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Limnol. Oceanogr., 51(6), 2006, 2849–2854
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The effect of light stress on the release of volatile iodocarbons by three species of marine microalgae

Abstract—We investigated the influence of high light stress on iodocarbon release by three species of marine phytoplankton from different algal classes: the prymnesiophyte *Emiliana huxleyi*, the prasinophyte *Tetraselmis* sp., and the diatom *Thalassiosira pseudonana*. Despite a pronounced decrease in the fluorescence-based maximum quantum yield for photosystem II (F_v/F_m), increased iodocarbon release relative to lower light controls was not observed in any of the experiments performed. These findings do not support the hypothesis that upper-ocean iodocarbon concentrations are influenced by light-induced algal stress.

The iodocarbons (e.g., CH_3I , $\text{C}_2\text{H}_5\text{I}$, CH_2ClI , and CH_2I_2) are a group of iodine-containing volatile organic compounds that are known to be produced naturally in seawater and are currently believed to be the most important vectors for the transfer of iodine from the ocean reservoir to the atmosphere (Vogt et al. 1999). Once across the sea surface the iodocarbons are easily photolysed (Roehl et al. 1997) and release reactive iodine species to take part in the biogeochemical cycle of iodine and atmospheric chemistry. In the atmosphere, the iodine released by iodocarbon photolysis rapidly interacts with ozone to form iodine oxides (IO/OIO), which then either react with themselves, NO_2 , or HO_x (Allan et al. 2000). This series of reactions has the potential to affect the oxidizing capacity of the troposphere (McFiggans et al. 2000) and, hence, the processing of greenhouse gases such as N_2O and CH_4 . Furthermore, iodine oxides are now believed to play a role in regulating planetary albedo by promoting new particle or potential cloud condensation nuclei formation in the atmosphere (O'Dowd et al. 2002). Despite their apparent importance, very little is known about the processes controlling iodocarbon production in the pelagic environment.

Marine algae have been the most extensively studied for oceanic iodocarbon production (e.g., Manley and Dastoor 1987; Nightingale et al. 1995; Moore et al. 1996; Manley and de la Cuesta 1997). Despite this focus, results obtained from experimental incubations indicate that neither macro- nor microalgae are a globally significant iodocarbon source. Global extrapolations of iodocarbon production rates observed in single-species incubations of marine macro- and microalgae yield emission rates of 10^{-8} to 10^{-3} Tg yr^{-1} , orders of magnitude lower than the 0.5 Tg I yr^{-1} suggested to be emitted from the oceans to the atmosphere (Miyake and Tsunogai 1963). However, in the

natural environment algal iodocarbon release could be influenced by environmental stresses, such as nutrient limitation, light inhibition, or grazing, that are usually omitted from laboratory culture studies. Some effort has been made to test this idea in incubations of marine macroalgae. For example, light intensity, desiccation, tissue wounding, and herbivore grazing (Nightingale et al. 1995) and hydrogen peroxide (H_2O_2) stress (Pedersen et al. 1996) have all been found to influence the rate of iodocarbon production by specific species of seaweeds. However, reports from studies on the effect of environmental stress on microalgal iodocarbon production are sparse in the literature, despite the likely more widespread importance of this phenomenon.

One possible influence on microalgal iodocarbon production with particular relevance in the surface mixed layer is light intensity. In nature, phytoplankton are likely to experience continuously fluctuating light levels, and exposure to excess light could have a deleterious impact on photosynthetic organisms by causing oxidative damage to the photosynthetic apparatus, thereby resulting in a decrease in photosynthetic efficiency. Damage to photosynthetic organisms caused by excess light is believed to result in part from damage to photosystem II (PSII) caused by the oxidation of lipids, proteins, and pigments by reactive oxygen species, such as singlet oxygen ($^1\text{O}_2$), hydrogen peroxide (H_2O_2), and the hydroxyl radical (OH^\bullet) (as reviewed by Niyogi 1999). A possible link between iodocarbon production and high light stress is the haloperoxidase enzymes that are known to be involved in algal halocarbon formation (e.g., Moore et al. 1996; Ohshiro et al. 2002) via a mechanism involving the breakdown of H_2O_2 (Butler and Walker 1993).

