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Open System Metabolism of the Grand River from Headwaters to Mouth

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Biological Assessment of Ecosystem Integrity in the Grand River, MI
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Student Summer Scholars (S3) Program
Grand Valley State University
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Open System Metabolism of the Grand River from Headwaters to Mouth
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Rapid Biological Assessments and Chlorophyll Quantification

Abstract

The Grand River is the longest river in Michigan and has been greatly impacted by human activities, particularly logging in the mid-1800s, which when coupled with 20th-century urbanization and continued agricultural use, led to historically poor river health. Despite this, actions throughout the past 50 years by federal, state, and local citizen involvement, have resulted in increased river health and broader water quality monitoring within the watershed. During the summer of 2021, rapid bioassessments targeting benthic macroinvertebrates were conducted along the Grand River, with the primary goal of following up on prior state-led surveys conducted at different locations along the mainstem of the river in 2009 and 2014. Using the Michigan standard rapid bioassessment protocols for both wadeable and non-wadeable streams (P51 and P22 methodologies), assessments at seven locations were carried out along with chlorophyll-*a* quantification. Initial macroinvertebrate and habitat survey results indicate a decline in general water quality as one travels downstream, likely due to riparian impacts and cumulative pollution. This pattern was consistent with the state survey data. However, we observed some improvements in the most upstream site (a shift from “acceptable” to “excellent”) and most downstream site (“poor” to “marginal”) and remained the same or slightly declined in all other sites. Taxa richness per site ranged from 14-24 in Grandville and Grand Rapids respectively, with a mean of 20 taxa at each site. Overall abundance was dominated by Ephemeroptera (25%), Trichoptera (19%) and Diptera (16%), with longitudinal changes in dominance. Habitat quality followed a similar pattern to the macroinvertebrate ratings in all sites when compared to most recent state surveys. Chlorophyll-*a* concentration was highest in the downtown Grand Rapids reach (9.353 ug/cm²) and lowest in our most upstream site (2.110

ug/cm²) and generally increased further downstream. Looking forward, this information provides a comparison for future biological monitoring efforts within the Grand River to assess changes in its ecological health.

Introduction

The Grand River is the largest river in the state of Michigan and its watershed the second largest in the state (Hanshue and Harrington 2017). Due to its length and size, the river was historically a key source of transport for early settlers and natives (Hanshue and Harrington 2017). With these utilizations, came the construction of numerous dams and other hydrological alterations for milling and other logging operations, especially in the lower Grand River area. These modifications, coupled with an increase in pollution during the industrial revolution in the 19th century and early 1900s led to historically poor river health and water quality. To combat these trends, actions like the implementation of the Clean Water Act as well as the formation of regional/local environmental organizations (see Grand Rapids White Water and Lower Grand River Watershed) have led to improved water conditions throughout the Grand River (U.S. Environmental Protection Agency (1972).

One way of monitoring and quantifying water quality changes throughout the Grand River is through biological assessments. With origins in early 20th century Europe, biological assessments frequently use the macroinvertebrate community to assess water quality at a given site (Cairns and Pratt 1993). Through the use of indicator species (most commonly Ephemeroptera, Plecoptera and Trichoptera) and a numerical metric score, assessing the general level of pollution and overall water quality can be easily and quickly repeated across sites or through time (Carter and Resh 2013, Lenat and Barbour 1994). The use of macroinvertebrates is

often preferred to chemicals or physical traits to assess water quality, because it provides a better sense of the past and present conditions, as compared to physicochemical characteristics (Hauer and Resh 2017).

Based on the ideals mentioned above, and in response to the desire for a standardized method for measuring the water quality in freshwater Michigan systems, the Water Resources Division implemented the *Qualitative Biological and Habitat Survey Protocols for Wadeable Streams and Rivers*, also known as Surface Water Assessment Section (SWAS) Procedure 51. (Hanshue and Harrington 2017, *Qualitative Biological and Habitat Survey Protocols for Wadeable Streams and Rivers* 2008). Implemented in 1990, this method uses a combination of fish, macroinvertebrates, and habitat assessments to assess the quality of a given site. Additionally, in 2013 the Water Resources Division constructed the *Qualitative Biological and Habitat Survey Protocols for Nonwadeable Rivers* (P22), as a method for better understanding larger non-wadeable rivers throughout the state (*Qualitative Biological and Habitat Survey Protocols for Nonwadeable Rivers* 2013). Similar to P51, this method uses macroinvertebrates as well as a habitat assessment to assess water and site quality.

Looking at a river from a longitudinal perspective, it is important to understand how the function, energetics and physical characteristics of the river changes, an idea detailed through the River Continuum Concept (RCC) (Vannote et al. 1980). In smaller order reaches, it is predicted that macroinvertebrate communities will largely be made up of “shredders” and “collector” functional feeding groups (FFG)’s due to the river's reliance on allochthonous leaf litter and other terrestrial energy inputs (Vannote et al. 1980). With increasing size, proportions of FFG’s change due to shifts in energy sources and availability, a change that is reflected by increases in “grazers” and “predators” along with declines in shredding macroinvertebrates (Vannote et al.

1980). In our study, macroinvertebrate communities should reflect these changes and corresponding shift throughout our reaches.

The main goal of our study was to conduct rapid bioassessments along the Grand River from headwaters to mouth. This will act as a continuation of the previous bioassessments conducted in the lower watershed in 2009 and 2014 as well as assessments in the upper watershed in 2011 and 2016 (Michigan Department of Environmental Quality (2011, 2012, 2016, 2017)). The advantage of this approach is the intentional focus on a single-season, headwaters-to-mouth sampling scheme. This allowed us to test some interesting river ecology theories and predictions, about how river ecosystems change from headwaters to mouth. In addition, we also collected algae to quantify organic biomass and chlorophyll-*a* concentrations. The hypotheses we specifically aimed to test were;

1. The aquatic insect community will shift from headwaters-to-mouth according to the predictions made by the RCC (Vannote et al. 1980). Specifically, shredders and consumers of coarse particulate organic matter will dominate in the headwaters, while mid-reaches will be dominated by filtering collectors and scrapers, and lower-order reaches will be dominated by gathering collectors.
2. Bioassessments will positively correlate with water chemistry such that indicators of cleaner water (less pollution) will be mirrored by better macroinvertebrate assessment scores (metrics will score higher in terms of taxa diversity, taxa richness, etc.).
3. Organic matter content and chlorophyll-*a* should correlate with biological and chemical indicators of river condition. Where condition is 'good' or 'healthy', chlorophyll and organic matter should be relatively high.

