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Vertical Community Structure of Bacteria and Phytoplankton in Lake Biwa Using Respiratory Quinone and Pigment Analysis

Hiroyuki TAKASU[1], Tadao KUNIHIRO[2] and Shin-ichi NAKANO[1]

[1]*Center for Ecological Research (CER), Kyoto University, Hirano-cho 2 chome, 509-3, Otsu, Shiga 520-2113, Japan*
[2]*Center for Marine Environmental Studies (CMES), Ehime University, Bunkyo-cho 2-5, Matsuyama, Ehime 790-8577, Japan*

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Abstract—We assessed vertical variability in biomass and community structures of both bacteria and phytoplankton in the mesotrophic, monomictic Lake Biwa (Japan) using bacterial respiratory quinone and phytoplankton pigment analysis. Relative biomass of *Alphaproteobacteria* (ubiquinone-10) and *Cytophaga-Flavobacterium-Bacteroidetes* (CFB, menaquinone-6, 7 and 8) decreased with reduction of content and richness of phytoplankton pigments. Compared to other bacterial groups, alphaproteobacterial- and CFB-biomass might be more susceptible to changes in quantity and/or quality of dissolved organic carbon (DOC) derived from variety of phytoplankton taxa. Pigment indicators for diatom (fucoxanthin) and cryptophyta (alloxanthin) were detected as major fractions of total pigment at all depths, especially in the sample from the hypolimnion. So, phytoplankton in Lake Biwa may supply organic matter produced from the epilimnion to the hypolimnion.

Keywords: bacterial community structure, phytoplankton community structure, quinone, photosynthetic pigment, Lake Biwa

INTRODUCTION

In the water column of freshwater systems, bacteria are numerically and functionally important components, and are major consumers of dissolved organic carbon (DOC), which dominates in organic carbon pool. Responses of bacterial groups at the broad phylogenetic level differ to different types of organic matter (Kirchman *et al*., 2004; Yokokawa and Nagata, 2010). Therefore, bacterial community structure and their function are susceptible to both quantity and quality of DOC. The labile fraction of DOC, which is readily used by bacteria, is primarily derived from phytoplankton and their chemical compositions are dependent on dominant phytoplankton taxa (Nakano, 1992, 1996; Hanamachi *et al*., 2008). Thus, community structure of phytoplankton may determine that of bacteria. However, we still have limited information about bacterial community structure in relation to that of phytoplankton.

In the present study, to identify the bacterial groups, which respond to changes in phytoplankton community structure, we assessed community structures of bacteria and phytoplankton in the epilimnion, metalimnion and hypolimnion of Lake Biwa, Japan.

Community structures of bacteria and phytoplankton were determined by quinone profiling and phytoplankton pigment analysis, respectively. Respiratory quinones are electron transport material in bacterial plasma membrane. Bacteria have only one dominant species of respiratory quinone (including ubiquinone: UQ and menaquinone: MK) at phylum or genus level. Thus, quinone is thought to be useful biomarkers to estimate community structure in the complex microbial community (Hedrick and White, 1986; Hiraishi, 1999). In many previous studies, bacterial abundance has been estimated as cell number although natural bacteria consist of various cell volume, they can contain more than hundredfold different cell size (Viles and Sieracki, 1992). Bacterial quinone content has strong positive correlations with their biomass rather than their cell abundance (Hiraishi *et al.*, 1998 and unshown our annual monitoring data), suggesting that it is a good indicator of bacterial biomass. Phytoplankton pigments have been used as markers for identifying different algal classes (reviewed by Jeffrey *et al.*, 1997). Advantages of these methods are to be able to assess biomass and community structure at the same time.

