Microb Ecol (1993) 26:9–20

MICROBIAL ECOLOGY

The Influence of Environmental Factors on Seasonal Changes in Bacterial Cell Volume in Two Prairie Saline Lakes

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Received: January 19, 1993; Revised: April 5, 1993

Abstract. Bacterial biovolumes of hypertrophic Humboldt Lake (total dissolved solids = 3.3 g liter^{-1}; 6 m deep) and oligotrophic Redberry Lake (total dissolved solids = 20.9 g liter^{-1}; 17 m deep), Saskatchewan, were measured concurrently with a variety of environmental variables to identify the major factors correlated with volume changes. There was no difference ($P > 0.05$) in mean bacterial volume between Redberry Lake (0.084 ± 0.034 μm^3 SD) and Humboldt Lake (0.083 ± 0.021 μm^3 SD). Statistical analyses suggested there were marked differences in the factors associated with the pronounced seasonality of bacterial cell volumes in these two lakes. Variance in bacterial volume in the epilimnion of Redberry Lake was best explained by a multivariate regression model which included ciliate abundance and chlorophyll concentration ($r^2 = 0.96$). The model accounting for changes in hypolimnetic bacterial volume included ciliate numbers and primary production ($r^2 = 0.94$), of the measured variables. Bacterial volume in Humboldt Lake was most highly correlated with primary production ($r^2 = 0.59$). Bacterial production (estimated as the rate of thymidine incorporation into DNA) and growth (thymidine incorporation rate normalized to cell numbers) were not correlated to cell volume, with the exception of cocci volume in Humboldt Lake.

Introduction

Spatial and temporal changes in bacterial cell volume have been observed in a variety of aquatic habitats. In order to model energy and nutrient flow, it is essential to know the factors which are related to changes in bacterial cell volume, because conversion of bacterial numbers to carbon, nitrogen, or phosphorus units is dependent on the derivation of accurate "numbers to volume" conversion factors. A

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major challenge for microbial ecologists is to identify factors controlling cell volume and hence bacterial biomass [14, 26].

Factors known to affect the volume and biomass of heterotrophic bacteria include carbon supply from algae, phosphorus concentration, predation, and water temperature [6, 13, 26]. Although strong positive relationships exist between bacterial abundance and chlorophyll concentration for a variety of aquatic systems [2], changes in bacterial volume over trophic gradients remain controversial. For example, Bird and Kalff's data [2] tended to support an inverse relationship between bacterial density and volume, whereas earlier studies demonstrated the opposite pattern [15].

Although the volume of saline lakes in the world is approximately equal to that of freshwaters [10], studies on the ecology of heterotrophic bacteria in saline lakes are limited. Saline lakes in western Canada are characterized by Mg^{2+} and SO_4^{2-} concentrations which are higher than in saline lakes elsewhere in the world [5]. This fact may account for low primary productivity in saline lakes of western Canada and high productivity in saline lakes elsewhere [5, 20]. To our knowledge there are no published studies on the bacterial ecology of western Canadian saline lakes.

In order to identify key factors influencing bacterial cell volume in prairie saline lakes, we related cell volume to biological, chemical, and physical data from two lakes with marked differences in these characteristics. We were particularly interested in the following questions: (1) is bacterial cell volume different in oligotrophic than in eutrophic lakes; (2) is water temperature an important determinant of bacterial cell volume; and (3) is the supply of labile dissolved organic carbon (as inferred from chlorophyll and primary production measurements) of equal significance in influencing bacterial cell volume in a higher salinity, oligotrophic saline lake compared to a lower salinity, hypertrophic saline lake?

Methods

Study Sites

Redberry Lake (52°43′N, 107°09′W) is saline [total dissolved solids (TDS), 20.9 g liter^{-1}], 17 m deep, and oligotrophic (maximum euphotic zone Chl, 5.5 mg m^{-3}). Sulfate is the dominant anion (93.1%) with a concentration of 130,208 μM [20]. Light usually penetrates to the bottom due to a mean euphotic zone (Z_{eu}) chlorophyll concentration of 1.7 mg m^{-3} [20]. Chlorophyll concentration is frequently greatest at the bottom. The lake develops a pronounced thermocline from May to September–October. Surface water temperatures range from −1.0°C (January) to 21.8°C (July). At 16 m the water temperature rarely exceeds 4°C, except during fall overturn when a temperature as high as 7.6°C can occur [20]. Mean euphotic zone primary production during 1989–1990 was 57.1 mgC m^{-2} h^{-1} [20].

