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Dynamic Carbon Cycling in Muskegon Lake – a Great Lakes Estuary

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Dynamic Carbon Cycling in Muskegon Lake – a Great Lakes Estuary

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Date

Dedication

This master's thesis is dedicated to my husband, Cameron Knapp, and my parents, Kerri and Jeff Ringler. Thank you for all the support and love you have shown as I've gone through this academic journey. It would not have been possible without you.

Acknowledgments

I want to thank Bopi Biddanda for all that he has done in advising and challenging me during my time at GVSU. He not only provided guidance but also friendship. The knowledge that I have learned from him has greatly impacted this thesis. I want to thank Tony Weinke for his guidance and friendship through this process as well. Bopi and Tony both made thoughtful additions to my scientific writing. My other committee members, Eric Snyder and Steve Ruberg also helped with valuable comments and any concerns or questions I had through the process. Val Klump and John Lenters advised me in the initial stages of this thesis work. Kurt Thompson helped create contour maps of lake metabolism for this thesis. I would like to thank several undergraduate students, Macy Doster, Rachel Ratliff, Tom Claffey, and Morgan Lindback, who provided field assistance. I would like to thank the other graduate students who have provided support through this journey. I would also like to thank my husband and family for their constant encouragement.

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Abstract

Ecosystem metabolism is the coupling of carbon and oxygen through photosynthesis and respiration. Gross primary production (GPP) is the carbon fixation by photosynthesis, ecosystem respiration (R) is carbon remineralization by bacterial and plankton respiration, and net ecosystem production (NEP) is the balance. Metabolism estimates determine if ecosystem is a sink or source of carbon to the atmosphere. When a lake has a positive NEP, or the GPP:R ratio is greater than 1, it is considered autotrophic and less carbon is being lost to the atmosphere than taken in, whereas if NEP is negative (GPP:R<1) it is considered heterotrophic and loses more carbon to the atmosphere. The two main objectives of my study were to: 1) estimate metabolism using 7 years of high frequency Muskegon Lake Observatory (MLO) data and a lower frequency biological oxygen demand (BOD) light-dark bottles data and 2) to determine if there was spatial heterogeneity in metabolism across Muskegon Lake using 4 buoy sites in 2016 and 2017. The first objective showed MLO 7-year average (±SD) of GPP, R, and NEP were 0.516 ± 0.466, -0.364 ± 0.341, and 0.028 ± 0.210 mg C L^{-1} d^{-1}, respectively and the BOD 7-year average (±SD) of GPP, R, and NEP was 0.332 ± 0.226, -0.117 ± 0.069, and 0.214 ± 0.177 mg C L^{-1} d^{-1}, respectively. The BUOY method consistently yielded higher rates for GPP and R and much lower rates of NEP compared to the BOD method. For the second objective, the spatial component of the study, GPP and R were significantly different across sites, but NEP was not significantly different. Our results suggest Muskegon Lake is annually a net sink of carbon. NEP may not vary much across the lake, but GPP and R and vary widely at each location. Our high frequency time-series data from multiple buoys demonstrates that freshwater lakes may display significant differences in metabolism across the ecosystem along with seasonally unequal rates

of metabolism. Muskegon Lake NEP rates were comparable to NEP rates at upwelling zones in the ocean indicating more focus should be placed on inland waters when researching global carbon cycles.

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Abbreviations

AOC – Area of Concern

AWRI – Annis Water Resources Institute

BOD – Biological Oxygen Demand

CDOM – Colored Dissolved Organic Matter

Chl – Chlorophyll

CNEP – Cumulative Net Ecosystem Production

Deep – Southerly and deepest buoy location

DO – Dissolved Oxygen

EPA – Environmental Protection Agency

GPP – Gross Primary Production

GPP:R – GPP and R ratio

MLO – Muskegon Lake Observatory

NEP- Net Ecosystem Production

NOAA – National Oceanic and Atmospheric Administration

PAR – Photosynthetically Active Radiation

Phyco – Phycocyanin

R – Respiration

SpCond – Specific Conductivity

Z_{mix} – mixed layer depth

Chapter 1 - Introduction

Introduction

"*The storage of carbon and release of free oxygen are the essence of life."*

- William Schlesinger (1997)

All life is carbon-based. Therefore, by tracking the flow of carbon within the carbon cycle one can gain a better understanding of life processes operating from the cellular to global scales. The movement of carbon is globally studied today due to the impact it has on climate change and how humans are altering biogeochemical cycles. The three large and reactive pools in which carbon is stored are: the atmosphere, ocean, and terrestrial land. Without human intervention, carbon can go through natural processes and be stored in each of these pools properly; however, with human intervention, we are increasingly moving and altering carbon from these storage places leading to excess amounts of carbon in the atmosphere. Within the three main carbon pools are smaller, more specific areas where carbon is stored. Of these smaller storage spaces, inland waters have historically been lumped into the terrestrial pool and not specifically included in global carbon budgets since they only comprise ~3% of the earth's surface and have been thought to serve only as passive pipes for the transit of carbon from the land to the ocean (Cole et al. 2007). However, recent studies are revealing that although inland waters take up a small area on Earth, they are hot spots of carbon cycling and serve as sensitive sentinels of climate change.

Inland waters, such as lakes and estuaries, serve as reactive hot spots of changing surrounding environments– thus integrating signals of change over huge terrestrial areas relative to their surface. By monitoring geographically distributed inland waters of various types, scientists can use them as indicators of local as well as global change (Cole et al 2007; Tranvik et

al. 2009). Changes in lakes can be indicators of changes in the watershed and climate through chemical, physical, and biological responses. Climate can drive the timing of ice formation and water levels. Other response variables that serve as indicators of change are patterns in water temperature, dissolved organic carbon (DOC), or plankton communities. Recent research and analysis of carbon cycling in inland waters shows that they process roughly 2.7 billion metric tons of carbon annually (Cole et al. 2007; Biddanda 2017). Of this 2.7 billion tons that enters inland waters, roughly half is respired to the atmosphere, 0.4 billion tons is buried in the sediment and 0.9 billion tons is exported to the ocean through the freshwater pathway (Cole et al. 2007; Biddanda 2017). Even with the obvious recognition of inland freshwaters as "hot-spots" of carbon processing, they are only recently starting to be acknowledged in global carbon budgets (Quéré et al. 2018).

The fate of carbon once it has entered the lake depends on various biological and physical processes including primary production, respiration, carbon burial in the bottom of the lake, and outflow (Figure 1). The balance between carbon fixation and biological carbon oxidation is the metabolism in an ecosystem. Primary production and respiration are the coupling of oxygen and carbon that sustain life. Oxygen does change through all of these processes in ~1:1 molar stoichiometry with carbon, and thus we can use that to estimate metabolism. Aquatic metabolism is commonly measured by tracking changes in dissolved oxygen (DO) and is then converted to carbon in equimolar terms (Biddanda et al. 1994). Components of metabolism include Gross Primary Production (GPP), Ecosystem Respiration (R), and Net Ecosystem Production (NEP). In aquatic ecosystems, GPP is the carbon fixation through photosynthesis, R is the respiration by all aerobic organisms, and NEP is the balance between GPP and R. Positive or negative NEP determines if an ecosystem is autotrophic or heterotrophic depending on if GPP or R dominate.

When an ecosystem is net autotrophic more carbon is taken up than released and when an ecosystem is net heterotrophic more carbon is released than taken up. These values will help in categorizing ecosystem's carbon cycling role in the global context.

There have been several methods which estimate the metabolism in aquatic ecosystems including the diel free water dissolved oxygen method often using buoy systems (FWDO or BUOY), biological oxygen demand (BOD) using light and dark bottles incubated for 24-hours, and carbon-14 methods which using light and dark bottles spiked with carbon-14 Sodium bicarbonate over a period of time (C-14). Sargent and Austin (1949) first used diel changes of oxygen in coral reefs and later Odum used diel changes of oxygen in rivers, lakes, and coral reefs using the BOD method and it became widely accepted (Odum 1956). Since then, others have used all three methods looking at the changes in oxygen or carbon over a 24-hour period to estimate production and respiration in aquatic ecosystems (Hall 1972; Smith and Key 1975; Cole et al. 2000; Hanson et al. 2007; LaBuhn and Klump 2016). Today, the BUOY method of tracking changes in dissolved oxygen is widely used allowing for global comparisons among different lake types. When estimating metabolism from sensors, there are several models which can result in different metabolism estimates for the same data. Different outcomes may result because the methods are based on different underlying statistics, including algebra, Bayesian, maximum likelihood and Kalman filter, maximum likelihood, and linear regression (McNair et al. 2013; Winslow et al. 2016). Recently, an R package was created to help make the process of estimating metabolism easier and it has 5 different models available, bookkeeping, Bayesian, Kalman, maximum likelihood, and ordinary least squares (Winslow et al. 2016). The most commonly used model is the bookkeeping model.

There are several uncertainties which still need to be resolved in metabolism studies. In the traditional methods, water was put into bottles and either oxygen or carbon was traced to determine the respiration rates. These lead to "container effects," which do not allow for physical processes to be included in the analysis, often did not have the correct light conditions, and container details were not included (Kemp et al. 1997; Staehr et al. 2010). A goal to fix these assumptions was the use of high frequency sensors; however, these sensors technologies have their own set of uncertainties.

When estimating metabolism using the BUOY method, dissolved oxygen is tracked over a long period at a high frequency. The increase in oxygen during the day represents the primary production, and the decrease at night represents respiration. It is assumed that respiration is equal during both night and day, and therefore we are able to estimate GPP from the daytime changes in dissolved oxygen. It is also assumed that the changes in DO represent the whole ecosystem. DO sensors are placed in the upper two meters of the water column, which represents the upper water column above the thermocline depth where gas exchange is occurring. Another assumption is that metabolism measurements from one location within a lake can represent the whole lake once scaled up. However, only a few studies have estimated metabolism at multiple locations within the same lake (Lauster et al. 2006; Van de Bogert et al. 2012; Vesterinen et al. 2017). These studies found that metabolism can vary widely at different locations within the same habitat and between different habitats.

With metabolism, GPP is only supposed to be positive, and R is only supposed to be negative; however, there are occasional times when inexplicably GPP is negative and R is positive, even though this is biologically impossible (Staehr et al. 2010; Winslow et al. 2016). These uncertain rates occur when physical processes are obscuring the dissolved oxygen sensor

causing GPP to appear negative and R to appear positive – such as when water masses with differing dissolved oxygen content move past the sensor during one diurnal cycle. Unfortunately, this issue has not been resolved and the suggested methods of correction to deal with this data challenge is not clear. For example, there is an R program which estimates metabolism, and one suggestion is to force a positive GPP and negative R; however, this could bias results, while the other suggestion is to exclude these from the results. Others found that excluding these data may lead to underestimates for metabolism (Brothers et al. 2017).

Muskegon Lake (43.23°N, 86.29°W) is a mesotrophic drowned river mouth freshwater estuary along the eastern shores of Lake Michigan with an area of ~17 km^2, mean depth of 7 m, maximum depth of 22 m, and a residence time of ~23 days (Figure 2). During certain wind events, upwelling occurs in Lake Michigan which forces oxygen rich, cold water through the navigation channel into Muskegon Lake (Liu et al. 2018). These cold-water intrusion events temporarily relieve bottom water hypoxic (DO <4 mg/L) conditions (Biddanda et al. 2018) and dilutes the nutrient rich water in Muskegon Lake (Liu et al. 2018). The lake was designed as an Area of Concern (AOC) by the Environmental Protection Agency (EPA) in 1985 based on nine beneficial use impartments such as eutrophication and habitat degradation due to legacy issues from sawmill and foundry industries in the 1800s and early 1900s (Steinman et al. 2008). Long term monitoring of Muskegon Lake began in 2003 to help delist Muskegon Lake from the AOC list. The Muskegon Lake Observatory buoy started in 2011 to have more monitoring on the lake at a mid-lake location. Three additional buoys have been deployed since 2016 to understand spatial heterogeneity and physical dynamics within the lake (Figure 1).

In Muskegon Lake there have been previous studies estimating metabolism. These studies used the BOD method at a relatively low frequency annually. Overall, these studies found

that there were significant spatial heterogeneity in metabolism rates within a land to lake gradient from the river to nearshore Lake Michigan making Muskegon Lake the 'Goldilocks Zone' for production and that there were seasonal shifts in production with mid-summer being the most productive (Weinke et al. 2014; Dila et al. 2015; Defore et al. 2016). These studies also concluded Muskegon Lake was net autotrophic except for a couple winter months. Although these earlier low-frequency (monthly and yearly) measurements revealed much about the seasonal carbon dynamics in this estuary, making more frequent daily rates of metabolism measurements at multiple locations in the lake would provide improved quantification of carbon cycling and better data for classifying this water body as a source or sink for carbon on an annual basis.

Purpose

The overall purpose of the present study was to estimate the metabolism within Muskegon Lake using the high-frequency free water dissolved oxygen measurements from the Muskegon Lake Observatory (MLO; www.gvsu.edu/buoy/), and to determine if Muskegon Lake is a source or sink of carbon to the atmosphere. Specific objectives for this study were: 1) use the MLO to determine long-term changes in metabolism and seasonal fluctuations using the BUOY and BOD method and identify the drivers of those changes, and 2) use additional buoys to estimate metabolism to explore the spatial heterogeneity of metabolism within Muskegon Lake. Resolving these objectives will provide insight into long-term patterns of seasonal metabolism and to further understand the spatial heterogeneity of metabolism in Muskegon Lake and similar lake/estuary ecosystems elsewhere.

Scope

The information gathered from this project can be applied to Muskegon Lake and for drowned river mouth lakes along the east shore of Lake Michigan. Additionally, this study will serve as a model for this specific lake type and potentially can be compared with coastal saltwater estuaries and other inland waters to understand what the differences may be. This study will also illustrate the importance of lake-wide measurements for carbon cycling rather than one location within the lake and the importance of including the world's lakes and estuaries in the global carbon budget.

Assumptions

A major assumption for this study is that the Muskegon Lake Observatory buoy and other buoy measurements represented the pelagic waters in Muskegon Lake. When the BOD method was used, we also assumed it represented the whole lake. When estimating metabolism, we assumed daytime respiration rates and nighttime respiration rates were equal, and that uncertain rates of metabolism were due to physical drivers overshadowing the biological DO measurements.

Hypothesis

We hypothesized the following for each objective: 1) metabolism will vary year to year depending on yearly environmental factors but will show a distinct seasonal pattern with increased metabolism rates in the summer and decreased metabolism rates in the spring and fall, 2) metabolism across Muskegon Lake will be significantly different.

Significance

This study will aid in the understanding the role inland waters play in the global carbon cycle. Findings from Muskegon Lake can be used as a model for similar lakes and estuaries when estimating the global contribution of carbon to or from the atmosphere from the world's freshwater bodies. This study will also help in informing the scientific community of the importance of lake wide heterogeneity in metabolism which has largely been overlooked and underplayed in the literature on ecosystem carbon dynamics.

Definitions

Metabolism – Balance of photosynthesis and respiration in an ecosystem.

Gross Primary Production – Carbon fixation by photosynthesis.

Respiration – Carbon remineralization by bacterial and planktonic respiration.

Net Ecosystem Production – The balance of gross primary production and respiration.

Figure Legends

Figure 1. Conceptual model of processes in lakes that affect carbon metabolism in the context of the present study in Muskegon Lake, MI. There will be a total of 4 buoy stations all of which could have same or different metabolism rates. In addition to the observatory-based BUOY method, the biological oxygen demand (BOD) method will be used. There are various ways in which metabolism could vary through the seasons and the lower left inset depicts some of these scenarios for GPP depending on the season, location, or method. Variables that may influence metabolism are river inputs and run off inputs of nutrients or dissolved organic matter (DOM). Additionally, wind may lead to more atmospheric exchange of gases and cause more mixing in the water column that could affect the metabolism in the surface waters. Phytoplankton that are produced will go through the food web and ultimately be respired by bacteria. Some carbon is expected to be buried by sedimentation and then possibly resuspended at times. Carbon can also leave Muskegon Lake through the channel to Lake Michigan. Question marks indicate where measurements of dissolved oxygen changes were made in the study.

Figure 2. Map of Michigan with the Muskegon River Watershed outlined and Muskegon Lake at its terminus, with insets showing the Great Lakes Basin and Muskegon Lake.

Figures

Figure 1

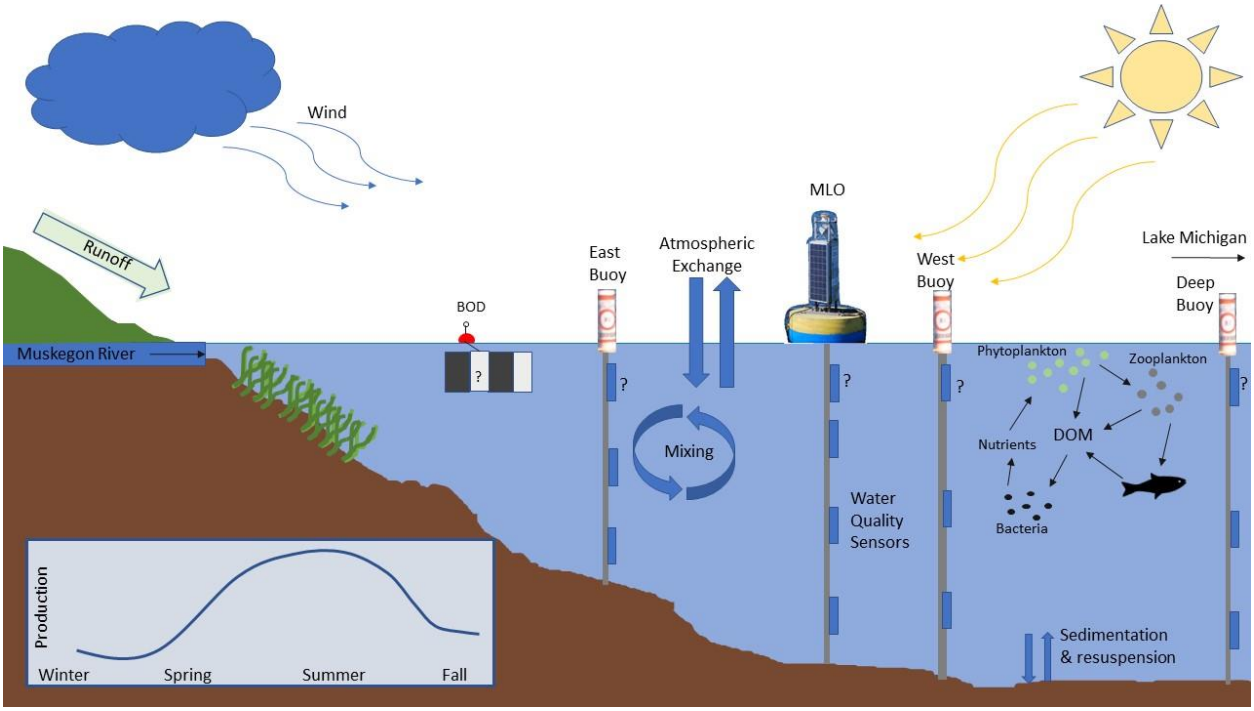
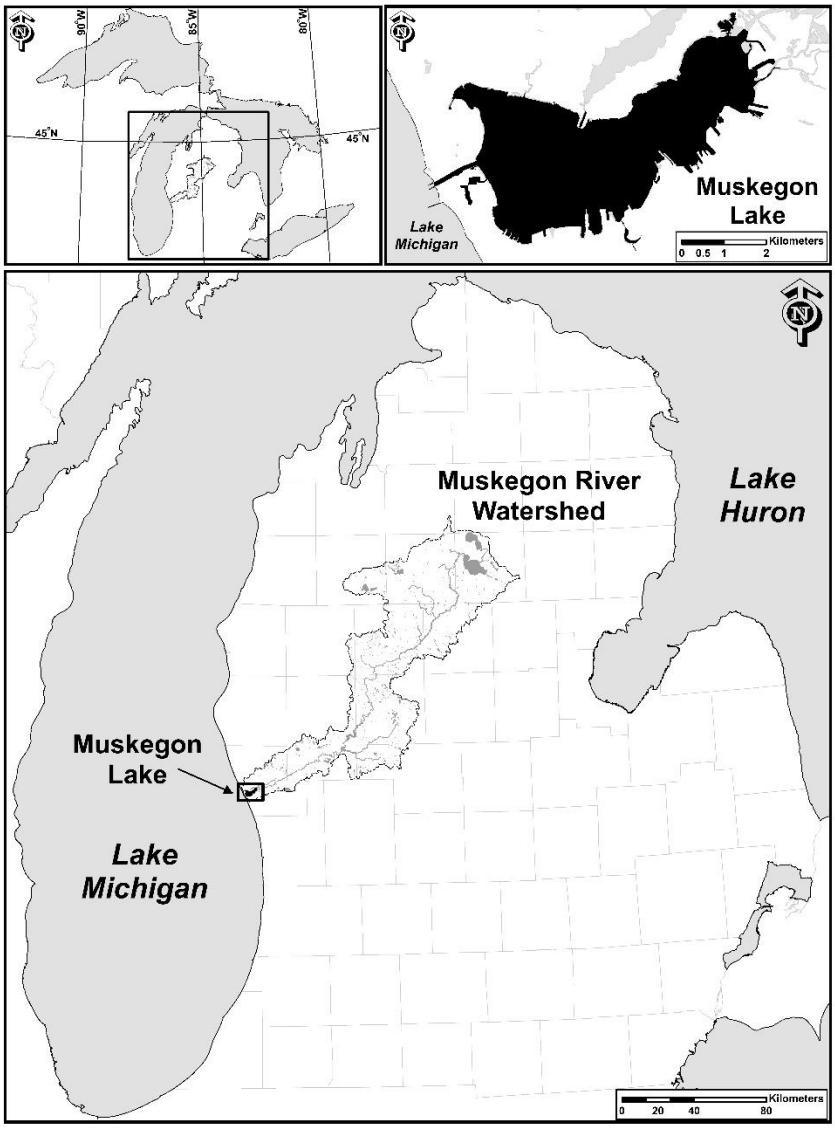


Figure 2


90°W
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Lake
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0 0.5 1 2
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Kilometers
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Chapter 2.1

Title

Multi-year Measurements in a Great Lakes Estuary Reveal Seasonally Variable Ecosystem Metabolism with Net Production

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Running Head

Measuring multi-year lake metabolism using time-series data

Keywords

Metabolism, carbon cycling, Muskegon Lake, primary production

Abstract

Ecosystem metabolism is comprises the storage and release of carbon through photosynthesis and respiration. Ecosystem metabolism was quantified in Muskegon Lake from 2011-2017 based on time-series data from the Muskegon Lake Observatory buoy (BUOY) and the biological oxygen demand (BOD) methods. BUOY provided continuous rates of metabolism, whereas the BOD method provided 3 – 8 discrete metabolism measurements a year. The BUOY 7-year average (±SD) of Gross Primary Production (GPP), Respiration (R), and Net Ecosystem Production (NEP) were 0.516 ± 0.466, -0.364 ± 0.341, and 0.028 ± 0.210 mg C L^{-1} d^{-1}, respectively. The BOD 7-year average (±SD) of GPP, R, and NEP was 0.332 ± 0.226, -0.117 ± 0.069, and 0.214 ± 0.177 mg C L^{-1} d^{-1}, respectively. ANOVA showed that there are significant differences between the BUOY and BOD methods for NEP ($p<0.001$) and R ($p<0.001$), but not GPP. Both methods showed a distinct seasonal pattern of GPP and R. Cumulative NEP ranged from 4.80 to 7.23 mg C L^{-1} y^{-1} with a 7-year average of 5.79 ± 0.88 mg C L^{-1} y^{-1}. Regression analysis indicated that GPP and R had a positive significant relationship with peak photosynthetic active radiation (PAR) days, and a negative relationship of GPP with river discharge. GPP:R ratios suggest most days were net autotrophic. Overall, metabolism showed a seasonal pattern for GPP and R, and a positive annual NEP trend – evidence that this Great Lakes estuary is both a seasonally productive ecosystem and annually a net sink for carbon.

Text

Introduction

Understanding the movement and processes that effect carbon are essential to understanding global change today. The global carbon cycle has been focused on the three largest pools: terrestrial, ocean and atmosphere. The terrestrial and ocean active pools exchange carbon with the atmosphere and with each other. Humans alter the interactions of these carbon pools through various land use changes, burning fossil fuels, and altering natural ecosystems. Within the terrestrial and ocean pools are multiple smaller pool types, each acting as a sink or source of carbon to the atmosphere. One of the smaller carbon pools, inland waters, have largely been ignored in the global carbon cycle estimates since they only take up ~3% of the Earth's surface. Inland waters have historically been thought of as passive pipes which only transport carbon from the terrestrial areas to the ocean (Cole et al. 2007; Biddanda 2017). Recent studies have shown that inland waters are disproportionately important in the global carbon cycle since they process just as much carbon as oceans on a global scale, and therefore, a new effort has been made to include inland waters into the global carbon cycle. Data regarding these smaller bodies of water is minimal and most studies have only investigated specific inland water bodies for short periods of time which may not represent the yearly or seasonal changes of the carbon pool.

There are various ways to understand what is occurring within the inland waters including creating a whole carbon budget measuring carbon entering, leaving, and within the water column, or measuring the primary production and respiration through oxygen changes in the water column (Field et al. 1998). Within inland waters, carbon can enter through

allochthonous loading from the watershed, atmospheric deposition, or autochthonous primary production. The fate of that carbon is either evaded to the atmosphere, downstream transport, or sediment burial. Ecosystem metabolism is collectively Gross Primary Production (GPP) – carbon fixation by photosynthesis, Ecosystem Respiration (R) – carbon remineralization by bacterial and planktonic respiration, and Net Ecosystem Production (NEP). It is the balance of GPP and R which determines if the lake is a sink or a source of carbon to the atmosphere. When NEP is negative, or the GPP:R ratio is less than 1, the lake is considered heterotrophic and loses more carbon to the atmosphere than is taken in, and when NEP is positive, or the GPP:R ratio is greater than 1, the lake is considered autotrophic and less carbon is lost to the atmosphere than is being taken in.

Metabolism can vary widely based on the type of inland water with GPP and R being highest in estuarine settings (Hoellein et al. 2013). It can also vary depending on the geographic location, nutrient availability, light attenuation, seasonality, and water column factors (Hoellein et al. 2013). The high variability in metabolism across inland water types has led to studies using high frequency buoy observing systems to monitor these systems *in situ.* These systems allow for high resolution time series data which give insights into the biological, chemical, and physical characteristics in a lake. Prior to the sensor technology, biological oxygen demand (BOD) bottles were used for gathering information on oxygen changes. The BOD method is done using light and dark bottles and incubating them for 24 hours under *in situ* conditions to determine the oxygen changes during the day and at night in light and dark bottles. This information gives insight into the daily DO changes which can give an estimate of primary production and respiration.

Three previous studies were done investigating the metabolism in Muskegon Lake using the BOD bottle method (Weinke et al. 2014; Dila et al. 2015; Defore et al. 2016). These previous studies found Muskegon Lake was a unique area where production was higher than the upstream river and the downstream Lake Michigan. These studies also reveal Muskegon Lake as an autotrophic ecosystem, except for some winter months (Weinke et al. 2014; Dila et al. 2015; Defore et al. 2016). The BOD method was employed either monthly or 3 times annually for these studies. The Muskegon Lake Observatory (MLO) was established in 2011 to take real time high frequency water quality and meteorological data. Today, the MLO enables us to estimate daily rates of metabolism through *in situ* measurements (BUOY).

The primary objective of this study was to estimate daily rates of metabolism using high frequency data buoy from 2011 to 2017. The second objective was to compare the high frequency data to the available discrete BOD data. The third objective of this study was to use other buoy parameters to find the drivers of metabolism during the 7 years of data. Implications from this study can be applied to similar freshwater estuaries and potentially salt-water estuaries around the world.

Methods

Study Site

Muskegon Lake (43.23°N, 86.29°W) is a mesotrophic drowned river mouth estuary on the east side of Lake Michigan and is the terminus for the 2nd largest watershed in Michigan – the Muskegon River Watershed (Figure 1). The watershed land use includes forest (53.2%), agriculture (23%), and urban (4.2%) (Marko et al. 2013). Muskegon Lake is ~17 km^2 with a residence time of ~23 days. The max depth is 22 m and the average depth is 7 m. Muskegon

Lake is connected to Lake Michigan through a navigation channel which allows the lake to act as a freshwater estuary. Water from Lake Michigan will intrude into Muskegon Lake during certain wind events temporarily relieving bottom water hypoxia (Biddanda et al. 2018; Liu et al. 2018). Muskegon Lake has a long historical use of sawmills in the 1800s and foundries in the 1900s along the south shore. Due to these historical industries surrounded the lake and watershed the EPA declared Muskegon Lake an AOC in 1985 due to poor water quality, eutrophication, and hypoxic conditions (Steinman et al. 2008). Along with various actions taken to improve the lake health, long-term monitoring has become a focus to delist Muskegon Lake as an AOC.

