

Production of bromoform and dibromomethane by Giant Kelp: Factors affecting release and comparison to anthropogenic bromine sources

Kelly D. Goodwin,¹ Wheeler J. North, and Mary E. Lidstrom²

California Institute of Technology, Environmental Engineering Science 138-78, Pasadena, California 91125

Abstract

Macrocystis pyrifera (Giant Kelp), a dominant macroalgal species in southern California, produced 171 ng per g fresh wt (gfw) per day of CHBr_3 and 48 ng gfw⁻¹ d⁻¹ of CH_2Br_2 during laboratory incubations of whole blades. Comparable rates were measured during in situ incubations of intact fronds. Release of CHBr_3 and CH_2Br_2 by *M. pyrifera* was affected by light and algal photosynthetic activity, suggesting that environmental factors influencing kelp physiology can affect halomethane release to the atmosphere. Data from H_2O_2 additions suggest that brominated methane production during darkness is limited by bromide oxidant supply. A bromine budget constructed for a region of southern California indicated that bromine emitted from the use of CH_3Br as a fumigant (1×10^8 g Br yr⁻¹) dominates macroalgal sources (3×10^6 g Br yr⁻¹). Global projections, however, suggest that combined emissions of marine algae (including microalgae) contribute substantial amounts of bromine to the global cycle, perhaps on the same order of magnitude as anthropogenic sources.

Investigations of brominated methane sources are motivated in part by concerns over the roles brominated species play in the atmosphere. Bromine delivered to the atmosphere from marine and terrestrial sources may participate in ozone-depleting chemistry (e.g. Yung et al. 1980). Changes in brominated methane concentrations also have the potential to affect hydroxyl radical ($\text{OH}\cdot$) concentrations, leaving $\text{OH}\cdot$ unavailable to destroy greenhouse gases or gases involved in stratospheric ozone depletion. An accurate biogeochemical picture of brominated methanes is needed to predict their role in changing tropospheric and stratospheric ozone concentrations, which in turn are predicted to affect climate (Ramanathan et al. 1985; Lacy et al. 1990). In addition, quantifying the biogenic sources of brominated methanes provides a context in which to evaluate the impacts of anthropogenic emissions.

Methyl bromide (CH_3Br) is known for its role in atmospheric chemistry (e.g. Butler and Rodriguez 1996), and, in addition, bromoform (CHBr_3) and dibromomethane (CH_2Br_2) are important to the bromine biogeochemical cycle (Reifenhäuser and Heumann 1992). Marine biogenic sources of CHBr_3 and CH_2Br_2 have been indicated from concentration patterns in air (Class and Ballschmiter 1988; Atlas et al. 1993) and seawater (e.g. Class and Ballschmiter 1988; Moore and Tokarczyk 1993). Specifically, seawater from macroalgal stands are associated with elevated concentra-

tions of these halomethanes (e.g. Manley et al. 1992; Klick 1992). Observations of Arctic ozone loss has stimulated investigations of brominated methane release by polar marine algae (e.g. Sturges et al. 1992; Schall et al. 1994; Laturnus et al. 1996). The potential role of biogenic halocarbons in atmospheric chemistry suggests a need to investigate temperate macroalgae as well as polar species. One of the predominant kelp species in California waters, *Macrocystis pyrifera* (L.) C. Agardh (Giant Kelp), forms extensive canopies that should be conducive to releasing halomethanes into the atmosphere.

Seaweeds probably produce halogenated organics as part of a defense system against microorganisms (McConnell and Fenical 1977), herbivores (Gschwend et al. 1985), or perhaps excess hydrogen peroxide (H_2O_2) (Collén et al. 1994). Polyhalogenated methanes are evidently produced by haloperoxidases, enzymes that catalyze halide oxidation in the presence of H_2O_2 , resulting in the halogenation of suitable organic compounds (Neidleman and Geigert 1986; Harper 1993). *M. pyrifera* contains a vanadium bromoperoxidase (V-BrPO) (Soedjak and Butler 1990), and vanadium bromoperoxidases have been shown to produce CHBr_3 and CH_2Br_2 in vitro (Itoh and Shinya 1994).

If H_2O_2 is used as a bromide oxidant in vivo, it could be supplied internally from photosynthetic activity (Eltner 1987) or externally from surface waters (Wever et al. 1991). Photooxidation of dissolved organic matter yields higher H_2O_2 concentrations in surface waters relative to dimly lit waters at depth (Cooper et al. 1988). Such a distribution could influence brominated methane production if bromoperoxidases rely primarily on external H_2O_2 supplies. In addition, significant amounts of H_2O_2 may be released to seawater from phytoplankton (Palenik et al. 1987). Elucidating the mechanisms of halomethane production is important to understanding the release of halomethanes and to predicting responses to environmental changes.

This study focused on production of CHBr_3 and CH_2Br_2 from *M. pyrifera* with an investigation of environmental factors that could influence the release of halomethanes. It spe-

¹ Present address: U.S. Geological Survey, 345 Middlefield Rd., MS 480, Menlo Park, California 94025.

² Present address: University of Washington, Department of Chemical Engineering, Box 351750, Seattle, Washington 98195-1750.

Acknowledgments

We are indebted to Amy Garner, Michael Mulqueen, Penelope Sherman, and Rashceda Abdush-Shahced for their laboratory assistance. Light transmission spectra for the plastic films used in incubation studies were graciously provided by George Rossman. This research was funded by grants from the Mellon Foundation, NSF (EID 90-23500), and DOE (W-GEC 91-089-CT). K. Goodwin was partially supported by an NIH training grant (T32 GM08346).

cifically examined H_2O_2 concentrations during algal incubations and the production of CHBr_3 and CH_2Br_2 in response to H_2O_2 addition.

Materials and methods

For in situ experiments, the upper 0.6–1.2 m of *M. pyrifera* fronds were enclosed with ambient seawater in either polyethylene (0.42×0.3 m) or mylar bags (typically 1.2×0.3 m) fitted with a septum port. Fronds from five plants growing at ~14-m depth were incubated in situ (2 h) in Laguna Beach or Dana Point, California, kelp beds on four separate days between July and August 1993. Bags containing 1,273–1,457 g of kelp (blades, bulbs, and stipes) were sealed around the stipes and tied to buoys to prevent sinking. Control bags without kelp were treated similarly. At the end of the experiment, fronds were severed from the plant and frond mass and bag water volume were measured. Surface seawater temperatures for all experiments were between 20 and 20.5°C. Seawater samples were removed by syringe via the septum and immediately transferred to glass bottles leaving no headspace (240- or 125-ml amber, screw-top bottles with Teflon/silicone septa). Bottles were transported on ice to the laboratory and stored upside down at 4°C. Within 48 h, one-sixth of the volume of seawater was displaced with nitrogen (N_2) at 1 atm. Bottles were shaken upside down at 28°C for at least 2 h. Headspace samples (100 μl) were analyzed on a Shimadzu GC-14A gas chromatograph equipped with an electron capture detector (ECD-GC) (Restek Rtx-502.2 column, $60 \text{ m} \times 0.32 \text{ mm} \times 1.8 \mu\text{m}$ film thickness) using 0.3-min splitless injections (Goodwin 1996). Standards for calibration were made in capillary chromatography-grade hexane from neat CHBr_3 and CH_2Br_2 .

For laboratory incubation experiments, samples of *M. pyrifera* were collected from surface waters in kelp beds off Dana Point and Laguna Beach on seven separate days between May 1994 and February 1995. The uppermost 1.2–1.5 m of fronds from several different plants were collected by hand in water depths of 14–20 m. Fronds were transported to the laboratory in chilled seawater (typically 15–18°C upon arrival). Ten blades with attached bulbs from two to four separate fronds were placed in a polyethylene bag (22.9×38.1 cm) fitted with a septum port and sealed with a glove bag closure. Bags containing 43–161 g of kelp were filled with 4 liters of seawater (15°C), sealed without headspace, and incubated for 2 h in a 15°C shaking water bath. The seawater was obtained from a distribution system at the Caltech marine laboratory. It was pumped from Newport Bay and charcoal filtered prior to distribution. Seawater was bubbled with N_2 to remove background halomethanes. The seawater was entrained with air while gravity fed into incubation bags from a large, open beaker and was thus exposed to halomethanes in the room air, but concentrations remained below detection (18 ng CHBr_3 liter⁻¹; 10 ng CH_2Br_2 liter⁻¹). Measurements of O_2 (~5 mg liter⁻¹; YSI electrode) and pH (~8) were similar for purged and unpurged waters. Seawater samples were removed through the bag septum by syringe and placed into glass bottles as noted below.

