Int. Revue ges. Hydrobiol. | 76 | 1991 | 4 | 603–616

GARY L. FAHNENSTIEL[1,4], HUNTER J. CARRICK[1,5], CLARE E. ROGERS[2], and LINDA SICKO-GOAD[3]

[1] Great Lakes Environmental Research Laboratory/NOAA, 2205 Commonwealth Blvd., Ann Arbor, MI 48105, USA
[2] Simpson Flow Cytometry Facility, Dept. Internal Medicine, The University of Michigan, Ann Arbor, MI 48109, USA
[3] Center for Great Lakes and Aquatic Science, The University of Michigan, Ann Arbor, MI 48109, USA

# Red Fluorescing Phototrophic Picoplankton in the Laurentian Great Lakes: What Are They and What Are They Doing?

*key words:* epifluorescence microscopy, flow cytometry, transmission electron microscopy, red fluorescing phototrophic picoplankton, Laurentian Great Lakes

## Abstract

Epifluorescence microscopy, flow cytometry, and transmission electron microscopy were used to characterize the community of red fluorescing (emission > 665 nm when excited with blue light) phototrophic picoplankton (RFPP) in lakes Huron and Michigan. A population of coccoid to ovate eukaryotic cells with a mean size of 1.2 µm dominated the RFPP community in both surface and deep water samples. Abundant prochlorophyte populations were not found in any samples. Comparisons of counts with epifluorescence microscopy and flow cytometry, revealed that RFPP were adequately enumerated with standard epifluorescence microscopy. These RFPP were significant contributors to total phototrophic picoplankton abundance in both lakes Michigan (24 %) and Huron (18 %), with maximum seasonal abundance during the May–June period (surface mixing layer temperatures, 3–9 °C). During thermal stratification, maximum vertical abundance was found in the metalimnion/hypolimnion at the 1–5 % isolumes. RFPP were only minor contributors (1–7 %) to total primary production. Growth rates of RFPP measured with dilution and small inocula growth experiments ranged from 0.05–1.0 $d^{-1}$. Microzooplankton grazing rates on RFPP measured with dilution experiments were similar to estimated growth rates, accounting for 52–280 % of growth on any given date.

## 1. Introduction

Phototrophic picoplankton are important components of freshwater and marine pelagic food webs (for review, see STOCKNER and ANTIA 1986). Several groups of algae can be represented in these communities including chlorophytes, prasinophytes, prochlorophytes, and cyanophytes (cyanobacteria) (STOCKNER and ANTIA 1986; CHISHOLM *et al.* 1988). Some of these groups can be identified by their fluorescent signatures with routine epifluorescence microscopy (TSUJI *et al.* 1986). The phycobilin-contraining cyanobacteria are easily identified by their yellow/orange emission when excited with blue light (phycoerythrin-dominant) or by their near-red emission (< 665 nm, phycocyani-dominant) when excited with green light. The remaining groups, chlorophytes, prochlorophytes and prasinophytes, fluoresce red (> 665 nm) when excited with blue or green light due to the dominance of chlorophyll or chlorophyll-like pigments (TSUJI *et al.* 1986; CHISHOLM *et al.* 1988). Until the recent discovery of abundant prochlorophytes by CHISHOLM *et al.* (1988), all red-fluorescing phototrophic picoplankton (RFPP) were thought to be eukaryotes (MURPHY and HAUGEN 1985).

---

[4] Also CGLAS, Univ. Michigan, Ann Arbor, Mi.
[5] Present address: Dept. Fisheries and Aquaculture, 7922 NW 71st St., Univ. Florida, Gainesville, FL. 32606

Despite the potential diversity of phototrophic picoplankton communities, most of the ecological and physiological work has focused on chroococcoid cyanobacteria of the genus *Synechococcus* (STOCKNER and ANTIA 1986). The RFPP (chlorophyll or chlorophyll-like pigment fluorescence) have received far less attention and very little physiological or ecological information exists (STOCKNER and ANTIA 1986). Not surprisingly, RFPP are more difficult to identify and count with epifluorescence microscopy due to their often weak and rapidly fading chlorophyll fluorescence (MURPHY and HAUGEN 1985; CHISHOLM *et al.* 1988). Nevertheless, RFPP are more abundant than *Synechococcus* in the lower euphotic zone of the North Atlantic and Pacific Oceans (MURPHY and HAUGEN 1985; CHISHOLM *et al.* 1988), and also may be important in freshwater environments.

This study was initiated to provide a first look at the RFPP in the Laurentian Great Lakes with particular regards to their characterization and dynamics. With the recent discovery of prochlorophytes in the ocean (CHISHOLM *et al.* 1988), the presence of RFPP in freshwaters is uncertain and potentially interesting. Because very little is known about the dynamics of RFPP in any environment, we also examined their dynamics in lakes Huron and Michigan as part of a larger study on phototrophic picoplankton in these lakes.

## 2. Description of Study Lakes

Lakes Huron and Michigan are two of the largest freshwater lakes in the world with surface areas and volumes exceeding 55,000 $km^2$ and 3,500 $km^3$, respectively (HERDENDORF 1982). The limnological characteristics of these lakes are relatively similar. Based on their biological and chemical characteristics, both of these lakes have been classified as oligotrophic (BEETON 1965; DOBSON *et al.* 1974). Phosphorus is the element that limits phytoplankton growth (SCHELSKE *et al.* 1974; LIN and SCHELSKE 1981). During the spring isothermal mixing period which lasts from March until late May/early June in the southern regions of these lakes, total phosphorus concentrations range from 0.1–0.3 μM (DOBSON *et al.* 1974; LESHT and ROCKWELL 1985). During summer thermal stratification, surface soluble reactive phosphorus concentrations are at or below the detection level (< 0.01 μM, LESHT and ROCKWELL 1985; LAIRD *et al.* 1987). Nitrate concentrations in both lakes generally exceed 15 μM throughout the year. Surface silica concentrations decrease from approximately 15–30 μM during the spring mixing period to ≤ 5 μM during thermal stratification in Lake Michigan and 5–15 μM in Lake Huron (LESHT and ROCKWELL 1985; LAIRD *et al.* 1987). In the surface-mixed layer phytoplankton chlorophyll concentrations range from 0.5–3.0 $mg \cdot m^{-3}$ with highest values during the spring mixing period (FAHNENSTIEL *et al.* 1989; LESHT and ROCKWELL 1985). The extinction coefficient of light (PAR) ranges from 0.14–0.23 $m^{-1}$: minimum values (0.14–0.17) occur in the July/early August period (SCAVIA *et al.* 1986; G. FAHNENSTIEL, unpubl. data).