Buoy Data Collection

The Muskegon Lake Observatory (MLO), located at 43.238239 N, 86.280532 W, was established in 2011. The buoy acts as the scientists and citizens' eyes on the lake for live updates in water quality, meteorological, and wave information available online. MLO was also deployed to assist in the long-term monitoring of Muskegon Lake as an AOC. The observatory has been deployed in April or May and retrieved in November or December since 2011. There were 3 winters (2012-2013, 2015-2016, and 2017) where an underwater buoy string was deployed to capture the winter conditions which normally go unrecorded. Meteorological data at MLO is recorded every 5 minutes and water quality data is recorded every 15 minutes. During the winters meteorological data were collected from the nearby NOAA station located at the navigation channel between Muskegon Lake and Lake Michigan. Hourly averages of meteorological and water quality data were used to estimate metabolism. An array of various sensors including a YSI Datasonde (Yellow Springs Instruments) went from the surface to the bottom of the water column in 4 main clusters. MLO data used in this paper includes temperature

at 2, 4, 6, 8, 10, and 11 m, dissolved oxygen (DO) at 2 m, conductivity at 2 m, wind direction, and photosynthetically active radiation (PAR) at 1 m. Other data collected at the observatory were phycocyanin, colored dissolved organic matter (CDOM), chlorophyll *a*, turbidity, nitrate, and pH. Sensors were maintained for biofouling on a monthly basis and additional precautions such as wipers and data QAQC was done to ensure data was accurate.

Free Water Dissolved Oxygen Metabolism Calculations

Ecosystem metabolism is estimated by the increase and decrease in oxygen over a 24-hour period. Production is occurring during daylight hours increasing oxygen, and respiration is always occurring. When production stops, oxygen decreases and only respiration is occurring. This change in rate gives the measure of production and respiration. With the free water dissolved oxygen method, atmospheric exchange of gas is also considered by using wind data. The equation for understanding these changes in dissolved oxygen is

$$\Delta O_2/\Delta t = GPP - R - F - A$$

where GPP is the gross primary production, R is respiration, F is physical gas flux, and A is advection or other processes and is considered negligible (Odum 1956; Staehr et al. 2010).

Data from the buoy were used to estimate metabolism from 2011 to 2017. Both meteorological and water quality data were averaged hourly for estimating metabolism. Raw data taken from the buoy was used to estimate various parameters and then input into the R package LakeMetabolizer (Winslow et al. 2016). There are now several methods to estimate metabolism including bookkeeping, Kalman, Bayesian, ordinary least squares, and maximum likelihood (McNair et al. 2015; Winslow et al. 2016). Each of these use different statistical methods; however, the easiest and most widely used is the bookkeeping method which was used

for this study. The various parameters needed to input into the R package for the bookkeeping method are observed DO, oxygen saturation, piston velocity, thermocline depth, and daytime hours.

The thermocline depth (Z_{mix}) is used when estimating NEP. Z_{mix} is the depth of the water column where water temperature decreases rapidly separating the warmer epilimnion water from the cooler hypolimnion water. This depth can change easily with the presence or absence of wind and changing air temperature. Before high frequency temperature loggers through the water column, Z_{mix} was estimated by weekly or monthly temperature profiles. With this study we were able to find daily averages of Z_{mix} by using temperature sensors at 2, 4, 6, 7, 9, and 11 m. The R package, rLakeAnlayzer was used to estimate daily $Z_{mix.}$

Piston velocity is a measure of the gas-exchange with the water and atmosphere. To estimate the piston velocity wind surface water temperature is needed. At MLO the wind sensor was at 2 m above the surface of the lake. For the piston velocity estimate, wind needed to be at 10 m above the surface. An empirical relationship was used to estimate wind from 2 to 10 m (Table 1; Equation 2) where z is the height the wind sensor, U_z is the wind speed at that height (m/s) and U_{10} is the wind speed at 10 m. U_{10} is then used to estimate K_{600} which is derived from Equation 3 in Table 1. The Schmidt Coefficient (Sc) is a function of surface temperature and denotes the ratio of kinematic viscosity and the diffusion coefficient. The piston velocity (K) is then estimated by using K_{600} and the Sc number (Table 1; Equation 3). The actual gas flux is then an estimate of the piston velocity and the observed oxygen and oxygen at saturation (Table 1; Equation 4). Oxygen saturation was derived as a function of salinity and water temperature (Table 1; Equation 5). Specific conductivity was used to estimate salinity. Oxygen saturation was then corrected for barometric pressure (Table 1; Equation 5).

Daylight can be estimated in a variety of ways. If a photosynthetically action radiation (PAR) sensor is unavailable, day of year and latitude can be used to determine number of daylight hours. However, if a PAR sensor is available, like in this study, hours were assigned a 1 or 0 if there was light or no light, respectively. This allowed for us to know when and what proportion of the day there was sunlight (Table 1; Equation 7).

Once these parameters were found, they were used as the input data for the bookkeeping model in the R package LakeMetabolizer. Data were outputted as mg O_2 L^{-1} d^{-1}. For most of the analysis in this study, metabolism was converted to mg C L^{-1} d^{-1} by dividing the O_2 measurements by 2.666 based on assumption of equimolar changes between the 2 elements (Biddanda et al. 1994).

BOD Measurements

Biological oxygen demand (BOD) method (also called the light – dark bottle method) was employed during seasonal monitoring from 2011 to 2017 in this study. BOD data were collected 3 times annually in the spring, summer, and fall as part of the long-term monitoring effort by Annis Water Resources Institute (AWRI) from 2011 to 2017. In 2017, additional BOD measurements were taken for this study. These sample dates were typically 3 weeks apart for a total of 8 sample dates. The 2011-2017 seasonal sampling was done at 3 locations within the lake, which were averaged to compare to the long-term method, however additional sampling in 2017 was done at the MLO site. Only the spring, summer, and fall was used for this method comparison because winter data was only available for high frequency buoy data, so it was excluded from this analysis. Water was collected at 2 m depth using a niskin bottle and put into a 20L acid-cleaned carboy. Carboys were kept on ice and were in a dark cooler while the

remaining sampling was occurring. Water was dispensed into quadruplicate light, dark, and initial 300 mL BOD bottles and incubated for 24 hours *in situ*. Incubations were done using a suspension system in Muskegon Lake where the bottles were kept at 1 m under the water, so they had the same conditions as they were in the lake (Biddanda et al. 2008). BOD bottles were able to have similar light and temperature conditions using this method.

Dissolved oxygen concentrations were determined with Winkler titrations after incubations using a Radiometer TitraLab(R) 860 automatic titrator with an automatic end point detection using an Ag/AgCl reference electrode (Granéli and Graneli 1991; Biddanda et al. 2001). Quadruplicate BOD bottles were used, and the most outlying replicate was removed from analysis. GPP was estimated from NEP and R using

$$GPP = NEP - R$$

where GPP is the gross primary production, NEP is the net ecosystem production, and R is the respiration.

Unrealistic Terms & Missing Data

Both methods sometimes generated unrealistic terms. For the buoy data, GPP would occasionally be negative and R would occasionally be positive. Typically, when this occurred both terms would be unrealistic on the same day and so that data was not used. This was also done for unrealistic BOD method terms. In addition to unrealistic measurements, buoy data had several missing periods when the buoy was undergoing maintenance or when the buoy was not in the water. These data gaps occur in all 7 years of data. A smoothing method was used to show continuous metabolism during these missing periods.

Statistics

The 7-year BUOY data was broken down into 5 groups to test for significant differences (Table 2). Group 1 was comparing yearly winter data (WG), group 2 was comparing yearly spring data (SG), group 3 was comparing yearly summer data (SUG), and group 4 was comparing yearly fall data (FG). Group 5 was comparing the 7-year seasonal averages to other seasons (SAG) (Table 2). The Season and Year labels are as follows: Winter 2012-2013 (W23), Winter 2015-2016 (W56), Winter 2016-2017(W7), Spring 2011 – 2017 are S1, S2, S3, S4, S5, S6, S7, respectively, Summer 2011 – 2017 are SU1, SU2, SU3, SU4, SU5, SU6, SU7, respectively, Fall 2011 – 2017 are F1, F2, F3, F4, F5, F6, F7, respectively, and Winter, Spring, Summer, and Fall are the 7 year averages for each season. Each of these groups were tested by using a Kruskal-Wallis test. If significant differences were found, a pairwise Wilcoxon test was done to determine what season or year were significantly different from each other. One-Way ANOVA was done to compare the rates of the BOD to BUOY method. Regression analysis was done using a subset of the 7-year BUOY data to understand what drives metabolism. Regression analysis was done with each of the metabolism parameters with water temperature, air temperature, wind, DO, pH, specific conductivity, thermocline depth, colored dissolved organic matter (CDOM), daily peak PAR, and turbidity. There were various missing days for each parameter and outliers more than 3 standard deviations were excluded. Since the regression data were only a subset, it was log transformed before analysis. Another subset of data was used for linear regressions of river discharge, rain, chlorophyll, phycocyanin, and GPP from the BUOY data.

Results

Seven Years of Metabolism

Metabolism was highly variable day to day during all years (Figure 2). The summer had the highest rates for GPP and R each year (Figure 3). Each year the spring had near zero GPP and R rates and they increased into the summer and then decreased in the fall and winter (Figure 3). NEP went from near zero in the spring to positive in the summer and the decreased again in the fall (Figure 3). GPP and R had mirror like daily rates for each year (Figure 4). Even with an increase in the summer, overall NEP remained near zero no matter the season (Figure 4).

Seasonal averages for each year show the same strong seasonal pattern as day to day data for GPP and R and a slight seasonal pattern for NEP (Figure 5). Kruskal Wallis results for WG, SG, SUG, and FG showed a significant difference in WG for GPP, FG for GPP and R, and SAG for GPP, R, and NEP (Table 2). The post-hoc pairwise Wilcoxon test for WG GPP showed that W23 and W7 were significantly different than W56 (Figure 5). GPP averages for W23, W56, and W7 were 0.117, 0.175, and 0.121 mg C L^{-1} d^{-1}, respectively. FG GPP pairwise Wilcoxon test results showed F7 was significantly different than all other years, F1 was significantly different with F2 and F3, and F3 was significantly different with all years except F2 (Figure 5). FG GPP averages for F1, F2, F3, F4, F5, F6, and F7, were 0.298, 0.162, 0.156, 0.272, 0.295, 0.408, and 0.552 mg C L^{-1} d^{-1}, respectively. FG R pairwise Wilcoxon test showed F7 being significantly different than all other years except F6, and F2 and F3 were significantly different than all other years (Figure 5). FG R averages for F1, F2, F3, F4, F5, F6, and F7 were -0.217, -0.102, -0.090, -0.189, -0.980, -0.296, and -0.387 mg C L^{-1} d^{-1}, respectively. Overall seven-year seasonal averages (SAG) for GPP and R pairwise Wilcoxon test showed a significant difference between all the seasons (Figure 6). SAG NEP pairwise Wilcoxon tests showed that winter was significantly different from all seasons (Figure 6).

GPP:R ratios showed interesting patterns for each season. All seasons were generally above the 1:1 ratio line indicating autotrophy. Winter data showed weekly averages near the 1:1 line in a tight cluster, whereas the summer data was more widespread and further from the 1:1 ratio line. Each season had a few days where the weekly average was heterotrophic (Figure 7). GPP:R ratios averaged at 1.42 ± 1.04, 1.37 ± 0.20, 1.50 ± 0.32, and 1.58 ± 0.71 for the winter, spring, summer, and fall, respectively (Figure 7).

Cumulative NEP

Cumulative NEP (CNEP) is a total measure of the biomass produced and respired through the year. Since each year had different starting dates and amounts of missing data, comparing the CNEP from year to year is not possible. Each year shows early spring with near zero rates of CNEP. Typically, early June is when CNEP began to show increasing trends and especially in July and August trends increased sharply (Figure 8). Each year had sharp increases and decreases in CNEP through the whole year. Toward the end of the year, well into the fall, CNEP begins to slow down for each year. The final CNEP totaling (mg C L^{-1} yr^{-1}) were 5.4, 6.1, 5.2, 4.8, 7.2, 6.6, and 5.4 for 2011, 2012, 2013, 2014, 2015, 2016, and 2017, respectively.

BOD & BUOY

Day to day comparisons were done to understand the differences between the BOD and BUOY methods. Day to day comparisons of the BOD and BUOY method showed that it can vary quite widely on a certain day, although generally following similar patterns (Figure 9-11). Overall the BUOY had higher average rates of GPP and R than the BOD method and for NEP the BOD method was typically higher than the BUOY method (Table 3). ANOVA results showed that NEP and R were significantly different between the methods, but GPP was not

significantly different (Table 3). NEP was typically higher with the BOD method than with the buoy method daily and even across the whole average and some days on method would give a positive result and the other method would give a negative result (Figure 11).

In addition to comparing the day to day comparisons, the overall seasonal BUOY averages were estimated to see how the BOD method represents each season. All BOD values were averaged for spring, summer, and fall through the 7 years in addition to the day to day comparisons were averaged and the whole season of buoy data were averages. GPP and R followed similar patterns through each of these averages, but NEP showed an increase with the BOD method in the summer and fall (Figure 12).

Metabolism Drivers

Linear regression was used to determine what MLO environmental parameters were potentially driving metabolism in Muskegon Lake (Table 4). When GPP was predicted, Z_{mix} (β = -0.372, $p < 0.001$, $R^2 = 0.080$) and daily peak PAR ($\beta = 0.195$, $p < 0.01$, $R^2 = 0.038$) were significant predictors (Figure 13). Daily maximum peak PAR and GPP follow the same seasonal pattern (Figure 14). R had significant relationships with water temperature ($\beta = -0.878$, $p < 0.01$, $R^2 = 0.037$), thermocline depth ($\beta = -0.409$, $p < 0.001$, $R^2 = 0.079$), and daily peak PAR (β = 0.157, $p = 0.039$, $R^2 = 0.020$). NEP had the most significant relationships with water temperature ($\beta = 0.579$, $p < 0.001$, $R^2 = 0.111$), air temperature ($\beta = 0.199$, $p = 0.023$, $R^2 = 0.024$), pH (β = 2.786, $p < 0.001$, $R^2 = 0.096$), and turbidity ($\beta = 0.115$, $p = 0.019$, $R^2 = 0.026$). The relationships that were significant had rather small R^2 values (Figure 13). Regression figures show the relationship with the predictors that were significant with each of the metabolism parameters (Figure 13).

Linear regression was also done with production (GPP, chlorophyll, phycocyanin) and precipitation (daily rain, rain accumulation, and river discharge). GPP had significant relationships with chlorophyll ($\beta = 0.203$, $p = 0.028$, $R^2 = 0.017$), phycocyanin ($\beta = 0.266$, $p < 0.001$, $R^2 = 0.058$), and river discharge ($\beta = -0.507$, $p < 0.001$, $R^2 = 0.456$) (Table 5). We then used chlorophyll and phycocyanin as the production response variables with rain and river discharge as the variables. Both chlorophyll and phycocyanin had significant relationships with accumulated rain (Chl: $\beta = 0.111$, $p < 0.01$, $R^2 = 0.027$; Phyco $\beta = 0.374$, $p < 0.001$, $R^2 = 0.152$) and river discharge (Chl: $\beta = -0.617$, $p < 0.001$, $R^2 = 0.164$; Phyco $\beta = -1.195$, $p < 0.001$, $R^2 = 0.309$) (Table 5). River discharge had the strongest relationship with all the production variables, despite it being a negative relationship (Figure 15). Chlorophyll and phycocyanin varied in their peak date depending on the year and spring rainfall (Figure 16).

Missing Data

Time series data often has periods of missing data. MLO is only typically in the water for only 6-7 months of the year due to difficulties with ice and boat logistics in the winter months. For 3 of the years we were able to deploy part of the buoy in the winter. Metabolism data were available from May 27th – December 11th 2011, April 7th – December 31st 2012, January 1st – November 3rd 2012, June 1st – November 1st 2014, May 2nd – December 31st 2015, January 1st – October 8th 2016, and January 1st – October 19th 2017. Within each of these date ranges are several gaps of missing data. In 2013, 2016, and 2017 there were a larger data gap between winter retrieval and spring deployment of the buoy. The equipment had to be serviced before and fully equipped to transition from winter to summer deployments. Other missing days in each

year is likely due to equipment temporarily being serviced for various reasons or for unrealistic values.

Discussion

Different Methods of Estimating Metabolism

Metabolism was originally measured in wastewater for sanitary purposes to understand the microbial metabolism of organic compounds in water. Later it was used by Odum (1956) in aquatic ecosystems. The carbon-14 method was also used. With the advent of oxygen sensors, more data was being collected in less time than with the BOD method. Remote sensing technologies are now also being used along with models to estimate metabolism other ways. This study has shown that metabolism rates between the BOD and BUOY method can vary greatly. Day to day comparisons showed that the BOD and BUOY method typically mirrored each other but could vary by many fold, depending on the day (Figures 9-11). GPP and R were both higher with the BUOY method. The R rates were significantly higher with the BUOY method which leads to the assumption that the BUOY method overestimates R. Since R was overestimated with the BUOY method, this led the NEP rates to be underestimated with the BUOY method. Interestingly, there were days where the BOD method would show a positive NEP but the BUOY method showed a negative NEP. Overall, the BOD method had significantly higher NEP rates compared to the buoy method. The average seasonal comparison of GPP and R showed similar results for each seasons average (Figure 12). Seasonal averages of NEP showed the spring to be practically the same, but the summer and fall were vastly different (Figure 11). These differences are likely influenced by the free water method accounting for atmospheric exchange and the number of days for each method. LaBuhn and Klump (2016) used both BOD

bottles and BUOY method and found the BOD sample compared well with the BUOY monthly average.

Each method had pros and cons. The BOD method has been classically used for over 50 years and is able to provide reliable biological signals for the ecosystem and is also used heavily for industrial wastewater treatment plants for water quality purposes. The method unfortunately suffers from container effects – where it cannot account for any physical processes within the ecosystem. The BOD method is also heavily labor intensive and infrequent measurements are often taken. The BUOY method has been used heavily for 25 years and is able to collect daily rates of metabolism over a longer period of time since the sensors are always collecting data. Therefore, with the BUOY method there is less work to do on a daily basis, but this data is not always as reliable. The sensors often malfunction and at times physical processes, such as wind which causes mixing, interprets the biological oxygen levels resulting in unrealistic GPP and R rates; therefore, the BUOY method results in more data overall, but it is not always reliable. Overall, if a long-term study is being done, the BUOY is the appropriate method and for a short term study the BOD method is appropriate.

Drawing Global Comparisons to Other Inland Waters and Ecosystems

Ecosystem metabolism can vary widely depending on the type of ecosystems, location, nutrient availably, or subtype of ecosystem (Table 6). Net ecosystem production in the lakes that are oligotrophic typically varies from 18-109 g C m^{-2} yr^{-1}, mesotrophic lakes vary from 90-365 g C m^{-2} yr^{-1}, and eutrophic lakes are typically greater than 365 g C m^{-2} yr^{-1} (Schlesinger and Bernhardt 2013). Lawrence Lake in Michigan and Mirror Lake in New Hampshire fall in at 191.4 g C m^{-2} yr^{-1} and 87.5 g C m^{-2} yr^{-1}, respectively (Schlesinger and Bernhardt 2013). The

ocean can vary in net ecosystem production from 130 to 420 g C m^{-2} yr^{-1} in the open and upwelling zones of the ocean, respectively (Schlesinger and Bernhardt 2013; Table 9.2). Seasonal changes like those seen in this study also occur in ocean production which can range from 10.9 to 13.0 Pg C per season (Field et al. 1998). Tropical forests have the highest rates of net ecosystem production at 1250 g C m^{-2} yr^{-1}, whereas the arctic tundra has the least with 90 g C m^{-2} yr^{-1} (Schlesinger and Bernhardt 2013; Table 5.3). These global ecosystem NEP rates can be vastly different depending on where they are in relation to the three distinct latitudinal bands (Field et al. 1998). In Muskegon Lake, the seven-year average of NEP for each season was found and then multiplied by the number of days in that season for a sum of all NEP for the year at 41 g C m^{-2} yr^{-1}. This is a rather conservative estimate since not every day was accounted for. Using the BOD method, the seven-year average annual NEP was 312 g C m^{-2} yr^{-1}. The vast differences between the BUOY and BOD method when comparing the NEP annually is a result of number of samples taken each year, container effects which influence the gas exchange in the BOD method, or model used to estimate NEP with the BUOY method, which can yield different results (McNair et al. 2013; Winslow et al. 2016). In addition, the BUOY method is quite different from the previously stated global comparisons for NEP in different lakes and this may be due to the difference in methods used in those averages. The BOD method lines up nicely with the mesotrophic NEP previously stated and that is likely due to use of similar methodologies. NEP is shown in this study to have higher rates using the BOD method versus the BUOY method (Figures 11-12). Other estuary systems around the world can have primary production rates from 29 to 603 g C m^{-2} y^{-1} depending on the location (Caffrey et al. 2014). Globally, Muskegon Lake estimates of GPP sit relatively in the middle with time of year and method taken into consideration (Table 6). Other estuaries have pelagic GPP ranging from 24 to

1800 mg C m^{-2} d^{-1} (Azevedo et al. 2006) whereas the Muskegon Lake freshwater estuary GPP ranged from 17 to 1900 mg C m^{-2} d^{-1} in the seven years of this study. Nevertheless, inland waters are disproportionately important compared to other ecosystems in the global carbon budgets since they bury more carbon than oceans and have high rates of respiration (Biddanda 2017; Battin et al. 2009; Cole et al. 2007).

Potential Drivers of Lake Metabolism

Ecosystem metabolism can vary widely between types of ecosystems and even types of inland waters. A number of variables, such as timeliness of nutrient cycling, residence time, vegetation, and watershed size, are known to contribute to the range of GPP rates globally (Hoellein et al. 2013). Metabolism estimates can vary depending on the time of year measurements are taken or by the method used (Table 6). Some have found a distinct increase in GPP from headwaters to the ocean, with streams being the lowest, then wetlands and ponds, lakes, and finally estuaries due to systematic changes in the variables listed above (Odum 1956; Hoellein et al. 2013). Most inland water types can be characterized as heterotrophic, with exceptions; however, estuaries are typically characterized half the time as autotrophic and half the time as heterotrophic (Hoellein et al. 2013). Although sometimes these metabolism drivers are not clear. For example, dissolved organic carbon (DOC) can both limit primary production or stimulate primary production by limiting light and adding nutrients, respectively (Seekell et al. 2015). R increases with increased DOC resulting in more heterotrophic lakes. Globally DOC is increasing in lakes making the threshold an important factor when considering inland waters role in global carbon cycles (Seekell et al. 2018; Zwart et al. 2016). A lake wide experiment of increasing DOC gradually showed a slight increase in GPP (due to more phosphorus) but also an

increase in R at nearly the same rate as DOC was being added. This increase in DOC resulted in a more heterotrophic lake (Zwart et al. 2016). Muskegon Lake is currently considered autotrophic in the mixed layer; however, depending on land use change in the watershed, it could continue to be autotrophic or become heterotrophic annually depending on DOC inputs.

Interestingly, metabolism variables were not strongly related to many of the individual parameters from the buoy data (Table 4). In this study GPP had a significant relationship with mixed depth layer and peak daily PAR, R had a significant relationship with water temperature, mixed layer depth, and peak daily PAR, and NEP had a significant relationship with water temperature, air temperature, pH, and turbidity (Table 4, Figure 13). NEP and turbidity had a positive relationship, as increasing NEP would lead to increases in particulate matter or algae causing autocorrelation between NEP and turbidity. Other studies found a significant relationship of GPP and water temperature in one estuary they were studying but not in another (Caffrey et al. 2014). We found it rather surprising we didn't find a relationship between GPP and temperature in the present study since GPP shows a strong seasonal pattern (Figure 3 and 4). However, we found a positive trend of water temperature and NEP which tends to agree with the slight seasonality of NEP becoming more autotrophic in the summer, but others found a negative trend of water temperature and NEP where the metabolism became more heterotrophic as water temperature increased (Caffrey et al. 2014). Perhaps these opposite findings are due to other environmental parameters in each watershed. We did see a strong relationship of GPP and R with daily maximum PAR and GPP and peak PAR followed closely seasonally (Figure 14). The mixed depth layer had a negative relationship with GPP and R (Figure 13). This result means that during summer months when the lake is stratified with a shallower mixed layer depth GPP and R are higher.

Regression analysis showed a significant relationship with GPP and chlorophyll, GPP and phycocyanin, and GPP with river discharge. A significant relationship was also found between chlorophyll and accumulated rain, chlorophyll and river discharge, phycocyanin and accumulated rain, and phycocyanin and river discharge (Table 5). Interestingly, each of the variables had a negative relationship with the river discharge. Azevedo et al. (2006) also found a negative relationship with river discharge and phytoplankton in a Portugal estuary. There may be a delay in the amount of rain or river discharge with and the response in Muskegon Lake production to the influx of water temporarily diluting the nutrient concentration or because of lower residence times. DOC decreases with high precipitation years because of lower residence times (de Wit et al. 2018) which may be why production was negatively related to discharge. Spring rainfall (rain before July) may influence algal blooms in Muskegon Lake. The average rainfall before July was 9.7 inches (Figure 17). It should be noted for these rainfall totals that each year had variable amounts of missing data and that could by why some years have more or less data. High discharge may make production increase later in the year after the residence time has increase again. Figure 16 shows a ~15-day lag time in phycocyanin spike after a rainfall even in the spring in 2011 and 2017. The other years did not have as large of rain in events in the spring (Figure 16), except in 2014.

Peak Dates

Peak GPP rates occurred as early as June 25th through September 18th throughout the 7 years of data. The highest peak GPP rate was 2.75 mg C L^{-1} d^{-1} on August 12th, 2015. There were 3 years when the GPP and R peak rates were the same day and with both instances the peak air temperature occurred within to 2 weeks of the peak GPP and R date. The highest peak NEP rate

was in 2017 at 1.02 mg C L^{-1} d^{-1}. Peak NEP and R dates ranged from July to September. In several of the years, peak NEP would be 1 or 2 weeks behind peak GPP and R. Peak water and air temperature were always in July or August, and the peak metabolism data was typically in August or September. Others found that peak NEP occurred in May before the summer solstice, that GPP peaked at the same time as water temperature, and respiration peaked several weeks after the water temperature peak (Laas et al. 2012). With a 7-year average of daily maximum peak PAR and GPP, we can see that peak GPP and peak PAR follow each other closely (Figure 14).

Unrealistic Estimates

There were several days of missing data throughout the 7 years due to sensor issues or unrealistic data. There were several days each year which metabolism estimates returned impossible values. Impossible values are negative GPP (oxygen consumption during the day) and positive R (oxygen production during the night) values show up. These days are likely from days where physical drivers, such as mixing and advection, were causing DO to change more rapidly than the biological signal could or there are unexpected increases od DO at night (Staehr et al 2010; Winslow et al. 2016). Many metabolism studies find the same issue with the GPP and R values and they are typically excluded from the study (Winslow et al. 2016; Brothers et al. 2017). Winslow et al. (2016) suggests to either remove the unrealistic estimates or run the metabolism model constrained where GPP and R are forced to be positive. Either way, the accuracy for both approaches will lead to bias in the data (Winslow et al. 2016). Brothers et al. (2017) compared the methods of excluding these data and including these data and found that

exclusion may lead to underestimations of metabolism. Many metabolism studies have excluded such data (Staehr et al. 2010), and we followed a similar protocol in the present study.

Management Implications

While this is mostly a basic science study, it does have several potential applications to land and water managers. Ecosystem metabolism studies can give insights into the net production that is fueling hypoxia during the summer months and driving cyanobacterial blooms during summer and fall season. Since Muskegon Lake is directly connected to Lake Michigan, nearshore water quality in the larger lake could be impacted by discharge from Muskegon Lake and its watershed. For example, contaminants from watershed runoff could cause some nearshore issues such as beach closures. On the other hand, net productivity in Muskegon Lake being transported out, may actively support nearshore food webs in lake Michigan. All of these positive and negative effects could be somewhat regulated by proper land use management in the watershed (Allen et al. 2013; Scavia et al. 2014). If excess nutrients, contaminants and organic matter runoff and loading is limited, that could potentially help in mitigating water quality deterioration and excessive production - which in turn creates algal blooms and excess respiration in bottom waters causing hypoxia in Muskegon Lake. In addition, Muskegon Lake has been an AOC since 1985, and the Muskegon Lake buoy is a way for continued monitoring while the lake is on the AOC list and after it has been removed. The high frequency data will allow managers and scientist to know the current status of the lake's restoration in comparison to the past.

