Lake Superior Zooplankton Community Grazing and Its Implications for the Deep Chlorophyll Maximum

A THESIS SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL
OF THE UNIVERSITY OF MINNESOTA

Bridget Noreen Seegers

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF MASTER OF SCIENCE

Adviser Dr. Robert Sterner

August 2009

Acknowledgments

I would like to first acknowledge Bob Sterner, my adviser, who supported me throughout this process and taught me what it means to be a scientist. My committee members, Jim Cotner and Katsumi Matsumoto, were important for discussions about my work. My labmate, Jim Hood, was wonderful support and helped me develop ideas and learn to create graphs using Statistica. My technition, Sandy Brovold, was a fabulous help. Also, I would like to acknowledge my brother, Brian Seegers, for his support and technical assistance. Greg Mitchell and Mattias Cape contributed with conversations. Carrie Otis spent long nights in the lab filtering and was always was willing to listen. My parents taught me to dream, laugh, and work hard. Their support has allowed me to succeed.

Abstract

Lake Superior, the world's largest freshwater lake, is cold, oligotrophic, and has a persistent deep chlorophyll maximum (DCM) during summer stratification. In oligotrophic systems, a DCM is often associated with a nutrient gradient, density gradient, or photoinhibition. However, no nutrient gradient has been observed in Lake Superior and the DCM is typically located below the strongest density gradient. Another possible explanation for a DCM would be grazing pressure. It has been observed that there is an increased abundance of zooplankton above the DCM. Enhanced grazing pressure by the zooplankton community in the relatively warm waters above the DCM could keep phytoplankton populations low. In the present study a series of dilution grazing experiments were conducted to determine grazing rates throughout the water column and to explore the potential grazing impacts on the DCM. The dilutions examined two size fractionations, whole water and <10 µm, because previous data indicated that small phytoplankton (<10µm) contribute a large amount to total primary production biomass. The crustacean macrozooplankton communities were surveyed above, within, and below the DCM. Additionally, algal bioassays were completed to look for evidence of nutrient enrichment by water from depths above, within, and below the DCM. Six cruises were completed over fourteen months in Lake Superior, and the mean grazing rate over all cruises was 0.19 d^{-1} and mean summer grazing rate above the hypolimnion was 0.32 d^{-1}. Surprisingly, the zooplankton communities were similar across depths, but showed differences between seasons. The two algal bioassay runs had contradictory results, which suggests a nutrient gradient may be present in August, but broken down by September. Substantial grazing rates suggest grazing dynamics are

critical to understanding the lake’s carbon and nutrient cycling. However, grazing pressure alone does not fully explain the DCM formation and maintenance.

Table of Contents

List of Tables

List of Figures

CHAPTER 1

Introduction

Lake Superior, headwaters of the Laurentian Great Lake system, holds nearly 10% of the Earth's surficial, liquid freshwater and is the world's largest lake by surface area (82,100 km^2; Herdendorf 1990). It has an annual mean temperature of 4ºC, an average depth of 127 m, and a maximum depth of 406 m (Hecky 2000). In spite of its global and regional importance, the Lake Superior ecosystem is still poorly understood in many respects.

The carbon cycle is central to understanding many of the biogeochemical dynamics within the system. The present understanding of organic carbon in Lake Superior yields a budget that is out of balance. Respiration and outputs greatly outweigh production and other estimated inputs (Cotner et al. 2004, Urban et al. 2005). The largest organic pool of carbon in Lake Superior is in the dissolved phase with a dissolved to particulate organic matter ratio greater than 10:1 (Biddanda et al. 2001). About half of the Lake Superior particulate organic carbon (POC) is in the < 1 μm size fraction and this fraction is primarily heterotrophic bacteria (Cotner et al. 2004). Primary production is thought to be the primary source of organic carbon to the system (Cotner et al. 2004, Urban et al. 2005, Sterner submitted).

Herbivory of algal populations in cold, oligotrophic systems like Lake Superior can remove large amounts of primary production (e.g. Calbet and Landry 2004, Strom et al. 2007). Grazing is a critical first step in transferring carbon and nutrients to the rest of the food chain. In spite of the potentially critical importance of grazing in the organic carbon cycle of Lake Superior, grazing rates in Lake Superior have seldom been explored. Schampel in his thesis work (2001) measured relatively high grazing rates (0.013 - 0.483 d^{-1}). Additionally, a single cruise in September, 2002 used the Landry-Hassett method (described below) and estimated substantial grazing rates (0.4 - 0.5 d^{-1}) suggesting that grazing could be an important unknown in the Lake Superior carbon cycle (Smutka 2005). The small number of grazing rate estimates does not allow for clear understanding of grazing relative to other ecosystem dynamics. However, these previous studies suggest that grazing is an important process occurring in the lake, and better understanding of grazing rates would lead to increased understanding of whole lake dynamics, such as the carbon cycle and nutrient cycles.

The grazer community in Lake Superior is composed of animals ranging in size from protozoan to crustacean zooplankton. The summer heterotrophic microorganism biomass in Lake Superior ranges from 12 -20 µg C L^{-1} (Fahnenstiel et al. 1998). . Bacteria account for nearly half of the heterotrophic microorganisms in the lake (Fahnenstiel et al. 1998, Cotner et al 2004). The grazers account for the remaining 50% of the heterotrophic microorganism biomass with protozoans, ciliates, and flagellates accounting for the vast majority, and rotifers contributing about 1% (Fahnenstiel et al. 1998). The summer crustacean zooplankton biomass averaged 28 µg C L^{-1} (Fahnenstiel et al. 1998). The crustacean community is dominated by copepods with the most common adult species in summer being *Leptodiaptomus.sicilis, Limnocalanus macrurus,* and *Diaptomus thomasi* (e.g., Barbiero and Tuchman 2001c, Brown and Branstrator 2004). Cladocerans are 10 to 20% of the crustacean zooplankton community reaching their highest abundance in late summer (Sprules and Jin 1990, Barbiero and Tuchman 2001c). A substantial increase in lake herring has lead to a change in the zooplankton community with reduction in the largest zooplankton size-class over recent decades (Link et al. 2004).

The phytoplankton community in Lake Superior is dominated by small species. Nearly three-quarters of the chlorophyll passes through a <5 µm filter, and picoplankton (<2 μm) account for half of Lake Superior autotrophs and the amount in this small fraction increases with depth (Fahnenstiel et al. 1998, Sterner submitted). Compared to the other Laurentian Great Lakes, Lake Superior has a relatively diverse phytoplankton community (Barbiero and Tuchman 2001). Diatoms represent about a third of the species (Barbiero and Tuchman 2001) with centric diatoms more abundant in surface waters and pennates numbers increasing below the thermocline (Fahnenstiel and Glime 1983, Barbiero and Tuchman 2004). Small flagellates account for nearly half of the phytoplankton species (Barbiero and Tuchman 2001) and cryptophyte genera Cryptomonas and Rhodomonas are especially abundant (Barbiero and Tuchman 2001, Sterner et al. submitted).

In addition to cold temperatures and low light during much of the year, Lake Superior has low nutrient levels that impact the phytoplankton community structure and primary production. Lake Superior algal communities are mostly P limited, however, when P enrichment is provided, primary production quickly become Fe limited (Sterner et al. 2004). P levels are very low in the lake and there is no evidence for a nutrient gradient with enriched waters at depth (Baehr and McManus 2003, Field and Sherrell 2003, Barbiero and Tuchman 2004, Anagnostou and Sherrel 2008). The nitrate: phosphate ratio is nearly 10,000 ensuring nitrate is not a limiting nutrient.

A prominent feature of the Lake Superior phytoplankton community is a persistent deep chlorophyll maximum (DCM) during summer stratification. Typically in the upper hypolimnion at a depth between 23 to 35 meters, the Lake Superior DCM chlorophyll concentrations are 1.7 to 2.7 times higher than chlorophyll concentrations above the DCM (Barbiero and Tuchman 2004). The DCM was first noted in late July and mid-August at 30m by Olson and Odlaug (1966) and has subsequently been documented throughout the decades

(Watson et al. 1975, Moll and Stroermer 1982, Fahnenstiel and Glime 1983, Barbiero and Tuchman 2004, Auer and Bub 2004, Russ et al. 2004). Using transmissometer profiles and grab samples, Watson et al. (1975) observed that the DCM established below the thermocline and covered large areas of the lake. Although the DCM is one of the most striking features of Lake Superior phytoplankton community, questions remain about the formation of the DCM and the biological significance of the DCM in terms of production and as a potential food source.

Development of a prominent DCM is not unique to Lake Superior, but is a widespread occurrence in clear and stratified bodies of fresh (e.g. Fee 1976, Barbiero and Tuchman 2001, Saros et al. 2005) and marine water (e.g., Cullen 1982, Marañón et al. 2000, Hense and Beckman 2008). Many theories have been advanced to explain the DCM in Lake Superior, including *in situ* productivity (Munawar and Munawar 1978, Moll and Stoermer 1982, Fahnenstiel and Glime 1983), reduced phytoplankton sinking (Watson et al. 1975), or zooplankton grazing (Olson and Odlaug 1966). Models produced to predict phytoplankton distribution and DCM formation in other oligotrophic systems typically combine biological, chemical, and physical variables (e.g., Klausmeier and Litchman 2001, Fennell and Boss 2003, Hodges and Rudnick 2004, Beckmann and Hense 2007). The contradictory conclusions and limited knowledge about important variables in Lake Superior, such as grazing rates, have allowed for continued debate about the exact nature of the DCM formation and maintenance.

In clear oligotrophic systems like Lake Superior, nutrient and light gradients often lead to DCM formation (Fee 1976). In these systems the primary production peaks in high quality environments where upwelling nutrient rich water encounters light from above, leading to enhanced growth and DCM formation (e.g., Sommer 1982, Olli and Seppala 2001, Yoshiyama et al. 2009). Moll and Stoermer (1982) conducted a study in Lake Michigan in July 1977 and used ^{14}C uptake to estimate primary production from eight depths from 5m – 40m. They determined that nearly 70% of net carbon fixation occurs in the DCM and predicted similar results in Lake Superior. Fahnenstiel and Glime (1983) concluded that the Lake Superior DCM development was the result of increased levels of subsurface *in situ* production, because of ^{14}C measurements of *in situ* production from early September. However, more recently, Sterner (submitted) measured *in situ* primary production using JGOFS ^{14}C protocols and found volumetric primary production peaks above, not within, the DCM in Lake Superior. To support the nutrient gradient theory the limiting nutrient concentrations should be low and constant above the DCM and increase with depth below the DCM. Lake Superior has not been found to have measurable gradients of the limiting nutrients, phosphorus or iron, to support the nutrient-light gradient theory, and the hypolimnion is not thought to be a significant source of dissolved nutrients (Baehr and McManus 2003, Field and Sherrell 2003, Barbiero and Tuchman 2004, Kumar et al 2007, Anagnostou and Sherrel 2008). Barbiero and Tuchman did find significantly lower particulate C:P in the DCM compared to above the DCM and suggested this could be indication of phosphorous enrichment at depth. However, Sterner (submitted) and Nalewajko et al. (1981) give evidence that lower C:P could be caused not by enriched P, but instead by low production, which reduces C. Low C:P typically is associated with low light (Sterner et al. 1990; Dickman et al. 2006), so this is not solid evidence for a P gradient in Lake Superior. The lack of a measurable nutrient gradient and the most recent detail profiles of in-situ primary production with production peaks above the DCM suggest that the Lake Superior DCM is not simply the result of increased production.

It has been suggested that a DCM may also be caused by reduced phytoplankton sinking rates caused by density gradients or lower sinking rates of nutrient deprived phytoplankton (Venrick et al. 1973, Watson et al. 1975). However, the Lake Superior DCM is typically in the upper hypolimnion, below the depth of greatest change in density, which suggests that DCM formation is not a result of reduced sinking (e.g., Auer and Bub 2004, Barbiero and Tuchman 2004, Russ et al 2004).

Another alternative is that DCM formation may result from photoinhibition at the surface or from increased chlorophyll content of algae at depth as an adaptation to the low light environment. In some systems the chlorophyll maximum and particulate organic carbon maximum are vertically separated (Fennel and Boss 2003). Previous studies of the Lake Superior DCM found it to be a chlorophyll maximum and to a lesser extent an actual deep biomass maximum or deep carbon maximum (DCarM), thus shade adaptation has not been thought to fully explain of the DCM (Barbiero and Tuchman 2004).

Another alternative is that the DCM results from higher levels of grazing loss in the warmer surface layer compared to at depth. Freshwater zooplankton grazers often exhibit top-down control on phytoplankton dynamics (Carpenter et al. 1987, Elser et al. 1988), and consumers have been found to play a role in maintaining DCMs (Pilati and Wurtsbaugh, 2003, Sawatzky et al. 2006). Pilati and Wurtsbaugh (2003) completed limnocorral experiments in Yellow Belly Lake, an oligotrophic mountain lake, with and without macrozooplankton and found that grazing combined with nutrient movement explained the persistence of the DCM. Sawatzky et al. (2006) in two oligotrophic mountain lakes, including Yellow Belly Lake, monitored the response of phytoplankton communities with *in situ* microcosms with and without zooplankton and found grazers are critical to the maintenance of the DCM. In Lake Superior increased crustacean zooplankton abundance above the DCM compared to within DCM have lead to speculation that grazing could contribute to DCM formation (Olson and Odlaug 1966, Anderson et al. 1972, Fahnenstiel and Glime 1983). However, zooplankton community grazing rates has received little attention in Lake Superior.

Grazing has been found to be an important influence on primary producer biomass across terrestrial and aquatic ecosystems with aquatic systems having significantly higher rates of herbivory (e.g., Cyr and Pace 1993, Shurin et al. 2006, Gruner et al. 2008, Sterner 2009). Cyr and Pace (1993) summarized the results from 44 aquatic studies and reported that 51% of primary production is removed by herbivores in aquatic ecosystems, which is about three times the amount in terrestrial systems. Gruner et al. (2008) also found that herbivory is especially important in aquatic systems and that consumption rates are positively correlated with the primary producer biomass. Herbivory is a significant loss of primary production in freshwater (e.g., Sterner 1989, Sawatzky et al. 2006) and marine systems (eg., Calbet and Landry 2004, Strom et al. 2007, Chen et al. 2009). Calbet and Landry (2004) completed an extensive review of nearly 800 oceanic autotropic growth and microzooplankton grazing experiments and found that overall 67% of growth is lost to grazing.

Grazing, in addition to being a substantial loss of phytoplankton biomass, can contribute nutrients directly available to support primary production (Peters 1973, Lehman 1980, Sawatzky et al. 2006). Oligotrophic environments like Lake Superior often have tight nutrient recycling loops, and grazing allows for rapid remineralization of minerals to be available to phytoplankton (Anderson et al. 1991). The potential importance of grazing in the Lake Superior carbon cycle by means of top-down control and nutrient recycling has not been closely examined

In the present study, I examined the zooplankton community grazing in Lake Superior, focusing on DCM formation, using the Landry-Hassett (L-H) (1982) dilution grazing technique. The crustacean zooplankton communities were sampled and enumerated above, within, and below the DCM to evaluate their role. Although evidence suggests microzooplankton are important grazers in the system, estimating microzooplankton abundance was out of the scope of the present study. Additionally, the potential role of a Lake Superior nutrient gradient in DCM formation was explored with a small number growth experiments completed with water from different depths. Regression models were developed to predict chlorophyll and POC concentrations during the summer stratified period combining biological and physical factors. My primary hypothesis was that zooplankton community grazing in Lake Superior would be substantial enough to exhibit top-down control on the phytoplankton communities, and thereby make grazing a mechanism contributing to DCM formation and maintenance.

Materials and Methods

Site and water collection

The CARGO project (CARbon Gain and LOss) focused on two goals: estimating lake-wide rates of primary production using in-situ ^{14}C uptake (Sterner, submitted) and estimating rates of grazing as a function of depth (this study). Six research cruises, CARGO1-6, were completed on western Lake Superior at deep off-shore stations between

July 2007 and September 2008 (Figure 1). Measurements and experiments were carried out over a range of stratified and unstratified conditions with three cruises completed during summer stratification (CARGO1,5,6), two cruises completed in fall (CARGO2, 3), and one cruise completed during early spring, inverse stratification (CARGO4) (Table 1, Figure 2). The DCM was clearly established during the summer stratified cruises (CARGO 1, 5, 6, Figure 2, 3). For most cruises, two different measurements of grazing were made for the sampling site CD-1 (47º3’54’’, 95º 25’ 55.2’’) (Figure 1, Table 1). One used an on-deck incubator, and the other was incubated in the laboratory. During CARGO4 weather conditions prevented sampling at CD-1 and WM water (47º19’58.8’’, 89º48’0”) was used for the St. Paul laboratory incubations. Based on past experience, these two sites are not normally greatly different from each other with respect to temperature and surface chlorophyll levels among other variables, and they represent the overall, offshore Lake Superior conditions. During CARGO2 no St. Paul incubations were completed, because severe weather prevented water sampling.

Water column profiles were collected using rosette casts with a Sea Bird 911plus CTD, which measured temperature and other physical and biological parameters. Water samples were collected in Si-O-ring equipped Niskin bottles. Particulate nutrient and chlorophyll samples were pre-filtered through 80-µm Nitex. Additional duplicate unfiltered chlorophyll sample were taken. Particulate organic carbon (POC), particulate organic nitrogen (PON), and particulate phosphorus (PP) were filtered onto pre-ashed 0.7-µm GF/F. Samples were initially frozen and for analysis were dried at 60ºC for 24 to 48 hours. POC and PON were analyzed using an acetanilide-standard calibrated Perkin Elmer 2400 CHN analyzer. After digestion with potassium persulfate, PP samples were analyzed using the ascorbic acid method.

Theory of Dilution-Based Measurement of Grazing –

The protocol for measuring community grazing rates was based on the principle originally described by Landry and Hassett (L-H) (1982). The L-H dilution-grazing technique has often been used to estimate community grazing rates in marine (e.g., Calbet and Landry 2004, Irigoien et al 2007, Strom et al. 2007) and freshwater (e.g., Fahnenstiel et al. 1991, Elser and Frees 1995, Pilati and Wurtsbaugh 2003) environments. The dilution method has the advantage of including the entire zooplankton community, including microzooplankton, as opposed to other methods that directly manipulate grazer density.

Landry and Hassett (1982) explain two key assumptions that support the original dilution-grazing technique. First, the L-H method assumes that phytoplankton growth is not density dependent, so that growth occurs at a constant rate under the nutrient-amended nutrient conditions of the experiment, and therefore the rate that phytoplankton gain biomass and grow is not influenced by the dilution. Second, grazing rate is assumed to be directly related to the rate of herbivore-prey encounters, which is thought to be an appropriate assumption below the incipient limiting concentration (ILC). The ILC is the food concentration where the grazers become satiated and maintain their maximum rate of food consumption even when food concentrations continue to increase. The basis of the method is that dilution decreases encounters such that the grazing rate can be estimated as the slope of the relationship between observed net growth rate (gains minus losses) and dilution (D), where D is the fraction of whole water as opposed to filtered water with both herbivores and prey removed. When these two assumptions hold, the rate of change at a given condition of dilution should follow:

$$Chl_t = Chl_0 \exp[(\mu_{max} - g)t] \tag{1a}$$

Where Chl is the chlorophyll concentration, a proxy for phytoplankton biomass or population number, Chl_0 is the initial chlorophyll concentration (time zero), μ_{max} is the phytoplankton maximum growth rate (d^{-1}), g is the zooplankton community grazing rate (d^{-1}), and t is time in days. Net phytoplankton growth ($_{net}$) is derived from equation (1a):

$$\mu_{net(D)}\,(d^{-1}) = \mu_{max} - g_{(D)} = \ln(Chl_t/Chl_0)/t. \tag{1b}$$

All terms are previously defined.

