit” samples showed an increase during daylight hours and a decrease at night. Notably, for “sunlit” samples, almost all of the CH₃I produced in the day was consumed during the night such that a long-term buildup of CH₃I in the flasks was small compared to the diurnal variations. For instance, the CH₃I concentration in the three morning samples (08:00) increased only slightly over the entire incubation experiment (average of increase
over 24 h of \sim 0.05 \text{ pmol L}^{-1} for NF samples and \sim 0.5 \text{ pmol L}^{-1} for F samples (Figure 1), compared to daytime variation of 1.35 \text{ pmol L}^{-1} for NF sample and 1.53 \text{ pmol L}^{-1} for F sample (from 08:00 to 17:00).

Both the concentration measured at each time-point (overall range of 0.67–5.84 pM) and the amplitude of the daytime increase (range of 0.18–1.53 pM from 08:00 to 17:00) varied from experiment to experiment. In order to characterize a typical daily cycle, the results from all (NF, F, and O samples) experiments (\( n = 24 \)) were normalized for each 24 h time period (from 08:00 to 08:00 on second day, and 08:00 on second day to 08:00 on third day). The normalized concentrations for the different time-points over a 24 h period were calculated as

\[
[\text{CH}_3\text{I}]_{\text{nor}} = \frac{[\text{CH}_3\text{I}] - [\text{CH}_3\text{I}]_{\text{initial}}}{[\text{CH}_3\text{I}]_{\text{peak}} - [\text{CH}_3\text{I}]_{\text{initial}}},
\]

where \([\text{CH}_3\text{I}]_{\text{nor}}\) is the normalized concentration of \text{CH}_3\text{I}, \([\text{CH}_3\text{I}]\) is the concentration measured at an individual time-point, \([\text{CH}_3\text{I}]_{\text{initial}}\) is the concentration in the “sunlit” samples analyzed at 08:00 for that 24 h time period, and \([\text{CH}_3\text{I}]_{\text{peak}}\) is the maximum concentration measured during the 24 h time period in the “sunlit” samples. For the “dark” samples, \([\text{CH}_3\text{I}]_{\text{initial}}\) is the concentration in the “dark” samples analyzed at 08:00 for each 24 h time period, and \([\text{CH}_3\text{I}]_{\text{peak}}\) is the maximum concentration in the “sunlit” samples during the same 24 h time period. Subsequently, average values of these normalized concentrations were calculated for each daily sampling time from all 24 incubation experiments.

The normalization procedure yields the typical daily cycle for the “sunlit” samples during incubations based on data collected over an entire annual cycle (Figure 2a). From early morning (08:00), the \text{CH}_3\text{I} concentration increases to a maximum at 17:00 within the “sunlit” samples. Hence, the maximal daily value of \([\text{CH}_3\text{I}]\) lagged the maximum of SSR in cycle by \sim 5 h. Sometime around 17:00 the \text{CH}_3\text{I} concentration started to decrease rapidly until near midnight (22:00). From 22:00 to 08:00 of the next day, the concentration of \text{CH}_3\text{I}
changed only slightly. Note however that the exact timing of peak and minimum concentrations are not well-defined due to the still-limited time resolution of the sampling. On average, over 24 h of incubation, the CH$_3$I concentration changed only slightly and the daily accumulations were small (see above and Figure 2a, red dashed line). The daytime increase and the net daily accumulation will be discussed in sections 3.2 and 4.2. Figure 2b illustrates the mean, normalized concentrations for the “dark” samples. The CH$_3$I concentration in the “dark” samples varied only very slightly over the entire 24 h period. Comparison of the “sunlit” samples and “dark” samples (Figure 2) indicates that samples exposed to sunlight had much higher daytime accumulations compared to samples kept in the dark.

Based on this “typical” average daily cycle, we calculated the daytime accumulation ($\Delta$[CH$_3$I]$_{day}$) in the “sunlit” samples for each experiment as the CH$_3$I concentration increase over the 9 h interval between 08:00 and 17:00. Similarly, the nighttime loss ($\Delta$[CH$_3$I]$_{night}$) was calculated as the concentration decrease measured between 17:00 and 08:00 on the second day.