## 3. Materials and Methods

Sampling was conducted at two offshore stations in Lake Huron (northern 45°25′ N, 82°55′ W, and southern 43°56′ N, 82°21′ W) and one offshore station in Lake Michigan (43°1′ N, 86°37′ W). The Lake Huron stations were sampled 26 times from 1986–1988 and the Lake Michigan station was sampled 17 times from 1986–1990. All water samples were collected with 5- or 30-L PVC Niskin bottles. Surface-mixed layer samples were collected at mid-depth if the surface mixed layer was < 20-m, and at 5–10 m if the surface mixed layer was > 20-m. Temperature was measured with a bucket thermometer and electronic bathythermograph. Underwater scalar irradiance was meas-

ured with a Licor LI-193SB sensor and LI-188B integrating meter. Chlorophyll concentrations were determined fluorometrically on 90 % acetone-extracted samples (STRICKLAND and PARSONS 1972).

Water samples for microscopical analysis were preserved with glutaraldehyde (1 % final conc.) buffered with sodium cacodylate (0.1 M). These picoplankton samples were refrigerated until duplicate slides were prepared within 24 h (WATERBURY *et al.* 1979) and were then frozen (–20 °C). All picoplankton slides were counted within a few days to minimize errors due to fading of autofluorescence.

Picoplankton abundance and composition was determined from each slide by enumerating a minimum of 500 cells using a Leitz Laborlux Microscope (mag. 1250X) or Jena Lumar Research Microscope (mag. 1400X). The length and breadth of at least 20 individuals of each population of RFPP were measured twice during each major season from projections of photomicrographs. Both microscopes used for epifluorescence enumerations were similarly equipped to distinguish the dominant autofluorescent emission of individual phototrophs with blue (450 nm) or green (530–560 nm) excitation. The RFPP were characterized by their red fluorescence (> 665 nm) when excited with blue light. For this paper, RFPP were defined as those cells that fluoresce red (emission > 665) when excited with blue light and measured < 3 µm in all dimensions. Phycocyanin-containing cyanobacteria were not included in this RFPP group.

Our standard procedure for counting preserved picoplankton samples may underestimate the abundance of RFPP due either to their weak autofluorescence (CHISHOLM *et al.* 1988) or to their destruction (MURPHY and HAUGEN 1985). To evaluate our standard counting procedure, we compared epifluorescence counts of preserved samples to counts of unpreserved samples with epifluorescence microscopy and flow cytometry. These comparisons were performed on both surface (5–10 m) and deep populations (50–60 m).

Flow cytometry was used to evaluate our standard counting procedure as well as to assist in the identification of RFPP. To maximize the autofluorescence of RFPP and thereby enhance their identification with flow cytometry, samples from both surface (5–10 m) and deep (40–60 m) regions were collected during low light periods (spring isothermal mixing and late thermal stratification). On April 8th, the surface mixed depth was equal to water column depth (100 m) and on November 7th, the surface mixed layer was approximately half the water column depth. These samples were filtered through a 3-µm Nuclepore filter prior to analysis. A Coulter EPICS (Coulter Cytometry, Hialeah. FL. USA) equipped with an argon laser operating at an excitation wavelength of 488 nm was used for all analysis and sorting. The standard 78-µm orifice tip was used, and the neutral density filter in front of the forward angle light scatter (FALS) detector was removed to enhance the signals of small particles (< 3 µm). Both FALS and 90° light scatter were collected. The light scatter of particles < 2 µm is barely above the background noise of our system; therefore, wide, unrestrictive gates were used to collect fluorescence histograms. Consequently, the number of particles having little or no autofluorescence (subpopulation c) may be overestimated. Two parameter (2P) histograms containing 64 channels on each axis were collected with logarithmic amplifiers and in list mode. Log-integrated red fluorescence (LIRFL) was defined as all light passed by a 665 nm long pass filter; log-intergrated orange fluorescence (LIOFL) was defined as the light that passed a 590 nm shortpass dichroic mirror. Standard programs provided by Coulter were used to determine cell numbers and mean fluorescence values for each subpopulation. Mean log values were converted to mean linear values using the method of MUIRHEAD *et al.* (1983). In order to compare sample runs from different days, fluoresbrite carboxylate microspheres of approximately 1.1 µm were added to each sample to serve as a standard. Relative counts, mean fluorescence intensity, and mean FLS of the individual cells were compared among the subpopulations. For flow cytometry, specific populations were identified by sorting and microscopic analysis.

An isolate of the RFPP population from southern Lake Huron was cultured in WC media (STANIER *et al.* 1971). Transmission electron microscopy and epifluorescence microscopy were used to characterize and identify this clone. For transmission electron microscopy, this clone was preserved with a mixture of 1 % glutaraldehyde and 1 % paraformaldehyde buffered with 0.1 M sodium cacodylate. TEM samples were then gently concentrated by centrifugation, rinsed four times with 0.1 M sodium cacodylate buffer (pH 7.2), and post-fixed with 1 % $OsO_4$ in cacodylate buffer for 1 h at 4 °C. These cells were dehydrated with a graded ethanol and propylene oxide series and then were embedded in Epon. Sections were cut with a diamond knife, collected on clean, 200 mesh copper grids, and were stained with aqueous uranyl acetate. Sections were then examined with a JEM 100B electron microscope operating at 80 kV.

Primary production was estimated with the $^{14}C$ technique as outlined in FAHNENSTIEL and SCAVIA (1987) and FAHNENSTIEL *et al.* (1989). Water collected from the surface mixed layer was immediately dispensed into shaded, 2-l polycarbonate bottles, inoculated with 200 μCi of $NaH^{14}CO_3$, and incubated for 1–2 h at 200–300 μ Einst. $\cdot m^{-2} \cdot s^{-1}$ in a shipboard incubator. Following incubation, three subsamples (100 ml) were filtered onto membrane filters (0.22-μm Millipore), decontaminated with 0.5 ml of 0.5 N HCl for 4–6 h, placed in scintillation vials with 12 ml of scintillation cocktail, and assayed with a Packard Tri-Carb scintillation counter. Also, a subsample was filtered through a 2-μm Nuclepore filter and the filtrate was retained for autoradiography.

Autoradiographs were prepared by preserving the < 2-μm fraction with 1 % glutaraldehyde (final conc.), buffered with 0.1 M sodium cacodylate. Subsamples of 5–20 ml were concentrated onto 0.2-μm Nuclepore filters, transferred onto subbed coverslips and then dipped and developed for track autoradiography (KNOECHEL and KALFF 1976; CARNEY and FAHNENSTIEL 1987). Tracks per cell were converted to carbon specific uptake rates using the equations of KNOECHEL and KALFF (1976) and CARNEY and FAHNENSTIEL (1987). The contribution of RFPP to total primary production was determined by estimating their population production (cellular rate from autoradiography × abundance) and then dividing by the total primary production rate.

