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Growth and production of planktonic protozoa in Lake Michigan: In situ versus in vitro comparisons and importance to food web dynamics

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Abstract

Growth of an entire planktonic protozoan community in Lake Michigan (nanoflagellates, microflagellates, and ciliates) was measured on 10 dates in 1988–1989 in fractionated lake water (<3, <8, <30, and <153 μm) incubated in bottles (in vitro), while in June and July 1989, in vitro growth rate estimates were compared with in situ growth rates derived from the mitotic index. Comparisons of in situ vs. in vitro growth rates for the taxa assayed were similar, indicating that in vitro experiments provide reasonable estimates of protozoan growth, and these rates were similar to those measured from other oligotrophic/mesotrophic habitats. However, containment artifacts in some bottle experiments influenced community growth as some taxa experienced mortality in bottles, thus underscoring the importance of censusing the entire community. Protozoa in Lake Michigan contribute significantly to both heterotrophic and phototrophic carbon pools; phototrophic flagellates represented 24% of primary production, while heterotrophic nanoflagellates and ciliates collectively constituted 40% of bacterial production. The fate of high bacterial production can be accounted for through grazing by both heterotrophic nanoflagellates and ciliates, indicating the trophic importance of protozoa in Lake Michigan.

Protozoa are important components of many aquatic ecosystems and exhibit a great range in form and function (e.g. Sherr and Sherr 1984). These organisms are the dominant trophic link through which the picoplankton and nanoplankton production observed in many ecosystems (e.g. Stockner and Antia 1986) can be transferred to higher trophic levels (e.g. Carrick et al. 1991). Little is known, however, about the population dynamics of naturally occurring protozoa and few estimates of protozoan growth have been made (e.g. Fenchel 1987).

Of the existing growth estimates for protozoa, most are laboratory observations of single species or small numbers of species (e.g. Caron et al. 1986). Although these investigations have suggested the quantitative importance of protozoa (e.g. Banse 1982), they probably represent an upper bound on growth rates for specific species due to idealized conditions in the laboratory. Most studies of protozoan growth made under field conditions focus on one component of the protozoan communities (e.g. Heinbokel 1988), and the natural variation in these estimates is largely unknown (e.g. Verity 1986*b*; Nagata 1988; Weisse 1991).

Because protozoa are extremely sensitive to containment and manipulation (e.g. Bloem et al. 1986), several methods have been used to estimate their growth in undisturbed natural plankton communities: porous polycarbonate cages (Stoecker et al. 1983; Landry et al. 1984), dialysis bags (Verity 1986*b*), and large carboys (Gilron and Lynn 1989). In each, the protozoa were incubated under in situ conditions. Some questions still exist as to whether methods involving containment of any kind (in vitro

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estimates) provide reasonable estimates of in situ (or gross) growth rates. Thus, in situ estimates have been measured by assaying the frequency of dividing cells of individual phytoflagellate populations sampled repeatedly from the water column (e.g. Swift and Durbin 1972; Braunwarth and Sommer 1985). Such estimates appear to be repeatable and to provide reasonable estimates of growth, as shown for tintinnid populations (Heinbokel 1988), but no estimates of heterotrophic nanoflagellate growth have been made with this technique and no direct comparison has been made between in situ and in vitro growth estimates (Furnas 1990).

In this study we measured the growth of the entire planktonic protozoan community (ciliates and flagellates) from 12 experiments conducted over a 2-yr period in Lake Michigan. Our objectives were threefold. First, we compared the growth rates of several protozoan populations both in situ and in vitro to assess containment effects. Methods of estimating in situ growth differed from previous studies in that populations from a specific water mass were tracked by a satellite drifter, and these measurements were compared with in vitro growth experiments carried out over the same time period for both heterotrophic and phototrophic taxa. Second, we assessed variation in taxon-specific in vitro growth estimates among sampling dates and size fractions for the entire protozoan community. Third, we calculated protozoan productivity and compared it to previous estimates of planktonic bacterial (Scavia and Laird 1987) and primary productivity (Fahnenstiel and Scavia 1987*a*) made in Lake Michigan to evaluate the contribution of protozoan production to heterotrophic and phototrophic carbon pools.

Methods

Sampling was conducted at a single offshore station in Lake Michigan (43°1′11″N, 86°36′48″W; max depth, 100 m) on four dates in 1988 (11 April, 10 May, 13 June, and 11 July) and six in 1989 (29 March, 19 April, 13 June, 10 July, 28 August, and 4 October). Water was collected from the surface mixing layer (5 m) with a clean 5- or 30-liter PVC Niskin bottle at sundown (2000–2200 hours). Temperature profiles were measured with an electronic bathythermograph, while surface water temperature was measured with a sensor attached to a National Oceanic and Atmospheric Administration Data Buoy (No. 45007) located at 42.7°N, 87.1°W. Abundances were determined from samples transferred into clean 250-ml amber bottles (acid washed and rinsed with deionized water) and preserved with either 1% Lugol's acid iodine solution (ciliate and microflagellate sample) or with 1% glutaraldehyde buffered with 0.1 M sodium cacodylate (nanoflagellate sample). Because of the wide range in both cell size and abundance among protozoa, the growth of microflagellates (composed entirely of Dinoflagellida) and ciliates (Ciliophora, most >20 and <200 μm in size) was measured separately from nanoflagellates (Chrysomonadida, Cryptomonadida, and Choanoflagellida >2 and <20 μm in size). Moreover, the potential trophic status of nanoflagellates was distinguished by the presence (phototrophic, Pnano) or absence (heterotrophic, Hnano) of pigment fluorescence (*see below*).

Nanoflagellates were enumerated with epifluorescent microscopy from slides prepared within 24 h of sampling. Subsamples (10–20 ml) were filtered onto prestained (Irgalan Black) 0.8-μm pore size Nuclepore filters and stained with primulin (Caron 1983). The filters were then mounted between a microscope slide and coverslip with immersion oil. Prepared slides were immediately stored at -20°C and counted within a month to minimize the fading of fluorescence. Biomass was estimated by enumerating 400–500 individuals from each prepared slide with a Jena Lumar microscope (1,000×) equipped for autofluorescence (450–490 excitation and >515 emission) and primulin analysis (320–380 excitation and >420 emission).

