

## Seasonal Dynamics of Phytoplankton and Planktonic Protozoan Communities in a Northern Temperate Humic Lake: Diversity in a Dinoflagellate Dominated System

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### Abstract

Species diversity and richness, and seasonal population dynamics of phytoplankton, planktonic protozoa, and bacterioplankton sampled from the epilimnion of Crystal Bog in 2000, were examined in order to test the hypothesis that these groups' diversity and abundance patterns might be linked. Crystal Bog, a humic lake in Vilas County, Wisconsin, is part of the North Temperate Lakes Long-Term Ecological Research Site. Phytoplankton and planktonic protozoa were identified and enumerated in a settling chamber with an inverted microscope. Bacterial cells were enumerated with the use of fluorescence 4', 6'-diamidino-2-phenylindole (DAPI)-staining procedures, and automated ribosomal intergenic spacer analysis (ARISA) was used to assess bacterioplankton diversity. Bacterial cell counts showed little seasonal variation and averaged  $2.6 \times 10^6$  cells/mL over the ice-free season. Phytoplankton and planktonic protozoan numbers varied by up to two orders of magnitude and were most numerous in late spring and summer. Dinoflagellates largely dominated Crystal Bog throughout the ice-free period, specifically *Peridiniopsis quadridens* in the spring, *Peridinium limbatum* in summer, and *Gymnodinium fuscum* and *P. quadridens* in fall. Brief blooms of *Cryptomonas*, *Dinobryon*, and *Synura* occurred between periods of dinoflagellate domination. The dominant dinoflagellate, *Peridinium limbatum*, was calculated to have a growth rate of  $0.065 \text{ day}^{-1}$  and a doubling time of 10.7 days. Heterotrophic nanoflagellates (HNFs) were a consistent component of the planktonic protozoa; seasonal patterns were determined for three

genera of HNFs (*Monosiga*, *Bicosoeca*, and *Desmarella moniliformis*). Three genera of ciliates (*Coleps*, *Strobilidium*, and *Strombidium*) comprised the greater part of the planktonic protozoa in Crystal Bog. The number of species of planktonic protozoa was too low to calculate a diversity index. Shannon–Weaver diversity indices for phytoplankton and bacterioplankton in the epilimnion followed very similar seasonal patterns in this lake, supporting the hypothesis that in freshwaters, diversity patterns of these groups are linked.

### Introduction

Numerous studies have documented the seasonal dynamics of planktonic algal and metazoan communities in temperate lakes of various trophic states. One particular class of temperate lakes, however, has been poorly studied. Oligotrophic, acidic lakes surrounded by wetlands dominated by *Sphagnum* moss are commonly referred to as bogs or fens and in the limnological literature as dystrophic lakes. Most limnologists would agree that these types of lakes are among the least understood aquatic systems and their microorganisms the most poorly described component of the food web. Prescott's [30] taxonomic reference *Algae of the Western Great Lakes Area* catalogues the microalgal flora of the region, but it does not identify the specific bodies of water in which collections were made or where specific species were found. Woelkerling [47] surveyed the algal flora of 28 acidic lakes, five alkaline lakes, and 12 closed bogs in Wisconsin with respect to summer phytoplankton and periphyton. Frost et al. [12] surveyed the phytoplankton communities in several northern Wisconsin lakes, including two acid lakes. Such surveys only indicate the common algal taxa on a few sample dates.

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Far less is known about the seasonal and spatial dynamics of planktonic protozoan communities in acidic lakes, possibly because planktonic protozoa are less easy to collect, preserve, and identify than algae or metazoa. Over the past three decades, research has demonstrated the roles of protozoa as consumers of bacteria and algae, as mineral recyclers in the microbial food web, and as prey consumed by the metazoa. In subtropical acidic lakes, ciliates belonging to the Oligotrichida are generally dominant [3]. In northern humic lakes heterotrophic nanoflagellates (HNFs) and ciliates have been reported infrequently [1, 2, 18, 20, 23].

Little has been known about the dynamics of bacterioplankton because there was no way to identify bacteria on morphological grounds and no generally accepted taxonomy of bacteria until the development of molecular methods. Van Hannen et al. [42] used denaturing gradient gel electrophoresis (DGGE) of 16S ribosomal DNA fragments to monitor changes in the bacterial community following a mass lysis of cyanobacteria. Höfle et al. [16] developed a community fingerprint based on extraction of bacterial community RNA and high-resolution electrophoresis of single 5S rRNA bands. Fisher and Triplett [8] developed a method for automated ribosomal intergenic spacer analysis (ARISA) that gives a rapid bacterial community fingerprint. This method has been applied to a northern temperate oligotrophic lake to examine the effects of resources and trophic interactions on freshwater bacterioplankton diversity [9].

Now that the tools for examining bacterioplankton diversity dynamics are available, it has become possible to evaluate their relationships to diversity changes in phytoplankton and planktonic protozoa, but such studies have not been previously performed during the same time period or in acidic water bodies. Höfle et al. [16] attempted to relate changes in bacterial diversity (based on 5S rDNA sequences) to changes in phytoplankton diversity in a eutrophic water body, and found some examples of possible linkages. However, in that study the phytoplankton and bacterial diversity data were not collected during the same time period, raising questions about the validity of the observed diversity relationships. The generality of diversity relationships among freshwaters of various types also remains uncertain.

As part of an NSF-sponsored Microbial Observatory (MO) program at the North Temperate Lakes Long-Term Ecological Research (NTL-LTER) Site, we monitored microbial community dynamics in the epilimnion of acidic Crystal Bog in northern Wisconsin throughout the season during the year 2000. Our initial objective under the MO program was to acquire a data set on the numbers, biovolumes, and diversity of phytoplankton and planktonic protozoa and the numbers and diversity of bacterioplankton in this humic lake. Our goal was to test the hypothesis that diversity patterns of bacterio-

plankton, phytoplankton, and planktonic protozoa may be linked in acid waters.

## Materials and Methods

**Site Description.** Crystal Bog Lake (89° 36' W long, 46° N lat) is a shallow humic lake located in the Northern Highlands State Forest in Vilas County, Wisconsin, where it is relatively undisturbed by human impact. As one of the NTL-LTER lakes, physical and chemical environmental data support the Microbial Observatory research program (<http://lter.limnology.wisc.edu/>). Crystal Bog has an area of 0.5 hectares (ha), a maximum depth of 2.5 m, and a mean depth of 1.7 m. Its average Secchi depth is 1.6 m. An extensive area of *Sphagnum* mat surrounds the lake, which has a pH of 5.1 and is classified as a poor fen. The *Sphagnum* mat controls the pH of the lake through cation exchange and organic anions and contributes significant amounts of dissolved organic carbon (DOC) to the system.

**Sample Collection and Processing.** For phytoplankton and planktonic protozoan community analyses, whole water samples of 500–1000 mL were collected from Crystal Bog in the fall of 1999 (September, October, November), the winter of 2000 (January and February), and biweekly throughout the ice-free period for 2000 (March 26 through November 17). Crystal Bog was sampled over the entire 2-m water column at the point of maximum depth using an integrated water column sampler consisting of a length of PVC pipe equipped with a ball-joint valve. A single sample from a station at the maximum depth of this small bog was collected on each sample date. Samples were preserved with 25% glutaraldehyde to a final concentration of 2% in each sample bottle. Samples were stored in the dark in a refrigerator until counted.