Small inocula growth experiments (SIG) were used to estimate growth rates of RFPP (FAHNENSTIEL and SCAVIA 1987). A 4-l polycarbonate bottle containing prefiltered lake water (< 0.2 μm, Gelman mini-capsule) was inoculated with lake water (100 ml) previously passed through a 3-μm Nuclepore filter. This bottle was incubated at ambient light (similar to mean depth of surface mixed layer) and temperature in a shipboard incubator and was sampled at 0, 24, 36, and 48 h. Growth rates were determined from changes in abundance.

Growth rates of RFPP and grazing rates by microzooplankton (< 153 μm) were estimated with the dilution technique (LANDRY and HASSETT 1982). Microzooplankton abundances were manipulated through a series of bottle dilutions after which changes in RFPP abundance were measured in each bottle. Bottle dilutions (10-, 8-, 5-, 3-, and 0-fold dilutions) were performed in 4-l polycarbonate bottles by mixing appropriate volumes of pre-screened lake water (< 153-μm) with filter sterilized lake water (< 0.2-μm; Gelman Mini-Capsules). Each bottle was augmented with phosphate (0.6 $\mu M \cdot l^{-1}$) to alleviate potential nutrient recycling between herbivores and algae. All bottles were incubated under ambient conditions in a shipboard incubator and sampled at 0 and 24 h. Because increasing bottle dilution alleviates grazing pressure, the slope of the growth rate of RFPP (dependent variable) across dilution treatments (independent variable) is an estimate of the grazing loss rate; the intercept is an estimate of the growth rate.

## 4. Results

### *a) Characterization and Enumeration*

A combination of epifluorescence microscopy, flow cytometry, and transmission electron microscopy was used to characterize and enumerate the population of RFPP in lakes Huron and Michigan. Four distinct groups of phototrophic picoplankton were noted with epifluorescence microscopy which was the principal method of enumeration. These four groups were distinguished by their dominant autofluorescent signal when excited with blue or green light. Only one of these groups was RFPP as defined by red emission > 665 nm when excited with blue light (Fig. 1); the others were phycobilin-containing cyanobacteria with dominant emissions < 665 nm. These RFPP fluoresced very weakly when excited with green light, which indicated limited amounts of accessory pigment.

Although this group of RFPP was temporally and spatially distributed in both lakes, they appear to constitute one distinct group. The cells were coccoid to ovate in shape and ranged in size from 0.8–1.7 μm with a mean of 1.2 μm (Fig. 1). The red fluorescence of these cells was particularly weak during the period of summer stratification; immediate slide preparation and counting was necessary. The only other red fluorescing cells observed from standard epifluorescence microscopy were somewhat larger 3–5 μm nanoflagellates (CARRICK and FAHNENSTIEL 1989).

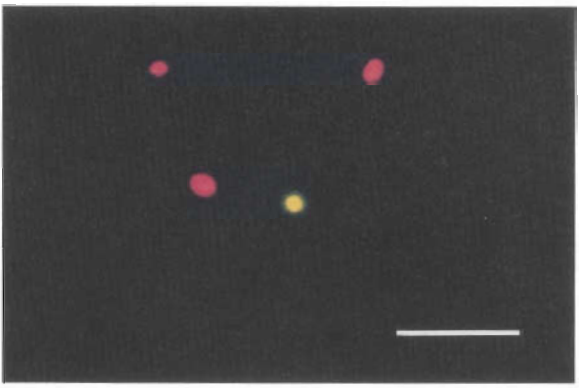

Figure 1. Epifluorescent photomicrograph of phototrophic picoplankton from Lake Michigan. Red-fluorescing cells are eukaryotic phototrophic picoplankton and yellow-fluorescing cells are the chroococcoid cyanobacterium *Synechococcus*. (Bar = 5 μm).

Flow cytometry distinguished three groups of picoplankton based on a combination of their orange and red autofluorescence (Groups *a–c*, Figs. 2 and 3). These three groups were consistently found in both surface and deep samples. Two of these groups were phototrophic picoplankton; one group was rich in orange fluorescence (emission < 590 nm, group *b* in Figs. 2 and 3), whereas the other group was rich in red fluorescence (emission > 665 nm, group *a* in Figs. 2 and 3). The group rich in orange fluorescence was phycoerythrin-containing cyanobacteria of the genus *Synechococcus*. This *Synechococcus* population had a orange to red fluorescence ratio of 5.

The group of cells rich in red fluorescence (group *a*, Figs. 2 and 3) appeared to be the same group routinely enumerated with epifluorescence microscopy. These cells comprised one population that had 14–25 times more red fluorescence than orange fluorescence (Figs. 2–3). Surface and deep water samples had similar populations (i.e. fluorescence properties, FALS) of RFPP. The main size of these RFPP as determined by comparisons to standard beads, was 1.2 μm. To confirm that this population (group *a*, Fig. 2 and 3) was the same population counted with routine epifluorescence microscopy, this population was sorted with flow cytometry and analyzed with epifluorescence microscopy; the flow cytometry population was identical to the population of RFPP routinely enumerated with epifluorescence microscopy.

The third group of picoplankton isolated with flow cytometry exhibited very little red or orange fluorescence (group *c* Figs. 2 and 3). These particles exhibited slightly more orange fluorescence than red fluorescence (ratio 1.5). We believe this population was comprised of particles having no natural autofluorescence and, therefore, were not phototrophic. When this population was sorted and then analyzed with epifluorescence microscopy, no autofluorescent organisms were noted. Most of the particles in this group appeared to be heterotrophic bacteria as determined by standard acridine orange counts. Because our flow cytometry system was working close to the background noise level, it is not unusual to count a large number of particles with little or no autofluorescence. The possibility that prochlorophytes were included in this group is unlikely, because these particles exhibited more orange fluorescence than red; prochlorophytes exhibit little or no orange fluorescence (CHISHOLM *et al.* 1988).