Ciliate and microflagellate biomass and community composition were determined with the Utermöhl technique (Utermöhl 1958). Subsamples (25–50 ml) were settled onto coverslips and systematically scanned, enumerating 400–1,000 individuals from each sample with an inverted microscope

(400×). Cell volumes were calculated for each taxon by measuring the cellular dimensions of at least 10 cells on four dates.

Cellular volumes for Pnano and microflagellates were converted to carbon based on Strathmann (1967) conversion factors; Hnano and ciliate cell volumes were converted to carbon with the conversion factor 0.15 g C ml^{-1} (Laws et al. 1984). Biomass estimates were not corrected for cell shrinkage due to preservation (e.g. Choi and Stoecker 1989). Protozoan systematics used here conform to those presented by Lee et al. (1985).

In vitro growth estimates—Protozoan growth rates in vitro were determined by filtering lake water to selectively remove potential predators (Sherr and Sherr 1983; Verity 1986*b*). For all experiments a darkened 20-liter carboy was gently filled with lake water from a depth of 5 m using a clean PVC Niskin bottle to avoid contamination (Fahnenstiel and Scavia 1987*a*). In addition, all carboys and polycarbonate bottles were acid washed, rinsed thoroughly with carbon-free deionized water, and rinsed a second time with lake water before use. The carboy containing collected lake water was mixed, and the water was passed through four different screens (3.0- and 8.0-μm Nuclepore filters, 30-μm and 153-μm Nitex screens) either by gravity or under low pressure (<5 mm of Hg). The resulting filtrates were dispensed into clean 4-liter polycarbonate bottles that were incubated at dusk in a deck-top incubator equipped with rotating racks; light (~20% surface irradiance) and temperature conditions in the incubator corresponded to those at 5 m in the water column.

Initial and final (24-h) subsamples for nanoflagellates (<3- and <8-μm treatments) and ciliates and microflagellates (<30- and <153-μm treatments) were removed from the bottles, preserved, and enumerated as described previously in order to estimate exponential growth by

$$r = \ln(N_t/N_0)/t$$

where r is the rate of population growth (d^{-1}), N_0 and N_t are initial and final cell densities, and t is duration of incubation. We sampled the bottle experiments after 24 h of incubation to keep incubation times as short as possible and avoid associated artifacts that can impact plankton growth (Fahnenstiel and Scavia 1987*a*; H. J. Carrick unpubl. data) and alleviate problems of sampling between cellular division cycles, as Lake Michigan populations appear to demonstrate synchronous division (*see Fig. 2*). Our in vitro growth rates most likely are a measure of rates ranging from gross to net growth, in that mortality unaccounted for within our bottles (e.g. grazing and cell damage) might reduce the observed growth rates measured with this technique.

In situ growth estimates—Experiments were conducted on 13 June and 10 July 1989 to estimate in situ growth rates of four dominant nanoflagellate taxa by means of the mitotic index (Swift and Durbin 1972). Water samples were collected over a 24-h period at 2-h intervals on 13 June and at 1-h intervals on 10 July near a satellite-tracked drifter equipped with a window-shade drogue. For the conditions of this study, the ability of this drifter to follow a specific water mass is quite good (McCormick et al. 1985).

Upon collection, water samples were immediately preserved in buffered glutaraldehyde and slides for epifluorescence microscopy were prepared within 24 h as described earlier, except that samples were double stained. Samples were treated for 8 min with the nuclear stain DAPI (4′,6′-diamidino-2-phenylindole; Porter and Fieg 1980) and for an additional 2 min with the protein stain Proflavin (3-6′-diamino-acridine hemisulfate; Haas 1982) before filtration onto prestained filters. Individual cells of the four taxa were located under blue light excitation (Proflavin: 450–490 excitation, >515 emission) and the cell contents were examined under UV light (DAPI: 320–380 excitation, >420 emission) in order to place them in one of three categories: cells with a single nucleus, cells with replicating nuclei, and paired cells (i.e. recently divided).

The duration of division (t_D) was estimated by the difference between the median time of occurrence for cells with double nuclei over the entire 24-h cycle and the me-

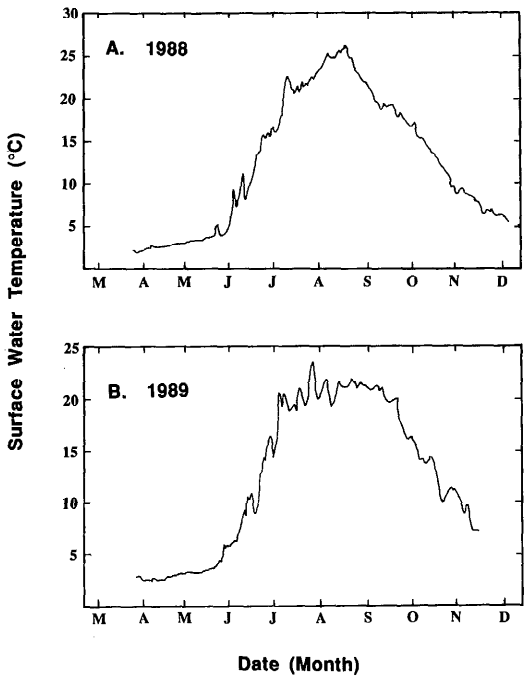

Fig. 1. Average daily surface water temperature (°C) for March–December 1988 (A) and 1989 (B) taken from a NOAA weather buoy located in Lake Michigan (42.7°N, 87.1°W).

dian time of occurrence for paired cells (Braunwarth and Sommer 1985). Growth rates for each population were then calculated by

$$\mu = \frac{1}{nt_D\,[\Sigma \ln(1 + P_A)]}$$

where μ is the in situ population growth rate (d^{-1}), t_D the duration of division, n the number of intervals sampled, and P_A the proportion of cells with paired nuclei at time t. Because loss processes (grazing, sedimentation, and cell lysis) probably do not affect the mitotic division cycle, we assume that our estimates of in situ growth approximate gross growth rates.

Daily protozoan production was calculated by converting exponential growth rates obtained from bottle experiments to linear rates. Linearized growth rates were then multiplied by protozoan biomass to yield production values. These estimates assume that biomass remains constant throughout the day.

Results

Ambient thermal conditions—Surface water temperatures over the 2 yr of study ranged from 2.5° to 26°C (Fig. 1). The timing of thermal stratification was similar in both years, as surface waters warmed from May to June and were maximal (24°–26°C) in July. Following the scheme of Fahnenstiel and Scavia (1987*a*), we defined three major thermal periods: isothermal mixing (temp. <4.0°C, March–May sampling); intermediate stratification (temp. > 4° and <15.0°C, June and October); and midstratification (temp. >15°C, July–September).