### 3.2. Seasonal Variability and Filtration Effects

Incubation experiments were conducted from spring through winter in order to examine seasonal variations of the CH$_3$I production. The daytime accumulations of CH$_3$I ($\Delta$[CH$_3$I]$_{day}$) from the 2.5 day incubation experiments (NF, F, and O samples) conducted from February to November were averaged and are shown in Figure 3d. The $\Delta$[CH$_3$I]$_{day}$ in sunlit samples ranged from 0.04 to 2.05 pmol L$^{-1}$ and from 0.33 to 0.45 pmol L$^{-1}$ in the dark samples. A seasonal cycle of $\Delta$[CH$_3$I]$_{day}$ with a clear maximum in summer and early fall (from June to September) and a minimum in winter (from November to February) was observed for the sunlit samples, whereas there was no apparent seasonality for the dark samples (Figure 3d).

For the Nonfiltered samples (NF), a maximum value of $\Delta$[CH$_3$I]$_{day}$ of 1.53 pmol L$^{-1}$ was observed in July with the minimum value of 0.04 pmol L$^{-1}$ observed in November (Figure 3a) (No incubation experiments with NF samples were conducted in August and September). The seasonal variation from the more limited number of experiments conducted with filtered samples (F) were consistent with the results of the nonfiltered (NF) experiments (Figure 3b). A maximum accumulation of 1.35 pmol L$^{-1}$ of CH$_3$I in filtered samples was observed in July (summer), whereas only 0.21 pmol L$^{-1}$ of CH$_3$I accumulated in experiments conducted at the end of October (fall). For experiments with the original samplers (O), the highest value of $\Delta$[CH$_3$I]$_{day}$ (2.05 pmol L$^{-1}$) was observed in September, with lower values found in winter time (from November to February).
Similar magnitudes of diurnal variability of CH$_3$I were observed for both NF and F samples during the incubations (Figure 3, in blue frame). In June, the D[CH$_3$I]$_{day}$ for NF samples was 0.89 and 0.76 pmol L$^{-1}$ for the F samples. The difference of D[CH$_3$I]$_{day}$ between the NF and F samples was small, with overlapping error bars (95% confidence).

4. Discussion

4.1. Diurnal Variation

The incubation experiments revealed a diurnal cycle of CH$_3$I in “sunlit” samples that appears closely linked to the daily light cycle but with a time-lag of ~5 h. A link to irradiance is consistent with the seasonal observations of in situ concentrations in the Kiel Fjord reported in the companion paper [Shi et al., 2014]. The ~5 h time-lag between peak irradiance and maximum daily [CH$_3$I] likely reflects a balance between light-dependent production and concentration-dependent loss processes (see below section 4.4).

Richter and Wallace [2004] and Moore [2006] also conducted incubation experiments in sealed bottles, but they did not resolve diurnal variability, because they analyzed the incubation samples over longer intervals (24 and 15 h, respectively). Our study has higher temporal resolution (5 h) than previous studies and can give new insight into diurnal production and cycling of methyl iodide. To our knowledge, there has been only one prior study to have examined diurnal variability of CH$_3$I concentrations in a natural system [Ekdahl et al., 1998]. They observed two maxima of CH$_3$I concentrations over 24 h (from 16:00 to 18:00 and from 2:00 to 5:00) in a rock pool containing macroalgae. They suggested that the first maximum was associated with photosynthesis by the algae in daytime and the second was suggested to have been associated with macroalgal respiration during nighttime.

4.2. Daytime and Daily Accumulation

The incubation experiments were conducted over a full seasonal cycle, and the amplitude of D[CH$_3$I]$_{day}$ showed a clear seasonal pattern (see Figure 3). It was notable that most of the CH$_3$I produced in sunlit samples during the day was subsequently degraded at night. In contrast, CH$_3$I concentrations remained invariant in samples that were kept in the dark (Figure 2b). The daily accumulation (D[CH$_3$I]$_{24h}$) was calculated as the variation of [CH$_3$I] between 08:00 and 08:00 of the following day and monthly averages are presented.
in Figure 4. Note, that there were $2 \times 24$ h cycles so that two values of $\Delta[CH_3I]_{24h}$ were resolved per incubation experiment. Overall, daily accumulation values in the sunlit samples were positive; however, the monthly averages were not statistically different from zero in most months, so that a seasonal signal could not be clearly identified.

