

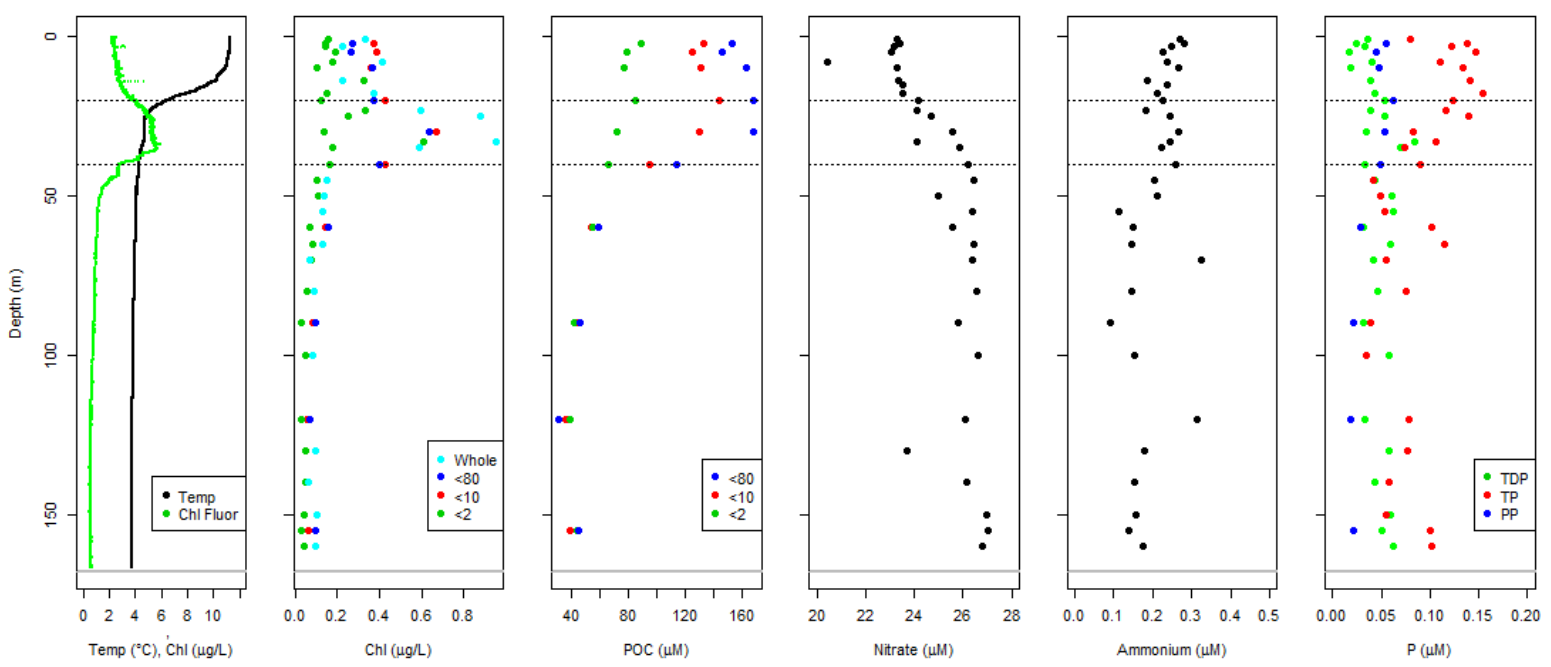
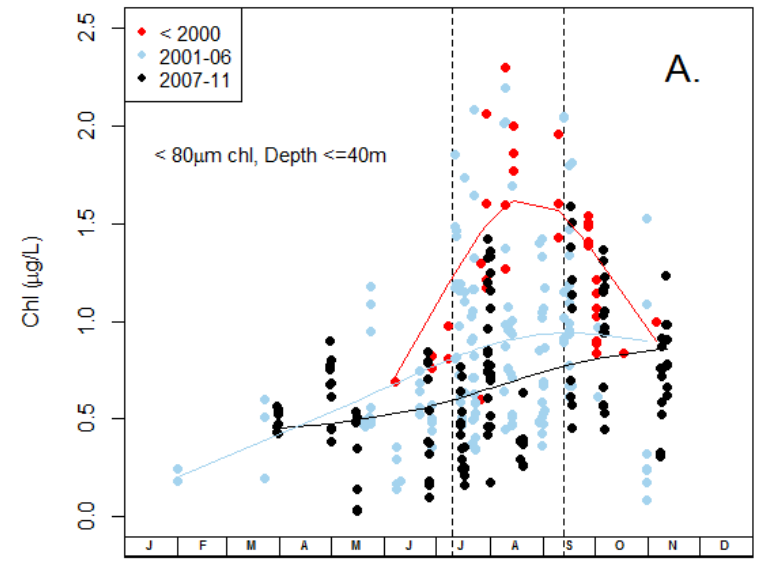
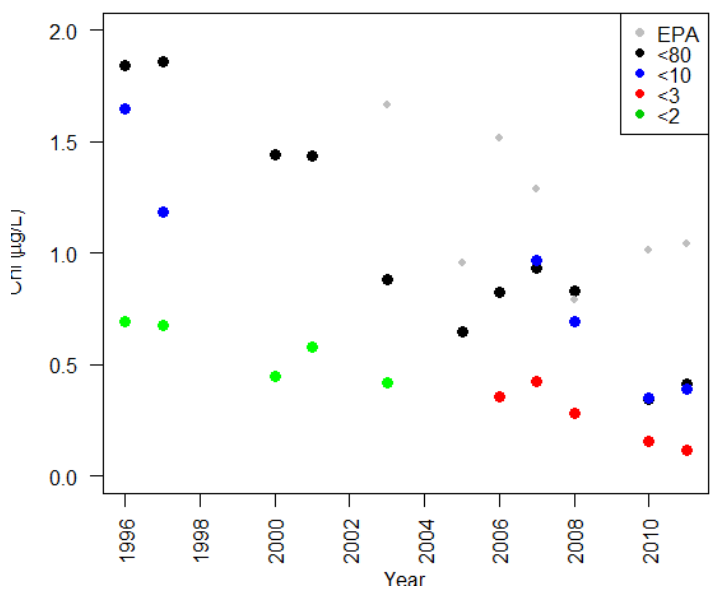
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Abstract: Lake Superior has been warming and its nitrate concentration has been increasing. Changes in other key parameters that would be most relevant in achieving an understanding of the carbon cycle and other ecosystem dynamics are still poorly documented. Here we utilize a 16-year record of offshore measurements to document for the first time some of the recent patterns in phosphorus pools and look retrospectively for changes in lower food web biomass over this time frame. The patterns of phosphorus pools with depth have been poorly resolved previously; we show that during both early and late stratification, total phosphorus is similar below the thermocline compared to above. This suggests thermal stratification does not inhibit upward movement of P, contradicting some of the models that have been put forward for how climate change affects lake and ocean productivity. We also find evidence that Lake Superior has been undergoing a similar direction of change as reported in the lower Laurentian Great Lakes. Between 1996 and 2010, chlorophyll and particulate carbon dropped significantly, especially in summer. Seasonality in surface chlorophyll has been much reduced. The largest size class of algae (10 - 80 μm) has nearly disappeared. These observations suggest that there is a regional signal of oligotrophication in all of the Laurentian Great Lakes. Mechanisms to explain lowered algal biomass in Lake Superior are still unclear but evidence points away from greater nutrient limitation due to more stable stratification. Increased sinking of large cells during longer stratified periods may play a role.



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LAKE SUPERIOR ALGAL BIOMASS AND NUTRIENTS: PATTERNS IN TIME AND SPACE

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6 **ABSTRACT**
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8 Lake Superior has been warming and its nitrate concentration has been increasing. Changes in
9 other key parameters that would be most relevant in achieving an understanding of the carbon
10 cycle and other ecosystem dynamics are still poorly documented. Here we utilize a 16-year
11 record of offshore measurements to document for the first time some of the recent patterns in
12 phosphorus pools and look retrospectively for changes in lower food web biomass over this
13 time frame. The patterns of phosphorus pools with depth have been poorly resolved
14 previously; we show that during both early and late stratification, total phosphorus is similar
15 below the thermocline compared to above. This suggests thermal stratification does not inhibit
16 upward movement of P, contradicting some of the models that have been put forward for how
17 climate change affects lake and ocean productivity. We also find evidence that Lake Superior
18 has been undergoing a similar direction of change as reported in the lower Laurentian Great
19 Lakes. Between 1996 and 2010, chlorophyll and particulate carbon dropped significantly,
20 especially in summer. Seasonality in surface chlorophyll has been much reduced. The largest
21 size class of algae (10 – 80 μm) has nearly disappeared. These observations suggest that there is
22 a regional signal of oligotrophication in all of the Laurentian Great Lakes. Mechanisms to
23 explain lowered algal biomass in Lake Superior are still unclear but evidence points away from
24 greater nutrient limitation due to more stable stratification. Increased sinking of large cells
25 during longer stratified periods may play a role.
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34 **Keywords – Trends, productivity, climate change, phosphorus**
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INTRODUCTION

Though less subjected to anthropogenic stress than the lower Laurentian Great Lakes (Allan et al., 2013) and often referred to as “pristine” (e.g. Munawar and Munawar, 2009), Lake Superior nevertheless has exhibited multiple significant recent multi-year trends (Kelly et al., 2011). Some of these changes clearly are anthropogenic in origin; others have unknown causes. Post-European major alterations to the mainly forested watershed involved logging and subsequent regrowth that over the past century has meant relatively lower coniferous and relatively more deciduous tree biomass (Schulte et al., 2007). Hydrologic changes include reduced stream flow on both the U.S. and Canadian shores over the past several decades (Ehsanzadeh et al., 2013); water levels are generally declining over the same time period (<http://www.glerl.noaa.gov/data/now/wlevels/dbd/>). The fish community has responded to multiple historic stressors including harvest, contaminants, and shoreline degradation (Kelso et al., 1996). In the early 1980s, native lake trout (*Salvelinus namaycush*), lake whitefish (*Coregonus clupeaformis*) and cisco (*Coregonus artedi*) stocks rebounded from chronically low levels in the 1960-1970s and peaked at historic levels in the early 1990s and declined thereafter (Bronte et al., 2003; Gorman and Hoff, 2009). Abundance of the dominant planktivorous fishes -- cisco, bloater (*Coregonus hoyi*), and rainbow smelt (*Osmerus mordax*) -- in nearshore waters (≤ 80 m depth), have declined to record low levels after 2008. The summer surface layer in Lake Superior has warmed and the length of the stratified season has increased (Austin and Colman, 2008). Wind speeds also increased between 1979 and 2006 (Desai et al., 2009) and with two opposing forcings (increased temperature/buoyancy vs. increased wind/stirring) changes in mixed layer depth are unresolved (Austin and Allen, 2011; Bennington et al., 2010). A steady and nearly linear increase in nitrate concentration has been underway for a century (Sternner et al., 2007). Most major ions (Ca, Cl, Mg, K) have been increasing at slow rates since the mid-1960s (Chapra et al., 2012).

