

Does dissolved organic matter impact primary production?

**A study on the effects of terrestrially derived dissolved organic matter on primary
production in nutrient-poor boreal lakes**

BRYANNA SHERBO

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Abstract

Over the past few decades, spatial and temporal variation in concentrations of allochthonous dissolved organic carbon (DOC) has been increasing in surface waters of boreal lakes around the world with climate change. Terrestrial landscapes export large amounts of organic matter into lakes, primarily as DOC. It is understood that DOC impacts primary production through its combined effects on light and nutrient availability. However, there remains uncertainty as to the mechanisms that cause DOC to have positive or negative effects on algal productivity. In a survey of 6-7 lakes spanning a DOC gradient (3.5-9.4 mg L⁻¹) at International Institute of Sustainable Development-Experimental Lakes Area (IISD-ELA) in northwestern Ontario, we determined the effects of DOC on whole-lake metabolism (gross primary production (GPP), respiration, and net ecosystem production (NEP)) and depth integrated net primary production (NPP). Using *in situ* diel free-water oxygen method and *in vitro* partial pressure of CO₂ incubations, we found that primary production was significantly impacted by DOC. In general, the effects of DOC on algal productivity was negative, although there was some evidence for the stimulation of whole ecosystem production by nutrients at low DOC concentrations. Epilimnetic chlorophyll, GPP, and community respiration increased significantly with DOC, and total depth integrated and sub-epilimnetic NPP decreased significantly with DOC. Lakes with the highest proportion of sub-epilimnetic production had well developed deep chlorophyll maxima (DCM). DCM were negatively correlated with DOC and are viewed as ecological hotspots for higher trophic levels. DCM and sub-epilimnetic productivity were eliminated in lakes with DOC values > 7.1 mg L⁻¹. These results substantiate the influence of allochthonous DOC on aquatic food webs and highlight the combined effects of DOC on light and nutrient availability. The association

of DOC with epilimnetic nutrients caused an increase in epilimnetic GPP (chapter 2) and decreasing light availability caused a decrease in total and sub-epilimnetic NPP (chapter 3). These contrasting effects of DOC on primary production provide further insight into the balance between nutrients and light with increasing concentrations of DOC.

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Chapter 1: Introduction and literature review

Introduction

Boreal lakes are the most numerous lake type in the world (Downing et al. 2006). They contain more than 60% of the world's freshwater (Schindler 2001). In Canada, the boreal region includes approximately one third of the world's boreal forest (Bryant et al. 1997), extending coast to coast. Terrestrially derived dissolved organic matter (DOM) is the most abundant of all complex particulate and dissolved substances discharged to lakes in the Canadian boreal forest (Ask et al. 2009b; Schindler et al. 1997). Terrestrially derived DOC is increasing in boreal lakes around the world and will continue to increase as a consequence of climate change (Clark et al. 2010; Seekell et al. 2015). While it is understood that DOC influences lake productivity there remains considerable uncertainty as to the conditions that cause DOC to have positive or negative effects on algal productivity, and the implications of these changes to lake food webs (Solomon et al. 2015).

Our study was part of a larger project at International Institute of Sustainable Development-Experimental Lakes Area (hereafter IISD-ELA), that looked at potential indicators of fish productivity in boreal lakes. A team of researchers looked at different trophic levels from 'Photons to Fish' to obtain a more holistic understanding of the effects of DOC on aquatic ecosystems. My thesis focused on the lowest trophic level, i.e., primary producers, to better understand the effects of DOC on primary production. Specifically, I looked at how DOC influenced whole-lake metabolism and phytoplankton production throughout the water column.

Background on dissolved organic matter

What is DOC?

Terrestrial DOC originates from decomposing organic plant matter and is introduced to aquatic systems by leaching or overland runoff (Sulzberger & Durisch-Kaiser 2009). Terrestrial landscapes deliver 5.1 Pg of carbon a year to inland waters, this amount is projected to increase by 0.3 Pg of carbon each year with climate change (Drake et al. 2017). DOC loading and concentrations vary greatly among regions and within lakes. For example, concentrations of DOC are highly variable spatially because of varying watershed characteristics (e.g., forest fires, proportion of wetlands in catchment, catchment size, development, etc.), temperature, and hydrological processes (Brett et al. 2017; Clark et al. 2010; Emmerton et al. 2018; Lepistö et al. 2014; Monteith et al. 2007).

Spatial Variation in DOC

Heterogeneity between watershed characteristics explains the majority of spatial variation in DOC loading and composition (Lapierre et al. 2015). Spatial variation in DOC loading and composition among lakes is dependent on soil composition, vegetation, geology, and topography, which affect hydrological and chemical regulation of solubility and transport (Clark et al. 2010; Lapierre et al. 2015). Within and among lakes, DOC can have both positive and negative effects on energy pathways and overall whole-lake production (Hanson et al. 2008; Prairie et al. 2002; Seekell et al. 2015a,b). DOC inputs locally are mainly influenced by watershed size, land use, residence time, and lake morphometry. Within lakes, DOC has the potential to control productivity because of its capacity for altering key physical (e.g., light attenuation, thermocline and photic depths), chemical (e.g. organic nutrients, pH),

and biological (e.g., chlorophyll *a*) attributes (Ask et al. 2009; Cole et al. 2006; Jones 1992; Solomon et al. 2015). Most of these relationships are non-linear and not well defined, and some have thresholds that are not well described. These interrelated variables make it difficult to partition direct and indirect effects of DOC.

As DOC is processed within lakes, it is consumed and degraded, which influences its stoichiometry, composition, and bioavailability. This change in composition and bioavailability of DOC can have cascading effects through lake food webs. DOC affects lake food webs by influencing fatty acid composition of zooplankton, toxin production, methylmercury concentration, and overall contaminant transfer through the food web (Creed et al. 2018).

Temporal Variation in DOC

Temporally, concentrations of DOC are primarily driven by climate change (precipitation, air and soil temperature) and atmospheric deposition of acidifying substances (e.g. sulfur dioxides). Current climate projections predict both increased surface temperatures and precipitation. Surface temperatures will increase by 1.5°C between 2030 and 2052 if warming continues at the current rate (Intergovernmental Panel on Climate Change, 2019). Precipitation will change with increased temperatures, with the largest increases in precipitation occurring at higher latitudes (Intergovernmental Panel on Climate Change, 2019). Precipitation is closely linked to runoff and concentrations of DOC in lakes (Raymond & Saiers 2010) (Figure 1.1).

Increases in DOC loading are more exaggerated in previously acidified watersheds recovering from sulphate deposition (Evans et al. 2006). Increases in DOC loading can be

explained by both deposition chemistry and catchment acid sensitivity (Monteith et al. 2007). As soil pH recovers in previously acidified watersheds, more organic nutrients are released by plant roots and soils (Clark et al. 2010). DOC can be envisaged as the link between terrestrial and aquatic environments. Concentrations of DOC have increased in the majority of acid-impacted lakes in North America and Europe and are predicted to increase to pre-industrial levels from decreased acid deposition and increases in precipitation (Clark et al. 2010; Monteith et al. 2007). This rise in DOC could have unprecedented effects on the carbon cycle.

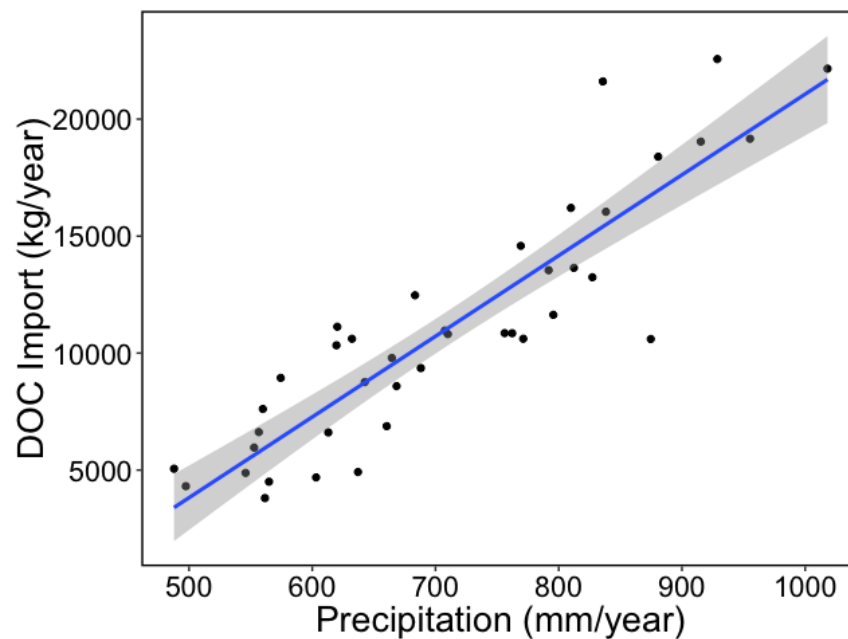


Figure 1.1 Yearly averages of terrestrial derived DOC imports into Lake 239 (L239) and precipitation (linear regression including an assessment of uncertainty as point-wise confidence intervals (grey shading) (Higgins IISD-ELA, unpublished).

Carbon Cycling

Carbon plays a fundamental role in the flow and transformation of materials within and across ecosystems, specifically between terrestrial and aquatic ecosystems. Water retention time facilitates transformation of carbon because, proportions of organic carbon loads and fates shift from allochthonous to autochthonous with an increase in water retention time (Hotchkiss et al. 2018). Organic carbon concentrations are highly variable in shorter water retention time systems, while long water retention time systems transform and breakdown allochthonous carbon lowering organic carbon concentrations. The carbon cycle links atmospheric greenhouse gases to boreal forest ecosystems, and can have significant implications on primary producers through community respiration, production and decomposition (Benoy et al. 2007).

DOC vs DOM?

DOC originates from decomposing plant or animal material into a dissolved form which can be used by aquatic organisms. Dissolved organic matter (DOM) represents a broader suite of dissolved organic compounds (i.e. carbon, nitrogen, and phosphorus). DOC increases proportionally with DOM and is often used as a surrogate (Solomon et al. 2015). Sobek et al. (2007) assembled a large database of DOC concentrations from 7,514 lakes across 6 continents, along with relevant parameters that could be used to classify lake types (i.e. catchment, soil, and climate). DOC concentrations ranged from 0.1–332 mg L⁻¹, with a median of 5.71 mg L⁻¹. Sobek et al. (2007) indicated that 87% of study lakes had DOC concentrations between 1-20 mg L⁻¹.

How does DOC influence lake processes?

DOC can be regarded as a state variable as a consequence of its ability to alter a number of fundamental lake processes (Fee et al. 1996). A state variable has significant implications which affect the functioning of a dynamic system (Beisner et al. 2003), and in this context, the state of freshwater ecosystems is heavily influenced by DOC through its effects on physical, chemical, and biological (i.e. algal productivity) properties. DOC is highly variable within and among lakes, yet its effects on primary production have not been fully explored and remain uncertain.

Physical Effects

DOC modifies light attenuation, thermocline, and photic depths due to its light absorbing properties (Figure 1.2). 'Brownification' (Brothers et al. 2014a; Kritzberg & Ekström 2012) is a process whereby inputs of terrestrially derived DOC from surrounding catchments stain lake water. These inputs are primarily made up of coloured or chromophoric DOM (cDOM) composed of aromatic and high molecular weight compounds which are highly photodegradable (Lindell et al. 2000). This interaction between DOC and light limitation causing brownification has been confirmed at IISD-ELA from historic data.

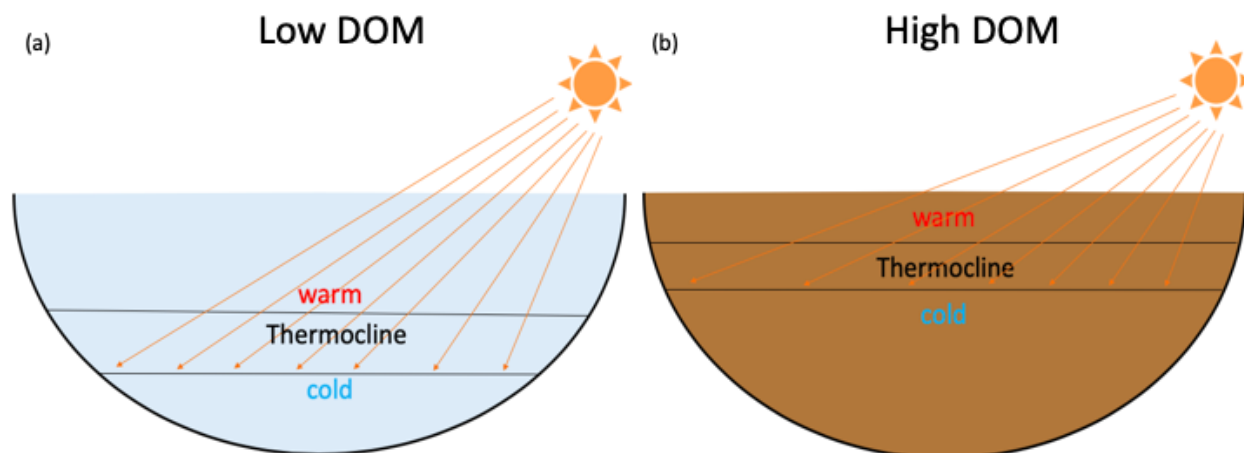


Figure 1.2 In lower DOC lakes, the thermocline and photic depth are deeper, resulting in a larger volume in which photosynthesis may occur *(a)*. In lakes with higher DOC, light is attenuated faster, resulting in a shallower thermocline and photic depths *(b)* (modified from Solomon et al. (2015)).

Chemical Effects

Terrestrially derived DOC can increase nutrient bioavailability (Daggett et al. 2015), stimulate microbial production (Tranvik 1988), and increase primary production throughout boreal lakes worldwide (Klug 2002; Algesten et al. 2003; Cole et al. 2006). DOC inputs may stimulate production because of its association with nutrients such as dissolved organic nitrogen and dissolved organic phosphorus (Daggett et al. 2015). Photodegradation of DOC makes nutrients more bioavailable (Kissman et al. 2013; Klug 2002; Lindell et al. 2000). As DOC goes through photochemical transformations, nutrients (e.g. total dissolved nitrogen and total dissolved phosphorus) become increasingly bioavailable for easier uptake by microbes and plankton (Berggren et al. 2014). In boreal lakes, since the majority of DOC

was from allochthonous origin, Tranvik & Bertilsson (2001) hypothesized that as DOC is photodegraded, microbial, primary, and secondary production should increase as a consequence of the added nutrients. In a nutrient addition study, Daggett et al. (2015) determined that photodegraded DOC increased soluble reactive phosphorus and dissolved organic nitrogen in DOC treatments, which nearly doubled phytoplankton biomass.

Effects of DOC on Primary Production

Primary production in nutrient-poor oligotrophic lakes in the Canadian boreal forest is primarily limited by phosphorus (Schindler 1977) and thus, should be stimulated by nutrients provided by DOM. Effects of DOC in oligotrophic boreal lakes can cause increases in dissolved nutrients and algal biomass (Daggett et al. 2015). Whereas, primary production in eutrophic lakes is not strongly impacted by DOC, despite DOC significantly altering water chemistry (Feuchtmayr et al. 2019). In eutrophic lakes with large external inputs of nutrients, any effects of DOC on productivity are more related to its effects on water clarity than nutrients. In shallow mesocosms the addition of DOC to above 10 mg L⁻¹ decreased phytoplankton growth likely due to decreases in light availability (Feuchtmayr et al. 2019). Therefore, nutrient-poor boreal lakes provide an excellent opportunity to examine the potential for nutrients associated with DOC to stimulate primary production.

It is difficult to tease apart how primary producers are affected by DOC, because DOC simultaneously effects interrelated variables, such as light and nutrients. Effects of DOC are dynamic and depend not only on the concentration of DOC but also where the DOC concentration of a particular lake fits within the broader range of concentrations (Figure 1.3a). For example, under some conditions DOC may increase primary production with

increased bioavailable nutrients, while under other conditions DOC may impose light limitation upon primary producers (Jones 1992), thereby limiting primary production (Brett et al. 2017). In aquatic ecosystems, a balance between nutrient stimulus and light limitation with DOC inputs has been proposed; this balance can result in a DOC threshold (Figure 1.3b) (Seekell et al. 2015a; Creed et al. 2018). As proposed by Solomon et al. (2015), the threshold marks a transition from where nutrients associated with DOC stimulate primary production to where reductions in light availability reduce primary production (Figure 1.3). For example, at lower concentrations of DOC below a DOC threshold the positive effects of nutrients on primary production may be stronger than the negative effects associated with reductions in light availability (Seekell et al. 2015a). Above the threshold, the effects of light on primary production have primacy (Hanson et al. 2003; Prairie et al. 2002; Seekell et al. 2015a).

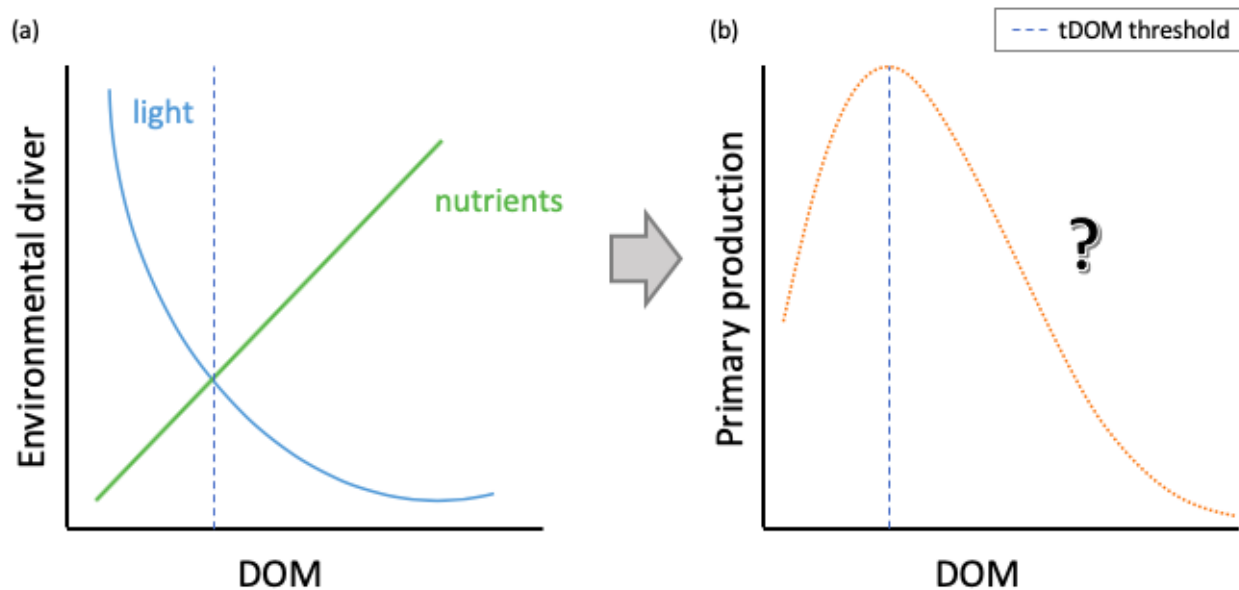


Figure 1.3 At the intersection of decreasing light and increasing nutrients a DOC threshold or peak primary production can be found (a). Primary production increases with nutrients associated with DOC until the threshold where it peaks, then decreases due to light limitations (b). Modified from Solomon et al. (2015).

Methods for assessing effects of DOC on primary production

Whole-Lake Manipulation Studies

Whole-lake manipulation studies address effects of DOC on whole-lake production, regionally and temporally (Vasconcelos et al. 2018; Zwart et al. 2016a). In a manipulated whole-lake study, where DOC was increased over two years, Zwart et al. (2016) observed an increase in pelagic primary production, possibly a consequence of an increase in phosphorus with no significant change in light attenuation. Respiration increased with DOC, resulting in whole-lake heterotrophy, i.e. negative net ecosystem production (Zwart et al. 2016). In a

long-term DOC enrichment study in a pond in Sweden, Vasconcelos et al. (2018) determined that DOC provided a nutrient subsidy to pelagic producers and caused light limitation on benthic algal communities, thereby inhibiting benthic algal photosynthesis. This caused a shift in primary production from benthic to pelagic algae at a whole-lake scale. Vasconcelos et al. (2018) concluded DOC inputs modified light and nutrients, thereby altering the relative contributions of benthic and pelagic algae to total primary production.

Mesocosm Studies

Mesocosm studies allow the manipulation of multiple factors but are limited in space and time. Vasconcelos et al. (2016) manipulated both water temperature and cDOM, to simulate effects of climate change. Vasconcelos et al. (2016) looked at the effects of temperature and browning (increasing DOC) on the competition between benthic and pelagic producers and overall lake production. This mesocosm study manipulated temperature (+3°C) and DOC, where DOC was more than two times higher in the high compared to low cDOM treatments and was slightly lower in the temperature manipulated warmed compared to ambient treatments. During this experiment, benthic algal production and biomass declined and pelagic algal production and biomass increased with increased cDOM. Nutrients increased with cDOM, increasing pelagic algal production and biomass. Warmed treatments (+3°C) had much lower benthic and pelagic primary production, pelagic algal biomass, and pelagic phosphorus than ambient temperature treatments (Vasconcelos et al. 2016). Vasconcelos et al. (2016) determined that climate change, with increased temperatures and DOC, will significantly alter primary production.

Spatial Studies

Many studies have used spatial approaches to assess the effects of DOC on lake processes and productivity using natural gradients of DOC, providing the opportunity to determine the existence of a DOC threshold (Ask et al. 2009; Seekell et al. 2015a; Seekell et al. 2015b). Such studies have benefits and limitations. They are beneficial when time is limited as they are simpler, less costly, and easier to undertake than ecosystem manipulations. Spatial studies include natural ecosystem complexity, generally a large range of DOC, and are common among researchers which allows for comparisons among different ecoregions and lake types (e.g. large or small lakes, stratified vs. polymictic lakes, oligotrophic vs. eutrophic lakes). However, they are limited because many variables in the environment cannot be completely controlled and there are a number of assumptions, not all of which are fully known, when extrapolating results to individual lakes over time to assess, for example, effects of future climate change.

In a spatial study, Prairie et al. (2002) determined a DOC threshold of 4-6 mg L⁻¹ in 33 lakes in Quebec and Hanson et al. (2003) found a DOC threshold >10 mg L⁻¹ in 25 lakes in Wisconsin. These thresholds refer to transition points where the effects of DOC on primary production shift from positive to negative. Both studies measured whole-lake metabolism using diel changes in dissolved oxygen (identical to our approach in chapter 2) to determine net ecosystem production (NEP), respiration (R), and gross primary production (GPP). The differences in thresholds between the two studies may be attributed to differing nutrient concentrations and light availability as the most influential variables (Table 1.1).

In a study of 4 unproductive shallow (2.8-4.6 m) subarctic lakes of varying DOC concentration in Sweden, Ask et al. (2009a) determined whole-lake production was

dominated by benthic habitats (86%), with 77% of production from benthic primary producers. Heterotrophic bacteria contributed to high amounts of production in both benthic and pelagic habitats and were fuelled by higher concentrations of DOC. Net ecosystem production was positive (autotrophic) in benthic habitats but negative (heterotrophic) in pelagic habitats, likely due to increased microbial activity in pelagic habitats (Ask et al. 2009a). No DOC threshold was identified in this study, potentially because the DOC concentrations were too low (2.4-4.1 mg L⁻¹ DOC).

Seekell et al. (2015a) examined algal productivity across lakes of varying DOC concentration and determined that DOC was positively and nonlinearly influenced by light and nutrients. These nonlinearities created a DOC threshold at ~4.8 mg L⁻¹. Below this threshold, primary production increased with DOC because of increases in nutrients associated with DOC. Above 4.8 mg L⁻¹ DOC the pattern reversed; primary production decreased as a result of decreased light. In a larger study of 703 subarctic and boreal lakes, Seekell et al. (2015b) observed a DOC threshold of 5.96 mg L⁻¹ DOC at the intersection of decreasing light and increasing nutrients.

While DOC inputs have been increasingly recognized for their influence on aquatic ecosystems, these effects are variable among and within lakes. Regarding the effects of DOC on primary production, studies have reported highly variable results. Primary production has been shown to increase with increasing DOC loads over time (Zwart et al. 2016a). As well, primary production has been influenced non-linearly by DOC in space-for-time study by Ask et al. (2009) and in model predictions by Kelly et al. (2018) and Solomon et al. (2015). Primary production has also been shown to both increase and decrease with a DOC

contributing to a DOC threshold (Hanson et al. 2003; Prairie et al. 2002; Seekell et al. 2015a; Seekell et al. 2015b).

Table 1.1 Studies examining DOC effects on primary production across lakes of varying DOC concentration. The DOC threshold represents the DOC concentration where primary production switches from increasing with nutrients to decreasing due to light limitation. These studies are referred to throughout this thesis.

Authors	Study site	Method	Nutrients	DOC Threshold
Prairie et al. 2002	33 lakes in Quebec	Metabolism study (NEP, GPP and R)	TP limited <20 $\mu\text{g L}^{-1}$	4-6 mg L^{-1}
Hanson et al. 2003	25 lakes in Wisconsin	Metabolism study (NEP, GPP and R)	TP limited >40 $\mu\text{g L}^{-1}$	>10 mg L^{-1}
Ask et al. 2009	4 lakes in Northern Sweden	Primary production was measured using ^{14}C	Low TN and TP	Not identified
Seekell et al. 2015a	28 subarctic and boreal lakes (Sweden and Alaska)	Modelled - Threshold occurred at intersection of light and nutrients	TN limited 80-700 $\mu\text{g L}^{-1}$	4.8 mg L^{-1}
Seekell et al. 2015b	703 subarctic and boreal lakes (Sweden and Alaska)	Modelled - Threshold occurred at intersection of light and nutrients	TN limited 221-311 $\mu\text{g L}^{-1}$	6.0 mg L^{-1}

Our study encompassed many aspects of the effects of DOC on primary production. Specifically, on the effects of DOC on whole-lake metabolism (chapter 2), phytoplankton primary production and deep chlorophyll maxima (chapter 3). Few studies on effects of DOC include all these aspects of primary production on the same study lakes over the same time period.

Method discussion

Chapter 2 examines the effects of DOC on heterotrophic and autotrophic production of all organisms (i.e. fish, zooplankton, algae, and microbes) using a ‘whole-ecosystem’ metabolism approach. Whole-lake metabolic parameters were estimated using a diel free-water dissolved oxygen technique where increases in dissolved oxygen are driven by autotrophic organisms and declines in dissolved oxygen are driven by respiration of all organisms, both of which are corrected for air-water gas exchange (Coloso et al. 2008; Odum 1956; Staehr et al. 2010). This method assumed that the change in dissolved oxygen in a lake reflects the biological balance between photosynthesis and respiration as well as the oxygen exchange between air and water (Odum 1956). The benefits to this method are that net ecosystem production was quantified throughout the epilimnion and included all organisms within benthic and pelagic habitats. The downsides to this method are its inability to assess productivity occurring below the thermocline and inability to partition benthic and pelagic primary production.

Chapter 3 focused on assessing DOC effects on phytoplankton primary production, which required sensitive methods in our low nutrient boreal lakes. Primary production in such low nutrient systems is typically measured using the standard ^{14}C incorporation method of radioactive carbon (e.g. Ask et al. 2009b; Schindler et al. 1972). However, the use of radioactive chemicals is currently not permitted at the IISD-Experimental Lakes Area (IISD-ELA) research station. As well, the ^{14}C method has potential drawbacks including the uncertainty whether results represent gross or net primary production, or some value in between, and it does not provide estimates of respiration (Davies et al. 2003). For these

reasons the ^{14}C method is not directly comparable with methods that utilize changes in O_2 , partial pressure of CO_2 , or DIC (e.g. Aberg & Wallin, 2014; Davies et al. 2003; Davies, 1997).

A novel method developed by Davies et al. (2003) demonstrated that small variations in dissolved inorganic carbon (DIC) during photosynthesis can be assessed within *in vitro* experiments by assessing changes in the partial pressure of CO_2 (pCO_2). This method is sensitive enough to measure low rates of primary production in boreal lakes. The approach by Davies et al. (2003) estimates the photosynthetic parameters ($P_{\max}$, α , respiration) using changes in DIC through the measurement of changes in pCO_2 throughout an incubation, and supplemental information on pH and alkalinity measured at the start and end of the incubation. The benefit of this method is its ability to measure phytoplankton photosynthesis in oligotrophic lakes throughout the depth of the water column using photosynthesis-irradiance parameters (Jassby & Platt 1976) and model calculations (Vadeboncoeur et al. 2001). A drawback of this method was its labour intensive nature and, consequently, our inability to run a larger number of concurrent incubations. As such, in our application of the pCO_2 method, we were unable to directly assess the photosynthetic response of algal communities below the thermocline and were forced to extrapolate photosynthesis-irradiance values determined in the epilimnion. While we concluded such errors were small (chapter 3), direct measurements of sub-epilimnetic productivity would have allowed more certainty in the calculation of depth integrated NPP. As well, Fee et al. (1987) showed high annual variability in the photosynthesis-irradiance response over 13 years in lakes at the IISD-ELA. Our study did not assess annual variability, it was performed as a snapshot in time to focus on the spatial differences in lake processes and DOC concentrations.

Thesis outline and structure

This thesis is written in manuscript style. Chapters 2 and 3 are written as stand-alone manuscripts for publication. There is some repetition because the background information on dissolved organic carbon and study site is the same in both chapters. These data chapters assess the effects of dissolved organic carbon on primary production.

We investigated the effects of terrestrially derived DOC on primary producers. My specific research questions were:

1. How does DOC influence whole-lake metabolism? (chapter 2)
 - Assessing the effects of DOC on gross primary production (GPP), net ecosystem production (NEP) and respiration (R) at a whole-lake scale measured using diel changes in dissolved oxygen.
2. How do effects of DOC influence phytoplankton productivity throughout the water column? (chapter 3)
 - Assessing net primary production throughout the water column to determine the influence of DOC on phytoplankton production and deep chlorophyll maxima.

Chapter 2: Effects of dissolved organic matter on whole-lake metabolism

Abstract

Terrestrial landscapes export large amounts of organic matter into lakes, primarily as dissolved organic carbon (DOC). Over the past few decades, concentrations of allochthonous DOC have been increasing in surface waters of boreal lakes. DOC impacts primary production through its combined effects on light and nutrient availability. We used a diel free-water oxygen method to measure primary production and community respiration to determine the influence of DOC on 7 boreal lakes spanning a DOC gradient (3.5-9.4 mg L⁻¹) at the IISD-Experimental Lakes Area in Northwestern Ontario. Gross primary production ($p < 0.05$) and community respiration ($p < 0.05$) increased significantly with DOC. The lakes were in near metabolic balance until DOC exceeded a threshold of 6 mg L⁻¹; then net ecosystem production declined as a consequence of increased respiration, becoming more heterotrophic. Below this threshold, effects of nutrients increased more rapidly than light attenuation, and above it, the opposite was true. These results substantiate the importance of allochthonous DOC as an energy subsidy by providing nutrients for primary producers and supporting aquatic food webs.

Introduction

Boreal lakes, the most numerous lake type in the world (Downing et al. 2006), receive large inputs of terrestrially derived organic matter, the most abundant of all complex particulate and dissolved substances discharged to lakes in the Canadian boreal forest (Ask et al. 2009b; Schindler et al. 1997). Dissolved organic matter (DOM) (measured as dissolved organic carbon (DOC)) can be regarded as a state variable due to its ability to alter lake

processes (Fee et al. 1996). DOC is a key factor controlling whole-lake productivity because of its potential for altering key physical (e.g., light attenuation, thermocline depths), chemical (e.g. organic nutrients, pH), and biological (e.g., chlorophyll *a* and primary production) attributes (Ask et al. 2009; Cole et al. 2006; Jones 1992; Solomon et al. 2015).

DOC is highly variable among and within boreal lakes due to hydrologic inputs, climate change, recovery from acidification, and changes in watershed characteristics (e.g., forest fires, development, etc.) (Brett et al. 2017; Clark et al. 2010; Emmerton et al. 2018; Lepistö et al. 2014; Monteith et al. 2007). Spatial variation of DOC among lakes is dependent on soil composition, vegetation, geology, and topography, which affect hydrological and chemical regulation of solubility and transport (Clark et al. 2010; Lapierre et al. 2015).

Terrestrially derived DOC is increasing in boreal lakes around the world (Clark et al. 2010; Seekell et al. 2015). Concentrations of DOC have increased in the majority of acid impacted lakes in North America and Europe over the last twenty years with decreasing atmospheric sulphate (Clark et al. 2010). As watersheds continue to recover from acid rain, DOC is predicted to increase to pre-industrial levels and increases in DOC loads via increased precipitation associated with climate change may drive DOC concentrations even higher; this widespread rise in DOC loads to freshwaters could have unprecedented effects on the carbon cycle (Monteith et al. 2007). Climate projections predict increasing variation in precipitation (wet and dry areas) and recovery from acidification, thereby further increasing variation of DOC among and within lakes. These increases in DOC within lakes have been colloquially referred to as 'brownification' (Brothers et al. 2014a; Kritzberg & Ekström 2012), and defined as a process whereby inputs of terrestrially derived DOC from surrounding catchments stain lake water. These inputs are primarily made up of colored DOM (cDOM)

which strongly absorbs light, and may impose light limitation upon primary producers (Jones 1992). In contrast, DOC inputs may stimulate primary production from its association with nutrients such as dissolved organic nitrogen and dissolved organic phosphorus (Creed et al. 2018). Thus, DOC could have either positive or negative effects on energy pathways and overall whole-lake production within and among lakes (Hanson et al. 2008; Prairie et al. 2002; Seekell et al. 2015a,b). This variability of DOC across the boreal landscape and within lakes illustrates the need for more research to tease apart varying effects of DOC on lake productivity.

As allochthonous DOC increases its contrasting effects on nutrients (increasing) and light availability (decreasing) can result in a potential threshold (Seekell et al. 2015a; Creed et al. 2018). The threshold marks a transition from where nutrients associated with DOC stimulate primary production to where reductions in light availability reduce primary production. At lower concentrations of DOC, effects of nutrients increase more rapidly than those attributable to light attenuation, and primary production increases (Seekell et al. 2015a). Above the threshold, at higher concentrations of DOC, nutrients continue to increase but light attenuation also increases, limiting the amount of light available for photosynthesis and thereby limiting primary production (Hanson et al. 2003; Prairie et al. 2002; Seekell et al. 2015a).

The primary production of lakes can be assessed as either gross or net primary production. Gross primary production (GPP) accounts for photosynthetic production; it is the gross fixation of inorganic carbon (CO_2) without consideration of respiratory losses. Net ecosystem production (NEP) represents GPP minus CO_2 losses via respiration (R) of all biota including microbes, plants, and animals (Equation 2.1) (Cole et al. 1994).

$$(2.1) \text{NEP} = \text{GPP} - R$$

An autotrophic lake has a positive NEP, indicating more photosynthesis than respiration ($\text{GPP} > \text{respiration}$), and a net CO_2 invasion into lakes from the atmosphere. A heterotrophic lake has a negative NEP, indicating more respiration than photosynthesis ($\text{GPP} < \text{respiration}$), resulting in net CO_2 evasion from lakes. Lakes can switch between states of autotrophy and heterotrophy. For example, increases in DOC can stimulate microbial respiration causing increased heterotrophy. Increased heterotrophy causes lakes to release CO_2 and potentially stored carbon from sediments into the atmosphere, leading to increased CO_2 emissions to the atmosphere (Molot & Dillon 1996; Sobek et al. 2005).

When assessing the effects of DOC on lake productivity and respiration it is advantageous to utilize approaches that include both benthic and pelagic habitats. Whole-lake metabolism represents the combined catabolism and anabolism of all organisms in lake surface waters, and can be calculated using the diel variation in dissolved oxygen concentration and several parameters to estimate air-water gas exchange (Staehr et al. 2010). Air-water gas exchange can be calculated using air and water temperatures, barometric pressure, thermocline depth, and wind speed (Staehr et al. 2010). DOC influences light attenuation and thermocline depth, further affecting whole-lake metabolism (Hanson et al. 2003). DOC can be used to help understand the carbon balance in lakes, because processes (e.g. respiration and photosynthesis) influence the carbon balance by storing or releasing CO_2 .

The primary objective of our study was to examine the effects of allochthonous DOC on epilimnetic metabolism for 7 oligotrophic boreal lakes along a DOC gradient. We hypothesized that: (1) DOC would provide a nutrient subsidy, leading to increased primary production; (2) increased DOC would reduce light intensity, thereby limiting the extent of the photic zone, leading to decreased primary production; (3) along our DOC gradient, a threshold concentration of DOC would be apparent, causing lake metabolism to switch from positive to negative. Nutrient subsidy and light limitation effects will act simultaneously over the DOC gradient; however, the effect of the nutrient subsidy would become overwhelmed by negative effects of light attenuation beyond the DOC threshold. That is, lower concentrations of DOC would stimulate primary production with increased nutrients while higher concentrations of DOC would decrease primary production as a result of increased light attenuation, offsetting effects on metabolic processes.