Conclusion

Understanding carbon dynamics in the world's ecosystems will be a vital part of future global change research. Sensor technology has allowed for higher frequency of metabolism studies in inland waters using the diel oxygen dynamics. Muskegon Lake had traditional BOD data and high frequency buoy data available from 2011 to 2017. Using this data, we were able to see the seasonal averages fluctuate throughout the years. GPP averages (±SD) went from 0.141 ± 0.13, 0.476 ± 0.33, 0.815 ±0.50, and 0.287 ± 0.31 mg C L^{-1} d^{-1} in the winter, spring, summer, and fall, respectively. R averages (±SD) went from -0.114 ± 0.12, -0.349 ± 0.25, -0.562 ±0.38, and -0.197 ± 0.23 mg C L^{-1} d^{-1} in the winter, spring, summer, and fall, respectively. GPP averages (±SD) went from 0.002 ± 0.08, 0.023 ± 0.16, 0.049 ±0.28, and 0.022 ± 0.18 mg C L^{-1} d^{-1} in the winter, spring, summer, and fall, respectively. Cumulative NEP reached 7.23 mg C L^{-1} d^{-1} in 2015. Cumulative NEP totals hovered near zero, and sometimes were even negative, until mid-spring where it began to become positive increasing more rapidly in the middle of the summer until late fall when it ceased increasing as quickly. Although the average NEP showed net autotrophy year-round, there were several days of negative NEP in all seasons. There were significant differences in BOD and buoy estimates of NEP and R, but not GPP. Regression models revealed NEP was most positively correlated with water temperature, air temperature, turbidity, and pH. GPP had significant positive relationship with peak daily PAR and a negatively significant relationship with mixed layer depth. R was also negatively related to mixed layer depth and water temperature but had a significant positive relationship with mixed layer depth. Additionally, productivity variables (GPP, chlorophyll, and phycocyanin) had a significant negative relationship with river discharge. Overall Muskegon Lake is an important water body in the Great Lakes region and represents similar freshwater estuaries around the world wherein similar metabolic phenomena may be occurring with seasonal and spatial

variability. We now have further understanding that this water type is generally net autotrophic in the mixed layer, but has strong seasonal patterns, and annually incorporates more carbon into the water body than is respired to the atmosphere. If other freshwater ecosystems follow similar patterns as Muskegon Lake, this could mean inland waters overall are sinks of carbon and potentially drawing more carbon from the atmosphere than they are releasing.

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Figure Legends

Figure 1. Map of Muskegon Lake with the Muskegon Lake Observatory (MLO) labeled as well as the main Muskegon River inlet, Bear Lake inlet, and the outlet channel leading to Lake Michigan.

Figure 2. Sample of metabolism data (from August 7th – August 20th, 2016) for Muskegon Lake by the BUOY method.

Figure 3. Smoothed daily metabolism for each year.

Figure 4. Smoothed daily rates of metabolism for each year 2011-2017.

Figure 5. Gross primary production (GPP; top), Respiration (R; middle), and Net Ecosystem Production (NEP; bottom) box and whisker plot of the seasonal and yearly averages for the 7 years of MLO BUOY data. Each season was considered a different group and tested for significance. Symbols above the figure represent significant differences in the winter and fall data.

Figure 6. 7-year seasonal averages for winter, spring, summer, and fall gross primary production (GPP; left), net ecosystem production (NEP; middle), and respiration (R; right). Symbols above the figure represent statistical significance, if seasons have different symbols, they are significantly different and if they share a symbol, they are not significantly different.

Figure 7. Weekly average GPP:R ratios for 2011-2017 during each of the seasons.

Figure 8. Cumulative net ecosystem production (CNEP) for each year. Three years (2013, 2016, and 2017) all began in January and the other years started in around May.

Figure 9. Gross primary production (GPP) biological oxygen demand (BOD) and buoy metabolism data for the same day. The 1st data point was typically taken in May, the 2nd in July, and the 3rd in September.

Figure 10. Respiration rates of metabolism using the biological oxygen demand (BOD) and buoy data methods. Each data point represents the same day for each method.

Figure 11. Net ecosystem production (NEP) rates for the biological oxygen demand (BOD) and buoy metabolism methods. Each data point represents the same day of data in May, July, and September.

Figure 12. Gross primary production (GPP) seasonal averages for the biological oxygen demand (BOD), average day to day match buoy data (Buoy DA), and total seasonal average (Buoy SE) data. All 3 versions follow a similar pattern increasing in the summer and returning to spring rates in the fall.

Figure 13. Linear regression of log transformed gross primary production (GPP; left row), net ecosystem production (NEP; middle row), and respiration (R; right row) (metabolism units mg C

L^{-1} d^{-1}) with air temperature (C), water temperature (C), max daily PAR (µmol/s/m²), Zmix (m), turbidity, and pH. Figures with R^2 and p values indicate significant relationships.

Figure 14. The seven-year average daily maximum PAR (µmol/s/m²) and GPP.

Figure 15. Log transformed gross primary production (GPP; mg C L^{-1} d^{-1}), chlorophyll (Chl; ug/L), and phycocyanin (Phyco; cells/mL) relationship with river discharge (ft^3/sec).

Figure 16. Chlorophyll (ug/L) and phycocyanin (cells/mL) daily averages for each year. Spring rain (inches) totals before July are in the top right corner for each plot.

Table 1: Required equations and parameters needed for estimating metabolism.

Equation Number	Parameter	Equation	Description	Observations Required
1	Schmidt Coefficient	$Sc=0.0476\ T^3+3.7818\ T^2-120.1\ T+1800.6$	Sc is used in estimating the gas flux.	Water temperature (T, °C) at DO sensor (units)
2	Wind speed at 10 m	$U_{10} = U_z \times 1.4125 \times z^{-0.15}$	U_z is the wind speed at the measured spot and z is the height of the sensor	Wind speed (U_z; m/s) Sensor height (z; m)
3	Piston velocity	$K_{600} = (2.07 + 0.215\ U_{10}^{1.7})/100$ $K = k_{600}\ x\ ([Sc/600]^{-0.5})$	Part of calculating piston velocity as a function of wind speed at 10 m above the surface. The piston velocity is a function of wind and temperature.	Wind speed (U_{10}; m/s) at 10 m
4	Physical Gas Flux	$F = k\ (O_{2meas} - O_{2sat})$	Using the piston velocity derived from wind speed and temperature along with the oxygen saturation and oxygen measured the gas flux can be calculated.	K (piston velocity; equation 3)
5	Oxygen Saturation	$O_{2sat\ (mg/L)} = (e^c) \times 1.423$ mg O_2 $C\ (ml\ O_2\ L^{-1}) = (-173.4292+249.6339 \times (100 / T) + 143.3483 \times In(T / 100) - 21.8492 \times (T / 100) + S \times [-0.033096 + 0.014259 \times (T / 100) - 0.0017 \times (T / 100)^2]$ Correction factor = $(BP \times 0.0987 - 0.0112)/100$ $O_{2sat\ (mg/L)}$ correction = $O_{2sat\ (mg/L)} \times$ correction factor	Oxygen saturation as a function of salinity and temperature. It was then corrected using barometric pressure. It could also be corrected using altitude if barometric pressure is not available. Salinity was found through measured conductivity.	Water temperature (T, kelvin) Salinity (S, estimated from conductivity) Barometric pressure (mbar)
6	Measured Oxygen	O_{2meas}	Measured from oxygen sensors in the water column	Dissolved oxygen (mg/L)
7	Dayfraction	Number of daylight hours Number of nighttime hours	Daylight hours	Photosynthetically Active Radiation

Table 2: Groups that were statistically tested. The first Groups 1-4 compared each year within the same season and Group 5 compared the 7-year average for each season. The Season and Year labels are as follows: Winter 2012-2013 (W23), Winter 2015-2016 (W56), Winter 2016-2017(W7), Spring 2011 – 2017 are S1, S2, S3, S4, S5, S6, S7, respectively, Summer 2011 – 2017 are SU1, SU2, SU3, SU4, SU5, SU6, SU7, respectively, Fall 2011 – 2017 are F1, F2, F3, F4, F5, F6, F7, respectively, and Winter, Spring, Summer, and Fall are the 7 year averages for each season. Bold indicates a significant difference and if there was a significant difference, a post hoc pairwise Wilcoxon test was done to determine which of the seasons and years within the groups were significantly different. These results are in Figures 5 (WG, SG, SUG, FG) and 6 (SAG).

Group	Season & Year	GPP	R	NEP
WG	W23, W56, W7	**<0.001**	0.293	0.889
SG	S1, S2, S3, S4, S5, S6, S7	0.290	0.353	0.977
SUG	SU1, SU2, SU3, SU4, SU5, SU6, SU7	0.243	0.344	0.981
FG	F1, F2, F3, F4, F5, F6, F7	**<0.001**	**<0.001**	0.705
SAG	Winter, Spring, Summer, Fall	**<0.001**	**<0.001**	**<0.01**

Table 3: Day to day comparison of rates of Metabolic parameters (GPP, NEP and R) derived from the BOD and MLO methods for 2011-2018. An ANOVA test was done to determine if there was a significant difference between the means of the two methods. ANOVA found a significant difference for NEP and R values between the BOD and BUOY method but not for GPP rates. These are averages (±SE) of the BOD and BUOY method for each metabolism rate in ug C L^{-1} d^{-1}.

	Method		ANOVA
MET	**BOD**	**BUOY**	**P-Value**
GPP	332.3±49.5	471.2±75.2	0.115
R	117.6±17.7	337.2±54.2	**<0.001**
NEP	214.7±42.9	2.80±41.6	**<0.001**

Table 4: Regression analysis for various water quality parameters and each of the metabolism variables.

	Variable	R^2	p-value			Intercept	Fstat	df	Beta
			Model	Intercept	Slope				
GPP	Water Temp	<0.001	0.646	0.991	0.646	-0.010	0.212	1, 210	-0.131
	Air Temp	<0.001	0.979	0.519	0.979	-0.402	<0.001	1, 210	-0.005
	Wind	0.002	0.547	**<0.01**	0.547	-0.541	0.363	1, 210	0.075
	DO	0.016	0.062	**0.024**	0.062	-2.347	3.513	1, 210	0.873
	pH	0.007	0.225	0.178	0.225	-4.216	1.479	1, 210	1.775
	SpCond	<0.001	0.892	0.795	0.892	-0.877	0.018	1, 210	0.078
	Zmix	0.080	**<0.001**	**<0.001**	0.262	0.158	18.27	1, 210	-0.372
	CDOM	0.003	0.427	0.646	0.427	-0.154	0.633	1, 210	-0.074
	Peak PAR	0.038	**<0.01**	**<0.001**	**<0.01**	-1.487	8.290	1, 210	0.195
	Turbidity	0.013	0.099	**<0.001**	0.098	-0.533	2.730	1, 210	0.192
R	Water Temp	0.037	**<0.01**	0.054	**<0.01**	1.865	8.045	1, 210	-0.878
	Air Temp	0.006	0.261	0.884	0.261	-0.100	1.269	1, 210	-0.258
	Wind	0.011	0.128	**<0.001**	0.128	-1.214	2.339	1, 210	0.209
	DO	0.011	0.119	**0.021**	0.119	-2.659	2.450	1, 210	0.809
	pH	0.002	0.549	0.728	0.549	1.206	0.359	1, 210	-0.971
	SpCond	<0.001	0.750	0.932	0.750	0.318	0.102	1, 210	-0.203
	Zmix	0.079	**<0.001**	0.129	**<0.001**	-0.237	18.01	1, 210	-0.409
	CDOM	<0.001	0.917	**0.026**	0.917	-0.834	0.011	1, 210	-0.011
	Peak PAR	0.020	**0.039**	**<0.001**	**0.039**	-1.733	4.302	1, 210	0.157
	Turbidity	0.001	0.598	**<0.001**	0.597	-0.912	0.279	1, 210	0.068
NEP	Water Temp	0.111	**<0.001**	**<0.001**	**<0.001**	-2.025	26.09	1, 210	0.579
	Air Temp	0.024	**0.023**	**<0.01**	**0.022**	-0.810	5.261	1, 210	0.199
	Wind	0.013	0.098	0.393	0.098	-0.075	2.767	1, 210	-0.087
	DO	0.012	0.108	**0.036**	0.108	-0.921	2.611	1, 210	0.319
	pH	0.096	**<0.001**	**<0.001**	**<0.001**	-6.178	22.43	1, 210	2.786
	SpCond	0.006	0.272	0.211	0.272	-1.782	1.213	1, 210	0.266
	Zmix	<0.001	0.864	**<0.001**	0.864	-0.227	0.029	1, 210	-0.007
	CDOM	0.003	0.378	0.515	0.378	-0.092	0.779	1, 210	-0.035
	Peak PAR	0.012	0.107	**<0.01**	0.107	-0.474	2.617	1, 210	0.047
	Turbidity	0.026	**0.019**	**<0.001**	0.019	-0.285	5.614	1, 210	0.115

Table 5: Production (GPP, Chl, and Phyco), rain and river discharge linear regression analysis. Gross primary production (GPP) was analyzed with chlorophyll (Chl), phycocyanin (PHYCO), daily rain (Rain), accumulated rain (Rain Ac), and river discharge. Then Chl and Phyco were analyzed with rain, accumulated rain, and river discharge. Bold indicates significant p-values.

Variable	**Predictor**	**R^2**	**p-value**			**Intercept**	**Fstate**	**Df**	**Beta**
			Model	Intercept	Slope				
GPP	Chl	0.017	**0.028**	**<0.001**	**0.028**	-1.137	4.895	1, 284	0.203
	PHYCO	0.058	**<0.001**	**<0.001**	**<0.001**	-2.882	17.54	1, 284	0.266
	Rain	0.001	0.503	**<0.001**	0.503	-0.718	0.451	1, 284	-0.179
	Rain Ac	0.002	0.448	**<0.01**	**<0.001**	3.012	13.57	1, 284	-0.507
	Discharge	0.456	**<0.001**	**<0.01**	**<0.001**	3.012	13.57	1, 284	-0.507
Chl	Rain	0.006	0.181	**<0.001**	0.181	1.923	1.8	1, 284	0.229
	Rain Ac	0.027	**<0.01**	**<0.001**	**<0.01**	1.719	7.76	1, 284	0.111
	Discharge	0.164	**<0.001**	**<0.001**	**<0.001**	6.526	55.65	1, 284	-0.617
Phyco	Rain	<0.001	0.884	**<0.001**	0.884	8.033	0.022	1, 284	0.352
	Rain Ac	0.152	**<0.001**	**<0.001**	**<0.001**	7.237	50.69	1, 284	0.374
	Discharge	0.309	**<0.001**	**<0.001**	**<0.001**	16.881	127	1, 284	-1.195

Table 6: Global comparisons of metabolism with MLO and other lakes or inland waters. BUOY is using the free water dissolved oxygen method from oxygen sensors in the water, BOD is using the biological oxygen demand method using light and dark bottles incubated for 24 hours and the Carbon-14 method is using radio isotopes to estimate metabolism.

GPP (mg C m^{-2} d^{-1})	**Location or ecosystem type**	**Method**	**Year**	**Reference**
21	Lake Michigan	BOD	July/August 2002-2013	Weinke et al. 2014
34	Lake Michigan	BOD	April/May 2010-2011	Dila & Biddanda. 2015
100	Open Ocean			Schlesinger 1991; Figure 9.7
372	Lake Superior	Carbon -14	Summer 2006-2008	Sterner. 2010
576	Emerald Lake	Incubation Chambers	July – Nov 2008	Sadro. et al. 2011
624	Muskegon Lake	BOD	Feb 2009 - Feb 2010	Defore et al. 2016
648	Lake Huron Wetlands	BUOY	May – Dec	Cooper et al. 2013
660	Emerald Lake	BUOY	July – Nov 2008	Sadro. et al. 2011
696	Crampton Lake	BUOY	June – Aug 2005	Coloso et al. 2008
1872	Muskegon Lake	BOD	April/May 2012-2011	Dila & Biddanda. 2015
1872	UK Colne Estuary	Carbon – 14	August 1995	Kocum et al. 2002
2004	Muskegon Lake	BOD	May – Sept 2011-2017	**This study**
2808	Muskegon Lake	BUOY	Jan – Dec 2011 -2017	**This study**
3012	Muskegon Lake	BOD	July/August 2002-2013	Weinke et al.2014
3288	Wetland/Pond	BUOY	June – August	Hoellein et al. 2013
3456	Green Bay	BUOY	June – Sept 2013-2015	Labuhn & Klump. 2012
5785	Lake	BUOY	June – August	Hoellein et al. 2013
10694	Castle Lake	BUOY	June – Aug 2004	Staehr & Sand-Jensen. 2007
10790	Estuary	BUOY	June – August	Hoellein et al. 2013
4296-15098	Nearshore Green Bay	BUOY	2010-2011	Althouse et al. 2014
8293-12854	Chesapeake Bay	Carbon – 14	Summer 1969 – 1970	Taft et al. 1980
20-5620	Streams & Rivers	Various	Various	Schlesinger and Bernhardt 2013; Table 8.7
720-10400	Estuaries	Various	Various	Schlesinger and Bernhardt 2013 Table 8.7
2500-4500	Shelf and Slope Gulf of Mexico	BOD	Various	Biddanda et al. 1994

Figure 1

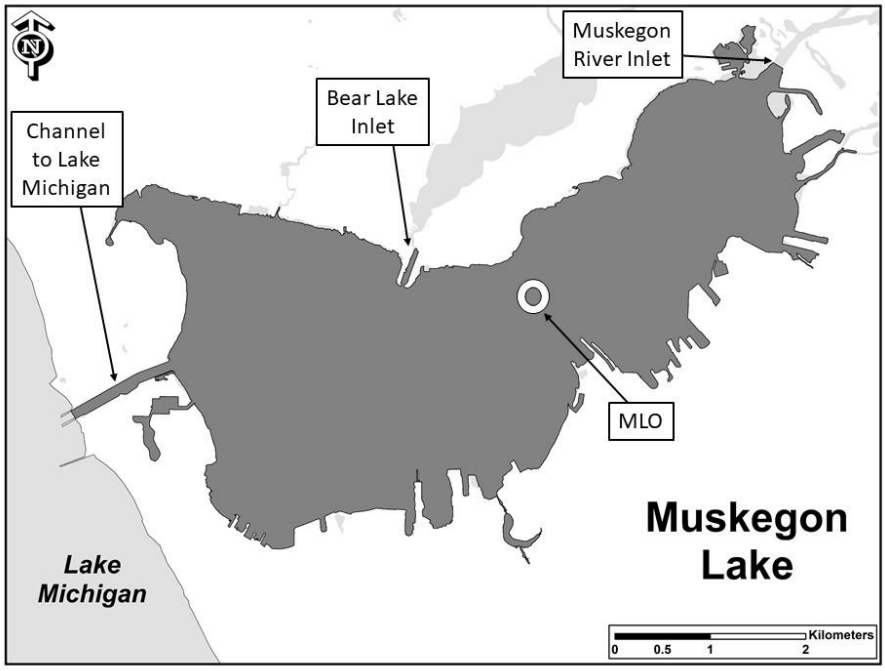

Muskegon
River Inlet
Bear Lake
Inlet
Channel
to Lake
Michigan
MLO
Muskegon
Lake
Lake
Michigan
Kilometers
0
0.5
1
2

Figure 2

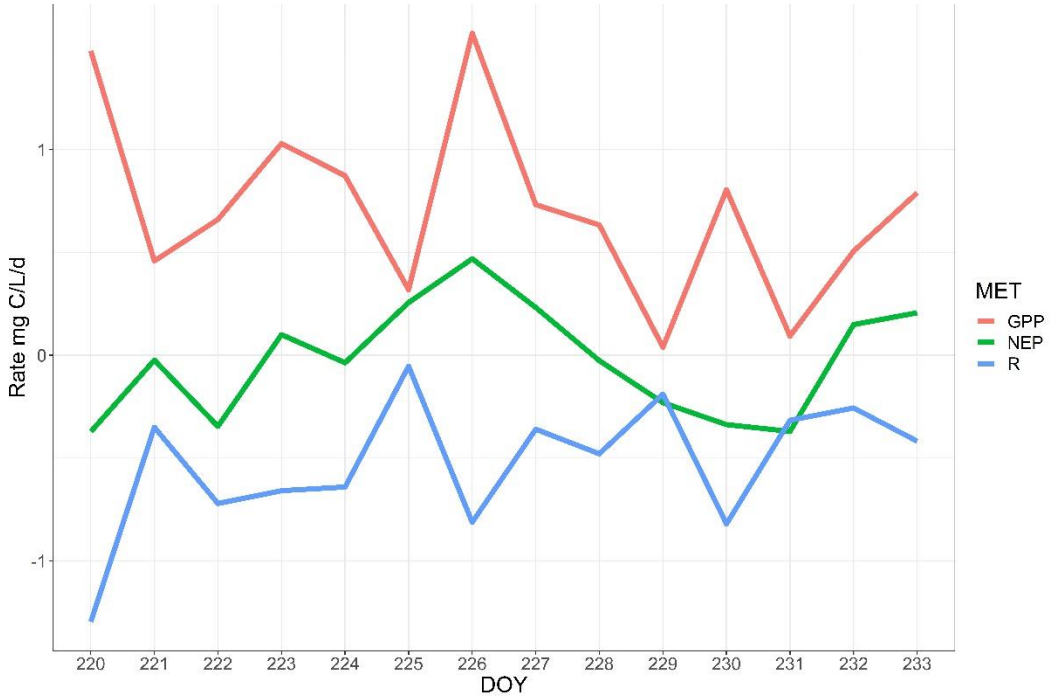
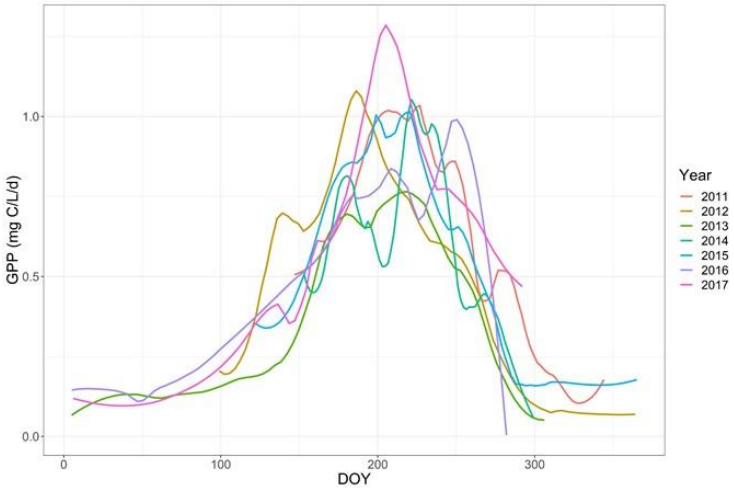
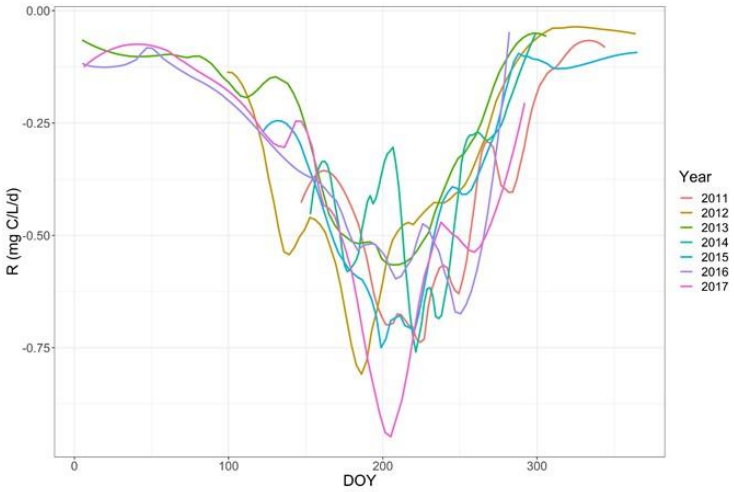

Rate mg C/L/d
1
0
-1
220
221
222
223
224
225
226
227
228
229
230
231
232
233
DOY
MET
GPP
NEP
R

Figure 3


GPP (mg C/L/d)
1.0
0.5
0.0
0
100
200
300
DOY
Year
2011
2012
2013
2014
2015
2016
2017


R (mg C/L/d)
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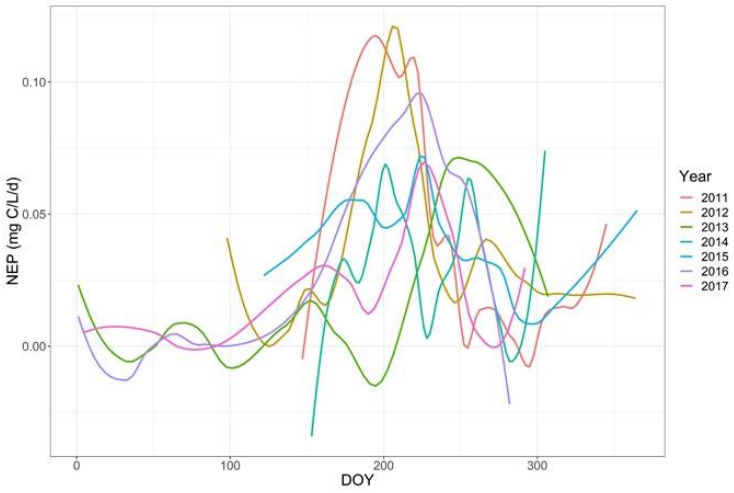
-0.75
0
100
200
300
DOY
Year
2011
2012
2013
2014
2015
2016
2017


NEP (mg C/L/d)
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0.00
0
100
200
300
DOY
Year
2011
2012
2013
2014
2015
2016
2017

Figure 4

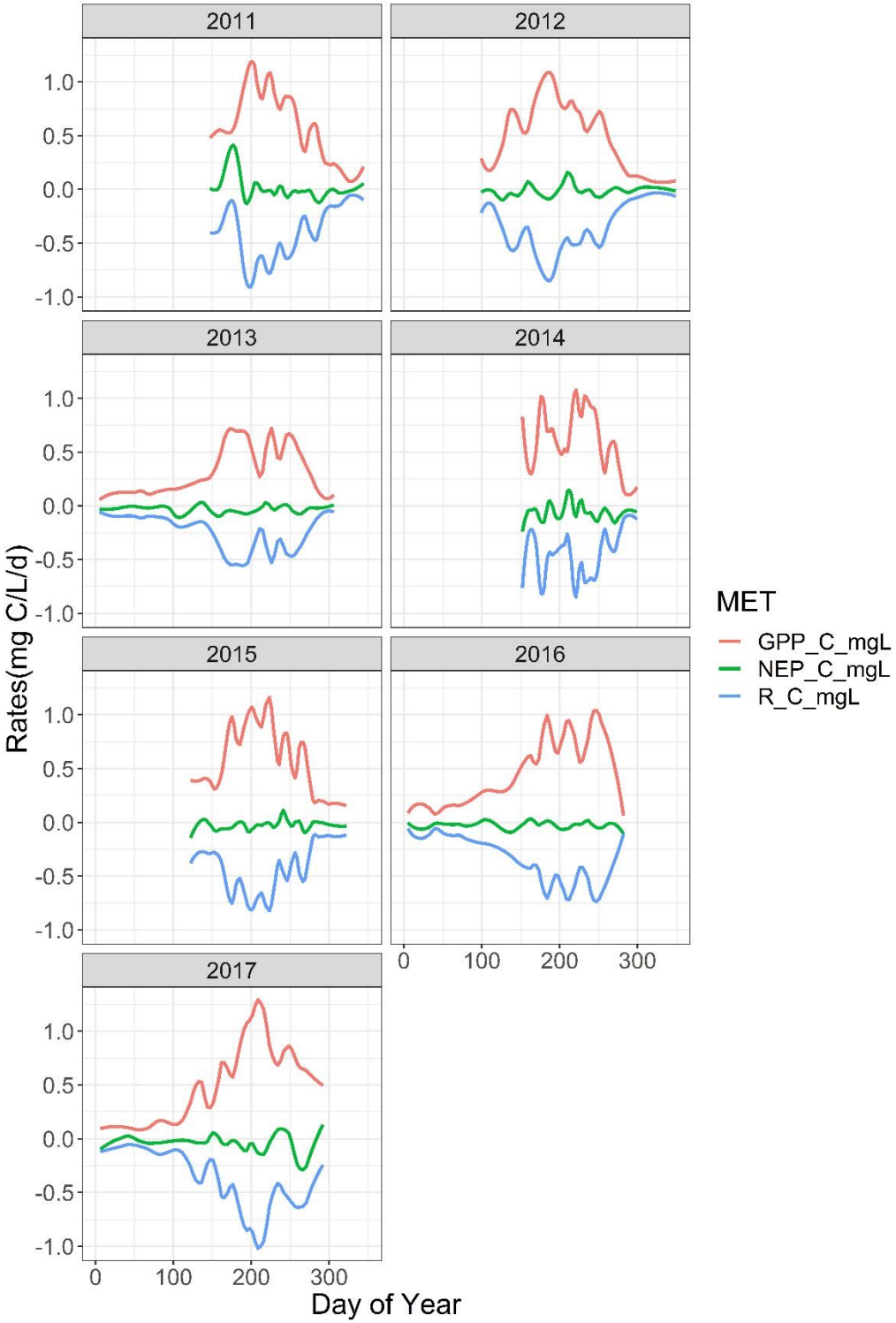
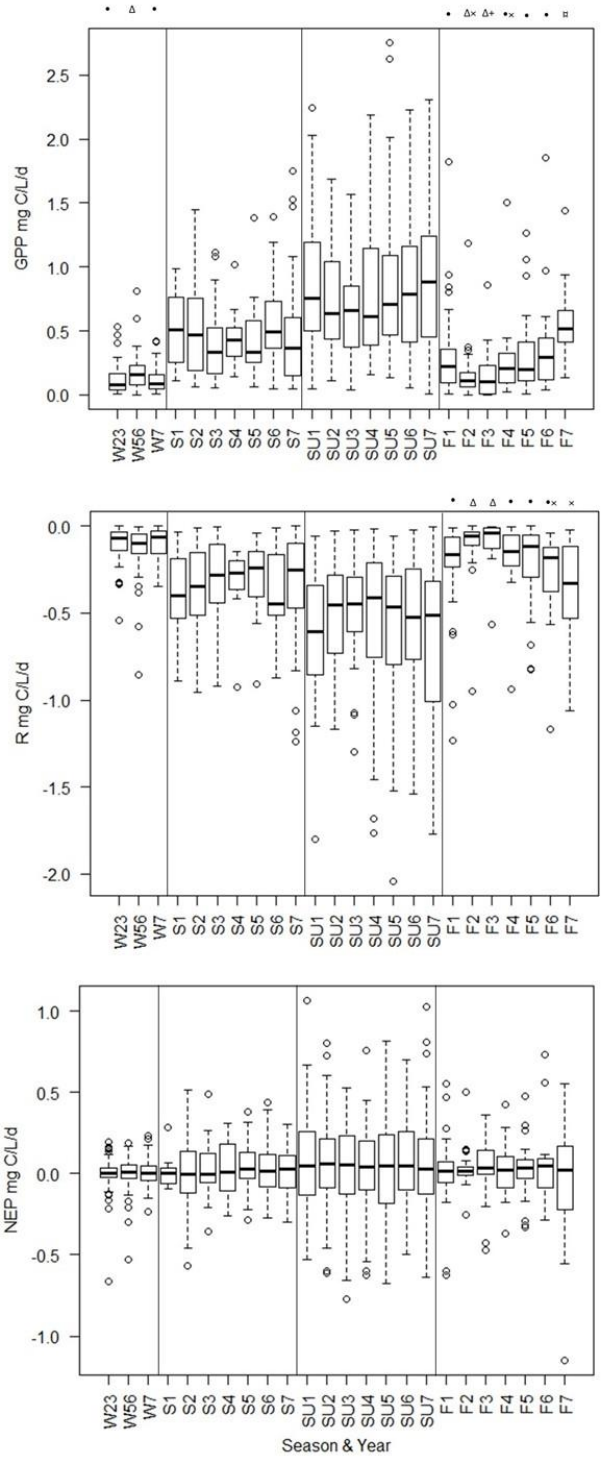