Given these known changes, it is important to ascertain whether there are corresponding changes in lake biomass or productivity potentially affecting water transparency, nutrient pools or the food web, including, perhaps, fish production which may co-vary with changing lake productivity (Stockner et al., 2000). One of the most potentially significant parameters that can affect lacustrine ecosystem dynamics is the concentration and size spectrum of the suspended biomass of microbes and phytoplankton. Chlorophyll concentrations are among a small handful of parameters that describe and integrate the state of a lake ecosystem. Unfortunately and somewhat surprisingly, knowledge regarding trends in these biomass pools in Lake Superior is fragmentary (Reavie and Allinger, 2011). Lake Superior phytoplankton are dominated by organisms in very small size classes (Fahnenstiel et al., 1986), especially PE-rich *Synechococcus* (Ivanikova et al., 2007) and flagellated species are an important component of the community (Munawar and Munawar, 1982; Munawar et al., 2009). These taxa are not easily quantified. During summer stratification a prominent deep chlorophyll maximum is ubiquitous throughout the offshore (Auer and Bub, 2004; Barbiero and Tuchman, 2001). Primary production is low, lower than many parts of the offshore ocean (Sternner, 2010), so changes in the availability of phytoplankton could easily affect higher trophic levels.

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4 Chlorophyll and biomass pools are known to have changed over the past one to several
5 decades in most of the Laurentian Great Lakes; these trends have been attributed to climate as
6 well as altered nutrient loads (Michalak et al., 2013) and the impact of exotic benthic filter
7 feeders (Hecky et al., 2004; Nicholls et al., 2011). Secchi transparency increased in Lakes Huron,
8 Erie and Ontario between 1968 and 2002 (Dobiesz and Lester, 2009; Dove, 2009). A 40-year
9 record of Lake Ontario conditions also shows declining phosphorus and some major ions and
10 increasing nitrate and silica (Dove, 2009). Chlorophyll in southeastern Lake Michigan declined
11 between 1995 and 2000 but only in early and not in late summer (Pothoven and Fahnenstiel,
12 2013). Patterns of seasonal silica depletion suggest a strong oligotrophication in Lakes
13 Michigan and Huron but not Superior (Evans et al., 2011). A comparison of direct observations
14 of diatom assemblages between the two periods of 1996-97 and 2007-08 indicated a general
15 decline in biovolume and cell densities in all the Laurentian Great Lakes, Lake Superior included
16 (Reavie and Allinger, 2011). Barbiero et al. (2012) showed that Lakes Huron and Michigan have
17 been undergoing significant oligotrophication. They documented reduced concentrations of
18 chlorophyll and phosphorus, increased silica concentrations, and a loss in cladocerans with
19 increased importance of calanoid copepods, with trends that converged on the levels
20 considered typical for Lake Superior. Unusually large summer algal blooms in Lake Erie in 2012
21 (Michalak et al., 2013) is an exceptional event, but a region-wide oligotrophication of most of
22 the Great Lakes is well-established. Most of what we know about trends in algal biomass has
23 been inferred from chlorophyll concentrations. There seem to have been no examinations of
24 long term POC values in any of the Laurentian Great Lakes.

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26 In the context of the well-documented decrease in biomass pools in the lower food web
27 in most of the Great Lakes, corresponding trends in Lake Superior are less certain. Changes in
28 biomass and nutrient pools in Lake Superior have been examined and discussed but results
29 have been somewhat contradictory. Schelske et al. (2006) inferred a decline in production in
30 Lake Superior as well as the other Laurentian Great Lakes since the mid-1800s from a sediment
31 record of silica deposition. Reavie and Allinger (2011) reviewed the paleo record as well as
32 diatom counts done from water column measurements and suggested that there has been a
33 decline, especially in spring diatom density, between 1996 and 2008. The biennial State of the
34 Great Lakes reports (<http://www.epa.gov/solec/index.html>) have not included chlorophyll as
35 one of the tracked indicators even though direct monitoring by federal agencies of chlorophyll
36 concentrations began in Lake Superior the 1970s. The use of chlorophyll data from the U.S. EPA
37 are limited because data prior to 2002 are probably unreliable (Barbiero et al., 2011). These
38 reports have included information on phytoplankton populations (based on microscope counts)
39 and in the most recent finalized report (Environment Canada and United States Environmental
40 Protection Agency, 2009), only four years of data for Lake Superior are represented. and
41 furthermore the existence of trends in the data from other lakes where the time span of
42 observations was longer was not clear (“problems exist with internal comparability “ of the
43 data, Environment Canada and United States Environmental Protection Agency, 2009, p. 75).
44 Urban (2009) showed that the Lake Superior chlorophyll data collected by Environment Canada
45 between 1973 and 1998 exhibits a highly significant downward trend. A study based on data
46 from 1973, 1983 and 2001, however, reported a ten-fold increase in phytoplankton biovolume
47 based on microscope analysis, but no change in chlorophyll (Munawar and Munawar, 2009). In
48 summary, some but not all data sources indicate that over the past one to several decades,
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4 Lake Superior has been behaving similar to the rest of the Laurentian Great Lakes in that algal
5 biomass has been declining. However, the sparseness of data and some questions about data
6 reliability make it difficult to ascertain with confidence any trend in the biomass of the lower
7 food web in Lake Superior. Beyond the existence of any such a long-term trend in chlorophyll
8 levels, details about vertical structure, about seasonality, about size structure changes, etc. are
9 almost totally unknown.

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12 Phosphorus is likely the single most limiting macronutrient in Lake Superior (Van Der
13 Werff, 2011), although low average light in the water column is an important factor overall in
14 primary production in this lake. Lake Superior exhibits an extremely imbalanced N:P
15 stoichiometry associated with its low TP and high pools of nitrate (Sterner, 2011). Nitrogen in
16 the form of nitrate is always in abundance relative to biological demand (Sterner et al., 2007)
17 whereas ammonium, the more preferred N source, is at potentially limiting levels throughout
18 the water column (Kumar et al., 2007). Micronutrients can become limiting when P is added
19 (Sterner et al., 2004). The low levels of TP, which are on the order of 2-5x the method
20 detection limit using standard approaches, mean that most existing P data have low
21 signal:noise, which challenges the documentation of spatial and temporal variability. As a
22 consequence, there are many significant unknowns about phosphorus pools in Lake Superior.
23 Total phosphorus concentrations in Lake Superior may be declining; this trend is suggested by
24 combined US and Canada monitoring data from 1970-2007 (Environment Canada and United
25 States Environmental Protection Agency, 2009, p. 79) (visual evidence suggests a downward
26 trend -- no formal statistical analysis was included). Declining TP concentration between the
27 early 1950s and the early 2000s was also observed by Urban (2009) by combining data from
28 multiple published and unpublished sources.

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31 Given the above-described limitations in available data and the sometime conflicting
32 results, there still is a need to resolve changes in biomass and nutrient pools in Lake Superior.
33 Further, any ability to examine biomass and nutrient pools with enhanced temporal or spatial
34 resolution, such as to document changes in vertical patterns or seasonality, will ultimately help
35 in assigning potential causes for these important environmental trends.

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38 Between 1996 and 2011, > 600 depth x date observations of chlorophyll and carbon
39 biomass in Lake Superior offshore waters were made by a single laboratory using consistent
40 methods (Supplemental Table 1). This work was not performed as a single monitoring program
41 – the goals of the field work and the spatial focus changed several times – but a single core set
42 of measurements was taken over all these projects. The emphasis was on the western ~1/3 of
43 the lake with a smaller number of samplings performed in the eastern portion of the lake.
44 Here, we combine the observations made over these multiple projects into a single
45 retrospective analysis.

46 47 48 49 50 51 **METHODS**

52 This paper examines a 16-year (1996-2011) record of ecosystem parameters measured
53 at 20 sites in offshore Lake Superior (Supplemental Table 1). All sites included here were > 5 km
54 from shore. The seasonal timing of samplings emphasized the summer stratified season, but
55 efforts were made to collect samples over as wide a range the annual cycle as possible. The
56 strengths of this dataset are in the detailed vertical information and the wide range of
57 conditions under which the lake was studied. Its weaknesses include limited spatial coverage
58 and lack of consistent timing of sampling through the year. These shortcomings prevent us
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4 from using some desirable statistical analyses to examine temporal trends. However, results
5 presented here seem to be very strongly supported by the data, and multiple checks were done
6 to attempt to confusing long-term changes with spatial variation.
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8 Typically, any given station occupation began by observing profiles of CTD-reported
9 temperature, chlorophyll fluorescence, and several other parameters, and sampling depths
10 were then chosen to target depths that would best describe the variation in CTD-reported
11 chlorophyll. In some years a consistent set of depths was sampled at some sites. Usually
12 several depths above, several within, and several below the CTD-reported deep chlorophyll
13 maximum were sampled. To more fully resolve vertical patterns in nutrients we also present
14 here two station occupations from 2010 which included a much more intensive sampling of
15 vertical structure.
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18 To measure chlorophyll and particulate carbon and nutrients, multiple size fractions
19 were first prepared using 142mm-diameter filters. Several different size-fraction cutoffs were
20 used over the years but a 10- μm fractionation was measured fairly consistently. Also,
21 depending on the year, either a 2- or 3- μm fractionation was done. Other size cutoffs,
22 performed less consistently, are not reported here. At the onset of this work, the focus was on
23 phytoplankton-zooplankton interactions and thus a < 80 μm size fraction was made with Nitex
24 mesh on the largest size fraction studied to eliminate potentially inedible colonial algae as well
25 as mesozooplankton from samples. Though the focus of the work shifted to whole-ecosystem
26 studies, the 80- μm fractionation was maintained for the largest size fraction routinely
27 measured. For chlorophyll measurement, lake water of given size fraction was filtered onto 0.2
28 μm nitrocellulose filters (25 or 47 mm diameter, depending on the project; typical filter
29 volumes = 100-1000 mL) folded, and placed in clean polypropylene plastic centrifuge tubes,
30 which were then frozen at -10 °C. Typically within 1 month, filters were thawed and 10-12 mL
31 of 90% acetone was placed in the tubes, which were hand shaken and left in the dark at ~5 °C
32 for 20-22 h. Chlorophyll a in the extract was then measured with a Turner 10-AU fluorometer
33 using narrow band filters (Welschmeyer, 1994). The fluorometer was re-calibrated with
34 commercial chlorophyll standards every 3-4 years, and a solid standard was used to correct for
35 instrument drift between recalibrations. We examined lab records of measurement of the solid
36 standard collected over this observation period and saw no evidence for instrument drift that
37 could account for the chlorophyll trends we are reporting.
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40 To measure Particulate Organic Carbon (POC), size-fractionated lake water was filtered
41 onto pre-combusted 25 mm GF/F filters at low vacuum (typical volumes = 500-2000 mL). Filters
42 were placed into clean plastic petri dishes, which were sealed and frozen. Thawed filters were
43 subsequently dried at 60 °C, wrapped in tin disks, and analyzed using a Perkin-Elmer 2400 CHN
44 analyzer, which was calibrated with acetanilide every run. To measure Particulate Phosphorus
45 (PP), size fractionated lake water was filtered onto acid-rinsed GF/F filters and frozen. These
46 were then thawed and digested in potassium persulfate (50g/L) in an autoclave for 30 m at 121
47 °C, and phosphate was measured using the Ascorbic Acid Method, Standard Methods 424F
48 (APHA, 1992) in a Shimadzu UV-VIS 160U Spectrophotometer.
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51 To measure NO_3^- , whole lake water was placed into 60 mL acid-rinsed plastic bottles and
52 frozen. These were then thawed and $\text{NO}_3^- + \text{NO}_2^-$ was measured with an autoanalyzer with Hach
53 Company NIST Standard used as a reference. Trials were run to compare filtered to unfiltered
54 samples and it was determined that filtering only added variation to the measurement in these
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4 waters of relatively low particle density and high nitrate. To measure NH_4^+ , GF/F-filtered water
5 was put into acid-rinsed 500mL plastic bottles and frozen. These were then thawed and the
6 OPA-fluorescence technique of Holmes (1999) was employed using the Protocol A variation. To
7 calculate ammonium concentration, the formula of Taylor et al. (2007) was used. The Taylor
8 formula gives varying lower ammonium concentrations in Lake Superior than the original
9 formula of Holmes, which was used by Kumar et al. (2007) in their investigation of ammonium
10 in Lake Superior. The concentrations reported here are considered more accurate.

