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Inhibitory effect of light on methane oxidation in the pelagic water column of a mesotrophic lake (Lake Biwa, Japan)

Abstract—Methane oxidation was studied in mesotrophic lake water (Lake Biwa, Japan) under thermally stratified conditions. Methane oxidation rates at in situ concentrations were very low in lake water from the epilimnion and thermocline but were high in hypolimnetic water. Incubation under light conditions ranging from 4.1 to 57 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ resulted in decreased methane oxidation in hypolimnetic water. This inhibition was more severe as the light intensity increased. Addition of inorganic nitrogen (ammonium and nitrate) did not promote methane oxidation in the thermocline but inhibited it in the hypolimnion. Methane oxidation activity in the thermocline was observed after 1 month of incubation under dark conditions. Our results suggest that the inhibitory effect of light was bacteriostatic for the methanotrophic population. The different rates of methane oxidation between the hypolimnion and epilimnion/thermocline may explain the surface maximum of dissolved methane during the period of thermal stratification.

Aerobic methane oxidation in aquatic environments occurs at oxic sites adjacent to areas of high methane concentrations, i.e., sediment surface or oxic water, affecting the methane cycle. Methane oxidation also may contribute to oxygen consumption in the sediment and water column (Rudd and Hamilton 1978; Gelda et al. 1995).

The distribution and oxidation of dissolved methane in lake water have been well documented for eutrophic envi-

ronments where the anoxic hypolimnion is developed permanently or seasonally due to chemical or thermal stratification. In such environments, the maximum methane concentration has been recorded in bottom water and the highest methane oxidation has been observed in the oxic–anoxic boundary in the metalimnion (Kiene 1991) and even in the apparently anoxic deep water (Kiene 1991; Bastviken et al. 2002; Liikanen et al. 2002). On the other hand, surface water contains much less dissolved methane and shows low methane oxidizing activity compared with deeper layers.

The methane distribution in oligotrophic to mesotrophic lakes, where an oxic hypolimnion is maintained throughout a stratified period, has not been widely studied so far. A limited number of studies have demonstrated that in oligotrophic to mesotrophic lakes, methane concentrations in the hypolimnion are low due to active oxidation in the surface sediment and bottom water, whereas surface water shows a relatively high concentration of dissolved methane (Schmidt and Conrad 1993; Murase et al. 2003). Inflow of river water with high methane concentration (Murase et al. 2003) and transportation from the littoral sediment (Sakai et al. 2002; Murase et al. 2003) are potential sources of the surface methane maximum. The methane oxidation process in the water column of oligotrophic or mesotrophic lakes is poorly understood (Lidstrom and Somers 1984), although in marine waters of Cape Lookout Bight, Kelley (2003) reported that

methane oxidation was not detectable, despite relatively high concentrations of methane (50–250 nmol L⁻¹).

In this study, methane oxidation activity in the water column at in situ methane concentrations and its controlling factors are examined in mesotrophic Lake Biwa, Japan, during a stratified period. Our results demonstrate that methane oxidation activity in the epilimnion and thermocline is very low compared with the hypolimnion and that light is an inhibiting factor of methane oxidation in lake water.

Materials and methods—Study site and sample preparation: Lake water was sampled from the epilimnion (5 m in water depth), thermocline (15 m in water depth), and hypolimnion (70 m in water depth) of a pelagic area of Lake Biwa (35°22.0'N and 136°6.00'E; water depth, 88 m) during a stratified period (August and November in 2000). The Secchi depth transparency at the sampling times was about 5.5 m. Lake water was sampled using a van Dorn water sampler. Serum vials (130 ml) were filled to overflowing with water samples, plugged with butyl rubber stoppers excluding any bubbles, and sealed with aluminum caps. To study the influence of inorganic nitrogen on methane oxidation, 1 ml of lake water was replaced with filter-sterilized NH₄NO₃ solution by injection on board the ship, giving a final concentration of 200 μmol L⁻¹ of inorganic nitrogen. This concentration was quite high compared with the in situ concentrations reported before (<1 μmol L⁻¹ for ammonium and <1–20 μmol L⁻¹ for nitrate; Miyajima et al. 1997; Urabe et al. 2000), although the concentration of ammonia and nitrate was not measured in this study. To examine the loss of methane due to leakage during the incubation period, lake water was sterilized by injection of 1 ml of NaN₃ solution, giving 2 mmol L⁻¹ final concentration. Water samples were transferred to the laboratory in an ice chest. No change in methane concentration during the transportation was observed in a preliminary test. Water temperature of the lake was measured at 20-cm intervals using an ACL1183-PDK sensor (Alec Electronic). Dissolved oxygen concentration in water samples was measured with a YSI 55 dissolved oxygen meter (Yellow Springs Instruments).