Methods

Site Description

Seven pristine boreal lakes spanning a DOC gradient (3.5-9.4 mg L⁻¹) at the International Institute of Sustainable Development-Experimental Lakes Area (hereafter IISD-ELA) in northwestern Ontario were selected for this study. These lakes were chosen for their DOC concentrations, similar lake morphometries (i.e. lake size), biotic similarities (fish and plankton species composition) and close geographical proximity (<30 km) to the IISD-ELA field station and meteorological station (map in appendix A1).

Watersheds

All watersheds in this region are dominated by coniferous forest and have been relatively protected from anthropogenic land-use impacts. These lakes experience similar climatic conditions, are small (max depth 7-30 m), nutrient-poor ($5.7\text{-}8.0\ \mu\text{g L}^{-1}$ TP) and phosphorus-limited (Schindler 1977). The lakes are dimictic, stratify throughout the summer, and are ice-covered between mid-November and late April. Watershed characteristics of the lakes in this study are typical of lakes in the Canadian boreal forest (Table 2.1).

Total watershed area (Table 2.1) included all terrestrial drainage, lake surfaces and tributary watersheds. Lake surface area and watershed area was determined using topographic maps. Lake volume was calculated from lake bathymetry. Drainage ratio was determined by watershed area divided by lake surface area ($A_d:A_0$). Retention time is the average theoretical water renewal time in years and was estimated by dividing lake volume (V_L) by outflow volume (V_{Outflow}). Outflow volume was measured on lakes with weirs (L239). For lakes without weirs, it was estimated from the relationships between precipitation, watershed area (A_d), and yield of precipitation using the equation:

$$(2.2) V_{\text{Outflow}} = \text{Precipitation} * A_d * \text{Yield}$$

Where precipitation and watershed drainage area (A_d) were measured, and yield was estimated at approximately 35%. Yield was determined using the long-term dataset for the L239 catchment, 35% of precipitation falling on the watershed reached L239 and 65% of precipitation was absorbed in the surrounding landscape (K. Beaty, unpublished).

Table 2.1 Watershed and morphological characteristics of lakes ordered by increasing DOC concentration. Symbols represent watershed drainage area (A_d), lake volume (V_L), lake surface area (A_0), outflow volume (V_{Outflow}), retention time (RT), A_d divided by V_L ($A_d:V_L$), and A_d divided by A_0 ($A_d:A_0$).

Lake	A_d (ha)	V_L (10^4 m^3)	A_0 (ha)	V_{Outflow} (10^5 m^3)	RT (years)	$A_d:V_L$ (ha/ 10^4 m^3)	$A_d:A_0$
L224	97.50	300	25.9	2.40	13	0.3	3.8
L373	80.60	301	27.3	1.98	15	0.3	3.0
L626	388.0	177	25.9	9.55	1.9	2.2	15
L223	260.0	195	27.3	6.40	3.1	1.3	9.5
L442	161.0	144	16.0	3.96	3.6	1.1	10
L658	57.00	54.6	8.00	1.40	3.9	1.0	7.1
L164	4950	100	20.3	122	0.2	49	240

Limnological Sampling

Lakes were sampled monthly from May-August throughout the open water season in 2018. Monthly sampling included physical, chemical, and biological sampling.

Physical parameters: Light profiles were measured as Photosynthetically Active Radiation (PAR) with an underwater LICOR flat plate PAR sensor (model LI 192 Underwater Quantum Sensor). Underwater readings of PAR were taken at 0.5 to 1 m depth intervals until the reading was less than 1% of surface PAR (photic depth). Maximum depth was measured once during the open water season in 2018 using a depth sounder and was assumed not to change significantly. The photic depth was used to represent the deepest depth receiving 1% surface irradiance. The photic zone represented the water column above the photic depth that received $>1\%$ of surface irradiance, below which photosynthesis is generally assumed to be negligible (Fee et al. 1996). The light attenuation coefficient (K_d) represents the rate

that light is absorbed with increasing depth throughout the water column. Light attenuation and photic depth were calculated for each lake over the open water season from light profiles (Appendix A1.2). Thermocline depth was calculated using LakeAnalyzer program in open source software R from monthly temperature profiles from June to August (Winslow et al. 2018).

Chemical: Water chemistry parameters were determined monthly from integrated epilimnetic water samples collected at the deepest point of each lake and analyzed by IISD-ELA chemistry lab staff using standard analytical methods. Dissolved and particulate nutrients (carbon, nitrogen and phosphorus), pH, conductivity and chlorophyll *a* were measured following the methods of Stainton et al. (1977). Water samples collected to determine DOC concentration were filtered through Whatman GF/C filters and the filtrates were analyzed at the Freshwater Institute (Fisheries and Oceans Canada) in Winnipeg on a Shimadzu Total Organic Carbon Analyzer. Dissolved inorganic carbon (DIC) was measured as micro moles per liter (mmol L^{-1}) on a Li-COR CO₂ gas analyzer model LI-820. Coloured or chromophoric dissolved organic matter (cDOM) was estimated as relative fluorescent units (RFU) with a fluorescent DOM sensor on a Yellow Springs Instrument (YSI model EX02). The fluorescent DOM sensor measures the fraction of dissolved organic matter which fluoresces, accounting for cDOM which absorbs UV light. Profiles of water temperature, dissolved oxygen, cDOM, and chlorophyll *a* were measured at 0.1 m depth intervals with YSI model EX02. DOC, cDOM, chlorophyll *a*, nitrogen (TN), and phosphorus (TP) were averaged over the open water season in 2018 from (4 monthly sampling events). TN and TP included dissolved and suspended particle fractions. TN and TP units were converted to moles per

liter before TN:TP ratios were determined in order to examine the potential for nutrient deficiency of the phytoplankton community (Guildford & Hecky 2000).

Lake Metabolism

Whole-lake metabolic parameters were estimated using a diel free-water dissolved oxygen technique (Coloso et al. 2008; Odum 1956; Staehr et al. 2010) using the LakeMetabolizer program model 1.5.0 (Winslow et al. 2016) in the statistical software R (R Core Team 2017). The method assumes that the change in oxygen in a lake reflects the biological balance between photosynthesis and respiration as well as the oxygen exchange between air and water (Odum 1956) (Equation 2.2).

$$(2.3) \text{ GPP} = \frac{\Delta \text{DO}_2}{\Delta t} + R + F$$

Where gross primary production (GPP, $\text{O}_2 \text{ mg L}^{-1} \text{ d}^{-1}$) equals the change in dissolved oxygen over time ($\Delta \text{DO}_2 / \Delta t$, $\text{mg L}^{-1} \text{ d}^{-1}$) plus respiration (R, $\text{O}_2 \text{ mg L}^{-1} \text{ d}^{-1}$) (from night time O_2 decrease) and oxygen flux between the atmosphere and water (F) (Cole & Caraco 1998b; Jähne et al. 1987; Staehr et al. 2010). The model assumes that changes in DO parameters are measured for all biota (e.g. microbial, phytoplankton, and zooplankton). NEP, GPP, respiration, and F were calculated using a Bayesian modeling approach (Winslow et al. 2016), and calculations of F followed the methods of Read et al. (2012) (see appendix A2).

Epilimnetic dissolved oxygen concentrations were measured from May-September at 15-minute intervals, using HOBO U26 dissolved oxygen and temperature probes. All HOBO U26 probes were deployed 0.5 m below the surface at the deepest point of the lake.

Automated measurements of wind speed, barometric pressure, and PAR were taken hourly at the IISD-ELA Rawson Lake Meteorological (MET) station, which was <30 km from all study lakes. A portable anemometer with automated measurements taken every 15 minutes was deployed on each lake for 1-2 weeks during the study period. Linear relationships between the daily averaged windspeeds at the meteorological site and those over each lake surface were used to establish lake specific correction factors. The correction factors allowed for the use of the anemometer at the meteorological station to be used to assess the wind speeds over each lake throughout the study period. Daily estimates for the lake metabolism parameters (GPP, NEP, and respiration) were calculated for all seven study lakes.

Model Assumptions

The LakeMetabolizer model 1.5.0 (Winslow et al. 2016) assumes that no photosynthesis occurs at night, so changes in DO concentrations at night occur solely as a result of respiration, after correction for air-water gas exchange. NEP and nighttime respiration (R_{night}) were determined directly from changes in DO concentrations but to determine GPP a value for daytime respiration (R_{day}) was required. GPP was determined by subtraction of NEP by R_{day} . It is widely assumed that R_{day} equals R_{night} for diel free-water O_2 studies (Cole et al. 2000; Coloso et al. 2008; Odum 1956; Staehr et al. 2010). It is possible that R_{day} was underestimated, as R_{day} likely exceeds R_{night} (Pace & Prairie 2005; Tobias et al. 2007). Thus, GPP and R_{day} could be underestimated (Staehr et al. 2010), but NEP or R_{night} are not affected by the potential underestimation of R_{day} because they are directly measured (Cole et al. 2000). As noted by Winslow et al. (2016) the LakeMetabolizer model can produce

unrealistic values (e.g. negative GPP or positive respiration) on days when physical processes (i.e. wave action and wind) overwhelm the biological changes in DO.

Statistical Analysis

We used Pearson's correlation analysis to evaluate how metabolism parameters (NEP, GPP, and respiration) and lake nutrients (TP and TN) varied with DOC. Pearson's was used for normally distributed variables, and the assumption of normality was assessed using the Shapiro-Wilk test. Spearman's correlation analysis was used for parameters that were not normally distributed ($A_d:A_0$, and $A_d:V_L$). Correlation analyses were conducted using the open source software R (R Team 2013). The relationship between NEP and DOC was fit to a polynomial curve using the trendline function in Microsoft Excel.

Results

DOC was strongly correlated with the physical properties of the 7 study lakes. As DOC increased from 3.5 to 9.4 mg L⁻¹, values of light attenuation increased from 0.3 to 1.5 m⁻¹. As a result, the photic depth declined by ~4x; from 14.4 m in the lake with the lowest DOC, to 3.5 m in the lake with the highest DOC (Table 2.3). Thermocline depths were also driven by changes in light attenuation associated with DOC (Fee 1996), with thermocline depths declining by ~2x; from 7.6 m in the lake with the lowest DOC concentration to 3.9 m in the lake with the highest DOC concentration. DOC was significantly correlated with variation in light attenuation ($r = 0.97$; $p < 0.001$), photic depth ($r = -0.98$; $p < 0.001$), thermocline depth ($r = -0.96$; $p < 0.001$) (Figure 2.1), lake volume ($r = -0.93$; $p < 0.005$), lake surface area (r

= -0.76; $p < 0.05$), maximum lake depth ($r = -0.77$; $p < 0.05$), and epilimnion depth ($r = -0.87$; $p < 0.05$).

Across our study lakes, lake DOC was significantly correlated with cDOM ($r = 0.92$; $p < 0.005$) and retention time ($r = 0.70$; $p = 0.07$). Retention time and cDOM were weakly correlated ($r = 0.69$; $p = 0.09$). L224 had the lowest concentration of DOC (3.5 mg L^{-1}), longest retention time (13 years) and the clearest water (K_d 0.3; cDOM 1.7 RFU). L164 had the highest concentration of DOC (9.4 mg L^{-1}), shortest retention time (0.2 years) and the darkest water (K_d 1.5; cDOM 16 RFU). In terms of light attenuation, cDOM and retention time, L224 and L164 represented opposite ends of the DOC gradient, and the other lakes fell in between.

Table 2.2 Physical characteristics of lakes ordered by DOC (mean $\pm$ SD 4 sampling dates in 2018): DOC, maximum depth ($Z_{\max}$), photic zone, light attenuation (K_d), and thermocline depth (mean $\pm$ SD 3 sampling dates).

Lake	DOC (mg L^{-1})	$Z_{\max}$ (m)	Photic Depth (m)	K_d (m^{-1})	Thermocline Depth (m)
L224	3.5 ± 0.15	27	14.4 ± 1.8	0.3 ± 0.0	7.6 ± 1.4
L373	4.1 ± 0.19	21	12.0 ± 1.5	0.4 ± 0.1	7.3 ± 0.2
L626	5.2 ± 0.22	11	10.7 ± 0.5	0.5 ± 0.1	5.9 ± 1.3
L223	5.6 ± 0.23	14	9.6 ± 1.2	0.5 ± 0.1	6.6 ± 0.3
L442	6.4 ± 0.26	18	7.6 ± 0.6	0.6 ± 0.1	6.4 ± 0.4
L658	9.1 ± 0.17	13	5.6 ± 0.1	1.0 ± 0.0	4.4 ± 0.8
L164	9.4 ± 0.26	7.0	3.5 ± 0.3	1.2 ± 0.1	4.0 ± 0.1

DOC was correlated with lake volume ($r = -0.93$; $p < 0.005$), lake surface area ($r = -0.76$; $p < 0.05$), maximum lake depth ($r = -0.77$; $p < 0.05$), and epilimnion depth ($r = -0.87$; $p < 0.05$). DOC was not correlated with watershed area ($r = 0.60$; $p = 0.149$), watershed

area to lake volume ($A_d:V_L$) ($r = 0.61$; $p = 0.17$) or watershed area to lake area ($A_d:A_0$) ($r=0.64$; $p = 0.14$). L164 had the highest concentration of DOC, highest watershed area to lake volume ratio ($A_d:V_L = 49 \text{ ha}/10^4 \text{ m}^3$), and a lower than average watershed area and the smallest lake volume. L224 and L373 had the lowest concentrations of DOC and the lowest watershed area to lake volume ratio ($A_d:V_L = 0.3 \text{ ha}/10^4 \text{ m}^3$).

The strong correlation between DOC and cDOM ($r = 0.92$; $p < 0.005$) in the study lakes indicated that the DOC was primarily coloured and of terrestrial origin (Table 2.3). DOC was significantly correlated with TP ($r = 0.84$; $p < 0.05$) (Figure 2.1) and not significantly correlated with TN (Appendix A2; $r = 0.69$; $p = 0.09$). TN:TP ratios in all study lakes were >85 indicating strong TP deficiency (Guildford & Hecky, 2000).

Table 2.3 Chemical characteristics (mean $\pm$ SD 4 sampling dates in 2018) of the seven study lakes: DOC, cDOM, total nitrogen (TN), total phosphorus (TP) and total nitrogen:total phosphorus ratios (TN:TP).

Lake	DOC (mg L ⁻¹)	cDOM (RFU)	TN ($\mu\text{g L}^{-1}$)	TP ($\mu\text{g L}^{-1}$)	TN:TP (molar)
L224	3.5 $\pm$ 0.15	1.70 $\pm$ 0.5	221.6 $\pm$ 14	5.6 $\pm$ 0.9	87.76
L373	4.1 $\pm$ 0.19	3.20 $\pm$ 0.4	268.2 $\pm$ 16	6.4 $\pm$ 1.0	92.73
L626	5.2 $\pm$ 0.22	4.40 $\pm$ 0.9	314.4 $\pm$ 21	6.1 $\pm$ 1.3	190.1
L223	5.6 $\pm$ 0.23	7.00 $\pm$ 1.8	438.0 $\pm$ 21	5.1 $\pm$ 1.1	114.5
L442	6.4 $\pm$ 0.26	15.7 $\pm$ 1.5	313.5 $\pm$ 15	6.2 $\pm$ 1.1	111.7
L658	9.1 $\pm$ 0.17	19.4 $\pm$ 1.0	375.1 $\pm$ 20	8.7 $\pm$ 1.5	107.5
L164	9.4 $\pm$ 0.26	15.5 $\pm$ 1.3	401.9 $\pm$ 11	8.0 $\pm$ 1.0	102.6

Many physical, chemical, and metabolic parameters measured in our study showed strong collinearity (Table 2.4). GPP and respiration were significantly correlated with TP,

chlorophyll *a*, DOC, thermocline, and light attenuation. DOC is significantly correlated to photic zone, chlorophyll *a*, TP, thermocline and light attenuation.

Table 2.4 Pearson correlation coefficients of physical, chemical and whole-lake metabolism parameters; net ecosystem production (NEP), respiration (R), gross primary production (GPP), dissolved organic carbon (DOC), total phosphorus (TP), total nitrogen (TN), photic depth, thermocline, and chlorophyll *a* (Chl-*a*). Significant relationships bolded ($r > 0.6$).

	GPP	R	NEP	DOC	TP	TN	Photic	Therm	Chl- <i>a</i>
GPP	1	0.96	-0.71	0.82	0.78	0.42	-0.74	-0.69	0.65
R		1	-0.88	0.83	0.77	0.41	-0.74	-0.74	0.72
NEP			1	-0.67	-0.61	-0.32	0.60	0.68	-0.69
DOC				1	0.84	0.68	-0.98	-0.96	0.89
TP					1	0.32	-0.75	-0.84	0.82
TN						1	-0.70	-0.65	0.39
Photic							1	0.92	-0.86
Therm								1	-0.94
Chl- <i>a</i>									1

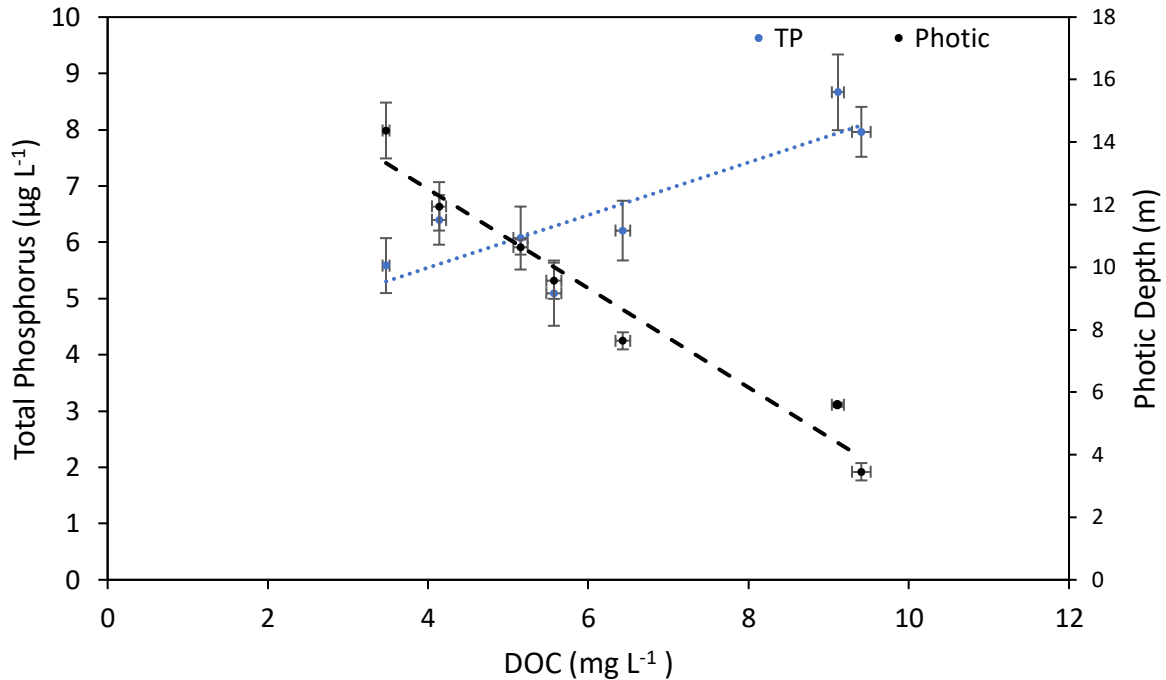


Figure 2.1 Relationships between photic zone and total phosphorus (mean $\pm$ SD) with increasing DOC. The trendlines represent the significant correlations (photic zone: $r = -0.98$, $p < 0.001$) (total phosphorus: $r = 0.84$, $p < 0.05$).

Lake Metabolism

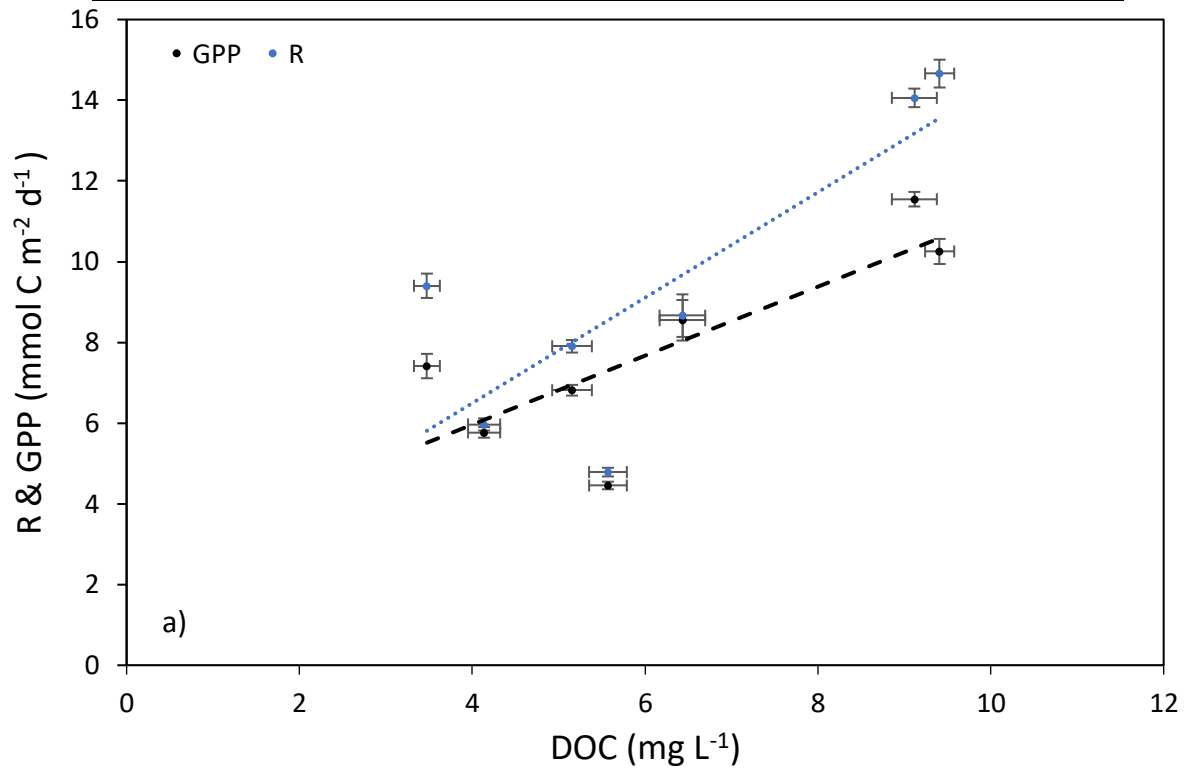
GPP and respiration both displayed strong positive relationships with DOC (Figure 2.2a). However, since NEP was a function of both GPP and respiration (Equation 2.1), the differing slopes of these relationships led the DOC vs. NEP relationship to be non-linear (Figure 2.2b). DOC was significantly correlated with GPP ($r = 0.82$; $p < 0.05$), respiration ($r = 0.83$, $p < 0.0$ (Figure 2.2a), and chlorophyll *a* ($r = 0.89$; $p < 0.01$). GPP was significantly correlated to respiration ($r = 0.96$; $p < 0.001$), light attenuation ($r = 0.86$; $p < 0.05$) (Figure 2.3d), but not with chlorophyll *a* ($r = 0.65$; $p = 0.12$), photic zone ($r = -0.74$; $p = 0.057$), or

thermocline depth ($r = -0.70$; $p = 0.09$). NEP was significantly correlated with respiration ($r = -0.88$; $p < 0.01$) but not significantly correlated with GPP ($r = -0.71$; $p = 0.07$).

The relationship between DOC and NEP (Figure 2.2b) was best described using a second order polynomial trendline which illustrated the increase in NEP with DOC until ~ 6 mg L^{-1} , above which NEP decreased. All lakes in this study were net heterotrophic ($\text{NEP} < 0$).

Table 2.5 Biological parameters of the seven study lakes: dissolved organic carbon (DOC), chlorophyll *a* (Chl-*a*), and metabolism parameters net ecosystem production (NEP), respiration (R), and gross primary production (GPP) (mean $\pm$ SD).

Lake	DOC (mg L ⁻¹)	Chl- <i>a</i> (μ g L ⁻¹)	NEP	R	GPP
			(mmol C m ⁻² d ⁻¹)		
L224	3.5 $\pm$ 0.15	1.4 $\pm$ 0.3	-1.99 $\pm$ 0.2	9.40 $\pm$ 0.3	7.41 $\pm$ 0.3
L373	4.1 $\pm$ 0.19	1.3 $\pm$ 0.2	-0.20 $\pm$ 0.1	5.97 $\pm$ 0.2	5.77 $\pm$ 0.1
L626	5.2 $\pm$ 0.22	2.0 $\pm$ 0.9	-1.17 $\pm$ 0.1	7.90 $\pm$ 0.2	6.81 $\pm$ 0.1
L223	5.6 $\pm$ 0.23	1.3 $\pm$ 0.4	-0.08 $\pm$ 0.1	4.79 $\pm$ 0.1	4.45 $\pm$ 0.1
L442	6.4 $\pm$ 0.26	1.8 $\pm$ 0.4	-0.11 $\pm$ 0.1	8.66 $\pm$ 0.5	8.55 $\pm$ 0.5
L658	9.1 $\pm$ 0.17	2.8 $\pm$ 1.0	-2.45 $\pm$ 0.2	14.1 $\pm$ 0.3	11.6 $\pm$ 0.3
L164	9.4 $\pm$ 0.26	2.3 $\pm$ 0.3	-4.73 $\pm$ 0.2	14.7 $\pm$ 0.2	10.3 $\pm$ 0.2



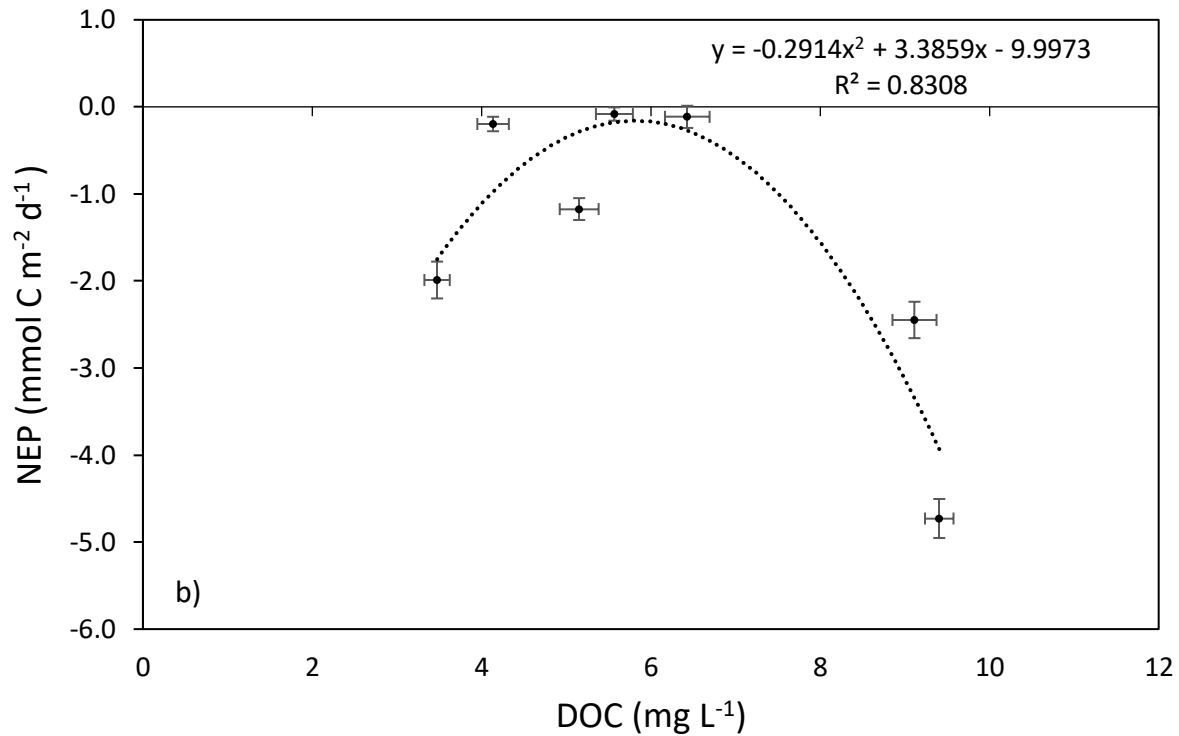


Figure 2.2 Primary production parameters with increased DOC within stratified lakes: (a) gross primary production (GPP) and respiration (R) with increasing DOC the solid lines represent the Pearson correlations (GPP: $r = 0.82$, $p < 0.05$) (R: $r = 0.83$, $p < 0.05$), and (b) net ecosystem production (NEP) with increasing DOC.

DOC was significantly correlated with TP ($r = 0.84$; $p < 0.05$) (Figure 2.3a). GPP increased significantly with TP ($r = 0.78$; $p < 0.05$) (Figure 2.3b). Respiration increased significantly with TP ($r = 0.77$; $p < 0.05$) (Figure 2.3c) and not significantly with TN ($r = -0.41$; $p = 0.36$). NEP decreased non significantly with TP ($r = -0.61$; $p = 0.15$) and TN ($r = -0.32$; $p = 0.48$).

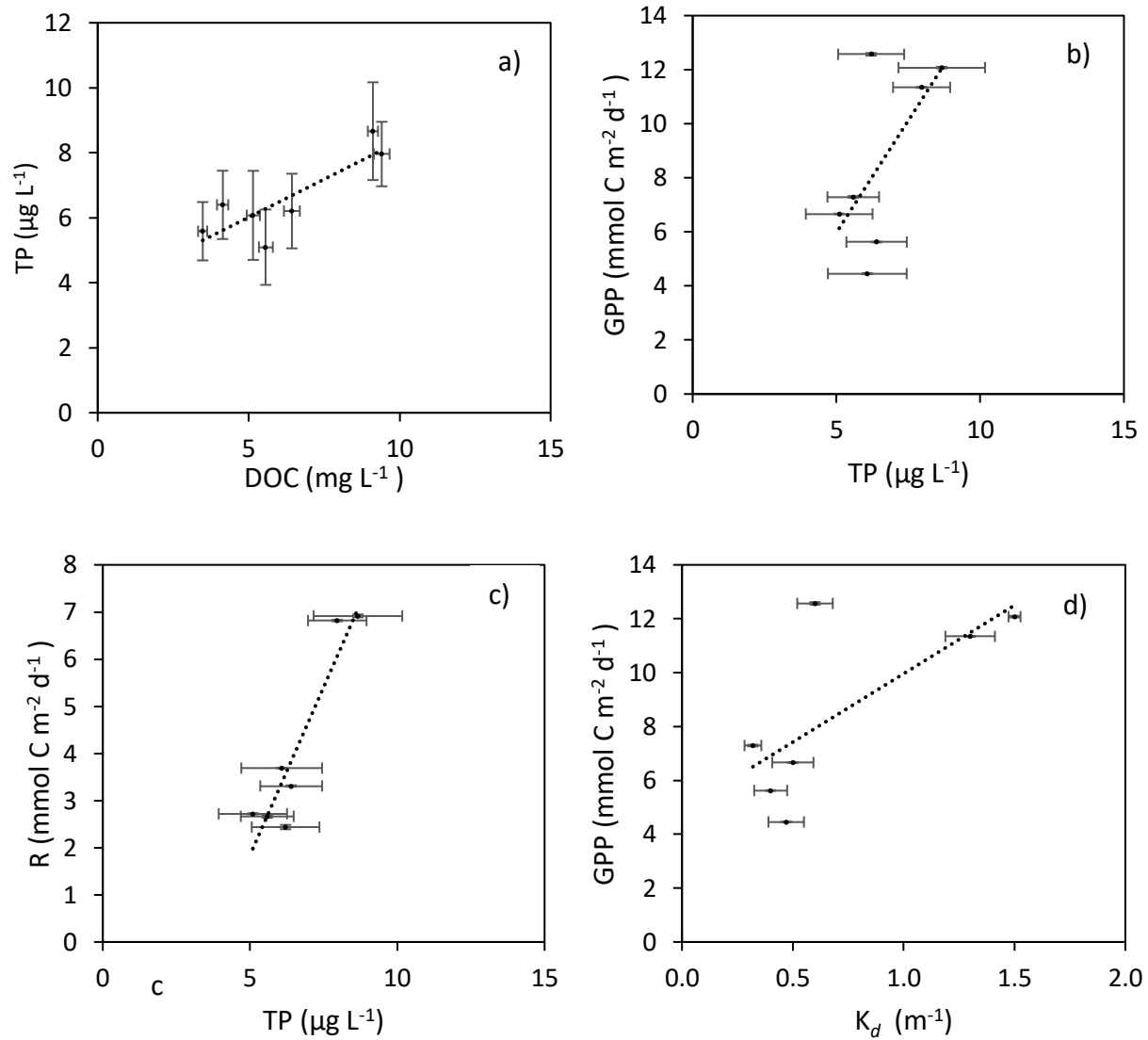


Figure 2.3 Nutrients and lake metabolism parameters related to light attenuation and DOC; (a) total phosphorus (TP) with increasing DOC ($n = 7$; $r = 0.84$; $p < 0.05$), (b) gross primary production with increasing TP ($r = 0.78$; $p < 0.05$) (c) respiration (R) with increasing TP ($r = 0.77$; $p < 0.05$), and (d) gross primary production with increasing light attenuation (K_d) ($r = 0.86$; $p < 0.05$).

Discussion

DOC is considered a state variable due to its capacity for altering key physical, chemical, and biological attributes of lakes (Ask et al. 2009; Cole et al. 2006; Jones 1992; Solomon et al. 2015). Within lakes, DOC concentrations and biological uptake are influenced by many factors, including: residence time, photodegradation (Klug 2002; Lindell et al. 2000), precipitation (Hall et al. 2018), and food web structure (Schindler et al. 1997). These factors can enhance the effects of DOC on primary producers and whole-lake metabolism. DOC is also highly variable across lakes. In a study of 7,514 lakes across 6 continents, a DOC gradient of 0.1-332 mg L⁻¹ was found, with 87% of lakes having a range of 1-20 mg L⁻¹ and a median concentration of 5.71 mg L⁻¹ (Sobek et al. 2007). Thus, the 7 lakes chosen for this study, which had a DOC range of 3.5-9.4 mg L⁻¹ and a median value of 5.57 mg L⁻¹, were highly representative of DOC concentrations in the majority of lakes globally.

Consistent with a growing number of studies (e.g. Ask et al. 2009; Hessen et al. 2017; Seekell et al. 2015), our results demonstrated the strong effects of allochthonous DOC in structuring lake properties and processes. The DOC in this study was predominantly allochthonous in origin as shown by the strong correlation between DOC and cDOM. Brownification increases with allochthonous DOC and cDOM (Brothers et al. 2014b), resulting in declining photic and thermocline depths (E. J. Fee et al., 1996) similar to the spatial (between lake) relationships found here. Temporally, in a manipulated lake basin experiment by Zwart et al. (2016) DOC concentrations increased 2.6 mg L⁻¹ in 4 years, light attenuation increased 1.05 m⁻¹ and thermocline became shallower by 0.30 m. In our study, as DOC increased from 3.5-9.4 mg L⁻¹, increases in light attenuation led to dramatic reductions in the photic depth (4x reduction) and thermocline depth (2x reduction).

In contrast to the negative effects of DOC on light transmission and photic depth, increases in DOC were positively correlated with key nutrients required for phytoplankton growth. Lakes with higher concentrations of DOC have more nutrients as a consequence of the photodegradation of terrestrial DOC (Molot & Dillon, 1997; Moran & Zepp, 1997; Zwart et al., 2016b) which can stimulate primary production (Daggett et al. 2015; Klug 2002). Terrestrially derived DOC and associated nutrients from the surrounding landscape is leached into boreal lakes following hydrologic events (Raymond & Saiers 2010). Evidence at IISD-ELA from the 51-year record of three inflow streams to L239, demonstrated the strong linkage between precipitation to DOC, and nutrient loads from watersheds in the region (Emmerton et al. 2018). This suggests that nutrients derived from allochthonous DOC strongly contributed to the stimulation of GPP.

Based on their mean nutrient (TN, TP) and chlorophyll *a* concentrations the lakes included in our study would be considered oligotrophic (Wetzel 2001). Primary production is often limited by nutrients, and phosphorus has been identified as the primary nutrient controlling primary production of boreal lakes in the region (Schindler 1977). Phosphorus limitation occurs when the TN:TP molar ratio is high (>50) (Guildford & Hecky 2000). Our study lakes had TN:TP ratios >85 . Thus, increased nutrient availability associated with DOC may be especially important in these pristine boreal lakes with low background nutrient concentrations. Lakes not limited by nutrients do not often find a positive correlation between DOC and primary production (Hanson et al. 2003).