Experiments in May 1994 were performed on a gas chromatograph requiring manual injection. Samples were placed in 32-ml amber, screw-top bottles with Teflon/silicone septa and stored without headspace at 4°C. Within 48 h, 4 ml of water was displaced with ultrahigh-purity helium at 1 atm. Bottles were shaken upside-down at 28°C for at least 1 h (bottles were previously found to reach 28°C within 15–20 min). Two headspace samples (100 μl) per vial were analyzed on a Hewlett-Packard 5880 ECD-GC (HP-5 column, $25 \text{ m} \times 0.32 \text{ mm} \times 1.05 \mu\text{m}$) using 1-min splitless injections (Goodwin 1996). Standards for calibration were made in hexane as described for in situ experiments. Remaining experiments were performed on a gas chromatograph equipped with a headspace autosampler (Hewlett-Packard 5890 series II plus ECD-GC, HP 7694 autosampler). The headspace autosampler was connected directly to the GC column, bypassing the inlet system (HP-624 column, $30 \text{ m} \times 0.32 \text{ mm} \times 1.8 \mu\text{m}$ film thickness; GC oven, 2.5 min at 40°C, 15°C min⁻¹, 0 min at 165°C, 70°C min⁻¹, 4 min at 220°C; 300°C detector; headspace autosampler, 95°C oven, 120°C sample loop, 160°C transfer line, 0.02-min loop equilibrium time, 0.09-min loop fill time, 0 psi vial fill pressure) (Goodwin 1996). Three replicate 15-ml samples were removed at each time point and placed into 20-ml autosampler vials. Trichlorobromomethane (CBrCl_3) was added as a reference peak prior to crimp sealing vials. Standards for calibration were made in seawater (seven concentrations, three replicates each) and run exactly as the samples themselves.

Laboratory control bags were incubated at 15°C in light without kelp and seawater was sampled at 0.25 and 2 h. No halomethane production was observed ($n = 8$). Incubation bags were spiked with 12 nM CH_2Br_2 and 10 nM CHBr_3 , incubated for 1 h, and samples were analyzed for recovery. The percent coefficient of variation for three replicate samples ranged from 2 to 10%. Recovery was ~100% for CH_2Br_2 (HP5880, $n = 9$, three separate bags; HP5890, $n = 20$, seven separate bags). Recovery for CHBr_3 was ~100% on the HP5890, but only 75% on the HP5880; results were corrected for recovery.

Experiments investigating the effects of light and photosynthetic state on CHBr_3 and CH_2Br_2 production were performed under three conditions: light, darkness, or light with the photosynthetic inhibitor DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea; Diuron). Experiments consisted of paired polyethylene bags incubated simultaneously under identical conditions except for the treatment under study (e.g. light vs. dark, light vs. DCMU). Paired bags contained 10 blades from the same fronds and of similar size and weight. DCMU is insoluble in water and it was necessary to dissolve it before administration to kelp (1 ml ethanol solution in 4 liters seawater, 50 μM DCMU final concn). As a control, ethanol (1 ml) was added to partner bags. Production of CHBr_3 and CH_2Br_2 did not differ significantly for kelp receiving ethanol compared to kelp that did not.

Experiments in light were conducted under cool-white fluorescent, full-spectrum fluorescent, or natural sunlight. Experiments in darkness were conducted with opaque plastic covering the incubation bags. Clear polyethylene bags used in incubation experiments were analyzed (Goodwin 1996) and shown to allow passage of ~66% of light in the bio-

logically relevant 400–750-nm range and 77% in the 800–1,070-nm range while the opaque plastic used to keep incubations dark essentially blocked transmission of light (<1% light passage at 400–750 nm and 4% at 800–1,070 nm).

Certain sets of blades were incubated for the standard 2 h and then H₂O₂ was added to achieve a concentration of 186 nM. Seawater was taken for CHBr₃ and CH₂Br₂ analysis ~25 min after the H₂O₂ addition. Hydrogen peroxide concentrations in seawater were analyzed using an HPPA (3-(*p*-hydroxyphenyl) propionic acid) assay (Palenik and Morel 1988) on a Shimadzu RF-540 spectrofluorophotometer.

Calculations

Dimensionless partition coefficients (concn gas/concn liquid) were obtained by interpolating the freshwater results of Tse et al. (1992) to 28°C. Salting-out coefficients were determined empirically in this laboratory by the method of Gossett (1987) and found to be 1.2 for both CHBr₃ and CH₂Br₂ (Goodwin 1996), yielding partition coefficients of 0.031 for CHBr₃ and 0.049 for CH₂Br₂ in seawater. All rates were normalized to kelp mass and used a 12-h photoperiod. Rates were calculated as follows for experiments requiring manual injection:

$$M_{bg} = C_{bg} V_{bg} \quad (\text{at each time point})$$

$$C_{bg} = \frac{M_{g+w}}{V_w}$$

$$M_{g+w} = C_g V_g + C_w V_w = C_g (V_g + V_w/H)$$

$$C_g = \frac{M_{inj}}{V_{inj}}$$

where C_{bg} is the concentration in bag (ng liter⁻¹); C_g is the concentration in bottle headspace at equilibrium with water (ng liter⁻¹); C_w is the concentration in bottle water at equilibrium with gas phase (ng liter⁻¹); M_{bg} is the mass of halomethane in bag (ng); M_{g+w} is the total mass (gas + water) of halomethane in bottle (ng); M_{inj} is the mass injected into GC (determined by standards) (ng); V_{bg} is the volume of bag (liters); V_g is the volume of headspace in bottle (liters); V_w is the volume of water in bottle after addition of headspace (liters); V_{inj} is the volume injected into GC (liters); and H is the dimensionless partition coefficient.

Calculations for experiments using the headspace autosampler did not require partition coefficients. Production rates were calculated by the following:

$$C_{bg} = \frac{M_{bt}}{V_{bt}}$$

where M_{bt} is the total mass halomethane in bottle (determined from standards) (ng) and V_{bt} is the volume of seawater in autosample bottle (liters).

Results and discussion

*Production rates and factors affecting production—*Whole blades of *M. pyrifera* incubated in the laboratory pro-

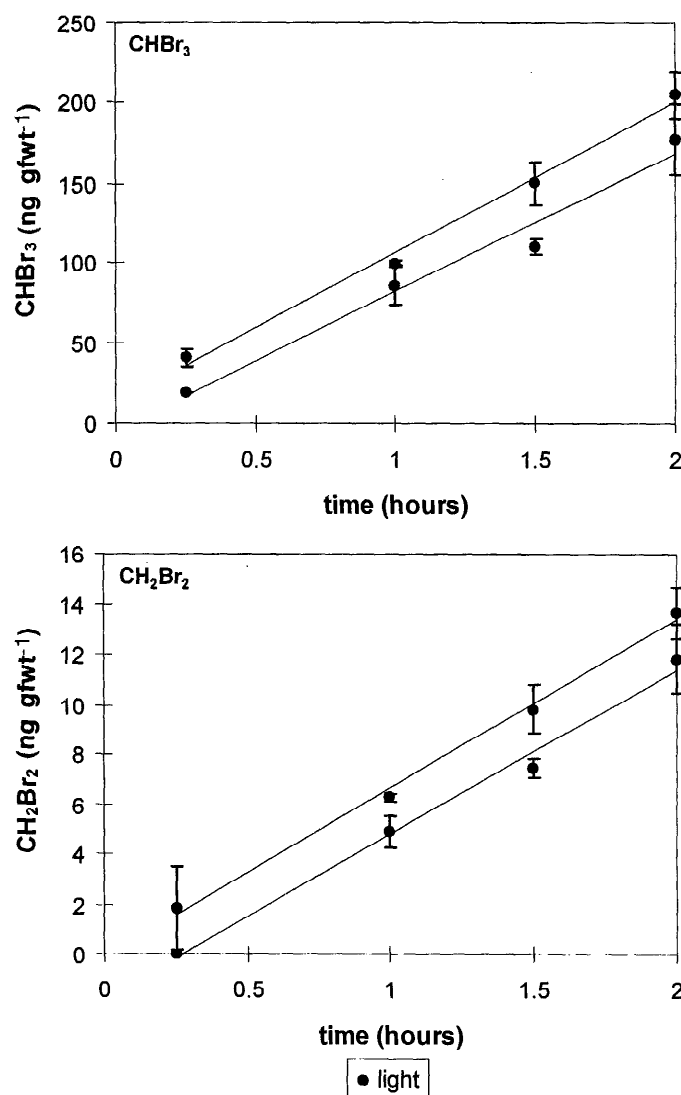


Fig. 1. Production of CHBr₃ and CH₂Br₂ by whole blades of *M. pyrifera*. Blades of similar size and weight were incubated simultaneously for 2 h in two separate bags under identical light and temperature conditions. Linear regression (solid lines) was used to determine production rates that were converted to daily rates using a 12-h photoperiod. Error bars are ± 1 standard deviation (SD) of three replicate samples from each bag.

