Variation in the Abundance of Pico, Nano, and Microplankton in Lake Michigan: Historic and Basin-wide Comparisons

Hunter J. Carrick[1,*], Emon Butts[1], Daniella Daniels[1], Melanie Fehringer[1], Christopher Frazier[1], Gary L. Fahnenstiel[2], Steven Pothoven[3], Henry A. Vanderploeg[3]

[1]Department of Biology & Institute for Great Lakes Research, Central Michigan University Biological Station, Central Michigan University, Mount Pleasant, MI 48859

[2]Great Lakes Research Center and Michigan Technological Research Institute, Michigan Technological University, Houghton, MI 49931

Water Center, University of Michigan, Ann Arbor, MI 48105

[3]Great Lakes Environmental Research Laboratory, National Oceanic and Atmospheric Administration, Ann Arbor, MI 48104

*Corresponding author: hunter.carrick@cmich.edu

Abstract: The Lake Michigan ecosystem has undergone numerous, systemic changes (reduced nutrient loading, changing climate, proliferation of invasive mussels) that have altered portions of the food web and thus, appear to have changed the lake's trophic state. That said, little is known about the components of the microbial food web (MFW, heterotrophic and phototrophic pico, nano, and micro-plankton), which we hypothesized have compensated as a food source for crustacean zooplankton given the recent declines in the biomass of large phytoplankton (mainly diatoms in the microplankton size category). Therefore, we measured the abundance of the entire MFW using complementary microscopic techniques, flow cytometry, and size fractionated chlorophyll concentrations at sites in northern and southern Lake Michigan, and one site in Lake Superior; the latter site served as a benchmark for oligotrophic conditions. In addition, a historic comparison was made between 1987 and 2013 for the southern Lake Michigan site. Ppico numbers (i.e., picocyanobacteria) in 2013 were lower compared with those in the 1980's; however, the percent contribution of the <2µm fraction increased 2-fold (> 50% of total chlorophyll). The abundance of small, pigmented chrysomonads and cryptomonads (Pnano size category) were not significantly different between 1987 and 2013 at the same time Pmicro did decline; this shift towards Ppico and Pnano dominance may be related to the recent oligotrophication of Lake Michigan. The abundance of ciliated protists (Hmicro size class) was 3-fold lower in 2013 compared with levels in 1987, while the abundance of both Hpico (eubacteria, range 0.24-1.36 x 10^6 cells mL^{-1}) and Hnano (mainly colorless chrysomonads; range 0.11-6.4 x 10^3 cells mL^{-1}) remained stable and reflected the resilience of bacteria-flagellate trophic linkage.

Introduction

The Lake Michigan ecosystem has undergone numerous, systemic changes that appear to have altered its trophic state (reduced nutrient loading, changing climate, proliferation of invasive mussels). Among these changes, has been a conspicuous decline in the typical, winter-spring phytoplankton bloom (mainly diatoms), now largely absent from the water column. The large decline (66-87%) in phytoplankton abundance and productivity was observed in 2007-08 as compared with the 1995-98 and 1983-87 time periods (Fahnenstiel et al., 2010). Loss of the bloom is alarming, because the spring diatom bloom sustained a large fraction of animal production (invertebrates and fish) in the lake (e.g., Gardner et al., 1990). Moreover, the winter-spring zooplankton assemblage also changed dramatically during the same time period, as native cyclopoids, cladocerans, and small copepod species declined by 50-90% in 2007-08 (Vanderploeg et al., 2012).

While the mechanism(s) driving these changes are difficult to ascertain, few human influences have had as large an impact on aquatic ecosystems over the short term as the introduction of dreissenid mussels in North America (Strayer et al., 2004). The quagga mussel expansion into mid-depth regions of Lake Michigan coincided with a shift towards smaller phytoplankton species in both surface and subsurface assemblages (Vanderploeg et al., 2010) with an overall decline in the magnitude of the subsurface chlorophyll layer (Pothoven and Fahnenstiel, 2013). Because changes in phytoplankton were mirrored by shifts in other elements of the food web, salmon stocking was reduced in 2013. While the re-engineering by non-indigenous bivalves in the Great Lakes has received much attention in terms of effects on larger conspicuous

changes such as these (e.g., Hecky et al., 2004), our knowledge of pelagic food web structure and dynamics in Lake Michigan after the recent zebra-quagga mussel shift remains unknown.

Given declines in phytoplankton biomass and primary production in the lake, we hypothesized that the abundance and size-specific composition of less conspicuous plankton has compensated for the decline in larger diatoms in Lake Michigan. Components of the microbial food web (MFW), namely bacteria, pico-sized algae, and flagellated and ciliated protists predators, have constituted alternative trophic pathways in other pelagic food webs (Calbet and Landry, 2004). Moreover, previous studies have shown the MFW to be represented throughout the Great Lakes prior to many of the systemic changes cited above (see Fahnenstiel et al., 1999). More recently, phototrophic picoplankton (Ppico) abundance in the western half of southern Lake Michigan appeared to decline below historic estimates in the 1980's, although their contribution to total pelagic chlorophyll concentrations has doubled since 2005 (Cuhel and Aguilar, 2013). The results were important, because they suggest that Ppico are now major contributors to carbon fixation and its subsequent transfer to higher trophic levels. Given this, the specific objectives this study were:

1. Assess spatio-temporal variation in size-specific phytoplankton biomass in Lake Michigan, particularly to determine if long term changes observed for the phytoplankton were limited to specific locations in the lake (north-south, and near-offshore) and/or thermal periods (mixing, periods of stratification).

2. Assess historic differences in the abundance of key heterotrophic plankton components in Lake Michigan by comparing our data collected here (2013) against identical measurements made in southern Lake Michigan (1987).
3. Discuss the possible factors that might account for the current abundance and size specific composition of phototrophic and heterotrophic plankton in Lake Michigan.

Materials and Methods

Lake Sampling

We sampled six sites distributed among three lake regions (northern Lake Michigan, southern Lake Michigan, and Lake Superior) to evaluate variation in the abundance of the entire microbial food web (Table 1). First, long-term trends in chlorophyll concentrations at the offshore station in southern Lake Michigan were evaluated using monthly data collected from 1987 to 2013 (see Pothoven and Fahnenstiel 2013). Second, variation in the MFW components (MFW, heterotrophic and phototrophic pico, nano and micro plankton) was evaluated among all three lake-regions and against data collected previously in 1987 (southern Lake Michigan) that served as a historical benchmark. This comparison was made on data collected using identical methodologies for the two time periods (see Fahnenstiel and Carrick, 1992; Carrick and Fahnenstiel, 1989, 1990). Third, variation in the phytoplankton assemblage in Lake Michigan was evaluated by assessing potential differences in size-fractionated chlorophyll among near and offshore locations and temporal periods (2013 data only).