2011
2012
2013
2014
2015
2016
2017
Rates(mg C/L/d)
1.0
0.5
0.0
-0.5
-1.0
0
100
200
300
Day of Year
MET
GPP_C_mgL
NEP_C_mgL
R_C_mgL

Figure 5


GPP mg C/L/d
R mg C/L/d
NEP mg C/L/d
W23
W56
W7
S1
S2
S3
S4
S5
S6
S7
SU1
SU2
SU3
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SU5
SU6
SU7
F1
F2
F3
F4
F5
F6
F7
Season & Year

Figure 6

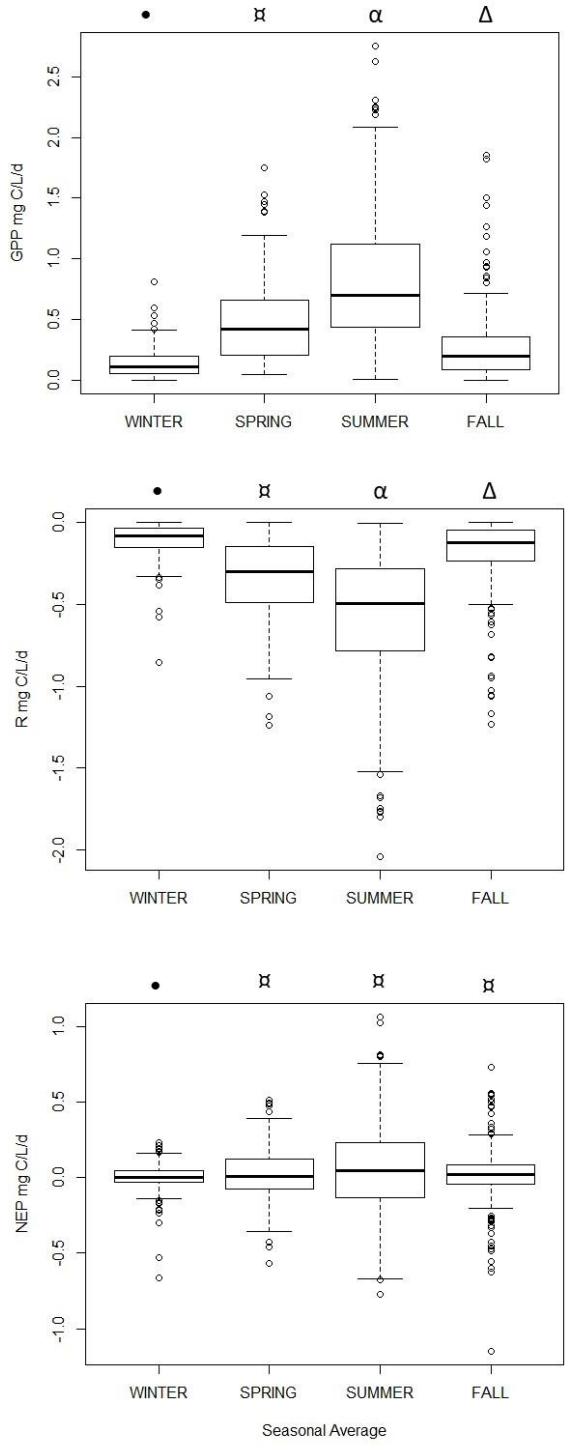

GPP mg C/L/d
R mg C/L/d
NEP mg C/L/d
WINTER
SPRING
SUMMER
FALL
Seasonal Average

Figure 7

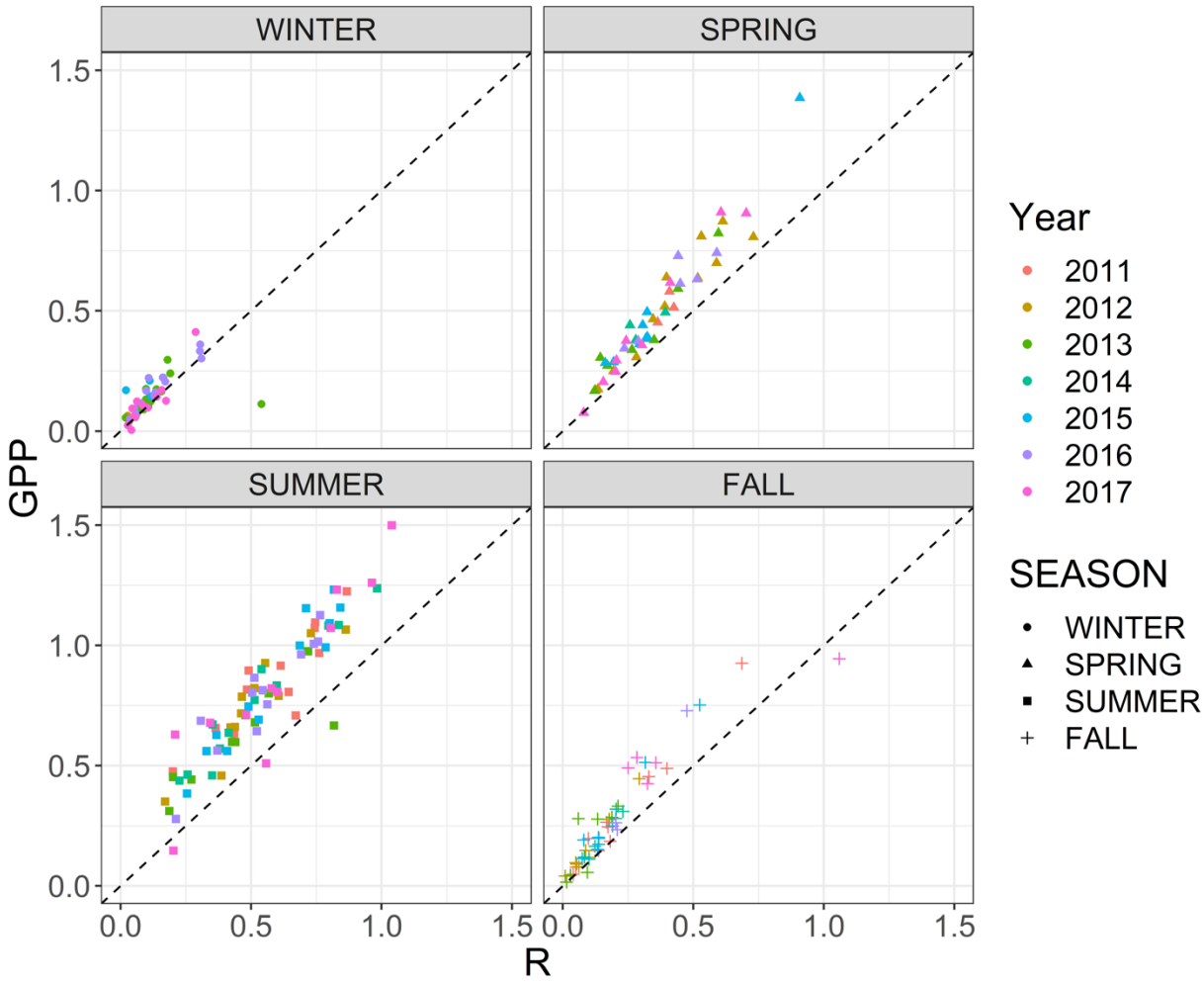

WINTER
SPRING
SUMMER
FALL
GPP
R
0.0
0.5
1.0
1.5
Year
2011
2012
2013
2014
2015
2016
2017
SEASON
WINTER
SPRING
SUMMER
FALL

Figure 8

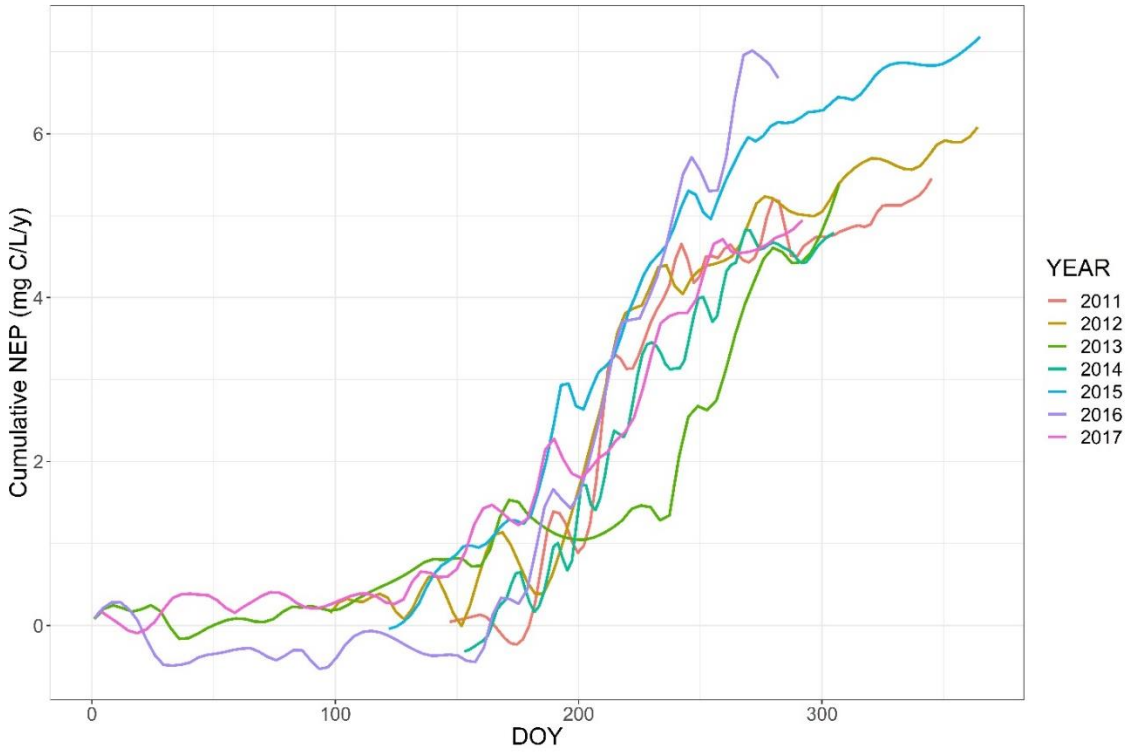

Cumulative NEP (mg C/L/y)
6
4
2
0
0
100
200
300
DOY
YEAR
2011
2012
2013
2014
2015
2016
2017

Figure 9

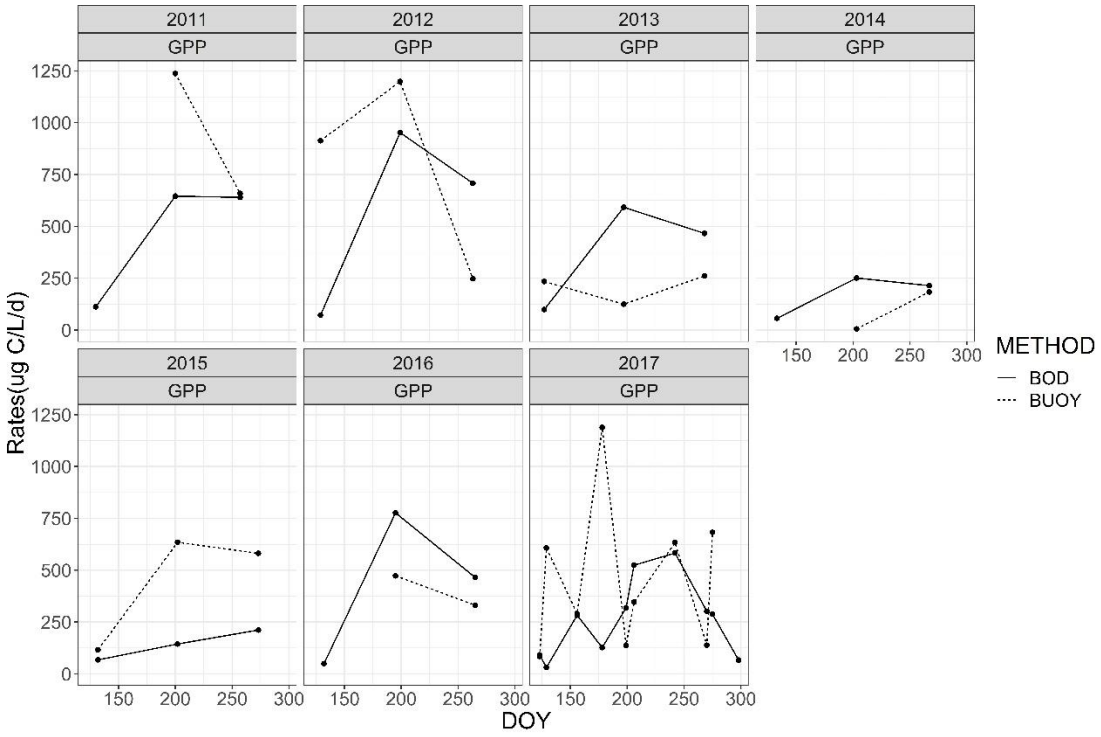

2011
2012
2013
2014
2015
2016
2017
GPP
Rates(ug C/L/d)
DOY
METHOD
BOD
BUOY

Figure 10

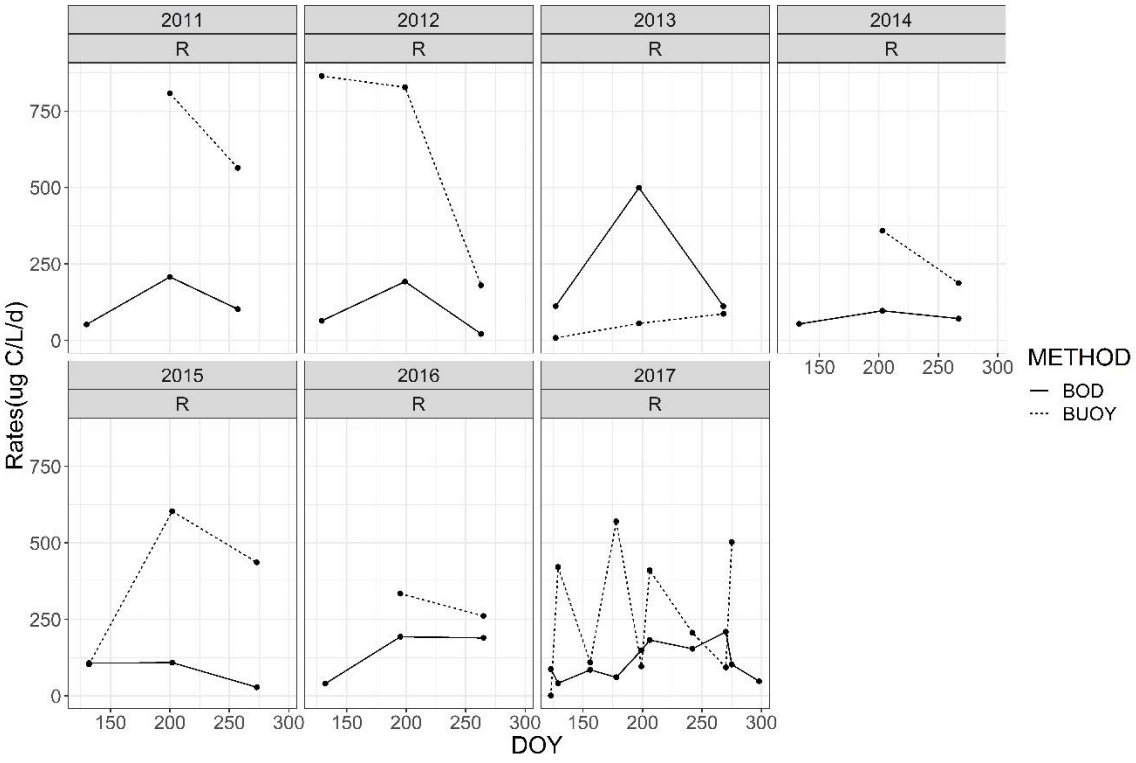

2011
2012
2013
2014
2015
2016
2017
R
Rates(ug C/L/d)
0
250
500
750
150
200
250
300
DOY
METHOD
BOD
BUOY

Figure 11

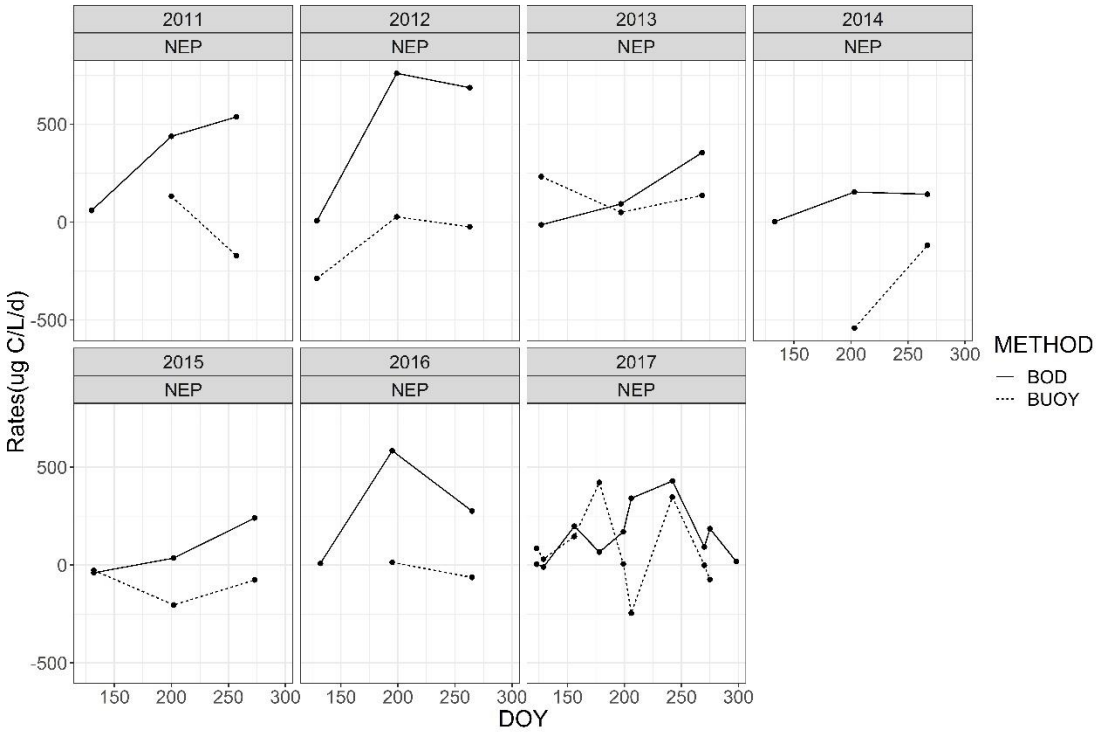

2011
NEP
2012
NEP
2013
NEP
2014
NEP
2015
NEP
2016
NEP
2017
NEP
Rates(ug C/L/d)
500
0
-500
150
200
250
300
DOY
METHOD
BOD
BUOY

Figure 12

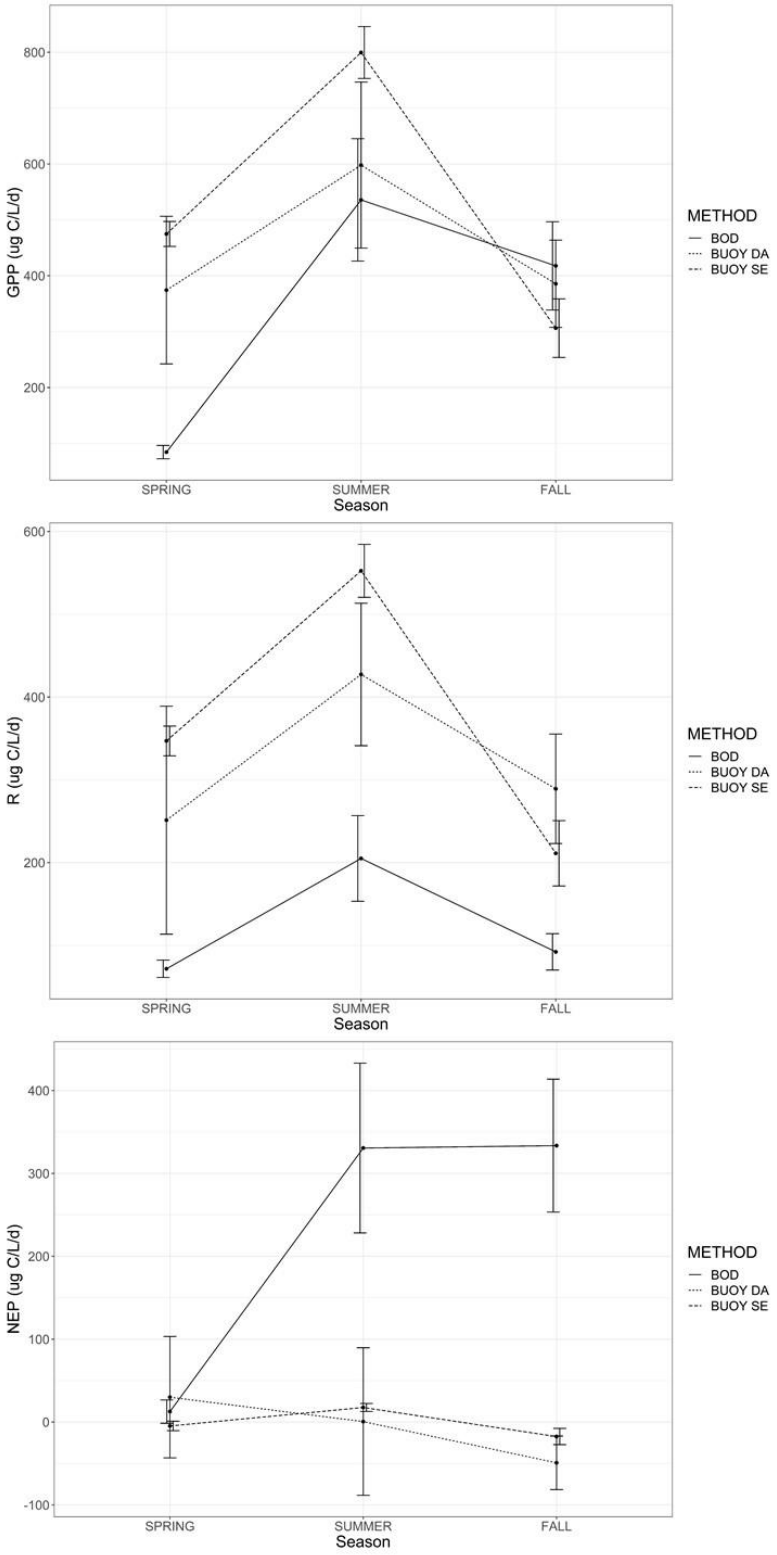

GPP (ug C/L/d)
800
600
400
200
SPRING
SUMMER
FALL
Season
METHOD
BOD
BUOY DA
BUOY SE
R (ug C/L/d)
600
400
200
SPRING
SUMMER
FALL
Season
METHOD
BOD
BUOY DA
BUOY SE
NEP (ug C/L/d)
400
300
200
100
0
-100
SPRING
SUMMER
FALL
Season
METHOD
BOD
BUOY DA
BUOY SE

Figure 13

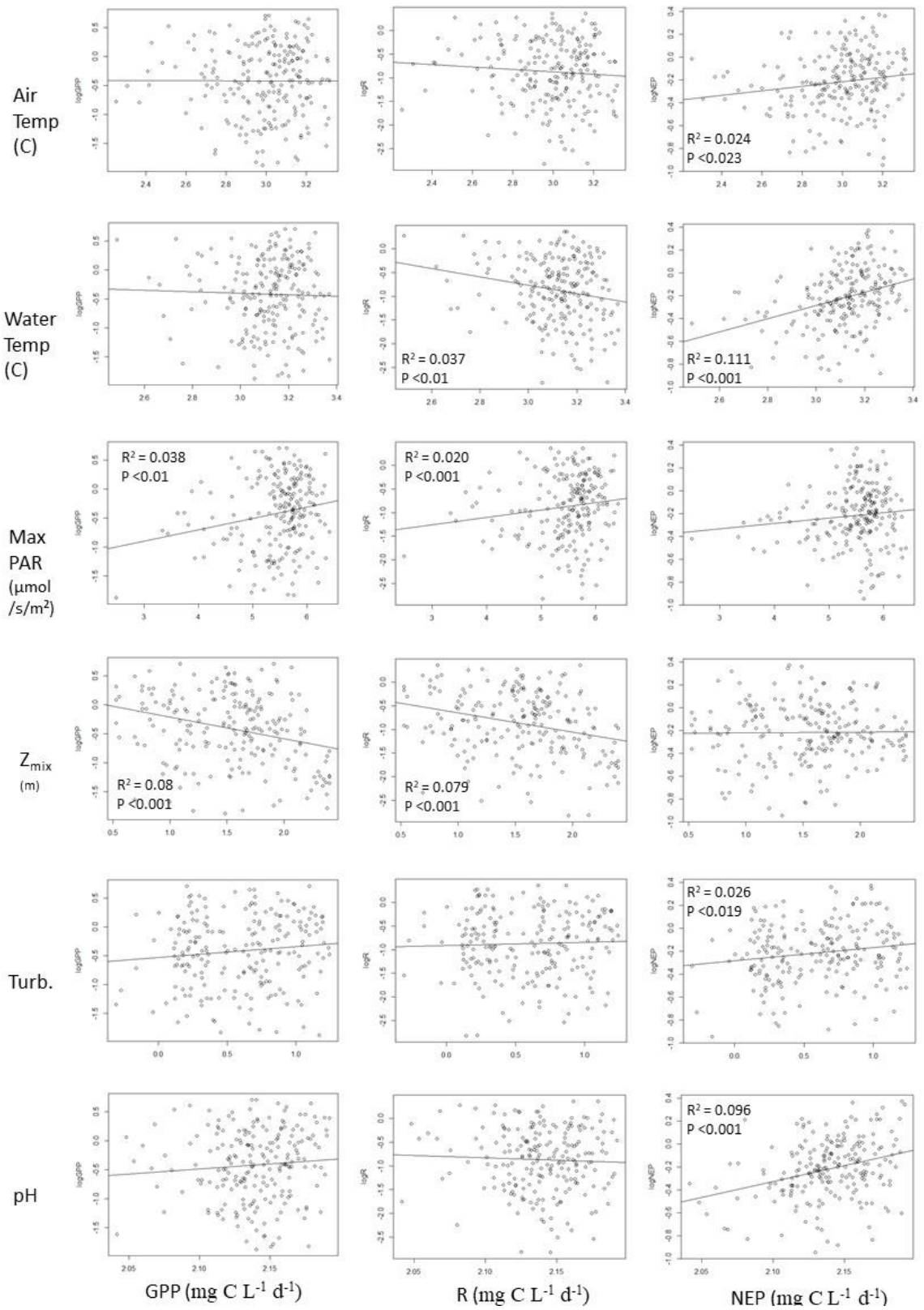

Air Temp (C)
Water Temp (C)
Max PAR (µmol /s/m²)
Z_mix (m)
Turb.
pH
R² = 0.024
P <0.023
R² = 0.037
P <0.01
R² = 0.111
P <0.001
R² = 0.038
P <0.01
R² = 0.020
P <0.001
R² = 0.08
P <0.001
R² = 0.079
P <0.001
R² = 0.026
P <0.019
R² = 0.096
P <0.001
GPP (mg C L-1 d-1)
R (mg C L-1 d-1)
NEP (mg C L-1 d-1)

