Limnol. Oceanogr., 46(3), 2001, 557–572

Unusual bacterioplankton community structure in ultra-oligotrophic Crater Lake

Ena Urbach,[1] *Kevin L. Vergin, Lei Young, and Ariel Morse*
Department of Microbiology, Oregon State University, Corvallis, Oregon 97331

Gary L. Larson
USGS Forest and Rangeland Ecosystems Science Center, Forest Science Laboratory, Oregon State University, Corvallis, Oregon 97331

Stephen J. Giovannoni
Department of Microbiology, Oregon State University, Corvallis, Oregon 97331

Abstract

The bacterioplankton assemblage in Crater Lake, Oregon (U.S.A.), is different from communities found in other oxygenated lakes, as demonstrated by four small subunit ribosomal ribonucleic acid (SSU rRNA) gene clone libraries and oligonucleotide probe hybridization to RNA from lake water. Populations in the euphotic zone of this deep (589 m), oligotrophic caldera lake are dominated by two phylogenetic clusters of currently uncultivated bacteria: CL120-10, a newly identified cluster in the verrucomicrobiales, and ACK4 actinomycetes, known as a minor constituent of bacterioplankton in other lakes. Deep-water populations at 300 and 500 m are dominated by a different pair of uncultivated taxa: CL500-11, a novel cluster in the green nonsulfur bacteria, and group I marine crenarchaeota. β-Proteobacteria, dominant in most other freshwater environments, are relatively rare in Crater Lake ($\leq$16% of nonchloroplast bacterial rRNA at all depths). Other taxa identified in Crater Lake libraries include a newly identified candidate bacterial division, ABY1, and a newly identified subcluster, CL0-1, within candidate division OP10. Probe analyses confirmed vertical stratification of several microbial groups, similar to patterns observed in open-ocean systems. Additional similarities between Crater Lake and ocean microbial populations include aphotic zone dominance of group I marine crenarchaeota and green nonsulfur bacteria. Comparison of Crater Lake to other lakes studied by rRNA methods suggests that selective factors structuring Crater Lake bacterioplankton populations may include low concentrations of available trace metals and dissolved organic matter, chemistry of infiltrating hydrothermal waters, and irradiation by high levels of ultraviolet light.

Molecular studies of bacterioplankton have identified a set of lake-water microorganisms that are surprisingly consistent, despite wide variation in trophic levels, geologic settings, and water chemistry. The β-proteobacterial division is dominant in all oxygenated lakes studied, including a suite of mostly small, oligotrophic lakes in the Adirondack Mountains (Hiorns et al. 1997; Methé et al. 1998), eutrophic Lake Loosdrecht (oxic due to constant, wind-induced turbulence; Zwart et al. 1998), immense Lake Baikal (Semenova and Kuznedelov 1998), an oligotrophic mountain lake (Alfreider et al. 1996), and lakes in the arctic (Bahr et al. 1996) and antarctic (Priscu et al. 1999) regions (Table 1). The lakes studied to date cover nearly the entire spectrum of conditions encountered among oxygenated lakes.

Crater Lake, located at the crest of the Cascade Mountains in southwestern Oregon, was chosen as a site for the present study of aquatic ecological processes because it is an isolated caldera lake with little input of allocthonous or anthropogenic materials. The oligotrophic lake is protected as a national park. About 78% of water entering the lake falls directly onto its surface as rain or snow, with the remainder entering as runoff from the lightly forested caldera walls or as a minor influx of hydrothermal fluid from the bottom (Collier et al. 1991). There is no surface outlet. Depletion of nitrogen compounds near the surface suggests that phytoplankton growth is N-limited, though bioassay studies imply colimitation with a trace metal (Groeger unpubl. data; Lane and Goldman 1984; Groeger and Teitjen 1993). The lake receives no anthropogenic nutrient inputs aside from low amounts in precipitation and waste from seasonal tour boats.

A phylogenetic study of bacteria in Crater Lake was undertaken as a preliminary step in the characterization of bacterioplankton processes in the lake. The primary objective of this work was to identify dominant bacterial and archaeal taxa by using small subunit ribosomal ribonucleic acid (SSU rRNA) gene clone library analyses, with confirmation by oligonucleotide probe hybridization to lake-water rRNA. A second objective was to compare Crater Lake's physical, chemical, and biological properties with those of other lakes

[1] Present address: Department of Microbiology, University of Tennessee, Knoxville, Tennessee 37996.

Acknowledgments

We thank M. Buktenica, A, Gibson, and S. Girdner of the National Park Service for sampling expeditions; R. Truitt, for phytoplankton and zooplankton data; R. Hoffman, S. Rumsey, and R. Collier for processing limnological data; J. Barrington for TOC analyses; N. Hamamura for *N. europea* RNA; M. Suzuki and E. DeLong for unpublished sequences; A. W. Groeger, B. W. Methé, and J. P. Zehr for unpublished data and probe sequences; N. Pace for bacterial divisions aligned sequence data; M. R. Fisk for geological data; A. Bernhard and M. Rappé for critical readings of the manuscript; the OSU Center for Gene Research and Biotechnology central services laboratory for DNA sequencing; and the OSU Biological Computing Consortium for computer facilities and software. This work was supported by NSF grant DEB-9709012 (S.J.G. and E.U.). Technical Paper 11729 from Oregon State University Extension and Experiment Station.

Table 1. Molecular studies of pelagic bacteria in oxygenated lakes.

Lake	Dominant taxa	Maximum depth (m)	Elevation (m)	Area (km^2)	Watershed area (km^2)	Trophic status	pH	Limiting nutrient(s)
Crater Lake	Ack4 actino. CL120-10 verruco.	589	1,882	53.2	15	oligo	6.9–8.0	N & trace metal
Gossenköllessee (water column)	β-proteo.	9.9	2,417	0.017	DNP	oligo	6.8	P
Gossenköllessee (snow cover)	β-proteo.							
Baikal (0.5 & 10 m Jul & Nov)	β-proteo.	1,637	456	31,502	540,000	oligo	7.1–7.2	N & P
Baikal (1,200 m Nov)	γ-proteo.							
Lake Vostoc (subglacial ice above lake)	β-proteo. (among sequenced clones)	670	−600	−14,000	DNP	oligo	DNP	P
Carry Pond	β-proteo.	5	550	0.028	0.2	oligo	4.9	N†
Dart's Lake	β-proteo. α-proteo.	18	536	0.52	11	oligo	6.0	P†
Grass Pond	β-proteo.	6	550	0.053	2.4	oligo	6.0	P†
Moss Lake	β-proteo.	13	536	0.46	5.5	oligo	6.7	P†
Sagamore Lake	β-proteo.	23	600	0.68	48	oligo	6.1	P†
Windfall Pond	β-proteo.	6	601	0.024	0.44	oligo	5.8	P†
Lake George	α-proteo. β-proteo.	58	150	114	492	meso-oligo	7.7	P
Lake Loosdrecht	β-proteo. (among sequenced clones)	2.5	−2	9.8	44	eutrophic	8.9	P
Toolik Lake	β-proteo.	25	720	1.49	65	oligo	7.1	N & P

* Estimates of 1% light penetration depths by various means. Wavelengths considered or justifications for ''probably low'' indications are in parentheses.
† Derived from molar N : P ratios calculated from data of Methé and Zehr 1999.
‡ Estimated from DOC concentrations by Williamson et al. 1996.
DNP, data not presented; DOC, dissolved organic carbon; SLB, sucrose lysis buffer (20 mM EDTA, 400 mM NaCl, 750 mM sucrose, 50 mM Tris-Hcl, pH 9.0); SDS, sodium dodecyl sulfate; ProtK, proteinase K; TRFLP, terminal restriction fragment length polymorphism; DGGE, denaturing gradient gel electrophoresis; proteo., proteobacteria.
References available at Web Appendix 3, (http://www.also.org/lo/pdf/vol_46/issue_3/0557a3.pdf).

to suggest selective forces that might determine the taxonomic structure of the bacterioplankton.

Methods

Limnological analyses—Measurements were made and samples collected from the deck of the RV *Neuston* at Sta. 13 (42°56′N, 122°06′W), over the deepest part of Crater Lake, on 19 August 1997. Samples were collected by using either 4-liter Van Dorn or 30-liter Niskin bottles, or by vertical sampling with a 64-μm mesh-size zooplankton net. Temperature and transmissivity (660 nm) measurements were made with a Seacat Profiler equipped with a Seatech 25-cm beam transmissometer. Nitrate-N and ammonia-N were measured by automated cadmium reduction and phenate colorimetric methods, respectively, and a Technicon autoanalyzer (Larson et al. 1996*a*). Chlorophyll concentrations were determined by fluorometry from samples collected onto 0.45-μm pore-size filters (Milli-

Table 1. Extended.

Ice cover (month yr^{-1})	Geology	Hydro-thermal influx	Age (yr)	UV light 1% attenuation depth (m)*	DOC (μM)	Sample processing	Extraction protocol
none	silicic volcano	yes	6,845 ± 50	70 (340 nm) 45 (304 nm)	≤16	none	SLB, SDS, ProtK
~8	crystalline metamor-phosed sediment	no	DNP	38 (340 nm) 19 (305 nm)	DNP	none	In situ hybridization
						filtered through 20 μm	In situ hybridization
5	continental rift, meta-morphic carbonates	yes	2.5×10^7	DNP	DNP	filtered through 2–3 μm	SLB, lysozyme, SDS, ProtK
						filtered through 1.5 μm	NaCl/EDTA, lysozyme, SDS, phenol
12	DNP	DNP	DNP	probably low (no light penetration through ice)	42 mM	none	cloned TRFLP bands; Chelex 100
yes	glaciated granite	no	DNP	0.79–1.60 (UVA) 0.34–0.66 (UVB)‡	50	none	SLB, lysozyme, SDS, ProtK
yes	glaciated granite	no	DNP	0.79–1.60 (UVA) 0.34–0.66 (UVB)‡	291	none	SLB, lysozyme, SDS, ProtK
yes	glaciated granite	no	DNP	0.79–1.60 (UVA) 0.34–0.66 (UVB)‡	357	none	SLB, lysozyme, SDS, ProtK
yes	glaciated granite	no	DNP	0.79–1.60 (UVA) 0.34–0.66 (UVB)‡	324	none	SLB, lysozyme, SDS, ProtK
yes	glaciated granite	no	DNP	0.79–1.60 (UVA) 0.34–0.66 (UVB)‡	540	none	SLB, lysozyme, SDS, ProtK
yes	glaciated granite	no	DNP	0.79–1.60 (UVA) 0.34–0.66 (UVB)‡	365	none	SLB, lysozyme, SDS, ProtK
yes	glaciated granite	no	DNP	0.79–1.60 (UVA) 0.34–0.66 (UVB)‡	DNP	none	SLB, lysozyme, SDS, ProtK
none	sphagnum peatland	no	200	probably low (average Sechi depth 0.3 m)	DNP	none	cloned DGGE bands; phenol beadbeating
9	glaciated Devonian conglomer-ate & sand-stone	no	12,400	probably low (1% PAR = 8–10 m)	575	filtered through 1 μm	French press