Comparison of in situ and in vitro growth—Growth of the four populations censused with the mitotic index demonstrated relatively synchronous division cycles (Fig. 2), allowing us to apply Braunwarth and Sommer's (1985) method to calculate in situ growth rates. The medians for dividing nuclei and paired cells for all four taxa on 13 June occurred between 0200 and 0400 hours and between 0444 and 0635 hours, respectively, with t_D values ranging from 1.55 to 2.50 h (Table 1). The medians for dividing nuclei and paired cells on 10 July occurred earlier than in June (2000–0100 hours); however, estimates of t_D were similar (1.01–2.46 h). On both dates, in situ growth estimates were higher than in vitro estimates (average in situ to in vitro ratio, 1.34); however, no significant differences were observed between the two estimates of growth for the four taxa examined ($t = 1.11$, $n = 8$, $P = 0.303$, Table 1). In addition, in situ to in vitro ratios for Pnano (ratio, 1.65) were higher than ratios for Hnano (ratio, 1.04).

Protozoan community growth in vitro—Growth rates were variable and exhibited different temporal patterns among the four groups (Hnano, Pnano, microflagellates, and ciliates) with rates ranging from 0 to 1.3 d^{-1} over the 2-yr study (Fig. 3). Hnano growth rates were similar between the <3- and <8-μm fractions, ranging from 0.04 to 0.29 d^{-1} during the spring isothermal period and increasing to >0.6 d^{-1} during thermal stratification. In contrast, Pnano in the <8-μm fraction showed net growth only on a few dates (range, 0–0.3 d^{-1}), and these tended to be lower than rates measured in the <3-μm fraction (0–0.69 d^{-1}). Microflagellate

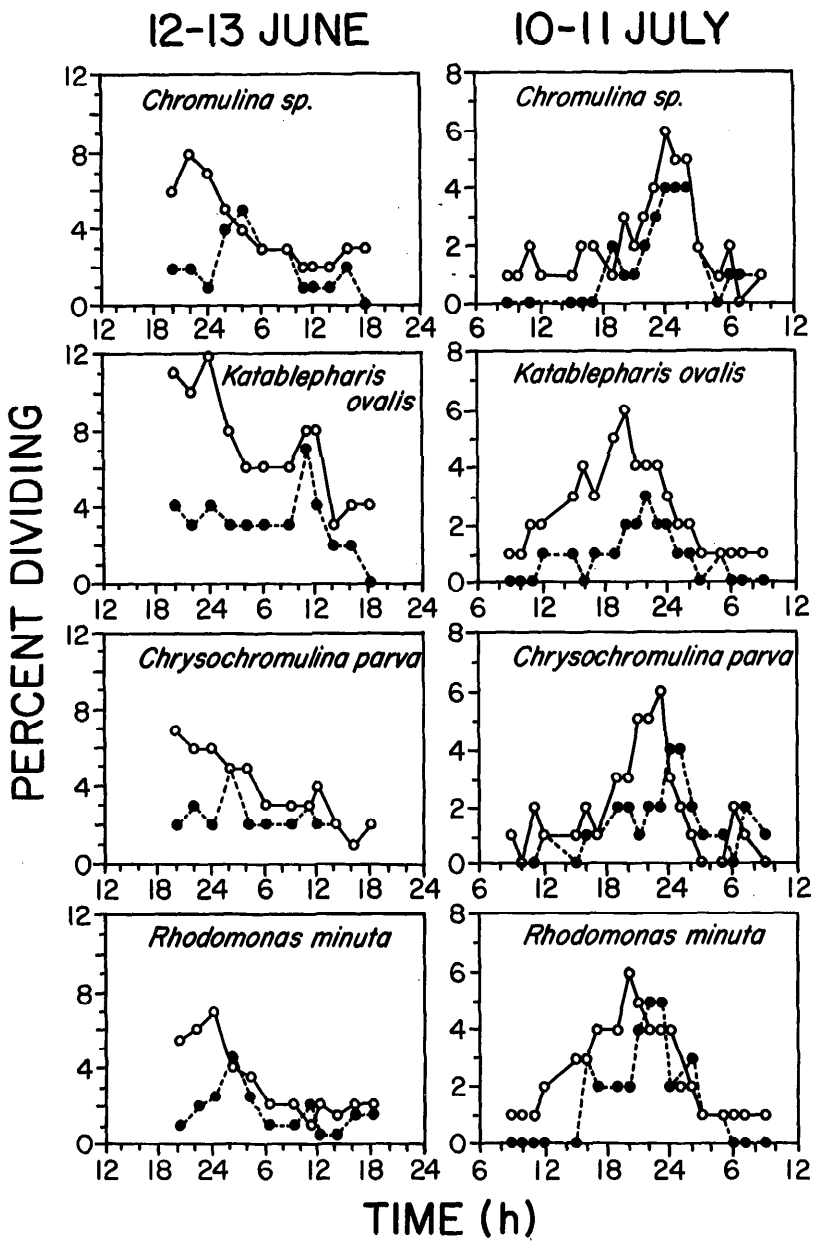

Fig. 2. Diel cycles determined on two dates for both dividing nuclei (○) and paired cells (●) of four protozoan taxa sampled from the surface waters (5 m) of Lake Michigan.

growth demonstrated little seasonality, with rates ranging from 0 to 0.64 d^{-1}, while growth in the <153-μm fraction was greater than that in the <30-μm treatment on most dates. Growth rates for ciliated protozoa were low in spring (0.01–0.23 d^{-1}), then increased in summer to values ranging from 0.27 to 1.48 d^{-1} in July. Prior to thermal stratification, ciliate growth tended to be higher in the <153-μm fraction than in the <30-μm fraction; the opposite was true after stratification.

Taxon-specific growth in vitro—The growth rates of individual taxa ranged from

Table 1. Comparisons of in situ vs. in vitro growth (d^{-1}) and duration of division estimates (t_D, h) as determined for four nanoflagellate taxa (trophic level in parentheses: H—heterotrophic; P—phototrophic) on two dates in the surface waters (5 m) of Lake Michigan. Values for in vitro growth are the mean (±1 SD) of replicate counts.