The observation of higher daytime and daily accumulation in sunlit samples is consistent with the observations from prior incubation experiments reported by Richter and Wallace [2004] and Moore [2006]. Richter and Wallace found that the accumulation of methyl iodide in dark samples over a 24 h period was only 0.72 pmol L$^{-1}$ (mean) compared with 4.08 pmol L$^{-1}$ (mean) in samples exposed to a natural light dark cycle [Richter and Wallace, 2004]. Moore [2006] reported CH$_3$I accumulation of 0.02 pmol L$^{-1}$ in dark samples after a 48 h incubation and 10 times higher accumulation of CH$_3$I in samples exposed to a natural light dark cycle [Moore, 2006]. All of these studies provide evidence that solar radiation promotes production of CH$_3$I in surface seawater. However, as noted, the earlier studies did not resolve possible diurnal variation of CH$_3$I.

The daytime accumulation rate was calculated based on the accumulation during the daytime period ($\Delta[CH_3I]_{day}$) only (i.e., over 9 h; from 08:00 to 17:00), whereas the daily accumulation rate was calculated based on the accumulation of methyl iodide over 24 h ($\Delta[CH_3I]_{24h}$), such that

$$Rate_{day} = \frac{\Delta[CH_3I]_{day}}{9h} \quad \text{or} \quad Rate_{24h} = \frac{\Delta[CH_3I]_{24h}}{24h}.$$ (2)

Both the daytime accumulation rates and the daily accumulation rates of different samples in different seasons were calculated as hourly rates and are presented in Tables 1 and 2. Here it should be noted that the daytime accumulation rates were 10 times higher than the daily accumulation rates because methyl iodide produced during day was degraded during night.

The daytime accumulation rates ($Rate_{day}$) in summer during this study (0.1–0.2 pmol L$^{-1}$ h$^{-1}$) are lower than the 1.0–1.5 pmol L$^{-1}$ h$^{-1}$ reported for short-term (<2 h) incubations conducted under artificial light by Moore and Zafiriou [1994]. The latter used artificial light with an intensity of 1325 W m$^{-2}$ over 280–1100 nm. In our study, the strongest light level (in summer) was 498 W m$^{-2}$ over 310–2800 nm (average from 08:00 to 17:00). The different light intensities might be one reason to explain the difference of the daytime (or short-term) accumulation rates in the presence of light (Table 1).

![Figure 4. Seasonal variation of the monthly average daily accumulations ($\Delta[CH_3I]_{24h}$) calculated from the incubation experiments. Error bars represent 95% confidence intervals. The method of calculation is presented in the main text.](image)

### Table 1. Comparison of Daytime Accumulation Rates ($Rate_{day}$ in pmol L$^{-1}$ h$^{-1}$) in Nonfiltered Samples (NF) or Filtered Samples (F)

<table>
<thead>
<tr>
<th>Rate$_{day}$ Moore and Zafiriou [1994]</th>
<th>This Study (2009–2010)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incubation time</td>
<td>2 h</td>
</tr>
<tr>
<td>Sample source</td>
<td>Coastal North seawater</td>
</tr>
<tr>
<td>Latitude</td>
<td>52.6° N</td>
</tr>
<tr>
<td>Irradiance (W m$^{-2}$)</td>
<td>1325 (artificial)$^a$</td>
</tr>
<tr>
<td>Samples</td>
<td>Light</td>
</tr>
<tr>
<td>F (pmol L$^{-1}$ h$^{-1}$)</td>
<td>1.11</td>
</tr>
<tr>
<td>NF (pmol L$^{-1}$ h$^{-1}$)</td>
<td>0.15</td>
</tr>
<tr>
<td>Latiradiance (W m$^{-2}$)</td>
<td>9 h</td>
</tr>
<tr>
<td>Sample source</td>
<td>Coastal Kiel Fjord water</td>
</tr>
<tr>
<td>Latitude</td>
<td>54.3° N</td>
</tr>
<tr>
<td>Irradiance (W m$^{-2}$)</td>
<td>498 (summer)$^b$</td>
</tr>
<tr>
<td>Samples</td>
<td>Light</td>
</tr>
<tr>
<td>F (pmol L$^{-1}$ h$^{-1}$)</td>
<td>0.14</td>
</tr>
<tr>
<td>NF (pmol L$^{-1}$ h$^{-1}$)</td>
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</tr>
<tr>
<td>Latiradiance (W m$^{-2}$)</td>
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<td>0.17</td>
</tr>
<tr>
<td>NF (pmol L$^{-1}$ h$^{-1}$)</td>
<td>0.04</td>
</tr>
</tbody>
</table>