Since the introduction of the L-H technique in 1982 it has often been applied to measure community grazing rates, especially in oligotrophic marine systems (Dolan et al. 2000) and increasingly in freshwater systems (e.g., Fahnenstiel et al. 1991, Pilati and Wurtsbaugh, 2003, Sawatzky 2006). The L-H is popular because it allows for little disturbance of the grazing community and is a relatively simple way to estimate both grazing and nutrient-saturated growth rates. However, the technique can sometimes produce uninterruptible, statistically problematic results (Dolan and McKeon 2005). The assumptions discussed above, and therefore, the results of the experiments have been central to debates in the literature (e.g., Dolan et al. 2001, Dolan and Mckeon 2005, Landry and Calbet 2005).

The assumptions of linear feeding response does not always hold true, but extensions of the theory have made it possible for grazing rates still to be estimated (e.g., Gallegos 1989, Evans and Paranjape 1992, Elser and Frees 1995, Sterner et al. 1995, Teixeira and Faguerias 2009). The explanations behind non-linear feeding include grazer satiation (Elser and Frees 1995, Sterner et al. 1995, Teixeira and Faguerias 2009), the ability of grazer communities to adjust clearance rates in response to different food concentrations (Gallegos 1989), and changes in the grazer community structure during incubations (e.g., Gallegos 1989, Landry et al. 1993, Dolan et al. 2000, First et al. 2009, Modigh and Franze 2009). First et al. (2007) examined the impacts of dilution-dependent changes in microzooplankton biomass and found that adjusting for the calculated grazer gradient did not significantly impact estimates of grazing rate. Modigh and Franze (2009) determined that the focus on chlorophyll as a proxy for prey biomass does not allow for an estimate of grazing on heterotrophic prey, which would lead to an underestimation of grazing especially in oligotrophic systems. The prey and predator communities are not static within the bottle and thus the results can be time dependent suggesting an importance of shorter incubation times (Landry et al. 1993). The non-linear dynamics could lead to concerns about the accuracy of grazing rate calculations. However, the grazing rate estimates that result from non-linear grazing patterns, regardless of the cause, have been determined to be accurate, especially when high level dilutions are completed (Gallegos 1989, Teixeira and Figueiras 2009). Despite the limitations, the L-H method continues to be the most widely accepted and used approach to estimate community grazing rates.

The Landry-Hassett (1982) dilution technique assumes a linear relationship of μ_{net} versus D. However, similar to studies discussed above, many of the results of the present study (Figure 3) exhibited a non-linear relationship of growth vs. D (e.g., Gallegos 1989, Elser and Frees 1995, Dolan et al 2000, Teixeira and Faguerias 2009). Therefore, we used either a linear or non-linear model to calculate grazing, depending on the results of the given experiment. The Statistica (7.0) least squares, Levenberg-Marquardt, non-linear estimation was used to complete a piecewise with breakpoint regression analysis to determine the best fit (Elser and Frees 1995, Sterner et al. 1995). The non-linear regression analysis estimated three variables (μ_{max}, the slope of the linear portion of the functional response, s, and the ILC) by finding the best fit two-line segment model that minimizes the sum of squares due to error (SSE). Community grazing for nonlinear cases was calculated by the difference between the growth at D = 1 (on the "flat" part of the curve) and growth at D =0 (μ_{max}) (see Figure 4). Specifically, grazing can be determined by

$$\mu_{net(D)} = \mu_{max} - g_{(D)} \tag{2}$$

for the linear case, and

$$\mu_{net(D)} = \mu_{max} - s*X, \quad \text{when } (D<ILC)$$

and

$$\mu_{net(D)} = \mu_{max} - s*ILC, \quad \text{when } (D\geq ILC) \tag{3}$$

for the nonlinear case.

Grazing measurements

For the first cruises, water samples were collected at 3 depths; two above and one within the DCM as determined in the field by CTD chlorophyll profiles (Figure 1). The measurements were expanded for the final two summer-stratified cruises to 6 depths; three above, one within, and two below the DCM. CARGO 4 was an early spring cruise with no DCM and inverse stratification. CARGO 4 water was collected at 5m, 20m, 60m. All water samples for grazing measurements were collected between 03:00 and 12:00.

Samples were transferred from Niskin bottles to trace metal cleaned (TMC) 20L carboys with TMC silicone tubing. A TMC protocol has been shown to be important for phytoplankton growth experiments on the Laurentian Great Lakes (Nriagu et al. 1996). For the trace metal protocol, all containers were soaked with 5% HCL overnight at 60ºC and subsequently thoroughly rinsed with Nanopure (Barnstead) and lake water before use. The appropriate volume of unfiltered lake water was measured for each dilution level, a nutrient spike was added (final concentration 50 nM $FeCl_3$, 7 nM $ZnSO_4$, 1 µM PO_4, and 60µM $C_6H_{12}O_6$), and the bottles were filled with 0.45 µm filtered lake water collected from >150m. Water was filtered through a TMC Geotech Environmental Equipment Inc. high capacity capsule 0.45 µm filter (model 73050004) by using a peristaltic pump. For CARGO1-3, incubations were completed in 1-liter TMC polycarbonate bottles at eleven dilution levels. The fractions of whole lake water (D) were 0.05, 0.10, 0.15, 0.20, 0.25, 0.30, 0.40, 0.50, 0.65, 0.80, and 1.00. Dilutions were reduced to 8 or 6 levels for CARGO4-6 depending on depth, and 2-liter TMC polycarbonate bottles were used to increase volume for the size-fractionation of final samples (whole water and 10 µm). Initial chlorophyll samples were taken from lake water samples for each depth. Initial chlorophyll concentration in each dilution bottle was then calculated from the fraction of whole water. The 0.45 µm filtered water used for dilutions had a mean initial chlorophyll concentration of 0.005μg L^{-1} for all cruises (see results for details). Bottles were incubated for 36 hours in an on-deck Plexiglas incubator which was maintained at lake surface water temperature. Mesh screening covered the incubator to reduce light to 25% of surface light levels. CARGO5-6, additionally, used a refrigerator set at 4ºC for the incubations of samples taken below the thermocline and light regimes that mimic the natural environment (Table 2). After the incubation, whole water samples from each bottle were filtered onto 0.2 μm cellulose nitrate filters. Filters were frozen for subsequent chlorophyll analysis. During the final three cruises, CARGO4-6, each 2-liter bottle had a final chlorophyll measurement from the whole water and the <10 μm fractions, because of an interest in the grazing impacts on different size fractions.

After each cruise ended, an second set of L-H dilutions was completed at the laboratory to gather a second set of grazing results. Therefore, on the final morning of the cruise sampling protocols were repeated. This water was stored in 20L carboys in coolers and transported to the laboratory in St. Paul. A full set of incubations were set-up in the laboratory within 18 hours of sampling. The samples were incubated at a temperature and light regime to mimic natural conditions (Table 2). Incubations ran for 48 hours. After the incubation, samples from each bottle were filtered onto 0.2 μm cellulose nitrate filters for chlorophyll analysis. Chlorophyll was extracted by soaking in 90% buffered acetone in the dark for 24 hours at 4ºC and measured on a Turner Designs model 10-AU fluorometer (Welschmeyer 1994).

Data Analysis of Grazing Measurements-

Net growth rates (μ_{net}) were calculated using initial chlorophyll concentration (Chl_0) and final chlorophyll concentration (Chl_t) in each bottle. Initially, it was assumed that chlorophyll in the 0.45 μm filtered water was zero. However, as indicated above, a mean chlorophyll concentration of 0.005μg L^{-1} was measured for the 0.45μm filtered water used for dilutions during CARGO6. During data analysis it was discovered that incorporation of this very small concentration altered significantly the growth rates calculated for the most dilute depths and most dilute treatment levels. The additional 0.005 μg L^{-1} chlorophyll in the filtered water changed the calculated grazing rates for both the whole and 10 μm fractions (e.g., Figure 4 presents some of the most extreme differences) and influenced the overall grazing calculations (Figure 5). Therefore, initial chlorophyll concentrations (Chl_0) at each dilution were determined from

$$Chl_0=D(Chl_1)+(1-D)Chl_{0.45filt.} \tag{4}$$

Where (D) is the dilution level in fraction of whole water; Chl_1 is the undiluted chlorophyll concentration (1=fraction of unfiltered lake water); and $Chl_{0.45filt}$ is the mean chlorophyll concentration in the 0.45μm filtered water (0.005 μg L^{-1}). The adjustment of an initial chlorophyll concentration of 0.005 μg chl L^{-1} in the dilution water reduced the mean grazing rate on the whole water fraction by 39% and grazing on the 10 μm fraction was reduced by 6%. The water for 0.45μm filtration was always collected from a depth >150 m where there was a relatively narrow range of total chlorophyll (0.062 – 0.118 μg L^{-1}, Table 3) with the exception of CARGO1, which had relatively high chlorophyll concentration (0.432 μg L^{-1}). Similar chlorophyll concentrations at depth increase the confidence of applying one correction to all measurements.

The community grazing rates calculated from the L-H dilution grazing experiments had a wide range of standard errors (SE). To account for widely varying levels of confidence for each point estimate of grazing rate, all analyses that combined grazing rates (means, ANOVA's, etc.) were completed using a weight of (1/SE) giving the results with the smallest SE the greatest weight. Additionally, any negative grazing results were interpreted as a grazing rate of zero (Landry and Calbet 2004). The grazing rate from CARGO5 on-deck incubations from 5 m was not used in the analysis, because the assumptions of the experimental design were not met and the results were well beyond reasonable limits of grazing rates.

Macrozooplankton

Zooplankton samples were taken from 3 depth intervals at CD-1 and WM for each cruise, except CARGO2 when stormy weather prevented tows. Depths were selected above the DCM, within the DCM, and below the DCM and the profiles were 10-15m long with four tows taken from each depth. Zooplankton tows occurred between 13:00 and 16:00. A closing 50 cm diameter net with 80 µm mesh was towed. The contents of the cod-end were preserved with formalin-free Lugols. Macrozooplankton were quantified and identified via microscope observation. At least 5 ml was sub-sampled and a minimum of 500 organisms per sample were counted and identified.

Phytoplankton Growth Potential with Water from Different Depths-

Nutrient gradients to support DCM formation have not been detected in Lake Superior. However, nutrients are often near analytical detection limits so it is difficult to state with assurance that water from depth is not enriched with some critical nutrient. An experimental approach was therefore attempted. During the summer-stratified cruises

CARGO5 and 6 growth experiments were run to look for indications of nutrient enrichment with water from different depths. Water from depths above, within and below the DCM (2m, 10m, 20m, 35m, 50m, 75m; CARGO6 included two additional depths 150m and 246m), were filtered through a TMC 0.45µm capsule filter by a peristaltic pump. Duplicate polycarbonate TMC 1-liter bottles for each depth were filled with 1000 ml of 0.45µm filtered water from the given depth and were topped off with of unfiltered water from 2m. The initial chlorophyll concentration of the 0.45µm filtered water from each depth was measured during CARGO6 and showed no significant difference. Therefore, the initial chlorophyll concentration was considered identical among the bottles. The bottles were incubated on-deck as described above for 36 hours. After incubation final chlorophyll values were measured as described above. Because the initial phytoplankton communities were considered identical between bottles any observed differences were attributed to nutrient enrichment, which resulted from a nutrient gradient with in the lake.

Results

Characteristics of the DCM -

In late July and early August chlorophyll concentrations measured by in-situ fluorescence and in lab with extracted pigments had maxima located between 32 and 36m. Chlorophyll maxima were in the upper hypolimnion below the greatest change in density (Figure 2). The September DCM was shallower at 20 m and was located at the thermocline (Figure 2). The POC maximum (DCarM) was in the lower thermocline closely coupled to the chlorophyll maximum. In September the POC maximum was located in the thermocline above the deep chlorophyll maximum.

Grazing-

Lake Superior is a dilute, cold, low productivity system. Nevertheless, significant grazing was observed throughout the year and throughout the water column (weighted mean: 0.19 ± 0.17 d^{-1}, Figure 6). Community grazing averaged across all depths was higher in the summer stratified season (0.23 d^{-1}) than in the non-stratified season (0.14 d^{-1}, t-test, $p < 0.001$, n= 73, Figure 7). The mean community grazing rate in the summer above the hypolimnion was 0.32 d^{-1}.

Lake Superior's annual mean temperature of 4ºC could be an important control of grazer activity both by inhibiting limiting grazer growth and by reducing per capita feeding rates. Community grazing was positively correlated with temperature throughout all seasons (Figure 8). Grazing on the whole water fraction was lower in the hypolimnion than the epilimnion and the thermocline ($p < 0.0001$, df = 40; $p = 0.02$, df = 27, respectively, Figure 9). Epilimnetic grazing rates on the <10 µm fraction were significantly higher compared to the thermocline and hypolimnion grazing rates (both $p < 0.0001$, Figure 9). Grazing rates for the whole and <10 µm fraction during the colder non-stratified cruises were not significantly different than hypolimnion grazing during summer stratification (Scheffe's, $p < 0.05$, n=73, Figure 8).

The influence of prey availability on grazing rates was tested, because community grazing rates could be expected to increase with prey availability if grazing communities expand when food abundance is high. The community grazing rate on <10 µm algal size fraction showed no significant relationship with initial <10 µm chlorophyll concentrations ($p = 0.117$, Figure 10b). During the non-stratified season, a slightly stronger positive correlation was seen between grazing rates on the whole water fraction and initial chlorophyll than during the stratified season ($r^2 = 0.103$, $p = 0.005$, Figure 10c). Analysis of the non-stratified season only considers the grazing on the whole water fraction, because in the <10 μm fraction grazing was only observed in 3 depths on a single cruise. The initial POC <80 μm concentration and grazing rates had no correlation in the yearly trend or during summer stratification (Figure 10). However, during the non-stratified season grazing rates showed a positive correlation with the initial <80 μm POC concentration ($r^2 = 0.247$, $p = 0.014$, Figure 11c). There were some weak correlations between food abundance and grazing rates, but these varied by season and did not explain much of the variation in grazing rates (Figure 10, 11).

The relationship between grazing rates and primary production could provide a better indication of the impacts of grazing on phytoplankton community structure. There was a positive relationship between grazing and production in the summer stratified period (Figure 12b), while no relationship was apparent in the non-stratified season (Figure 12c). The positive correlation between grazing rates and production suggests that grazing could be playing a role keeping phytoplankton abundance relatively low, even when primary production is relatively high.

A total of 66% of the L-H dilution experiments resulted in non-linear relationships with dilution. When this is the case, the dilution gradient method allows for calculation of not only community grazing rates but also of the incipient limitation concentration (ILC), the food abundance at which grazers find satiation. Therefore, at food quantities at or above the ILC grazers are consuming at their maximum rate and grazing rates do not continue to increase with food supply indicated by the flat part of the growth curve (Figure 4). The high occurrence of satiation in the grazing experiment indicates that grazers in Lake Superiors are often grazing at their maximum rate at ambient food concentrations.

The occurrence of a satiated grazer community was no more likely during summer stratification than during the non-stratified period (χ^2, p= 0.126, df =1, Table 4). The occurrence of satiation was also not related to size fractionation (χ^2, p= 0.890, df =1, Table 4). There appeared to be a pattern of increased initial chlorophyll and POC concentrations and the increased likelihood of satiation, but these differences were only significant during the non-stratified season with initial chlorophyll concentration (Figure 13). However, the non-stratified results had significantly higher mean chlorophyll concentrations when satiation occurred (t-test, p = 0.043, df = 19, Figure 13). Throughout all seasons and size fractions there were no correlations between ILC dilution values and the initial chlorophyll concentration or <80 µm POC concentrations, the proxies for phytoplankton biomass (Table 5).

Grazing patterns related to the DCM were analyzed for stratified season cruises when the DCM was clearly established in July –September (CARGO1, 5, and 6). Grazing rates on the whole water fraction were significantly higher above the DCM compared to grazing rates below the DCM (Sheffe, p < 0.001, df = 94, Figure 14). In contrast, the grazing on the <10 µm size fraction was significantly higher within the DCM compared to above and below the DCM (Sheffe, p = 0.004, df = 56, Figure 14). There was often vertical separation between the DCM and the deep carbon maximum (DCarM). The DCarM in Lake Superior is often located above the DCM (Figure 2). Grazing above the DCarM was significantly higher than grazing within or below the DCarM (Figure 15). The grazing rates on the whole water size fraction were significantly different between the DCarM layers, and grazing rates on the < 10 μm size fraction was significantly higher above the DCarM (Figure 15).

The relationship between estimates of grazing (g) and phytoplankton growth (μ_{max}) rates is often examined to estimate the percentage of primary production that is consumed by the zooplankton community (e.g., Calbet and Landry 2004, Irigoien et al. 2007, Strom et al. 2007). This comparison is only approximate, however, because μmax measured under nutrient amended conditions is likely to exceed in situ growth rates. However, Calbet and Landry (2004) found a strong 1:1 relationship between dilution estimated primary production from μ_{max} ($\mu_{max} *$ phytoplankton biomass) and ^{14}C primary production (r =0.89) giving confidence to using dilution-grazing estimated maximum growth rate (μ_{max}). My results, in contrast, do not produce a clear relationship between ^{14}C primary production (Sterner submitted) and maximum growth rate (μ_{max}) (r =0.13, Figure 16). Calbet and Landry (2004) estimated phytoplankton biomass from volumetric estimates of the phytoplankton community. My results did not have an estimate of phytoplankton biomass and used the more inclusive biomass estimate of <80 μm POC concentration as a proxy. Calbet and Landry (2004) had only a small number of negative μ_{max} (29 of 788) estimates and they changed these values to 0.01 d^{-1} in their analysis to avoid having to divide by zero. However, 60% of my results had negative μ_{max} indicating high grazing pressure even at the highest dilutions, and therefore it did not seem appropriate to change all the values to 0.01 d^{-1} for a grazing rate to growth rate estimate. Ultimately, the large range of error, high p-values, and often negative results of μ_{max} in our L-H dilution-grazing results did not allow for confident analysis of grazing rate to growth rate using μ_{max}.

We examined the factors predicting chlorophyll and POC concentrations during the summer stratified period with multiple linear regression. A statistical model was created using a combination of biological and physical parameters based on theories that have explained DCM formation. Chlorophyll and POC concentrations were modeled as a function of primary production, grazing, temperature, chlorophyll to carbon ratio, and PAR. In-situ primary production was measured during the CARGO cruises using JGOFS protocols (Knap et al. 1996). Temperature and PAR came from CTD casts. Grazing and production were normalized to constant temperature using residuals to create variable independence. The statistical model used grazing rates from the whole water fraction and on-deck experiments, because the primary production was measured in the whole water fraction. The model chosen by backwards stepwise variable selection (F to enter and remove was 1.0, $p < 0.05$) was:

$$\mathrm{Chl} = 0.188 - 0.114T + 0.063(\mathrm{Chl{:}C}) + 0.185\log(\mathrm{PAR}) \qquad (3)$$

Where: Chl = Chlorophyll concentration ($\mu g\ l^{-1}$)

T = Temperature (°C)

Chl:C = Chlorophyll concentration to POC concentration ratio ($\mu g\ l^{-1} : mg\ l^{-1}$)

PAR = Photosynthetic active radiation

The model had good predictive power ($r^2 = 0.89$) and appeared unbiased (Figure 17).

