

UNIVERSITÉ DU QUÉBEC À MONTRÉAL

EFFECTS OF ELEVATED CARBON DIOXIDE ON FRESHWATER PLANKTON
COMMUNITY DYNAMICS

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN BIOLOGY

BY
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UNIVERSITÉ DU QUÉBEC À MONTRÉAL

LES EFFETS D'UNE HAUSSE DU DIOXYDE DE CARBONE SUR LES
INTERACTIONS DES COMMUNAUTES PLANCTONIQUE EN EAU DOUCE

MÉMOIRE PRÉSENTÉ COMME EXIGENCE PARTIELLE DE LA MAÎTRISE
EN BIOLOGIE

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JULIA HART

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LIST OF ABBREVIATIONS AND ACRONYMS

CO₂ – Carbon dioxide

ppm – parts per million

CCM – Carbon Concentrating Mechanism

Chl*a* – Chlorophyll *a*

GREENS – chlorophytes measured by Fluoroprobe

CYANOS – cyanophytes with pigment phycocyanin measured by Fluoroprobe

BROWNS – diatoms, dinoflagettes and chrysophytes measured by Fluoroprobe

CRYPTOS – cryophytes and cyanophytes with pigment phycoerythrin measured by Fluoroprobe

DO – dissolved oxygen

FP - Fluoroprobe

RÉSUMÉ

Les changements climatiques engendreront une augmentation des précipitations et une augmentation de la vitesse du vent. Ces changements physiques sont, à leur tour, susceptibles d'augmenter le transport de carbone organique dans les lacs par le ruissellement, entraînant une augmentation des concentrations de dioxyde de carbone dissous (CO_2) par le processus de respiration microbienne. Des expériences de laboratoire effectuées récemment suggèrent que l'augmentation du CO_2 dans les écosystèmes d'eaux douces aurait des effets importants sur la production primaire et sur les dynamiques de la communauté planctonique. Cependant, peu de travaux existent dans les milieux naturels qui appuient ces résultats. Pour intégrer les études de laboratoires précédentes, nous avons effectué une manipulation expérimentale à grande échelle sur l'ensemble d'un l'écosystème lacustre stratifié situé dans la région nord-tempérée. En utilisant de grandes quantités de glace sèche, nous avons doublé les niveaux de pCO_2 épilimnétiques (de 700 ppm à 1400ppm). Modélisation de la productivité primaire nette basée sur les valeurs mesurées aux côtés PAR niveaux de pCO_2 intégrés montré la productivité primaire nette devrait avoir augmenté dans le bassin expérimental au cours de la manipulation. Cette augmentation du CO_2 à entrainer des grands changements dans les communautés phytoplanctoniques et zooplanctoniques, comparativement à ceux du bassin de contrôle. Le changement le plus significatif et inattendu a été la perte totale du maximum profond de chlorophylle, qui est toujours présent au milieu de l'été dans ce lac. Des changements imprévus dans la composition de la communauté phytoplanctonique ont également été observées. La biomasse des cyanobactéries a bénéficié de l'augmentation du CO_2 tandis que la biomasse de groupes dominants, tels que les chlorophytes et les chrysophytes, a diminué. Ces changements sont attribués à l'augmentation rapide de la biomasse de zooplancton suite à la manipulation du pCO_2 . Cette nouvelle biomasse zooplanctonique réduit la biomasse du phytoplancton, malgré des niveaux élevés de production primaire, et semble favoriser la dominance des cyanobactéries, qui sont difficiles à brouter. Le zooplancton ont également changé leur comportement en modifiant leur position typique dans la colonne d'eau et en choisissant des profondeurs autres que ceux où le maximum profond de chlorophylle serait normalement situé. Les résultats de cette expérience à grande échelle indiquent que des quantités élevées de pCO_2 pourraient avoir des impacts sur les communautés et les écosystèmes qui dépendent fortement de la structure et du comportement des organismes dans la chaîne alimentaire d'une manière qui ne peut pas être entièrement prévisibles lors des études à plus petites échelles.

ABSTRACT

Increased precipitation and wind speeds anticipated with climate change are likely to augment the runoff of organic carbon to north temperate lakes, resulting in greater dissolved concentrations of carbon dioxide (CO₂) via microbial respiration. Recent lab experiments indicate significant effects of such CO₂ increases on freshwater ecosystems, especially with respect to phytoplankton community dynamics; however, little *in situ* work exists to substantiate these results. In a whole-ecosystem experimental manipulation of a stratified lake, we demonstrated that when epilimnetic dissolved CO₂ levels were doubled (pCO₂ raised from 700ppm to 1400ppm) both phytoplankton and zooplankton experienced significant community changes, relative to those observed in a control lake basin. Modelling of net primary productivity based on measured PAR values alongside integrated pCO₂ levels showed net primary productivity should have increased in the experimental basin during the course of the manipulation. An unexpected change was the large decline of phytoplankton biomass, primarily at the location of the perennially present deep chlorophyll maximum. Significant unanticipated changes in phytoplankton community composition were also observed with cyanobacteria benefitting the most, and normally dominant groups like chlorophytes and chrysophytes declining. These changes were attributable to changes in the trade-off between light and CO₂ alongside rapid increases in zooplankton biomass following CO₂ manipulation, resulting in large reductions in phytoplankton community biomass, despite evidence of higher levels of primary productivity. The results of this large-scale experiment indicate that elevated dissolved CO₂ could have community and ecosystem impacts that are highly dependent on behavior and food web structure, in ways that could not be anticipated from smaller-scale studies.

INTRODUCTION

Climate change is anticipated to be one of the biggest environmental problems affecting the entire globe in the near future. The scientific community has always been interested in understanding and attempting to predict how climate change will affect ecosystems and communities. Aquatic ecosystems are vital for all life and alterations to these very important ecosystems could lead to significant impacts. Inland waters play a major role in both resources and recreation. It is essential to understand how aquatic systems and all the encompassed biota will react to future climate change. Carbon dioxide has been increasingly studied in scientific literature as the continually rising atmospheric levels are alarming. While many freshwater lakes may not be directly affected by rising atmospheric levels, lakes will likely exhibit an increase of carbon dioxide levels in the water column due to indirect effects of climate change including altered precipitation and wind speeds. Changes in carbon dioxide levels are expected to have significant effects on the phytoplankton community, which exists as the base on the aquatic food web. Any changes in phytoplankton species diversity, biomass or spatial distribution may have the potential to cause significant changes for the entire aquatic food web, starting with the zooplankton who feed directly upon the phytoplankton.

The goal of this research was to assess the effects of elevated carbon dioxide levels on freshwater plankton community dynamics. The effects of high levels of carbon dioxide had previously only been studied under highly controlled laboratory conditions. This research project involved a unique experiment that applied previous laboratory experimental manipulations to a large-scale lake basin manipulation. Having such a large-scale experiment allowed for the complexity of natural systems to be taken into account.

This experiment was conducted in Lac Croche at the Station de Biologie des Laurentides, St. Hippolyte, Quebec, Canada during summer 2013 (June-September). This introductory section contains current knowledge on the subject matter. The thesis consists of one chapter in the form of a scientific article for publication. The second author is Yves Prairie who has been vital in the planning and execution of the experiment and in giving advice to interpret results throughout the writing process. The third author is my supervisor Beatrix Beisner who commented, corrected and gave advice throughout the research project and writing process.

0.1 Literature Review

0.1.1 Aquatic ecosystems and climate change

Scientists have been striving to better understand how climate change will occur and the impacts it will have on ecosystems across the globe. One of the most pervasive aspects of climate change is the atmospheric increase in the greenhouse gas carbon dioxide (CO₂). Atmospheric carbon dioxide (CO₂) levels have continued to rise since pre-industrial times and a record-high reading of over 400ppm has recently been observed (Showstack, 2013). Without major changes in human activities, these levels are not expected to decrease, but rather, to almost double in the next 100 years (Solomon *et al.* 2007). A large proportion of research dedicated to this increase in CO₂ has involved monitoring the responses of terrestrial ecosystems and land plants (Cure & Acock 1986; Agrell *et al.* 2000). Such research has suggested that high atmospheric CO₂ should increase growth and photosynthesis, with species-specific responses (Ainsworth & Long, 2005). However, there is little experimental data on the changes expected in the structure and function of freshwater communities (but see: Urabe *et al.* 2003; Low-Decarie *et al.* 2011). According to Rost *et al.* (2007),

extensive experiments are required to assess and predict the response of aquatic communities of the future.

In unproductive freshwater ecosystems, carbon has long been thought to have little effect over biological processes, unlike the effects of the limited supplies of nitrogen and phosphorus. After the pioneering work of Schindler (1977) and Shapiro (1988), there was a general school of thought amongst freshwater biologists that carbon did not play much of a role as a limiting nutrient for primary production (Jansson *et al.* 2012). However, recent studies of freshwater ecosystems show that rates of photosynthesis in the phytoplankton actually increased with augmented levels of dissolved carbon indicating that carbon can be in limiting supply and thus, phytoplankton must have some competitive interactions around it (Urabe *et al.* 2003; Spijkerman, 2010; Jansson *et al.* 2012).

0.1.2 Carbon in freshwater ecosystems

Inorganic carbon is found in freshwater ecosystems in three main forms: free carbon dioxide, bicarbonate and carbonate. The three forms exist in an equilibrium controlled by pH levels (Wetzel, 2001). The equilibrium favors free CO₂ when pH < 6.3, bicarbonate is favoured when pH is between 6.3 and 10.2 and carbonate is favoured when pH > 10.2 (Holland *et al.* 2012). The majority of freshwater carbon is found in the form of bicarbonate (Wetzel, 2001). Phytoplankton utilize free carbon dioxide for photosynthesis. Few phytoplankton are able to utilize other forms of inorganic carbon.

Recent studies have shown that the majority of freshwater lakes are actually supersaturated with carbon dioxide (Cole *et al.* 1994; Jonsson *et al.* 2003; Downing *et al.* 2006; Jansson *et al.* 2012). This means that lakes have greater concentrations of CO₂ than the atmosphere. Cole *et al.* (1994) found that of 1835 lakes sampled worldwide, less than 10% were close to equilibrium with the atmosphere. A second worldwide survey of 4902 lakes found mean pCO₂ values of 1287ppm (Sobek *et al.*

2005). A survey of 33 unproductive boreal lakes found the mean pCO₂ level to be even higher at 1762 ppm (Sobek *et al.* 2003). The source of supersaturation can be high rates of bacterial decomposition (Cole & Caraco, 2001; Prairie *et al.* 2002), driven by external inputs from streams (Jones & Mulholland, 1998) or a vast majority comes from terrestrial primary production in the surrounding watershed (Cole & Caraco, 2001). This high level of carbon dioxide (supersaturation) does not mean that levels have reached a peak, but many systems have the potential to continue to rise even higher (Holland *et al.* 2012).

The direct effects of elevated atmospheric carbon dioxide on freshwater lakes are not expected to be significant as the majority of these lakes have concentrations of CO₂ greater than the atmosphere. Therefore a slight increase in atmospheric levels should not impact levels within the water column. However, climate change is anticipated to *indirectly* impact lake carbon dioxide levels. In the northern hemisphere, wind speeds are expected to increase through time (Collins *et al.* 2013). Changes to the wind regimes will influence the stratification of lakes, which have been shown recently to alter the pCO₂ dynamics of lakes (Mercier-Blais *et al.* 2014). Higher temperatures are also expected to occur throughout the northern hemisphere. Higher temperatures allow for longer growing periods and can be expected to increase photosynthesis and plant growth on land. This combined with increased precipitation will likely increase the amount of organic matter being brought into aquatic systems. Organic matter will stimulate bacterial respiration, which produces carbon dioxide. The bacterial loop is known to be one of the main factors contributing to the supersaturation of carbon dioxide in unproductive lakes. Therefore, in the future, if these changes do take place, it can be expected that the stimulated bacterial loop will increase its production of carbon dioxide. And, if the pool of inorganic carbon changes in an aquatic ecosystem, the primary producers (phytoplankton) that rely on carbon as sources of energy, may see some community changes.

0.1.3 Phytoplankton ecology

Phytoplankton is the broad umbrella term that encompasses the photoautotrophic primary producers of aquatic systems (Reynolds, 2006). The term photoautotroph refers to organisms that can take light energy and convert it into useable chemical energy (Reynolds, 2006), a process known as photosynthesis. In order for photosynthesis to occur, phytoplankton require light and CO₂. A few phytoplankton species are heterotrophic or mixotrophic. Heterotrophy refers to when microalgae use carbon obtained from the breakdown of an organic substance (Wetzel, 2001), allowing them to survive in the dark. Mixotrophic phytoplankton can assimilate carbon dioxide for photosynthesis simultaneously with organic compounds (Wetzel, 2001). Phytoplankton encompasses a very diverse group and can be further divided into categories based on size as well as based on physiological characteristics. They are the base of the aquatic food web, providing available chemical energy for higher trophic levels. They are the producers of close to half the global primary production (Falkowski *et al.* 2008). Thus, they are vital to consider when assessing aquatic community dynamics.

The main groups of phytoplankton that this research focused on are cyanobacteria (blue-green algae), chrysophytes (golden-brown algae), bacillariophytes (diatoms), chlorophytes (green algae), and cryptophytes. The major species of phytoplankton found in the study lake (Lac Croche) can be found in Appendix A. All phytoplankton contain the photosynthetic pigment chlorophyll *a*, making the assessment of its concentration in a water sample an ideal method to quantify phytoplankton biomass. Individual species of phytoplankton contain specific pigments other than chlorophyll *a* such as carotenoids, xanthophylls and biliproteins (Wetzel, 2001).

