

# Alphaproteobacterial dominance in a large mesotrophic lake (Lake Biwa, Japan)

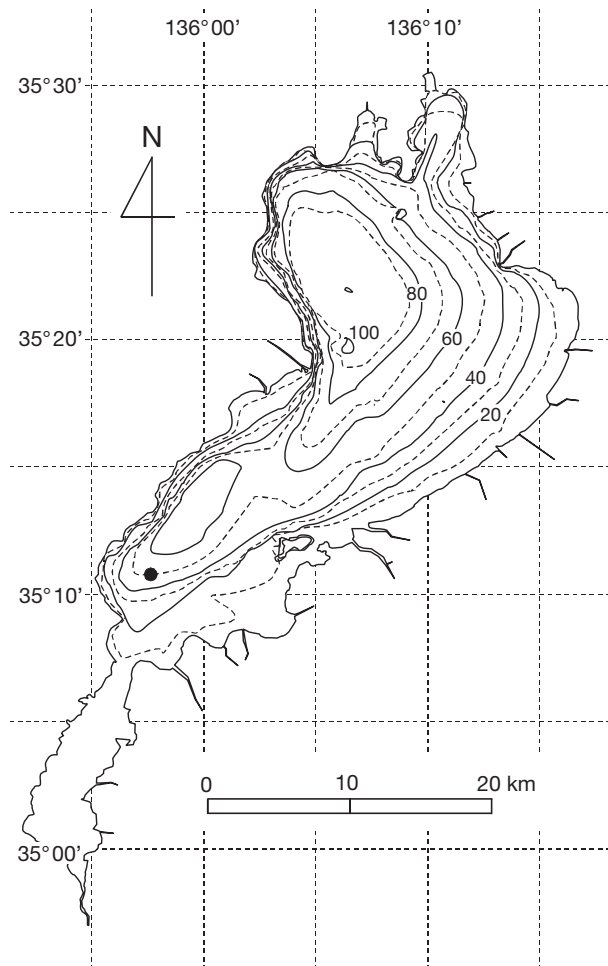
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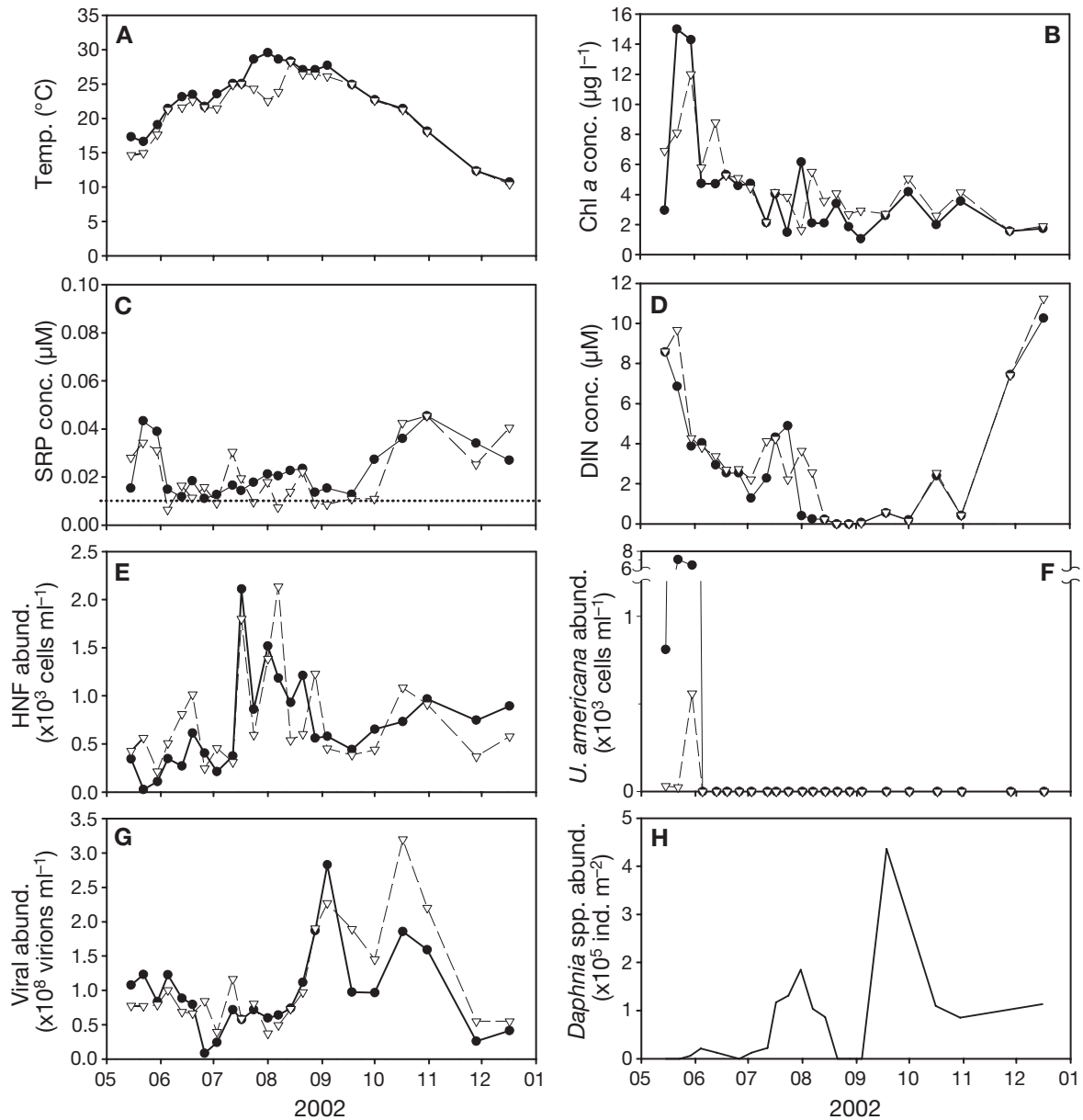
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**Appendix 1.** Lake Biwa and the sampling station (●)



**Appendix 2.** Seasonal changes in (A) temperature, (B) chl *a* concentration, (C) soluble reactive phosphorus (SRP) concentration, (D) dissolved inorganic nitrogen (DIN) concentration, (E) abundance (abund.) of heterotrophic nanoflagellates (HNF), (F) abundance of *Uroglena americana*, (G) abundance of viruses, and (H) abundance of *Daphnia* spp. Data were collected at a depth of 1 m (●) and in subsurface layers (▽). For *Daphnia* spp., total abundance in the epilimnetic water column is shown. (C) Dotted line shows the detection limit. (A) Water temperature varied in the range of 10.4 to 29.6°C, with the maximum on 26 August. (B) Chl *a* concentrations were high in May (max. 15.0  $\mu\text{g l}^{-1}$ ) and low in other months (1.1 to 8.8  $\mu\text{g l}^{-1}$ ). (C,D) Average concentrations of SRP and DIN were  $0.021 \pm 0.011 \mu\text{M}$  (range, 0.006 to 0.045  $\mu\text{M}$ ) and  $3.10 \pm 3.08 \mu\text{M}$  (range, 0 to 11.24  $\mu\text{M}$ ), respectively. (D) The high DIN to SRP ratio (average, 158) indicates general deficiencies in P (Nishimura et al. 2005, Kim et al. 2006), although DIN concentrations were low ( $< 1 \mu\text{M}$ ) during the late stratification period (August and September). (E) Abundance of HNF varied in the range of  $0.02 \times 10^3$  and  $2.1 \times 10^3 \text{ cells ml}^{-1}$ , with 2 peaks on 17 July and 7 August. (F) *U. americana*, a mixotrophic colonial (colony diameter, around 300  $\mu\text{m}$ ) chrysophyte (Kimura & Ishida 1985), were abundant in May ( $8.1 \times 10^2 - 7.0 \times 10^3 \text{ cells ml}^{-1}$ ), especially in the surface layer relative to the subsurface layer; the abundances in both layers were low ( $< 4.8 \text{ cells ml}^{-1}$ ) in other months. (G) Abundances of viruses varied in the range of  $8.4 \times 10^6$  to  $3.2 \times 10^8 \text{ virions