The importance of watershed characteristics on lake DOC has been well studied, with watershed area, mean annual run off, and soil carbon density cited as contributing to DOC loads (Sobek et al. 2007; Lapierre et al. 2015). In addition, the relationship between

residence time, DOC and cDOM have been evaluated (Mari et al. 2007), with longer residence times reducing DOC concentrations via UV and microbial degradation. As DOC is broken down or transformed, organic nutrients can become more bioavailable for nutrient uptake by phytoplankton (Berggren et al. 2014). Once within lakes, the fate of DOC is reliant on residence times, water temperature, and microbial processes (Creed et al. 2018). Thus, the variation in watershed and lake properties contributes to spatial variability of DOC between lakes. In our study, spatial heterogeneity between lake size and watershed area explained the majority of variation in residence time, DOC, and nutrient concentrations. In a study of 239 temperate and boreal lakes with DOC concentrations ranging from 1-15 mg L⁻¹ Lapierre et al. (2015), showed that watershed characteristics and climate (mean annual temperature and precipitation) were the most significant factors in predicting lake carbon concentrations.

Whole-Lake Metabolism

In a recent whole lake DOC addition experiment, Zwart et al. (2016) reported an 'unexpected' increase in GPP that was attributed to increased phosphorus associated with DOC loading. Based on results from smaller scale experiments and modelling, Zwart et al. (2016) originally hypothesized that productivity would decrease in response to reductions in water clarity and thermocline depths. However, nutrients (TP and TN) associated with DOC superseded the negative effects of DOC on water clarity, consistent with results in our study. Thus, it would appear that in natural lake ecosystems, particularly those which are oligotrophic, nutrients associated with DOC can increase epilimnetic primary production and whole-lake metabolism (Zwart et al. 2016). These studies (Prairie et al. 2002; Hanson

et al. 2003; Zwart et al. 2016; ours) evaluate epilimnetic metabolism, there is a potential for loss of productivity in the metalimnion of lakes due to reductions in water clarity associated with DOC.

In a whole lake manipulation study on four Wisconsin lakes that included nutrient enrichment and food web manipulations, Cole et al. (2000) reported that the variation in GPP and respiration (in the absence of strong planktivory) was primarily driven by nutrients. These four lakes had a similar range of DOC concentrations (5.3 – 13.6 mg L⁻¹) as that in our study. Cole et al. (2000) found net heterotrophy with NEP values ranging from -40 to -20 mmol C m⁻² d⁻¹. Carignan et al. (2000) and Prairie et al. (2002) also found NEP values ranging from -40 to -20 mmol C m⁻² d⁻¹ in a study of 33 lakes in Quebec with DOC concentrations ranging from 2-11 mg L⁻¹. The variation in NEP values among lakes was attributed to the differences in nutrient availability (Cole et al. 2000), three of four lakes had nutrient (inorganic N and P) additions from 1993-1997.

In a whole lake experimental DOC enrichment study, Zwart et al. (2016) increased DOC concentrations from 8 to 11 mg L⁻¹ over 4 years in a lake in Michigan. DOC was correlated with GPP, NEP and respiration, consistent with our study. GPP increased 9 mmol C m⁻² d⁻¹, respiration increased 28 mmol C m⁻² d⁻¹ and NEP decreased 19.2 mmol C m⁻² d⁻¹ (Zwart et al. 2016). The range of GPP (4.5 to 11.6 mmol C m⁻² d⁻¹) in our study was comparable to other studies.

Hanson et al. (2003) found a similar decline in NEP with increasing concentrations of DOC in a study of 25 boreal lakes in Wisconsin. At lower DOC concentrations (0-10 mg L⁻¹), GPP, respiration and NEP were in near metabolic balance; at DOC concentrations > 10 mg L⁻¹, respiration increased significantly, causing NEP to become increasingly heterotrophic

(Hanson et al. 2003). Our study lakes were in near metabolic balance until $\sim 6 \text{ mg L}^{-1}$ DOC, comparable to Prairie et al. (2002) who found decreasing NEP in lakes $> 6 \text{ mg L}^{-1}$ DOC. This supports our hypothesis that the increase in GPP and NEP until 6 mg L^{-1} DOC was most likely related to nutrients associated with DOC.

Similar to other studies (Hanson et al. 2003; Zwart et al. 2016) respiration increased more rapidly than GPP in response to increasing DOC. In a similar study, Ask et al. (2009) showed that such increases in respiration were related to microbial respiration of organic matter. Respiration can be related to increased microbial activity with increased allochthonous DOC. Microbes often rely on carbon as a substrate for respiration (Hanson et al. 2007; Tranvik et al. 1999). Tranvik (1992) found that between 5-40% of total DOC was biologically labile and utilized by bacteria within days or weeks of input. Allochthonous DOC can account for almost 90% of the carbon required to support bacterial growth (Hessen 1992). In our study, after DOC exceeded 6 mg L^{-1} , respiration surpassed GPP likely due to increased microbial growth and respiration associated with DOC (Hanson et al. 2007; Tranvik et al. 1999). Whole-lake metabolism parameters were correlated with DOC. The strong correlations between DOC, GPP, and nutrients were consistent with our first hypothesis that nutrients associated with DOC would drive increases in algal productivity.

DOC Threshold

In our study NEP reached a threshold at a DOC concentration near 6 mg L^{-1} . Consequently, NEP increased until $\sim 6 \text{ mg L}^{-1}$ DOC. Initially, NEP increased with increasing DOC, but then decreased once the DOC threshold ($\sim 6 \text{ mg L}^{-1}$) was exceeded. GPP and respiration increased significantly along the DOC gradient. These responses were all

consistent with other studies (Ask et al. 2009; Hanson et al. 2008; Prairie et al. 2002; Seekell et al. 2015b; Solomon et al. 2015; Zwart et al. 2016)

Following the DOC threshold $>6 \text{ mg L}^{-1}$ DOC the differences between respiration and GPP were much larger and values of NEP were more negative. Increases in respiration exceeded increases in GPP in lakes with DOC concentrations greater than 6 mg L^{-1} . The increase in respiration with DOC was consistent with other studies (Ask et al. 2009; Cole et al. 2000) where respiration was driven by increased microbial growth with increasing DOC. The exceedence in respiration over GPP led to a non-linear decrease in NEP consistent with our third hypothesis that a DOC threshold would cause lake metabolism to switch from positive to negative.

The threshold marks differences between GPP and respiration. The presence of a DOC threshold is consistent with Prairie et al. (2002), Hanson et al. (2003), and Seekell et al. (2015a, 2015b), which all had similar DOC gradients ($\sim 1\text{-}25 \text{ mg L}^{-1}$). Prairie et al. (2002) determined a DOC threshold of $4\text{-}6 \text{ mg L}^{-1}$ in 33 lakes in Quebec with DOC concentrations between $3.5\text{-}11.2 \text{ mg L}^{-1}$. Seekell et al. (2015a) found a threshold of 4.8 mg L^{-1} DOC in 28 nitrogen-limited boreal and arctic lakes in Sweden and Alaska with DOC concentrations between $1.8\text{-}21 \text{ mg L}^{-1}$. Seekell et al. (2015b) found a mean threshold of 6.0 mg L^{-1} DOC in 703 boreal and arctic lakes in Sweden with DOC concentrations between $0.3\text{-}18.5 \text{ mg L}^{-1}$. And Hanson et al. (2003), found a DOC threshold $>10 \text{ mg L}^{-1}$ in 25 lakes in Wisconsin with DOC concentrations between $1.6\text{-}24.6 \text{ mg L}^{-1}$.

Below the DOC threshold, NEP was fairly consistent, after it declined steadily with increasing respiration. Increases in respiration, most likely driven by increased microbial respiration (Zwart et al. 2016), exceeded GPP. Above the threshold, primary production

decreased with increased light attenuation (Prairie et al. 2002; Hanson et al. 2003; Seekell et al. 2015a, 2015b). In our study, GPP and respiration increased across the DOC gradient, although thermocline and photic depth decreased, suggesting our DOC gradient (3.5-9.4 mg L⁻¹) was too small to capture a larger change.

The differences between DOC thresholds may be related to ambient nutrient concentrations. Lakes in Quebec (Prairie et al. 2002) had lower concentrations of TP (<20 µg L⁻¹) than lakes in Wisconsin (Hanson et al. 2003) (>40 µg L⁻¹ TP). Our concentrations of TP were < 13 µg L⁻¹, and more similar to the boreal lakes in Quebec studied by Prairie et al. (2002). Boreal and arctic lakes in Sweden and Alaska were limited by nitrogen (Seekell et al. 2015a, 2015b), not phosphorus. Thresholds identified by our study and Prairie et al. (2002), are similar to Seekell et al. (2015a, 2015b) even though Canadian boreal lakes are phosphorus-limited.

Other factors such as methods used to measure lake metabolism and geographic location are also likely contributing factors to differences between studies. Prairie et al. (2002), Hanson et al. (2003), and our study used diel changes in dissolved oxygen to determine metabolic parameters. Whereas, Seekell et al. (2015a, 2015b) used elasticity relationships between DOC, TN, and light attenuation to determine a DOC threshold. Even though the degree of potential nutrient limitation, methods, and geographic location were different among studies a comparable DOC threshold was still found, providing an increasing degree of confidence in this concept.

Why did light availability not have a stronger effect on GPP? Photosynthetic organisms rely on sunlight as their primary energy source. However, rather than having a negative effect, in our study GPP was positively correlated with light attenuation. However,

while DOC reduced the photic depth, primary production in the epilimnion was generally light saturated. It is expected that this increase in GPP was driven by DOC and TP, which are intercorrelated with light parameters. Epilimnetic waters were light-saturated, similar to Zwart et al. (2016). Where, DOC concentrations increased from 8-11 mg L⁻¹ in 4 years, light attenuation increased 1.05 m⁻¹ and thermocline became shallower by 0.30 m, light climate in the epilimnion did not change significantly (Zwart et al. 2016). Perhaps, if the DOC gradient was larger (>13 mg L⁻¹) a shift to declining GPP due to light limitation would have been more evident. GPP continued to increase with DOC likely due to increased nutrient bioavailability (Berggren et al. 2014). However, a DOC threshold of NEP at 6 mg L⁻¹ was evident.

Gross primary production increased with allochthonous DOC, providing energy for higher trophic levels supporting the aquatic ecosystem. These results substantiate the importance of DOC as a nutrient subsidy and validate its influence on primary production. However, more studies are needed on the effects of DOC on primary production throughout the water column including metalimnion and hypolimnion. The open water lake metabolism approach is becoming a widely used. It is an accepted method of estimating epilimnetic production, which was useful for comparisons of metabolism across lakes of differing DOC. Particularly because this method integrated metabolism from both pelagic (i.e. phytoplankton) and benthic (i.e. periphyton) habitats. However, such an approach would not account for the potential contribution of deep chlorophyll peaks that occurred in the metalimnion or hypolimnion of some lakes. Deep chlorophyll peaks have been observed in clearer lakes with thermoclines shallower than the photic zone at IISD-ELA (Fee et al. 1976) and were observed in lakes L224, L223, L626, 442, and L373 in July and August 2018 (Tonin

pers. com.). If high amounts of primary production by both phytoplankton and benthic algae were occurring below the epilimnion, whole lake productivity could be underestimated. As well, we recognize that more studies are needed to determine year round whole-lake metabolism. Welch et al. (1976) determined that whole-lake respiration rates in the winter were more than one order of magnitude lower than summer epilimnetic rates at comparable chlorophyll *a* concentrations. And Karlsson et al. (2008) determined that winter respiration equalled 35% of benthic and pelagic respiration in the summer and 26% of annual lake respiration on a lake in subarctic Sweden.

The results from our study, support our hypotheses. Nutrients increased with DOC, providing a nutrient subsidy, which lead to increased GPP (hypothesis 1). Effects of DOC causing decreased light availability caused NEP to decrease as DOC increased past $\sim 6 \text{ mg L}^{-1}$ however, GPP was not impacted (hypothesis 2). A threshold concentration of DOC was determined, limiting NEP at $\sim 6 \text{ mg L}^{-1}$ causing production to decrease (hypothesis 3).

Conclusion

In our study DOC was significantly correlated to GPP, respiration, maximum depth, thermocline depth, photic depth, cDOM, chlorophyll *a*, nitrogen, and phosphorus. While more studies are needed to further tease apart the relative importance of these variables, my results and those of others (e.g. Zwart et al. 2016) demonstrate that in natural lake ecosystems changes in DOC drive concurrent variations in a variety of factors (e.g. light, nutrients, thermocline depth, etc.) that drive lake productivity. Our study was able to establish that GPP and respiration were strongly responsive to increased nutrients associated with DOC. However, these factors and others influencing DOC require further

research to better understand the complex metabolic interrelationships that exist in boreal lakes. Further efforts are needed to evaluate these relationships in lakes with higher background nutrient concentrations. DOC was shown to stimulate respiration and primary production via an increase in nutrients (TN and TP) in the seven lakes in our study. In these oligotrophic lakes, primary production is limited by primarily by nutrients. Lakes with higher concentrations of DOC have more bioavailable nutrients. After DOC became $>6 \text{ mg L}^{-1}$ increases in respiration surpassed GPP causing a decrease in NEP, indicating the presence of a DOC threshold. This threshold is significant to help predict the effects of DOC on whole-lake production in other lakes.

Concentrations of DOC will continue to increase in boreal lakes around the world (Clark et al. 2010; Seekell et al. 2015a,b), with increasing temperatures, precipitation, and recovery from acidification. Climate change will increase spatial variability of DOC among and within lakes. Increasing DOC could have major implications on lake food webs starting with the increase in primary production.

Chapter 3: How terrestrially derived organic matter influences phytoplankton primary production

Abstract

Boreal lakes, the most numerous lake type in the world, receive large inputs of terrestrially derived dissolved organic carbon (DOC). DOC impacts primary production through its combined effects on light and nutrient availability. Our study examined the effects of DOC on 6 pristine boreal lakes spanning a DOC gradient (3.5-9.4 mg L⁻¹ DOC). Phytoplankton primary production was assessed using *in vitro* partial pressure of CO₂ incubations. Our results showed strong effects of allochthonous DOC on lake processes (i.e. light and nutrient inputs). Despite increases in epilimnetic phytoplankton biomass, phytoplankton productivity was negatively correlated with increasing DOC ($r = -0.87$; $p < 0.05$). Depth integrated chlorophyll, which declined with DOC, was a better predictor of phytoplankton productivity. Lakes with the highest proportion of production below the epilimnion had well developed deep chlorophyll maxima (DCM). DCM are viewed as ecological hotspots for nutrient cycling, primary production, and zooplankton grazing. The depth of peak chlorophyll, which occurred near 1% surface irradiance, was negatively correlated with DOC ($r = -0.95$; $p < 0.005$). The presence of DCMs and sub-epilimnetic productivity were eliminated in lakes with DOC values > 7.1 mg L⁻¹. Contrary to our hypothesis primary production was limited by light and not nutrients. The primary effect of allochthonous DOC on phytoplankton productivity occurred via its light attenuating properties.

Introduction

Photosynthetic algae are the energetic base of aquatic food webs, providing energy, fatty acids, and essential nutrients to higher trophic levels, including many species of zooplankton and fishes (Ask et al. 2009; del Giorgio & Peters 1994; Gelda & Effler 2002; Sargent et al. 1999; Vadeboncoeur et al. 2008; Wetzel 2001). In boreal lakes, the most abundant of all complex particulate and dissolved substances discharged to lakes is terrestrially derived organic matter (Ask et al. 2009b; Schindler et al. 1997), primarily measured and reported as dissolved organic carbon (DOC) (Emmerton et al. 2018; Schindler et al. 1997; Seekell et al. 2015). While DOC values represent the analytical measurement of organic carbon, the term DOC often encompasses the broader complex of dissolved organic matter that includes organic carbon, nitrogen and phosphorus. Many algal species in boreal lakes have a demonstrated capacity for mixotrophy, utilizing agglomerated DOM particles or bacteria as sources of energy and nutrients (Findlay et al. 2001). Mixotrophic algae can use different sources of energy and carbon (i.e. autotrophy and heterotrophy) (Graham et al. 2009). Other species, while autotrophic, may utilize nutrients released during the degradation of DOM by UV radiation or microbial activity (Jansson et al. 2008; Vasconcelos et al. 2018).

Primary production can be stimulated or suppressed by DOC as a consequence of its simultaneous, but contrasting, effects on light and nutrients (Creed et al. 2018; Seekell et al. 2015; Zwart et al. 2016a). Allochthonous DOC is chromophoric and has strong light attenuating properties that reduce availability of photosynthetically active radiation (PAR) in the water column, thereby shoaling both the photic and thermocline depth (Zwart et al. 2016). Allochthonous DOC increases brownification, where inputs of terrestrially derived

organic matter from surrounding catchments stain lake water with brown-coloured compounds (Williamson et al. 2015). Thus, due to their strong effects on water column transparency, inputs of allochthonous DOC may impose light limitation theoretically suppressing primary production (Jones 1992).

DOC also influences the chemical characteristics of lakes. The addition of allochthonous DOC can lead to more bioavailable nutrients (i.e. nitrogen and phosphorus) associated with bacterial degradation and photodegradation of organic matter (Moran & Zepp 1997), which are readily used by aquatic organisms (Klug 2002). Nutrient-poor boreal lakes are primarily limited by phosphorus and sensitive to increases in nutrient bioavailability (Schindler 1977). The bioavailability of nutrients associated with DOC is further affected by water temperature, pH, water and soil chemistry, and photolytic and microbial degradation processes (Creed et al. 2018; Hessen et al. 2017; Leenheer & Croué 2003; Solomon et al. 2015). Increased nutrient bioavailability associated with the degradation of DOC can stimulate primary production (Creed et al. 2018; Daggett et al. 2015; Klug 2002).

DOC concentrations are highly variable spatially and temporally among and within boreal lakes due to variation of hydrologic inputs (e.g. precipitation), climate change (e.g. temperature), recovery from acidification, and changes in watershed and lake characteristics (e.g. forest fires, development, wetlands, watershed area, lake size, etc.) (Brett et al. 2017; Clark et al. 2010; Emmerton et al. 2018; Lepistö et al. 2014; Monteith et al. 2007). Spatial and temporal variability of DOC make it challenging to identify how DOC influences primary production. Often phytoplankton productivity is assessed only in the epilimnion, under the assumption that the vast majority of primary production occurs within

the upper mixed layer (Kelly et al. 2014; Leach et al. 2018). Such studies overlook primary production occurring below the epilimnion but still within the photic zone (Fee 1980), as well as the potential importance of deep chlorophyll maxima (DCM) to support higher trophic levels (Tonin 2019). This study quantifies the effects of DOC on phytoplankton productivity and its drivers within 6 boreal lakes with similar lake morphometries, spanning a DOC gradient (3.5-9.4 mg DOC L⁻¹). This study partitions the effects of DOC on phytoplankton productivity within the epilimnion, throughout the photic zone (which can extend well below the depth of the thermocline), and within the metalimnion that may include DCM (Fee 1976).

The purpose of this study was to determine the effects of terrestrially derived DOC on phytoplankton primary production. We examined the effects of DOC on depth integrated primary production using 6 oligotrophic boreal lakes along a DOC gradient. We hypothesized that: (1) nutrients associated with DOC would increase depth-integrated primary production despite reductions in light availability and shallower photic zones; (2) DCM will correlate with light attenuation and photic depth, DOC will cause reductions in light and photic depth, such that DOC will decrease or eliminate sub-epilimnetic productivity and DCM presence.

Methods

Study Description

We studied 6 oligotrophic boreal lakes spanning a DOC gradient (3.5-9.4 mg L⁻¹) at the International Institute of Sustainable Development-Experimental Lakes Area (IISD-ELA) in northwestern Ontario. These lakes were chosen for their DOC concentrations, biotic

similarities (fish and plankton species composition), similar lake morphologies, and close geographical proximity (<30 km) to the IISD-ELA field station (Appendix A1). The lakes experience similar climatic conditions, are small (max depth 7.0-30 m), and nutrient-poor (5.7-8.0 $\mu\text{g L}^{-1}$ total phosphorus). The lakes are dimictic, they thermally stratify throughout the summer, and are ice-covered between mid-November and late April. All catchments are typical boreal forest, dominated by jack pine (*Pinus banksiana*), red pine (*Pinus resinosa*), and black spruce (*Picea mariana*) (Schindler et al. 1990). IISD-ELA is far from urban centers (>50 km) and has minimal watershed disturbance, excluding forest fires in 1974 and 1980 (Schindler et al. 1990).

Limnological Sampling

Lakes were sampled monthly from May-August throughout the open water season of 2018. Monthly sampling included physical, chemical, and biological sampling.

Physical parameters: Light profiles were measured as Photosynthetically Active Radiation (PAR) with an underwater LICOR flat plate sensor (model LI 192 Underwater Quantum Sensor). Underwater readings of PAR were taken at 0.5 to 1 m depth intervals until the reading was less than 1% of surface PAR (photic depth), below which photosynthesis is generally assumed to be negligible (Fee et al. 1996). The light attenuation coefficient (K_d) represents the rate that light is attenuated with increasing depth in the water column (Swinehart 1962). Light profiles during the open water season were used to calculate light attenuation and determine photic depths (Appendix A1.2). Maximum lake depth was measured once during the open water season in 2018 using a depth sounder and was assumed not to change significantly. Thermocline depth was calculated based on changes in

water temperature and density from monthly temperature profiles from June to August using LakeAnalyzer package in the statistical software R (Winslow et al. 2018).

Chemical: Water samples were collected monthly (May-August 2018) from the epilimnion of each lake using a depth integrated sampler. The epilimnion was defined as the upper water layer of uniform temperature, this ignored any shallow, temporary, diurnal stratification phenomena. Chemical analyses were conducted by IISD-ELA water chemistry staff. Dissolved and particulate nutrients (carbon, nitrogen, and phosphorus), pH, conductivity, and chlorophyll *a* were measured following the methods of Stainton et al. (1977). Water samples collected for DOC measurements were filtered through Whatman GF/C filters and the filtrates were analyzed at the Freshwater Institute (Fisheries and Oceans Canada) in Winnipeg on a Shimadzu Total Organic Carbon Analyzer. Dissolved inorganic carbon (DIC) as micro moles per liter (mmol L^{-1}) was measured on a Li-COR CO₂ gas analyzer model LI-820. Coloured or chromophoric dissolved organic matter (cDOM) was estimated as relative fluorescent units (RFU) with a fluorescent DOM sensor on a Yellow Springs Instrument (YSI model EXO2) sonde. The fluorescent DOM sensor emits ultraviolet light to measure the fraction of DOM which fluoresces. Fluorescent DOM is an estimate of cDOM (Courtois et al. 2017; Xylem 2014). Profiles of water temperature, dissolved oxygen, cDOM, and chlorophyll *a* were measured at 0.1 m depth intervals with YSI model EXO2. Values of DOC, cDOM, chlorophyll *a*, total nitrogen, and total phosphorus from four monthly sampling events were averaged.

Primary Production

Measuring phytoplankton photosynthesis in low nutrient systems required sensitive methods, and historically it was measured using the standard ^{14}C incorporation method (e.g. Ask et al. 2009b; Schindler et al. 1972). A novel method developed by Davies et al. (2003) demonstrated that pCO_2 (partial pressure of CO_2) methods have the sensitivity to measure low rates of primary production in boreal lakes; an alternative method to ^{14}C incorporation which uses radioactive isotopes (e.g. Ask et al. 2009b; Schindler et al. 1972). As well, the ^{14}C method is not directly comparable with other methods that utilize changes in O_2 , pCO_2 or DIC, whereas, the latter methods are comparable to each other (Davies et al. 2003). Another advantage of the pCO_2 method is its potential to directly measure phytoplankton respiration which the standard ^{14}C method cannot (e.g. Aberg & Wallin 2014; Davies et al. 2003; Davies 1997).

Phytoplankton community composition and biomass was determined via microscopy on a on a monthly basis during the ice-free season (May through October) using consistent methods throughout the 51-year dataset by Dave Findlay at Plankton R Us (Findlay et al. 1994; Findlay & Kling 1979). Cell measurements were performed on each species to a maximum of 50 individuals. Cell density was calculated by multiplying the cell count by the ratio of the area counted to the total area of the counting chamber. Cell numbers were then converted to phytoplankton biomass (mg m^{-3}) by multiplying cell density by cell volume to the 10^{-6} .

Phytoplankton primary production was determined by *in vitro* incubations along a light gradient following a similar method to Davies et al. (2003). We improved this method by incorporating a Varian micro-gas chromatograph (GC), fitted with a CO_x column for

assessing low molecular weight compounds. The GC minimizes headspace volume within the injection loop and GC column, and we used a tracer gas (neon) to correct for small variations in headspace volume of the sample or GC performance. Water from integrated epilimnion samples was incubated for 6-hours within 160-mL airtight bottles containing 30 mL of gaseous (air) headspace. A small amount (5 mL) of tracer gas was injected into the headspace to add positive pressure (required by GC) and to correct for small variations of water and headspace volumes or gas chromatograph performance. The bottles were placed in a water bath kept within $\pm 1^{\circ}\text{C}$ of epilimnetic lake temperature, along a light gradient from a sodium halide light source (Figure 3.1).

Following the first hour of incubation ($T=0$), 5 mL of headspace from each bottle was sampled hourly using a glass syringe and injected into 7 mL vacutainers filled with helium at room pressure. The vacutainers were stored under 5 mL of positive pressure. Samples were measured on the GC within 3 weeks to determine concentrations of CO_2 . Standard curves relating GC output in microvolts to CO_2 concentration were undertaken each day that samples were analyzed on the GC. CO_2 standards (0 ppm CO_2 , 50.36 ppm CO_2 , 499.6 ppm CO_2 , and 5004 ppm CO_2) obtained from Praxair were used to develop these standard curves. Linear regression equations relating GC peak areas (in microvolts) to CO_2 concentrations standards ($r^2 > 0.8$) were used to estimate concentrations of CO_2 within the incubated samples. Aqueous DIC concentrations at each time step were calculated from changes in pCO_2 using CO2SYS software (Lewis & Wallace, 1998) with the dissociation constants K_1 and K_2 from Millero (1979), KHSO_4 dissociation constant after Dickson (1990), and NBS scale ($\text{mol L}^{-1} \text{H}_2\text{O}$).

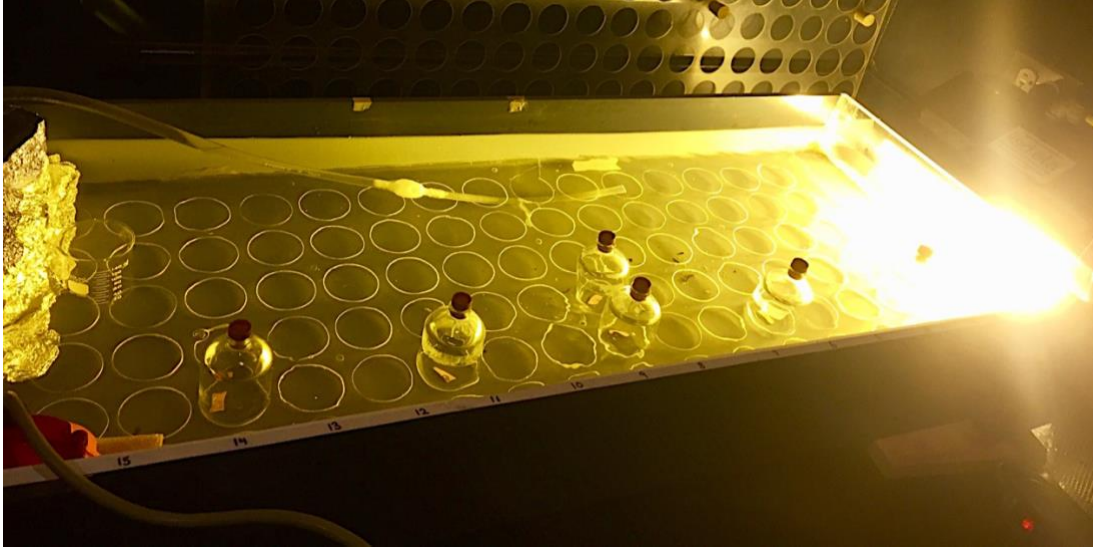


Figure 3.1 *In vitro* incubations along a light gradient with a duplicate and two dark vials wrapped in tin foil.

Net photosynthesis (P_{net}) was measured in clear incubation bottles, and dark respiration was measured within two completely opaque incubation bottles wrapped in foil. This method assumes that dark respiration is equivalent to respiration (R_0) occurring in the light bottles (Davies et al. 2003). Net photosynthesis and respiration were determined by the change in DIC (mg m^{-3}) from the headspace, plus changes in DIC dissolved in the water fraction (DIC_{aq}) of each bottle, divided by the incubation time (Equation 3.1). Respiration was calculated the same as P_{net} (Equation 3.1) for dark bottles, both respiration and P_{net} have units of $\text{mg m}^{-3} \text{ h}^{-1}$. These estimates assume that changes in DIC were from autotrophic primary producers not from bacterial production.

$$(3.1) \quad P_{\text{net}} \text{ or } R = \frac{(\text{DIC}_i - \text{DIC}_f)_{\text{headspace}} + \text{DIC}_{\text{aq}}}{\text{incubation time (in hours)}}$$

Photosynthesis-irradiance curves

Photosynthesis-irradiance (P-I) curves were developed by fitting incubator values of gross primary production vs. irradiance to the Jassby & Platt (1976) equation (Appendix 2.1) using TableCurve 2D v5.01.02, developed by Systat (Systat Software, San Jose, CA). While some other P-I curve fitting equations include a photoinhibition parameter, photoinhibition was not observed in any of the P-I relationships in our study (Appendix 2.1). The Jassby & Platt (1976) equation provides estimates for maximum photosynthesis ($P_{\max}$), the slope of the light limited portion of the P-I response curve (α), and light saturating irradiance (I_k) (Figure 3.2). P-I curve parameters were used only if r^2 coefficient of the fit was > 0.85 .

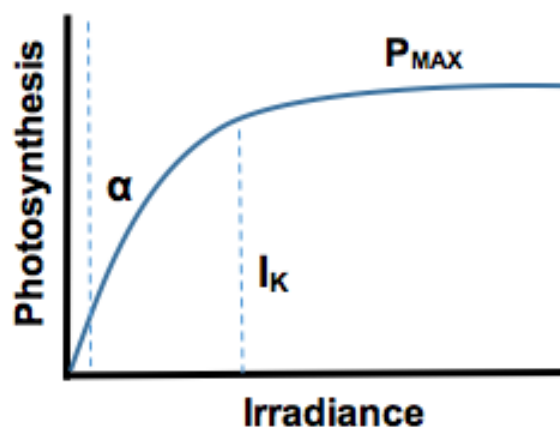


Figure 3.2 Schematic of the different photosynthetic parameters determined from a P-I curve. $P_{\max}$ represents the maximum photosynthesis rate above the light saturating irradiance. Alpha represents the maximum light utilization coefficient represents the initial slope of the P-I curve under light limiting conditions. The value of the light saturating irradiance (I_k) was calculated as $P_{\max}/\alpha$. The first dotted line refers to the 1% light level.

Depth Integrated Primary Production

Net primary production was calculated at 0.5 m depth intervals in lakes using the P-I parameters normalized to chlorophyll *a* (P_{max}^b , α^b), and light intensity using equation 3.2 (Vadeboncoeur et al. 2001). Photosynthetic parameters α , P_{max} , and I_k were assumed to remain constant throughout the water column, consistent with other studies (Ask et al. 2009; Fee 1973; Jassby & Platt 1976a; Stojanovic et al. 2019) (Appendix 3.2). Normalizations to chlorophyll *a* allowed for the dynamic calculation of photosynthesis and respiration *in situ* under variable chlorophyll *a* concentrations. See table 3.1 for units of equation 3.2.

$$(3.2) \quad NPP_z^b = P_{max}^b \times \tanh \left[\frac{\alpha^b \times I_z}{P_{max}^b} \right] - R_0^b$$

Where NPP_z^b represents net primary production normalized to chlorophyll *a* (superscript *b*) at depth interval subscript *z* (e.g. between 0 – 0.5 m depth). P_{max}^b and α^b represent values derived from laboratory incubations for each lake, fitted to the Jassby & Platt (1976) equation and normalized to chlorophyll *a*, as described previously. Values of NPP_z^b within each depth interval were then multiplied by the corresponding chlorophyll *a* concentration at each depth interval to achieve NPP_z , which represents the volumetric rate of primary production within each depth layer.

Chlorophyll *a* at each depth interval was measured using the YSI model EXO2 and converted from relative fluorescence units to chlorophyll ($\mu\text{g L}^{-1}$) at each depth interval. Chlorophyll *a* measurements from the sonde were corrected *post hoc* by linear regression between sonde estimates and laboratory based fluorometric estimates (C. Desjardins pers. com.) (Appendix A3.2). DCM was assessed as maximum chlorophyll *a* concentration below

the thermocline. Depth integrated chlorophyll estimates were summed from the surface (0 m) to the photic depth (depth of 1% surface irradiance). Light at depth (I_z) was calculated for every 0.5 m depth interval ($I_z = PAR_{max}^{(-K_d \times Z)}$).

Table 3.1 Parameters from equation 3.2, calculating net primary production (NPP) per unit chlorophyll (b) at depth (z).

Parameter	Description	Units
NPP^b_z	net primary production	$\text{mg C L}^{-1} \text{ h}^{-1}$
$P^b_{\max}$	Photosynthesis maximum	$\text{mg C L}^{-1} \text{ h}^{-1}$
α^b	Alpha	$\mu\text{mol photons m}^2 \text{ sec}^{-1} / \text{mg C L}^{-1} \text{ h}^{-1}$
I_z	Light at depth	$\mu\text{mol photons m}^2 \text{ sec}^{-1}$
$Tanh$	Hyperbolic tangent function	
b	Normalization to chlorophyll at depth	$\mu\text{g L}^{-1}$
R_0	Respiration	$\text{mg C m}^{-2} \text{ h}^{-1}$

Statistical Analysis

We used Pearson's correlation analysis (parametric) to evaluate how phytoplankton primary production varied with light and DOC. Pearson's was used for normally distributed variables. Spearman's correlation analysis (non-parametric) was used for parameters that were not normally distributed. Correlation analyses were conducted using the open source software R (R Team 2013). Logarithmic transformations were used for variables with non-linear trendlines (NPP total, epilimnetic, and sub-epilimnetic). The assumption of normality was assessed using the Shapiro-Wilk test and by visualizing data as a histogram and normal quantile plot (Appendix A3).

Results

DOC was strongly correlated with physical properties of the 6 study lakes. As DOC increased across the lake gradient from 3.5 to 9.4 mg L⁻¹, light attenuation increased while thermocline and photic depth decreased. DOC was positively correlated with light attenuation coefficient ($r = 0.96$; $p < 0.01$) and negatively correlated with photic depth ($r = -0.96$; $p < 0.01$), thermocline depth ($r = -0.98$; $p < 0.001$). Many of these variables were intercorrelated. Light attenuation coefficient was significantly correlated to photic depth ($r = -0.89$; $p < 0.05$) and thermocline depth ($r = -0.91$; $p < 0.05$). Photic depth declined by $\sim 4\times$; from 14.4 m in L224 the lowest DOC lake, to 3.5 m in L164 the highest DOC lake. Thermocline depth declined by $\sim 2\times$; from 7.6 m in L224 to 3.9 m in L164. These relationships between DOC and photic and thermocline depth were non-linear, following a hyperbolic 'rise to max' response; photic and thermocline depths converged as DOC approached 9 mg L⁻¹ (Figure 3.3).

Table 3.2 Physical characteristics of the 6 study lakes, ordered by dissolved organic carbon (DOC) (mean $\pm$ SD, 4 sampling dates in 2018), including maximum depth (Z_{max}), light attenuation coefficient (K_d), photic depth, and thermocline depth (mean $\pm$ SD 3 sampling dates).