A statistical model was also created to predict POC concentrations during the summer stratified period. The chosen model by stepwise variable selection was:

$$POC = 0.128 - 0.017T - 0.008(Chl:C) + 0.030\log(PAR) \qquad (4)$$

Where: POC = Particulate organic carbon (mg l^{-1})
All other variables are defined above. The statistical model has moderate predictive power ($r^2 = 0.56$) and also appeared unbiased (Figure 18).

Phytoplankton Growth Potential with Water from Different Depths

If a nutrient gradient exists, then water from depth should increase phytoplankton growth rates compared to surface water. CARGO5 growth experiments showed a positive correlation between growth measured by final chlorophyll concentration and the depth of filtered water ($r^2 = 0.435$, $p = 0.002$, Figure 19). The mid-September CARGO6 phytoplankton growth experiments showed no significant relationship between final chlorophyll and filtered water depth ($p = 0.057$, Figure 19).

Zooplankton Community-

There were distinct patterns found in the zooplankton communities. The zooplankton community structure changed throughout the cruises (ANOVA, $p < 0.05$, Figure 20, Table 6). Immature crustaceans make up most of the population across all cruises with nauplii being the majority (Figure 20). Throughout all seasons the adult crustacean community is dominated by copepods with the most common species being *Limnocalanus macrurus, Diaptomus sicilis*, and *Diacyclops thomasi* (Figure 20, 21). The zooplankton community was similar with depth during the summer stratified season (Figure 21). Above the DCM there were significantly more *Diaptomus* copepodites than below the DCM (ANOVA, $p < 0.05$, Figure 21). There was increased zooplankton abundance in the summer stratified season and at depth (DCM). Immature zooplankton, nauplii and copepodites, were consistently a major contribution to the zooplankton communities. The nauplii were the most abundant single group of zooplankton over all depths during summer stratification (Figure 20).

Discussion

Grazing was found to be significant in Lake Superior throughout the year. Grazing rates during the summer stratified period throughout the upper 75 m of the water column averaged 0.23 d^{-1}, and the warmer waters above the hypolimnion had an average grazing rate of 0.32 d^{-1}. During the non-stratified period grazing rates averaged 0.14 d^{-1} (Figure 9). Smutka (2005) also used the LH method and reported higher September grazing rates of 0.45 d^{-1} in the upper hypolimnion from a single cruise. Grazing estimates for other Laurentian Great Lakes are consistent with our Superior results. A Lake Heron and Lake Michigan study used three approaches (L-H dilutions, ^{14}C-labeled Synechococcus grazing, and ampicillin) to measure grazing on *Synechococcus*, an important primary producer in Lake Heron and Lake Michigan, showed slightly higher summer grazing rates (0.33-0.54 d^{-1}) and non-stratified season grazing ranged from 0.11-0.22 d^{-1} (Fahnenstiel et al. 1991). The grazing rates I found in this study are within the range of 0.16-0.38 d^{-1} seen in subarctic coastal and open ocean studies (e.g., Landry et al. 1993; Liu et al. 2002, Olson and Strom 2002, Strom et al. 2007). Grazing is an integral part of the Lake Superior carbon cycle throughout the year.

The cold mean annual temperature of 4ºC Lake Superior could be an important factor limiting grazing. Lake Superior has seen significant warming especially in summer with surface temperatures increasing 3.5 ºC over the past century (Austin and Colman 2007, Austin and Colman 2008). Community grazing rate was positively correlated with temperature (Figure 8). Grazing rates during cold non-stratified conditions (0.14 d^{-1}) and within the hypolimnion during the stratified season (0.13 d^{-1}) were not significantly different. Rates in the upper water column during summer stratification were significantly higher than these, which suggests that at first approximation the main seasonal signal in grazing dynamics is for an enhancement in the warm layer during summer stratification. The summer allows for increased grazing rates in warmer waters as stratification occurs. Enhanced grazing at warmer temperature may result from higher populations of grazers, higher specific activity of grazers, or both. Temperature serves as a good predictor for crustacean zooplankton abundance in large lakes because it reacts both to direct metabolism effects and indirect food web shifts (Patalas 1990, Patalas and Salki 1992). Warming water temperatures of Lake Superior could release grazers from temperature restraints causing increased grazing rates. Any increase in grazing could influence the carbon and nutrient cycles of Lake Superior.

Grazing rates on the whole and <10 µm fractions were not significantly different from each other (Figure 9). Sterner (submitted) found that three quarters of the Lake Superior chlorophyll is in the < 5 µm fraction. Picoplankton (<2 µm) account for half of Lake Superior autotrophs (Fahnenstiel et al. 1998, Sterner submitted). In Lake Michigan and Lake Huron dominant consumers of Synechococcus were in the 2-8 µm fraction (Fahnenstiel et al 1991). Grazing on small fractions likely dominate grazing dynamics and therefore, significant differences are not seen grazing rates on the whole and <10 µm fractions. Non-linear results allow for the estimation of the ILC, which can be interpreted to characterize feeding responses of the grazer community. Sixty-six percent of the L-H dilutions showed saturation. Surprisingly, there was no relationship between the occurrence of feeding saturation and either the initial chlorophyll or POC concentrations during all cruises (Table 3). Strom et al. (2007) reported a similar lack of pattern between feeding saturation and initial chlorophyll concentration throughout the year in the Gulf of Alaska. The lack of relationship between algal abundance and ILC indicates that the ILC may have more to do with characteristics of the grazer community than with overall food abundance. The average ILC value across cruises occurred at the dilution level 0.28 (fraction whole water). The grazer community begins consuming at the maximum grazing rate well-below ambient food concentrations. The saturated feeding could indicate that the Lake Superior grazers are adapted to the low food environments.

The non-linear results in the L-H experiments could indicate changes in grazer dynamics within the dilution bottles. Patterns that develop because of "bottle effects" may make the previous interpretation of the non-linear breakpoint being the ILC incorrect. Predation on the small herbivores by larger zooplankton could lead to decreased grazing on the phytoplankton community at the lowest dilutions, and lead to the flattening of the grazing curve that looks like satiation (e.g., Gallegos 1989, Dolan et al. 2000, Agis et al. 2007, Modigh and Franze 2009, Teixeira and Figueiras 2009). Heterotrophic nanoflagellates and ciliates concentrations are known to change over the time period of L-H experiment (Dolan et al. 2000, Agis et al 2007). Shifts in grazer communities during L-H experiments could be important in Lake Superior, because ciliates and flagellates are major members of the Lake Superior grazing community contributing nearly fifty percent of the heterotrophic microorganism biomass (Fahnenstiel 1998). Non-linearities in the grazer concentrations can lead to non-linearities in grazing impacts (Dolan et al. 2000, Teixeira and Figueiras 2009). In my experiments the grazer community concentrations were not monitored. Therefore, grazer dynamics within the bottles are unknown.

Although grazing rates were saturated in sixty six percent of experiments, the grazing rates still increased with chlorophyll concentration, which suggests that the Lake Superior grazing community reacts positively to an increase in food supply. The relationship is weak during summer stratification ($r^2 = 0.04$) and a stronger positive correlation occurs during the non-stratified season. ($r^2 = 0.103$, Figure 10). Both freshwater and marine systems have shown a positive correlation between grazing rates and primary producer biomass (Gruner et al. 2008). Microzooplankton communities in the North Atlantic can respond quickly to an increase in phytoplankton biomass in the summer when warm temperatures do not limit zooplankton growth and grazing (Pomeroy and Deibel 1986). However, the positive correlation is not always seen between grazing rates and producer biomass and North Pacific studies demonstrated that zooplankton grazing rates had no correlation with changes in prey biomass (Strom et al. 2001). Lake Superior grazing rates have very weak correlations with biomass.

Grazing rates in Lake Superior had a stronger positive correlation with primary production than with phytoplankton biomass (Figure 12). High grazing pressure associated with high production may be preventing the accumulation of phytoplankton biomass and thus preventing a strong relationship between food abundance and grazing rates. Grazing, therefore, has the ability to uncouple the relationship between primary production and biomass and be an important top-down control in the community. Sawatzky et al. (2006) observed that production losses due to high grazing pressure in an oligotrophic system can be offset by gains in nutrient recycling and reduce competition for available nutrient, which allows for increases in primary production. The increase in primary production produces more resources that then can support additional grazers (Irigoien et al. 2005, Chen et al. 2009). My results in Lake Superior relating grazing rates and primary production suggest a similar balance, with losses in biomass due to high algal mortality from consumption and gains in primary production potentially from grazing nutrient enrichment.

The DCM and DCarM are distinctly different layers in Lake Superior during summer stratification. The DCM typically developed in the upper region of the hypolimnion below any dramatic changes in density. In contrast the DCarM usually established in the thermocline near the greatest change in density, which suggests reduced sinking could be important. The vertical separation between the DCM and DCarM in Lake Superior indicates that photoadaptation of the phytoplankton at depth is contributing to the DCM formation. Although both chlorophyll and carbon are proxies for phytoplankton biomass, the grazing response to the biomass estimates was not always identical (Figure 10, 11). Teixeira and Figueiras (2009) attributed different grazing rate trends associated with chlorophyll and carbon concentration to changing carbon to chlorophyll ratios within the water column. Fennel et al. (2007) concluded in Crater Lake that a vertical separation of the DCM and DCarM is a sign of photoadaptation, and the depth of the DCM is ultimately dependent on the phytoplankton community’s ability to adjust its carbon to chlorophyll ratio.

Numerous statistical models have been created to predict phytoplankton distribution and DCM formation in oligotrophic systems, combining biological, chemical, and physical variables. Two statistical models were created to predict Lake Superior phytoplankton biomass with one focusing on chlorophyll and the second on POC as proxies for phytoplankton biomass. Models often find light and nutrient gradients are among the important factors for predicting phytoplankton concentrations (eg., Klausmeier and Litchman 2001, Fennell and Boss 2003, Yoshiyama et al. 2009). The Lake Superior model is similar, because it uses light to predict chlorophyll concentration. However, the Lake Superior chlorophyll model does not use nutrients as a predictor. Additionally to light, the model shows that temperature and the chlorophyll to carbon ratio are the best indicators of chlorophyll and POC concentrations. The models are dominated by physical variables, and these variables greatly influence the biological dynamics in the lake. Temperature and light have been found to be good indicators for both primary production in Lake Superior (Sterner submitted) and grazing rates in the Laurentian Great Lakes (McNaught 1980). Nutrients are not a variable in the statistical model, but the strong relationship between grazing and production rates suggests that nutrient availability is influenced by grazing, which results in the availability of recycled nutrients. The importance of the chlorophyll to carbon ratio supports that photoadaptation is an important factor in Lake Superior. Both statistical models reflect the importance of physical characteristics in Lake Superior, which seem to drive the biological dynamics that shape the phytoplankton community structure and impact the flow of carbon within the system.

Phytoplankton Growth Potential with Water from Different Depths-

Often in oligotrophic systems light and nutrient gradients contribute to the formation of a DCM (eg., Klausmeier and Litchman 2001, Fennell and Boss 2003, Yoshiyama et al. 2009). The low nutrient conditions of Lake Superior are known to limit phytoplankton and cyanobacteria growth (Sterner et al. 2004, Ivanikova et al. 2007), and therefore nutrient enrichment could be expected to increase growth rates. Growth experiment results looking for evidence of nutrient enrichment in deep waters differed greatly from CARGO5 in late July 2008 to CARGO6 in mid September 2008. CARGO5 results showed a significant and strong relationship between growth and filtered water depth ($r^2 = 0.44$), but CARGO6 had no significant trend (Figure 19). The increased growth with water from depth during CARGO5 suggests that there could be some nutrient enrichment with depth in mid-summer. The CARGO6 results suggest that by mid-September any previously established nutrient gradient has broken down and water from depth is not enriched compared to surface waters. Lake Superior's low nutrient levels are difficult to measure and clear nutrient gradients have not been detected (Baehr and McManus 2003, Field and Sherrell 2003, Barbiero and Tuchman 2004, Kumar et al 2007, Anagnostou and Sherrel 2008). Summer stratification begins to break down in September (e.g., Auer and Bub 2004, Barbiero and Tuchman 2004, Russ et al. 2004) A seasonal shift could weaken gradients which establish during stratification. Baehr and McManus (2003) measured a weak phosphorus gradient in early summer and found the gradient had disappeared by September. Several years of nitrate drawdown results show the drawdown is most prevalent in mid-July to late August and less apparent by mid-September (Sterner, in review). Unfortunately, these particular experiments were only completed on the last two cruises of the study. Therefore, results can only suggest a potentially important nutrient gradient during summer stratification which breaks down by mid-September, but can not firmly establish trends.

Zooplankton Community-

Zooplankton abundances in Lake Superior have been reported to have increased populations in the epilimnion compared to the depth of the DCM, and led to the theory that reduce zooplankton grazing could be a factor contributing to DCM formation (Olson and

Odlaug 1966, Anderson et al. 1972, Fahnenstiel and Glime 1983). However, the current results found the zooplankton communities were very similar above, within, and below the DCM (Figure 21). The crustacean community dominated by the copepods species *L. macrurus, D. sicilis*, and *D. thomasi* (Figure 20) are consistent with other Lake Superior zooplankton surveys, which found clear dominance of these copepods (Barbiero and Tuchman 2004, Brown and Branstrator 2004). There were significant changes in the crustacean community over the cruises. Other surveys have reported high numbers of cladocerans in late summer and have even found late summer co-dominance of cladocerans and copepods (Brown and Branstrator 2004). My results found an increase in cladocerans in during CARGO6, in late summer, but never co-dominance of cladocerans and copepods (Figure 20, Table 6).

More recent surveys found evidence of diurnal migration in deep calanoid populations, which indicates that these grazers could take advantage of abundant food in the DCM (Barbiero and Tuchman 2004). Barbiero and Tuchman (2001) reported the most abundant crustacean species occupy much deeper depths during the day. McNaught et al. (1980) compared diurnal and nocturnal grazing rates in Lake Heron and found that grazing rates increased nearly 3-fold at night, primarily because of diurnal vertical migration. Copepods demonstrate the strongest DVM patterns, while cladocerans tend to stay in the warmer surface layers. Lake Superior is dominated by copepods, so DVM could be an important grazing dynamic overlooked by this study. The zooplankton tows during the present study were done between 13:00 and 17:00 and went to a depth of 50 m. Therefore, the tows completed in mid-day may have missed important dynamics of the zooplankton community. It is possible that our diurnal grazing rates could be underestimating overall grazing rates, because nocturnal rates were not measured.

Conclusions

Grazing is a significant loss to primary production in Lake Superior especially in the epilimnion during summer stratification. Lake Superior has a background grazing rate (0.19 d^{-1}) throughout the cold non-stratified seasons, which is also maintained in the cold hypolimnion in the stratified summer months, but increased grazing is seen in the warmer summer waters (0.32 d^{-1}). Future grazing studies could focus on the potential of warming water temperatures impacting and most likely increasing grazing rates. Grazing is the first step in carbon and nutrient transfer up the food web, therefore any knowledge of consumer response to warming would allow for better understanding of the whole lake dynamics reaction to warming.

It has been shown that the DCM is not an actual biomass maximum. An increase in the chlorophyll to carbon ratio in the DCM suggests that photoadaptation is an important feature of the DCM phytoplankton community. The establishment of the DCarM occurs below the highest grazing rate and grazing could be important factors in DCarM formation. Grazing is a significant factor contributing to the phytoplankton communities we see in the lake, but the DCM and DCarM formation could not be fully explained by grazing pressures. The models show that physical characteristics are important in shaping the phytoplankton community. Grazing appears to be an important factor in the Lake Superior carbon cycle. Grazing has been established as a significant loss to primary production in Lake Superior. My results show a relationship between grazing rates and primary production and suggest a balance between grazing and growth. Grazing induced high algal mortality from consumption could be preventing biomass accumulation while simultaneously grazing supports increasing primary production by recycling nutrients and reducing phytoplankton competition for limited nutrients. Grazing has previously received little attention in Lake Superior, but can now be recognized as an important factor influencing the lake’s nutrient and carbon cycles.

Table 1. Cruise summary and data associated with grazing measurements. Values in bold represent the maximum for that date. Grazing rate is an average for the two experiments. For latitude and longitude of sampling sites, see text.

Date Cruise	Station Sampled On-deck Incubation	Station Sampled St. Paul Incubation	Depth (m)	Temp (ºC)	Chl (µg L^{-1})	<80 µm POC (mg L^{-1})	Grazing (d^{-1})
July 31, 2007 CARGO1	CD-1	CD-1	5	16.8	0.042	**0.152**	0.354
			20	4.1	1.245	0.139	**0.378**
			32	4.7	**1.332**	0.118	0.155
October 6, 2007 CARGO2	CD-1	None	5	11.2	0.568	0.124	**0.398**
			15	9.7	**1.36**	**0.178**	0.242
			25	5.5	1.03	0.143	0.170
November 8, 2007 CARGO3	CD-1	CD-1	5	8.3	0.329	0.127	0.154
			15	8.4	0.311	0.115	**0.477**
			25	8.3	**0.761**	**0.132**	0.137
April 30, 2008 CARGO4	CD-1	WM	5	1.8	**0.900**	0.113	**0.127**
			20	1.8	0.778	0.109	0
			60	1.8	0.582	**0.119**	0.051
July 31, 2008 CARGO5	CD-1	CD-1	2	14.1	0.606	0.122	0.350
			10	11.4	0.849	0.138	**0.410**
			20	7.7	0.780	0.145	0.229
			35	5.2	**1.326**	**0.156**	0.110
			50	4.4	0.3057	0.064	0.258
			75	4.0	0.2637	0.084	0.160

September 17, 2008 CARGO6	CD-1	CD-1	2	10.9	0.616	0.105	0.135
			10	8.8	1.383	**0.168**	0.178
			20	5.8	**1.587**	0.160	0.051
			35	4.6	0.696	0.091	**0.358**
			50	4.2	0.354	0.065	0.053
			75	3.9	0.209	0.059	0.244

Table 2. The conditions of the incubations completed in St. Paul or in the ship refrigerator to better mimic natural temperature conditions.

Cruise	Location	Sample water layer	Temperature (ºC)	Light Level µE/m-2/sec-1	Hours Light:Dark
CARGO1	St. Paul	Epilimnion	13	53.2	16:08
CARGO1	St. Paul	Thermocline	13	53.2	16:08
CARGO1	St. Paul	Hypolimnion	4	53.2	16:08
CARGO3	St. Paul	Non-stratified	4	53.2	10:14
CARGO4	St. Paul	Non-stratified	4	53.2	10:14
CARGO5	On-Ship	Hypolimnion	3.5	46.5	14:10
CARGO5	St. Paul	Epilimnion	10	66.4	14:10
CARGO5	St. Paul	Thermocline	10	66.4	14:10
CARGO5	St. Paul	Hypolimnion	4	54.8	14:10
CARGO6	On-Ship	Hypolimnion	4	46.5	12:12
CARGO6	St. Paul	Epilimnion	10	66.4	12:12
CARGO6	St. Paul	Thermocline	6	66.4	12:12
CARGO6	St. Paul	Hypolimnion	6	66.4	12:12

Table 3. Initial <80 µm chlorophyll concentrations from measured depth closest to 150m where water was sampled for 0.45um filtration for dilutions.

CRUISE	**<80um Chlorophyll (µg L^{-1})** **at measured depth closest to 150m**
CARGO1	0.432
CARGO2	0.089
CARGO3	0.062
CARGO4	0.188
CARGO5	0.164
CARGO6	0.149

Table 4. Dependence of the occurrence of satiated feeding response on a variety of categorical variables.

Variable	Chi-square	P	df
Stratified or Non	2.34	0.126	1
Fraction	0.02	0.890	1

Table 5. Relationship between the ILC value related to phytoplankton biomass estimated by the initial concentration of chlorophyll and <80 µm POC. The initial chlorophyll concentration of each fraction was used.

