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Phytoplankton Seasonality Along a Trophic Gradient of Temperate Lakes: Convergence in Taxonomic Compostion during Winter Ice-Cover

Emon Butts[1] and Hunter J. Carrick[1,*]

Abstract - A gap in our understanding of phytoplankton seasonality in temperate lakes exists mainly due to the lack of information collected during the winter months. We summarized seasonal changes of phytoplankton biomass and taxonomic composition relative to water-column biogeochemical conditions in 6 lakes located on Beaver Island and 1 site in Lake Michigan in close geographic proximity to each other (<20 km apart). A number of physical–chemical parameters (e.g., temperature, DOC) were similar between lakes, but lakes towards the interior of the island had lower pH, alkalinity, and conductivity. Moreover, lakes at the interior of the island supported 2-fold greater phytoplankton-chlorophyll and carbon compared with perimeter lakes, and phytoplankton taxonomic composition differed considerably during the ice-free period (April–December). Interestingly, the winter phytoplankton assemblages were strikingly similar in all 7 lakes, when large populations of phyto-flagellates (Chrysophyceae and Cryptophyceae) occurred under the ice at low light and temperatures < 4 ºC. Given the mixotrophic capabilities of these phytoflagellates, we suggest seasonal convergence reflects the community response to under-ice conditions, which promotes the occurrence of an important component of annual phytoplankton biomass.

Introduction

The factors that regulate phytoplankton seasonality (wax and wane) can be difficult to predict due to the many biotic and abiotic interactions that act singly or in concert, and influence population growth, dispersal, and survival (e.g., Reynolds 2006). As with most organisms, specific phytoplankton populations are more likely to dominate assemblages when environmental factors favor their key natural-history requirements; in extreme cases, these peak conditions can lead to the occurrence of seasonal blooms (Carrick 2011). In addition to increased nutrient availability, phytoplankton blooms have shown to be induced by physical factors such as water-column light availability and stability (e.g., Millie et al. 2014, Sandgren 1988). Seasonal phytoplankton blooms and sensitivity to changing climatic conditions have been documented in both marine and freshwater ecosystems (Winder et al. 2012), which seems logical, given that most seasonal algal blooms are associated with reoccurring environmental conditions that promote of the growth of native species, the presence of which, supports predictable features such as fisheries production in lakes and marine ecosystems (see Wetzel 2001). These seasonal phytoplankton assemblages need to be contrasted against harmful algal bloom events

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that are assocated with declines in ecosystem aesthetics, production of secondary metabolites harmful to vertebrates, and imbalances in primary productivity and community respiration (Azanza et al. 2005, Burkholder et al. 1992, Kirkpatrick et al. 2004, Rinta-Kanto et al. 2009).

The seasonal variation in phytoplankton biomass and taxonomic composition in temperate lakes has not been fully documented or understood. Most sampling regimes do not include winter collections because this period is assumed to be one with biological inactivity (e.g., Salonen et al. 2009). This situation has created a bias in the data collected to characterize the annual plankton cycle in most lakes (Twiss et al. 2012). Data from the winter months is important because ice-cover duration can constitute 4–6 months of the year, and thereby promotes strong changes in physical drivers such as light and temperature, which are sustained for longstanding periods and can be sensitive to changing climate (see Hampton et al. 2015). Given that light and temperature are key regulators of phytoplankton growth, dispersal, and survival (Huisman 1999, Interlandi and Kilham 2001, Litchman 1998, Sommer 1985), it stands to reason that the intensity and duration of winter conditions could greatly influence changes in phytoplankton biomass and species composition.

Lakes in the temperate zone (northern hemisphere) undergo seasonal changes that cause them to exhibit considerable variation in the timing and occurrence of specific phytoplankton assemblages (Reynolds 2006). In many lakes, plankton survive during ice-cover periods and can act to oxygenate the water column, apparently because production is sufficient to outweigh respiratory losses (e.g., Phillips and Fawley 2002). The phytoplankton that survive under ice-cover can serve as a food for higher trophic levels, acting to sustain metazoan populations when the overall plankton biomass is typically low. For instance, Vanderploeg et al. (1992) showed that diatom blooms in Lake Michigan fed and sustained multi-voltine, crustacean zooplankton assemblages under the ice. Townsend and Cammen (1988) demonstrated that, in some shallow lakes, winter phytoplankton blooms influenced calanoid copepod populations and that the timing of the blooms affected juvenile fish recruitment. Decomposition of winter–spring phytoplankton blooms have been shown to sustain future phytoplankton assemblages during much of the summer growing season, via the slow regeneration of nutrients to the water column (Falkowski et al. 1988), which can fuel hypoxia in some sensitive lakes (e.g., Lashaway and Carrick 2010, Reavie et al. 2016, Wilhelm et al. 2014). Interestingly, physical conditions in extreme, polar environments (i.e., Arctic and Antarctic regions) created extended periods of ice-cover, during which phytoplankton blooms occurred in and under the ice and constituted the bulk of annual primary production (Arrigo et al. 2012, Berman et al. 2005,).

Here we present data that show that phyto-flagellates dominated winter plankton assemblages in 6 lakes of strongly divergent biogeochemistry on Beaver Island, MI, and 1 in nearby Lake Michigan. These winter blooms occurred during harsh conditions of darkness and low temperature, in synchrony, independent of lake biogeochemistry and trophic state. Our research addressed 3 specific questions: (1) What biogeochemical patterns exist along the transition from lakes in Beaver Island's to near-shore Lake Michigan? (2) What are the seasonal shifts

in phytoplankton biomass and species composition among lakes that share a similar regional climate? and (3) What is the relative contribution of winter phytoplankton assemblages to annual phytoplankton biomass? Herein, we have restricted our analysis to surface mixed-water assemblages, which excludes information on subsurface assemblages that is likely to be more important in deeper, clearwater lakes such as Lake Geneserath and Lake Michigan.