pore), extracted with 90% (v/v) acetone, and total P values were determined by the ascorbic acid method after persulfate digestion (American Public Health Association 1985). Dissolved oxygen concentrations were determined by the modified azide Winkler technique (McManus et al. 1996). Phytoplankton were preserved with Lugol's solution and concentrated by gravity settling (96 h total). Cells >1 μm were identified and counted by means of an inverted microscope, and biovolume conversion factors were determined for each taxon by geometric approximation (McIntire et al. 1996). Phytoplankton biovolumes reported for broad taxonomic groups are sums of determinations for several species, each scaled by an appropriate conversion factor. Zooplankton were diluted with lake water prefiltered through 0.2-μm pore-size filters, preserved with 4% formaldehyde/4% sucrose, concentrated by gravity settling (24 h), and counted by means of an inverted microscope (Larson et al. 1996*b*). Zooplankton weight conversion factors were estimated for each taxon from dried animals and were used to convert organism densities to units of biomass per unit volume (Larson et al. 1993). Bacterioplankton cell densities were determined for cells preserved in 2% formaldehyde, stained with 4′6′-diamidino-2-phenylindole (DAPI), and examined by fluorescence microscopy (Porter and Feig 1980). More than 300 cells were counted for all microscopic plankton counts. Primary productivity was measured as incorporation of ^{14}C into biomass collected onto 0.45-μm pore-size filters (Millipore) after ~3 h in situ incubations in the presence of ^{14}C-bicarbonate (Goldman 1963). Bacterial productivity was measured as incorporation of ^{3}H-thymidine and ^{3}H-leucine into triplicate samples incubated in situ for <1 h (Smith and Azam 1992). Incubations for pro-

ductivity measurements bracketed local noon as closely as possible.

Light penetration and total organic carbon (TOC) measurements were made on 11 August 1998. Light penetration was recorded for 2-nm bandwidths between 300 and 850 nm by means of a LiCor LI-1800 UW spectral radiometer, which was lowered through the water column near mid-day. The reading at 1 cm below the surface was used as the reference for 100% surface irradiance. Samples for TOC determinations were collected by using acid-washed, 2-liter Niskin samplers. Triplicate samples (~5 ml) from 13 depths (0–500 m) were transferred to acid-washed and combusted glass vials containing 25 μl 50% (v/v) phosphoric acid and were sealed with Teflon® closures. Samples and potassium phthalate standards were bubbled with CO_2-free gas for 10 min prior to analysis on a Shimadzu TOC-500.

Sampling and nucleic acid purification—Lake-water samples (0, 20, 40, 60, 80, 100, 120, 140, 160, 180, 300, and 500 m) were collected with 30-liter Niskin bottles and immediately filtered through 142-mm, 0.2-μm pore-size SUPOR filters (Gelman). Filters were bathed in sucrose lysis buffer (0.75 M sucrose, 20 nM EDTA, 400 mM NaCl, and 50 mM Tris HCl [pH 9.0]) and stored in liquid nitrogen for transport to the laboratory, then stored at −80°C. Deoxyribonucleic acid (DNA) and RNA fractions were prepared as described (Giovannoni et al. 1990*b*).

Clone library analysis—SSU rRNA clone libraries were prepared from 0-, 120-, and 500-m lake plankton DNA samples. Polymerase chain reaction (PCR) products were produced either by amplification with bacterial-specific primers 27F (AGA GTT TGA TCM TGG CTC AG; Lane 1991) and 1518R (AAG GAG GTG ATC CAN CCR CA; Gordon and Giovannoni 1996) or the archaeal-specific primer pair ARCH25F (TCY GKT TGA TCC YGS CRG), specific for archaea, and 1492R (GGT TAC CTT GTT ACG ACT T; Gordon and Giovannoni 1996), specific for archaea and bacteria. Amplification products were cloned into vector pGEM-T Easy (Promega) according to the manufacturer's instructions. Full-length clones were identified by agarose gel electrophoresis of amplification products made with vector-specific primers M13 forward and M13 reverse, and the same products were used to sort clones into *Hae*III restriction fragment length polymorphism (RFLP) groups, with the aid of FragmeNT software (Molecular Dynamics). A representative of each RFLP group was partially sequenced by using primer 27F or 700R (CTA CGC ATT TCA CYG CTM CAC) and an ABI 373A or 377 automated DNA sequencer with BigDye chemistry (Perkin-Elmer). RFLP group representatives were given preliminary phylogenetic identities by comparison to a database of SSU rRNA sequences by using ARB software (*see* Strunk et al. 1996). Chimeric sequences were identified by using the CHECK CHIMERA utility of the Ribosomal Data Project (Maidak et al. 1999) and by separate phylogenetic analyses of the ends of suspect sequences; these were removed from the data set. Representatives of nonplastid phylogenetic groups without full-length representatives in GenBank were sequenced in their entirety by using vector-specific primers T7 and SP6, SSU rRNA-specific primers 700R and 700F (GTG KAG CRG TGA AAT GCG TAG), and other SSU rRNA-specific primers as necessary (Lane 1991).

Phylogenetic analysis—SSU rRNA sequences were automatically aligned by using ARB software and the alignments refined by eye by using an aligned sequence editor (Genetics Computer Group). Masks were designed for each data set to include only nucleotide sites at which all sequences were unambiguously aligned and determined. For most clones, phylogenetic relationships were inferred by neighbor-joining with Kimura 2-parameter genetic distances (2 : 1 transition : transversion ratio), with bootstrap proportions calculated by neighbor-joining and maximum parsimony as implemented in PHYLIP (*see* Felsenstein 1993). For candidate division ABY1, inference of the phylogenetic tree and bootstrap proportions for neighbor-joining with Kimura distances were as above, with bootstrap proportions for neighbor-joining with maximum likelihood distances (empirically determined Γ shape factor 0.66 and fraction of invariant sites 0.1), and for maximum parsimony was calculated by using PAUP* software (*see* Swofford 1996), as were reliability values for maximum likelihood quartet-puzzling. In all cases, bootstrap proportions were inferred from 100 resampled data sets.

Oligonucleotide probe hybridization—Oligonucleotide probes complementary to rRNA sequence clusters and subclusters found in Crater Lake libraries were either designed by using ARB software or derived from the literature (Table 2). Stringent wash temperatures were determined empirically for each bacterial SSU rRNA probe by hybridization to synthetic rRNAs (Polz and Cavanaugh 1997), transcribed in vitro from clones with known sequences, or to cellular RNAs from *Urechis caupo* and *Strongylocentrotus purpuratus* (EUK1209), *Haloferax volcanii* (ARC915), or *Nitrosomonas europea* (Bet42a). In each case, hybridization was followed by test washes at successively higher temperatures. Dot blot arrays of RNAs from all 12 depths were prepared and analyzed as previously described (Giovannoni et al. 1990*a*, 1996), with degenerate oligonucleotide 338PL as bacterial-specific reference probe. Synthetic and cellular RNAs that were used to determine stringent wash temperatures were also employed as standards for quantification of specific phylogenetic groups.

Results

Limnological data—Depth profiles of Crater Lake presented typical August conditions (Figs. 1, 2). Surface waters were thermally stratified, with a maximum temperature of 16°C and a thermal gradient to ~80 m. A slight increase in water temperature below 350 m (3.55–3.62°C) reflects hydrothermal activity at the bottom of the lake (McManus et al. 1993). Chlorophyll concentrations revealed a deep chlorophyll maximum (DCM) at 140 m, with a lesser peak at 30 m. Phytoplankton populations were dominated by chlorophytes (an undescribed taxon of ~100 μm^3 $cell^{-1}$) and dinoflagellates (*Gymnodinium inversum*), with bacillariophytes (mainly *Nitzschia* sp.) as minor species. The peak of chlo-

Table 2. Phylogenetic group-specific oligonucleotide probes used in this study.

Probe	Specificity: Division	Specificity: Cluster, subcluster, or taxonomic group	Sequence	Wash temp. (°C)	Reference
1406R	universal		ACG GGC GGT GTG TRC	37	Lane 1991
ARCH915	archaea		GTG CTC CCC CGC CAA TTC CT	50	Krumholz and Stahl 1990
338PL	bacteria		GCW GCC WCC CGT AGG WGT	45	this work
EUK1209	eukaryotes		GGG CAT CAC AGA CCTG	37	Giovannoni et al. 1988
C111-662	actinomycetes	C111	GAA TTC CCC RCT CCC CT	45	this work
ACK4-380	actinomycetes	ACK4	GCG TCG CTG GGT CAG AGT TT	50	this work
DE-33-649	α-proteobacteria	freshwater SAR11	TTC CTC TAT CAA ACT CTA GTC	40	Methé and Zehr pers. comm.
Bet42a*	β-proteobacteria		GCC TTC CCA CTT CGT TT	55	Manz et al. 1992
PLAST542A	cyanobacteria/plastids	stramenopiles	TCC GGA TAA CAC TTG CAT	40	this work
PLAST542G	cyanobacteria/plastids	haptophytes, chlorophytes,† cryptomonads	TCC GGA TAA CGC TTG CAT	45	this work
CLGNS-584	green nonsulfur	CL500-11	GCC GAC TTG CCC AAC CTC	45	this work
OP10-439	OP10	CL0-1	CAA ATT TCT TCC CAC TCA	37	this work
CLPLA-348	planctomycetes	CL500-3	ATT CGT TAC TGC AGC CT	37	this work
CL120-10-447	verrucomicrobia	CL120-10	CCA GGG TAT GAG CCT GGC CAT-TAG T	45	this work

* LSU rRNA-specific probe.
† Except *Chlamydomonas*.

Table 3. Summary data for Crater Lake clone libraries.*

Library	Clones	RFLP groups	Sequences	Phylog. clusters
Bacterial				
0 m	98	60	60	17
120 m	64	45	48	15
500 m	75	49	51	21
Archael				
500 m	17	2	2	1

* Chimeric sequences are excluded.

rophyte biovolume appeared immediately above the DCM, a lesser peak for dinoflagellate biovolume above the 30-m chlorophyll peak, and a minor peak for bacillariophytes at 10 m. Zooplankton biovolume was dominated by phytophagous rotifers (mostly *Kellicottia longispina*) and by cladocerans *Daphnia pulicaria* and *Bosmina longirostris.* Nitrate-N and ammonia-N were depleted at the surface, with a shallow nitricline between 100 and 300 m. A peak of ammonia-N (9 μg L^{-1}) appeared at 60 m. Total phosphate (16–19 mg L^{-1}) concentrations were nearly unchanged with depth (data not shown). Dissolved oxygen (0.55–0.63 μM) and transmissivity (82.6–90.9%) exhibited minimum values at the surface but were otherwise nearly unchanged with depth (Fig. 1). Primary productivity exhibited a peak of 167 ng C L^{-1} h^{-1} at 120-m depth. Bacterial productivity varied from 1 to 10 ng C L^{-1} h^{-1} over the depth profile, cell numbers from 2×10^8 to 1×10^9 cells L^{-1}, and bacterial specific productivity from 3.2 to 25.6 ag C $cell^{-1}$ h^{-1} (Fig. 2). Although it is not known whether the bacterial number and productivity values are typical, other data are characteristic of the lake during late summer and early fall (Larson et al. 1996*a*,*b*; McIntire et al. 1996).

