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Methyl chloride (CH_3Cl) production in phytoplankton cultures

Abstract—Unialgal nonaxenic cultures of seven species of phytoplankton, including both warm- and cold-water organisms, were examined under halocarbon-clean conditions for the production of CH_3Cl. Incubations of all species showed CH_3Cl increases significantly greater than in the control medium. The magnitude of increase varied with the phytoplankton species present and in some cases also with growth stage. Longer time series showed that CH_3Cl continued to increase after the death of all the phytoplankton cells. Although direct production as a by-product of phytoplankton metabolism cannot be ruled out earlier in the time series, indirect chemical and (or) biotic reaction of algal-associated organic matter is required to explain this continued increase. Characterization of the role of bacteria in both pre- and postsenescent stage CH_3Cl production will require comparable experiments carried out under axenic conditions.

Recognition of the role played by volatile organohalogen compounds in the regulation of stratospheric ozone (Molina and Rowland 1974) prompted extensive study into cycles of both anthropogenic and natural halomethanes in the environment. CH_3Cl was found to be the dominant organochlorine compound in the troposphere (Singh et al. 1977; Rasmussen et al. 1980). The lack of interhemispheric gradient in the free tropospheric mixing ratio of CH_3Cl (Rasmussen et al. 1980; Koppmann et al. 1993) suggested a widespread natural source. Both CH_3Cl and CH_3Br have lifetimes within the troposphere (1.7 yr—Koppmann et al. 1993; Mellouki et al. 1992) sufficient to allow some fraction of surface inputs to reach the stratosphere.

Measurements by Lovelock (1975) first indicated that

biology within the oceans might be an important source of organohalogens to the troposphere. Concentrations of CH_3I were 1,000-fold greater in a *Laminaria digitata* bed compared with open-ocean surface waters (Lovelock 1975). A more recent investigation of macroalgal production of monohalomethanes (Manley and Dastoor 1987) used *Macrocystis pyrifera,* a giant kelp. These results suggested that the methyl halide release occurred as a direct by-product of kelp metabolism. Microbial degradation of kelp organic matter has also been implicated in CH_3I production (Manley and Dastoor 1988). Wuosmaa and Hager (1990) described the isolation of a methyltransferase enzyme from the rhodophyte *Endocladia muricata* capable of methylating halide ions. When incubated in seawater, *E. muricata* whole cells and cell-free extracts showed only CH_3Cl formation. A widespread distribution of "methyl chloride transferase" activity in macroalgae was suggested by the detection of CH_3Cl production in 50% of species randomly collected from Monterey Bay, California. Activity of this methyltransferase enzyme was also identified in a terrestrial fungus and a higher plant. Emission of some polyhalogenated compounds, most notably bromoform ($CHBr_3$), dibromomethane (CH_2Br_2), bromodichloromethane ($CHBrCl_2$), chlorodibromomethane ($CHBr_2Cl$), and diiodomethane (CH_2I_2), by some species of macroalgae is well documented (e.g Gschwend et al. 1985; Manley et al. 1992). Macroalgal production of polybrominated methanes is an important source of organobromine to the troposphere (Gschwend et al. 1985; Manley et al. 1992). However, as calculated by Manley and Dastoor (1987), release of monohalomethanes by coastal macroalgae is unlikely to be important on a global scale.