ILC value related to variable	r	p
Whole Chlorophyll	0.054	0.786
10 µm Chlorophyll	-0.085	0.567
Whole POC	0.124	0.635
10 µm POC	-0.033	0.865

Table 6. Comparison of taxonomic crustacean macrozooplankton community overall all cruises. Species listed showed significant shifts between cruises. Bold numbers represent populations that are significantly different (Sheffe's, $p < 0.05$). Weather prevented sampling during CARGO2.

		CARGO1	CARGO3	CARGO4	CARGO5	CARGO6
	ANOVA	Individuals m^{-3}	Individuals m^{-3}	Individuals m^{-3}	Individuals m^{-3}	Individuals m^{-3}
Leptodiaptomus sicilis	0.003	1.47	**11.89**	3.45	4.96	10.38
Diaptomus thomasi	0.000	0.04	**10.56**	4.80	0.32	2.61
Nauplii	0.000	**110.90**	14.40	20.62	**116.15**	36.90
Cyclopoida copepodites	0.000	3.70	**14.80**	0.56	1.50	1.70
Skistodiapotomus oregonensis	0.001	**0.09**	0.01	0.00	0.00	0.00
Bosmina longimanus	0.001	0.00	**0.34**	0.00	0.00	0.00
Daphinia mendote	0.005	0.00	0.14	0.00	0.01	**3.27**
Holopedium gibberum	0.009	0.00	0.00	0.00	0.00	**0.20**
immature daphnia	0.000	0.00	0.00	0.00	0.00	**0.97**

Figure 1. Map of Lake Superior with cruise stations CD-1 and WM.

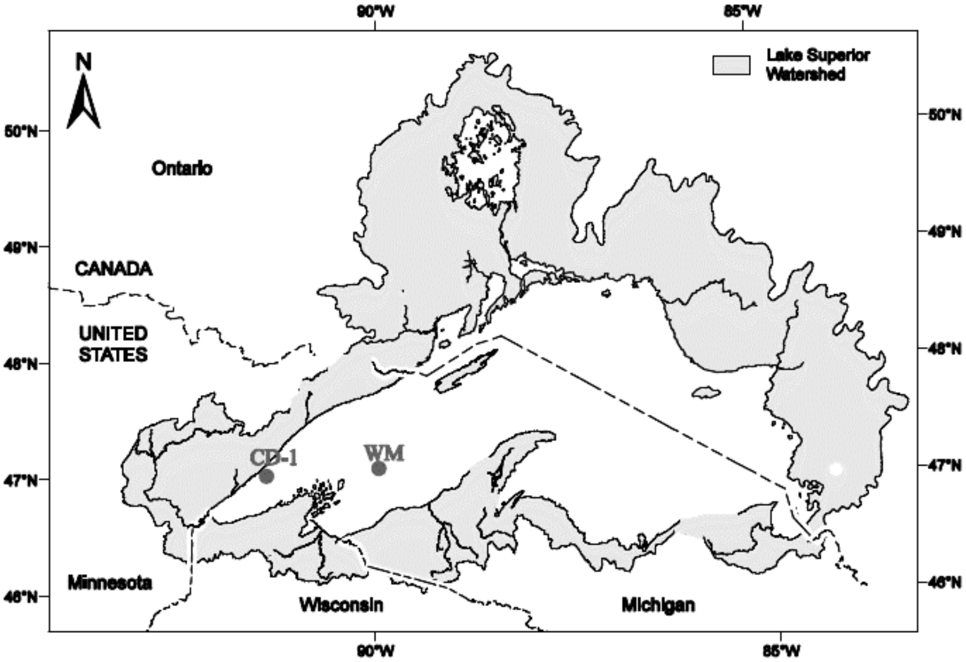


Figure 2. CARGO1-6 profiles showing temperature change in density per meter increase in depth, in situ chlorophyll, and POC profiles in Lake Superior during summer cruises 2007 – 2008 (CARGO1, July 2007; CARGO2, October 2007; CARGO3, November 2007, CARGO4, April 2008; CARGO5, July 2008; CARGO6, September 2009).

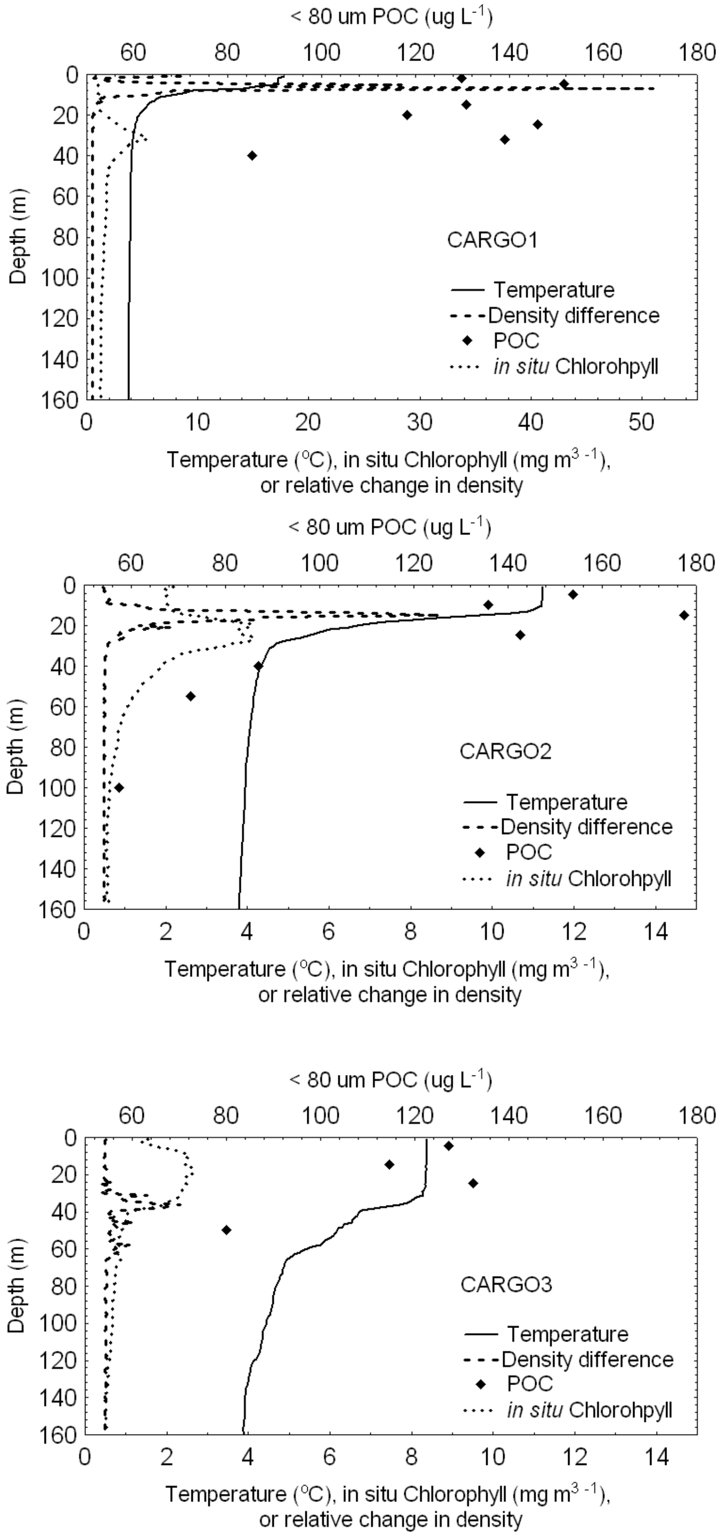


Figure 2 Continued.

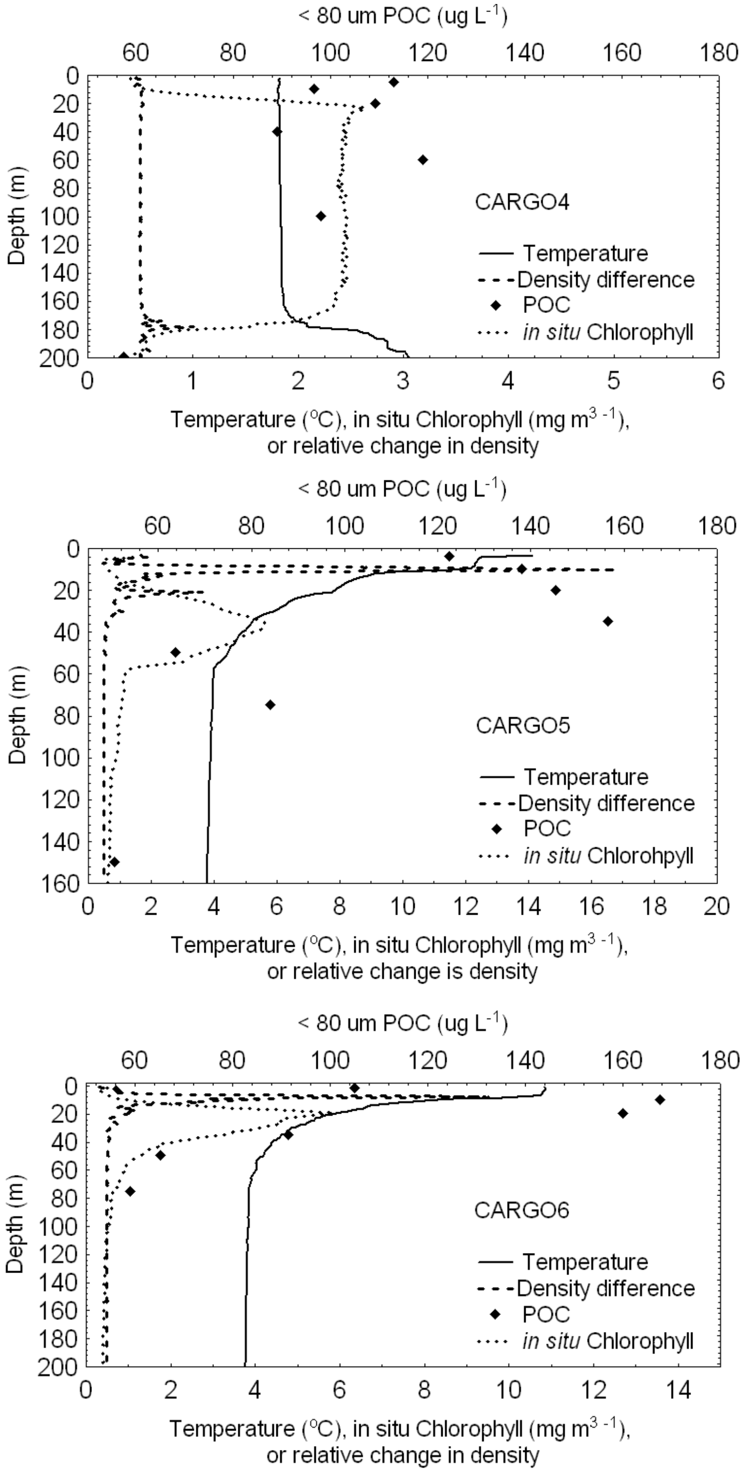


Figure 3. Temperature (^{o}C, —), relative change in density (- -), POC (μg L^{-1}, ◆), in situ chlorophyll (mg m$^{3\ -1}$, • • •) profiles of the top 40 meters during summer cruises CARGO1 (a), CARGO2 (b), CARGO3 (c).

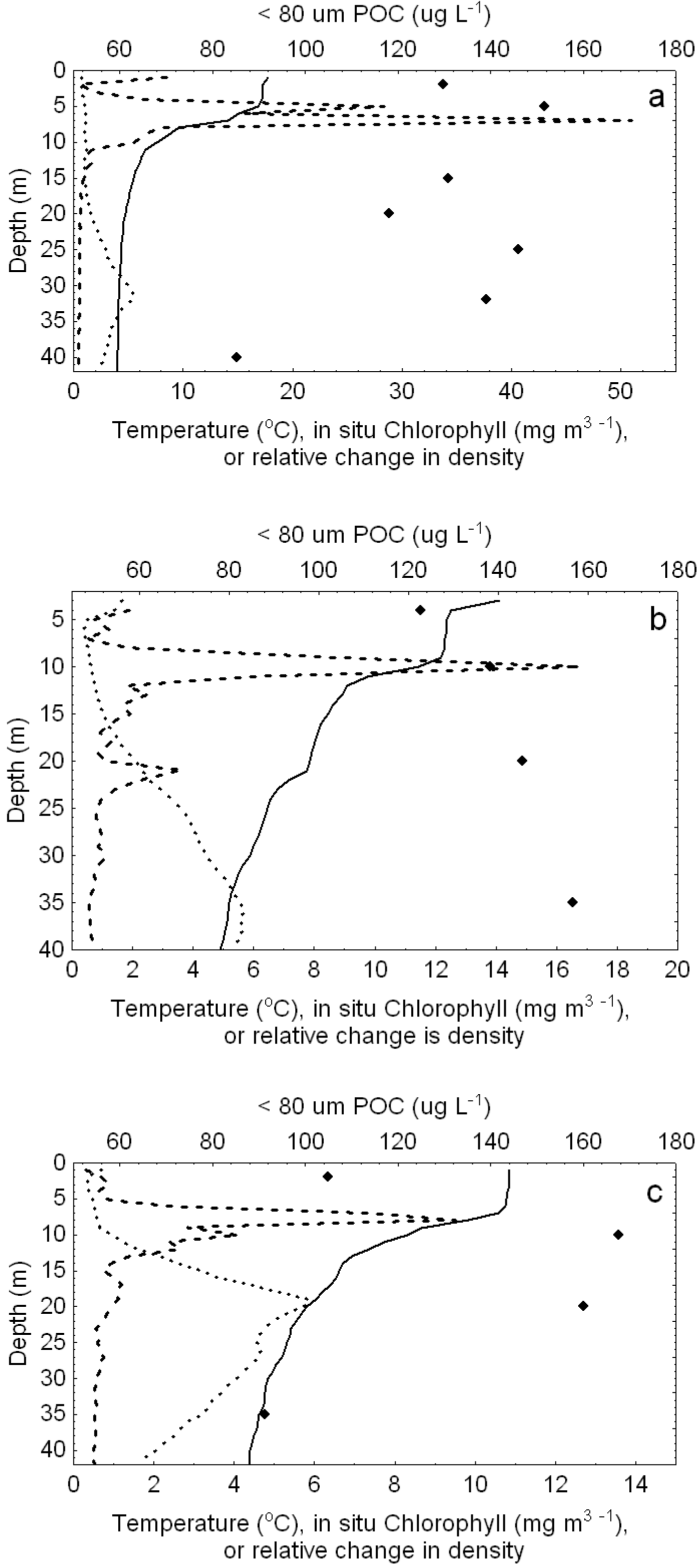


Figure 4. Example of grazing results as influenced by chlorophyll concentration in the 0.45 µm filtered water for dilutions. On the left are rates assuming 0.00 µg Chl L^{-1} and on the right are results assuming 0.005 µg Chl L^{-1}. A, B: CARGO6 whole water; C,D: CARGO6 75m 10um

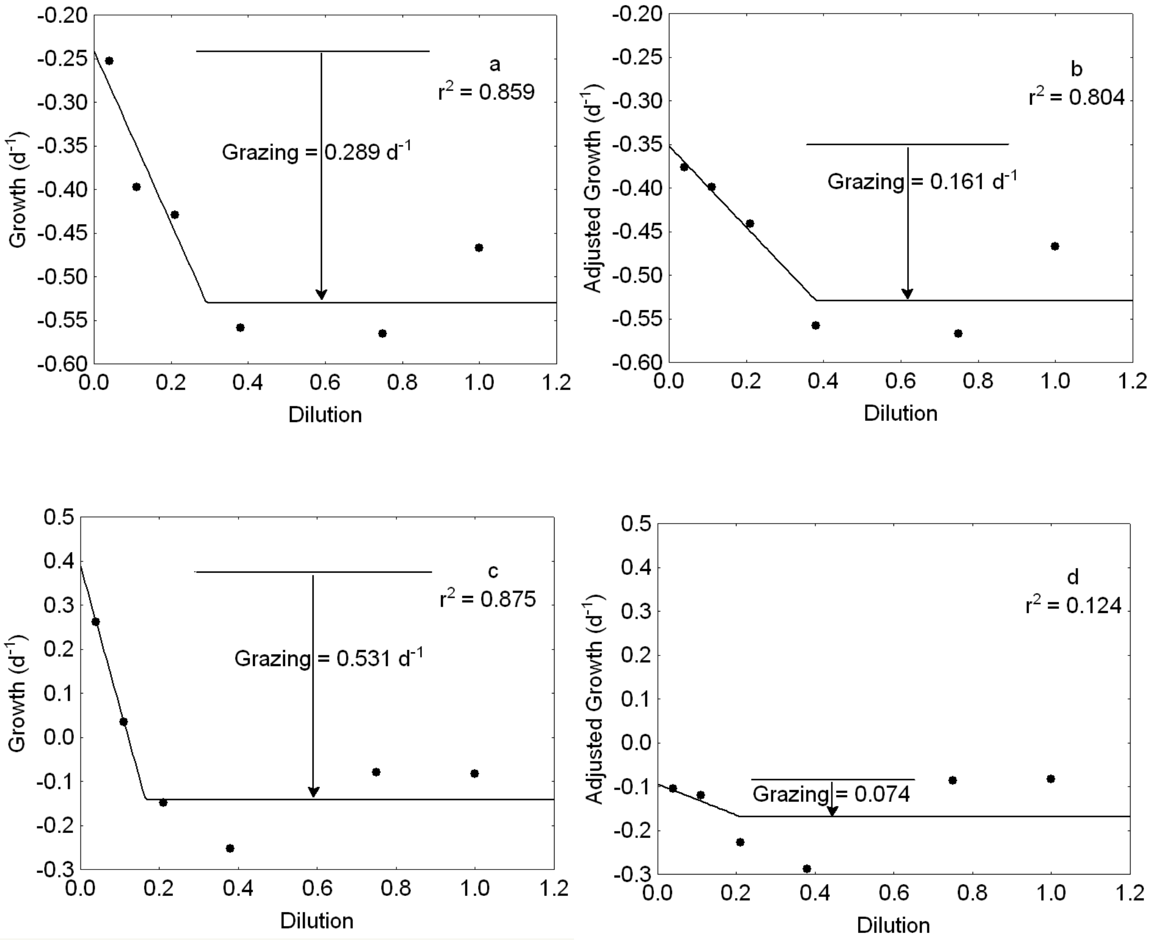


Figure 5. CARGO6 grazing on whole and 10um fractions comparing impacts of an initial 0.005 µg Chl L^{-1} in the 0.45 µm filtered dilution water with an assumption of 0.00 µg Chl L^{-1} in the 0.45 µm filtered dilution water.

