Importance of Picoplankton in Lake Superior[1]

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In Lake Superior, approximately 50% of total primary production is attributable to phytoplankton that pass through a 3-μm screen. The <3-μm size class is dominated by eukaryotic flagellates, nonmotile eukaryotic cells (1 μm), and chroococcoid cyanobacteria. Approximately 20% of total primary production is attributable to orange autofluorescent chroococcoid cyanobacteria (mean size = 0.7 μm) as determined by size fractionation and track autoradiography. These small prokaryotes exhibited abundances of 42 000 and 56 000 cells·mL^{-1}, maximum photosynthetic rates of 7 and 6 fg·cell^{-1}·h^{-1}, and growth rates of 1.5 and 0.8·d^{-1} in the epilimnion and hypolimnion, respectively. A significant portion of this picoplankton (<1 μm) production may be consumed by heterotrophic protozoans in a "microbial loop."

Environ 50 % de la production primaire totale du lac Supérieur a pour origine des organismes phytoplanctoniques pouvant traverser un tamis à mailles de 3 μm. La classe de taille des <3 μm est dominée par des flagellés eucaryotiques, des cellules eucaryotiques non motiles (1 μm) et des cyanobactéries chroococcoïdes. La répartition selon les tailles et l'étude des tracés autoradiographiques a montré qu'environ 20 % de la production primaire totale était due à des cyanobactéries chroococcoïdes à autofluorescence orange (taille moyenne de 0,7 μm). Ces petits procaryotes présentaient respectivement, selon qu'ils se trouvaient dans l'épilimnion ou l'hypolimnion, des abondances de 42 000 ou 56 000 cellules·mL^{-1}, des taux maximums de photosynthèse de 7 et 6 fg·cellule^{-1}·h^{-1} et des taux de croissance de 1,5 ou 0,8·jour^{-1}. Une partie appréciable de la production de ce picoplancton (<1 μm) peut être consommée par des protozoaires hétérotrophes en « boucle microbienne ».

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Picoplankton (<1 μm) are important contributors to oceanic primary production (Li et al. 1983) and are usually dominated by chroococcoid cyanobacteria and small eukaryotes (Johnson and Sieburth 1979, 1982). The recognition of autotrophic picoplankton as an important part of the oceanic system has forced a reorganization of contemporary models of plankton dynamics, including both methodological impacts for primary production and theoretical impacts on pathways of carbon and energy flow (Sherr and Sherr 1984; Smith et al. 1984). However, the importance of picoplankton has not been determined in the upper Great Lakes, except for abundance estimates for phytoplankton <3 μm from Saginaw Bay (Sicko-Goad and Stoermer 1984). We investigated the abundance and production of <3- and <1-μm phytoplankton in Lake Superior and their possible role in the food web.

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Methods

A 230-m-deep station in central Lake Superior (47°27′48″N, 88°34′42″W) was sampled 10 times between May 1 and October 15 in 1979 and once on September 28 in 1983.

Primary production was measured with the ^{14}C technique (Vollenweider 1974). In 1979, all incubations were carried out in situ for 4 h as described in Fahnenstiel and Glime (1983). In 1983, laboratory incubations were conducted for 1 h in 2-L polycarbonate bottles at 150 and 35 μEin·m^{-2}·s^{-1}. The higher light intensity is saturating for both epilimnetic and hypolimnetic phytoplankton in Lake Superior (G. L. Fahnenstiel, unpubl. data). After incubation, the contents of the incubation bottles were combined and split into several fractions. Whole water samples were filtered directly onto 0.45-μm Selectron membrane filters in 1979 and 0.22-μm Millipore filters in 1983. The remaining sample was fractionated by screening (3, 5, 10, 20, and 64 μm and 1, 3, and 10 μm were used in 1979