Competition is an interaction that shapes the composition of all ecosystems. In aquatic ecosystems, phytoplankton, like any other organism, compete for limiting resources such as nutrients and light. It is this competition along with other biological processes, and resistance to predators that define the niches of phytoplankton

(Margalef, 1978). The principle of *competitive exclusion* would suggest that in homogeneous environments, species that are in competition for the same resources cannot co-exist and one species will dominate (Hardin, 1960; Armstrong & McGehee, 1980). However, phytoplankton seemingly defy this principle and as Hutchinson (1961) outlined in what is known as “the paradox of the plankton”, many aquatic systems can have numerous species of phytoplankton all competing for very similar resources.

As higher levels of CO₂ are expected to occur in freshwater ecosystems, the competitive interactions for carbon will change. Because phytoplankton represent a very functionally diverse assemblage, different species have varying abilities to take up carbon (Low-Decarie *et al.* 2011). If carbon is no longer limiting, species that had a competitive edge owing to faster carbon uptake rates for photosynthesis, will no longer have the upper hand. These shifts in interactions may produce significant changes in terms of overall biomass as well as relative species abundance. Phytoplankton can respond to an environmental change through altering community composition, along with total biomass (Li *et al.* 2009). Thus, it is crucial to understand possible patterns in such community composition and growth of phytoplankton in order to predict any changes associated with climate change (Litchman *et al.* 2012).

0.1.4 Trade-Off between Light and Carbon Dioxide

A sufficient supply of CO₂ is required for phytoplankton to perform continuous photosynthesis. Despite the majority of lakes actually having levels of CO₂ higher than the atmosphere, carbon dioxide can still be limiting (Jansson *et al.* 2012). Additionally, carbon dioxide is not the only limiting factor that phytoplankton must obtain. Light is compulsory for photosynthesis and can exert significant control over phytoplankton communities. Light declines with depth (Kirk, 1994) and conversely CO₂ and other nutrients increase with depth (Zehr & Ward, 2002). Thus,

there exists a trade-off for phytoplankton. This relationship is well documented in stratified systems (Yoshiyama *et al.* 2009) and it is just one relationship that can significantly influence phytoplankton communities throughout the water column.

One such way that light and carbon dioxide are seen to influence phytoplankton community structure is in the location of phytoplankton across the water column. Often light and carbon dioxide are both optimized near the metalimnion. The metalimnion is considered favorable as nutrients like CO₂ are high, and light levels are still intense enough for rapid productivity. This is often seen to be a favorable location for phytoplankton. When phytoplankton biomass is highly concentrated a deep chlorophyll maximum (DCM) is formed (Fee, 1976). And due to these factors, DCMs are often located near the metalimnion.

0.1.5 Carbon acquisition

Phytoplankton use the enzyme ribulose biphosphate carboxylase-oxygenase (Rubisco) in the carbon fixing process of photosynthesis (Holland *et al.* 2012). Rubisco is located in micro-compartments called carboxysomes (Price, 2011). In most phytoplankton, Rubisco is only at half capacity with CO₂ levels equal to atmospheric levels, which indicates that carbon can limit photosynthetic efficiency (Badger *et al.* 1998). Many species have developed mechanisms to overcome the low levels of CO₂ and to use other carbon sources (Maberly *et al.* 2009). These mechanisms are called carbon concentrating mechanisms (CCMs). Many freshwater phytoplankton, along with all cyanobacteria are known to possess CCMs (Price, 2011). CCMs are active processes requiring energy that can be utilized if sufficient nutrients and light are present. These processes, if employed, may reduce energy available for growth and development.

One common CCM involves the ability to access the large pool of dissolved bicarbonate in the water column, which is usually much larger than the pool of

dissolved CO₂ (Wetzel, 2001). The CCM involves actively pumping bicarbonate into the algal cell and using the enzyme carbonic anhydrase to convert bicarbonate into carbon dioxide (Urabe *et al.* 2003; Maberly *et al.* 2009). These processes create elevated levels of CO₂ in the carboxysomes, which Rubisco is able to utilize (Price, 2011). Although energetically expensive, these processes do provide an advantage in competition for available carbon sources. Cyanobacteria are one group known to possess this CCM and have been shown to succeed in environments with lower concentrations of CO₂ (Shapiro, 1997). However, not all phytoplankton are able to use bicarbonate.

Chrysophytes, a group of diverse phytoplankton, have been found to not possess the CCM allowing the use of bicarbonate (Maberly *et al.* 2009). This forces chrysophytes to be more reliant on the levels of dissolved CO₂ than other phytoplankton with CCMs, like cyanobacteria. Higher CO₂ concentrations in the water column will decrease the need to run CCMs, which creates less energetically demanding growing conditions (Giordano *et al.* 2005; Hollan *et al.* 2012). With increased CO₂, phytoplankton will not need to depend on CCMs to fulfill carbon requirements. This could negate the advantage that phytoplankton with CCMs have over those that do not, such as chrysophytes (Maberly *et al.* 2009). As competition for carbon changes, there may be a shift in the phytoplankton community composition. Given that phytoplankton form the base of the aquatic food web, any changes to the phytoplankton community can be passed onto other trophic levels, (Sterner & Elser, 2002; Van de Waal *et al.* 2010) and potentially cascade throughout the entire freshwater ecosystem. A very pronounced effect can be seen on zooplankton that feed directly on phytoplankton. Zooplankton rely on phytoplankton for nutrition, and therefore any change that occurs in phytoplankton abundance has potential to alter zooplankton populations as well.

0.1.6 Zooplankton ecology

Zooplankton are a diverse group of heterotrophic plankton that make up the second trophic level of the aquatic food web. They rely heavily on phytoplankton for chemical energy and play a very important role in the aquatic food web as they pass energy from the primary producers to consumers in higher trophic levels (primarily fish). Zooplankton are often classified based on taxonomy as well as size. The two major size groups often are: microzooplankton (<200 μm long) and macrozooplankton (>200 μm long) representing largely the rotifers in the former case and crustaceans in the latter (Wetzel, 2001). This study focused on crustacean zooplankton, which are further divided into two main groups: the cladocerans and the copepods (Kalff, 2003; Wetzel 2001).

Cladocerans (suborder Cladocera) are generally covered with a rigid coat of chitin, called the carapace. Most cladocerans are herbivorous filter feeders, but some can utilize selective feeding to choose particles based on their size, availability, shape and nutritional quality (Wetzel, 2001). Only a few species, including *Leptodora*, are predatory on smaller plankton. Cladocerans have a shorter life cycle than do copepods (Kalff 2003; Wetzel 2001), which allows them to respond to changing environments very quickly. Copepods (Class Copepoda) have a longer life cycle, which includes numerous nauplii and copepodite stages. Copepods are divided into three Orders: cyclopoids, calanoids and harpacticoids with only the former two being planktonic. Cyclopoids are often carnivorous to some degree, but their diet can also be composed of algae, bacteria and detritus. Long considered exclusively herbivorous, the calanoids are now mostly classified as omnivores, feeding on rotifers and ciliates as well as algae, bacteria and detritus. Species and genera of all the different groups of zooplankton in the study lake are presented in Appendix B.

0.1.7 Previous experiments

A number of laboratory experiments have investigated the effects of higher concentrations of CO₂ on phytoplankton. Urabe *et al.* (2003), used flasks of phytoplankton grown in growth chambers of differing CO₂ levels and found those in higher CO₂ levels demonstrated increased algal biomass. A similar bottle experiment by Jansson *et al.* (2012), found water samples aerated with high CO₂, had phytoplankton respond with increased primary production. This supports other experimental data showing the same increase in primary production in marine environments (Kim *et al.* 2006 ; Tortell *et al.* 2008 ; Yoshimura *et al.* 2009). A comparison of the relative success of phytoplankton species in a higher CO₂ environment was performed by Low-Decarie *et al.* (2011). Six species of phytoplankton from three major groups (cyanobacteria, diatoms and chlorophytes) were grown at elevated CO₂ levels. Although all species showed an increase in growth, the relative success differed between species with cyanobacteria increasing the least. Despite multiple in lab experiments, almost no data has been collected from in situ experiments which provide the best knowledge of “real-world” effects.

Many species of phytoplankton have differing abilities to take up and effectively use CO₂ (Rost *et al.* 2003; Riebesell 2004; Maberly *et al.* 2009). These differences can affect competitive interactions (Tortell *et al.* 2002) leading to the differential success by species seen in the experiment by Low-Decarie *et al.* 2011. And these physiological differences can lead to shifts in phytoplankton community composition in responses to the increasing CO₂ environment (Riebesell *et al.* 2007; Tortell *et al.* 2008).

0.2 Research goals

As global climate change continues to modify environments, it is important to understand the effects on freshwater ecosystems. This study involved experimentally increasing the concentration of dissolved CO₂ in one basin of a three-basin lake and

examining the effects on the phytoplankton community. Assessment of biomass, production and relative abundance were also done. Secondly, the study determined whether the changes initiated in the phytoplankton community had repercussions for zooplankton growth and species abundance. The core goal was to assess the effect of increased concentration of dissolved carbon dioxide on the phytoplankton community of a freshwater ecosystem.

0.3 Hypotheses and Predictions

The increase of lake carbon dioxide levels may affect phytoplankton biomass and species composition but may also have the potential to affect zooplankton growth and species richness, as well as the bacterial food web. It was suspected that such changes associated with climate change may not be observed merely within the phytoplankton community, but could affect other parts of the aquatic food web. We had a set of initial hypotheses:

H1: We would observe an increase in phytoplankton primary productivity in the experimental basin compared to the control basin, as the manipulation should increase the saturation of Rubisco.

H2: We would observe an increase in phytoplankton biomass in the experimental basin compared to the control basin due to the elimination of CO₂ as a potential limiting nutrient of growth. With more carbon in a readily available form, the phytoplankton community as a whole would benefit.

H3: We would observe a shift in the phytoplankton community structure (relative biomass between phytoplankton species) in the experimental basin, as species' success would vary based on physiological traits and the ability to utilize carbon dioxide. Chrysophytes should benefit the most with success declining with chlorophytes, diatoms and cyanobacteria. Phytoplankton without CCMs would benefit the most as they are heavily reliant on carbon dioxide levels. The control basin would not experience a shift in the relative biomass other than normal seasonal

changes.

H4: We would observe a shift in the zooplankton community structure in the experimental basin in response to the changing phytoplankton community.

CHAPTER I

EFFECTS OF ELEVATED CARBON DIOXIDE ON FRESHWATER PLANKTON COMMUNITY DYNAMICS

This chapter was written in the form of a scientific paper and will be submitted to a peer-reviewed journal in November 2015.

1.1 Abstract

Increased precipitation and wind speeds anticipated with climate change are likely to augment the runoff of organic carbon to north temperate lakes, resulting in greater dissolved concentrations of carbon dioxide (CO₂) via microbial respiration. Recent lab experiments indicate significant effects of such CO₂ increases on freshwater ecosystems, especially with respect to phytoplankton community dynamics; however, little in situ work exists to substantiate these results. In a whole-ecosystem experimental manipulation of a stratified lake, we demonstrated that when epilimnetic dissolved CO₂ levels were doubled (pCO₂ raised from 700ppm to 1400ppm) both phytoplankton and zooplankton experienced significant community changes, relative to those observed in a control lake basin. Modelling of net primary productivity based on measured PAR values alongside integrated pCO₂ levels showed net primary productivity should have increased in the experimental basin during the course of the manipulation. Instead however there was an unexpected large decline of phytoplankton biomass, primarily at the location of the perennially present deep chlorophyll maximum. Significant unanticipated changes in phytoplankton community composition were also observed with cyanobacteria benefitting the most, and normally dominant groups like chlorophytes and chrysophytes declining. These changes were attributable to changes in the trade-off between light and CO₂ alongside rapid increases in zooplankton biomass following CO₂ manipulation, resulting in large reductions in phytoplankton community biomass, despite evidence of higher levels of primary productivity. The results of this large-scale experiment indicate that elevated dissolved CO₂ could have community and ecosystem impacts that are highly dependent on behavior and food web structure, in ways that could not be anticipated from smaller-scale studies.

1.2 Introduction

Carbon dioxide (CO₂) levels have continued to rise since pre-industrial times. Record-breaking values of over 400ppm have been recently observed (Showstack, 2013) and increases are expected to continue steadily over the next century (Collins *et al.* 2013). Atmospheric changes are unlikely to have a detectable impact on the partial pressure of carbon dioxide (pCO₂) in lake water, given that atmospheric increases are small relative to the degree of CO₂ supersaturation naturally found in lakes (Kling *et al.* 1991; Cole *et al.* 1994; Jonsson *et al.* 2003). However, indirect effects are likely in pCO₂ levels, mainly through increased precipitation (Zhang *et al.* 2007) resulting in elevated organic carbon loads, that will stimulate bacterial respiration of organic carbon (del Giorgio *et al.* 1997; Cole & Caraco, 2002; Prairie *et al.* 2002; Sobek *et al.* 2003) entering lakes via streams (Jones *et al.* 1998). Similarly, anticipated changes in wind regimes (Collins *et al.* 2013) will influence the stratification of lakes, altering pCO₂ dynamics (Mercier-Blais *et al.* 2014).

A steady supply of CO₂ is required by phytoplankton for photosynthesis. Despite the fact that the majority of lakes are CO₂ supersaturated, levels may still be limiting the efficiency of phytoplankton productivity (Jansson *et al.* 2012). Phytoplankton use the ribulose biphosphate carboxylase-oxygenase (Rubisco) enzyme for carbon fixation (Holland *et al.* 2012). For most phytoplankton, Rubisco is only at half capacity when pCO₂ is at equilibrium with atmospheric levels, suggesting that carbon-availability can limit photosynthetic efficiency (Badger *et al.* 1998). Most phytoplankton species require 25-35 μmol L⁻¹ CO₂ for Rubisco saturation (Raven & Johnston, 1991). However, current-day atmospheric levels near 380 ppm CO₂ only supply 14 μmol L⁻¹ of pCO₂ at 20°C (Song *et al.* 2014). Supersaturated lake water samples bubbled with CO₂, experienced increased primary productivity (Jansson *et al.* 2012), supporting experiments demonstrating similar increases in marine environments (Tortell *et al.* 2008).