The first direct evidence of phytoplankton involvement in the natural production of some volatile organohalogens was $CHBr_3$ emission observed in laboratory incubations of microalgal communities collected from beneath arctic annual ice (Sturges et al. 1992). Release of CH_2Br_2, $CHBr_2Cl$, and $CHBrCl_2$ has also been detected in laboratory incubations of ice-algae from the antarctic (Sturges et al. 1993) and in situ incubations in the arctic (Moore et al. 1994). The influence of bacteria and grazers present in these natural populations on halocarbon emission is not yet clear. Production of $CHBr_3$, $CHBr_2Cl$, $CHBrCl_2$, and CH_2ClI has been observed in unialgal laboratory cultures of two cold-water phytoplankton species—*Porosira glacialis* and *Nitzschia* sp. (CCMP 580) (Tokarczyk and Moore 1994). To our knowledge, only circumstantial evidence has been published supporting a phytoplankton role in the production of the monohalomethanes. The widespread supersaturation of CH_3Cl (Singh et al. 1983; Tait et al. 1994) and CH_3Br (Singh et al. 1983; Khalil et al. 1993) in open-ocean surface water cannot be explained by release from coastal macroalgae. Above-average concentrations of CH_3I have been observed in seawater and air in regions typified by high bioactivity (Rasmussen et al. 1982). We report here the production of CH_3Cl by unialgal cultures of several species of phytoplankton—the first direct evidence supporting a phytoplankton role in oceanic CH_3Cl production.

Nonaxenic cultures of several species of phytoplankton have been examined for the production of CH_3Cl. About 450 ml of autoclaved f/2 or f/8 medium (Guillard 1975) in a glass vessel was purged with ultra-high purity (UHP) air/CO_2 (~0.1% CO_2) for 36 h to remove halocarbon contaminants. Following inoculation with 2–3 ml of algal culture, gentle purging was continued for a further 3 h, then the vessel was sealed. Warm-water species (diatoms: *Odontella mobiliensis, Phaeodactylum tricornutum, Thalassiosira weissflogii*; prymnesiophyte: *Isochrysis galbana*) were incubated at 20°C under "cool-white" fluorescent tubes (~70 μmol quanta m^{-2} s^{-1}) with a diel L/D cycle of 16:8. Cold-water phytoplankton (diatoms: *Porosira glacialis, Nitzschia seriata, Nitzschia* sp. CCMP 580) were held under constant illumination of 10 μmol quanta m^{-2} s^{-1} and incubated at 4°C. Samples of medium were withdrawn every 2–3 d through a metal needle into vacuum-baked (100°C, minimum 24 h) all-glass syringes for immediate determination of CH_3Cl. During sample removal, a low flow of air/CO_2 was maintained to prevent entrance of contamination from the atmosphere. The gas flow was continued for a further 3 min to supply CO_2. Care was taken to minimize the numbers of phytoplankton removed from the vessel. In the case of the cold-water species and *O. mobiliensis,* the cells settled at the bottom of the vessel, and negligible numbers were withdrawn during sampling. *T. weissflogii, P. tricornutum,* and *I. galbana,* however, were dispersed throughout the medium volume, and removal of cells could not be avoided. It is unknown whether the physical disruption of the cells of these three species during the purge step of the analysis results in additional release of CH_3Cl. All species except *N. seriata* were originally obtained from the Provasoli-Guillard Center for the Culture of Marine Phytoplankton.

Analysis of CH_3Cl was by purge-and-trap gas chromatography with oxygen-doped electron capture detection (ECD). The method used was modified from Tait et al. (1994) (Fig. 1). A 10-ml sample of culture medium was injected directly through a multiport valve into the purge vessel where it was purged for 5 min (40 ml min^{-1}) with UHP helium onto a Porapak-Q trap held at 0°C (ice/water mixture). The trap was desorbed (100°C) into the gas chromatograph (HP 5890), where it was separated isothermally (70°C) on a 30 m GS-Q megabore column (J&W Scientific) with a helium carrier flow of 6 ml min^{-1}. We doped the UHP N_2 make-up gas with 0.5% oxygen (Grimsrud and Miller 1978, 1980; Rasmussen et al. 1980) to increase the sensitivity of the ECD (275°C) to CH_3Cl and extend the linear calibration range. The resulting detection limit was 7 pM, and precision was ±4% at the 200 pM level. The presence of CH_3Cl in our phytoplankton cultures has been confirmed by GCMS.

