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Genetic Structure and Community DNA Similarity of Picoplankton Communities from the Laurentian Great Lakes

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ABSTRACT. *The similarity of picoplankton assemblages in the epilimnia (5 m) and mid-hypolimnia (45 to 70 m) of several Laurentian Great Lakes was compared by quantitative 16S rRNA-based and community DNA hybridizations to determine if different picoplankton communities develop during the summer in these water masses. Total nucleic acids were extracted and purified from picoplankton collected in Lakes Erie, Ontario, Huron, Michigan, and Superior during July and August 1992. Based on 16S rRNA hybridizations, the majority of picoplanktonic nucleic acids (91% to 98%) were from bacteria. Microeucaryotes accounted for less than 3% of the total nucleic acids. Up to 2% of the picoplanktonic nucleic acids were contributed by archaea. The genetic similarity of picoplankton communities was estimated by pair wise hybridization of heterogeneous DNA samples. This similarity is an estimate of the fraction of total DNA shared in common between two communities. Total picoplanktonic DNA in the epilimnia of all lakes except Lake Superior was similar during August (≥ 70% similarity). At most sites in these lakes, however, the community DNA of epilimnetic and hypolimnetic picoplankton was different. A high abundance of cyanobacterial nucleic acids in Lake Ontario's epilimnion may explain why the community DNA of epilimnetic and hypolimnetic picoplankton was different in this lake. Together, the results verify that bacteria account for the majority of picoplankton in these great lakes but also indicate that different picoplankton communities can form in the epilimnion and hypolimnion during summer after these lakes become thermally stratified.*

INDEX WORDS: *Picoplankton, 16S rRNA, DNA-DNA hybridization, bacteria, archaea, Laurentian Great Lakes.*

INTRODUCTION

Unique microbial communities often develop in different aquatic habitats. Physical and chemical differences in the waters of most oceans and freshwater lakes affect the abundance and distribution of microorganisms (Mitchell and Fuhrman 1989, Owen 1989, Sieberth and Donaghay 1993). Gradients of temperature, dissolved oxygen, and suspended particles are often found in waters of the Laurentian Great Lakes during summer (Bertram 1993, Hicks and Owen 1991). Previous studies in these lakes focused on the abundance and physiological responses of bacterioplankton along these gradients, during different seasons, or in different lakes (Scavia and Laird 1987, Munawar and Munawar 2001).

Specific communities of phytoplankton and bacterial grazers have been reported in different Laurentian Great Lakes (Munawar and Munawar 1986, 2001; Hwang and Heath 1997, 1999). Phytoplankton and bacterial production are tightly coupled in most oceans and lakes (Cole *et al*. 1988) and biological mechanisms like size-selective grazing may influence the composition of aquatic microbial communities (Pernie *et al.* 1990, Sherr *et al.* 1992, Cotner *et al.* 1995). However, no studies exist on the diversity or genetic composition of heterotrophic picoplankton (0.2 to 2 μm sized organisms) in different water masses of the Laurentian Great Lakes, especially on large spatial scales.

The diversity of picoplankton communities has only been investigated in some aquatic environments. Initially, these investigations depended on identifying bacterial strains that were isolated on media (Satoh *et al*. 1989, McArthur *et al*. 1992).

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Subsequently, it was recognized that many environmental strains of bacteria and archaea have not yet been cultured in the laboratory (Giovannoni *et al*. 1990, DeLong 1992, Fuhrman *et al*. 1993), indicating that our understanding of microbial communities may be biased when only culture-based approaches are used. More recently, rRNA-based hybridization methods have been used extensively to document the composition of microbial communities in oceans and lakes (Glöckner *et al.* 1999, Karner *et al.* 2001, MacGregor *et al.* 1997, Pernthaler *et al.* 1998, Urbach *et al.* 2001). Alternatively, community DNA-DNA hybridization provides a convenient way of comparing microbial communities based on genomic abundance (Lee and Fuhrman 1990, 1991; Sinsabaugh *et al*. 1992; Lambert *et al*. 1993; Yeager and Sinsabaugh 1998).

In this study, 16S rRNA-based and whole community DNA-DNA hybridization methods were used together to investigate the diversity of picoplankton communities at two spatial scales in the Laurentian Great Lakes. The goals were to identify the relative contributions of the major domains of life (i.e., *Bacteria*, *Archaea*, *Eucarya*) in picoplankton communities, determine the genetic similarity of epilimnetic and hypolimnetic picoplankton communities, and evaluate environmental factors that may control the similarity of picoplankton communities during the summer in the Laurentian Great Lakes.