To date the only study to address the hypothesis that microalgal iodocarbon release is increased during periods of high light stress is that of Scarratt and Moore (1999). In their study, cultures of the marine microalga *Porphyridium purpureum* (a unicellular Rhodophyte) are exposed to a light intensity ($800 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$) in excess of the acclimated irradiance level ($20 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$), and the resulting effect on aqueous iodomethane (CH_3I) concentrations was explored. Their investigation revealed no increase in the CH_3I release rate in the high light-exposed cultures relative to the lower light controls. Although this provides an important first step toward determining if high light exposure influences iodocarbon release, this study did not include any measure of the level of microalgal stress imposed by the applied treatment. The

Table 1. Target iodocarbon compounds extracted from seawater by purge and cryogenic trap and analyzed using gas chromatography–mass selective detector (GC-MSD) during this study. Associated retention times in minutes and quantifying ions (m/z) are also presented.

Compound	Structure	Retention time (min)	Quantifying ion (m/z)
Iodomethane	CH ₃ I	6.11	142
Iodomethane (deuterated)	CD ₃ I	6.15	145
Iodoethane	CH ₃ CH ₂ I	8.24	156
2-Iodopropane	CH ₃ CHICH ₃	9.30	170
2-Iodopropane (deuterated)	CD ₃ CDICD ₃	9.30	177
1-Iodopropane	CH ₃ CH ₂ CH ₂ I	10.13	170
Chloriodomethane	CH ₂ ClI	10.15	176
Bromiodomethane	CH ₂ BrI	11.43	222
Diiodomethane	CH ₂ I ₂	12.82	268

incorporation of such a measurement is essential to confirm if the high light exposure did actually cause an increased level of stress in the organisms (Davison and Pearson 1996).

Here we address the hypothesis that microalgal iodocarbon release is increased or induced during microalgal stress by exposing three unialgal laboratory cultures from different algal classes (the prymnesiophyte, *Emiliania huxleyi*, CCMP 379; the diatom, *Thalassiosira pseudonana*, CCMP 1335; and the prasinophyte, *Tetraselmis* sp., CCMP 961) to photoinhibiting levels of light. Of the studied microalgae, specific strains of *E. huxleyi* and *T. pseudonana* have been investigated previously for CH₃I release (Manley and de la Cuesta 1997). The results of this study show that while *T. pseudonana* (CCMP 1335) produces this compound in batch culture under noninhibiting light conditions, *E. huxleyi* (CCMP 370) does not. However, as algal defenses are often inducible (reviewed by Jones 1988), it is important to study iodocarbon release under both normal metabolism and during periods of stress. The level of imposed photoinhibition is assessed here by measuring the fluorescence-based maximum quantum yield for PSII (F_v/F_m), based on fluorescence with and without the photosynthetic inhibitor 3'-(3,4-dichlorophenyl)-1',1'-dimethyl urea (DCMU), a method used previously in studies of microalgal physiology (e.g., Parkhill et al. 2001)

Materials and methods: iodocarbon analysis—All iodocarbon analyses were carried out using a purge-and-trap sample preparation system with a Hewlett-Packard 6890A gas chromatograph and 5973 mass selective detector (GC-MSD) fitted with a (J & W) 60-m DB-VRX capillary column (film thickness, 0.32 μ m). The specific compounds included in this investigation are listed in Table 1. For sample preparation, 40-mL subsamples were gently filtered (0.7- μ m GF/F, Whatman) directly from the incubation syringes into 100-mL glass syringes and purged for 20 min using oxygen-free nitrogen (OFN) at a flow rate of

40 mL min⁻¹ as a purge gas. Particles/aerosols were removed from the purge gas stream using glass wool held in glass tubing. Additionally, water vapor was removed using a section of Nafion tubing (International Science Consultants) held in a molecular sieve followed by a counter-flow Nafion dryer (Perma-Pure) using OFN at a flow rate of 100 mL min⁻¹. Target analytes were then trapped and cryo-focused in a section of open stainless-steel tubing held at a temperature of -150°C using liquid nitrogen and a thermostatted heating device. The samples were then desorbed by immersing the trap in boiling water, and samples were introduced into the GC using helium as a carrier gas at a flow rate of 2.6 mL min⁻¹. Following the start of the run, the oven was held at 36°C for 5 min and then heated up to 200°C at 20°C min⁻¹, held at 200°C for 2 min, and then heated up to 240°C at 40°C min⁻¹. The MSD was operated in Single Ion Mode and was configured to collect data between 3 and 18 min of the run. All system calibrations were carried out using liquid standards (Sigma Chemical) gravimetrically prepared in high-performance liquid chromatography-grade methanol (Fisher) injected into seawater samples. Deuterated surrogate analytes (CD₃I and CD₃CDICD₃) were added to each sample analyzed to monitor and correct for system sensitivity drift. Detection limits, determined by examining the signal-to-noise ratio and external calibration using liquid standards injected into previously purged seawater samples, were on the order of 1 fmol for CH₃I and C₂H₅I and 10 fmol for CH₃CH₂CH₂I, CH₃CHICH₃, CH₂ClI, CH₂BrI, and CH₂I₂. Exact values are subject to system sensitivity drift.