Field-site Description

Beaver Island is located in northern Lake Michigan 51 km west of Charlevoix, MI. The island is roughly 145 km^2 in surface area and 9.6 km wide x 21 km long (Fig. 1). It offers a unique opportunity to study seasonal dynamics of phytoplankton

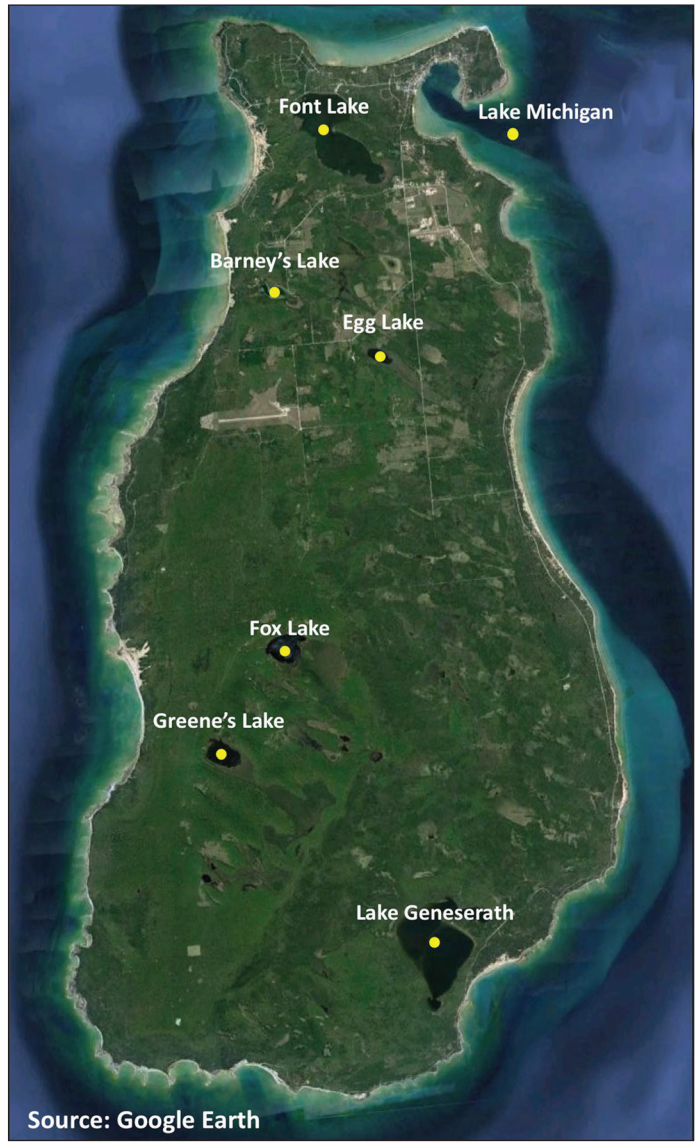

Figure 1. Map of the study sites.

because the island supports 6 inland lakes in relatively close proximity, allowing easy access for frequent sampling. These lakes are also noteworthy in that they experience relatively little anthropogenic impact in comparison to lakes on Michigan's mainland, thus making them valuable for comparisons to more-disturbed ecosystems (see Calabro et al. 2013). We sampled northern Lake Michigan at sites offshore of the northeast side of Beaver Island (45.55817°N, 85.47470°W). We retrieved water samples from under the ice during the winter months.

Methods

Sampling and ambient lake-conditions

We sampled 7 lakes on 10 dates over a 1-y period at approximately monthly intervals excluding the months of May and August (October 2011–September 2012). We sampled Lake Michigan from January to September 2012; this sampling excluded May and August. The 6 inland lakes were sampled at a single, offshore site, over the deep-water contour in each lake (at maximum depth); Lake Michigan was sampled offshore of the northeast end of Beaver Island (Fig. 1). Sampling dates covered all 4 seasons, which we defined as: winter (December, January, February); spring (March, April, June); summer (July, August, September); and fall (October, November). During winter ice-cover periods (January–March), we employed an ice auger to bore 4 successive holes in the ice to create 1 large opening. We trimmed the ice with a handheld axe and took water samples through the opening; ice thickness was measured using a handheld measuring tape. In each lake, we collected each sample at 0.5 m depth in 3.0-L Van Dorn bottles; immediately transferred the water into shaded, 10-L polycarbonate carboys; and transported them to the laboratory for further analysis and sample preservation.

We measured physical and chemical conditions in the field at each lake using a hand-held meter (model 880, YSI, Inc., Yellow Springs, OH) to measure temperature, conductivity, and dissolved oxygen content (DOC). We measured photosynthetically active radiation (PAR) on 3 occasions (December, June, July) in each lake using a Licor 1000 (Li-Cor, Lincoln, NE) equipped with underwater up-welling and down-welling radiometers (2 pi probes). We took readings at successive depths to estimate extinction coefficients (see Wetzel and Likens 2000). In the laboratory, we determined several other lake-water parameters from the samples collected on all 10 sampling dates. We employed a bench-top meter (Thermo Fisher OrionVstar, Thermo Fisher Scientific, Waltham, MA) to measure hydrogen-ion concentrations (pH). Alkalinity was estimated as ug/L $CaCO_3$ by titrating lake-water subsamples (100 ml) with 0.1 N HCl (Wetzel and Likens 2000). We dispensed surface-water subsamples into 2 bottles (250-ml polyethylene, amber): 1 preserved with 2% acid Lugol's solution and another with 1% gluteraldehyde; these samples were subsequently used to estimate phytoplankton taxonomic composition under the microscope (see below).

Phytoplankton chlorophyll and phosphorus content

We determined phytoplankton biomass on each date (n = 10) using 2 independent estimates: chlorophyll-*a* concentrations and estimated algal carbon from

phytoplankton-cell counts (see below). We measured chlorophyll concentrations fluorometrically on both raw and size-fractionated water. We conducted fractionations by filtering raw water through membrane filters with pore sizes that selected for organisms <2 µm and <20 µm in size. The filtrates were concentrated onto Whatman GF/F filters (effective pore size, 0.7 µm) and pigments were extracted using 50:50 acetone:DMSO without grinding (Carrick et al. 1991, Shoaf and Lium 1976). We measured fluorescence of the extracted pigments using a 10-AU fluorometer (Turner Designs, San Jose, CA).

We measured the total phosphorus (TP) and intracellular phosphorous concentration, in the form of intracellular polyphosphates bodies (poly-P), by concentrating the seston in lake water samples collected from each lake–date combination onto 0.2-µm membranes (Millipore GWSP; EMD Millipore, Billerica, MA) or analyzing whole lake-water, respectively. We determined total P content by treating subsamples of whole lake-water to a potassium persulfate extraction (final concentration of 2.4 mM), followed by autoclaving at 100 ºC for 60 min. The poly-P content of plankton material was measured by heating samples at 100 ºC for 60 min, thereby liberating soluble reactive P (PO_4^{-3}) from the condensed, inorganic polyphosphate compounds (poly-P) that can occur in either cyclic, linear, or cross-linked bonds with oxygen (Fitzgerald and Nelson 1966, Harold 1966). We then estimated both poly-P and total P concentrations as soluble reactive P measured colorimetrically using a spectrophotometer (method 365.1; USEPA 1997, 2002).