duced CHBr₃ and CH₂Br₂, and linear production was generally observed (Fig. 1). Blades produced more CHBr₃ than CH₂Br₂ in all but 1 of the 20 incubations conducted. Preliminary in situ measurements of CHBr₃ and CH₂Br₂ production by portions of intact *M. pyrifera* fronds were similar to laboratory measurements of production by whole blades (Table 1), supporting the validity of laboratory measurements. In no instance was halomethane production observed in seawater controls. The mean CHBr₃ concentration measured in in situ control bags was 254 ± 82 ng liter⁻¹ (\pm SD, $n = 3$) and was below detection (50 ng liter⁻¹) for CH₂Br₂. The detection limit for CHBr₃ was 160 ng liter⁻¹ for the system used for in situ experiments.

Production rates were measured in the laboratory for

Table 1. Comparison of *M. pyrifera* CHBr_3 and CH_2Br_2 production rates estimated by laboratory whole blade incubations ($n = 20$; 200 blades) and in situ frond incubations ($n = 4$) (gfw = g fresh weight).

	CHBr_3	CH_2Br_2
Laboratory production (ng gfw $^{-1}$ d $^{-1}$), whole blades		
Median	171	48
Range	25–1,126	22–173
In situ production (ng gfw $^{-1}$ d $^{-1}$), intact fronds		
Median	256	41
Range	136–304	14–88

whole blades of *M. pyrifera* on seven different occasions over 9 months to assess seasonal variability. Highest production rates were measured in midsummer, particularly for CHBr_3 (Fig. 2). In general, the pattern observed here for *M. pyrifera* suggests the need to assess seasonal fluctuations for macroalgae in order to understand biogenic release of halogenated methanes. The high rates measured in midsummer occurred during visibly poor canopy conditions (Goodwin 1996), suggesting that algal physiological state may affect halomethane release. Klick (1992) also observed peak halomethane concentrations during summer concurrent with visible degradation of seaweeds.

Nutrient depletion in waters above the thermocline contribute to summertime canopy deterioration in southern California (Jackson 1977), and conditions vary daily due to thermocline movement (Zimmerman and Kremer 1986). To address the relationship between kelp health and halometh-

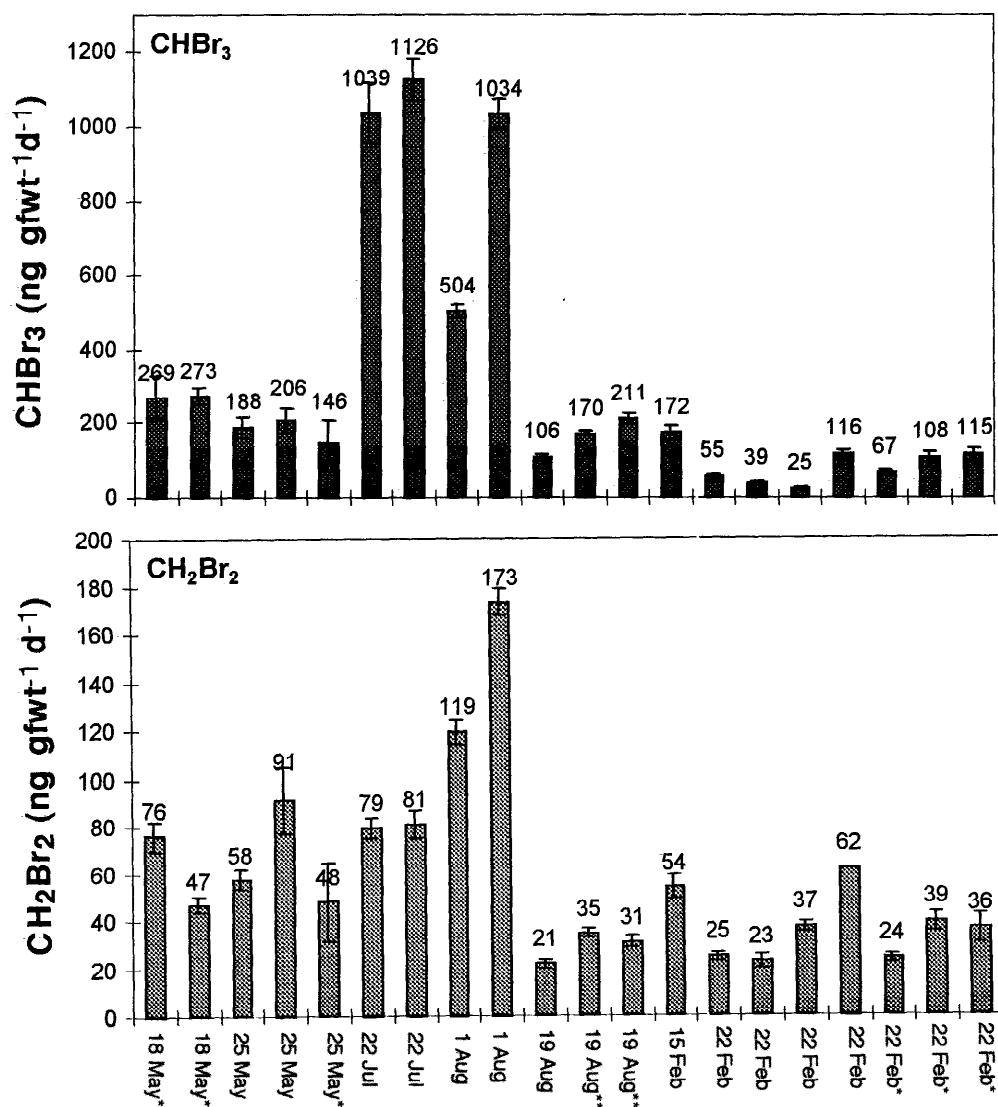


Fig. 2. Production rates of CHBr_3 and CH_2Br_2 for whole blades of *M. pyrifera* collected May 1994 to February 1995. Blades were incubated under cool-white fluorescent, full-spectrum fluorescent (*), or sunlight (**). Error bars are ± 1 SE of regression.

ane production, we measured production rates from highly tattered and unhealthy-looking blades collected in late spring. Thallus surfaces are one location with which V-BrPO is associated (Butler et al. 1990); thus, it was unknown whether brominated methane production would be disrupted due to blade degradation. The unhealthy-looking blades were capable of linear CHBr_3 and CH_2Br_2 production and produced CHBr_3 at a somewhat higher rate (235 ± 36 ng per g fresh wt [gfw] per day) and CH_2Br_2 at a similar rate (43 ± 0.6 ng gfw $^{-1}$ d $^{-1}$) (\pm SE) relative to healthier counterparts incubated simultaneously (CHBr_3 , 146 ± 58 ; CH_2Br_2 , 48 ± 17 ng gfw $^{-1}$ d $^{-1}$ \pm SE; 25 May 1994). These results are generally consistent with the observed pattern of higher CHBr_3 rates associated with canopy conditions during summer (Fig. 2).

Production rates of polybrominated methanes for different algal species vary widely (see Goodwin 1996). Production rates of CHBr_3 and CH_2Br_2 measured here for *M. pyrifera* were similar to those reported for *Ascophyllum nodosum* and *Fucus vesiculosus* by Gschwend et al. (1985). Our median CH_2Br_2 production rate for whole blades was similar to the mean CH_2Br_2 production rate of Manley et al. (1992) for blade disks of *M. pyrifera*, adjusting that rate for a 12-h photoperiod (see below) and the CH_2Br_2 partition coefficient from Tse et al. (1992). Our highest rates of CHBr_3 production for whole blades were similar to the Manley et al. (1992) mean rate for blade disks (adjusting that rate for a 12-h photoperiod), but our median rate was lower. Rate differences for CHBr_3 may have arisen in part from differences in kelp tissues caused by environmental or physiological factors (Goodwin 1996). Median values are given (Table 1) because they provide a better estimate of the central value than the mean (299 CHBr_3 ng gfw $^{-1}$ d $^{-1}$; $58 \text{ CH}_2\text{Br}_2$ ng gfw $^{-1}$ d $^{-1}$) for small observational sets (Kennedy 1984).