Methods

Site Selection

Surveying was conducted from June through September of 2021, with the majority of sampling occurring from mid-July through early August. Sites were targeted based on the historical locations of assessments conducted by the Department of Environmental Quality (DEQ). In areas/sections where multiple studies had been conducted, the middlemost site was selected, unless a nearby site was more accessible to sample. A total of 7 sites were selected (Figure 1), with an attempt to encompass the entire Grand River from headwaters to mouth, yet still be feasible to sample in our given time frame.

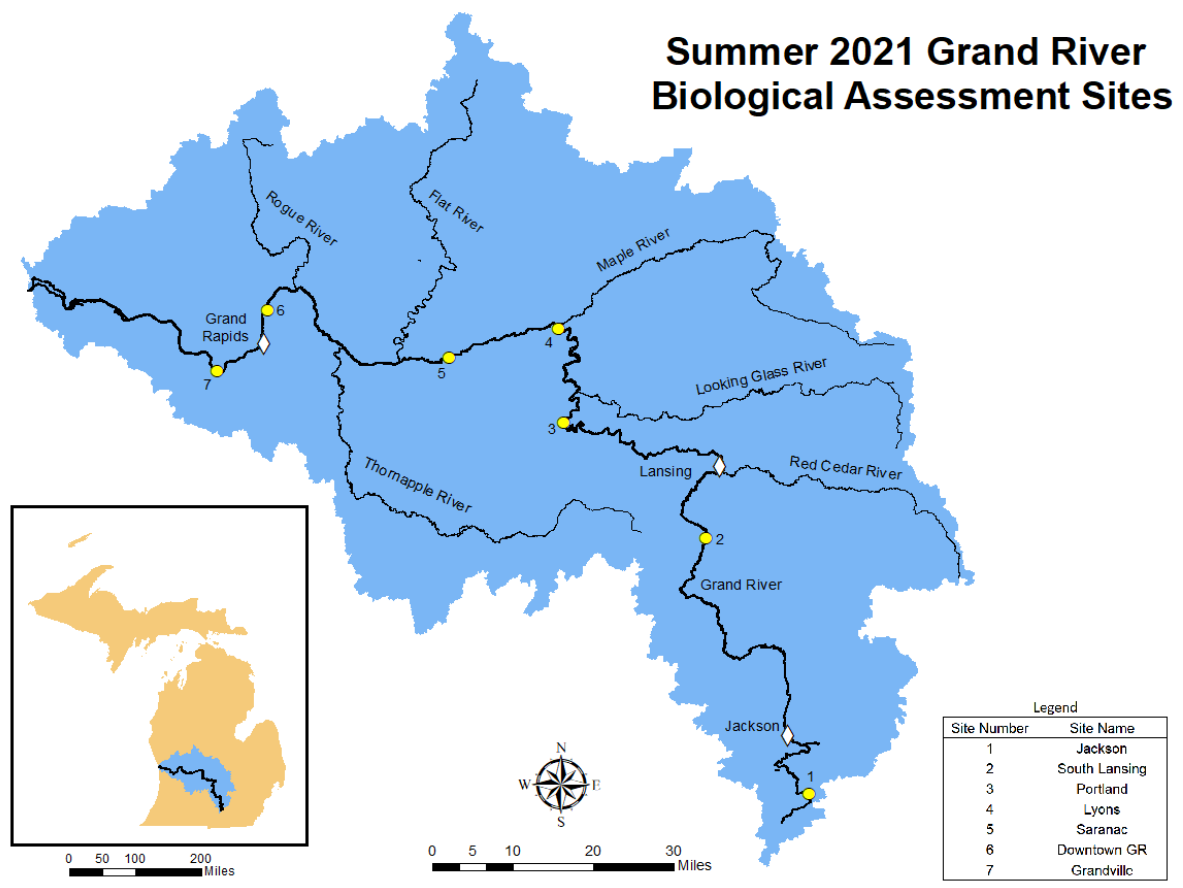


Figure 1: Summer 2021 biological assessment study sites along the main stem of the Grand River, MI.

Wadeable (P51) Biological Assessments

Macroinvertebrate collection, analysis, and identification for the upper watershed sites (1-3) were followed as detailed in section VI of the P51 methodology document (Qualitative Biological and Habitat Survey Protocols for Wadeable Streams and Rivers, 2008). This involved a total of 20 minutes of macroinvertebrate netting, using a D-frame kick-net (500 μ m). In lieu of field identification, the macroinvertebrates sample was sieved (500 μ m) and placed in a whirl pak with 95% ethanol, which was decanted and re-filled with 75% ethanol the following day for preservation. Each sample was processed in lab, as detailed in steps A3-C (Qualitative Biological and Habitat Survey Protocols for Wadeable Streams and Rivers, 2008), with sub-sampling carried out using a bucket pour rather than a small net as stated. Family level identification was reached, mainly using two taxonomic guides (Voshell Jr. 2003, Merritt et al. 2019) and we identified 300 +/- 60 individual macroinvertebrates. Macroinvertebrate scores were calculated as outlined in the supplemental scores interpretation report (see: Update of GLEAS Procedure 51 Metric Scoring and Interpretation, 2018). Habitat assessments were performed and scored according to section VII of the P51 method, with the glide-pool metric being used in all sites.