Phytoplankton biomass taxonomic composition

We estimated phytoplankton biomass (as cellular carbon) and taxonomic composition using complementary enumeration techniques (Booth 1993, Carrick and Schelske 1997). We enumerated the abundance and general taxonomic composition of phototrophic picoplankton from subsamples preserved with 1% gluteraldehyde that were filtered onto 0.2-µm black nuclepore membrane filters. The filters were then mounted onto glass slides with immersion oil, stored at -20 °C, and counted within one week to reduce fading of fluorescence (see Carrick and Fahnenstiel 1989). We used a Leica DMR 5000 research-grade microscope equipped for chlorophyll-*a* fluorescence (blue light 450–490-nm excitation and >515 nm emission) to performed the counts at 1000x magnification, and enumerated 250 individuals from 2 duplicate slides to determine phycobilin proteins (green light 530-560-nm excitation and >580 nm emission). We assigned general taxonomic (division) position and morphological categories (e.g., spherical, rod-shaped, colonial) based on dominant pigment fluorescence of individual picoplankton cells.

We enumerated the abundance and taxonomic composition of nano- and microsized phytoplankton from subsamples preserved with 2% Acid Lugol's; aliquoits were dispensed into settling chambers (10–50 ml volume) and allowed to settle for 24 h on coverslips (Utermöhl 1958). We counted a total of 300–400 cells by random fields under a Leica DMI 5000 research-grade, inverted microscope at both 400x and 630x magnification. The appropriate taxonomic references were used to

enumerate, to their lowest taxonomic position, the phytoplankton taxa encountered (Prescott 1962, Skuja 1956). Water-column cell densities and species-specific carbon were calculated using standard equations and conversion factors (e.g., Carrick and Fahnenstiel 1989, 1990). For both methods of microscopy, we calculated cell biovolumes (μm^{-3}) for each taxon by the cellular dimensions of at least 10 cells on at least 2 dates, took the average, and expressed the result as the equivalent spherical diameter (μm^{-3}). We converted taxon-specific biovolumes to carbon using equations of Strathmann (1967) for diatoms (0.1 pg C per μm^{-3}), the Verity et al. (1992) equation for nanoplankton (0.433 $[\mu m^{-3}]^{0.863}$), and the Laws et al. (1984) conversion factor for picoplankton (0.28 pg C per μm^{-3}).

Statistical analyses

We used factor analysis to evaluate environmental variation among lakes, where water-column sampling events (lake-date combinations) were considered observations and key biogeochemical parameters were considered variables. These data were log transformed to meet assumptions of normality, and then assembled into a 66 x 4 data matrix through an iterative process, whereby variables with the greatest explanatory power were retained for final analysis. We conducted factor analysis on the correlation matrix among variables (principal components analysis, PCA), retained the resulting factors with eigenvalues > 1.0 for interpretation, following an axis rotation procedure using the Varimax method (see Manly 1986). We scored the observations (sampling events) into the space defined by the newly derived factors, and grouped them by their proximity to one another according to visual inspection and subsequent statistical evaluation (see below). Differences in 9 major biogeochemical parameters (physical, chemical, biological) were evaluated among lake types (perimeter, interior) through a series of pair-wise comparions using a Mann-Whitney *U* test (significancnt at alpha = 0.05). Spatio-temporal variation in chlorophyll and phytoplankton carbon among lakes (perimeter, interior) and sampling periods (winter, spring, summer, fall) were evaluated using 2-way, multivariate analysis of variance (MANOVA; Zar 2009). We made pairwise comparisons with Tukey's multiple means comparisons to isolate pair-wise differences (alpha = 0.05). The data was log transformed to attain a normal distribution, and the assumption of equal variance was broken.

Results

Ambient conditions

The 7 lakes varied considerably in terms of their biogeochemistry and trophic status (Table 1). The range of pH among lakes was 3 units (Min–max = pH 5.47–8.56); conductivity values typically ranged almost 10-fold in magnitude (min–max = 29–286 $\mu S\ cm^{-1}$; see Table 1 for mean values). Mean alkalinity varied from 6.1 to 138.0 mg $CaCO_3\ L^{-1}$ among lakes, indicating broad differences in their buffering capacities; alkalinity was lowest in Greene's Lake and the highest in Barney's Lake. The average DOC varied from 8.84 mg L^{-1} to 10.20 mg L^{-1}. Total phosphorus concentrations ranged about 2-fold among lakes, with mean values from 10.61 to

19.77 µg L^{-1} (Table 1) and polyphosphate concentrations varied from 2.69 to 6.29 µg L^{-1}. Additionally, variation in the amount of visual color and productivity created nonuniform light penetration among the lakes, with attenuation coefficients of 0.13 to 2.77 m^{-1} (Table 1). During the winter period (December–February), ice thickness among all the Beaver Island lakes varied from 1.9 to 32 cm in thickness; however, mean ice thickness was not very different among the 6 island lakes (overall mean ± 1 SD = 15.6 ± 8.4 cm). Ice cover on Lake Michigan was variable due to movement of ice sheets throughout the lake. The mean water temperature, TP, and poly-P concentrations were not statistically different across the 7 lakes, which provided evidence that they experience similar climate conditions (1-way ANOVA: $F = 0.01$, $P > 0.05$).

The ordination of water-column variables produced 2 principal components (PC) with eigenvalues > 1.00 (Table 2, Fig. 2A). Both components were correlated strongly with original variables and collectively accounted for > 84% of the variation in the dataset. Once scored into the space defined by the 2 PCs, the 66 sampling events clustered into 3 discernable groups that corresponded well with the sampling periods (summer, spring/fall, winter; Fig. 2A). PC-1 accounted for 47.1% of the variation; this axis correlated positively with pH and conductivity ($r > 0.92$ for both). In general, sampling events were broadly distributed along PC-1; this result was expected given the wide range among lakes in their dissolved inorganic substances as reflected in the pH and conductivity measurements (carbon and total substances; Fig. 2, Table 2). PC scores among seasons exhibited complete overlap along this axis suggesting that variation was consistent among seasons (see Fig. 2A). Samples with higher pH and higher conductivity scored positively with PC-1; these samples were collected from Barney's Lake, Font Lake, Lake Geneserath, and Lake Michigan (lakes located around the perimeter of Beaver Island; Table 2). Samples with lower-conductance water had negative scores along this axis; these observations corresponded with samples collected from Greene's, Fox, and Egg Lakes (interior; Table 2). The pH of these lakes was generally <7 (Table 2). PC-2

Table 1. Mean values of key biogeochemical conditions measured from October 2011 to September 2012 among 7 lakes: 6 lakes on Beaver Island and nearshore Lake Michigan. Attenuation coefficients were measured on 3 occasions (December, June, July) , while ice thickness was measured on 3 dates (December, January, February). Water depths are estimates of the average depth. Temperature is represented as minimum and maximum values observed.