Standards were prepared by injecting 20 μl of pure CH_3Cl vapor (Aldrich Chem. Co.) through a Teflon-backed septum into 500 ml of prepurged (UHP N_2), cooled (2–3°C) Super-Q water contained in a sealed glass vessel. A stir bar ensured rapid mixing and enhanced the rate of vapor dissolution. The low temperature and the high ratio of liquid to headspace volume within the vessel (50) resulted in negligible partitioning of the injected CH_3Cl into

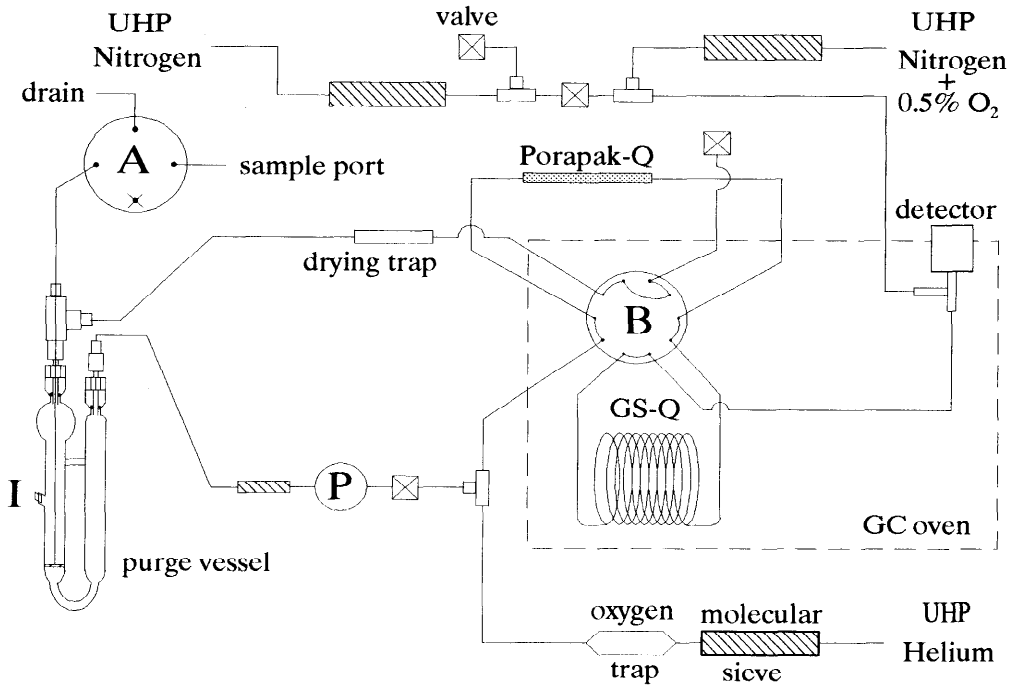

Fig. 1. Schematic of purge-and-trap gas chromatography system for the analysis of CH_3Cl in seawater: A—four-port Valco distribution valve; B—10-port two-position Valco valve; I—injection port for standard; P—pressure regulator.

the gas phase. A time series of the standard that started 5 min after injection of the pure vapor into the vessel showed no significant change in peak area over a 12-h period, indicating that not only was equilibrium reached quickly within the vessel but also that the integrity of the standard was maintained over this time period. Precision of standards day to day was ±7% (C.V.).

After gentle agitation to disperse cells, samples were also withdrawn from the incubation vessels for light microscope (Fuchs Rosenthal counting grid) and Coulter Counter cell counts to follow the growth of the cultures. Acridine orange epifluorescence microscopy (Hobbie et al. 1977) was used on several occasions to determine numbers of bacteria.