$^a$At a solar zenith angle of 48°, over 280–1100 nm.

$^b$Average from 08:00 to 17:00 over 310–2800 nm.
The *daily* accumulation rates (*Rate*$_{24h}$) in this study ranged from 0.008 to 0.03 pmol L$^{-1}$ h$^{-1}$ (in Table 2). Our results are similar in magnitude to the results of multiday incubations reported by Moore [2006]. Moore [2006] conducted two incubation experiments using offshore waters (station T5: 50°N–55°N) during two cruises in the North Atlantic in spring and summer of 2003. They observed daily accumulation rates (*Rate*$_{24h}$) of only 0.005 to 0.015 pmol L$^{-1}$ h$^{-1}$ in “sunlit” samples (50 h incubation). However, both sets of rates are ~10 times lower than *daily* rates reported by Richter and Wallace [2004] (0.17 pmol L$^{-1}$ h$^{-1}$ for NF samples and 0.10 pmol L$^{-1}$ h$^{-1}$ for F samples) in the tropical Atlantic. Moore [2006] explained their lower daily accumulation rates possibly due to the average light level during their incubations being reduced by frequent fog.

There were several differences between all of these studies. As noted above, the different light intensities might be one factor. Richter and Wallace [2004] conducted incubation experiments at 10°N under clear skies. They did not report actinic measurements; however, the irradiation at 10°N is typically 8 times higher than at 50°N. The difference might also be attributable, at least in part, to the different water types used in these studies. In our study, coastal water was used, compared with offshore North Atlantic water used by Moore [2006] and oligotrophic water of the tropical Atlantic used by Richter and Wallace [2004]. The different water types might have impacted light absorption and/or photochemical precursor concentrations and hence production rates, but they might also have impacted chemical or biological loss processes (see below section 4.4).

**4.3. Filtration Effects**

As discussed in section 1, the relative importance of abiotic and biotic processes underlying methyl iodide production in the ocean continues to be debated [Stemmler et al., 2014]. In order to determine if methyl iodide production is mainly associated with biological activity or photochemistry, a comparison between incubations of filtered and unfiltered natural seawater samples was performed. Note that whereas our sampling location lies well outside the natural region of Prochlorococcus occurrence [Urbach et al., 1992; Partensky et al., 1999], diatoms are present in Kiel Fjord [Sommer, 1996].

No significant difference of the daytime accumulations of CH$_3$I (*Δ[CH$_3$I]$_{day}$*) was observed between NF and F samples (Figure 3, blue frame). June and July were chosen for the filtered/nonfiltered comparison, because these months experience strongest solar radiation intensity and also higher biological activity. Our observations appear to be consistent with the arguments expressed in earlier studies [e.g., Moore and Zafiriou, 1994; Happell and Wallace, 1996; Richter and Wallace, 2004; Moore, 2006; Stemmler et al., 2014; Shi et al., 2014] that the production of methyl iodide in the ocean is not directly biological (i.e., metabolic) but rather photochemical. Nonetheless, Richter and Wallace [2004] observed that the CH$_3$I accumulation in filtered samples from the tropical Atlantic was ~30% lower than in untreated samples. They suggested this may have been a result of organic precursor removal via filtration, rather than a difference in biological activity due to filtration per se. Compared with the 0.1 μm membrane filter used in their study, a 0.2 μm membrane filter was used in our study. The daily accumulation (*Δ[CH$_3$I]$_{24h}$*) in filtered samples was 11% (mean) lower than that in the unfiltered samples but this difference was not statistically significant.