Light penetration and TOC measurements were made ~1 yr after the clone library sampling date. Photosynthetically available radiation (PAR) was attenuated to 1% of surface irradiance at 95-m depth, UVA (340 nm) at 70 m, and UVB (304 nm) at 45 m, and TOC values varied between 6 and 16 μM C (Table 1). The light penetration data are typical of August measurements made during other years at Crater Lake (Crater Lake National Park unpubl. data; Larson et al. 1996*a*).

Clone libraries—Four SSU rRNA gene clone libraries were prepared from unfractionated Crater Lake plankton: bacterial libraries from 0, 120, and 500 m, and an archaeal library from 500 m. The 0-, 120-, and 500-m samples were chosen to represent surface, phytoplankton maximum, and abyssal populations, respectively. Nearly full-length SSU rRNA gene clones were obtained by PCR amplification with primers near the ends of the gene and cloning into a TA cloning vector. No products were obtained when archaeal-specific primers were used to amplify 0- or 120-m samples, so only a single archaeal library was prepared using DNA from 500 m.

RFLP analyses were used to identify groups of closely related sequences in each clone library, and at least one clone from each RFLP group was partially sequenced at the

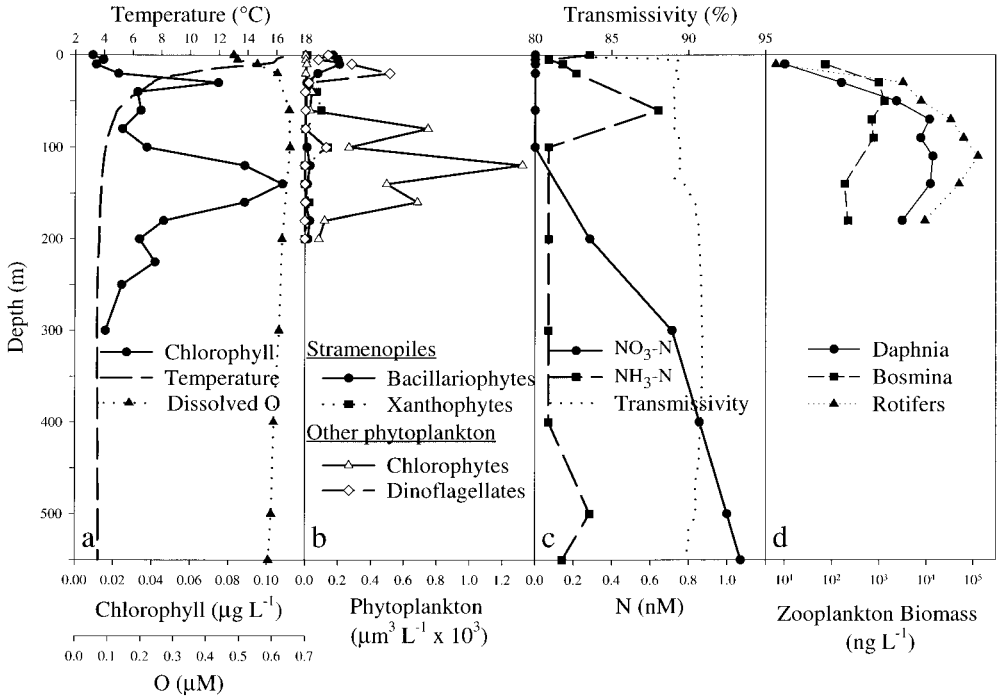


Fig. 1. Limnological data. Depth profiles for (a) total chlorophyll, temperature, and dissolved oxygen, (b) phytoplankton biovolume, (c) nitrate and ammonia nitrogen, and transmissivity, and (d) zooplankton biomass.

5′ end of the gene. Eleven chimeric clones were identified and removed from the data set, either at this stage or after additional sequencing. The resulting data set included 252 clones, of which 161 were sequenced (Table 3).

Phylogenetic analyses comparing Crater Lake sequences to GenBank data distributed the clones into 37 phylogenetic groups, including clades shared with cultured organisms and phylogenetic ''clusters'' of currently uncultivated microbes (Table 3; Web Appendix 1, http://www.aslo.org/lo/pdf/vol_46/issue_3/0557a1.pdf). A cluster is a group of sequences from uncultivated organisms that are more closely related to each other than to genes from any cultured organism (Giovannoni et al. 1990*a*). Clusters unaffiliated with any of the deeply branching ''divisions'' of the bacteria (equivalent clades of cul-

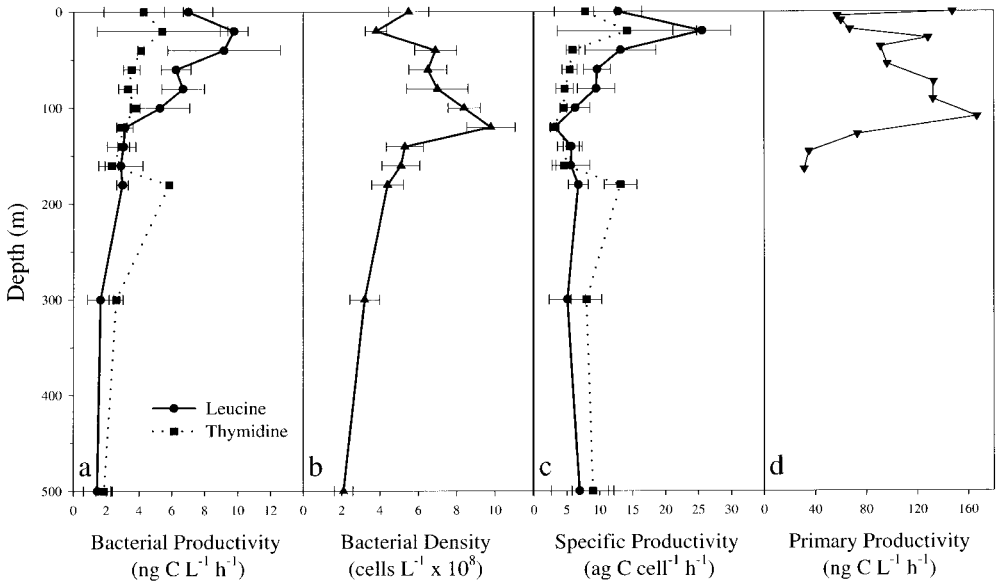
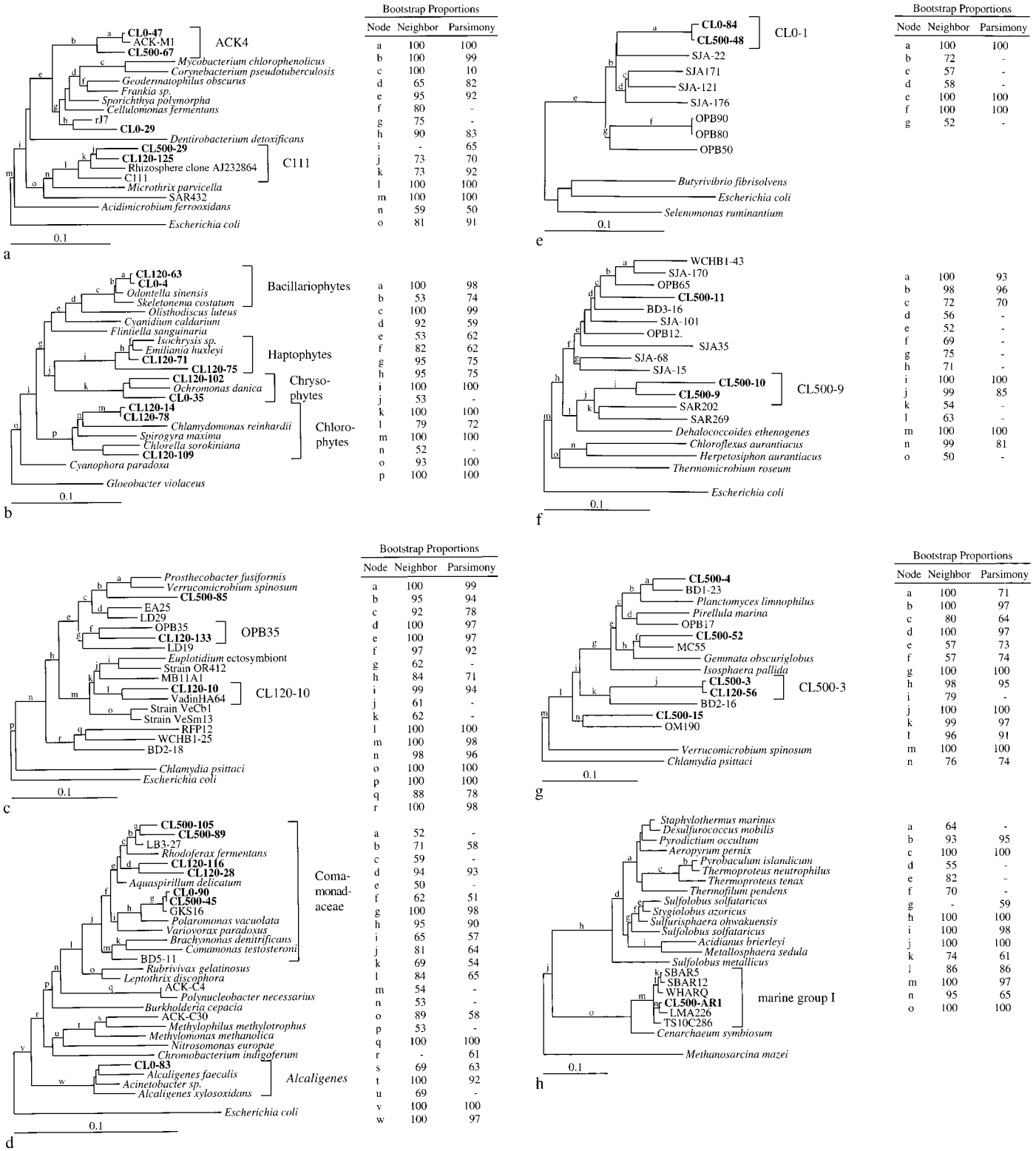


Fig. 2. Productivity and related data. Depth profiles for (a) bacterial productivity determined by ^{3}H-thymidine and ^{3}H-leucine incorporation, (b) bacterial cell density, (c) bacterial-specific productivity, calculated as the ratio of (a)/(b), and (d) primary productivity.