Lake	Type	Water depth (m)	Temp. (°C)	Total Phosphorus (µg L^{-1})	Conductivity (µS cm^{-1})	pH	Ligtht attenuation coeff. (m^{-1})	Ice thickness (cm)
Greene's Lake	Interior	3	5.4, 8.3	15.1	28.7	6.00	2.33	16.0
Fox Lake	Interior	6	4.9, 28.0	10.6	31.1	6.71	2.33	16.1
Egg Lake	Interior	2	5.3, 26.2	16.9	93.1	7.87	2.77	11.5
Lake Geneserath	Perimeter	10	5.1, 27.1	11.7	165.3	7.83	0.26	19.8
Font Lake	Perimeter	3	4.5, 25.4	15.5	182.1	8.14	1.12	10.9
Barney's Lake	Perimeter	3	4.9, 25.6	19.8	200.2	8.29	0.48	19.3
Lake Michigan	Perimeter	100	7.7, 23.5	10.9	228.9	8.14	0.13	Open

accounted for 33.4% of the variation. This axis correlated positively with temperature and internal phosphorus storage (as poly-P) in the plankton ($r > 0.81$ for both). In general, PC-2 seemed to define a phytoplankton physiological gradient defined by changes in the water-column temperature and storage of poly-P. This result makes sense given that the summer assemblages occurred at higher temperatures and stored larger concentrations of poly-P compared with the winter period (lowest scores). Interestingly, the spring and fall assemblages transitioned between the

Table 2. Pair-wise comparisons for biological, chemical, and physical variables measured among perimeter (Barneys, Font, Geneserath, Michigan) and interior (Egg, Fox, Greene's) lakes on Beaver Island, MI. Lakes were sampled on 10 occasions during October–September 2011–2012. Values (± SD) are averages for each lake type and comparions were performed using a series of Mann-Whitney *U* Tests (where, $^{**}P < 0.01$; ns = not significant).

Parameter (units)	Perimeter lakes	Interior lakes	Comparison
Biological			
Chlorophyll-*a* (µg/L)	5.1 ± 0.8	11.5 ± 1.4	Perimeter < interior**
Phytoplankton C (µg/L)	158.1 ± 19.0	390.5 ± 88.8	Perimeter < interior**
Chemical			
Alkalinity (µ/S/cm)	107.2 ± 5.5	19.7 ± 4.3	Perimeter > interior**
Conductivity (µ/S/cm)	184.6 ± 8.8	49.6 ± 5.5	Perimeter > interior**
Oxygen (mg/L)	9.7 ± 0.5	9.3 0.5	Perimeter = interior ns
pH	8.2 ± 0.1	6.9 ± 0.2	Perimeter > interior**
Poly-P (µg/L)	3.1 ± 1.3	4.3 ± 0.5	Perimeter = interior ns
Total P (µg/L)	14.6 ± 0.3	14.2 ± 1.2	Perimeter = interior ns
Physical			
Temperature (°C)	13.4 ± 1.2	13.3 ± 1.5	Perimeter = interior ns

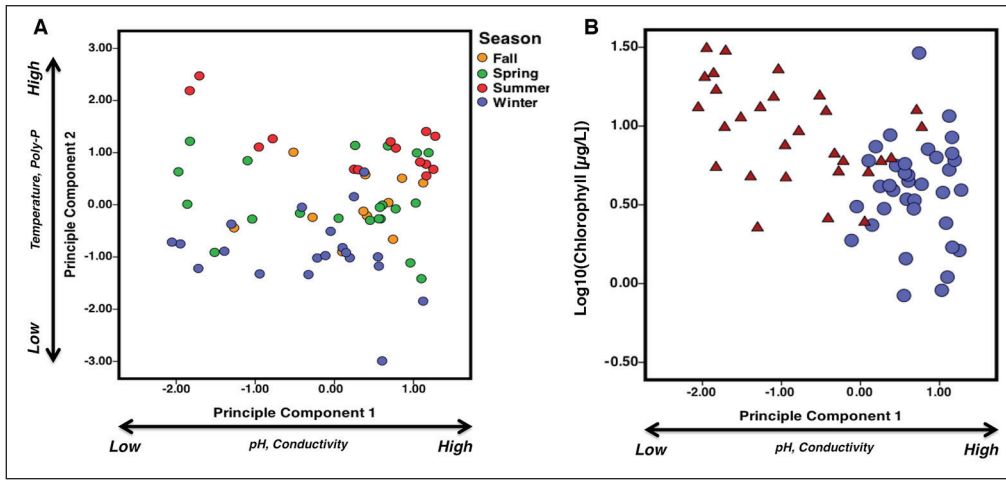

Figure 2. (A) Principal component analysis (PCA) ordination, where PC-1 accounted for 47.1% of the variation and was correlated positively with pH and conductivity. PC-2 accounted for 33.4% of the variation and correlated positively with temperature and internal phosphorus storage (as poly-phosphorus; Poly-P). Site–date samples were plotted against the PCs and distinguished by season. (B) Log chlorophyll plotted against component PC-1. Triangles represent interior lakes; circles represent perimeter lakes.

summer (high scores) and winter conditions (low scores). When PC-1 was correlated with chlorophyll concentrations, it showed that interior lakes supported greater phytoplankton biomass relative to the perimeter lakes and was statistically significant (Fig. 2B, Table 2).

Spatio-temporal variation in phytoplankton

The chlorophyll-*a* concentration among the 7 lakes (n = 10 for each) varied from 0.8 to 31.1 µg L^{-1}, indicating a trophic status spanning from oligotrophic to eutrophic (Table 2, Fig. 2). Lake Michigan (average ± 1 standard deviation, 1.18 ± 0.11 µg L^{-1}) had the lowest average concentrations of chlorophyll, whereas Greene's Lake (17.7 ± 2.9 µg L^{-1}) had the greatest chlorophyll concentrations. In terms of size structure, >60% of the chlorophyll in Lake Michigan was <2 µm in size; in the other 6 lakes, this fraction contributed <20% to the assemblages' overall mean biomass. In Barney's, Egg, Font, and Geneserath lakes, the the 2–20 µm and >20-µm fractions contributed similarly to the chlorophyll totals. The assemblage in Greene's Lake was dominated by chlorophyll from phytoplankton in the 2–20-µm size range, while the chlorophyll from cells in the >20 µm fraction was most prevalent in Fox Lake.

Total phytoplankton biomass (as estimated cellular carbon) exhibited a similar trophic gradient among lakes (range = 8.6–2451.7 µg C L^{-1}) and showed good agreement when compared with chlorophyll concentrations determined from paired samples (r = 0.76 $P < 0.0001$, n = 66). In general, phytoplankton biomass and chlorophyll-*a* were greater in the interior lakes than in the perimeter lakes (Tables 2, 3). Total phytoplankton carbon varied significantly among the 4 seasons; carbon levels were greater in the spring and fall periods compared with those in the summer and winter (Table 3).