In terms of logistics, near and offshore waters (locations) in Lakes Superior (LS), northern Lake Michigan (LMN), and southern Lake Michigan (LMS) were sampled in

2013 (Table 1). Lake Superior was sampled from a small research vessel (R/V Agassiz,) on four dates at an offshore station north of the Keweenaw peninsula within the 300 m contour (LS1) and on one date (July) in Keweenaw Bay near Houghton, MI at a nearshore station (LS6). On four dates, near and offshore stations in northern Lake Michigan were sampled from a small research vessel (M/V Chippewa) in the vicinity of Beaver Island west of the Straits of Mackinaw; the nearshore station was located eastward off the north shore of the island (LMN1) and the offshore sites was located within the 100 m contour eastward off the south shore (LMN8). Southern Lake Michigan was sampled from the research vessel R/V Laurentian on four dates along a historic transect from Muskegon within the 15 m contour (LMS15) and 100 m contour (LMS110). Finally, the offshore LMS110 station was previously sampled on four dates in 1987 aboard the R/V Shennehon.

At all stations (including LMS in 1987), the water column was sampled during four major thermal periods that included: mixing (April-May), early (June), mid (July), and late (August, September) stratification (see Scavia and Fahnenstiel, 1987). The early June sample at the northern Lake Michigan sites served as the mixing period, because the water column had not stratified there. Water column conditions were measured for key physical-chemical parameters (e.g., temperature, conductivity, PAR) using either a Seabird CTD or a handheld YSI-8800 meter along with an underwater PAR sensor (Li-cor LI-1000). Whole water samples were retrieved from 5 m depth in the surface mixed layer (5 m depth, except LMN1, 1 m depth). This depth was chosen because it is a mid-depth in the surface mixed layer for Lake Michigan and was the same depth sampled in previous studies (see Carrick and Fahnenstiel 1989, 1990). All

water samples were collected using a trace metal clean, modified 5-L Niskin bottles poured into 10-L carboys and then dispensed into a dark 4-L bottles (polycarbonate). Two subsamples were removed and preserved (1% Lugols, 1% glutarladehyde) to enumerate plankton and for flow cytometry analysis, while the remaining water was placed in coolers and transported back to the laboratory for subsequent analysis (see below).

Size-Specific Chlorophyll

In 2013 only, size-specific plankton biomass was estimated from duplicate chlorophyll-a measurements made on water collected (seasonally, 4 thermal periods) at near and offshore locations in both LMN and LMS (2 regions, 2 locations, 4 periods, 2 replicates, n=32). Duplicate water samples were passed separately through three screens with specific pore sizes (2.0-µm Nuclepore filters, 20-µm Nitex mesh, and raw unfiltered water, see Fahnenstiel and Carrick, 1992). The filtrate was subsequently concentrated onto filter membranes (Whatman GFF, 0.7 µm pore size) and the pigments extracted for 1 h in a 50:50 mixture of Acetone:DMSO (Shoaf and Lium 1976) without grinding (Carrick et al., 1993). Chlorophyll-a concentrations were corrected for phaeopigments and chlorophyll-b interference (Welschmeyer, 1994) and coefficients of variation among samples were typically <5%. Chlorophyll concentrations were estimated for three major plankton size categories (picoplankton 2-µm; nanoplankton 2-20 µm; microplankton >20 µm).

Plankton Abundance and Taxonomic Composition

The abundance and taxonomic composition of the entire microbial food web was measured using a series of complementary enumeration techniques (see Carrick and Schelske 1997). The abundance (cells mL^{-1}) of plankton groups ranged in size from bacteria to microzooplanton was estimated for all samples collected at three offshore stations in 2013. The techniques used here were identical to those used to analyze samples collected in 1987 at the SLM offshore station, thereby providing a unique opportunity to evaluate historic changes in MFW components (4 regions x 4 periods, n=16).

The abundance and taxonomic composition of pico and nanoplankton were measured on preserved samples (1% glutaraldehyde) by concentrating the water onto 0.2-µm and 0.8-µm nominal pore-size black polycarbonate filters (25-mm diameter; Poretics) respectively; these samples were subsequently analyzed using epifluorescence microscopy (Booth 1993; Carrick and Schelske, 1997). Heterotrophic picoplankton (Hpico) abundance was measured by direct counts (0.1-1.0 mL) using the acridine orange method (Hobbie et al., 1977). Phototrophic picoplankton (Ppico) abundance was estimated from direct counts (5-20 mL) of unstained water samples (Fahnenstiel and Carrick, 1992). The abundance of heterotrophic (Hnano, colorless) and phototrophic (Pnano, pigmented) nanoplankton was measured by direct counts (20-40 mL) using the primuline staining method (Caron, 1983; Carrick and Fahnenstiel, 1989). For all preparations, filters were mounted between glass slides and coverslips with immersion oil and stored at -20 °C; these samples were counted within two-weeks to minimize the fading of autofluorescence (Fahnenstiel and Carrick, 1992).

Individual organisms present on the microscope slides were enumerated by counting random fields until a total of 400-500 cells was reached; this was performed using a research grade, Leica DMR 5000 (Wetzlar, Germany) research microscope (1000× magnification) equipped for chlorophyll and acridine fluorescence (blue light 450-490 nm excitation and > 515 nm emission), as well as, determination of phycoerythrin pigments (green light 530-560 nm excitation and > 580 nm emission). Dominant pigment fluorescence of individual cells was used to assign general taxonomic (phylum) position (Tsuji et al., 1986; Carrick and Schelske, 1997). Counting error was estimated from Poisson counting statistics (Carrick and Fahnenstiel, 1989). Ppico cells were enumerated and placed into categories based upon gross cell morphology and colony arrangement as outlined by Wehr et al. (2015), Krienitz et al. (1996), Henley et al. (2004), as well as, the phylogeny proposed by Komarek and Anagnostidis (1998) for cyanobacteria. Water column cell densities of all four groups were calculated and reported in cells mL^{-1}

The abundance and taxonomic composition of heterotrophic (Hmicro, Ciliophora) and phototrophic (Pmicro, Pyrrophyceace) micro-plankton were enumerated from subsamples preserved with acid Lugol's that were dispensed into settling chambers (40-100 ml volume) and were allowed to settle for 24 hours onto coverslips (Utermöhl, 1958; Carrick and Fahnenstiel, 1990). Cells present were enumerated using a research grade, inverted microscope (Leica DMI 4000) at both 400x and 630x magnification. Individuals were counted by random fields until a total of 300-400 cells were counted. All nano and microplankton individuals encountered were enumerated to their lowest taxonomic

position (Skuja, 1956; Lee et al., 2000). Water column cell densities of both groups were calculated and reported in cells mL^{-1}.