Incubation of lake water: Methane oxidation was measured in the laboratory by monitoring the temporal change of the initial methane concentration at the time of sampling. Lake water samples in the vials were incubated at 15°C for 6–10 d or 90 d (long-term incubation) in an incubator with an illuminator (MIR-220RL, Sanyo). Lighting was provided by white fluorescent lamps with a Perspex ultraviolet (UV) shield at 57 μmol m⁻² s⁻¹ for 12 h d⁻¹. Different levels of light intensity were prepared by wrapping the vials with different layers of shading, which gave 4.1, 12, 23, and 57 μmol m⁻² s⁻¹ of light intensity. The light intensity (photosynthetic photon flux density) in the incubation experiment was measured using a LI-192SA underwater quantum sensor with a LI-250 light meter (Li-Cor). For dark incubation, the vials were wrapped with aluminum foil.

Gas analysis: After incubation, duplicate samples were sacrificed for determination of methane concentration. A portion of the water sample (40 ml) in the vials was replaced

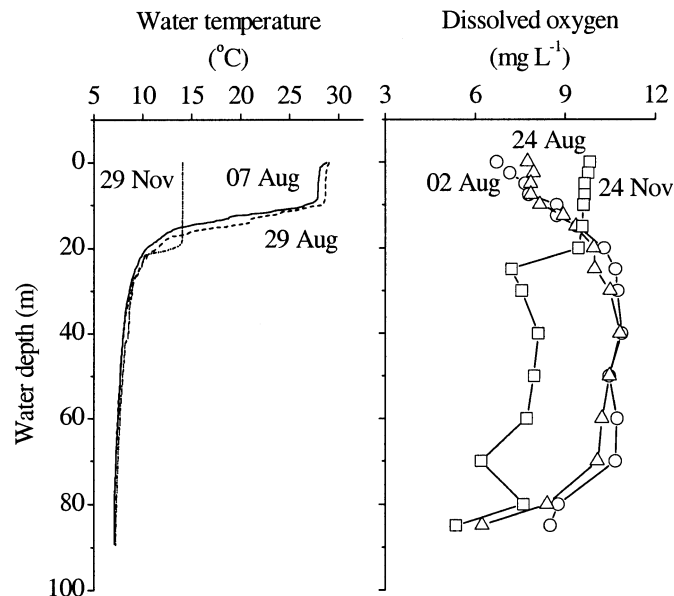


Fig. 1. Depth profiles of water temperature and dissolved oxygen content in a pelagic water column.

with the same volume of He (>99.9999%) by injection to make a gas headspace. The vials were vigorously shaken for at least 20 s. Methane concentrations in the headspace were determined on a gas chromatograph equipped with a flame ionization detector (GC14B, Shimadzu) and a 3-m Porapaq N column. Methane contents in water samples were calculated using the Bunsen solubility coefficient (Yamamoto et al. 1976).

Results—Strong thermal stratification was observed in August and November (Fig. 1). Lake water was mostly saturated (90–100%) with atmospheric oxygen throughout the water column in August, and oxygen content in the epilimnion was less than in the hypolimnion because of the higher water temperature. In November, the water temperature of the epilimnion decreased and consequently contained higher amounts of dissolved oxygen compared with August. The hypolimnion still contained 6–8 mg L⁻¹ of dissolved oxygen in November, although about 30–50% of the oxygen in hypolimnetic water was consumed during the stratified period.

Methane concentrations in the water samples were oversaturated to the atmospheric methane and ranged from 19 to 207 nmol L⁻¹ (see Figs. 2–5 for the initial methane concentrations). The high methane concentration in the lake was consistent with previous studies (Miyajima et al. 1997; Murase et al. 2003) except that methane concentration of the hypolimnion on 7 August was higher (207 nmol L⁻¹, Fig. 2) than previous observation (from less than 10 to 80 nmol L⁻¹; Miyajima et al. 1997; Murase et al. 2003).