Freshwater phytoplankton must balance the need for CO₂ with other requirements such as light. The trade-off between light and nutrients is well

documented in stratified systems (Yoshiyama *et al.* 2009) with nutrients increasing with depth (Zehr & Ward, 2002), and light declining with depth (Kirk, 1994). It is these trade-offs that can influence plankton community positions throughout the water column. A deep chlorophyll maximum (DCM) is an area of highly concentrated phytoplankton biomass that is often located near the metalimnion (Fee, 1976). The metalimnion is considered favorable as nutrients, including carbon in the form of CO₂ are high, and light levels are still sufficient enough for efficient productivity.

As pCO₂ levels increase in lakes, it is expected that phytoplankton taxa will benefit differentially because of varying physiological affinities for CO₂, leading to community composition changes. In particular, some species rely on Carbon Concentrating Mechanisms (CCMs) to deal with restricted CO₂ availability, enabling their use of other C-sources (Maberly *et al.* 2009). One prominent CCM used especially by cyanobacteria (Shapiro, 1997), permits utilization of the bicarbonate pool, which is usually larger than that of dissolved CO₂ (Wetzel, 2001), for pH above 6.5. Although energetically expensive, such processes do provide competitive advantage through utilization of multiple inorganic carbon sources. With increased pCO₂, the need for CCMs to fulfill photosynthetic requirements decreases, potentially negating advantages to CCM-possessing phytoplankton because of the associated high energetic burden (Maberly *et al.* 2009; Wolfe & Siver, 2013). Thus, as the competitive arena for carbon changes, shifts in the phytoplankton community composition are to be expected (Fee, 1976; Riebesell *et al.* 2007). To investigate such shifts in community composition, a recent study compared the relative success of three major phytoplankton groups (cyanobacteria, diatoms and chlorophytes) in elevated pCO₂ environments (Low-Decarie *et al.* 2011). While all groups experienced increased growth, the relative values differed between groups, with cyanobacteria increasing the least. This differential success can largely be attributed to different capacities to take up and effectively use CO₂ (Maberly *et al.* 2009; Rost *et al.* 2003; Riebesell, 2004). However, despite several lab experiments, data from *in situ* experiments at large-scales are lacking and are critical to assess the potential

importance of multiple interactions between groups responding differently to elevated CO₂.

As climate change modifies aquatic environments, it is important to understand and predict community changes, including those occurring across trophic levels. In lakes, phytoplankton form the base of the food web and changes in their communities can influence higher trophic levels, especially the dominant crustacean zooplankton herbivores (Sterner & Elser, 2002; Van de Waal *et al.* 2010), with multiple feedbacks between groups possible. To determine the potential responses of plankton communities to increased pCO₂ levels, we experimentally increased pCO₂ concentrations in the epilimnion of a lake basin and compared the response to a separate control basin in the same lake. Responses examined included phytoplankton and crustacean zooplankton production and composition. The vertical spatial distribution patterns of the plankton communities were also considered, as a reflection of behavioral responses. Based on previous lab studies, we hypothesized that elevated CO₂ would stimulate primary productivity, thereby increasing phytoplankton biomass in the epilimnion following CO₂ addition. Phytoplankton without CCMs such as chrysophytes, were expected to benefit the most. In addition, the change in CO₂ in the epilimnion was suspected to have the potential to alter the phytoplankton trade-off between CO₂ and light and alter the spatial distribution of phytoplankton in the water column. A predictive model was also produced using measured irradiance and pCO₂ levels to estimate net primary productivity (NPP) changes expected based on bottom-up processes alone. Our study is the first to examine the responses to increasing pCO₂ concentrations on phytoplankton communities in a whole-lake food web context.

1.3 Materials and methods

1.3.1 Study Site

The study site was the north temperate lake, Lac Croche (Station de Biologie des Laurentides), St-Hippolyte, Quebec, Canada. This protected headwater lake is composed of three relatively isolated basins (Fig. 1). Basin 1 (B1) has always been used as a control in recent studies on this lake, and is separated from the adjacent central basin (B2) by a shallow channel <2m deep. The Experimental basin (B3) is separated from B2 by an island, and two shallow channels on either side. The south channel is 1m deep and the north channel varies from 1 to 6m in depth. A black polyethylene curtain was installed in November 2007 across the northern channel to isolate B3. All basins have a maximum depth of 10-12m and sampling docks were anchored at the deepest point in each.

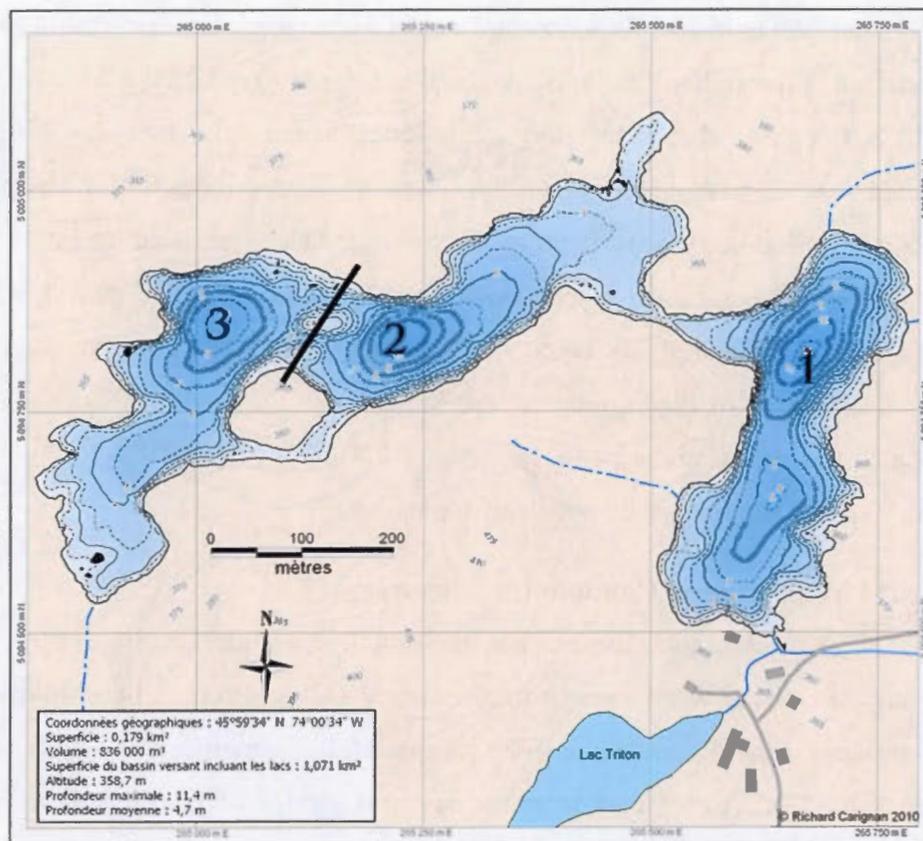


Fig. 1 Bathymetric map of Lake Croche (courtesy of Richard Carignan, 2010). The three basins are labelled 1, 2 and 3 and the polyethylene curtain is shown by the black line.

1.3.2 Sampling

Sampling began on June 18th, 2013 (day 169), prior to the experiment, to establish baseline activity for the Experimental (B3) and Control (B1) basins. The four week experiment began on July 25th, 2013 (day of year 206), when 500kg of dry ice pellets was added to the shallows of B3 to increase the pCO₂ concentration. Dry ice was added at depths of less than 3m to ensure that only the epilimnion was being manipulated, as this is where the impacts of climate change associated increases in pCO₂ through runoff should most greatly be experienced. To maintain the elevated

levels of $p\text{CO}_2$ in the Experimental basin, subsequent smaller additions of dry ice were made throughout the four weeks of the experiment: 255kg added Aug. 2nd (day 214), 200kg added Aug. 8th (day 220), 250kg added Aug. 14th (day 226) and 400kg added Aug. 21st (day 233). Note, a trial run of the dry ice addition was performed to ensure methodology was effective. 250kg of dry ice was added on July 15 (day 196). Each basin was sampled twice weekly during the experimental period, with a variety of variables measured, but zooplankton were only sampled once per week. Following the termination of the experiment on August 25th, 2013 (day 237), weekly sampling of all variables continued until September 17th 2013 (day 260) to follow the return of the Experimental basin to normal $p\text{CO}_2$ levels.

1.3.3 Phytoplankton Community Assessment

Phytoplankton biomass was measured in vertical profile in 50cm depth bins using an underwater spectrofluorometer (FluoroProbe, bbe-Moldaenke, Kiel, Germany). The FluoroProbe (FP) measures fluorometrically the concentration of chlorophyll *a* (Chl*a*) of four major spectral groups of phytoplankton, representing broadly the taxonomic classes of: diatoms + dinoflagellates + chrysophytes (BROWNS), chlorophytes (GREENS), cyanophytes containing phycocyanin (CYANOS), and cryptophytes + cyanophytes containing phycoerythrin (CRYPTOS), after subtracting fluorescence from dissolved organic matter ('yellow substances') using an ultraviolet-B excitation source. We focused on the dominant FP groups (BROWNS, GREENS, CYANOS). Previous work in this lake indicates that the chrysophytes form the dominant component of the BROWNS at the point in the summer at which this experiment was carried out. Peak total biomass was calculated by taking the maximum total chlorophyll value and subtracting the minimum across the photic zone (0-7m). FP data was examined in profile across time, but also by taking depth-integrated averages to determine total volumetric Chl*a* levels by date using 0.5m intervals across the photic zone (0 to 7m). Areal Chl*a* was calculated across the epilimnetic (0-4m) and metalimnetic (4-7m) depths in each basin by using

the FP values at the mid-point of each 1m depth interval, multiplied by the volume of water in the corresponding depth layer; the sum of these was then divided by the area of the basin.

For phytoplankton composition, integrated samples were collected across the photic zone using a 1cm-diameter PVC tube and preserved in Lugol's solution. Species were identified and counted using an inverted microscope at 400x magnification using the Utermohl method in a subset of samples: two prior to manipulation (July 2, July 18), four during (July 26, August 2, August 9, August 20) and one post-manipulation (August 30). Approximately 15 cells of each species were measured and values were used to convert counts to biovolume (biomass) using geometric formulae (Hillebrand *et al.* 1999).

1.3.4 Zooplankton Community Assessment

Vertical net hauls using a 54 μ m-mesh net (30cm diameter x 150cm length) were used to sample crustacean zooplankton communities. Zooplankton were anesthetized with soda water before being preserved in a final-concentration 75% ethanol solution. In the lab, repeated 2 mL sub-samples (to a minimum of 400 individuals) were taken using a Hensen-Stempel pipette from a well-mixed 100mL sample. Crustaceans were identified and counted at 100x magnification and biomass estimated using standard length-dry weight relations (McCauley, 1984).

1.3.5 Abiotic Variables

Temperature, pH and dissolved oxygen (DO) were measured in profile (every 0.5m) using a YSI6600. pCO₂ levels were measured in profile at the same depths using an environmental gas analyzer (EGM-4, PP Systems, Amesbury, MA, USA). Photosynthetically active radiation (PAR) was measured in profile at each 0.5m using a Li-Cor submersible light sensor (LI-192) coupled to a surface quantum sensor (LI-190) (LI-COR Inc. Lincoln NE, USA) on each sampling date and used to estimate the depth of the light extinction coefficient (K_d or depth of 1% surface light). Surface

irradiance (at 0.5 m depth), reflecting ambient light levels, was also estimated at 10 minute intervals using a Li-Cor submersible light sensor attached to an instrumented buoy in the Control basin.

Estimates of net primary productivity (NPP) were also done on several occasions (Aug. 2, 9, 16, 23 2013). Incubation experiments were carried out by taking water from 0.5m depth in each basin using a dark 4L Nalgene bottle and splitting each set across 24 light and dark 300-ml Pyrex glass bottles. Duplicate bottles were then incubated for 6 hours on a wheel within a temperature-controlled (20°C) light gradient incubator as described in Carignan *et al.* (2000). The incubator was filled with lake water to ensure a corresponding light regime and bottles from each basin were exposed to six different irradiance levels (between 30-800 $\mu\text{E m}^{-2} \text{ s}^{-1}$) to enable the development of a photosynthesis-irradiance (PI) curve. Dissolved oxygen levels were measured before and after incubation using membrane inlet mass spectrometry (MIMS) to determine net oxygen changes in both light and dark bottles to estimate NPP and GPP. Aliquots were drawn from the bottom of the incubation bottles of a 60mL syringe with a small tube and transferred to the bottom of the MIMS glass vials letting the water overflow with twice the volume of the vials. They were then poisoned with Mercury and capped with a glass stopper. Two MIMS replicates per incubation bottles were done, with two replicate incubation bottles per light level treatments. Values are presented in $\mu\text{mol O}_2 \text{ produced} / \text{L} / \text{hr}$.

1.3.6 Statistical Analyses

To determine the effect of the manipulation on observed variables, we subtracted the value in the Control (B1) from that observed in the Experimental B3 on each sampling date. To determine statistical differences between the mean variation in the Experimental and Control basin across the pre-experimental period and during the experimental period, the nonparametric Wilcoxon-Mann-Whitney U-test, was performed using JMP 10.0 (SAS Institute Inc., Cary, NC, USA), because for

all variables, normality was not satisfied and could not be achieved through transformation.