Flow Cytometry

Paired water samples collected from LMS were analyzed using flow cytometry and direct counts to estimate Hpico abundance (n=12). Briefly, preserved water samples (1% glutaraldehyde) were stained with acridine orange (as described above) and analyzed by flow cytometry to estimate the abundance of Hpico using a FACSAria III (model-type 650110) from Becton Dickinson Biosciences. This instrument has 3 lasers (excitation in Violet, Blue, Green) and 9 detectors plus forward scatter (FS) and side scatter (SS). Water samples were analyzed when excited using the blue laser (488 nm). A series of bivariate plots (FL2 versus FL3) were generated using BD FACSDiva™ software, to estimate the number of bacteria sized cells. The beginning and ending volume of samples was used to estimate the actual volume of sample analyzed. Background electronic noise was minimized with a threshold setting of 300.

Statistical Analyses

Variation in the abundance of the six, size-specific plankton groups were evaluated using one-way, multivariate analysis of variance, where lake region was considered a fixed factor (MANOVA using Wilks Lambda, see Zar 2009). The four lake

region treatments consisted of three regions sampled in 2013 (Lake Superior, Lake Michigan-North, LMN; Lake Michigan-South, LMS) and one region previously sampled in 1987 (Lake Michigan-south, LMS) that offered a unique, historic comparison.

Spatio-temporal variation in chlorophyll (size categories and total concentrations) among lake locations and sampling periods (2013) in Lake Michigan were evaluated using two-way, multivariate analysis of variance (MANOVA using Wilks Lambda, see Zar 2009). Locations (near and offshore) and sampling periods (mixing, early, mid, late stratification) were considered fixed factors in the analysis. While we admit that the existence of clear independence among environmental samples can be rare in nature (see Kruskal 1988), we treated individual periods as independent samples, given the time scale of sampling (weeks to months) versus that of factors governing the population dynamics of plankton (hours to days). Furthermore, separate analyses were performed for data collected at LMN and LMS regions. Subsequent 1-way ANOVA was performed to evaluate time-space interactions. Pairwise comparisons were made using the Student Newman Keuls (SNK) multiple means test to isolate pair-wise differences (alpha 0.05) for analyses that yielded significant interaction terms. For all analyses, data were log transformed (+1) to meet assumptions of normality and equality of variance. All data met assumptions for normality (Kolmogorov-Smirnov test) and homoscedasticity (Levene's test for equality of error variances).

Results

Long-term Trends in Chlorophyll

Chlorophyll concentrations varied considerably at the offshore station in southern Lake Michigan over the 26-year period addressed in this study (1987-2013, Fig. 1). Spring chlorophyll values were significantly higher compared with summer values (paired t-test, $t=4.52$, $p<0.0001$, $df=21$). Spring chlorophyll concentrations ranged from 0.67 to 3.50 (April-May), with an average (+/1 one std. deviation) concentration of 1.88 +/ 0.89 µg L^{-1}. As such, spring values were nearly a 2-fold greater over this 26-year period compared with summer values. Interestingly, the difference between spring and summer values was not evident after 2000, the period when dreissenid mussels (particularly *Dreissena bugensis*) colonized and established populations in southern Lake Michigan. Summer chlorophyll concentrations ranged from 0.53 to 1.61 (June-July), with an average concentration of 1.01 +/ 0.30 µg L^{-1}; these values were relatively constant over the period of record.

Variation in Size-specific Chlorophyll

Using fractionated chlorophyll as a proxy, concentrations ranged from 0.48 to 7.00 µg L^{-1} among the 32 samples collected from Lake Superior, LMN, and LMS in 2013 (Table 2). Chlorophyll in the nanoplankton size fraction (2-20-µm) contributed > 50% to the phytoplankton assemblage in Lake Superior, while chlorophyll in the picoplankton (< 2-µm) size class contributed 15-20% and microplankton (>20-µm) contributed 21-29%. At the LMN station, picoplankton contributed more than 60% of chlorophyll to the phytoplankton assemblage, followed by nanoplankton (23%) and microplankton (~15%). At the LMS, conspicuous near to offshore differences were noted in the size structure of the phytoplankton assemblage. Nearshore, phytoplankton chlorophyll was distributed

evenly among Ppico, Pnano, and Pmicro size classes. At the offshore waters, the phytoplankton assemblage was composed mainly of Ppico (50%) with Pnano- and Pmicro constituting the remaining half of chlorophyll concentrations.

In northern Lake Michigan (LMN), the size structure of phytoplankton chlorophyll varied significantly among sampling intervals but not spatially between near and offshore locations (two-way MANOVA; Fig. 2, Table 3). Specifically, Ppico chlorophyll was greatest during the spring mixing period compared with levels measured during mid and late stratification periods (Table 4); levels were lowest during the early stratification period (26 June). The temporal trend in total chlorophyll mirrored this pattern, which should not be surprising given the Ppico fraction was dominant in this portion of the lake (Tables 3, 4).

In the southern Lake Michigan (LMS), the size structure of phytoplankton chlorophyll varied significantly among sampling intervals, locations, and the interaction between the two (two-way MANOVA; Fig. 2, Table 3). Specifically, picoplankton, nanoplankton, and total chlorophyll concentrations were greatest during the spring mixing, and declined during mid, late and early stratification (Table 4). These trends corresponded with a large spring bloom that took place at the nearshore station during the spring mixing period (24-April); these levels were several times greater than concentrations offshore (7.0 μg L^{-1}). Interestingly, phytoplankton in the > 20 μm size class reached its peak during mid-stratification (months after the nearshore spring bloom), while concentrations were lower during mixing, late, and early stratification, respectively (Table 4).

Variation in Size-specific Plankton Abundance

The abundance of the plankton enumerated from these samples exhibited considerable variation among the six, size-specific categories (heterotrophic and phototrophic pico, nano, and microplankton); these abundance estimates spanned 5-orders of magnitude (Fig. 3). In terms of phototrophic plankton, Ppico abundances spanned more than 3-orders of magnitude among all samples, with peak abundances observed during mid-stratification (2.8 to 200 x 10^3 cells mL^{-1}). Taxonomically, this group was composed of small cyanobacteria (*Synechococcus* sp*., Snowella, Cyanobium*) and eukaryotes (*Nanochloris [Choricystis]*, *Gloeocystis*) that were present in all the lake samples (Table 5). Ppico numbers were lowest in samples collected at LMN, occurred to intermediate levels in LMS (2013) and Lake Superior, and were greatest in LMS during the 1987 (Fig. 3). The abundance of Pnano spanned more than 2-orders of magnitude from 22 to 2,115 cells mL^{-1}. Pnano numbers were not different in LMN and LMS compared with historic values in 1987 LMS; these values were all higher than those measured for populations in Lake Superior (Table 5). The Pnano assemblage was composed of taxa from several groups of phytoplankton that included chrysophytes (e.g., *Ochomonas* sp., *Dinobryon serularia*), cryptophytes (e.g., *Rhodomonas minuta*, *R. lens*, *Cryptomonas ovata*) and haptophytes (*Chrysochromulina parva*, *Micromonas* sp.). Pmicro were present in relatively low in numbers among all lake regions, such that their abundance ranged from 0.10 to 5.90 cells mL^{-1}. Pmicro abundance was greater in LMS-1987 compared with the other three lake regions. Taxonomically, the Pmicro assemblage was composed of pyrrophyta (dinoflagellates), whose dominant taxa were *Gymnodium varians* and *Ceratium hirudinella* (Table 5).