Lake	DOC (mg L ⁻¹)	Z_{max} (m)	K_d (m ⁻¹)	Photic Depth (m)	Thermocline Depth (m)
L224	3.5 $\pm$ 0.15	27	0.3 $\pm$ 0.0	14.4 $\pm$ 1.8	7.6 $\pm$ 1.4
L373	4.1 $\pm$ 0.19	21	0.4 $\pm$ 0.1	12.0 $\pm$ 1.5	7.3 $\pm$ 0.2
L223	5.6 $\pm$ 0.23	14	0.5 $\pm$ 0.1	9.6 $\pm$ 1.2	6.6 $\pm$ 0.3
L239	7.1 $\pm$ 0.23	30	0.7 $\pm$ 0.1	5.5 $\pm$ 0.3	5.9 $\pm$ 0.2
L658	9.1 $\pm$ 0.17	13	1.0 $\pm$ 0.0	5.6 $\pm$ 0.1	4.4 $\pm$ 0.8
L164	9.4 $\pm$ 0.26	7.0	1.2 $\pm$ 0.1	3.5 $\pm$ 0.3	4.0 $\pm$ 0.1

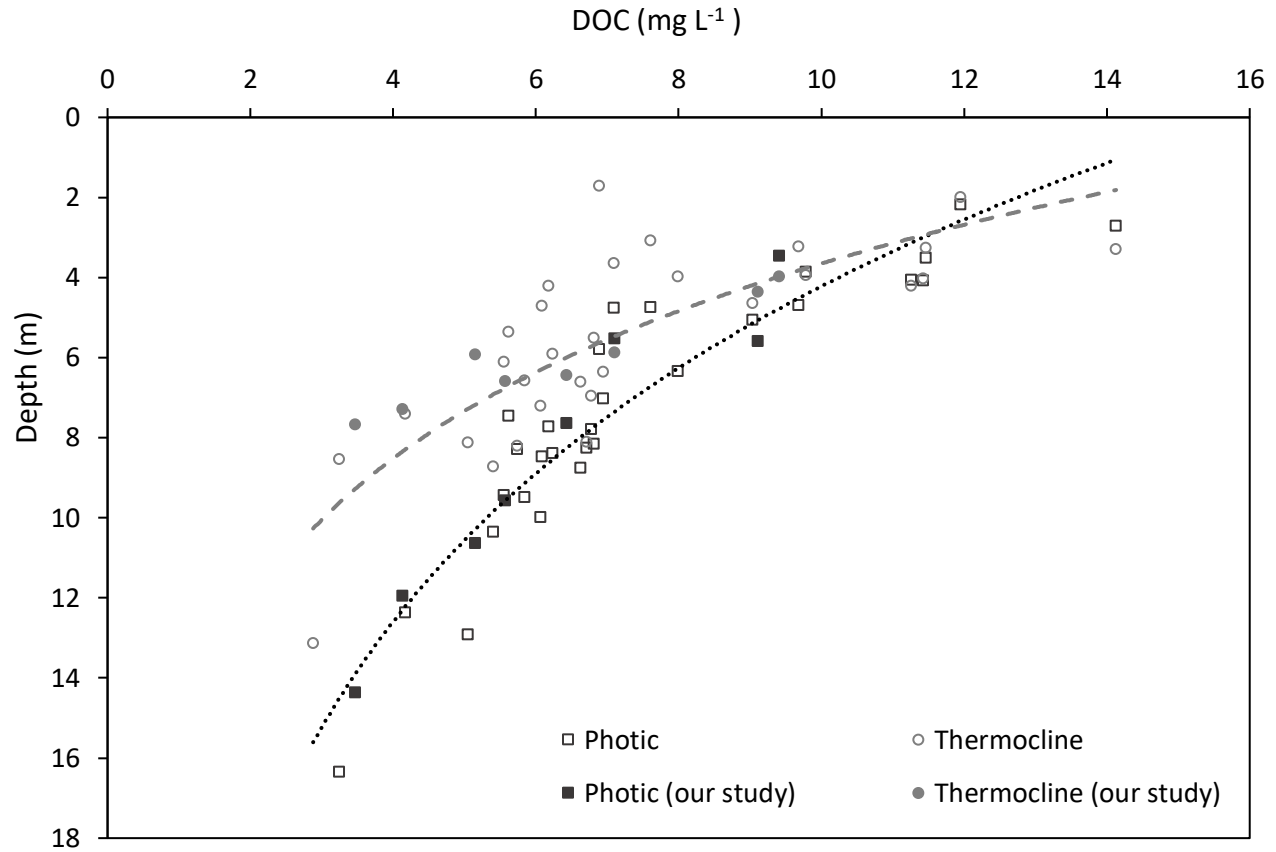


Figure 3.3 Relationship between DOC and photic and thermocline depths. Historic data (open symbols) from IISD-ELA database and data from our study lakes (closed symbols).

DOC was positively correlated with cDOM ($r = 0.93$; $p < 0.01$), indicating its chromophoric properties. Light attenuation coefficient was strongly correlated to cDOM ($r = 0.98$; $p < 0.001$). Epilimnetic nutrient (TN, TP) concentrations across the study lakes increased significantly with DOC. DOC was positively correlated with total phosphorus ($r = 0.88$; $p < 0.05$) and total nitrogen ($r = 0.95$; $p < 0.01$). DOC was correlated to epilimnetic chlorophyll *a* ($r = 0.93$; $p < 0.01$) and not correlated to depth integrated chlorophyll (0.01 m to Z_{photic}) ($r = -0.76$; $p = 0.08$) (Figure 3.5).

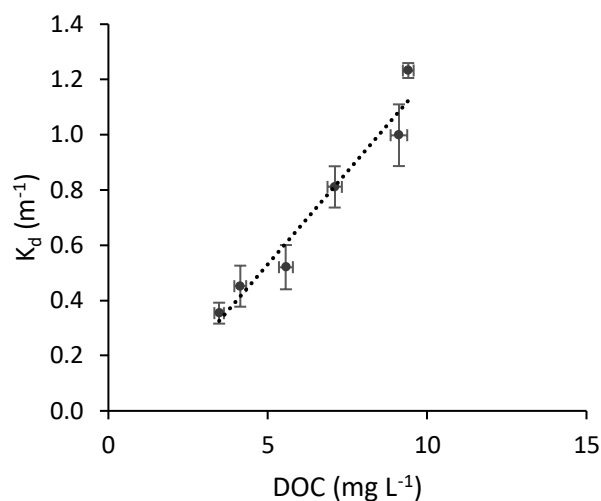


Figure 3.4 Relationship between DOC and light attenuation coefficient ($r = 0.96$; $p < 0.01$).

Table 3.3 Chemical characteristics (mean $\pm$ SD 4 sampling dates in 2018) of the study lakes: dissolved organic carbon (DOC), dissolved inorganic carbon (DIC), chromophoric dissolved organic matter (cDOM), pH, chlorophyll *a* (Chl-*a*), total nitrogen (TN), and total phosphorus (TP). Lakes are ordered by increasing DOC concentration.

Lake	DOC (mg L ⁻¹)	DIC (μ M)	cDOM (μ g L ⁻¹)	pH (SU)	Chl- <i>a</i> (μ g L ⁻¹)	TN (μ g L ⁻¹)	TP (μ g L ⁻¹)
L224	3.5 $\pm$ 0.15	123 $\pm$ 22	1.7 $\pm$ 0.5	7.1 $\pm$ 0.1	1.4 $\pm$ 0.3	222 $\pm$ 14	5.6 $\pm$ 0.9
L373	4.1 $\pm$ 0.19	199 $\pm$ 14	3.2 $\pm$ 0.4	7.3 $\pm$ 0.2	1.3 $\pm$ 0.2	268 $\pm$ 16	6.4 $\pm$ 1.0
L223	5.6 $\pm$ 0.23	150 $\pm$ 12	4.4 $\pm$ 0.9	7.0 $\pm$ 0.2	1.3 $\pm$ 0.4	438 $\pm$ 21	5.1 $\pm$ 1.1
L239	7.1 $\pm$ 0.23	179 $\pm$ 29	17 $\pm$ 1.4	7.1 $\pm$ 0.1	2.4 $\pm$ 0.9	306 $\pm$ 25	6.3 $\pm$ 1.0
L658	9.1 $\pm$ 0.17	159 $\pm$ 8.4	19 $\pm$ 1.0	6.9 $\pm$ 0.5	2.3 $\pm$ 0.3	375 $\pm$ 20	8.7 $\pm$ 1.5
L164	9.4 $\pm$ 0.26	111 $\pm$ 13	16 $\pm$ 1.3	6.8 $\pm$ 0.2	2.8 $\pm$ 1.0	402 $\pm$ 11	8.0 $\pm$ 1.0

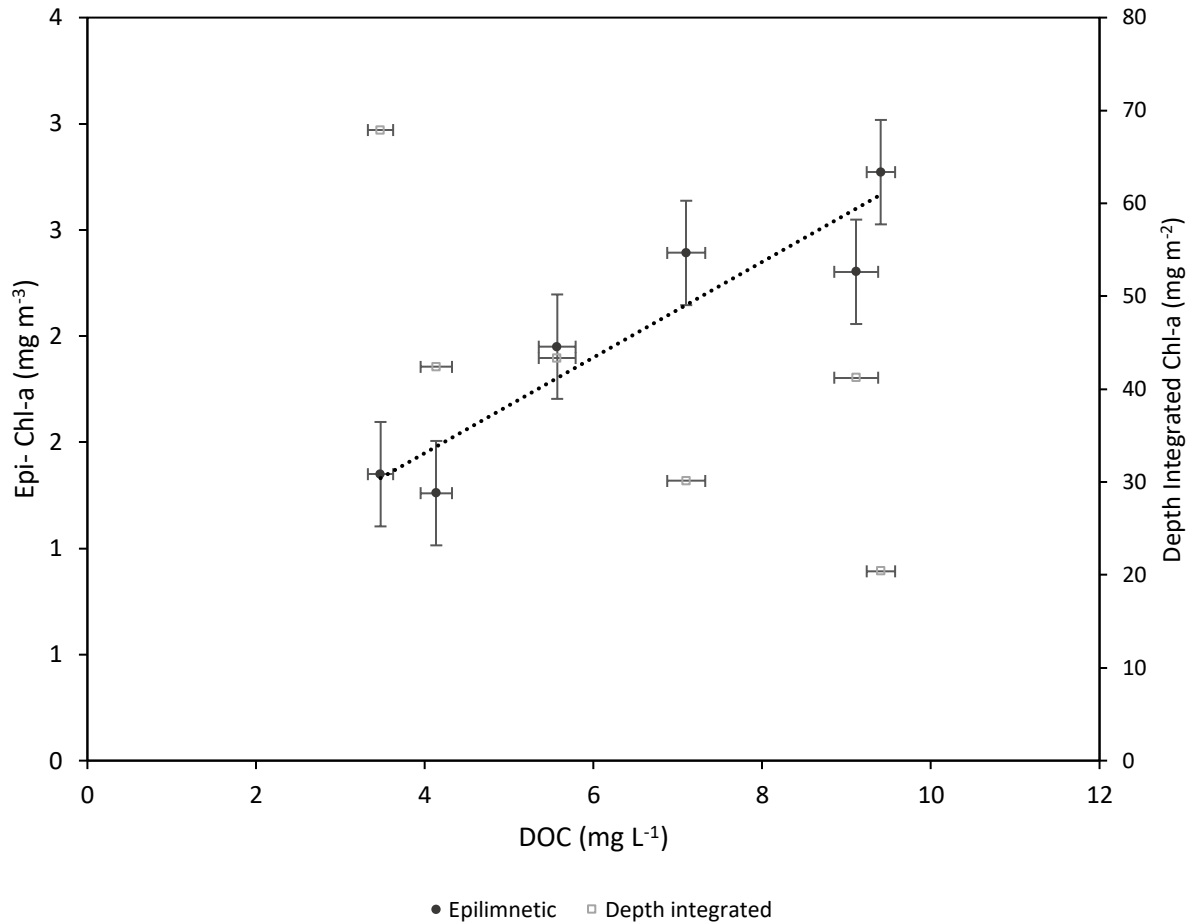


Figure 3.5 Relationship between DOC and epilimnetic ($r = 0.93$; $p < 0.01$) and depth integrated chlorophyll a (0.01 m to Z_{photic}) ($r = -0.76$; $p = 0.08$) (mean $\pm$ SD 4 sampling dates in 2018). Trendline indicates significance.

DOC was not significantly correlated to P_{max}^b ($r = -0.46$; $p = 0.36$), α^b ($r = 0.22$; $p = 0.67$), I_k ($r = -0.63$; $p = 0.17$), or R^b ($r = 0.50$; $p = 0.31$) (Figure 3.6). Phytoplankton biomass was positively correlated to P_{max} ($r = 0.90$; $p < 0.05$) (Figure 3.7), but not α ($r = -0.03$; $p = 0.95$) or I_k ($r = 0.52$; $p = 0.29$). DOC was not significantly correlated to phytoplankton biomass ($r = 0.50$; $p = 0.31$).

Table 3.4 Pelagic primary production P-I curve parameters normalized to chlorophyll *a*: photosynthesis maximum ($P^b_{\max}$), alpha (α^b), light saturating irradiance (I_k), respiration (R_0), and goodness of fit (r^2) between raw incubator data and the Jassby & Platt (1976) equation.

Lake	$P^b_{\max}$ (mg C m ⁻³ h ⁻¹)	α^b (μmol photons m ² sec ⁻¹ /mg C L ⁻¹ h ⁻¹)	I_k (μmol photon m ² sec ⁻¹)	R_0 (mg C m ⁻³ h ⁻¹)	r^2
L224	0.733	0.007	106.7	4.9x10 ⁻⁵	0.93
L373	0.384	0.003	131.0	2.1x10 ⁻⁵	0.99
L223	0.223	0.010	17.02	0.042	0.85
L239	0.557	0.008	29.70	0.007	0.99
L658	0.433	0.010	45.43	0.071	0.98
L164	0.265	0.003	80.22	0.005	0.91

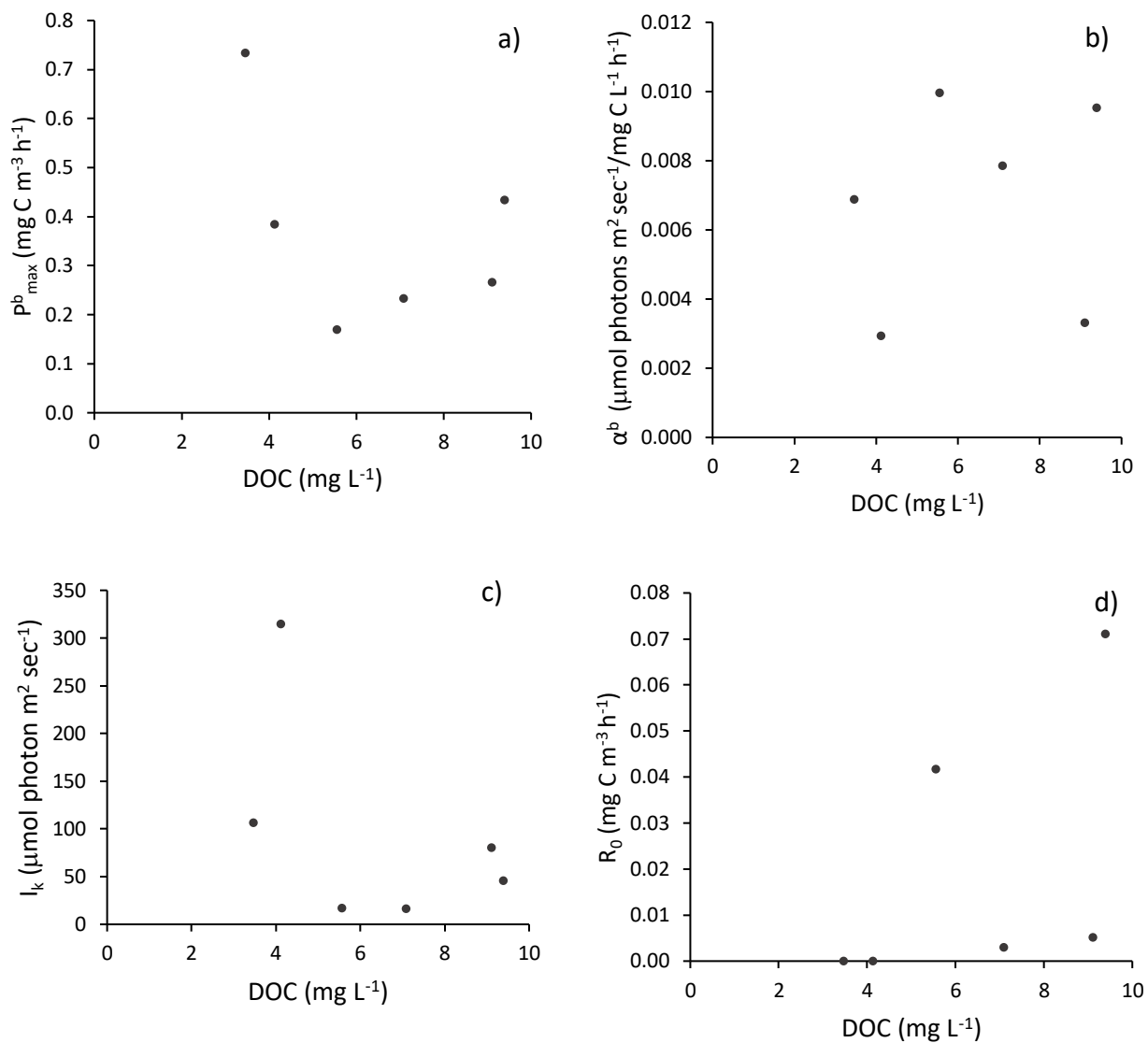


Figure 3.6 Relationships between DOC and P-I parameters normalized to chlorophyll *a*; (a) photosynthesis maximum ($P_{\max}^b$); (b) alpha (α^b); (c) light saturating irradiance (I_k); (d) respiration (R_0).

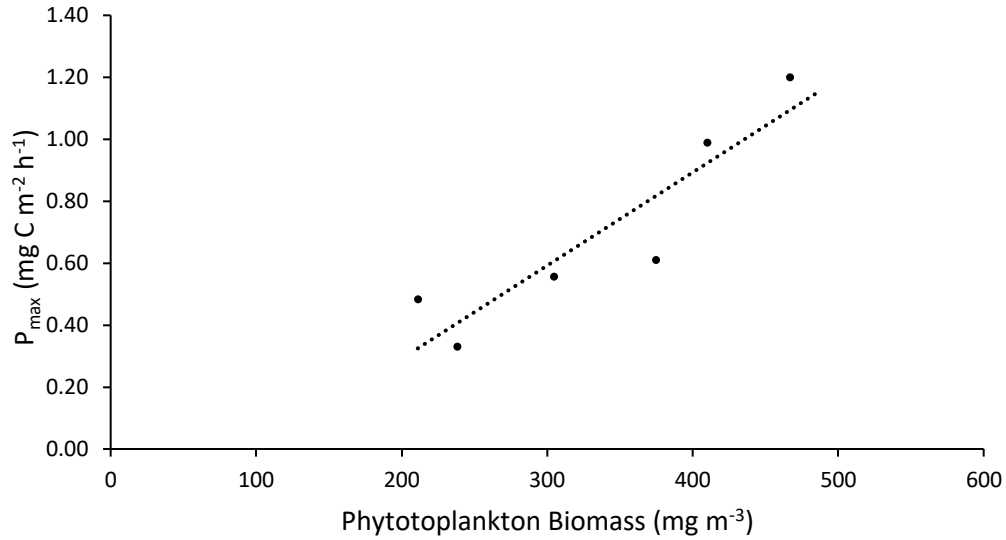


Figure 3.7 Relationship between phytoplankton biomass and photosynthesis maximum ($P_{\max}$) not normalized to chlorophyll *a* ($r = 0.90$; $p < 0.05$).

Primary production

Across the range of DOC, total depth integrated (0 m- Z_{photic}) NPP varied by $\sim 7x$, ranging from 1.93 to 14.9 mg C m⁻² h⁻¹, and from 1.93 to 12.1 mg C m⁻² h⁻¹ for the epilimnion (Figure 3.8). Negligible NPP occurred below the thermocline in lakes with DOC $> 7\text{--}8$ mg L⁻¹, therefore total depth integrated and epilimnetic NPP estimates overlap. Average volumetric rates of NPP ranged from -0.02 to 0.71 mg C m⁻³ h⁻¹. DOC was significantly correlated to log transformed total NPP ($r = -0.87$; $p < 0.05$), NPP_{epi} ($r = -0.85$; $p < 0.05$), and sub-epilimnetic NPP $Z_{\text{thermocline}}$ to Z_{photic} ($r = -0.92$; $p < 0.05$). DOC was not significantly correlated to volumetric NPP ($r = -0.77$; $p = 0.07$). Light attenuation was significantly correlated to log transformed total NPP ($r = -0.85$; $p < 0.05$), epilimnetic NPP_{epi} ($r = 0.83$; $p < 0.05$), and sub-epilimnetic NPP $Z_{\text{thermocline}}$ to Z_{photic} ($r = -0.91$; $p < 0.05$). L164 was not included in sub-epilimnetic NPP $Z_{\text{thermocline}}$ to Z_{photic} correlations

because the photic zone is above the thermocline depth. Photosynthesis maximum was significantly correlated with total NPP ($r = 0.87$; $p < 0.05$), $NPP_{\text{epi-}}$ ($r = 0.87$; $p < 0.05$), and sub-epilimnetic NPP $Z_{\text{thermocline}}$ to Z_{photic} ($r = 0.86$; $p < 0.05$), but not with volumetric NPP ($r = 0.62$; $p = 0.19$). Log transformed total NPP was not significantly correlated with epilimnetic chlorophyll ($r = -0.77$; $p = 0.08$), depth integrated chlorophyll ($r = -0.80$; $p = 0.06$), or phytoplankton biomass ($r = -0.01$; $p = 0.98$).

Table 3.5 Net primary production as areal and volumetric measures across study lakes. Areal rates are sums of total NPP (NPP_{total}), epilimnetic NPP ($NPP_{\text{epi-}}$) (sum NPP from 0m- $Z_{\text{thermocline}}$), sub-epilimnetic NPP ($Z_{\text{thermocline}}$ - Z_{photic}), and areal proportion of epilimnion NPP ($NPP_{\text{epi-}}$ divided by NPP_{total}). The volumetric rate is the average NPP in the epilimnion.

Lake	Areal NPP_{total}	Areal $NPP_{\text{epi-}}$	Areal NPP $Z_{\text{thermocline}}-Z_{\text{photic}}$	Areal Proportion $NPP_{\text{epi-}}$	Volumetric Average $NPP_{\text{epi-}}$
	(mg C m ⁻² h ⁻¹)			(%)	(mg C m ⁻³ h ⁻¹)
L224	14.9±0.3	12.1±0.0	2.78±0.1	81.29	0.71±0.0
L373	5.26±0.2	4.68±0.1	0.58±0.0	94.43	0.31±0.1
L223	3.68±0.1	3.31±0.0	0.38±0.1	89.76	0.25±0.0
L239	3.47±0.2	3.45±0.3	0.02±0.0	98.33	0.25±0.1
L658	1.93±0.2	1.93±0.3	-0.15±0.1	100.0	0.19±0.2
L164	2.70±0.4	2.70±0.4	n/a	99.80	-0.02±0.4

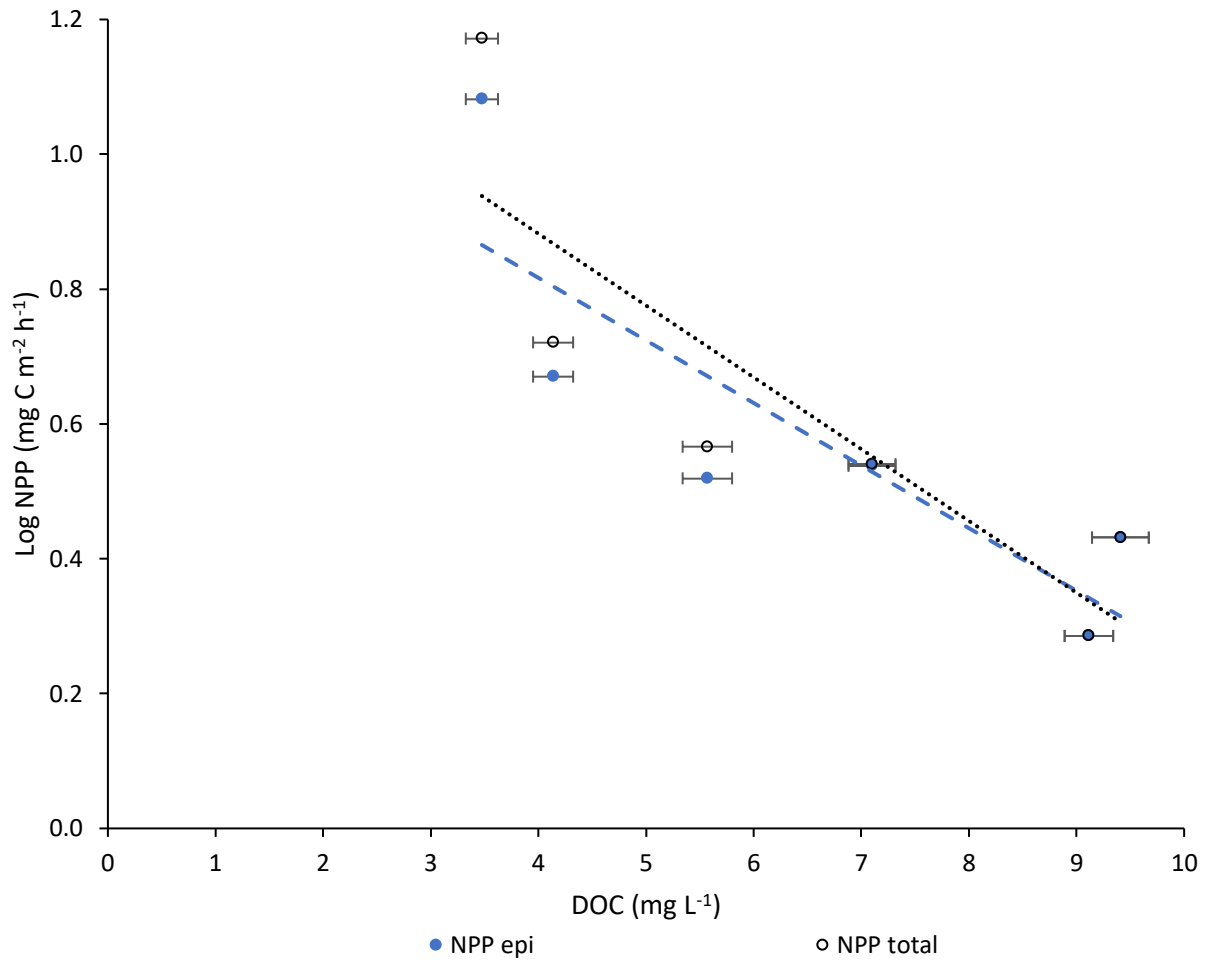


Figure 3.8 Relationship between log transformed areal NPP (total NPP and epilimnetic NPP) with DOC (mean $\pm$ SD). Total and epilimnetic NPP represent depth integrated values from the surface to the depth of the photic zone (Z_{photic}) or thermocline (Z_{therm}) respectively. Epilimnetic NPP overlaps NPP total for lakes with DOC values $> 7 \text{ mg L}^{-1}$.

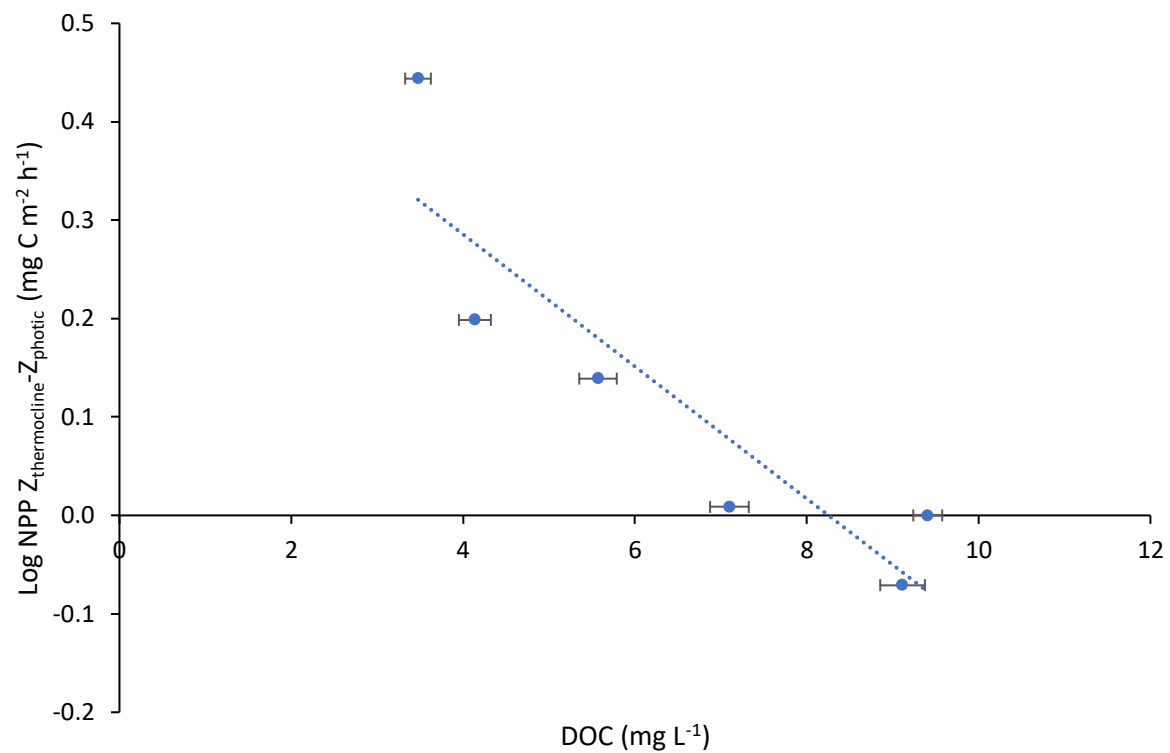


Figure 3.9 The relationship between log transformed areal sub-epilimnetic NPP ($Z_{\text{thermocline}} - Z_{\text{photic}}$) with DOC (mean $\pm$ SD). A constant value (1.00) was added to all values prior to logarithmic transformation to account for the negative values.

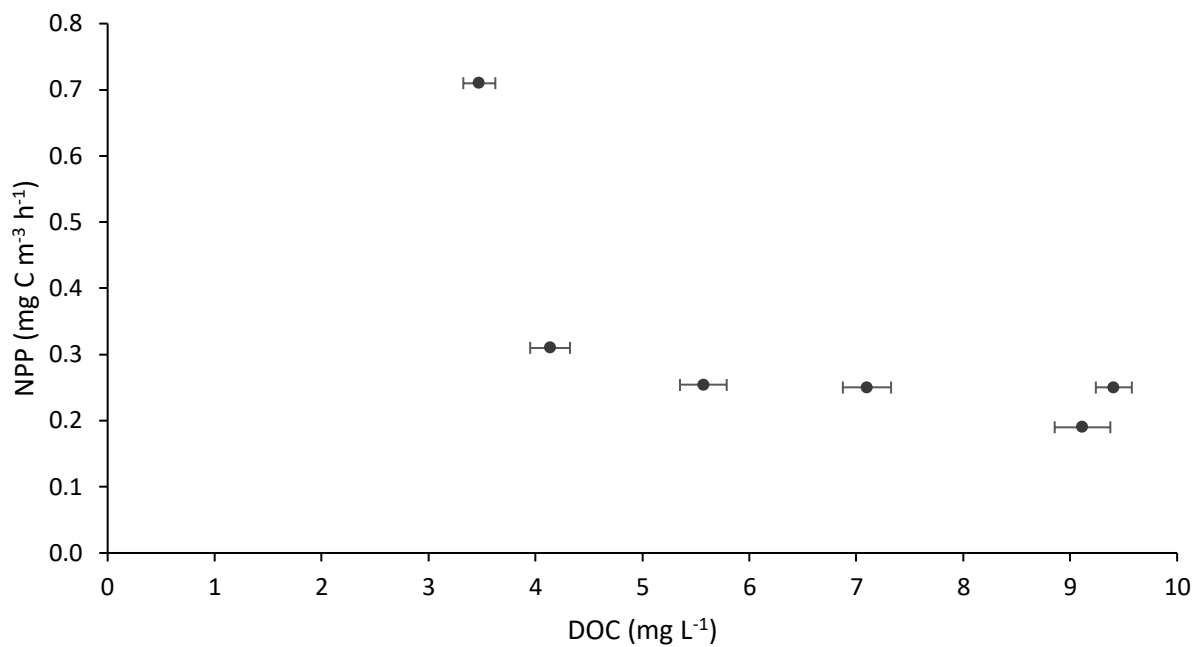


Figure 3.10 Relationship between volumetric NPP (average NPP in the epilimnion) and DOC (mean $\pm$ SD).

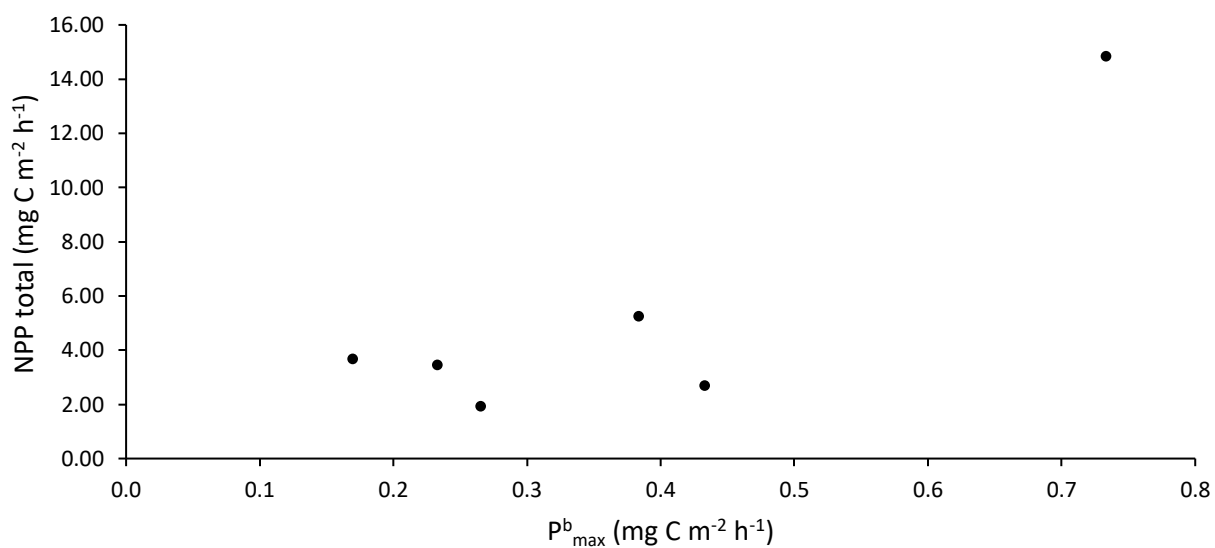


Figure 3.11 Relationship between total NPP and photosynthesis maximum (P^b_{max}).

The metalimnion of low DOC lakes contained DCM in July when primary production was estimated (Figure 3.12). The depth of the DCM was negatively correlated with DOC ($r = -0.95$; $p < 0.005$). Lakes with the highest sub-epilimnetic NPP (i.e. L224 and L223) had well developed DCM. L224 and L223 had 17-21% of NPP occurring below the thermocline. L224 had the highest proportion of NPP occurring below the thermocline (21%). L224 is the second deepest lake ($Z_{\max} = 27$ m), clearest lake ($K_d = 0.03 \text{ m}^{-1}$), with the lowest DOC (3.5 mg L^{-1}), and cDOM (1.7 RFU). L224 has a very deep photic depth (14.5 m) and a developed DCM at 13.5 m, deeper than its thermocline (7.7 m). L223 had the second highest proportion of NPP occurring below the thermocline (17%). L223 has a deep photic depth (10.7 m) and a developed DCM at 7.0 m, deeper than its thermocline (5.9 m). L658 and L164 had the highest proportion of NPP occurring in the epilimnion ($>99\%$). These lakes were the darkest ($K_d > 1.0$), shallowest ($Z_{\max} < 13$), and highest DOC lakes ($>9 \text{ mg L}^{-1}$). L164, the highest DOC lake, had 0% NPP occurring below the thermocline. This was expected as the photic depth (3.5 m) was shallower than the thermocline (3.5 m). Neither of these high DOC lakes (i.e. L164 and L658) had DCM present.