Taxon	t_D	In situ	In vitro	Ratio
12–13 June 1989				
Chromulina sp. (H)	2.24	0.41	0.50(0.19)	0.82
Katablepharis ovalis (H)	2.50	0.66	0.68(0.05)	0.97
Chrysochromulina parva (P)	1.55	0.56	0.40(0.08)	1.40
Rhodomonas minuta (P)	2.38	0.56	0.41(0.01)	1.37
10–11 July 1989				
Chromulina sp. (H)	1.01	0.53	0.34(0.11)	1.56
K. ovalis (H)	1.17	0.49	0.62(0.06)	0.79
C. parva (P)	2.46	0.19	0.08(0.05)	2.38
R. minuta (P)	2.00	0.30	0.21(0.08)	1.43
Mean		0.46	0.41	1.34
SD		0.15	0.20	0.51

0 to 2.28 d^{-1} and in most cases growth was restricted to a particular thermal period (Fig. 1). The two dominant Pnano, *Chrysochromulina parva* Lackey (Chrysomonadida) and *Rhodomonas minuta* Skuja (Cryptomonadida) was >0.4 d^{-1} during the periods of isothermal mixing and intermediate stratification, but little or no growth was observed during midstratification (Fig. 4, Table 2). Hnano such as *Chromulina* sp. (Chrysomonadida) and *Katablepharis ovalis* Skuja (Cryptomonadida) grew throughout the

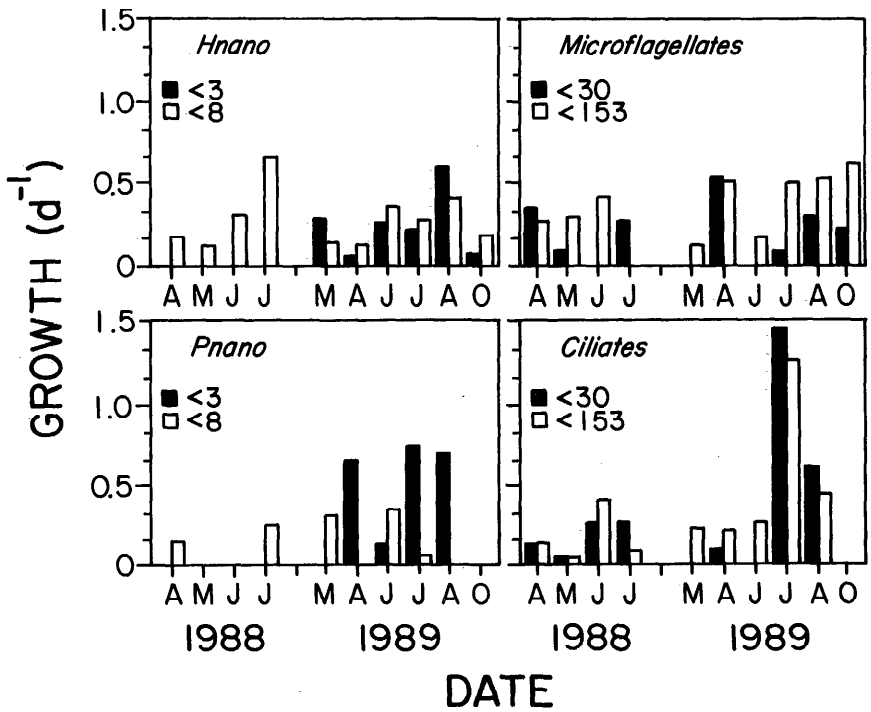

Fig. 3. Growth rates of four protozoan groups in the surface waters (5 m) of Lake Michigan determined from bottle experiments on four dates in 1988 and six in 1989.

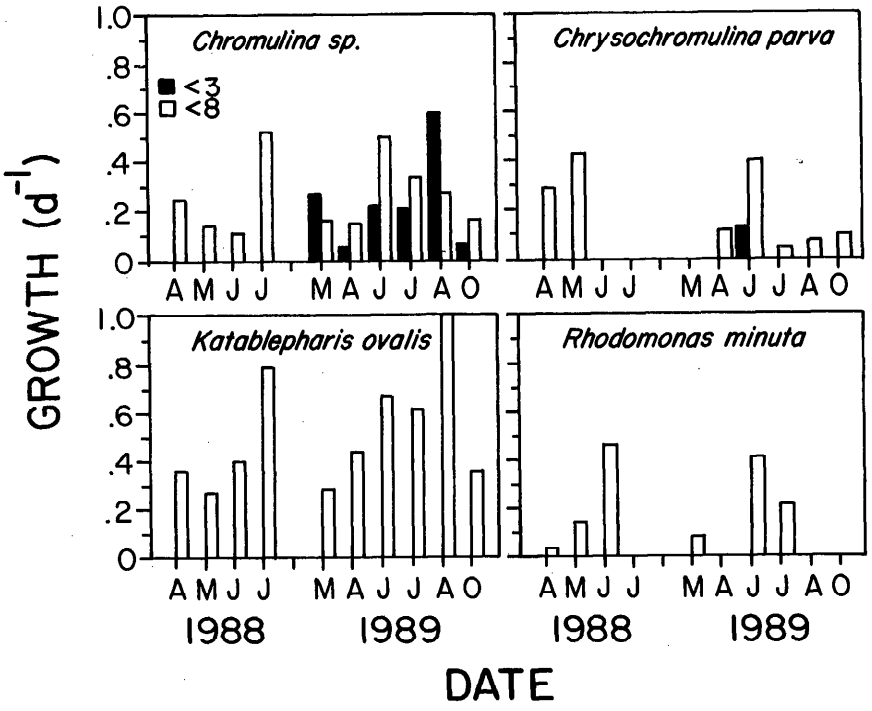

Fig. 4. As Fig. 3, but for abundant heterotrophic nanoflagellates (left) and phototrophic nanoflagellates (right).

year and achieved rates higher than 0.5 and 1.0 d^{-1} during late stratification (Fig. 4, Table 2).

The microflagellate assemblage in Lake Michigan was composed entirely of individuals belonging to the Dinoflagellida (Fig. 5, Table 2). Among the actively growing microflagellata taxa, *Glenodinium* sp. (Gymnodiniidae) exhibited high growth rates (max, 0.69 d^{-1}) during isothermal mixing. The growth of *Gymnodium helveticum* (Gymnodiniidae) was greatest during intermediate stratification (0.47 d^{-1}), while *Ceratium hirudinella* (Muller) Dujardin (Peridiniidae) grew only during mid-stratification (July–October), when it achieved maximal growth rates near 0.5 d^{-1}. The smaller microflagellate taxa *Gymnodinium varians* Maskell (Gymnodiniidae) and *Peridinium* sp. (Peridiniidae), grew throughout the year with rates ranging from 0 to 0.82 d^{-1}.