Table 3. Two-way MANOVA results coupled with Tukey's pairwise comparisons that assessed variation in phytoplankton biomass (as celluar carbon, ug L^{-1}) among taxonomic categories, and for estimates of total phytoplankton. Lake type (perimeter or interior, see Table 1) and season (winter, spring, summer, fall) were considered fixed factors, where ns = not significantly different, * = $P < 0.05$, ** = $P < 0.01$, and *** = $P < 0.001$. No significant interactions were observed.

Factor	Group	*F*-value	Tukey's pairwise tests
Lake	Diatom	1.55 ns	No difference
	Chlorophyte	0.06 ns	No difference
	Chrysophyte	0.01 ns	No difference
	Cyanobacteria	0.02 ns	No difference
	Other	6.24*	Perimeter < interior
	Total phytoplankton	6.21*	Perimeter < interior
	Total chlorophyll	6.96*	Perimeter < interior
Season	Diatom	3.68*	Winter = summer < fall = spring
	Chlorophyte	2.40 ns	No difference
	Chrysophyte	4.71**	Summer < fall = winter = spring
	Cyanobacteria	10.97***	Winter < spring = fall = summer
	Other	5.96**	Winter < summer = spring = fall
	Total phytoplankton	6.02**	Summer = winter < spring = fall
	Total chlorophyll	0.22 ns	No difference

Key taxonomic groups (algal divisions) were not different between interior and perimeter lakes, although the biomass in 3 of the 5 groups varied among seasons (Table 3). The biomass of cyanobacteria, cryptophytes, dinoflagellates, and euglenoids was lowest in the winter compared with the other 3 seasons, whereas chrysophyte biomass was lowest in the summer compared with levels present in the other 3 seasons, and no seasonal differences among seasons were observed for diatoms and chlorophytes (Table 3, Fig. 3). Seasonal trends in phytoplankton taxonomic composition appeared to be lake-specific and showed considerable compositional changes from month to month (Fig. 3). Greene's, Fox, and Barney's lakes and Lake Michigan showed unimodal peaks in phytoplankton biomass over the year. Interestingly, the perimeter lakes supported spring or early summer phytoplankton blooms (March, April, June), all of which were composed of diatoms and chrysophytes. All 7 lakes supported mixed assemblages during the summer months (June, July, September). In the late summer–early fall period, cyanobacteria dominated in 6 of 7 lakes.

Despite the apparent seasonal differences in phytoplankton observed among lakes, a distinct environmental gradient of increasing temperature and poly-P storage (PC-2) were evident among the lakes, which corresponded with shifts in phytoplankton biomass and taxonomic composition (Fig. 4). The biomass of

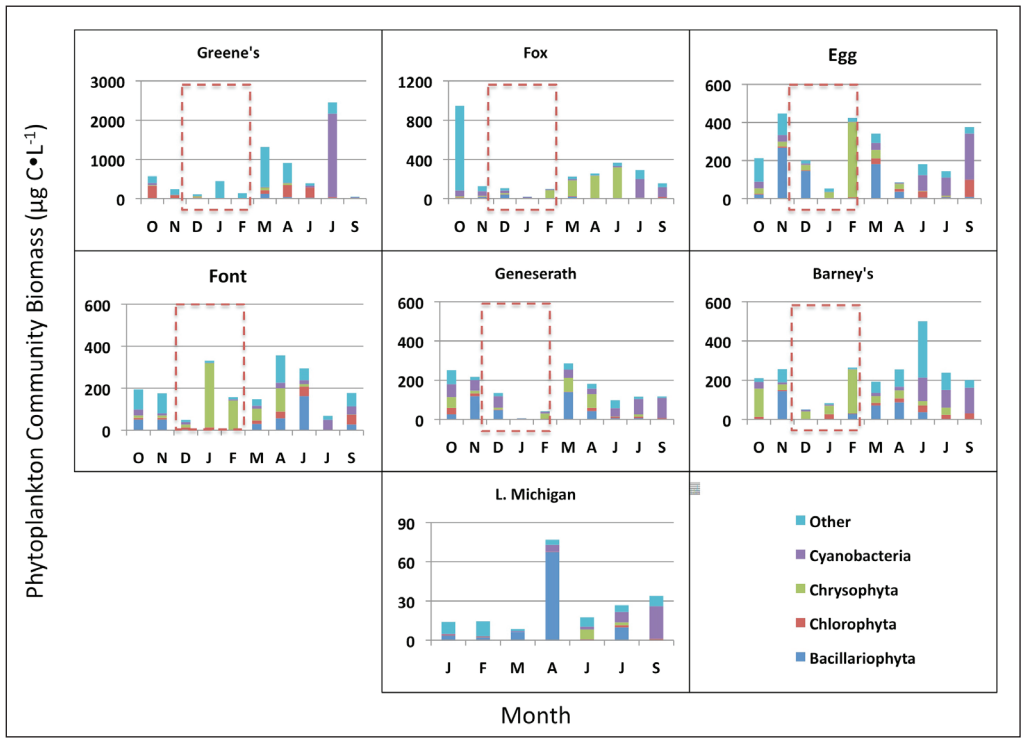

Figure 3. Estimates of phytoplankton biomass and taxonomic composition (as cellular carbon) plotted for the 10 seasonal sampling dates. The red box denoted the 3-month winter period when the lakes were ice covered (the other category includes Cryptophyta and Pyrrophyta).

cyanobacteria, chlorophytes, and the cryptophyte/dinoflagellate group all increased with temperature and poly-P concentrations in the plankton assemblage (Fig. 4). The biomass of diatoms increased with pH and conductivity, although this relationship was not significant (r = 0.25, P=0.57, n =57). In contrast, chrysophyte carbon exhibited an abrupt decline along a gradient of increasing temperature and poly-P storage (Fig. 4). This result made sense because the winter (December–February)