To evaluate the possibility that RFPP abundance is underestimated with epifluorescence microscopy (CHISHOLM *et al.* 1988; LI and WOOD 1988), we compared standard epifluorescence counts from preserved samples with counts of live samples with flow

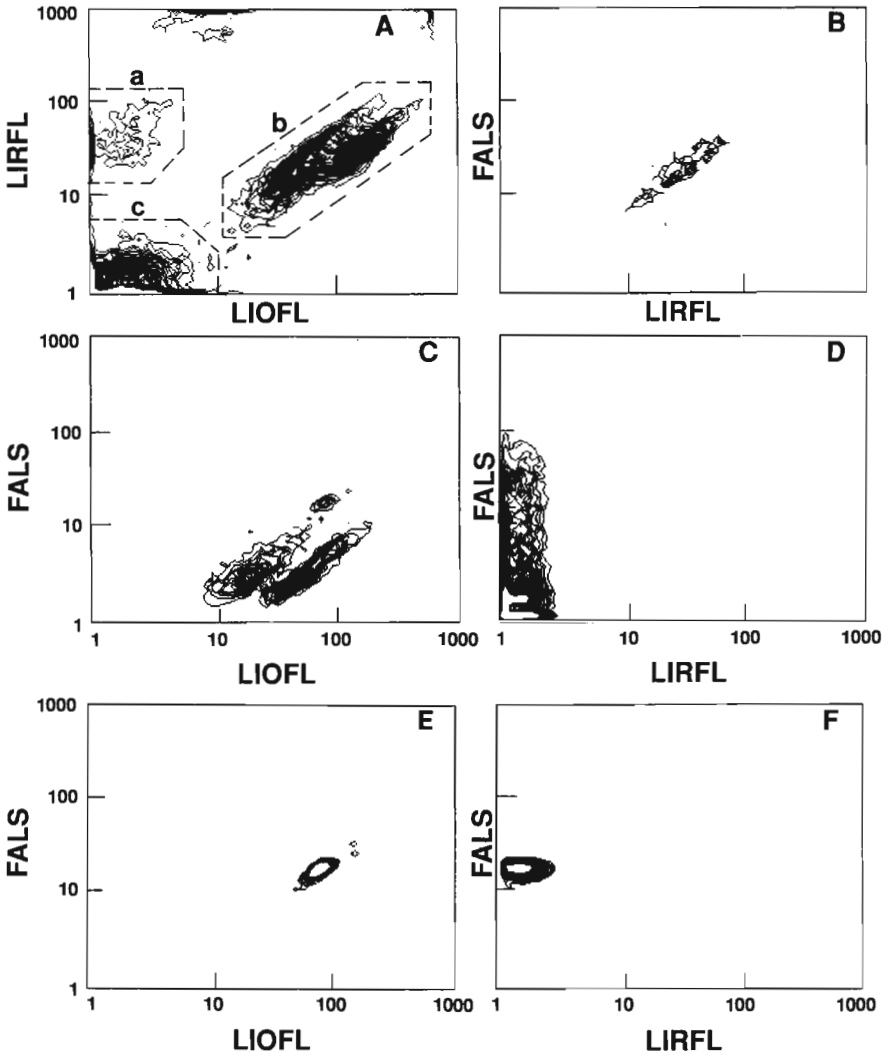


Figure 2. Flow cytometric signature of Lake Michigan picoplankton (< 3 μm) from November 11, 1989. *A*) Two-dimensional histogram of log-integrated red fluorescence (LIRFL) and log-integrated orange fluorescence (LIOFL). Three populations of particles were noted (dashed lines): (*a*) red-fluorescing phototrophic picoplankton (RFPP), (*b*) chroococcoid cyanobacteria, and (*c*) particles with little fluorescence (heterotrophs) and background. *B*) Two-dimensional histogram of forward angle light scatter (FALS) and LIRFL for the population of RFPP (Group *a*, Fig. 2*A*). *C*) Two-dimensional histogram of FALS and LIOFL for the group of chroococcoid cyanobacteria (Group *b*, Fig. 2*A*). *D*) Two-dimensional histogram of FALS and LIRFL for particles designated Group *c*, Fig. 2*A*. *E*) Two-dimensional histogram of FALS and LIOFL for 1.1 μm fluorescent beads. *F*) Two-dimensional histogram of FALS and LIRFL for 1.1 μm fluorescent beads.

cytometry. Flow cytometry is a particularly useful approach for enumerating weakly fluorescent particles like RFPP (CHISHOLM *et al.* 1988). However, quantitative flow cytometry requires knowledge of the exact volume of suspension analyzed, and this information was not collected during our analyses. To compare flow cytometry and epifluorescence

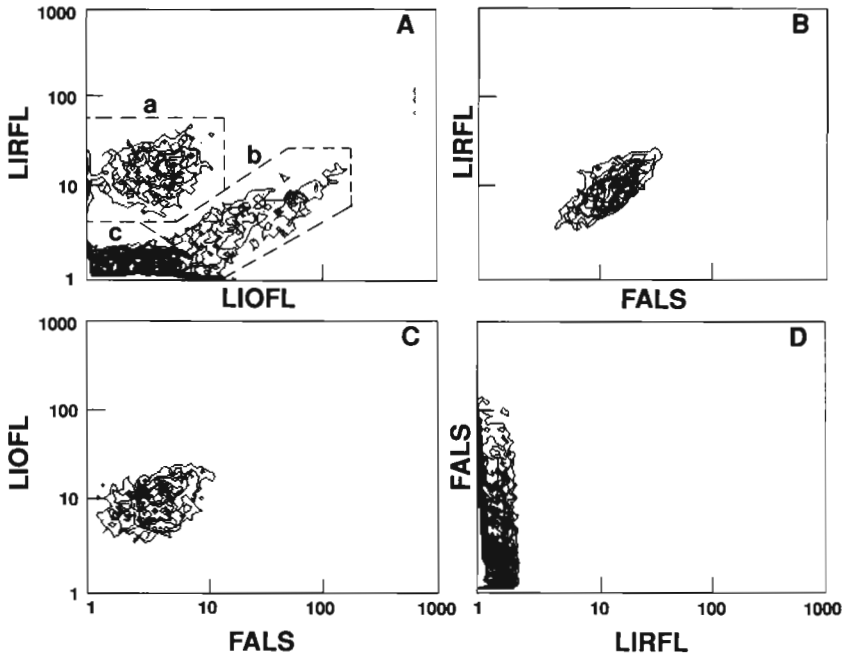


Figure 3. Flow cytometric signatures of Lake Michigan picoplankton (<3 µm) from April 8, 1990. *A*) Two-dimensional histogram of LIRFL and LIOFL (Note the same three groups of particles found in Figure 2, similarly designated as Groups *a–c*). LIRFL, LIOFL and other abbreviations as defined in Figure 2. *B*) Two-dimensional histogram of LIRFL and FALS for population of RFPP (Group *a*, Fig. *3A*). *C*) Two-dimensional histogram of LIOFL and FALS for chroococcoid cyanobacteria (Group *b*, Fig. *3A*). *D*) Two-dimensional histogram of LIRFL and FALS for particles designated Group *c*, Fig. *3A*.

microscopy, we used the ratio of RFPP: yellow/orange phototrophs. Yellow/orange-fluorescing chroococcoid cyanobacteria are relatively robust cells that are easily enumerated with both epifluorescence microscopy and flow cytometry; therefore, they can serve as a standard (Li and Wood 1988). On the two dates examined, the ratio of RFPP: yellow/orange phototrophs was similar between techniques (flow cytometry-0.019 and 4.2; epifluorescence microscopy-0.026 and 3.0). Also, on several occasions we compared our standard epifluorescent counts of preserved samples to immediate epifluorescent counts of live samples. Our standard epifluorescent counts averaged 83 % of the immediate live counts with a range of 65–118 %. Thus, the results from these two comparisons, epifluorescence vs. flow cytometry and preserved epifluorescence vs. live epifluorescence, confirmed that most, if not all, RFPP were enumerated with epifluorescence microscopy. If some RFPP were missed at certain times of the year, they are only a minor fraction of the total population.