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13 To measure the low concentrations of Total Dissolved Phosphorus (TDP) and Total
14 Phosphorus (TP) in Lake Superior, in recent years a freeze-dry concentration step was
15 developed and employed. It is described here for the first time. First, either whole water (not
16 80- μm fractionated) or 0.2- μm filtered lake water was frozen in Trace Metal Cleaned, MilliQ-
17 rinsed plastic bottles (typical volume = 500 mL). These were subsequently thawed and 50-80
18 mL was transferred into acid-soaked, TMC-cleaned glass bottles. These were capped and
19 frozen in a standard laboratory freezer until solid. Bottles were then uncapped and covered
20 with a Nitex mesh held on with rubber bands or zip ties and placed in a -40 °C freeze drier for 3
21 d until the water was fully sublimated. The resulting powder was resuspended in 7 mL of
22 potassium persulfate solution and autoclaved for 30 m at 121 °C. Phosphate was then
23 determined by the Ascorbic Acid Method (per above). Nanopure blanks and empty bottles
24 were routinely run through the freeze-drier to determine blanks and the detection limit. The
25 Method Detection Limit (Average blank + 3.29 x SD) was 2 nmol/L, approximately a 10-fold
26 improvement over standard persulfate digestion methods
27 (http://www.epa.gov/greatlakes/monitoring/sop/chapter_2/LG204.pdf).

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29 Statistical analyses were run in R (R Development Core Team, 2008). Significant
30 temporal trends were examined using linear regression and by calculation of Kendall's tau.
31 Data are archived at www.bco-dmo.org.

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33 Trend analysis using data taken at different locations over time risks aliasing temporal
34 with spatial variation. There are two things that lower this risk in our analyses. First, in spite of
35 Lake Superior's vast area, horizontal variation of offshore surface chlorophyll seems to be small
36 enough to not greatly affect the trend analysis. For example, both the U.S. Environmental
37 Protection Agency and Environment Canada perform spatially intensive sampling which
38 includes measurement of chlorophyll concentration at multiple sites. The EC collects vertically
39 integrated samples (usually 50 m to the surface) in August of selected years. EC data from
40 1996, 1997, 2001 and 2005 were provided to us (Alice Dove, EC, pers. comm.). We looked at
41 site averages of data reported as "CHLOROPHYLL A CORRECTED INTEGRATED" and found no
42 strong E/W or N/S gradients (Supplemental Fig. A). The EC chlorophyll concentration data are
43 reported in relatively large increments (0.1 $\mu\text{g/L}$ against a mean of 0.6 $\mu\text{g/L}$ with $\sim \frac{1}{2}$ of the
44 values reported as 0.1 $\mu\text{g/L}$. These characteristics suggest that these measurements may not
45 be highly precise, which may contribute to some of the site-to-site variation observable in
46 Supplemental Fig. A. We accessed US-EPA data at
47 http://www.epa.gov/glnpo/monitoring/data_proj/glenda/, which included data from 1992-
48 2008. However, data prior to 2002 are suspected to have a low bias owing to problems with
49 the filters used in the fluorometer (R. Barbiero pers. comm., see also Barbiero et al., 2011).
50 Hence, only data from 2002-2008 are used in this paper. We also selected only samples coded
51 as "surface" depths (due to strong variation of chlorophyll with depth in Lake Superior) and for
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4 the months of August and September (to match up with our “summer” observations described
5 below). We then averaged the resulting subset of data by site. No clear spatial trends in
6 summer surface chlorophyll between 2002-2008 were apparent (Supplemental Fig. A), though
7 only the western half of the lake was represented in this particular subset of data. More
8 refined spatial information on chlorophyll concentration is potentially available from satellite-
9 derived remote sensing (e.g. Shuchman et al., 2013), but these algorithms are still in active
10 development (Lesht et al., 2012, 2013; Mouw et al., 2013) and use is not yet standard or
11 routine. The second protection against aliasing comes from examination of temporal trends for
12 data collected at a single site in the lake (station CD-1). The trends we observed at this one site
13 were similar to those observed in the more comprehensive analysis using all sites, so here we
14 generally present only the more comprehensive analysis.
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21 RESULTS

22 *Nutrients and Biomass, Recent State*

23 Long-term changes in lacustrine biomass pools may be driven by many mechanisms, and
24 assigning causality to temporal trends is difficult. Vertical profiles of potentially limiting
25 elements can be helpful in evaluating which mechanisms may be at work. Owing to difficulties
26 with measuring small quantities, little information has been available about the vertical
27 distribution of the main limiting element, phosphorus, in Lake Superior. Detailed vertical
28 profile samplings done during two occupations of Station WM during 2010 are presented here
29 (Figs. 1 and 2). WM is one of the more heavily represented sites in the long-term chlorophyll
30 record analyzed here. Primary productivity was measured at this location using *in situ* ¹⁴C
31 techniques (Sterner, 2010). One of these two occupations occurred early in the stratified
32 season (surface temperature = 7.6 °C; Fig. 1) and the other occurred late in the stratified season
33 (surface temperature = 11.3°C ; Fig. 2).
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38 During the June (Fig. 1) sampling, there were large vertical gradients in biomass but
39 small to negligible gradients in nutrients. A thermocline was present at ~15m and a Deep
40 Chlorophyll Maximum was observable in both the CTD fluorescence (max value at 25 m) and
41 the filtered/extracted chlorophyll measures (max value at 10 m), with a large vertical offset
42 between the two perhaps owing to physiological or photochemical quenching. POC also
43 peaked below the surface (max value at 10 m) though the deep carbon maximum was much
44 less apparent than the chlorophyll maximum recorded with either method. The phytoplankton
45 at depths < 40m were dominated by organisms in the 2-10 μm range, with picophytoplankton
46 present throughout the water column. Chlorophyll in the 10-80 μm range was virtually absent
47 in most depths, and similarly there was relatively small amounts of POC in this size range. Both
48 [NO₃⁻] and [NH₄⁺] were slightly lower at depths < 40 m compared to deeper water layers. [TDP]
49 exhibited no detectable vertical gradient at all. Aside from two possibly spurious observations
50 around 40 m, [TP] also exhibited no strong vertical structure. Finally, [PP] also showed no
51 obvious vertical patterns. Most of the phosphorus throughout the water column was in the
52 dissolved pool.
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57 In August (Fig. 2) the thermocline was present at ~ 16m. A prominent DCM was
58 observed in both measures of chlorophyll (maximum CTD fluorescence at 22m, maximum
59 filtered/extracted chlorophyll at 33m). Picoplankton did not account for much if any of the
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4 DCM, with larger size fractions predominating at this depth. Significant amounts of chlorophyll
5 > 80 μm in size was limited to DCM depths. More vertical structure in nutrient profiles was
6 observed than in the June sampling. By this time of the season, surface $[\text{NO}_3^-]$ drawdown was
7 $\sim 3\mu\text{M}$, similar to typical seasonal maximum drawdown (Sterner, 2010). Concentrations of NH_4^+
8 were slightly elevated in depths < 50m than below. There was a clear, but small, decrease in
9 [TDP] at depths < 20m compared to the rest of the water column. In contrast, [PP] and [TP]
10 were higher at depths < 40 m. Concentrations of dissolved P were similar to concentrations of
11 particulate P, with dissolved P higher in deeper samples and particulate P higher in shallower
12 depths. Comparing the directly measured PP to the difference between TP and TDP in Fig 2, it
13 appears there could have been significant phosphorus at depths < 40 m that passed through a
14 GF/F filter (thus not accounted for in PP) but was retained on a 0.2 μm filter (and thus not
15 accounted for in TDP).
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21 *Chlorophyll and Biomass, Temporal Trends*