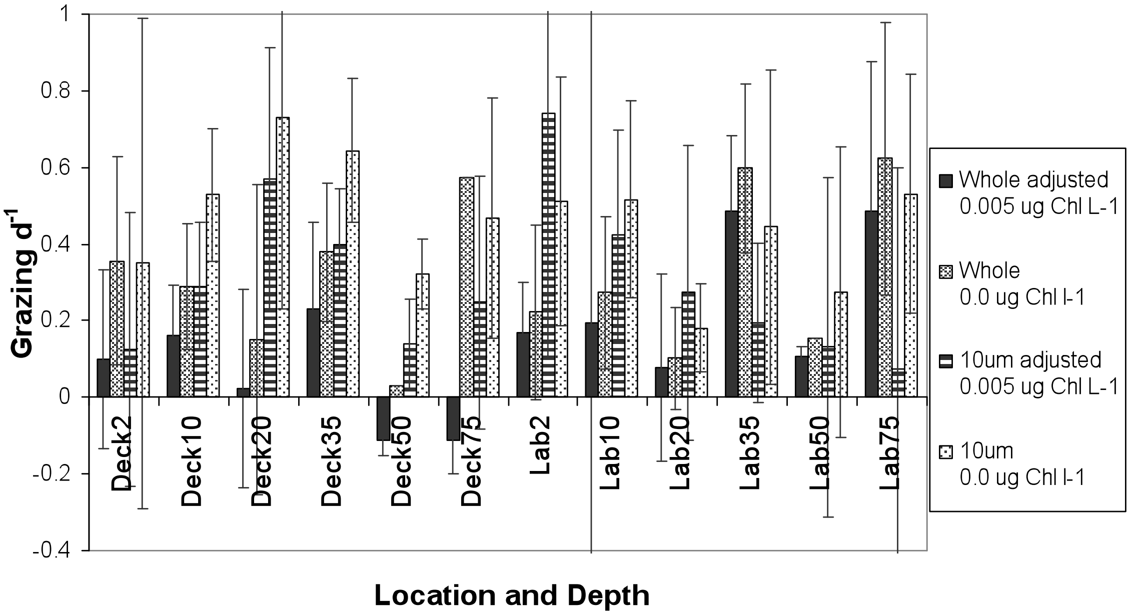


Figure 6. Grazing rates estimated for each measurement for all six cruises, as indicated by the cruise, incubation location, and depth of sampling as labeled.

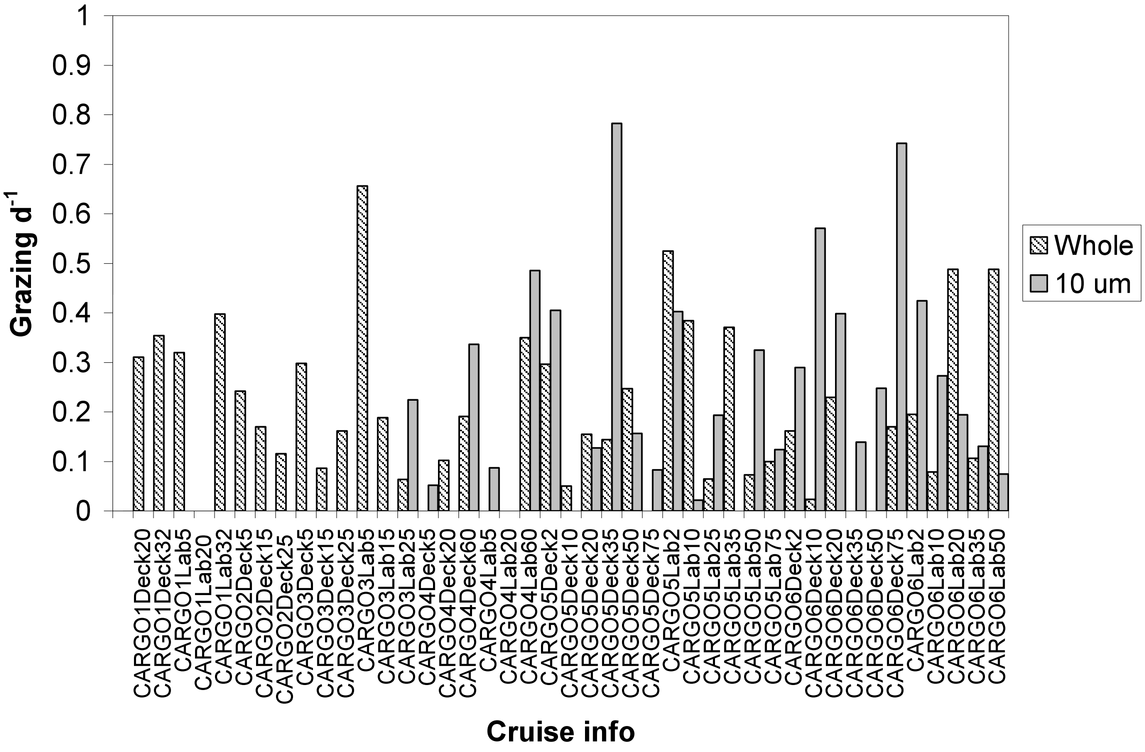


Figure 7. Weighted comparison of stratified (S) season grazing to non-stratified (N) for all depths. T-test comparison showed weighted summer stratified grazing significantly higher than non-stratified season grazing. (p<0.001, n = 73).

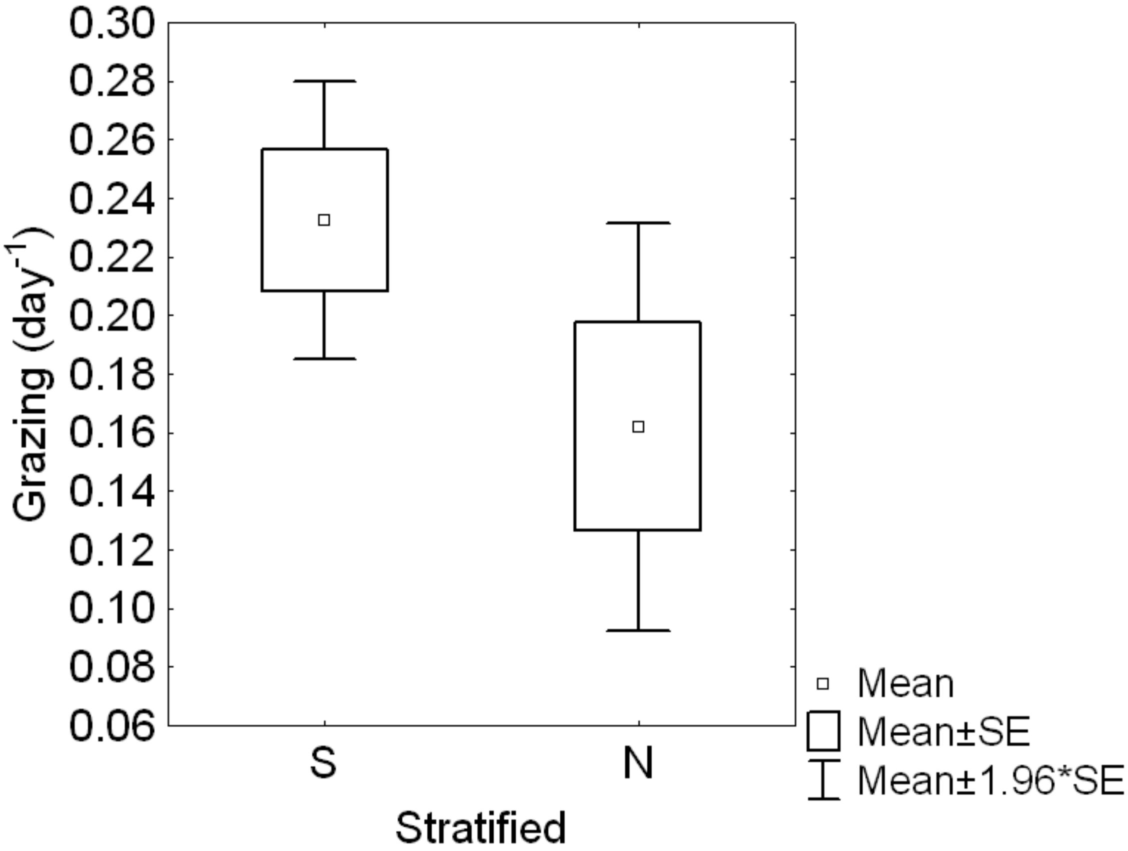


Figure 8. Grazing vs. temperature. Top row: summer stratified, whole water (a) and 10 µm (b). Bottom row: non-stratified, whole water (c) all seasons, whole water (d). Statistical

results based on weighted regressions such that not all data points are equally influential to the fitted lines.

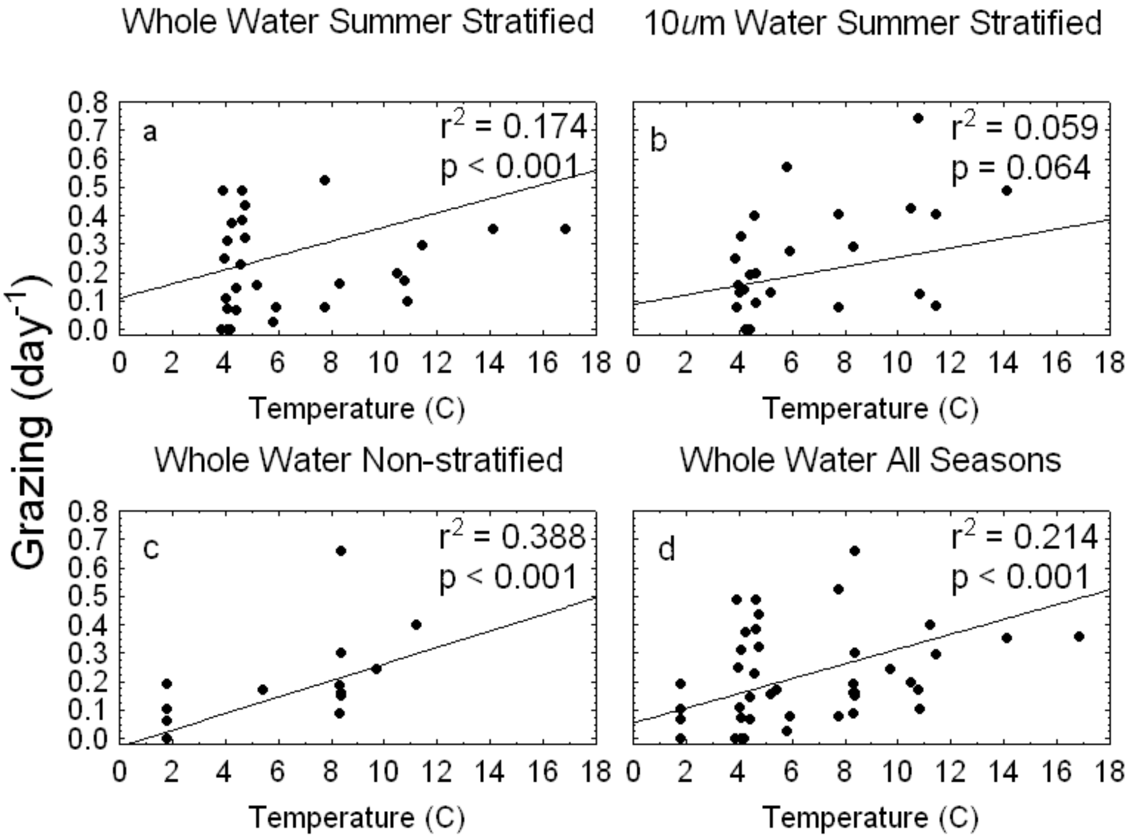


Figure 9. Comparison of weighted grazing rates on whole and < 10 μm algal fractions under summer stratification (CARGO 1, 5, 6) (a,b) and under non-stratified (CARGO 2, 3, 4) (c). Means are marked with dash. The box marks ± 1 SD and the whiskers are 95% confidence intervals. The P-value given is the ANOVA value for within fraction comparisons; horizontal bars below join not significantly different from each other based on Scheffe's comparison test within each panel ($p < 0.05$, n = 28; n = 24; n=21).

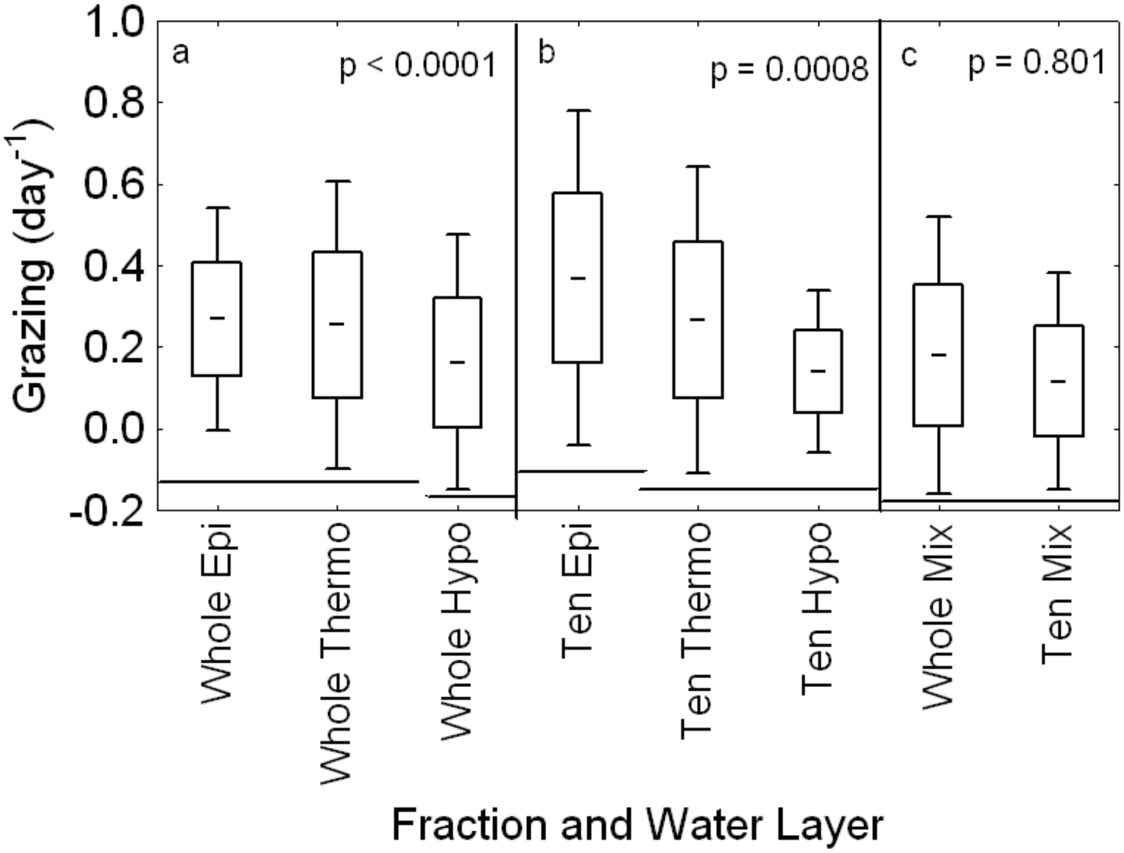


Figure 10. Grazing vs. initial chlorophyll concentration. Top row: summer stratified, whole water (a) and < 10 µm (b). Bottom row: non-stratified, whole water (c) all seasons, whole water (d). The fraction of the initial chlorophyll concentrations values match the size fraction of the grazing rates. Statistical results based on weighted regressions such that not all data points are equally influential to the fit.

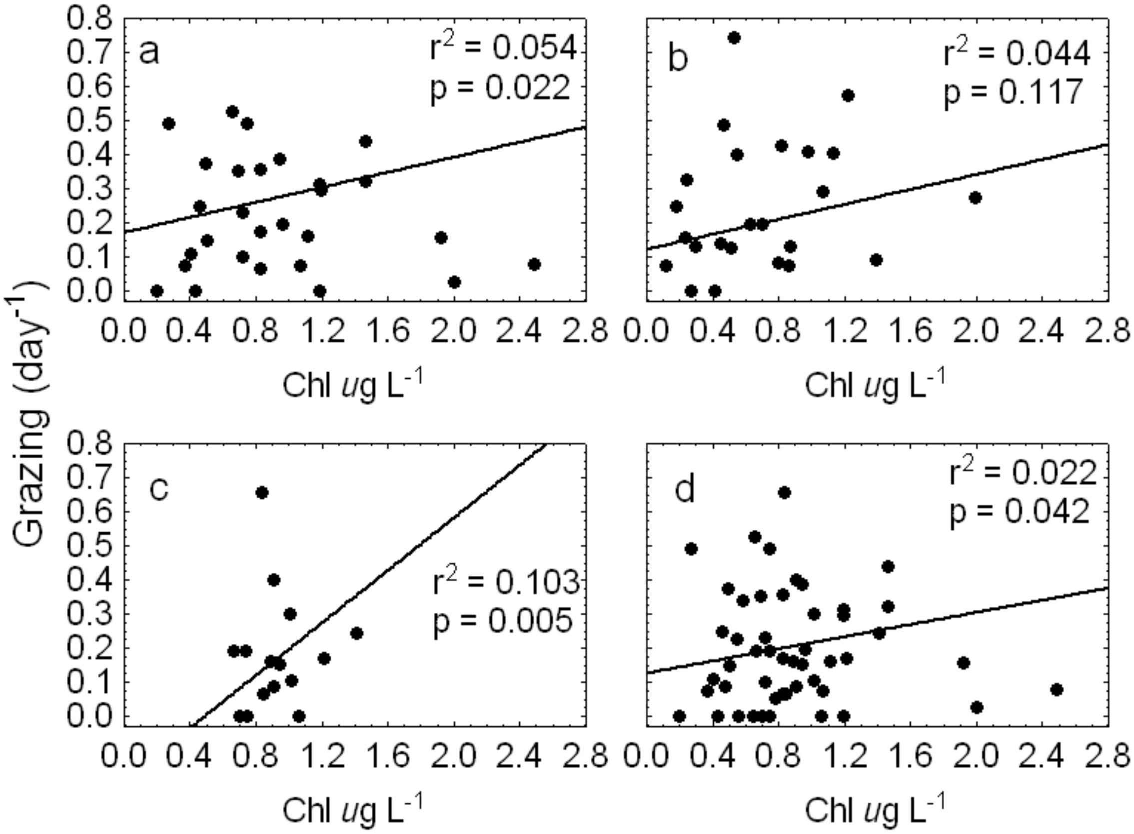


Figure 11. Grazing vs. initial < 80 µm POC concentration. Top row: summer stratified, whole water (a) and < 10 µm (b). Bottom row: non-stratified, whole water (c) all seasons, whole water (d). Statistical results based on weighted regressions such that not all data points are equally influential to the fit.

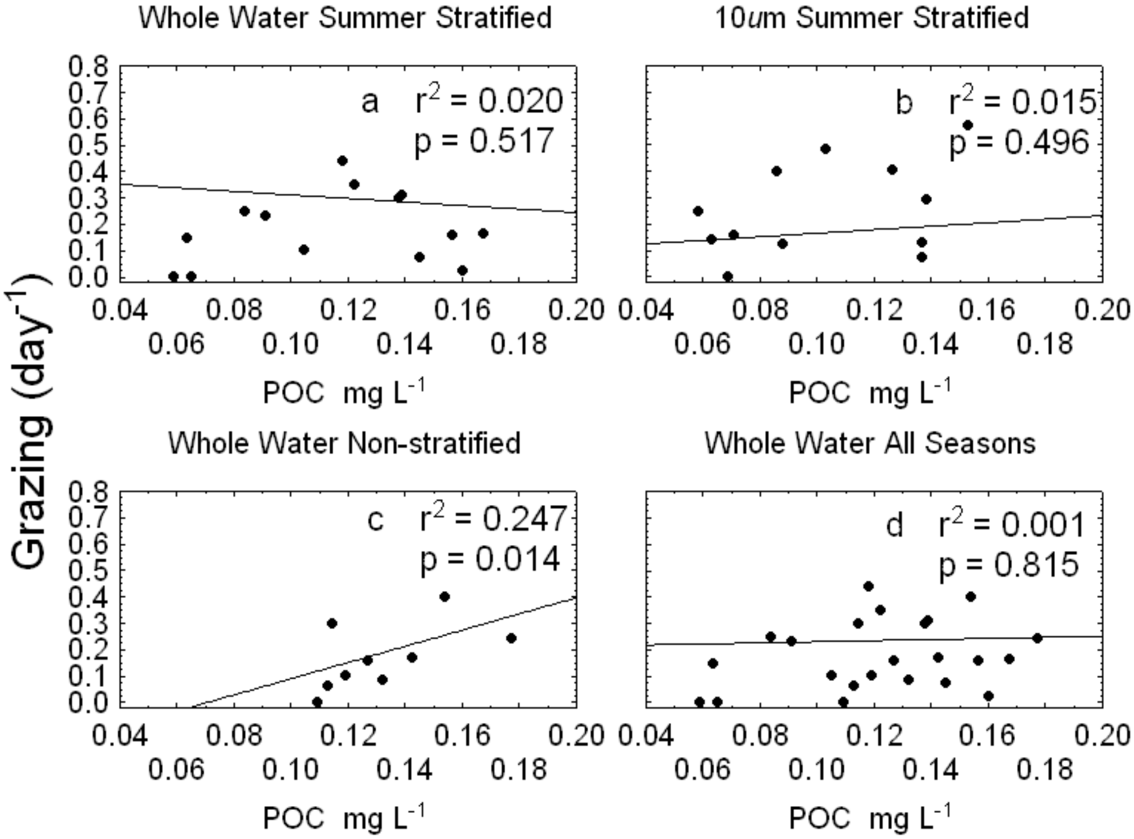


Figure 12. Grazing vs. primary production rates during all seasons (a), summer stratification (b), and the non-stratified season (c). Grazing rates are on the whole water fraction. The primary production rates are from *in situ* ^{14}C estimation (Sterner submitted). Statistical results based on weighted regressions such that not all data points are equally influential to the fit.