MATERIALS AND METHODS

Sample Collection

Water samples from the epilimnion (5 m) and mid-hypolimnion (45 to 70 m) in Lakes Erie, Ontario, Huron, Michigan and Superior were collected from offshore sites during July and August 1992 (Table 1). The depths for the hypolimnetic samples were chosen by examining water temperature and transparency profiles (Hicks and Pascoe 2001). Hypolimnetic samples were taken from clear water (near the highest water transparency) in each lake. Water was collected using submersible pumps (2 or 3 independent samples per depth) as described by Hicks and Pascoe (2001). The pumping system was flushed for at least 10 min at each depth (> 2 tube volumes) prior to taking samples. Water for prokaryotic cell counts was placed in sterile polypropylene centrifuge tubes (n = 2), preserved with 37% formaldehyde (0.22 μm filtered, 2% final concentration) and refrigerated until counted (within 2 weeks). Numbers of total prokaryotic cells were determined by epifluorescence direct-counts of DAPI-stained cells (Porter and Feig 1980).

Additional water (2 or 3 independently collected replicates) was prefiltered by pressure filtration (< 5 psi) through pre-ashed glass fiber filters (Gelman A/E, 1.2 μm nominal pore size) to remove larger eucaryotic cells (Hicks and Pascoe 2001). DAPI-stained prokaryotic cells were counted in all filtrates to determine cell losses after using different filters. On average 86% (2% standard error, 11 comparisons) of the prokaryotic cells passed this prefiltration step. No eucaryotic cells with discernable nuclei were identified by microscopy in the prefiltrates but procaryotic cells attached to particles were also probably removed. Picoplankton in 8 to 42 L of prefiltered water from each site were captured on Durapore filters (Millipore Corp.; 142 mm diameter, 0.22 μm pore) by pressure filtration (N_2 gas, 5 psi). Replicate filters (n = 2–3) were immediately placed in sterile Whirlpak bags and frozen (–20°C) until the nucleic acids were extracted. At least 99% (0.1% standard error, 14 comparisons) of the prokaryotic cells in the glass fiber filtrates were captured on the Durapore filters (as measured by DAPI counts). Thus, on average 85% of the procaryotic picoplankton cells in the original water samples were

TABLE 1. Locations in the Laurentian Great Lakes where picoplankton samples were obtained during July and August 1992.

Site	Date	Lake	Location
E15M	7 Aug 92	Erie	42°31.00′N, 79°53.36′W
O33M	9 Aug 92	Ontario	43°35.48′N, 78°48.06′W
O64	10 Aug 92	Ontario	43°31.5′ N, 76°55′ W
H45M	16 Aug 92	Huron	45°08.12′N, 82°59.00′W
M18M	22 Aug 92	Michigan	42°44.00′N, 87°00.00′W
M47M	18 Aug 92	Michigan	45°10.42′N, 86°22.30′W
SGM-1	14 Jul 92	Superior	46°56.31′N, 91°31.92′W
SGM-2	19 Aug 92	Superior	46°56.31′N, 91°31.92′W

TABLE 2. Procaryotic cell densities of unfiltered water, Gelman A/E filtrates, and in water passing the Durapore extraction filters (0.22 µm pore) from sites in the Laurentian Great Lakes. Cell recoveries represent the percentages of prokaryotic cells in original water samples that were captured on the Duropore extraction filters. Standard error of the mean is shown in parentheses (n = 2–3).

Site	Depth (m)	Total Procaryotic Cells (10^6/mL)			Cell Recovery (%)
		Unfiltered	Gelman Filtrate	Durapore Filtrate	
E15M	5	nd*	3.54 (0.51)	0.007 (nd)	nd
	45	2.62 (0.13)	0.74 (0.03)	0.001 (0.001)	28.2
O33M	5	6.79 (0.70)	6.07 (0.39)	0.010 (0.006)	89.2
	65	1.35 (0.02)	1.39 (0.10)	0.042 (0.007)	99.9
O64	5	1.70 (0.01)	1.93 (0.08)	0.004 (0.001)	113.3
	65	1.29 (0.04)	1.15 (0.02)	0.006 (0.002)	88.7
H45M	5	0.87 (0.02)	0.92 (0.04)	0.013 (0.003)	104.3
	62	1.26 (0.13)	1.13 (0.08)	0.042 (0.021)	86.3
M18M	5	3.57 (0.05)	2.91 (0.05)	0.005 (0.004)	81.4
	65	1.16 (0.08)	0.82 (0.02)	0.000 (0.000)	70.7
M47M	5	3.54 (0.66)	3.16 (0.02)	0.045 (0.035)	88.0
	65	1.57 (0.06)	1.37 (0.17)	0.005 (0.002)	86.9
SGM-1	5	nd	1.47 (0.09)	0.003 (nd)	nd
	70	nd	1.40 (0.17)	0.001 (0.001)	nd
SGM-2	5	0.88 (0.04)	nd	nd	nd