1.3.7 Net Primary Productivity Model

The series of oxygen evolution/consumption (PI) estimates from the incubations on water taken from the different basins with their varying dissolved CO₂ concentrations, allowed the development of a simple model to predict changes to net primary productivity (NPP) as a function of both light and CO₂ concentrations. The model captured well substantial photoinhibition observed at high light levels, as well as the positive effect of CO₂ on NPP (Fig. S9). The model was then applied to the continuous (every 10 minutes) underwater irradiance levels coupled with interpolated epilimnetic pCO₂ (from measured weekly pCO₂ vertical profiles and light extinction coefficients, K_d , m⁻¹) to estimate the NPP vertical profile (0.5 m depth resolution) of the phytoplankton communities of each basin every 10 minutes. The same interpolation was done for total chlorophyll profiles measured with the FP to provide biomass estimates. These values were then integrated to produce a time series of daily specific NPP in the epilimnion of each basin.

1.4 Results

The dry-ice additions successfully increased the carbon dioxide partial pressure in the epilimnion of the manipulated basin (Fig. 2a). Carbon dioxide levels spiked after each addition, declining slightly until the next addition. While the peak surface pCO₂ recorded (1567 ppm) is double natural levels in the experimental lake, it is not beyond those values commonly found naturally in north temperate lakes (Sobek *et al.* 2003). Mean levels were significantly higher in the Experimental basin during the manipulation (Table S1). Initial pH values prior to the start of the experiment were slightly lower by about 0.5 in the Experimental basin (Fig. 2b), becoming more positive during the experiment, although not significantly so (Table S1). Temperature profiles throughout the entire sampling period did not differ significantly (Fig 2c; Table S1). Dissolved oxygen (DO) also showed similar profiles

in both basins (Table S1), with the notable exception of two weeks into the experimental period, at which point the manipulated basin showed a pronounced epilimnetic increase (Fig. 2d).

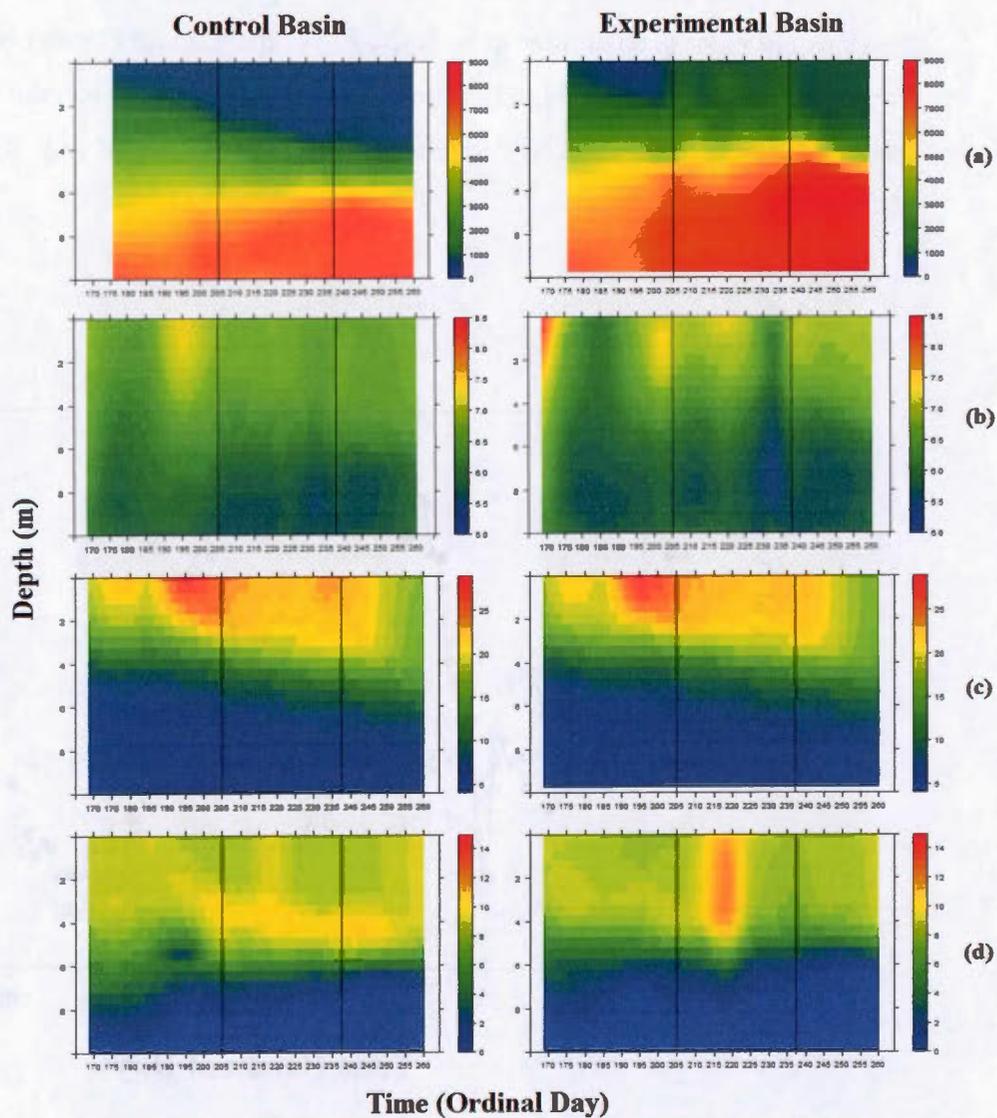


Fig. 2 Surface plots showing time series differences of the vertical profiles of the main abiotic parameters: $p\text{CO}_2$ (ppm) (a), pH (b), temperature ($^{\circ}\text{C}$) (c), dissolved oxygen (DO; mg/L) (d) in both the Experimental and Control basins. Dry ice additions occurred repeatedly between days 206 to 237 as indicated by the vertical black lines delimiting the experimental period.

The modelled time series of predicted net primary productivity indicated that levels would have been similar in both the Experimental and Control basins prior to the start of the experiment (Fig. 3). After the addition of the dry ice, the Experimental basin had elevated predicted NPP levels compared to the Control (Fig. 3).

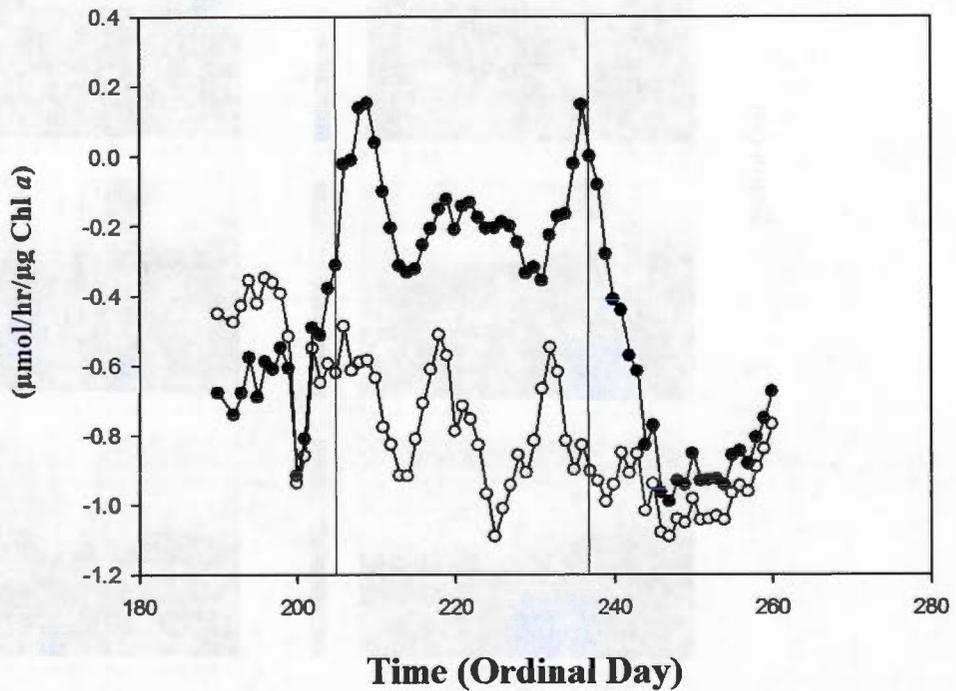


Fig. 3 Predicted net primary production ($\mu\text{mol/hr}/\mu\text{g Chl } a$) in the Experimental basin (black symbol) and the Control basin (white symbol). The vertical black lines delimit the experimental period.

The peak (maximum - minimum) phytoplankton biomass (as measured with the FluoroProbe; FP) increased over the entire survey period in the Control, but it failed to do so in the Experimental basin and declined during the experimental period (Fig. 4). This decline of biomass was characterized with a loss of the deep chlorophyll maximum (Fig 5).

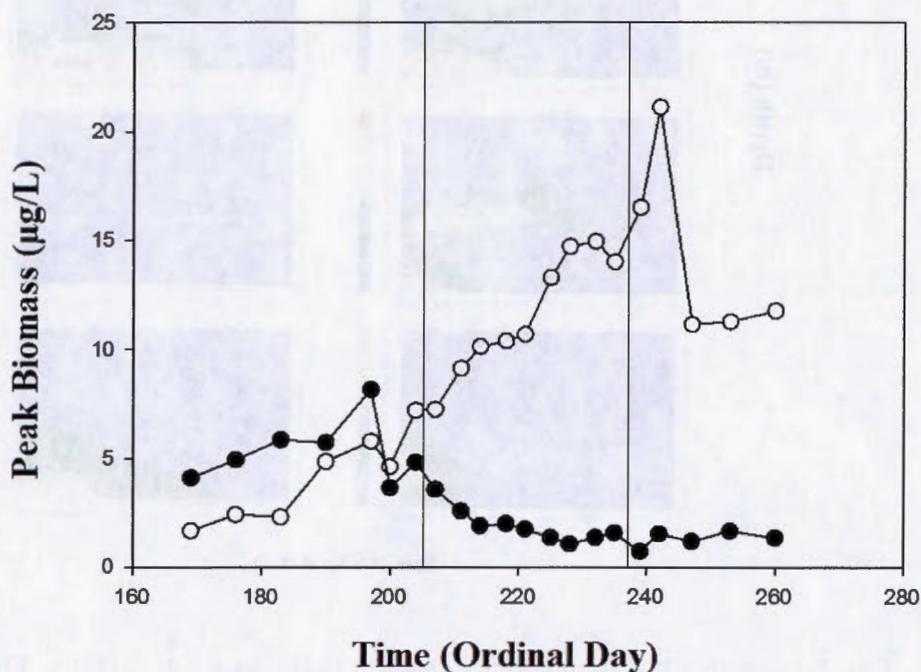


Fig. 4 Peak total biomass across the water column (maximum biomass – minimum biomass) ($\mu\text{g/L}$) as measured by the Fluoroprobe in both the Experimental basin (black symbol) and the Control basin (white symbol). The vertical black lines delimit the experimental period.

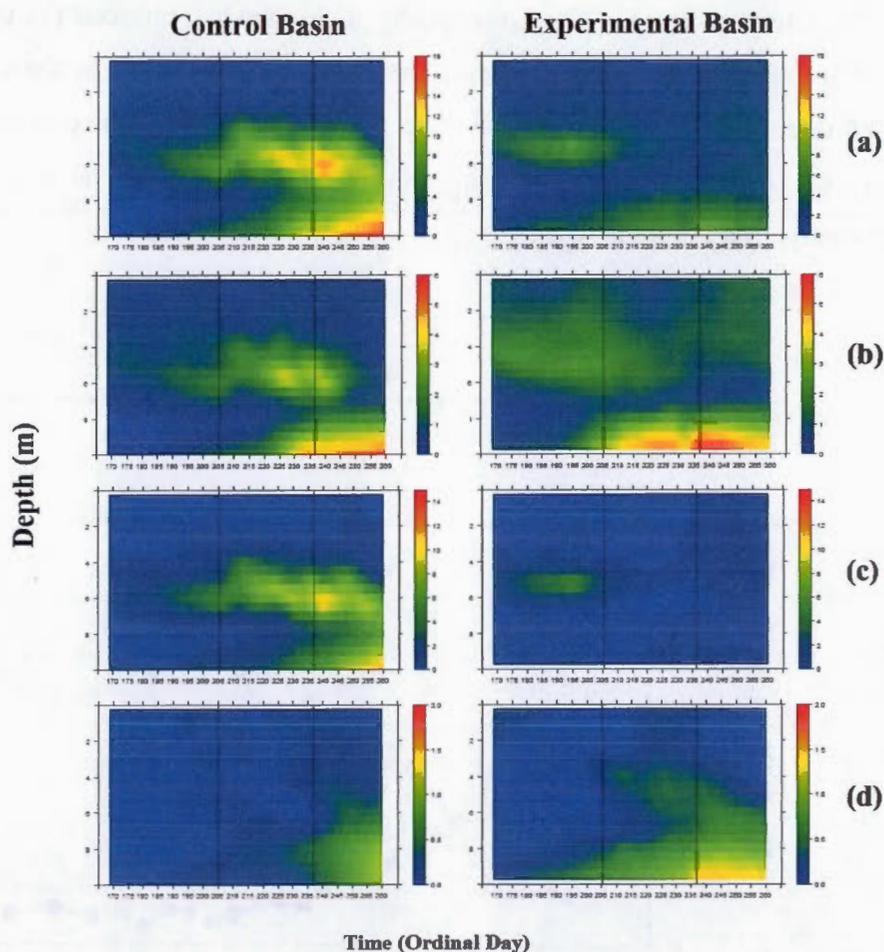


Fig. 5 Contour plots of phytoplankton biomass time series of the Experimental and Control basins (mg/L) as measured with the FP for total biomass (a) and the dominant phytoplankton spectral groups: GREENS (b), BROWNS (c), and CYANOS (d). The vertical black lines delimit the experimental period.