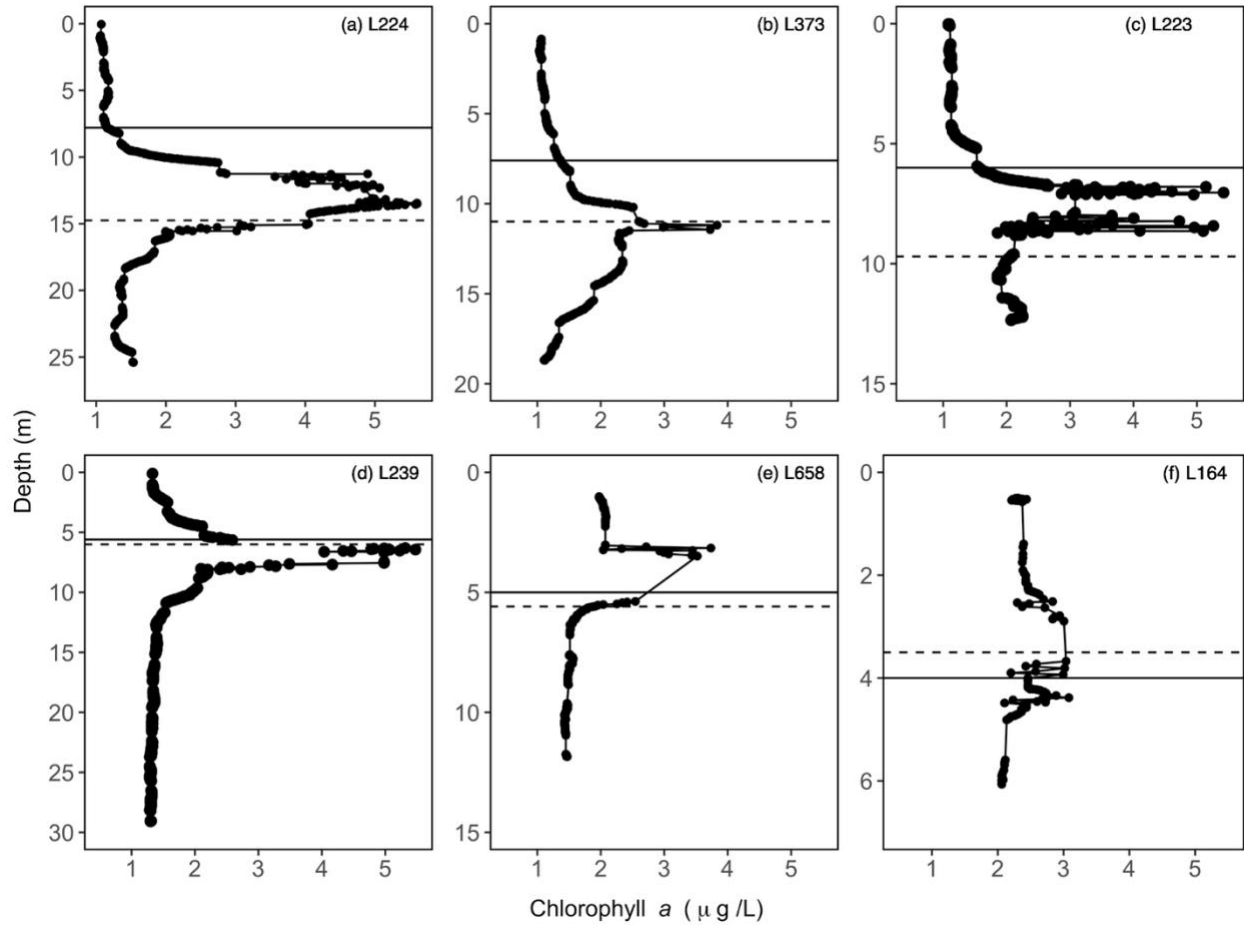


Figure 3.12 Chlorophyll *a* profiles showing DCM depths in our study lakes with panels ordered by increasing DOC concentration. The dotted horizontal line indicates the photic zone and the black horizontal line indicates the thermocline.

NPP steadily declined with depth in all lakes reaching $\sim 0 \text{ mg C m}^{-2} \text{ h}^{-1}$ near the photic depth. NPP was not correlated with light attenuation rate ($r = -0.44$; $p = 0.38$) or photic depth ($r = -0.46$; $p = 0.36$). Figure 3.13 suggests that NPP was not strongly influenced by chlorophyll because NPP did not increase with DCM.

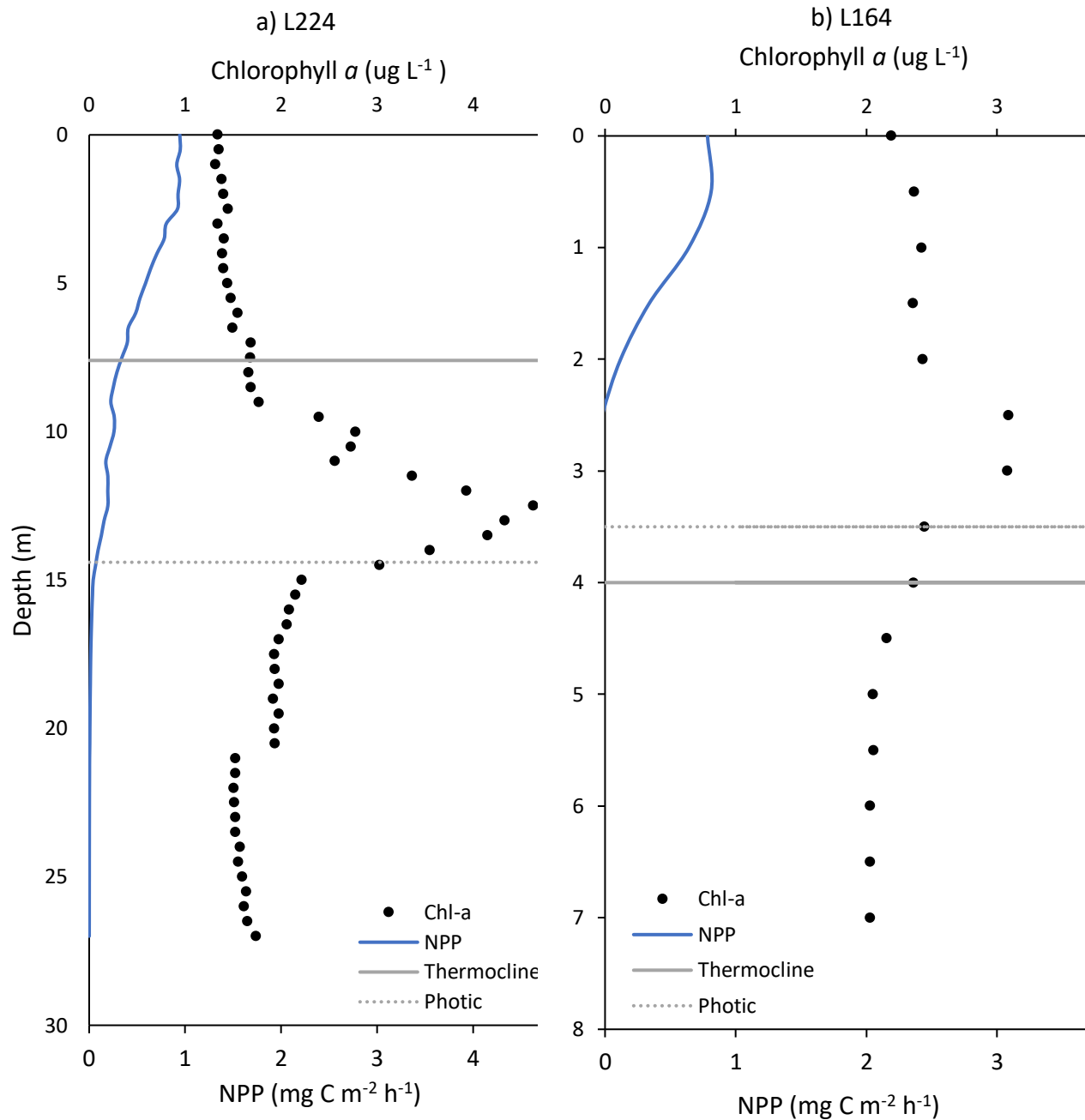


Figure 3.13 Relationships between volumetric NPP and chlorophyll *a* with depth: (a) the lowest DOC lake (L224); and (b) the highest DOC lake (L164). Negative values of NPP in L164 below 2 meters are not included. Other lakes are included in appendix A3.

Discussion

Our study focused on a central question: Do variations in allochthonous DOC significantly impact phytoplankton biomass and productivity in boreal lakes? Our results showed strong effects of allochthonous DOC on phytoplankton net primary production. Phytoplankton productivity (NPP total, epilimnetic, and sub-epilimnetic) decreased non-linearly with DOC, refuting hypothesis 1. Total NPP declined by 87% from the lowest DOC lake to the highest DOC lake. Sub-epilimnetic NPP also declined as DOC increased, after DOC reached 7-8 mg L⁻¹ there was insufficient light below the thermocline to support phytoplankton productivity. This result is consistent with other studies that demonstrate strong relationships between primary production, DOC, water clarity, and light limitation (Kelly et al. 2018; von Einem & Granéli 2010).

Climatic shifts toward higher rainfall and increased predominance of terrestrially derived dissolved organic matter, which is more biorefractory, aromatic, larger in size, and higher in molecular weight than autochthonous DOM will greatly influence aquatic ecosystems (Benoy et al. 2007; Creed et al. 2018). Our results suggest that increases in such biorefractory components of DOM leading to brownification will likely accentuate declines in NPP, even in low nutrient systems.

Decreasing NPP with DOC could have implications on the rest of the food web as found by Tonin (2019) on the same study lakes. In that study, increasing DOC led to decreased zooplankton biomass and an increased reliance of zooplankton on terrestrial sources of DOC (Tonin 2019). Using stable isotopes of carbon, nitrogen, and hydrogen, Tonin (2019) indicated that DCM likely played a disproportional role in meeting the energetic and

nutritional requirements of zooplankton. DCM layers were an important driver of the declines in secondary production (Tonin 2019).

Contrary to our first hypothesis where increased nutrient bioavailability associated with increased DOC was predicted to increase primary production, NPP actually declined by 5 to 7 times across the DOC gradient. The decline in NPP with DOC was initially surprising given that, epilimnetic nutrients and phytoplankton biomass increased with DOC, and maximum rates of photosynthesis increased with phytoplankton biomass. Often epilimnetic chlorophyll *a* and NPP show parallel responses (Schindler et al. 1978), but not always (del Giorgio & Peters 1993). Our results demonstrated that while appearing intuitive, increases in epilimnetic phytoplankton biomass (as chlorophyll *a*) with DOC were actually misleading. As DOC increased, phytoplankton were concentrated into a smaller volume of epilimnetic water as the thermocline depth became shallower. Similar to Fee et al. (1996), light attenuating properties of DOC reduced the epilimnion thickness by ~50% (from ~8 m to ~4 m) and reduced the photic depth by ~75% (from ~14 m to ~3.5 m) across the DOC gradient. Thus, while epilimnetic chlorophyll *a* concentrations increased, depth-integrated chlorophyll actually decreased with DOC. These results suggest that epilimnetic phytoplankton biomass should not be used as an indicator of productivity without accounting for declines of epilimnetic volume associated with DOC. Further, these results demonstrate the primacy of DOC's negative effects on light, in contrast to its positive effects on nutrients even within pristine boreal lakes where nutrient concentrations are low and primary production is generally considered nutrient limited (Schindler 1977). These conclusions are generally consistent with other studies (Karlsson et al. 2009b; Kunz & Diehl 2003; Seekell et al. 2015; Solomon et al. 2015).

Drivers

In our study, increases in DOC led to 1) decreases in depth-integrated chlorophyll *a* concentrations; 2) reduced productivity with depth and restrictions of primary production to shallower depths due to increased light attenuation. These factors are important outcomes of increased DOC that influenced primary production.

The effects of DOC on light and nutrients have contrasting implications for primary producers (Seekell et al. 2015). Oligotrophic lakes with lower concentrations of DOC generally have a low bioavailability of nutrients and high light availability (Zwart et al. 2016; Molot & Dillon 1997; Moran & Zepp 1997). As allochthonous DOC increases, light availability decreases in response to the chromophoric properties of DOC and its strong effects on light attenuation. While others (Hanson et al. 2003; Prairie et al. 2002; Seekell et al. 2015a; Seekell et al. 2015b) have found a threshold effect of DOC on NPP, we did not in this chapter. NPP consistently declined with DOC across the DOC gradient in our study lakes. Whole-lake NPP is significantly influenced by benthic production, these studies (Hanson et al. 2003; Prairie et al. 2002) included benthic production in their calculations of primary production (using the same method used in the previous chapter). Both studies by Seekell et al. (2015a,b) modelled trends in light and nutrients to identify a DOC threshold. While focusing solely on trends of light and nutrients in our study, they suggests a DOC threshold is present because increases in DOC were associated with rising nutrients and declining light, having opposing effects on primary production (chapter 2).

Phytoplankton have the ability to acclimate physiologically to their environment (i.e. temperature, light, and nutrients) by adjusting their photosynthetic apparatus which, in

turn, alters their photosynthesis-irradiance response parameters (e.g. $P_{\max}$, α , I_k) (Köhler et al. 2018; Morán & Estrada 2001; Sakshaug et al. 1997). Conversely, environmental factors (i.e. temperature, light, and nutrients) can influence photosynthetic activity, metabolic processes, and the rate of cell division (Jodłowska & Śliwińska 2014). Light saturating irradiance (I_k) can be an indicator of acclimation because it can change depending on the previous light history of algal cells (Sakshaug et al. 1997). In this study, I_k was highest in low DOC lakes that had high water clarity and declined non-linearly with increases in DOC. Relationships between DOC and $P_{\max}$ and I_k were non-linear negative trends and the relationship between DOC and α was a non-linear positive trend. This suggests algal cells may have acclimated to higher DOC conditions. Photosynthetic parameters were measured once in July and did not capture seasonal variability.

DOC effects on primary production at depth

Sub-epilimnetic primary production is strongly affected by the vertical distribution of temperature and light (Camacho 2006; Fee et al. 1996; Fee 1976; Leach et al. 2018). DOC affects the vertical response of NPP through its effects on light attenuation, which decreases the depth of the photic zone and sub-epilimnetic chlorophyll *a* concentrations. In some instances sub-epilimnetic NPP has been shown to contribute significantly to whole-lake production (Giling et al. 2017). However, in our study sub-epilimnetic NPP contributed a maximum of 19% of total depth integrated NPP, and its contribution declined rapidly with increases in DOC. As DOC concentrations reached 7-8 mg L⁻¹, positive net primary production below the thermocline could not be maintained due to insufficient light. The elimination of sub-epilimnetic NPP coincided with the convergence of photic and thermocline depths

where K_d values were $\sim 1 \text{ m}^{-1}$ and 9 mg L^{-1} DOC. Brothers et al. (2014) found that strong terrestrial DOC fluxes restricted sub-epilimnetic primary production, which decreased completely with DOC greater than 7 mg L^{-1} . NPP was negatively influenced by both light and DOC; however, they are strongly interrelated making it difficult to tease apart the main driver of decreasing NPP (Seekel et al. 2015).

DOC effects on deep chlorophyll maxima (DCM)

Boreal lakes, including those at the IISD-ELA, are typically small and deep with sharp thermoclines (Schindler 1971) and stable stratification (Hesslein & Quay 1973). Low DOC lakes in the region are clear with deep photic zones, optimal for the development of DCM (Fee 1976; Leach et al. 2018). The depths at which DCM are located are directly related to light attenuation, generally occurring at the limits of the photic zone (i.e. depths where light is $\sim 1\%$ of surface irradiance) (Fee 1976; Knapp et al. 2003; Leach et al. 2018). Our results were entirely consistent with those previous studies (Fee 1976; Knapp et al. 2003; Leach et al. 2018). DCM were deepest in clearer lower DOC lakes with deep photic depths and low light attenuation.

DCM are ecologically important hot spots for nutrient cycling and primary production (Leach et al. 2018). In a companion study on the same suite of boreal lakes used here, Tonin (2019) determined that phytoplankton within deep chlorophyll layers were an important dietary resource for zooplankton and ultimately fish. Increases in DOC eliminated DCM, caused declines in zooplankton biomass, and ultimately caused declines in fish growth rates (Tonin 2019).

Assumptions

Assumptions of our study included: omission of basin morphometry, overestimated sub-epilimnetic productivity, using photosynthesis-irradiance parameters from epilimnetic incubations, and not correcting for water volume in depth layers. Our intention was to isolate the effects of DOC on primary production, we chose not to include morphometric corrections that would have introduced additional explanatory variables for lake to lake differences in NPP. Fee (1978) noted the lack of morphometric corrections for these boreal lakes likely resulted in the overestimation of sub-epilimnetic NPP by ~11%. While these effects were modest, they would also be offset to an unknown extent by our use of photosynthesis-irradiance parameters from epilimnetic incubations throughout the water column. Since our estimates showed that >80% of the depth-integrated productivity occurred within the epilimnion, if there was an overestimation it would affect at most the 20% of depth-integrated sub-epilimnetic production. Sub-epilimnetic NPP was $<1 \text{ mg C m}^{-2} \text{ h}^{-1}$ in 5 out of 6 lakes; therefore, the magnitude of error would be relatively small. If sub-epilimnetic production was overestimated, the decreasing trend of NPP with DOC would still be present. In our study we did not correct NPP with water volume at each depth layer. Water volume at depth layers declines at deeper depths due to the concave nature of lake basins. In order to standardize NPP calculations water volume at depth layers were not accounted to better compare between lakes of different shapes and sizes. If water volume depth was accounted for differences between NPP in study lakes may have been due to morphological differences and not strictly related to DOC.

Conclusion

The effects of DOC on NPP represent a balance between the positive effect of nutrient enrichment and the negative effect of light limitation. Our study indicated that the effects of DOC on primary production was primarily driven by light limitation. Originally, we hypothesized that the addition of organic nutrients associated with DOC to low nutrient boreal lakes would stimulate primary production. However, our results were clear: light was a stronger driver than nutrients as phytoplankton productivity declined by 87% across the DOC gradient. Further, the strong light attenuating properties of DOC appear the key driver for losses of DCM and sub-epilimnetic productivity, that may play a disproportional role in supplying energy and essential nutrients to higher trophic levels (Tonin 2019).

While more studies are needed to further tease apart the relative importance of light and nutrients from increased DOC, our results and those of others (e.g. Giling et al. 2017) demonstrate that changes in DOC drive phytoplankton productivity with concomitant effects on lake food webs. With terrestrially derived DOC inputs on the rise in many lakes in North America and Eurasia from increased temperatures, precipitation and recovery from acidification (Brett et al. 2017; Clark et al. 2010; Evans et al. 2005; Lepistö et al. 2014; Monteith et al. 2007) it is important to understand the effects DOM will have on lake type (i.e. dimictic, polymictic), trophic status, and to examine higher trophic levels.

Chapter 4: General discussion and conclusion

General discussion

In our study terrestrially derived dissolved organic carbon (DOC) had significant impacts on primary production within boreal lakes. We hypothesized that nutrients (i.e. nitrogen and phosphorus) from photodegraded dissolved organic matter (DOM) would stimulate primary production because the study lakes were oligotrophic, nutrient limited (Schindler 1977) with very low concentrations of bioavailable nutrients. This hypothesis was partially supported in chapter 2 where net ecosystem production (NEP) increased as DOC increased from 3 to 6 mg L⁻¹ DOC. However, NEP declined rapidly as DOC surpassed 6 mg L⁻¹ DOC. This hypothesis was refuted in chapter 3, when net primary production declined with DOC.

In chapter 2, we used a diel free-water oxygen method to measure primary production and community respiration. Gross primary production and community respiration increased significantly with DOC. Net ecosystem production increased until DOC exceeded a threshold of 6 mg L⁻¹; then net ecosystem production declined as a consequence of respiration exceeding gross primary production causing lakes to become more heterotrophic.

In chapter 3, net primary production and sub-epilimnetic production decreased with DOC, despite epilimnetic chlorophyll increasing. Depth integrated chlorophyll, which declined with DOC, was a better predictor of phytoplankton productivity. The presence of deep chlorophyll maxima and sub-epilimnetic productivity were eliminated in lakes with DOC values > 7 mg L⁻¹. Primary production decreased by 87% across the DOC gradient

primarily from the strong effects of DOC on the light attenuation and its availability for photosynthesis.

Key differences between chapters are, chapter 2 included all producers (i.e. phytoplankton and benthic algae) in the epilimnion and chapter 3 focused on phytoplankton throughout the water column (excluding sediments).

In chapter 2, respiration exceeded gross primary production as DOC surpassed the threshold of 6 mg L^{-1} causing NEP to become increasingly negative. A DOC threshold was not apparent in chapter 3 potentially because benthic production and respiration were not studied. Benthic respiration from sediments has been shown to surpass production (Jansson et al. 2008).

The contribution of benthic primary producers and sediment respiration in the littoral zone can explain the contradictory results between chapters. Benthic primary production contributes significantly to whole-lake production in small to moderate sized lakes (Vadeboncoeur et al. 2003, Vadeboncoeur et al. 2008). Originally our study included benthic primary production, we found that gross primary production was much larger in the littoral zone than the water column. Phytoplankton required much more sensitive methods (i.e. measuring the change in pCO_2) whereas benthic production was measured by the change in dissolved inorganic carbon. Benthic production was not included in this thesis due to time constraints.

Across the DOC gradient, the photic depth declined from $\sim 15 \text{ m}$ to 2 m , representing a loss of $> 7\text{x}$ of habitat available for photosynthesizing primary producers. Chapter 2 was not impacted by the decrease in light availability and photic depth because it focused on the light saturated epilimnion. Whereas, chapter 3 sub-epilimnetic primary production declined

linearly by 74% across the DOC gradient, likely due to decreasing light causing a decrease in habitat available for photosynthetic algae. Light availability can help explain the contradictory results between chapters.

The effects of DOC on primary production were hard to tease apart because many variables were intercorrelated. DOC was significantly correlated to GPP, respiration, NPP, chlorophyll (epilimnetic and depth integrated), thermocline depth, photic depth, coloured dissolved organic matter (cDOM), nitrogen, and phosphorus. While more studies are needed to further determine the relative importance of these variables, my results and those of others (e.g. Zwart et al. 2016) demonstrate that in natural lake ecosystems changes in DOC drive concurrent variations that drive lake primary production. With concentrations of DOC on the rise in boreal lakes around the world due to climate change (Clark et al. 2010; Seekell et al. 2015a,b) it is necessary to determine qualitative predictions on the impacts DOC will have on lake food webs. Qualitative predictions of DOC impacts will help inform policy to better prevent the major implications of DOC on aquatic ecosystems.

Study Limitations

The limitations of this study are its assumptions of using space-for-time substitutions, incomplete range of DOC to represent all lake types (i.e. shallow polymictic lakes and lakes of different trophic status), not accounting for mixotrophic algal species, and not quantifying sub-epilimnetic benthic algal production or heterotrophic production.

In our study, we examined how spatial variations in DOC across boreal lakes influenced primary productivity. Given the effects of climate change and recovery from acid rain on DOC loading to lakes, there is also an interest in understanding how these temporal

changes in DOC will influence aquatic ecosystems. Space-for-time substitutions have been widely used but do contain a number of assumptions. We selected lakes very carefully based on their similarities in basin shape, size, watershed, and dimictic properties, however, variation still exists. With the nature of this study, temporal factors were not studied. Temporal changes in DOC associated with climate change are much smaller than the gradient of lakes in this study. DOC is changing temporally and spatially and is a significant variable in influencing aquatic ecosystems (Clark et al. 2010). DOC is affected by many variables; it would be impossible to control all variables in a field experiment.

Our DOC gradient ($3.5\text{-}9.4\text{ mg L}^{-1}$) compared to other literature is fairly small, despite these DOC concentrations being very high for lakes at IISD-ELA. In a study by Hanson et al. (2003) with a $1.6\text{-}25\text{ mg L}^{-1}$ DOC gradient, declines in NEP following a DOC threshold were not evident until 10 mg L^{-1} DOC. However, the lakes studied by Hanson et al. (2003) included polymictic lakes. Had our DOC gradient been larger perhaps a clearer DOC threshold and shift in NPP, GPP, and NEP might have been more evident. However, our study focused on stratified boreal lakes of which our DOC gradient encompasses the average concentrations of DOC (Sobek et al. 2007).

Algal species that both photosynthesize and consume other algae (i.e. mixotrophs) have been found at IISD-ELA (IISD-ELA historic data, unpublished). If time permitted it would have interesting to separate the mixotrophic algal species from the autotrophic species. We could have examined the extent to which mixotrophic algae compensate for the losses of autotrophic productivity in general, and the overall role they play in subsidizing the nutrient and energy requirements of higher trophic levels. The presence of mixotrophic algal

species could have provided insight to why DCM peaks did not contribute as much as expected to net primary production in chapter 3.

Benthic algal and heterotrophic production are influenced by DOC and contribute significantly to aquatic ecosystems. Benthic algal production plays a huge role in whole-lake metabolism within the littoral zone and likely contributed to increasing GPP and respiration in chapter 2 (Vadeboncoeur et al. 2003). We are uncertain the degree to which benthic production contributed to our estimates of primary production. As with the unknown contribution of benthic algal production, the importance of heterotrophic bacteria has been shown to increase with DOC, better compensating for light limitation than primary producers (Ask et al. 2009; Giling et al. 2017; Karlsson & Byström 2005). If time permitted it would have been beneficial to quantify benthic and heterotrophic production to better understand the drivers of NEP, GPP, and respiration.

Future directions

Our study added valuable data and insights to a growing number of studies examining the effects of terrestrially derived DOC on the primary productivity of boreal lakes. However, more research is still needed to better understand these effects across a broader range of lake types (e.g. shallow polymictic systems, mesotrophic to eutrophic systems) and to clarify these effects on heterotrophic bacteria and DCM. DOC affects many variables simultaneously, additional research is needed to tease apart these interactions among interrelated variables.

The effects of DOC heterotrophic bacteria should be further investigated to determine its influence on primary production. Our chapter 2 results showed a large increase in respiration which could be associated with microbial degradation of organic matter in

sediments. However, it is unknown and highly contested the degree to which energy and nutrients from the microbial loop were incorporated into higher trophic levels. On the same study lakes Tonin et al. (2019) determined that the incorporation of terrestrial DOC via microbial food web led to declines in zooplankton and fish productivity, suggesting that heterotrophy did not compensate for losses in autotrophic production.

Our results highlight the relationship between DOC and primary producers throughout the water column (i.e. epilimnetic and sub-epilimnetic). However, due to differing conclusions regarding the influence of DOC on DCM (i.e. Tonin et al. (2019) and our study) the importance of DCM should be further investigated to determine its contribution to whole-lake production as well how it is influence by DOC.

It is pertinent to tease apart the interrelated variables associated with DOC as many of these variables are non-linearly related, making them very difficult to predict. In our study light and nutrients were the most significant interrelated variables associated with DOC that had opposing effects on primary production. There are many studies on the effects of light and nutrients on primary producers (Dubourg et al. 2015; Karlsson et al. 2009; Seekell et al. 2015), however, additional studies are still needed to quantify bioavailable nutrients associated with photodegradation of DOC that can be used by primary producers.

Temporal effects of DOC associated with climate change or recovery from acidification may be influenced by additional variables such as increased temperatures, land-use changes, precipitation, and status of catchment soils associated with recovery from acidification. To assist with qualitative predictions and directly study temporal changes these variables should be studied over time as whole ecosystem experiments. Future studies on qualitative predictions of DOC in specific regions will be necessary for management and

environmental protection; especially with terrestrially derived DOC inputs on the rise in many lakes in North America and Eurasia from increased temperatures, land-use changes, precipitation and recovery from acidification (Brett et al. 2017; Clark et al. 2010; Evans et al. 2005; Lepistö et al. 2014; Monteith et al. 2007).

References

- Åberg, J., & Wallin, M. B. (2014). Evaluating a fast headspace method for measuring DIC and subsequent calculation of pCO₂ in freshwater systems. *Inland Waters*, 4(2), 157–166.
<https://doi.org/10.5268/IW-4.2.694>
- Ask, J., Karlsson, J., Persson, L., & Ask, P. (2009). Terrestrial organic matter and light penetration: Effects on bacterial and primary production in lakes. *Limnol. Oceanogr*, 54(6), 2034–2040.
- Ask, J., Karlsson, J., Persson, L., Ask, P., Byström, P., & Jansson, M. (2009). Whole-lake estimates of carbon flux through algae and bacteria in benthic and pelagic habitats of clear-water lakes. *Ecology*, 90(7), 1923–1932. <https://doi.org/10.1890/07-1855.1>
- Beisner, B. E., Haydon, D. T., & Cuddington, K. (2003, September 1). Alternative stable states in ecology. *Frontiers in Ecology and the Environment*. John Wiley & Sons, Ltd.
[https://doi.org/10.1890/1540-9295\(2003\)001\[0376:ASSIE\]2.0.CO;2](https://doi.org/10.1890/1540-9295(2003)001[0376:ASSIE]2.0.CO;2)
- Benoy, G., Cash, K., McCauley, E., & Wrona, F. (2007). Carbon dynamics in lakes of the boreal forest under a changing climate. *Environmental Reviews*, 15(NA), 175–189.
<https://doi.org/10.1139/A07-006>
- Berggren, M., Sponseller, R. A., Alves Soares, A. R., & Bergström, A. K. (2014). Toward an ecologically meaningful view of resource stoichiometry in DOM-dominated aquatic systems. *Journal of Plankton Research*, 37(3), 489–499.
<https://doi.org/10.1093/plankt/fbv018>
- Brett, M. T., Bunn, S. E., Chandra, S., Galloway, A. W. E. E., Guo, F., Kainz, M. J., ... Wehr, J. D. (2017). How important are terrestrial organic carbon inputs for secondary production

in freshwater ecosystems? *Freshwater Biology*, 62(5), 833–853.

<https://doi.org/10.1111/fwb.12909>

Brothers, S., Köhler, J., Attermeyer, K., Grossart, H. P., Mehner, T., Meyer, N., ... Hilt, S.

(2014). A feedback loop links brownification and anoxia in a temperate, shallow lake.

Limnology and Oceanography, 59(4), 1388–1398.

<https://doi.org/10.4319/lo.2014.59.4.1388>

Camacho, A. (2006). On the occurrence and ecological features of deep chlorophyll maxima

(DCM) in Spanish stratified lakes. *Limnetica*, 25(1–2), 453–478. Retrieved from

<https://ddd.uab.cat/record/27928>

Carignan, R., Planas, D., & Vis, C. (2000). Planktonic production and respiration in

oligotrophic shield lakes. *Limnology and Oceanography*, 45(1), 189–199.

<https://doi.org/10.4319/lo.2000.45.1.0189>

Clark, J. M., Bottrell, S. H., Evans, C. D., Monteith, D. T., Bartlett, R., Rose, R., ... Chapman, P. J.

(2010). The importance of the relationship between scale and process in

understanding long-term DOC dynamics. *The Science of the Total Environment*,

408(13), 2768–2775. <https://doi.org/10.1016/j.scitotenv.2010.02.046>

Cole, J. J., & Caraco, N. F. (1998). Atmospheric exchange of carbon dioxide in a low-wind

oligotrophic lake measured by the addition of SF₆. *Limnology and Oceanography*,

43(4), 647–656. <https://doi.org/10.4319/lo.1998.43.4.0647>

Cole, J. J., Caraco, N. F., Kling, G. W., & Kratz, T. K. (1994). Carbon dioxide supersaturation in

the surface waters of lakes. *Science-AAAS-Weekly Paper Edition*, 265(5178), 1568–

1569.

Cole, J. J., Carpenter, S. R., Pace, M. L., Bogert, M. C. Van De, Kitchell, J. L., & Hodgson, J. R.

- (2006). Differential support of lake food webs by three types of terrestrial organic carbon. *Ecology Letters*, 9, 558–568.
- Cole, J. J., Pace, M. L., Carpenter, S. R., & Kitchell, J. F. (2000). Persistence of net heterotrophy in lakes during nutrient addition and food web manipulations. *Limnology and Oceanography*, 45(8), 1718–1730. <https://doi.org/10.4319/lo.2000.45.8.1718>
- Cole, J. J., Pace, M. L., Carpenter, S. R., Kitchell, J. F., & Cole, J. (2000). Persistence of net heterotrophy nutrient addition and food web manipulations. *Limnology and Oceanography*, 45(8), 1718–1730.
- Coloso, J. J., Cole, J. J., Hanson, P. C., & Pace, M. L. (2008). Depth-integrated, continuous estimates of metabolism in a clear-water lake. *Canadian Journal of Fisheries and Aquatic Sciences*, 65(4), 712–722. <https://doi.org/10.1139/f08-006>
- Courtois, S., Steinmann, D., Cajon, A., & Van Der Linden, L. (2017). Continuous monitoring of cyanobacterial blooms: benefits and conditions for using fluorescence probes. *Revue Des Sciences de l'Eau*, 30(2), 149–155. <https://doi.org/10.7202/1042923ar>
- Creed, I. F., Bergström, A.-K. K., Trick, C. G., Grimm, N. B., Hessen, D. O., Karlsson, J., ... Weyhenmeyer, G. A. (2018). Global change-driven effects on dissolved organic matter composition: Implications for food webs of northern lakes. *Global Change Biology*, 24(8), 3692–3714. <https://doi.org/10.1111/gcb.14129>
- Creed, I. F., Bergström, A.-K., Trick, C. G., Grimm, N. B., Hessen, D. O., Karlsson, J., ... Weyhenmeyer, G. A. (2018a). Global change-driven effects on dissolved organic matter composition: {Implications} for food webs of northern lakes. *Global Change Biology*. <https://doi.org/10.1111/gcb.14129>
- Creed, I. F., Bergström, A. K., Trick, C. G., Grimm, N. B., Hessen, D. O., Karlsson, J., ...

- Weyhenmeyer, G. A. (2018b). Global change-driven effects on dissolved organic matter composition: Implications for food webs of northern lakes. *Global Change Biology*.
<https://doi.org/10.1111/gcb.14129>
- Creed, I. F., Trick, C. G., Grimm, N. B., Hessen, D. O., Kidd, K. A., Kritzberg, E., ...
 Weyhenmeyer, G. A. (2018). Global change-driven effects on dissolved organic matter composition: Implications for food webs of northern lakes. *Global Change Biology*,
 24(8), 3692–3714. <https://doi.org/10.1111/gcb.14129>
- Daggett, C., Saros, J., Lafrancois, B., Simon, K., & Amirbahman, A. (2015). Effects of increased concentrations of inorganic nitrogen and dissolved organic matter on phytoplankton in boreal lakes with differing nutrient limitation patterns, 77.
<https://doi.org/10.1007/s00027-015-0396-5>
- Daggett, C. T., Saros, J. E., Lafrancois, B. M., Simon, K. S., & Amirbahman, A. (2015). Effects of increased concentrations of inorganic nitrogen and dissolved organic matter on phytoplankton in boreal lakes with differing nutrient limitation patterns. *Aquatic Sciences*, 77(3), 511–521. <https://doi.org/10.1007/s00027-015-0396-5>
- Davies, John-mark Mark, Hesslein, R. H., Kelly, C. A., & Hecky, R. E. (2003). PCO₂ method for measuring photosynthesis and respiration in freshwater lakes. *Journal of Plankton Research*, 25(4), 385–395. <https://doi.org/10.1093/plankt/25.4.385>
- Davies, John Mark. (1997). *An investigation and evaluation of photosynthetic and respiratory measurements as determined from pCO₂ changes of incubated culture and lake water samples*. University of Manitoba.
- del Giorgio, P. A., & Peters, R. H. (1993). The influence of DOC on the bacteria-chlorophyll relationship in lakes. *SIL Proceedings, 1922-2010*, 25(1), 359–362.