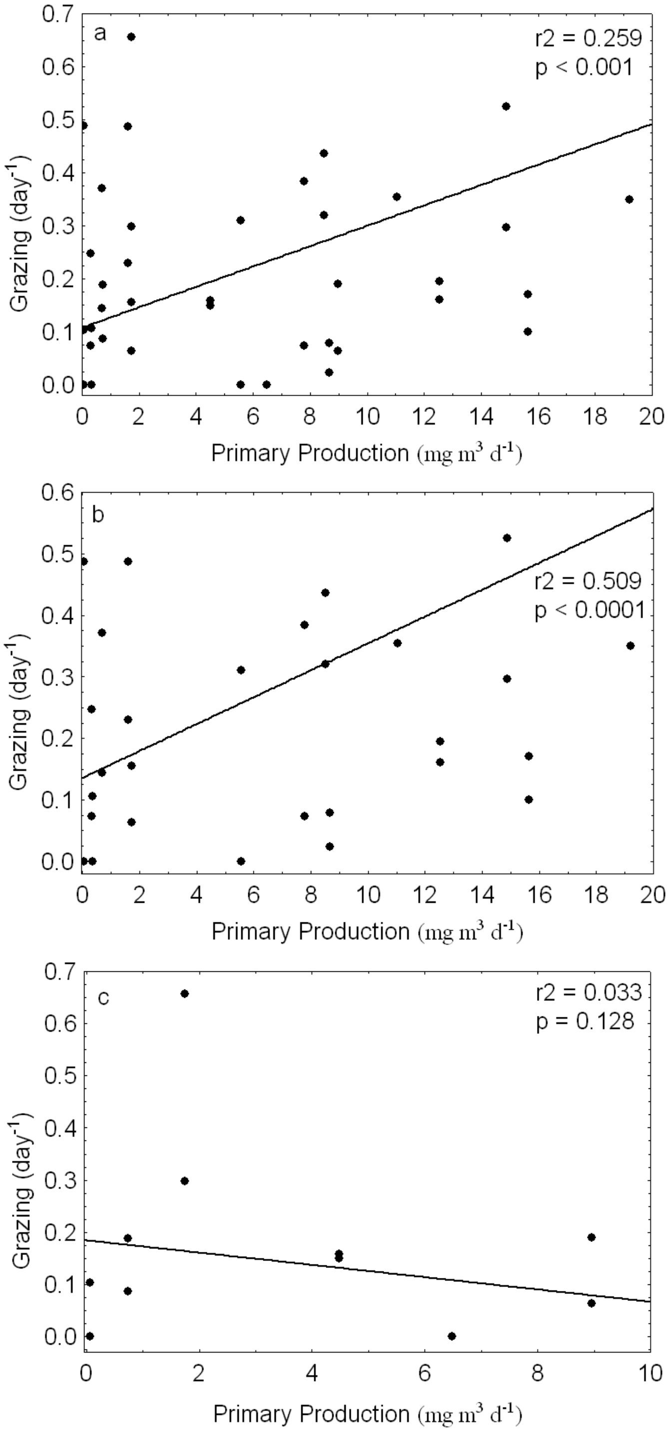


Figure 13. Comparison of mean initial chlorophyll and mean initial <80 µm POC in the absence (N) or presence (Y) of feeding satiation (ILC, measured as fraction whole water) throughout all seasons (**a**), during summer stratification (**b**), and during the non-stratified period (**c**). The box marks ± 1 SD and the whiskers are 95% confidence intervals. T-test p-value is given. (n = 73; n = 52; n = 21).

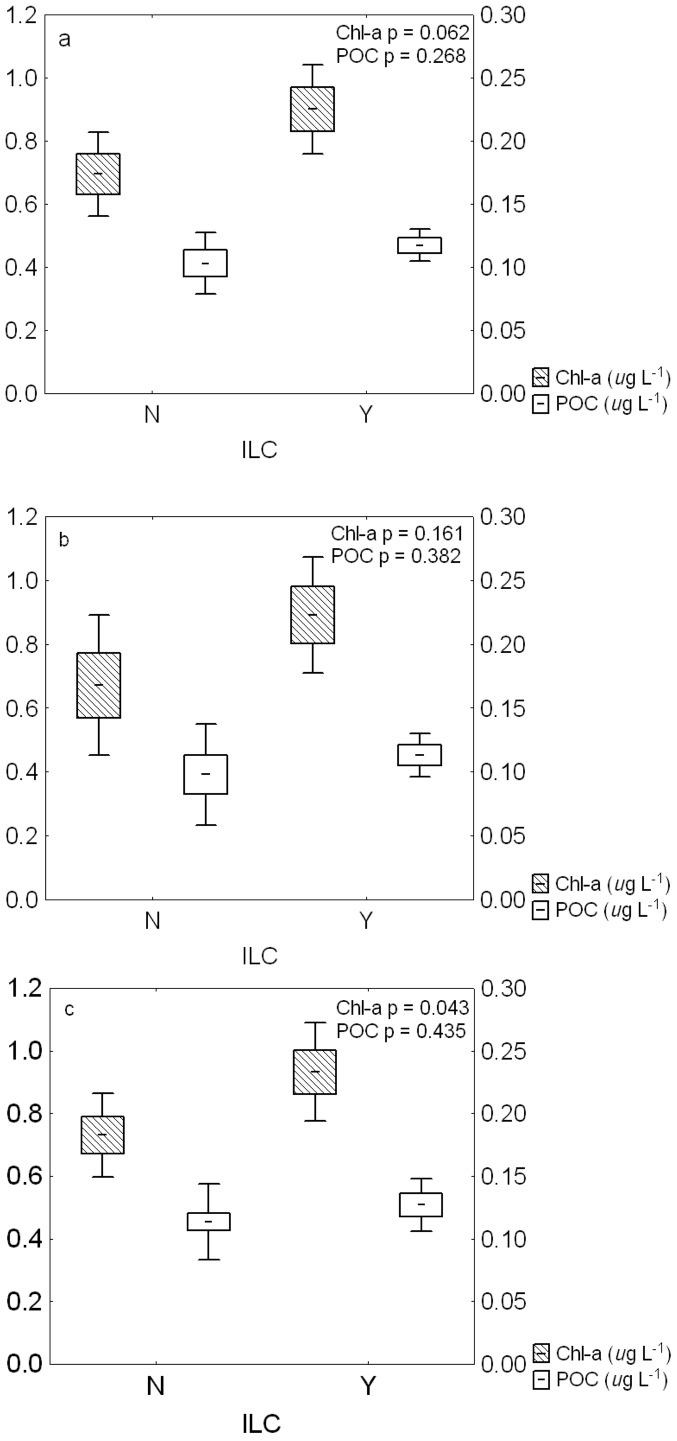


Figure 14. Comparison of summer stratified grazing above (A), within (D), and below (B) the DCM. The box marks ± 1 SD and the whiskers are 95% confidence intervals. The P-value given is the ANOVA value for within fraction comparisons; horizontal bars below join not significantly different from each other based on Scheffe's comparison test ($p < 0.05$; whole n = 28, 10μm n = 24).

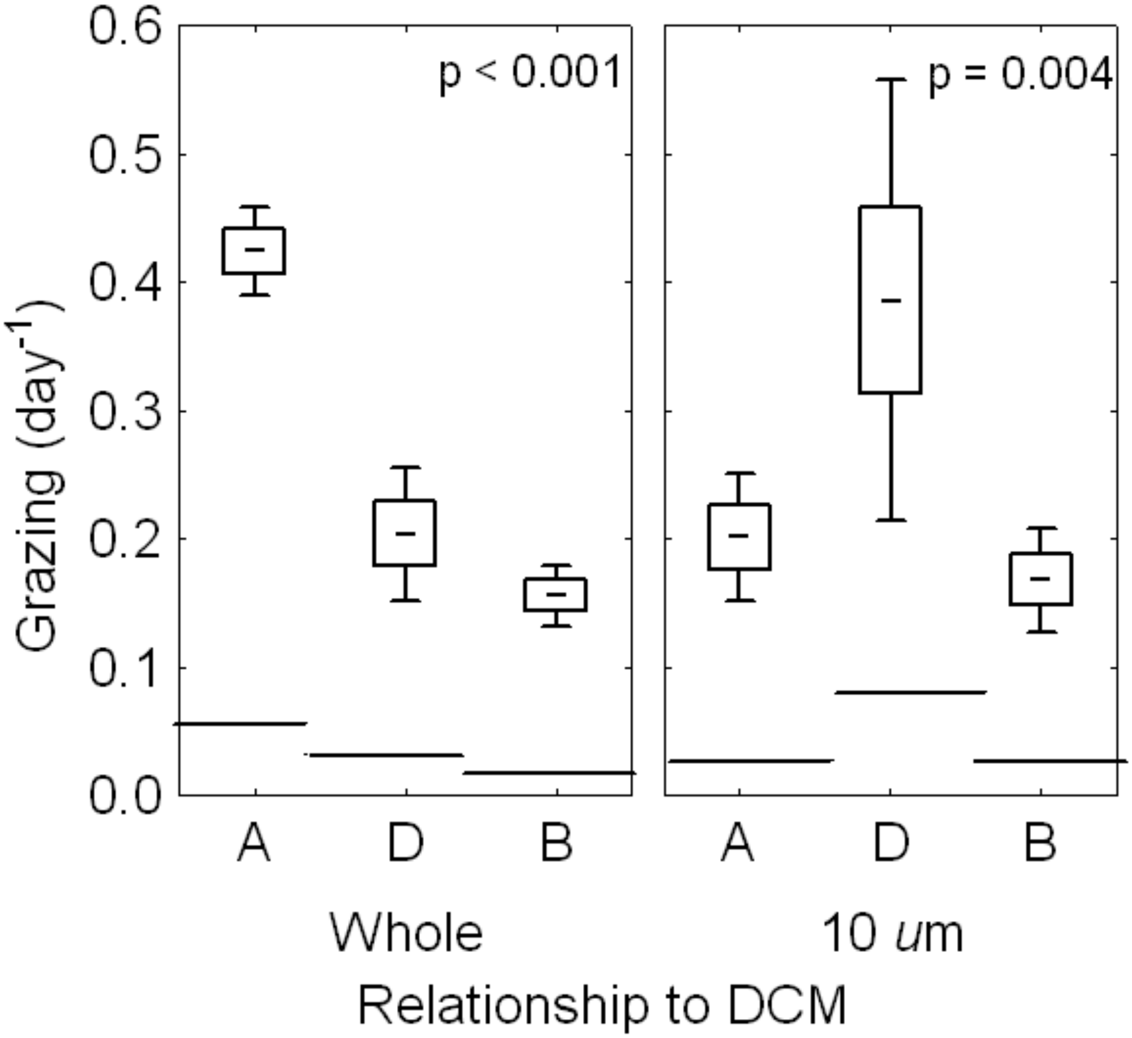


Figure 15. Weighted comparison of summer stratified grazing above (A), within (C), and below (B) Deep Carbon Maximum (DCarM). The box marks ± 1 SD and the whiskers are 95% confidence intervals. The P-value given is the ANOVA value for overall comparisons; horizontal bars below join not significantly difference from each other based on Scheffe's comparison test ($p < 0.05$, $n = 28$; $n = 24$)

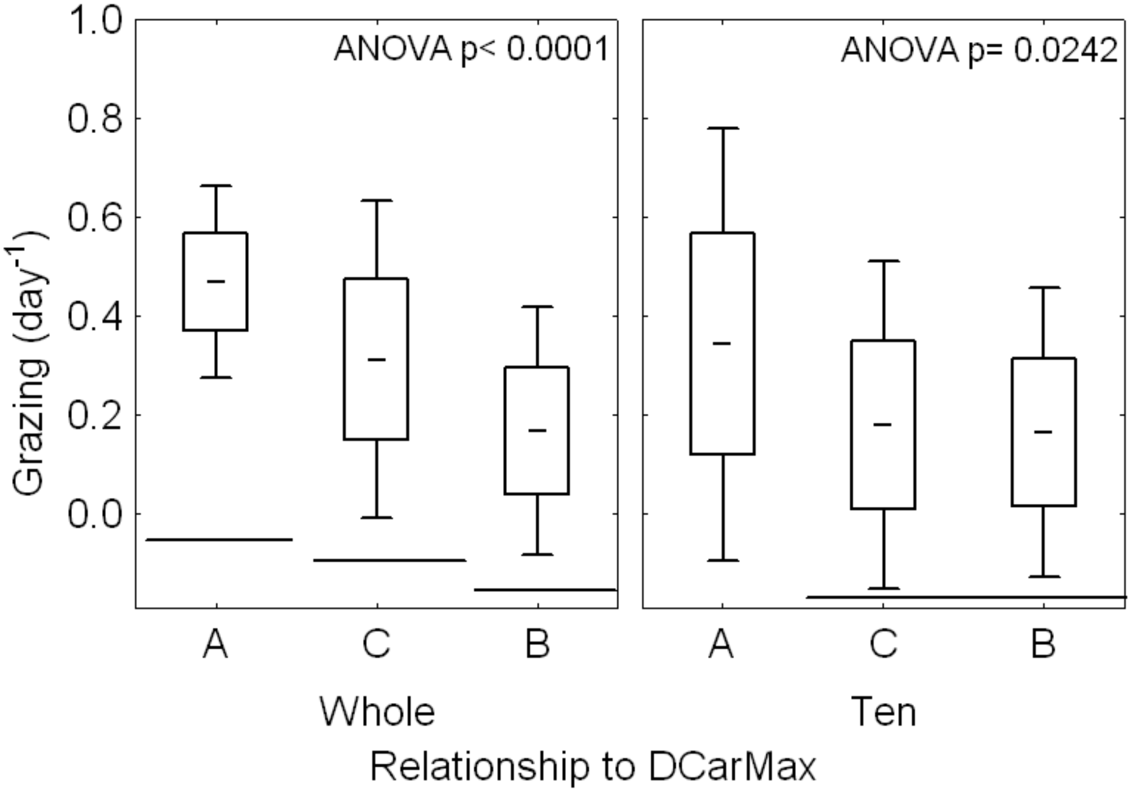


Figure 16. Relationship between ^{14}C-based estimates of growth and L-H calculated estimates of growth (μ_{max}). The ^{14}C-based estimates are from Sterner (submitted).

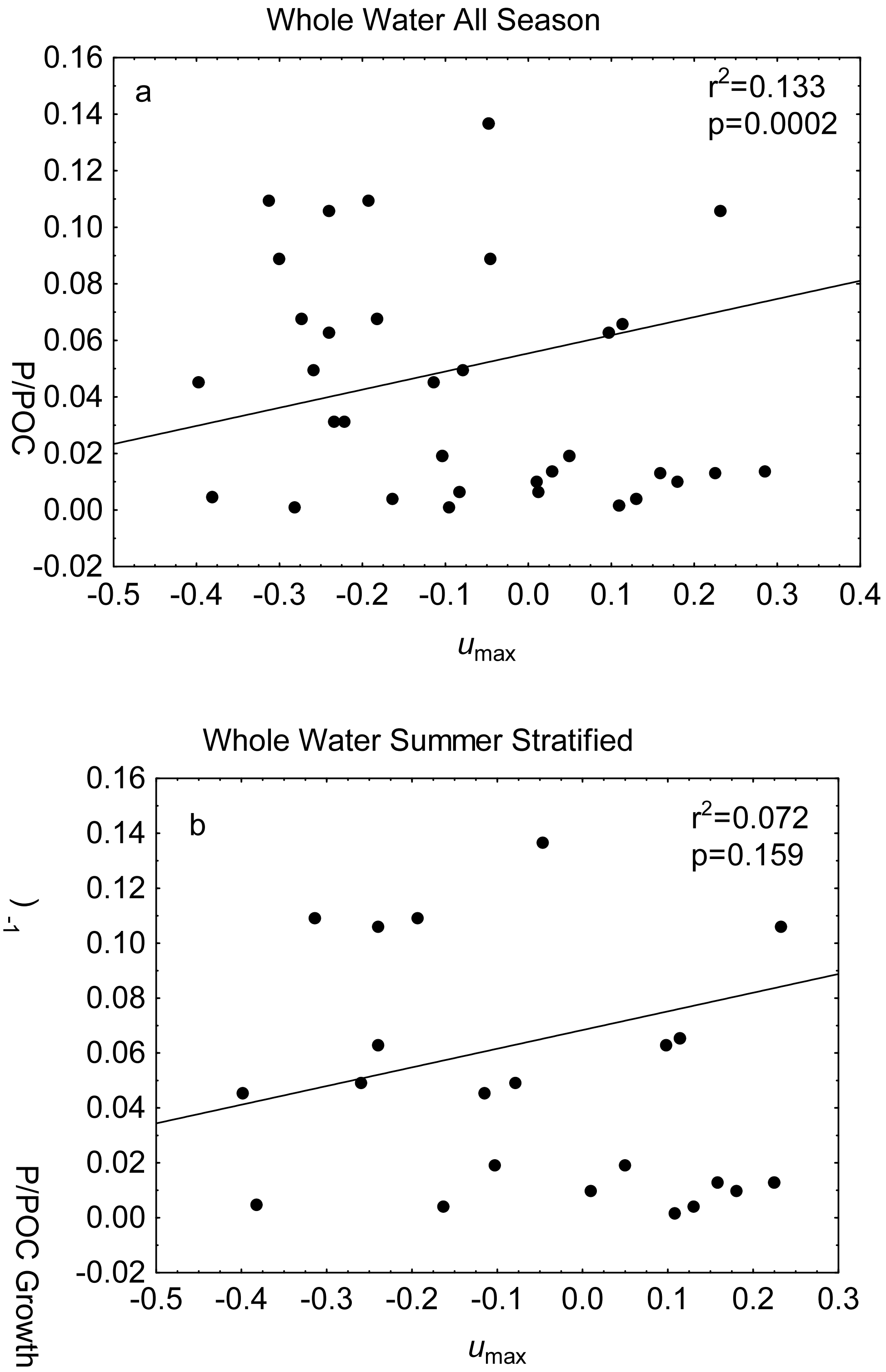


Figure 17. Residual plot resulting from the regression model of chlorophyll concentration during summer stratified conditions as a function of temperature, log(PAR), and the chlorophyll to POC ratio (Equation 3). Solid line is model prediction. Dashed line is 1:1.