Similar to flagellate populations, growth of individual ciliate taxa was restricted to specific periods during the study (Fig. 6, Table 2). For example, the growth of the tintinnids *Tintinnidium* sp. and *Codonella* sp. (Choreotrichida) was restricted almost entirely to isothermal mixing conditions, whereas *Halteria* sp. (Oligotrichida) grew fastest during intermediate stratification. Although *Strobilidium* sp. (Choreotrichida) and *Urotricha* sp. (Prorodontida) exhibited positive growth in nearly all the experiments, they achieved highest growth rates, exceeding 1.0 d^{-1}, during midstratification. Moreover, these taxa had higher growth rates in the <30-μm treatments in midsummer, which suggests that they may have experienced predation by other microzooplankton >30 and <153-μm in size (presumably other ciliates, rotifers, and nauplii). The growth of *Vorticella* sp. (Sessilida) varied and coincided with the availability of suitable substrata; this taxon was found growing attached to diatoms (*Fragilaria* and *Tabellaria*) during isothermal periods or in association with masses of blue-green

Table 2. Growth rates (avg ±1 SD) of abundant heterotrophic nanoflagellate (Hnano), phototrophic nanoflagellate (Pnano), microflagellate, and ciliate taxa in fractionated Lake Michigan surface waters (5 m). The thermal period in which maximal growth occurred is indicated; thermal periods are defined in the text.

Dominant taxon	Cell vol. (μm^3)	Size fraction		Range (d^{-1})	Thermal period
		<3 μm	<8 μm		
Hnano					
Chromulina sp.	7.9	0.24±0.20	0.26±0.15	0–0.60	Mid
Katablepharis ovalis	127.1	0	0.53±0.25	0–1.05	Mid
Pnano					
Chrysochromulina parva	26.4	0	0.15±0.17	0–0.43	Mix
Rhodomonas minuta	175.0	0	0.14±0.18	0–0.47	Int
		<30 μm	<153 μm		
Microflagellates					
Glenodinium sp.	20,000	0.10±0.17	0.12±0.22	0–0.69	Mix
Gymnodinium helveticum	10,856	0.01±0.02	0.09±0.15	0–0.47	Int
Ceratium hirudinella	47,032	0	0.12±0.18	0–0.49	Mid
Gymnodinium varians	816	0.24±0.26	0.32±0.28	0–0.82	Var
Peridinium sp.	6,259	0.19±0.23	0.27±0.36	0–0.78	Var
Ciliates					
Tintinnidium sp.	8,134	0.14±0.29	0.19±0.24	0–0.84	Mix
Codonella sp.	12,185	0	0.06±0.18	0–0.556	Int
Halteria sp.	3,063	0.21±0.27	0.33±0.47	0–1.45	Int
Strobilidium sp.	1,024	0.63±0.70	0.30±0.28	0–2.28	Mid
Urotricha sp.	1,450	0.42±0.45	0.39±0.20	0–1.25	Mid
Vorticella sp.	8,538	0	0.41±0.31	0–0.98	Var

(cyanobacteria) *Anabaena* during thermal stratification.

Protozoan production—Production values for both heterotrophic and phototrophic protozoa were compared with previous measurements of planktonic bacterial (Scavia and Laird 1987) and primary (Fahnenstiel and Scavia 1987*a*) production over a similar range in temperature in Lake Michigan (Table 3). Heterotrophic protozoan production (Hnano and ciliates) constituted 40% of average bacterial production in the lake, with Hnano and ciliate production representing 10 and 30%. Phototrophic protozoan (Pnano and microflagellates) production represented 24% of total planktonic primary production in the epilimnion, with Pnano and microflagellate production contributing 14 and 10% to this estimate.

Discussion

Evaluation of bottle experiments: Comparison of in vitro and in situ growth—The comparison between in situ and in vitro growth estimates for the populations evaluated indicates that in vitro measurements provide reasonable estimates of in situ growth (average in situ to in vitro growth ratio, 1.34). Although no overall difference between in situ and in vitro growth was observed, the growth ratio for heterotrophic nanoflagellates (Hnano ratio, 1.04) was lower than that for phototrophic nanoflagellates (Pnano ratio, 1.64). If we assume that our in situ growth rates are an estimate of gross growth rates (growth independent of loss factors), which is probably a robust assumption given that the mitotic index should not be affected by predation, sedimentation, and cell lysis, then our in vitro growth rates most likely measure a rate between gross and net growth.

Our in situ to in vitro ratio for Pnano (1.64) is similar to ratios of in situ to in vitro primary production (measured as O_2 evolution) estimates made in Lakes Huron and Michigan (average ratio, 2.00, Fahnenstiel and Carrick 1988), which may indicate a sensitivity to containment for phototrophs; the heterotrophs tested showed no differences (ratio, 1.04). Neutral density screening was used to adequately adjust light