Heterotrophic picoplankton (Hpico) abundance exhibited less variation relative to the other size categories, ranging from 0.24 to 1.36 x 10^6 cells mL^{-1} among all samples (Fig. 3). Hpico abundance was not different among the four lake regions (one-way ANOVA, $p > 0.75$). Hnano abundance exhibited a range in abundance among samples (range 0.13 to 6.4 x 10^3 cells mL^{-1}) and varied significantly among lake regions. Hnano population densities were lowest in Lake Superior, moderate in both LMN and LMS, and were significantly higher in 1987 samples from LMN. The Hnano assemblage was composed of taxa from several taxonomic groups that included choanoflagellates (e.g., *Desmerella*), chrysomonads (e.g., *Chromulina*, *Ochomonas*, and *Kephrion*), cryptomonads (*Katablepharis ovalis*, *Cryptaulax* sp.). Lastly, Hmicro also demonstrated a large range among samples, with values spanning from 0.83 to 17.2 cells mL^{-1}. Hmicro numbers were significantly higher in samples collected in 1987 LMS compared with the other three lake regions. The Hmicro assemblage consisted of a relatively diverse mix of ciliated protists (Table 5) including choreotrichs (e.g., *Strobilidium*, *Codonella*), haptorids (*Askenasia*, *Mesodinium*, *Monodinium*), oligotrichs (*Pelaostrombidium*, *Pelagohalteria*), and prorodonids (e.g., *Urotricha*, *Pseudobalanion*).

Discussion

The abundance for five of six, size-specific plankton groups varied significantly among the lake regions sampled here, although the pattern was not consistent (see Fig. 3). Overall, the current Lake Michigan plankton assemblage was more similar to the one present in Lake Superior compared with the historic assemblage in Lake Michigan (e.g., Scavia et al., 1986; Fahnenstiel and Carrick, 1992; Carrick and Fahnenstiel, 1989,

1990). For instance, populations of Hmicro and Pmicro in the SLM 2013 exhibited dramatically lower numbers relative to their abundance in 1987. Conversely, populations of Pnano and Hnano were not different among all three Lake Michigan sampling regions, although these estimates were all greater than numbers in Lake Superior. Ppico numbers at both LMN and LMS and Lake Superior were depressed relative to 1987 Lake Michigan, while no significant changes in Hpico abundance were observed among the four lake regions. These Hpico abundance estimates determined from direct cell counts agreed well with the abundance determined on paired samples using flow cytometry (person rank correlation $r=0.732$, $p<0.01$, $n=12$); these results indicated that our counting procedures were sound. That said, the small sample size available for comparison was a reality of this study and this restricted the power of some of our analyses (Zar, 2009). The statistical power for five of the MANOVA comparisons were unaffected by sample size (alpha ~1.000), although the power for the Hpico comparison exhibited particularly low statistical power (alpha 0.122) limiting our ability to detect differences if they existed (inflated Type II error). Interestingly, the limited statistical power for the Hpico comparison could have occurred for relevant ecological reasons, given their great abundance relative to other plankton (on the order of 10^6 cells mL^{-1}), limited seasonal abundance (e.g., Scavia et al., 1986), and their apparent spatial constancy among lake regions as observed here (Fig. 3). As such, the relative constancy of planktonic bacteria and their microbial consumers has been shown to provide stability among a range of lake and oceanic ecosystems during periods of change (see below).

A Shift Towards Pico and Nano-sized Phytoplankton

The relative importance of both phototrophic nano and picoplankton seemed evident given their relatively high abundance in all our samples relative to the lower abundance of Pmicro. The shift in phytoplankton towards smaller sized organisms and the 2-fold decline in chlorophyll (Fahnenstiel et al. 2010) seems to be indicative of a new trophic status for Lake Michigan, where the current productivity of the lake is now lower (see Barberio et al., 2012). Along these lines, the abundance of Ppico at both LMN and LMS were lower compared with estimates made in the 1980's in southern Lake Michigan (LMS), although the percent contribution of the Ppico <2µm fraction has increased and now constitutes > 50% of total, pelagic chlorophyll in Lake Michigan. This same phenomenon has been documented for the western portion of the southern basin of Lake Michigan, where Ppico numbers have declined over time, while their % contribution to total chlorophyll has increased by 2-fold to nearly 50% (Cuhel and Aguilar, 2013). In a broader sense, a comparative survey of lakes and marine ecosystems also showed an increase in the contribution of Ppico with declining total chlorophyll (see Calleri and Stockner, 2000). Ppico abundance in LMS and Lake Superior (range 21.4 to 45.0 x 10^3 cells mL^{-1}) were comparable with those reported from other oligotrophic lakes and marine systems (Callieri, 2007) and fall within the range reported for lakes with similar chlorophyll concentrations of ~1 µg L^{-1} (see regression model in Callieri and Stockner, 2002). Moreover, our Ppico population densities compare well with previous population estimates first reported for the central basin of Lake Superior in 1979 (Fahnenstiel et al., 1986), as well as, those reported from samples collected more recently from the western basin of Lake Superior (Ivanokova et

al., 2007). That said, the present abundance of Ppico in LMS and Superior was considerably lower when compared with Ppico population estimates in the other three Great Lakes: Lake Ontario in 1985 (Caron et al., 1985), Lake Erie in 1998 (Carrick, 2004), and Lake Huron in 1988-90 (Fahnenstiel and Carrick, 1992).

The abundance of Pnano was not significantly different between 1987 and 2013; however, their abundance was in fact higher than historic levels (Fig. 3). Pnano has always been a key component of plankton assemblages throughout the Great Lakes (Fahnenstiel et al., 1998; Munawar and Munawar, 2000; Reavie et al., 2014). Their resilience to changes over the past 20-30 years is both interesting and important given the dramatic decline in other primary producers such as diatoms (see Fahnenstiel et al., 2010) and other Pmicro as measured here (see Fig. 3). That said, if Pnano now fill the niche once occupied by diatoms (compensation effect), this has implications for food web dynamics in the lake. First, diatoms were an important seasonal food source for benthic animals, because they sink at high rates and thereby contribute a significant fraction of carbon to the benthos annually (Gardner et al., 1990). The sinking rates of Pnano and Pmicro are minimal in comparison (Scavia and Fahnenstiel, 1987 and Carrick, 2005, respectively), and thus they would not likely represent a significant source of food to the benthos. Second, many of the dominant Pnano and Pmicro species present have known capabilities for mixotrophy; these include the chrysomonad *Dinobryon* (Bird and Kalff 1987), the cryptomonads *Rhodomonas* and *Crytomonas* (Tranvik et al., 1989), and the dinoflagellate *Gymnodinium helveticum* (Frey and Stoermer 1980). Carbon obtained through mixotrophy could further augment production by these phytoplankton groups during periods of limited nutrient availability; this

phenomenon that has been quantitatively important in oceanic systems (e.g., Jost et al., 2004; Pernice et al., 2014) and the global carbon cycle as a whole (Hartmann et al., 2012; Mitra et al., 2014). Finally, Pnano were the dominant phytoplankton groups that grew during the runoff event that took place in April 2013, which led to a nearshore bloom of 7 µg L-1 chlorophyll in the 2-20 µm size class. This type of bloom has been observed previously in Lake Michigan, when episodic run-off events promoted growth of nano-sized phytoplankton within the coastal region of the lake (Millie et al. 2002, 2003). Interestingly, a secondary bloom was observed at the 110 m contour in July, which may have been "seeded" by this nearshore event months earlier. If so, episodic events such as these could represent important augmentations to both near and offshore primary production (see below).