Humboldt Lake (52°09′N, 105°06′W) is hypertrophic (maximum euphotic zone Chl, 839 mg m^{-3}), 6 m deep, and slightly saline (TDS, 3.3 g liter^{-1}). The water column remains aerobic throughout the year. Sulfate is the dominant anion (87.3%) with a concentration of 27,274 μM [20]. Humboldt Lake's mean Z_{eu} is 3.4 m, with a mean chlorophyll concentration of 62.6 mg m^{-3} and a mean euphotic zone primary productivity of 230.2 mgC m^{-2} h^{-1} during 1989–1990 [20]. Wind activity keeps the lake mixed.

Sampling

Water samples were collected from 2 m and 16 m in Redberry Lake and 0.5 m in Humboldt Lake using an opaque 8-liter Niskin sampler. Samples were collected on a monthly basis from Redberry Lake

(n = 8 for each depth) and from Humboldt Lake (n = 13), monthly in May and June, biweekly from July to October 1989, and monthly from January to March 1990 (March data for Redberry Lake was lost). No samples were collected in November and December from either lake because of unstable ice conditions.

Bacterial Numbers, Size, and Volume

Water samples (10 ml) for the determination of bacterial numbers and size were placed in sterile glass tubes and preserved with Lugol's iodine solution. Bacteria were stained with DAPI and counted with an epifluorescence microscope [16]. Bacteria were also stained with DAPI and collected on black polycarbonate membrane filters (pore-size, 0.2 μm) for size determinations. Photographs were taken to include ≥200 cells per sample. The size distribution of bacteria was obtained using an image processing program (Ultimage, version 1.3.4, GTFS Incorporated, Santa Rosa, CA, U.S.A.). The accuracy of the system was tested using fluorescent latex beads of 0.28, 0.51, 0.97, 1.16, 1.29, and 1.98 μm diameters (Polyscience Inc., Warrington, PA, U.S.A.). Bacterial cell volume was calculated as volume = $8.5 \times AR^{2.5} \times CP^{-2}$, where AR equals the area and CP the convex perimeter [3]. This formula was chosen because it is valid for spheres, rods, spirals, and comma shaped cells with an error of <10% for cells with length/width ratios of up to 5. Bacterial biomass was converted to carbon units using a power function calculated from Simon and Azam's [24] data for cell volume and carbon content.

Bacterial Production

Bacterial production was estimated as the rate of [*methyl*-^{3}H]thymidine (TdR) incorporation into bacterial DNA. The rate of TdR incorporation normalized to cell number (10^{-21} mol cell^{-1} h^{-1}) was used as an index of bacterial growth rate. Water samples (10 ml) were transferred to sterile screw-top glass tubes. For each sample there were one control and two live tubes. Controls were prepared by the addition of 0.5 ml 5 M NaOH [17].

Working solutions of TdR (80–85 Ci nmol^{-1}; The Radiochemical Centre, Amersham, U.K.) were prepared daily with sterile distilled water. TdR was added to each tube to give a final concentration of 18 nM. Tubes were attached to an incubation harness and returned to the sampling depth. Incubation times were 15 min in summer, 30 min to 1 h in spring and fall, and up to 3 h in winter. Preliminary experiments indicated that the TdR concentration used was high enough to saturate, but not inhibit, uptake of TdR, and should have prevented extracellular isotope dilution, but possibly not intracellular dilution [19]. The rate of TdR incorporation was linear over the incubation periods [19].

Thymidine incorporation was stopped and the samples preserved by the addition of 0.5 ml 5M NaOH. The tubes were then stored on crushed ice until labeled macromolecules were collected on membrane filters in the laboratory, usually within 3 h, but always within 24 h, of NaOH addition to the samples. The filters were treated with phenol/chloroform and ethanol so that only labeled DNA was radioassayed in a liquid scintillation counter [17].