Phytoplankton spectral groups, as defined by the FP and corresponding to major phytoplankton taxonomic classes, responded differently to elevated CO_2 in terms of biomass, and the biomass depth-distribution (Fig. 5b-d). Significant reductions in biomass were observed in the GREENS and BROWNS, especially around the metalimnion (Fig. 5b, c). By contrast, CYANO biomass increased in the

Experimental basin following the CO₂ addition (Fig. 5d). Areal chlorophyll *a* in the epilimnion (0 – 4m) of the Experimental basin declined slightly during the experimental period whereas the Control basin had a small but distinct increase over the same time period (Fig. 6a). The areal Chl*a* of the metalimnion (4 -7m) differed vastly between the two basins with a decline in the Experimental basin and a large increase in the Control basin (Fig. 6b).

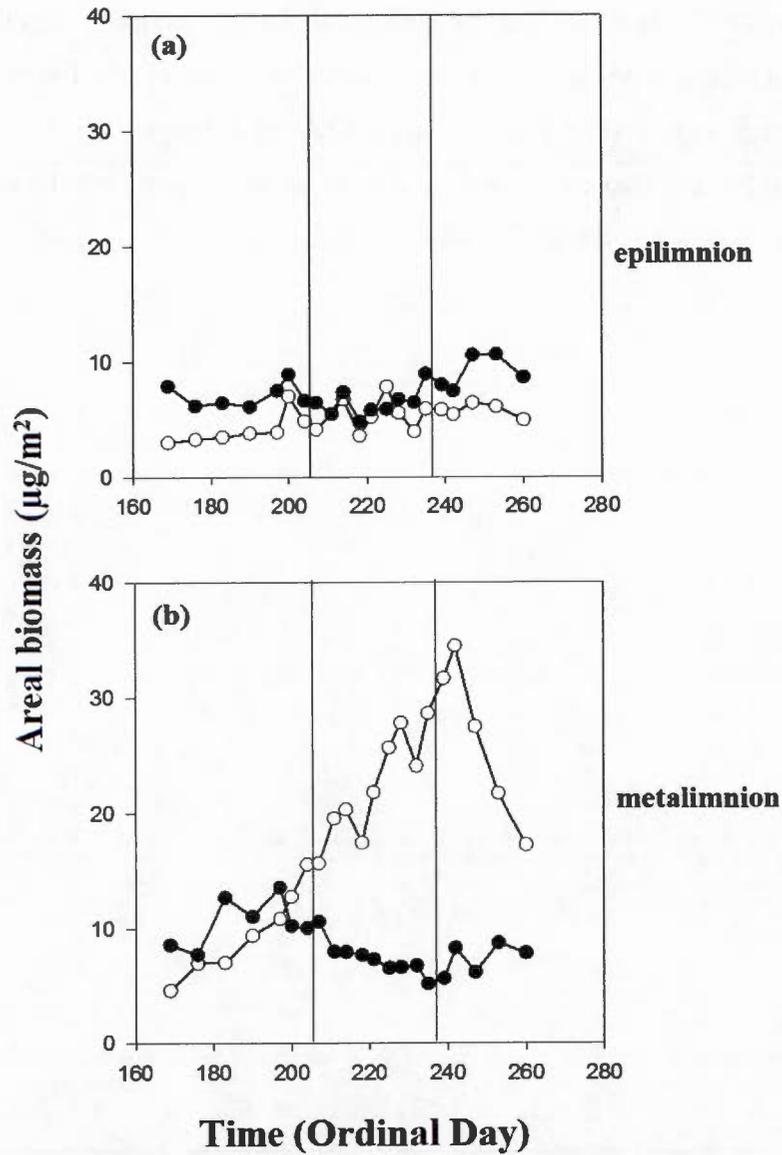


Fig. 6 Areal phytoplankton biomass ($\mu\text{g}/\text{m}^2$) across the epilimnetic (0-4m) and metalimnetic (4-7m) depths observed in the Experimental basin (black symbol) and the Control basin (white symbol) as measured with the FP. The vertical black lines delimit the experimental period.

The zooplankton dynamics differed between the two basins, especially in relation to the phytoplankton (Fig. 7). In the Experimental basin, zooplankton initially increased rapidly, reaching a peak on day 218 (August 6th), about two weeks following the initial dry ice addition (Fig. 7a). In contrast, zooplankton declined slowly from their early summer peak in the Control basin, while phytoplankton was steadily increasing (Fig. 7b).

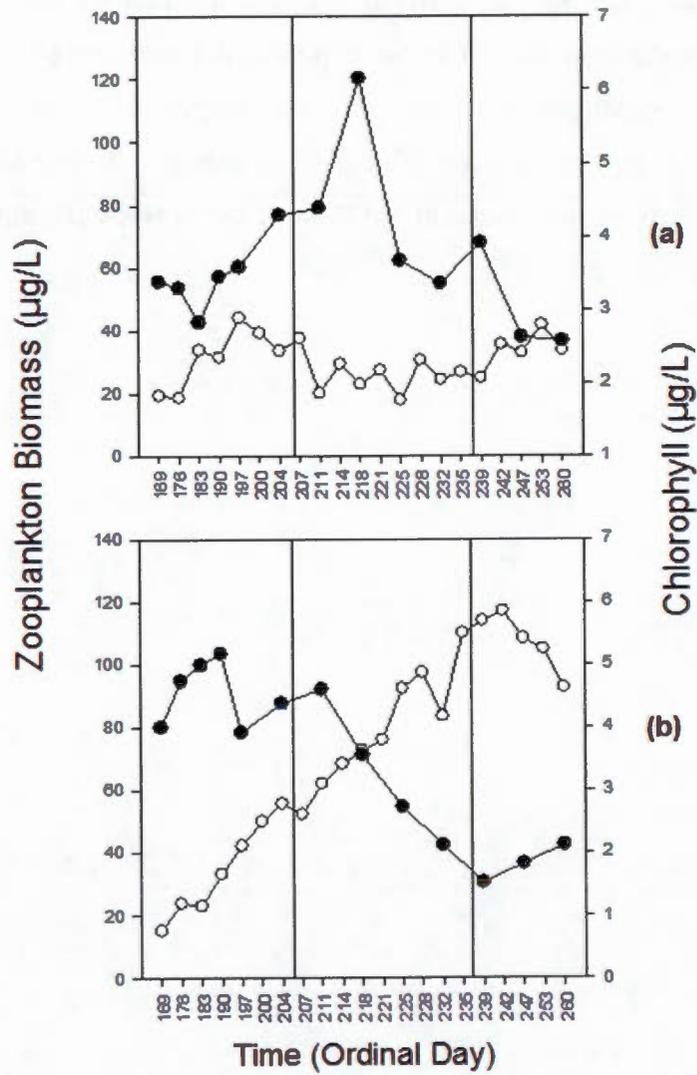


Fig. 7 Time series of zooplankton biomass ($\mu\text{g/L}$, dry weight) (black symbol) and total phytoplankton biomass (chlorophyll *a* in $\mu\text{g/L}$) (white symbol) in the Experimental (a), and in the Control (b) basins. The vertical black lines delimit the experimental period.

1.5 Discussion

Through differential effects on CCMs, lab studies to date have demonstrated large phytoplankton community shifts with increases in pCO₂ (Low-Decarie *et al.* 2011; Riebesell, 2004). However, the results of our whole-lake manipulation demonstrate that community structure will be influenced by several other factors that can counteract and even reverse the conclusions from lab competition studies.

The model using irradiance and pCO₂ levels to estimate net primary productivity (NPP) predicted that increasing pCO₂ should lead to greater NPP. In Lac Croche, the metalimnion has a high concentration of CO₂, which could be one of many factors that leads to the normal chlorophyll peak at this depth, with the majority of the phytoplankton congregating at these depths. However, being situated at these deeper depths implies reduced access to light for these phytoplankton. During our experimental manipulation, the epilimnetic pCO₂ was virtually doubled. This increase makes the epilimnion more favorable for phytoplankton to occupy with both higher light and greater pCO₂. Based on this model, we would have expected the phytoplankton community to migrate upwards towards the epilimnion with additional CO₂ and light, leading to increased overall primary productivity.

Our results support this mechanism for increased productivity in the epilimnetic waters. In the epilimnion of the Experimental basin, a pronounced increase in DO occurred shortly after the CO₂ addition. Oxygen is a by-product of photosynthesis and an indicator of primary productivity in lakes (Staehr & Sand-Jensen, 2007). Furthermore, the elevated DO levels observed occurred in conjunction with reduced epilimnetic phytoplankton biomass (volumetric and areal) indicating that the remaining phytoplankton were significantly stimulated by the addition of pCO₂ to produce such elevated DO levels. Lastly, the NPP model indicates the Experimental basin had consistently higher NPP levels than the Control basin. Thus, our study provides new whole-lake experimental support for primary productivity increases with elevated pCO₂ as observed previously (Tortell *et al.* 2008; Sobrino *et al.* 2009).

However, the model does not predict the observed dramatically lower peak levels of chlorophyll in the Experimental basin relative to the Control. This decline of biomass was characterized with a loss of the DCM with augmented $p\text{CO}_2$; a biomass peak normally found between 4 and 6m depths (Ouellet & Beisner, 2014). Associated with this were unexpected shifts in the phytoplankton community composition, favouring cyanobacteria but negative responses in other dominant groups: chlorophytes, chrysophytes and diatoms.

One possible explanation for the lack of correspondence between the epilimnetic NPP predictions and peak chlorophyll levels would be that a dilution of the peak biomasses owing to a migration of some phytoplankton toward highly CO_2 saturated epilimnetic waters, as highly motile species are known to modify behaviours in this way (Clegg *et al.* 2007). However, if this migration occurred, while we would have expected to see a dilution effect, owing to the loss of the highly concentrated DCM, total phytoplankton biomass (estimated areally) would have remained constant. However, although areal $\text{Chl}a$ in the metalimnion greatly declined throughout the Experimental period (supporting the migration theory) there was no corresponding increase in the areal epilimnetic values, but instead, a slight decrease. Thus, there must be a further mechanism invoked to explain the reduction of phytoplankton biomass when NPP would have increased.

The most likely mechanism for these paradoxical observations is an increase in top-down forces leading to the decline in biomass and phytoplankton community shifts. Zooplankton can exert tremendous population and compositional control over the phytoplankton (Sterner & Elser, 2002). During the experimental period, phytoplankton biomass declined, and only began increasing again after the herbivorous zooplankton community (cladocera and calanoid copepods) had declined toward the last part of the experiment. With the $p\text{CO}_2$ addition, zooplankton biomass peaked at almost double the biomass present in the control basin midway through the experiment before declining again. Further support for a top-down effect of zooplankton is also evident from phytoplankton community structure shifts with the

more edible phytoplankton such as chlorophytes and chrysophytes being rapidly depleted and less desirable cyanobacteria flourishing. These observations indicate differential survival to intense zooplankton grazing. Furthermore, it is possible that such grazing effects acted in conjunction with differential carbon utilization to affect community structure.

Based on previous lab studies, we expected taxa such as chrysophytes, without CCMs and relying solely on the pool of free CO₂, would benefit the most from supplementation. The other phytoplankton (chlorophytes, cryptophytes and diatoms) were expected to benefit less, and cyanobacteria the least. However, our observations were in the opposite order, with only cyanobacteria increasing and other groups declining. Such results might be expected however, if the added CO₂ decreased the need for cyanobacteria to run their CCMs, lowering their overall energy expenditure (Song *et al.* 2014; Giordano *et al.* 2005), allowing more energy to be allocated to growth and division. Furthermore, while affinity for CO₂ varies between phytoplankton species, cyanobacteria as a whole have been found to have greater affinities than other phytoplankton groups (Kirk, 1983). While this might explain why cyanobacteria were favored in conjunction with high herbivory, it does not explain why other groups of phytoplankton experienced declines; greater herbivory on these groups does. As the addition of dry ice is not suspected to harm or cause physical damage to the phytoplankton, these unexpected declines indicate that factors other than pCO₂ levels must be considered. Overall, our results point to a strong top-down food web effect, wherein crustacean zooplankton show negative feeding selectivity toward cyanobacteria relative to other prey options (Porter & Orcutt, 1980; de Bernardi & Giussani, 1990; Sarnelle *et al.* 2010). What remains to be examined in future detailed studies is the triggering mechanism permitting zooplankton populations to increase so rapidly in response to a CO₂ addition.

While the evidence for increased primary productivity in our study was not surprising, the ultimate outcome in terms of biomass stocks and community composition, were generally unexpected, with the effects of the herbivores

outweighing, but also interacting with, the direct effects of CO₂. To better understand how aquatic ecosystems will react to future changes, some large-scale studies are needed to tease apart these complex interactions and point to unexpected response at the behavior, population and community level. A future step to increase this understanding will be to implement changes in lake color in addition to pCO₂, as lakes will most likely experience a darkening due to increased organic matter deposition in the future. While larger-scale experiments such as ours can suffer from a lack of replication and a relatively coarse time scale, the biologically-significant changes observed warrant serious consideration and provide important new insights that could not have been gained in other ways.

2.0 CONCLUSION

2.1 Conclusion of Chapter I

In the past decade, the number of studies examining the effects of future climate change has greatly increased. Climate change is expected to greatly impact aquatic systems, including freshwater systems such as lakes. Lakes are a vital part of our landscape and understanding how they will change in the future will allow policy makers to make the best decisions in order to protect these important ecosystems. One aspect of climate change that is already occurring is the increase in atmospheric carbon dioxide. While lakes may not be directly impacted by this particular change in the atmosphere, there are numerous aspects of climate change that are anticipated to increase carbon dioxide within freshwater lakes (increased wind speeds, increased temperature). And this increase in carbon dioxide is expected to impact the biota in lake systems.