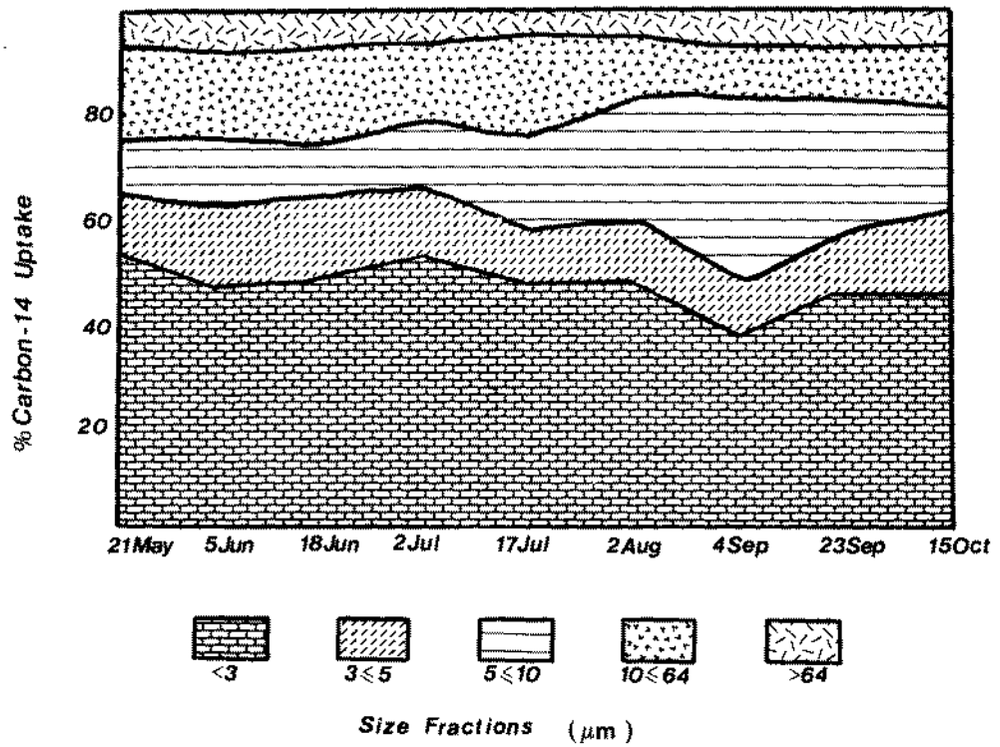

FIG. 1. Seasonal variation of ^{14}C fixation by percent contribution of various size classes in 1979 as determined by filter fractionation.

and 1983, respectively) and then final-filtering through 0.45-μm filters in 1979 and 0.22-μm filters in 1983. Nuclepore polycarbonate filters were used for the 1-, 3-, and 5-μm fractions and Nitex screens for the 10-, 20-, and 64-μm fractions.

In 1983, samples were also taken from the ^{14}C experiments and prepared for epifluorescence autoradiography (Fuhrman and Azam 1982). The remaining contents of the ^{14}C incubation bottles (whole and <1 μm) were preserved with either paraformaldehyde, glutaraldehyde, and sodium cacodylate (Lazinsky and Sicko-Goad 1979) or 1% formalin. Samples were in contact with the preservative for only a few seconds, at which time samples were filtered onto 0.22-μm Nuclepore filters. These filters were then inverted onto subbed cover slips (Fuhrman and Azam 1982) and dipped and developed for track autoradiography (Knoechel and Kalff 1976). Fading of autofluorescence can occur with prolonged storage and possibly during autoradiographic development. To minimize this fading we kept exposure time to a minimum (1−3 d) and performed replicate developments with and without the stop bath treatment. Some autofluorescent fading was noted relative to undeveloped samples, but this fading was not severe enough to cause significant problems. Loss of ^{14}C during the preservation, filtering, and autoradiographic preparation steps was monitored by filtration and did not exceed 10% with paraformaldehye and glutaraldehyde and 30% with 1% formalin. Number of tracks and cell size, shape, and color were noted for autofluorescent cells.