F_v/F_m determinations—Samples from each culture were dark adapted for 30 min prior to F_v/F_m determinations. Following this step, fluorescence was measured using a Turner Designs fluorometer on at least duplicate 5–7-mL subsamples before and 60 s after the addition of 50 μ L of 3 mmol dm⁻³ DCMU (Sigma Chemical) prepared in ethanol (Fisher). F_v/F_m was calculated using Eq. 1.

$$\frac{F_v}{F_m} = \frac{(F_{DCMU} - F_0)}{F_{DCMU}} \quad (1)$$

Where, F_0 and F_{DCMU} are the fluorescence readings before and after DCMU addition, F_v is variable fluorescence, and F_m is maximal fluorescence. For a complete description of the theory behind this measurement, see Parkhill et al. (2001).

Light stress experiments—The three microalgal strains used during this study (*Emiliania huxleyi*, CCMP 379; *Tetraselmis* sp., CCMP 961; and *Thalassiosira pseudonana*, CCMP 1335) were obtained from the Provasoli–Guillard Centre for the Culture of Marine Algae and Protozoa (CCMP, West Boothbay Harbor). Batch cultures for use in each experiment were initially grown in bulk (i.e., 1–1.5 liters) in seawater enriched with $f/2$ nutrients (Guillard 1975) in a Sanyo incubation cabinet maintained at 15°C. Light was supplied by fluorescent tubes (FL40SS W/37, Sanyo) at an intensity of 47 μ mol photons m⁻² s⁻¹ (measured using a Macam Photometrics Spectroradiometer with

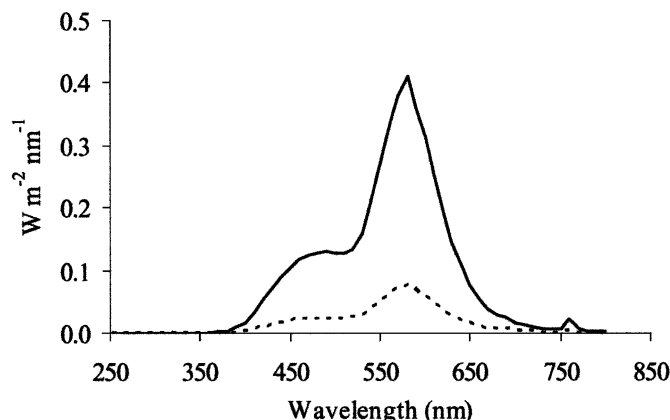


Fig. 1. Irradiance spectra for the FL40SS W/37 (Sanyo) fluorescent tubes used as a light source during this study. The solid line shows the spectrum obtained at $250 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ and the dashed line shows the measurement at $47 \mu\text{mol photons m}^{-2} \text{s}^{-1}$.

a flat cosine irradiance sensor) and a light:dark (LD) rhythm of 14:10 h. An irradiance spectrum of the light source is shown in Fig. 1. To determine growth stage, cell counts were carried out periodically in at least duplicate on 0.3–2.0-mL aliquots of each culture using a Beckman Coulter Multisizer fitted with a 100- μm aperture. Dense cultures were diluted with 0.2 μm filtered seawater prior to counting to prevent blockage of the aperture. Some additional cell counts were carried out manually using a microscope and a hemocytometer to ensure that Multisizer counts were correct. All cultures were handled in a Category 2 Microbiology Cabinet. Checks on bacterial contamination were made using an acridine orange staining technique (Hobbie et al. 1977).