Importance of the Bacteria-Nanoflagellate Trophic Linkage

The relative constancy in Hpico numbers appear to reflect the resilience of bacteria populations to the changes that have occurred in Lake Michigan over the past 26 years. The abundance of Hpico measured here (range 0.24 to 1.36 x 10^6 cells mL^{-1}) were similar to populations measured in oligotrophic lakes and low productivity sites in the ocean (Sanders et al., 1992; Li, 1998). The abundance of Hpico were comparable with those made by Scavia et al., (1986) in 1985 at the same offshore station in Lake Michigan; their seasonal range of 0.60 to 1.10 x 10^6 cells mL^{-1} was also very similar to our estimates for LMS in 1987. The chlorophyll concentrations measured during the 1986-87 survey in Lake Michigan (range: 1.0 to 3.0 µg L^{-1}; see Fig. 1) were higher than those measured at offshore LMS in 2013 as measured here (Table 2; 0.5 to 1.5 µg L^{-1}).

Finally, our 2013 values were lower compared with a lake-wide survey made by Munawar et al., (2005) in October 1988, who measured bacteria abundance at 14 stations including near and offshore locations (range 0.700 to 2.900 x 10^6 cells mL^{-1}). These results seem to suggest that more extensive surveys that include a wide range of environmental conditions may capture greater variation in Hpico abundance estimates.

The abundance of Hmicro in LMS and LMN were relatively low (0.3 to 6.5 cells • mL^{-1}) and were 2-fold lower in 2013 compared with their numbers in 1987. As such Hmicro numbers in Lake Michigan are now more comparable with Hmicro populations in Lake Superior. Choreotrich ciliates exhibited the greatest reduction in abundance between 1987 and 2013 (e.g, *Tintinnidium* sp. and *Codonella cratera);* these ciliates typically feed on diatoms and produce silica-based lorica (e.g., Lee et al., 2000). Historically, the peak abundance of these ciliates coincided with the spring diatom bloom in Lake Michigan (Carrick and Fahnenstiel 1990), so it seems likely their reduced numbers have been driven in part by bottom up influences such as the reduction in their key food. *Also, d*espite the potential for high growth rates by many protists, their numbers and diversity were reduced by 70-80% and 30-50%, respectively when exposed to mussel grazing (Lavrentyev et al., 1995). More recent experimental results indicated that ciliates were grazed at high rates by mussels in Saginaw Bay (Lake Huron), and the production of lorica or tests offered limited protection from mussel grazing, particularly if these species had low growth rates and limited motility (Lavrentyev et al., 2014); this may be the case for taxa such as *Tintinnidium* and *Codonella* in Lake Michigan (see Carrick et al., 1992).

In contrast, Hnano abundance did not exhibit significant variation among the three sampling regions in Lake Michigan (range 0.4 to 6.4 x 10^3 cells mL^{-1}), whereby the LMS populations 2013 were very similar to their numbers in 1987 (Carrick and Fahnenstiel 1989). Given that Hnano are key consumers of bacteria in the Great Lakes, their stability may in part account for their relatively constancy in Hpico abundance through tight trophic coupling (Laird et al., 1990). Microcosm experiments performed in 1987-88 showed that crustacean zooplankton (mixed copepod assemblage) effectively graze Hnano in southern Lake Michigan (Carrick et al., 1991; Bundy et al., 2005), and that the carbon flux via this trophic linkage rivaled the flux from phytoplankton to crustacean zooplankton (Carrick 2005). Thus, the relative stability in both Hpico and Hnano numbers in southern Lake Michigan suggests that this linkage has remained viable under the new trophic conditions after 2000. Furthermore, the importance of this trophic linkage has been observed among widely varying ecosystems (freshwater and marine) and may represent a universal feature of aquatic food webs, whereby dissolved organic matter is returned to crustacean zooplankton via the bacteria-Hnano linkage (see Sanders et al., 1992).

Possible Factors Contributing to Food Web Changes in Lake Michigan

The underlying mechanism(s) that could account for the differences in MFW structure observed among lake regions sampled herein are potentially numerous (invasive mussels, nutrient declines, food web interactions). Nutrient concentrations have declined by nearly 2-fold in northern and southern Lake Michigan over past 30 years (1983 to 2010) coinciding with reductions in external nutrient loadings, such that

current day total phosphorus (TP) concentrations in the offshore region of the lake are now very similar to those in Lake Superior (Barbiero et al., 2012). Given that P has long been recognized as a key limiting nutrient in Lake Michigan (e.g., Schelske and Stoermer, 1971), further reductions in ambient P concentrations could further limit phytoplankton growth and biomass. Moreover, Pauer et al., (2011) estimated that current, ambient P concentrations were lower than would be expected when estimated from load reductions (4.3 versus 3.1 µg L^{-1}, see discussion in Barbiero et al., 2012). Interestingly, mussel feeding can reduce available P to plankton and subsequently lower nutrient uptake rates (Johengen et al., 2013). It stands to reason that these conditions would further reduce growth rates of bacteria and phytoplankton (Heath et al., 1995), because considerable quantities of P have been sequestered in mussel tissue (Nalepa et al., 2010). Either way, the reductions in P could favor smaller phytoplankton < 20 µm in size, following the argument that smaller cells generally have superior uptake kinetics for inorganic P compared with larger cells such as diatoms (e.g., Grover 1989).