**4.4. Loss Processes**

In our experiments, the loss of CH$_3$I during nighttime was much larger in samples that had been incubated in the light. As a consequence, the daily accumulation of CH$_3$I over a full 24 h period (*Δ[CH$_3$I]$_{24h}$*) was low (Table 2 and Figures 1 and 4). Figure 5 shows the correlation between CH$_3$I concentrations and loss rates in
were calculated. The average for Kiel Fjord waters from May 2009 to July 2010 was 0.002 pmol L\(^{-1}\). However, during our incubation experiments, a sea-to-air loss was excluded because the flasks were sealed. However, the nighttime loss rates for our "sunlit" incubations were higher than predicted from chloride substitution, averaging 0.11 pmol L\(^{-1}\) (range: 0.003–0.07 pmol L\(^{-1}\)). These results suggest that additional loss pathways were operating during our incubations. There are two main possibilities:

1. Bacterial degradation: some bacteria are likely to have passed through the 0.2 \(\mu\)m filters or remained on bottle walls, and our incubation experiments were not axenic. As noted above, Moore [2006] reported "non-chloride loss" rates ranging from <1 to 15% d\(^{-1}\) (mean: 7%) based on incubation experiments using \(^{13}\)C labeled methyl iodide in open ocean waters. However, the nighttime loss rates for our "sunlit" incubations were >80% d\(^{-1}\). Bacterial degradation is one possible mechanism to explain this large loss. Richter and Wallace [2004] conducted incubation experiments with alternative treatments, including samples that had been filtered in parallel to samples that had been poisoned with mercury (II) chloride (the poisoned water still contained bacterial cells and organic matter, but without metabolism). Their poisoned samples had slightly higher CH\(_3\)I accumulation, which might have been consistent with reduced bacterial degradation, but the difference was not statistically significant. They speculated that removal of organic precursors led to the decreased accumulation of CH\(_3\)I in their filtered samples. However, their experiments were not designed to resolve diurnal variability. As a result, the experiments did not provide any information on nighttime losses.

2. Chemical degradation: alternatively, CH\(_3\)I might be lost via an additional, as-yet unknown, chemical degradation pathway. Figure 5 suggests first-order (or higher) kinetics for the loss process in the "sunlit" samples, but not in the "dark" samples. We find this difference between the sunlit and dark incubations hard to reconcile with a bacterial degradation process. We speculate that the higher removal rates in the "sunlit" samples may be the result of reaction with an unknown, photochemically produced, reactive species. Clearly, an explanation for this behavior requires further experimental investigation.

\[\text{CH}_3\text{I} (\text{pM}) \text{ vs. CH}_3\text{I (pM)}\]

**Figure 5.** Loss rates of CH\(_3\)I as a function of peak daytime concentrations in both (a) "sunlit" samples (circles) and (b) "dark" samples (stars). Solid lines represent linear regressions. The dashed line in Figure 5b depicts the loss rates expected due to chloride substitution as calculated for the maximum temperatures (20°C) encountered during the study.
4.5. Correlations With Seasonally Varying Parameters

The time series of incubation experiments presented here were conducted over the same time period as a study of in situ variations published in the companion paper of Shi et al. [2014]. The annual cycles of irradiance, CDOM, nutrients, SST, salinity, and Chl are for the same sampling location are presented in Shi et al. [2014], where they were used to investigate correlations with monthly mean concentrations of in situ [CH$_3$I] and estimates of daily production rates. They found that solar radiation had the strongest positive correlation ($R^2 = 0.93$) of all variables with [CH$_3$I], which appeared to be consistent with the hypothesis that SSR is the primary forcing of CH$_3$I production in surface seawater, through a photochemical pathway. However, they also found that the daily net production rates (P$_{net}$) of CH$_3$I derived from the seasonal time series was correlated at zero lag with SST, SSR, and Chl (there was no correlation of [CH$_3$I] or P$_{net}$ with nutrients, salinity, or CDOM). The broad seasonal peak of P$_{net}$ made it impossible to distinguish the key factor controlling CH$_3$I net production using the in situ concentration data alone.