Non-wadeable (P22) Biological Assessments

Lower watershed sites (4-7) were assessed using the P22 methodology and accessed via motorboat or canoe and kayak (Qualitative Biological and Habitat Survey Protocols for Non Wadeable Rivers, 2013). Methods were followed as detailed in sections III through IV, with the modification of macroinvertebrates being preserved in 70% ethanol and identified to family using Voshell Jr. 2003 and Merritt and Cummins 2019, in a lab rather than in the field. Habitat assessments were carried out at each transect site, following the procedure outlined in section V

of the P22 methodology. A range finder was used to assess river width when available or estimated on Google Earth when necessary.

Algae Collection & Chlorophyll-a Quantification

For algae collection, five rocks were randomly selected from the reach area and 4.91 cm² were scraped and vacuum dried onto 1.5 µm pore glass fiber filter paper. The filter paper and any remaining algae and sediment were transferred to a 15 mL plastic screw cap vial and stored on ice in the dark until being stored in a dark freezer at 4°C in the lab. Samples were processed within 28 days of collection, starting with a chlorophyll extraction in 10 mL of 90% buffered acetone for 24 hours at 4°C (Steinman et al. 2017). Once ready for analysis, vials were transferred to a Fisher Scientific Centrifuge centrifuge, and run for 20 min at 500g (Standard Methods for the Examination of Water & Wastewater, 2005). While in the dark, 3mL of extract were transferred to a 1 cm glass cuvette and its optical density read at 750 nm & 664 nm using an Orion AquaMate 7000 VIS spectrophotometer (Steinman et al. 2017). The sample was then acidified using 0.1 mL of 0.1 N HCl and mixed (Steinman et al. 2017). After 90 seconds the optical density was once again read at 750 nm as well as 665 nm (Steinman et al. 2017). Before each reading at 750 nm, a blank cuvette containing 90% buffered acetone or 90% acetone acidified with 0.1 mL of HCl were used to re-zero the spectrophotometer.

Organic Matter Content- Ash Free Dry Mass (AFDM)

Once chlorophyll concentration was determined, each sample was poured into a 25 mL Fisherbrand ceramic crucible and placed in a fume hood to evaporate the acetone. Samples were then transferred to a 70°C VWR drying oven for 24 hours. After cooling to room temperature, the mass of each crucible was recorded to the nearest 0.001 g using a OHAUS Analytical

Standard balance. Samples were then placed in a Isotemp Programmable muffle furnace set to 500°C for 1 hour. Once cooled each crucible was re-weighed with mass loss equal to the organic matter content.

Analysis

Macroinvertebrate assemblages were compared using a multivariate non-metric multidimensional scaling (NMDS) test to evaluate whether the communities were more or less similar to one another using a “vegan” package (*Vegan: Community Ecology Package*, 2020). The robustness of this NMDS was tested using a stress test, where a value of <0.05 indicates ‘excellent’ representation (e.g. a ‘satisfactory’ reduction from multi-dimensions down to 2) and a value of >0.3 is ‘bad’. The statistical significance of potential differences in community composition was tested using a permutation multivariate analysis of variance test called “adonis” (Anderson 2001), wherein a p-value of ≤ 0.05 indicates significance. Finally, we identified macroinvertebrate taxa that contributed most to the differences between sites using a similarity percentage or simper test (Clarke 1993). All multivariate tests were done using R studio (v. 2021.09.2+382). Additional macroinvertebrate analysis was performed using Excel (v. 16.58). Chlorophyll data was tested for normality using a Shapiro Wilks test using SPSS (v. 27.0.1.0). Further testing was conducted using a one-way ANOVA (analysis of variance) with LSD posthoc pairwise comparisons to test for significant differences between sites. Site distance from Lake Michigan along the river was determined using Google Earth.

Results

Rapid Bioassessments

Using the raw macroinvertebrate data, a series of metrics were calculated based on the P51 method. These metrics were then composited into an overall macroinvertebrate score and rating for each site (Table 1). In a similar fashion, habitat survey information was also used to

determine a habitat rating and score. This information was then directly compared to the most recent state-led assessments for comparison (Table 1).

Table 1: Overall Macroinvertebrate and Habitat scores and ratings for each site. Most recent historical sampling information is also given for comparison.

* “-” indicates that this score or rating was not calculated for that site during that time

Site	Jackson	Lansing	Portland	Lyons	Saranac	Grand Rapids	Grandville
Macroinvertebrate Past Score	4	5	5	62	66	42	14
Macroinvertebrate 2021 Score	7	4	3	62	58	38	33
Macroinvertebrate Past Rating	Acceptable	Excellent	Excellent	Good	Good	Fair	Poor
Macroinvertebrate 2021 Rating	Excellent	Acceptable	Acceptable	Good	Good	Marginal	Marginal
Habitat Past Score	125	124	173	-	-	-	-
Habitat 2021 Score	139	144	166	44	53	55	42
Habitat Past Rating	Good	Good	Excellent	-	-	-	-
Habitat 2021 Rating	Good	Good	Excellent	Marginal	Marginal	Marginal	Marginal
Year Last Sampled	2011	2016	2011	2009	2014	2014	2009

To understand the health and quality of the Grand River compared to other similar watersheds, our scores were compared to other large river watersheds in Michigan (Table 2). Sites used in calculating other rivers' score and ratings were all from mainstem sample sites to allow for a more accurate comparison.

Table 2: Comparison of previous macroinvertebrate and habitat scores among other large rivers in Michigan. Yellow highlighted rows indicate sample efforts from this study and a “-” indicates that this score or rating was not calculated for that site during that time. Note that these values represent average scores based on the number of sites surveyed in the given year. N-sizes are listed.