22 Similar trends were seen when examining all of the offshore sites combined as when
23 using a narrower subset of the data using only the single site with the most extensive samplings
24 (site CD-1), so the comprehensive dataset is presented here. The period from JD 190 and 255
25 (early July to mid-September) was chosen by visual inspection to bracket the period when
26 chlorophyll concentrations were highest, especially in early years, and this period will be
27 referred to here as “summer.” The largest changes in chlorophyll in Lake Superior between
28 1996 and 2011 were seen in the < 80 μm size fraction, in the summer, and at depths above the
29 typical DCM depth. For example, chlorophyll measured at depths $\leq 40\text{m}$ during three time
30 windows (1996-97, 2000-2006, and 2007-2011, chosen subjectively based on examination of
31 the data) were clearly distinct but only during summer (Fig. 3A). Fig. 3A suggests that
32 chlorophyll since 1996 has exhibited a much reduced seasonality (note cubic spline fits).
33 Differences were considerable: summertime, shallow chlorophyll in the 2007-11 window was \sim
34 $1/3$ of that observed in the < 2000 window. Similar, but less distinct differences were observed
35 in POC (Fig. 3B).
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41 Reduced levels of summer chlorophyll from the beginning to the end of the observation
42 period were observed over the whole water column, although these reductions were apparent
43 at depths > 40 m only in the third time window (2007-11) compared to the previous two (data
44 not shown). Compared to the period 1996-1999, chlorophyll measured in 2000-2006 appeared
45 to show a deepening of the deep chlorophyll maximum; a very large diminishment of
46 chlorophyll above 20m was also seen (Fig. 4). By 2007-2011, A DCM was still observable but its
47 magnitude was much diminished. POC values were different across years mainly in a near
48 complete absence of values > 15 μM in 2007-11 whereas such values were observed in earlier
49 periods. Changes in POC vs. depth were not obvious (Fig. 4). Similar to what was observed
50 with season, the variability of chl and POC with depth was less in later observations than in
51 early ones.
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55 Somewhat different size fraction cutoffs were studied during the different time
56 segments over the whole observation period. Nevertheless, a seeming robust set of changes
57 involving size structure were apparent (Fig. 5A). Decreasing trends were well supported
58 statistically (Table 1). Decreases in chlorophyll over time were seen throughout the size
59 spectrum. The smallest size fraction (either 2 or 3 μm , depending on the project) seems to form
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4 a single, continuous decreasing trend through time from ~ 0.7 to $0.2 \mu\text{g L}^{-1}$. During the two
5 earliest observation years, chlorophyll in the 10-80 μm size range was readily measurable.
6 However, this size fraction has been absent or nearly so in all years since 2007. While
7 performing additional sampling using zooplankton nets during 1996-97 (results not reported
8 here), we observed that nets were frequently clogged with colonial diatoms (likely *Alauocosira*),
9 whereas in recent years nets have not clogged to nearly the same extent. Thus, decadal-scale
10 decreases characterize phytoplankton of all sizes, but the largest size class of algae has been
11 reduced to nearly undetectable levels. Chlorophyll data from the US-EPA for years 2002-2008
12 (“whole” water fraction) are presented for comparison (panel A). The EPA data do not form a
13 statistically significant trend by themselves, but those observations are generally consistent
14 with our measurements. EPA data may run a little higher than our measurements because they
15 are not fractionated or due to other methodological considerations. Measurements of POC in
16 different size fractions are not as extensive as for chlorophyll (Fig. 5B) but nevertheless are
17 consistent with the chlorophyll data in exhibited downward trends. Fitted slopes of POC over
18 time all were negative, with statistical significance observed in the trend associated with the
19 largest size fraction (Table 1),
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21 We tested for shifts in seston composition by examining annual means of C:P, C:N and
22 C:chl (first taking means by cruise and then means by year), and plotting against year with four
23 subsets of data (all combinations of summer/winter and shallow/deep where season is defined
24 as elsewhere in this paper and 40m was the depth cutoff) (Supplemental Fig. B). The date
25 range and numbers of years differed among these tests due to sparseness of data in some
26 measures. Among all those tests, only the C:chl ratio for summer shallow observations was
27 significantly related to year, showing a ~ 6 -fold rise from the beginning to the end of the
28 observation period (Fig. 6). This shift in ratio is to be expected given the larger changes in
29 chlorophyll than observed for POC. Composition data were further examined by performing
30 MANOVA tests on pairs of composition measures for individual observations, subsetting the
31 data as above (Supplemental Fig. C). P-values were low for this set of analyses in spite of great
32 overlap in early vs. late observations for all data subsets. However, multiple observations for
33 given station occupations are presented here; thus these hypothesis tests might be considered
34 pseudoreplicated. The data are presented this way to display the variation in the data of the
35 individual observations. Evidence was strongest that there were differences between early and
36 late observation periods for summer, shallow samples. Overall, except for C:chl, any shifts in
37 seston ratios were subtle at best.
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48 DISCUSSION

49 Chlorophyll in Lake Superior declined between 1996-2011, a trend consistent with what
50 has happened throughout the other Laurentian Great Lakes (Barbiero et al., 2012; Dobiesz and
51 Lester, 2009; Evans et al., 2011; Nicholls et al., 2011; Pothoven and Fahnenstiel, 2013; Reavie
52 and Barbiero, 2013). Biomass as measured by carbon also declined but at a slower rate.
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54 This decline was very selective in terms of spatial (vertical dimension) and temporal
55 extent as well as what subset of the algal community was involved. The trend was greatest for
56 the larger phytoplankton in the summer, surface layer. Total summer, surface chlorophyll
57 dropped by ~ 3 x (from 1.5 to $0.5 \mu\text{g/L}$). By simple subtraction of the slopes given in Table 1, we
58 estimate that chlorophyll in the 10 to 80 μm size fraction dropped by $0.17 \mu\text{g/L/y}$, chlorophyll in
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4 the 2 (or 3) to 10 μm size fraction dropped by 0.109 $\mu\text{g/L/y}$, and chlorophyll in the smallest size
5 fraction, less than 2 or 3 μm , dropped at the lowest rate, 0.039 $\mu\text{g/L/y}$. The largest size fraction
6 thus decreased at an approximately 4-fold faster rate than the smallest size fraction. The trend
7 was not present or was less apparent for other subsets of the community.
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10 These shifts greatly altered the seasonality of chlorophyll in the lake. Between the mid
11 to late 1990s and the early 2010s, the seasonal waxing and waning of surface chlorophyll
12 diminished greatly such that by the end of the period, summer chlorophyll was perhaps no
13 different from winter chlorophyll. Though the data from the winter season is sparser than from
14 summer, it seems as though there has been little to no change to chlorophyll or to POC in the
15 winter. Thus, what changed most is the reduction of the several-month summertime increase
16 then decline of larger algae. At the end of this observation period it was difficult to observe any
17 seasonal pattern in surface chlorophyll at all. Patterns in DOC were similar but smaller in
18 magnitude.
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21 A summer Deep Chlorophyll Maximum is a ubiquitous feature in the Laurentian Great
22 Lakes (Barbiero and Tuchman, 2001). Previous observations in Lake Superior performed in
23 1996-2001 (Barbiero and Tuchman, 2004) indicated that the DCM was located between 23 and
24 35m depth, above the strongest vertical gradient in temperature. The diatom Cyclotella was
25 reported to make up considerable portion of the DCM biomass (Fahnenstiel and Glime, 1983).
26 However, little to no maximum in POC corresponded with the chlorophyll maximum, indicating
27 that shade adaptation of phytoplankton (enhanced chlorophyll:carbon ratios) was likely a
28 principal cause (Barbiero and Tuchman, 2004). The Lake Superior DCM formation has been
29 modeled and mechanisms inferred to be important included photoadaptation, nutrient delivery
30 through the thermocline, and grazing (White and Matsumoto, 2012). Our newly reported
31 observations are consistent with these made earlier regarding changes in chl vs. carbon with
32 depth. The data here also agree that most of the observed DCM in Lake Superior is an
33 adjustment in the chl:carbon ratio, and we observe that this takes place mainly in cells larger
34 than 2 μm in size. There is evidence in our data of lower POC in depths < 10m (see, e.g. Fig. 4,
35 lower middle panel) relative to DCM depths, so the deep chlorophyll maximum is reflected to
36 some extent in POC. Carbon incorporation in Lake Superior at depths above 10m sometimes
37 shows strong photoinhibition, potentially contributing to reduced biomass in the shallowest
38 depths (Sterner, 2010). There also seems to be some evidence of changing DCM depth with
39 time over our observation period. In addition, there has been a very large change in summer,
40 surface chl:POC over our observation period such that surface particulate matter was much
41 lower in chlorophyll in 2011 than it was in 1996. From the beginning to the end of this
42 observation period, biomass as represented by POC did decline, but chlorophyll content
43 (chl:POC) did as well. This shift in carbon:chlorophyll ratio could indicate an increasing
44 importance of tripton (nonliving particulate matter), an increasing fraction of heterotrophs in
45 the community compared to autotrophs, an increased representation of high C:chl
46 cyanobacteria (Sathyendranath et al., 2009) or a “bleaching” of the extant autotrophs.
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49 The details of the chlorophyll decline we observed, together with our new observations
50 of vertical nutrient gradients, provide some guidance to begin to sort out possible causal
51 reasons behind this trend. In looking for explanations for reduced chlorophyll in Lake Superior,
52 we need to focus especially on those mechanisms that would affect summer, surface, large
53 phytoplankton and would tend to lower chlorophyll content of biomass. Potential reasons will
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4 be discussed here, though definitive conclusions are not possible. Mechanisms that have been
5 hypothesized to account for lowering algal biomass in the other Laurentian Great Lakes include
6 food web changes (Cuhel and Aguilar, 2013), reduced nutrient loads, and climate. TP mass
7 balance modeling throughout the Laurentian Great Lakes suggests a dominant role of reduced
8 nutrient loading until ~1990, with a strong food web effect after that (Chapra and Dolan, 2012).
9 Extending this known multi-year trend for the other lakes to Lake Superior indicates either that
10 there are multiple lake-specific factors having similar influence throughout the Great Lake
11 region or that a single, large-scale factor is at work.

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14 Temperature and carbon:chlorophyll. An obvious place to begin is to question whether
15 there may be a direct relationship between the warming surface layer (Austin and Colman,
16 2007) and increasing C:chl (Fig. 6). C:chl varies by more than an order of magnitude in living
17 algae. The range here was ~six-fold. C:chl in general is responsive to light, to nutrients, to
18 species identity and other factors. The effect of temperature on the chlorophyll content of
19 nutrient-saturated algae exposed to low to moderate light (i.e. are non-photoinhibited) is well
20 worked out (Geider, 1987). C:chl decreases exponentially with increased temperature at
21 constant light (Geider, 1987). Warm-temperature adapted algae acclimate in similar ways to
22 low-light adapted algae by increasing chlorophyll content (Maxwell et al., 1995). Extrapolation
23 to nutrient-limited conditions or to conditions of surplus light may be somewhat questionable,
24 but these reported effects of temperature run contrary to our observations of increased C:chl in
25 a warming surface layer. We therefore reject this potential direct mechanism. Further, warmer
26 temperatures increase algal susceptibility to photoinhibition (Falk et al., 1990), so all else
27 being equal we might expect an intensification of the DCM over time, also contrary to
28 observations.