The goal of this M.Sc. project was to integrate previous knowledge of carbon dioxide and plankton into a large scaled full lake basin manipulation experiment. Lac Croche was an ideal study lake due to its natural morphometry of three distinct basins. This allowed for us to manipulate one full basin and monitor another basin as a control. This was the first project of its kind and has resulted in unique results that have not been previously found. This is the first experiment to perform a full ecosystem manipulation and include two levels in the food web (phytoplankton and zooplankton). From the results we can clearly see how important the food web interactions are. The food web interactions were more important in determining plankton community size and composition than the manipulation itself.

Our goal of elevating $p\text{CO}_2$ in the epilimnion of the experimental basin was accomplished. We virtually doubled the $p\text{CO}_2$ from 700ppm to 1400ppm. We did so

by adding large quantities of dry ice (solid CO₂) in the shallows of the lake basin. This elevated carbon dioxide was anticipated to stimulate primary production in the phytoplankton community, and this was clearly seen with a pulse-like increase of dissolved oxygen. Oxygen, a by-product of photosynthesis, is an excellent indicator of primary productivity. This result mirrored results determined in previous studies (Jansson *et al* 2012). The increased productivity was also reflected in the results of the model predicting net primary productivity. Basin 3 consistently had higher productivity than Basin 1. We can attribute this increase in productivity, to an increase of saturation of Rubisco leading to an increased efficiency of the photosynthetic pathway. However, we found that this increase in primary production did not translate into an increase in biomass, contrary to what we had predicted. Instead, the phytoplankton in the experimental basin experienced a dramatic decline in biomass. And most notably, the deep chlorophyll maximum (DCM) was virtually eliminated in the experimental basin.

The deep chlorophyll maximum has been observed in previous years of study in this lake. To have it completely eliminated means a dramatic shift in the experimental basin's phytoplankton community. Based on the increases in epilimnetic CO₂, we had determined that after the addition of dry ice, the epilimnion in the Experimental basin would have become more favorable for phytoplankton to inhabit. However, we did not see any evidence of phytoplankton migrating upward leading to a dilution of the deep chlorophyll maximum. This dilution would have simply distributed the total phytoplankton biomass across a greater distance in the water column. Rather, what occurred was a reduction in total phytoplankton biomass. We have attributed this massive change in the phytoplankton community to be due to intense zooplankton grazing. The zooplankton community experienced a large increase in biomass, while the phytoplankton community declined. Correspondingly, the elimination of the DCM led to alterations in zooplankton behaviour and we found that zooplankton occupied different depths in the water column. In the control basin,

the zooplankton tended to congregate around the depth of the DCM as that is where the majority of phytoplankton were. But in the experimental basin, the DCM depths were where the fewest zooplankton could be found and therefore, zooplankton had to move throughout the water column.

Both the communities of phytoplankton and zooplankton experienced changes within their composition. Based on previous experiments, we had expected the chrysophytes to benefit from the additional carbon, however, all major groups (chlorophytes, chrysophytes, cryptophytes) declined in biomass except for the cyanobacteria. Cyanobacteria were the only group to actually increase throughout the experimental period. Again, these changes in composition have been attributed to zooplankton grazing. The edible groups of phytoplankton (greens, browns) were quickly depleted by zooplankton leaving the less edible cyanobacteria to thrive. We saw the most efficient zooplankton grazers such as *Daphnia* quickly increase their biomass, followed by a slower increase of biomass of the copepods. However, due to the rapid decline of phytoplankton biomass, the phytoplankton community could no longer support the zooplankton biomass, leading to a rapid crash of the zooplankton. These results have not been discussed in previous literature, mostly due to the lack of experiments that include multiple trophic levels. Our results show the need for large scale studies in order to fully understand how freshwater systems are anticipated to change with any future changes to the carbon pool.

2.2 Future Recommendations

This was the first study of its kind to perform a full ecosystem manipulation and more large-scale studies are needed. Full basin manipulations allow the complexity of freshwater ecosystems to be included in the experiment and the results are more easily applied to natural settings. However, a finer resolution of results would be a recommendation for the future. Our sampling was twice a week, and changes occurred very rapidly, and more frequent sampling (daily) would allow these changes to be tracked much more clearly. Upon completion of this project, we

realized how much influence food web interactions had on community size and composition. More intensive sampling of zooplankton throughout the entire experimental period, including their water column position would allow for better understanding of their behaviour. Lastly, if possible, inclusion of fish and bacterial sampling would give a clearer picture of how the entire food web would be affected by increasing carbon dioxide. It would also be interesting to perform a similar study in different kinds of lakes including highly productive (eutrophic) or arctic ones to better grasp how lakes with different properties would react to greater dissolved CO₂.

APPENDIX A

PHYTOPLANKTON PRESENT IN LAC CROCHE

Table S1: Major phytoplankton genera found in Lac Croche.

Species	Species	<i>Chrysophytes</i>	<i>Cryptophytes/Euglenoids/Dinoflagellates</i>	<i>Cyanobacteria</i>
<i>Diatoms</i>	<i>Chlorophytes</i>	<i>Bitrichia</i>	<i>Peridinium</i>	<i>Coelomonon</i>
<i>Achnantheidium</i>	<i>Planctonema</i>	<i>Chrysidiastrum</i>	<i>Chroomonas</i>	<i>Merismopedia</i>
<i>Actinella</i>	<i>Quadrigula</i>	<i>Chromulina</i>	<i>Cryptomonas</i>	<i>Snowella</i>
<i>Asterionella</i>	<i>Gleocystis</i>	<i>Chrysosphaerella</i>	<i>Kathablepharis</i>	<i>Chroococcus</i>
<i>Cyclotella</i>	<i>Dictyosphaerium</i>	<i>Dinobryon</i>	<i>Kephyrion</i>	<i>Woronichinia</i>
<i>Euonotia</i>	<i>Sphaerocystis</i>	<i>Epipyxis</i>	<i>Rhodomonas</i>	<i>Microcystis</i>
<i>Fragilaria</i>	<i>Oocystis</i>	<i>Uroglena</i>	<i>Euglena</i>	<i>Radiocystis</i>
<i>Navicula</i>	<i>Coelastrum</i>	<i>Mallomonas</i>	<i>Gymnodinium</i>	<i>Oscillatoria</i>
<i>Pinnularia</i>	<i>Ellipsoidion</i>	<i>Synura</i>	<i>Peridiniopsis</i>	<i>Planktothrix</i>
<i>Stauronesis</i>	<i>Golenkinia</i>	<i>Spiniferomonas</i>		<i>Limnothrix</i>
<i>Synedra</i>	<i>Chlamydomonas</i>			<i>Aphanizomenon</i>
<i>Tabellaria</i>	<i>Polytoma</i>			<i>Aphanothece</i>
<i>Urosolenia</i>	<i>Scourfieldia</i>			<i>Gleothece</i>
	<i>Crucigenia</i>			
	<i>Scenesdesmus</i>			
	<i>Tetrastrum</i>			
	<i>Pediastrum</i>			
	<i>Tetraedron</i>			
	<i>Cosmarium</i>			
	<i>Euastrum</i>			
	<i>Staurastrum</i>			
	<i>Staurodesmus</i>			
	<i>Mougeotia</i>			

APPENDIX B

ZOOPLANKTON PRESENT IN LAC CROCHE

Table S2: Zooplankton species found in Lac Croche

Species
<i>Cladocera</i>
<i>Bosmina longirostris</i>
<i>Daphnia ambigua</i>
<i>Daphnia catawba</i>
<i>Daphnia longiremis</i>
<i>Diaphanosoma brachyurum</i>
<i>Holopedium gibberum</i>
<i>Leptodora kindtii</i>
<i>Calanoida</i>
<i>Epischura lacustris</i>
<i>Leptodiptomus minutes</i>
<i>Cyclopoida</i>
<i>Cyclops scutifer</i>
<i>Mesocyclops edax</i>
<i>Orthocyclops modestus</i>
<i>Tropocyclops prasinus</i>
<i>Diptera</i>
<i>Chaoborus flavicans</i>
<i>Chaoborus punctipennis</i>

APPENDIX C

ADDITIONAL PHYTOPLANKTON COMMUNITY RESPONSES

Major taxon biovolume estimates of integrated water column phytoplankton biomass from microscope counts showed phytoplankton classes responded differently to elevated CO₂ in terms of biomass (Fig. S1). Biomass of the chlorophytes, chrysophytes, cryptophytes and diatoms were initially higher in the Experimental basin, but all declined to a much larger degree relative to what was observed in the Control basin during the experiment (Fig. S1a-c,e). The only group that increased in biomass throughout the experiment was the cyanobacteria (Fig. S1d). Euglenoids and dinoflagellates were also enumerated microscopically, but they were always very rare in both basins (data not shown).

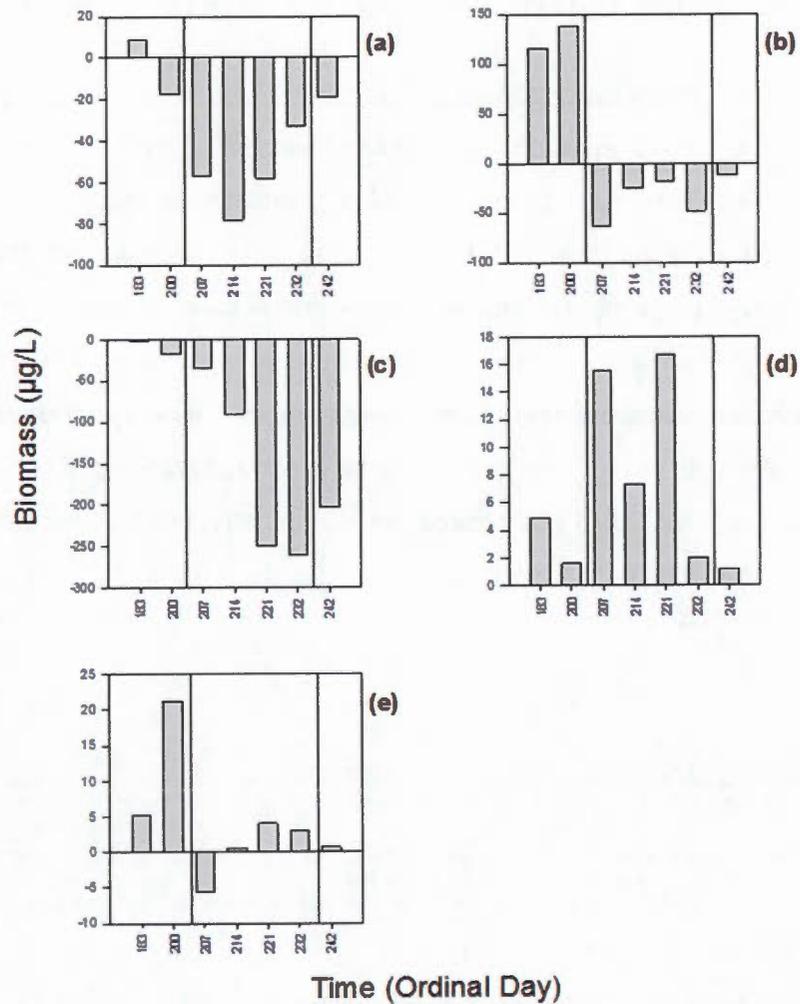


Fig. S1 Differences in phytoplankton biomass ($\mu\text{g/L}$) by taxonomic group observed in the Experimental minus the Control, based on biovolumes calculated from microscope counts. Taxonomic groups represent cryptophytes (a), chlorophytes (b), chrysophytes (c), cyanobacteria (d), diatoms (e). The vertical black lines delimit the experimental period.

APPENDIX D

ADDITIONAL ZOOPLANKTON COMMUNITY RESPONSES

Zooplankton community composition also showed different trends in the two basins (Fig. S2). Larger peak biomass values were observed in the Experimental basin in the dominant grazer *Daphnia* on day 211 (Fig. S2a), and in all other groups one week later (day 218; Fig. S2b-d), creating also a corresponding peak in total zooplankton biomass (Fig. 7). While the cladocerans (including *Daphnia*) and calanoid copepods ultimately declined over the experimental and post-experimental periods, cyclopoid copepods increased steadily over time in the Experimental basin (Fig. S2c), as they also did in the Control basin.