In 1979, phytoplankton samples were preserved with Lugol's solution and counted on an inverted microscope. In 1983, two sets of phytoplankton samples were taken. The first set was preserved and counted as in 1979. The second set was preserved with paraformaldehyde, glutaraldehyde, and sodium cacodylate for fluorescent and electron microscopy (Lazinksy and Sicko-Goad 1979). These preserved phytoplankton samples were stored on ice and returned to the laboratory for further processing. The TEM samples were concentrated by gentle centrifugation, rinsed four times in 0.1 mol cacodylate buffer·L^{-1} (pH 7.2), and then postfixed with 1% OsO_4 (osmic acid anhydride) in cacodylate buffer for 1 h at 4°C. The cells were then dehydrated in a graded ethanol and propylene oxide series and embedded in Epon (Luft 1961). Thin sections were cut with a diamond knife, collected on cleaned Formvar-coated 200-mesh copper grids, and stained with aqueous uranyl acetate (Watson 1958). Sections were examined with a JEOL JEM 100B electron microscope operating at 80 kV. Heterotrophic and autotrophic cells were counted on a Laborlux 12 microscope equipped for fluorescent microscopy (excitation 450−500 nm; emission >515 nm). Heterotrophic cells were also enumerated under light microscopy with a differential staining technique that consisted of imbedding 1-μm sections stained with toluidine blue-O (McCully et al. 1980).

Results

The 1979 results from three depths were averaged because vertical trends were not apparent (Fahnenstiel 1980). The <3-μm size class accounted for 40−54% (mean = 47%) of the total carbon fixed, while the <10-μm size class accounted for 78% (Fig. 1). Few seasonal trends were found in any size class. The 5- to 10-μm size class appeared to fix more carbon during the thermally stratified period (August−September) when there was a rapid increase in the diatom *Cyclotella* (Fahnenstiel and Glime 1983).

In 1983, the <3-μm size class was studied in more detail. Since little seasonal variability was found in 1979, only one period was sampled in 1983. The amount of carbon fixed in the <3- and <10-μm size classes was similar to measurements in 1979, and the <1-μm size class accounted for approximately 20% of the total carbon fixed. Following are the percentages of