				Mainstem River Site Average					
Wadable River Segments	River Length (mi)	Year Sampled	# of Sites	Macroinvertebrate Score	Macroinvertebrate Rating	Habitat Score	Habitat Rating	Method Used	Flows into Lake
Upper Grand River	252	2021	3	4.6	Acceptable	150	Good	P51	Michigan
Upper Grand River	252	2009 & 2014	8	4.8	Acceptable	127	Good	P51	Michigan
St. Joseph River	206	2015-2016	2	8	Excellent	159	Excellent	P51	Michigan
Upper Manistee River	190	2014	7	4.7	Excellent	158	Excellent	P51	Michigan
River Raisin	139	2013	8	3.5	Acceptable	141	Good	P51	Erie
Au Sable River	138	2017-2018	5	5.2	Excellent	175	Excellent	P51	Huron
Upper Huron River	130	2012	9	1	Acceptable	126	Good	P51	Erie
Kalamazoo River	130	2014	3	2	Acceptable	162	Excellent	P51	Michigan
Shiawassee River	120	2015	10	4	Acceptable	131	Good	P51	Huron
Non-wadable River Segments	River Length (mi)	Year Sampled	# of Sites	Macroinvertebrate Score	Rating	Habitat	Rating	Method Used	Flows into
Lower Grand River	252	2021	4	48	Fair	48.5	Marginal	P22	Michigan
Lower Grand River	252	2011 & 2016	16	33	Marginal	-	-	P22	Michigan
Lower Manistee River	190	2014	4	75	Excellent	-	-	P22	Michigan
Lower Huron River	130	2012	3	35	Marginal	-	-	P22	Erie
Kalamazoo River	130	2014	1	20	Poor	-	-	P22	Michigan
Shiawassee River	120	2015	1	52	Good	-	-	P22	Huron
Menominee River	116	2012	3	59	Good	-	-	P22	Michigan

A total of 1744 macroinvertebrates were collected and identified across all 2021 sites, including 44 different taxa. Taxa composition by order (or other higher classification) is illustrated in Figure 2 and is organized by site as well as a comprehensive total collection.

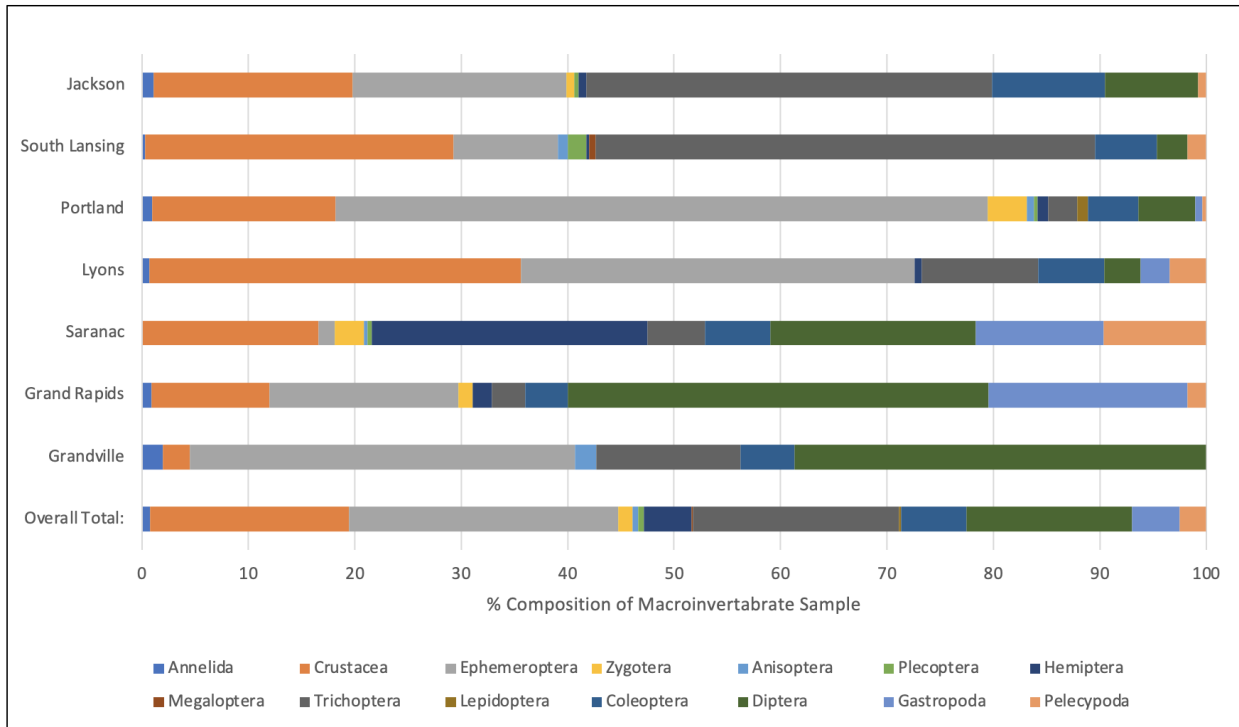


Figure 2: Percent composition of various macroinvertebrate orders (and some higher classifications) that were identified, organized by site as well as an overall collection effort. Total collection sizes; Jackson (273), South Lansing (345), Portland (297), Lyons (146), Saranac (259), Grand Rapids (225), Grandville (199), and Overall (1744).

Focusing in on what are considered “indicator taxa”, Ephemeroptera, Plecoptera and Trichoptera were compared separately. The total number of taxa identified in each group was determined and then plotted against the sites corresponding distance from the mouth of the Grand River to depict the longitudinal change in taxa richness (Figure 3). Number of Diptera taxa were also plotted as it’s another taxon group of interest when assessing water quality.