* nd = not determined

captured on the extraction filters (0.22 to 1.2 µm size fraction, Table 2, Hicks and Pascoe 2001).

Nucleic Acid Extraction and Purification

The extraction and purification of picoplanktonic nucleic acids followed Fuhrman *et al*. (1988) with some modifications (for a complete description see Hicks and Pascoe 2001). Briefly, frozen filters were cut into small pieces and incubated in STE buffer [pH 8.0] with lysosyme followed by SDS/proteinase-K solution. Nucleic acids and protein in this lysis solution were precipitated overnight, pelleted by centrifugation, rinsed with ethanol and redissovled in 0.5 mL of TE buffer [pH 8.0]. The nucleic acids were purified by sequentially extracting with Tris-saturated phenol (BRL, Inc.), phenol:chloroform: isoamyl alcohol (24:6:1), and chloroform:isoamyl alcohol (24:1). The nucleic acid extract was precipitated overnight at –20°C by adding 5 M NaCl (0.04 volume) and ice-cold 100% ethanol (2 volumes). The nucleic acids were pelleted by centrifugation (16,000 × *g*, 10 min, 4°C), rinsed with ice-cold 70% ethanol, and redissolved in 300 µl of TE buffer [pH 8.0]. All nucleic acid extracts were further purified using Sephadex G-200 columns to help remove humic materials that were present in several samples (Tsai and Olson 1992).

Nucleic acid concentrations were estimated by measuring absorbance at 260 nm and purity was monitored by A_{260}:A_{280} ratios (ratios ranged from 1.9–2.2) and A_{260}:A_{230} ratios (ranging from 1.5–2.4). Losses of nucleic acids during the extraction and purification procedures were estimated by adding calf thymus DNA (Sigma Chemical Co.) to a clean Durapore filter and following the same procedures. Fifty-two percent of calf thymus DNA (5% standard error, n = 6) was recovered after the extraction procedure. Only 53 percent (4% standard error, n = 6) of the calf thymus DNA was recovered after the Sephadex purification. Considering these recoveries, about 28% of the nucleic acids from captured cells would have been conserved during these procedures. This estimate does not consider the efficiency of removing nucleic acids from natural cells, so we are unsure if differential extraction or recovery of nucleic acids from some picoplankton occurred.

These purified nucleic acid extracts were used for various community DNA and 16S rRNA-based hybridizations until all analyses were completed or the nucleic acid extracts were depleted. In most cases, enough nucleic acid was extracted so that all analysis could be completed. However, smaller amounts of nucleic acids were extracted from some Lake Huron and Lake Superior samples. Lake Huron was excluded from the 16S rRNA-based and epilimnion-hypolimnion community DNA hybridizations because not enough nucleic acid was recovered from

the hypolimnetic samples at this site. After epilimnetic nucleic acids obtained from Lake Superior in August (SGM-2) were depleted by the epilimnetic DNA comparisons, nucleic acids were extracted from July samples (SGM-1) so that DNA in epilimnetic and hypolimnetic picoplankton communities could be compared in this lake and 16S rRNA hybridizations could be completed.

Quantitative Hybridizations With 16S rRNA Probes

Quantitative filter hybridizations were performed as previously described (DeLong 1992). Nucleic acid extracts from picoplankton and cultures were denatured with glutaraldehyde (0.5%) and blotted in a series of dots (25 ng to 1 μg) on nylon hybridization membranes (MagnaCharge, Micron Separations, Inc) and immobilized by baking (80°C, 1 h). Nucleic acids from different microorganisms representative of the target taxa (*Bacteria*, *Archaea*, and *Eucarya*) were included on all filters and used as authentic standards to quantify taxon-specific and non-specific binding of the oligonucleotide probes. These nucleic acids were extracted and purified from cultured cells of *Pseudomonas fluorescens* (*Bacteria*; ATCC 13525), *Saccharomyces cerevisiae* (*Eucarya*; strain SAC-1 from Dept. of Microbiology Culture Collection, Michigan State University), and *Haloarcula marismortui* (*Archaea*; Mylvaganam and Dennis 1992) with the same procedures used for natural picoplankton samples.