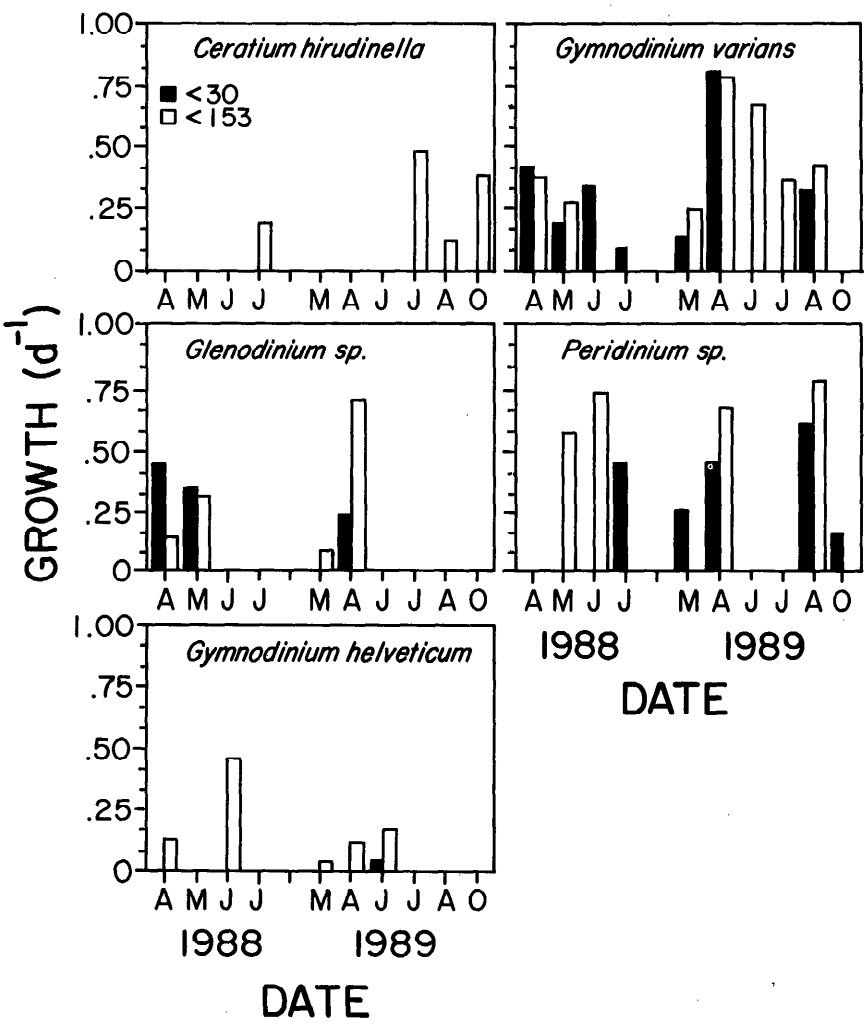

Fig. 5. As Fig. 3, but for abundant phototrophic microflagellate taxa (all Dinoflagellida).

quantity (from incident irradiance) in our incubator to levels occurring at 5 m in the water column; however, this technique may have produced differences in light quality in our bottles which adversely affected plankton, particularly phototrophs. Among protozoa, however it is not unusual for predators to be similar in size to their prey (Fenchel 1987). This phenomenon might apply here, as indicated by lower growth of both Hnano and Pnano in the <8-μm relative to the <3-μm fraction observed on several dates (*see Table 2*).

We acknowledge that sensitivity to containment can be species-specific and may have affected taxa other than those tested. For example, *Strombidium* sp. disappeared from our bottles on several dates at a rate (range, −0.77 to −0.20 d^{-1}) greater than previous measures of grazing loss (Carrick et al. 1991; W. D. Taylor unpubl. data). Taylor and Johannsson (1991) also obtained low rates of increase for this species on many occasions and speculated that containment effects were responsible. For this reason, a check on in vitro methods for ciliates and microflagellates, as was done for nanoflagellates, would be valuable.

Assuming the four taxa compared here are representative of most protozoa in the

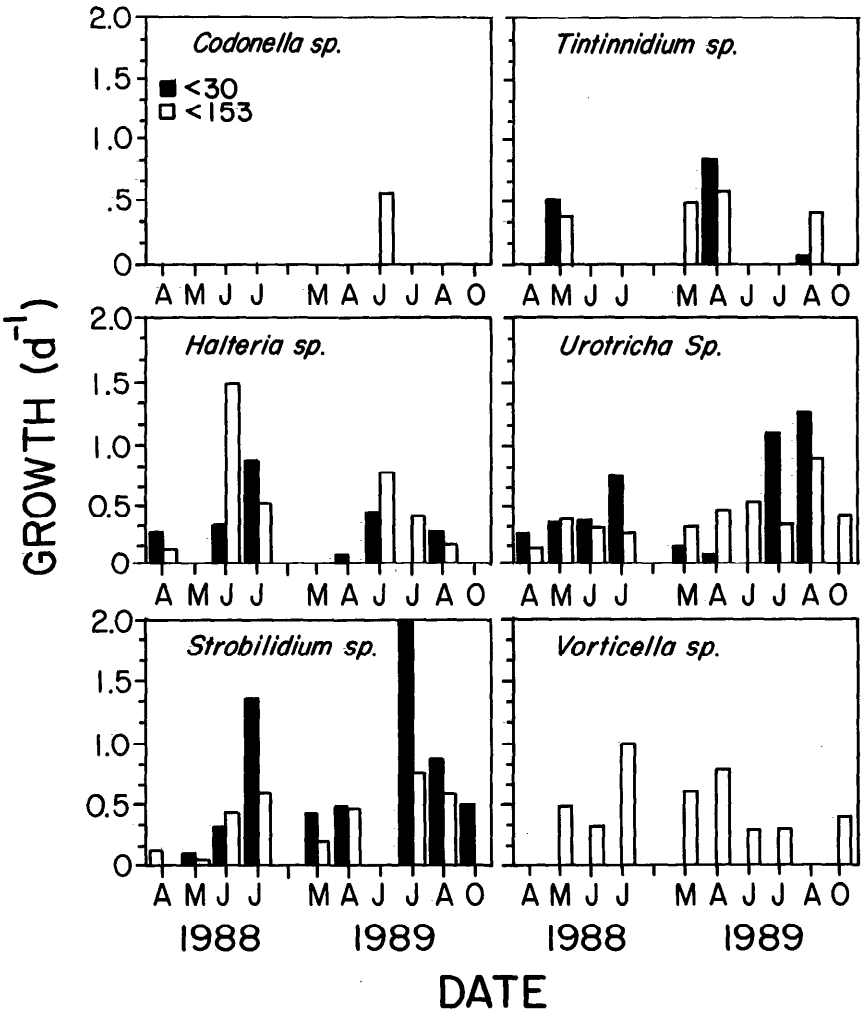

Fig. 6. As Fig. 3, but for abundant ciliated protozoan taxa.

Great Lakes in terms of their sensitivity to containment, our average estimates of in vitro growth may be low by 25–40%, which is reasonable given typical error associated with these methods. The close agreement between in situ and in vitro growth estimates was expected given that fairly large containers were used (4-liter polycarbonate bottles) and incubation times were kept relatively short (~24 h). Fahnenstiel and Scavia (1987*a*) found that containment effects for Lake Michigan phytoplankton, manifested as decreased ^{14}C uptake and changes in chlorophyll concentrations, generally were not detectable for incubations of 24 h, but were significant for long incubation periods (>48 h). They also determined that underestimation of phytoplankton growth due to containment was greatest during thermal stratification (July). Hence, comparisons here are probably robust, because both took place during thermal stratification (June and July).