Methane oxidation in water samples from different water depths: In the dark, lake water from the hypolimnion (70 m depth) showed a rapid decrease in methane concentration with time, demonstrating active methane oxidation (Fig. 2C). In contrast, water from the thermocline (15 m depth) showed only a small decrease in methane concentration, and methane

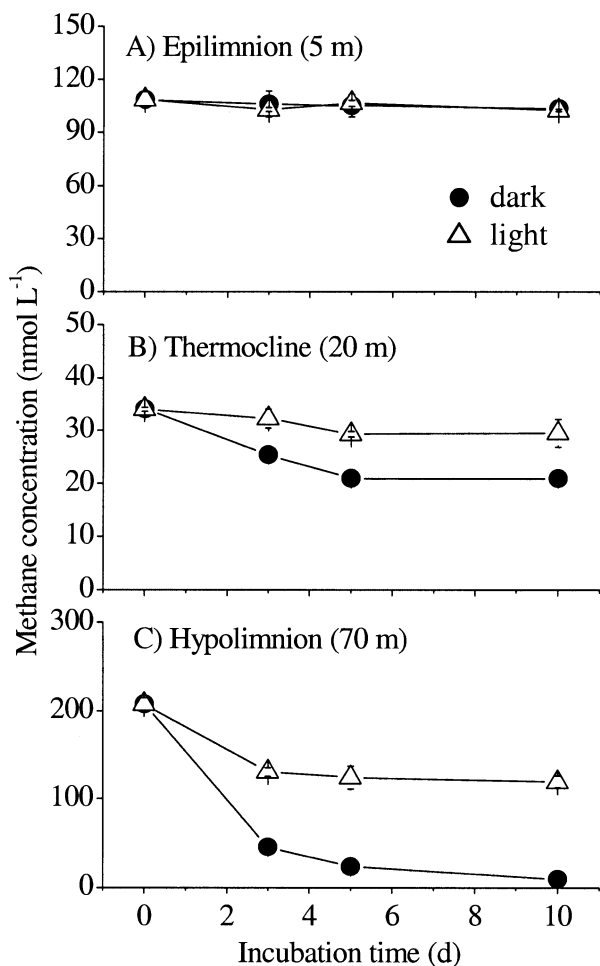


Fig. 2. Temporal change in methane concentration of lake water during incubation under dark or light conditions (7 August 2000). The bars show the range of duplicate samples.

concentration in the epilimnetic water (5 m depth) was unchanged over the incubation time (Fig. 2A,B).

Incubation under light resulted in markedly inhibited methane consumption in hypolimnetic water. This inhibition started from the beginning of the incubation, and no decrease of methane concentration was observed after 2 d (Fig. 2C). The methane consuming activity in the thermocline was also almost completely inhibited by illumination (Fig. 2B). In epilimnetic water, which showed no methane consumption in the dark, no influence of light on methane consumption was observed (Fig. 2A).

The effect of inorganic nitrogen: Addition of NH_4NO_3 to the lake water from the thermocline had no effect on methane consumption either under dark or light conditions (Fig. 3A). On the other hand, addition of inorganic nitrogen to hypolimnetic water resulted in significant inhibition of methane oxidation, especially under dark conditions (Fig. 3B).

The effect of different light intensities: The extent of the inhibitory effect of light on methane consumption in hypolimnetic water depended on the light intensity (Fig. 4). Light