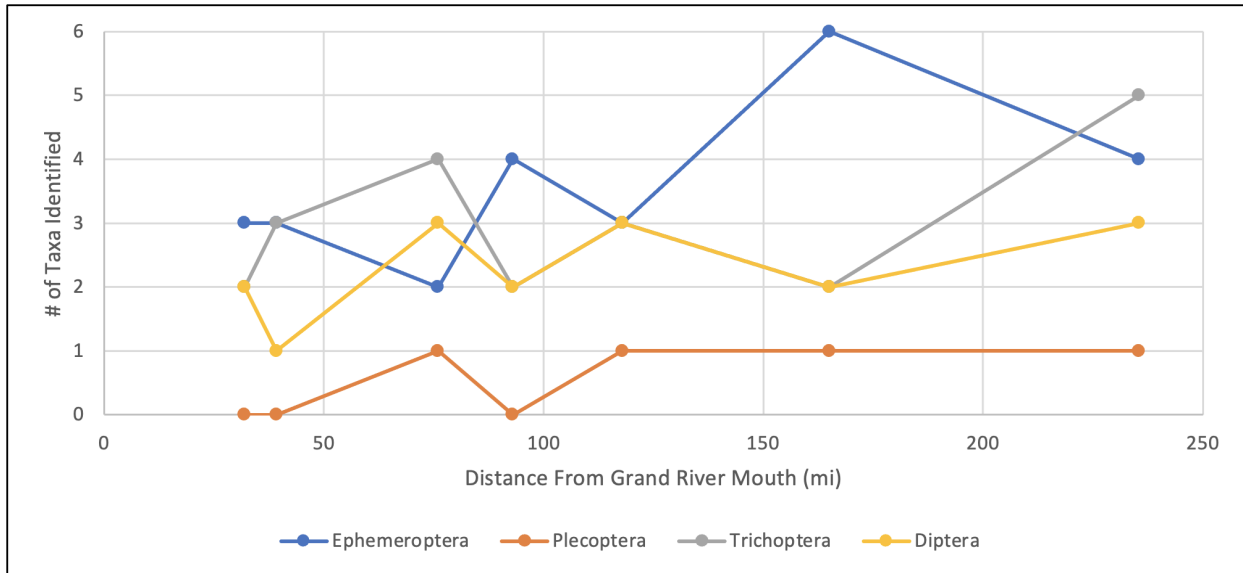


Figure 3: Family-level richness (e.g., number of Families) of dominant macroinvertebrate taxa compared longitudinally based on their site location along the Grand River mainstem.

To compare the differences or similarities between the entire macroinvertebrate composition of each site, an NDMS was constructed and analyzed using an adonis test (Figure 4).

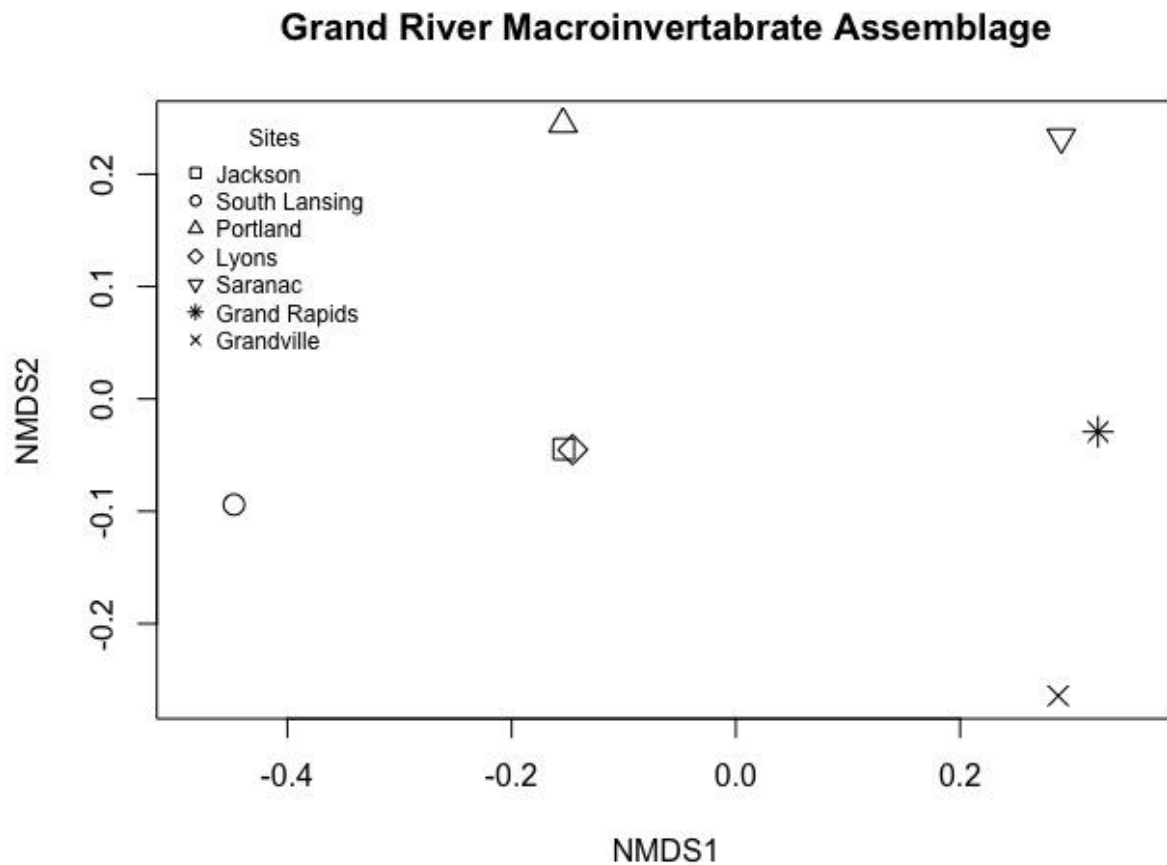


Figure 4: Comparison of macroinvertebrate assemblages at each site, which are differentiated by shape (stress =0.045; $r^2=0.327$)

Chlorophyll A Quantification

Mean chlorophyll concentration was determined at each site and plotted by site number as indicated in Figure 5. Chlorophyll data was transformed (log base 10) to meet assumptions of normality (tested using Levene's test), followed by a post-hoc LSD (least significant difference) test for pair-wise statistical contrasts.