During our incubation experiments, Δ[CH$_3$I]$_{day}$ also showed a pronounced seasonal cycle with higher daytime accumulations in summer months (Figure 3). Weak but significant correlations were observed of Δ[CH$_3$I]$_{day}$ with both SSR and SST ($R^2 = 0.45$ and 0.49, respectively, $p << 0.01$, Figure 6). These regressions confirm that SSR and/or SST are closely linked to the daily production of methyl iodide in the incubation experiments. As discussed in the companion paper of Shi et al. [2014], the temporal (and geographical) variation in SST is determined strongly by irradiance. Thus, the positive correlation with SST could also be due to the influence of light and remain consistent with SSR as the primary forcing of CH$_3$I production. In the case of this incubation study, the lack of difference of Δ[CH$_3$I]$_{day}$ between F and NF samples lends some further support for the photochemical production pathway.

4.6. Insight Into the Mass Balance of CH$_3$I in the Kiel Fjord

A seasonal mass balance of CH$_3$I in the Kiel Fjord was examined using monthly in situ time series study [Shi et al., 2014], collected in parallel with these incubation studies. The results of the incubation experiments can be compared with this in situ mass balance. In Shi et al. [2014], the daily (24 h) mass balance for CH$_3$I for surface waters in Kiel Fjord was given as

$$\Delta[CH_3I]_{24h} = P_{net} - L_{sea-air} - L_{SN2} - L_{Mix}.$$  

where $L_{sea-air}$ is the daily sea-to-air flux of CH$_3$I, $L_{SN2}$ is the “chemical” loss due to nucleophilic substitution of Cl$^-$ for I$^-$, $P_{net}$ is the daily net production of CH$_3$I (in units of pmol L$^{-1}$ over a 24 h period). However, the diurnal data from the incubation experiments revealed additional, uncharacterized losses that are not

![Figure 6. Scatterplots of the daytime accumulation of CH$_3$I (Δ[CH$_3$I]$_{day}$) versus (a) SSR and (b) SST during the incubations. Red triangles denote NF-samples, black circles are F-samples, and blue stars are O-samples.](image-url)
represented in equation (3), which implies that the overall production of CH$_3$I must be considerably larger than P$_{net}$. In analogy with biological production we refer to this as gross production (P$_{gross}$) defined as

$$P_{gross} = P_{net} + L_{other}.$$  (4)

When applying equation (3) to the sealed flasks of the incubation experiments it is obvious that L$_{sea} = L_{mix} = 0$, so that equation (3) reduces to

$$D_{1/2}CH_3I/C_{138}^{24hr} = P_{net} - L_{SN2};$$  (5)

where L$_{SN2}$ is calculated using the corresponding daily average temperature, salinity, and CH$_3$I concentration. Although the monthly averages of CH$_3$I accumulation over 24 h ($\Delta[CH_3I]_{24h}$) were not statistically significant in most months because of experimental and measurement error, an estimate of P$_{net}$ was calculated using equation (5) in order to allow comparison with the P$_{net}$ values calculated from the in situ study.

Further, the accumulation in the incubation flasks during the daytime can be defined as

$$\Delta[CH_3I]_{day} = P_{gross(day)} - L_{other(day)} - L_{SN2(day)}.$$  (6)

where $\Delta[CH_3I]_{day}$ represents accumulation during the daytime period only (i.e., over 24 h period). Note that here, P$_{gross}$ is expressed on a per day basis; however, we have assumed that all production takes place during the daytime hours.

$$\Delta[CH_3I]_{night} = - L_{SN2(night)} - L_{other(night)}.$$  (7)

Here L$_{other(night)}$ and L$_{SN2(night)}$ are calculated for a 15 h period (from 17:00 to 08:00 of the second day). L$_{other(night)}$ can therefore be estimated directly from the nighttime decreases in CH$_3$I concentration observed during the incubations. While the loss rate from “sunlit” samples appears to correlate with CH$_3$I concentration (Figure 5), the estimate of L$_{other(night)}$ represents an average loss rate for the range of CH$_3$I concentrations present in the flasks over the nighttime period. The daytime concentration increase and range of CH$_3$I concentrations is similar to the nighttime concentration decrease/range and we therefore assume that average L$_{other(night)}$ is appropriate also for the loss over the daytime period, L$_{other(day)}$ as well. Given these assumptions, P$_{gross}$ was calculated using equation (6) and its monthly average values are plotted in Figure 7 (red line).