Unequivocal interpretation of field results is often hindered by the complex, dynamic nature of the ocean waters being sampled. Monospecific incubations of phytoplankton offer a more controlled environment in which to investigate possible ways phytoplankton may contribute to the CH_3Cl pool. The primary goal of these experiments was to determine whether any CH_3Cl release could be detected. Initially, high nutrient concentrations (f/2) were used to promote elevated cell densities. Later incubations with nutrient levels reduced to a quarter the former level allowed shorter experiment times without early nutrient limitation. The pH in all cultures at time zero was between 7.6 and 7.9. Media were not buffered, and the pH rose to 9.0 during the exponential and early stationary phases in the densest cultures.

Figure 2A shows the concentration of CH_3Cl measured in cultures of *T. weissflogii* and *P. tricornutum*. Production was slower during the exponential growth phase and increased with the onset of the stationary phase. Highest rates of CH_3Cl increase in the *T. weissflogii* culture were seen during and following phytoplankton senescence. No significant CH_3Cl production was observed in the control medium. Slow increases during the phytoplankton exponential growth phase compared with later in the time series were seen in repeat cultures of *P. tricornutum* and *T. weissflogii* and also in a culture of *Nitzschia* sp. (CCMP 580) (Fig. 2B). Unlike the *T. weissflogii* culture, however, production of CH_3Cl in the presence of CCMP 580 leveled off during the senescent phase. Incubations of *N. seriata* and *P. glacialis* that were run parallel to CCMP 580, although showing CH_3Cl levels significantly greater than the control, exhibited a lower, more uniform rate of CH_3Cl increase (Fig. 2B). The magnitude and pattern of CH_3Cl production depended on the species of phytoplankton present in the vessel.

Analysis of cultures of two of the warm-water species, *O. mobiliensis* (Fig. 3) and *T. weissflogii* (Fig. 2A), were continued after the death of all the phytoplankton cells. In both cases, CH_3Cl levels continued to increase. The extended incubation of *O. mobiliensis* still showed further CH_3Cl increase 2–3 weeks later. A dense culture (36,000 cells ml^{-1}) of *O. mobiliensis* was filtered (3-μm Nuclepore) under gentle vacuum to remove the phytoplankton cells (dimensions, ~50 × 75 μm) and leave only bacteria and algal exudates. The resulting filtrate was then purged of volatile halocarbons (air/CO_2) and incubated in a closed culture vial. The filtration step removed 80–90% of the bacteria, probably due to adhesion to phytoplankton cells. However, CH_3Cl showed a significant increase relative to a similarly filtered control medium (Fig. 4). Although