Bootstrap Proportions		
Node	Neighbor	Parsimony
a	100	100
b	100	99
c	100	10
d	65	82
e	95	92
f	80	-
g	75	-
h	90	83
i	-	65
j	73	70
k	73	92
l	100	100
m	100	100
n	59	50
o	81	91

Bootstrap Proportions		
Node	Neighbor	Parsimony
a	100	98
b	53	74
c	100	99
d	92	59
e	53	62
f	82	62
g	95	75
h	95	75
i	100	100
j	53	-
k	100	100
l	79	72
m	100	100
n	52	-
o	93	100
p	100	100

Bootstrap Proportions		
Node	Neighbor	Parsimony
a	100	99
b	95	94
c	92	78
d	100	97
e	100	97
f	97	92
g	62	-
h	84	71
i	99	94
j	61	-
k	62	-
l	100	100
m	100	98
n	98	96
o	100	100
p	100	100
q	88	78
r	100	98

Bootstrap Proportions		
Node	Neighbor	Parsimony
a	52	-
b	71	58
c	59	-
d	94	93
e	50	-
f	62	51
g	100	98
h	95	90
i	65	57
j	81	64
k	69	54
l	84	65
m	54	-
n	53	-
o	89	58
p	53	-
q	100	100
r	-	61
s	69	63
t	100	92
u	69	-
v	100	100
w	100	97

Bootstrap Proportions		
Node	Neighbor	Parsimony
a	100	100
b	72	-
c	57	-
d	58	-
e	100	100
f	100	100
g	52	-

Bootstrap Proportions		
Node	Neighbor	Parsimony
a	100	93
b	98	96
c	72	70
d	56	-
e	52	-
f	69	-
g	75	-
h	71	-
i	100	100
j	99	85
k	54	-
l	63	-
m	100	100
n	99	81
o	50	-

Bootstrap Proportions		
Node	Neighbor	Parsimony
a	100	71
b	100	97
c	80	64
d	100	97
e	57	73
f	57	74
g	100	100
h	98	95
i	79	-
j	100	100
k	99	97
l	96	91
m	100	100
n	76	74

Bootstrap Proportions		
Node	Neighbor	Parsimony
a	64	-
b	93	95
c	100	100
d	55	-
e	82	-
f	70	-
g	-	59
h	100	100
i	100	98
j	100	100
k	74	61
l	86	86
m	100	97
n	95	65
o	100	100

Fig. 3. Phylogenetic trees relating cloned SSU rRNA sequences from Crater Lake (bold) to cultured isolates and sequences from other clone library studies, calculated by neighbor-joining. Phylogenetic clusters and subclusters are bracketed in the figure. The number of nucleotide positions considered in phylogenetic analyses are indicated parenthetically: (a) actinomycetes (493); (b) plastid clade within the cyanobacteria (509), deep-branching stramenopile clone CL0-95 branches below *Olisthodiscus luteus* (not shown); (c) verrucomicrobiales (1,118); (d) β-proteobacteria (476); (e) candidate division OP10 (1,255), (f) green nonsulfur bacteria (1,141); (g) planctomycetales (916); and (h) crenarchaeota (357).

tured and/or uncultured organisms, named incongruously with rankings from phylum to class; Stackebrandt in press) are termed ''candidate divisions'' (Hugenholtz et al. 1998). ''Subclusters'' (Field et al. 1997) within ordinary clusters or candidate divisions may have equivalent phylogenetic depth to entire clusters in divisions containing diverse cultivated members. Among Crater Lake sequences, we identified four clusters or subclusters that were novel, comprised only of sequences from Crater Lake clones, and three clusters or subclusters that were newly identified, comprised of Crater Lake clones plus short and/or misclassified sequences from previous clone library studies. Representative, full-length sequences were obtained for each of these groups to enable higher resolution phylogenetic analysis and to facilitate probe design. Full-length sequences were obtained for 14 clones in 10 clusters or subclusters. Crater Lake plankton clone sequences fell into 13 bacterial and archaeal divisions: actinomycetes; cyanobacteria; verrucomicrobiales; candidate division OP10; green nonsulfur bacteria; planctomycetales; α-, β-, δ-, and γ-proteobacteria; chlorobiaceae; new candidate division ABY1; and crenarchaeota. Phylogenetic relationships in highly represented divisions and candidate division ABY1 are described below; identities of clones belonging to chlorobiaceae, α-, δ-, and γ-proteobacteria, in which Crater Lake phylogenetic groups each represent $<5\%$ of the combined data set, are tabulated (Web Appendix 1).

Actinomycetes—The largest number of Crater Lake clones fell into the actinomycete division (75 clones; Web Appendix 1), mostly in the previously identified ACK4 and C111 clusters (Fig. 3a). The ACK4 cluster appears to be a cosmopolitan group of freshwater microorganisms, because members have been found in several lakes and the Columbia River in Oregon (Hiorns et al. 1997; Crump et al. 1999). Cluster C111 includes clones found in both soil and lake libraries (Kuske et al. 1997; Semenova and Kuznedelov 1998). A single clone from the Crater Lake 0-m library (CL0-29) groups with an isolate from an activated sludge digester supplemented with phenol (rJ7). Thus, all Crater Lake actinomycetes belong to previously identified phylogenetic groups, with the two dominant clusters also found in other lake libraries.

Cyanobacteria—The second largest number of clones belongs to the plastid clade within the cyanobacterial division (45 clones). Members of the plastid clade include clones clustering with the stramenopiles (bacillariophytes, haptophytes, and chrysophytes) and chlorophytes (Fig. 3b). These clones are likely derived from eukaryotic phytoplankton, which dominate photosynthetic plankton in the lake (McIntire et al. 1996). As this study was focused on bacterioplankton, no full-length sequences were determined for Crater Lake plastid clones, although some formed phylogenetic groups distinct from known sequences.

Verrucomicrobiales—The verrucomicrobiales were also a major group in the bacterial clone libraries (29 clones). Most Crater Lake verrucomicrobiales belonged to the CL120-10 cluster, which included 26 Crater Lake clones, 4 short sequences recovered from the Columbia River (Crump et al. 1999), and 1 clone from a winery waste bioreactor (VadinHA64, previously misidentified as a green nonsulfur bacterium). In phylogenetic analyses of short sequences, the CL120-10 clones formed a distinct cluster (not shown), while complete sequences revealed a specific relationship between CL120-10 and cultured strains from anoxic rice paddy soil (VeCb1 and VeSm13) and oxic waters off the Oregon coast (OR412; Giovannoni unpubl. data), as well as cloned sequences from Monterey Bay, California (MB11A1; DeLong and Suzuki unpubl. data), and an ectosymbiont of a cultured ciliate (Fig. 3c). Other verrucomicrobia identified in Crater Lake libraries were members of the OPB35 cluster (two clones) and CL120-85 (one clone), specifically related to *Prosthecobacter fusiformis*, *Verrucomicrobium spinosum*, and clones from soil, lake water, and hot spring clone libraries. In summary, verrucomicrobia were a dominant microbial group in the Crater Lake libraries, and most belonged to a newly identified phylogenetic cluster, CL120-10.

β-Proteobacteria—Sixteen Crater Lake clones were identified as belonging to the β-proteobacteria, the division that is most common at other freshwater sites (Methé et al. 1998; Semenova and Kuznedelov 1998; Zwart et al. 1998; Crump et al. 1999) (Fig. 3d). However, the two β-proteobacterial groups identified as ''globally distributed'' freshwater clades, freshwater cluster B (including *Polynucleobacter necessarius*, also known as ACK1; Zwart et al. 1998; Methé and Zehr 1999) and freshwater cluster C (including *Methylophilus methylotrophus;* Zwart et al. 1998), were not found in the Crater Lake libraries. Instead, β-proteobacteria in Crater Lake belonged either to the comamonadaceae (13 clones) or to the *Alcaligenes* clade (three clones). Crater Lake clones in the comamonadaceae (also known as ACK2; Methé and Zehr 1999) were closely related to *Polaromonas vacuolata* or *Rhodoferax fermentans* and were similar to sequences found as 14% of a series of Adirondack Lake clone libraries (Methé et al. 1998), though they were not identified as an important clade in Lake Loosdrecht (Zwart et al. 1998). The *Alcaligenes* clade includes isolates of *Alcaligenes* and *Acinetobacter* and has been found at low frequency in other freshwater clone libraries. Thus, Crater Lake β-proteobacteria were members of groups that are common in some lakes, but they did not belong to the dominant clusters that can represent up to 72% of lake-water rRNA at other sites (Höfle et al. 1999).

Candidate division OP10—Crater Lake clones belonging to candidate division OP10 fell into a single group, the CL0-1 subcluster (Fig. 3e). To date, this subcluster has only been detected in Oregon; it includes 14 Crater Lake clones (two complete sequences) and 2 short sequences from the Columbia River (CRE-PA63 and CR-PA13, not shown; Crump et al. 1999). In the Columbia River study, these clones were erroneously classified as Gram-positive bacteria. Other, distantly related members of OP10 include clone library sequences from an anaerobic bioreactor and a hot spring. Thus, Crater Lake candidate division OP10 bacteria belonged to a newly identified subcluster, CL0-1, in a candidate division containing members from widely disparate environments.

Green nonsulfur bacteria—Fourteen clones in the Crater Lake libraries belonged to the green nonsulfur division.