The clone of RFPP isolated from Lake Huron had similar pigment fluorescence and morphology as the natural population of RFPP. The cultured population consisted of coccoid to ovate cells approximately 1.2 µm in diameter that had a red to orange fluorescence ratio of 20. Thus, this isolate appears to be a representative of the dominant population of RFPP in lakes Huron and Michigan and can be used to provide identification of the natural population. Transmission electron micrographs of this cultured isolate showed it to be eukaryotic (Fig. 4). These RFPP were *Chlorella*-like cells that possessed single

membrane-bound chloroplast, nucleus, and mitochondria (Fig. 4). The chlorophyte designation of these cells is also supported by their lack of accessory pigment (reduced red emission when excited with green light).

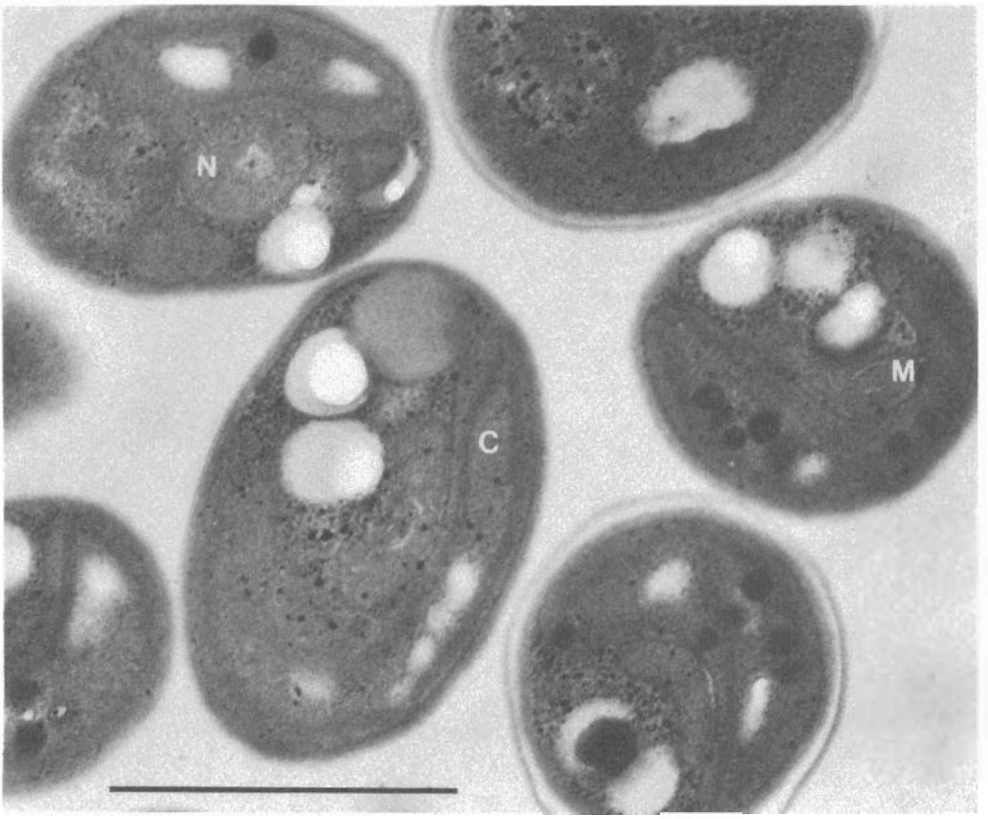


Figure 4. Transmission electron micrograph of cultured isolate of red-fluorescing phototrophic picoplankton from Lake Huron. These *Chlorella*-like eukaryotic cells possess a single chloroplast (C), nucleus (N) and mitochondria (M). Horizontal bar = 1 μm.

### *b) Abundance, distribution, growth and grazing*

RFPP were abundant throughout the year in both lakes Huron and Michigan (Fig. 5 and 6), with maximum abundance occurring in both lakes during the late spring period of isothermal mixing to the onset of thermal stratification (May–June). In Lake Huron RFPP abundance ranged from 165 cells · ml$^{-1}$ at 5 m in December 1986 to 19,300 cells · ml$^{-1}$ at 5 m in May 1988 (Fig. 5). Mean abundance during the 3-yr sampling period was 4,900 cells · ml$^{-1}$. In Lake Michigan RFPP abundance ranged from 1,000 cells · ml$^{-1}$ at 35 m in October 1987 to 16,900 cells · ml$^{-1}$ at 20 m in June 1987 (Fig. 6), with mean abundance of 7,100 cells · ml$^{-1}$.

During the periods of isothermal mixing, abundance of RFPP was uniform with depth. During periods of thermal stratification, a subsurface peak in abundance was noted in the lower metalimnion/upper hypolimnion at depths receiving 1–5 % of surface irradiance (Fig. 7).

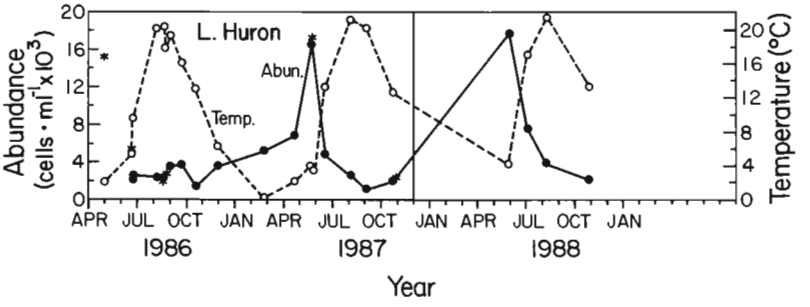


Figure 5. Seasonal pattern of red-fluorescing phototrophic picoplankton abundance (—) and temperature (- -) from the surface mixed layer of southern Lake Huron. Abundance estimates from a northern Lake Huron station also are provided (*).

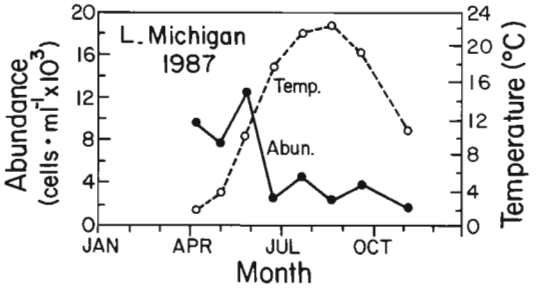


Figure 6. Seasonal pattern of red-fluorescing phototrophic picoplankton abundance (—) and temperature (- -) from the surface mixed layer of Lake Michigan.