Statistical Analyses

Bacterial cell volume data were statistically analysed as: rods (rods, spiral- and comma-shaped cells), cocci (spheres and spheroids), and total volume (rods + cocci). Data from 2 and 16 m depths in Redberry Lake were analyzed separately. A parallel study [20] generated data on: water temperature; particulate and dissolved carbon, nitrogen, and phosphorus; ciliate numbers; and rates of primary production. Data on the abundance and species of heterotrophic nanoflagellates, which are known to be major consumers of bacteria in aquatic ecosystems (e.g., [9, 22]), are not available for Humboldt and Redberry Lakes. Spearman rank correlation, ANOVA, and step-wise multiple regression tests were performed (Statgraphics; Manugistics, Inc., Rockville, Maryland, U.S.A.) to correlate changes in bacterial cell volume with these data.

Results

Near-surface water temperatures of Redberry and Humboldt lakes were similar, but the hypolimnion of Redberry Lake was markedly colder (Table 1). Nitrogen and phosphorus concentrations were significantly higher in Humboldt Lake than in Redberry Lake, while the dissolved organic carbon (DOC) concentration was greater in Redberry Lake (Table 1).

Algal standing crop (chlorophyll *a*), primary production, bacterial numbers, bacterial production, and ciliate numbers were all significantly greater in Humboldt Lake than in Redberry Lake (Figs. 1–3). Ciliate numbers at 16 m were greater than at 2 m in Redberry Lake. The ciliate populations in Redberry Lake were dominated by species of *Strombidium, Strobilidium, Balanion, Urotricha, Holteria,* and *Cyclidium,* while in Humboldt Lake the dominant genera were *Strobilidium, Urotricha, Cyclidium,* and *Epistylis*.

Bacterial Cell Volume and Size

In the epilimnion (2 m) of Redberry Lake, mean cell volume peaked (0.109 μm^3) in June and again in September (0.141 μm^3) with the phytoplankton fall bloom (Fig. 1). The changes in mean bacterial cell volume tracked similar changes in the number of ciliates. Coccoid cells in the epilimnion varied in size from monthly mean diameters of 0.43 μm in winter to 0.56 μm in spring, while rods had a mean width of 0.35 μm (range 0.16–0.57 μm) and a mean length of 1.37 μm (range 0.97–2.02 μm). In the hypolimnion, mean cell volume was greatest (0.173 μm^3) in May following the spring algal bloom and again in September (0.103 μm^3) with the fall bloom (Fig. 2). Coccoid cell size had monthly mean diameters varying from 0.37 μm in winter to 0.70 μm in spring, while rod cells had a monthly mean width of 0.30 μm (range 0.16–0.49 μm) and a mean length of 1.47 μm (range 0.88–2.18 μm).

The mean cell volume of bacteria in Humboldt Lake was greatest (0.137 μm^3) in August during the summer bloom of *Aphanizomenon flos-aquae* (Fig. 3; [20]). A second peak in mean cell volume (0.104 μm^3) occurred in October with the fall diatom bloom. The mean coccoid cell diameter varied from 0.39 μm to 0.55 μm ($\bar{x}$ = 0.43 μm), while the mean width of rods varied from 0.75 to 0.94 μm ($\bar{x}$ = 0.87 μm) with lengths of 1.11 μm to 2.86 μm ($\bar{x}$ = 1.70 μm).

There was no difference (ANOVA, $P > 0.05$) in mean cell volume between the epilimnion and hypolimnion of Redberry Lake, and no difference in mean cell volume between the populations of Redberry and Humboldt lakes.

Correlation of Bacterial Cell Volume and Environmental Data

In the epilimnion of Redberry Lake, changes in cocci cell volume were inversely correlated with changes in DOC, phosphorus, and nitrogen concentrations (Table 2). Bacterial and primary production were not correlated with bacterial cell volume ($P > 0.08$ and $P < 0.40$, respectively). The number of ciliates were positively correlated with rod, but not cocci, cell volumes. In the hypolimnion no correlations

Table 1. Some physical and chemical characteristics of Redberry and Humboldt lakes from May 1989 to March 1990 [20]. Additional details can be found in [20]. DOC = dissolved organic carbon, TP = total phosphorus, TDP = total dissolved phosphorus, and SRP = soluble reactive phosphorus. Data given are the mean and range; the mean for $NO_3 + NO_2$-N in Redberry Lake is not given since it was usually <2 μg liter^{-1}, the detection limit of the method. Number of measurements was 8 for each depth in Redberry Lake and 13 for Humboldt Lake