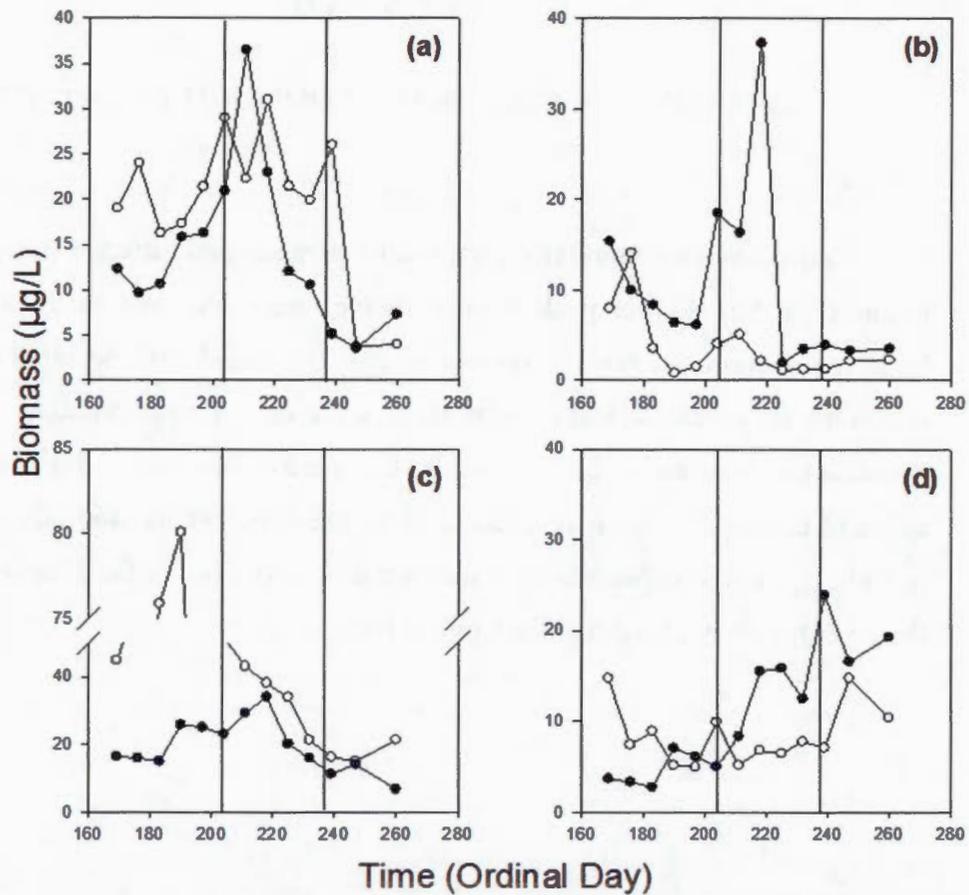


Fig. S2 Time series of zooplankton biomass in the Experimental (black symbols) and Control basins (white symbols) by major taxonomic group: (a) *Daphnia*, (b) Other cladocera (*Bosmina* and *Holopedium*), (c) calanoid copepods, and (d) cyclopoid copepods. The vertical black lines delimit the experimental period.

The most important grazers in the community (*Daphnia*) were the first zooplankton group to respond with increasing biomass, reaching peak values just five days after the experiment began. We also observed that the other groups, composed of less efficient grazers also responded with increased biomass, several days later. In all zooplankton groups (with the exception of the cyclopoids), population biomass peaks were followed by sharp crashes. This resembles a typical predator-prey

dynamic with peak herbivore levels severely depleting prey biomass, at which point a productive phytoplankton prey community could no longer support the inflated zooplankton biomass.

Zooplankton in the Experimental basin also altered their vertical spatial position (Fig. S3). Zooplankton were less abundant at the usual phytoplankton DCM depth (around 4m) in the Experimental Basin, unlike the Control basin, where the highest levels of biomass occurred at these depths. In the Experimental basin after the DCM had disappeared, fewer zooplankton were found at the depth at which the normal DCM is found, likely because the phytoplankton in this area were completely depleted. In the Control, zooplankton biomass was concentrated near the still-present DCM. Apparently, once edible phytoplankton were no longer concentrated in the DCM, being replaced by cyanobacteria, zooplankton were no longer choosing to occupy those depths. The idea of altered organism behavior resulting from changes in the carbon pool has not been addressed in previous climate change studies in plankton. This was not a result that had been anticipated prior to the study, and provides exciting future avenues for research, and showing again the value of the “real-world” context in building predictive capacity for lakes under climate change scenarios.

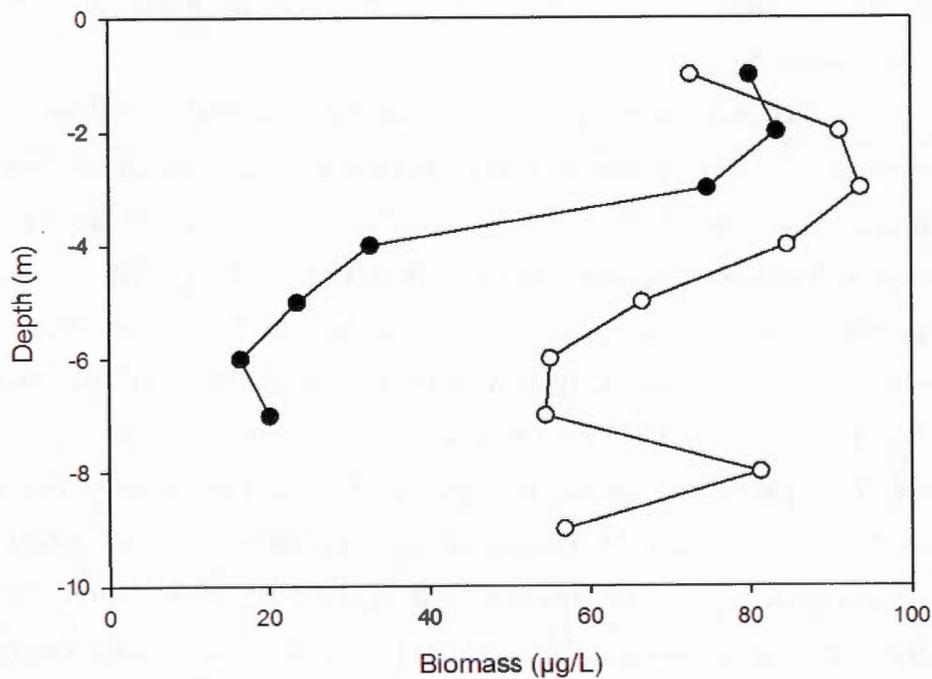


Fig. S3 Profile of the total zooplankton biomass difference in the water column as assessed on day of year 263 by the Laser Optical Plankton Counter (LOPC) in the Experimental basin (black symbol) and the Control basin (white symbol).

APPENDIX E

INTERANNUAL COMPARISON

Data collected from Lac Croche during a year in which no manipulation took place (2011) acted as a secondary control to compare the experimental results of 2013. $p\text{CO}_2$ in 2011 (Fig. S4a) shows comparable levels to the control in 2013 (Fig. 2a). Primary abiotic data in 2011 (Fig. S4) includes pH (b), temperature (c) and dissolved oxygen (d). The plot of pH (Fig. S4b) shows both basins with similar patterns throughout the summer, however, this is an example of the interannual variability that exists in Lac Croche, as pH levels in 2013 (Fig. 2b) differ quite significantly. Comparable with temperature (Fig. S4c), the two basins exhibit virtually the same temperature profiles throughout the summer, but the profiles are different in 2013 (Fig. 2c). Dissolved oxygen in 2011 (Fig. S4d) is distinctly unique in the Control basin, and the high levels of oxygen near the metalimnion can most likely be attributable to high phytoplankton productivity in that region.

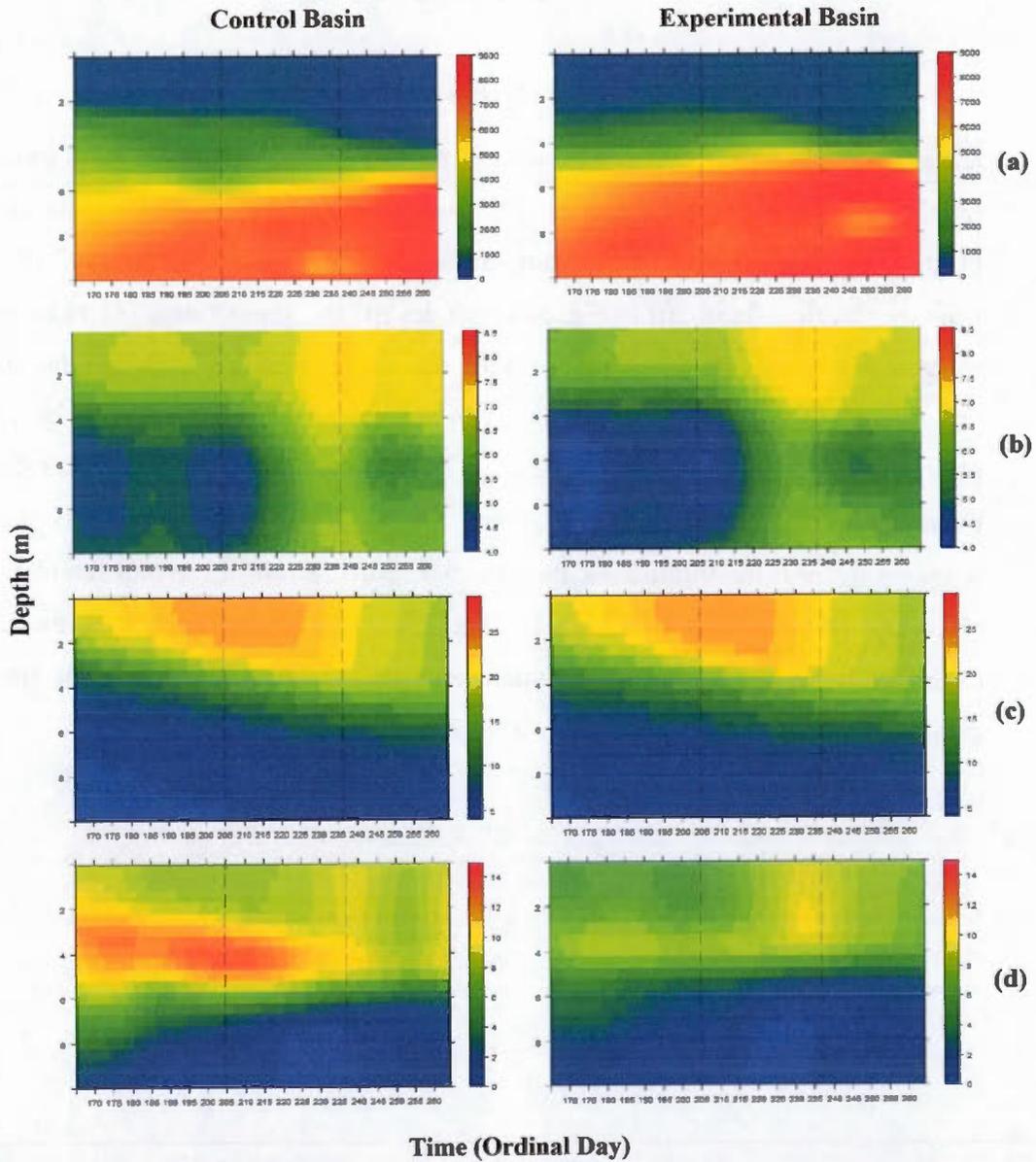


Fig. S4 Surface plots showing time series differences of the vertical profiles of the main abiotic parameters: pCO₂(ppm) (a), pH (b), temperature (°C) (c), dissolved oxygen (DO; mg/L) (d) in both the Experimental and Control basins during the control year (2011). The vertical dashed lines delimit the dates corresponding to the experimental period in 2013.

Peak phytoplankton biomass (maximum-minimum)($\mu\text{g/L}$) (Fig. S5) in both basin 1 and basin 3 followed very similar patterns. As the trend in the control year does not match the control basin in 2013, it once again suggests large interannual variation in Lac Croche. Similarly, the zooplankton dynamics of 2011 (Fig. S6) exhibit interannual variation, but both basins have similar dynamics (Fig. 4). This interannual variation continues to be present in areal chlorophyll levels (Fig. S7) and zooplankton spatial position in the water column (Fig. S8).

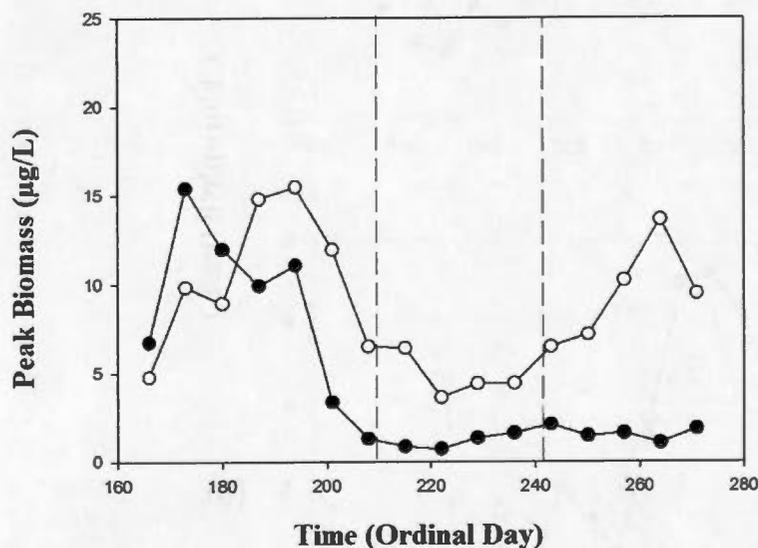


Fig. S5 Peak total phytoplankton biomass across the water column (maximum biomass – minimum biomass) ($\mu\text{g/L}$) as measured by the Fluoroprobe in both the Experimental basin (black symbol) and Control basin (white symbol) during the control year (2011). The vertical dashed lines delimit the dates corresponding to the experimental period in 2013.