Between 12 and 15 h prior to the start of each experiment the cultures were transferred to $4 \times 250\text{-mL}$ autoclaved glass syringes to allow time for physiological acclimation to the new culture vessels. All experiments were carried out on late-logarithmic phase cultures with F_v/F_m values of approximately 0.50–0.60. T_0 samples for iodocarbon analysis, CPC, and cell counts were taken from each of the four syringes while all cultures were held at the acclimated light intensity. Starting cell densities (\pm standard deviation) of *E. huxleyi*, *Tetraselmis* sp., and *T. pseudonana* were $1.80 (\pm 0.03)$, $0.16 (\pm 0.01)$, and $1.09 (\pm 0.05) \times 10^6 \text{ cell mL}^{-1}$, respectively. Following this step, two culture syringes were transferred to a second Sanyo incubation cabinet with the same temperature setting and LD cycle but were held at a higher light intensity of $250 \mu\text{mol photons m}^{-2} \text{s}^{-1}$. Given that the iodocarbons are known to be susceptible to photolysis (Martino et al. 2005), one concern could be that the loss rate in the high light treatments is greater than that at the lower light intensity. However, a supplementary experiment revealed that none of the iodocarbons of interest are subject to photolysis following exposure to the light source used in this investigation at either 47 or $250 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ (see Fig. 2 for data obtained for CH_2I_2 and CH_2ClI). Given the absorption spectra for the iodocarbons

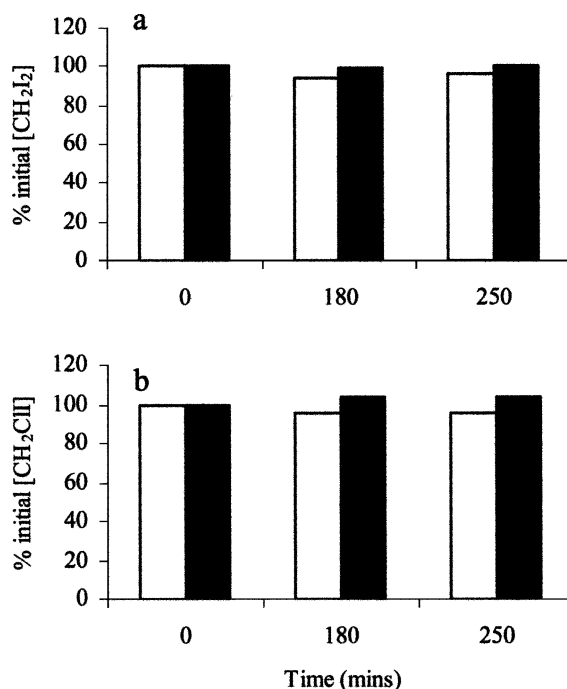


Fig. 2. The results of a supplementary experiment designed to examine the rate of iodocarbon photolysis at $250 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ and $47 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ using FL40SS W/37 (Sanyo) fluorescent tubes as a light source. The percentage changes in (a) CH_2I_2 and (b) CH_2ClI concentrations during 250 min of exposure are shown. The white and black bars show the data obtained at 47 and $250 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, respectively.

presented by Roehl et al. (1997), it is likely that significant photolysis of these compounds only takes place in the presence of light of shorter wavelengths. In addition to $T=0$, samples were taken at no fewer than three time points from each syringe over the course of the experiments, which were 24–25 h in duration. Samples for iodocarbon analysis and F_v/F_m determinations were taken at each of these time points, and samples for cell counts were taken at the beginning and end of each experiment.

Results: physiological measurements—Figure 3 shows the observed changes in F_v/F_m in both control and high light treatments of cultures of *E. huxleyi*, *Tetraselmis* sp., and *T. pseudonana*. Although some changes in F_v/F_m values were observed in the low light controls, these were in all cases substantially less pronounced than those seen in the replicate cultures exposed to the higher light intensity. The different responses to the applied light treatments observed between cultures are likely due to interspecific variations in the ability to adapt to exposure to varying irradiance levels. Good agreement was observed between replicate cultures and duplicate analyses.

Iodocarbon analyses—Figure 4 shows that despite the observed changes in F_v/F_m in the high light-exposed cultures (see Fig. 1), no increase in the concentrations of CH_3I and CH_2ClI relative to the controls was observed in