MATERIALS AND METHODS

Study site and sampling

Lake Biwa is a large (surface area, 674 km^2), deep (maximum depth, 104 m), monomictic and mesotrophic lake located in the central part of Honshu Island, Japan. We set a sampling site, a pelagic station (35°12′58″ N, 135°59′55″ E; maximum depth 72 m) in the north basin of the lake, and collected water samples on 28 July 2010. Depth profiles of water temperature and dissolved oxygen concentration were obtained using a CTD probe (SBE 911 plus; Sea Bird Electronics). The depth profile of water temperature was shown in Fig. 1. Samples were collected from 5 m (the epilimnion), 20 m (the metalimnion) and 70 m (the hypolimnion) water depths (Fig. 1) with a 5 L Niskin X bottle (General Oceanics). Samples for quinone and pigment analyses were taken in 10 L-capacity of polyethylene bags, and those for analysis of dissolved organic carbon (DOC) in 500 mL polycarbonate bottles washed with 1.2M HCl and rinsed several times with ultrapure water (Advantec, Japan) before use. For enumeration of bacteria, picophytoplankton and nanoflagellates, water samples were poured into 100 mL polypropylene bottles, and then glutaraldehyde was immediately added to bottles (final conc., 1%) to fix microbial cells.

Dissolved Organic Carbon (DOC)

Samples for DOC were filtered through 0.2 μm polycarbonate filters (Whatman) pre-soaked in 1.2M HCl and rinsed with ultrapure water. The DOC

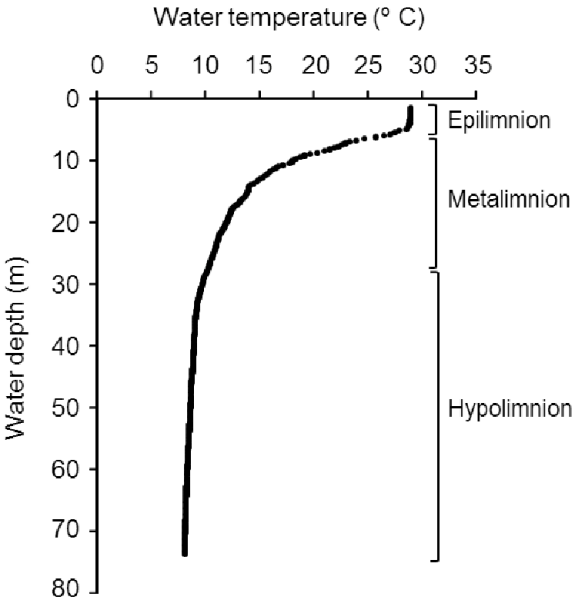

Fig. 1. Depth profile of water temperature.

concentration was determined using a total carbon analyzer (TOC-5000A, Shimadzu).

Enumeration of bacteria, picophytoplankton and nanoflagellates

Two mL of a fixed water sample was used for enumeration of bacteria, 10 mL for that of picophytoplankton and 25 to 50 mL for that of nanoflagellates. Bacteria were counted with an epifluorescence microscopy (BX60, Olympus) under ultraviolet excitation by the DAPI method (Porter and Feig, 1980) using 0.2 μm pore size black polycarbonate filters (Millipore). Nanoflagellates were respectively counted as heterotrophic nanoflagellates (HNF) and autotrophic nanoflagellates (ANF) with the epifluorescence microscopy under both ultraviolet and green excitation by the primulin method (Caron, 1983), using 0.8 μm pore size black polycarbonate filters (Corning). Picophytoplankton was counted under green excitation using 0.2 μm pore size black polycarbonate filters (Millipore). More than 300 cells of bacteria and picophytoplankton were counted at least in random 20 fields. For HNF and ANF, at least 100 fields were randomly inspected.

Quinone profiling

For quinone analysis, bacteria-sized particles in the water sample were collected with 0.2 μm pore size teflon filters (Advantec) after passing through 2.0

Table 1. Hydrological and biological variables.

	Water depth (m)	Water temp. (°C)	DO[1] (mg L^{-1})	DOC[2] (mg C L^{-1})	Bacteria ($\times 10^9$ cells L^{-1})	Picophytoplankton ($\times 10^8$ cells L^{-1})	HNF[3] ($\times 10^6$ cells L^{-1})	ANF[4] ($\times 10^6$ cells L^{-1})
Epilimnion	5	28.6	8.2	1.16	3.9	2.8	3.1	0.3
Metalimnion	20	12.0	6.9	0.94	1.6	0.3	0.8	0.1
Hypolimnion	70	8.2	6.9	0.92	0.8	0.2	0.8	—

[1]Dissolved oxygen.
[2]Dissolved organic carbon.
[3]Heterotrophic nanoflagellates.
[4]Autotrophic nanoflagellates.