Nucleic acids were hybridized with 16S rRNA-based oligonucleotide probes that were labeled on the 5′ end with [^{32}P] following DeLong's (1992) procedure. Radiolabeled probes specific for the domains *Bacteria* (EUB338; Amman *et al*. 1990), *Archaea* (ARC915; Stahl and Amman 1991), and *Eucarya* (EUK1195; Giovannoni *et al*. 1988) were prepared. All filters were prehybridized at 45°C for 30 min, hybridized overnight at 45°C after fresh hybridization buffer and the appropriate radiolabeled probe were added, washed three times for 30 min at 37°C, and then washed for an additional 30 min to provide stringency (56°C for EUB338; 45°C for ARC915; and 40°C for EUK1195). Specifically bound probe was quantified using a radioanalytic imaging system (Ambis System; Scanalytics, Billerica, MA).

The slope of probe bound per unit rRNA was determined from 4 to 6 points in the linear portion of the binding curve. The portions of picoplanktonic nucleic acid that were contributed by each domain were determined by dividing the nmols of group-specific probe bound per unit nucleic acid by the sum of bacterial + archaeal + eucaryal probes bound per unit nucleic acid (Giovannoni *et al.* 1990, DeLong *et al.* 1994). After the hybridization signal from each sample was normalized to the total response from all three domain probes and corrected for the specific activity of the probes, it were normalized to the response of authentic nucleic acids and corrected for any non-specific binding (Giovannoni *et al.* 1990). The percentages of nucleic acid contributed by members of the *Bacteria*, *Archaea*, and *Eucarya* were calculated from these corrected hybridization signals.

Community DNA Similarity

Whole community DNA probes were prepared using purified nucleic acid extracts from picoplankton communities following Lee and Fuhrman's (1990) procedure). DNA probes were labeled with [α-^{35}S]dCTP (DuPont, NEN Research Products, Boston, MA) by nick translation as described in accompanying literature (Amersham Life Science). These probes were purified using NENSORB™ columns (DuPont Research Products) to remove unincorporated nucleotides and enzymes, recovered in 50% methanol, and frozen until needed (–20°C). The probes were dried in a SpeedVac, reconstituted in 100 μL of Milli-Q water, and denatured by boiling for 10 min before they were added to the hybridization solution. Probe specific activities ranged from 0.2 to 1.2×10^8 CPM/μg nucleic acid.

Target DNAs from picoplanktonic nucleic acid extracts were loaded onto nylon membrane filters (n = 2 or 3 per sample; MagnaGraph, Micron Separations, Inc.) and hybridized as previously described (Lee and Fuhrman 1990). Reciprocal hybridizations (i.e., probe and target DNAs reversed) were also performed to check for asymmetrical hybridizations. Filters were prehybridized for 6 to 8 h at 68°C. Samples on these filters were hybridized for 48 to 50 h at the same temperature followed by several washes (each done in duplicate). The first and third washes were done at room temperature in 2X SSC for 5 min. During the second wash, filters were rinsed at 68°C for 30 min with 0.5% SDS in 2X SSC (Lee and Fuhrman 1990). The washed filters were air dried and exposed to X-ray film (Konica Medical Corp.). After the film was developed, the autoradiographic images were transferred to a Macintosh computer using a high-resolution video camera. Images of the hybridization dots were quantified with NIH Image (v. 1.44) software. Indi-

vidual dots were cut from the membrane and the radioactivity counted with a Packard liquid scintillation counter (model 2200CA) when this image analysis system was not sensitive enough to capture autoradiographic images.