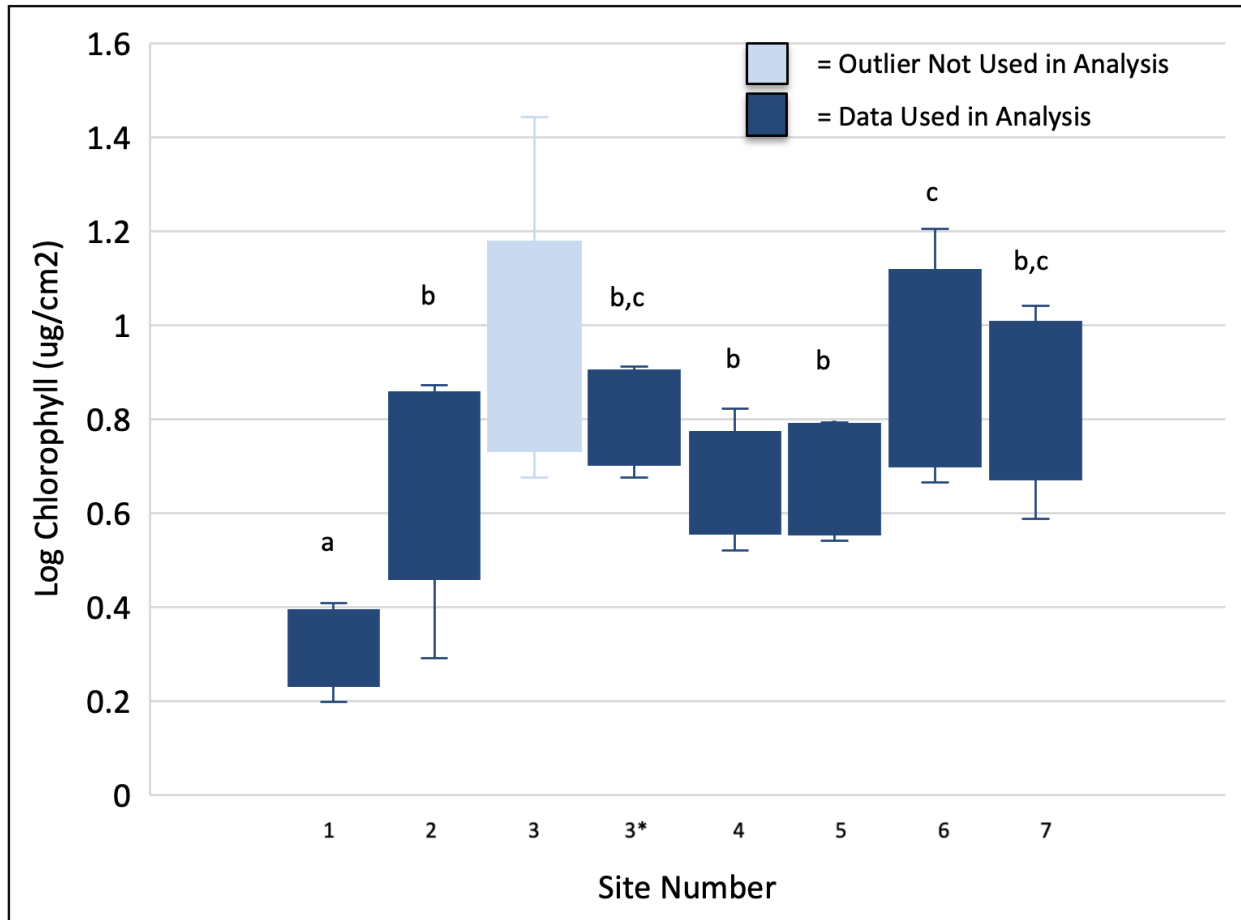


Figure 5: Log₁₀ chlorophyll concentration by site; 1-Jackson (n=5) , 2-South Lansing (n=5), 3-Portland (n=5), 3*-Portland (n=4), 4-Lyons (n=5), 5-Saranac (n=5), 6-Grand Rapids (n=5), and 7-Grandville (n=5). Only indicated data was used for ANOVA analysis. Boxes with the same assigned letters are not statistically significantly different. One Way ANOVA, $p < 0.001$, Levene's test value after transformation $p = 0.117$.

* Indicates distribution with removal of a suspected outlier from Site 3.

Ash-Free Dry Mass (AFDM)

Similarly to chlorophyll-*a* concentration, AFDM was also calculated for each site and its mean concentration determined (Figure 6). No further analysis was performed on this data due to large variations.