μm nominal pore size GMF-2UM glass fiber filters (Whatman) to remove large particles such as phytoplankton and zooplankton. The quinone content and profile were determined by the method of Kunihiro *et al*. (2008, 2011) with slight modification. The molecular species and concentrations of quinones were determined using HPLC equipped with an ODS column (pore size 3.5 μm, Eclipse Plus C18, 3.0 × 150 mm, Agilent) and a photodiode array detector (SPD-M20A, Shimadzu). A mixture of 20% isopropylether in methanol was used as the mobile phase at a flow rate of 0.5 mL min^{-1}. The column oven temperature was maintained at 35°C.

In this paper, we refer to the quinones according to the following abbreviations (ubiquinone: UQ, menaquinone: MK, plastoquinone: PQ). The number indicates that of isoprene units in the side chain of the quinone. For example, UQ-10 represents a ubiquinone with 10 isoprenoid units, and MK-9 (H_2) represents a menaquinone with 9 isoprenoid units where one of the 9 units is hydrogenated with 2 hydrogen atoms.

Pigment analysis

Samples for pigment analysis were filtered through 0.3 μm nominal pore size GF-75 glass fiber filters (Advantec). The filter was dipped into 2 mL *N*,*N*-dimethylformamide (DMF) and then sonicated for 10 minutes, and kept in the dark at 4°C for 2 hours. The extract was filtered through 0.2 μm pore size PTFE filter (Puradisc 25 PP, Whatman) to remove fine particles. One hundred μL of 0.5 M ammonium acetate was added to the filtered extract as an ion-pair solution. An aliquot of the mixture was injected into the HPLC system with an ODS column (pore size 3.5 μm, Synmmetry C8 column, 3.0 × 100 mm, Waters). Pigment separation were performed with ternary solvent system based on Zepata *et al*. (2000), namely, solvent A [methanol: acetonitrile: 0.25 M pyridine solution = 50:25:25 (v/v/v)], solvent B [methanol: acetonitrile: acetone = 20:60:20 (v/v/v). The linear gradient of solvents was as follow: 0 min: 100% A; 22 min: 60% A; 27 min: 5% A; 40 min: 100% A. The solvent flow rate was 0.5 mL min^{-1}. The column oven temperature was maintained at 30°C. Pigments were identified by comparison with retention times and were quantified from their peak areas and the extinction coefficients listed in Jeffrey *et al*. (1997).

RESULTS

Hydrological and biological parameters

Some hydrological and biological parameters of each sample were summarized in Table 1. Dissolved oxygen concentration was the highest at 5 m depth (8.2 mg L^{-1}), and was at the same level at 20 m and 70 m depths (6.9 mg L^{-1}). The whole water column was well oxygenated. Chlorophyll *a* concentration and bacterial abundance were decreased with depth. DOC concentration was the highest at 5 m depth (1.16 mg C L^{-1}), and similar DOC concentrations were detected at 20 m (0.94 mg C L^{-1}) and 70 m depths (0.92 mg C L^{-1}). Cell densities

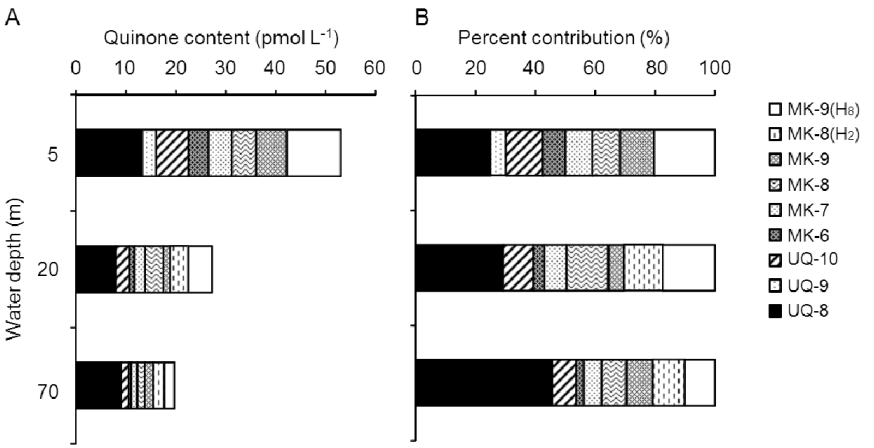

Fig. 2. Respiratory quinone contents (A) and percent contribution (B).