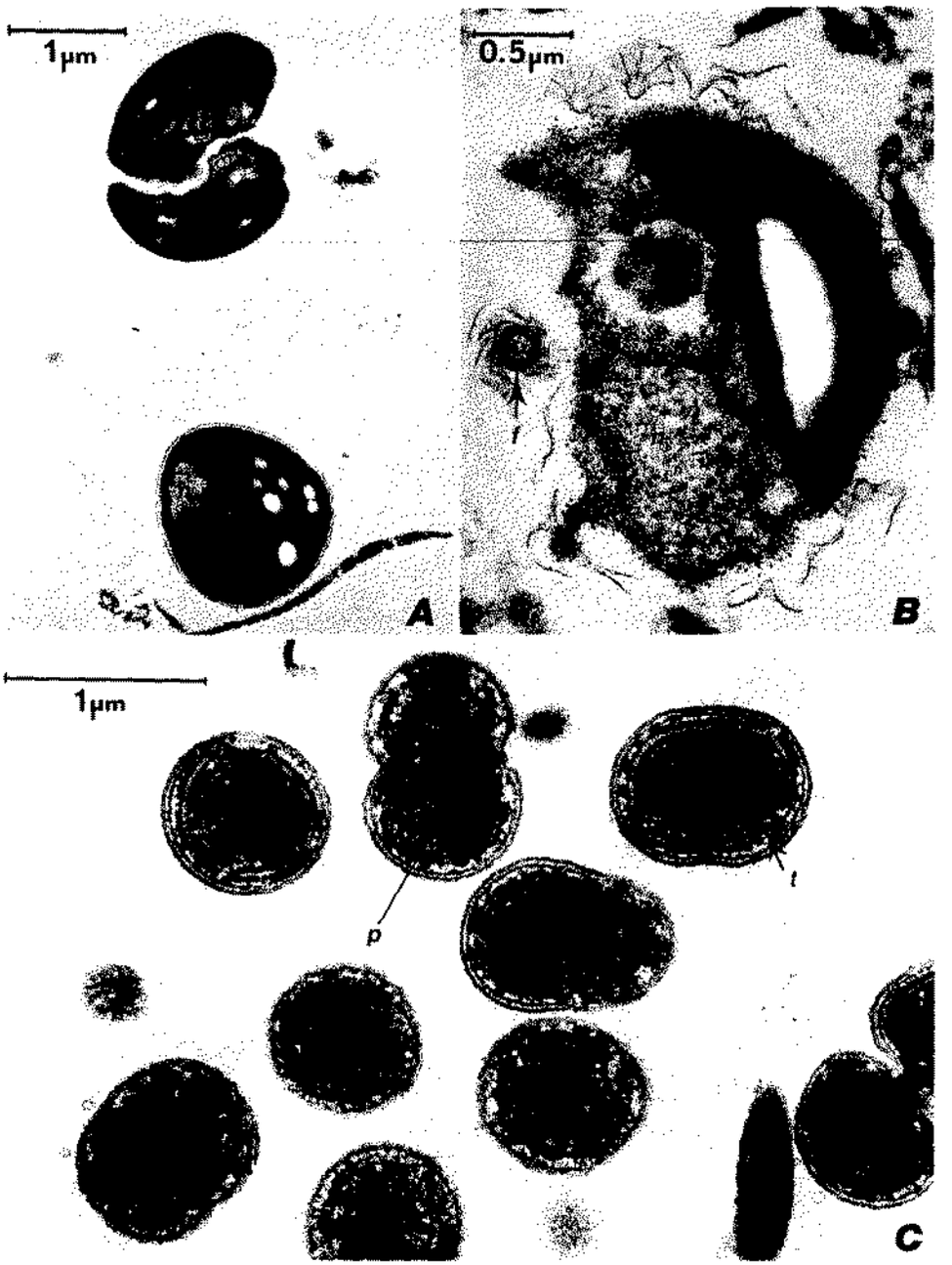

FIG. 2. Transmission electron micrographs of Lake Superior picoplankton (A) Eukaryotic green cell. This tiny nonflagellated phototroph has visible chloroplast (c) and nucleus (n). (B) Eukaryotic microflagellate. This small flagellate has visible nucleus, chloroplast, and flagella (f), with scales on cell and flagella. (C) Orange autofluorescent chroococcoid cyanobacteria. These small prokaryotes have polyhedral bodies (p) and peripheral arrangement of thylakoids (t), which are separated by dense cytoplasmic material.

total carbon fixed in the <1-, <3-, and <10-μm size fractions in 1979 and 1983:

	1979 (yearly average)	1983 (September)
<1	—	20
<3	47	49
<10	78	76

The predominant organisms in the 1- to 3-μm size category were green eukaryotic cells, cyanobacteria, and small flagellates, while the predominant organisms in the <1-μm size class were small (range = 0.4–1.0 μm; mean = 0.7 μm) orange autofluorescent chroococcoid cyanobacteria (Fig. 2; Table 1). The orange autofluorescent color of these cyanobacteria when excited with blue light indicates the presence of phycoerythrin (Waterbury et al. 1979). These chroococcoid cyanobacteria were present at abundances of 42 000 and 56 000 cells·mL^{-1} and exhibited light-saturated carbon fixation rates of 7 and 6 fg·cell^{-1}·h^{-1} for samples from the epilimnion and hypolimnion, respectively. Approximately 77 and 91% of the carbon fixed in the <1-μm size class from the epilimnion and hypolimnion, respectively, were fixed by these