Lake	Depth	Temperature (°C)	DOC (mg liter^{-1})	TP μg liter^{-1}	TDP μg liter^{-1}	SRP μg liter^{-1}	$NO_3 + NO_2$-N μg liter^{-1}	NH_3-N μg liter^{-1}
Redberry	2m	11.3	35.3	44	39	21	–	26
		−1.0 to 21.8	32.3–38.3	27–57	27–52	2–30	<2–12	14–50
	16m	1.7	37.4	58	46	27	–	26
		−0.7 to 4.0	35.5–38.6	45–101	35–56	19–41	<2–15	14–46
Humboldt	0.5m	12.2	23.4	316	265	213	46	192
		−0.5 to 22.8	19.9–27.9	220–435	220–435	130–314	<2–136	45–518

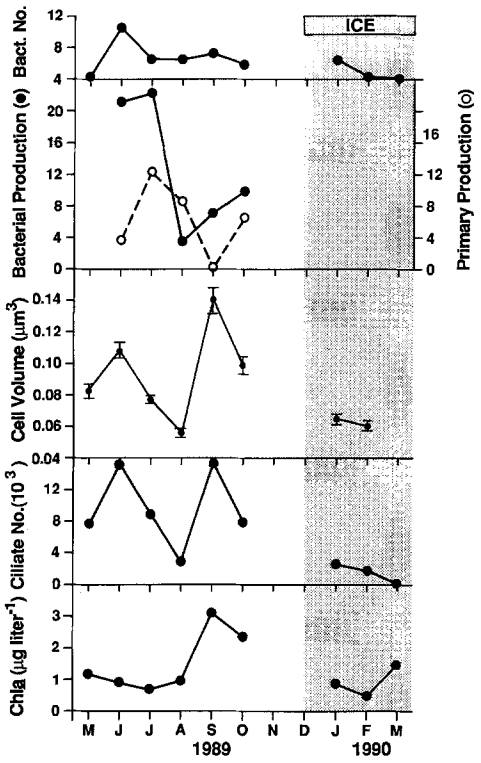

Fig. 1. Seasonal changes in bacterial numbers (cells $\times 10^6$ ml^{-1}), bacterial production (pmol TdR liter^{-1} h^{-1}), primary production (mgC m^{-3} h^{-1}), total bacterial cell volume (vertical bars are ± 1 SE of the mean when greater than the symbol), ciliate numbers (cells liter^{-1}), and chlorophyll *a* concentration in the epilimnion (2 m) of Redberry Lake. Primary production and chlorophyll data are from [20].

were found between bacterial cell volume and environmental parameters. In Humboldt Lake, although nitrogen concentration was correlated with cocci cell volume, the strongest correlations for bacterial volumes were with biological parameters and water temperature (Table 3).

Ciliate number was the dominant factor in the multivariate models of total cell volume changes in Redberry Lake (Table 4). Multiple regression analysis did not produce a multivariate model for bacterial volume changes in Humboldt Lake, but did isolate primary production as the dominant correlate.

Bacterial Biomass

Bacterial biomass at 2 m in Redberry Lake varied from 50.9 μgC liter^{-1} under the ice in February to 224.6 μgC liter^{-1} in June (Fig. 4). In the hypolimnion, bacterial biomass was similar to that of the epilimnion but only reached a peak of 161.5 μgC liter^{-1} in June. Bacterial biomass was significantly higher for most of the year in Humboldt Lake, especially during and after the August peak in cyanobacteria (Figs. 3 and 4).

Epilimnetic bacterial biomass of Redberry Lake was positively correlated with ciliate numbers ($r = 0.95$, $P < 0.01$) and inversely with NH_3-N concentration ($r = -0.84$, $P < 0.03$). In the hypolimnion, biomass was correlated only with DOC concentration ($r = -0.95$, $P < 0.01$). In Humboldt Lake, bacterial biomass