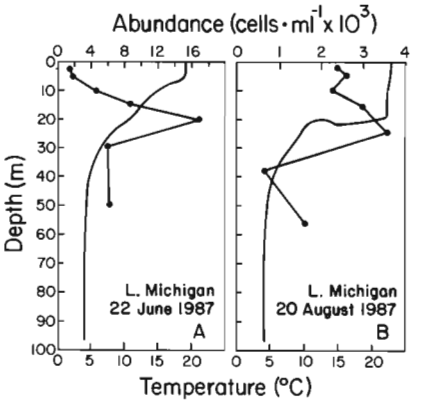


Figure 7. Vertical profiles of red-fluorescing phototrophic picoplankton abundance (•—•) and temperature (—) during the period of thermal stratification in Lake Michigan. *A*) 22 June 1987, *B*) 20 August 1987.

On five dates in 1988, the cellular primary production rates of RFPP were determined with track autoradiography (Table 1). Cellular production rates varied from 3.2 to 11.6 fg C · cell$^{-1}$ · h$^{-1}$, with highest values during the period of spring isothermal mixing/ early stratification. The contribution of RFPP to total primary production varied from 0.9–7.3 % (Table 1).

Table 1. Cellular primary production rates, abundance, and contribution to total primary production of RFPP in lakes Huron (LH) and Michigan (LM).

| Date | Abundance (cells · m$^{-1}$) | Production Rate (fg · cell$^{-1}$ · h$^{-1}$) | Contribution (%) |
|---|---|---|---|
| 11/5/1988 LM | 12,240 | 11.6 | 2.3 |
| 14/6/1988 LM | 12,764 | 10.6 | 7.3 |
| 22/6/1988 LH | 7,647 | 4.0 | 2.0 |
| 26/6/1988 LH | 5,602 | 3.2 | 1.6 |
| 13/10/1988 LH | 3,048 | 8.5 | 0.9 |

Growth rates of RFPP determined with two techniques, SIG and dilution, varied from 0.05–1.00 d$^{-1}$ with a mean of 0.36 d$^{-1}$ (Table 2). Highest growth rates were found during periods of spring isothermal mixing (April–May) and transitional thermal stratification (May–June and October–December). Although there was much variability between estimates on any one date, overall the two techniques exhibited reasonably good agreement (paired $t$-test: $t = 1.28$, $n = 8$, $p = 0.24$).

Grazing by microzooplankton (< 153 µm) was a major loss for RFPP (Table 2). Grazing loss rates ranged from –0.11 to –0.89 d$^{-1}$ with a mean of –0.32 d$^{-1}$. Microzooplankton grazing accounted for 52–280 % of growth on the dates examined (Table 2).

## 5. Discussion

Until the recent discovery of abundant prochlorophytes in the Atlantic and Pacific Oceans (CHISHOLM *et al.* 1988), RFPP were presumed to be eukaryotes (GLOVER *et al.* 1985; MURPHY and HAUGEN 1985; LI and WOOD 1988). The discovery of these small prochlorophytes has forced us to revaluate our ideas on the structure and abundance of phototrophic picoplankton communities. For example, if prochlorophytes are abundant, then previous estimates of phototrophic picoplankton abundance are probably low because standard epifluorescence microscopy likely underestimates prochlorophyte abundance (CHISHOLM *et al.* 1988). Yet, in freshwater ecosystems little is known about the abundance and distribution of these prochlorophytes.

In this study, a combination of epifluorescence microscopy, flow cytometry and transmission electron microscopy was used to characterize the RFPP community which allowed for more detailed descriptions than has hitherto been possible. From examination of natural samples with epifluorescence microscopy and flow cytometry, it was clear that the RFPP community in lakes Huron and Michigan was dominated by coccoid to ovate cells approximately 1.2 µm in diameter which contained limited amounts of accessory pigment. Moreover, these RFPP were adequately enumerated with standard epifluorescence microscopy. With transmission electron microscopy, these cells were found to be eukaryotic and relatively similar to other previously described *Chlorella*-like cells from other environments (DEMPSEY *et al.* 1980; JOHNSON and SIEBURTH 1982; JOINT and PIPE 1984). Throughout our study, prochlorophytes did not appear to be present. Our evidence

Table 2. Growth rates, microzooplankton (< 153 µm) grazing loss rates ($d^{-1}$), and percent of growth balanced by microzooplankton grazing for RFPP populations from lakes Huron (LH) and Michigan (LM). Growth rates were determined from dilution and small inocula growth (SIG) experiments and microzooplankton grazing rates from dilution experiments.

| Date | Growth Rates ($d^{-1}$) | | Grazing Rates ($d^{-1}$) | Graz./Growth as % |
|---|---|---|---|---|
| | SIG | Dilution | Dilution | |
| 29/4/86 LH | 0.61 | | | |
| 30/4/86 LH | 0.66 | | | |
| 22/6/86 LH | 0.48 | | | |
| 17/8/86 LH | 0.57 | | | |
| 1/5/87 LM | | 0.39 | −0.22 | 56 |
| 18/5/87 LH | | 0.39 | −0.23 | 59 |
| 23/6/87 LH | 0.60 | 0.30 | −0.19 | 42 |
| 29/7/87 LH | 0.20 | 0.28 | −0.52 | 217 |
| 20/8/87 LM | 0.10 | 0.20 | −0.42 | 280 |
| 15/10/87 LH | 1.00 | 0.58 | −0.89 | 113 |
| 2/11/87 LM | 0.12 | 0.26 | −0.16 | 84 |
| 11/4/88 LM | 0.17 | 0.21 | −0.28 | 147 |
| 10/5/88 LM | 0.37 | 0.05 | −0.11 | 52 |
| 18/5/88 LH | 0.23 | 0.11 | −0.19 | 112 |

for the dominance of the RFPP community by *Chlorella*-like cells does not preclude the presence of prochlorophytes in the Laurentian Great Lakes; rather it suggest that if prochlorophytes are present, they are but a minor component of the RFPP community.

RFPP are a significant component of the phototrophic picoplankton community in lakes Huron and Michigan, constituting approximately 18 % and 24 %, respectively, of total phototrophic picoplankton abundance. During the period of spring isothermal mixing, RFPP abundance exceeded 15,000 cells · $ml^{-1}$ in both lakes, and constituted over 50 % of the total phototrophic picoplankton abundance (G. FAHNENSTIEL, unpubl. data).