Figure 14

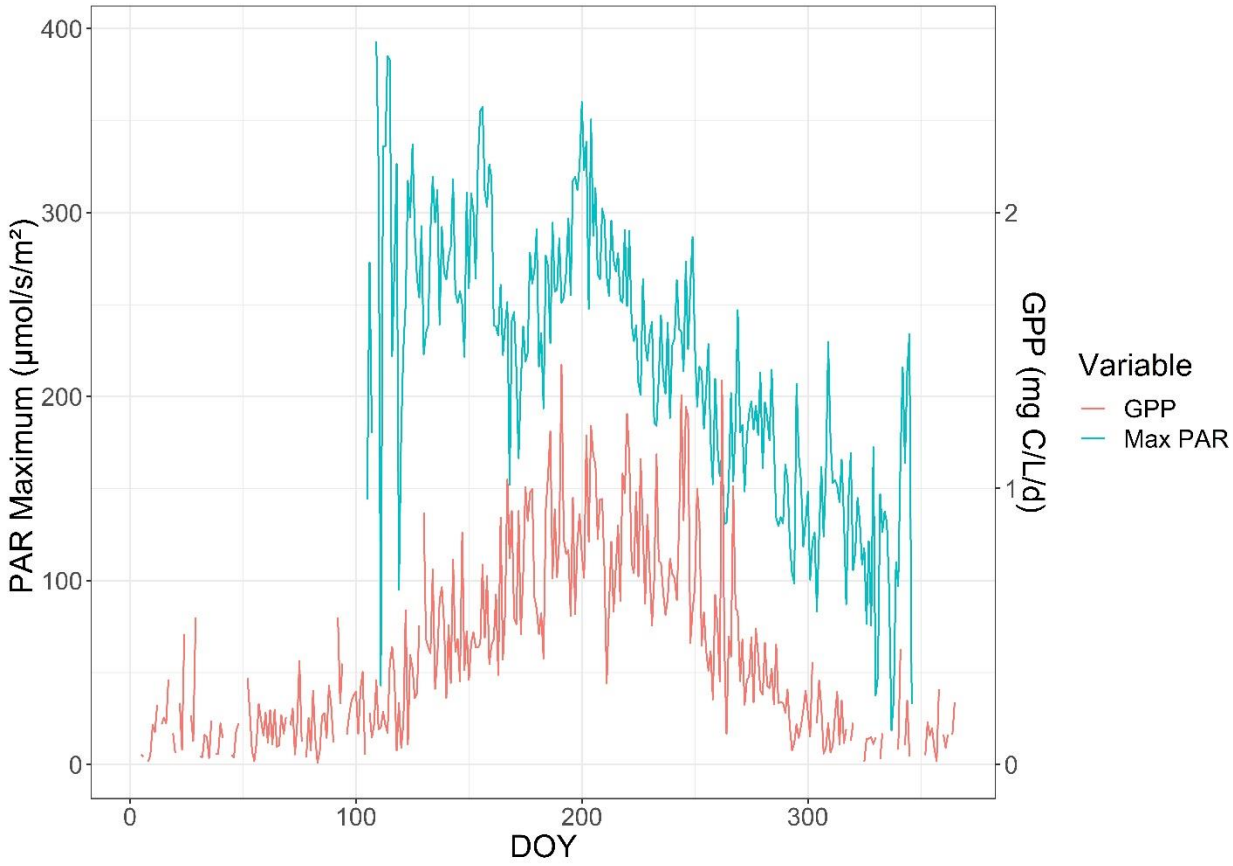

400
300
200
100
0
PAR Maximum (µmol/s/m²)
0
100
200
300
DOY
2
1
0
GPP (mg C/L/d)
Variable
GPP
Max PAR

Figure 15

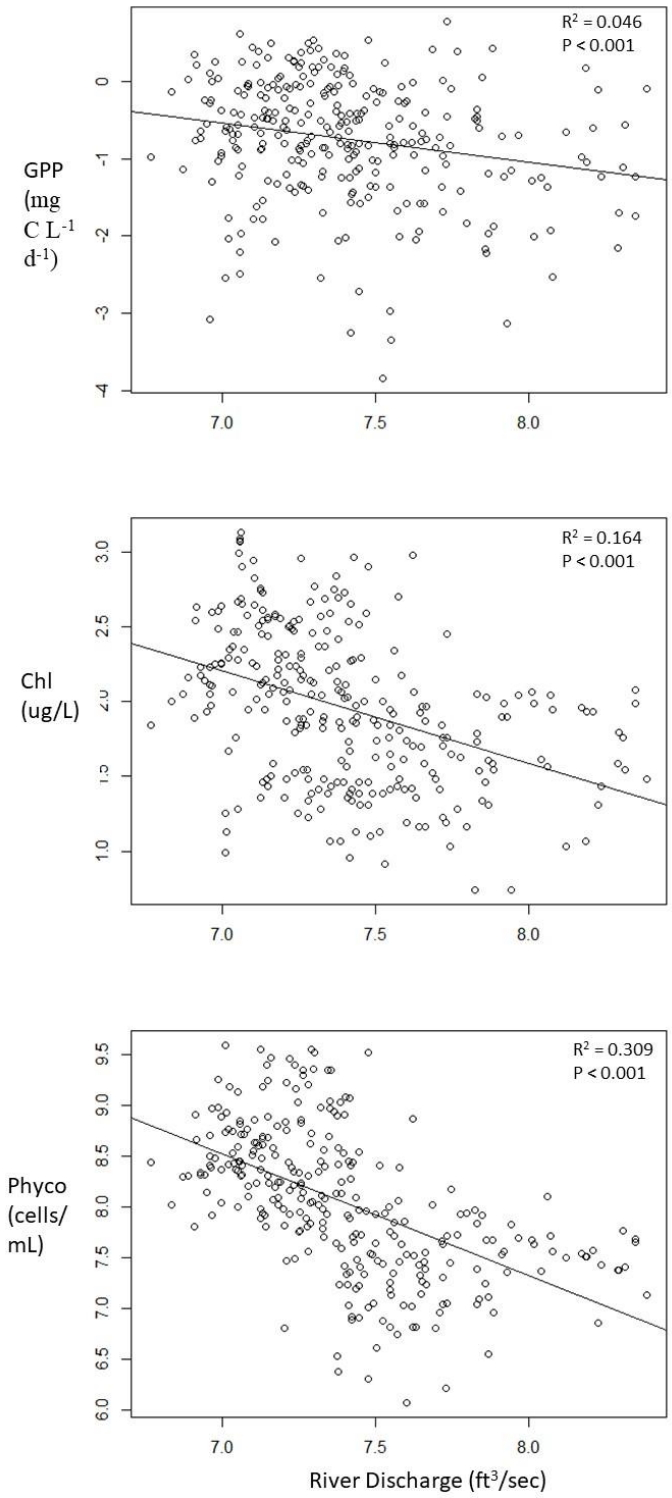
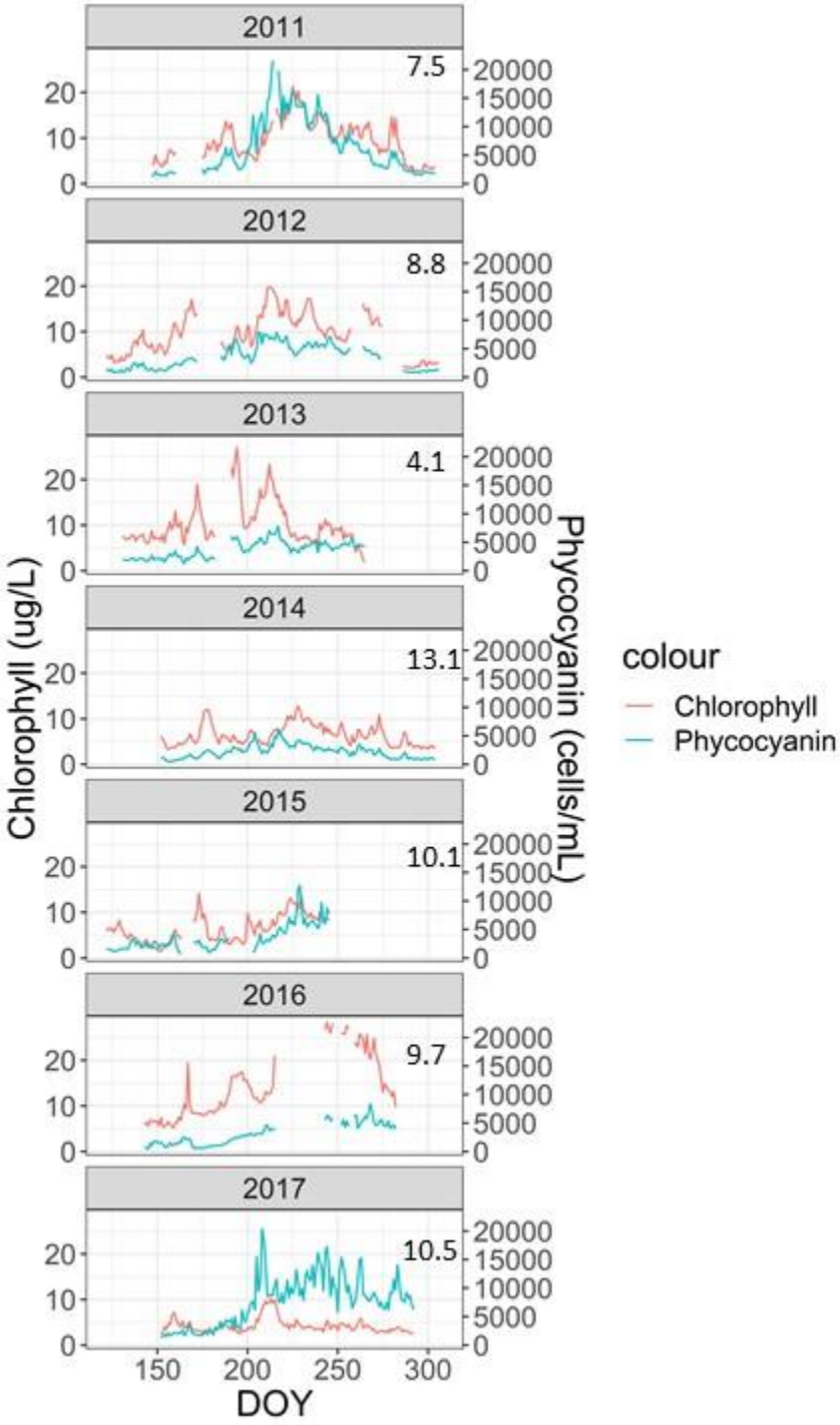

R² = 0.046
P < 0.001
GPP (mg C L-1 d-1)
0
-1
-2
-3
-4
7.0
7.5
8.0
R² = 0.164
P < 0.001
Chl (ug/L)
3.0
2.5
1.0
7.0
7.5
8.0
R² = 0.309
P < 0.001
Phyco (cells/ mL)
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
7.0
7.5
8.0
River Discharge (ft3/sec)

Figure 16


2011
7.5
2012
8.8
2013
4.1
2014
13.1
2015
10.1
2016
9.7
2017
10.5
Chlorophyll (ug/L)
Phycocyanin (cells/mL)
DOY
150
200
250
300
colour
Chlorophyll
Phycocyanin

Chapter 2.2

Title

Time-series Data from Multiple Buoys Reveal Spatial Heterogeneity in Metabolism in a Great Lakes Estuary

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Running Head

Multiple location metabolism

Keywords

Metabolism, Muskegon Lake, Carbon Cycling, Spatial Heterogeneity

Abstract

We utilized high-frequency time-series data of surface water dissolved oxygen (DO) concentrations from multi-sensor buoy deployments to estimate rates of metabolism in Muskegon Lake, a freshwater estuary in 2016 and 2017. Ecosystem metabolism was quantified at 4 locations: Muskegon Lake Observatory (MLO, central location), East, West, and Deep (southerly location), to determine if there were significant differences in metabolism due to measurement location or seasonal patterns. Kruskal-Wallis tests showed significant differences in Gross Primary Production (GPP) and Respiration (R) between seasons and sites. Net Ecosystem Production (NEP) was only significantly different at the East site through the seasons. GPP and R peaked in the summer with the Deep site having the highest rates. Spring and fall seasonal averages of NEP are sometimes negative depending on the site, whereas summer NEP is mostly positive at all sites, indicating there may be seasonal switches from heterotrophy to autotrophy to heterotrophy. NEP (mg C m^{-2} d-1) averaged -25.7, -5.8, 4.6, 11.5 in the spring, 24.3, 16.6, 50.8, 35.3 in the summer, and -45.8, -12.1, - 19.4, -12.0 in the fall at the East, MLO, West, and Deep sites, respectively. Overall there was high variability between sites; therefore, single point measurements may not adequately represent lake-wide metabolism. Our high frequency time-series data from multiple buoys demonstrates that freshwater lakes may display significant differences in metabolism across the ecosystem along with seasonally unequal rates of metabolism. Ecosystem level metabolism studies should incorporate multiple locations to ensure a complete estimate of whole system metabolism is assessed.

Text

Introduction

Recent measurements and models indicate that inland waters linking land and the ocean are highly sensitive, globally distributed ecosystems that can respond quickly to a variety of anthropogenic and environmental stressors. Due to their intense reactivity, they may serve as important sentinels of change at local and global scales (Adrian et al. 2009). Recent research has shown that of the ~3 billion metric tons of carbon that annually enters inland waters, roughly half is respired to the atmosphere (an amount equivalent to the sequestration of carbon in soils and the net primary production of oceans) while about a tenth is sequestered in sediment (equivalent to the burial of carbon in oceanic sediments). This suggests that freshwater bodies, which cover only ~3% of Earth's surface area, may have a disproportionately large influence on the global carbon cycle (Cole et al. 2007; Biddanda 2017). Indeed, it is only recently that anthropogenic changes in the inland water carbon cycle have begun to be considered in quantifying the global carbon budget (Quéré et al. 2018). To adequately understand the role of inland waters in the global carbon cycle, ecosystem metabolism studies need to be carried out to determine the fate of carbon individual freshwater ecosystems that serves as models for other freshwater ecosystems.

Ecosystem metabolism, a fundamental property of all ecosystems, comprises the storage and release of carbon through photosynthesis and respiration. In aerobic systems, metabolism involves complementary and coupled dynamics of both O_2 and CO_2. Metabolism is therefore commonly measured by tracking changes in dissolved oxygen (DO) that are converted to corresponding carbon units on an equimolar basis (Biddanda et al. 1994). Thus, estimates of ecosystem metabolism collectively provide a measure of the Gross Primary Production (GPP) –

carbon fixation by photosynthesis, Ecosystem Respiration (R) – carbon remineralization by bacterial and planktonic respiration, and Net Ecosystem Production (NEP) – the balance between GPP and R. NEP determines if a lake is considered a sink of carbon from the atmosphere or a source of carbon to the atmosphere. When a lake has a positive NEP, or the GPP:R ratio is greater than 1, it is considered autotrophic and less carbon is being lost to the atmosphere than taken in, whereas if NEP is negative (GPP:R<1) it is considered heterotrophic and loses more carbon to the atmosphere. Inland waters have generally been thought of to be heterotrophic; however, depending on the inland water type it may be more autotrophic if it is more nutrient rich like most shallow coastal waters are (Hoellein et al. 2013). Others have found a seasonal switch between autotrophy and heterotrophy (Lass et al. 2012; Defore et al. 2016). Understanding the temporal as well as spatial variability of metabolism in lakes is critical to quantifying the role of inland waters on the global carbon cycle.

Metabolism studies have been limited by the cost of equipment and accessibility to field sites. Therefore, most metabolism studies only utilize one sensor location within a lake rather than multiple sensor locations. This limitation has led to the major assumption that one sensor location can represent metabolism at a lake-wide level (Cole et al. 2000, Staehr et al. 2010). Most often, sensors are placed in the central limnetic area of the lake when only one location is used. A few studies have been performed in the littoral and limnetic zones and have shown significant differences in lake metabolism depending on location of measurement (Lauster et al. 2006; Vesterinen et al. 2017). Including littoral zones has shown to increase GPP and NEP overall in lakes (Lauster et al. 2006; Cooper et al. 2013; Vesterinen et al. 2017). A study in northern Wisconsin used 27-35 sensors in 2 lakes to study the spatial heterogeneity and found

that each location within an individual lake could vary by 1-2 orders of magnitude in metabolism for the same day (Van de Bogert et al. 2012).

In Muskegon Lake, three earlier studies collected samples at multiple locations for metabolism, but they were done either once a month for a year or only 3 times a year using a traditional biological oxygen demand (BOD) bottle method (Weinke et al. 2014; Dila et al. 2015; Defore et al. 2016). Weinke et al. (2014) found a decreasing gradient in GPP and R from Muskegon Lake inlet out to an offshore Lake Michigan site during the summer. Defore et al. (2016) found strong seasonality in Muskegon Lake metabolism where metabolism peaks in the summer and then NEP becomes negative in the winter. Muskegon Lake was named a "Goldilocks Zone" because the lake had more autotrophy compared to the upstream river and offshore Lake Michigan zones due to its higher residence time and nutrient availability (Dila et al. 2015). These three studies concluded that Muskegon Lake is generally net autotrophic and a carbon sink from the atmosphere. Since each Muskegon Lake study found spatial and seasonal heterogeneity, further research is needed to understand this variability. Muskegon Lake now has high frequency data available which will help in determining if the patterns of the previous studies will match daily measurements.

Muskegon Lake has had high frequency buoy data available since 2011 at a mid-lake location. In 2016 three additional buoys were placed strategically throughout the lake based on past sampling sites, which enabled lake metabolism to be estimated individually at all 4 sites in 2016 and 2017. The objectives of the present study using high frequency data were to: 1) determine if there was a significant difference in metabolism among the sites, 2) understand the seasonality in metabolism across Muskegon Lake, and 3) determine if Muskegon Lake is annually autotrophic or heterotrophic.

Methods

Study Site

Muskegon Lake (43.23°N, 86.29°W) is a mesotrophic drowned river mouth freshwater estuary located along the eastern shores of Lake Michigan (Figure 1). The lake is the end point for the Muskegon River Watershed, the second largest in Michigan at 6,822 km^2. Land use in the watershed is mostly forest (53.2%) with some agriculture (23%), and only small percent urban (4.2%) (Marko et al. 2013). Muskegon Lake has an area of ~17 km^2 with a mean depth of 7 m, maximum depth of 22 m, and a residence time of ~23 days. Muskegon Lake is hydrologically connected to Lake Michigan through a navigation channel which allows water to enter Muskegon Lake from Lake Michigan on occasion, acting as a freshwater estuary system. This water intrusion from Lake Michigan only happens during certain wind events, where upwelling occurs in Lake Michigan which forces oxygen rich, cold water through the navigation channel into Muskegon Lake (Liu et al. 2018). These cold-water intrusion events temporarily relieve bottom water hypoxic conditions (DO <4 mg/L; Biddanda et al. 2018) and brings in nutrient-poor water diluting Muskegon Lake nutrient rich water potentially affecting lake metabolism rates (Liu et al. 2018).

Muskegon Lake was designated as an Area of Concern (AOC) by the Environmental Protection Agency (EPA) in 1985 due to historical impairments. In the 1880s there were 47 sawmills surrounding Muskegon Lake and was known as the "Lumber Queen of the World" (Steinman et al. 2008). After the lumber industry declined, industrial activity began in Muskegon shifted mainly to foundries and paper mills in the 1900s (Steinmann et al. 2008). The EPA based the AOC listing on nine beneficial use impairments. One of these historical impairments

included historic hypereutrophic conditions caused by excess nutrient loading. Muskegon Lake has had issues with eutrophication, but restoration efforts have reduced the nutrient levels and harmful algal blooms (for example, total phosphorus levels have dropped from 68 to 27 µg/L from 1972 to 2005, respectively (Steinman et al. 2008)).

Data Collection

The Muskegon Lake Observatory (MLO), centrally located at 43°14'17.66"N, 86°16'49.92"W and three other buoys – West (west end of lake near the navigation channel and Bear Lake inlet), Deep (southern area of lake and the deepest location), and East (east end of lake near the Muskegon River inlet) located at 43°13'57.57"N, 86°18'33.75"W, 43°13'26.40"N, 86°17'49.92"W, 43°14'43.89"N, 86°15'49.57"W, respectively, were used in 2016 and 2017 to study the spatial heterogeneity in lake-wide metabolism (Figure 1). MLO was placed in the central part of the lake in 2011 to represent the whole lake in monitoring efforts and the other locations were selected because they were used in previous studies for ongoing monitoring efforts for the AOC and to understand the physical and biological spatial heterogeneity of the lake. In 2016, data were collected from August through November and in 2017 data were collected from May through October. Although MLO data has been available much longer, the focus of this study was to understand the spatial heterogeneity and only dates when all buoys were deployed were considered. Water quality data measured by MLO was recorded every 15 minutes and every hour at the other sites. Hourly averages for MLO were used to match the same frequency as the other sites. At all sites, DO and conductivity were measured using YSI (Yellow Springs Instruments) 6600/6920 datasondes at 2 m. In addition, temperature sensors were placed through the water column at increments of 2-3 m to estimate the thermocline depth.

Meteorological data were recorded at MLO by the meteorological station placed 2 m above the water surface and a photosynthetically active radiation (PAR) sensor was located at MLO at 1 m under the water. Sensors were cleaned monthly for biofouling prevention and YSI wipers were installed which cleaned the sensor every 15 minutes. If wipers were not working properly, they were replaced or fixed as needed. Sensors were calibrated together at the beginning of the season and checked together at the end of the season for drift.

Metabolism Estimation

The free water dissolved oxygen method was used to estimate metabolism at the 4 locations in Muskegon Lake. This method uses high frequency diel oxygen curves to estimate the GPP, R, and NEP for each day (Figure 2). To estimate the metabolism, the R package LakeMetabolizer was implemented for all data. Within this package there are 5 metabolism models: bookkeeping, ordinary least squares, maximum likelihood, kalman filter, and bayesian (Winslow et al. 2016) and were outline in McNair et al. (2013). The bookkeeping model is the most commonly used and simplest model to use and originated from Odum (1956) and used extensively after Cole et al. (2000). The governing equation to calculate the metabolism in an environment is:

$$\Delta O_2 = GPP - R - F - A$$

where ΔO_2 is the change in DO, GPP is the gross primary production, R is the respiration, F is the atmospheric exchange coefficient, and A accounts for other processes, such as advection, non-aerobic consumption of oxygen, or photochemical oxidation, which could cause changes in DO (Staehr et al. 2010). A is dropped in this study, and others, since it is considered negligible compared to other sources of oxygen change (Odum 1956, Staehr et al. 2010; LaBuhn and

Klump 2016). Although the equation above seems simple, many additional models are needed to estimate metabolism. NEP is the balance between GPP and R where a negative NEP means R is greater than GPP making the ecosystem heterotrophic or if NEP is positive GPP is greater and the ecosystem is autotrophic. An assumption when estimating metabolism is that R is equal during the day and night. As oxygen increases during the day, we can estimate the GPP by the change in oxygen, and at night as oxygen decreases, we can determine what the R rates are based on the decrease of oxygen. The decrease of oxygen is the R rate is applied to the day and night as it is assumed. Other uncertainties and assumptions when estimating metabolism include daytime and nighttime R is equal, sensors represent DO changes in the entire mixed layer, physical movements may obscure the biological processes (such as when GPP is negative and R is positive), and the horizontal, vertical, and temporal metabolism is heterogeneous (Staehr et al. 2010).

During 2016 and 2017 there are several missing data due to unrealistic data values and sensor issues. The East site sonde stopped recording data from the end of June through mid-August in 2017. Data gaps were present at all locations during different time periods due to sensor issues or sensors temporarily being removed from the lake for another project (Figure 2). Given these circumstances, 2016 and 2017 data were merged for some of the seasonal analysis. Negative GPP and positive R values were considered unrealistic estimates, because they are not possible biologically, but occur due to the physical mixing of water masses with different dissolved oxygen concentrations (Winslow et al. 2016; Brothers et al. 2017).

To use the R package, LakeMetabolizer, water temperature at multiple depths, DO near the surface, conductivity, barometric pressure, PAR, and wind speed data is needed. This raw data is needed to estimate the inputs for the LakeMetabolizer bookkeeping model. The data input

into the LakeMetabolizer package are gas flux, DO at saturation, observed DO, mixed layer depth, and daytime fraction (light hours and dark hours). There are several models to estimate the gas exchange coefficient; this study used the k.cole method from rLakeAnalyzer which is based on the method outlined in Cole and Caraco (1998). Each of these parameters were estimated using the R package rLakeAnalyzer (Winslow et al. 2017) and then input into the LakeMetabolizer package (Winslow et al. 2016). The output of LakeMetabolizer includes GPP, R, and NEP in mg O_2 L^{-1} d^{-1}. Metabolism rates were converted into carbon units by dividing the mg O_2 L^{-1} d^{-1} rate by 2.666 to get metabolism in mg C L^{-1} d^{-1}. Following the O_2 to C conversion, metabolism was converted to mg C m^{-3} d^{-1} and then metabolism rates were then converted aerial rates of mmol C m^{-2} d^{-1} by multiplying the daily metabolism rate by the daily average thermocline depth. Daily rates of GPP, R, and NEP were estimated following this procedure.

Lake Wide Estimates and Seasonal Patterns

Metabolism was estimated at 4 locations across Muskegon Lake in limnetic zones. Estimates from these sites were compared to determine if they were significantly different from each other and aimed to determine if the assumption of 1 sensor in metabolism studies being sufficient. Weekly averages were calculated from daily rates. Data were tested for normality using a Shapiro-Wilk test and then analyzed for significant differences using Kruskal-Wallis. Following the Kruskal-Wallis test, a post hoc Pairwise Wilcoxon was performed to determine which sites and seasons were significantly different from each other. Comparisons were done among sites in the same season and at the same site across seasons. Sites and seasons were given the following labels: Spring East (SE), Spring MLO (SM), Spring West (SW), Spring Deep (SD), Summer East (SUE), Summer MLO (SUM), Summer West (SUW), Summer Deep (SUD),

Fall East (FE), Fall MLO (FM), Fall West (FW), and Fall Deep (FD). Data were put into seven groups for analysis: Group 1 or spring sites (SS) – SE, SM, SW, SD; Group 2 or summer sites (SUS) – SUE, SUM, SUW, SUD; Group 3 or fall sites (FS) – FE, FM, FW, FD; Group 4 or east seasonal (ES) – SE, SUE, FE; Group 5 or MLO seasonal (MS) – SM, SUM, FM; Group 6 or west seasonal (WS) – SW, SUW, FW; and Group 7 or deep seasonal (DS) – SD, SUD, FD.

Weeks were combined into seasonal sections of spring (weeks 18-28), summer (weeks 29-38), and fall (weeks 39-48. Data for 2016 and 2017 were merged in the seasonal averages for contour mapping because seasonal patterns in the different years were generally similar, so data for the two years were merged for contour mapping and left for their respected years for other analysis. Seasonal contour maps of Muskegon Lake were created to visualize the spatial heterogeneity using Surfer 8 mapping software. The (GPP, R, and NEP) data values measured at the four sample locations (MLO, Deep, West, and East) within the Muskegon Lake basin were segmented into three seasonal data groups based on their sample collection times (Spring, Summer, and Fall) and then an average was determined for each set of seasonal data. The average data at each of the four sample locations were then assigned their respective location coordinates (in decimal degrees) for MLO, Deep, West, and East, so that the data could then be imported into Surfer 8 (a grid-based graphics program) as a data worksheet. An additional set of four data points were also included in each average data series worksheet (without any values) to restrict the area of the calculated grid surface to the extent of the Muskegon Lake basin.