Our estimates of in situ growth rate are similar to those measured in previous studies for the same taxon. Growth of *R. minuta* determined from two experiments were 0.30 and 0.56 d^{-1}. These results are similar to those measured with the mitotic index for *R. minuta* populations in Lake Constance,

with growth rates ranging from 0.20 to 0.34 d^{-1} (Braunwarth and Sommer 1985). In addition to the good agreement in growth rates for *R. minuta* between Braunwarth and Sommer (1985) and this study, duration of division times (t_D) measured for Lake Michigan (range, 2.00–2.38 h) were similar to those determined for Lake Constance populations (1.00–1.72 h). The main difference between the division cycle for the two populations was that a small percentage of the Lake Michigan population divided throughout the day, and the Lake Constance population divided only between 1400 and 0800 hours.

Interpretation of protozoan growth: Importance of measuring taxon-specific growth—Our results indicate that containment artifacts can influence growth rates derived from bottle experiments. First, the potential for predation among similar-sized organisms in bottles and the sensitivity of individual taxa to experimental protocol underscores the importance of measuring taxon-specific growth rates across the entire protozoan community. On several occasions, differences in growth among various fractions were noted for nanoflagellate and small ciliate taxa, particularly during mid-stratification (July–August). For example, growth of *Chromulina* sp. during this period was nearly 10% higher in <3-μm relative to <8-μm water, while the ciliates *Strobilidium* sp. and *Urotricha* sp. exhibited 53% and 55% greater growth in <30-μm compared with that in <153-μm filtrate. We also observed no net growth in the <30-μm and <153-μm filtrates for most nanoflagellate taxa on most dates tested (Carrick 1990). These observations agree with the notion that grazing occurs among similar-sized organisms (e.g. Rassoulzadegan and Sheldon 1986) and may lead to reduced efficiency of trophic transfer from protozoa to metazoa.

Second, analysis of the entire community provides data on sensitive taxa that are adversely affected by containment. The adverse response of *Strombidium* to bottle enclosure again emphasized the importance of censusing all components of the assemblage, as inclusion of an abundant community component like *Strombidium* in our initial counts for growth calculations knowingly yielded conservative estimates of community growth (Furnas 1990). These findings underscore the difficulty in estimating protozoan growth from bottle incubations. Errors in estimating community growth associated with sensitive taxa may be unavoidable, but errors related to grazing among similar sized organisms can in part be evaluated by measuring growth within a fractionation series. This is not to say other techniques such as dilution experiments provide any better approximation of gross growth rates, as the dilution method can suffer from other specific difficulties (Furnas 1990; Li 1990).

Table 3. Comparison of heterotrophic [nanoflagellate (Hnano) and ciliate] and phototrophic [microflagellates (Pmicro) and nanoflagellates (Pnano)] protozoan production with epilimnetic (5 m) bacterial and primary production in Lake Michigan.

Carbon pool	Protozoan group	Proto. prod. (μg C liter $^{-1}$ d $^{-1}$)	Bact.-prim. prod. (μg C liter $^{-1}$ d $^{-1}$)	Ratio
Hetero	Hnano	2.68	25.72*	0.10
	Ciliates	7.83		0.30
	Total			0.40
Photo	Pnano	3.58	25.04†	0.14
	Pmicro	2.55		0.10
	Total			0.24

* Bacterial production calculated from figure 6A of Scavia and Laird (1987), assuming cell volume of 0.081 μm^3 and carbon conversion of 0.154 pg C μm^3.
† Primary production calculated from Fahnenstiel and Scavia (1987*a*).

Variation in protozoan growth—Growth rates of protozoa in Lake Michigan were similar to those reported from other oligotrophic-mesotrophic habitats, although differences in experimental protocol and environmental conditions make direct comparison difficult. For instance, growth rates for Hnano (range, 0.12–0.67 d^{-1}; mean, 0.28 d^{-1}) are at the low end of the range of those measured in Lake Biwa (Nagata 1988), Georgia coastal waters (Sherr and Sherr 1983; Sherr et al. 1984), and offshore Hawaiian waters (Landry et al. 1984; Laws et al. 1984).

Lower Hnano growth rates observed in Lake Michigan might be ascribed to environmental differences (Table 4). In particular, the range in temperature among the sites mentioned above was 12°–29°C, while the range in temperature over the course of

Table 4. Comparisons of water temperature and corresponding heterotrophic nanoflagellate growth rates determined by one of two experimental methods (Fra—fractionation; Dil—dilution) across several study sites.

Temp. (°C)	Growth (d^{-1})	Method	Study site	Reference
2–20	0.22–0.60	Fra	Lake Michigan	This study
19–27	0.30–1.20	Fra	Lake Biwa	Nagata 1988
26	0.91–1.71	Fra	Georgia coast	Sherr and Sherr 1983
12–29	0.60–1.71	Fra	Georgia coast	Sherr et al. 1984
27	0.93–1.28	Dil	Hawaiian waters	Landry et al. 1984
27	0.90–1.28	Dil	Hawaiian waters	Laws et al. 1984

our study in Lake Michigan was 2°–20°C. These differences in Hnano growth rates in Lake Michigan and other sites occur independent of the methods used. In addition, both Hnano and ciliate growth in Lake Michigan increased with temperature (r = 0.78, $P < 0.005$ and $r = 0.61$, $P < 0.05$, respectively), but the growth of phototrophic nanoflagellates and microflagellates did not. The relationship between heterotrophic protozoan growth rates and temperature may reflect the temperature dependence of heterotrophic processes such as grazing (Caron et al. 1986) and digestion of bacterial prey (Sherr et al. 1988).

The determined growth of ciliates (range, 0–1.4 d^{-1}; mean, 0.32 d^{-1}) was similar to estimates from Lake Ontario (0–0.85 d^{-1}; mean, 0.20 d^{-1}: Taylor and Johannsson 1991) and agreed well with growth rates determined by Stoecker et al. (1983), who measured growth of an entire ciliate community in <40-μm filtrate collected from an estuarine pond (range, 0–1.64 d^{-1}). Estimates were also similar to those determined for a tintinnid-dominated community censused weekly over a 2-yr period in Narragansett Bay, Rhode Island (range, 0–2.3 d^{-1}; mean, 0.55 d^{-1}: Verity 1986*b*), across a similar range in temperature (0°–24°C). The more narrow range in ciliate growth observed in our study compared to that in Narragansett Bay may be a function of our limited temporal resolution. Our estimates of microflagellate growth (primarily dinoflagellates, range, 0–0.6 d^{-1}; mean, 0.36 d^{-1}) were similar to those determined from Santa Monica Bay, California (range, 0.16–0.50 d^{-1}: Weiler and Chisholm 1976), and Kaneohe Bay, Hawaii (average, 0.24 d^{-1}: Landry et al. 1984), whereas the growth of Lake Michigan Pnano (0–0.34 d^{-1}) was similar to populations in Narragansett Bay (range, 0–0.33 d^{-1}: Verity 1986*a*) and Kaneohe Bay (0.14 d^{-1}: Landry et al. 1984).