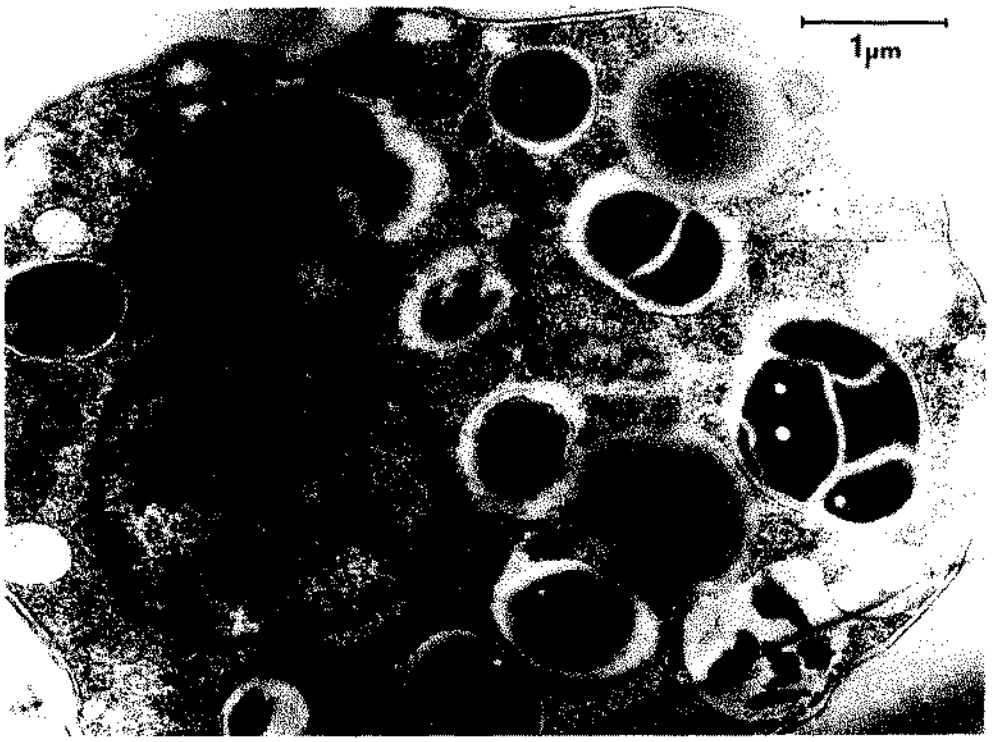

FIG. 3. Transmission electron micrograph of heterotrophic protozoan in Lake Superior. This protozoan has ingested many cyanobacteria (cb) which can be seen in food vacuoles.

small prokaryotes. Since 20% of these cyanobacteria were removed by 1-μm fractionation (Table 1), total cyanobacteria production was calculated by multiplying cyanobacteria abundance and specific production rate. Thus, cyanobacteria were responsible for 16–24% of the total primary production (Table 1). Growth rates for cyanobacteria were calculated from carbon fixation expressed on a daily basis and an estimate of their carbon content. The 1-h photosynthetic rates were converted to daily rates based on typical incident and depth-attenuated light intensity using the numerical model of Fee (1973). Carbon content was determined from average cell volume assuming 121×10^{15} μg C · μm^{-3} (Watson et al. 1977). The calculated exponential growth rates for these cyanobacteria were 1.5 · d^{-1} in the epilimnion and 0.8 · d^{-1} in the hypolimnion.

Electron microscopy performed in 1983 revealed abundant heterotrophic protozoans in the 3- to 20-μm size range (Fig. 3). The term heterotrophic protozoans refers to microheterotrophs (<20 μm), which are flagellated, ciliated, and ameboid organisms. Preliminary abundance estimates from epifluorescent and differential stained counts for these protozoans are in the range of 400–500 cells · mL^{-1}, with an estimated biomass of 49–60 mg · m^{-3} and carbon content of 7.35–9 mg C · m^{-3}. Many of these protozoans from both epilimnetic and hypolimnetic samples had ingested cyanobacteria, as can be seen in Fig. 3 where approximately 10 cyanobacteria (some partially digested) are contained in food vacuoles of a protozoan.

TABLE 1. Abundance and primary production of chroococcoid cyanobacteria in the epilimnion and hypolimnion of Lake Superior in September 1983.

	Epilimnion	Hypolimnion
Total autofluorescent cells (cells · mL^{-1})	44 001	60 799
Chroococcoid cyanobacteria (cells · mL^{-1})	40 471	54 312
Chroococoid Cyanobacteria in <1-μm size fraction (cells · mL^{-1})	34 214	41 504
Maximum primary production rate[a] (fg C · cell^{-1} · h^{-1})	7	6
Exponential growth rate[a] (d^{-1})	1.5	0.8
Primary production in <1-μm size fraction[b] (μg C · L^{-1} · h^{-1})	0.31	0.27
Calculated cyanobacteria production in <1-μm size fraction (abundance × production[a] = μg C · L^{-1} · h^{-1})	0.24	0.25
Total cyanobacteria production (abundance × production[a] = μg C · L^{-1} · h^{-1})	0.28	0.30
Percent of total primary production attributable to cyanobacteria	16	24

[a]Determined by track autoradiography.
[b]Determined by filter fractionation.