The Surfer 8 program interpolates irregularly spaced X, Y, Z values from a data worksheet into a regularly spaced grid using a variety of gridding methods. The gridding method used for this set of data was Inverse Distance to a Power, with which the data are weighed during interpolation such that the influence of one data point relative to another declines with distance

from the grid node. The Inverse Distance to a Power gridding program was run on each of the 9 sets of seasonal/data averages (GPP Spring, GPP Summer, GPP Fall, R Spring, R Summer, R Fall, and NEP Spring, NEP Summer, NEP Fall) at each of the four sample locations. Also, because Muskegon Lake is a natural feature which has a distinct physiological boundary trend that runs from Northeast to Southwest, an adjustment of the search ellipse in the Inverse Distance to a Power algorithm was used to mimic this trend to accurately capture the position of each of the sampling locations. Once the 9 grids were created, they were then opened individually in Surfer and a New Contour Map routine was run to numerically differentiated seasonal/data (GPP, R, and NEP) averages across the Muskegon Lake basin into individual gradient-base (contour) vector layers. The contour maps for each of the 9 sets of seasonal/data averages were then masked with the Muskegon Lake polygon boundary and the contour levels were set using a data range that captured the entire set of average values for each of the 9 seasonal/data average (GPP, R, and NEP) groups.

Autotrophic vs. Heterotrophic?

The weekly averages of lake metabolism were used for each location to determine if the lake was net autotrophic or net heterotrophic. GPP:R ratios and NEP are commonly used for determining this. If the GPP:R ratio is greater than 1, then the system is considered autotrophic and dominated by GPP and if the GPP:R ratio is less than 1, then the system is considered heterotrophic and dominated by R. NEP can either be positive or negative. When NEP is positive the lake is considered autotrophic and when NEP is negative the lake is considered heterotrophic.

Results

Average Environmental Factors

During the sampling period in 2016 and 2017, the average (±SD) wind speed was 9.314±6.442 knots, the average (±SD) barometric pressure was 1019.07±5.86 mb, and the average (±SD) daily maximum PAR was 215.02±87.97 µmol/s/m². Each site had independent measurements of mixed layer depth, surface temperature, conductivity, DO, and DO saturation (Table 1). Kruskal-Wallis tests for all environmental data revealed there was a significant difference among all sites in thermocline depths (p-value <0.001), surface temperature (p-value<0.001), specific conductivity (p-value<0.001), and DO at saturation (p-value<0.001). DO was not significantly different for the Deep and MLO site (p-value = 0.324) but all other sites were significantly different (p-value<0.001). DO showed high daily variability at surface concentrations (Figure 2).

Lake Wide and Seasonal Patterns

Metabolism rates varied seasonally and spatially within Muskegon Lake (Figure 3). Untransformed and log transformed data were not normally distributed according to a Shapiro-Wilk test; therefore, a Kruskal-Wallis non-parametric test was done to determine if there was a significant difference among the different groups (Table 2). The 7 groups were tested among the same season between sites (Groups SS, SUS, and FS) and among the same site during different seasons (Groups ES, MS, WS, and DS) and tested for with GPP, R, and NEP. All groups had significant differences for GPP and all groups, except SUS, had significant differences in R (Table 2). NEP was only significantly different for ES, otherwise all NEP was not significantly different among sites and seasons (Table 2).

Pairwise Wilcoxon tests were done with the groups that showed significant differences for GPP, R, and NEP to determine what season and sites within that group were significantly different. G1 consisted of the spring data at each site. Among this data, the East and West sites were significantly different from all sites but the MLO and Deep sites were not significantly different from each other (Figure 4). The MLO and Deep sites had the highest GPP rates, followed by the West site and then the East site. In the summer (SUS) the East, MLO, and West sites were all the same and only the Deep site was significantly different for GPP. In the Fall the East and MLO sites were the same and the West and Deep sites were statistically the same for GPP. The Deep site had the highest rates of GPP in the spring and summer. FS data in the fall showed MLO had the highest rates of GPP. Each site was significantly different seasonally for GPP except the West site. The West site spring and fall were the statistically the same but significantly different than summer (Figure 4).

R followed similar patterns as GPP. In the spring, the East and West sites were statistically different from all sites but the MLO and Deep sites were statistically the same. In the summer, there were no statistical differences among sites. In the fall, the East and MLO sites were the same and statistically different than the West and Deep sites. The West and Deep sites were not statistically different in the fall. The East and Deep sites were statistically different each season for R. The MLO site in the fall was statistically different from the spring and summer. The West site in the summer was statistically different from the spring and fall. MLO and Deep had the highest R rates in the spring. In the summer East and Deep sites had the highest R rates, and in the fall, MLO and East sites had the highest R rates (Figure 4).

Although GPP and R can be significantly different among sites and seasons, NEP interestingly only had significant differences among the East site. The summer at the East site

was significantly different than the spring and fall. In the spring and fall there was much less variance in NEP rates whereas the summer could vary much more, negatively and positively (Figure 4).

Autotrophic or Heterotrophic?

The weekly averages of GPP:R reveal that Muskegon Lake was net autotrophic at most locations during our study, with a few exceptions (Figure 5). The Deep and West site had the highest GPP:R average at 1.53 (±0.29) and 1.50 (±0.41), respectively. MLO had a 1.41 (±0.32) and East had a 1.37 (±0.72) GPP:R average. There was a total of 10 weekly averages with a negative GPP:R ratio. The East site had 6 weeks with negative GPP:R ratios in May and June 2017. MLO had 3 weeks with negative GPP:R ratios in May, July, and September 2017. The West site had one week in July 2016.

Discussion

Varying Location Based Metabolism Estimates

The primary objective of this study was to determine if there was spatial heterogeneity in metabolism rates across four limnetic locations within Muskegon Lake. Muskegon Lake had statistically different rates of GPP and R among most seasons and sites. Despite these differences in GPP and R, NEP was only significantly different at the East site in spring and fall. NEP seemed to always have similar averages no matter the site or season (Figure 4). While NEP was significantly different in the summer for the East site, the averages in the boxplot do not appear to be (Figure 4). The East site had fewer data in the spring than all other sites and seasons due to sensor failure. This lower sample size could influence the results among seasons for the East site.

The distance from the Muskegon River inlet and site depths could influence metabolism trends. Allochthonous organic carbon sources are key drivers in lake metabolism by causing light extinction limiting GPP but still allowing for high respiration rates due to the higher amounts of allochthonous organic carbon entering from upstream (Toming et al. 2013). In addition to the allochthonous influence, sites closer to the river inlet, may have a shorter residence time than sites away from main river inlets. The East site is the closest at 1,500 m, the MLO site is 3,100 m, the Deep site is 5,200 m, and the West site is 5,400 m. Although the West site is further from the Muskegon River mouth, it is also closest to the second largest inlet, the eutrophic Bear Lake channel. All sites in this study were deeper than the average depth of Muskegon Lake (7 m) at 11, 12, 15, and 22 m in the limnetic zone. The East and MLO sites had the lowest rates of NEP in 2016 and 2017 averages (Figure 6). The East site was never the highest for GPP or R according to the seasonal averages. The East site likely had the lowest rates of NEP since it had the greatest influence from the river inputs of organic matter. This additional organic matter near this site may have stimulated more respiration and lower NEP rates. The Deep site consistently had the higher rates of GPP and R in 2016 and 2017, except in the fall where MLO had the highest rates (Figure 7 & 8). Typically, the west side of the lake had higher rates of GPP (Figure 7) and NEP (Figure 9). While the contour maps (Figure 7-9) show that the Deep site typically always had higher rates of metabolism, this may be due to the areal conversions. Areal conversions were done by using the mixed layer depth for each site. The Deep site consistently had deep mixed layer depths (Table 1) – which may account for the higher rates of areal metabolism observed here.

Other studies have found significant differences between sites when monitoring multiple locations within lakes. These are sometimes broken down between littoral and limnetic sites. A

study where two lakes were studied with either 27 or 35 individual sensors were placed in multiple locations within the same lake was done in Wisconsin. This study showed that depending on the sensor location, metabolism could vary up to 1 – 2 orders of magnitude (Van de Bogert et al. 2012). They found that GPP and R differences were related to location in the lake, whereas NEP was not as affected by lake location but rather day of deployment. They point out that single sonde studies can have errors of over an order of magnitude and that risks the actual measure of GPP or R mischaracterizing the trophic status of the lake. They suggest using at least 4 sensors randomly placed throughout the lake (Van de Bogert et al. 2012), and we concur with this strategy.

Seasonal Changes in Autotrophy and Heterotrophy

Autotrophy or heterotrophy are important because it describes the fate of carbon in an ecosystem. If more carbon is leaving the lake through respiration, then heterotrophy dominates, and NEP is negative. If more carbon is being produced than respired, then NEP is positive, and the system is considered autotrophic. NEP has been shown to indicate heterotrophy when there are higher amounts of dissolved organic carbon (Anderson & Sobek 2006; Laas et al. 2012). Seasonal averages of NEP show that during the summer months the lake is autotrophic but, in the spring, and fall this could vary depending on sites and year (Figure 9). Some studies have found a seasonal switch from autotrophy to heterotrophy when looking at metabolism year-round (Lass et al. 2012; Defore et al. 2016). A study done in 2009 and 2010 estimated metabolism in a lake for 2 years. From this they found that the lake begins as heterotrophic in the spring and switched to autotrophy in early spring and then back to heterotrophy in August or September (Laas et al. 2012). They found that warmer springs caused the peak of metabolism rates to occur

earlier in the year and that half of the year was heterotrophic, and half was autotrophic making it CO_2 neutral. This study was done in a much shallower lake than Muskegon Lake, with a mean depth of only 2.8 m (Laas et al. 2012).

Weekly averages show the peak GPP and R date for all sites in Muskegon Lake were around mid-July in 2017 for all sites. Weekly averages of NEP did not show a distinct peak date; however, seasonal averages showed that NEP peaked during the summer (Figure 5). Peak average daily air temperature in 2017 were in mid-July at 24.3°C. The peak date was not identified in 2016 since data was missing for the first half of the season for the East, West, and Deep sites. The timing of peak temperature may follow the peak rates of metabolism as well, specifically GPP and R. GPP usually peaks around the same time that water temperature does (Laas et al. 2012). If they water temperature peak shifts, so will GPP. Therefore, if a warmer spring causes warmer water and an early peak you would expect to find high rates of GPP and potentially earlier algal blooms during the year. R was found to peak shortly after the water temperature peak (Laas et al. 2012).

The weekly GPP:R ratios averages tell a slightly different story in this study. The East site had a GPP:R value of less than 1 in six weeks in the spring. MLO had three weeks in with GPP:R ratios less than 1. In July, 2016 the West site had one week with GPP:R less than 1. The GPP:R ratio for all 6 of the weeks with a GPP:R less than 1 ranged from 0.91 to 0.97. The sample sizes during these weeks were lower due to unreasonable GPP and R values (negative GPP and positive R), however those values were removed from analysis. These weeks with lower ratios had higher than average wind speeds and rain accumulation up to an inch. There is a distinct pattern where the shallower sites closer to the river had a greater chance of having heterotrophic weeks. The weekly GPP:R and seasonal NEP rates could be telling slightly

different stories based on how they are averaged. Looking at Figure 3, it appears that NEP can vary widely between weeks but overall average near zero. GPP and R start low in the spring, increase in the summer, and return closer to zero in the fall. These seasonal patterns may have been missed in other studies that were carried out during only a few weeks in the summer.

Time-series vs point measurements in Muskegon Lake Previous Muskegon Lake Studies

Prior to the high frequency data available now, other metabolism studies were conducted in Muskegon Lake where the light and dark biochemical oxygen demand (BOD) bottle method was used. Weinke et al. (2014) found a land-to-lake gradient in metabolism with the estuary being a net carbon sink and offshore in Lake Michigan being a net carbon source. Higher autochthonous and allochthonous inputs from the watershed provide nutrients but may limit light in the estuary and coastal zones metabolism (Weinke et al. 2014). Dila and Biddanda (2015) named Muskegon Lake the 'goldilocks zone' when also looking at this gradient from river to estuary to lake. Weinke et al. (2014) found a range of GPP at ~0.50 to 0.75 mg C L^{-1} d^{-1} within Muskegon Lake sites. Both Weinke et al (2014) and Dila and Biddanda (2015) found NEP to be positive indicating autotrophy and negative NEP in the offshore Lake Michigan. Since the study sites were all within Muskegon Lake for the current study, we did not see this gradient as much, but we did see that the Deep and West locations typically had higher rates of GPP and R than East and MLO. A year-long metabolism study with monthly sampling in Muskegon Lake revealed that most months were net autotrophic except for the winter months (Defore et al. 2016). Defore et al. (2016) also revealed that the peak metabolism rates were in the summer months which aligns with what this study found for all sites. Dila and Biddanda (2015) found GPP rates as high as 1.0 µg C L^{-1} d^{-1} during a September bloom event. This study found weekly GPP average can go as high as 2.30 µg C L^{-1} d^{-1}. Overall, all three studies found that Muskegon

Lake is net autotrophic. Daily rates of metabolism showed high variability across GPP, R, and NEP from a day to day basis compared to the monthly sampling done in the previous Muskegon Lake studies. The high frequency data revealed that the fall and spring heterotrophy and autotrophy varied depending on the location. Seasonal switches in heterotrophy and autotrophy were also found in another study (Laas et al. 2012).

As previously discussed, Muskegon Lake is an AOC according to the EPA. Part of the delisting process involves extensive research, restoration, best practice management, and monitoring. Total Phosphorus (TP) levels have decreased since 1972 from 58 µg/L to 26 µg/L in 2005 (Steinman et al. 2008). Additionally, chlorophyll a decreased from 24.7 µg/L in 1972 to 5.9 µg/L in 2005 (Steinman et al. 2008). GPP and R both decrease with lake area and depth but increase with algal biomass, dissolved organic carbon, and total phosphorus (Staehr et al. 2012). Long term trends in metabolism in Muskegon Lake may have a decreasing trend following the decrease in TP since 1972.

Low NEP Summer Days

Several daily rates of NEP in the summer were negative. These negative NEP days in the summer could be due to several reasons including lower PAR, high wind events, or rain events. It is not unusual to find negative NEP days in the summer, as other studies have found too (Lass et al. 2012). There were a total of 8 days in summer 2017 that had 3 or 4 sites with negative NEP, and several other days with 1 site having negative NEP. The overall summer 2017 average daily maximum PAR was 227 ± 76, the average rain was 0.05 ± 0.13 inches, average wind was 7.8 ± 2.6 knots, and the average maximum daily wind was 9.5 ±3.2 knots. During these 8 negative NEP days in the summer the average maximum daily PAR was 174 ± 64 which was

quite a bit lower than the overall summer average. Half of these days had rain the day of the negative NEP. Two of the days that did not have rain on the same day did have rain on the days leading up to the negative NEP days. And the other two days that did not have rain leading up to the negative NEP even. All of the days had a higher than average wind speed with the average wind speed being 10 ± 2.8 knots and the average maximum wind speed was 13 ± 3.6. With this, the wind speed and maximum daily PAR may have had a bigger influence than rain did.

Problem of Uncertain Rates and Missing Time-Series Data

There were several days at each location where the GPP rates returned a negative value and the R rates returned a positive value. These days can occur because of unexpected increases of DO at night from mixing (Brothers et al. 2017). These negative GPP rates and positive R rates are not ecologically possible since GPP must be positive and R must be negative. These types of rates have been discussed in other papers as well since many metabolism studies face this issue (Staehr et al 2010; Winslow et al. 2016). There are several approaches when considering these rates. For the purpose of this paper, these rates were excluded from analysis. Winslow et al. (2016) discusses that these unrealistic estimates can be dealt with using two approaches: the LakeMetabolizer model can be run unconstrained and these unrealistic estimates can be removed, or the model can be run constrained and these estimates that would normally be unrealistic are forced into being positive for GPP or negative for R. Both methods have their drawbacks. Forcing them into a positive GPP or negative R may cause masking of the data to return the correct sign which would not improve the accuracy or leaving it unconstrained may create bias as well (Winslow et al. 2016). Brothers et al. (2017) concluded that excluding these values may lead to underestimations of metabolism. Brothers et al. (2017) found that with the

standard exclusion of the uncertain terms metabolism was significantly higher than when these terms were included. They found that these uncertain rates were more frequent in the winter and that increasing DO concentrations at night occurred 45% of the time (Brothers et al. 2017). The inclusion of negative GPP values resulted in GPP averages 4 times lower than when they were excluded (Brothers et al. 2017). This study had several days of missing data due to these uncertain terms at all sites. Each site had various amounts of missing data due to sensor issues or unrealistic estimates. The MLO site had 40 missing days in 2016 and 73 missing days in 2017; the East site had 24 missing days in 2016 and 75 missing days in 2017; the West site had 28 missing days in 2016 and 49 missing days in 2017; and the Deep site had 20 missing days in 2016 and 26 missing days in 2017. Today, the problem of missing data with time-series observatories continues – but any remaining and validated data stream can contribute so much more for advancing ecosystem change science than a few good fair-weather daily discrete measurements.

Conclusion

Four buoys located in the limnetic zones in Muskegon Lake in 2016 and 2017 tracked the spatial heterogeneity in metabolic rates. GPP and R had significant differences among groups, whereas NEP was only significantly different among one group. Seasonal analysis showed that in general, metabolic rates were low in the spring, increased in the summer, and decreased in the fall. During the summer in 2016 and 2017 all sites had positive NEP rates indicating autotrophy. In fall 2016 all sites had negative NEP rates. Fall 2017 showed negative NEP averages for the East and West site and the MLO site averages was positive overall but with high variability and the Deeps site was positive. In the spring 2017, East and MLO had negative NEP rates and West

and Deep had positive NEP rates. The Deep site consistently had the higher rates of metabolism due to the higher residence time and distance from the river inlet. Weekly averages of GPP:R ratios show the lake was generally autotrophic throughout the year with a few exceptions at the East site in the spring and MLO and West site during the year. Such variability may be due to the East site's proximity to the main river inlet with higher amounts of allochthonous organic matter that may cause more turbidity diminishing GPP.

Overall there was a seasonal shift from heterotrophy-autotrophy-heterotrophy through the year in this Great Lakes estuary, and the Deep site had higher rates of metabolism than others. The overall annual net autotrophy of the lake indicates that there is more carbon being sequestered than being respired to the atmosphere. This pattern may be similar in lakes and estuaries globally, which could affect the global carbon cycle. Estimating metabolism at different locations has shown to be important since each site varied depending on its residence time, depth, and proximity to the main river inlet. Future studies estimating metabolism should include a longer data sets instead of focusing only on a short period in the summer and include multiple sensor locations since the rates can vary widely. Given that sensor technology cost is decreasing, more time-series multi-sensor lake metabolism studies in lakes across the globe can be carried out in the future that can then be integrated into the global carbon budget. Understanding the spatial heterogeneity of ecosystem metabolism is key to appreciating the reactive role of freshwater ecosystems in Earth's carbon cycle.

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Figure Legends

Figure 1. Map of Muskegon Lake showing locations of each lake metabolism sampling location – East, Muskegon Lake Observatory (MLO), West, and Deep. The Muskegon River Inlet and Bear Lake Inlet are the two main inlets and the Channel to Lake Michigan is the main outlet for the Lake.

Figure 2. Daily Average Dissolved Oxygen (DO) surface concentrations for 2016 (top) and 2017 (bottom) at each location.

Figure 3. Weekly average GPP, R, and NEP (mg C L^{-1} D^{-1}) rates for each location and year in Muskegon Lake, Michigan. In 2016, data were only available for August and September. In 2017, gaps in data were the result of sensor malfunction or use of sensors for other studies.

Figure 4. Seasonal and site GPP (top figure), NEP (middle figure), and R (bottom figure) boxplots. Above each box plot are symbols and letters showing statistical test results. If two boxplots share the same symbol or letter, they are statistically the same. The letters show spatial statistical test results among sites during a season. Symbols show statistical test results among the same site across seasons.

Figure 5. The weekly average GPP and R (mg C L^{-1} D^{-1}) relationship for each site. Each point represents a weekly average. The diagonal dashed line represents the 1:1 relationship. Points above this line indicate GPP dominates and the system is considered autotrophic and points below the line indicate the system is heterotrophic for that week. Most weeks were autotrophic

everywhere, with exception of six weeks at the East station, one week at the MLO station, and one week at the West site.

Figure 6. Seasonal mean ± SE of Gross Primary Production (GPP), Ecosystem Respiration (R), and Net Ecosystem Production (NEP) (mmol C m^{-2} D^{-1}) for each location during the spring, summer, and fall.

Figure 7. Seasonal contour maps of Gross Primary Production (GPP) for spring (top) summer (middle), and fall (bottom).

Figure 8. Seasonal contour maps of Respiration (R) for spring (top) summer (middle), and fall (bottom).

Figure 9. Seasonal contour maps of Net Ecosystem Production (NEP) for spring (top) summer (middle), and fall (bottom).

Table 1: Average characteristics (±SD) for each site during the study period time in 2016 and 2017. The general characteristics here are included hourly in the metabolism estimations. These data were available at each location within Muskegon Lake. MLO represents the Muskegon Lake Observatory located centrally in the lake and Deep is the southerly buoy at the deepest location in the lake.

Variable	MLO	East	West	Deep
Depth (m)	12	11	15	22
Thermocline depth (m)	6.0±3.2	8.1±2.7	9.5±4.3	11.4±7.1
Surface temperature (°C)	21.5±3.3	17.9±5.3	18.7±5.0	19.0±5.1
Sp. Conductivity (µS/cm)	339.0±30.3	369.4±34.5	355.8±32.8	351.7±33.0
Dissolved Oxygen (mg/L)	9.4±1.0	9.6±0.9	9.4±0.9	9.4±0.9
DO Saturation (mg/L)	8.6±0.6	10.8±2.4	9.9±1.8	9.5±1.4

Table 2: Kruskal-Wallis test results for each group of data. The first three groups are each season compared by site and the last 4 data groups are each site compared by season. With this we can understand the spatial differences among seasons and the seasonal differences among sites. The season/site labels are as follows: Spring East (SE), Spring MLO (SM), Spring West (SW), Spring Deep (SD), Summer East (SUE), Summer MLO (SUM), Summer West (SUW), Summer Deep (SUD), Fall East (FE), Fall MLO (FM), Fall West (FW), and Fall Deep (FD). Bolding indicates a significant difference. If a significant difference was found, a pairwise Wilcoxon Test was done and the results of this post hoc test are shown in Figure 4.

Group	Season and Site	GPP	R	NEP
SS	SE, SM, SW, SD	**<0.001**	**<0.001**	0.238
SUS	SUE, SUM, SUW, SUD	**0.010**	0.057	0.689
FS	FE, FM, FW, FD	**0.005**	**0.005**	0.307
ES	SE, SUE, FE	**<0.001**	**<0.001**	**0.013**
MS	SM, SUM, FM	**0.001**	**0.003**	0.732
WS	SW, SUW, FW	**<0.001**	**<0.001**	0.192
DS	SD, SUD, FD	**<0.001**	**<0.001**	0.101

Table 3: Seasonal averages (±SE) of volumetric and aerial rates of metabolism in 2016 and 2017 for each site. Oxygen equivalents are in the supplemental material.

Seasonal averages of metabolism at each site (mg C L^{-1} d^{-1} (SE))												
Year	**Season**	**MLO**			**EAST**			**WEST**			**DEEP**		
		GPP	**R**	**NEP**	**GPP**	**R**	**NEP**	**GPP**	**R**	**NEP**	**GPP**	**R**	**NEP**
2016	Spring	.654±.055	-.487±.046	-.021±.031	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Summer	.842±.082	-.579±.059	.010±.0360	.915±.072	-.631±.054	.006±.046	.925±.057	-.637±.043	.005±.045	1.068±.076	-.720±.056	.030±.045
	Fall	.249±.059	-.206±.051	-.064±.043	.328±.038	-.248±.033	-.055±.025	.242±.027	-.172±.022	-.022±.012	.244±.036	-.170±.029	-.017±.014
2017	Spring	.608±.063	-.456±.050	-.024±.029	.084±.022	-.088±.016	-.040±.009	.404±.048	-.291±.039	-.002±.022	.662±.078	-.475±.065	.001±.003
	Summer	.923±.121	-.627±.090	.027±.057	1.164±.15	-.780±.111	.044±.045	1.157±.14	-.736±.093	.112±.096	1.176±.08	-.778±.063	.060±.036
	Fall	.543±.053	-.363±.062	-.011±.066	.552±.113	-.400±.078	-.060±.039	.348±.049	-.251±.036	-.036±.031	.419±.061	-.287±.048	-.017±.032
Seasonal averages of metabolism at each site (mmol C m^{-2} d^{-1} (SE))													
2016	Spring	179±18	-130±13	-0.1±9	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Summer	277±32	-185±21	9±11	371±33	-247±20	12±20	362±30	-254±18	14±17	404±29	-264±20	21±18
	Fall	165±30	-136±21	-42±32	285±33	-219±30	-51±23	384±29	-189±23	-22±13	392±57	-271±44	-24±22
2017	Spring	339±32	-253±25	-11±16	56±14	-58±11	-26±5	269±30	-190±21	5±13	501±72	-353±53	11±22
	Summer	531±75	-352±50	28±38	661±55	-435±40	37±27	637±77	-379±34	95±74	745±67	-483±48	48±25
	Fall	422±45	-273±46	4±50	327±40	-237±30	-37±25	253±32	-174±19	-15±19	336±34	-215±25	7±23

Figure 1

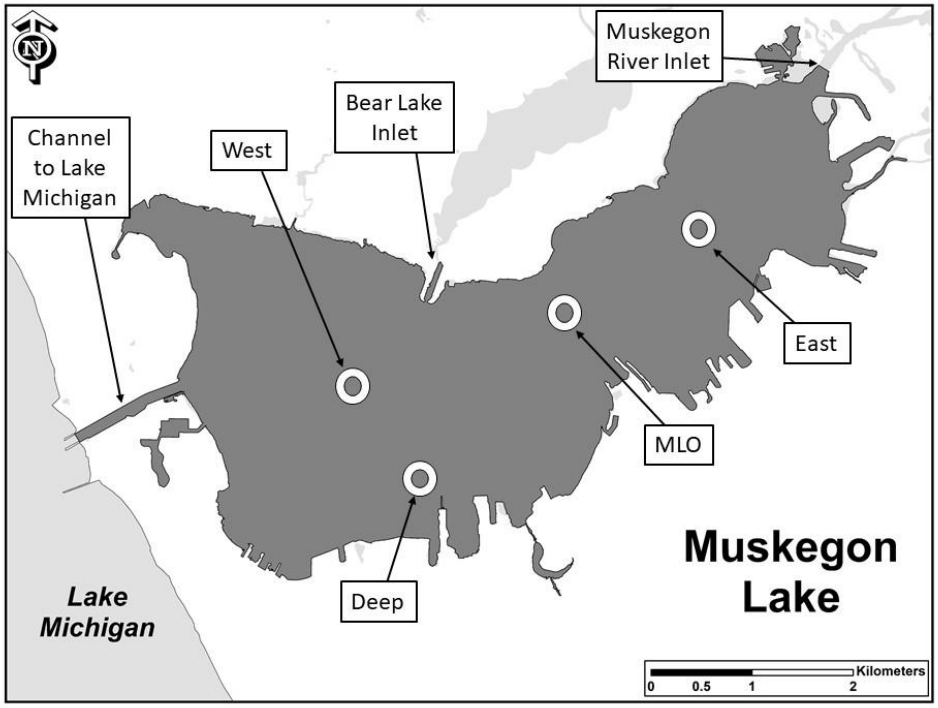

Muskegon River Inlet
Bear Lake Inlet
Channel to Lake Michigan
West
East
MLO
Deep
Lake Michigan
Muskegon Lake
Kilometers
0 0.5 1 2