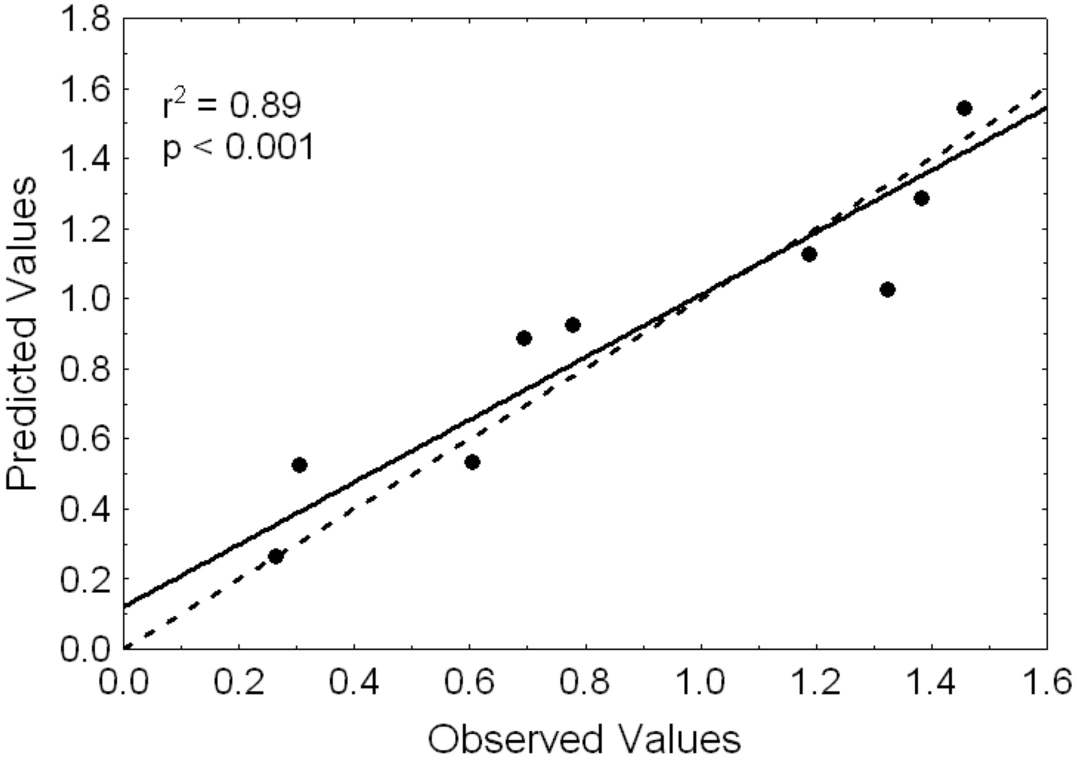


Figure 18. Residual plot resulting from the regression model of POC concentration during summer stratified conditions as a function of temperature, log(PAR), and the chlorophyll to POC ratio (Equation 4). Solid line is the model prediction. Dashed line is 1:1.

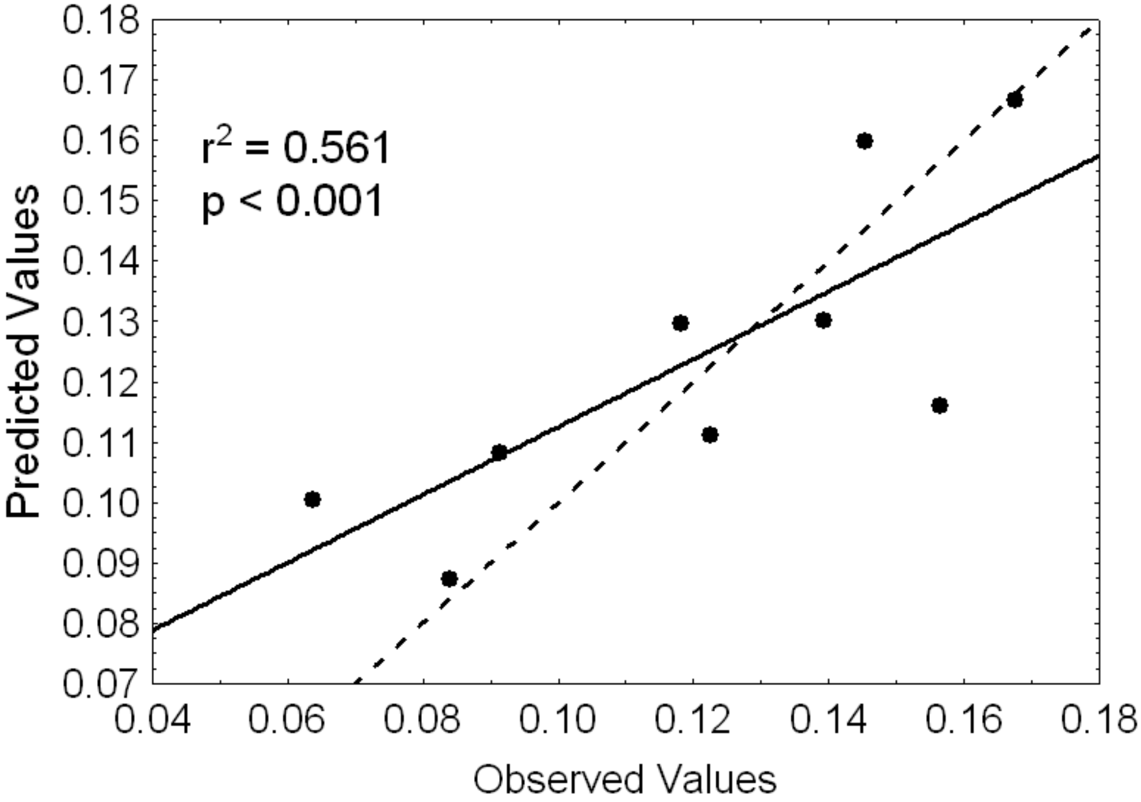


Figure19. Surface water growth experiments mixed with water from depth. The net growth was measured after 36 hours incubation. CARGO5 (a); CARGO6 (b).

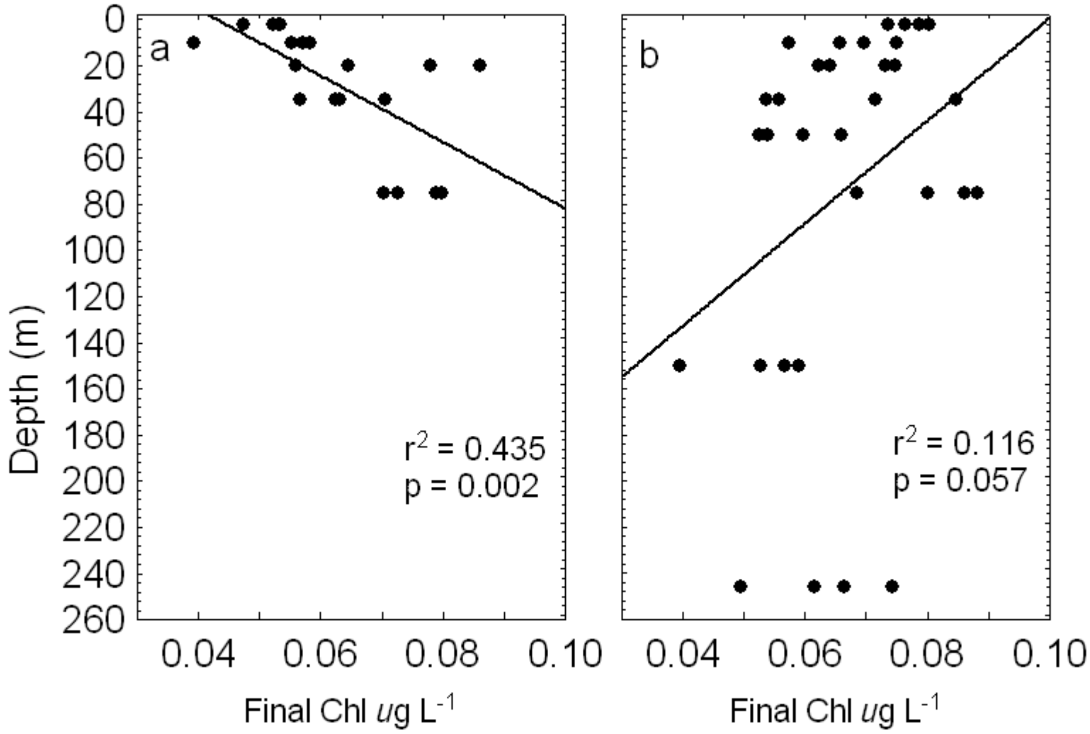


Figure 20. Taxonomic compositions of crustacean macrozooplankton community through cruises. Weather prevented sampling during October 2007 (CARGO2).

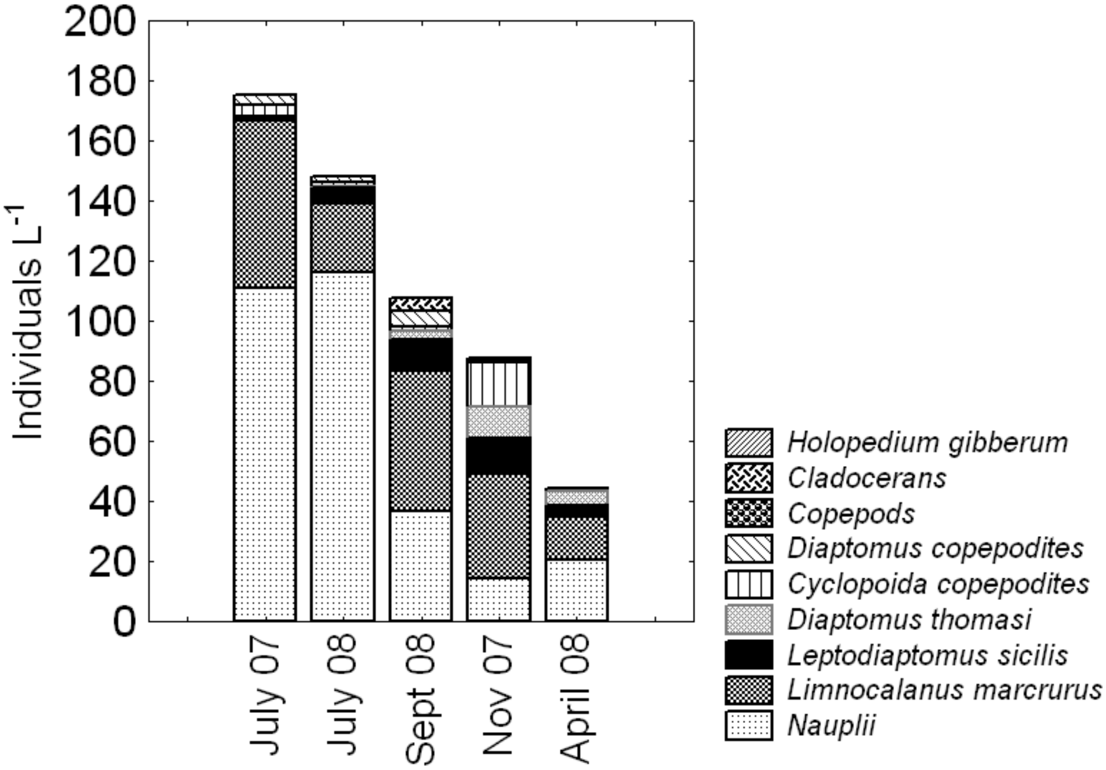
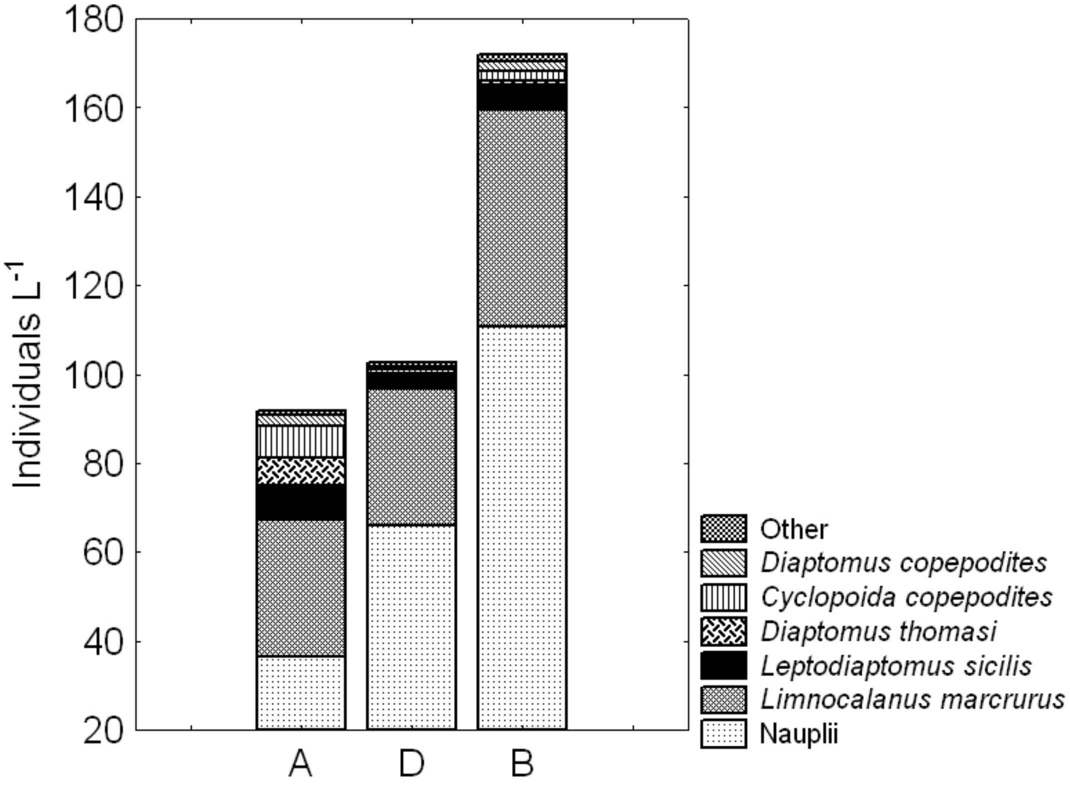


Figure 21. Taxonomic compositions of crustacean macrozooplankton community during summer stratification above (A), within (D), and below (B) the DCM.



Literature Cited

Agis, M., A. Granda, J.R. Dolan 2007. A cautionary note: examples of possible microbial community dynamics in dilution grazing experiments. Journal of Experimental Marine Biology and Ecology. **341**: 176–183.

Andersen, T., A. K. L. Schartau, and E. Paasche. 1991. Quantifying external and internal nitrogen and phosphorus pools, as well as nitrogen and phosphorus supplied through remineralization, in coastal marine plankton by means of a dilution technique. Marine Ecology-Progress Series. **69**: 67-80.

Anagnostou E., R. Sherrell. 2008. A MAGIC method for sub-nanomolar orthophosphate determination in freshwater. Limnology and Oceanography: Methods **6**:64-74.

Auer, M. T., and L. A. Bub. 2004a. Selected features of the distribution of chlorophyll along the southern shore of Lake Superior. Journal of Great Lakes Research. **30:** 269-284.

---. 2004b. Selected features of the distribution of chlorophyll along the southern shore of Lake Superior. Journal of Great Lakes Research **30:** 269-284.

Auer, M. T., and K. D. Powell. 2004. Heterotrophic bacterioplankton dynamics at a site off the southern shore of Lake Superior. Journal of Great Lakes Research **30:** 214-229.

Austin, J.A., and S.M. Colman. 2007. Lake Superior summer water temperatures are increasing more rapidly than regional air temperatures: A positive ice-albedo feedback. Geophysical Research Letters. **34**: L06604, doi:10.1029/2006GL029021.

Austin, J.A., and S.M. Colman. 2008. A century of temperature variability in Lake Superior. Limnology and Oceanography. **53**:2724-2730.

Baehr, M. M., and J. Mcmanus. 2003. The measurement of phosphorus and its spatial and temporal variability in the western arm of Lake Superior. Journal of Great Lakes Research **29:** 479-487.

Barbiero, R. P., R. E. Little, and M. L. Tuchman. 2001. Results from the US EPA's biological open water surveillance program of the Laurentian Great Lakes: III. Crustacean zooplankton. Journal of Great Lakes Research **27:** 167-184.

Barbiero, R.P., L.L. Schacht, M.A. DiMartino. 2000. Effects of the Vertical Distribution of Zooplankton on the Estimation of Abundance and Biovolume Using Deep and Shallow Tows. U.S. EPA, Great Lakes National Program Office, Chicago, IL.

Barbiero, R. P., and M. L. Tuchman. 2001a. Results from the US EPA's biological open water surveillance program of the Laurentian Great Lakes: I. Introduction and phytoplankton results. Journal of Great Lakes Research **27:** 134-154.

---. 2001b. Results from the US EPA's biological open water surveillance program of the Laurentian Great Lakes: II. Deep chlorophyll maxima. Journal of Great Lakes Research **27:** 155-166.

---. 2004. The deep chlorophyll maximum in Lake Superior. Journal of Great Lakes Research **30:** 256-268.

Beckmann, A.,and I. Hense. 2007. Beneath the surface: characteristics of oceanic ecosystems under weak mixing conditions—a theoretical investigation, *Progress in Oceanography.* **75**: 771–796.

Biddanda ,B. Ogdahl, M., Cotner, J., 2001. Dominance of bacterial metabolism in oligotrophic relative to eutrophic waters. Limnology and Oceanography. **46**: 730-739.

Brown, M. E., and D. K. Branstrator. 2004. A 2001 survey of crustacean zooplankton in the western arm of Lake Superior. Journal of Great Lakes Research **30:** 1-8.

Calbet, A. and M.R. Landry. 2004. Phytoplankton growth, microzooplankton grazing, and carbon cycling in marine systems. Limnology and Oceanography. **49:** 51-57.

Carpenter, S. R., Kitchell, J. F., Hodgson, J. R. et al. (1987) Regulation of lake primary productivity by food web structure. Ecology. **68**: 1863–1876.

Carrick, H. J., G. L. Fahnenstiel, E. F. Stoermer, and R. G. Wetzel. 1991. The importance of zooplankton-protozoan trophic couplings in Lake Michigan. Limnology and Oceanography. **36:** 1335-1345.

Chowfraser, P., and W. G. Sprules. 1992. Type-3 functional-response in limnetic suspension-feeders, as demonstrated by in situ grazing rates. Hydrobiologia **232:** 175-191.

Cyr, H., and M.L. Pace, 1993. Magnitude and patterns of herbivory in aquatic and terrestrial ecosystems. Nature. **361**:148–150.

Cullen, J.I. 1982. The deep chlorophyll maximum: comparing vertical profiles of chlorophyll a. Canadian Journal of Fisheries and Aquatic Sciences. **39**: 791 -803.

Dolan, J. R., C. L. Gallegos, and A. Moigis. 2000. Dilution effects on microzooplankton in dilution grazing experiments. Marine Ecology-Progress Series **200:** 127-139.

Dolan J.R., K. McKeon. 2004. The reliability of grazing rate estimates from dilution experiments: Have we over-estimated rates of organic carbon consumption? Ocean Science. **1**:21–36

Dickman, E. M., M. J. Vanni, and M. J. Horgan. 2006. Interactive effects of light and nutrients on phytoplankton stoichiometry. Oecologia **149:** 676-689.