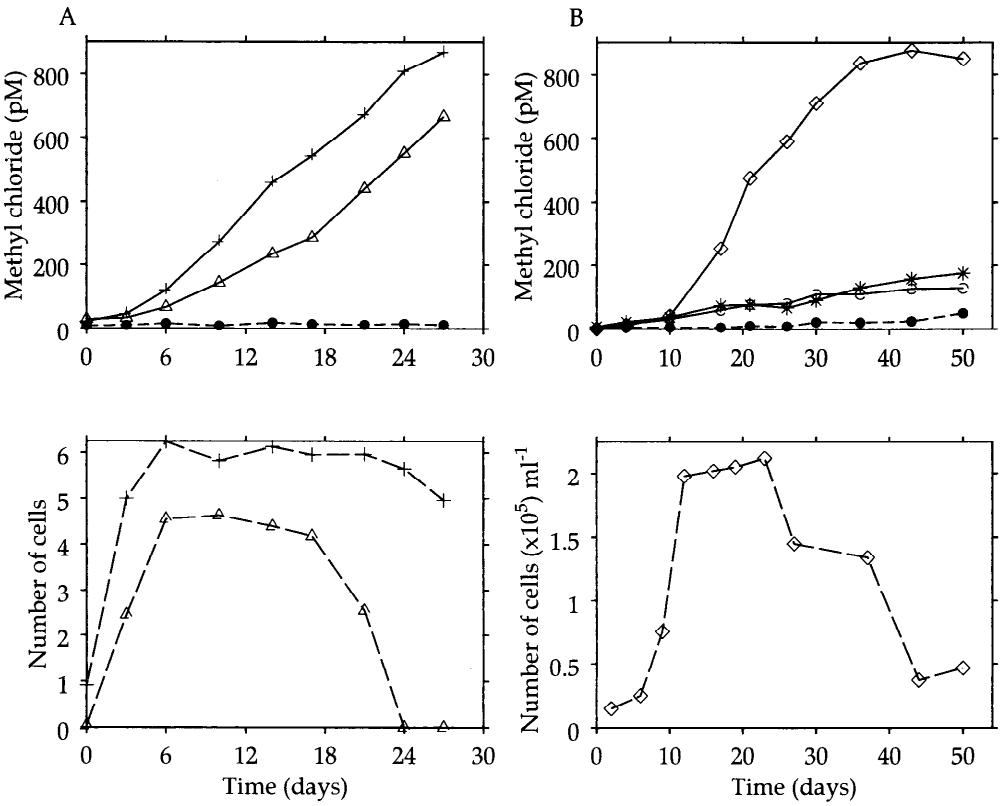

Fig. 2. A. Time series of CH_3Cl (above) and cell numbers (below) for two warm-water diatoms: *Thalassiosira weissflogii* (△) and *Phaeodactylum tricornutum* (+). Cell numbers for *P. tricornutum* are $\times 10^6$ ml^{-1} and for *T. weissflogii* $\times 10^5$ ml^{-1}. Concentration of CH_3Cl measured in a control vessel containing only culture medium—●. B. As panel A, but in media from cultures of cold-water diatoms; CCMP 580 *Nitzschia* sp. (◇), *Porosira glacialis* (*), and *Nitzschia seriata* (○). Control—●. Lower panel shows cell numbers in the CCMP 580 culture.

direct production of CH_3Cl by the phytoplankton cannot be ruled out in the full culture, indirect reactions (chemical or bacterially mediated) of algal organic compounds are required to account for these results.

Two indirect pathways of CH_3Cl production in seawater have been suggested. Zafiriou (1975) demonstrated that chloride substitution of CH_3I was a mechanism of CH_3Cl formation in seawater. However, when the rate constants he measured in the laboratory are applied to the average level of CH_3I throughout much of the open ocean (1–3 pM; Lovelock 1975; Singh et al. 1983), <5% of the CH_3Cl flux estimated to come from oceanic sources (Singh et al. 1983; Tait et al. 1994) can be produced by this reaction. CH_3I was not significantly different from the control in cultures of both *N. seriata* and *P. glacialis* and was only slightly higher in *Nitzschia* sp. CCMP 580 (R. Tokarczyk pers. comm.). Unless more rapid alternative pathways of CH_3I transformation to CH_3Cl exist, CH_3I cannot be the source of the CH_3Cl observed in our cultures.

DMSP (β-dimethylsulphoniopropionate), the biological precursor of DMS in seawater (Andreae 1990), can react with chloride ion to produce CH_3Cl (White 1982). An experiment that used high levels of DMSP (400 nM) in 0.2-μm filtered seawater (pH 8.0) sealed in a covered incubation vessel showed insignificant CH_3Cl production over a 1-week interval (Tait and Moore unpubl. results). One of the species we have cultured (*P. tricornutum*) has been shown to contain negligible amounts of DMSP in batch culture conditions (Keller et al. 1989). However, the greatest CH_3Cl production to date was found in vessels containing this species. It is unlikely that the chemical DMSP+chloride pathway of CH_3Cl formation can explain the CH_3Cl production observed in cultures of *P. tricornutum*.