Figure 2

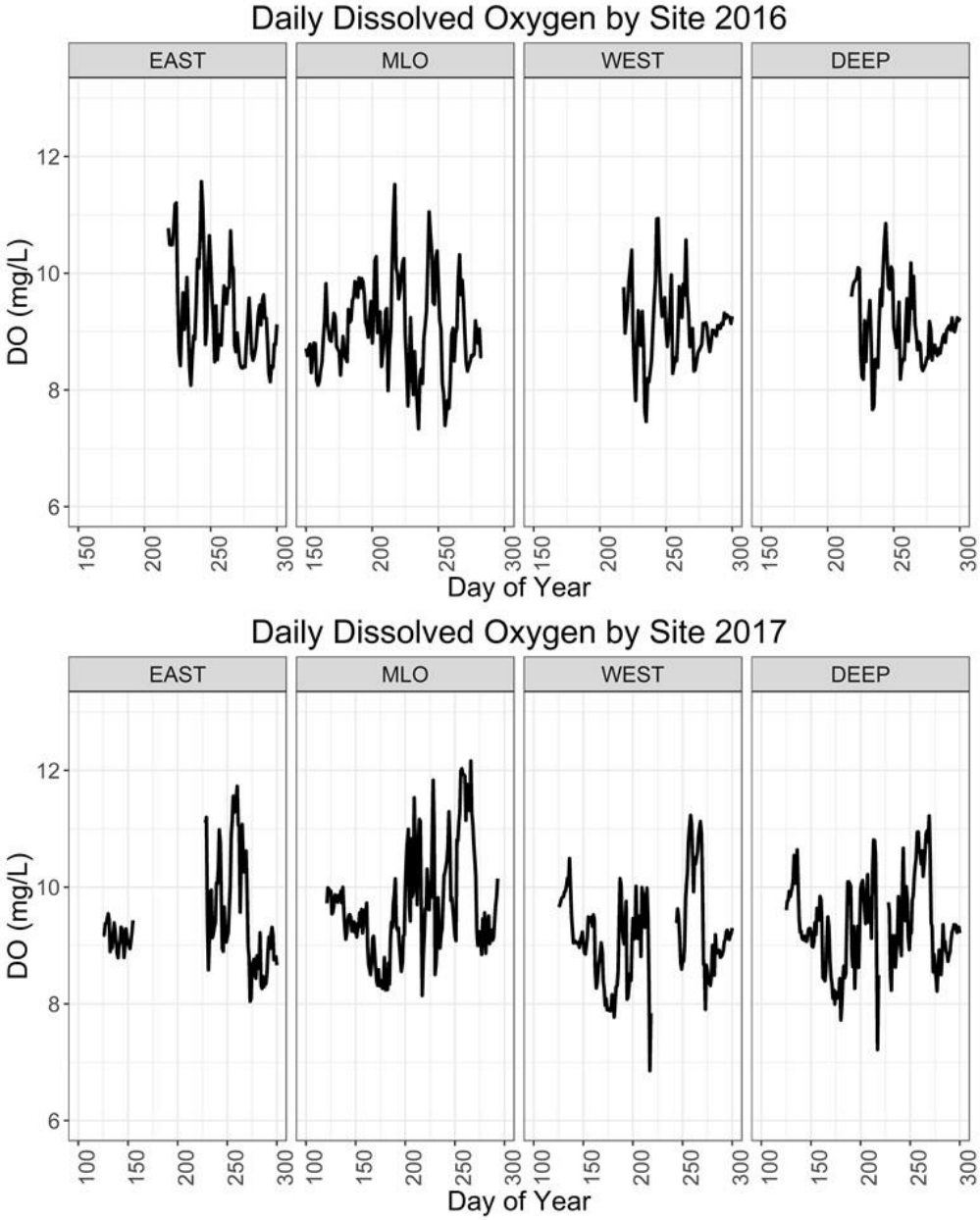

Daily Dissolved Oxygen by Site 2016
EAST
MLO
WEST
DEEP
DO (mg/L)
Day of Year
Daily Dissolved Oxygen by Site 2017
EAST
MLO
WEST
DEEP
DO (mg/L)
Day of Year

Figure 3

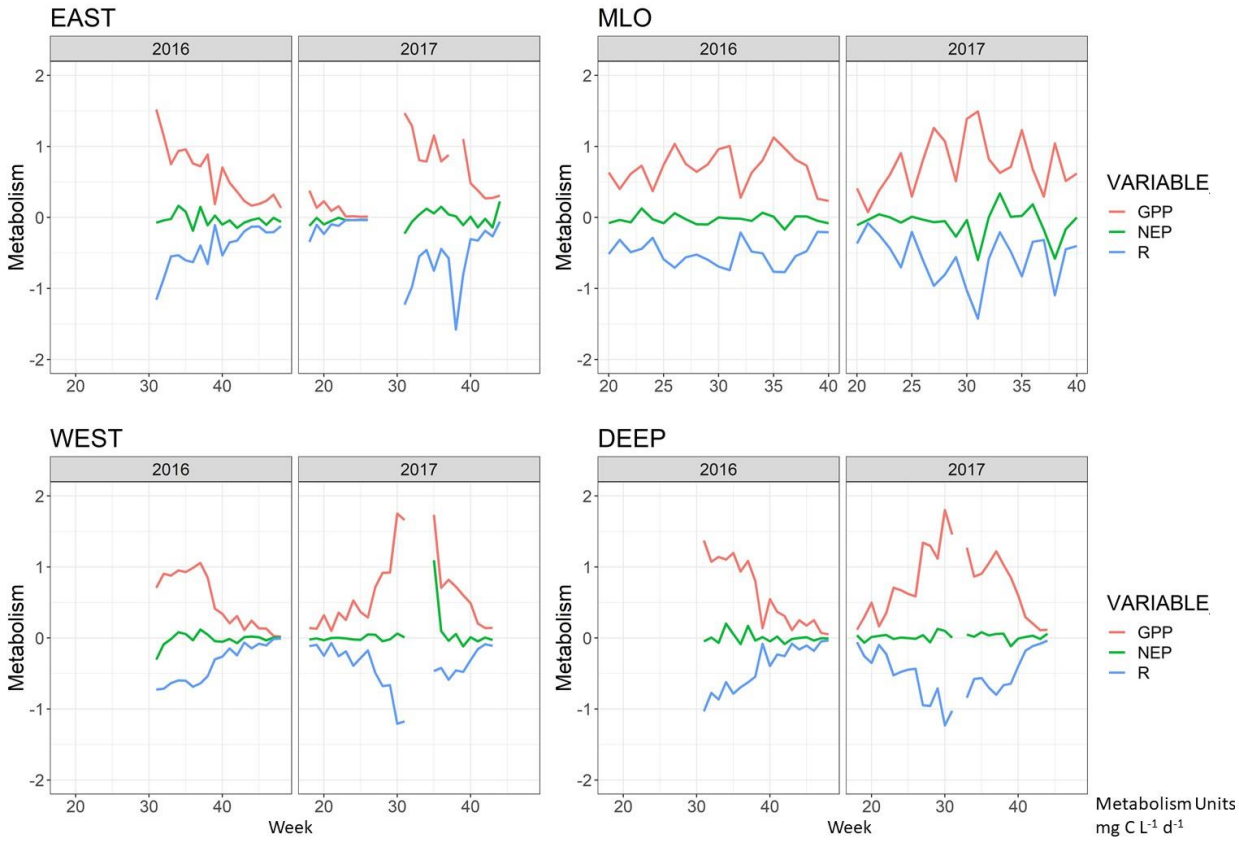

EAST
MLO
WEST
DEEP
2016
2017
Metabolism
Week
VARIABLE
GPP
NEP
R
Metabolism Units
mg C L-1 d-1

Figure 4

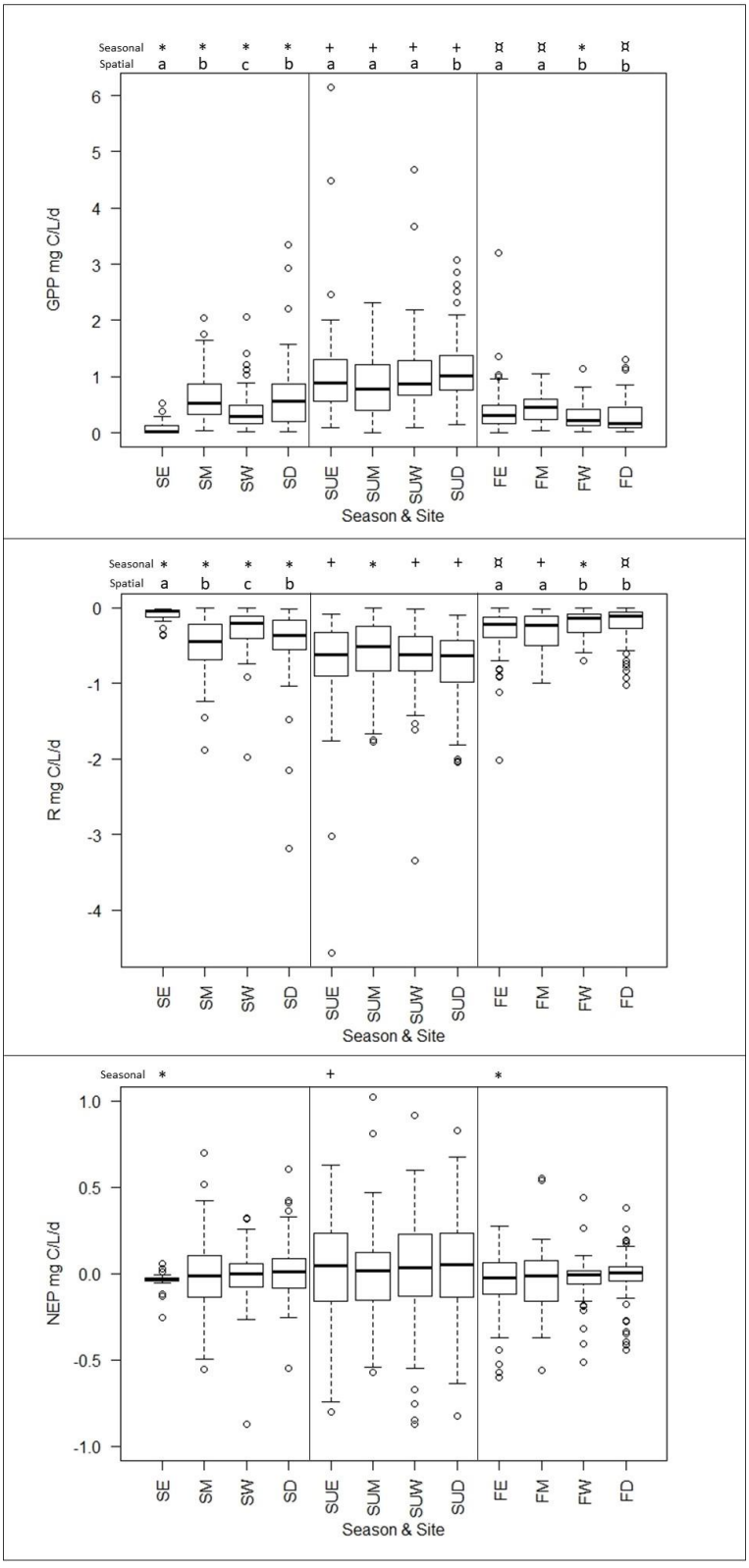

Seasonal * * * * + + + + ¤ ¤ * ¤
Spatial a b c b a a a b a a b b
GPP mg C/L/d
0
1
2
3
4
5
6
SE SM SW SD SUE SUM SUW SUD FE FM FW FD
Season & Site
Seasonal * * * * + * + + ¤ + * ¤
Spatial a b c b a a b b
R mg C/L/d
0
-1
-2
-3
-4
SE SM SW SD SUE SUM SUW SUD FE FM FW FD
Season & Site
Seasonal * + *
NEP mg C/L/d
1.0
0.5
0.0
-0.5
-1.0
SE SM SW SD SUE SUM SUW SUD FE FM FW FD
Season & Site

Figure 5

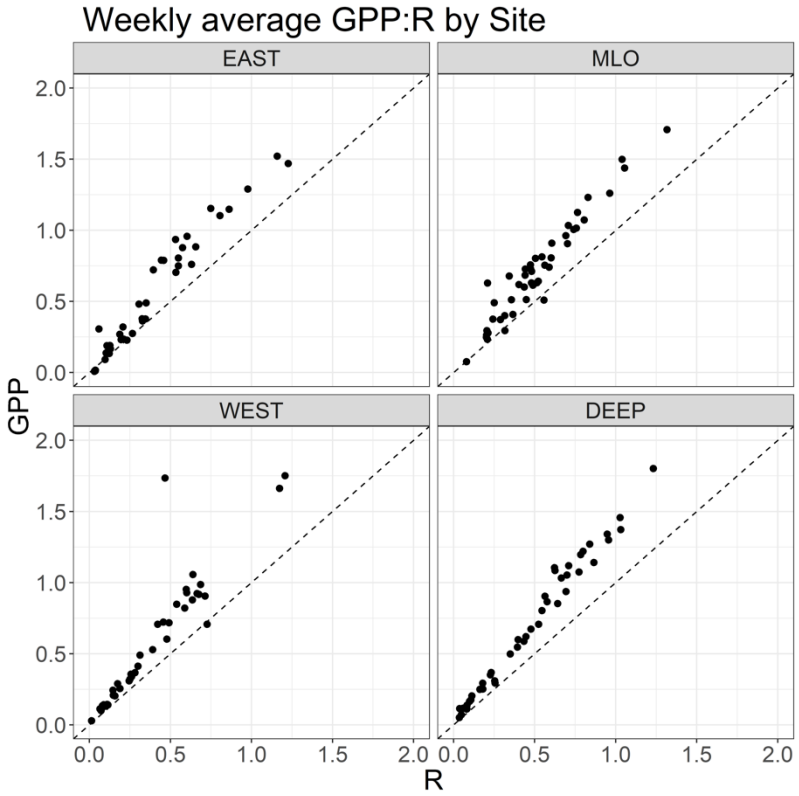

Weekly average GPP:R by Site
EAST
MLO
WEST
DEEP
GPP
R
0.0
0.5
1.0
1.5
2.0

Figure 6

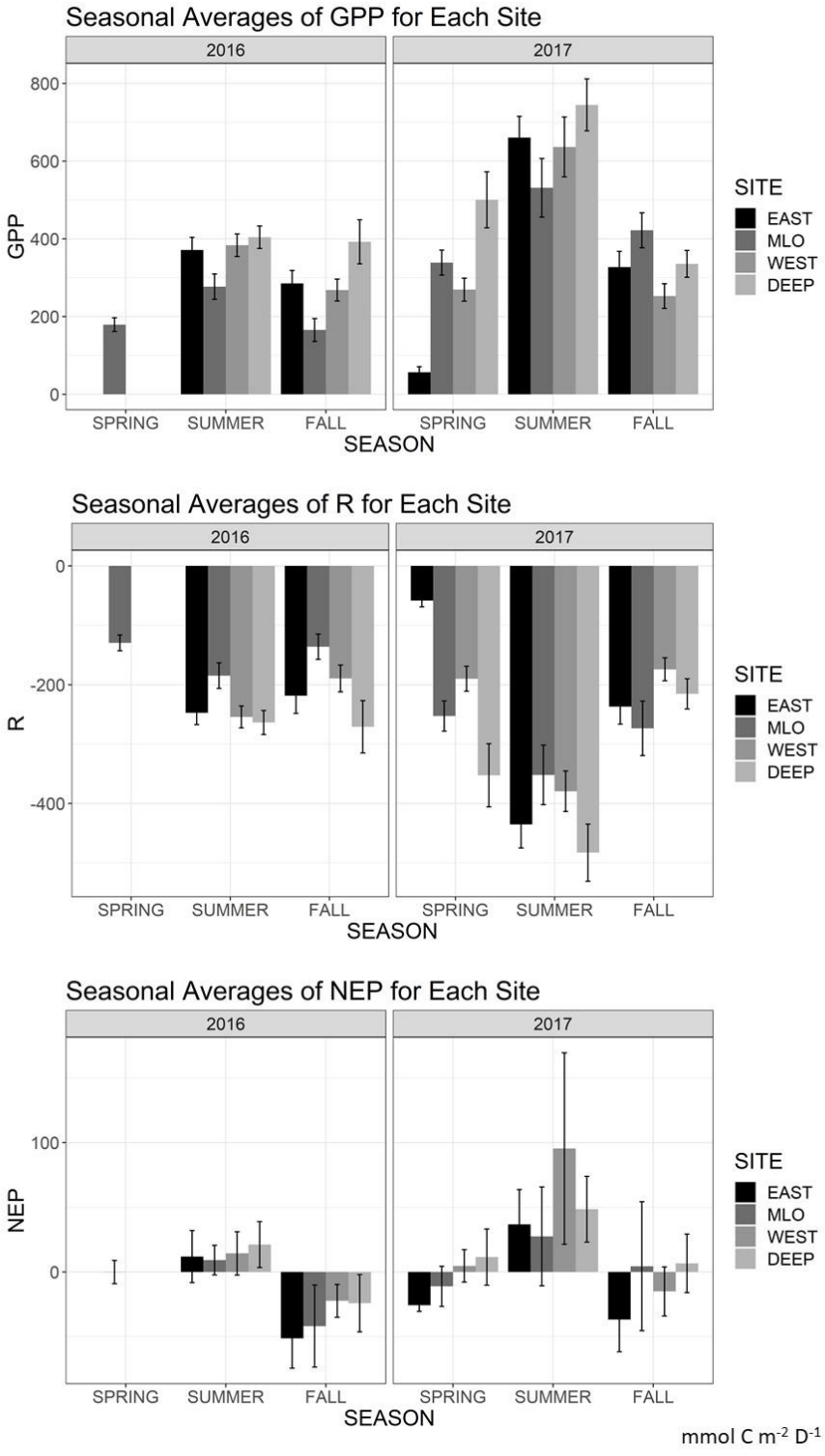

Seasonal Averages of GPP for Each Site
2016
2017
GPP
800
600
400
200
0
SPRING
SUMMER
FALL
SEASON
SITE
EAST
MLO
WEST
DEEP
Seasonal Averages of R for Each Site
R
0
-200
-400
Seasonal Averages of NEP for Each Site
NEP
100
0
mmol C m-2 D-1

Figure 7

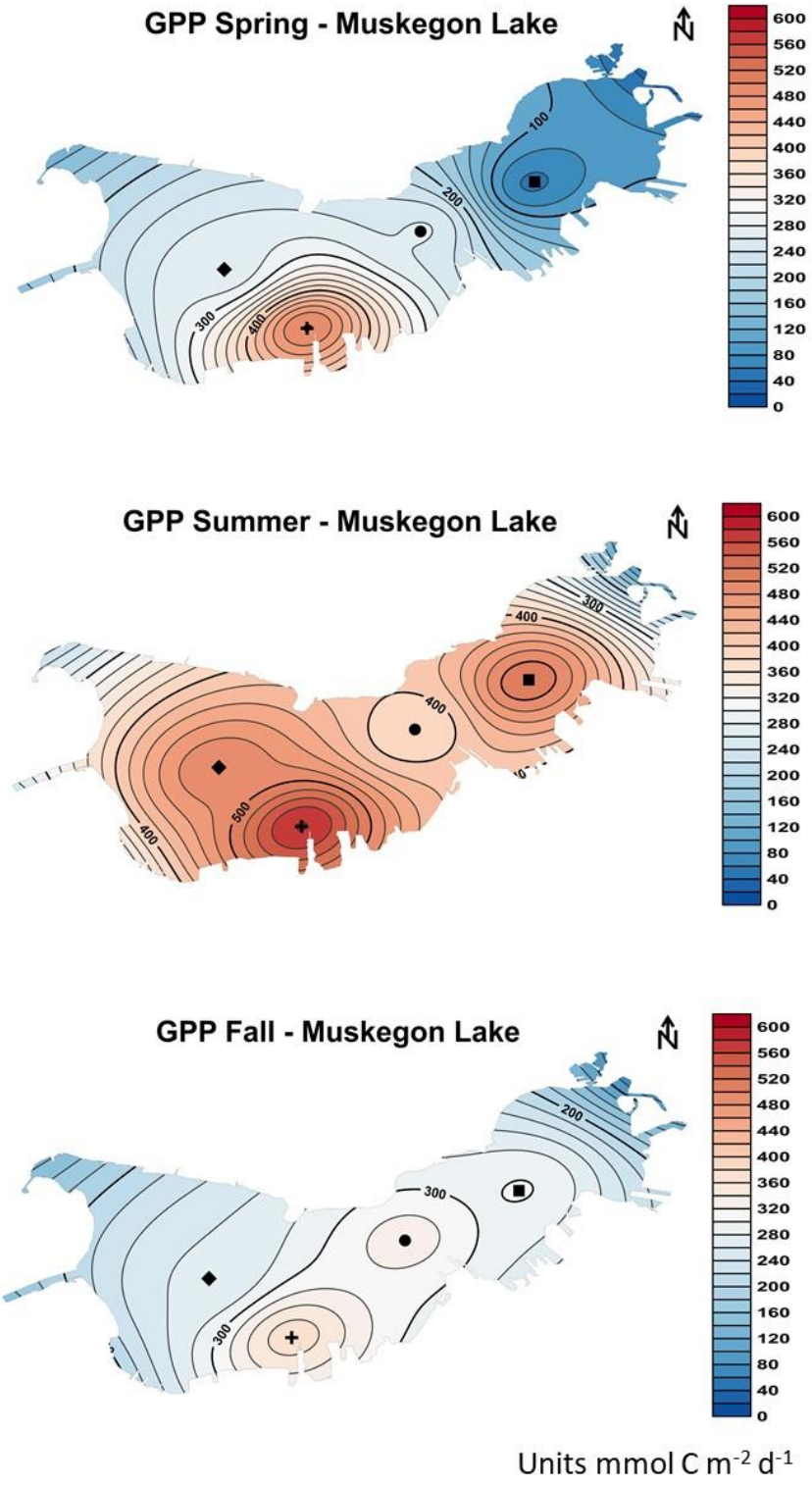

GPP Spring - Muskegon Lake
N
600
560
520
480
440
400
360
320
280
240
200
160
120
80
40
0
100
200
300
400
GPP Summer - Muskegon Lake
N
600
560
520
480
440
400
360
320
280
240
200
160
120
80
40
0
300
400
400
500
400
GPP Fall - Muskegon Lake
N
600
560
520
480
440
400
360
320
280
240
200
160
120
80
40
0
200
300
300
Units mmol C m-2 d-1

Figure 8

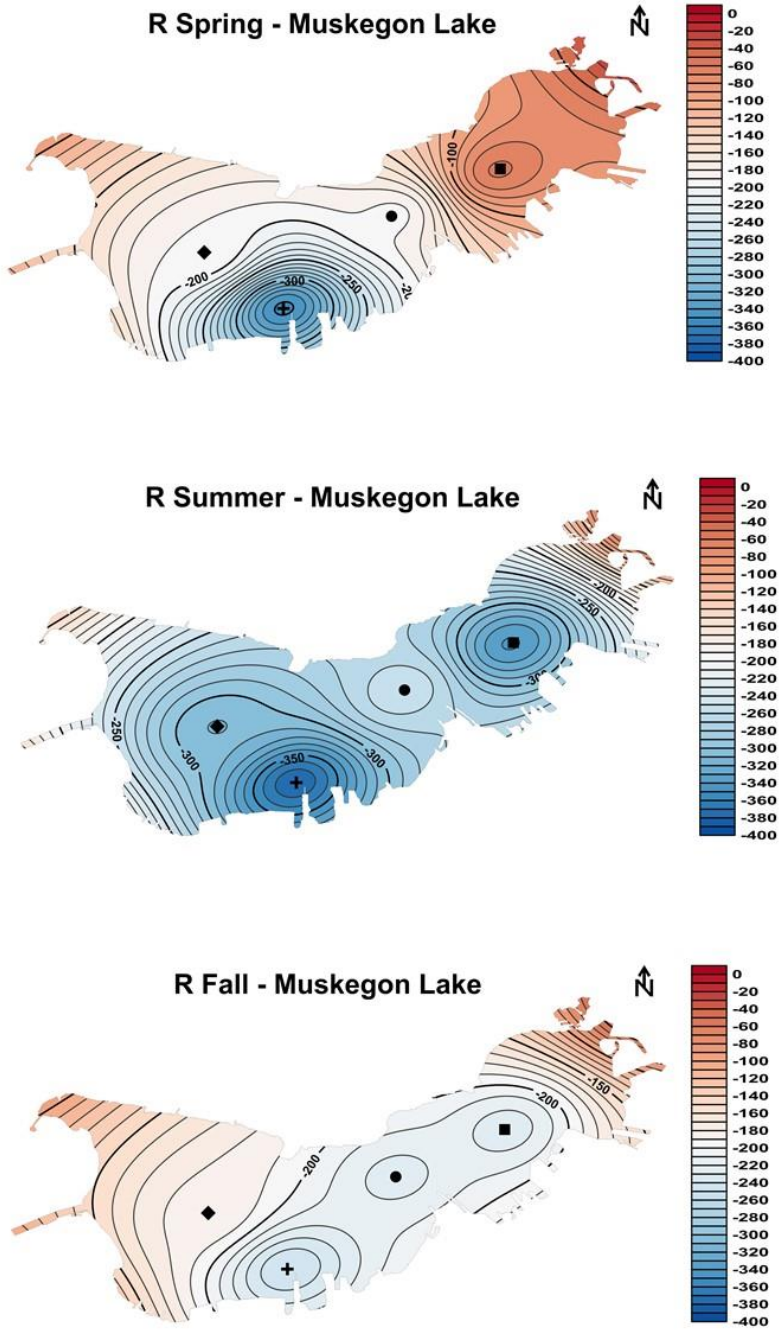
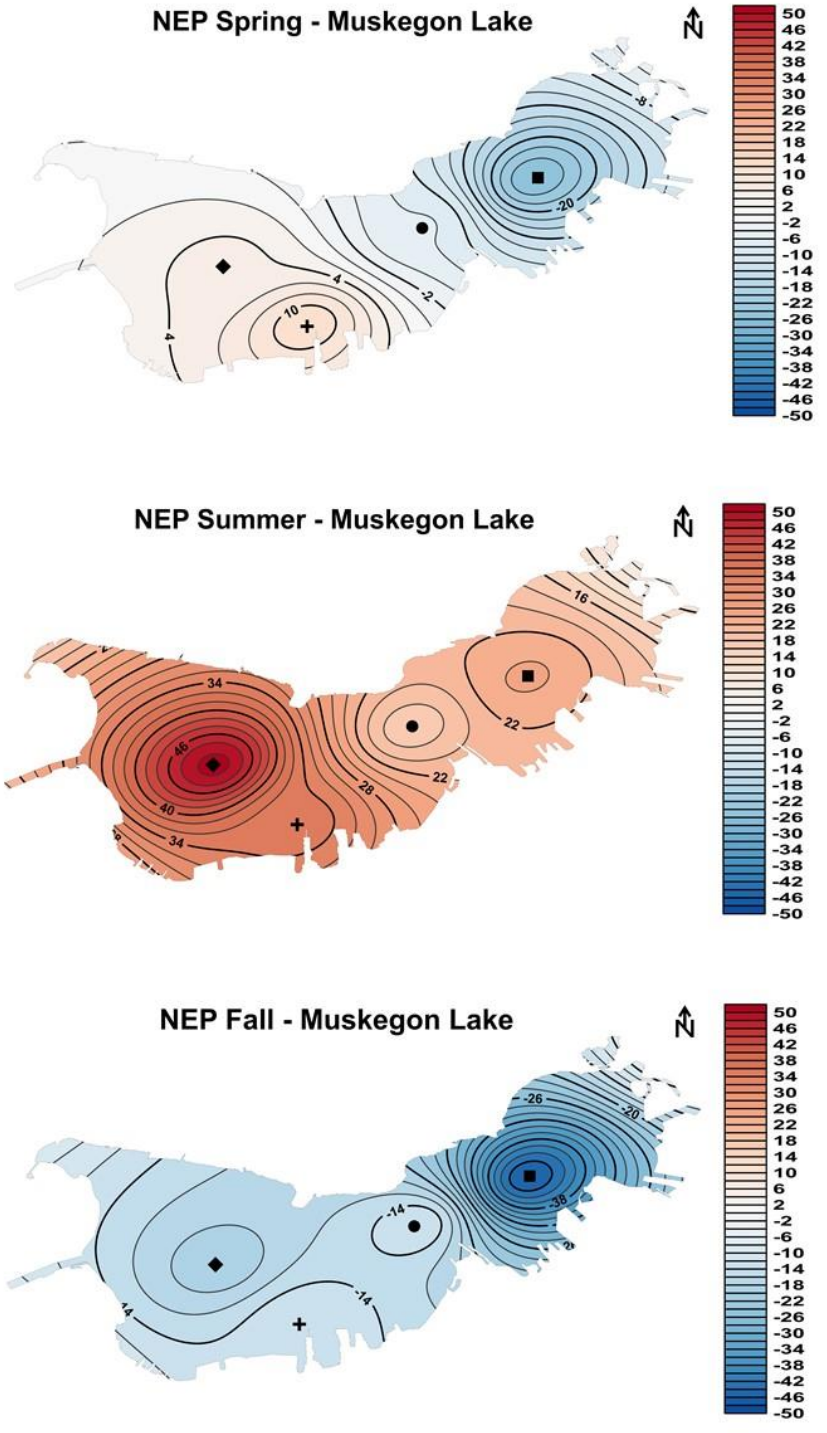

R Spring - Muskegon Lake
N
0
-20
-40
-60
-80
-100
-120
-140
-160
-180
-200
-220
-240
-260
-280
-300
-320
-340
-360
-380
-400
-100
-200
-300
-250
R Summer - Muskegon Lake
N
-200
-250
-250
-300
-350
-300
R Fall - Muskegon Lake
N
-150
-200
-200
Units mmol C m-2 d-1

Figure 9



Units mmol C m^{-2} d^{-1}

Chapter 3

Extended Literature Review

A Review of Carbon Cycling in Inland Waters

"Production and respiration are two sides of the same metabolic coin – the yin and yang of the biosphere." – Bopi Biddanda (2006)

Abstract

Primary production and respiration drive life and the carbon cycle on Earth. Today human intervention through burning fossil fuels and land use change, is altering the natural carbon cycle. These anthropogenic alterations have led to numerous studies estimating carbon storage and fluxes in various natural and anthropogenic ecosystems. While the three main carbon storage pools, ocean, atmospheric, and terrestrial, have been researched extensively, inland waters tend to be ignored in the big picture because they only comprise ~3% of the Earth's surface. Recent research has found that inland waters are "hot spots" for carbon processing instead of only acting as a passive pipe for the transit of carbon form the land to the ocean. Going forward, it is important to fill in uncertainties associated with carbon cycling in various types of inland waters so that they can be integrated into the global budget.