The presence of light was an important factor in the production of CHBr_3 and CH_2Br_2 by *M. pyrifera* (Fig. 3). In one instance, blades incubated in darkness produced no measurable CHBr_3 or CH_2Br_2 . Otherwise, CHBr_3 production was reduced 5–19-fold in darkness while CH_2Br_2 production was reduced 3–6-fold compared to incubations in light under otherwise identical conditions. *M. pyrifera* production rates based on short-term incubations should thus include a photoperiod; a 12-h photoperiod was used for calculations in this study.

Abiotic photochemical production of H_2O_2 (Cooper et al. 1988) did not seem to explain the effect of light on brominated methane production because H_2O_2 concentrations were similar in both light and dark incubations of seawater without kelp (light, 23.7 ± 2.2 nM H_2O_2 ; dark, 23.3 ± 2.7 nM H_2O_2) (average over 2-h incubation \pm SD; $n = 12$, four time points). This result suggests that kelp-supplied bromide oxidant was involved in brominated methane production during these experiments.

Different light sources were tested for their effect on brominated methane production. Production rates in sunlight were only about 1.6 times higher relative to a cool-white fluorescent incubation performed on the same day (Fig. 2; 19 August), despite substantially higher light fluxes (17 – $40\times$ higher) and fluctuating light conditions (927 – $2,166 \mu\text{Einst m}^{-2} \text{ s}^{-1}$). A no-kelp control in full sunlight showed

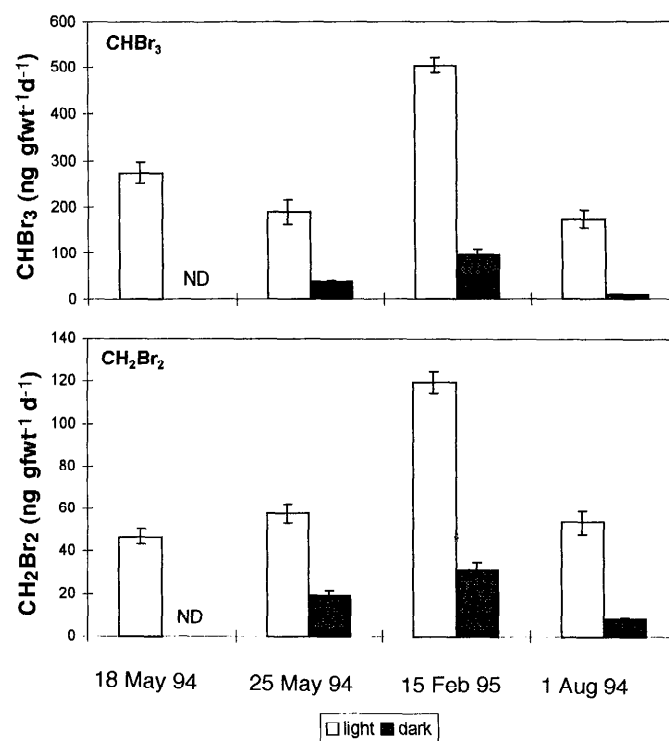


Fig. 3. Production rates of CHBr_3 and CH_2Br_2 for whole blades of *M. pyrifera* incubated under fluorescent light or in darkness. Paired columns represent production during simultaneous incubations in otherwise identical conditions. ND = production not detected for incubations in darkness. Error bars are ± 1 SE of regression.

no brominated methane production. Although full-spectrum fluorescent light simulates natural sunlight more closely than does cool-white fluorescent light (Vita-lite technical data), light fluxes were similar under these two conditions (52 – $56 \mu\text{Einst m}^{-2} \text{ s}^{-1}$), and CHBr_3 and CH_2Br_2 production rates did not differ significantly for incubations performed under either type of light (Fig. 2; 18 and 25 May, 22 February). This production behavior seems consistent with the light intensity required for photosynthetic saturation of *M. pyrifera*. Laboratory fluorescent light fluxes were typically two-thirds the saturating light intensity of $80 \mu\text{Einst m}^{-2} \text{ s}^{-1}$ (Arnold and Manley 1985), whereas sunlight was consistently above saturating light intensity.

In contrast to the demonstrated light effect for *M. pyrifera* CHBr_3 and CH_2Br_2 production, other investigators (Manley and Dastoor 1987) have shown that light does not affect *M. pyrifera* methyl halide production (e.g. CH_3Br). This difference may be due to the enzymes involved in that a methyltransferase, instead of a peroxidase, apparently catalyzes methyl halide production (Wuosmaa and Hager 1990; Harper 1993). Light has been observed to affect CHBr_3 and CH_2Br_2 production in the rockweeds *Fucus vesiculosus* (Klick 1993) and *A. nodosum* (Nightingale et al. 1995), and light affected bromination of phenol red by V-BrPO for *A. nodosum*, but not for seven other algal species tested (Wever et al. 1991).

Results of adding the photosynthetic inhibitor, DCMU, further indicated that decreased release of CHBr_3 and CH_2Br_2

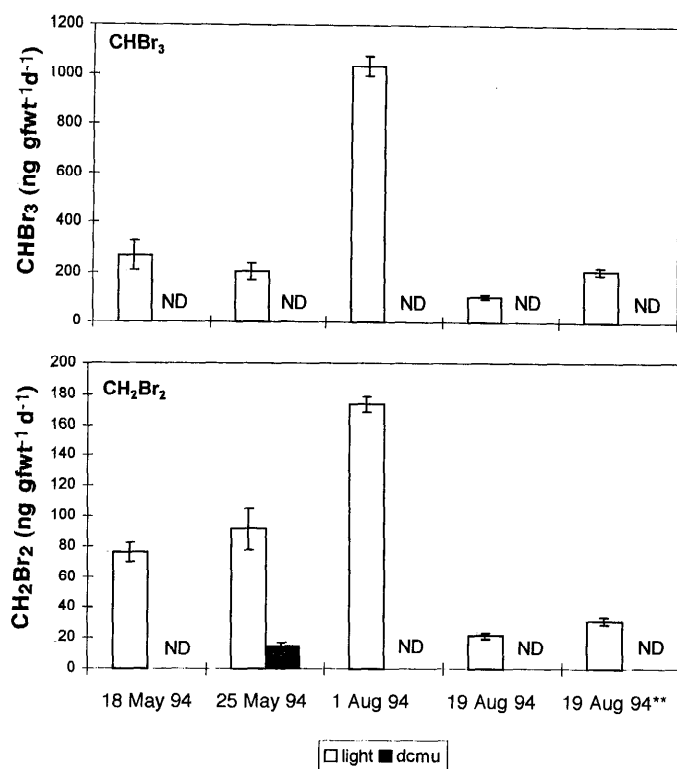


Fig. 4. Production rates of CHBr_3 and CH_2Br_2 for whole blades of *M. pyrifera* incubated under fluorescent light or in sunlight (***) with or without the photosynthetic inhibitor DCMU. Paired columns represent production during simultaneous incubations in otherwise identical conditions. ND = production not detected for DCMU-treated incubations. Error bars are ± 1 SE of regression.

during darkness was not a photochemical effect but was most likely due to physiological factors. Release of CHBr_3 and CH_2Br_2 was inhibited for blades incubated in light with DCMU compared to blades incubated in light under otherwise identical conditions (Fig. 4). Photosynthetic inhibition by DCMU was indicated by a decline in pH and O_2 levels, as expected from cellular respiration (pH, 8.18 to 8.09; O_2 , 5.0 to 3.4 mg liter⁻¹), whereas pH and O_2 levels increased during incubation of kelp not treated with DCMU, as expected from photosynthesis (pH, 8.22 to 8.34; O_2 , 5.6 to 5.9 mg liter⁻¹). Also, inhibition of photosynthesis by DCMU was suggested by a lack of bubble formation (presumably O_2) in the incubation bags.