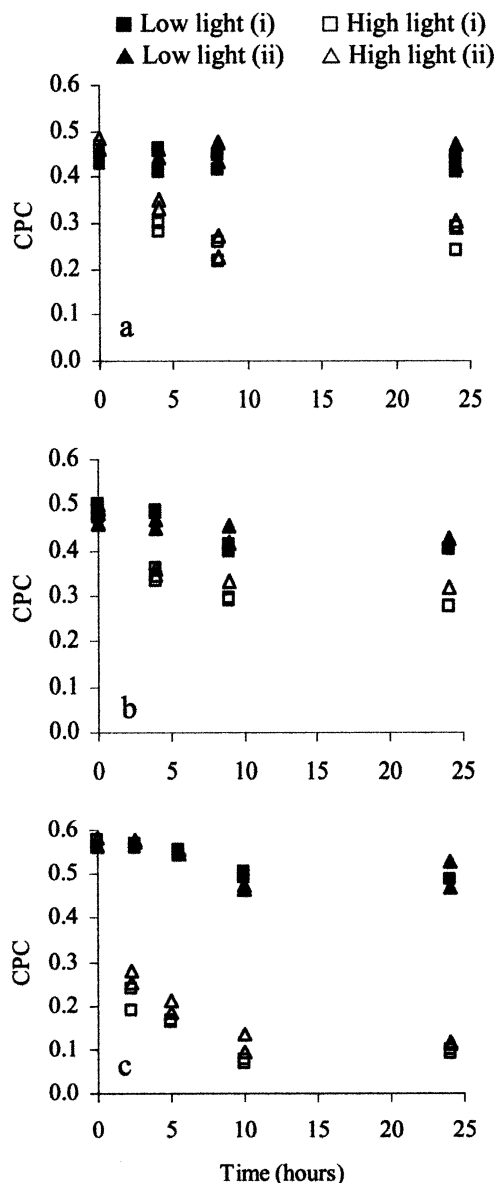


Fig. 3. Changes in F_v/F_m estimated using the DCMU method in high- ($250 \mu\text{mol m}^{-2} \text{s}^{-1}$; open symbols) and low light- ($47 \mu\text{mol m}^{-2} \text{s}^{-1}$; closed symbols) exposed cultures of (a) *Emiliana huxleyi* (CCMP 379), (b) *Tetraselmis* sp., (CCMP 961), and (c) *Thalassiosira pseudonana* (CCMP 1335). The results shown are duplicate analyses from each replicated culture (i and ii) held at low and high light intensities.

any culture. In addition, no production of $\text{C}_2\text{H}_5\text{I}$, $\text{CH}_3\text{CH}_2\text{CH}_2\text{I}$, $\text{CH}_3\text{CHICH}_3$, CH_2I_2 , and CH_2BrI relative to control cultures was observed (data not shown). While the focus of this study was primarily on volatile iodocarbons, it is worth reporting that peak area data were also obtained for some bromo- and chlorocarbon compounds (CH_2Br_2 , CHBr_3 , and CHBr_2Cl), but no changes in the peak areas of any of these compounds were observed in either high or low light treatments. Given that these data were uncalibrated, they will not be discussed further here.

Discussion—Cultures of *E. huxleyi* (CCMP 379), *Tetraselmis* sp. (CCMP 961), and *T. pseudonana* (CCMP 1335) were examined for the influence of high light stress on microalgal iodocarbon release. In agreement with the study of Scarratt and Moore (1999), light-induced stress did not induce iodocarbon release in any of the cultures examined, despite obvious decreases in the fluorescence-based maximum quantum yield for PSII (F_v/F_m), indicative of oxidative damage to the photosynthetic apparatus (reviewed by Niyogi 1999). These findings, together with the results of our preliminary experiments, which eliminated the possibility of increased photolysis in the high light treatments (see Fig. 2), show that iodocarbon release is not associated with light stress and is unlikely to be linked with protection against oxidative damage in the microalgae studied.

The phytoplankton species used in this study were chosen to represent three distinctly different algal groups that are common to the marine environment. If the findings presented here are representative of a wide range of marine phytoplankton, they indicate that turbulence-driven, seasonal, and climate change-induced variations in irradiance flux to surface ocean waters will not induce increased iodocarbon release.

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Acknowledgments

This work was funded by the United Kingdom Natural Environment Research Council (NERC grants NER/S/A/2000/03326, GT5/98/8/MS, NER/G/S/2003/00024, and NE/B501039/1). Financial support for some of the equipment was provided by the Laboratory for Global Marine and Atmospheric Chemistry (LGMAC), NERC grant NER/H/S/1999/00176. The authors would like to acknowledge the technical support of Gareth Lee and Liz Rix and the helpful comments of two anonymous reviewers.