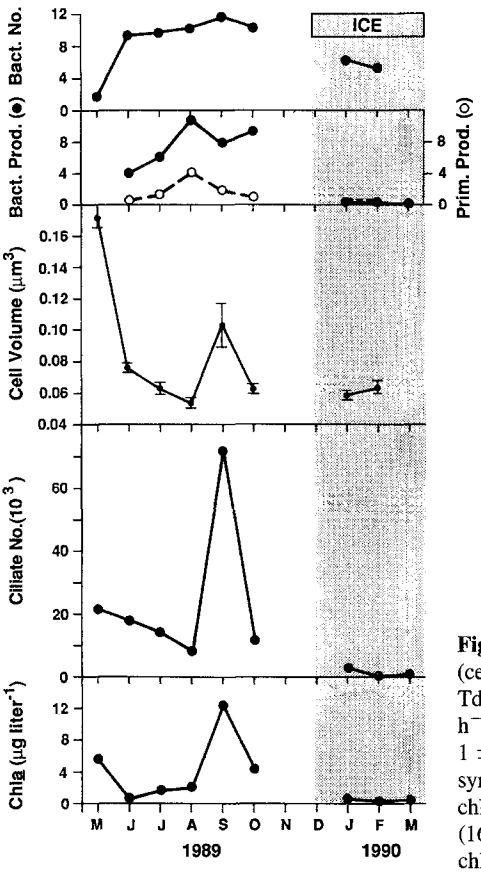

Fig. 2. Seasonal changes in bacterial numbers (cells $\times$ 10^6 ml^{-1}), bacterial production (pmol TdR $liter^{-1}$ h^{-1}), primary production (mgC m^{-3} h^{-1}), total bacterial cell volume (vertical bars are 1 $\pm$ SE of the mean when greater than the symbol), ciliate numbers (cells $liter^{-1}$), and chlorophyll *a* concentration in the hypolimnion (16 m) of Redberry Lake. Primary production and chlorophyll data are from [20].

was correlated with ciliate numbers ($r = 0.76$, $P < 0.008$) and particulate organic carbon ($r = 0.65$, $P = 0.05$).

Discussion

Mean seasonal bacterial volumes (0.082–0.086 μm^3) from Redberry and Humboldt lakes (Figs. 1–3) fall within the range of values from the few studies already published on the seasonal changes in planktonic bacterial cell volumes [23]. Previously reported mean bacterial cell volumes range from 0.013 μm^3 [18] to 0.170 μm^3 [6].

Comparison of the biomass data from Redberry and Humboldt lakes with other studies using a constant conversion factor must be done cautiously. Our conversion factors varied with volume but averaged 2.5×10^{-13} gC μm^{-3}. Bacterial biomass of Lake Arlington, Texas ranged from 73–200 μgC $liter^{-1}$ based on a conversion factor of 0.94×10^{-13} gC μm^{-3} [6]. Allowing for the difference in conversion factors, the bacterial biomass of Redberry Lake (Fig. 4) was generally lower than that of Lake Arlington. Bacterial biomass of Humboldt Lake (Fig. 4) also over-

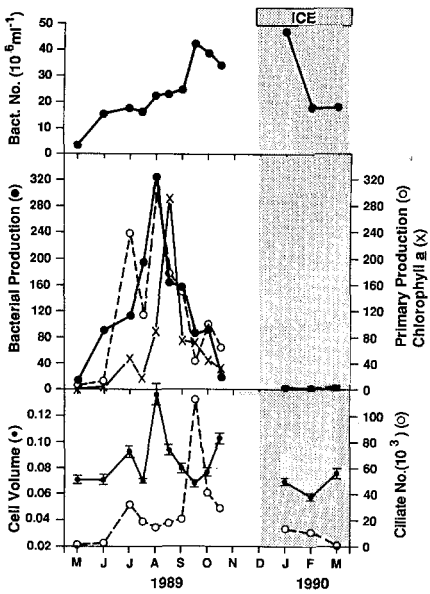

Fig. 3. Seasonal changes in bacterial numbers, bacterial production (pmol TdR liter^{-1} h^{-1}), primary production (mgC m^{-3} h^{-1}), total bacterial cell volume (vertical bars are ± 1 SE of the mean when greater than the symbol), ciliate numbers (cells liter $^{-1}$), and chlorophyll *a* concentration in Humboldt Lake. Primary production and chlorophyll data are from [20].