Discussion

Small phytoplankton are an important part of the Lake Superior ecosystem. Phytoplankton that passed through 3- and 1-μm screens were responsible for 48 and 20% of the primary production, respectively. Their importance appears to be a consistent feature of Lake Superior, since little seasonal or yearly variability was found. Our work is also consistent with that of Munawar et al. (1978), who found that phytoplankton <5 μm were the major primary producers in Lake Superior.

Fractionation experiments are relatively easy to perform, but can often be difficult to interpret without microscopic analysis

of fractionated size classes (Runge and Ohman 1982). We examined the components of <3- and <1-μm size classes by light, fluorescent, and electron microscopy after they had been fractionated. The <3-μm size class was dominated by microflagellates, eukaryotic green cells, and cyanobacteria (Fig. 2). The composition of this size class is very similar to that found in certain oceanic plankton communities (Johnson and Sieburth 1982). Chroococcoid cyanobacteria (mean size = 0.7 μm) dominated the <1-μm size class. Similar cyanobacteria were found in Lake Ontario (Caron et al. 1985) and in Grand Traverse Bay of Lake Michigan (Sicko-Goad and Stoermer 1984), suggesting that cyanobacteria may be a ubiquitous part of the Great Lakes ecosystem. In Lake Superior, these small organisms were responsible for 16–24% of the total primary production and exhibited relatively fast growth rates of 1.5 and $0.8 \cdot d^{-1}$ in the epilimnion and hypolimnion, respectively. Landry et al. (1984) found comparable growth rates of $1.4-1.7 \cdot d^{-1}$ for cyanobacteria at stations off Hawaii. The cyanobacteria from Lake Superior probably belong to the genus *Synechococcus*, since they are morphologically similar to those described as Type 1 by Johnson and Sieburth (1979).

The exact role of these small cyanobacteria in the Lake Superior food web is still uncertain; inevitably some portion of this production must be consumed directly. However, Lake Superior's zooplankton community is dominated by calanoid copepods (Watson and Wilson 1978), which are unable to effectively graze particles of this size (Vanderploeg 1981; Johnson et al. 1982). We believe that a sizeable fraction of picoplankton production is consumed by heterotrophic protozoans. We found considerable evidence that protozoans can ingest cyanobacteria in Lake Superior (i.e. Fig. 3). Although our evidence is primarily for ingestion, Johnson et al. (1982) found that two microheterotrophs could use and subsist on similar sized cyanobacteria. Furthermore, heterotrophic protozoans are a common part of the Lake Superior plankton community. Munawar and Munawar (1978) found Lake Superior to be dominated by flagellates, many of which are heterotrophs (e.g. *Katablepharis*, *Cryptaulax*, and some *Ochromonas* spp.). Our preliminary estimates of protozoan (flagellates and nonflagellates) abundance are $400-500$ cells $\cdot mL^{-1}$. If we assume that these protozoans have a maximum filtering rate of $1.9\ L \cdot cell^{-1} \cdot d^{-1}$ (Fenchel 1982), we can expect a maximum loss rate of $0.76-0.95 \cdot d^{-1}$. This loss rate would be a significant fraction of the cyanobacteria growth rate and suggests that protozoans may be significant grazers of cyanobacteria.

Our evidence for the importance of protozoans in grazing chroococcoid cyanobacteria in Lake Superior is not very complete; however, our contention has support from work in other aquatic environments. In many marine systems, protozoans are an important link in the food web as part of the so-called "microbial loop" (Azam et al. 1983). Heterotrophic protozoans provide the link between small producers and larger multicellular zooplankton (Haas and Webb 1979; Linley et al. 1983; Sherr and Sherr 1984). This same link may also be an important part of the Lake Superior food web. Since approximately 20% of the primary production originates in phyoplankton that are not readily consumed by copepods, protozoans in the 3- to 20-μm range may be important in passing this production to the "classical" food web. Conventional thought regarding carbon and nutrient cycling would be profoundly affected by this "microbial loop" (Sherr and Sherr 1984).

Much more work is needed to confirm this hypothesis, but our results clearly indicate the importance of chroococcoid cyanobacteria and their ingestion by protozoans.

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