On the same collection dates throughout the ice-free period of the year 2000, water samples for bacterial community fingerprint analysis were obtained from Crystal Bog and filtered through autoclaved 10- $\mu$ m nylon mesh screening (Spectrum) to remove eukaryotic cells. Samples were cooled on ice for transport back to the Trout Lake field station. Water samples were filter-concentrated in aliquots of 250 or 500 mL onto sterile 0.2- $\mu$ m filters (Supor-200, Gelman). Filters were then placed in cryovials, frozen in liquid nitrogen, and stored at  $-80^{\circ}\text{C}$  until DNA could be extracted with a FastPrep DNA purification kit (BIO 101). In addition, 250 mL of unfiltered water was also preserved in 2% glutaraldehyde for later enumeration of bacterial cells.

**Identification and Enumeration of Algae and Protozoa.** Twenty-mL aliquots of preserved sample were

settled in chambers for  $\geq 48$  h prior to counting. Counting was performed on an Olympus IX-50 inverted microscope at 200 $\times$  and 400 $\times$ . Algae and protozoa were identified and counted in one half the surface area of the settling chamber, equivalent to 10 mL of sample. The remaining half of the chamber was scanned at 200 $\times$  for additional counts of larger phytoplankton and protozoan species present at low densities.

Identifications of phytoplankton were based on Smith [39] and Prescott [30] plus additional specialized texts for dinoflagellates [29], desmids [7, 31–34], and chrysophytes [4]. Identifications of protozoa were based on Kudo [21], Patterson [24], and particularly Foissner and Berger [10], together with their associated taxonomic volumes [11]. Identifications were made to species where possible. Abundance of each species was expressed as number of cells, colonies, or filaments per liter. For mean cell, colony, or filament volume estimates, at least 10 individuals (when available) were measured for size with a calibrated ocular micrometer on each sample date, and volumes were calculated based on standard geometric formulas [14]. Novel geometric formulas were devised for some taxa, for example, those shaped like a cone of elliptic cross section or a cylindrical filament wound into a coiled spring. Biovolume of each species was the product of the count/liter and the mean volume.

**Bacterial Abundance Analysis.** Bacterial abundance was determined by staining 2 mL of unfiltered preserved water from Crystal Bog with 4', 6'-diamidino-2-phenylindole (DAPI) according to the procedures given in Porter and Feig [28]. The stained bacterial cells were filtered onto black 25-mm 0.2- $\mu\text{m}$  pore size PCTE filters, mounted on slides, and examined under oil immersion with a Nikon Diaphot epifluorescence microscope. The numbers of bacterial cells were then counted in 10 random Whipple grids per slide on two perpendicular transects. Additional information on bacterial enumeration is available in the on-line methods manual for the Microbial Observatory for the NTL-LTER site (<http://microbes.limnology.wisc.edu/methods.htm>).

**Community Fingerprint Analysis of Bacteria.** Bacterial DNA was extracted from 500 mL of filtered lake water using the FastPrep DNA purification kit (BIO101). Bacterioplankton diversity was assessed by automated ribosomal intergenic spacer analysis (ARISA). PCR for ARISA was performed following the method of Fisher and Triplett [8] with modifications. PCR reactions contained PCR buffer consisting of 50 mM Tris (pH 8.0), 250  $\mu\text{g}$  of bovine serum albumin per mL and 3.0 mM  $\text{MgCl}_2$  (Idaho Tech), 250  $\mu\text{M}$  of each dNTP, 10 pmol of each primer, 1.25 U of *Taq* polymerase (Promega), and 1

$\mu\text{L}$  of lake-extracted DNA in a final volume of 25  $\mu\text{L}$ . The primers used for ARISA were 1406f (universal 16S rRNA gene; 5'-TGYACACACCGCCCGT-3') labeled with 6-FAM, and 23Sr (bacteria-specific, 23S rRNA gene; 5'-GGGTTBCCCCATTCRG-3'). All PCR was carried out in an Eppendorf MasterCycler Gradient (Eppendorf). The initial denaturation was performed at 94°C for 2 min, followed by 30 cycles of 94°C for 35 s, 55°C for 45 s, and 72°C for 2 min, with a final extension carried out at 72°C for 2 min.

Denaturing capillary electrophoresis was carried out for each PCR reaction using an ABI 310 Genetic Analyzer (PE Biosystems). Electrophoresis conditions were 60°C and 15 kV with a run time of 50 min using the POP-4 polymer. A custom 200- to 2000-bp rhodamine X-labeled size standard (Bioventures) was used as the internal size standard for each sample. The data were analyzed using GeneScan 3.1 software (PerkinElmer). To include the maximum number of peaks while excluding background fluorescence, a fluorescence cutoff of 500 fluorescence units was used.

**Diversity Analysis.** For algal and protozoan species, species richness was the total number of species identified in the bog on each sample date during the season. For bacteria the total number of distinct fluorescent peaks in the ARISA data for each sample was taken as an estimate of species diversity [8, 9]. Diversity of microbial communities was calculated and compared by using the Shannon–Weaver index [25]:

$$H' = - \sum (n_i/N) \ln (n_i/N)$$

For algae and protozoa a diversity index was calculated for both cell numbers and biovolumes;  $n_i$  was either the number of cells, colonies or filaments of each species or the biovolume of each species, and  $N$  was the total count or the total biovolume of all the phytoplankton or planktonic protozoa in a liter. For bacterial diversity  $n_i$  was the fluorescent peak area of the  $i$ th peak and  $N$  was the total fluorescence in the electropherogram.

The maximum diversity of a microbial community occurs when all the species are equally abundant in numbers or contribute equally to the total planktonic biovolume. Maximum diversity is given by:

$$H_{\max} = \ln S$$

$S$  is the total number of species observed on a sample date. The evenness ( $E$ ) of the microbial communities was calculated by comparing the actual diversity to this maximum diversity where

$$E = H'/H_{\max}$$

The value of  $E$  ranges from 0 to 1.0.