The filtering activity of dreissenid mussels on plankton cells cannot be ignored as a contributing factor that could explain observed differences the lower abundance of most plankton groups observed here. The decline measured for phytoplankton biomass and primary production points to filtering by invasive dreissenid mussels as a likely causal mechanism, given that the relative abrupt decline in phytoplankton coincided with the timing of the mussel colonization of Lake Michigan (see Figure 1; Pothoven and Fahnenstiel 2013). Furthermore, the dramatic increase in water clarity and reduction in the density of phytoplankton observed for Lake Michigan has been confirmed by more

than one study (e.g., Barbiero et al., 2012; Reavie et al. 2014). Enclosure experiments have shown that mussels cleared relatively large volumes of water (10-400 mL • h^{-1}) and consumed an array of particles from the water column at both high and low temperatures (Baldwin et al., 2002, Vanderploeg et al., 2010). Fixed volume experiments also showed that mussels removed a range of seston at clearance rates of 51 to 339 mL h^{-1} (Horgan and Mills, 1997), although their preferred prey size range lies between 5 to 35 µm (Sprung and Rose, 1988) which corresponded with Hmicro and Pmicro (ciliates and dinoflagellates, respectively; Carrick and Fahnenstiel 1990). Given their flexible prey selection and high clearance rates (range 0.2 - 1.3 d^{-1}; Fanslow et al., 1995), mussel feeding can overcome plankton growth under most circumstances, because these clearance rates rival or exceed typical phytoplankton (range 0.1 - 0.3 d^{-1}; Scavia and Fahnenstiel, 1987) and protistan (range 0.2 - 0.6 d^{-1}; Carrick et al., 1992) growth rates. Thus, it stands to reason that filtering by these mussels could remove an array of heterotrophic and phototrophic cells, in addition to diatoms, from the water column and therefore impart significant changes to the plankton assemblage in a collective sense. These comparisons assume that the growth of phytoplankton and protists have not increased since 1987, which seems reasonable given the reduction in nutrients (for phototrophs) and potential prey (for heterotrophs) in the water column of Lake Michigan.

The changing climate in the Great Lakes region coincides with higher water temperatures in these lakes and an increase in the summer stratification period by more than 2-weeks (McCormick and Fahnenstiel 1999). Changes such as these could influence lower food web dynamics in Lake Michigan as well. For instance, we observed

an extreme rainfall event in April 2013 that produced heavy rains (75-175 mm in 36 hours) and a near a 100-year run-off event for the Grand River, Michigan (Grumm and Ross, 2013). This run-off coincided with a large, nearshore phytoplankton bloom that affected a substantial stretch of coastline in southern Lake Michigan (Carrick and Vanderploeg, personal observation). While these rainfall-runoff events can occur with some seasonal regularity during the spring period (e.g., Millie et al., 2002, 2003), the magnitude of this event was unusual. The trend towards greater warming and higher precipitation delivered in events of greater intensity, has already been documented for the Great Lakes region (see Pryor et al., 2014), and may prove to be of heightened importance in a lake, whose productivity has declined over the past 26 years.

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Figure Legends

Fig. 1. Chlorophyll concentration measured for spring (April-May) and summer (June-July) phytoplankton assemblages in southern Lake Michigan (at LMS, 110 m depth) over a period spanning from 1987 to 2013.

Fig. 2. Average chlorophyll concentrations distributed among plankton size groups (pico-, nano-, and micro-plankton) collected on four dates at near and offshore locations in northern Lake Michigan and southern Lake Michigan (2013).

Fig. 3. Size-specific abundance (average +/- one standard error) for phototrophic and heterotrophic plankton (pico, nano, micro-plankton, cells mL^{-1}) measured among four lake regions in the upper Great Lakes.

Table 1. A summary of locations and dates sampled during the present study in 2013. Previous data collected at the offshore station in southern Lake Michigan was reanalyzed herein and served as a historic benchmark (offshore, 1987).

Lake Region	Location Station ID	Longitude	Latitude	Depth (m)	Dates Sampled
Northern Lake Michigan	Nearshore LMN1	85.44712	45.75000	10	12-June, 26-June, 22-July, 5-August (2013)
	Offshore LMN8	85.47470	45.55817	100	12-June, 26-June, 22-July, 5-August (2013)
Southern Lake Michigan	Nearshore LMS15	86.34972	43.19139	15	24-April, 15-May, 16-July, 23-September (2013)
	Offshore LMS110	86.53778	43.19139	110	24-April, 15-May, 16-July, 23-September (2013)
	Offshore LMS110	86.53778	43.19139	110	7-April, 1-May, 21-July, 9-September (1987)
Central Lake Superior	Nearshore LS6	88.57537	47.46459	80	26-July (2013)
	Offshore LS1	88.47073	46.80396	150	24-May, 25-June, 26-July, 6-September (2013)

Table 2. Percent chlorophyll distributed among plankton size groups (pico-, nano, and micro-plankton) collected from near and offshore locations in three lake regions in 2013 (Lake Superior; northern Lake Michigan, LMN; southern Lake Michigan, LMS). Total chlorophyll (mean +/- one standard deviation, µg L^{-1}) is the average concentration in each lake region.

Lake Region	**Transect Location**	**<2 µm %**	**2-20 µm %**	**>20 µm %**	**Total µg L^{-1}**
Superior	Nearshore	15.0	56.0	29.0	1.21 +/- 0.23
	Offshore	20.6	58.2	21.2	1.23 +/- 0.46
LMN	Nearshore	60.2	23.2	16.6	0.95 +/- 0.17
	Offshore	61.0	23.4	15.6	1.04 +/- 0.36
LMS	Nearshore	30.4	37.6	32.0	3.29 +/- 2.41
	Offshore	50.8	26.5	22.7	1.76 +/- 1.74

Table 3. Results for two-way MANOVA results that assessed variation in plankton chlorophyll-a for samples collected at near and offshore stations (location) during mixing, early, mid, and late stratification (period). Variation was determined among plankton size categories (pico, nano, micro, and total plankton, see Table 4). The analysis was repeated for northern (LMN) and southern (LMS) Lake Michigan regions.

Lake Region	Test	df	F-value	p-value
LMN	Location	4	3.70	0.0920
	Period	12	9.48	0.0001
	Interaction	12	2.56	0.0510
LMS	Location	4	44.30	0.0001
	Period	12	16.32	0.0001
	Interaction	12	9.66	0.0001

Table 4. One-way MANOVA results coupled with Student-Newman-Keuls pairwise tests to assess variation in chlorophyll-a biomass for plankton size fractions. The analysis was blocked for the fixed factors (Location, Period), to evaluate differences among temporal periods (mixing, early, mid, and late stratification) at near and offshore stations (location). Comparisons were made separately for two regions in Lake Michigan; ns= not different, ** p<0.01, *** p<0.0001.

Region	Factor	Fraction	F-Value	SNK Pairwise Tests
LMN	Location	<2 µm	1.54 ns	No difference
		2-20 µm	1.00 ns	No difference
		>20 µm	0.29 ns	No difference
		Total	3.82 ns	No difference
	Period	<2 µm	22.01***	Early < Late = Mid < Mix
		2-20 µm	3.74 ns	No difference
		>20 µm	3.00 ns	No difference
		Total	51.39 ***	Early < Late = Mid < Mix
LMS	Location	<2 µm	9.08 **	Near > Off
		2-20 µm	55.01 ***	Near > Off
		>20 µm	37.43 ***	Near > Off
		Total	219.61 ***	Near > Off
	Period	<2 µm	26.23 ***	Late = Early < Mid < Mix
		2-20 µm	24.63 ***	Early < Late < Mid < Mix
		>20 µm	55.75 ***	Early < Late < Mix < Mid
		Total	213.93 ***	Early < Late < Mid = Mix

Table 5. Heterotrophic and phototrophic plankton in Lake Michigan organized among six size-specific groups with corresponding list of dominant taxa.