<https://doi.org/10.1080/03680770.1992.11900134>

del Giorgio, P. A., & Peters, R. H. (1994). Patterns in planktonic P:R ratios in lakes: Influence of lake trophic and dissolved organic carbon. *Limnology and Oceanography*, 39(4), 772–787. <https://doi.org/10.4319/lo.1994.39.4.0772>

Del Giorgio, P. A., & Peters, R. H. (1994). Patterns in planktonic P:R Ratios in lakes: influence of lake trophic and dissolved organic carbon. *Limnology and Oceanography*, 39(4), 772–787. Retrieved from <https://aslopubs.onlinelibrary.wiley.com/doi/pdf/10.4319/lo.1994.39.4.0772>

Dickson, A. G. (1990). Standard potential of the reaction: $\text{AgCl(s)} + 12\text{H}_2\text{(g)} = \text{Ag(s)} + \text{HCl(aq)}$, and the standard acidity constant of the ion HSO_4^- in synthetic sea water from 273.15 to 318.15 K. *The Journal of Chemical Thermodynamics*, 22(2), 113–127. [https://doi.org/10.1016/0021-9614\(90\)90074-Z](https://doi.org/10.1016/0021-9614(90)90074-Z)

Dillon, P. J., & Molot, L. A. (1997). Dissolved organic and inorganic carbon mass balances in central Ontario lakes. *Biogeochemistry*, 36(1), 29–42. <https://doi.org/10.1023/A:1005731828660>

Dubourg, P., North, R. L., Hunter, K., Vandergucht, D. M., Abirhire, O., Silsbe, G. M., ... Hudson, J. J. (2015). Light and nutrient co-limitation of phytoplankton communities in a large reservoir: Lake Diefenbaker, Saskatchewan, Canada. *Journal of Great Lakes Research*, 41, 129–143. <https://doi.org/10.1016/j.jglr.2015.10.001>

Emmerton, C. A., Beaty, K. G., Casson, N. J., Graydon, J. A., Hesslein, R. H., Higgins, S. N., ... Tardif, J. C. (2018). Long-Term Responses of Nutrient Budgets to Concurrent Climate-Related Stressors in a Boreal Watershed. *Ecosystems*, 1–16. <https://doi.org/10.1007/s10021-018-0276-7>

- Evans, C. D., Monteith, D. T., & Cooper, D. M. (2005). Long-term increases in surface water dissolved organic carbon: observations, possible causes and environmental impacts. *Environmental Pollution*, 137(1), 55–71.
- Evans, Chapman, Clark, Monteith, & Cresser, M. S. (2006). Alternative explanations for rising dissolved organic carbon export from organic soils. *Global Change Biology*, 12(11), 2044–2053. <https://doi.org/10.1111/j.1365-2486.2006.01241.x>
- Fee, E. J. (1973). Modelling Primary Production in Water Bodies: A Numerical Approach that Allows Vertical Inhomogeneities. *Journal of the Fisheries Research Board of Canada*, 30(10), 1469–1473. <https://doi.org/10.1139/f73-236>
- Fee, E. J. (1980). Important Factors for Estimating Annual Phytoplankton Production in the Experimental Lakes Area. *Canadian Journal of Fisheries and Aquatic Sciences*, 37(3), 513–522. <https://doi.org/10.1139/f80-066>
- Fee, E. J., Hecky, R. E., Kasian, S. E. M., & Cruikshank, D. R. (1996). Effects of lake size, water clarity, and climatic variability on mixing depths in Canadian Shield lakes. *Limnology and Oceanography*, 41(5), 912–920. <https://doi.org/10.4319/lo.1996.41.5.0912>
- Fee, J. E. (1976). The Vertical and Seasonal Distribution of Chlorophyll in Lakes of the Experimental Lakes. *Limnology and Oceanography*, 21(6), 767–783. Retrieved from <https://aslopubs.onlinelibrary.wiley.com/doi/pdf/10.4319/lo.1976.21.6.0767>
- Feuchtmayr, H., Pottinger, T. G., Moore, A., De Ville, M. M., Caillouet, L., Carter, H. T., ... Maberly, S. C. (2019). Effects of brownification and warming on algal blooms, metabolism and higher trophic levels in productive shallow lake mesocosms. *Science of the Total Environment*, 678, 227–238. <https://doi.org/10.1016/j.scitotenv.2019.04.105>

- Findlay, D. L., Hecky, R. E., Hendzel, L. L., Stainton, M. P., & Regehr, G. W. (1994). Relationship between N₂-fixation and heterocyst abundance and its relevance to the nitrogen budget of Lake 227. *Canadian Journal of Fisheries and Aquatic Sciences*, 51(10), 2254–2266. <https://doi.org/10.1139/f94-229>
- Findlay, D. L., & Kling, H. J. (1979). A species list and pictorial reference to the phytoplankton species of central and northern Canada. Parts I and II. *Can. Fish. Mar. Serv. Manuscript Rep.*, 1503, 619p. Retrieved from <https://mspace.lib.umanitoba.ca/handle/1993/30398>
- Gelda, R. K., & Effler, S. W. (2002). Metabolic rate estimates for a eutrophic lake from diel dissolved oxygen signals. *Hydrobiologia*, 485(1), 51–66. Retrieved from <http://www.springerlink.com/index/UG31554524433106.pdf>
- Giling, D. P., Staehr, P. A., Grossart, H. P., Andersen, M. R., Bohrer, B., Escot, C., ... Obrador, B. (2017). Delving deeper: Metabolic processes in the metalimnion of stratified lakes. *Limnology and Oceanography*, 62(3), 1288–1306. <https://doi.org/10.1002/lno.10504>
- Graham, L. E., Graham, J. M., & Wilcox, L. W. (2009). *Algae*. Pearson. Retrieved from <https://dl.uswr.ac.ir/handle/10026.1/6775>
- Guildford, S. J., & Hecky, R. E. (2000). Total nitrogen, total phosphorus, and nutrient limitation in lakes and oceans: {Is} there a common relationship? *Limnology and Oceanography*, 45(6), 1213–1223. <https://doi.org/10.4319/lo.2000.45.6.1213>
- Hall, B. D., Hesslein, R. H., Emmerton, C. A., Higgins, S. N., Ramlal, P., & Paterson, M. J. (2018). Multi-decadal carbon sequestration in a headwater boreal lake., (1), 1–16. <https://doi.org/10.1002/lno.11060>
- Hanson, P. C., Bade, D. L., Carpenter, S. R., Kratz, T. K., & May, N. (2003). Lake Metabolism:

- Relationships with dissolved organic carbon and phosphorus. *Limnology and Oceanography*, 48(3), 1112–1119.
- Hanson, P. C., Carpenter, S. R., Kimura, N., Wu, C., Cornelius, S. P., & Kratz, T. K. (2008). Evaluation of metabolism models for free-water dissolved oxygen methods in lakes. *Limnol. Oceanogr: Methods*, 6, 454–465. Retrieved from <http://lter.limnology.wisc.edu>.
- Hessen, D. O. (1992). *Dissolved Organic Matter in Lacustrine Ecosystems: Energy Source and System Regulator. Hydrobiologia* (Vol. 229). Retrieved from <https://link-springer-com.uml.idm.oclc.org/content/pdf/10.1007/BF00006995.pdf>
- Hessen, D. O., Håll, J. P., Thrane, J. E., & Andersen, T. (2017). Coupling dissolved organic carbon, CO₂ and productivity in boreal lakes. *Freshwater Biology*, 62(5), 945–953. <https://doi.org/10.1111/fwb.12914>
- Hotchkiss, E. R., Sadro, S., & Hanson, P. C. (2018). Toward a more integrative perspective on carbon metabolism across lentic and lotic inland waters. *Limnology and Oceanography Letters*, 3(3), 57–63. <https://doi.org/10.1002/lol2.10081>
- Jähne, B., Heinz, G., & Dietrich, W. (1987). Measurement of the diffusion coefficients of sparingly soluble gases in water. *Journal of Geophysical Research: Oceans*, 92(C10), 10767–10776. <https://doi.org/10.1029/JC092iC10p10767>
- Jansson, M., Bergström, A.-K., Blomqvist, P., & Drakare, S. (2000). Allochthonous organic carbon and phytoplankton/bacterioplankton production relationships in lakes. *Ecology*, 81(11), 3250–3255. [https://doi.org/10.1890/0012-9658\(2000\)081\[3250:AOCAPB\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2000)081[3250:AOCAPB]2.0.CO;2)
- Jansson, M., Hickler, T., Jonsson, A., & Karlsson, J. (2008). Links between terrestrial primary

- production and bacterial production and respiration in lakes in a climate gradient in {Subarctic} {Sweden}. *Ecosystems*, 11(3), 367–376. <https://doi.org/10.1007/s10021-008-9127-2>
- Jassby, A. D., & Platt, T. (1976a). Mathematical formulation of the relationship between photosynthesis and light for phytoplankton. *Limnology and Oceanography*, 21(4), 540–547.
- Jassby, A. D., & Platt, T. (1976b). Mathematical forumulation of the relationship between photosynthesis and light. *Limnology and Oceanography*, 21(July).
- Jodłowska, S., & Śliwińska, S. (2014). Effects of light intensity and temperature on the photosynthetic irradiance response curves and chlorophyll fluorescence in three picocyanobacterial strains of *Synechococcus*. *Photosynthetica*, 52(2), 223–232. <https://doi.org/10.1007/s11099-014-0024-y>
- Jones, R. I. (1992). The influence of humic substances on lacustrine planktonic food chains. *Hydrobiologia*, 229(1), 73–91. <https://doi.org/10.1007/BF00006992>
- Karlsson, J., & Byström, P. (2005). Littoral energy mobilization dominates energy supply for top consumers in subarctic lakes. *Limnology and Oceanography*, 50(2), 538–543. <https://doi.org/10.4319/lo.2005.50.2.0538>
- Karlsson, J., Byström, P., Ask, J., Ask, P., Persson, L., & Jansson, M. (2009). Light limitation of nutrient-poor lake ecosystems. *Nature*, 460(7254), 506–509. <https://doi.org/10.1038/nature08179>
- Kelly, P. T., Solomon, C. T., Weidel, B. C., & Jones, S. E. (2014). Terrestrial carbon is a resource, but not a subsidy, for lake zooplankton. *Ecology*, 95(5), 1236–1242. <https://doi.org/10.1890/13-1586.1>

- Kelly, P. T., Solomon, C. T., Zwart, J. A., & Jones, S. E. (2018). A Framework for Understanding Variation in Pelagic Gross Primary Production of Lake Ecosystems. *Ecosystems*, 21(7), 1364–1376. <https://doi.org/10.1007/s10021-018-0226-4>
- Kissman, C. E. H., Williamson, C. E., Rose, K. C., & Saros, J. E. (2013). Response of phytoplankton in an alpine lake to inputs of dissolved organic matter through nutrient enrichment and trophic forcing. *Limnology and Oceanography*, 58(3), 867–880. <https://doi.org/10.4319/lo.2013.58.3.0867>
- Klug, J. L. (2002). Positive and negative effects of allochthonous dissolved organic matter and inorganic nutrients on phytoplankton growth. *Canadian Journal of Fisheries and Aquatic Sciences*, 59(1), 85–95. <https://doi.org/10.1139/f01-194>
- Knapp, C. W., Graham, D. W., Steedman, R. J., & DeNoyelles, F. (2003). Deep chlorophyll maxima in small boreal forest lakes after experimental catchment and shoreline logging. *Boreal Environment Research*, 8(1), 9–18.
- Köhler, J., Wang, L., Guislain, A., & Shatwell, T. (2018). Influence of vertical mixing on light-dependency of phytoplankton growth. *Limnology and Oceanography*, 63(3), 1156–1167. <https://doi.org/10.1002/lno.10761>
- Kritzberg, E. S., & Ekström, S. M. (2012). Increasing iron concentrations in surface waters - A factor behind brownification? *Biogeosciences*, 9(4), 1465–1478. <https://doi.org/10.5194/bg-9-1465-2012>
- Kunz, T. J., & Diehl, S. (2003). Phytoplankton, light and nutrients along a gradient of mixing depth: A field test of producer-resource theory. *Freshwater Biology*, 48(6), 1050–1063. <https://doi.org/10.1046/j.1365-2427.2003.01065.x>
- Lapierre, J. F., Seekell, D. A., & del Giorgio, P. A. (2015). Climate and landscape influence on

- indicators of lake carbon cycling through spatial patterns in dissolved organic carbon. *Global Change Biology*, 21(12), 4425–4435. <https://doi.org/10.1111/gcb.13031>
- Leach, T. H., Beisner, B. E., Carey, C. C., Pernica, P., Rose, K. C., Huot, Y., ... Verburg, P. (2018). Patterns and drivers of deep chlorophyll maxima structure in 100 lakes: The relative importance of light and thermal stratification. *Limnology and Oceanography*, 63(2), 628–646. <https://doi.org/10.1002/lno.10656>
- Leenheer, J. A., & Croué, J.-P. (2003). *Peer reviewed: characterizing aquatic dissolved organic matter*. ACS Publications.
- Lepistö, A., Futter, M. N., & Kortelainen, P. (2014). Almost 50 years of monitoring shows that climate, not forestry, controls long-term organic carbon fluxes in a large boreal watershed. *Global Change Biology*, 20(4), 1225–1237. <https://doi.org/10.1111/gcb.12491>
- Lewis, E., & Wallace, D. W. R. (1998). CO2SYS-Program developed for the CO2 system calculations. Carbon Dioxide Inf Anal Center Report ORNL/CDIAC-105.
- Lindell, M. J., Granéli, H. W., & Bertilsson, S. (2000). Seasonal photoreactivity of dissolved organic matter from lakes with contrasting humic content. *Canadian Journal of Fisheries and Aquatic Sciences*, 57(5), 875–885. <https://doi.org/10.1139/f00-016>
- Mari, X., Rochelle-Newall, E., Torréton, J. P., Pringault, O., Jouon, A., & Migon, C. (2007). Water residence time: A regulatory factor of the DOM to POM transfer efficiency. *Limnology and Oceanography*, 52(2), 808–819. <https://doi.org/10.4319/lo.2007.52.2.0808>
- McCallister, S. L., & del Giorgio, P. A. (2012). Evidence for the respiration of ancient terrestrial organic {C} in northern temperate lakes and streams. *Proceedings of the*

- National Academy of Sciences*, 109(42), 16963–16968.
- Millero, F. (1979). The thermodynamics of the carbonate system in seawater. *Geochimica Et Cosmochimica Acta*, 43(1), 1651–1661. [https://doi.org/10.1016/0016-7037\(79\)90184-4](https://doi.org/10.1016/0016-7037(79)90184-4)
- Molot, L. A., & Dillon, P. J. (1996). Storage of terrestrial carbon in boreal lake sediments and evasion to the atmosphere. *Global Biogeochemical Cycles*, 10(3), 483–492. <https://doi.org/10.1029/96GB01666>
- Molot, L. A., & Dillon, P. J. (1997). Photolytic regulation of dissolved organic carbon in northern lakes. *Global Biogeochemical Cycles*, 11(3), 357–365. <https://doi.org/10.1029/97GB01198>
- Monteith, D. T., Stoddard, J. L., Evans, C. D., De Wit, H. A., Forsius, M., Høgåsen, T., ... Vesely, J. (2007). Dissolved organic carbon trends resulting from changes in atmospheric deposition chemistry. *Nature*, 450(7169), 537–540. <https://doi.org/10.1038/nature06316>
- Moran, M. A., & Zepp, R. G. (1997). Role of photoreactions in the formation of biologically labile compounds from dissolved organic matter. *Limnology and Oceanography*, 42(6), 1307–1316. <https://doi.org/10.4319/lo.1997.42.6.1307>
- Morán, X. A. G., & Estrada, M. (2001). Short-term variability of photosynthetic parameters and particulate and dissolved primary production in the Alboran sea (SW Mediterranean). *Marine Ecology Progress Series*, 212, 53–67. <https://doi.org/10.3354/meps212053>
- Odum, H. T. (1956). Primary production in flowing waters. *Limnology and Oceanography*, 1(2), 102–117. <https://doi.org/10.4319/lo.1956.1.2.0102>

- Pace, M. L., & Prairie, Y. T. (2005). Respiration in lakes. In *Respiration in Aquatic Ecosystems* (pp. 103–122).
<https://doi.org/10.1093/acprof:oso/9780198527084.003.0006>
- Prairie, Y. T., Bird, D. F., & Cole, J. J. (2002). The summer metabolic balance in the epilimnion of southeastern Quebec lakes. *Limnology and Oceanography*, 47(1), 316–321. <https://doi.org/10.4319/lo.2002.47.1.0316>
- Raymond, P. A., & Saiers, J. E. (2010). Event controlled DOC export from forested watersheds. *Biogeochemistry*, 100(1), 197–209. <https://doi.org/10.1007/s10533-010-9416-7>
- Read, J. S., Hamilton, D. P., Desai, A. R., Rose, K. C., MacIntyre, S., Lenters, J. D., ... Wu, C. H. (2012). Lake-size dependency of wind shear and convection as controls on gas exchange. *Geophysical Research Letters*, 39(9), 9405.
<https://doi.org/10.1029/2012GL051886>
- Sakshaug, E., Bricaud, A., Dandonneau, Y., Falkowski, P. G., Kiefer, D. A., Legendre, L., ... Takahashi, M. (1997). Parameters of photosynthesis: Definitions, theory and interpretation of results. *Journal of Plankton Research*, 19(11), 1637–1670.
<https://doi.org/10.1093/plankt/19.11.1637>
- Sargent, J., Bell, G., McEvoy, L., Tocher, D., & Estevez, A. (1999). Recent developments in the essential fatty acid nutrition of fish. *Aquaculture*, 177(1–4), 191–199.
[https://doi.org/10.1016/S0044-8486\(99\)00083-6](https://doi.org/10.1016/S0044-8486(99)00083-6)
- Schindler, D. W., Beaty, K. G., Fee, E. J., Cruikshank, D. R., DeBruyn, E. R., Findlay, D. L., ... Turner, M. A. (1990). Effects of climatic warming on lakes of the central boreal forest. *Science*, 250(4983), 967–970. <https://doi.org/10.1126/science.250.4983.967>

- Schindler, D. W., Fee, E. J., & Ruszczynski, T. (1978). Phosphorous input and its consequences for phytoplankton standing crop and production in the experimental lakes area and in similar lakes. *J. FISH. RES. BOARD CANADA*, 35(2), 190–196.
<https://doi.org/10.1139/f78-031>
- Schindler, D W, Schmidt, R. V, & Reid, R. A. (1972). Acidification and bubbling as an alternative to filtration in determining phytoplankton production by the ^{14}C method. *Journal of the Fisheries Board of Canada*, 29(11), 1627–1631.
- Schindler, David W. (1977). Evolution of phosphorus limitation in lakes. *Science*, 195(4275), 260–262.
- Schindler, David W, Curtis, P. J., Bayley, S. E., Parker, B. R., Beaty, K. G., & Stainton, M. P. (1997). Climate-induced changes in the dissolved organic carbon budgets of boreal lakes. *Biogeochemistry*, 36(1), 9–28. <https://doi.org/10.1023/A:1005792014547>
- Seekell, D. A., Lapierre, J. F., Ask, J., Bergstrom, A. K., Deininger, A., Rodriguez, P., & Karlsson, J. (2015). The influence of dissolved organic carbon on primary production in northern lakes. *Limnology and Oceanography*, 60(4), 1276–1285.
<https://doi.org/10.1002/lno.10096>
- Seekell, D. A., Lapierre, J., & Karlsson, J. (2015). Trade-offs between light and nutrient availability across gradients of dissolved organic carbon concentration in Swedish lakes: implications for patterns in primary production. *Canadian Journal of Fisheries and Aquatic Sciences*, 72(11), 1663–1671. <https://doi.org/10.1139/cjfas-2015-0187>
- Sobek, S., Tranvik, L. J., & Cole, J. J. (2005). Temperature independence of carbon dioxide supersaturation in global lakes. *Global Biogeochemical Cycles*, 19(2), 1–10.
<https://doi.org/10.1029/2004GB002264>

- Sobek, S., Tranvik, L. J., Prairie, Y. T., Kortelainen, P., & Cole, J. J. (2007). Patterns and regulation of dissolved organic carbon: An analysis of 7,500 widely distributed lakes. *Limnology and Oceanography*, 52(3), 1208–1219.
<https://doi.org/10.4319/lo.2007.52.3.1208>
- Solomon, C. T., Jones, S. E., Weidel, B. C., Buffam, I., Fork, M. L., Karlsson, J., ... Saros, J. E. (2015). Ecosystem Consequences of Changing Inputs of Terrestrial Dissolved Organic Matter to Lakes: Current Knowledge and Future Challenges. *Ecosystems*, 18(3), 376–389. <https://doi.org/10.1007/s10021-015-9848-y>
- Staehr, P. A., Bade, D., Bogert, M. C. Van De, Koch, G. R., Williamson, C., Hanson, P., ... Kratz, T. (2010). Lake metabolism and the diel oxygen technique: State of the science. *Limnol. Oceanogr: Methods*, 8, 628–644. <https://doi.org/10.4319/lom.2010.8.628>
- Stojanovic, T., Pinter, G., Lauko, I., & Myers, N. (2019). Parameter identification and sensitivity analysis for a phytoplankton competition model. *Quarterly of Applied Mathematics*, 77(1), 1–18.
- Swinehart, D. F. (1962). The Beer-Lambert law. *Journal of Chemical Education*. Division of Chemical Education . <https://doi.org/10.1021/ed039p333>
- Team, R. C. (2017). R: A language and environment for statistical computing. Vienna, Austria. Retrieved from
<ftp://ftp.uvigo.es/CRAN/web/packages/dplR/vignettes/intro-dplR.pdf>
- Tobias, C. R., Böhlke, J. K., & Harvey, J. W. (2007). The oxygen-18 isotope approach for measuring aquatic metabolism in high productivity waters. *Limnology and Oceanography*, 52(4), 1439–1453. <https://doi.org/10.4319/lo.2007.52.4.1439>
- Tonin, J. (2019). *The effects of dissolved organic carbon on pathways of energy flow*,

resource availability, and consumer biomass in nutrient-poor boreal lakes. University of Manitoba.

Tranvik, L. (1992). Allochthonous dissolved organic matter as an energy source for pelagic bacteria and the concept of the microbial loop. In *Dissolved Organic Matter in Lacustrine Ecosystems: Energy Source and System Regulator* (pp. 107–114).

Dordrecht: Springer Netherlands. https://doi.org/10.1007/978-94-011-2474-4_8

Tranvik, L., Downing, J. A., Cotner, J. B., Loiselle, S. A., Striegl, R. G., Ballatore, T. J., ... Knoll, L. B. (2009). Lakes and reservoirs as regulators of carbon cycling and climate. *Limnology and Oceanography*, 54(6part2), 2298–2314.

Tranvik, L. J. (1988). Availability of dissolved organic carbon for planktonic bacteria in oligotrophic lakes of differing humic content. *Microbial Ecology*, 16(3), 311–322. <https://doi.org/10.1007/BF02011702>

Tranvik, L. J., Olofsson, H., & Bertilsson, S. (1999). Photochemical Effects on Bacterial Degradation of Dissolved Organic Matter in Lake Water. *Proceedings of the 8th International Symposium on Microbial Ecology*, 1–8. Retrieved from [papers3://publication/uuid/0C413F04-3D8D-4D66-95D8-677540A05BC9](https://doi.org/10.1007/978-94-011-2474-4_8)

Vadeboncoeur, Y., Jeppesen, E., Vander Zanden, M. J., Schierup, H. H., Christoffersen, K., & Lodge, D. M. (2003). From Greenland to green lakes: Cultural eutrophication and the loss of benthic pathways in lakes. *Limnology and Oceanography*, 48(4), 1408–1418. <https://doi.org/10.4319/lo.2003.48.4.1408>

Vadeboncoeur, Y., Lodge, D. M., & Carpenter, S. R. (2001). Whole-lake fertilization effects on distribution of primary production between benthic and pelagic habitats. *Ecology*, 82(4), 1065–1077. Retrieved from

[http://onlinelibrary.wiley.com/doi/10.1890/0012-9658\(2001\)082\[1065:WLFEOD\]2.0.CO;2/full](http://onlinelibrary.wiley.com/doi/10.1890/0012-9658(2001)082[1065:WLFEOD]2.0.CO;2/full)

- Vadeboncoeur, Y., Peterson, G., Vander Zanden, M. J., & Kalff, J. (2008). Benthic algal production across lake size gradients: Interactions among morphometry, nutrients, and light. *Ecology*, *89*(9), 2542–2552.
- Vasconcelos, F. R., Diehl, S., Rodriguez, P., Hedstrom, P., Karlsson, J., Bystrom, P., ... Byström, P. (2016). Asymmetrical competition between aquatic primary producers in a warmer and browner world. *Ecology*, *97*(10), 2580–2592. <https://doi.org/10.1002/ecy.1487>
- Vasconcelos, F. R., Diehl, S., Rodríguez, P., Karlsson, J., & Byström, P. (2018). Effects of Terrestrial Organic Matter on Aquatic Primary Production as Mediated by Pelagic–Benthic Resource Fluxes. *Ecosystems*, *21*(6), 1255–1268. <https://doi.org/10.1007/s10021-017-0217-x>
- von Einem, J., & Granéli, W. (2010). Effects of fetch and dissolved organic carbon on epilimnion depth and light climate in small forest lakes in southern Sweden. *Limnology and Oceanography*, *55*(2), 920–930. <https://doi.org/10.4319/lo.2010.55.2.0920>
- Welch, H. E., Dillon, P. J., & Sreedharan, A. (1976). Factors Affecting Winter Respiration in Ontario Lakes. *Journal of the Fisheries Research Board of Canada*, *33*(8), 1809–1815. <https://doi.org/10.1139/f76-232>
- Wetzel, R. G. (2001). *Limnology: lake and river ecosystems*. gulf professional publishing.
- Wilkinson, G. M., Carpenter, S. R., Cole, J. J., Pace, M. L., & Yang, C. (2013). Terrestrial support of pelagic consumers: patterns and variability revealed by a multilake study. *Freshwater Biology*, *58*(10), 2037–2049.

Williamson, C. E., Overholt, E. P., Pilla, R. M., Leach, T. H., Brentrup, J. A., Knoll, L. B., ...

Moeller, R. E. (2015). Ecological consequences of long-term browning in lakes.

Scientific Reports, 5(1), 18666. <https://doi.org/10.1038/srep18666>

Winslow, L. A., Zwart, J. A., Batt, R. D., Dugan, H. A., Iestyn Woolway, R., Corman, J. R., ...

Read, J. S. (2016). LakeMetabolizer: An R package for estimating lake metabolism from free-water oxygen using diverse statistical models. *Inland Waters*, 6(4), 622–636.

<https://doi.org/10.5268/IW-6.4.883>

Winslow, M. L., Winslow, A. L., Read, J., Woolway, R., Brentrup, J., Zwart, J., ... Collinge, D.

(2018). rLakeAnalyzer.

Winslow, M. L., Winslow, A. L., Zwart, J., Batt, R., Corman, J., Dugan, H., ... Woolway, R.

(2016). Package ‘LakeMetabolizer’.

Xylem. (2014). EXO fDOM Assessment. *Xylem News Brief*, NB16, 1–4.

<https://doi.org/10.1101/gr.094052.109>

Zwart, J. A., Craig, N., Kelly, P. T., Sebestyen, S. D., Solomon, C. T., Weidel, B. C., & Jones, S. E.

(2016a). Metabolic and physiochemical responses to a whole-lake experimental increase in dissolved organic carbon in a north-temperate lake. *Limnology and Oceanography*, 61(2), 723–734. <https://doi.org/10.1002/lno.10248>

Zwart, J. A., Craig, N., Kelly, P. T., Sebestyen, S. D., Solomon, C. T., Weidel, B. C., & Jones, S. E.

(2016b). Metabolic and physiochemical responses to a whole-lake experimental increase in dissolved organic carbon in a north-temperate lake. *Limnology and Oceanography*, 61(2). <https://doi.org/10.1002/lno.10248>

Appendix A1: Background information

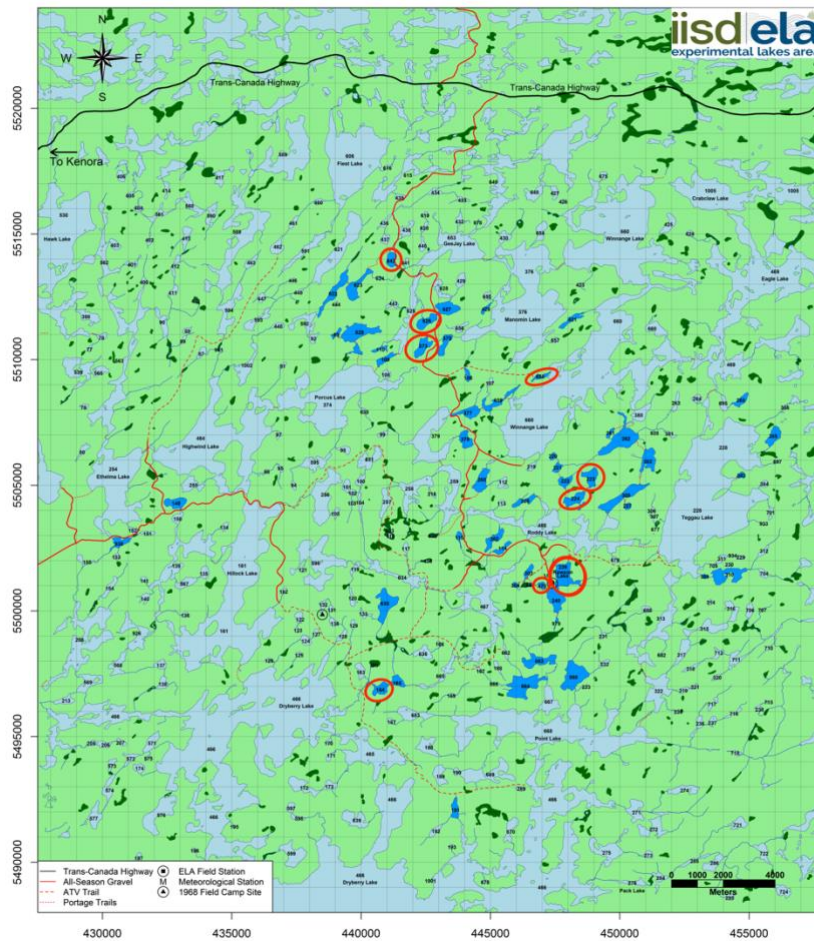


Figure A1.1 Map of IISD-ELA area, including the 9 PHISH lakes in this study circled in red.

A1.2 Light attenuation coefficient and photic depth

Example for L658. Percent light over the open water season in 2018 was averaged per depth and graphed, if R^2 was not close to 1.0 outliers were removed. Light attenuation coefficient is the exponent on the e function (1.03). Photic depth is 4.1 m determined using the calculations in the table below or by inputting 1% light as the y-value to determine x-value.

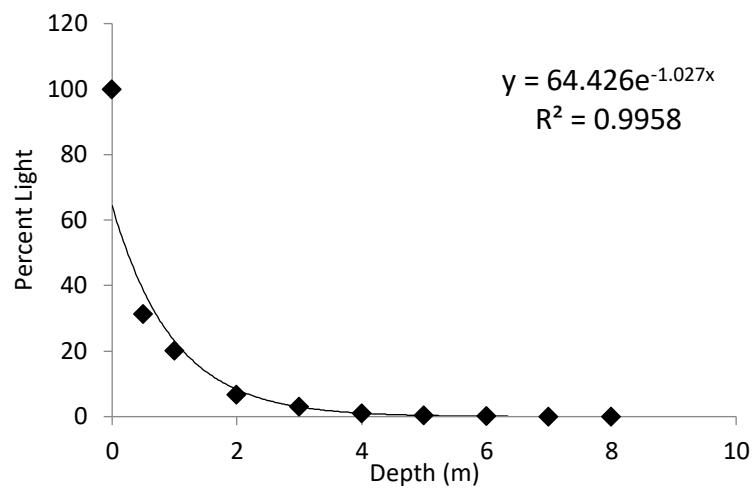


Figure A1.2 Decline in light availability in L658 graphed as percent light with depth.

Table A1.2 Calculation of photic depth (m) for L658.

	Calculation	Value
1/function	1/64.426	0.0155
LN above	ln (0.0155)	-4.165
Depth of photic zone	-4.165/1.027	4.056

Appendix A2: Effects of dissolved organic matter on whole-lake metabolism

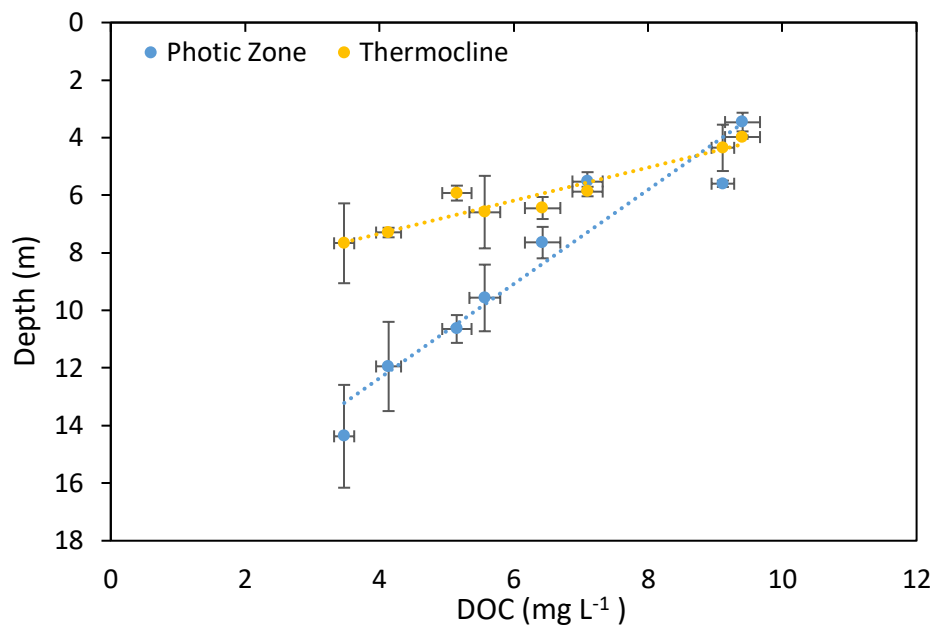


Figure A2.1 Relationships between DOC, photic zone and thermocline depth.

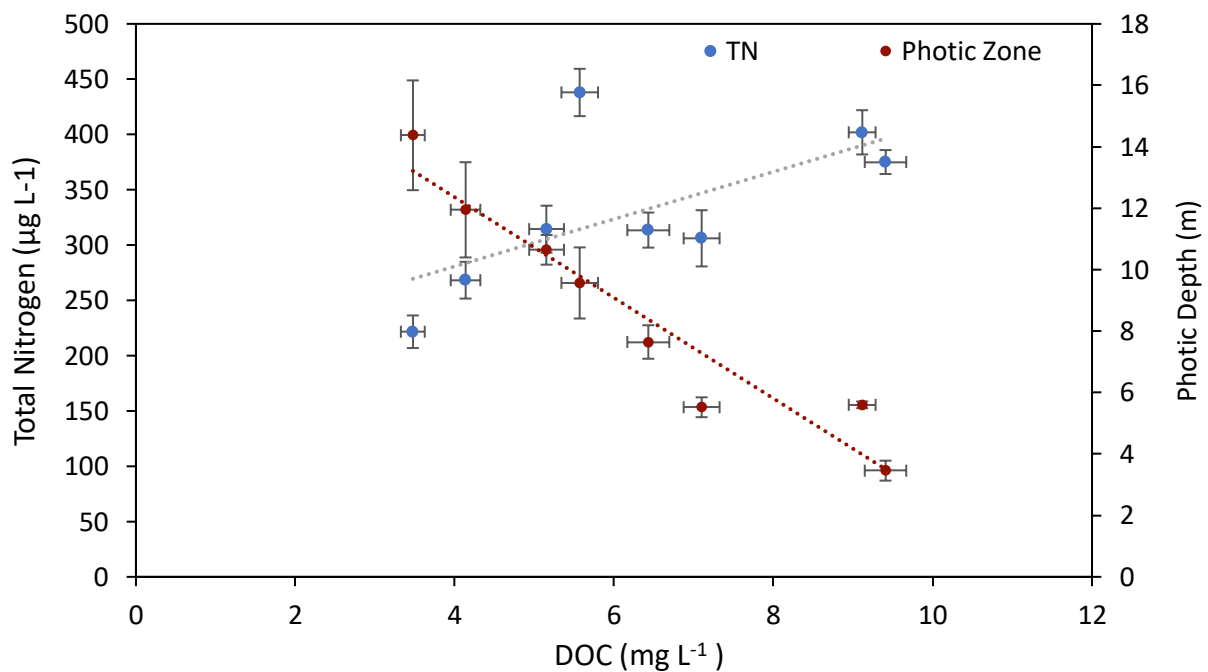


Figure A2.2 Relationships between photic zone and total nitrogen with increasing concentrations of dissolved organic carbon (Photic: $r = 0.98$; $p < 0.001$; TN $r = 0.68$; $p = 0.09$).

A2.3 Biological characteristics

Phytoplankton Biomass and community composition

Phytoplankton biomass (as Chl-*a*) was positively correlated with DOC, increasing by ~2x from the lake with the lowest DOC to the lake with the highest DOC. Phytoplankton biomass from microscopy was not correlated with DOC. The phytoplankton community composition was DOCinated by chrysophytes accounting for 62% of the total phytoplankton biomass in all lakes except L373. In L373 phytoplankton species were more evenly distributed, chrysophytes accounted for 28% and diatoms 34% of the total phytoplankton biomass.

Table A2.3. Phytoplankton community composition determined by microscopy Plankton R Us (Dave Findaly) in July 2018.

Lake	Biomass (mg m ⁻³)						Total
	Cyano-bacteria	Chloro-phyte	Chryso-phyte	Diatoms	Crypto-phytes	Dino-flagellates	
224	15.3	19.5	298	15	7.2	21.4	376
373	35.3	19.2	83.9	67	3.2	9.50	219
626	11.6	25.2	338	8.5	9.0	378	770
223	25.6	6.30	219	22	10	21.5	304
442	12.5	11.1	283	41	10	40.3	398
239	6.10	29.0	177	34	25	48.5	319
164	1.60	8.60	370	10	47	98.5	536
658	20.2	27.9	204	23	33	58.0	366
L470	13.5	8.70	270	31	21	3.80	348

A2.4 Metabolism model

Lake metabolism was determined by including relevant parameters (dissolved oxygen, water temperature, wind speed, irradiance, thermocline depth, and air-water gas exchange) in a model. Each parameter (Tables 2.1-2.4) was uploaded into 'LakeMetabolizer'. Using the governing equation (Equation 2.2), metabolism parameters (NEP, GPP and R) were determined (Table 2.5).