**Table 1. Species of phytoplankton in Crystal Bog (1999–2000)**

Dinoflagellates	Cryptomonads	<i>Arthrodesmus constrictus</i>
<i>Peridinium limbatum</i>	<i>Cryptomonas</i> (small)	<i>Arthrodesmus octocornis</i>
<i>Peridinium inconspicuum</i>	<i>Cryptomonas</i> (large)	<i>Tetmemorus brebissonii</i>
<i>Peridinium willei</i>		<i>Closterium lunula</i>
<i>Peridinium cinctum</i>	Cyanobacteria	<i>Closterium macilentum</i>
<i>Gymnodinium fuscum</i>	<i>Merismopedia elegans</i>	<i>Euastrum elegans</i>
<i>Gymnodinium cneoides</i>	<i>Merismopedia glauca</i>	<i>Xanthidium aculeatum</i>
<i>Hemidinium nasutum</i>	<i>Chroococcus turgidus</i>	<i>Cosmarium</i>
<i>Glenodinium armatum</i>	<i>Chroococcus prescottii</i>	<i>Spondylosium planum</i>
<i>Peridiniopsis quadridens</i>	<i>Chroococcus limneticus</i>	<i>Bambusina brebissonii</i>
	<i>Marssonella elegans</i>	<i>Scenedesmus quadricauda</i>
Chrysophytes	<i>Cyanarcus hamiformis</i>	<i>Scenedesmus bijuga</i>
<i>Uroglenopsis americanum</i>	<i>Aphanocapsa elachista</i>	<i>Ankistrodesmus falcatus</i> 1
<i>Dinobryon sertularia</i>	<i>Aphanocapsa delicatissima</i>	<i>Ankistrodesmus falcatus</i> 2
<i>Dinobryon bavaricum</i>	<i>Gloeotheca linearis</i>	<i>Chlamydomonas sphagnicola</i>
<i>Dinobryon divergens</i>	<i>Gomphosphaeria lacustris</i>	<i>Chlamydomonas</i> sp.
<i>Dinobryon</i> sp.	<i>Coelosphaerium pallidum</i>	<i>Botryococcus sudeticus</i>
<i>Mallomonas caudatum</i>	<i>Rhabdoderma lineare</i>	<i>Dictyosphaerium ehrenbergianum</i>
<i>Mallomonas alpina</i>	<i>Spirulina nordstedtii</i>	<i>Crucigenia tetrapedia</i>
<i>Chromulina</i>	<i>Anabaena</i> sp.	<i>Crucigenia fenestrata</i>
<i>Ophiocytium capitatum</i>	<i>Oscillatoria</i> sp.	<i>Tetraedron trigonum</i>
<i>Synura</i>		<i>Dispora crucigenoides</i>
<i>Epipyxis ramosa</i>	Chlorophytes	<i>Quadrigulla lacustris</i>
<i>Chryamoeba</i>	<i>Oocystis pusilla</i>	<i>Mougeotia</i>
<i>Rhipidodendron</i>	<i>Oocystis solitaria</i>	<i>Zygnema</i>
	<i>Sphaerocystis schroeteri</i>	
Diatoms	<i>Gloeocystis gigas</i>	Euglenoids
<i>Asterionella formosa</i>	<i>Gloeocystis planctonica</i>	<i>Euglena acus</i>
<i>Tabellaria</i>	<i>Schroederia setigera</i>	<i>Euglena acutissima</i>
<i>Synedra</i>	<i>Closteridium lunula</i>	<i>Euglena minuta</i>
<i>Navicula</i>	<i>Staurastrum tetracerum</i>	<i>Trachelomonas volvocina</i>
<i>Fragilaria</i>	<i>Staurastrum furcatum</i>	<i>Leptocinclis sphagnophila</i>
<i>Pinnularia</i>	<i>Staurastrum pentacerum</i>	<i>Phacus</i>
<i>Aulocoseira</i>	<i>Staurastrum vestitum</i>	<i>Astasia klebsii</i>
<i>Cyclotella</i>	<i>Staurastrum clevei</i>	<i>Entosiphon sulcatum</i>
<i>Meridion</i>	<i>Staurastrum cuspidatum</i>	
<i>Actinella punctata</i>		

## Results

**Species Richness of Phytoplankton and Protozoa.** In Crystal Bog 118 taxa of phytoplankton and planktonic protozoa were identified over the period from 18 September 1999 to 13 November 2000. This number included 96 species of phytoplankton (Table 1) and 22 of planktonic protozoa (Table 2). The distribution of numbers of phytoplankton species among the major taxonomic categories was dinoflagellates 9, chrysophytes 13, diatoms 10, cryptomonads 2, cyanobacteria 16, chlorophytes 38, and euglenoids 8. Planktonic protozoan species fell into three major categories: amoebae with four species, heterotrophic flagellates three, and ciliates with 15 recognized species.

Chlorophytes and chrysophytes were richest in species numbers in the summer. Cyanobacteria had their highest number of species in the fall, while diatoms were most abundant and species rich in the spring and fall when the bog was mixing. Euglenoids were most abundant in the winter. Amoebae were never common in the

plankton, but one species (*Chlamydomonas*) occurred in higher numbers in the fall from August to October than other periods of the year. Heterotrophic nanoflagellates were present all year, but because of their small size and lack of morphologically distinguishing features, only three genera could be identified. Plastid-bearing nanoflagellates such as *Epipyxis* and *Chromulina* were listed under phytoplankton. Among the genera of ciliates counted, only *Strombidium* and *Strobilidium* were consistently present in the epilimnion of Crystal Bog.

**Abundance of Bacteria, Phytoplankton, and Protozoa.** The numbers of bacterial cells and phytoplankton and protozoan cells, colonies, or filaments are given in counts per mL in Table 3. No bacterial counts were performed for February or March. Bacterial counts were the mean of three replicates and show very little variation with sample date. The counts ranged from a high of  $3.91 \times 10^6$  cells/mL to a low of  $1.34 \times 10^6$  cells/mL with a mean of  $2.57 \times 10^6$  cells/mL for the whole season. In

**Table 2. Species of protozoa in Crystal Bog (1999–2000)**

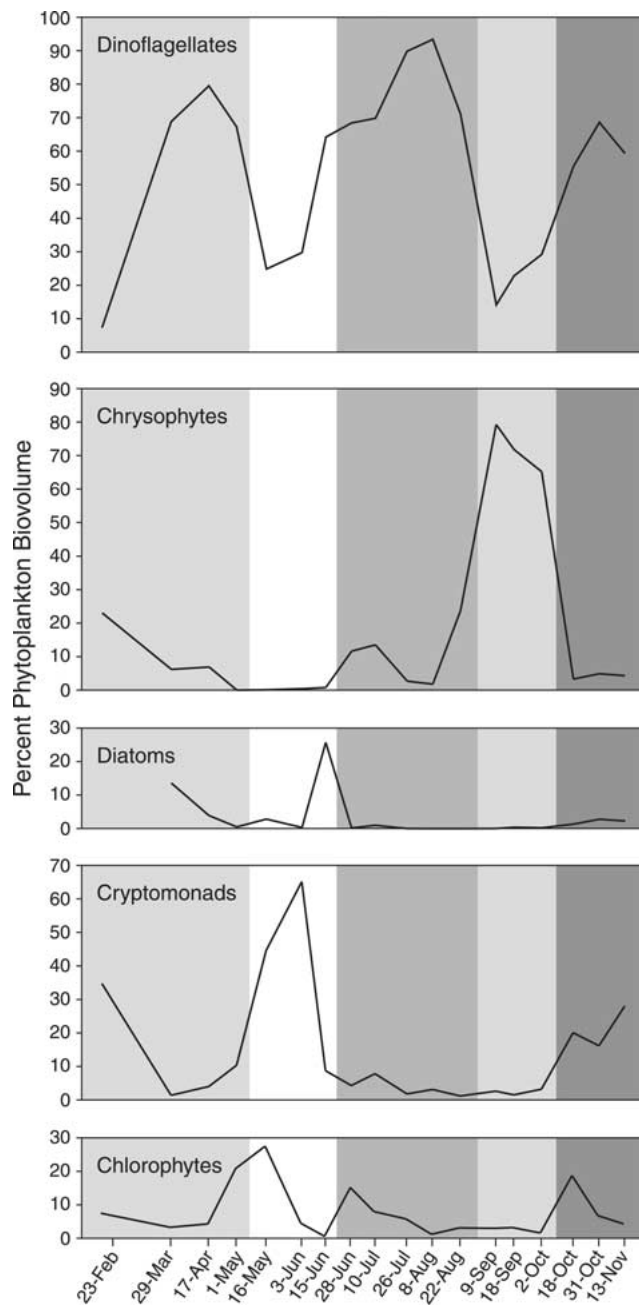
Amoebae	Ciliates
<i>Arcella hemisphaerica</i>	<i>Strombidium</i>
<i>Actinophrys</i>	<i>Strobilidium</i>
<i>Chlamyaster</i>	<i>Cyclidium</i>
<i>Diffugia</i>	<i>Urotricha</i>
	<i>Prorodon</i>
	<i>Podophrya</i>
Flagellates	<i>Colpidium</i>
<i>Monosiga</i>	<i>Coleps</i>
<i>Bicosoeca</i>	<i>Vaginicola annulata</i>
<i>Desmarella moniliformis</i>	<i>Paramecium</i>
Other nanoflagellates	<i>Phialina</i>
	<i>Stentor</i>
	<i>Uroleptus</i>
	<i>Aspidisca</i>
	<i>Bursaridium pseudobursaria</i>
	<i>Rhabdostyla</i>

contrast phytoplankton cell numbers ranged over more than two orders of magnitude from a low of 36 cells/mL to a high of 4246 cells/mL. The counts of protozoa were less variable, ranging over an order of magnitude from 17 to 377 cells/mL. Both phytoplankton and protozoa were most abundant in late spring (June 15 to 28).