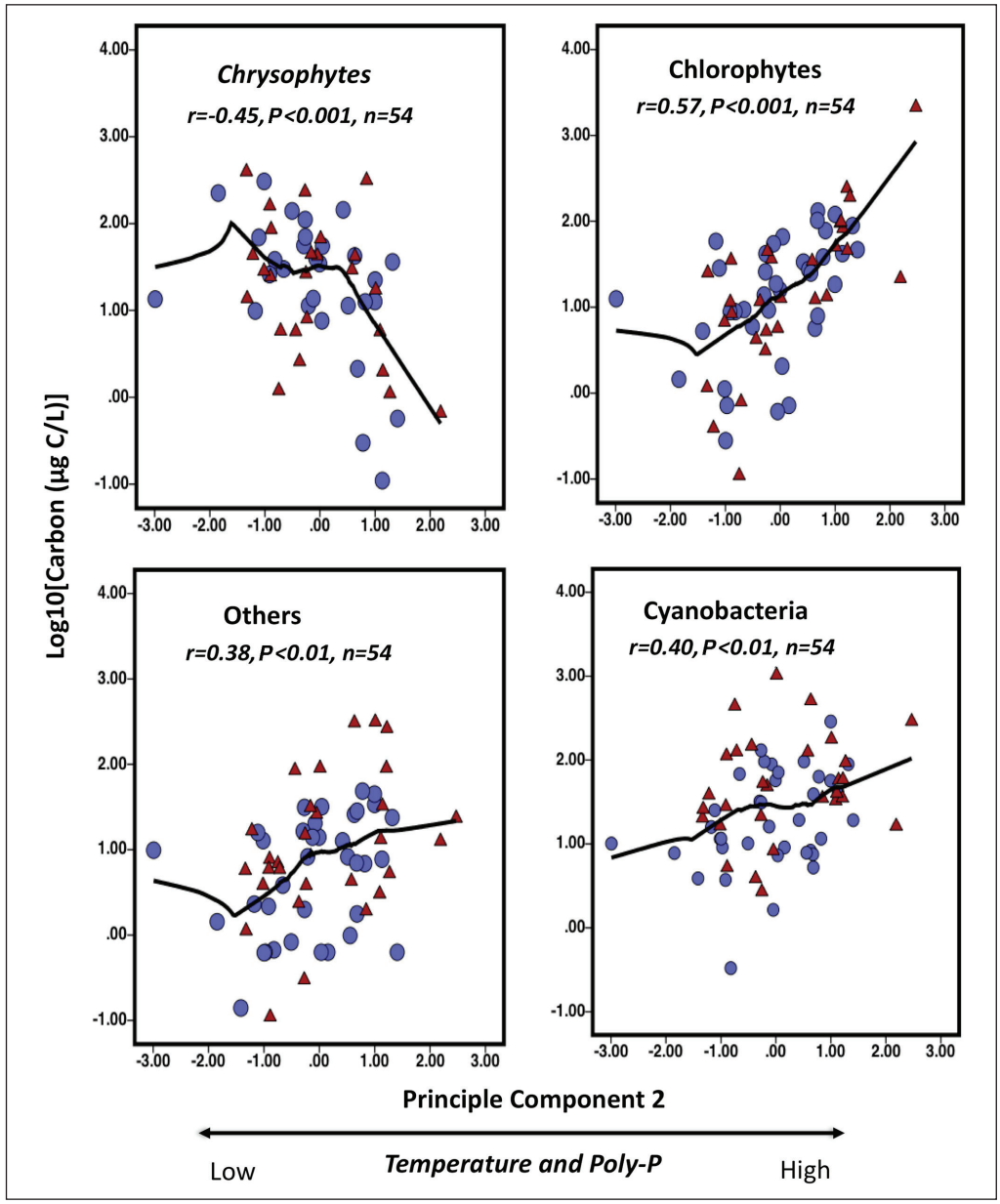

Figure 4. Phytoplankton biomass (as carbon) for each of the 4 major taxonomic groups (Chrysophyta, Chlorophyta, Cyanobacteria) and Others (Cryptophyta, Pyrrophyta) plotted against PC-2. Triangles represent interior lakes; while circles represent perimeter lakes.

phytoplankton assemblages in all 7 lakes were dominated by chrysophyte phyto-flagellates that reached their greatest biomass in 4 lakes during this time (Fig. 4). Interestingly, the 6 inland lakes were completely frozen during this period, while Lake Michigan was inundated with floating ice sheets at our sampling locations during the winter 2011–2012 (NOAA-GLERL 2017).

Contribution of winter assemblages

Our data showed that the biomass of winter phytoplankton assemblages was contributed mainly by the presence of phyto-flagellates (Fig. 3). The dominant flagellates occurring in the inland lakes were composed of chrysophytes, specifically several species of *Dinobryon* (*D. cylindricum* O.E Imhof, *D. bavaricum* O.E Imhof, *D. divergens* O.E Imhof, and *D. sertularia* Ehrenberg). In Lake Michigan, several phyto-flagellate species dominated the winter assemblage, including the cryptophytes *Rhodomonas minuta* Skuja and dinoflagellate *Gymnodinium varians* Maskell. This trend was surprising given that the lakes were ice covered, creating a seemingly harsh set of environment conditions for 3 months, when the environment was typified by low light (no light penetrated under the snow and ice in the 6 inland lakes) and low temperature (1.5–3.5 °C).

During ice cover, cumulative chlorophyll biomass was considerable in all of the lakes. When comparing phytoplankton biomass in the 6 island lakes over the entire year versus the biomass in chlorophyll that occurred during ice cover (December–February), winter assemblages contributed 24.8 ± 7.2% (mean ± 1 SD) of the annual biomass (Fig. 5). The winter assemblage in Fox Lake showed the smallest contribution (12% of its annual biomass), while Barney's Lake displayed the largest contribution (31.5%) (Fig. 5). In Lake Michigan, the contribution by

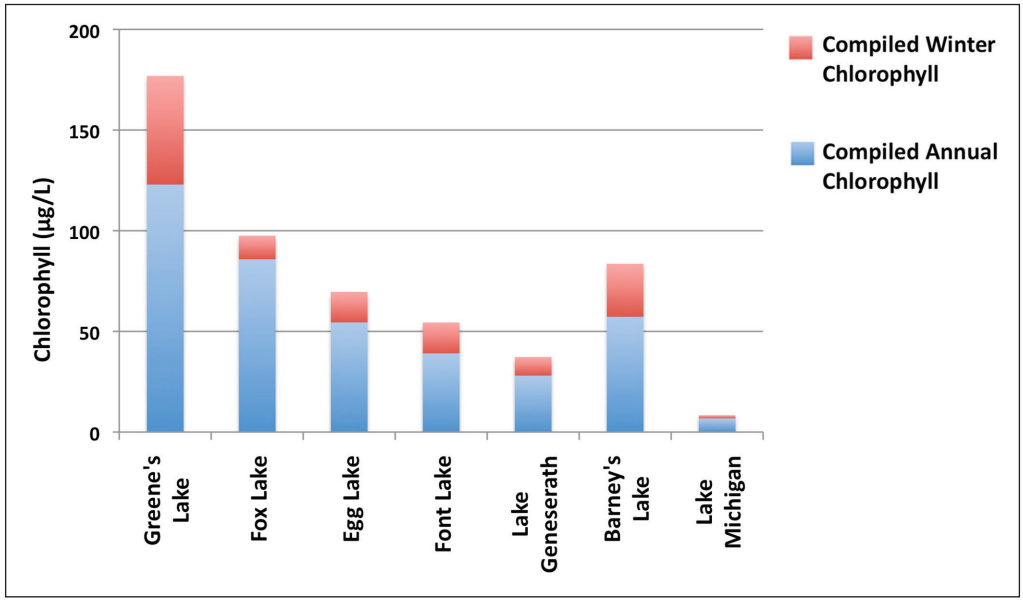

Figure 5. Compilation of all biomass in carbon of sampling dates highlighting the contribution by winter assemblages to the annual biomass.

winter assemblages was about ~7 %, but this total also excluded the 3 months (October–December) that were taken into account for the island lakes (Fig. 5). Thus, the winter phytoplankton community may play a key, but understudied, role in the overall function of the microbial food-web dynamics.