Sample metabolism model parameters

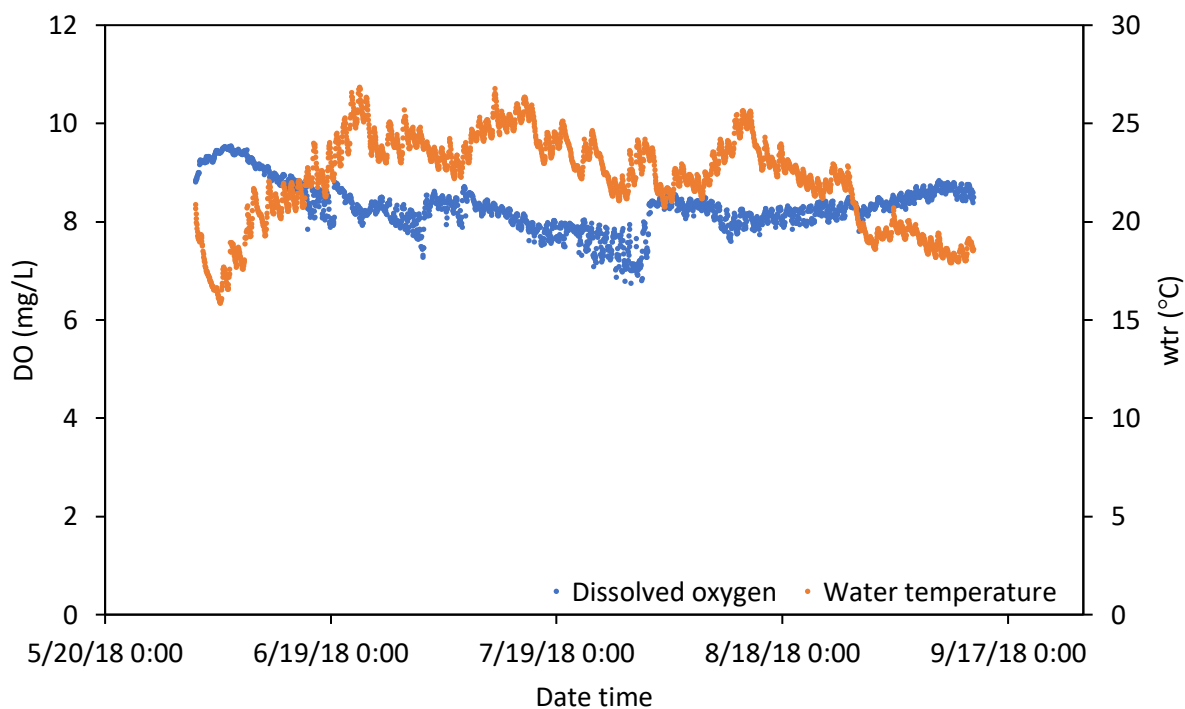


Figure A2.41 Dissolved oxygen (DO) (mg/L) and water temperature (°C) at hourly intervals throughout the open water season May 20th-September 17th, 2018.

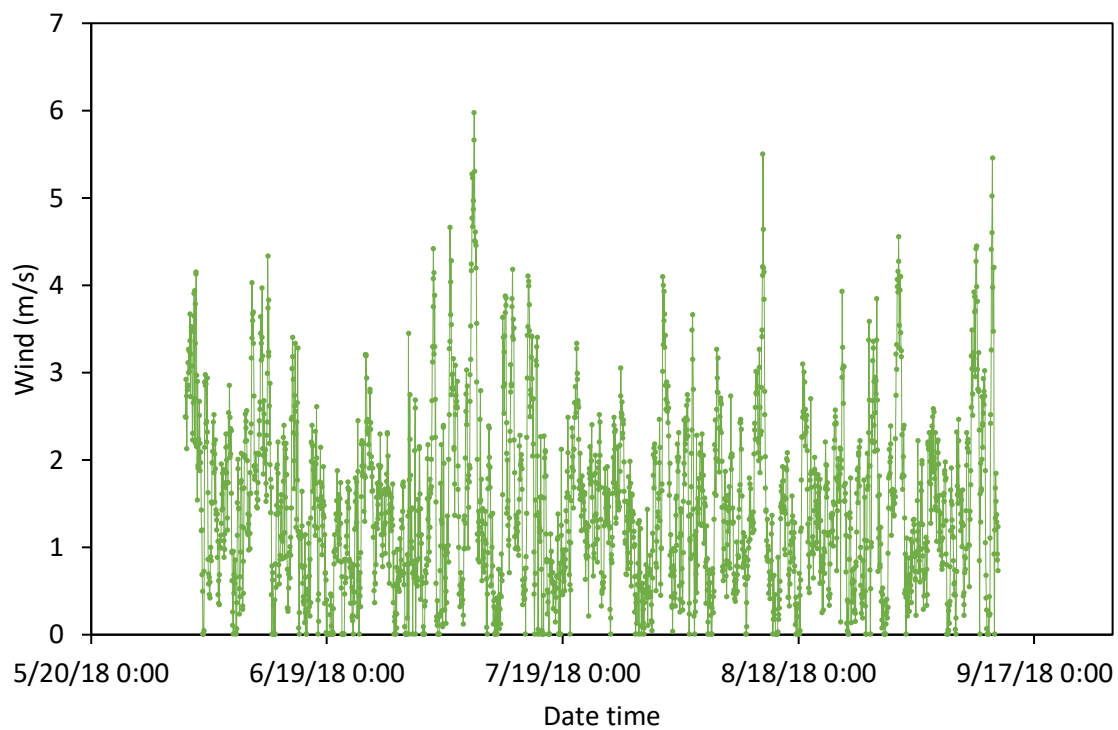


Figure A2.42 Wind (m/s) at hourly intervals throughout the open water season May 20th-
September 17th.

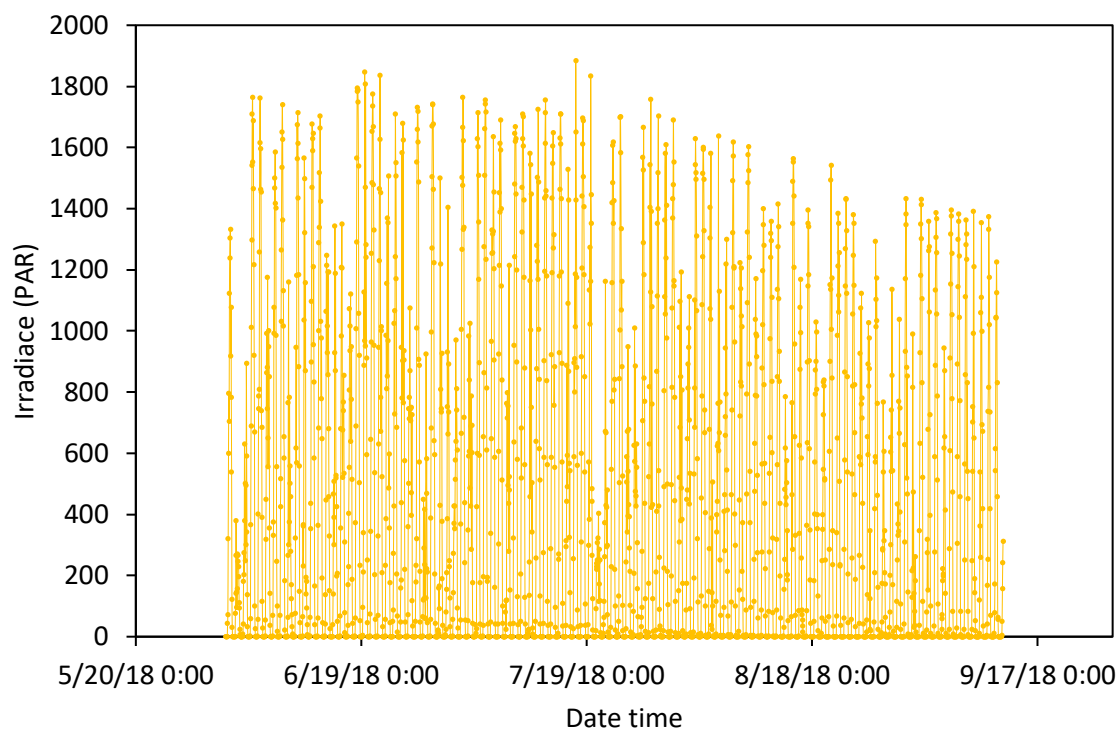


Figure A2.43 Irradiance (PAR) at hourly intervals throughout the open water season May 20th-September 17th.

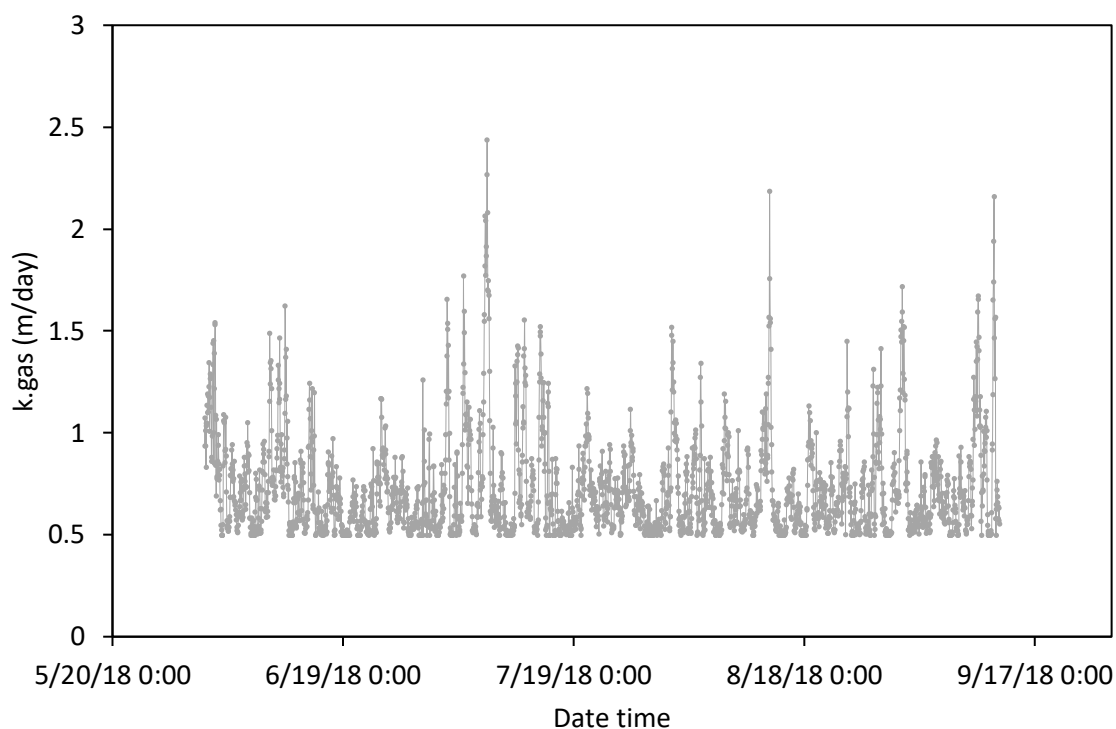


Figure A2.44 Gas exchange coefficient (k.gas) at hourly intervals throughout the open water season May 20th-September 17th.

Daily metabolism values for all lakes (Figures A2.4a-g)

In L224, days 181 to 228 were omitted to remove unrealistic values of negative GPP or positive respiration when physical processes (i.e. wave action and wind) overwhelm the biological changes in DO.

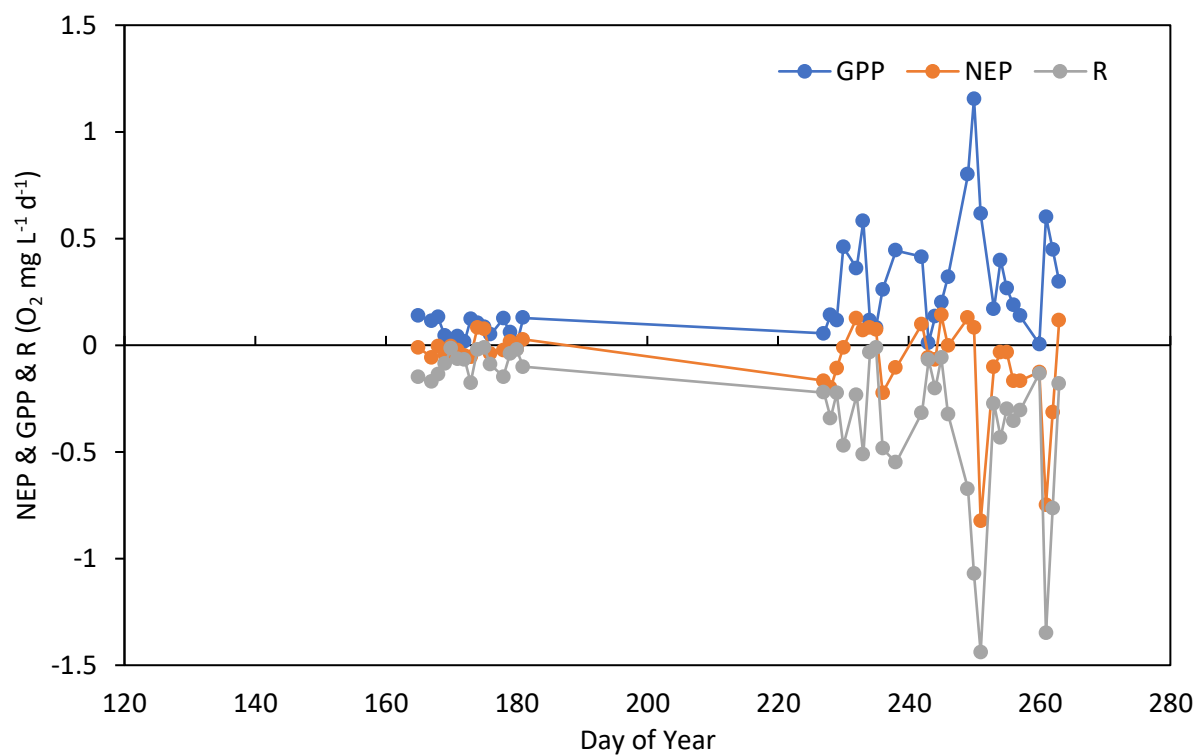


Figure A2.4a Daily metabolism parameters throughout the open water season of L224.

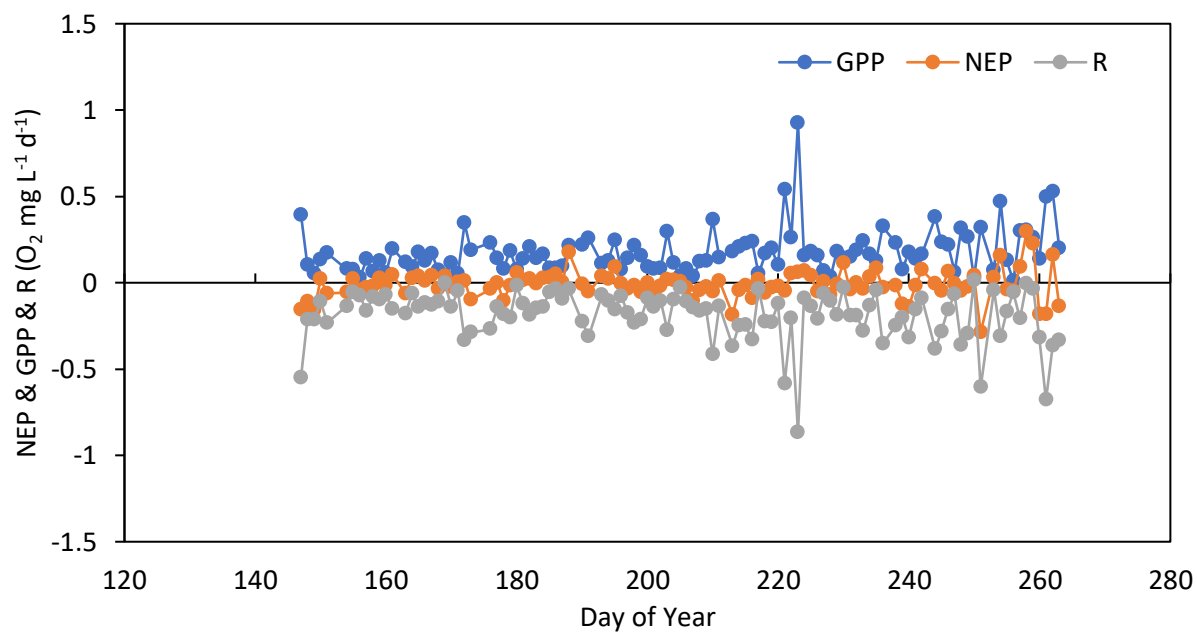


Figure A2.4b Daily metabolism parameters throughout the open water season of L373.

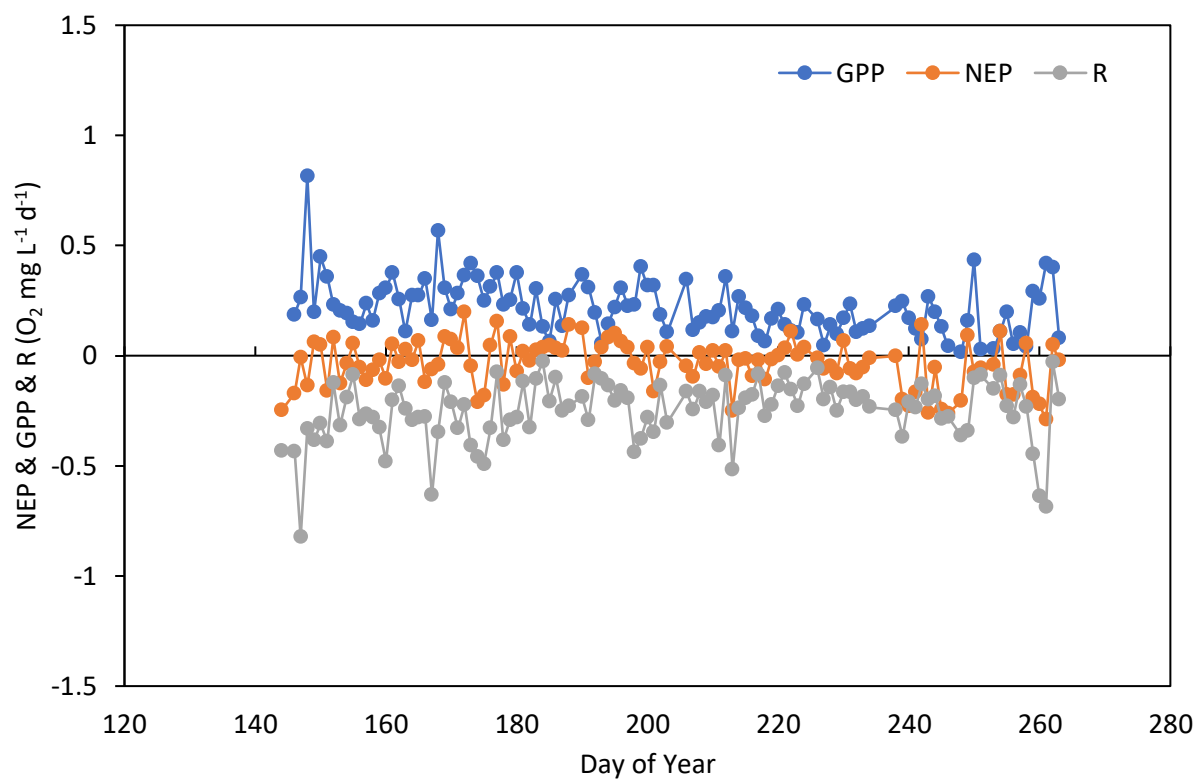


Figure A2.4c Daily metabolism parameters throughout the open water season of L626.

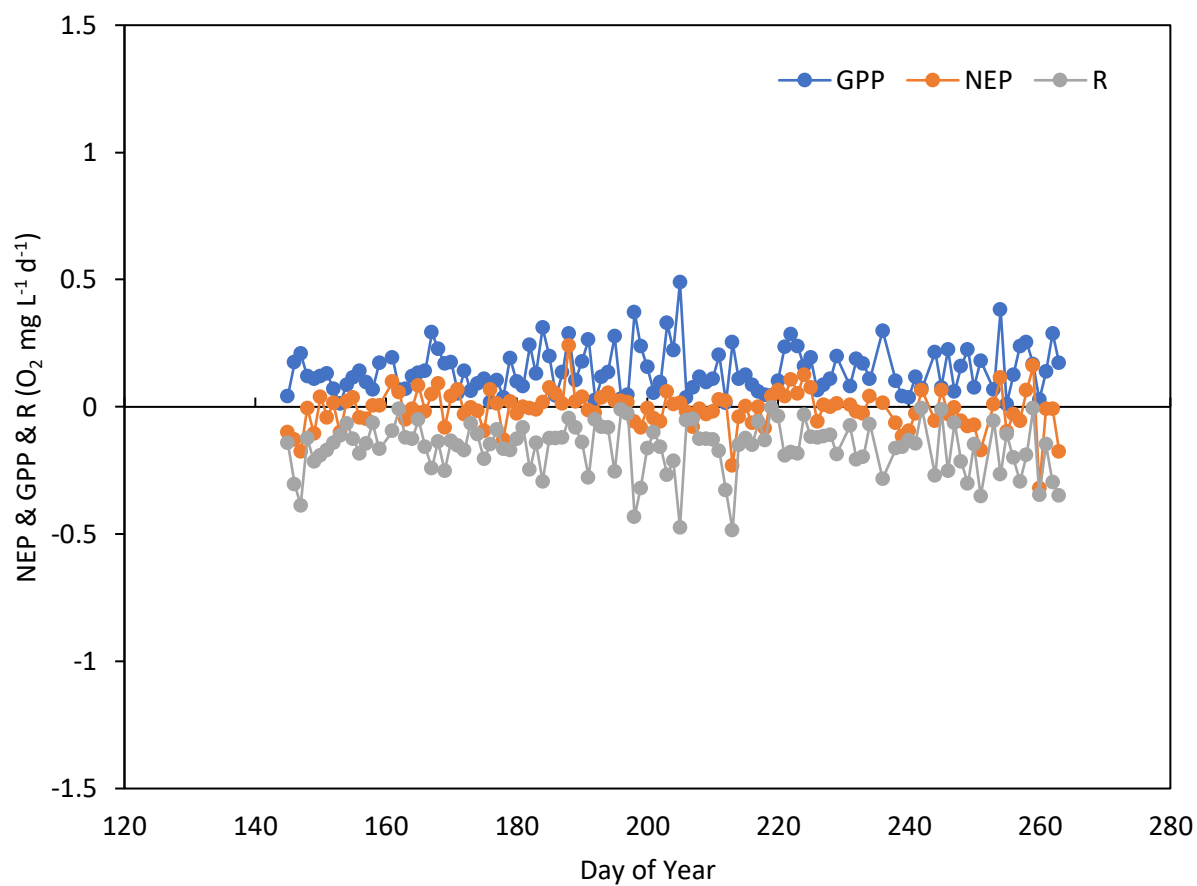


Figure A2.4d Daily metabolism parameters throughout the open water season of L223.

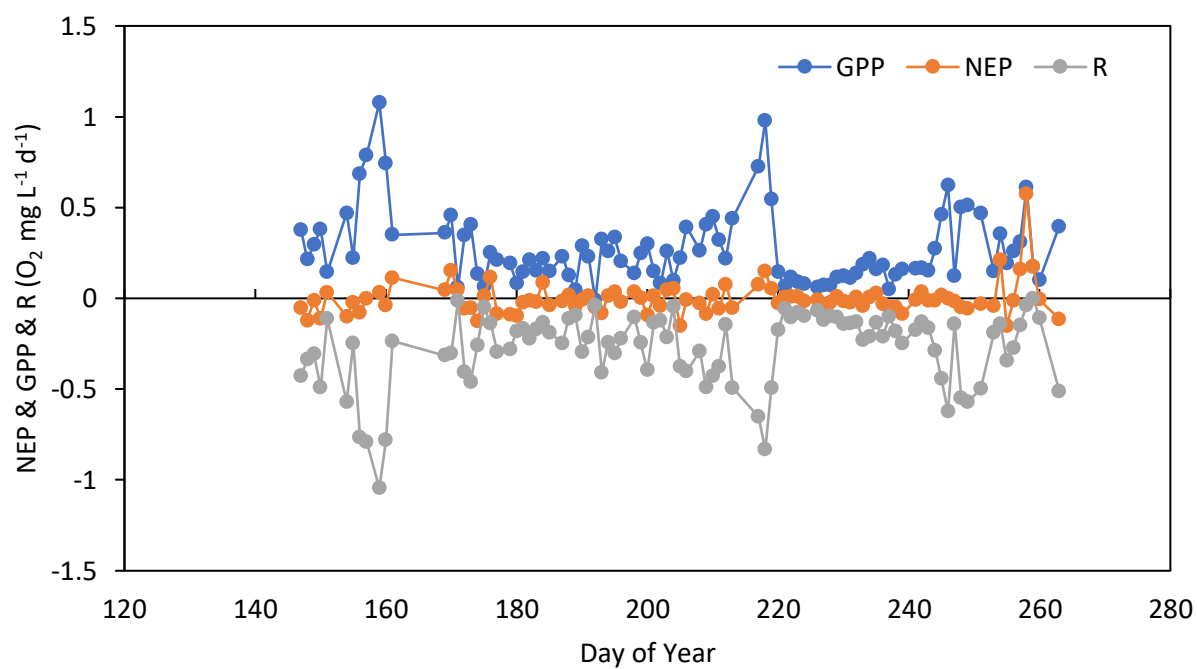


Figure A2.4e Daily metabolism parameters throughout the open water season of L442.

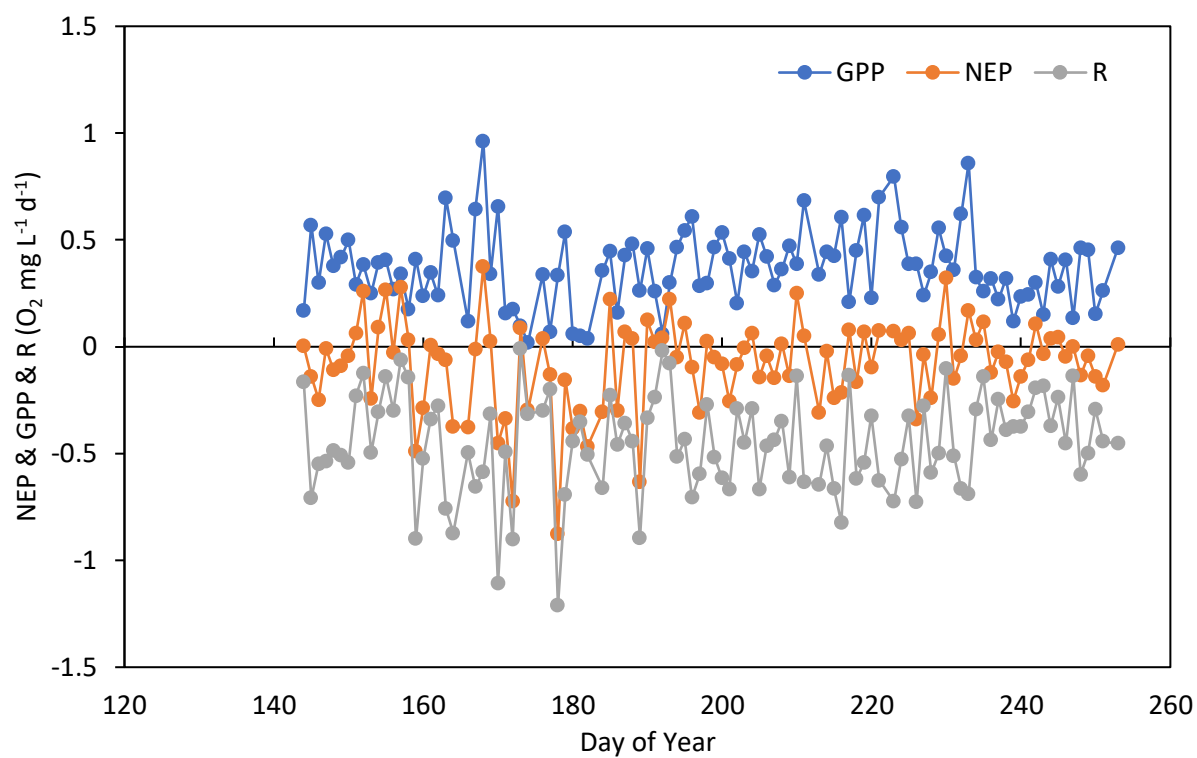


Figure A2.4f Daily metabolism parameters throughout the open water season of L658.

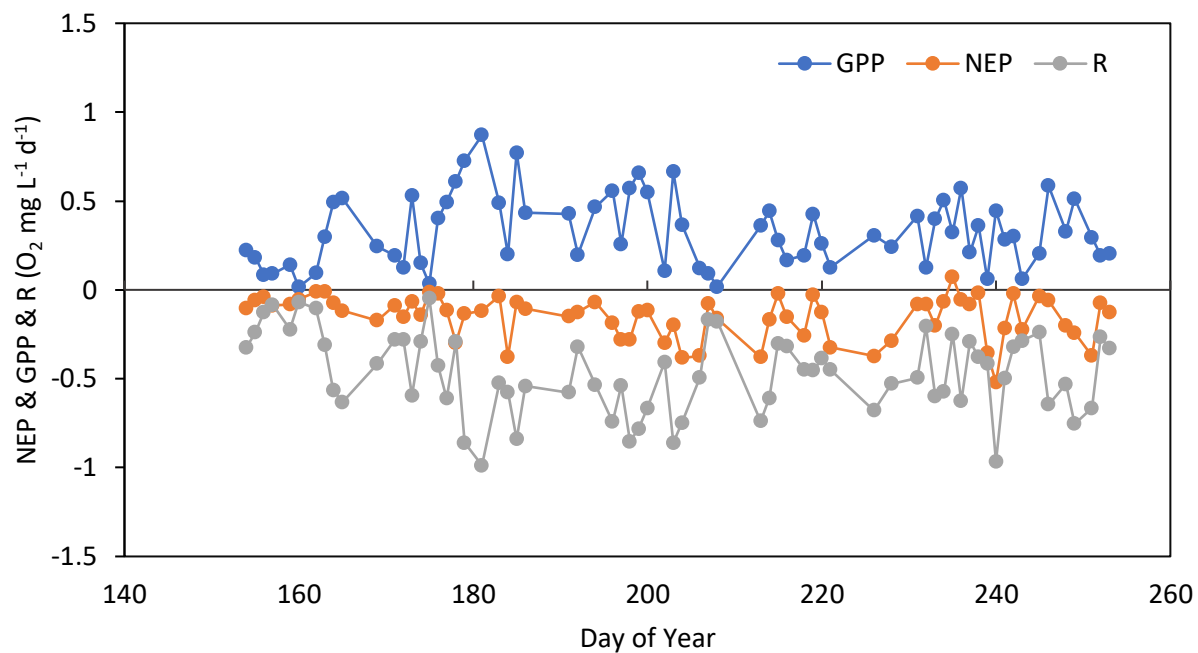


Figure A2.4g Daily metabolism parameters throughout the open water season of L164.

Appendix A3: How terrestrially derived organic matter influences phytoplankton primary production

A3.1 Checking assumptions for data transformation

Shapiro-Wilk normality test was conducted on total NPP values to assess normality and had a p-value of 0.0078. Log transformed total NPP had a had a p-value of 0.41. Both p-values are greater than 0.001 and are therefore normally distributed. The histogram of total NPP shows a left-skewed frequency distribution. The corresponding quantile plot shows majority of points close to the straight line and one-point deviating.

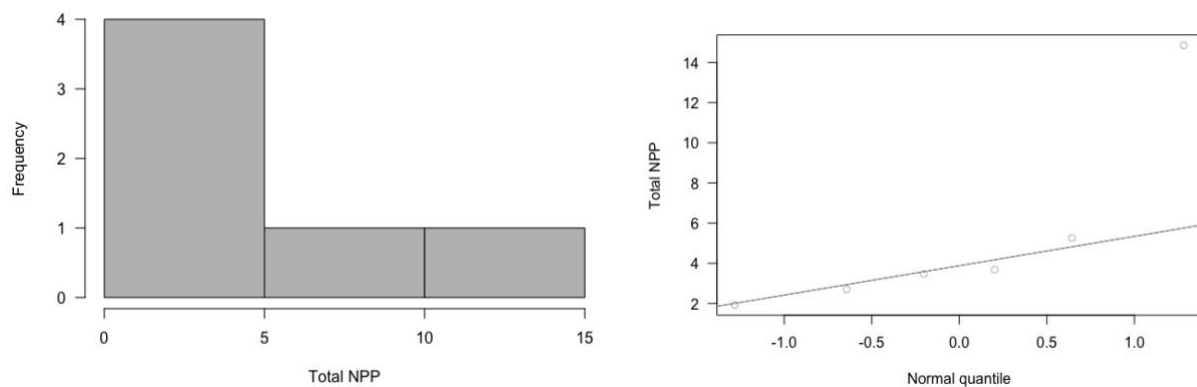


Figure A3.1a The frequency distribution of total net primary production (NPP) (left); and the corresponding normal quantile plot (right).

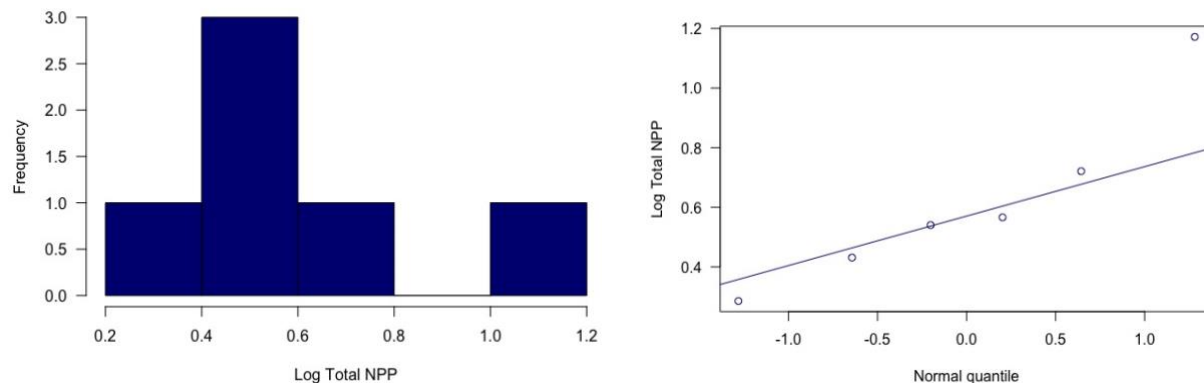


Figure A3.1b The frequency distribution of Log transformed total net primary production (NPP) (left); and the corresponding normal quantile plot (right).

A3.2 Chlorophyll measurements

Chlorophyll a comparison between YSI EXO2 chlorophyll values to IISD-ELA's chemistry lab. YSI EXO2 measured chlorophyll using relative fluorescence units (RFU), which is a relative number based on the concentration range of the calibration standards. Standards of 625 $\mu\text{g/L}$ standard and a 0 $\mu\text{g/L}$ standard were used. Chlorophyll a measurements from the sonde were corrected by linear regression below between sonde estimates and laboratory based fluorometric estimates.