In contrast to our findings, eukaryotic RFPP do not appear to be a significant component of the phototrophic picoplankton communities in other freshwater environments. In Lake Constance the phototrophic picoplankton community was almost exclusively chroococcoid cyanobacteria (WEISSE 1988) and phycobilin-containing cyanobacteria also dominated in several other lakes (CARON *et al.* 1985; HARDY *et al.* 1986; NAGATA 1986; PICK and CARON 1987; KENNAWAY and EDWARDS 1989). Eukaryotic RFPP were not observed as significant components of the phototrophic picoplankton community in any of these lakes. Red-fluorescing spherical cells 2–3 µm in diameter were noted in Lake Biwa, but these cells were only a very minor component of the picoplankton, with a maximum abundance of 1,800 cells · $ml^{-1}$. Because our RFPP were adequately enumerated with epifluorescence microscopy, it is unlikely that these cells were missed in the other freshwater environments. The lack of RFPP abundance in these other environments is noteworthy.

Abundant populations of RFPP are a common feature of many marine environments (GLOVER *et al.* 1985; MURPHY and HAUGEN 1986; CHISHOLM *et al.* 1988; JOCHEM 1988; LI and WOOD 1988), and in this respect lakes Huron and Michigan are more similar to these marine systems than to other freshwater environments. However, several populations of red-fluorescing cells including prochlorophytes may be present in some marine environments (CHISHOLM *et al.* 1988; LI and WOOD 1988), whereas in lakes Huron and Michigan only one population of eukaryotic cells was found. More work is needed on the

abundance and composition of RFPP communities, particularly in freshwater environments.

The pattern of seasonal RFPP abundance is one of the first noted for RFPP. The seasonal abundance peak occurred during spring isothermal mixing/early thermal stratification, when surface water temperatures were between 3 and 9 °C. Environmental conditions appear to favor growth during this period as growth rates at this time were among the highest observed (Table 2). This seasonal abundance pattern is in marked contrast to the seasonal abundance peak noted for *Synechococcus* in temperate freshwater and marine environments: *Synechococcus* typically reaches maximum abundance in midsummer to early fall, when surface water temperatures are at or near their maximum (KREMPIN and SULLIVAN 1981; CARON *et al.* 1985; JOINT 1986; JOCHEM 1988).

During the period of thermal stratification, RFPP exhibited a subsurface abundance maximum similar to those noted for other populations of RFPP in marine environments (MURPHY and HAUGEN 1985; GLOVER *et al.* 1986). However, the maximum in lakes Huron and Michigan occurred at the 1–5 % isolumes whereas in the marine environment the maximum occurred near the 0.5 % isolume. These slight differences between isolumes of abundance maxima are likely due to differences in the spectral quality of light with depth. Picoplankton-sized eukaryotes are particularly well-adapted to harvesting blue light (GLOVER *et al.* 1986), and in the oceanic environment more blue light is found at depth than in the Great Lakes.

Although RFPP are relatively abundant and significant contributors to phototrophic picoplankton abundance, they contribute only a minor fraction (range 1–7 %) of total primary production in lakes Huron and Michigan. This contribution is in marked contrast to oceanic environments, where RFPP communities dominated by prochlorophytes contributed 15–60 % of the total primary production (CHISHOLM *et al.* 1988).

Growth rates of RFPP demonstrate their capability for rapid population increase as rates ranged from 0.05–1.00 $d^{-1}$. These growth rates are in the range of values reported for *Synechococcus* populations from the Great Lakes (0.1–1.5; FAHNENSTIEL *et al.* 1986, FAHNENSTIEL *et al.* 1991) and Lake Constance (0.1–1.2; WEISSE 1988) and for prochlorophyte populations from the oceans (0.07–0.35; CHISHOLM *et al.* 1988).

Most of the RFPP production appears to be consumed within the microbial foodweb; grazing by microzooplankton (< 153 µm) accounted for 42–280 % of the growth rate on any given date. Although we do not have data on the role of specific micrograzers for RFPP populations, grazing experiments on *Synechococcus* which is similar in size to RFPP, suggest that the dominant grazers in this fraction (< 153 µm) are heterotrophic flagellates and ciliates (FAHNENSTIEL *et al.* 1991). It is likely that these same flagellates and ciliates are the dominant grazers of RFPP.

In conclusion, the RFPP in lakes Huron and Michigan are primarily coccoid to oval eukaryotic cells with a mean size of 1.2 µm. These cells are important components of the phototrophic picoplankton communities in both lakes, but contribute only a small fraction (1–7 %) to total primary production. Like many other picoplankton-sized organisms, these cells exhibit relatively fast growth rates and most of their production appears to be consumed by micrograzers.

## 6. Acknowledgements

We would like to thank the crew of the RV/Shenehon for their assistance and support and J. STOCKNER and M. GOLNER for their helpful comments on the manuscript. Contribution No. 718 from Great Lakes Environmental Research Laboratory.

## 7. References


BEETON, A. M., 1965: Eutrophication of the St. Lawrence Great Lakes.–Limnol. Oceanogr. **10**: 240–254.

CARNEY, H. J., and G. L. FAHNENSTIEL, 1987: Quantification of track and grain density autoradiography and evaluation of $^{14}C$ loss on preservation.–J. Plankton Res. **9**: 41–50.

CARON, D. A., F. R. PICK, and D. R. S. LEAN, 1985: Chroococcoid cyanobacteria in Lake Ontario: Vertical and seasonal distributions during 1982.–J. Phycol. **21**: 171–175.

CARRICK, H. J., and G. L. FAHNENSTIEL, 1989: Biomass, size structure, and composition of phytotrophic and heterotrophic nanoflagellate communities in lakes Huron and Michigan.–Can. J. Fish. Aquat. Sci. **46**: 1922–1928.

CHISHOLM, S. W., R. J. OLSON, E. R. ZETTLER, R. GOERICKE, J. B. WATERBURY, and N. A. WELSCHMEYER, 1988: A novel free-living prochlorophytes abundant in the oceanic euphotic zone.–Nature **334**: 340–343.

DEMPSEY, G. P., D. LAWRENCE, and V. CASSIE, 1980: The ultrastructure of *Chlorella minutissima* FOTT et NOVAKOVA (Chlorophyceae, Chlorococcales).–Phycologica **19**: 13–19.

DOBSON, H. F. M., M. GILBERTSON, and P. G. SLY, 1974: A summary and comparison of nutrients and related water quality in Lakes Erie, Huron and Superior.–J. Fish. Res. Bd. Can. **31**: 731–738.

FAHNENSTIEL, G. L., H. J. CARRICK, and R. ITURRIAGA, 1991: Physiological characteristics and food web dynamics of *Synechococcus* in lakes Huron and Michigan.–Limnol Oceanogr. **36**: 219–234.