Size Category	Taxonomic Group	Dominant Taxa
Hpico	Eubacteria	Cocci, bacillus (morphology)
Ppico	Cyanobacteria	*Synechococcus, Snowella, Cyanobium*
	Chlorophyta	*Nanochloris (Chloricystis), Gloeocystis*
Hnano	Choanoflagellida	*Desmerella*
	Chrysomodadida	*Chromulina, Ochromonas, Kephrion*
	Cryptomondadida	*Katablepharis, Cryptaulax*
Pnano	Chrysophyta	*Ochromonas, Dinobryon*
	Cryptophyta	*Rhodomonas, Cryptomonas, Chroomonas*
	Haptophyta	*Chrysochomulina*
Pmicro	Pyrrophyta	*Gymnodinium, Glenodinium, Ceratium*
Hmicro	Choreotrichida	*Strobilidium, Codonella, Tintinnidium*
	Haptorida	*Askenasia, Mesodinium, Monodinium,*
	Oligotrichida	*Pelagostrombidium, Pelagohalteria*
	Prorodontida	*Urotrichia, Pseudobalanion, Coleps, Cyclidium*
	Sessilida	*Vorticella, Vaginacola*

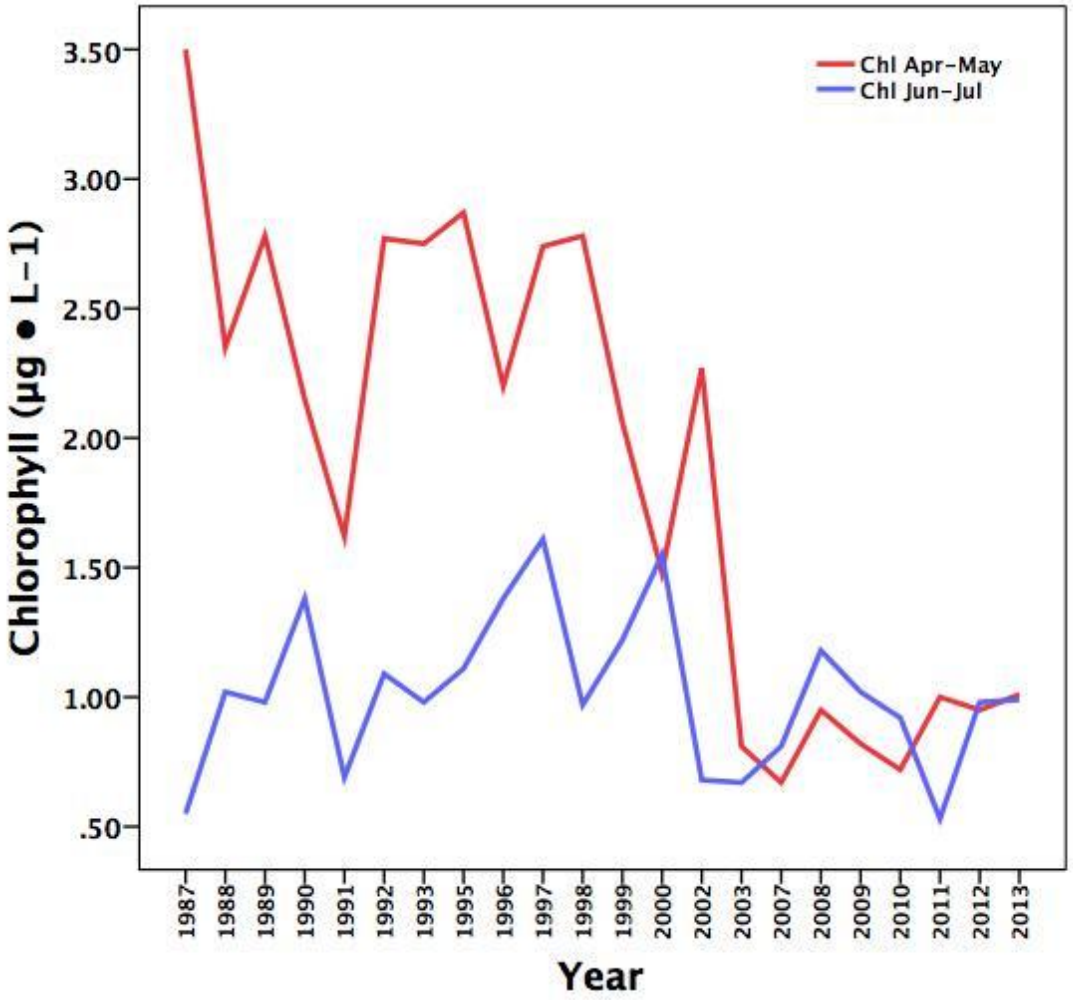
Chl Apr-May
Chl Jun-Jul
Chlorophyll (µg • L-1)
3.50
3.00
2.50
2.00
1.50
1.00
.50
1987
1988
1989
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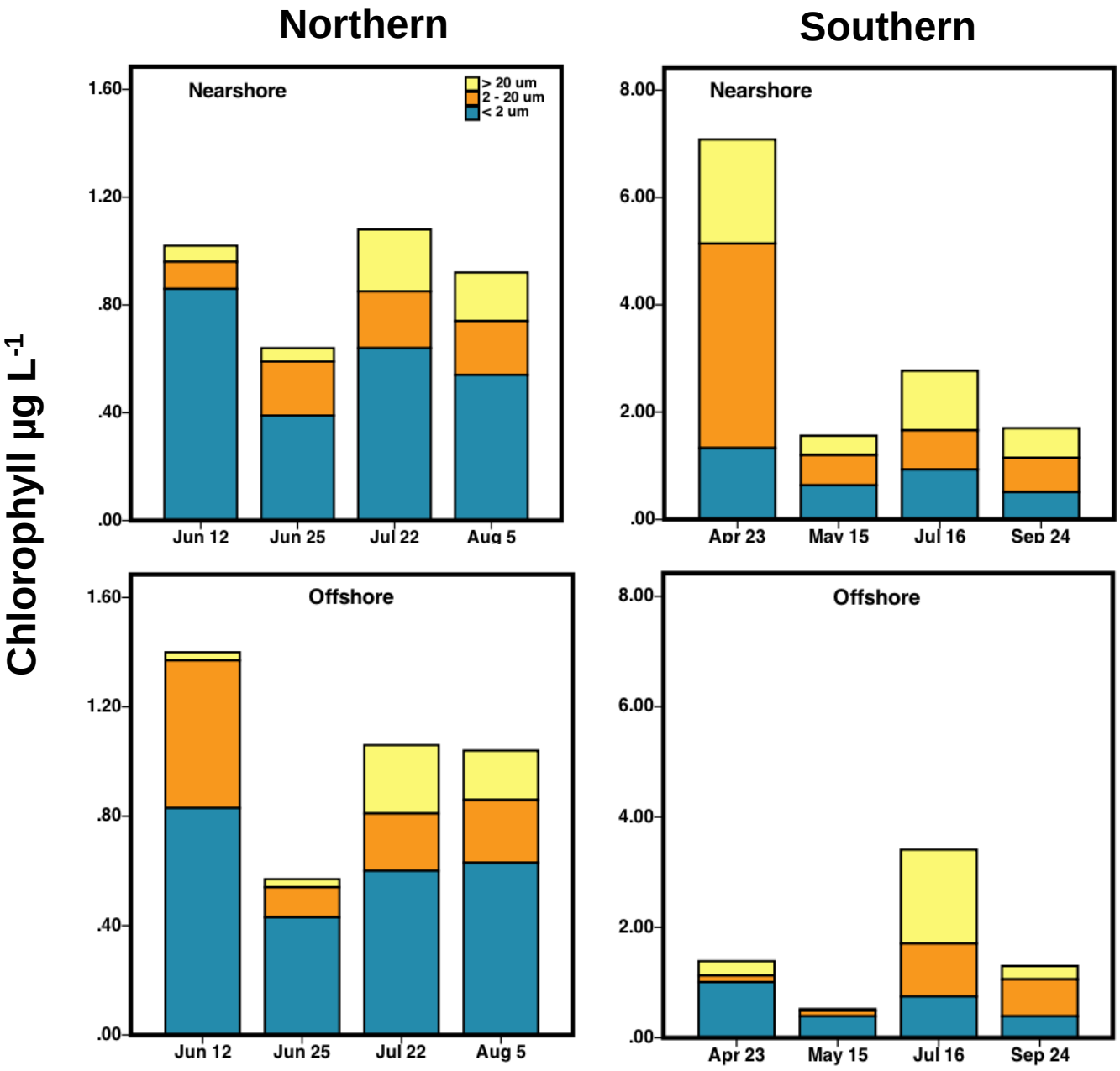
Figure 1