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31 External nutrient loading. Could the trends we observed represent a nutrient-based
32 oligotrophication of Lake Superior, a mechanism that has resulted in lowered algal biomass in
33 other large lakes of the world (e.g. Lake Constance, Stich and Brinker, 2010). Some of the
34 dissolved substances in Lake Superior are changing over time. Nitrate is rising steadily, though
35 it is unlikely that is having an influence on total algal biomass given the low TP concentration in
36 the lake. Most major ions also are slowly increasing (Chapra et al., 2012). Stream flow has
37 declined (Ehsanzadeh et al., 2013), which indirectly suggests that loading of nutrients from land
38 could also be declining. In addition, stream water P concentrations have recently declined in
39 forested watersheds in the Great Lakes region (Eimers et al., 2009; O'Brien et al., 2013).

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42 Because of the aforementioned issues with signal:noise, any trends in phosphorus
43 concentrations in the lake are difficult to state confidently. Historic data on Lake Superior P
44 concentrations are sparse, but several authors have attempted to deduce [TP] trends.
45 Combining different data sources and taking the reported values at face value indicates a very
46 substantial drop of ~4x in TP concentration from the 1950s to the early 2000s (Urban, 2009). In
47 another effort, data solely from the U.S. EPA's GLNPO monitoring between 1992-2010 indicates
48 a spike in [TP] in 1996, a rapid falloff of 2-3x until ~2000 and an approximately constant
49 concentration since (Barbiero et al., 2012). Within the observational window examined in this
50 paper, the first of these analyses suggest the period of observation is part of a long-term TP
51 decline and the other analysis suggests that the early part of the observational window was
52 marked by unusually high TP concentrations. Either analysis provides some support for
53 declining TP concentration for at least part of the observational window considered here. It is

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4 possible that a declining TP concentration in Lake Superior is linked indirectly to the nitrate
5 buildup via linkages to carbon and oxygen cycles and changed N retention (Finlay et al., 2013)
6 though we are aware of no historic TP data for the first half of the twentieth century that would
7 help confirm this linkage in element cycles.
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10 P loading estimates (Dolan and Chapra, 2012) average 3242 metric tonnes/y and using a
11 mean [TP] of 2.6 µg/L from the same reference, the total P inventory of Lake Superior can be
12 calculated as 30,800 tonnes. Governmental data sources place total municipal contribution at
13 2.7%, and total industrial contribution at 5.6% of the total (using averages reported for the
14 period 1994-2008) (Dolan and Chapra, 2012). No significant linear upward or downward trend
15 of total P loading over that observation window was reported (Dolan and Chapra, 2012), but
16 relatively high loadings in 1996, 1997, 2001, 2006 and 2008 were observed in response to
17 overall high stream flows those years. The relatively low impact of direct human contribution
18 to P loading in Lake Superior, and the expected inherent long response times to loading in this
19 lake, given loading vs. inventory make it seem quite unreasonable to suggest that a 3x
20 summertime drop in chlorophyll over 16 years is a direct response to loading, anthropogenic or
21 otherwise. We conclude that unlike what has been documented in other large lakes, the
22 changing biomass pools we observed in Lake Superior are not responding mainly to nutrient
23 management changing direct human input.
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26 Proper assessment of changing TP as a factor connected to changing biomass pools
27 should rest on a good understanding of how TP might determine algal biomass through the
28 year. Should one expect lower chlorophyll year-round in response to lower TP concentration?
29 Or are certain seasons or depths more likely to respond to changing concentration? Both P and
30 light are important limiting factors in Lake Superior (Nalewajko et al., 1981). For example, it is
31 perhaps the case that cold temperature and relatively deep mixing increases the importance of
32 light such that algal biomass is relatively insensitive to phosphorus during colder seasons. In
33 contrast, warm and relatively well-lit summer conditions could allow for greater algal growth to
34 where they would be expected to increase to a TP-determined limit (Guildford et al., 2000).
35 Surface seston C:P ratios are higher in Lake Superior in the summer mixed layer than at other
36 depths or other seasons (Sterner, 2011). There is also biological reasoning to think that
37 decreased nutrient availability would disfavor larger cells, which all else being equal are less
38 competitive than small cells (Grover, 1989). On balance it would seem that the details of the
39 trend we observed could be considered consistent with a nutrient-based mechanism.
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42 We therefore cannot categorically accept or reject a loading-based explanation. Historic
43 TP data do support decreased nutrient pools, and for reasons discussed above it is reasonable
44 to conclude that nutrients would be most strongly linked to larger algae in the upper stratified
45 water column. Although we saw no evidence for increasing C:P ratios to indicate greater P
46 stress with time, reduced nutrient pools are possibly a viable explanation for these trends. Our
47 almost total ignorance of the internal dynamics of P in Lake Superior make it impossible to
48 comment further on what could be determining interannual variation in TP in the lake since
49 watershed P loading does not appear to be changing.
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52 Climate and mixing. Our observation of greatest pace of change in chlorophyll during
53 summer in the surface layer seems to point generally to climate as a driver. Summer
54 epilimnetic temperature show a strong increase (Austin and Colman, 2007) which is likely to
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4 influence algal dynamics Changing climate affects lakes in complex ways (Adrian et al., 2009;
5 Moss, 2012). Climate changes the seasonality and timing of processes (Winder and Schindler,
6 2004). The changes observed here weren't so much in timing but an overall dampening of
7 variation.
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10 Higher air temperatures would increase the length and stability of stratification, which
11 hypothetically would lower the mixing-related supply of nutrients from below the thermocline.
12 At the same time though, increases in wind could moderate or overcome this effect. Depressed
13 productivity due to climate-related nutrient reductions has been hypothesized for Lake
14 Tanganyika (O'Reilly et al., 2003), an hypothesis that drew rebuttal and reply (Eschenbach,
15 2004; O'Reilly et al., 2004). Similar processes have been invoked to explain reduced chlorophyll
16 in the stratified ocean (Polovina et al., 2008; Sarmiento et al., 2004). Under both higher CO₂
17 and warmer temperatures, greater C:nutrient ratios could result both due to increased
18 availability of carbon and reduced nutrient supply associated with enhanced thermal
19 stratification (van de Wall et al., 2010). There are two reasons though why climate-induced
20 reduction of mixing is an unsatisfactory explanation for our observations for Lake Superior.
21 First, as documented here for the first time, there are very small gradients in TDP in the lake,
22 and observed gradients in TP are opposite in direction to those that would support this
23 hypothesis. Though slight in magnitude, we observed higher TP in the surface layer than in
24 strata below it during stratification. Indeed, given the Lake Superior P profiles we observed,
25 decreased mixing across the thermocline would increase, not reduce TP in the surface layer.
26 This would increase, not decrease algal biomass in the summer surface layer. More
27 information on P movement and distribution in Lake Superior is vitally needed but the
28 information available runs contrary to the mixing hypothesis. Second, we observed no
29 convincing trend in C:P ratios, which are one potential diagnostic tool for P limitation.
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32 A different effect of enhanced stratification though is on likely changes in algal sinking
33 from the trophogenic zone. Increased stability of stratification would disfavor cells with higher
34 sinking rates. Such a process would differentially harm large phytoplankton because their
35 sinking losses would be elevated. Such a processes has been invoked for Lake Tahoe (Winder et
36 al., 2008). Our observations of changed size structure are consistent with expectations for
37 climate-induced increases in sinking rates of large algae.
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40 Food web. Another major loss for phytoplankton is grazing. What evidence is there
41 that a changing food web could have depressed algal biomass in Lake Superior between the
42 mid 1990s and early 2010s? Though invasive mussels likely have had a pronounced effect in
43 some of the lower Laurentian Great Lakes, their biomass in Lake Superior is much lower
44 (Benson, 2013; Vanderploeg et al., 2002) and impacts on offshore Lake Superior algal biomass
45 are probably negligible (see, e.g., Nicholls et al., 1999).
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48 Planktonic grazing pressure is a significant loss term for phytoplankton populations in
49 Lake Superior (Seegers, 2009) as in many lakes. Changes in micrograzer abundance, which are
50 probably the main grazer on the bulk algal community, are unknown, but changes in the
51 crustacean grazers as well as forage fish that consume them for the period 1989-2000 (covering
52 the initial part of our observational window) have been described (Gorman et al., 2009). These
53 observations provide evidence for top-down control of larger zooplankton in Lake Superior --
54 zooplankton biomass and mean size covaried with populations of lake herring (Coregonus
55 artedi). Further, the abundance of zooplankton is now quite high compared to the mid-1990s,
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during a peak in abundance in planktivore fish (Owen Gorman, USGS Pers. Comm.). Thus, there is some evidence for food web changes that could result in elevating grazing loss rates during this observational period. However, proper evaluation of grazing pressure as a mechanism requires better understanding of the specific linkage of large copepod grazers, which are increasing, to summer, surface large phytoplankton. Evidence for or against the mechanism of increased grazing is therefore highly incomplete.

To summarize this consideration of mechanisms, we find support for one (sinking), incomplete or mixed support for some (grazing, loading), and reasons to reject others (temperature effects on C:chl, vertical mixing). Though the direction of change of chlorophyll in Lake Superior is the same as in the lower Great Lakes, we cannot invoke invasive mussels as an important mechanism in Lake Superior. Our work expands the scope and range of the oligotrophication that is being discussed for other lakes but the mechanisms accounting for changes in Lake Superior do not seem to line up well with those discussed for the lower lakes.

Finally, we consider how our observations may relate to questions about the whole-lake carbon cycle. A salient issue is whether the ~3x decrease in chlorophyll we observed in summer surface samples may relate to whole lake production? The observed change in POC, albeit relatively smaller in magnitude than chlorophyll, does suggest that there is more occurring than shifts in pigment content. Given the low standing stocks of biomass already in Lake Superior, further declines may well be relevant to the sustainability of the fisheries.

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Table 1. Statistics on trends for mean summer, shallow chlorophyll and POC by year over time. Data for all sites are presented in Fig. 5.