ml}^{-1}$ . (H) *Daphnia* spp. showed 2 peaks on 1 August ( $1.9 \times 10^5 \text{ ind. m}^{-2}$ ) and 18 September ( $4.4 \times 10^5 \text{ ind. m}^{-2}$ ). Phytoplankton community was generally dominated by *Chlorophyceae* (mean contribution,  $74.2 \pm 21.5\%$ ); other major taxa included *Chrysophyceae* (max. 59.1% on 22 May), *Cyanobacteria* (max. 54.5% on 4 September) and *Bacillariophyceae* (max. 57.0% on 18 September) (data not shown)



**Appendix 3.** Relative abundance of the major phylogenetic groups of bacteria in freshwater lakes, ponds and reservoirs. Phylogenetic groups include *Alpha-* ( $\alpha$ ), *Beta-* ( $\beta$ ), and *Gamma-* ( $\gamma$ ) subdivisions of *Proteobacteria*, the *Cytophaga-Flavobacteria* cluster (CF), and *Actinobacteria* (Actino). Relative abundances of EUB-probe positives are also shown. Values are averages and ranges (in parentheses) of the abundances relative to total prokaryotes. All data were obtained by FISH using oligonucleotide probes targeted to rRNA. –: group was not determined

Lake/Pond/ Reservoir	Country	Dominant	Abundance relative to total prokaryotes (%)						Source
			EUB	$\alpha$	$\beta$	$\gamma$	CF	Actino	
Biwa	Japan	$\alpha$	59.1 (34.5–73)	41.7 (20.1–66.9)	11.8 (5.4–36.7)	2.2 (0–6.8)	5.8 (1.6–18.1)	14.3 (2.7–38.4)	This study
Baikal	Russia	$\beta$	58.7	1	16.2	1.2	8.2	–	Glöckner et al. (1999)
Baikal	Russia	$\beta$	72.5	–	15	–	14	9.5	Glöckner et al. (2000)
Tibetan lakes <sup>a</sup>	PR China	$\beta$ or Actino	60 (51.9–66.5)	1.5 (0.3–4.5)	21.8 (14.3–26.6)	1.0 (0–4.6)	8.7 (1.6–20.6)	17.7 (5.2–35.1)	Wu et al. (2006)
Lago di Cadagno	Switzerland	$\beta$	51 (46–61)	1 (0–2)	19.3 (17–21)	2.3 (0–5)	7.7 (3–12)	–	Glöckner et al. (1999)
Lago di Cadagno <sup>b</sup>	Switzerland	$\gamma$	(53–66)	(0–1)	(1–2)	(29–36)	–	–	Bosshard et al. (2000)
Grosser Ostersee	Germany	$\beta$	52.2 (46–61)	2 (1–3)	9.8 (3–16)	<1 (0–1)	3.6 (1–7)	–	Glöckner et al. (1999)
Constance	Germany	$\beta$	37.5	4.1	12	2.2	5.4	–	Zwisler et al. (2003)
Fuchskuhle	Germany	Actino	68.5	–	18.5	–	2	22	Glöckner et al. (2000)
Zwischenahner Meer	Germany	$\beta$ or Actino	43.2	1.3	11.25	0.45	2.9	16.7	Bruns et al. (2003)