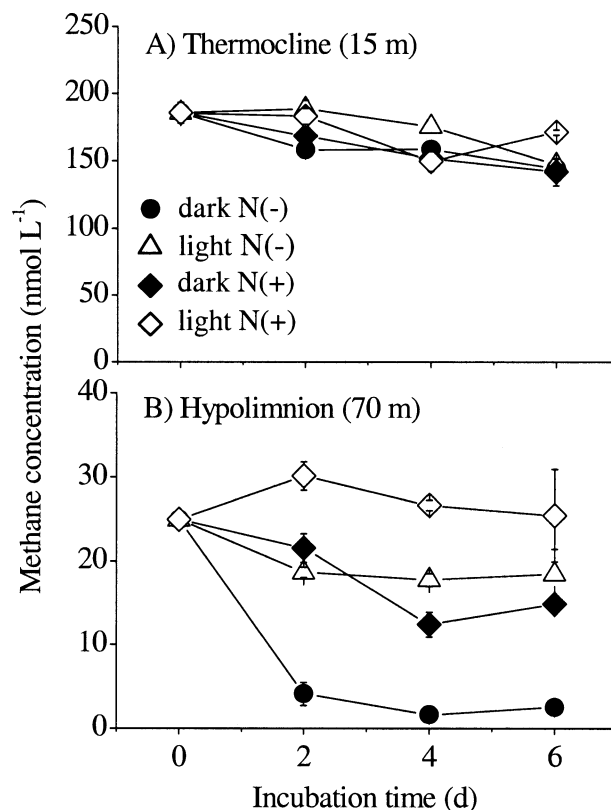


Fig. 3. The effect of light and inorganic nitrogen on methane oxidation in lake water (28 August 2000). The bars show the range of duplicate samples.

intensity up to $12 \mu\text{mol m}^{-2} \text{s}^{-1}$ resulted in almost completely inhibited methane consumption after 2 d. Even the lowest light intensity ($4.1 \mu\text{mol m}^{-2} \text{s}^{-1}$) in this study partly inhibited methane consumption.

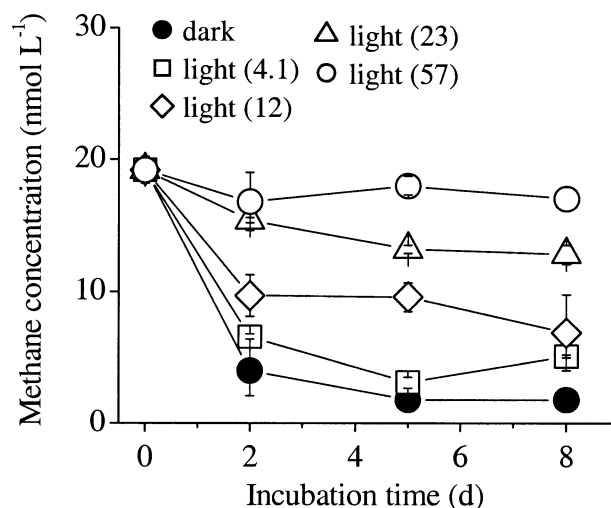


Fig. 4. Effect of different light intensities on methane oxidation in hypolimnetic water (29 November). Numbers in parentheses indicate the light intensity ($\mu\text{mol m}^{-2} \text{s}^{-1}$). The bars show the range of duplicate samples.

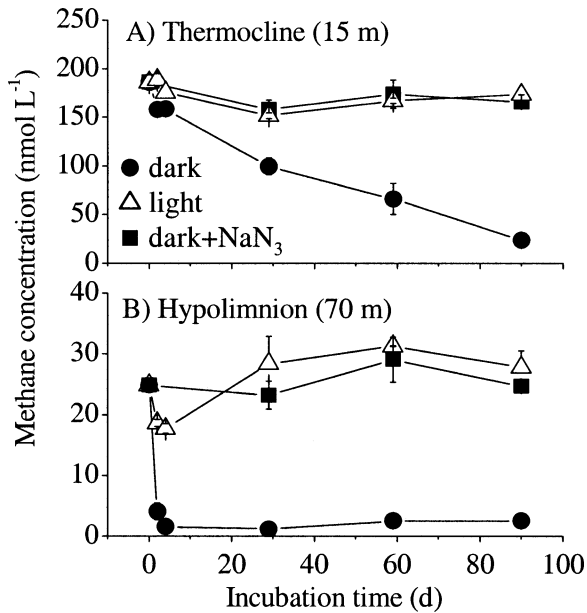


Fig. 5. Temporal change in methane concentration in lake water during a long-term incubation (28 August 2000). The bars show the range of duplicate samples.

Long-term incubation of lake water: A long-term incubation of water from the thermocline under dark conditions resulted in a gradual decrease in methane concentration at a slowly increasing rate. There was no change in the methane concentration of water under light (Fig. 5). Addition of NaN_3 completely inhibited methane oxidation in both epilimnetic and hypolimnetic water under dark conditions, ensuring that methane oxidation was a biological process.

Discussion—In this study, methane oxidation was determined by monitoring temporal decrease in methane from the initial in situ concentration. The approach was simple, but the results clearly demonstrate the difference of methane oxidation activity in different depths of lake water. No change in methane concentration was observed when we incubated the epilimnetic water collected from the different point of the pelagic area with 2-bromoethanesulfonic acid (BES), a specific inhibitor of methane production (Murase unpubl. data). It is, therefore, unlikely that methane consumption is balanced with methane production in the epilimnion.