Temporal variation in protozoan community growth can be influenced by the growth of individual populations, which again indicates the importance of censusing members of the entire community (Furnas 1990). The abundance of ciliates during isothermal conditions was dominated by fast-growing loricate ciliates (*Tintinnidium* sp. and *Codonella* sp.), whereas these organisms showed little or no growth in the surface waters during thermal stratification. Tintinnid populations are most abundant during mixing periods or are components of deep communities following stratification in Lakes Ontario (Taylor and Heynen 1987), Huron, and Michigan (Carrick and Fahnenstiel 1990). Also, a distinct assemblage of small ciliates (*Strobilidium* sp. and *Urotricha* sp.) and Hnano (primarily *Chromulina* sp.) were present during mid-stratification and achieved their highest growth rates at this time. This assemblage appeared to be characteristic of summer epilimnetic plankton communities in Lakes Michigan and Huron (Carrick and Fahnenstiel 1989, 1990) and may be associated with the abundance of picoplankton prey at this time of the year (Scavia and Laird 1987; Fahnenstiel and Carrick 1992).

Production of Lake Michigan protozoa: Quantitative importance to food web dynamics—The potential importance of protozoa as consumers of microbial productivity in the Great Lakes has been emphasized since the discovery that phototrophic picoplankton biomass and production is considerable in these lakes (Fahnenstiel et al.

1986; Pick and Caron 1987). Also, high bacterial production with relatively constant bacterial abundance in Lake Michigan suggests that protozoan grazing balances growth and regulates biomass (Scavia and Laird 1987).

Hnano productivity in Lake Michigan (range, 0.8–8.4 μg C liter^{-1} d^{-1}) determined in this study is similar to that determined elsewhere (Sherr et al. 1984; Nagata 1988); however, our Hnano production estimates are low compared to bacterial production estimates in Lake Michigan (Scavia and Laird 1987). If we assumed 30% Hnano growth efficiency (Fenchel 1982), Hnano could consume ~40% of bacterial carbon production. If we calculate Hnano production from the <3-μm fraction and assume 30% efficiency, only 9% of bacterial productivity can be consumed by this fraction of Hnano.

These observations tend to conflict with the idea that bacterioplankton in Lake Michigan are under grazer control by flagellates of about 2 μm in size (Laird Pernie et al. 1990). The differences may be in part resolved by the idea that organisms >2 μm in size can pass through the polycarbonate filters used in the above studies, due to the flexibility of these organisms and to irregularities in nominal pore size of the filters (Stockner et al. 1990). In any case, other organisms in addition to Hnano may be active bacterial grazers in Lake Michigan (Carrick and Fahnenstiel 1989). If we include ciliate production in our comparison with bacterial production, 135% of bacterial production (again assuming 30% efficiency) could be consumed. Although this comparison is not ideal because bacterial production was not measured at the same time protozoan production was estimated, it does suggest that some ciliates in addition to Hnano are active grazers of bacteria and that bacterial production could support a significant portion of heterotrophic protozoan productivity in the lake.

Recent studies have shown the quantitative importance of ciliate grazing impact on phototrophic picoplankton in Lake Michigan (Fahnenstiel et al. 1991) and ciliate grazing on bacteria in some marine systems (Sherr and Sherr 1987); our results add to this contention. Although bacterial growth was substantially higher than the average protozoan growth rates presented here, some small ciliate taxa (*Halteria* sp., *Strobilidium* sp., and *Urotricha* sp.) that are known to be bacterivores (Fenchel 1987; Sanders et al. 1989) did achieve maximal rates comparable to those of bacteria (max growth rates of 1.28, 1.25, and 2.28 d^{-1}, respectively). It is also important to note that mortality of these small ciliates due to predators of similar size will reduce the efficiency with which production is transferred to metazoa. These findings concur with the idea that Hnano are grazed by macrozooplankton at higher rates compared with ciliates (Carrick et al. 1991).

The quantitative importance of small (<20 μm) phototrophs to primary production has been demonstrated in various habitats (*see* Stockner and Antia 1986). In the upper Great Lakes, >50% of primary productivity is attributable to organisms that pass a 10-μm screen (Fahnenstiel et al. 1986; Fahnenstiel and Carrick 1992). Our estimates of Pnano and microflagellate production (average equivalent spherical diameters, 7 and 25 μm) show that phototrophic flagellates contribute an average of nearly 25% to pelagic primary production in Lake Michigan and closer to 50% during midstratification (Table 3). These results suggest that despite their lower biomass in comparison to algae (i.e. diatoms, chlorophytes, and cyanobacteria: Fahnenstiel and Scavia 1987*b*), phototrophic flagellates contribute significantly to annual primary production in Lake Michigan.

We admit that our delineation of trophic level assumes that an individual is either heterotrophic or phototrophic, although some individuals may be mixotrophic and contribute significantly to both carbon pools. Ciliates in the Great Lakes are known to contain pigmented endosymbionts (Taylor and Heynen 1987; Carrick and Fahnenstiel 1990) and these, whether whole cells or chloroplasts, can be actively photosynthetic (Stoecker et al. 1989). Additionally, some phototrophic flagellates can supplement photosynthesis by ingesting picoplankton (e.g. Bird and Kalff 1986), although this phenomenon is believed to be of minor sig-

nificance in the Great Lakes (Fahnenstiel et al. 1991).

Nonetheless, we do demonstrate that protozoa contribute significantly to both the heterotrophic and phototrophic carbon pools in Lake Michigan based on cellular production estimates. Our estimates may be conservative because we did not take into account the potential cell shrinkage resulting from preservation, nor as stated previously, the predation that probably occurred in our bottles and produced lower realized growth rates. Given these factors, the quantitative importance of protozoa in the Great Lakes is quite evident and protozoan production is sufficiently large to account for high bacterial production in Lake Michigan.

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