Farm pond	Germany	$\beta$	77	0	23	0	6	–	Jürgens et al. (1999)
Gossenköllesee	Austria	$\beta$	63.8 (63–65)	10.5 (10–11)	19 (12–26)	– (<1)	5.5 (5–6)	–	Alfreider et al. (1996)
Gossenköllesee	Austria	$\beta$	21 (7–45)	11 (4–23)	24 (5–41)	– (<2)	3.5 (<10)	–	Pernthaler et al. (1998)
Gossenköllesee	Austria	$\beta$	51	10	18	1	16	–	Glöckner et al. (1999)
Gossenköllesee	Austria	$\beta$ or Actino	79.5 (47–96)	12.5 (5–22)	23.5 (11–40)	3.9 (2–10)	–	35.8 (15–60)	Glöckner et al. (2000)
High mountain lakes	Austria	$\beta$ or Actino	–	–	(7–38)	–	–	(3–68)	Warnecke et al. (2005)
Rimov Reservoir	Czech Rep.	$\beta$	77.3	<1	22	2.5	7	–	Šimek et al. (2001)
Rimov Reservoir	Czech Rep.	$\beta$	50	<2	23	5	20	–	Šimek et al. (2003)
Rimov Reservoir	Czech Rep.	$\beta$	62.1 (40–83)	–	22.6 (8–52)	–	27.2 (8–50)	–	Masin et al. (2003)
Rimov Reservoir	Czech Rep.	$\beta$	58	–	18	8	15	18	Šimek et al. (2005)
Orlik Reservoir	Czech Rep.	$\beta$ or CF	48.2 (30–62)	7.2 (<12)	12.4 (7–18)	5.2 (<18)	15.1 (5–25)	–	Masin et al. (2003)
Sep Reservoir	France	$\beta$	(26.2–62.8)	5.9	14.2	–	4.1	–	Jardillier et al. (2004)
Sau Reservoir	Spain	CF	43.4	2.6	10.5	0.7	10.7	–	Gasol et al. (2002)
Tiete Reservoirs	Brazil	$\alpha$	(34.5–53.4)	14.8 (11.8–19.2)	10.8 (7.8–13.3)	(1.1–3.5)	(4.5–12.8)	–	Abe et al. (2003)
Moss	Antarctica	$\beta$	55.1 (49.6–84)	4.3 (2.3–10.6)	24.3 (26.4–71.5)	2.7 (0–29.6)	5.3 (1.8–13.5)	–	Pearce (2003)
Range of means (Lake Biwa not included)				0–15	10–24	0–5	2–27	10–36	
Range of means (Lake Biwa included)				0–42	10–24	0–5	2–27	10–36	

<sup>a</sup>Data for freshwater lakes (<1.0 g l<sup>-1</sup> salinity) only  
<sup>b</sup>High abundance of *Gammaproteobacteria* was detected in the anoxic chemocline. This value is not included in the 'range of means' and range presented in Fig. 4

## LITERATURE CITED

- Abe DS, Matsumura-Tundisi T, Rocha O, Tundisi JG (2003) Denitrification and bacterial community structure in the cascade of six reservoirs on a tropical river in Brazil. *Hydrobiologia* 504:67–76
- Alfreider A, Pernthaler J, Amann R, Sattler B, Glöckner FO, Wille A, Psenner R (1996) Community analysis of the bacterial assemblages in the winter cover and pelagic layers of a high mountain lake by in situ hybridization. *Appl Environ Microbiol* 62:2138–2144