Discussion

Spatial patterns in lake biogeochemistry and phytoplankton biomass

The Beaver Island lakes share a historic relationship with Lake Michigan because their formation was thought to be regulated by the geologic history of the Laurentian Great Lakes some 12,600 years ago (see Lewis et al. 2010). Thus, the Beaver Island archipelago and specific landscape features were likely produced during the last North American glaciation, which formed the Laurentian Great Lakes through sequential fluctuations in lake water levels and the isostatic rebound of the landmass (Colman et al. 1994, Ewert et al. 2004). This scenario seems reasonable given the timeline for the formation of other landscape and geologic features that currently exist on Beaver Island (e.g., Angeline's Bluff and Bonner's Bluff; see Dietrich 1988). Furthermore, the lakes on Beaver Island were formed during different geologic periods, indicating that their ages differ, which may explain the divided spatial variation in biogeochemistry observed between the interior and perimeter lakes. For instance, the interior lakes include Greene's, Fox, and Egg lakes (Dietrich 1988) that were formed earlier in the natural history of the island, while the perimeter lakes— Barney's, Font and Geneserath Lakes—consisted of more recently formed embayments of Lake Michigan (Leuck et al. 2007).

The large degree of variation in lake biogeochemistry that we observed among the lakes on Beaver Island was atypical considering their relative proximity (see Wetzel 2001). This result suggests a relatively unusual scenario in comparison to those in other regions in North America. Specifically, lakes in the interior of Beaver Island exhibited low pH and conductivity, and appeared to be dystrophic in their trophic status, with higher levels of dissolved organic carbon and lower light-penetration compared to the perimeter lakes (see Tables 1, 2); these lake conditions are typical of older and more productive lakes (see Williamson et al. 1999). Interestingly, we detected little difference among lakes in terms of total phosphorus, oxygen, or temperature, while average chlorophyll and phytoplankton biomass was significantly greater in the interior compared with the perimeter lakes (Table 2, Fig. 2B). These findings are somewhat surprising, given that phytoplankton biomass often correlates with increasing total phosphorous content (Dillon and Rigler 1974, Filstrup et al. 2014, Van Nieuwenhuyse and Jones 1996). We expected the lack of temperature variation among lakes because of their proximity and similar climate regimes. Although we did not measure nitrogen during this study, we acknowledge the role of N, P, and trace elements in contributing to the seasonal changes in phytoplankton biomass and taxonomic composition that were observed in other lakes in the Great Lakes region, such as Lake Erie (Moon and Carrick 2007).

In looking at chlorophyll as a proxy for lake trophic state (Nürnberg 1996), the interior lakes contained more chlorophyll compared with the perimeter lakes, and

our data showed that the interior lakes and perimeter lakes were different from one another in terms of their mean chlorophyll concentrations ranging from oligotrophic to eutrophic (range = 0.8–31.1 μg L^{-1}). Phytoplankton carbon from cell counts showed the same pattern, thereby corroborating the chlorophyll data (r = 0.76 $P < 0.0001$, n = 66). The idea that lake trophic status increases with geologic age has been well established in work on succession theory (Wetzel 2001). Moreover, Kalff (2002) and Nünberg and Shaw (1998) compared 600 freshwater lakes and found that lakes with higher dissolved organic carbon content (stained) generally exhibited higher primary production and bacteria productivity compared with clear-water lakes. More recently, Solomon et al. (2015) suggested several mechanisms that can regulate variation in phytoplankton biomass and productivity among lakes of varying DOC concentrations, including trade-offs between light penetration and higher nutrients with increasing DOC. In either case, enhanced plankton biomass and production in higher DOC lakes, which can support enhanced biodiversity (del Georgio and Peters 1994, Young et al. 2005), has been attributed to the alternative energy source that added DOC provides. Although our results are first-order estimates, they support this idea.

Seasonal phytoplankton blooms and their contribution to taxonomic composition

Many temperate lakes support relatively discrete phytoplankton bloom events during thermal-mixing periods, with coinciding shifts in taxonomic composition (Reynolds 2006, Wetzel 2001). This type of temporal variation in phytoplankton assemblages likely reflects the balance between many interacting forces that impinge on individual populations (Reynolds 2006). Of the 7 lakes we evaluated, only nearshore Lake Michigan supported a well-defined spring bloom, which was composed mostly of diatoms. Historical trends in that lake indicate that predictable spring diatom blooms generally made up over 50% of the carbon in the phytoplankton assemblage during the March–April period (Carrick et al. 2001). This annual spring diatom bloom in Lake Michigan has been shown to fuel benthic production in the lake (Gardner et al. 1990). Interestingly, this spring bloom has not been observed in southern Lake Michigan since 2004, due mainly to the expansion of invasive mussels (Fahnenstiel et al. 2010). Our data suggest that specific areas in Lake Michigan may act as refugia where spring diatom blooms still occur, and that this disappearance may not be a complete, basin-wide feature (Carrick et al. 2015).

Perhaps the most stiking result we observed in contrasting the phytoplankton among lakes was their temporal dissimilary from month to month, particularly during the ice-free months, when a mixed assemblage was present, the specific composition of which was unique to each lake (Figs. 3, 4). Despite these considerable shifts in taxonomic composition during the ice-free period, we unexpectedly observed an overwhelming dominance by phyto-flagellates in all 7 lakes during winter. The physical conditions in each lake were harsh; the light and temperature conditions hardly seemed conducive to supporting phytoplankton under the ice (Table 1). However, all of the inland lakes exhibited dominance by chrysophytes,

specifically 4 species of *Dinobryon* (*D. bravaricuum, D. divergens, D. cylindricum, D. sertularia*), while Lake Michigan was dominated by the cryptophyte, *Rhodamonas minuta*. Thus, these conditions appeared to select for chrysophytes like *Dinobryon* regardless of the biogeochemical conditions and trophic states of each lake. Interestingly, *Dinobryon* is commonly found in ice-covered phytoplankton communities, where it has exhibited blooms and is capable of grazing on pelagic bacteria (Abgeti and Smol 1995, Berninger et al. 1992, Thomas et al. 1991, Watson et al. 2008). Our data suggest that this lake feature may be widespread and occur with some level of temporal synchrony. Our data were not of high-enough resolution to evaluate short-term variation among lakes or changes in abundance with residence time under the ice. However, it was evident that, as observed elsewhere (Vanderploeg et al. 1992), conditions under the ice lacked any significant light penetration, likely due to the snow cover on top of the ice.