DCMU inhibition of CHBr_3 and CH_2Br_2 apparently is not caused by direct reaction with H_2O_2 or brominated methanes. First, proton nuclear magnetic resonance spectroscopy (NMR) as described in Goodwin (1996) showed no change in DCMU chemical shifts at a H_2O_2 :DCMU ratio approximating incubation conditions (170 nM:0.17 nM) or at an equimolar ratio (0.17 nM each) in the presence or absence of horseradish peroxidase. Second, DCMU (50 μM) did not affect amounts of CHBr_3 (100 nM) or CH_2Br_2 (50 nM) added to seawater (data not shown). In addition, DCMU is a quinone analogue acting at the herbicide binding protein in the electron transport chain (Hsu et al. 1986) and is thus unlikely to interact directly with the *M. pyrifera* V-BrPO, which is

an acidic glycoprotein (Soedjak and Butler 1991) with no organic cofactors being reported.

To further assess inhibition of CHBr_3 and CH_2Br_2 production by DCMU, aniline was substituted for DCMU during an incubation. Whether bromoperoxidases produce brominated methanes by forming an enzyme-bound halogenating intermediate or by generating hypobromous acid (HOBr) that then reacts nonspecifically with nucleophilic acceptors is an issue of debate (Harper 1993). However, bromine species such as HOBr would have oxidized aniline, potentially leaving them unavailable to halogenate organics. In addition, aniline is known to scavenge H_2O_2 during incubations of algae in light (Zepp et al. 1987), and sufficient aniline was added (55 μM) to deplete existing H_2O_2 (<50 nM); thus, external H_2O_2 concentrations should have been low. Nonetheless, significant CHBr_3 and CH_2Br_2 production was observed for kelp incubated with aniline after a lag period of 1.5 h (CHBr_3 , 20 ± 8.7 ; CH_2Br_2 , 21 ± 2.2 ng gfw t⁻¹ d⁻¹ \pm SE). As expected, aniline did not appear to inhibit photosynthesis as indicated by the formation of bubbles in the incubation bag.

These data suggest that photosynthesis-dependent mechanisms are important for production of CHBr_3 and CH_2Br_2 by *M. pyrifera*. If DCMU inhibition of brominated methane production was from DCMU reacting with H_2O_2 or an oxidized bromine species such as HOBr, aniline would have been similarly oxidized and should have caused similar inhibition of CHBr_3 and CH_2Br_2 production. That DCMU completely inhibited brominated methane production, whereas aniline did not, suggests that the action of DCMU was related to its effect on photosynthesis. The connection between photosynthesis and halomethane production may be the internal supply of bromide oxidant (e.g. H_2O_2) to V-BrPO. Both darkness and DCMU cut off H_2O_2 produced during photosynthesis (Elstner 1987) and photorespiration (Tolbert 1974). Although photorespiration was unlikely to occur under incubation conditions (Bidwell and McLachlan 1985), it may occur in the kelp canopy where blades are exposed to high oxygen concentrations (S. L. Manley pers. comm.).

Hydrogen peroxide, CHBr_3 , and CH_2Br_2 concentrations were monitored concurrently during laboratory kelp incubations (15 February 1995) and were found to be correlated during light incubations. The seawater H_2O_2 concentration was 44 ± 0.75 nM prior to incubation but dropped below detection (10 nM) after kelp was added for both light and dark incubations. Biological processes seem to dominate H_2O_2 decomposition in coastal marine waters (Moffett and Zafriou 1993) and probably account for the lowered H_2O_2 concentration in this case. Hydrogen peroxide increased over time during kelp incubations in light (from below detection to 31 ± 0.75 nM H_2O_2 at 2.2 h), as did CHBr_3 and CH_2Br_2 (CHBr_3 , 172 ± 19 ; CH_2Br_2 , 54 ± 5.2 ng gfw t⁻¹ d⁻¹ \pm SE). Hydrogen peroxide concentrations remained below detection during the dark incubation, and, as previously observed, production of CHBr_3 and CH_2Br_2 was reduced relative to light (CHBr_3 , 9.0 ± 1.2 ; CH_2Br_2 , 8.5 ± 0.15 ng gfw t⁻¹ d⁻¹ \pm SE). Hydrogen peroxide concentrations remained below detection (in this case 50 nM) during a light incubation with DCMU-treated blades, and no release of CHBr_3 or CH_2Br_2 was measured. The detection limit for H_2O_2 increased from 10 to 50

Table 2. Amounts of CHBr_3 and CH_2Br_2 (ng gfw $^{-1}$ \pm SD, $n = 3$) measured after addition of H_2O_2 compared to amounts expected from linear regression of earlier values (ng gfw $^{-1}$ \pm standard error of estimate ([SE] $_y$, $n = 9$). Significant enhancement of CHBr_3 and CH_2Br_2 with addition of H_2O_2 was observed for kelp incubated in darkness (bold) (i.e. confidence interval and prediction interval do not overlap, $\alpha = 0.05$). Paired bags were incubated simultaneously under identical conditions except for the treatment under study. Kelp was incubated for 2 h prior to H_2O_2 addition under cool-white fluorescent light (light, aniline, DCMU), full-spectrum fluorescent (*), or in darkness.

Treatment	CHBr_3		CH_2Br_2	
	ng gfw $^{-1}$ \pm SE $_y$ expected	ng gfw $^{-1}$ \pm SD actual after H_2O_2	ng gfw $^{-1}$ \pm SE $_y$ expected	ng gfw $^{-1}$ \pm SD actual after H_2O_2
	Light	44 \pm 3.5	51 \pm 6.7	14 \pm 0.98
Dark	4.3 \pm 0.22	10\pm0.59	3.6 \pm 0.03	5.2\pm0.32
Aniline	7.1 \pm 1.6	9.3 \pm 0.48	6.4 \pm 0.14	6.2 \pm 0.35
DCMU	0.84 \pm 0.29	0.85 \pm 0.04	0.52 \pm 0.06	0.73 \pm 0.01
Light	21 \pm 2.5	24 \pm 0.54	10 \pm 0.63	12 \pm 0.44
Light (*)	19 \pm 1.3	24 \pm 1.4	6.2 \pm 0.38	7.9 \pm 0.53

nM when DCMU was present due to increased variability of readings that decreased the ability to distinguish low values of H_2O_2 .

If inhibition of halomethane production by darkness and DCMU was due to a decreased availability of oxidant (e.g. H_2O_2), then it might be possible to reverse these inhibitory effects by adding external H_2O_2 to kelp incubations. This hypothesis was tested by adding peroxide to kelp incubations at concentrations (186 nM H_2O_2) similar to those found in well-lit surface waters (100 nM; Moffett and Zafriou 1990). Peroxide addition significantly increased the amount of CHBr_3 and CH_2Br_2 released from kelp blades incubated in darkness (Table 2), and no H_2O_2 was detected 25 min after the addition. Peroxide addition did not significantly increase CHBr_3 and CH_2Br_2 release from kelp blades treated with aniline, as would be expected from aniline consumption of H_2O_2 (Table 2). Peroxide addition also did not stimulate halomethane production for DCMU-treated blades (Table 2). Hydrogen peroxide could not be detected in this incubation even 8 min after peroxide addition. If DCMU acts by inhibiting bromide oxidant generated through photosynthesis, the amount of added H_2O_2 was insufficient to reverse this action. Peroxide addition to kelp blades incubated in cool-white or full-spectrum fluorescent light did not cause CHBr_3 and CH_2Br_2 quantities to be higher than would be expected based on linear regression of earlier values (Table 2). Approximately 100 nM H_2O_2 was detected 5 min after peroxide addition for light incubations. These data suggest that production in darkness may have been limited by bromide oxidant supply, while production in light under these conditions was not. Collén et al. (1994) observed increased halomethane production in light upon peroxide addition for the red alga *Meristiella gelidium*; however, substantially higher peroxide concentrations were applied (1 mM H_2O_2).

Table 3. Estimated bromine emissions from *M. pyrifera* compared to selected anthropogenic sources in Orange and San Diego counties.

Regional bromine emission estimates			
Biogenic		Anthropogenic	
Bromine source	g Br yr $^{-1}$ *	Bromine source	g Br yr $^{-1}$
<i>M. pyrifera</i>			
CH_3Br	5.1 \times 10 4	CH_3Br fumigation†	1.2 \times 10 8
CH_2Br_2	6.2 \times 10 5	Water chlorination	
CHBr_3	2.3 \times 10 6	by-products‡	7.9 \times 10 6
Sum	3.0 \times 10 6	Sum	1.3 \times 10 8

* CH_3Br values based on Manley and Dastoor (1987); CH_2Br_2 and CHBr_3 from Table 1.

† State of California (1995) assuming half of application is emitted to the atmosphere (U.N. Environmental Programme 1992).