- Bosshard PP, Santini Y, Gruter D, Stettler R, Bachofen R (2000) Bacterial diversity and community composition in the chemocline of the meromictic alpine Lake Cadagno as revealed by 16S rDNA analysis. *FEMS Microbiol Ecol* 31:173–182
- Bruns A, Nubel U, Cypionka H, Overmann J (2003) Effect of signal compounds and incubation conditions on the culturability of freshwater bacterioplankton. *Appl Environ Microbiol* 69:1980–1989
- Gasol JM, Comerma M, Garcia JC, Armengol J, Casamayor EO, Kojecka P, Šimek K (2002) A transplant experiment to identify the factors controlling bacterial abundance, activity, production, and community composition in a eutrophic canyon-shaped reservoir. *Limnol Oceanogr* 47:62–77
- Glöckner FO, Fuchs BM, Amann R (1999) Bacterioplankton compositions of lakes and oceans: a first comparison based on fluorescence in situ hybridization. *Appl Environ Microbiol* 65:3721–3726
- Glöckner FO, Zaichikov E, Belkova N, Denissova L, Pernthaler J, Pernthaler A, Amann R (2000) Comparative 16S rRNA analysis of lake bacterioplankton reveals globally distributed phylogenetic clusters including an abundant group of actinobacteria. *Appl Environ Microbiol* 66:5053–5065
- Jardillier L, Basset M, Domaizon I, Belan A, Amblard C, Richardot M, Debrosses D (2004) Bottom-up and top-down control of bacterial community composition in the euphotic zone of a reservoir. *Aquat Microb Ecol* 35:259–273
- Jürgens K, Pernthaler J, Schalla S, Amann R (1999) Morphological and compositional changes in a planktonic bacterial community in response to enhanced protozoan grazing. *Appl Environ Microbiol* 65:1241–1250
- Kim C, Nishimura Y, Nagata T (2006) Role of dissolved organic matter in hypolimnetic mineralization of carbon and nitrogen in a large, monomictic lake. *Limnol Oceanogr* 51:70–78
- Kimura B, Ishida Y (1985) Photophagotrophy in *Uroglena americana*, Chrysophyceae. *Jpn J Limnol* 46:315–318
- Masin M, Jezbera J, Nedoma J, Straskrabova V, Hejzlar J, Šimek K (2003) Changes in bacterial community composition and microbial activities along the longitudinal axis of two canyon-shaped reservoirs with different inflow loading. *Hydrobiologia* 504:99–113