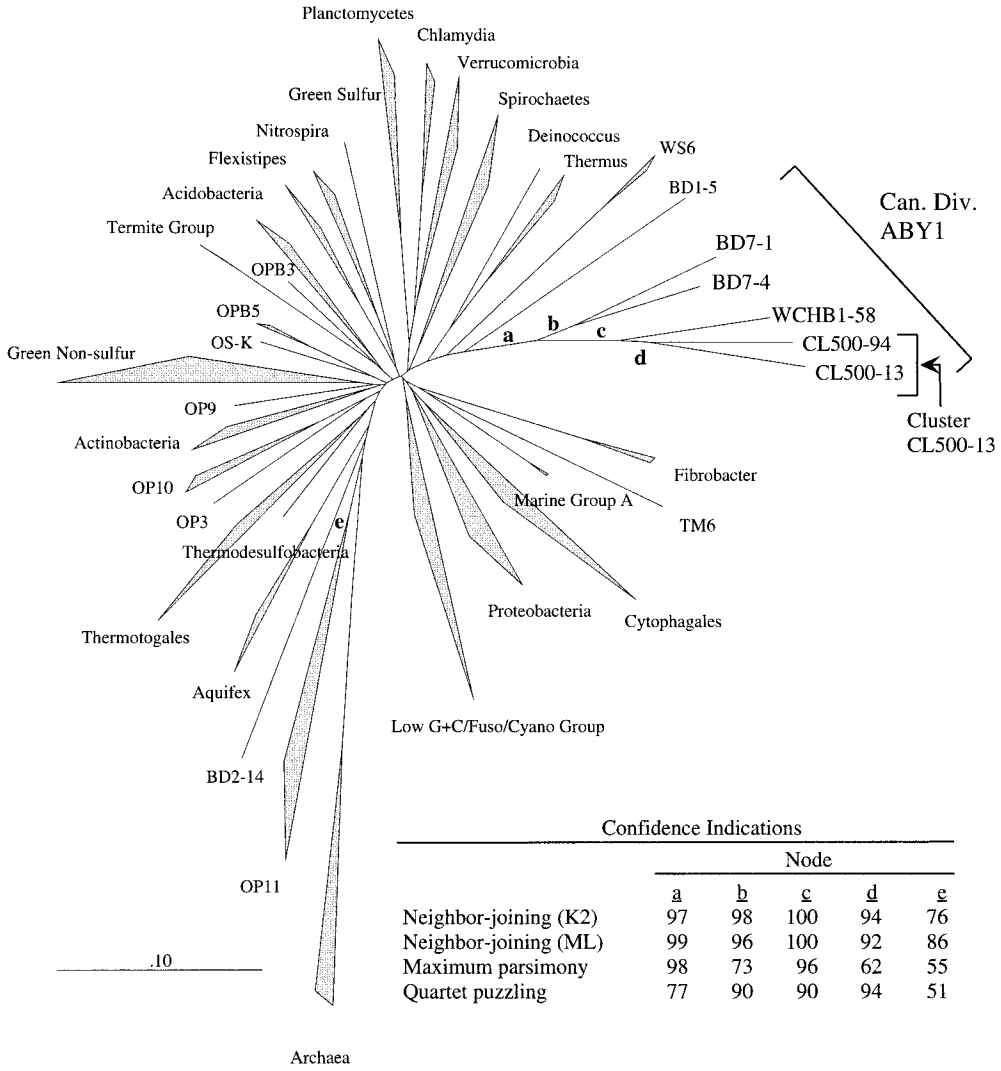


Fig. 4. Phylogenetic tree illustrating unity and deep branching of newly identified candidate division ABY1 and its distinction from candidate division OP11. The phylogenetic framework was inferred by neighbor-joining from 766 aligned nucleotide positions in 117 SSU rRNA gene sequences. Branching patterns within most division-level taxonomic groups are collapsed for clarity, with wedges proportional to the number of sequences considered in each division. Bootstrap proportions and quartet puzzling confidence levels are shown for bifurcations of interest (node e refers to candidate division OP11). K2, Kimura 2-parameter genetic distances; ML, maximum likelihood genetic distances; BD1-5 and BD2-14, single sequences unaffiliated with known bacterial divisions.

These included members of the novel cluster CL500-11 (12 clones) and the novel subcluster CL500-9 (two clones), which falls within the marine SAR202 cluster (Fig. 3f). SAR202 was first identified in a clone library made with 250 m water from the oligotrophic open ocean (Giovannoni et al. 1996) and has also been found in clone libraries made from deeper marine waters; it has not previously been identified in freshwater samples. Thus, green nonsulfur bacteria in Crater Lake included the dominant, novel phylogenetic cluster CL500-11 and a novel freshwater subcluster within the marine SAR202 cluster.

Planctomycetales—Thirteen planctomycetales clones were identified in the Crater Lake clone libraries (Fig. 3g). Most fell into the CL500-3 subcluster (eight clones), a newly identified clade that also includes short sequences from pelagic ocean water and an antarctic lake (short sequences not shown). Analyses of full-length sequences revealed that CL500-3 belonged to a cluster shared with a cloned sequence from marine sediments collected at 1,521-m depth (BD2-16). A second planctomycete cluster, CL500-15, was novel; it formed a deep branch within the planctomycetales division and included only three Crater Lake clones. Finally, two planctomycete clones, CL500-52 and CL500-4, were strongly allied with the *Gemmata* and *Planctomyces* genera, respectively, groups that also included cloned sequences from soils (MC55) and deep marine sediments (BD1-23).

Thus, planctomycetales from Crater Lake included a newly identified subcluster that also contained short sequences from marine and other lake samples, a novel cluster found only in Crater Lake, and representatives of cultured groups.

Crenarchaeota—Archaeal sequences could be amplified and cloned only from the 500-m sample, and all 17 clones in this library belonged to the marine group I archaeal cluster (Fig. 3h). Members of this phylogenetic group are related to the symbiotic *Cenarchaeum symbiosum* and environmental clones from a wide variety of marine environments, freshwater sediments, rice paddies, terrestrial soils, winery by-products, and the gut of a sea cucumber (DeLong 1998). Limited diversity in the Crater Lake archaeal library was similar to the low variety of sequences generally seen in planktonic marine archaeal libraries (Massana et al. 2000). Crater Lake archaea thus all appeared to belong to the same cluster, previously identified in marine and other environments.

Candidate division ABY1—Two Crater Lake clones formed a novel subcluster within a newly identified candidate division (Fig. 4). The clones CL500-13 and CL500-94 grouped with sequences obtained from sediments in the Japan trench (BD7-1 and BD7-4) and a contaminated aquifer in Michigan, U.S.A. (WCHB1-58), to form a well-supported, deep branch within the bacteria. The aquifer sequence had previously been placed in candidate division OP11 on the basis of marginal bootstrap support. Addition of new sequences to the data set appears to have transferred it into a new, better supported deep branch. Phylogenetic analyses using a variety of methods confirmed the distinctiveness of this clade, which did not form well-supported alliances with other phyla or deeply branching clades of uncultivated bacteria. Following the example of Hugenholtz et al. (1998), we propose that the new clade be named a candidate division and dubbed ABY1, for abyssal division I bacteria. Crater Lake sequences in candidate division ABY1 formed the phylogenetic cluster CL500-13.

Frequencies of taxonomic groups in clone libraries from different depths—Representation of the different phylogenetic clusters and subclusters differed markedly among the three bacterial clone libraries (Web Appendix 2, http://www.aslo.org/lo/pdf/vol_46/issue_3/0557a2.pdf). Because high copy-number plastid rRNA genes can skew the frequency analysis of lower copy-number genes from prokaryotic bacterioplankton, frequencies of the nonplastid phylogenetic groups were calculated without plastid clones for comparison among libraries. In this analysis, the 0- and 500-m libraries were dominated by clones in the actinomycete division, with codominance of actinomycetes and verrucomicrobia in the 120-m library. Taxonomic groups in the 0-m library included actinomycetes (47.3%, mostly cluster ACK4), candidate division OP10 (13.5%, all CL0-1), chlorobiaceae (12.2%, various clusters within the cytophagales), verrucomicrobiales (9.5%, all CL120-10), β-proteobacteria (9.5%, both comamonadaceae and *Alcaligenes*), and α-proteobacteria (8.1%, various clusters). In the 120-m library were actinomycetes (39.6%, ~2 : 1 ratio for C111 : ACK4 clusters), verrucomicrobiales (39.6%, mostly CL120-10), β-proteobacteria (8.3%, both comamonadaceae and *Alcaligenes*), and other minor groups. The 500-m bacterial library featured actinomycetes (30.9%, ~4 : 1 ACK4 : C111), green nonsulfur bacteria (19.1%, mostly cluster CL500-11), planctomycetales (16.2%, mostly CL500-3 and CL500-15), α-proteobacteria (8.8%, various groups), β-proteobacteria (7.4%, all comamonadaceae), and minor groups. In the archaeal library, all clones were members of the group I crenarchaeota, similar to clones from marine samples. Variation in the frequencies of taxonomic groups among the clone libraries and the ability to amplify archaeal rRNA genes only from 500 m suggest a stratified distribution of bacterial and archaeal taxa in the summer water column of Crater Lake.

Oligonucleotide probe hybridization—Hybridization of phylogenetic group-specific oligonucleotide probes to RNA from a Crater Lake depth profile confirmed vertical stratification and relative dominance of bacterial and archaeal groups (Fig. 5). This type of analysis makes use of unamplified RNA and gives a more accurate picture of the relative importance of taxonomic groups than can be obtained from their frequencies in clone libraries. Hybridization of kingdom-specific probes showed a bipartite division of the water column, with the euphotic zone (0–160 m) exhibiting a nearly uniform 70 : 30 : 0 split among eukaryote, bacterial, and archaeal rRNA concentrations, and deep waters (300–500 m) showing a 50 : 35 : 15 distribution (Fig. 5a). Among preserved heterotrophic bacteria, euphotic zone waters were dominated by CL120-10 verrucomicrobia and ACK4 actinomycetes, each of which represented 21–55% of the nonplastid bacterial rRNA in the upper water column. The deep-water assemblage was dominated by green nonsulfur bacterium CL500-11 (44–49% at 300–500 m), with ACK4 and CL120-10 each representing ~15% of nonplastid bacterial rRNA (Fig. 5c). Relative hybridization of probes binding to rRNA of the three kingdoms and heterotrophic bacterial groups thus show a clear differentiation between microbial assemblages in the euphotic zone and in deeper waters.

Hybridization of plastid-specific probes was consistent with the distribution of phytoplankton groups as determined by microscopic quantification (Figs. 1, 5b). The stramenopile-specific probe indicated high numbers of bacillariophyte, chrysophyte, and/or xanthophyte plastids in surface waters (0–40 m), coincident with maximum biovolume concentrations of bacillariophyte and xanthophyte phytoplankton. The probe recognizing rRNA from other (nonstramenopile) plastids showed a peak at the DCM (120–160 m), where chlorophyte phytoplankton were dominant. Deep-water samples (300–500 m) showed low amounts of plastid sequences (9–14% of bacterial rRNA), consistent with low densities of phytoplankton plastids in detrital particles. Probes directed against rRNA from phytoplankton taxa thus were consistent with data for the distribution of living phytoplankton and detrital export to deeper waters.

Probes binding to the rRNA of β-proteobacteria and minor bacterial species showed various patterns in their distributions with depth (Fig. 5c,d). β-Proteobacterial rRNA was most abundant at the surface (16% at 0 m) and otherwise relatively constant (6–9% of nonplastid bacterial rRNA).