Chlorophyll, Linear regression, all sites.

| Variable | Slope±SE (µg/L/y) | n | p-value | r-squared (adj) |
|----------|-------------------|----|---------|-----------------|
| 80 µm | -0.100 ± 0.011 | 11 | <0.001 | 0.89 |
| 10 µm | -0.070 ± 0.014 | 6 | 0.007 | 0.83 |
| 2, 3 µm | -0.039 ± 0.004 | 10 | <0.001 | 0.89 |

Chlorophyll, Kendall Tau, all sites.

| Variable | Mann Kendall Tau | n | p-value (2-sided) |
|----------|------------------|----|-------------------|
| 80 µm | -0.562 | 11 | 0.009 |
| 10 µm | -0.905 | 6 | 0.007 |
| 2, 3 µm | -0.727 | 10 | 0.001 |

Chlorophyll, Linear regression, CD-1 only.

| Variable | Slope±SE (ug/L/y) | n | p-value | r-squared (adj) |
|----------|-------------------|----|---------|-----------------|
| 80 µm | -0.095 ± 0.010 | 11 | <0.001 | 0.90 |

POC, Linear Regression, all sites.

| Variable | Slope±SE (µg/L/y) | n | p-value | r-squared (adj) |
|----------|-------------------|---|---------|-----------------|
| 80 µm | -0.253 ± 0.102 | 8 | 0.048 | 0.42 |
| 10 µm | -0.079 ± 0.058 | 6 | 0.25 | 0.14 |
| 2, 3 µm | 0.077 ± 0.195 | 5 | 0.712 | -0.20 |

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4 Figure legends
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7 Fig. 1. Vertical profiles of various parameters at site WM on June 26, 2010. Bottom (gray line) =
8 164m. Dotted lines indicate depths of 20 and 40m.
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10 Fig. 2. Vertical profiles of various parameters at site WM on August 19, 2010. Bottom (gray
11 line) = 168. Dotted lines indicate depths of 20 and 40m.
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14 Fig. 3. Changes in seasonality in biomass pools in multiple offshore stations. A. Chl *a* in the < 80
15 μm size class and within the 0-40m depth zone vs. day of year. Vertical lines at JD 190 (date)
16 and 255 (date) demark the period considered summertime. Fitted curves are cubic smoothing
17 splines. B. POC for the same size fraction and depth interval as in A.
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20 Fig. 4. Chlorophyll (A, B, C) and POC (D, E, F) as a function of depth for summer observations.
21 All values are for the < 80 μm size fraction. Data were binned into 5m depth strata starting 0m
22 and are plotted at the midpoint of the depth interval. Dark bars represent medians, boxes
23 represent upper and lower quartiles, and whiskers represent ranges with outliers (plotted as
24 individual points) excluded. Box widths are proportional to the square root of the sample sizes.
25 Depths of 20 and 40m are marked for reference to other figures.
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30 Fig. 5. Mean summer (190<JD<255), shallow (depth \leq 20 m) chlorophyll (A) or POC (B) of
31 different size fractions (see legend) for all offshore sites (includes years with a minimum of 6
32 observations/year). Regression statistics are given in Table 1.
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35 Fig. 6. Mean C:chl ratio for summer shallow observations by year. The line is from a linear
36 regression ($Y = -548 + 0.29X$, $p < 0.05$).
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Figure 1
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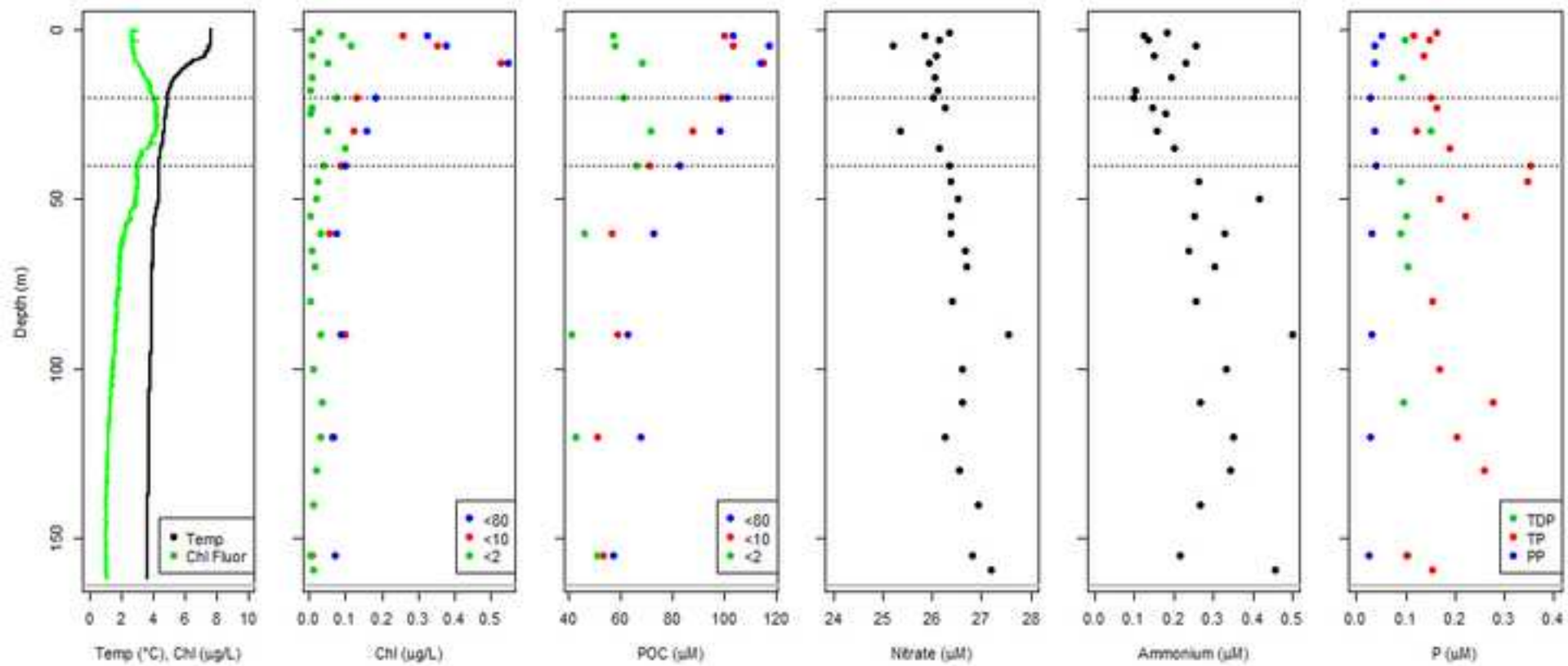


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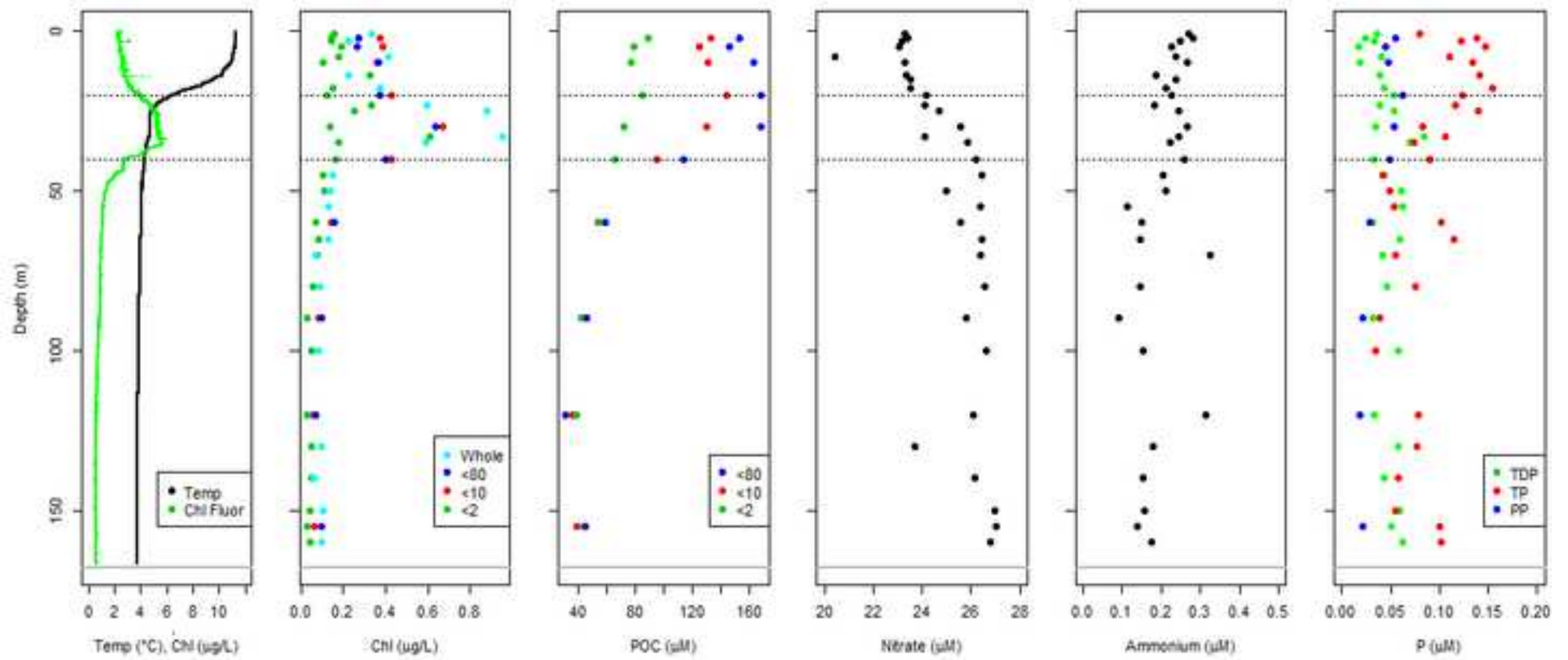