‡ Goodwin (1996). CHBr_3 is a major product (e.g. Bean et al. 1980; Jolley et al. 1990).

Regional and global estimates—A useful but often neglected tool for understanding and constraining global bromine budgets is constructing a regional budget for comparison. Constructing a budget from laboratory production measurements requires an estimate of macroalgal biomass. *M. pyrifera* coverage along the coasts of Orange and San Diego Counties (~100-mi. coastline) has fluctuated more than an order of magnitude between 1963 and 1991, with minimum and maximum standing crops corresponding with strong El Niño and La Niña conditions (North et al. 1993). Biomass values (4.2 \times 10 9 –9.0 \times 10 10 ; avg of 3.9 \times 10 10 g) were obtained by multiplying area coverage (North et al. 1993) by a typical bed density of 6 kg $^{-2}$ m $^{-2}$ (Goodwin 1996). *M. pyrifera* in this region thus produces 3.0 \times 10 6 g Br yr $^{-1}$ on average (3.2 \times 10 5 –6.9 \times 10 6 g Br yr $^{-1}$) from CHBr_3 and CH_2Br_2 (Table 1 values) and from CH_3Br based on laboratory measurements of Manley and Dastoor (1987). CHBr_3 contributes 77% of this bromine while CH_3Br contributes <2% (Table 3). Release rates exclude contribution by holdfasts that have been observed to release halomethanes in certain species of Antarctic macroalgae (Laternus et al. 1996). If other regional macroalgal species release CHBr_3 and CH_2Br_2 at rates similar to *Macrocystis*, macroalgal bromine release would approximately double (Goodwin 1996).

In this region, anthropogenic emissions seem to dominate macroalgal bromine emissions (Table 3). The regional bromine release by *M. pyrifera* was similar to that estimated for byproducts of water chlorination (~8 \times 10 6 g Br yr $^{-1}$ in 1995) (Goodwin 1996). However, CH_3Br fumigation alone released an estimated 10 8 g Br yr $^{-1}$ in this region during 1993 (state of California, 1995), substantially greater than the highest estimate for *M. pyrifera*. The dominance of anthropogenic emissions over macroalgal ones is not expected for all coastal regions (e.g. polar regions), but it may represent other areas with dense urban and agricultural development. Coastal phytoplankton of the Southern California Bight could be important contributors of biogenic bromine if they produce CH_3Br in quantities similar to that reported for *Phaeocystis* sp. by Scarratt and Moore (1996) (~1 \times 10 7

Table 4. Estimated global bromine emissions from selected algal groups compared to anthropogenic sources.

Global bromine emission estimates			
Biogenic		Anthropogenic	
Bromine source	g Br yr ⁻¹ *	Bromine source	g Br yr ⁻¹
Kelp (CHBr ₃ , CH ₂ Br ₂ , CH ₃ Br) ^{a,c,†}	3.1×10 ⁹ –4.8×10 ¹⁰	CH ₃ Br fumigation and industrial emissions ^{g,h}	3.5×10 ¹⁰ –6.4×10 ¹⁰
Nonkelp macroalgae (CHBr ₃ , CH ₂ Br ₂) ^{b,d}	8.2×10 ⁸ –3.2×10 ¹⁰	CH ₃ Br biomass burning ⁱ	2.1×10 ¹⁰
Ice algae community (CHBr ₃) ^e	9.5×10 ⁹ –1.4×10 ¹¹	C ₂ H ₂ Br ₂ ^j	1.7×10 ⁹ –2.7×10 ¹⁰
Coastal phytoplankton (CH ₃ Br) ^f	3.6×10 ⁸ –1.4×10 ¹⁰	CF ₃ Br + CF ₂ ClBr ^k Water chlorination by-products ^l	6.2×10 ⁹ 4.6×10 ⁹
Sum	1×10 ¹⁰ –2×10 ¹¹	Sum	7×10 ¹⁰ –1×10 ¹¹

* Rates converted to g Br yr⁻¹ based on 40 Tg kelp, 20 Tg nonkelp macroalgae (Tg = 10¹² g; De Vooy 1979).

† CHBr₃ and CH₂Br₂ from ^athis study and ^bManley et al. (1992), divided by two to adjust for a 12-h photoperiod with CH₂Br₂ rates an additional one-third to adjust for Tse et al. (1992) partition coefficient (Goodwin 1996); ^cCH₃Br (5.3×10⁷ g Br yr⁻¹) based on Manley and Dastoor (1987) laboratory measurements; ^dNightingale et al. (1995), converted from % dry weight (Goodwin 1996); ^eSturges et al. (1992); ^fScarratt and Moore (1996); ^gWMO (1994); based on *P. tricornutum* or *Phaeocystis* sp.; ^hButler and Rodriguez (1996); ⁱWMO (1994), natural fires contribute an addition 4.3×10⁹ g Br yr⁻¹; ^jWMO (1994), assuming all the bromine from ethylene dibromide (4.0×10⁹–6.2×10¹⁰ g) emitted to atmosphere; ^kMcCulloch (1992). Note that the U.S. manufacture of CF₃Br was halted in 1994; ^lGschwend et al. (1985).

g Br yr⁻¹ assuming 4.8 × 10¹⁰ m² and 65 mg Chl m⁻²; Morel and Berthon 1989).

Constructing a global bromine budget is more tentative than is the regional budget owing to limited data on biomass and species-specific production. Assuming a kelp standing crop of 40 Tg (De Vooy 1979) and production based on measurements of *M. pyrifera* in this study, kelp is estimated to produce 3.0 × 10⁹ g Br yr⁻¹ from CHBr₃ and CH₂Br₂ worldwide. CHBr₃ contributes ~79% of this bromine. Based on laboratory measurements of Manley and Dastoor (1987), kelp emissions of bromine from CH₃Br are small relative to CHBr₃ and CH₂Br₂ (0.11–1.7%) (Table 4). Combining bromine emissions from kelp with those of nonkelp macroalgae (Manley et al. 1992; Nightingale et al. 1995), ice algae communities (Sturges et al. 1992), and coastal phytoplankton (Scarratt and Moore 1996), estimated bromine emissions are 1 × 10¹⁰–2 × 10¹¹ g Br yr⁻¹ for just these three brominated methanes (Table 4). CHBr₃ contributes ≥88% of this bromine. The estimated global input of CHBr₃ to the atmosphere based on atmospheric measurements is on the order of 10¹² g CHBr₃ yr⁻¹, which is greater than anthropogenic inputs (Penkett et al. 1985; Krysell 1991); therefore, CHBr₃ sources are not yet fully budgeted, as observed for CH₃Br (Lobert et al. 1995). Phytoplankton (e.g. Tokarczyk and Moore 1994) and perhaps cyanobacteria (Schall et al. 1996) may be additional CHBr₃ sources, but more information is required before an adequate assessment can be made. In addition, other brominated compounds are produced by marine algae at rates comparable to CH₂Br₂ production (e.g. dibromochloromethane, dichlorobromomethane) (Nightingale et al. 1995; Laturus et al. 1996), which will further contribute biogenic bromine to the global cycle.

Most of the CHBr₃ produced in the water column should eventually reach the atmosphere because aquatic sinks are slow relative to volatilization. The half-life of CHBr₃ due to volatilization is on the order of days to months, depending

on mixed-layer depth (Helz and Hsu 1978). However, the half-life of CHBr₃ due to halide exchange is 1.3–18.5 yr for 25°C seawater (Geen 1992), and the half-lives of CHBr₃ and CH₂Br₂ due to hydrolysis range from 128 to 6,850 yr, depending on temperature (Mabey and Mill 1978; Washington 1995). Adsorption of CHBr₃ and CH₂Br₂ to humic materials is thought to be negligible (Helz and Hsu 1978), while data for aqueous-phase photoreactions appear to be lacking. Microbial degradation of CHBr₃ in seawater appears to be insignificant (Goodwin et al. 1997).

In contrast to CHBr₃, several aquatic sinks compete with CH₃Br volatilization. The half-life of CH₃Br is 4 d in 22°C seawater due to a combination of hydrolysis and halide exchange (Jeffers and Wolfe 1996), and reaction with organic components and photodegradation may also be important (Gentile et al. 1989). Biological removal of CH₃Br is suspected to occur at high latitudes (Moore and Webb 1996; Lobert et al. 1997). Microbial oxidation of CH₃Br and CH₂Br₂ in a seawater enrichment has been observed (Goodwin et al. 1997).