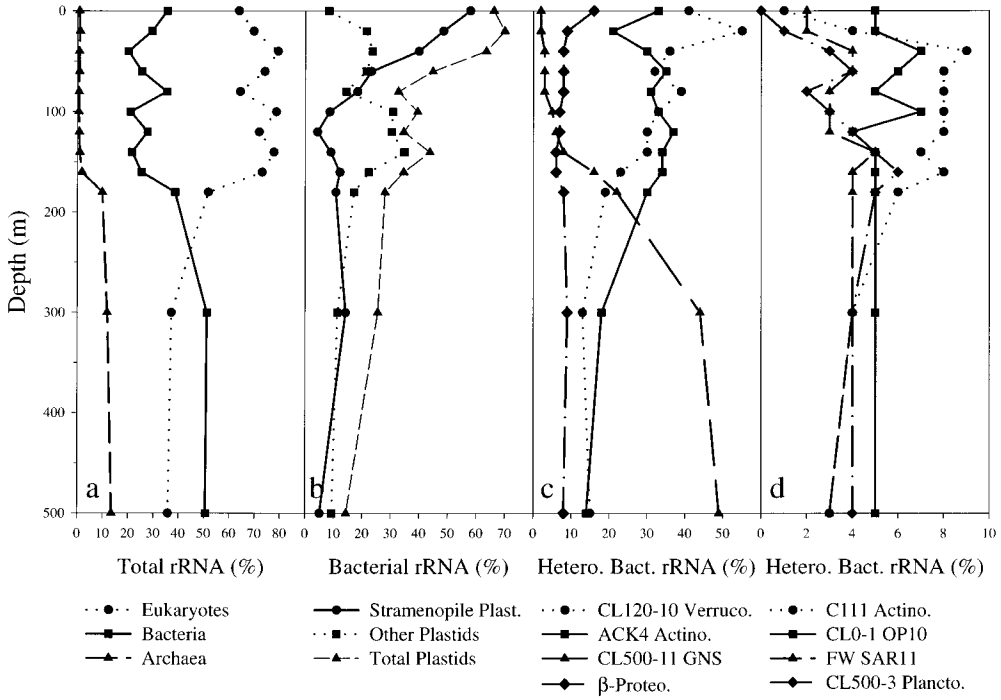


Fig. 5. Depth distribution of phylogenetic groups in Crater Lake as determined by relative hybridization of group-specific oligonucleotide probes: (a) three kingdoms of life relative to total rRNA, (b) plastid groups relative to total bacterial rRNA, (c) dominant bacterial taxa relative to heterotrophic bacterial rRNA (total bacterial minus plastid rRNA), and (d) minor bacterial taxa relative to heterotrophic bacterial rRNA. FW SAR11, freshwater subclade of SAR11.

rRNA from CL500-3 planctomycetes was absent at the surface but increased in relative concentration over the upper water column to form a peak of 6% at 160 m, followed by an apparent decline to 4% in deep waters. Actinomycete C111 accounted for only 1% of nonplastid bacterial rRNA at the surface, was relatively constant at 7–9% from 40 to 160 m, and decreased to 3% at 500 m. rRNA from the CL0-1 subcluster in candidate division OP10 varied little with depth, comprising 4–7% of nonplastid bacterial rRNA over the entire water column. Distribution of these bacterial taxa reflected physical and chemical characteristics of Crater Lake, the activity of bacterial predators, and physiological capabilities of these currently unculturable species. These data confirmed the inference from clone library analysis that β-proteobacteria were not dominant in Crater Lake on the sampling date and argue against any consistent biases in rRNA extraction from different samples.

Discussion

Differences between Crater Lake populations and other freshwater bacterioplankton—Clone libraries and oligonucleotide probe hybridizations have identified CL120-10 verrucomicrobiales and ACK4 actinomycetes as the dominant bacterioplankton taxa in Crater Lake surface waters and CL500-11 green nonsulfur bacteria and group I marine crenarchaeota in deep waters. These findings contrast with data from nine other studies in which bacterioplankton in 13 lakes were identified by using rRNA methods (Table 1). These investigations include clone library analyses similar to the present study (Bahr et al. 1996; Bel'kova et al. 1996; Hiorns et al. 1997; Methé et al. 1998; Semenova and Kuznedelov 1998), cloning and sequencing of major bands from denaturing gradient gel electrophoresis (Zwart et al. 1998) or terminal restriction fragment length polymorphism (RFLP) gels (Priscu et al. 1999), and in situ hybridization with group-specific oligonucleotide probes (Alfreider et al. 1996). In all but two of these lakes, β-proteobacteria were identified as the single dominant taxonomic group, with codominance of α- and β-proteobacteria reported in the remainder. β-Proteobacteria were dominant in euphotic zone samples from Lake Baikal, though deep waters (1,200 m) held predominantly γ-proteobacteria (Bel'kova et al. 1996). In contrast, Crater Lake libraries contained few clones in the β-proteobacteria (6.3% of all clones), and the 500-m library contained only a single clone in the γ-proteobacteria. These data are confirmed by hybridization of oligonucleotide probes to RNA from lake-water samples collected on the same day as the clone library samples (Fig. 5). Differences in prefiltration protocols or extraction methods cannot account for discrepancies between Crater Lake surface populations and other lake bacteria, because several studies followed the same procedures as were used in this study (Table 1). The unusual composition of Crater Lake bacterioplankton must be due to some atypical characteristic(s) of the Crater Lake environment.

Selective factors—Several morphometric, physical, and ecological characteristics may be ruled out as selective fac-

tors determining the composition of Crater Lake bacterioplankton because they are shared with lakes dominated by β-proteobacteria (Table 1). For example, the great depth of Crater Lake cannot be the sole determinant of bacterioplankton population structure because Lake Baikal (1,637 m) is dominated by β-proteobacteria. Similarly, Lake Vostoc (670 m, but 1,000 m according to more recent estimates; Priscu pers. comm.) may be dominated by β-proteobacteria, since at the base of its permanent ice cover members of this group dominate (Priscu et al. 1999). Lack of seasonal ice cover and relatively recent lake formation are ruled out as selective factors because these properties are shared with Lake Loosdrecht. Zooplankton populations in Crater Lake are also not unusual. Comparisons of physical and chemical properties of Crater Lake to lakes dominated by β-proteobacteria do, however, suggest several unusual characteristics that may account for its unique bacterioplankton.

Colimitation by N and trace metals is a property unique to Crater Lake among the tabulated lakes and is therefore a potential selective factor. Other possible factors include chemical properties of the silicic igneous rock substratum, which may be associated with the paucity of trace metals. The potential for trace metal limitation of phytoplankton growth in Crater Lake has been demonstrated by a small number of bioassay experiments (Groeger unpubl. data; Lane and Goldman 1984; Groeger and Teitjen 1993). However, identification of the limiting element and its effects on bacterioplankton growth require further investigation. The potential for trace metal limitation of phytoplankton and perhaps bacterioplankton in Crater Lake is a property shared with marine high nutrient–low chlorophyll regions, where productivity of some taxa is limited by the availability of iron (Cavender-Bares et al. 1999). Thus, iron limitation could be a selective factor structuring bacterioplankton populations in both marine and Crater Lake systems.

The influx of hydrothermal water is another potential selective factor affecting bacterioplankton in the lake. Although hydrothermal influx is a property shared with Lake Baikal, the relative influence of hydrothermal water on the chemistry of the two lakes is different. The hydrology of Lake Baikal is dominated by riverine influx, with the chemistry of hydrothermal springs having little impact on the budget of dissolved minerals (Faulkner et al. 1991). In contrast, hydrothermal input dominates the flux of most dissolved chemicals into Crater Lake, including Ca, Mg, K, Na, SO_4, Cl, B, HCO_3, and Si (Nelson et al. 1996). Warm, saline pools at the bottom of the lake are associated with lush microbial mats (Dymond et al. 1989); these mats and the chemistry of escaping hydrothermal waters may influence growth and food web interactions of pelagic microorganisms in Crater Lake.

Evidence for two other potential selective factors, low dissolved organic carbon (DOC) concentrations and high ultraviolet (UV) light exposure, is found in our data (Table 1). We chose to measure TOC, rather than filtered DOC, to reduce the possibility of sample contamination. However, it is likely that a significant fraction of Crater Lake TOC is dissolved, i.e., DOC. During August 1998, we measured TOC concentrations of 6–16 μM over the Crater Lake water column, with concurrent UV light penetration to 45 m for UVB (1% of surface irradiation for 304 nm) and 70 m for UVA (1% of surface irradiation for 340 nm) (Table 1). The Crater Lake TOC and UV penetration data are extremely low and high, respectively, compared to typical values for DOC and UV penetration in lakes (261–517 μM DOC, 0.28–0.61 m for 1% UVB and 0.67–1.47 m for 1% UVA; Williamson et al. 1996). Crater Lake UV penetration is comparable to UV penetration in antarctic lakes during austral summer, although Crater Lake TOC values are below DOC concentrations in these lakes (Vincent et al. 1998). Relatively low concentrations of DOC in Crater Lake would be consistent with the lake's low primary productivity and low influx of allocthonous carbon. However, these factors alone do not account for the extreme values observed. Because UV light destroys DOC, and DOC is the primary absorptive material attenuating UV light in oligotrophic lakes, it is likely that the low DOC and high UV light penetration are causatively linked (Morris and Hargreaves 1997). The interaction of UV light with DOC releases organic compounds that may be more or less degradable by microbial taxa. In addition, low concentrations of chelating compounds in DOC may contribute to potential trace metal limitation. Thus, selective effects of Crater Lake DOC on euphotic zone bacterioplankton may be to favor taxa able to metabolize low concentrations of DOC, including compounds released from DOC by UV light, as well as potential enhancement of trace metal limitation.

In addition to indirect effects on bacterioplankton growth by alteration of DOC, UV light may have a direct selective effect on microorganisms in the water column. Organisms near the surface of Crater Lake must be able to withstand unusually high doses of UV light for aquatic environments. UV light damages DNA by altering adjacent thymidine nucleotides into mutagenic cyclobutane ''dimers.'' Mechanisms of UV resistance in bacteria include enzymatic repair of DNA by photoreactivation and by RecA-dependent dark repair. In addition to robust repair systems, bacteria may also resist UV damage by minimizing the number of adjacent thymidine residues in their DNA, accomplished by increasing the ratio of G+C : A+T nucleotides. Near the surface of Crater Lake, dominant taxa ACK4 actinomycetes and CL120-10 verrucomicrobia are both members of bacterial divisions characterized by high ratios of G+C : A+T in their DNA. This suggests that the dominance of these groups may result, to some degree, from inherent resistance to damage by UV light.

Colimitation by N and trace metals, chemical properties of hydrothermal waters, low DOC concentrations in the presence of UV light, and direct selection for UV resistance are several factors that may determine the taxonomic structure of microbial communities in Crater Lake, either alone or in combinations. In accordance with some of these hypotheses, Methé and Zehr (1999) found positive correlations between the relative abundance of β-proteobacteria in their clone libraries and DOC, total iron, and chlorophyll *a* (Chl *a*) concentrations. Not only are bacterioplankton potentially affected by these factors, but the phytoplankton in Crater Lake may exhibit some unusual features as well. An unidentified chlorophyte dominated the photosynthetic biovolume in our depth profile, while at other times of the year, an unidentified

chrysophyte predominates (McIntire et al. 1996). These data imply two alternative explanations: (1) these phytoplankters may be common lake-water taxa that have yet to be described, or (2) Crater Lake is home to unusual phytoplankton, potentially due to the same selective factors as act upon bacterioplankton. Properties of unusual phytoplankton may, in turn, influence bacterioplankton population structure. Further research will be required to identify the most important selective factors and to demonstrate their effects on Crater Lake phytoplankton, bacterioplankton, and their food web.