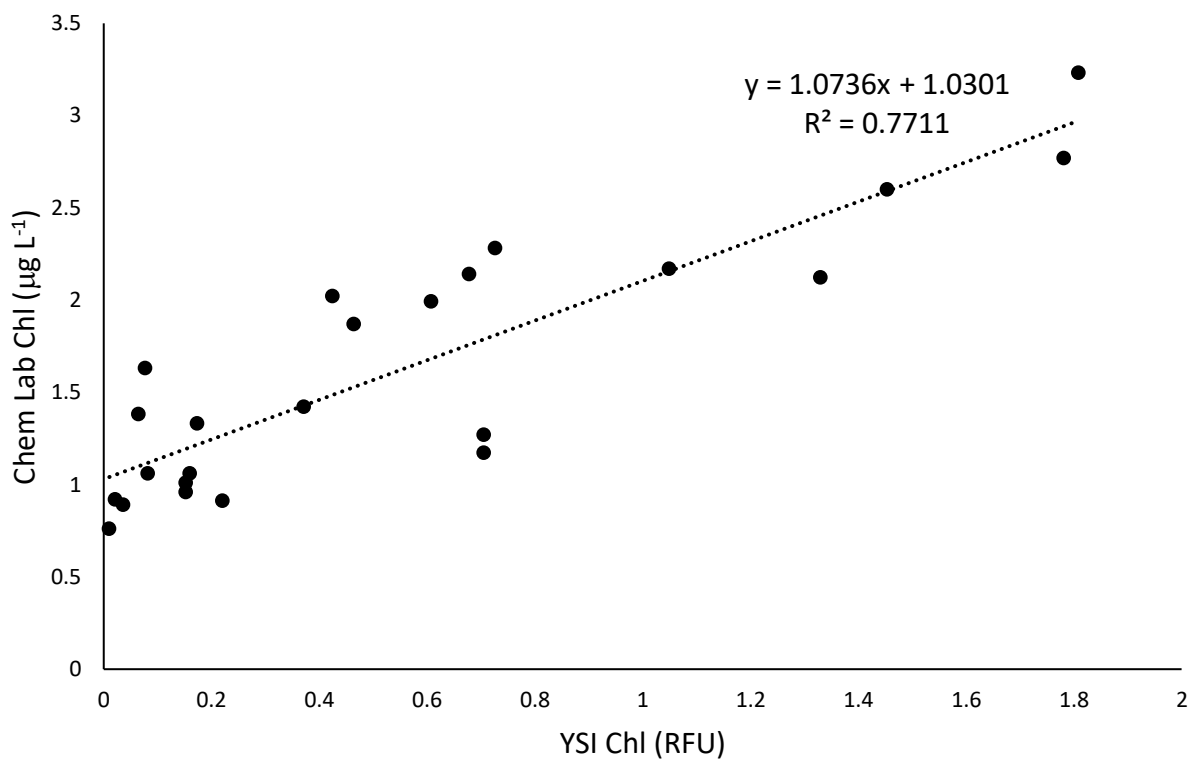


Figure A3.2 Linear regression equation comparing epilimnetic chlorophyll measured using the YSI EXO2 to the IISD-ELA's water chemistry lab (C. Desjardins pers. com.).

A3.3 Photosynthesis-irradiance curves

Photosynthesis-irradiance curves were plotted with the Jassby & Platt (1976) primary production equation and measured values from incubations. Photosynthesis-irradiance curve for L442 and L626 could not be fit with an $r^2 > 0.7$ and were omitted from this chapter.

Table A3.3 Pelagic primary production P-I curve parameters: photosynthesis maximum ($P_{\max}$), alpha, transition irradiance, respiration (R_0), and parameter fit r^2 .

Lake	$P_{\max}^b$ (mg C $m^{-3} h^{-1}$)	α^b ($\mu\text{mol photons } m^2$ $\text{sec}^{-1}/\text{mg C } L^{-1} h^{-1}$)	I_k ($\mu\text{mol photon}$ $m^2 \text{ sec}^{-1}$)	R_0 (mg C $m^{-3} h^{-1}$)	r^2
L224	0.733	0.007	755.0	4.9×10^{-5}	0.93
L373	0.484	0.002	302.2	2.1×10^{-5}	0.99
L223	0.169	0.010	120.4	0.042	0.85
L239	0.557	0.004	135.8	0.007	0.99
L658	0.433	0.010	221.3	0.071	0.98
L164	0.265	0.003	276.9	0.005	0.91

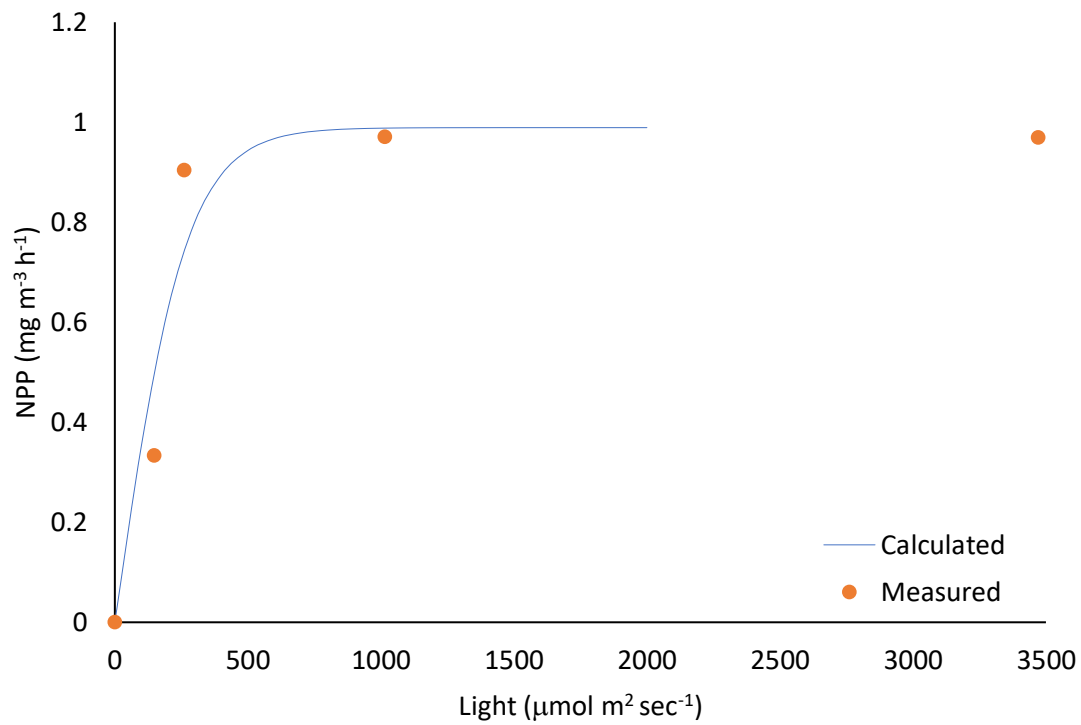


Figure A3.3a P-I curve of measured and calculated values for L224.

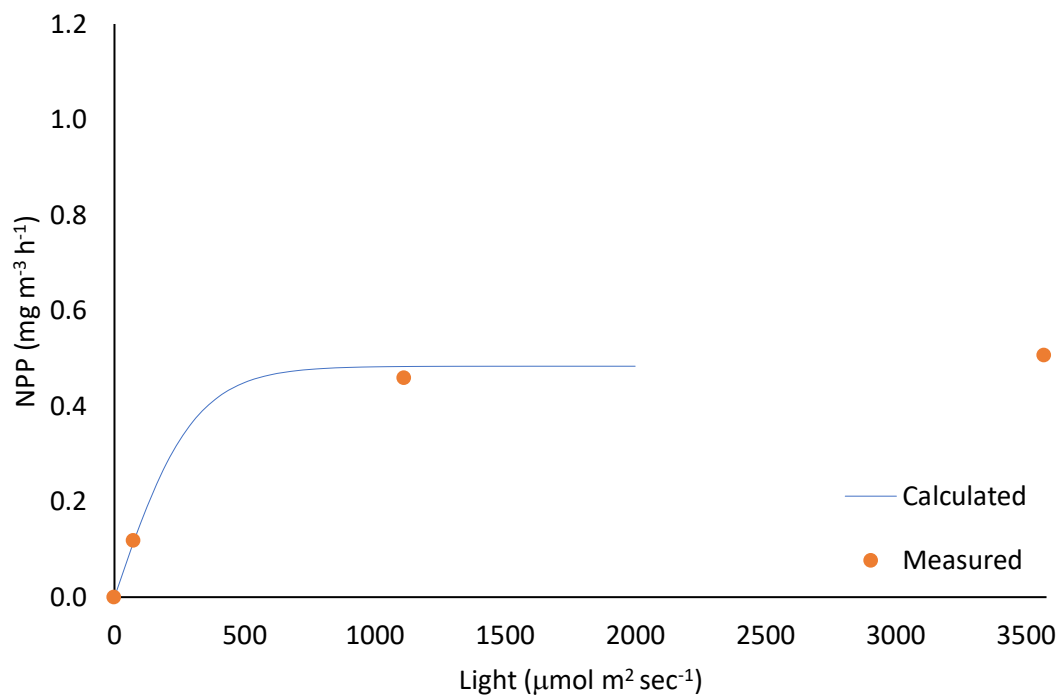


Figure A3.3b P-I curve of measured and calculated values for L373.

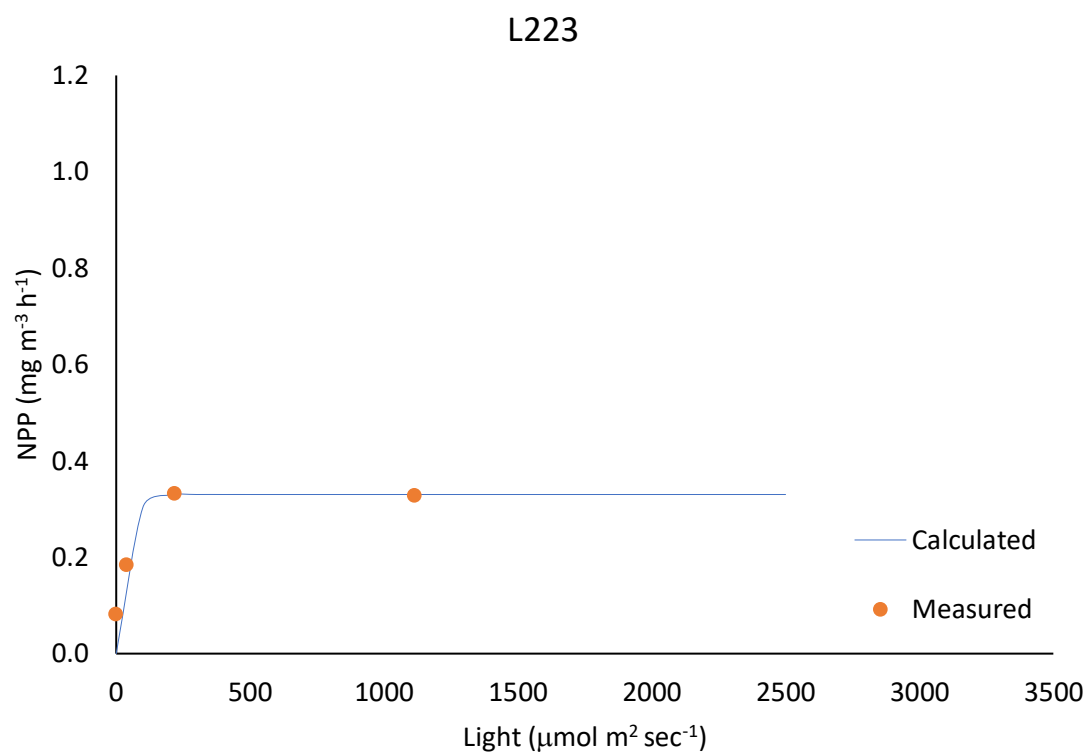


Figure A3.3c P-I curve of measured and calculated values for L223.

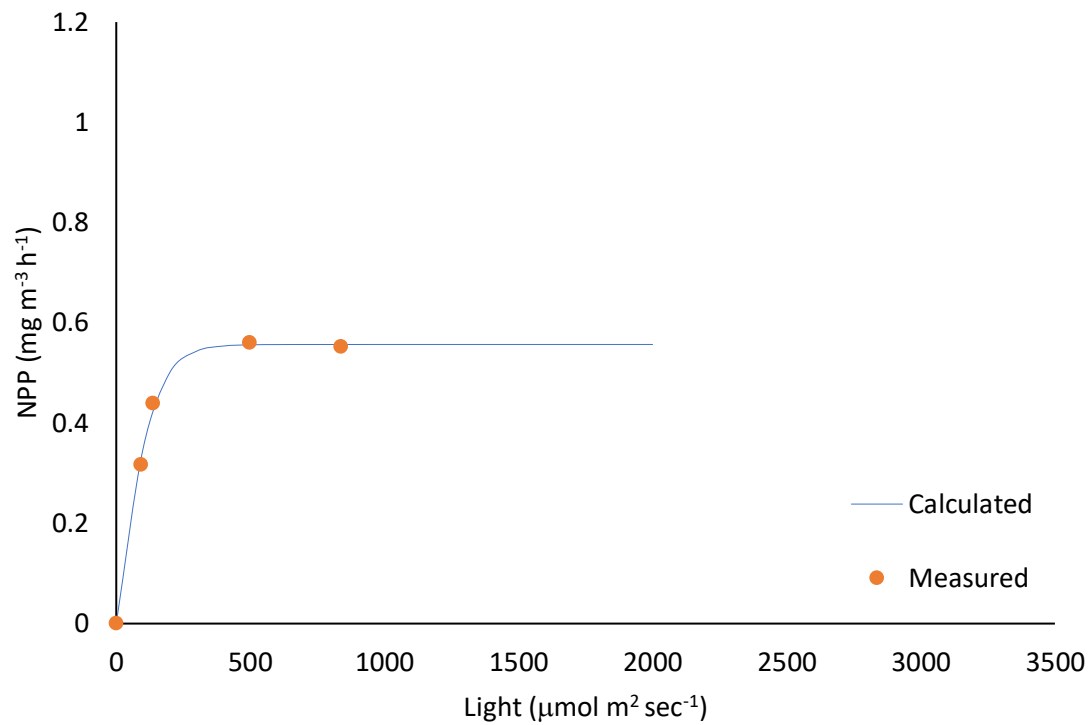


Figure A3.3d P-I curve of measured and calculated values for L239.

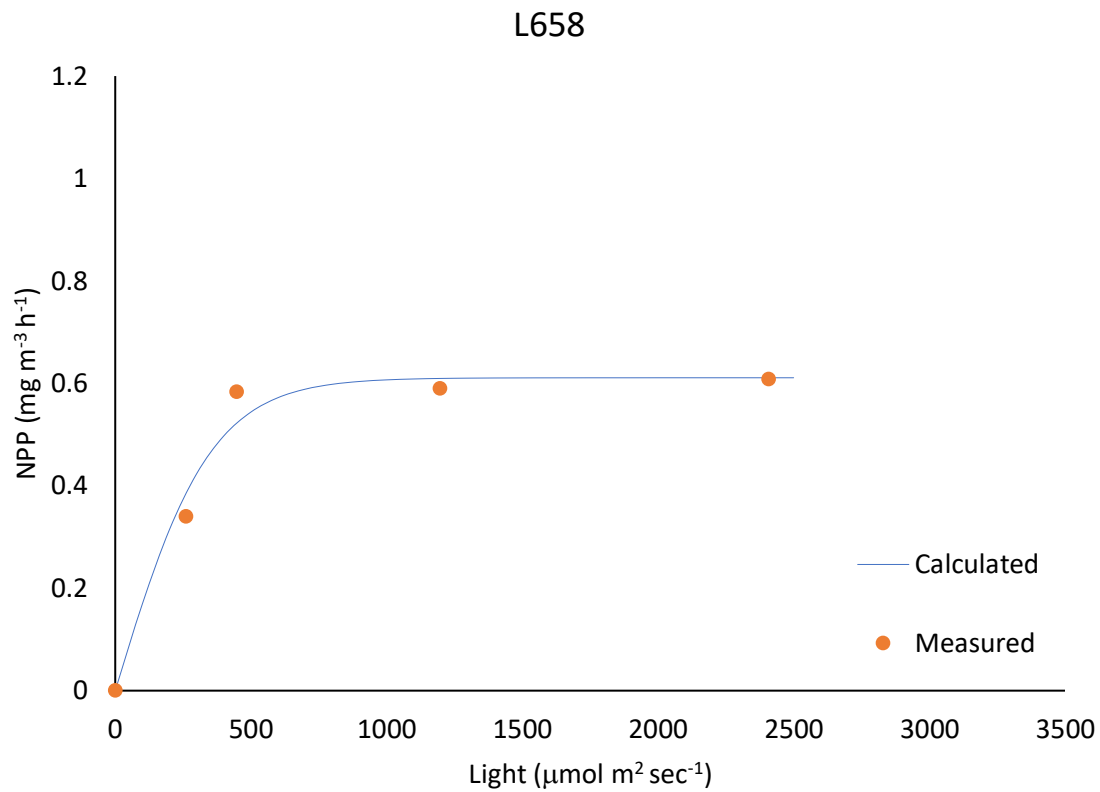


Figure A3.3e P-I curve of measured and calculated values for L658.

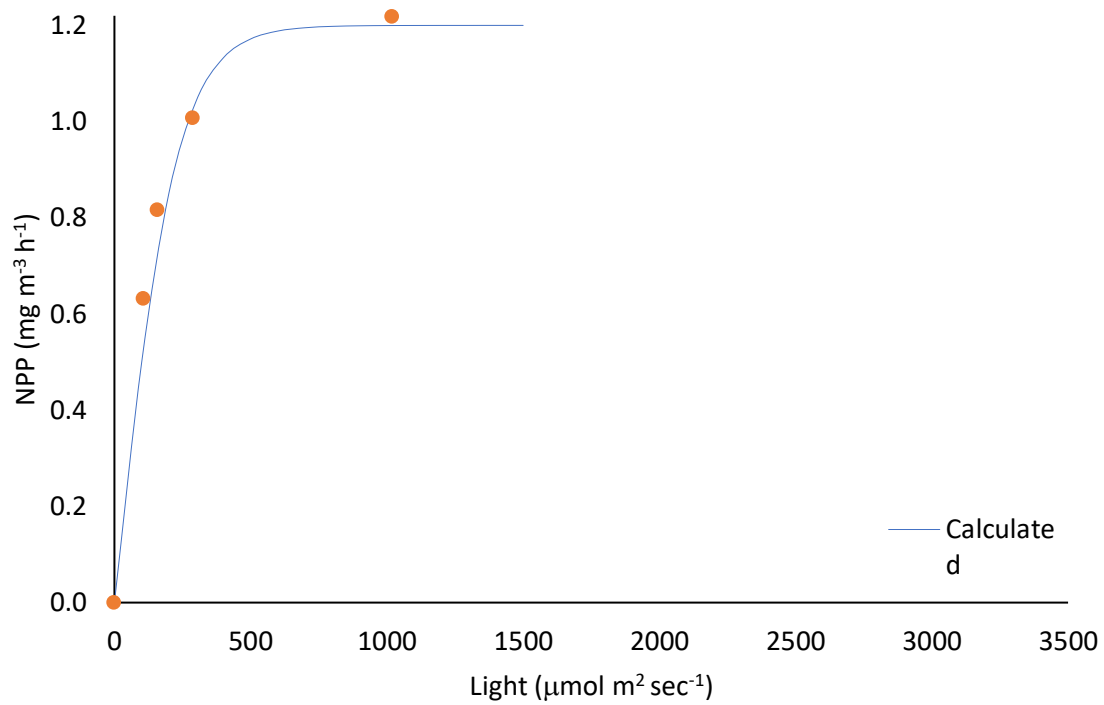


Figure A3.3f P-I curve of measured and calculated values for L164.

A3.4 Depth integrated primary production

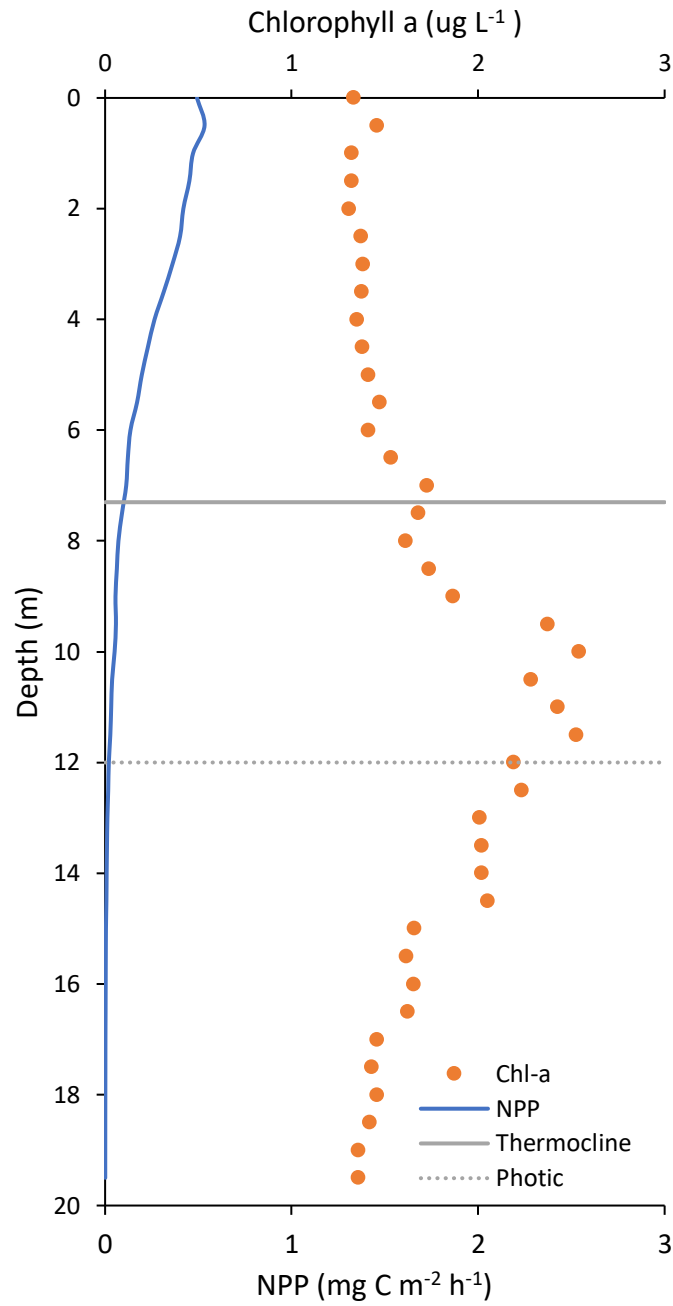


Figure A3.4a Relationships between NPP and chlorophyll with depth for L373. Negative values of NPP were not included.

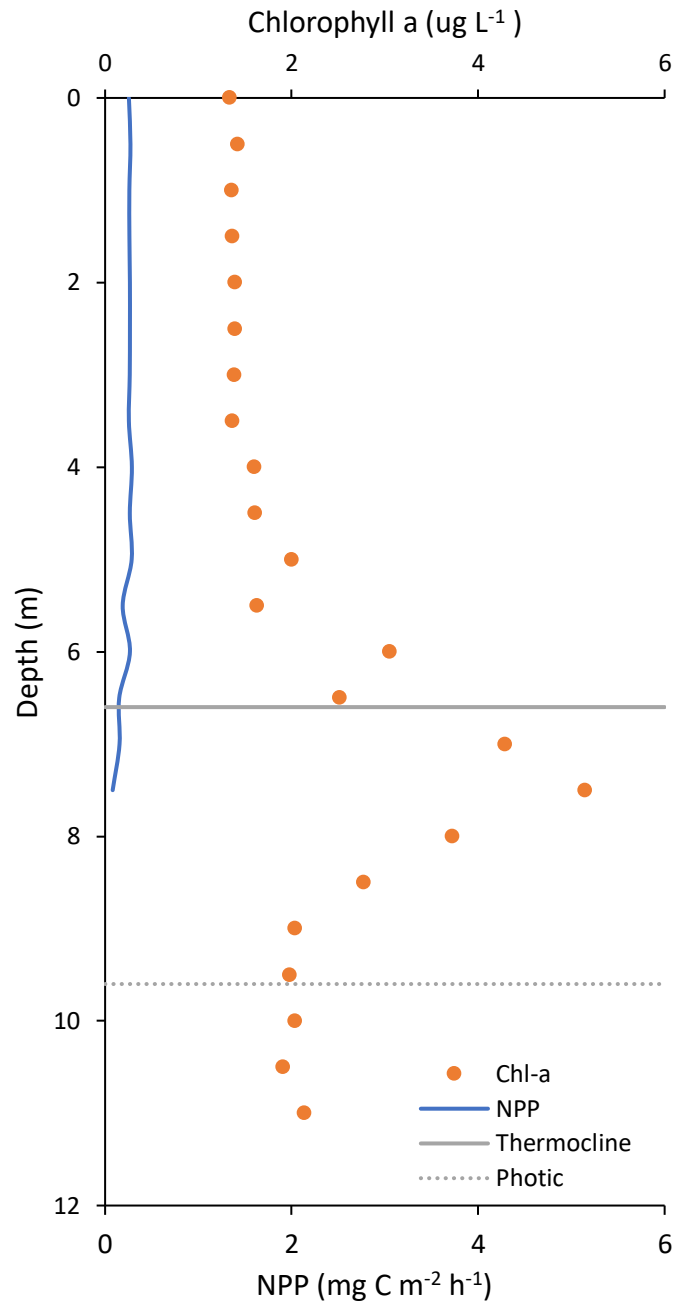


Figure A3.4b Relationships between NPP and chlorophyll with depth for L223. Negative values of NPP were not included.

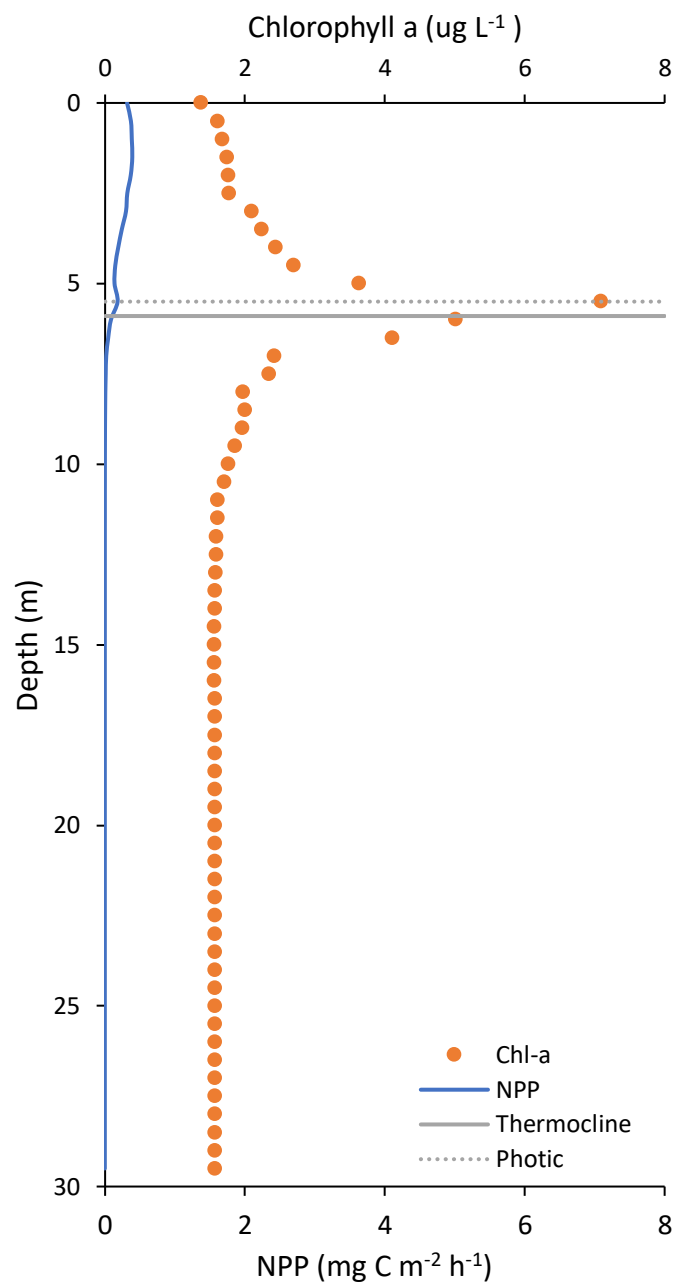


Figure A3.4c Relationships between NPP and chlorophyll with depth for L239. Negative values of NPP were not included.

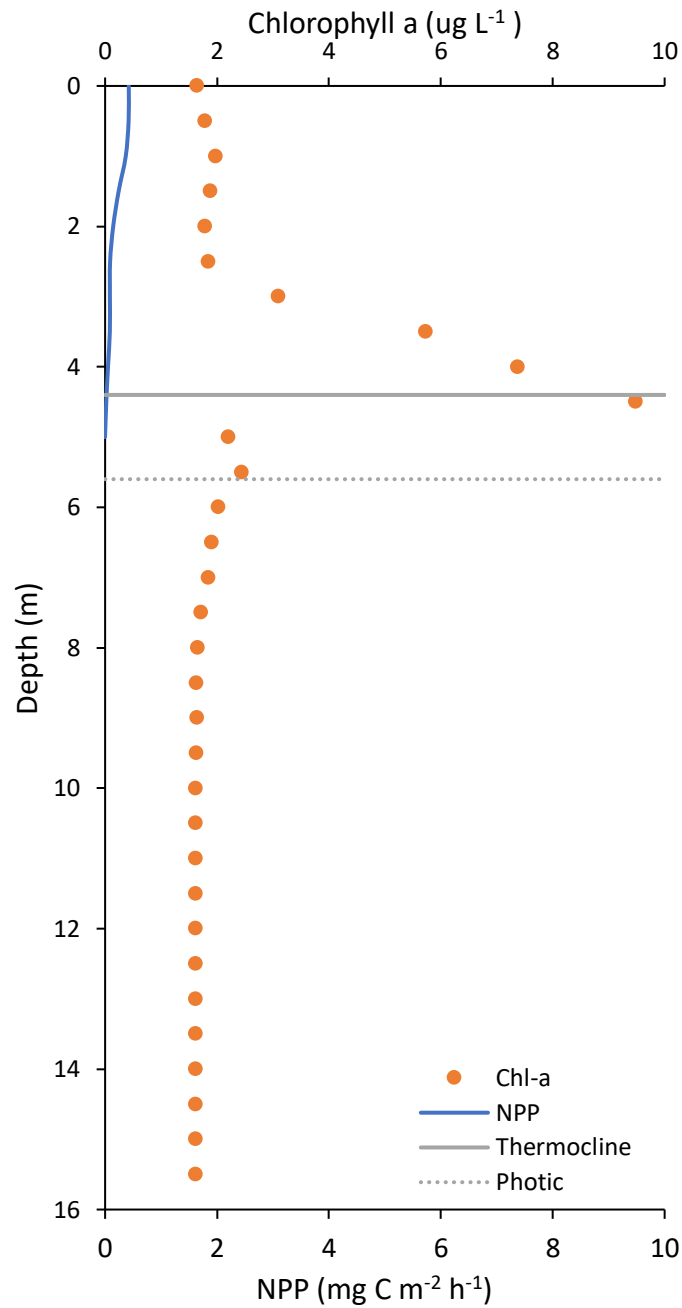


Figure A3.4d Relationships between NPP and chlorophyll with depth for L658. Negative values of NPP were not included.

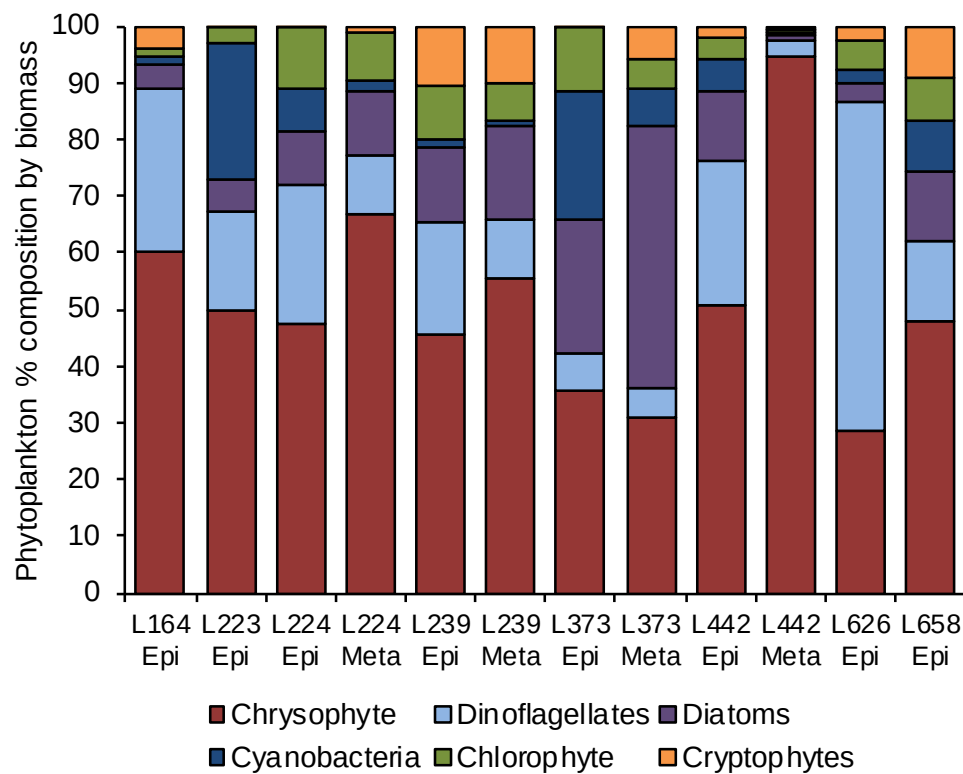


Figure A3.6 Phytoplankton percent composition (by biomass) across eight study lakes in mid-August, at the time of zooplankton collections. Note: Euglenophytes were present in epilimnetic L658 samples but were removed from this plot because they composed <.2% of the phytoplankton community by biomass.

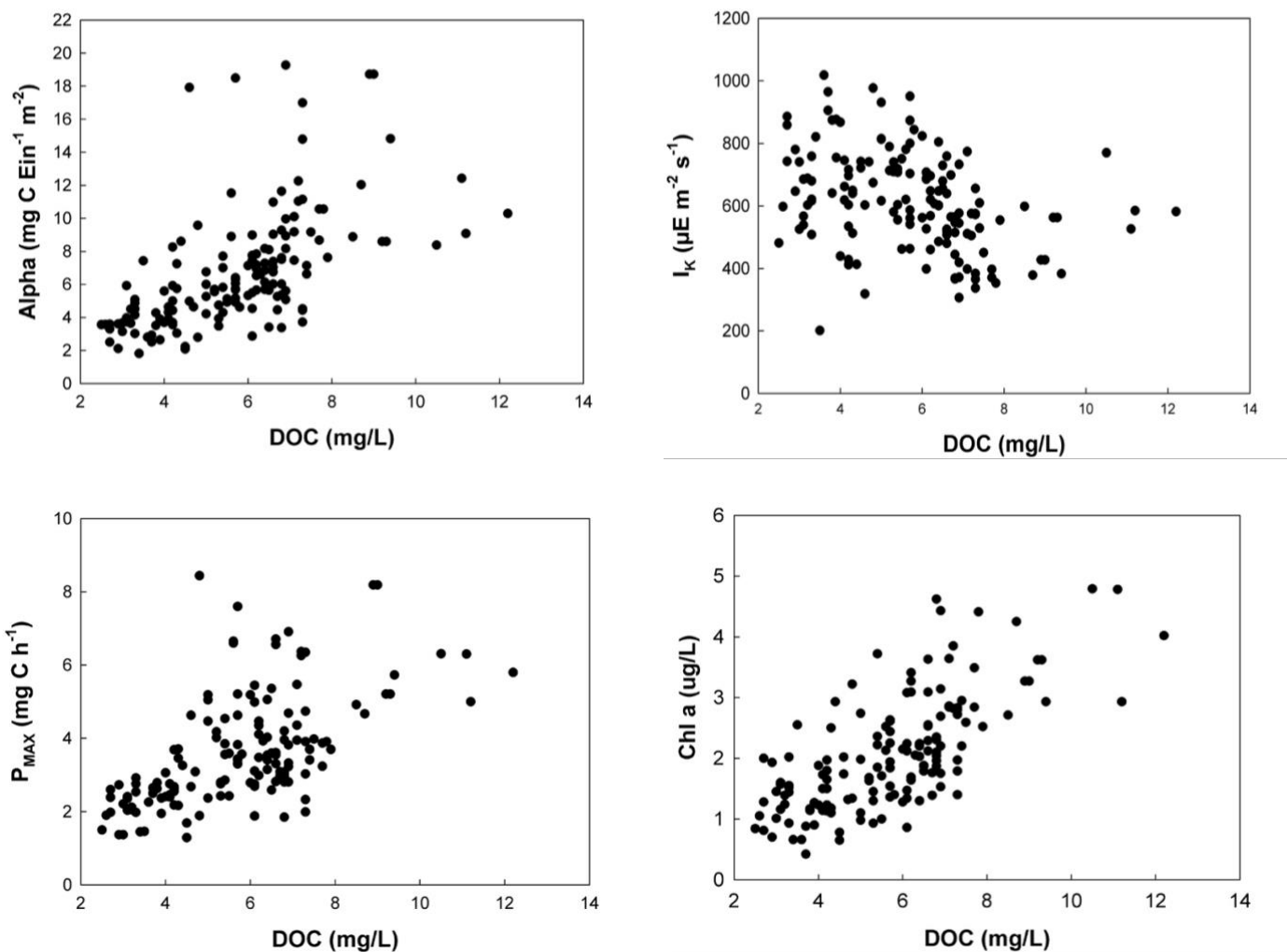


Figure A3.7 Historic data from IISD-ELA of pelagic phytoplankton P-I curve parameters: alpha, transition light intensity (I_k), photosynthesis maximum (P_{max}), and chlorophyll *a* (Chl-*a*) (Higgins et al. unpublished).