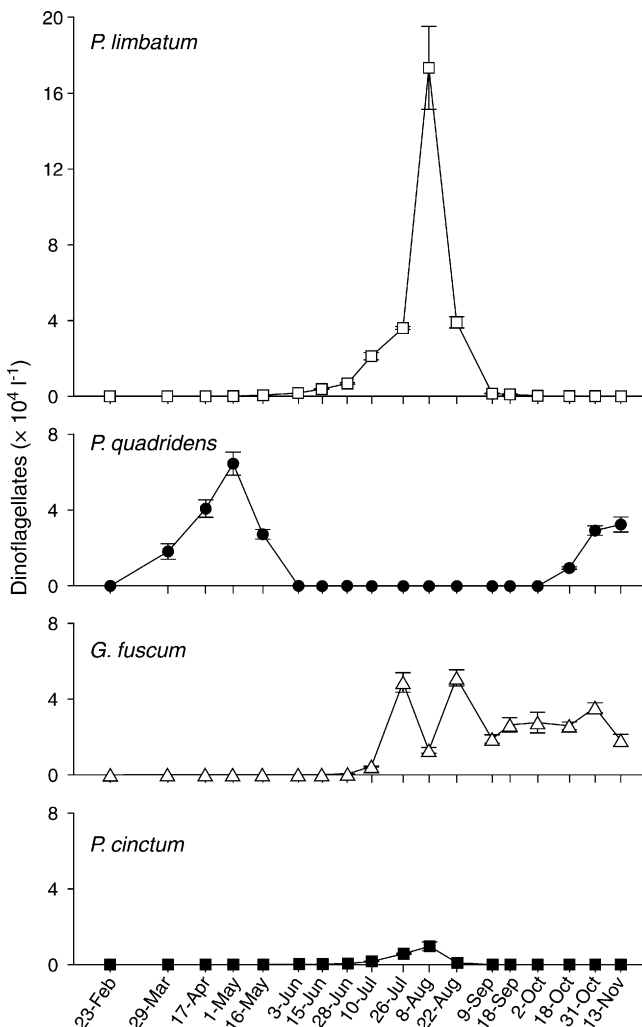
**Seasonal Patterns in Phytoplankton Assemblages.** Five taxonomic groups of phytoplankton (dinoflagellates, chrysophytes, diatoms, cryptomonads, and chlorophytes) were dominant in the sense that they represented at least 20% of the total phytoplankton biovolume for one or more sample dates during the season (Fig. 1). Dinoflagellates represented >20% of the total phytoplankton biovolume on every sample date except September 9. The chrysophytes comprised >20% of total phytoplankton biovolume on four sample dates

**Table 3. Cell numbers of bacteria, phytoplankton, and protozoa in samples from the epilimnion of Crystal Bog**

Sample date	Bacteria (cells $\times 10^6$ /mL)	Phytoplankton (cells/mL)	Protozoa (cells/mL)
23 Feb		36	17
29 Mar		313	243
17 Apr	2.74	205	190
1 May	2.08	377	110
17 May	2.58	1914	85
3 Jun	3.91	1484	65
15 Jun	2.36	4246	331
28 Jun	1.34	2787	377
10 Jul	2.27	1752	150
26 Jul	2.19	2137	106
8 Aug	2.61	1197	196
22 Aug	2.69	3747	79
9 Sep	2.9	1855	62
18 Sep	3.76	2275	96
2 Oct	2.87	331	138
18 Oct	2.17	706	161
31 Oct	2.1	811	191
13 Nov	2.58	1346	175

**Figure 1.** The pattern of dominant phytoplankton groups in the ice-free season for Crystal Bog in 2000. Of the five phases shown three phases were dominated by dinoflagellates. All values are expressed as percent of total phytoplankton biovolume.

(22 August through 10 October). Cryptomonads also represented >20% on four sample dates but at separate times in the season (15 May and 3 June and again on 18 October and 13 November). Chlorophytes reached 20% of total phytoplankton biovolume from 1 to 16 May, and diatoms exceeded 20% only on 15 June. The cyanobacteria never reached more than ~5% of total phytoplankton biovolume. Dinoflagellates were clearly the



**Figure 2.** The seasonal abundance (cells  $\times 10^4 \text{ L}^{-1}$ ) of the four most common dinoflagellates in Crystal Bog in 2000.

most dominant taxonomic group in Crystal Bog in terms of biovolume.

The ice-free period in Crystal Bog was divided into five phases, each exhibiting characteristic phytoplankton populations. The first phase extended from ice-out up to early May. The phytoplankton community was initially rich in species but that diversity declined as dinoflagellates increased in numbers, especially *Peridiniopsis quadridens*, which reached 65,700 cells/liter on 1 May when it comprised 60% of the total phytoplankton biovolume.

The second phase began after 1 May, as increasing water temperatures appeared to bring down the spring bloom of dinoflagellates. *P. quadridens* fell from its peak abundance on 1 May to zero on 15 June 2000 (Fig. 2). Other spring dinoflagellate species (*Glenodinium armatum* and *Hemidinium nasutum*) also declined and disappeared from the epilimnion. During this spring turnover of species, when dinoflagellates lost their

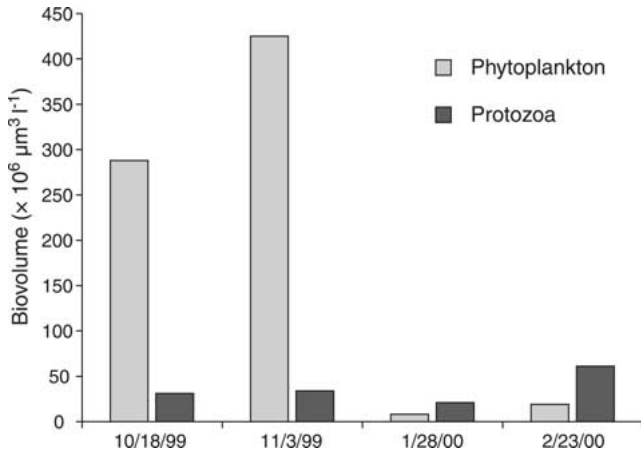
dominance, there was a series of pulses as other phytoplankton groups briefly achieved large numbers. Chlorophytes reached  $1.25 \times 10^6$  cells/liter and comprised 28% of total phytoplankton biovolume on 16 May, but a dense population of cryptomonads quickly replaced them. Cryptomonads reached 539,000 cells/liter and 65% of phytoplankton biovolume on 3 June (Fig. 1). By the following sample date the cryptomonads had declined to only 105,000 cells/liter, but the diatoms bloomed to  $3.53 \times 10^6$  cells/liter due to a surge in numbers of the centric diatom *Cyclotella*, which represented 26% of total phytoplankton biovolume on 15 June. The second phase ended with the collapse of this diatom bloom.

The emergence of a new summer assemblage of dinoflagellates, including *Peridinium cinctum*, *Gymnodinium fuscum*, and dominated by the large, armored *Peridinium limbatum*, marked the third phase (Figs. 1 and 2). From June 15 to July 10 these three species of dinoflagellates represented >64% of total phytoplankton biovolume. At this same time there was a surge in the mixotrophic flagellates *Dinobryon sertularia* ( $1.67 \times 10^6$  cells/liter), *D. bavaricum* (81,900 cells/liter), and *D. divergens* (10,100 cells/liter). Subsequently the dinoflagellate assemblage rose to 90% and 94% of total phytoplankton biovolume on 26 July and 8 August, respectively. On 8 August *P. limbatum* alone accounted for 89.8% of total phytoplankton biovolume when its abundance in the water column reached 176,000 cells/liter.