New phylogenetic groups of uncultivated bacteria in Crater Lake—Analysis of cloned sequences from Crater Lake has identified seven new bacterial clades. These are represented in the database by nearly complete SSU rRNA sequences from Crater Lake clones and, in some cases, by additional short or misclassified sequences from other studies (Web Appendix 1; Fig. 3; clades represented by single sequences are not counted). These new phylogenetic clusters and subclusters include CL120-10 verrucomicrobiales, CL0-1 candidate division OP10 bacteria, CL500-11 and CL500-9 green nonsulfur bacteria, CL500-3 and CL500-15 planctomycetales, and CL500-13 candidate division ABY1 bacteria. Discovery of these new groups increases the number of uncultivated microbial taxa known from molecular studies of natural populations, each of which plays an unknown role in biogeochemical processes.

Habitats of candidate division ABY1 bacteria—The geographic distribution of collection sites for candidate division ABY1 clones suggests some properties of the physiology of these bacteria and perhaps their habitat and mode of dispersal. This deep-branching bacterial clade includes clones derived from both deep freshwaters and deep marine sediments (500 m Crater Lake water and 6,379 m Japan Trench sediments; Li et al. 1999). Thus, ABY1 organisms must be at least barotolerant, if not obligate barophiles. A single clone (WCHB1-58) was recovered from a shallow depth (6.2 m) in a phreatic aquifer (Dojka et al. 1998); this implies, alternatively, that (1) ABY1 bacteria may be barotolerant but not obligate barophiles, or (2) the WCHB1-58 sublineage of ABY1 is more tolerant of lower pressures than other ABY1 sublineages. With the exception of WCHB1-58, the distribution of ABY1 organisms at deep, hydrothermally active sites suggests that these bacteria may form part of a deep subsurface biosphere (Pace 1997). Hydrothermal waters may distribute these microorganisms to accessible, favorable habitats worldwide.

Biogeography of CL120-10 verrucomicrobiales and CL0-1 OP10 bacteria—CL120-10 verrucomicrobia and CL0-1 OP10 bacteria have not been found in other lakes studied by rRNA methods. However, these clades do occur at low frequency in a library made from Columbia River water collected near its ocean outlet (Crump et al. 1999). The Columbia forms part of the border between Oregon and Washington states, and Crater Lake does not contribute to its waters. However, the occurrence of these two bacterial groups in river water implies that Crater Lake-type bacterioplankton may occur at additional sites in the Columbia River watershed, a 58-million-ha area located mostly between the Cascade Mountains of Oregon and Washington and the Rocky Mountains of Montana and Wyoming. Studies mapping the occurrence of bacterioplankton dominated by CL120-10 verrucomicrobiales and ACK4 actinomycetes, or other unusual assemblages, could help identify factors promoting the growth of unusual bacterioplankton communities.

Stratification of microbial groups—Microbial taxa in Crater Lake are vertically stratified, as demonstrated by oligonucleotide probe hybridization to lake-water RNA and the frequencies of clusters in clone libraries. The euphotic zone waters of Crater Lake are dominated by CL120-10 verrucomicrobia and ACK4 actinomycetes, which suggests that they participate in recycling of carbon and nutrients coupled with phytoplankton growth and a food web based on primary productivity. The rRNA from C111 actinomycetes is maximal between 40 and 160 m, which suggests that these organisms may play similar roles but that they are sensitive to UV light, predation, or other limiting factors that are maximal at the surface. In deeper waters, group I marine crenarchaeota account for 19–27% of Crater Lake prokaryotic rRNA below 300 m (assuming all archaea belong to group I), and CL500-11 green nonsulfur bacteria account for 17–19% (44–49% of nonplastid bacterial rRNA; Fig. 5). Maximal populations of archaea, CL500-11 green nonsulfur bacteria, and CL500-3 planctomycetes occur below 140 m, suggesting that they function in the remineralization of detrital particles or processes associated with sediments or with hydrothermal waters. The vertical distribution of microbial groups in Crater Lake is consistent with a division of labor among taxa linked to different biogeochemical processes at different depths in the lake.

Similarities between Crater Lake and open-ocean bacterioplankton—There are striking parallels between microbial population structures in Crater Lake and in the oligotrophic, open ocean. These parallels include the relationships between oligotrophic freshwater and marine euphotic zone assemblages and cognate communities in more nutrient-rich environments, taxonomic similarities among deep-water assemblages, and the partition into euphotic zone and deep-water populations.

The open ocean and Crater Lake are both nutrient-depleted environments and can be compared along a trophic gradient to the coastal ocean and more productive lakes, respectively. In the marine euphotic zone, the major difference between coastal and open-ocean populations is the presence of β-proteobacteria in coastal areas. rRNA clone library studies of the coastal ocean have identified β-proteobacteria that belong to freshwater cluster C, the comamonadaceae, and other groups, and account for 10–16% of nonplastid clones (Rappé et al. 1997). The lesser diversity and abundance of β-proteobacteria in Crater Lake relative to other lakes parallels their absence in the open ocean and suggests that Crater Lake may be an oligotrophic, freshwater counterpart to open-ocean systems. However, β-proteobacteria in coastal habitats may derive from freshwater runoff, and β-proteobacteria predominate in other oligotrophic lakes (Table

1). Thus, although the parallels are intriguing, there is no simple correspondence between euphotic zone bacterioplankton populations in Crater Lake and the open ocean.

Marine aphotic zone bacterioplankton are taxonomically similar to deep-water populations in Crater Lake. In the open ocean, bacterioplankton at 200–250 m are dominated by three groups: SAR11 α-proteobacteria, SAR202 green nonsulfur bacteria, and marine group I crenarchaeota (Giovannoni and Rappé 2000; Giovannoni and Urbach unpubl. data; Massana et al. 2000). The latter two groups are related to Crater Lake deep-water taxa. Archaeal clones from Crater Lake are close relatives to clones from both coastal and open-ocean regions (Fuhrman et al. 1993; Massana et al. 2000; Fig. 3h). In coastal marine populations, they account for up to 30% of prokaryotic rRNA below 100 m (Massana et al. 1997; Murray et al. 1998), comparable to their representation in Crater Lake. Similarly, green nonsulfur bacteria in Crater Lake include members of the marine SAR202 cluster. In the open ocean, these bacteria form a peak immediately below the euphotic zone (Giovannoni et al. 1996; Giovannoni unpubl. data), while in Crater Lake, they were found only in the 500-m library, which implies that they, too, reside in the aphotic zone. Also in the green nonsulfur division, Crater Lake CL500-11 bacteria account for a large proportion of prokaryotic rRNA in deep waters. The phylogenetic relatedness of several Crater Lake microbial groups to bacteria identified in marine aphotic zone clone libraries, and the similarity of their vertical distributions, suggests that Crater Lake deep-water populations are similar to their counterparts in oligotrophic marine environments.

The recurrence of stratified community structures in marine and freshwater environments implies that the same principles of aquatic ecosystem function apply to both systems. As in Crater Lake, ocean taxa are divided into euphotic and aphotic zone assemblages. It is likely that phylogenetically related microbes that occur in both environments, like marine group I crenarchaeota and green nonsulfur bacteria, perform the same or similar biogeochemical roles. Additional functions common to the two systems are likely to be performed by unrelated microbes belonging the same functional groups, or ''guilds,'' but specifically adapted to marine or freshwater conditions. Comparative investigation in vertical and temporal structure of microbial communities in oligotrophic aquatic environments will shed further light on the roles of different microbial taxa.

Conclusion

Crater Lake is home to a unique bacterioplankton assemblage, a finding that suggests a number of hypotheses. A comparison of physical, chemical, and biological properties among lakes suggests that Crater Lake microbial populations, including bacterioplankton and phytoplankton, may be structured by potential selective factors, including low concentrations of DOC, N, and trace metals; chemical properties of hydrothermal waters; and effects of high UV light penetration. The stratified distribution of Crater Lake taxonomic groups suggests different biogeochemical functions for the different evolutionary clusters, with surface groups like CL120-10 verrucomicrobiales and ACK4 actinomycetes processing carbon and nutrients from photosynthetic activity or compounds produced by from DOC by UV light, and deep-water groups, such as CL500-11 green nonsulfur bacteria and group I marine crenarchaeota, processing detritus, transforming chemicals in hydrothermal fluids, or participating in a food web based on these materials. The co-occurrence of phylogenetic groups in both Crater Lake and oligotrophic marine systems, and similarities in their vertical distributions, suggests further that this division of labor pertains to both ecological systems. The occurrence of Crater Lake CL120-10 verrucomicrobia and CL0-1 OP10 bacteria in Columbia River water suggests that other waterbodies in its watershed harbor bacterioplankton similar to Crater Lake's. Measurements of UV flux, nutrient limitation, DOC concentration, and hydrothermal influx at such sites may narrow the number of potential selective factors structuring these unusual populations. Finally, the occurrence of ABY1, a candidate division of barotolerant bacteria in sediments of a marine, plate tectonic trench and in deep waters of a hydrothermal caldera lake of recent vintage, suggests that these bacteria may be disseminated from a deep subsurface biosphere. Future work in Crater Lake and identification of additional sites with similar planktonic taxa will increase understanding of many bacterial processes in aquatic systems.

References

ALFREIDER, A., J. PERNTHALER, R. AMANN, B. SATTLER, F-O. GLÖCKNER, A. WILLE, AND R. PSENNER. 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.

AMERICAN PUBLIC HEALTH ASSOCIATION. 1985. Standard methods for examination of water and wastewater, 16th ed. APHA.

BAHR, M., J. E. HOBBIE, AND M. L. SOGIN. 1996. Bacterial diversity in an arctic lake: A freshwater SAR11 cluster. Aquat. Microb. Ecol. **11:** 271–277.

BEL'KOVA, N. L., L. Y. DENISOVA, E. N. MANAKOVA, E. F. ZAICHIKOV, AND M. A. GRACHEV. 1996. Species diversity of deep-water microorganisms of Lake Baikal: Analysis of 16S rRNA sequences. Dokl. Biol. Sci. **348:** 692–695.

CAVENDER-BARES, K. K., E. L. MANN, S. W. CHISHOLM, M. E. ONDRUSEK, AND R. B. BIDIGARE. 1999. Differential response of equatorial Pacific phytoplankton to iron fertilization. Limnol. Oceanogr. **44:** 237–246.

COLLIER, R., J. DYMOND, AND J. MCMANUS. 1991. Studies of hydrothermal processes in Crater Lake, OR. College of Oceanography Report #90 7. Oregon State University.

CRUMP, B. C., E. V. ARMBRUST, AND J. A. BAROSS. 1999. Phylogenetic analysis of particle-attached and free-living bacterial communities in the Columbia River, its estuary, and the adjacent coastal ocean. Appl. Environ. Microbiol. **65:** 3192–3204.

DELONG, E. F. 1992. Archaea in coastal marine environments. Proc. Natl. Acad. Sci. USA **89:** 5685–5689.

———. 1998. Archaeal means and extremes. Science **280:** 542–543.

DOJKA, M. A., P. HUGENHOLTZ, S. K. HAAK, AND N. R. PACE. 1998. Microbial diversity in a hydrocarbon- and chlorinated-solvent–contaminated aquifer undergoing intrinsic bioremediation. Appl. Environ. Microbiol. **64:** 3869–3877.