Community DNA similarity is an estimate of the fraction of identical DNA shared in common between two communities (Lee and Fuhrman 1990). It was calculated using the degree of cross hybridization between target and control (i.e., probe) DNAs. All hybridization results with the same DNA probe (i.e., constructed from control DNA) were normalized to the control DNA response (e.g., similarity = [target DNA binding/control DNA binding] × 100) after subtracting any non-specific binding to the membrane filter. Self-hybridization of a probe to control DNA on a membrane was theoretically defined as 100% similarity. The similarity of replicate DNA samples (n = 2 or 4) from the same community was compared to identify a level below which two communities could be operationally defined as different. DNAs from both the epilimnion and hypolimnion at one site in each lake (excluding Huron) were used for these comparisons.

RESULTS

Description of Sites

Epilimnia had formed in all the Laurentian Great Lakes by the time these lakes were sampled (July and August, 1992). The temperatures in the epilimnia (5 m) ranged from 10.6°C to 20.3°C and the temperatures where the hypolimnetic samples were taken ranged from 3.8°C to 5.1°C (Hicks and Pascoe 2001). The water column in each lake was aerobic at the depths sampled. Chlorophyll *a* (0.3 to 6.5 μg/L; Hicks and Pascoe 2001) and prokaryotic cell abundances (Table 2, unfiltered samples) were generally greater in the epilimnion than in the hypolimnion of these lakes.

Quantitative Hybridizations with 16S rRNA Probes

Bacteria contributed the majority of nucleic acids recovered from picoplankton (91 to 98%) at each site from these lakes (Table 3). Microeucaryotes accounted for less than 3% of the total nucleic acids. Up to 2% of the picoplanktonic nucleic acids in the epilimnia and hypolimnia of these lakes were from procaryotic archaea.

TABLE 3. Relative binding of taxon-specific 16S rRNA probes to picoplankton nucleic acids from the Laurentian Great Lakes. Taxon-specific responses were normalized to the sum of responses to 16S rRNA domain probes (Bacteria+Archaea +Eucarya). Standard error of the mean for replicate samples is shown in parentheses (n = 2).

Site*	Depth (m)	Bacteria (%)	Archaea (%)	Eucarya (%)
E15M	5	97.1 (0.5)	0.7 (0.03)	2.2 (0.5)
	45	97.8	0.7	1.5
O33M	5	98.0 (0.7)	1.0 (0.1)	1.1 (0.5)
	65	97.3 (0.6)	1.3 (0.0)	1.4 (0.6)
O64	5	97.9 (0.1)	0.1 (0.02)	2.0 (0.1)
	65	97.8 (0.02)	0.8 (0.01)	1.4 (0.01)
M18M	5	97.9	1.6	0.5
	65	97.5	0.7	1.9
M47M	5	95.5 (0.3)	1.5 (0.1)	3.1 (0.4)
	65	95.6 (1.1)	1.6 (0.03)	2.8 (0.2)
SGM-1	5	90.9	1.9	7.3

*Sites were in Lakes Erie (E15M), Ontario (O33M, O64), Michigan (M18M, M47M), and Superior (SGM).

Community DNA Similarity

Calibration of Community DNA Similarity

High community DNA similarities, usually > 90%, were obtained when DNA from replicate samples was cross-hybridized (see values along diagonals of Tables 4 and 5). The community DNA hybridization method has been thoroughly tested with single bacterial strains and artificial bacterial assemblages (Wayne *et al*. 1987, Lee and Fuhrman 1990, Stackebrandt and Goebel 1994). Based on calibration experiments with constructed bacterial assemblages, Lee and Fuhrman (1990) suggested that two bacterial communities be operationally interpreted as different communities if DNA cross-hybridization was less than 70%. The higher DNA similarities between replicate samples from the epilimnia or the hypolimnia of the Laurentian Great Lakes (usually > 90%, Tables 4 and 5) indicate that 70% similarity may be too low for classifying picoplankton communities as different assemblages in this study. The more conservative 70% DNA cross-hybridization level, however, was used to operationally define different communities.

Reciprocal hybridizations (i.e., probe and target DNAs switched) were often asymmetric. Asymmetry is indicated by a large difference between two community DNA similarity values derived from recipro-

TABLE 4. Community DNA similarities (%) between picoplankton assemblages in the epilimnia of the Laurentian Great Lakes. All water was collected at 5 m and prefiltered through Gelman A/E glass fiber filters to remove larger eucaryotic cells. Values are means of replicate hybridizations (n = 2–3 targets, 1 probe). The standard error of the mean is shown in parentheses. The results of reciprocal hybridizations can be seen across the diagonal of the matrix. The smaller similarity value for reciprocal hybridizations is shown in bold type. Values in brackets are DNA similarities between replicate samples taken from the same community as the probe DNA.