Elser, J.J and Frees, D.L. 1995. Microconsumer Grazing and Sources of Limiting Nutrients for Phytoplankton Growth: Application and Complications of a Nutrient-Deletion/Dilution-Gradient Technique. Limnology and Oceanography. **40.** 1-16

Elser, J. J., Elser, M. M., MacKay, N. A. et al. (1988) Zooplankton mediated transitions between N- and P-limited algal growth. Limnology and Oceanography. **33**: 1–14.

Fahnenstiel, G. L., H. J. Carrick, and R. Iturriaga. 1991. Physiological-characteristeics and food-web dynamics of *Synechococcus* in Lake Huron and Michigan. Limnology and Oceanography. **36:** 219-234.

Fahnenstiel, G.L., J.M. Glime. 1983. Subsurface chlorophyll maximum and associated *Cyclotella* pulse in Lake Superior, USA. Internationale Revue der Gesamten Hydrobiologie. **68**:605-616.

Fahnenstiel, G. L., A. E. Krause, M. J. Mccormick, H. J. Carrick, and C. L. Schelske. 1998. The structure of the planktonic food-web in the St. Lawrence Great Lakes. Journal of Great Lakes Research **24:** 531-554.

Fee, E. J. 1976. The vertical and seasonal distribution of chlorophyll in lakes of the Experimental Lakes Area, northwestern Ontario: implications for primary production estimates. Limnology and Oceanography. **32**:1154–1159.

Fennel, K., and E. Boss. 2003. Subsurface maxima of phytoplankton and chlorophyll: Steady-state solutions from a simple model. Limnology and Oceanography **48:** 1521-1534.

Fennel, K., R. Collier, G. Larson, G. Crawford, and E. Boss. 2007. Seasonal nutrient and plankton dynamics in a physical-biological model of Crater Lake. Hydrobiologia **574:** 265-280.

Field M.P., R.M. Sherrell. 2003. Direct determination of ultra-trace levels of metals in fresh water using desolvating micronebulization and HR-ICP-MS: application to Lake Superior waters. Journal of Analytical Atomic Spectrometry **18**:254-259.

First, M. R., H. L. Miller, P. J. Lavrentyev, J. L. Pinckney, and A. B. Burd. 2009. Effects of microzooplankton growth and trophic interactions on herbivory in coastal and offshore environments. Aquatic Microbial Ecology **54:** 255-267.

Gutierrez-Rodriguez, A., M. Latasa, B. Mourre, and E. A. Laws. 2009. Coupling between phytoplankton growth and microzooplankton grazing in dilution experiments: potential artefacts. Marine Ecology-Progress Series **383:** 1-9.

Gruner, D. S., J. E. Smith, E. W. Seabloom, S. A. Sandin, J. T. Ngai, H. Hillebrand, W. S. Harpole, J. J. Elser, E. E. Cleland, M. E. S. Bracken, E. T. Borer, and B. M. Bolker. 2008. A cross-system synthesis of herbivore and nutrient resource control on producer biomass. Ecology Letters. **11**:740-755.

Hense, I., and A. Beckmann. 2008. Revisiting subsurface chlorophyll and phytoplankton distributions. Deep-Sea Research Part I-Oceanographic Research Papers **55:** 1193-1199.

Hillebrand, H. and others 2009. Herbivore metabolism and stoichiometry each constrain herbivory at different organizational scales across ecosystems. Ecology Letters **12:** 516-527.

Irigoien, X., K. J. Flynn, and R. P. Harris. 2005. Phytoplankton blooms: a 'loophole' in microzooplankton grazing impact? Journal of Plankton Research **27:** 313-321.

Ivanikova, N. V., R. M. L. Mckay, G. S. Bullerjahn, and R. W. Sterner. 2007a. Nitrate utilization by phytoplankton in Lake Superior is impaired by low nutrient (P, Fe) availability and seasonal light limitation - A cyanobacterial bioreporter study. Journal of Phycology **43:** 475-484.

---. 2007b. Nitrate utilization by phytoplankton in Lake Superior is impaired by low nutrient (P, Fe) availability and seasonal light limitation - A cyanobacterial bioreporter study. Journal of Phycology **43:** 475-484.

Klausmeier, C. A., and E. Litchman. 2001. Algal games: The vertical distribution of phytoplankton in poorly mixed water columns. Limnology and Oceanography **46:** 1998-2007.

Klausmeier, C. A., E. Litchman, and S. A. Levin. 2004. Phytoplankton growth and stoichiometry under multiple nutrient limitation. Limnology and Oceanography **49:** 1463-1470.

Kumar, S., R. W. Sterner, and J. C. Finlay. 2008. Nitrogen and carbon uptake dynamics in Lake Superior. Journal of Geophysical Research-Biogeosciences **113**.

Landry, M. R., and A. Calbet. 2005. Reality checks on microbial food web interactions in dilution experiments: response to the comments of Dolan and McKeon. Ocean Science. **1**:39-44.

Landry, M. R., and R. P. Hassett. 1982. Estimating the grazing impact of marince micro-zooplankton. Marine Biology. **67:** 283-288.

Landry, M.R., J. Kirshtein, and J.Constantinou. 1995. A refined dilution technique for measuring the community grazing impact of microzooplankton, with experimental tests in the central equatorial Pacific. Marine Ecology Progress Series. **120**: 53-63.

Landry, M.R., B.C. Monger and K.E. Selph. 1993. Time-dependency of microzooplankton grazing and phytoplankton growth in the subarctic Pacific. Progress in Oceanography. **32**: 239-258.

Lessard, E.J., and M.C. Murrell. 1998. Microzooplankton herbivory and phytoplankton growth in the northwestern Sargasso Sea. Aquatic Microbial Ecology. **16**: 173–188.

Link J., J.H. Selgeby, R.E. Keen. 2004. Changes in the Lake Superior Crustacean Zooplankton Community.Journal of Great Lakes Research. **30**:327-339.

Marañón, E., P.M. Holligan, M. Varela, B. Mouriño and A.J. Bale. 2000. Basin-scale variability of phytoplankton biomass, production and growth in the Atlantic Ocean. Deep-Sea Research Part I. **47:** 825–857.

McNaught, D. C., M. Buzzard, D. Griesmer, and M. Kennedy. 1980. Zooplankton grazing and population dynamics relative to water quality in southern Lake Huron. U.S. Environmental Protection Agency, Ecological Research Service. EPA-600/3-80-069.

Modenutti, B., E. Balseiro, C. Callieri, C. Queimalinos, and R. Bertoni. 2004. Increase in photosynthetic efficiency as a strategy of planktonic organisms exploiting deep lake layers. Freshwater Biology. **49:** 160-169.

Modigh, M. and G. Franze. 2009. Changes in phytoplankton and microzooplankton populations during grazing experiments at a Mediterranean coastal site. Journal of Plankton Research. **31**: 853-864.

Moll, R. A. and E. F. Stoermer. 1982. A hypothesis relating trophic status and subsurface chlorophyll maxima of lakes. Archiv für Hydrobiologie. **94**:425–440.

Munawar, M., and Munawar, I.F. 1978. Phytoplanktonof Lake Superior 1973. Journal of Great Lakes Research. **4**:415–442.

Nalewajko, C., and D. Voltolina. 1986. Effects of environmental variables on growth-rates and physiological-characteristics of Lake Superior phytoplankton. Canadian Journal of Fisheries and Aquatic Sciences **43:** 1163-1170.

Nriagu, J. O., G. Lawson, H. K. T. Wong, and V. Cheam. 1996. Dissolved trace metals in Lakes Superior, Erie, and Ontario. Environmental Science and Technology. **30**: 178-187.

Patalas, K. 1990. Diversity of the zooplankton communities in Canadian lakes as a function of climate. Internationale Revue der Gesamten Hydrobiologie. 24:360-368.

Patalas. K. and A. Salki. 1992. Crustacean Plankton in LakeWinnipeg: variation in space and time as a function of lake morphology, geology, and climate. Canadian Journal of Fisheries and Aquatic Sciences. **49**: 1035–1059.

Pilati, A. and W.A. Wurtsbaugh. 2003. Importance of zooplankton for the persistence of a deep chlorophyll layer: a limnocorral experiment. Limnology and Oceanography. **48**: 249–260.

Pomeroy, L.R. and D. Deibel. 1986. Temperature regulation of bacterial activity during the spring bloom in Newfoundland coastal waters. Science. **223**: 359-36.

Olli, K., and J. Seppala. 2001. Vertical niche separation of phytoplankton: large-scale mesocosm experiments. Marine Ecology Progress Series **217**:219-233.

Olson, T.A., T.O. Odlaug. (1966) Limnological observation on Western Lake Superior. University of Michigan. Great Lakes Res. Div. Publ. **15**:109-118.

Olson, M.B. and S.L. Strom. 2002. Phytoplankton growth, microzooplankton herbivory and community structure in the southeast Bering Sea: insight into the formation and temporal persistence of an *Emiliania huxleyi* bloom. Deep-Sea Research II. **49**: 5969-5990.

Russ, M. E., N. E. Ostrom, H. Gandhi, P. H. Ostrom, and N. R. Urban. 2004. Temporal and spatial variations in R : P ratios in Lake Superior, an oligotrophic freshwater environment. Journal of Geophysical Research-Oceans **109**.

Saros, J. E., S. J. Interlandi, S. Doyle, T. J. Michel, and C. E. Williamson. 2005. Are the deep chlorophyll maxima in alpine lakes primarily induced by nutrient availability, not UV avoidance? Arctic Antarctic and Alpine Research **37:** 557-563.

Sawatzky, C. L., W. A. Wurtsbaugh, and C. Luecke. 2006. The spatial and temporal dynamics of deep chlorophyll layers in high-mountain lakes: effects of nutrients, grazing and herbivore nutrient recycling as growth determinants. Journal of Plankton Research **28:** 65-86.

Schampel, J.H. 2001. Herbivory and bacterivory in the pelagic food web of western Lake Superior. M.S. University of Minnesota, St. Paul.

Sommer, U. 1982. Vertical niche separation between two closely related planktonic flagellate species (Rhodomonas lens and Rhodomonas minuta var. nannoplanctica). Journal of Plankton Research. 4:137–142.

Sprules, W. G., and J. D. Stockwell. 1995. Size-based biomass and production models in the St. Lawerence Great Lakes. ICES Journal of Marine Science. **52**: 705-710.

Sterner, R.W. 1989. The role of grazers in phytoplankton succession. In U. Sommer [ed.], Plankton ecology: Succession in plankton communities. Springer.107-l 70.

Sterner, R.W. 2009. Role of Zooplankton in aquatic ecosystems. Encyclopedia of Inland Waters, Elsevier.

Sterner, R.W. Submitted. In situ measured primary production in Lake Superior. Journal of Great Lakes Research. GLR-D-09-00084

Sterner R.W., Chrzanowski T.H., Elser J.J., George N.B. (1995). Sources of nitrogen and phosphorus supporting the growth of bacterio- and phytoplankton in an oligotrophic Canadian shield lake. Limnology and Oceanography. **40**:242-249.

Sterner, R. W., T. M. Smutka, R. M. L. Mckay, X. M. Qin, E. T. Brown, and R. M. Sherrell. 2004. Phosphorus and trace metal limitation of algae and bacteria in Lake Superior. Limnology and Oceanography **49:** 495-507.

Strom, S. L., M. A. Brainard, J. L. Holmes, and M. B. Olson. 2001. Phytoplankton blooms are strongly impacted by microzooplankton grazing in coastal North Pacific waters. Marine Biology **138:** 355-368.

Sprules, W.G., and Jin, E.H. 1990. Composition and size structure of zooplankton communities in the St. Lawrence Great Lakes. Verhandlungen der Internationalen Vereinigung fur Theoretische und Angewandte Limnologie. **24**:379–382.

Teixeira, I.G., and Figueiras, F.G. 2009. Feeding behaviour and non-linear responses in dilution experiments in a coastal upwelling system. Aquatic Microbial Ecology. **55**: 53 -63.

Urban, N. R., D. S. Apul, and M. T. Auer. 2004. Community respiration rates in Lake Superior. Journal of Great Lakes Research **30:** 230-244.

Urban, N. R., M.T. Auer, S.A. Green, X. Lu, D.S. Apul, K.D. Powell, L Bub. 2005. Carbon cycling in Lake Superior. Journal of Geophysical Research-Oceans. **110**: C06S90.

Venrick, E.L., McGowan, J.A., and Mantyla, A.W. 1973. Deep maxima of photosynthetic chlorophyll in the Pacific Ocean. Fisheries Bulletin. (U.S.) **71**:41–52.

Watson, N.H.F., K.P.B. Thomson, and F.C. Elder.1975. Sub-thermocline biomass concentration detected by transmissometer in Lake Superior. . Verhandlungen der Internationalen Vereinigung fur Theoretische und Angewandte Limnologie. **19**:682–688.

Welschmeyer, N. 1994. Fluorometric analysis of Chlorophyll a in the presence of Chlorophyll b and pheopigments. Limnology and Oceanography. **39**: 1985-1992.

Yoshiyama, K., J. P. Mellard, E. Litchman, and C. A. Klausmeier. 2009. Phytoplankton Competition for Nutrients and Light in a Stratified Water Column. American Naturalist **174:** 190-203.

Appendix

Table 1. Grazing sample summary and data associated with grazing measurements. For latitude and longitude of sampling sites, see text.

Cruise	Inc. Located	Depth (m)	Size Frac. (μm)	Grazing (Linear or Non)	Related to DcarM	Related to DCM	Grazing (day-1)	Weight (1/SE)
CAR GO1	Deck	20	Whole	L	DcarM	Above	0.44	5.10
CAR GO1	Deck	32	Whole	L	Below	DCM	0.31	9.70
CAR GO1	Lab	5	Whole	L	Above	Above	0.35	4.41
CAR GO1	Lab	20	Whole	N	DcarM	Above	0.32	1.25
CAR GO1	Lab	32	Whole	L	Below	DCM	0.00	4.69
CAR GO2	Deck	5	Whole	N	Non-Strat	Non-Strat	0.40	2.27
CAR GO2	Deck	15	Whole	N	Non-Strat	Non-Strat	0.24	1.23
CAR GO2	Deck	25	Whole	N	Non-Strat	Non-Strat	0.17	0.62
CAR GO3	Deck	5	Whole	L	Non-Strat	Non-Strat	0.16	16.48
CAR GO3	Deck	15	Whole	N	Non-Strat	Non-Strat	0.30	1.61
CAR GO3	Deck	25	Whole	N	Non-Strat	Non-Strat	0.09	0.66
CAR GO3	Lab	5	Whole	L	Non-Strat	Non-Strat	0.15	17.90
CAR GO3	Lab	15	Whole	N	Non-Strat	Non-Strat	0.66	3.63
CAR GO3	Lab	25	Whole	N	Non-Strat	Non-Strat	0.19	0.68
CAR GO4	Deck	5	Whole	N	Non-Strat	Non-Strat	0.06	0.15
CAR GO4	Deck	20	Whole	N	Non-Strat	Non-Strat	0.00	0.69
CAR GO4	Deck	60	Whole	N	Non-Strat	Non-Strat	0.10	0.21

CAR GO4	Lab	5	Whole	L	Non-Strat	Non-Strat	0.19	4.08
CAR GO4	Lab	20	Whole	L	Non-Strat	Non-Strat	0.00	15.02
CAR GO4	Lab	60	Whole	N	Non-Strat	Non-Strat	0.00	9.47
CAR GO5	Deck	2	Whole	N	Above	Above	0.35	0.62
CAR GO5	Deck	10	Whole	N	Above	Above	0.30	0.21
CAR GO5	Deck	20	Whole	N	DcarM	Above	0.07	1.99
CAR GO5	Deck	35	Whole	N	Below	DCM	0.16	1.16
CAR GO5	Deck	50	Whole	N	Below	Below	0.14	0.82
CAR GO5	Deck	75	Whole	N	Below	Below	0.25	1.01
CAR GO5	Lab	10	Whole	N	Above	Above	0.52	16.48
CAR GO5	Lab	25	Whole	N	Above	DCM	0.38	0.26
CAR GO5	Lab	35	Whole	N	DcarM	Below	0.06	0.38
CAR GO5	Lab	50	Whole	N	Below	Below	0.37	1.15
CAR GO5	Lab	75	Whole	N	Below	Below	0.07	0.11
CAR GO6	Deck	2	Whole	N	Above	Above	0.10	0.43
CAR GO6	Deck	10	Whole	N	DcarM	Above	0.16	1.24
CAR GO6	Deck	20	Whole	N	DcarM	DCM	0.02	0.09
CAR GO6	Deck	35	Whole	N	Below	Below	0.23	1.02
CAR GO6	Deck	50	Whole	L	Below	Below	0.00	0.40
CAR GO6	Deck	75	Whole	L	Below	Below	0.00	0.04
CAR GO6	Lab	2	Whole	L	Above	Above	0.17	1.20
CAR GO6	Lab	10	Whole	N	DcarM	Above	0.20	0.00
CAR GO6	Lab	20	Whole	N	DcarM	DCM	0.08	0.32

CAR GO6	Lab	35	Whole	N	Below	Below	0.49	2.48
CAR GO6	Lab	50	Whole	L	Below	Below	0.11	40.87
CAR GO6	Lab	75	Whole	L	Below	Below	0.49	2.58
CAR GO4	Deck	5	Ten	N	Non-Stratified	Non-Stratified	0.22	1.31
CAR GO4	Deck	20	Ten	N	Non-Stratified	Non-Stratified	0.05	0.42
CAR GO4	Deck	60	Ten	L	Non-Stratified	Non-Stratified	0.00	0.32
CAR GO4	Lab	5	Ten	L	Non-Stratified	Non-Stratified	0.34	5.33
CAR GO4	Lab	20	Ten	N	Non-Stratified	Non-Stratified	0.09	0.14
CAR GO4	Lab	60	Ten	L	Non-Stratified	Non-Stratified	0.00	12.43
CAR GO5	Deck	2	Ten	N	Above	Above	0.49	0.87
CAR GO5	Deck	10	Ten	N	Above	Above	0.41	1.50
CAR GO5	Deck	20	Ten	L	DcarM	Above	0.07	14.87
CAR GO5	Deck	35	Ten	N	Below	DCM	0.13	0.92
CAR GO5	Deck	50	Ten	L	Below	Below	0.00	4.98
CAR GO5	Deck	75	Ten	N	Below	Below	0.16	1.09
CAR GO5	Lab	2	Ten	L	Above	Above	0.08	3.50
CAR GO5	Lab	10	Ten	N	Above	Above	0.40	1.91
CAR GO5	Lab	25	Ten	N	Above	DCM	0.09	0.46
CAR GO5	Lab	35	Ten	N	DcarM	Below	0.19	1.02
CAR GO5	Lab	50	Ten	L	Below	Below	0.00	5.42

CAR GO5	Lab	75	Ten	L	Below	Below	0.32	5.49
CAR GO6	Deck	2	Ten	N	Above	Above	0.12	0.35
CAR GO6	Deck	10	Ten	N	DcarM	Above	0.29	1.74
CAR GO6	Deck	20	Ten	N	DcarM	DCM	0.57	1.66
CAR GO6	Deck	35	Ten	N	Below	Below	0.40	2.72
CAR GO6	Deck	50	Ten	N	Below	Below	0.14	1.19
CAR GO6	Deck	75	Ten	N	Below	Below	0.25	0.75
CAR GO6	Lab	2	Ten	N	Above	Above	0.74	1.17
CAR GO6	Lab	10	Ten	N	DcarM	Above	0.42	1.55
CAR GO6	Lab	20	Ten	N	DcarM	DCM	0.27	0.71
CAR GO6	Lab	35	Ten	L	Below	Below	0.19	4.81
CAR GO6	Lab	50	Ten	N	Below	Below	0.13	0.29
CAR GO6	Lab	75	Ten	N	Below	Below	0.07	0.14