The values of P$_{net}$ derived from the incubation experiments can be compared with P$_{net}$ values derived from the field time series study. The latter exhibited strong seasonality, varying from near-zero in winter months to maximum values of 0.57 ± 0.15 pmol L$^{-1}$ d$^{-1}$ in summer (Figure 7, black line). P$_{net}$ derived from incubation experiments (Figure 7, blue line) was 0.46 ± 0.19 pmol L$^{-1}$ d$^{-1}$ in summer (June to August) and 0.14 ± 0.33 pmol L$^{-1}$ d$^{-1}$ in winter (November to February). Hence, although it was not possible to detect a seasonal cycle, the overall magnitude of P$_{net}$ derived from the incubations is in good agreement with P$_{net}$ values derived from the field study [Shi et al., 2014]. P$_{gross}$ is much higher than both estimates of P$_{net}$ in summer (from July to October). The difference reflects the excess of CH$_3$I production over loss by uncharacterized processes, such as those listed and discussed in section 4.4. Both the incubation experiments and the time series revealed a close, seasonal relationship between CH$_3$I production and light intensity.
5. Summary

“Whole-bottle” (quartz-flask) long-term (57 h) incubation experiments were conducted to examine the production of methyl iodide in nearshore, brackish waters from July 2009 to July 2010. A strong diurnal variation of methyl iodide was observed in the samples incubated under natural light conditions. This has not been observed before, likely because prior incubation experiments did not resolve the diurnal cycle. On average, the concentration of methyl iodide in the sunlit samples increased between 08:00 and 17:00, and decreased from 17:00 to 08:00. Compared to these sunlit samples, the CH$_3$I concentration in dark control samples varied only slightly over 24 h. This, and lack of a significant difference between filtered and nonfiltered samples implies that solar radiation plays an important role in the CH$_3$I production process.

Methyl iodide loss during nighttime was unexpectedly large in the “sunlit” samples. The large nighttime losses caused the daily net accumulation of CH$_3$I over 24 h periods to be low. During our incubation experiments, the calculated loss rate due to chloride substitution was 1–2 orders of magnitude lower than the observed loss rate of CH$_3$I. The loss rates of CH$_3$I correlated with its concentrations in the sunlit samples, but not in the dark samples. For the dark samples, loss rates were also higher than predicted due to chloride substitution alone. These results suggest that additional loss pathways were operating during our incubations. One possibility is bacterial degradation, because our incubation flasks were not axenic. However, the difference of loss rates between the sunlit and dark incubations is hard to reconcile with a bacterial degradation processes alone. Another possibility for the higher removal rates in the sunlit samples may be reaction with an unknown, photochemically produced, reactive species. The mechanisms of loss during nighttime require further experimental investigation.

A mass balance for CH$_3$I was calculated based on the results of incubation experiments, assuming that $P_{\text{gross}}$ is zero in the night, and $L_{\text{other}}$ is an average loss rate for the range of [CH$_3$I] during nighttime, which is also appropriate as daytime loss rate. Given these assumptions, $P_{\text{gross}}$ and $P_{\text{net}}$ were calculated on a monthly basis. $P_{\text{gross}}$ showed a strong seasonal variation with a maximum in summer and a minimum in winter, but it was not possible to resolve seasonal variable in $P_{\text{net}}$. The values of $P_{\text{net}}$ ranged from $-0.69$ to $0.67$ pmol L$^{-1}$ d$^{-1}$, consistent with $P_{\text{net}}$ values derived from the accompanying field study [Shi et al., 2014]. $P_{\text{gross}}$ is much higher than both estimates of $P_{\text{net}}$, especially in summer months. The difference implies an additional (uncharacterized) loss pathway for CH$_3$I, such as microbial degradation or “nonchloride” chemical degradation.

In summary, the diurnal variability observed in the incubation flasks shows that short-term production (and loss) rates can be very much higher than the rates estimated from long-term, low frequency trends and budgets. This has important implications for the interpretation of rates calculated from prior incubation studies using natural light, which have not resolved the diurnal cycle, as well as for the design of future field and experimental studies.

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