	Probe DNA				
Target DNA	E15M	033M	H45M	M18M	SGM
E15M†	[97 (nd*)]	**88** (0)	108 (0)	107 (19)	**44** (3)
O33M‡	106 (5)	[98 (8)]	103 (2)	88 (3)	**44** (2)
H45M†	**89** (5)	**89** (5)	[117 (nd)]	**101** (17)	**47** (5)
M18M‡	**97** (5)	**85** (3)	107 (6)	[97 (11)]	**47** (3)
SGM-2 (19 Aug 92)†	108 (2)	92 (nd)	114 (8)	102 (nd)	[73 (nd)]

*nd = not determined
†n = 2 target DNAs, if one replicate target was lost then the standard error was not determined (nd)
‡n = 3 target DNAs

cal hybridizations. Interestingly, reciprocal hybridizations were fairly symmetric when replicate DNA samples from the same location and depth were cross-hybridized as indicated by the low standard error (SE) for the mean of these values (Tables 4 and 5). Reciprocal hybridizations between samples from different locations were noticeably asymmetric though (Tables 4 and 5). Lee and Fuhrman (1990, 1991) also observed this pattern when comparing natural samples or mixtures of known bacteria. They demonstrated that asymmetry in reciprocal hybridizations was due to the relative complexity of different DNA samples. Their suggestion that the lower DNA similarity from reciprocal hybridizations is a better estimate of the true similarity was adopted to interpret data in this study.

Comparisons across Epilimnetic Communities in Different Lakes

DNA similarities between epilimnetic communities in the different Laurentian Great Lakes ranged from 44% to 117% (Table 4). DNA from epilimnetic communities in Lakes Erie, Ontario, Huron, and Michigan was very similar (≥ 85%), but different from epilimnetic DNA taken from Lake Superior (≤ 47%, Table 4). The differences between reciprocal DNA similarities from these epilimnetic communities were generally smaller than the differences observed when epilimnetic and hypolimnetic bacterioplankton were compared. Comparisons to the Lake Superior epilimnetic community were the only exceptions (Table 4).

Comparisons between Epilimnetic and Hypolimnetic Communities within Lakes

Community DNA similarities between epilimnetic and hypolimnetic communities ranged from 27% to 80% (Table 5). The smallest similarity between epilimnetic and hypolimnetic communities (27%) was observed in Lake Erie. Intermediate similarities (40% to 62%) were observed when comparing epilimnetic and hypolimnetic DNAs from Lakes Ontario and Michigan (Table 5). The most similar community DNAs (>70% similarity) comparing epilimnetic and hypolimnetic communities were observed at one site in Lake Ontario (O33M) and in Lake Superior (Table 5). Large asymmetries in community DNA similarities were often observed during the comparison of epilimnetic and hypolimnetic communities when the probe and target DNAs were reversed (Table 5). The greatest asymmetry was observed in the comparison of Lake Erie communities. In these cases, the smaller of the two similarity values was used as the best estimate of community DNA similarity (see discussion in Lee and Fuhrman 1990).

DISCUSSION

Quantitative 16S rRNA hybridizations indicated that procaryotic nucleic acids (bacterial + archaeal) accounted for at least 91% and usually greater than 97% of the total nucleic acids that were extracted

TABLE 5. Community DNA similarities (%) between picoplankton assemblages from the epilimnia and hypolimnia in Lakes Erie (E15M), Ontario (O33M, O64), Michigan (M18M, M47M) and Superior (SGM-1). All lakes were sampled during August 1992 except Lake Superior (14 July 1992). The values shown are means of duplicate hybridizations (n = 2 targets, 2 probes) except for Lake Superior samples (n = 4 targets, 4 probes). The standard error of the mean is shown in parentheses. The results of reciprocal hybridizations can be seen across the diagonal of the matrix. The smaller similarity value for reciprocal hybridizations is shown in bold type. Values in brackets are DNA similarities between replicate samples taken from the same community as the probe DNA.