Winter ice-cover conditions and the role of phyto-flagellates: An hypothesis

When phyto-flagellates dominate winter assemblages, they have the ability to employ mixotrophy, which should afford them the physiological flexibility to enhance their likelihood of surviving harsh conditions of low light and temperature (Fig. 6). In Lake Michigan, although there was not 100% ice-cover, phyto-flagellates still dominated during cold temperatures. This unique adaptation could provide a competitive advantage, thereby expanding their tolerance so that they could supplement limited resources through the consumption of bacteria. Mixotrophy has relatively high metabolic costs in order to maintain the necessary enzymes and cellular structures that facilitate both modes of nutrition (Tranvik 1989). Its wide geographic and taxonomic distribution suggest that mixotrophy confers an adaptive advantage despite these costs (Bird and Kalff 1987, Raven 1997). Mixotrophy has been observed and documented across 5 classes of phytoplankton, among ciliates (Boraas et al. 1988), and in ecosystems of varying climate. As such, we developed a heuristic model to visually display how phyto-flagellates might employ mixotrophy in temperate lakes in both scenarios of ice-cover and ice-free conditions (Fig. 6). In scenario A, winter conditions in ice-covered lakes result in low (or no) light that limits photosynthesis. Under these conditions, phyto-flagellates decrease the size of their chloroplast, decrease their uptake of dissolved inorganic carbon (DIC) due to depressed photosynthesis, and instead enhance their intake of bacteria. The carbon source that fuels excretion under these conditions originates from particulate organic matter in the form of living bacteria in the water column. Under typical ice-free lake conditions (scenario B), ample light is available and phyto-flagellates increase their chloroplast size, increase DIC intake, and decrease their consumption of bacteria. The carbon source fueling excretion under these conditions originates from dissovled inorganic matter that has been remineralized by bacteria in the water column.

Many lakes around the world have been shown to exhibit algal growth during ice cover (Hegseth 1998, Legendre et al. 2011, Smith and Nelson 1986,), although few studies have documented synchrony among lakes of varying condi-

tion. Additionally, we appear to have identified a unique phyto-flagellate assemblage present in these lakes that is particularly adapted to these highly selective conditions (mainly chrysophytes). Previous studies have demonstrated that phyto-flagellates were able to survive longstanding periods of little or no light (Berge et al. 2008, Tittel et al. 2003, Watson et al. 2008) because they employ unique adaptations to perform heterotrophy or mixotrophy to maintain viable populations (see Fay et al. 2013). Recent studies have shown mixotrophy to be a significant component of carbon cycling in the oligotrophic ocean, with plastid-bearing protists experimentally exhibiting higher rates of bacterivory than aplastidic protists (Hartmann et al. 2012, Moorthi et al. 2009). Arenovski et al. (1995) and Hartmann et al. (2012) hypothesized that plastidic protists compensate for the insufficient amount of inorganic nutrients in oligotrophic ecosystems by consuming bacteria. Thus, it is possible that the scarcity of resources during harsh winter conditions has caused selection for planktonic organisms with physiological capabilities to not only withstand harsh conditions, but that may actually act opportunistically on the conditions of low light and temperature.

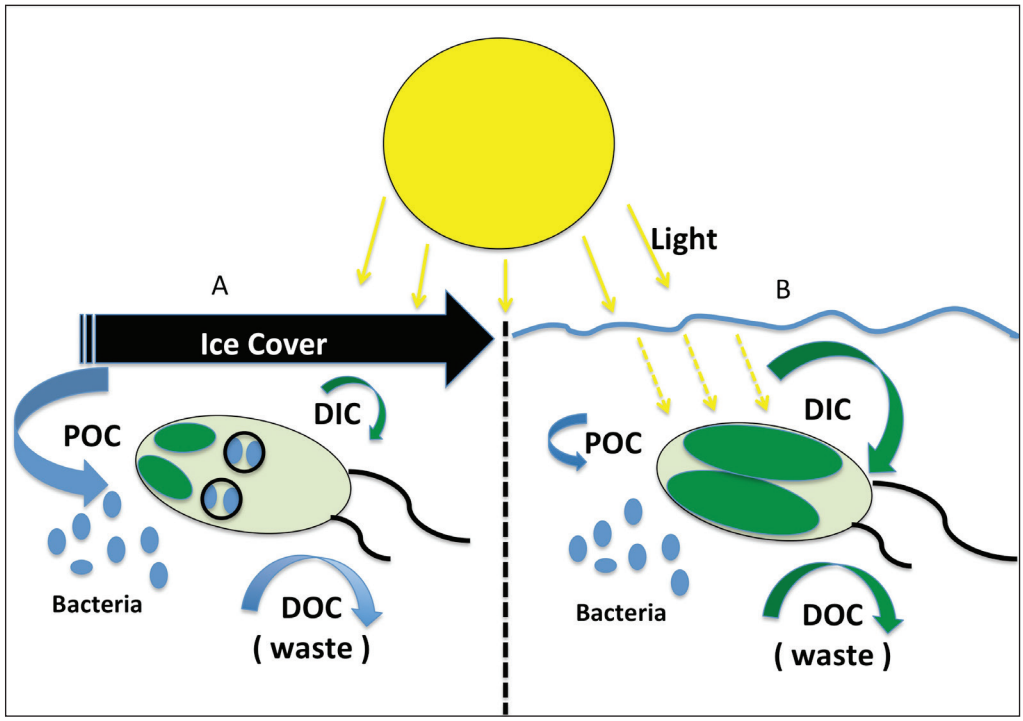

Figure 6. A heuristic model outlining the potential importance of mixotrophy during ice-cover and ice-free periods. (A) During ice-cover period, phyto-flagellates rely most heavily on bacterivory relative to photosynthesis; ice cover reduces light availability leading to increased uptake of bacteria with a reduction in the size of plastids. (B) During ice-free periods, light availablity is relatively high, prompting the full development of plastids and enhanced uptake of dissolved inorganic carbon (DIC) by phyto-flagellates with little to no bacterivory (where, POC = particulate organic matter, DOC = dissolved organic matter).

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