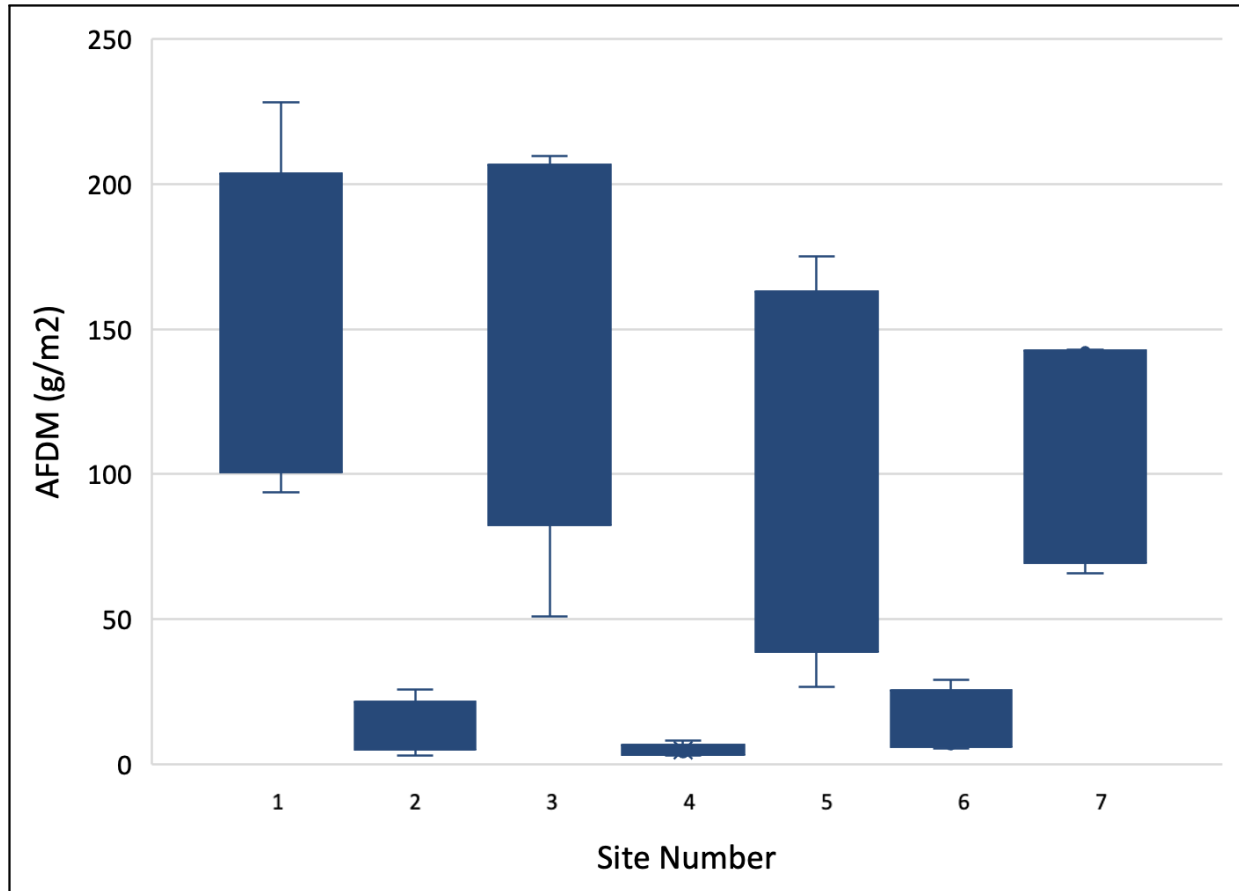


Figure 6: Mean AFDM concentration (n=5) by site; 1-Jackson, 2-South Lansing, 3-Portland, 4-Lyons, 5-Saranac, 6-Grand Rapids, and 7-Grandville.

Discussion

Calculated macroinvertebrate scores and ratings from 2021 sampling indicate a general decline in water quality as one travels downstream. Since the macroinvertebrate scores are not directly comparable longitudinally due to different numeric scoring (e.g., wadeable (P51) vs.

non-wadeable (P22)), macroinvertebrate qualitative ratings were used to reflect this trend. Ratings begin with “excellent” in the Jackson reach and gradually decline down to “marginal” in the Grandville reach, with in-between sites conforming to the declining trend (Table 1). Habitat ratings also follow a similar trend, but only decline from “good” to “marginal” and remain fairly consistent across all sites. In direct site by site comparison to previous assessments, the majority of sites show slight numeric score decline but the same overall rating, with the exception being Jackson and Grandville, both of which improved in terms of macroinvertebrates and habitat (Table 1). Some of this variation in direct comparison could be due to slight differences in sampling location, in particular the Jackson reach since this upstream site had lots of variation in channel substrate and habitat in the surrounding area.

The Grand River is comparable to other larger rivers in Michigan, falling in the middle of these, both for macroinvertebrate and habitat assessment. This comparison was done by calculating average scores for both the wadeable and non-wadeable bioassessment surveys. The upper Grand River had a mean macroinvertebrate rating of “acceptable”, a rating held by 6 other rivers in wadable areas. Habitat rating was similar to other large Michigan rivers, being the same amongst 5 of the 8 previous assessments on other rivers, all of whom fall within the “good” rating range (Table 2). Within this, assessments from 2021 actually showed an increase of 23 points numerically, which indicates an increase in quality despite remaining in the same habitat rating as past years. The lower Grand River had a mean macroinvertebrate rating of “fair”, similar to the scores and ratings of other non-wadeable river sections in Michigan. It is interesting to note that both the mean macroinvertebrate numerical score and its corresponding rating increased from 2009 & 2014 sampling to sampling in 2021, rising from 33 to 48 and “marginal” to “fair”, respectively (Table 2). This is a trend that could be due to upstream

restoration and water quality improvement efforts as well as decreased cumulative impacts throughout the watershed. Due to our laboratory identification of macroinvertebrates, vs the standard DEQ field identification for procedures 22 and 51, there may be slight variations in our scores. That being said, there is no indication that this would have favored a general increase or decrease in any particular site or taxa.

In looking at the abundance of various families and orders within our macroinvertebrate samples, they were largely dominated by Crustacea (19%), Ephemeroptera (25%), Trichoptera (19%) and Diptera (16%) (Figure 2). This is not surprising since these are commonly the most dominant macroinvertebrate groups, but it is interesting to note how their abundance changed longitudinally between sites. Upstream sites showed higher percent abundance of Ephemeroptera as well as Trichoptera, with our Portland sample comprising 61% Ephemeroptera and South Lansing containing 46% Trichoptera (Figure 2). Further downstream, these groups make up a smaller proportion and a decrease in abundance, reaching almost 40% composition in Grand Rapids and Grandville (Figure 2). In looking at these groups further, total number of taxa in each group was plotted against the longitudinal position of each site along the mainstem of the Grand River (Figure 3). This plot shows a general decline in taxa richness amongst all the sites as one travels downstream, in particular the Ephemeroptera. Since these are considered to be good taxa indicators for water quality due to their pollution sensitivity, their decline in richness further supports the conclusions based on the bioassessment macroinvertebrate scores and ratings.

To better compare each site against one-another, an NMDS was run, looking at the entire macroinvertebrate assemblage of each site in comparison to other site assemblages. As seen in Figure 4, the upper river and lower river sites separate along the first axis to form two distinct groupings. It also shows that each site had a unique macroinvertebrate assemblage indicating that

each insect community is unique and distinct when compared longitudinally. In Figure 4, the Jackson and Lyons sites fall atop each other/overlap, indicating they are quite similar.