Northern
Southern
Nearshore
Nearshore
Offshore
Offshore
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2 - 20 um
< 2 um
1.60
1.20
.80
.40
.00
8.00
6.00
4.00
2.00
Jun 12
Jun 25
Jul 22
Aug 5
Apr 23
May 15
Jul 16
Sep 24
Chlorophyll µg L-1
Sampling Date (2013)

Figure 2

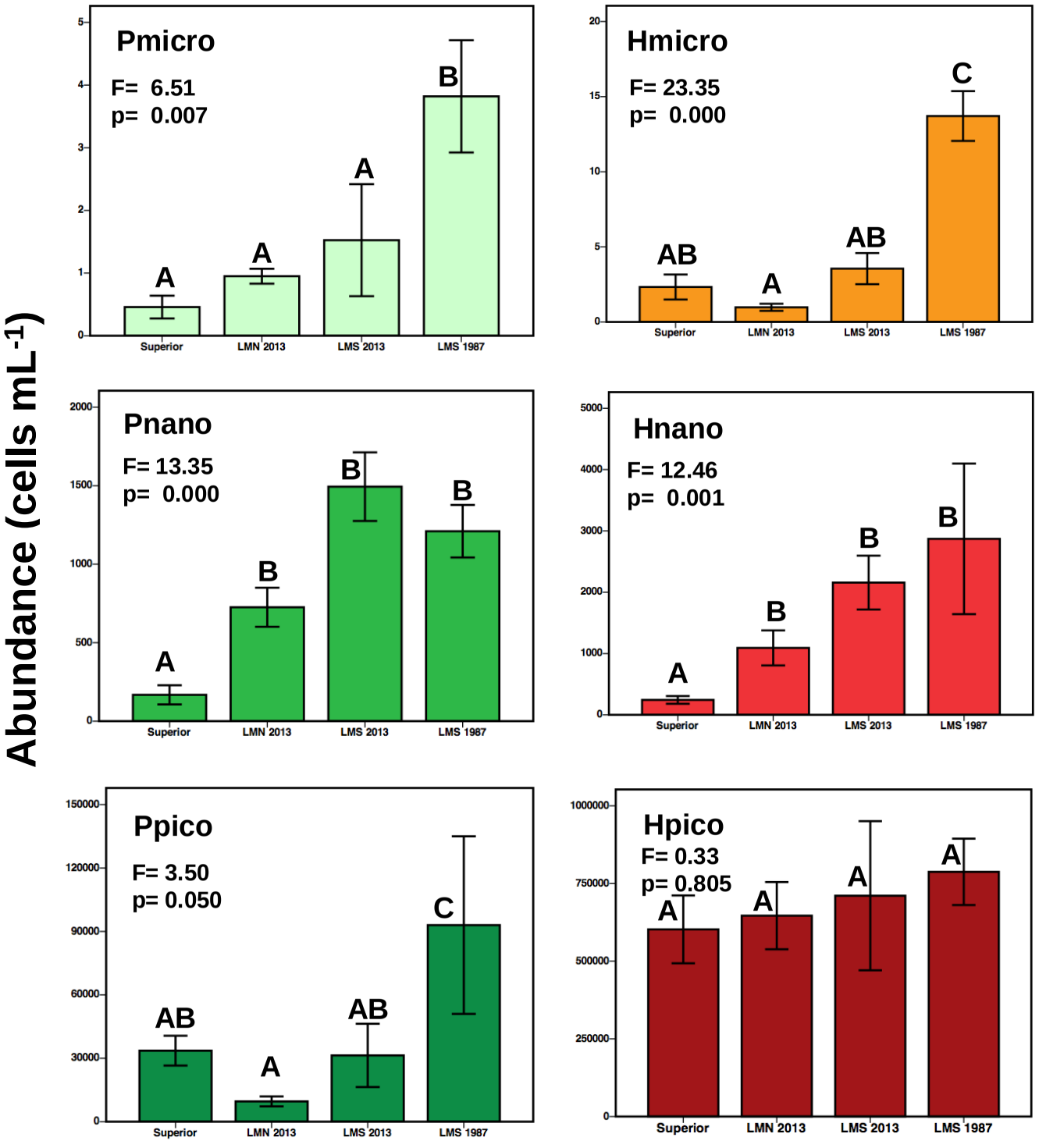
Pmicro
F= 6.51
p= 0.007
A
A
A
B
Hmicro
F= 23.35
p= 0.000
AB
A
AB
C
Pnano
F= 13.35
p= 0.000
A
B
B
B
Hnano
F= 12.46
p= 0.001
A
B
B
B
Ppico
F= 3.50
p= 0.050
AB
A
AB
C
Hpico
F= 0.33
p= 0.805
A
A
A
A
Superior
LMN 2013
LMS 2013
LMS 1987
Abundance (cells mL-1)
Sampling Regions

Figure 3