Antibiotics (~1.0 mg ml^{-1} streptomycin:2.0 mg ml^{-1} penicillin) were added to the extended *O. mobiliensis* culture (Fig. 3) at 61 d. No further significant increase in either CH_3Cl or bacterial numbers was observed. Although this result suggests that bacteria may have contributed to CH_3Cl production after the death of all the phytoplankton cells, the leveling off may have resulted from exhaustion of an algal-produced substrate that reacted chemically to give CH_3Cl. We propose that the increase in CH_3Cl production after the death of all the phytoplankton cells occurs via precursors that are released by the phytoplankton and that are bacterially or chemically broken down to give CH_3Cl. Earlier in the

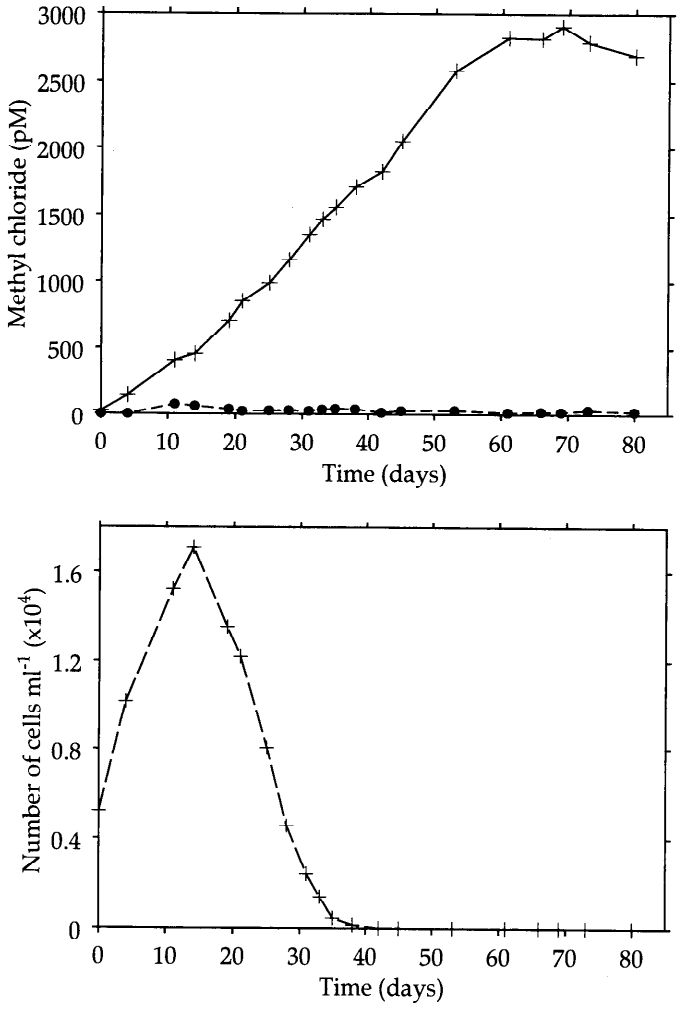

Fig. 3. Time series of CH_3Cl concentration in medium from a culture of *Odontella mobiliensis* (+) and a control vessel containing medium alone (●). Antibiotics (penicillin/streptomycin) were added at 61 d. Lower panel shows cell numbers.

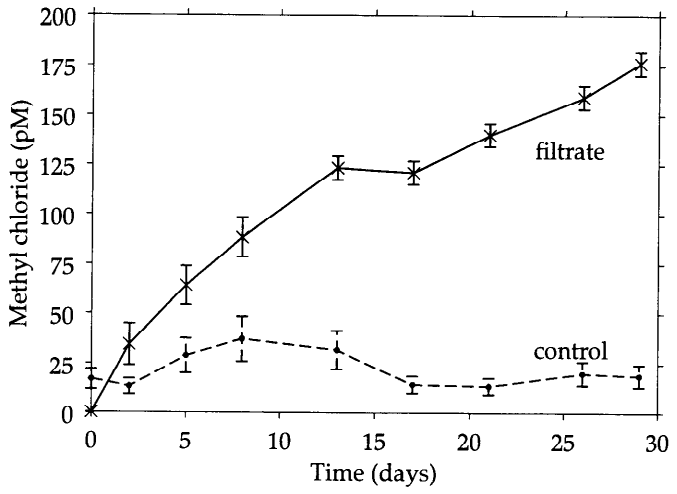

Fig. 4. "Indirect" CH_3Cl production in culture medium from which *Odontella mobiliensis* cells were removed (3-μm Nuclepore). Error bars include uncertainty in the measured concentration from run-to-run precision at low concentrations of CH_3Cl (n = 9) and day-to-day variation of standards during the time series.