The fourth phase occurred during late summer and fall, as the dominance of *P. limbatum* declined (Fig. 1). There was a strong peak of the colonial chrysophyte *Synura* (113,000 colonies/liter on 18 September) together with two species of *Mallomonas* (*M. caudatum* and *M. alpina*). All three were likely to be highly grazing resistant due to large size or the presence of long sharp spines. Dinoflagellates returned to dominance in the fifth phase on 18 October. At this time only a few cells of *P. limbatum* (about 75 cells/liter) remained in the water column. *Gymnodinium fuscum* and a return of *Peridiniopsis quadridens* marked this fall resurgence of dinoflagellates (Fig. 2).

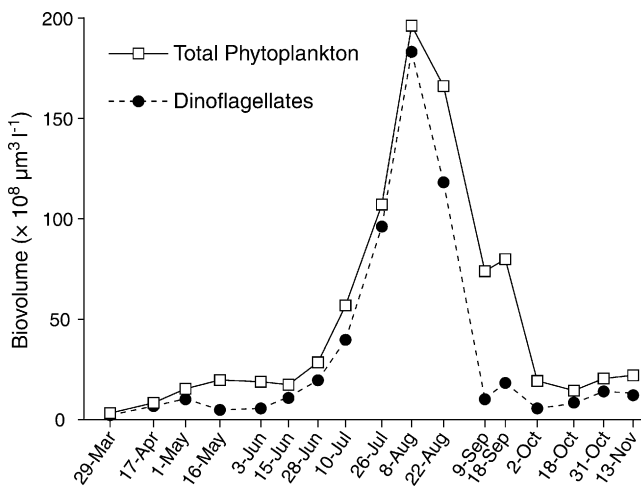
The winter period of ice cover can be treated as a separate microbial phase. Phytoplankton species richness in Crystal Bog declined during winter, but the species richness of protozoa increased over the same time period. Heterotrophic microorganisms dominated the bog as the biovolume of protozoa exceeded that of the phytoplankton in January and February (Fig. 3). The planktonic biovolume became dominated by cryptomonads, euglenoids, and ciliates, which were the most abundant group through the winter months.

**Dinoflagellate Dominance.** When total phytoplankton biovolume was plotted against total dinoflagellate biovolume for the ice-free season, the dominance

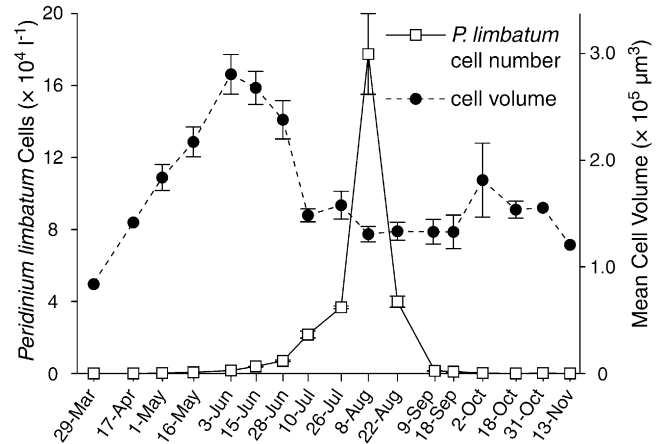


**Figure 3.** In winter, planktonic protozoa became the dominant eukaryotic microorganisms in terms of biovolume under the ice and snow.

of dinoflagellates in Crystal Bog in 2000 became evident (Fig. 4). The pattern for dinoflagellates mirrored that for the total phytoplankton. The largest discrepancy occurred from 9 September through 2 October, when a large bloom of *Synura* and significant numbers of *Mallomonas* dominated the fourth phase. Among the dinoflagellates, three species were the most abundant. *Peridiniopsis quadridens* dominated the first phase in spring. *Peridinium limbatum* dominated the summer or third phase and was the most abundant dinoflagellate in Crystal Bog. *Gymnodinium fuscum* was a strong presence in the third phase but became even more abundant in the fifth phase when *Peridiniopsis quadridens* returned (Figs. 1 and 2).



**Figure 4.** Dinoflagellates so dominated the phytoplankton of Crystal Bog that their seasonal pattern of biovolume largely determined the pattern for total phytoplankton biovolume over the entire ice-free season.



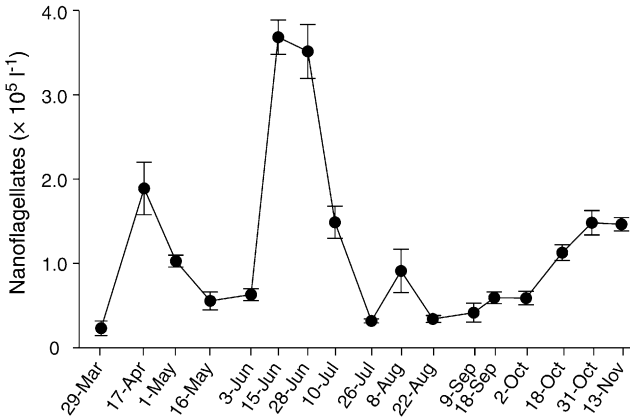
**Figure 5.** Growth of *Peridinium limbatum* in Crystal Bog was similar to the growth of a laboratory batch culture in terms of both cell numbers and changes in mean cell size. Error bars are too small to show beyond the symbols.

The number of *P. limbatum* cells/liter steadily increased from March through July and reached a maximum on 8 August (Fig. 5). The subsequent decline was far more rapid as the *P. limbatum* population fell from 176,000 cells/liter on 8 August to an average level of 75 cells/liter over the last three sampling dates. In the spring mean cell size increased during what could be termed a lag phase in the growth of *P. limbatum*. As the population began to grow exponentially, mean cell size declined to a stable level in August and September. After the crash in *P. limbatum* population, mean cell size rose among the remaining cells, as presumably more nutrients were available per cell with less intraspecific competition. When cell numbers were plotted as natural logarithms, linear regression gave a value of  $r = 0.065 \text{ day}^{-1}$  and a corresponding doubling time of  $t_d = 10.7 \text{ days}$  ( $n = 7$ ,  $r^2 = 0.99$ ).

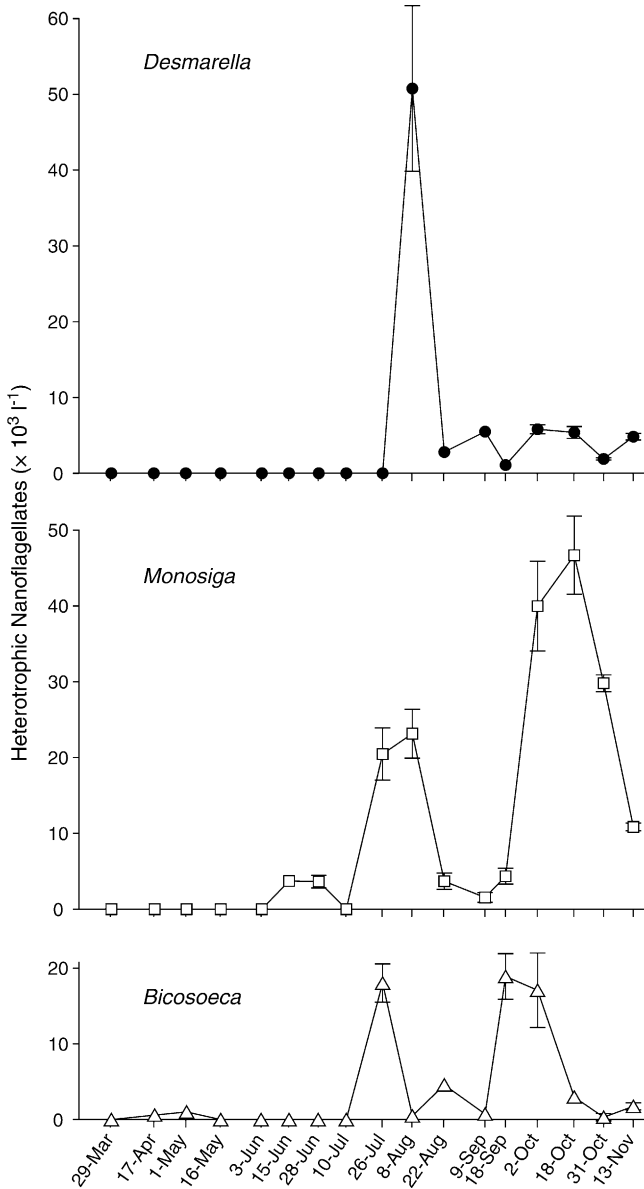
**Seasonal Patterns in Planktonic Protozoa.** Only one genus of amoebae occurred in the plankton with any consistency: *Chlamydomonas*, a small heliozoan, occurred in low numbers in the summer and fall (Table 2). Large testate amoebae were uncommon, and when they occurred, they probably represented washout from the *Sphagnum* mat.