Table 2. Spearman rank coefficients between monthly mean bacterial cell volume and enviromental variables in the epilimnion of Redberry Lake. Only variables with $0.02 \leq P \leq 0.05$ are listed, NS = $P > 0.05$, $n = 8$. Variable abbreviations are defined in the text

Variable	Cocci	Rods	Total
DOC	−0.83	NS	NS
TDP	−0.77	NS	NS
NH_3-N	−0.78	NS	NS
Ciliates	NS	0.76	0.86

lapped the Lake Arlington values and was generally higher than most reported values (cf. [6]).

Although the data on bacterial volume and biomass from Redberry and Humboldt lakes are similar to those recorded in other studies, there are important differences in the factors influencing bacterial volume and biomass from previous studies. For example, one study [15] found that bacteria were larger in eutrophic as compared to oligotrophic lakes, whereas a survey study [2] showed a general trend of decreasing cell volume with increasing cell number. Mean cell volume was not significantly different between oligotrophic Redberry Lake and hypertrophic Humboldt Lake. Further, there was no significant correlation between bacterial volume and bacterial numbers in Humboldt Lake or at either depth in Redberry Lake. Our data are in agreement with those recently collected from a series of Canadian and Danish lakes which showed no clear trend towards either larger or smaller cell volumes across trophic gradients [12].

Table 3. Spearman rank coefficients between monthly mean bacterial cell volume and environmental variables in Humboldt Lake. Only variables with coefficients at $P \leq 0.05$ are listed. NS = $P > 0.05$, $n = 13$

Variable	Cocci	Rods	Total
Temperature	0.80**	NS	NS
Chlorophyll *a*	0.72**	0.58*	0.66*
Primary production	0.92***	0.60*	0.74**
Bacterial production	0.86**	NS	NS
$NO_2 + NO_3$-N	−0.63*	NS	NS

* = $P \leq 0.05$
** = $P \leq 0.01$
*** = $P \leq 0.001$

Table 4. Multiple regression coefficients for total bacterial cell volume versus environmental covariates in Redberry Lake. Equation constants were 0.056 at 2m and 0.062 at 16 m. *P*-values from ANOVA tests were ≤0.002

Depth	Covariate	Coefficient	r^2	Total r^2
2 m	Ciliates	4.65×10^{-6}	0.81	
	Chlorophyll	0.012	0.15	0.96
16 m	Ciliates	6.73×10^{-7}	0.86	
	Primary production	−0.004	0.08	0.94

Water temperature was not a dominant factor correlated with bacterial cell volume in either Redberry or Humboldt lakes (Tables 2–4). No correlation between water temperature and bacterial cell volume during diel studies in Lake Aydat (France) was found, but a positive correlation in Lake Cromwell (Canada) was calculated [23]. The latter observation agrees with results obtained by others (e.g., [6, 7]).

The dominant correlate with bacterial cell volume, particularly with coccoid volume, in Humboldt Lake was primary production (Table 3), suggesting the availability of labile dissolved organic carbon influenced bacterial cell volume. Although it is presently impossible to quantify precisely either the supply or standing stock of labile dissolved organic carbon, these can be indirectly estimated by measures of chlorophyll and primary production [8]. It is well known that bacteria increase cell size in response to increases in availability of dissolved organic carbon (reviewed in [21]) and quality [26].

A striking feature of Redberry and Humboldt lakes is that the waters are not highly colored and light usually penetrates to the bottom of Redberry Lake [20], despite the high DOC concentrations (Table 1). As far as we are aware there are no published studies on the quality or microbial degradation of DOC in saline lakes. Unlike the labile dissolved organic carbon produced by algae, DOC is generally considered to consist largely of refractory compounds. The inverse correlation between DOC and cocci cell volume in Redberry Lake (Table 2) may be an artifact