of HNF were higher (5 m; 3.1×10^6 cells L^{-1}, 20 m; 0.8×10^6 cells L^{-1}, 70 m; 0.8×10^6 cells L^{-1}) than that of ANF in each depth (5 m; 0.3×10^6 cells L^{-1}, 20 m; 0.1×10^6 cells L^{-1}, 70 m; not observed).

Distribution of respiratory quinones

Total respiratory quinone contents of 5 m, 20 m and 70 m were 53.0, 27.2 and 19.7 pmol L^{-1}, respectively (Fig. 2). Vertical distribution of respiratory quinone content was similar to that of bacterial number. UQ-8, which is a major member of the *Betaproteobacteria*, dominated in the mole fraction of quinone at all depths, and its mole percentages in the respiratory quinone composition at 5 m, 20 m and 70 m were 24.9, 29.3 and 45.8%, respectively. MK-9 (H_8), which is one of the hydrogenated MKs and mainly derived from the members of the phylum *Actinobacteria*, presented in the second largest percentage at 5 m (20.2%) and 20 m (17.4%). UQ-10, which is mainly due to member of *Alphaproteobacteria*, and MK-8, which is present in the member of *Cytophaga-Flavobactrium-Bacteroides* (CFB) cluster, Low G+C content gram positive bacteria, *Delta-* and *Epsilonproteobacteria*, ubiquitously distributed at all depths around 10% of total respiratory quinones.

Distribution of phytoplankton pigments

Total pigment contents of 5 m, 20 m and 70 m were 2.29, 1.55 and 0.15 μg L^{-1}, respectively (Fig. 3). Zeaxanthin, which is an indicator for cyanobacteria (*Synechococcus* sp.), predominated in the pigment content at 5 m depth (25.9%). Peridinin, which is an indicator for dinophyta, was major pigments at 5 m (11.9%) and 20 m (13.0%) depth. Diadinoxanthin was also major pigments at 5 m (11.9%) and 20 m (13.0%), and it is an indicator pigment for haptophyta. Lutein,

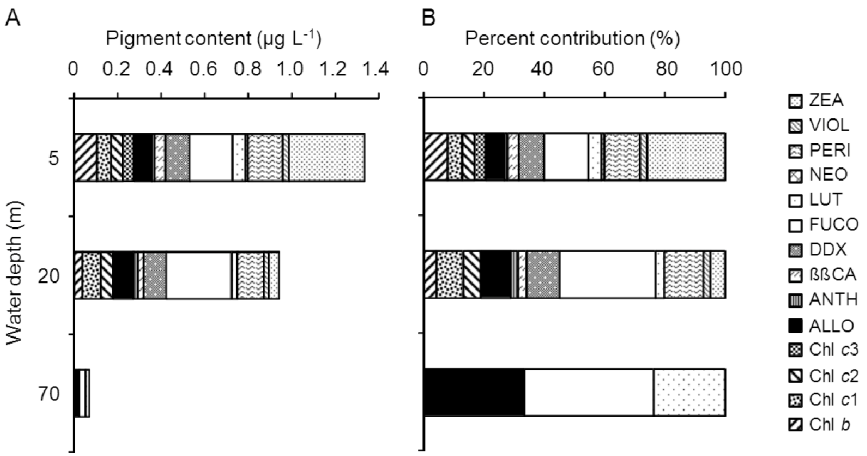

Fig. 3. Pigment contents (A) and percent contribution (B). ZEA: Zeaxanthin; VIOL: Violaxanthin; PERI: Peridinin; NEO: Neoxanthin; LUT: Lutein; FUCO: Fucoxanthin; DDX: Diadinoxanthin; *ββ*CA: *ββ* Carotene; ANTH: Astaxanthin; ALLO: Alloxanthin; Chl *c*3: Chlorophyll *c*3; Chl *c*2: Chlorophyll *c*2; Chl *c*1: Chlorophyll *c*1; Chl *b*: Chlorophyll *b*.