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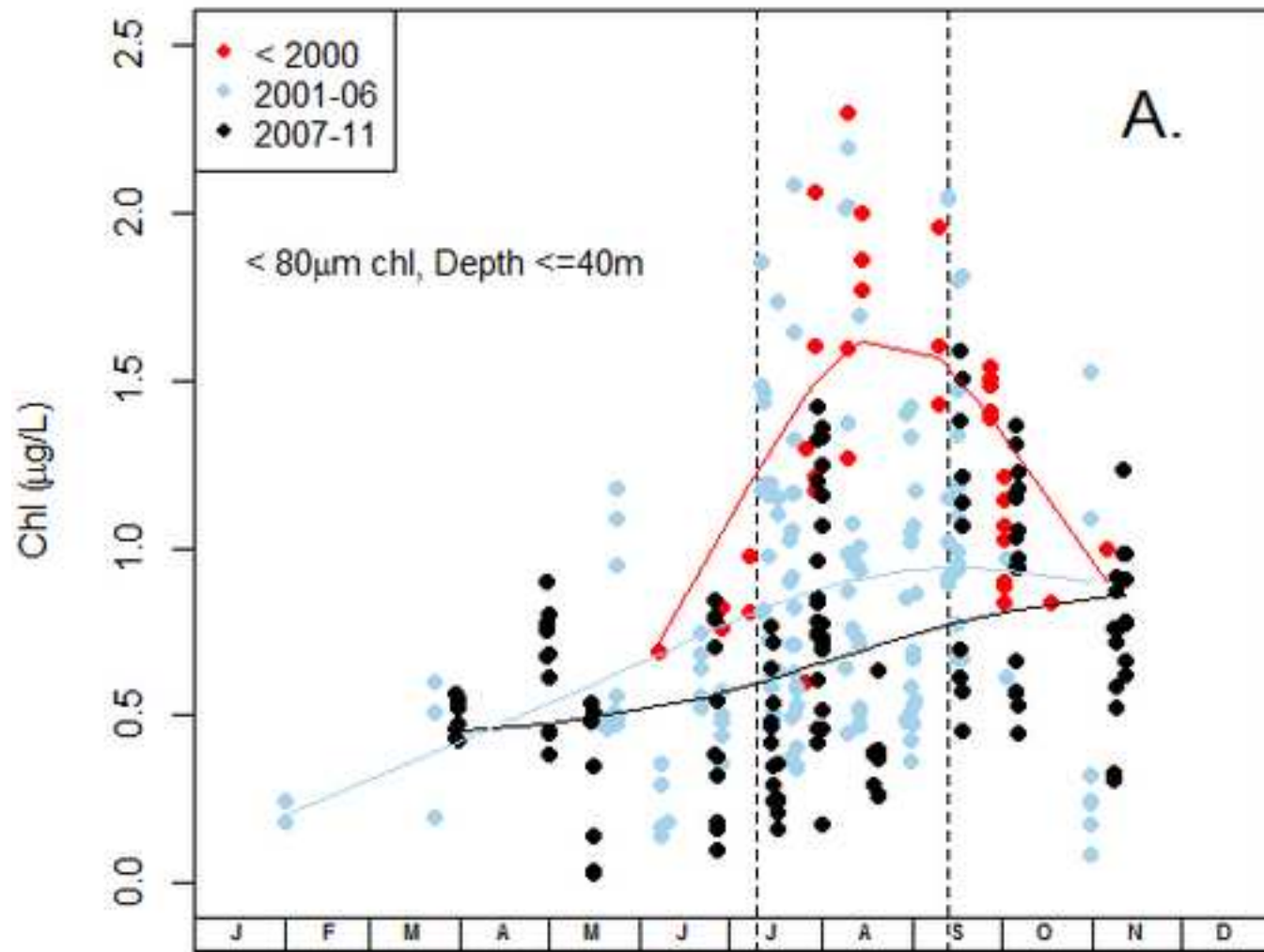


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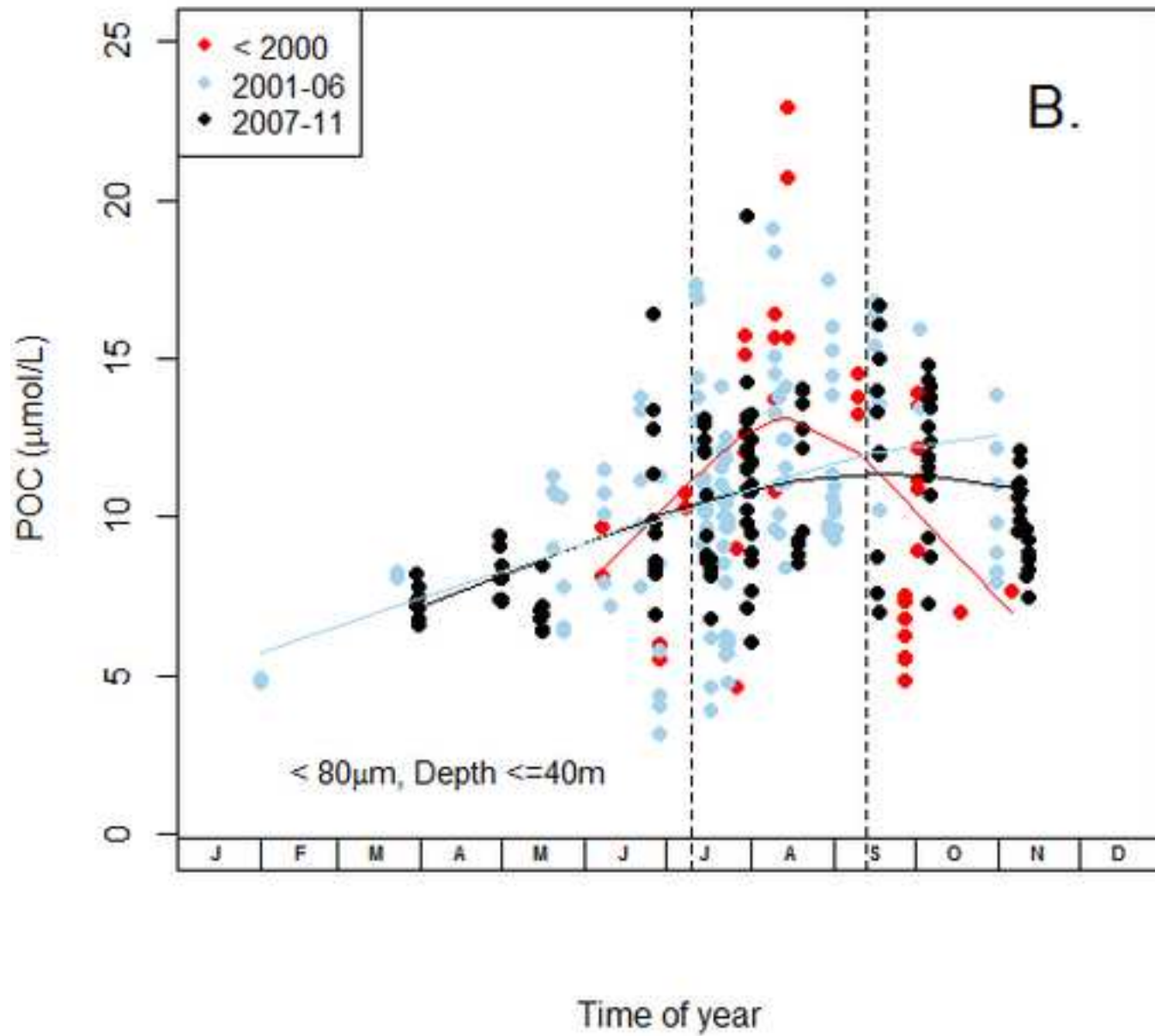


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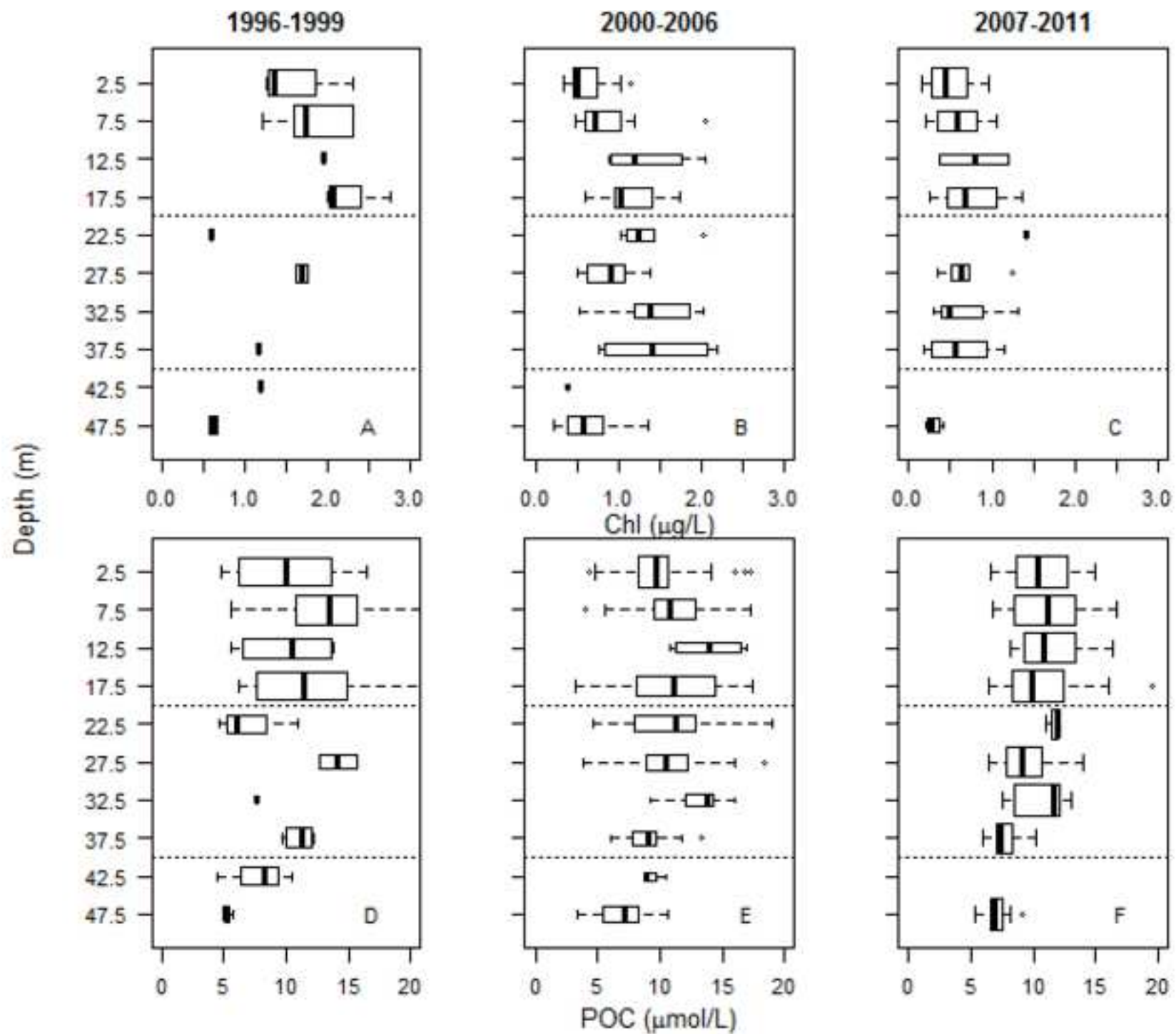