Heterotrophic nanoflagellates (HNFs) occurred throughout the ice-free season in Crystal Bog (Fig. 6). Most nanoflagellates could not be identified to genus with the inverted microscope and magnification that was used; however, a few recognizable genera (*Monosiga*, *Bicosoeca*, and *Desmarella moniliformis*) began to appear in increasing numbers in late June and July (Fig. 7). *Monosiga* was the most abundant recognized HNF in Crystal Bog and reached its peak in autumn.

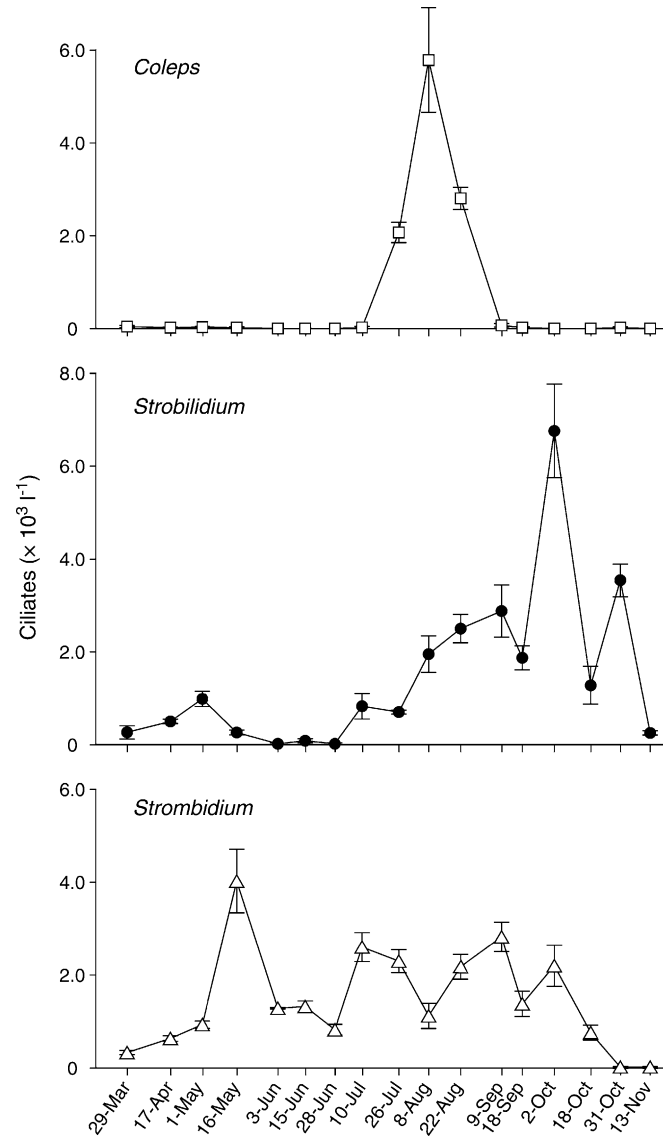
The majority of the identified genera of ciliates occurred only sporadically in the epilimnion of Crystal Bog.



**Figure 6.** Seasonal pattern in abundance of heterotrophic nanoflagellates in Crystal Bog, 2000.

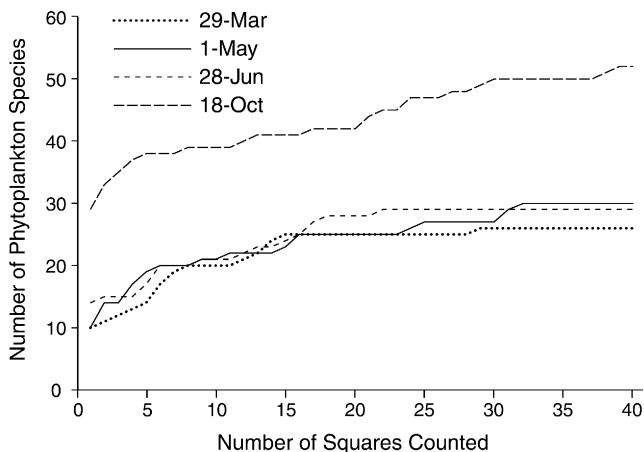


**Figure 7.** Seasonal abundance of three genera of heterotrophic nanoflagellates in Crystal Bog.



**Figure 8.** Seasonal pattern of abundance of the three common epilimnetic ciliates in Crystal Bog.

Detection of the genus *Rhabdostyla*, a solitary peritrich that is phoretic on the carapace of aquatic crustaceans, depends on settling an encumbered host. *Vaginicola annulata* normally occurs attached to a substratum, so its presence in the epilimnion in early spring (March and April) and again in fall (October and November) suggested that it washed into the water column when the lake mixed. Two ciliate genera were consistently present in the epilimnion—*Strobilidium* and *Strombidium* (which are phagotrophic on bacteria) (Fig. 8). A third ciliate *Coleps* produced the largest biovolume ( $2.26 \times 10^8 \mu\text{m}^3\text{L}^{-1}$ ) of any protozoan in Crystal Bog. The rise and decline in abundance of *Coleps* paralleled that of *Peridinium limbatum*.

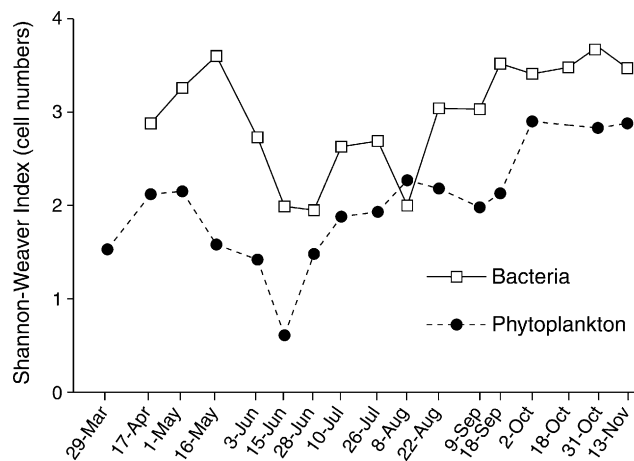


**Figure 9.** Accumulation curves for the number of species of phytoplankton as a function of counting effort.

#### Phytoplankton and Planktonic Protozoan Species Diversity.

A total of 10 mL of sample volume were counted for each replicate count (3–6) on each sample date. The surface area of the settling chamber was divided into square grids, each 3 mm on a side (9 mm<sup>2</sup>). As squares were counted, the number of species counted initially rose steeply but eventually reached a plateau as the number of species in the community on that date was approached as an asymptote. Such an accumulation curve is a plot of the cumulative number of types observed as a function of sampling effort (Fig. 9). The more concave-downward the accumulation curve the better sampled the community [17]. Plots of the values of the Shannon–Weaver index for phytoplankton against species numbers showed that the index first rose linearly with addition of species but then leveled out beyond 35 to 40 recognized species. This result reinforced the accumulation curves in Fig. 9. While further sampling would doubtless add a few more species, the effect on the Shannon–Weaver index should be negligible when counting effort had reached the plateau region because any additional species detected will be at such low numbers and biovolumes as to make very little change in the index.