DYMOND, J., R. W. COLLIER, AND M. E. WATWOOD. 1989. Bacterial

mats from Crater Lake, Oregon and their relationship to possible deep-lake hydrothermal venting. Nature **342:** 673–675.

FAULKNER, K. K., C. I. MEASURES, S. E. HERBELIN, J. M. EDMOND, AND R. F. WEISS. 1991. The major and minor element geochemistry of Lake Baikal. Limnol. Oceanogr. **36:** 413–423.

FELSENSTEIN, J. 1993. PHYLIP (Phylogeny Inference Package). Version 3.5c. Univ. of Washington-Seattle.

FIELD, K. G., D. GORDON, M. RAPPÉ, E. URBACH, K. VERGIN, AND S. J. GIOVANNONI. 1997. Diversity and depth-specific distribution of SAR11 cluster rRNA genes from marine planktonic bacteria. Appl. Environ. Microbiol. **63:** 63–76.

FUHRMAN, J. A., K. MCCALLUM, AND A. A. DAVIS. 1993. Phylogenetic diversity of subsurface marine microbial communities from the Atlantic and Pacific Oceans. Appl. Environ. Microbiol. **59:** 1294–1302.

GIOVANNONI, S. J., T. B. BRITSCHGI, C. L. MOYER, AND K. G. FIELD. 1990*a*. Genetic diversity in Sargasso Sea bacterioplankton. Nature **345:** 60–62.

———, E. F. DELONG, T. M. SCHMIDT, AND N. R. PACE. 1990*b*. Tangential flow filtration and preliminary phylogenetic analysis of marine picoplankton. Appl. Environ. Microbiol. **56:** 2572–2575.

———, AND M. RAPPÉ. 2000. Evolution, diversity, and molecular ecology of marine prokaryotes, p. 47–84. *In* D. L. Kirchman [ed.], Microbial ecology of the oceans. Wiley.

———, M. S. RAPPÉ, K. L. VERGIN, AND N. L. ADAIR. 1996. 16S rRNA genes reveal stratified open ocean bacterioplankton populations related to the Green Non-Sulfur bacteria. Proc. Natl. Acad. Sci. USA **93:** 7979–7984.

GOLDMAN, C. R. 1963. The measurement of primary productivity and limiting factors in freshwater with carbon-14, p. 103–113. *In* M. S. Doty [ed.], Primary productivity measurement, marine and freshwater. U.S. Atomic Energy Commission TID-7633.

GORDON, D. A., AND S. J. GIOVANNONI. 1996. Detection of stratified microbial populations related to *Chlorobium* and *Fibrobacter* species in the Atlantic and Pacific Oceans. Appl. Environ. Microbiol. **62:** 1171–1177.

GROEGER, A. W., AND T. C. TEITJEN. 1993. Physiological responses of nutrient-limited phytoplankton to nutrient addition. Verh. Internat. Verein. Limnol. **25:** 370–372.

HIORNS, W. D., B. A. METHÉ, S. A. NIERZWICKI-BAUER, AND J. P. ZEHR. 1997. Bacterial diversity in Adirondack mountain lakes as revealed by 16S rRNA gene sequences. Appl. Environ. Microbiol. **63:** 2957–2960.

HÖFLE, M., H. HAAS, AND K. DOMINIK. 1999. Seasonal dynamics of bacterioplankton community structure in a eutrophic lake as determined by 5S rRNA analysis. Appl. Environ. Microbiol. **65:** 3164–3174.

HUGENHOLTZ, P., C. PITULLE, K. L. HERSHBERGER, AND N. R. PACE. 1998. Novel division level bacterial diversity in a Yellowstone hot spring. J. Bacteriol. **180:** 366–376.

KRUMHOLZ, L., AND P. A. STAHL. 1990. Fluorescent-oligonucleotide probing of whole cells for determinative, phylogenetic, and environmental studies in microbiology. J. Bacteriol. **172:** 762–770.

KUSKE, C. R., S. M. BARNS, AND J. D. BUSCH. 1997. Diverse uncultivated bacterial groups from soils of the arid southwestern United States that are present in many geographic regions. Appl. Environ. Microbiol. **63:** 3614–3621.

LANE, D. J. 1991. 16S/23/S rRNA sequencing, p. 115–175. *In* E. Stackebrandt and M. Goodfellow [eds.], Nucleic acid techniques in bacterial systematics. Wiley.

LANE, J. L., AND C. R. GOLDMAN. 1984. Size-fractionation of natural phytoplankton communities in nutrient bioassay studies. Hydrobiologia **118:** 219–223.

LARSON, G. L., C. D. MCINTIRE, M. HURLEY, AND M. W. BUKTENICA. 1996*a*. Temperature, water chemistry, and optical properties of Crater Lake. Lake Reservoir Manag. **12:** 230–247.

———, ———, R. TRUITT, M. BUKTENICA, AND E. K. THOMAS. 1993. Zooplankton assemblages in Crater Lake. U.S. Department of the Interior. Report NPS/PNROSU/NRTR-93/03.

———, ———, R. E. TRUITT, M. W. BUKTENICA, AND E. DARNAUGH-THOMAS. 1996*b*. Zooplankton assemblages in Crater Lake, Oregon, USA. Lake Reservoir Manag. **12:** 281–297.

LI, L., C. KATO, AND K. HORIKOSHI. 1999. Bacterial diversity in deep-sea sediments from different depths. Biodivers. Conserv. **8:** 659–677.

MAIDAK, B. L., AND OTHERS. 1999. A new version of the RDP (Ribosomal Database Project). Nucleic Acids Res. **27:** 171–173.

MANZ, W., R. AMANN, AND W. LUDWIG. 1992. Phylogenetic oligodeoxynucleotide probes for the major subclasses of Proteobacteria: Problems and solutions. Syst. Appl. Microbiol. **15:** 593–600.

MASSANA, R., E. F. DELONG, AND C. PEDRÓS-ALIÓ. 2000. A few cosmopolitan phylotypes dominate planktonic archaeal assemblages in widely different oceanic provinces. Appl. Environ. Microbiol. **66:** 1777–1787.

———, A. E. MURRAY, C. M. PRESTON, AND E. F. DELONG. 1997. Vertical distribution and phylogenetic characterization of marine planktonic *Archaea* in the Santa Barbara Channel. Appl. Environ. Microbiol. **63:** 50–56.

MCINTIRE, C. D., G. L. LARSON, R. E. TRUITT, AND M. K. DEBACON. 1996. Taxonomic structure and productivity of phytoplankton assemblages in Crater Lake, Oregon. Lake Reservoir Manag. **12:** 259–280.

MCMANUS, J., R. COLLIER, J. DYMOND, C. G. WHEAT, AND G. L. LARSON. 1996. Spatial and temporal distribution of dissolved oxygen in Crater Lake, Oregon. Limnol. Oceanogr. **41:** 722–731.

———, R. W. COLLIER, AND J. DYMOND. 1993. Mixing processes in Crater Lake, Oregon. J. Geophys. Res. **98:** 295–307.

METHÉ, B. A., W. D. HIORNS, AND J. P. ZEHR. 1998. Contrasts between marine and freshwater bacterial community composition: Analyses of communities in Lake George and six other Adirondack lakes. Limnol. Oceanogr. **43:** 368–374.

———, AND J. P. ZEHR. 1999. Diversity of bacterial communities in Adirondack lakes: Do species assemblages reflect lake water chemistry? Hydrobiologia **401:** 77–96.

MORRIS, D. P., AND B. R. HARGREAVES. 1997. The role of photochemical degradation of dissolved organic carbon in regulating the UV transparency of three lakes on the Pocono Plateau. Limnol. Oceanogr. **42:** 239–249.

MURRAY, A. E., C. M. PRESTON, R. MASSANA, L. T. TAYLOR, A. BLAKIS, K. WU, AND E. F. DELONG. 1998. Seasonal and spatial variability of bacterial and archaeal assemblages in the coastal waters near Anvers Island, Antarctica. Appl. Environ. Microbiol. **64:** 2585–2595.

NELSON, P. O., J. F. REILLY, AND G. L. LARSON. 1996. Chemical solute mass balance of Crater Lake, Oregon. Lake Reservoir Manag. **12:** 248–258.

PACE, N. R. 1997. A molecular view of microbial diversity and the biosphere. Science **276:** 734–740.

POLZ, M. F., AND C. M. CAVANAUGH. 1997. A simple method for quantification of uncultured microorganisms in the environment based on in vitro transcription of 16S rRNA. Appl. Environ. Microbiol. **63:** 1028–1033.

PORTER, K. G., AND Y. S. FEIG. 1980. The use of DAPI for identifying and counting aquatic microflora. Limnol. Oceanogr. **25:** 943–948.

PRISCU, J. C., AND OTHERS. 1999. Geomicrobiology of subglacial ice above Lake Vostok, Antarctica. Science **286:** 2141–2144.

RAPPÉ, M. S., P. F. KEMP, AND S. J. GIOVANNONI. 1997. Phylogenetic diversity of marine coastal picoplankton 16S rRNA genes cloned from the continental shelf off Cape Hatteras, North Carolina. Limnol. Oceanogr. **42:** 811–826.

SEMENOVA, E. A., AND K. D. KUZNEDELOV. 1998. A study of the biodiversity of Baikal picoplankton by comparative analysis of 16S rRNA gene 5′-terminal regions. Mol. Biol. **32:** 754–760.

SMITH, D. S., AND F. AZAM. 1992. A simple, economical method for measuring bacterial protein synthesis rates in seawater using ^{3}H-leucine. Mar. Microb. Food Webs **6:** 107–114.

STACKEBRANDT, E. In press. Defining taxonomic ranks. *In* M. Dworkin [ed.], The prokaryotes: An evolving electronic resource for the microbiological community. http://www.prokaryotes.com [6 July 2000]. Springer.

STRUNK, O., AND OTHERS. 1996. ARB—a software environment for sequence data. Version 2.5b. Technical Univ. of Munich.

SWOFFORD, D. L. 1996. PAUP*: Phylogenetic analysis using parsimony (and other methods). Version 4.0. Sinauer.

VINCENT, W. F., R. RAE, I. LAURION, C. HOWARD-WILLIAMS, AND J. C. PRISCU. 1998. Transparency of Antarctic ice-covered lakes to solar UV radiation. Limnol. Oceanogr. **43:** 618–624.

WILLIAMSON, C. E., R. S. STEMBERGER, D. P. MORRIS, T. M. FROST, AND S. G. PAULSEN. 1996. Ultraviolet radiation in North American lakes: Attenuation estimates from DOC measurements and implications for plankton communities. Limnol. Oceanogr. **41:** 1024–1034.

ZWART, G., AND OTHERS. 1998. Nearly identical 16S rRNA sequences recovered from lakes in North America and Europe indicate the existence of clades of globally distributed freshwater bacteria. Syst. Appl. Microbiol. **21:** 546–556.

Received: 26 July 2000
Accepted: 12 December 2000
Amended: 10 January 2001