Introduction

Global Carbon Cycle

Since all life is composed of carbon, it is essential to understand how carbon is transported and processed on the everchanging Earth. Photosynthesis fuels the biosphere by capturing carbon and releasing oxygen to the atmosphere and respiration is the breakdown of

organic carbon consuming oxygen, which together links the oxygen and carbon cycles on Earth. By understanding the carbon cycle globally, we can also understand the other biogeochemical reactions of other elements of Earth such as Nitrogen, Phosphorus, and Sulfur through stoichiometry because their movement is coupled of that of carbon. Carbon moves from the atmosphere, ocean, and land through various transport mechanisms. Overall the Earth has 32 x 10^{23} g of carbon, but only 40 x 10^{18} g C is in the active pools. Within these active pools the ocean has 38,000 x 10^{15} g C, the soils have 1,500 x 10^{15} g C, the atmosphere has 750 x 10^{15} g C, and living plants have 560 x 10^{15} g C (Schlesinger and Bernhardt 2013). Fossil fuels are currently adding 9.1 x 10^{15} g C to the atmosphere annually; however only 56% of this released carbon remains in the atmosphere and 32% is absorbed into the ocean (Schlesinger and Bernhardt 2013). The terrestrial land is the biggest absorber of carbon followed by the ocean (Biddanda 2017). The overall residence time of carbon in the atmosphere before photosynthesis captures it on land or in the ocean is ~5 years (Schlesinger and Bernhardt 2013). Seasonal oscillations are present each year by the ramp up and slowdown of production due to changes in temperature and light with the seasons and buffering of CO_2 with the oceans. Today, the global carbon cycle is increasingly perturbed due to industrial scale anthropogenic activities leading to climate change.

Role of Humans

The Anthropocene is the new age of human domination ever since the advent of agriculture and fire, although many argue over the exact starting period of the Anthropocene (Erle 2018). Since then, anthropogenic alterations of biochemical cycles have interrupted the natural cycle of carbon and other elements. Humans have cut down great forests, prevented rivers from forming their natural paths through damming and channelization, drained wetlands,

mined for precious materials, created insoluble surfaces with concrete, applied fertilizer and pesticides, and of course burning fossil fuels has greatly impacted natural biogeochemical cycles for centuries. The Keeling Curve has shown that carbon concentrations in the atmosphere continue to rise since the 1960s with CO_2 surpassing 400 ppm. With the help of ice coring technology, we know CO_2 ranged from 275-285 ppm in the pre-industrial age – but are above 400 ppm today (Forster et al. 2007; Schlesinger and Bernhardt 2013; Bernola et al. 1995). With CO_2 levels rising in the atmosphere come increasing temperatures and altered ocean currents creating negative feedback loops that could cause even greater damage to the biosphere. Therefore, it is it increasingly important to understand the anthropogenically altered biogeochemical cycling of elements on Earth.

Role of Inland Waters

Inland waters comprise of rivers, lakes, estuaries, wetlands, and reservoirs and overall comprise roughly ~3% of the Earth's surface. And while they make up only a small footprint on Earth, they have been shown to be sentinels of change as they are highly reactive to chemical, biological, and physical changes in the surrounding environments (Tranvik et al 2009; Battin et al. 2009; Cole et al. 2007; Biddanda 2017). Inland waters were historically thought of as passive pipes which only transport water from the land to the ocean; however, recent research has shown that more than half of the carbon absorbed in the land is processed in freshwater ecosystems (Battin et al. 2009; Biddanda 2017). Carbon flux from land into freshwater ecosystems is roughly 2.7-2.9 Pg C yr^{-1} from four main sources: particulate and dissolved organic carbon from soil, chemical weathering, sewage, and carbon fixation (Batting et al. 2009). Of this carbon that is imported into freshwater ecosystems, roughly 0.4 Pg C yr^{-1} is buried into sediments, 0.9 Pg C yr^{-}

^{1} is transported to oceans, and 1.4 Pg C yr^{-1} is emitted to the atmosphere (Battin et al. 2009; Biddanda 2017). While we know inland waters process a large fraction of carbon that the land inputs to the inland waters, there is still little presence of this in global carbon budgets (Regnier et al. 2013; Quéré et al. 2018).

Metabolism

What is metabolism?

Collectively primary production and respiration is metabolism in any type of ecosystem. Gross primary production (GPP) is the sequestration of carbon by capturing the energy of the sun into organic compounds which fuels the Earth. Respiration (R) is the breakdown of organic carbon by bacteria and other organisms which can release carbon to various storage pools. The difference between GPP and R is the net ecosystem production (NEP) which determines if an ecosystem is releasing more carbon to the atmosphere or taking in more carbon than is released to the atmosphere. Ecosystems can be considered autotrophic (NEP > 1) or heterotrophic (NEP < 1) depending on the resulting NEP.

There have been various methods for estimating metabolism. Traditional methods include using the biological oxygen demand (BOD) and carbon – 14 (^{14}C) methods. Both the BOD and ^{14}C method measure either oxygen or carbon over a 24-hour period to determine production and respiration rates. Light and dark bottles are used to estimate production and respiration, respectively by looking at the changes in oxygen. The ^{14}C is done by using an isotope tracer (^{14}C Sodium bicarbonate a radiolabeled proxy for dissolved carbon dioxide in water) in bottles of water. The ^{14}C is taken up by phytoplankton during the incubation period. ^{14}C is measured at an initial start time and at the end to determine how much of the total pool of dissolved carbon

dioxide has been take up by phytoplankton. For the BOD method, changes in oxygen are due to primary production by benthic plants and phytoplankton increasing oxygen, benthic respiration uptake of oxygen decreasing overall oxygen, exchange of oxygen with the air which could increase or decrease oxygen, and possible influx of oxygen from groundwater (Odum 1956; Sargent and Austin 1954; Sargent and Austin 1949). Since oxygen and carbon are paired the carbon flux would be the same but with the opposite sign. Sargent and Austin (1949) were the first to use changes in dissolved oxygen over a 24-hour period to measure production in an Atoll at the Marshall Islands (Sargent and Austin 1949; Sargent and Austin 1954). In 1956, Odum used the BOD method in flowing waters to determine how different communities are supported (Odum 1956). Since Sargent and Austin (1954) and Odum (1956) using diel measurements of oxygen has been widely used in aquatic ecosystems to estimate metabolism (Hall 1972; Smith and Key 1975; Cole et al. 2000; Hanson et al. 2007; LaBuhn and Klump et al. 2016; and others). These methods have been refined through the years and are accurate in measuring oxygen or carbon changes. The downfall is that they have "container effects", which do not account for atmospheric exchange or groundwater influx (Bender et al. 1987). Every step of these methods can be challenging and time consuming which do not allow for a high frequency of measurements.

The advent of sensor technology helped alleviate some of the challenges associated with the BOD and ^{14}C methods and the free water dissolved oxygen method became mainstream (Staehr et al. 2010; Cole et al. 2000). Using oxygen sensors, diel measurements are able to be taken for long periods of time with little intervention and rather than spending time doing Winkler Titrations or using isotopes to get the resulting oxygen concentrations as data is downloaded straight from the sensor. While this may sound like the perfect solution, it has its

challenges as well. Sensors are known to drift and will have problems with biofouling and other maintenance requirements. Since the sensor is placed freely in the water column, the gas exchange with the atmosphere must also be taken into consideration and oxygen may decrease or increased based on mixing events within the water column rather than the biological signal. Placing the sensor in the top part of the water column captures surface production and respiration. There have been several models for estimating the metabolism from *in situ* sensors (McNair et al. 2013; McNair et al. 2015; Cole et al. 2000; Winslow et al. 2016). Each of the methods have different underlying statistics (algebra, Bayesian, maximum likelihood, likelihood filter, and linear regression) which can result in slightly different estimates of metabolism despite using the same data. The algebraic model is often called the bookkeeping model or accounting approach which is the most widely used and easiest to use (McNair et al. 2013; McNair et al. 2015; Winslow et al. 2016).

Uncertainties and assumptions in metabolism

Despite the fact that these methods have been used for 60+ years, there are still several uncertainties associated with the methods. One of the first uncertainties is that one testing location within a lake is often used and representative of the whole lake metabolism. This testing locations is typically in the middle and deepest spot in a lake but the sensor is located at the surface of the water. This uncertainty has been tested in a few studies and in this thesis. Including littoral zones instead of just the pelagic zones was shown to increased net primary production (Lauster et al. 2006; Vesterinen et al. 2017). One study used 27-35 sensors in two lakes to determine how many sensors are needed to accurately represent a lake and if there were significant differences. They found metabolism varied by 1-2 orders of magnitude between sites

and that using 4 sensors resulted the best measurements without having to deploy too many sensors within the lake (Van de Bogert et al. 2012). It is assumed sensors are the surface of the water is able to detect changes of the mixed layer but that also leaves out the other layers in a lake. Vertical metabolism is driven mainly by light availability which causes an increase in respiration with depth (Coloso et al. 2008; Obrador et al. 2014). Since R occurs at all depths, but GPP may slow down with depth due to light, when considering the whole water column lakes may have more heterotrophy occurring (Coloso et al. 2008; Obrador et al. 2014). These studies have shown that is important to understand metabolism at various locations within the same ecosystem. Another uncertainty within lake metabolism studies is that occasionally GPP measurements are negative and R measurements are positive, which is biologically impossible. The main suggestion when this happens is to exclude this data from analysis as it could create an underestimation (Staehr et al. 2010; Winslow et al. 2016; Brothers et al. 2017). Another assumption is that respiration is equal during the day and at night (Staehr et al. 2010; Hanson et al 2003; Cole et al. 2000). Some of these uncertainties have been looked at, but at large they need continuous research.

Drivers of Ecosystem Metabolism

Metabolism is driven by a variety of variables including dissolved organic carbon (DOC), nutrient availability, light availability, watershed size, weather events, seasons, geographical locations, and inland water type. With an increasingly changing world, the relationship of these variables needs to be understood. DOC for example is flushed into the aquatic ecosystems from the surrounding landscape and is having an increasing trend in lakes globally (Seekell et al. 2015; Clark et al. 2010; Monteith et al. 2007). DOC is closely knit with light availability and

with nutrient availability. Nutrients are bound in humic compounds that are found in DOC which may stimulate production (Seekell et al. 2015). In some lakes DOC limits lights, but in others that have low ambient DOC, it can increase production (Kissman et al. 2013). Other used a lake wide experiment with DOC additions and they found that pelagic primary production increased, likely due to the slight increase of phosphorus and only a small change of epilimnetic light availability (Zwart et al. 2016). Zwart et al. (2016) found the respirations rates also increased at the same rate DOC was added to the ecosystem creating a more heterotrophic ecosystem, despite the slight increase in production. These results indicate increases in DOC in lakes globally may create more heterotrophic aquatic ecosystems (Zwart et al. 2016; Kissman et al. 2013) and DOC is controlled by slope, wetland area, precipitation, watershed size, and the area of the lake (Hall et al. 2015).

Several other variables could influence metabolism. Residence time within lakes can be altered by rainfall during storm events. In the present study, river discharge had a negative relationship with GPP, likely due to the shortened residence time during increased river flows. Metabolism rates were highest in the area of the lake with the longest residence time. In this study and others increasing water temperatures are often correlated to increased metabolism (Caffrey 2003). Primary production is also driven by the amount of light available in a day creating daily and seasonal changes where the winter primary production is lower than in the summer or a cloudy day brings lower rates. Metabolism can be highly variable day to day likely due to available light, wind, and other environmental parameters.

Muskegon Lake History and Metabolism

Previous Muskegon Lake Studies

Muskegon Lake has been listed as an Area of Concern (AOC) by the Environmental Protection Agency (EPA). Because of this, there has been extensive monitoring and restoration efforts for the lake. Seasonal monitoring beginning in 2003 found that occasionally Muskegon Lake would be hypoxic (oxygen < 4 mg/L), and metabolism was estimated during these monitoring dates using the BOD method. Following this, the Muskegon Lake Observatory (MLO) was established to monitor the lake daily from the central location. So far, we have found that Muskegon Lake experiences seasonal hypoxia for 29-85 days a year (Biddanda et al. 2018). However, since Muskegon Lake is hydrologically connected to Lake Michigan, cold oxygen rich water from Lake Michigan enters Muskegon Lake during northerly wind events temporarily relieving bottom water hypoxia (Liu et al. 2018). Additionally, three studies have been done using the BOD metabolism method and all resulting in net autotrophy in Muskegon Lake (Weinke et al. 2014; Dila et al. 2015; Defore et al. 2016). A gradient was found with Muskegon Lake being most productive, thus a "goldilocks zone," and Lake Michigan being net heterotrophic with less production occurring (Weinke et al. 2014; Dila et al. 2015; Defore et al. 2016). This thesis found that with high frequency data Muskegon Lake as still net autotrophic with significant differences between seasons and it was sometimes heterotrophic, depending on the year, in the fall and winter. Our main objectives for this study were to understand the seasonal metabolism patters, determine if there were spatial differences in metabolism, and if the traditional BOD method compared to the BUOY method. We found a season change of metabolism where it was low in the winter, increased in the spring, peaked in the summer, and began to decrease again in the fall, we found that spatially metabolism was significantly different indicating one sensor may not be enough to represent lake wide metabolism, and that traditional

BOD method typically had higher rates of metabolism but with the same seasonal pattern (Figure 1).

Past and Future of Muskegon Lake

Land use may alter biogeochemical processes in Muskegon Lake in the future by increased runoff from more impervious surfaces adding additional pollutants to the watershed. Land use historically in the Muskegon River Watershed originally was heavily forested prior to the logging industry in the 1800s where the lumber industry crashed due to unsustainable practices (Steinman et al. 2008). As of 1978 the land use in the Muskegon River Watershed was mainly forested (53.2%); agriculture as the second largest (23.0%); grass/pasture (9.9%); water and wetlands (9.7%); and finally, urban (4.3%) (Tang et al. 2005; Marko et al. 2013; Freedman et al. 1979). Urban areas are predicted to increase in the watershed from 4.2% to 7.1% by 2040 and with this there is expected to be increased runoff and pollution from these urban areas by 5-12% (Tang et al. 2005). Nitrogen and phosphorus losses are expected to increase by 3% mainly due to loss of agriculture land. Forested areas are predicted to have the largest area losses by 3.7% and wetlands are predicted to have the smallest loss by 0.6% by 2040 (Tang et al. 2005). Long term monitoring has been done in Muskegon Lake since 2003 where various parameters at six locations in the lake and in 1972 additional testing was done in the lake as part of the Area of Concern (AOC) delisting efforts (Steinman et al. 2008). Between 1972 study and 2003-2005 study soluble reactive phosphorus (SRP) and total phosphorus (TP), and chlorophyll *a* were significant reduced from 20 µg/L to 5 µg/L, 58 µg/L to 26 µg/L, and 24.7 µg/L to 5.9 µg/L respectively. Secchi disk increased from 1.5 to 2.3 m from 1972 to 2003-2005 data. Nitrate concentrations increased significantly from 1972 to 2002-2005 (Steinman et al. 2008).

Metabolism across world-wide ecosystems

Since metabolism can vary so widely depending on environmental and geographical factors, it's interesting to know how other ecosystems shape up. Within aquatic ecosystems oligotrophic, mesotrophic, and eutrophic lakes primary production varies from 50-300, 250-1000, and >1000 mg C m^{-2} d^{-1}, respectively (Schlesinger and Bernhardt 2013). Within ocean ecosystems, primary production ranges from 130 to 270 to 820 mg C m^{-2} d^{-1} in the ocean, shelf, and upwelling zones, respectively (Schlesinger and Bernhardt 2013). Estuaries cover only 0.3% and lakes cover 2% (total freshwater is 3% of the Earth's surface) of the worlds surface water but process carbon at higher per unit area rates than the ocean ecosystems previously stated. In temperate estuaries metabolism can range from 24 to 2740 mg C m^{-2} d^{-1} (Azevedo et al. 2006; Schlesinger and Bernhardt 2013). Specifically, primary production in Bristol Channel was 204 mg C m^{-2} d^{-1} (Joint 1978), St. Lawrence was 10-800 mg C m^{-2} d^{-1} (Sinclair et al. 1978), and Apalachicola Bay production ranges from 90 – 1800 mg C m^{-2} d^{-1} (Mortazavi et al. 2000; Azevedo et al. 2006). Muskegon Lake primary production ranged from 17 to 1900 mg C m^{-2} d^{-1} depending on the time of year. Comparing estuaries and Muskegon Lake to ocean measurements shows that although they take up a very small space on Earth, they are having primary production rates ranges within the same range as the oceans.

Each ecosystem has differing amounts of carbon storage and processing ability. Globally there are three distinct latitudinal changes of primary production where ecosystems closer to the equator have the highest rates of primary production at , then the midtemperate latitudes are driven by terrestrial production in the northern hemisphere and oceanic production in the southern hemisphere, and then at low latitudes production is uniform and the lowest (Field et al.

1998). Overall the Earth net primary production is 104.9 x 10^{15} g C yr^{-1} with 53.8% being from land and 46.2% being from oceans (Field et al. 1998). Within different ecosystems the open ocean has a net global production of 42 x 10^{15} g C y^{-1} and is 90% of the ocean surface, coastal zones are 9 x 10^{15} g C y^{-1} and are 9.9% of ocean ecosystems and upwelling zones produce 0.15 x 10^{15} g C y^{-1} and are 0.1% of the ocean ecosystems. On land the total forests, shrublands, deserts, tundra, and crops total 58.9 x 10^{15} g C y^{-1} of net production each year (Schlesinger and Bernhardt 2013). If Muskegon Lake were representative of all inland waters on Earth which comprise 4,000 x 10^{3} km^{2} with an annual rate of 41 g C m^{-2} y^{-1}, the total net primary production for inland waters would be 0.164 x 10^{15} g C y^{-1} by the BUOY method. If the BOD method is used to represent net production for inland waters, the total would be 1.25 x 10^{15} g C y^{-1} (Table 1). These rates are comparable with the upwelling zones in oceans (0.15 x 10^{15} g C y^{-1}), Mediterranean shrublands (1.3 x 10^{15} g C y^{-1}), and the arctic tundra (0.5 x 10^{15} g C y^{-1}) (Table 1). Since these inland waters are comparable to other terrestrial and aquatic ecosystems, that means they are important contributors in the global carbon context and should be included in global estimates. Ultimately, comparisons of gross primary production across ecosystems types would be the best indicator for comparing global carbon cycling across ecosystems and that may be pertinent to study in the future with the carbon cycling being increasingly important.

Overall respiration (R) is the dominant component of global metabolism and therefore plays an over-size role in carbon cycling in all ecosystems. However, R is seriously understudied. Since R accounts for most of ecosystem metabolism, small changes its rate can result in very large changes of NEP and GPP. With the current changing climate, R is likely to increase substantially; however, GPP and NEP may not increase or change correspondingly (Dila et al. In Review). Increased surface water stratification due to increased temperatures may also

reduce overall GPP or NEP by reducing nutrient flux form deeper water, leaving R to dominate metabolism even more (Dila et al. In Review). In a world undergoing rapid warming and anthropogenic stress, R is a critically important metabolic variable that deserves greater scrutiny (Yvon-Durocher et al. 2010; Coelho et al. 2013; O'Rilley et al. 2014; Duarte et al. 2014).

Conclusion

Inland waters were first inventoried from 1914 through 1925 by Halbfass and Thienmann which concluded lakes cover 1.8 % of the Earth's surface or 2.5 million km^2 (Halbfass 1914; Thienemann 1925; Downing 2010). However, additional surveys were done 70 years later have now conclude that there are 4.2 million km^2 or 304 million natural lakes on Earth (Downing et al. 2006; Downing 2010). Even though inland waters are small in comparison to the rest of the world, they are disproportionately important in the global carbon context (Downing 2010; Biddanda 2017; Cole et al. 2007; Tranvik et al 2009; Battin et al. 2009). While Muskegon Lake itself may only take up a tiny bit of the Earth's surface, it may represent other freshwater ecosystems similar to it that add up to 3% of the planet. If Muskegon Lake did represent all inland waters around the world, the net primary production would be similar to upwelling zones in the oceans with Muskegon Lake ranging from 0.109×10^{15} to 0.835×10^{15} g C y^{-1} with the BUOY and BOD methods, respectively, and upwelling zones having 0.15×10^{15} g C y^{-1} total net primary production. In a world where humans are increasingly altering ecosystems, it is important to thoroughly understand what is happening on a small scale so that it can be applied to larger scales for gaining a better understanding of how current and future ecosystems will respond to increasing anthropogenic stress and climate change.

Extended Methodology

Study Site

Muskegon Lake is the end point for the 2nd largest watershed in Michigan, the Muskegon River Watershed. The lake is roughly ~17 km^2 with an average depth of 7 m and a maximum depth of 22 m. Muskegon Lake is on the EPA's AOC list due to historical impairments from the logging industry in the 1800s and other industrial activities in the 1900s. Muskegon Lake is a freshwater estuary since it is connected to Lake Michigan through a navigation channel and water from Lake Michigan goes through this channel in Muskegon Lake (Liu et al. 2018). Although remediation actions have been taken which has greatly improved the lake quality, it still suffers from algal blooms and hypoxia annually. As remediation efforts continue, eventually Muskegon Lake will be removed from the AOC list.

Muskegon Lake Observatory Buoy and other buoys

The Muskegon Lake Observatory began in 2011 for continuous monitoring of the lake. The buoy is centrally located and is at a depth of 12 m. There are several sensors location from the surface water to the bottom of the water column measuring oxygen, temperature, specific conductivity, chlorophyll, turbidity, and pH. All the water quality data is taken every 15 minutes. There is a meteorological station on the buoy which measures temperature, wind, barometric pressure, and rain every 5 minutes. The three additional buoys, East, West, and Deep, were used in 2016 and 2017. These buoys had temperature, dissolved oxygen, and conductivity at various depths every hour. These additional buoys allowed us to see patterns across the lake.

Metabolism Estimates

Metabolism can be estimated using multiple methods and models. We used both the free water dissolved oxygen method from a buoy (BUOY) and the biological oxygen demand method (or light – dark bottle; BOD). The bookkeeping model was used to estimate metabolism from the BUOY data. Both methods use the 24-hour cycle of oxygen increases and decreases to estimate production and respiration rates. Atmospheric exchange of gases, driven by physical processes, is also included in the metabolism estimates along with the biological changes. Hourly averages were used to estimate metabolism for the daily rates. The data were input into the R program LakeMetabolizer and rLakeAnalyzer to estimate metabolism. The buoys required frequent maintenance to ensure data were accurate. Monthly cleaning and as needed repairs were done to ensure the maximum amount of data were collected with accuracy. The BOD method required sampling 3 times a year (except in 2017 there were 8 sampling dates). These water samples were placed in light and dark bottles for an initial, 24 hour light, and 24 hour dark bottles. Following the incubation period, each sample underwent a Winkler titration to determine the oxygen levels. The changes in oxygen were then used to estimate the metabolism rates.

Statistical Methods

Several statistical methods were used for this thesis. In long term chapter, a Kruskal Wallis test was used to determine the significant differences between several groups of data. These data groups were broken down by season and year and then also the average for each season. Several of these groups had significant differences, and so a posthoc pairwise Wilcoxon test was used to determine what groups within the groups were significantly different. These were all nonparametric tests because this data were not normal. Also, in this long term chapter, an ANOVA test was done to compare the traditional BOD method to the BUOY method. This

test result found significant differences among the respiration and net ecosystem production rates. Regression analysis was done with each of the metabolism estimates (GPP, R, NEP) and the environmental data (wind, rain, river discharge, chlorophyll, pH, ect.). These results found several significant relationships with weak R^2 values. The spatial chapter broke each site and season up into several groups where the data was nonparometic. A Kruskal Wallis test was done to compare each of these groups. Groups that had significant differences were tested again with the posthoc pairwise Wilcoxon test. This data analysis was all done using R.

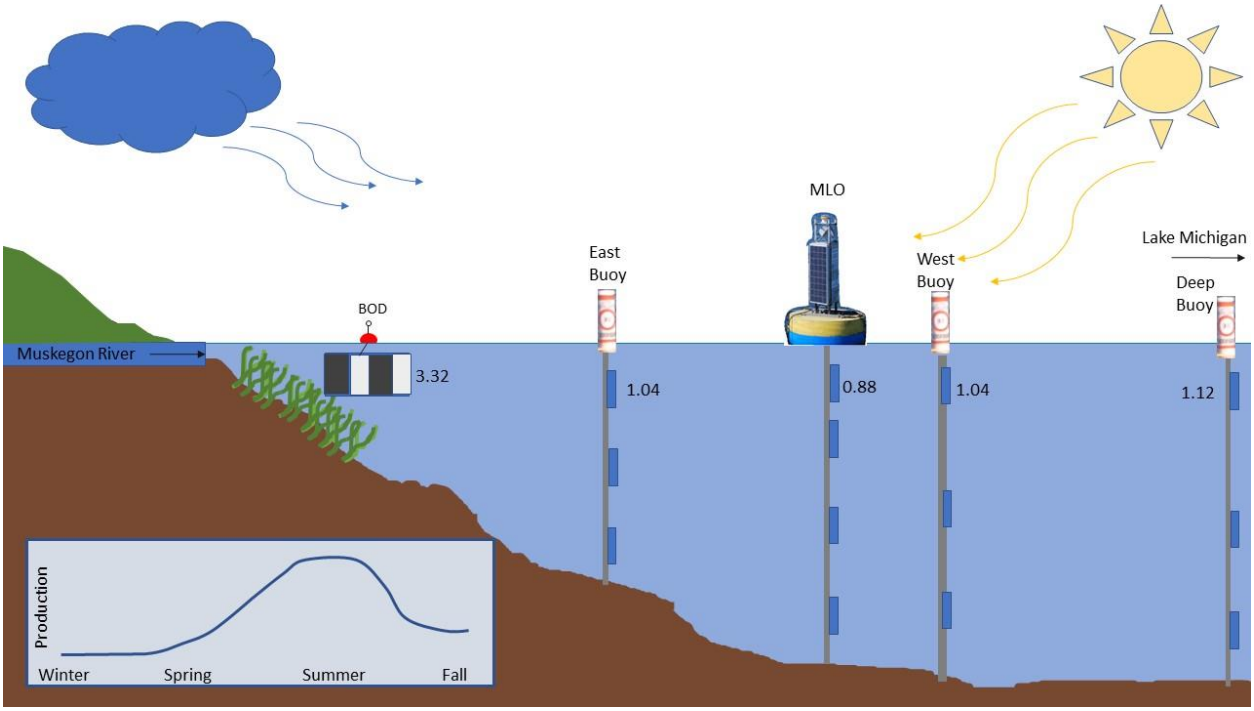
Figure Legends

Figure 1. Conceptual figure of Muskegon Lake ecosystem with some of the study questions answered. Each buoy represents the East, MLO, West, and Deep location in the lake with the average summer GPP values in mg C L^{-1} d^{-1} made by the BUOY method. There are differences depending on where in the lake you take metabolism measurements and that is likely due to distance from river input, depth and residence time of the particular area. The BOD method proved to be different than the BUOY method yielding metabolism rates generally higher than the BUOY method. We saw a seasonal pattern of metabolism being low in the winter where not much is going on and then it is spiking in the summer and falling again into the fall.

Table 1: Global comparisons of primary production with other aquatic ecosystems and other ecosystems from around the world. Estimates from Schlesinger and Bernhardt (2013). Inland water estimates were done using two methods (BOD and BUOY) with Muskegon Lake as the model to represent all inland waters. Bold indicates comparable ecosystems to inland waters and italics indicates data from this study.

Biomes	**Area (10^6 km^2)**	**NEP (g C m^{-2} y^{-1})**	**Total NEP (10^{15} g C y^{-1})**
Open Ocean	326	130	42
Tropical Forest	17.5	1250	20
Tropical savanna	27.6	540	14
Coastal Zone	36	250	9.0
Temperate Forest	10.4	775	7.6
Temperate grassland	15.0	375	5.3
Crops	13.5	305	3.9
Deserts	27.7	125	3.3
Boreal Forest	13.7	190	2.4
Mediterranean Shrubland	**2.8**	**500**	**1.3**
Inland Waters BOD	***4***	***310***	***1.25***
Arctic Tundra	**5.6**	**90**	**0.5**
Inland Waters BOUY	***4***	***41***	***0.164***
Upwelling Ocean	**0.36**	**420**	**0.15**
Ice	15.5		

Figure 1


MLO
East
Buoy
West
Buoy
Lake Michigan
Deep
Buoy
BOD
Muskegon River
3.32
1.04
0.88
1.04
1.12
Production
Winter
Spring
Summer
Fall

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