		Probe DNA											
		E15M		O33M		O64		M18M		M47M		SGM-1	
Target DNA		5 m	45 m	5 m	65 m	5 m	65 m	5 m	65 m	5 m	65 m	5 m	70 m
E15M	5 m	[148 (97)]	178 (85)										
	45 m	**27** (8)	[48 (8)]										
O33M	5 m			*	96 (18)	nd†	nd						
	65 m			**71** (26)	*	nd	nd						
O64	5 m			nd	nd	[103 (2)]	56 (3)						
	65 m			nd	nd	**40** (2)‡	[108 (3)]						
M18M	5 m							*	85 (9)	nd	nd		
	65 m							**62** (7)	*	nd	nd		
M47M	5 m							nd	nd	[91 (0)]	94 (4)		
	65 m							nd	nd	**62** (8)‡	[95 (7)]		
SGM-1	5 m											[95 (6)]	83 (1)
	70 m											**80** (5)	[102 (14)]

* Self-hybridization was defined as 100% similarity.
† nd = not determined
‡ Similarity between epilimnetic and hypolimnetic samples was significantly different from similarity between epilimnetic replicates ($p < 0.05$)

from these picoplankton samples, while eucaryotic nucleic acids accounted for less than 3% (Table 3). These results were consistent with our microscopic examinations of these samples. Some investigators believe that extraction methods may contribute to the selective extraction of nucleic acids from some microbial cells. Hicks and Pascoe (2001) demonstrated that the percentage of cyanobacterial nucleic acids (relative to total picoplanktonic nucleic acids) was correlated with the relative abundance of autofluorescent picoplanktonic cells in these same samples. This finding indicates that the relative abundance of at least one bacterial taxon was preserved after nucleic acids were extracted from these picoplankton samples.

As expected, the majority of the picoplanktonic nucleic acids (91% to 98%) were contributed by bacteria in these lakes during August (Table 3). It is unlikely that these microorganisms were present on particles because all water was prefiltered prior to extracting nucleic acids. Bacterial nucleic acid percentages were similar at the majority of sites sampled, although less bacterial nucleic acid was present at one site in Lake Michigan (i.e., M47M) than in Lake Ontario (Hicks and Pascoe 2001). Prefiltering water probably removed the majority of eucaryotic cells because less than 3% of the total nucleic acids was contributed by microeucaryotes. Although archaeal nucleic acids were not expected, they accounted for up to 2% of the total nucleic acids recovered from the epilimnia and hypolimnia of these lakes. The presence of archaea in pelagic picoplankton assemblages has been reported in some marine environments and inland lakes, but has heretofore never been documented in the large lakes of the world (DeLong *et al*. 1994, Fuhrman *et al*. 1993, Jurgens *et al*. 2000, Pernthaler *et al*. 1998). For example, Glöckner *et al.* (1999) did not detect archaeal cells by FISH analysis in Lake Baikal. Keough *et al*. (2003) provide a more thorough analysis of archaeal nucleic acids detected in the Laurentian Great Lakes and their potential sources. They demonstrated that unrecognized members of the Group I Crenarchaeota contributed some of the ar-

chaeal nucleic acids in similar samples collected from the Laurentian Great Lakes during 1993.

The community DNA composition of epilimnetic picoplankton communities in the different Laurentian Great Lakes was remarkably similar (≥ 70% DNA similarity) even though the spatial scale of these comparisons was hundreds of kilometers (Table 4). Munawar and Munawar (1986, 2001) have reported numerous phytoplankton in common and similar phytoplankton biomasses during the summer in these large lakes. Although the Laurentian Great Lakes have many phytoplankton species in common, these species are often found at different times of the year and in different abundances in different lakes (Munawar and Munawar 1986, 2001). A picoplankton community response to a different phytoplankton community in Lake Superior during August might explain the different epilimnetic community DNA composition in this lake compared to the other lakes. The similarity of epilimnetic picoplankton communities in the other Laurentian Great Lakes during August is contrary to investigations in marine environments where different epilimnetic bacterial communities have been observed in different oceans as well as a few kilometers apart in the same ocean basin (Lee and Fuhrman 1990, 1991).