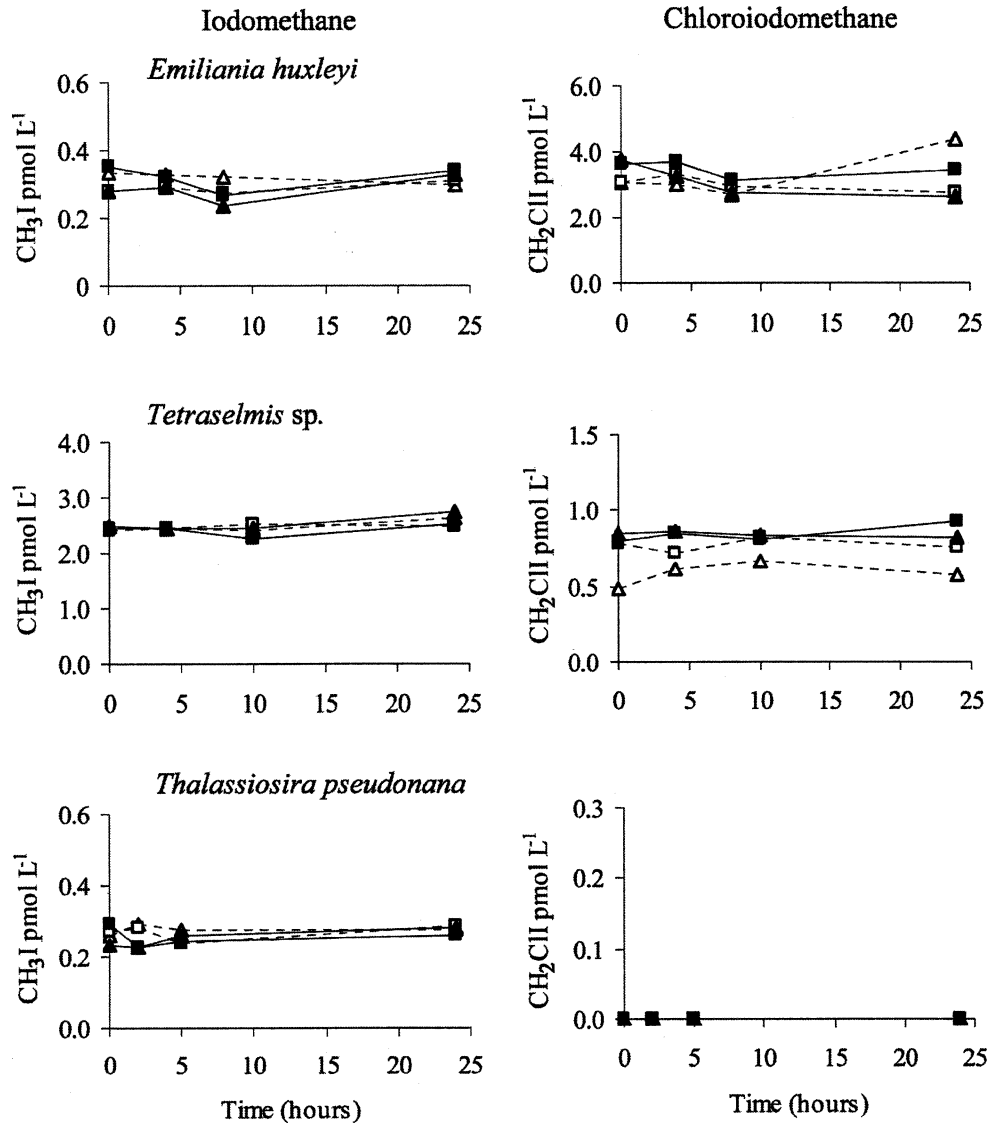


Fig. 4. Changes in the concentrations of iodomethane (CH_3I) and chloriodomethane (CH_2ClI) in high- ($250 \mu\text{mol m}^{-2} \text{s}^{-1}$; dashed lines, open symbols) and low light ($47 \mu\text{mol m}^{-2} \text{s}^{-1}$; solid lines and closed symbols) exposed cultures of *Emiliana huxleyi* (CCMP 379), *Tetraselmis sp.* (CCMP 961), and *Thalassiosira pseudonana* (CCMP 1335). CH_2ClI peak areas were consistently below detection limits in the *T. pseudonana* cultures. The results shown were obtained from replicated cultures held at each light intensity.

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Received: 21 November 2005

Accepted: 14 June 2006

Amended: 5 July 2006