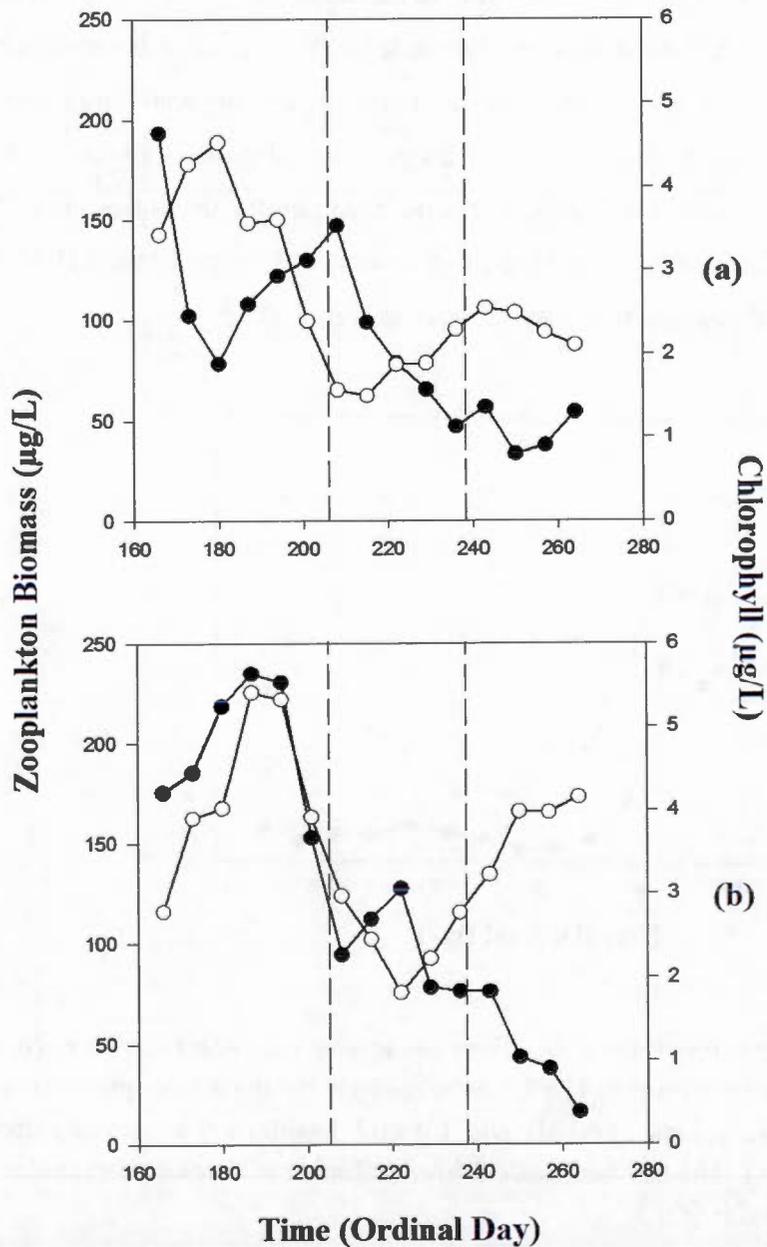


Fig. S6 Time series of zooplankton biomass ($\mu\text{g/L}$, dry weight) (black symbol) and total phytoplankton biomass (chlorophyll *a* in $\mu\text{g/L}$) (white symbol) in Experimental basin (a), and in Control basin (b) during the control year (2011). The vertical dashed lines delimit the dates corresponding to the experimental period in 2013.

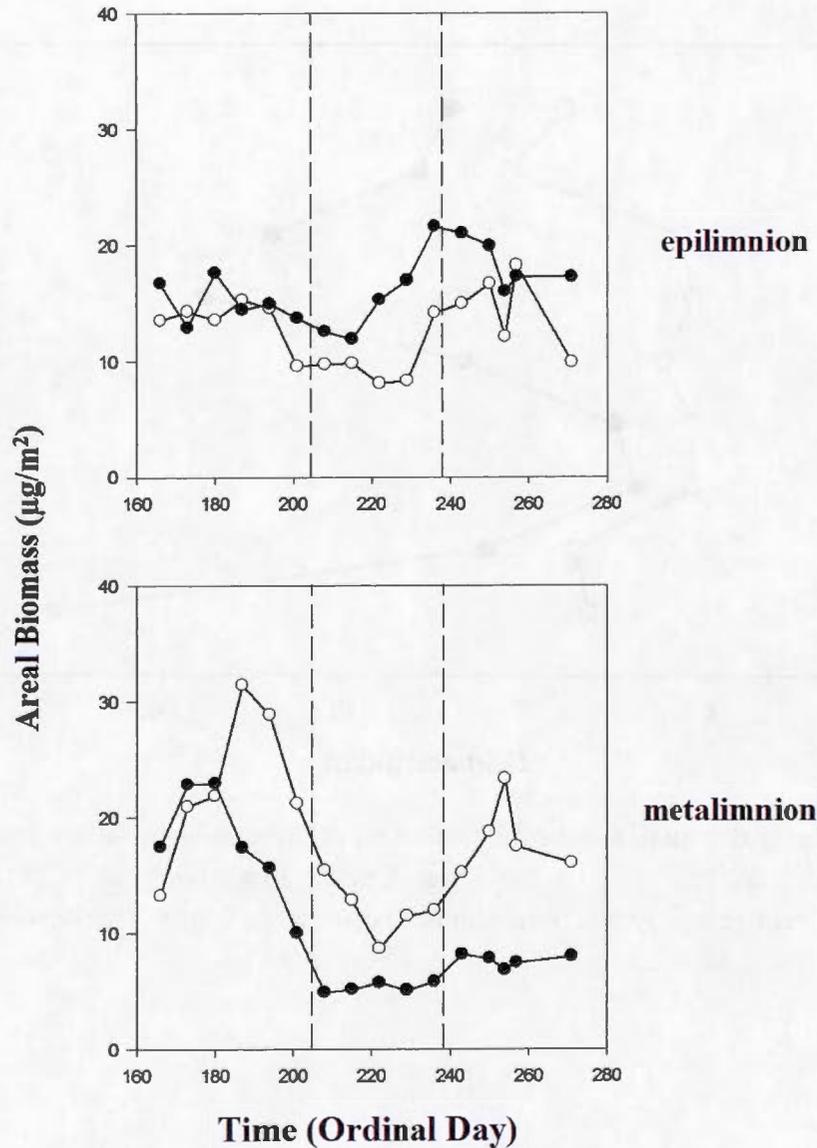


Fig. S7 Areal phytoplankton biomass ($\mu\text{g}/\text{m}^2$) across the epilimnetic (0-4m) and metalimnetic (4-7m) depths observed in Experimental basin (black symbol) and Control basin (white symbol) as measured with the FP during the control year (2011). The vertical dashed lines delimit the dates corresponding to the experimental period in 2013.

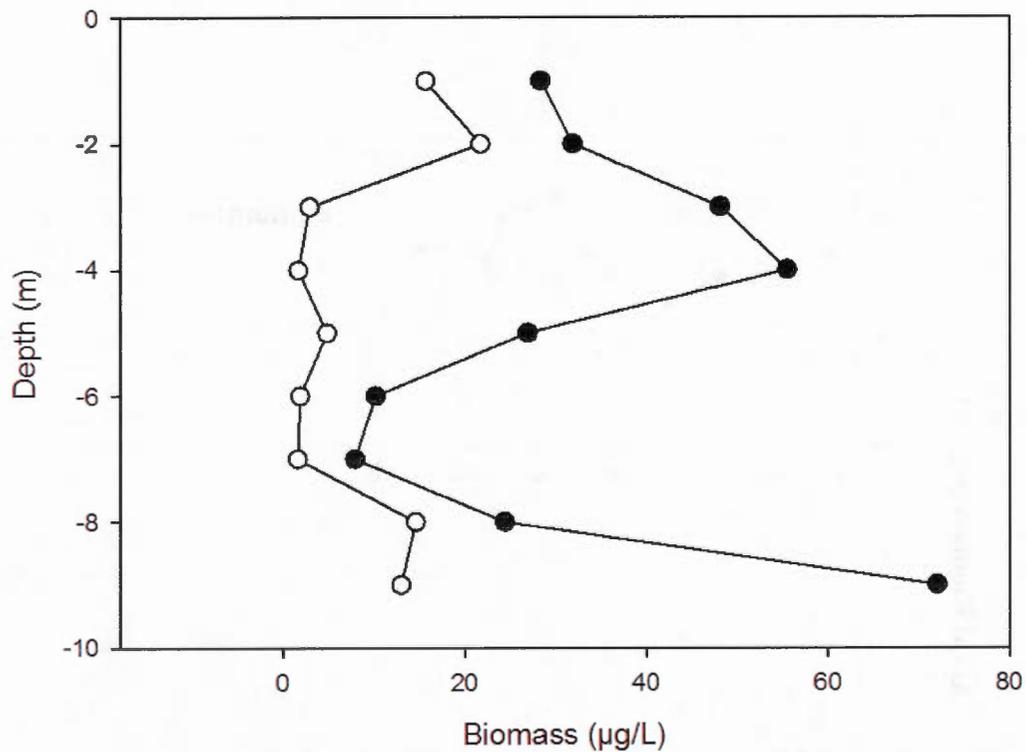


Fig. S8 Profile of the total zooplankton biomass difference in the water column as assessed on day of year 260 by the Laser Optical Plankton Counter (LOPC) in Experimental basin (black symbol) and Control basin (white symbol) during the control year (2011).

APPENDIX F

STATISTICAL ANALYSIS

Table S3: Results of the analysis of variance using the non-parametric Wilcoxon test for non-normally distributed data. Tests were done for differences of time-averaged means between the two focal basins of Lac Croche across two time periods: prior (pre-experimental) and during the experiment. Variables analyzed are for depth-integrated FP values for total Chla, GREENS, CYANOS, BROWNS, CRYPTOS and depth-integrated YSI values for temperature, dissolved oxygen (DO) and pH

Variable	Pre-experiment Mean Difference ±SEM	Experimental period difference ±SEM	U-test P-value
pCO ₂ (ppm)	233.91 ± 121.32	868.79 ± 67.40	<0.0001
Total Chla (µg/L)	0.590 ± 0.255	-1.769 ± 0.230	<0.0001
GREENS (µg/L)	0.592 ± 0.076	0.103 ± 0.689	<0.0001
CYANOS (µg/L)	0.014 ± 0.013	0.169 ± 0.011	<0.0001
BROWNS (µg/L)	-0.034 ± 0.198	-1.956 ± 0.179	<0.0001
CRYPTOS (µg/L)	0.017 ± 0.025	-0.084 ± 0.023	0.2076
Temperature (°C)	0.775 ± 0.829	0.569 ± 0.626	0.8431
DO (mg/L)	-0.401 ± 0.440	-0.353 ± 0.332	0.5919
pH	0.037 ± 0.063	0.879 ± 0.048	0.7859

APPENDIX G

NET PRIMARY PRODUCTIVITY MODEL

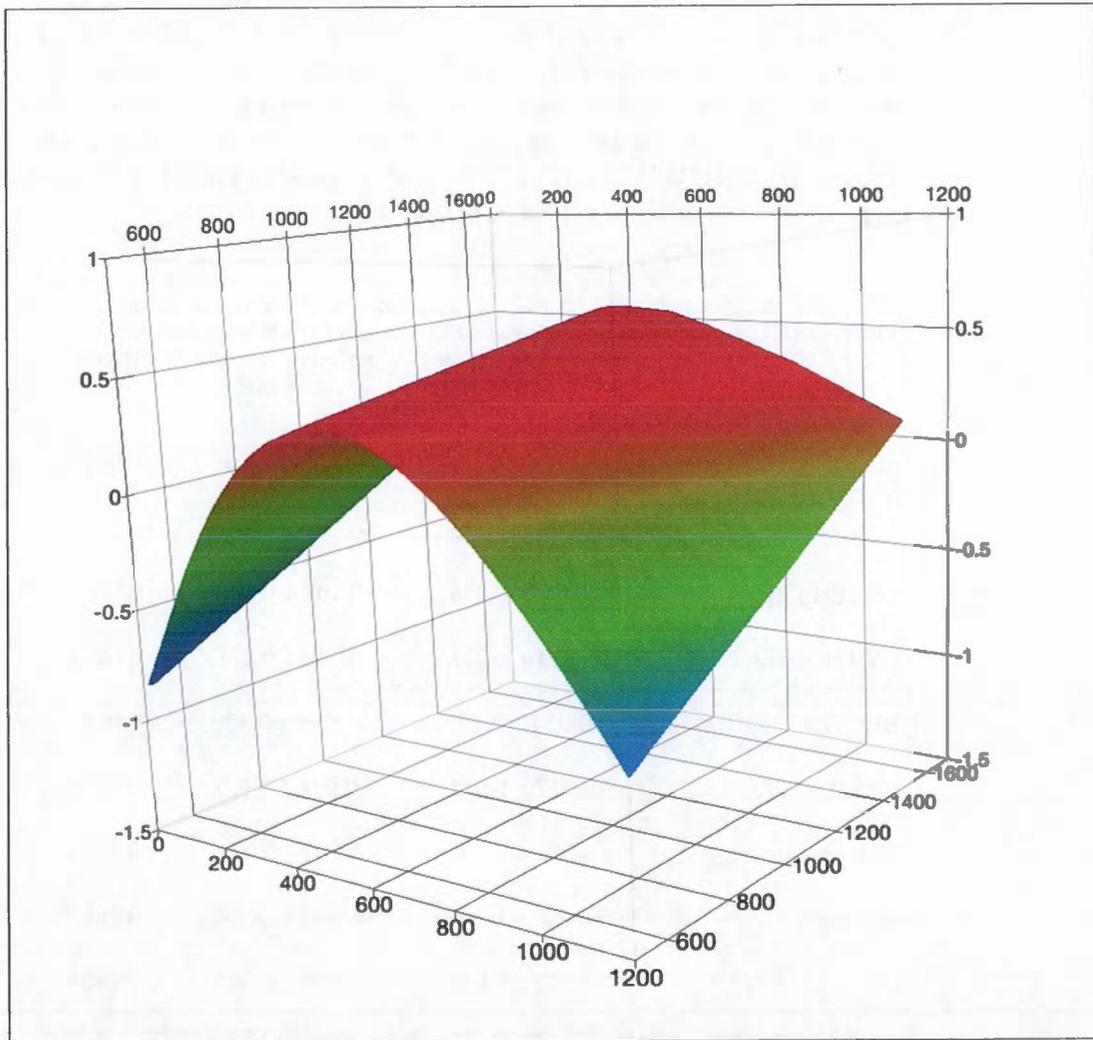


Fig. S9 Model of net primary productivity based on light and carbon dioxide levels.

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