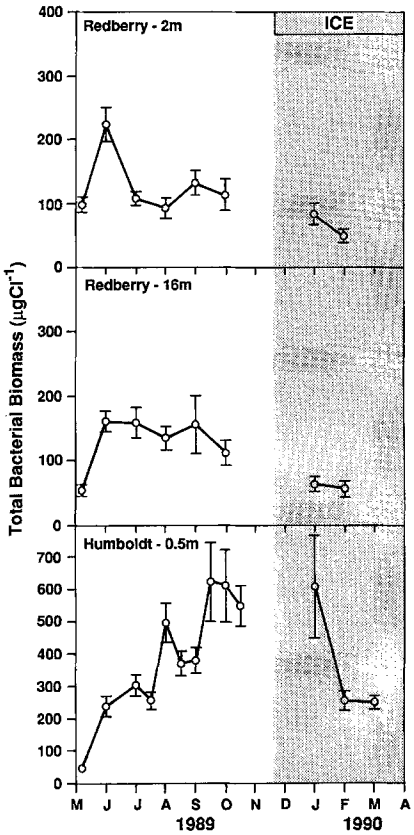

Fig. 4. Seasonal changes in total (cocci + rods) bacterioplankton biomass in Redberry and Humboldt lakes. Vertical bars represent ± 1 SE of the mean when they are greater than the symbol.

because current methodologies do not allow a partitioning between total and labile DOC fractions.

We estimated bacterial production and growth as the rate of thymidine incorporation into DNA and as this rate normalized to cell numbers, respectively [19]. Mean cell volume is proportional to growth rate under steady state conditions [11]. With the exception of cocci volume in Humboldt Lake, our estimates of bacterial growth ($P > 0.08$) and production (Table 2–4) were not correlated to cell volume, which is generally in accordance with other field studies [4, 11, 23, 25].

While ciliates can feed on bacterioplankton, they are currently not considered obligate bacterivores, except in special circumstances, and are more likely consumers of cyanobacteria or nanoplankton [9]. Despite this, ciliates can exert considerable influence on bacterial populations through size-selective grazing of the largest bacterial size classes, thereby biasing the size distribution of bacterial cells towards smaller cell size [9]. The positive correlations between bacterial cell volume and ciliate number in Redberry Lake (Fig. 1, Tables 2 and 4) may have been due to: (1) ciliates grazing other protozoan bacterivores, which primarily consumed small cells, resulting in increased bacterial cell volume; or (2) ciliate numbers increasing due to an increase in large bacterial cells, which they then preferentially grazed.

Epstein and Shiaris [9] measured an average bacterial ingestion rate by ciliates (which included species dominant in Redberry Lake) of 5,400 cells ciliate^{-1} day^{-1}, resulting in a removal rate of about 6% of the bacterial standing crop in an estuary. Assuming the ciliates in Redberry Lake have a similar bacterial grazing rate, they could remove between 16.2 and 83.3, and 84.6 $\times$ 10^3 bacterial cells ml^{-1} day^{-1} in the epilimnion in August, May, and September, respectively, or 0.2%, 2.0%, and 1.1% of the bacterial population in those months (Fig. 1, the months of lowest and highest bacterial cell volumes and ciliate numbers). In the hypolimnion the ciliate population could theoretically graze 45.5–388.8 $\times$ 10^3 bacterial cells ml^{-1} day^{-1} or 0.5–3.3% of the bacterial population in August and September, respectively. On the basis of these calculations ciliates were not major grazers of bacteria in Redberry Lake.

Bacterial biomass increased, following the wane of the *Aphanizomenon* bloom in Humboldt Lake (Fig. 4), similar to the observations of others [15, 22]. Although bacterial biomass increased during this period in Humboldt Lake, mean bacterial volume decreased until late October, and was coincident with large density increases of *Chydorus sphaericus* and *Diaphanosoma leuchtenbergianum,* which are known bacterivores [1], and ciliates (Fig. 3). Of the five dominant zooplankton species in Humboldt Lake, areal energy reserves for *C. sphaericus* and *D. leuchtenbergianum* were higher than for any of the other species at a time when edible algal biomass was low, suggesting bacteria and/or protistan bacterivores were a major food source [1]. The relative importance of ciliates versus zooplankton grazers of bacteria in Humboldt Lake needs to be ascertained.

In conclusion, statistical analysis of data from two saline lakes have implied that, while there was no significant difference in the mean cell volume of bacteria between oligotrophic Redberry Lake and hypertrophic Humboldt Lake, there were marked differences in the dominant factors influencing seasonal changes in bacterial volumes in the two lakes. The data from field studies, such as ours, highlight the complexity and our incomplete understanding of processes which control bacterial cell volume in natural systems.

Acknowledgments. We thank Marley Waiser for technical assistance, Dick A. Snyder for advise regarding the image analysis system, and anonymous referees for critical comments. Ciliate identifications and numbers were provided by C. Earle under an NHRI/Environment Canada contract.

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