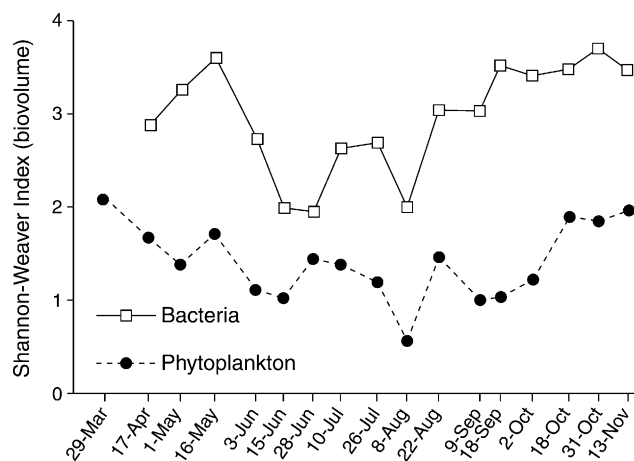
The diversity index for the phytoplankton of Crystal Bog was calculated on the basis of both cell or colony numbers (Fig. 10) and biovolumes (Fig. 11). Both diversity indices responded to changes in the microbial populations. The diversity index for cell numbers initially rose then declined in response to peaks in abundance of chlorophytes (16 May), cryptomonads (3 June), and diatoms (15 June). The peak in the abundance of the diatom *Cyclotella* ( $3.53 \times 10^6$  cells/liter) produced the greatest drop in the diversity index of cell numbers. After 15 June, the diversity index for cell numbers rose and remained at or above 2.0 for the remainder of the season. The diversity index based on species biovolumes pre-



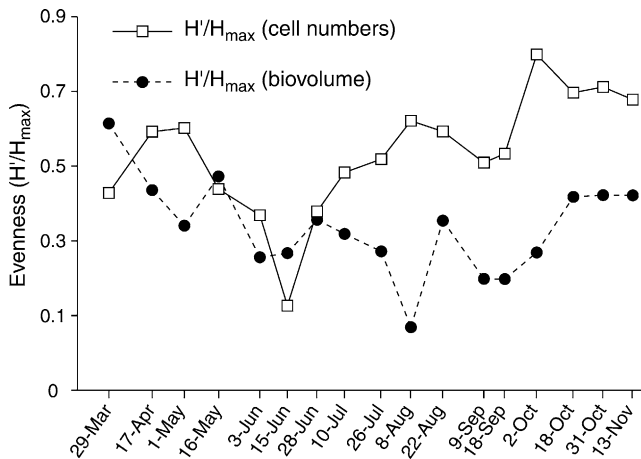
**Figure 10.** The Shannon–Weaver indices for bacterioplankton and phytoplankton in terms of cell numbers in Crystal Bog.

sented a somewhat different pattern (Fig. 11). The biovolume index declined from an initial value of 2.1 on 29 March and reached a low of 1.0 during the blooms of cryptomonads and diatoms on 3 June and 15 June, respectively. The diversity index for biovolume rose as dinoflagellates increased in abundance and biovolume but then dropped sharply to only 0.53 when *Peridinium limbatum* reached almost 90% of total phytoplankton biovolume. The crash in the *P. limbatum* population produced a rise in the biovolume diversity index, but the subsequent bloom in the colonial chrysophyte *Synura* led to another drop. As the bloom of *Synura* declined in October, the diversity index rose and returned to the level first seen on 29 March.

There were too few species of protozoa present in the epilimnion on a consistent basis to permit the calculation of a diversity index.



**Figure 11.** The Shannon–Weaver indices for bacterioplankton and phytoplankton in terms of biovolume in Crystal Bog, 2000.



**Figure 12.** The evenness indices for phytoplankton communities in terms of cell numbers and biovolume compared.

**Bacterial Diversity.** In Crystal Bog the Shannon–Weaver index began at 2.84 on 17 April and rose moderately to 3.57 by 16 May (Figs. 10 and 11). Thereafter the index dropped to 1.94 on 15 June rose to 2.57 on 10 July, and then fell to 2.0 on 8 August. The index jumped from 2.0 to 3.0 on 22 August, and then slowly rose to a plateau averaging 3.51 over the period from 18 September through the last sample on 13 November 2000.

The drop in the bacterial diversity index from 17 May through 15 June seemed to coincide with the decline in abundance of *P. quadridens* and the subsequent blooms of *Cryptomonas*, *Cyclotella*, and *Dinobryon*. The following rebound in the bacterial diversity index seemed to parallel the rise in the phytoplankton index. The phytoplankton index rose as a result of increasing numbers of species of chrysophytes and chlorophytes in the epilimnion. As *P. limbatum* dominated the system, however, both indices dropped in synchrony. The bacterial diversity index rebounded in response to the crash in *P. limbatum* on 22 August. After 8 August, the bacterial diversity index increased in a more consistent manner than the index for the phytoplankton community. In general the phytoplankton diversity index based on species biovolumes tracked the changes in the bacterial diversity index more closely than the index based on cell numbers.

Evenness was calculated on the basis of both cell numbers and biovolume (Fig. 12). Both evenness indices followed the same patterns as their corresponding diversity indices (Figs. 10 and 11). The evenness index for cell numbers was consistently higher than that for species biovolumes, suggesting that the phytoplankton community is more uneven in terms of biovolume than cell numbers.

## Discussion

Dinoflagellates are a recognized component of the phytoplankton communities of many freshwater lakes.

Watson et al. [43] examined the reported taxonomic composition of phytoplankton communities across temperate lakes of differing phosphorus nutrient status and showed that dinoflagellates are generally absent from hypereutrophic lakes but become more common, although never dominant, as lake trophic status declines through eutrophic to mesotrophic and oligotrophic. In mesotrophic and eutrophic lakes, dinoflagellates may occur as blooms, particularly the large armored genera *Ceratium* [13] and *Peridinium* [27]. In the present study dinoflagellates dominated Crystal Bog throughout most of the ice-free season. In one other study of a deep humic lake in France (Lake Vassivière), dinoflagellates were abundant in summer, but diatoms dominated the lake phytoplankton [1]. In previous studies single phytoplankton samples from lake surveys had indicated that dinoflagellates could be common in acidic lakes in Wisconsin [47]. Frost et al. [12] had even reported that *P. limbatum* represented 87% of phytoplankton biovolume in Crystal Bog on 23 August 1996, but no comprehensive seasonal data were collected.

In Crystal Bog the seasonal dynamics of the phytoplankton community were divided into five phases, three of which were dominated by dinoflagellates. Phases are commonly used to describe the annual pattern of seasonal succession in phytoplankton communities [44]. The most familiar pattern applies to temperate eutrophic lakes where the breakup of winter ice is followed by a spring diatom bloom. As silica concentrations in the water are depleted, diatoms decline and are replaced by first green algae and then cyanobacteria. The cyanobacteria are in turn replaced by diatoms when water temperatures decline and the lakes mix in the fall. Patterns of seasonal succession in phytoplankton vary with lake trophic status and latitude. The pattern described for Crystal Bog appears to be different from other published patterns. To determine how consistent this pattern is within Crystal Bog, the bog would have to be sampled over additional seasons. Additional bogs in northern Wisconsin would need to be sampled over entire ice-free seasons to establish how widespread this dinoflagellate-dominated pattern was in acidic systems.