time series, when living phytoplankton were present, direct production of CH_3Cl as a by-product of phytoplankton metabolism is a possibility. Further experiments under axenic conditions are underway to determine whether phytoplankton themselves are capable of producing CH_3Cl, directly or indirectly, via the release of a chemically labile precursor. CH_3Br, although not quantified, was detected in cultures of *O. mobiliensis* and *P. tricornutum*.

Due to the unknown effects of bacteria present in these cultures, our results must be defined as net CH_3Cl production. The sustained nature of CH_3Cl increase after the death of all the phytoplankton cells in the *O. mobiliensis* incubation suggests that in this case bacterial processes may contribute to net CH_3Cl production. However, evidence of microbial degradation of CH_3Cl has been reported. Fortuitous co-oxidation (see Stirling and Dalton 1979) of both CH_3Cl and CH_3Br has been shown to occur in cell-free suspensions of the broadly specific methane-oxygenase enzyme system (Stirling and Dalton 1979, 1980). There is also evidence (Hyman and Wood 1984) that ammonia mono-oxygenase from *Nitrosomonas europaea*, an autotrophic ammonia-oxidizing bacterium, is capable of performing similar co-oxidations.

The artificial conditions of culture (enclosure within a small volume, elevated nutrient levels, and high monospecific algal cell densities) may favor dominance of bacterial species and strains not abundant in natural marine populations. Both methane-oxidizing bacteria (Sieburth et al. 1993) and strains of *N. europaea* (Austin 1988) have been isolated from aerobic upper open-ocean waters. Microbial degradation of CH_3Cl and CH_3Br is possible within the aerobic upper layer of the oceans. Identification of species of bacteria present in the cultures will ultimately be necessary to determine whether bacterial processes occurring in the vessels are likely to occur in the environment. Phytoplankton might also be expected to behave differently in batch culture than in the natural environment. No fungi have been observed in any of the laboratory incubations.

The oceans are thought to be the source of ~80% of the flux of CH_3Cl to the troposphere (Crutzen et al. 1979; Rasmussen et al. 1980), but little is known about its dominant formation pathways in seawater. The unialgal culture experiments described here offer one means of investigating the nature of the marine biological source. Although the rates of halocarbon production observed under such conditions should be applied to the marine environment with extreme caution, a rough comparison has proved informative.

When scaled for the three orders of magnitude greater Chl *a* concentrations in culture compared with the marine environment, rates of CH_3Cl increase measured in our experiments (avg ~25 nmol m^{-3} d^{-1}) can account for <0.5% of the average net CH_3Cl required to sustain the

estimated sea-to-air flux. Therefore, although the production of CH_3Cl in phytoplankton culture has been clearly demonstrated, it is not yet clear how significant this production might be in the environment. The list of examined organisms is far from extensive and is dominated by diatoms. Despite this, a dependence of CH_3Cl increase on the phytoplankton species cultured is evident. It is possible that other phytoplankton species not yet studied contribute more significantly to the oceanic pool of CH_3Cl. The effect of the culture conditions also requires attention. In the northwest Atlantic, the highest supersaturations of CH_3Cl were found at lower latitudes, within nutrient-depleted, surface layers above the seasonal pycnocline (Tait et al. 1994). First, the phytoplankton species which have been studied to date, although easy to grow in culture, are not typical of such areas; second, the low nutrient, high light levels of the northwest Atlantic differ dramatically from the culture conditions. Whether the bacteria present in the cultures play a role in production or consumption of CH_3Cl is presently under investigation.