Estimates of global bromine emissions from anthropogenic (8 × 10¹⁰–1 × 10¹¹ g Br yr⁻¹) and combined algal sources (~10¹⁰–10¹¹ g Br yr⁻¹) are of similar magnitude (Table 4). Algae thus appear to contribute significantly to the global bromine cycle. Nonetheless, natural and anthropogenic emissions are different in important ways. For example, biogenic brominated methane emissions may vary due to season, species distribution, and climatic variation (e.g. El Niño). In addition, biogenic emissions seem to consist primarily of CHBr₃, whereas CH₃Br dominates anthropogenic emissions. CH₃Br is more likely to play a significant role in stratospheric ozone depletion because of its longer atmospheric lifetime (0.8 yr; Shorter et al. 1995) compared to CHBr₃ (0.05 yr; Ramanathan et al. 1985) and CH₂Br₂ (0.40 yr; Mellouki et al. 1992). However, total organic bromine, not just CH₃Br, should be salient to ozone cycling (Li et al. 1994)

partly because compounds may reach higher altitudes than expected from their atmospheric lifetimes (e.g. Kley et al. 1996). Indeed, CH_2Br_2 has been measured in the lower stratosphere and may deliver $\sim 10\%$ of the bromine entering there (Kourtidis et al. 1996). Defining the role of biogenic brominated methanes will surely continue as understanding of the global bromine cycle progresses.

Conclusions

Light and photosynthetic activity affected macroalgal CHBr_3 and CH_2Br_2 release, suggesting that environmental factors that influence kelp physiology (e.g. light, health, season, climate) may ultimately affect release of halomethanes into the atmosphere. Bromine emissions from CH_3Br fumigation seem to dominate emissions from macroalgae in southern California. A comparison of global anthropogenic and biogenic bromine sources suggest that combined macro- and microalgal emissions are an important part of the global bromine cycle. Biogenic bromine emissions seem to be dominated by CHBr_3 , whereas anthropogenic emissions appear to be dominated by CH_3Br .

References

- ARNOLD, K. E., AND S. L. MANLEY. 1985. Carbon allocation in *Macrocystis pyrifera* (Phaeophyta): Intrinsic variability in photosynthesis and respiration. *J. Phycol.* **21**: 154–167.
- ATLAS, E., AND OTHERS. 1993. Alkyl nitrates, nonmethane hydrocarbons, and halocarbon gases over the equatorial Pacific ocean during Saga 3. *J. Geophys. Res.* **98**: 16933–16947.
- BEAN, R. M., R. G. RILEY, AND P. W. RYAN. 1980. Investigation of halogenated components formed from chlorination of marine water, p. 223–234. In R. L. Jolley, H. Gorchev, and D. H. Hamilton Jr. [eds.], *Water chlorination environmental impact and health effects*. V. 2. Ann Arbor Science Publishers.
- BIDWELL, R. G. S., AND J. MCLACHLAN. 1985. Carbon nutrition of seaweeds: Photosynthesis, photorespiration and respiration. *J. Exp. Mar. Biol. Ecol.* **86**: 15–46.
- BUTLER, A. AND OTHERS. 1990. Studies of vanadium-bromoperoxidase using surface and cortical protoplasts of *Macrocystis pyrifera* (Phaeophyta). *J. Phycol.* **26**: 589–592.
- BUTLER, J. H., AND J. M. RODRIGUEZ. 1996. Methyl bromide in the atmosphere, p. 27–90. In C. H. Bell, N. Price, and B. Chakrabarti [eds.], *The methyl bromide issue*. John Wiley & Sons.
- CLASS, T., AND K. BALLSCHMITER. 1988. Chemistry of organic traces in air VIII: Sources and distribution of bromo- and bromochloromethanes in marine air and surfacewater of the Atlantic ocean. *J. Atmos. Chem.* **6**: 35–46.
- COLLÉN, J., AND OTHERS. 1994. The involvement of hydrogen peroxide in the production of volatile halogenated compounds by *Meristiella gelidium*. *Phytochemistry* **36**: 1197–1202.
- COOPER, W. J., AND OTHERS. 1988. Photochemical formation of H_2O_2 in natural waters exposed to sunlight. *Environ. Sci. Technol.* **22**: 1156–1160.
- DE VOOYS, C. G. N. 1979. Primary production in aquatic environments, p. 259–292. In B. Bolin et al. [eds.], *The global carbon cycle*. John Wiley & Sons.
- ELSTNER, E. F. 1987. Metabolism of activated oxygen species, p. 253–315. In P. K. Stumpf and E. E. Conn [eds.], *The biochemistry of plants a comprehensive treatise*. V. 11. Biochemistry of metabolism. Academic.
- GEEN, C. E. 1992. Selected marine sources and sinks of bromoform and other low molecular weight organobromines. M.Sc. thesis, Dalhousie Univ.
- GENTILE, I. A., AND OTHERS. 1989. The degradation of methyl bromide in some natural fresh waters influence of temperature, pH, and light. *Pestic. Sci.* **25**: 261–272.
- GOODWIN, K. D. 1996. Natural cycles of brominated methanes: Macroalgal production and marine microbial degradation of bromoform and dibromomethane. Ph.D. thesis, California Institute of Technology.
- , M. E. LIDSTROM, AND R. S. OREMLAND. 1997. Marine bacterial degradation of brominated methanes. *Environ. Sci. Technol.* **31**: 3188–3192.
- GOSSETT, J. M. 1987. Measurement of Henry's law constants for C_1 and C_2 chlorinated hydrocarbons. *Environ. Sci. Technol.* **21**: 202–208.
- GSCHWEND, P. M., J. K. MACFARLANE, AND K. A. NEWMAN. 1985. Volatile halogenated organic compounds released to seawater from temperate marine macroalgae. *Science* **227**: 1033–1035.
- HARPER, D. B. 1993. Biogenesis and metabolic role of halomethanes in fungi and plants. *Metal Ions Biol. Syst.* **29**: 345–388.
- HELZ, G. R., AND R. Y. HSU. 1978. Volatile chloro- and bromocarbons in coastal waters. *Limnol. Oceanogr.* **23**: 858–869.
- HSU, B.-D., J.-Y. LEE, AND R.-L. PAN. 1986. The two binding sites for DCMU in photosystem II. *Biochem. Biophys. Res. Commun.* **141**: 682–688.
- ITOHI, N., AND M. SHINYA. 1994. Seasonal evolution of bromomethanes from coralline algae (Corallinaceae) and its effect on atmospheric ozone. *Mar. Chem.* **45**: 95–103.
- JACKSON, G. A. 1977. Nutrients and production of giant kelp, *Macrocystis pyrifera*, off southern California. *Limnol. Oceanogr.* **22**: 979–995.
- JEFFERS, P. M., AND N. L. WOLFE. 1996. On the degradation of methyl bromide in sea water. *Geophys. Res. Lett.* **23**: 1773–1776.
- JOLLEY, R. J., AND OTHERS [EDS.]. 1990. *Water chlorination chemistry, environmental impact and health effects*, v. 6. Lewis.
- KENNEDY, J. H. 1984. *Analytical chemistry*. Harcourt-Brace-Jovanovich.
- KLEY, D., AND OTHERS. 1996. Observations of near-zero ozone concentrations over the convective Pacific: Effects on air chemistry. *Science* **274**: 230–232.
- KLICK, S. 1992. Seasonal variations of biogenic and anthropogenic halocarbons in seawater from a coastal site. *Limnol. Oceanogr.* **37**: 1579–1585.
- . 1993. The release of volatile halocarbons to seawater by untreated and heavy metal exposed samples of the brown seaweed *Fucus vesiculosus*. *Mar. Chem.* **42**: 211–221.
- KOURTIDIS, K., R. BORCHERS, AND P. FABIAN. 1996. Dibromomethane (CH_2Br_2) measurements at the upper troposphere and lower stratosphere. *Geophys. Res. Lett.* **23**: 2581–2583.
- KRYSELL, M. 1991. Bromoform in the Nansen Basin in the Arctic ocean. *Mar. Chem.* **33**: 187–197.
- LACIS, A. A., D. J. WUEBBLES, AND J. A. LOGAN. 1990. Radiative forcing of climate by changes in the vertical distribution of ozone. *J. Geophys. Res.* **95**: 9971–9981.
- LATURNUS, F., C. WIENCKE, AND H. KLÖSER. 1996. Antarctic macroalgae—sources of volatile halogenated organic compounds. *Mar. Environ. Res.* **41**: 169–181.
- LI, S.-M., AND OTHERS. 1994. Organic and inorganic bromine compounds and their composition in the Arctic troposphere during polar sunrise. *J. Geophys. Res.* **99**: 25415–25428.
- LOBERT, J. M., AND OTHERS. 1995. A net sink for atmospheric CH_3Br in the East Pacific ocean. *Science* **267**: 1002–1005.
- , AND OTHERS. 1997. Undersaturation of CH_3Br in the Southern Ocean. *Geophys. Res. Lett.* **24**: 171–172.
- MABEY, W., AND T. MILL. 1978. Critical review of hydrolysis of