In Crystal Bog *Peridinium limbatum* was the dominant dinoflagellate in the sense that it persisted throughout the entire open-water season and dominated total phytoplankton biovolume in the summer. Counts of *P. limbatum* abundance were taken at sufficiently close intervals (2 weeks) to permit calculation of the maximum growth rate ( $0.065 \text{ days}^{-1}$ ) and doubling time (10.7 days). The average specific growth rate of *Peridinium* species in cultures using a number of different media was  $0.06 \text{ days}^{-1}$  with a generation time of 11 days [26]. Pollinger [26] described the growth of a bloom of *Peridinium* in Lake Kinneret as resembling the growth curve of a batch culture.

In Crystal Bog heterotrophic nanoflagellates (HNFs) were a persistent part of the protozoan community in the epilimnion. With the limitations imposed by the magnification of the inverted microscope and the lack of epifluorescence, only three genera of HNFs could be recognized. The lorica of *Bicosoeca* was readily detected, as was the collar and flagellum of *Monosiga*. *Desmarella moniliformis* could be recognized by its collar and occurrence in groups of four to 12 cells. Jansson et al. [18] counted and determined biomasses for *Bicosoeca* and Monosigales in samples from humic Lake Öträskelet using an inverted microscope. Arvola and Salonen [2] found *Desmarella moniliformis* in polyhumic lake Mekkojarvi in southern Finland and counted them with an inverted microscope at 400 $\times$ . There are few published reports on ciliates in humic lakes, but Kalinowska [20] studied ciliates in a number of small humic lakes in Poland. *Strombidium*, *Strobilidium*, *Coleps*, and *Urotricha* were common, as in Crystal Bog, and their abundances were similar. In some of these lakes in Poland, these genera were the only ciliates present.

The Shannon–Weaver index accounts for both species richness and evenness of the microbial community, but it is sensitive to the total number of species identified in the community. When a phytoplankton count is done as part of a limnological study, the goal is usually to identify and count only the dominant taxa in pelagic systems. A sample is collected and placed in some type of settling chamber where at least 100 individuals of each of the important species are counted. A count of 100 individuals ensures that the true count lies between 80 and 120 with 95% confidence [45]. Such a count requires surveying only a small volume of sample, usually <0.5 mL. While this procedure gives the dominant taxa of phytoplankton and their abundance and biomasses (if sizes are also measured) to a known accuracy, it provides no information about diversity. Since the measurement of microbial diversity was a prime goal of the MO program, a different approach to counting phytoplankton had to be adopted. For the Crystal Bog samples all the phytoplankton in at least 10 mL of sample were counted. For many species of phytoplankton and protozoa that were rare, the count of sample volume extended to 100 mL. The effect of this high level of counting effort was reflected in the accumulation curves, which asymptotically approached a plateau region for each sample date. Because the cumulative number of species counted was in this plateau region, the values of the Shannon–Weaver index stabilized.

The Shannon–Weaver diversity index has been used a number of times on phytoplankton communities in marine and freshwater systems [6, 36, 37]. Beaver and Crisman [3] reported Shannon–Weaver indices for protozoan communities ranging from 2.82 in hyper-eutrophic lakes to 1.53 in acidic, oligotrophic lakes. The

values of the Shannon–Weaver index generally fall between 1.5 and 3.5 for most eukaryotic microbial communities. In Crystal Bog the values of the Shannon–Weaver index for cell numbers varied from 0.5 to 3.0. When expressed on the basis of biovolume, the index varied from 0.5 to 2.1. For limnological studies biovolume is preferable to cell numbers because cell numbers do not represent biomass due to the extreme variation in cell sizes among algal species. The Shannon–Weaver index for biovolumes of phytoplankton tracked the changes in the bacterial index more closely than the index based on cell numbers. The Shannon–Weaver indices for the Crystal Bog bacterial and phytoplankton assemblages were calculated from data obtained by very different methods. The similarity of the two patterns suggests that diversity in bacterioplankton and phytoplankton communities of acidic water bodies is linked.

Most studies of bacterial diversity have been accomplished in marine or eutrophic freshwater systems, and a variety of techniques have been used to measure diversity, including cloning and sequencing of 16S rRNA genes [15, 46], density gradient gel electrophoresis (DGGE) [22, 42], and automated ribosomal intergenic spacer analysis [9, 49]. That of Höfle et al. [16] on eutrophic Lake Plußsee is particularly interesting because it is the only study that attempted to relate changes in bacterial diversity (as determined by 5S rRNA bands) to changes in phytoplankton species composition. The study, however, did not gather data on both phytoplankton and bacterioplankton simultaneously. Höfle et al. [16] obtained their phytoplankton data from Sommer [40], who collected the data in 1989. The study by Höfle et al. [16] did not detect any close correlation between phytoplankton and bacterioplankton community structures over the entire growth season. There were, however, similar declines in diversity of phytoplankton and bacterioplankton in the spring clear-water phase and the late August decline when a dinoflagellate *Ceratium hirundinella* dominated the phytoplankton. The bacterial diversity decline in Lake Plußsee in late August when *C. hirundinella* dominated seems very similar to our observations that Crystal Bog bacterial diversity declined as *Peridinium limbatum* populations increased.

There are three possible explanations for the apparent effect of *Peridinium limbatum* on bacterial diversity. When *P. limbatum* dominates the phytoplankton biovolume in July and August, it may release a restricted set of dissolved organic substrates that favor the growth of only a restricted set of bacterioplankton species. *P. limbatum* might directly consume bacterioplankton, but it is not known to be phagotrophic [38]. Those species of *Peridinium* that are known to be phagocytic are marine species and consume larger prey than bacteria [41]. A third possible mechanism for this effect on bacterial diversity could be the release of some toxic or allelopathic

agent into the water when the density of *P. limbatum* becomes very high. Toxic dinoflagellates are well known for marine species [5], and a few cases of toxic freshwater dinoflagellate blooms have been reported [19]. Rengefors and Legrand [35] reported that the winter-blooming dinoflagellate *Peridinium aciculiferum* killed a potential competitor *Rhodomonas lacustris* in two Swedish lakes. Wu et al. [48] found that *Peridinium bipes* produced a substance that was toxic for the cyanobacterium *Microcystis aeruginosa*. *Peridinium bipes* is closely related to *P. limbatum*.

The question of why dinoflagellates should be dominant in acidic systems may have more than one answer. The data from Crystal Bog suggest that acid waters are not favorable to blooms of diatoms and cyanobacteria. One hypothesis, therefore, could be that dinoflagellates are dominant because neither diatoms nor cyanobacteria are capable of competing with them in acid waters. Another hypothesis could arise from the nature of the food web in acid lakes; fish are often absent, so zooplankton species are often the dominant predators impacting the microbial food web. Any phytoplankton species that are essentially immune to predation by zooplankton could potentially form extensive blooms in an acid lake. *Peridiniopsis quadridens*, *Peridinium cinctum*, and *Peridinium limbatum* are heavily armored, and *Gymnodinium fuscum* has a special predator avoidance mechanism. It can jet away from any disturbance like a microbial squid by expelling mucocysts. Such a rapid flight response should be highly effective in avoiding crustacean predators. After 8 August, the decline in *P. limbatum* was so rapid as to suggest the action of some as-yet-undefined predator (that might also consume the bloom of *Coleps* at the same time).

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