Active methane oxidation was observed only in the hypolimnion, although the activity may have been accelerated due to the incubation temperature (15°C), which was higher than the in situ temperature ($7.2\text{--}7.5^\circ\text{C}$). That methane oxidation occurs in hypolimnetic water is in agreement with $\delta^{13}\text{CH}_4$, which seasonally increased in the hypolimnion during a stratified period (from -62.6 to -21.8% , Murase et al. 2003). Initial methane concentration, which was reported to be positively correlated with methane oxidation (Ward et al. 1987), is unlikely to be the reason for the different activities among the samples from the different layers because the initial methane concentrations in the epilimnion and thermocline were comparable to and even higher than those in the hypolimnion on 8 August (Fig. 2) and on 28 August

(Fig. 3). In addition, hypolimnetic water showed active oxidation of methane at the low initial concentration (25 nmol L^{-1}) observed on 28 August.

The results clearly demonstrate that methane oxidation in the hypolimnion was completely inhibited by light at $57 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ for 12 h d^{-1} . UV irradiation is not the reason for the inhibition, since most of the UV irradiation from the lamp was absorbed with a Perspex shield and also the glass serum vial can intercept UV irradiation. Thus, the result suggests that methane oxidation was inhibited by visible light. Increased methane oxidation in the thermocline after a long-term incubation under dark conditions supports this conclusion. The inhibitory effect of visible light on growth and activity of methanotrophs has been reported before (Dumestre et al. 1999) with higher light intensity (complete inhibition was observed at more than $300 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ for 12 h d^{-1}). Methane oxidation in the hypolimnion was increasingly inhibited by increased intensities of light, but even the lowest light intensity ($4.1 \mu\text{mol m}^{-2} \text{ s}^{-1}$) affected methane oxidation to some extent. If we assume that the light intensity at the water surface of the lake is $2,000 \mu\text{mol m}^{-2} \text{ s}^{-1}$, and the diffuse attenuation coefficient of this lake ranges between 0.30 and 0.44 (Tsuda and Nakanishi 1992), the water depth at which the light intensity is $12 \mu\text{mol m}^{-2} \text{ s}^{-1}$ is between 12 and 17 m. This suggests that light may be an important controlling factor of methane oxidation in the surface water of the lake.

The long-term incubation under dark conditions, showing methane oxidation in lake water from thermocline, suggests that the effect of light may be bacteriostatic for the methanotrophic population. Nitrifying bacteria can also oxidize methane (Bedard and Knowles 1989), and nitrifying activities in lake and oceans are reversibly inhibited by light intensity (Horrigan et al. 1981; Yoshioka and Saijo 1985; Guerrero and Jones 1996). It is unclear whether methane in the lake water is oxidized by methanotrophs or nitrifiers, although the affinity of nitrifying bacteria to methane is reported to be low compared with methanotrophs (Hanson and Hanson 1996).

It is well recognized that ammonium inhibits methane oxidation (de Angelis and Scranton 1993; Nold et al. 1999), but some studies have reported that methane oxidation is stimulated by addition of ammonium to oxygen-saturated lake water (Rudd et al. 1976). Thus, we tested the hypothesis that light inhibits methane oxidation by affecting the competition for inorganic nitrogen between methanotrophs and phototrophs. The results, however, suggest that methane oxidation in thermocline is not limited by inorganic nitrogen (NH_4^+ , NO_3^-). In contrast, the result in the hypolimnion demonstrated that inorganic nitrogen, probably ammonium, is the potential controlling factor of methane oxidation in the lake water. Increased salt concentration (de Angelis and Scranton 1993) and high oxygen concentrations (Rudd et al. 1976) are other potential inhibitory factors of methane oxidation in water, but these are unlikely in this study, since there was no remarkable difference in salinity and oxygen content among different water depths.

Our results demonstrated that methane oxidation may be limited in the euphotic zone of the entire lake. In fact, methane oxidation in bottom water of the littoral zone (10 m of

water depth) was negligible (Murase unpubl. data). The littoral zone can be a significant source of high methane concentrations in the epilimnion and thermocline (Sakai et al. 2002; Murase et al. 2003), and thus light inhibition of methane oxidation in the littoral zone may be an important factor controlling dynamics of methane in the lake.

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