- Nishimura Y, Kim C, Nagata T (2005) Vertical and seasonal variations of bacterioplankton subgroups with different nucleic acid contents: possible regulation by phosphorus. *Appl Environ Microbiol* 71:5828–5836
- Pearce DA (2003) Bacterioplankton community structure in a maritime Antarctic oligotrophic lake during a period of holomixis, as determined by denaturing gradient gel electrophoresis (DGGE) and fluorescence in situ hybridization (FISH). *Microb Ecol* 46:92–105
- Pernthaler J, Glöckner FO, Unterholzner S, Alfreider A, Psenner R, Amann R (1998) Seasonal community and population dynamics of pelagic bacteria and archaea in a high mountain lake. *Appl Environ Microbiol* 64:4299–4306
- Šimek K, Pernthaler J, Weinbauer MG, Hornak K, Dolan JR, Nedoma J, Masin M, Amann R (2001) Changes in bacterial community composition and dynamics and viral mortality rates associated with enhanced flagellate grazing in a mesoeutrophic reservoir. *Appl Environ Microbiol* 67:2723–2733
- Šimek K, Hornak K, Masin M, Christaki U, Nedoma J, Weinbauer MG, Dolan JR (2003) Comparing the effects of resource enrichment and grazing on a bacterioplankton community of a meso-eutrophic reservoir. *Aquat Microb Ecol* 31:123–135
- Šimek K, Hornak K, Jezbera J, Masin M, Nedoma J, Gasol JM, Schauer M (2005) Influence of top-down and bottom-up manipulations on the R-BT065 subcluster of beta-proteobacteria, an abundant group in bacterioplankton of a freshwater reservoir. *Appl Environ Microbiol* 71:2381–2390
- Warnecke F, Sommaruga R, Sekar R, Hofer JS, Pernthaler J (2005) Abundances, identity, and growth state of actinobacteria in mountain lakes of different UV transparency. *Appl Environ Microbiol* 71:5551–5559
- Wu QL, Zwart G, Schauer M, Kamst-van Agterveld MP, Hahn MW (2006) Bacterioplankton community composition along a salinity gradient of sixteen high-mountain lakes located on the Tibetan Plateau, China. *Appl Environ Microbiol* 72:5478–5485
- Zwisler W, Selje N, Simon M (2003) Seasonal patterns of the bacterioplankton community composition in a large mesotrophic lake. *Aquat Microb Ecol* 31:211–225