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Enhanced bacterial growth in response to photochemical transformation of dissolved organic matter

Abstract—We tested the hypothesis that light, especially UV-B radiation, increases the availability of dissolved organic matter (DOM) to pelagic bacteria in lake water. Filtered (0.2 μm) and autoclaved humic lake water (water color 70 mg Pt liter^{-1}, 12 mg dissolved organic C liter^{-1}) was exposed to simulated sunlight (UV-B, 1.14 W m^{-2}; UV-A, 3.87 W m^{-2}; and PAR, 20 W m^{-2}) for various periods of time (0–100 h). Irradiated water was then inoculated with a natural bacterial assemblage (0.6-μm filtered water) and bacterial yield was measured in the stationary phase of the resulting batch cultures. Both bacterial numbers and cell volumes increased (numbers by 65% and volumes as much as 360%) with increasing UV radiation, resulting in an almost sixfold increase in bacterial biomass. Our experiment shows that light exposure can enhance availability of natural lake DOM to bacteria, possibly through cleavage of macromolecules into smaller units, and may influence both carbon cycling and food webs in lake water.

Dissolved organic matter (DOM) in lake water can be divided into labile and recalcitrant moeities. Labile DOM originates primarily from autochthonous primary producers (e.g. phytoplankton and submersed macrophytes). DOM from allochthonous sources, on the other hand, is generally considered more recalcitrant because it has been exposed to microbial degradation and transformation before reaching the lake and because it, to a large extent, is based on recalcitrant support tissues of terrestrial vascular plants (Hobbie 1988). Nevertheless, high biomass of pelagic bacteria can be found in humic lakes, despite low or moderate autochthonous production of bacterial substrates. It has been suggested that this high biomass is due to extensive bacterial utilization of allochthonous DOM (Hessen 1985; Tranvik 1988).

The availability of recalcitrant DOM may be increased by photochemical reactions (Kieber et al. 1989). Exposure of humic water to short wavelength light bleaches the brown color, due to cleavage of large organic molecules and aromatic structures into smaller units (Strome and Miller 1978).

Depletion of the stratospheric ozone layer (Gleason et al. 1993), resulting in increased UV-B irradiation, has stimulated studies of the role of UV-light in ecosystem processes. Considerable work has been done on the direct harmful effects of UV-B radiation on organisms in water (e.g. bacteria: Herndl et al. 1993; phytoplankton: Karentz et al. 1994; zooplankton: Thomsen 1986; fish: Hunter et al. 1979) and also on such indirect effects as altered community succession and food-web dynamics (Bothwell et al. 1994).

UV-light may, by transforming recalcitrant DOM into labile forms (Kieber et al. 1989), have positive effects on bacterial production. We hypothesize that photochemical reactions have a significant impact on the carbon cycle of pelagic waters, working in concert with bacteria in the degradation of DOM. Our study describes the availability of DOM from a humic lake as a function of exposure to short wavelength light similar to sunlight.

Surface water was sampled from mesohumic Sjättesjön (pH 7.16; conductivity, 10.4 mS m^{-1}; water color, 70 mg Pt liter^{-1}; absorbance at 430 nm, 0.045 cm^{-1}; DOC, 12 mg liter^{-1}) in southern Sweden in April 1992. The water was transported in a 5-liter polyethylene container to the laboratory and stored cold (+4°C) for a few hours until further processing. All glassware, filtration equipment, etc. was acid rinsed and autoclaved.

To avoid limitation of bacterial growth by inorganic nutrients, we enriched the water with inorganic P and N (final concn, 1 μM P and 10 μM N) with solutions of NH_4NO_3 and KH_2PO_4. The concentrations are within the range typically found in mesotrophic lakes. Thus, potential involvement of the added nutrients in the photochemical reactions under study would probably be com-