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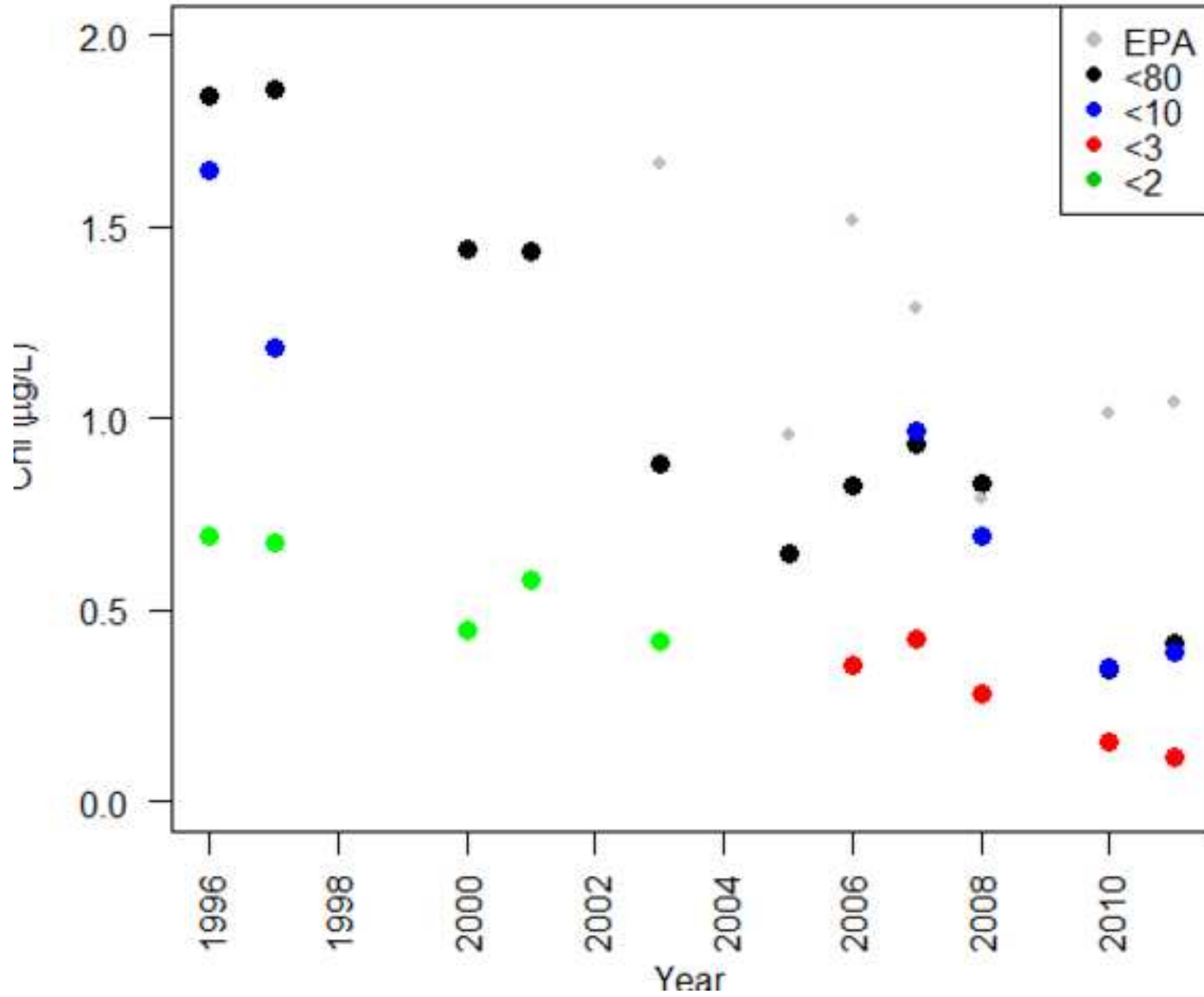


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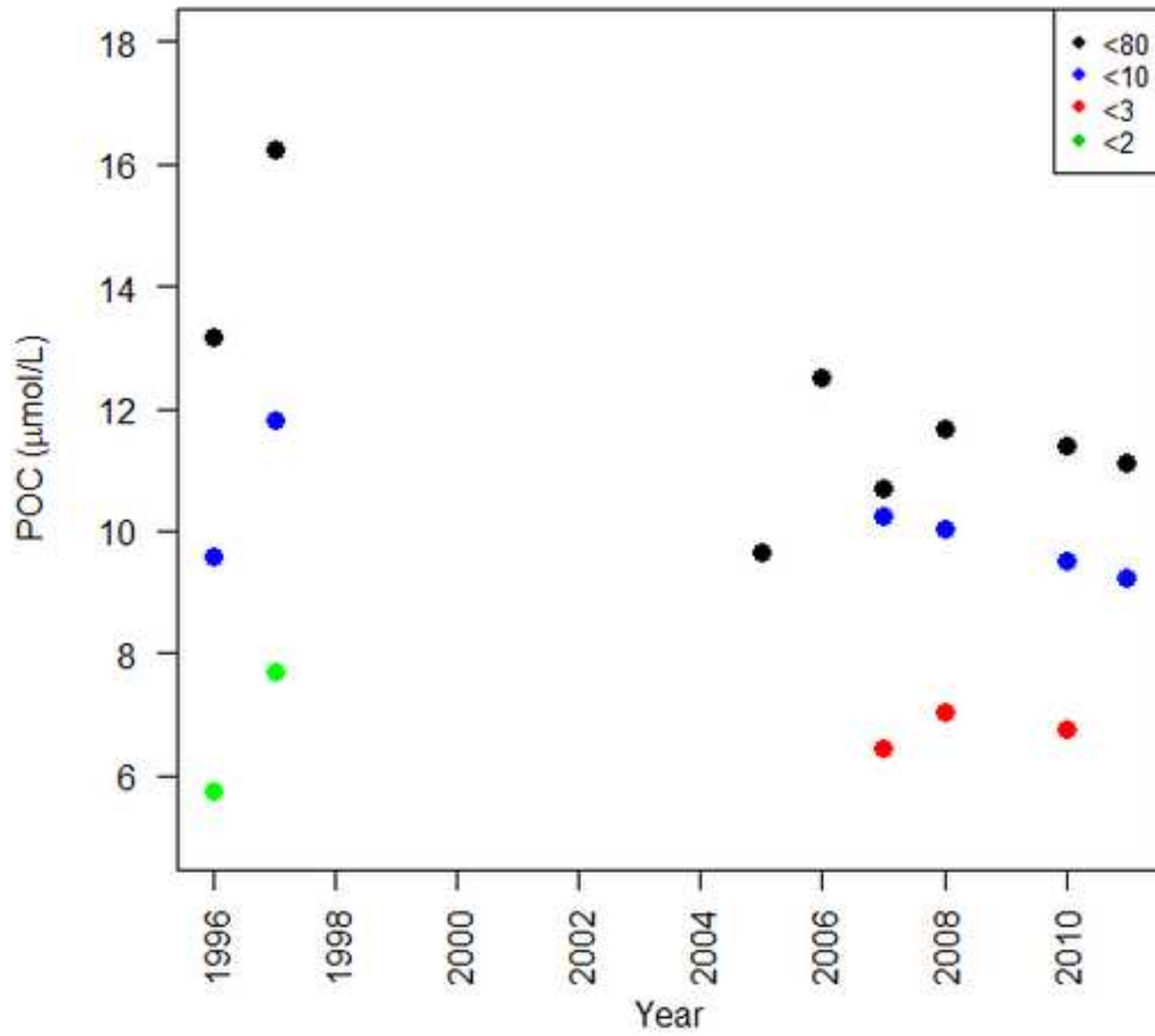
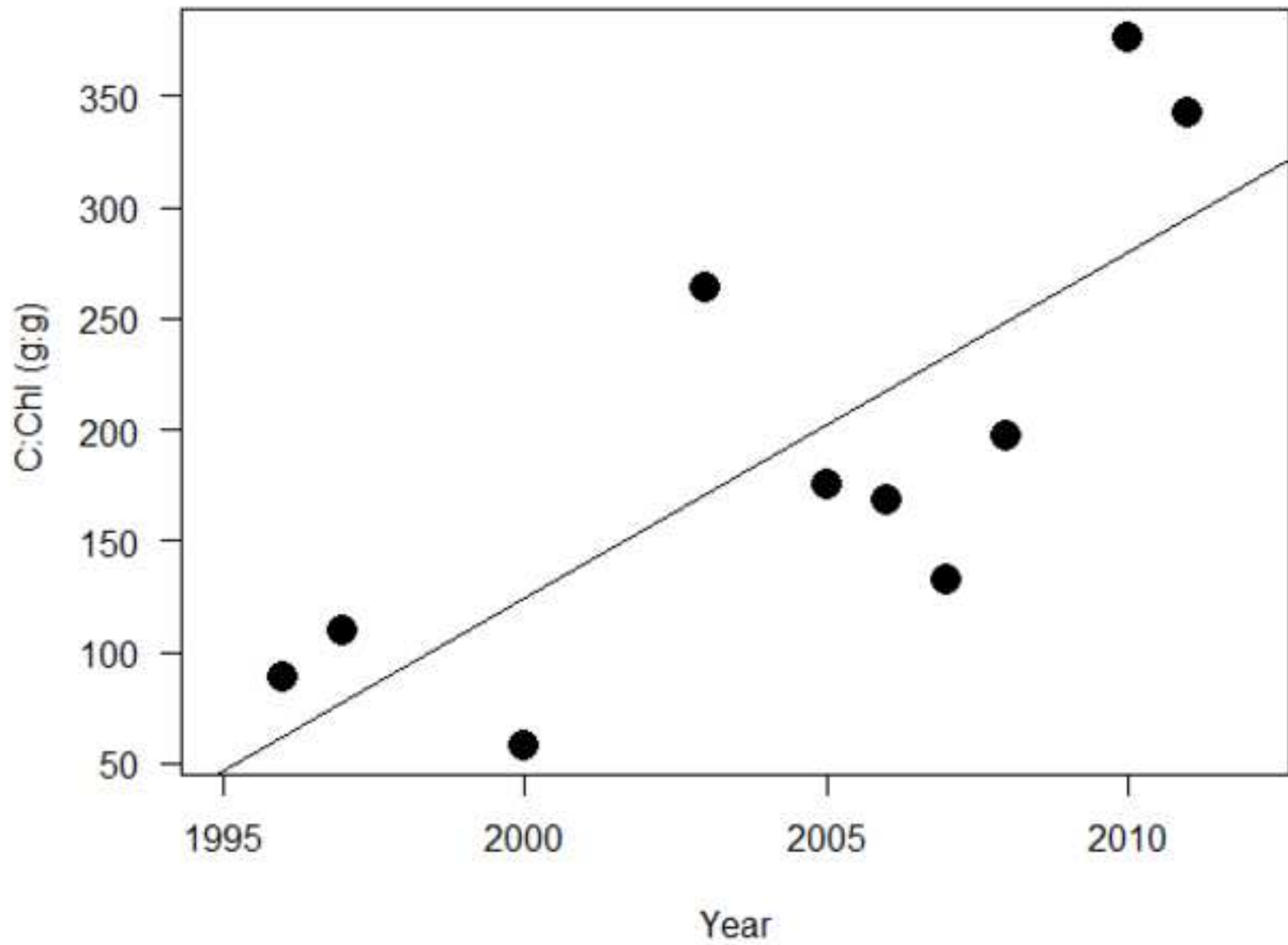


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