FAHNENSTIEL, G. L., J. F. CHANDLER, H. J. CARRICK, and D. SCAVIA, 1989: Photosynthetic characteristics of phytoplankton communities in lakes Huron and Michigan: P-I parameters and end-products.–J. Great Lakes res. **15**: 394–407.

FAHNENSTIEL, G. L., and D. SCAVIA, 1987: Dynamics of Lake Michigan phytoplankton: primary production and growth.–Can. J. Fish. Aquat. Sci. **44**: 499–508.

FAHNENSTIEL, G. L., L. SICKO-GOAD, D. SCAVIA, and F. STOERMER, 1986: Importance of picoplancton in Lake Superior.–Can. J. Fish. Aquat. Sci. **43**: 235–240.

GLOVER, H. E., A. E. SMITH, and L. SHAPIRO, 1985: Diurnal variations in photosynthetic rates: Comparisons of ultraphytoplankton with a larger phytoplankton size fraction.–J. Plankton Res. **7**: 519–533.

GLOVER, H. E., M. D. KELLER, and R. R. L. Guillard, 1986: Light quality and oceanic ultraphytoplankters.–Nature **319**: 142–143.

HARDY, F. J., K. S. SHORTREED, and J. G. STOCKNER, 1986: Bacterioplankton, phytoplankton, and zooplankton communities in a British Columbia coastal lake before and after nutrient reduction.–Can. J. Fish. Aquat. Sci. **43**: 1504–1514.

HERDENDORF, C. E., 1982: Large lakes of the world.–J. Great Lakes Res. **8**: 379–412.

JOCHEM, F., 1988: On the distribution and importance of picocyanobacteria in a boreal inshore area (Kiel Bight, Western Baltic).–J. Plankton Res. **10**: 1009–1022.

JOHNSON, P. W., and J. MCN. SIEBURTH, 1982: *In situ* morphology and occurrence of eucaryotic phytotrophs of bacterial size in the picoplankton of estuarine and oceanic waters.–J. Phycol. **18**: 318–327.

JOINT, I. R., and R. K. PIPE, 1984: An electron microscope study of a natural population of picoplankton from the Celtic Sea.–Mar. Ecol. Prog. Ser. **20**: 113–118.

JOINT, I. R., 1986: Physiological ecology of picoplankton in various oceanographic provinces.–p. 287–309. *In:* T. PLATT and W. K. W. LI (eds.), Photosynthetic Picoplankton.–Can. Bull. Fish. Aquat. Sci. **214**: Ottawa.

KENNAWAY, G. M. A., and G. EDWARDS, 1989: Seasonal and vertical distribution of picoplankton in Llyn Padarn, Gwynedd.–Br. Phycol. J. **24**: 375–384.

KNOECHEL, R., and J. KALFF, 1976: Track autoradiography: a method for the determination of phytoplankton species productivity.–Limnol. Oceanogr. **21**: 590–596.

KREMPIN, D. W., and C. W. SULLIVAN, 1981: The seasonal abundance, vertical distribution, and relative microbial biomass of chroococcoid cyanobacteria at a station in southern California coastal waters.–Can. J. Microbiol. **27**: 1341–1344.

LAIRD, G. A., D. SCAVIA, G. L. FAHNENSTIEL, L. A. STRONG, J. M. MALCZYK, G. A. LANG, and W. S. GARDNER, 1987: Southern Lake Michigan nutrients, temperature, chlorophyll, plankton, and water movement during 1983 and 1984.–NOAA Technical Memo. ERL GLERL-67.

LANDRY, M. R., and R. P. HASSETT, 1982: Estimating the grazing impact of marine micro-zooplankton.–Mar. Biol. **67**: 283–287.

LESHT, B. M., and D. C. ROCKWELL, 1985: The state of the middle Great Lakes: Results of the 1983 water quality survey of lakes Erie, Huron, and Michigan.–ANL/ER-85-2 Argonne National Laboratory.

LI, W. K. W., and A. M. WOOD, 1988: Vertical distribution of North Atlantic ultraphytoplankton: Analysis by flow cytometry and epifluorescence microscopy.–Deep-Sea Res. **35**: 1615–1638.

LIN, C. K., and C. L. SCHELSKE, 1981: Seasonal variation of potential nutrient limitation to chlorophyll production in southern Lake Huron.–Can. J. Fish. Aquat. Sci. **38**: 1–9.

MUIRHEAD, K. A., T. C. SCHMITT, and A. R. MUIRHEAD, 1983: Determination of linear fluorescence intensities from low cytometric data accumulated with logarithmic amplifiers.–Cytometry **3**: 251–256.

MURPHY, L. S., and E. M. HAUGEN, 1985: The distribution and abundance of phototrophic ultraplankton in the North Atlantic.–Limnol. Oceanogr. **30**: 47–58.

NAGATA, T., 1986: The seasonal abundance and vertical distribution of the < 3-μm phytoplankton in the North Basin of the Lake Biwa.–Ecol. Res. **1**: 207–221.

PICK, F. R., and D. A. CARON, 1987: Picoplankton and nanoplankton biomass in Lake Ontario: Relative contribution of phototrophic and heterotrophic communities.–Can. J. Fish. Aquat. Sci. **44**: 2164–2172.

SCAVIA, D., G. L. FAHNENSTIEL, M. S. EVANS, D. J. JUDE, and J. T. LEHMAN, 1986: Influence of salmonid predation and weather on long-term water quality trends in Lake Michigan.–Can. J. Fish. Aquat. Sci. **43**: 435–443.

SCHELSKE, C. L., E. D. ROTHMAN, E. F. STOERMER, and M. A. SANTIAGO, 1974: Responses of phosphorus limited Lake Michigan phytoplankton to factorial enrichments with nitrogen and phosphorus.–Limnol. Oceanogr. **19**: 409–419.

STANIER, R. Y., R. KUNISWAWA, R. MANDEL, G. COHEN-BAZIRE, 1971: Purification and properties of unicellular blue-green algae (Order Chroococcales).–Bacteriol. Rev. **35**: 171–205.

STOCKNER, J. G., and N. J. ANTIA, 1986: Algal picoplankton from marine and freshwater ecosystems: a multidisciplinary perspective.–Can. J. Fish. Aquat. Sci. **43**: 2472–2503.

TSUJI, T., K. OHKI, and Y. FUJITA, 1986: Determination of photosynthetic pigment composition in an individual phytoplankton cell in seas and lakes using fluorescent microscopy; properties of the fluorescence emitted from picophytoplankton cells.–Mar. Biol. **93**: 343–349.

WEISSE, T., 1988: Dynamics of autotrophic picoplankton in Lake Constance.–J. Plankton Res. **10**: 1179–1188.