Comparisons between epilimnetic and hypolimnetic bacterial communities in each of the Laurentian Great Lakes were made over relatively small distances (40 to 65 m range). Yet, the community DNA composition of picoplankton communities in the epilimnion compared to the hypolimnion was different (< 70% similarity) at four of six sites that were investigated (E15M, O64, M18M, and M47M; Table 5). The epilimnetic and hypolimnetic communities were similar at only two sites in two different lakes (O33M and SGM-1). At one of these sites, O33M, a large enough proportion of DNA was shared in common between the epilimnetic and hypolimnetic assemblages for these communities to only be considered marginally similar (71% similarity). These communities would have been defined as dissimilar if a less conservative similarity value than 70% had been used to define similar community DNA compositions. At the other site in Lake Superior (SGM-1; July 14), the temperature difference between the epilimnion and hypolimnion (about 5°C) was the smallest difference observed in all the lakes. It is conceivable that not enough time had elapsed for a different picoplankton community to develop in the epilimnion of Lake Superior because it was not stratified as long as the other lakes that were sampled later in August. The oligotrophic nature of this lake as well as the earlier time when samples were taken could be responsible for the similar DNA composition of epilimnetic and hypolimnetic picoplankton at this site in Lake Superior.

The photic zone provides an opportunity for phototrophic procaryotes (e.g., cyanobacteria) to proliferate, which would not occur deeper in the hypolimnia of these lakes. Chroococcoid cyanobacteria may have contributed DNA disproportionally to the epilimnetic samples because of their small size (0.7 to 1.3 μm) and abundance in some lakes (Caron *et al*. 1985, Fahnenstiel and Scavia 1987). In fact, cyanobacterial nucleic acids in the epilimnion of Lake Ontario accounted for a major portion (57% to 65%) of the picoplanktonic nucleic acids in the same samples as this study, unlike the hypolimnetic samples (Hicks and Pascoe 2001). Chlorophyll concentrations were also measured at one of two sites examined in this lake. At site O64, the chlorophyll *a* concentration was significantly greater at depths where picoplankton samples were taken in the epilimnion (6.5 μg/L) compared in the hypolimnion (1.6 μg/L; Hicks and Pascoe 2001). The great abundance of cyanobacterial nucleic acids in the epilimnion probably contributed to the different community DNA compositions observed in the epilimnion and hypolimnion at site O64 in Lake Ontario.

Differences detected between picoplanktonic DNA from the epilimnion and hypolimnion in Lakes Erie and Michigan could not be explained by differences in the abundance of cyanobacterial nucleic acids. So, other factors that may control the diversity of picoplankton communities need to be considered. Several physical and biological forces, including temperature (Simon *et al*. 1999, Lindström 2001), pH (Lindström and Leskinen 2002), grazing by predators (Jürgens and Matz 2002), and eucaryotic phytoplankton biomass and composition (Muylaert *et al*. 2002) can control the composition of aquatic bacterial communities (Nold and Zwart 1998).

Differences in the picoplanktonic community DNA compositions we observed in the Laurentian Great Lakes point to two factors that can exert strong influences on the structure of aquatic bacterial communities: phytoplankton communities and temperature. Differences in the abundance or composition of phytoplankton between the epilimnia and hypolimnia of these lakes may have also led to some heterotrophic members of the epilimnetic picoplankton flourishing as well (Aas and Hicks 1993, Muylaert *et al*. 2002). Bacterioplankton production is strongly related to phytoplankton production in aquatic systems (Cole *et al*. 1988). Differences in

available organic matter between the epilimnia and hypolimnia may select for different bacteria; copiotrophic versus oligotrophic forms (Poindexter 1981). Labile DOM from phytoplankton production may lead to higher abundances of a few heterotrophic species in epilimnetic picoplankton communities.

The growth of bacterioplankton (e.g., thymidine incorporation rate) is related to temperature in the Laurentian Great Lakes, especially below 10°C (Scavia and Laird 1987). Even though phytoplankton blooms occur at cold temperatures in temperate areas, bacterial production often only increases after waters warm (Pomeroy and Deibel 1986). The difference in water temperature between the epilimnia and hypolimnia of the Laurentian Great Lakes may allow heterotrophic picoplankton to grow more quickly in the epilimnia compared to the hypolimnia and cause divergence in the genetic diversity of picoplankton communities.

Two important findings about picoplankton communities in the Laurentian Great Lakes resulted from this investigation. First, even though bacteria contributed the majority of nucleic acids found in the picoplankton, the presence of archaeal and eucaryotic nucleic acids indicates that these microorganisms are also members of these communities. Second, epilimnetic picoplankton communities of similar genetic composition can be present over large distances in different Laurentian Great Lakes after the epilimnia form during the summer but these communities are often genetically different from hypolimnetic picoplankton a smaller distance away at the same site. The development of picoplankton communities within these lakes may be primarily influenced by local environmental conditions like temperature and the abundance and composition of phytoplankton assemblages.

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