- organic compounds in water under environmental conditions. *J. Phys. Chem. Ref. Data* **7**: 383–409.
- MANLEY, S. L., AND M. N. DASTOOR. 1987. Methyl halide (CH₃X) production from the giant kelp, *Macrocystis*, and estimates of global CH₃X production by kelp. *Limnol. Oceanogr.* **32**: 709–715.
- , K. GOODWIN, AND W. J. NORTH. 1992. Laboratory production of bromoform, methylene bromide and methyl iodide by macroalgae and distribution in nearshore southern California waters. *Limnol. Oceanogr.* **37**: 1652–1659.
- MCCONNELL, O., AND W. FENICAL. 1977. Halogen chemistry of the red alga *Asparagopsis*. *Phytochemistry* **16**: 367–374.
- MCCULLOCH, A. 1992. Global production and emissions of bromochlorodifluoromethane and bromotrifluoromethane (Halons 1211 and 1301). *Atmos. Environ.* **26**: 1325–1329.
- MELLOUKI, A., AND OTHERS. 1992. Atmospheric lifetimes and ozone depletion potentials of methyl bromide (CH₃Br) and dibromomethane (CH₂Br₂). *Geophys. Res. Lett.* **19**: 2059–2062.
- MOFFETT, J. W., AND O. C. ZAFIRIOU. 1990. An investigation of hydrogen peroxide chemistry in surface waters of Vineyard Sound with H₂¹⁸O₂ and ¹⁸O₂. *Limnol. Oceanogr.* **35**: 1221–1229.
- , AND ———. 1993. The photochemical decomposition of hydrogen peroxide in surface waters of the eastern Caribbean and Orinoco River. *J. Geophys. Res.* **98**: 2307–2313.
- MOORE, R. M., AND R. TOKARCZYK. 1993. Volatile biogenic halocarbons in the northwest Atlantic. *Glob. Biogeochem. Cycles* **7**: 195–210.
- , AND M. WEBB. 1996. The relationship between methyl bromide and chlorophyll a in high latitude ocean waters. *Geophys. Res. Lett.* **23**: 2951–2954.
- MOREL, A., AND J.-F. BERTHON. 1989. Surface pigments, algal biomass profiles, and potential production of the euphotic layer: Relationships reinvestigated in view of remote-sensing applications. *Limnol. Oceanogr.* **34**: 1545–1562.
- NEIDLEMAN, S. L., AND J. GEIGERT. 1986. Biohalogenation: Principles, basic roles and applications. Ellis Horwood.
- NIGHTINGALE, P. D., G. MALIN, AND P. S. LISS. 1995. Production of chloroform and other low-molecular-weight halocarbons by some species of macroalgae. *Limnol. Oceanogr.* **40**: 680–689.
- NORTH, W. J., D. E. JAMES, AND L. G. JONES. 1993. History of kelp beds (*Macrocystis*) in Orange and San Diego Counties, California. *Hydrobiologia* **260/261**: 277–283.
- PAJENIK, B., AND F. M. M. MOREL. 1988. Dark production of H₂O₂ in the Sargasso Sea. *Limnol. Oceanogr.* **33**: 1606–1611.
- , O. C. ZAFIRIOU, AND F. M. M. MOREL. 1987. Hydrogen peroxide production by a marine phytoplankter. *Limnol. Oceanogr.* **32**: 1365–1369.
- PENKETT, S. A., AND OTHERS. 1985. An interhemispheric comparison of the concentrations of bromine compounds in the atmosphere. *Nature* **318**: 550–553.
- RAMANATHAN, V., AND OTHERS. 1985. Trace gas trends and their potential role in climate change. *J. Geophys. Res.* **90**: 5547–5566.
- REIFENHÄUSER, W., AND K. G. HEUMANN. 1992. Bromo- and bromochloromethanes in the Antarctic atmosphere and the south polar sea. *Chemosphere* **24**: 1293–1300.
- SCARRATT, M. G., AND R. M. MOORE. 1996. Production of methyl chloride and methyl bromide in laboratory cultures of marine phytoplankton. *Mar. Chem.* **54**: 263–272.
- SCHALL, C., F. LATURNUS, AND K. G. HEUMANN. 1994. Biogenic volatile organoiodine and organobromine compounds released from polar macroalgae. *Chemosphere* **28**: 1315–1324.
- , AND OTHERS. 1996. Biogenic brominated and iodinated organic compounds in ponds on the McMurdo Ice Shelf, Antarctica. *Antarct. Sci.* **8**: 45–48.
- SHORTER, J. H., AND OTHERS. 1995. Rapid degradation of atmospheric methyl bromide in soils. *Nature* **377**: 717–719.
- SOEDJAK, H. S., AND A. BUTLER. 1990. Characterization of vanadium bromoperoxidase from *Macrocystis* and *Fucus*: Reactivity of vanadium bromoperoxidase toward acyl and alkyl peroxides and bromination of amines. *Biochemistry* **29**: 7974–7981.
- , AND ———. 1991. Mechanism of dioxygen formation catalyzed by vanadium bromoperoxidase from *Macrocystis pyrifera* and *Fucus distichus*: Steady state kinetic analysis and comparison to the mechanism of V-BrPO from *Ascophyllum nodosum*. *Eiochim. Biophys. Acta* **1079**: 1–7.
- STATE OF CALIFORNIA DEPARTMENT OF PESTICIDE REGULATION. 1995. Annual pesticide use report by county, January to December 1993 (draft).
- STURGES, W. T., G. F. COTA, AND P. T. BUCKLEY. 1992. Bromoform emission from Arctic ice algae. *Nature* **358**: 660–662.
- TOKARCZYK, R., AND R. M. MOORE. 1994. Production of volatile organohalogens by phytoplankton cultures. *Geophys. Res. Lett.* **21**: 285–288.
- TOLBERT, N. E. 1974. Photorespiration, p. 474–504. *In* W. P. D. Stewart [ed.], *Algal physiology and biochemistry*. Univ. California Press.
- TSE, G., H. ORZEY, AND S. I. SANDLER. 1992. Infinite dilution activity coefficients and henry's law coefficients of some priority water pollutants determined by a relative gas chromatographic method. *Environ. Sci. Technol.* **26**: 2017–2022.
- U.N. ENVIRONMENT PROGRAMME. 1992. Montreal protocol assessment supplement, methyl bromide: Its atmospheric science, technology and economics. Nairobi, Kenya.
- WASHINGTON, J. W. 1995. Hydrolysis rates of dissolved volatile organic compounds: Principles, temperature effects and literature review. *Ground Water* **33**: 415–424.
- WEVER, R., AND OTHERS. 1991. Brominating activity of the seaweed *Ascophyllum nodosum*: Impact on the biosphere. *Environ. Sci. Technol.* **25**: 446–449.
- WMO, SCIENTIFIC ASSESSMENT OF OZONE DEPLETION. 1994. World meteorological organization global ozone research and monitoring project. Report no. 37. Geneva.
- WUOSMAA, A.-M., AND L. P. HAGER. 1990. Methyl chloride transferase: A carbocation route for biosynthesis of halometabolites. *Science* **249**: 160–162.
- YUNG, Y. L., AND OTHERS. 1980. Atmospheric bromine and ozone perturbations in the lower stratosphere. *J. Atmos. Sci.* **37**: 339–353.
- ZEPP, R. G., Y. I. SKURLATOV, AND J. T. PIERCE. 1987. Algal-induced decay and formation of hydrogen peroxide in water: Its possible role in oxidation of anilines by algae. *Am. Chem. Soc. Symp. Ser.* **327**: 215–216.
- ZIMMERMAN, R. C., AND J. N. KREMER. 1986. *In situ* growth and chemical composition of the giant kelp *Macrocystis pyrifera*: Response to temporal changes in ambient nutrient availability. *Mar. Ecol. Prog. Ser.* **27**: 277–285.

Received: 14 November 1996

Accepted: 5 June 1997

Amended: 22 July 1997