fucoxanthin and alloxanthin were indicator pigments for chlorophyta, diatom and cryptophyta, and these pigments were detected from all samples. Fucoxanthin accounted for large fraction of total pigments at all depths, its relative composition of 5 m, 20 m and 70 m were 14.7, 31.8 and 42.9%, respectively. Percentages of alloxanthin at 5 m, 20 m and 70 m were 6.3, 10.0 and 33.4%, respectively. Lutein accounted for 23.7% of total pigments at 70 m sample, but it was a minor pigment at 5 m (4.5%) and 20 m (3.0%).

DISCUSSION

It is well known that bacterial biomass or production is closely coupled with that of phytoplankton in freshwater and marine systems. Little is known about how bacterial community changes with phytoplankton composition, though few studies examined genetic diversity of bacteria and phytoplankton (Riemann *et al*., 2000; Rooney-Varga *et al*., 2005). However, quantitative measurements of bacterial- and phytoplankton-community structure are quite rare. We could successfully collect the information about biomass and community structure of both bacteria and phytoplankton at the broad taxonomic level (Figs. 2 and 3). Differences of bacterial community structures were successfully detected at each depth, although it was not so remarkable relative to that of phytoplankton.

Biomass of individual bacterial groups showed significant positive correlation with water temperature at the similar correlation coefficient values in the epilimnion of our annual monitoring data (data not shown). It suggests that effect of water temperature was not group specific. Relative biomass of alphaproteobacterial- (UQ-10) and CFB- (MK-6, 7 and 8) decreased with reduction

of content and richness of pigments, though such tendency was not found in biomass of other bacterial groups (Fig. 2). It has been reported that abundances or growth rates of alphaproteobacteria and CFB had correlation with DOC and/or Chl. *a* concentrations (Yokokawa and Nagata, 2005; Pérez and Sommaruga, 2011 and unshown our annual monitoring data from the sampling site). Substrate preferences of these two groups have also been reported; *Alphaproteobacteria* preferentially utilize low-molecular weight DOM such as amino acids, and CFB conduct degradation of high-molecular weight DOM, such as chitin, *N*-acetylglucosamine and protein (Cottrell and Kirchman, 2000). Therefore, compared to other bacterial groups, *Alphaproteobacteria* and CFB might be more susceptible to changes in quantity and/or quality of DOC, which derived from variety of phytoplankton taxa.

It was reasonable that we found an indicator for diatom (fucoxanthin) in all samples (Fig. 3). In Lake Biwa, large diatoms such as *Fragilaria crotonensis* and *Asterionella formosa* often dominate (Nakano, 1992; Kagami *et al*., 2006 and unshown our data from microscopic observation). Those large phytoplankton sink faster than small phytoplankton (Kiørboe, 1993). They might contribute largely to the vertical flux of organic matter from the epilimnion to the hypolimnion. An indicator for cryptophyta (alloxanthin) was also detected in all samples. Urabe *et al*. (2000) reported that one of the cryptophyta, *Cryptomonas* sp. dominated in both epilimnion and metalimnion in Lake Biwa and it ingested bacteria. The phagotrophic mode of *Cryptomonas* sp. is induced by light regime and nutrient concentration (Urabe *et al*., 2000). Thus, *Cryptomonas* sp. might serve not only as a DOC producer, but also as a bacterial grazer depending on environmental conditions, especially in the aphotic hypolimnion.

Attempts are underway to clarify seasonal variations of biomass and community structure of both bacteria and phytoplankton for further understanding the importance of phytoplankton composition as a determinant of bacterial community structure.

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H. Takasu (e-mail: htakasu@ecology.kyoto-u.ac.jp)