Chlorophyll-*a* concentration increased from up- to down-stream. Jackson had the lowest mean at 2.110 ug/cm² and increased up to 9.353 ug/cm² in the Grand Rapids reach (Figure 5). High chlorophyll-*a* concentration aligns with physical observations in Grand Rapids, which had many areas of shallower water which allows for more sunlight penetration to the river bottom. Areas of larger substrate, namely Portland, Grand Rapids and Granville, corresponded with higher concentrations of chlorophyll-*a*, suggesting a possible correlation. Durling analysis, one sample from the Portland site gave an abnormally high concentration, believed to be due to error during the spectroscopy reading and was removed from statistical analysis.

With the quantification of chlorophyll-*a*, it was also hoped that ash-free dry mass could be calculated and a comparison could be made between the two. Ultimately, this information was non-conclusive nor reliable due to wide fluctuations in the calculated concentration (Figure 6). It is believed that variations in collection methods could have accounted for these differences. Chlorophyll collection from sites South Lansing, Lyons and Grand Rapids were done by scraping the surface of a submerged rock, while collection at sites Portland, Saranac, and Grandville also collected travertine and collection from Jackson was done using a sediment sample due to a lack of sampleable rocks. This type of collection should be repeated in a consistent fashion, for example with artificial substrate samplers, to get comparable results between sites.

Conclusions

Overall the Grand River remains in relatively good health in comparison to other larger rivers within Michigan. Generally water quality decreases from the headwaters to mouth most likely due to cumulative pollution along with riparian impacts. Compared to previous state-led assessments, macroinvertebrate scores slightly decreased in all sites except in Jackson and Granville which saw large increases in quality. As an overall composite, Ephemeroptera, Trichoptera and Diptera were the most dominant taxonomic group with the highest abundances in Portland, South Lansing and Grand Rapids respectively. Longitudinal comparisons of the macroinvertebrate communities fit within the river continuum concept, indicating changes in river structure and function along our sites. Chlorophyll-*a* generally increased downstream, but was more reliant on substrate size as well as water depth. Habitat assessments were comparable with prior surveys when applicable and were largely based on the reach location. For example, the Portland site was located within a state game area and correspondingly had the highest habitat rating. With this in mind, a site like Portland could help serve as a “river baseline” moving forward to help gauge negative impacts throughout the Grand River. Future assessments are required throughout the watershed to help monitor continued changes in water quality and overall river health.

Open System Metabolism of the Grand River from Headwaters to Mouth

Abstract

The Grand River historically was a key waterway for transport due to its length and overall watershed size and served as a key resource for early settlers. With this, continued development and industrialization led to poor water quality in the early 1900's, a trend which has since been positively combated due to efforts like the clean water act and citizen involvement to improve the rivers overall water quality. To better understand the health of the Grand River, monitoring efforts can be conducted, including measuring metabolism, which is considered a measure of the river's ability to support life and provides insight into the energetics of the river. Using a one station open system method, the metabolism of the Grand River was measured in seven different reaches ranging from headwaters to mouth during the summer of 2021. Metabolism estimations using an energy dissipation model follow closely what is predicted by the river continuum concept, with the headwaters and mouth/lower segments being largely heterotrophic, while the middle orders/segments are mainly autotrophic. GPP peaked at 7.254 $\text{gO}_2/\text{m}^2/\text{day}$ in the Saranac reach and was lowest in the South Lansing reach measured at $-0.151 \text{ gO}_2/\text{m}^2/\text{day}$, with other metabolism parameters following similar trends. This information can provide a baseline or comparable measurements for future metabolism work within the Grand River watershed or other large river systems within Michigan.

Introduction

For a history of the Grand River, see page 3. In order to understand and monitor the water quality of a river like the Grand River, a variety of biological and physical measurements can be used, one of which is river ecosystem metabolism. River metabolism is a method of measuring

the amount of organic carbon that is fixed by photosynthesis (in-stream production by algae and macrophytes) and respired by organisms within a riverscape and provides insight into the energetics of the food base and its capacity to support life (Young et al. 2008). To assess metabolism, five main parameters have been established; net primary production (NPP), ecosystem respiration (ER), gross primary production (GPP) and net daily metabolism (NDM) and production to respiration ratio (P/R) (Odum 2003, Hall, Jr. and Hotchkiss 2017). These parameters are calculated based on the rate of dissolved oxygen change, which can then be compared between reaches or sample sites.

Like other monitoring parameters, metabolism is affected by a variety of biotic and abiotic factors including; climate, pollution, temperature, and light (Young et al. 2008). In particular, light abundance was found to be one of the most important factors affecting metabolism equations (Bott 1985). In addition to the biotic and abiotic factors, longitudinal position along the river also plays a key role in the metabolic activity of a given river segment. As detailed in Vannote (1980), the river continuum concept predicts how a river changes energetically with increasing size and order, transitioning from mainly heterotrophic to autotrophic and eventually back to heterotrophic. These transitions in the energy base have been supported by numerous studies who have found similar changes longitudinally along a river (Bott 1985, McTammany et al. 2003, Minshall et al. 1992). Changes in energy are largely affected by changes in primary production within the river through changes in depth and turbidity of the water (Vannote et al. 1980).

Unlike some other measurements and metrics, metabolism is holistic, representing and accounting for the entire reach and the variety of habitats that are present within and around the surrounding area (Young et al. 2008). Additionally, the balance of energy supply and demand as

well as the sources of energy that a given reach is reliant on can be determined, which helps to better understand the key components of that given food web (Young et al. 2008). Reaches with higher carbon production than consumption are categorized as an autotrophic or self-sustaining food web, while areas with more carbon consumption than production rely on allochthonous carbon inputs like leaf litter (Young et al. 2008). Measuring metabolism is relatively simple and can be done through monitoring dissolved oxygen (DO) within the water, which can then be used to determine metabolic activity (Young et al. 2008).

There are a variety of techniques for measuring metabolism (open vs closed system, one vs two station, etc.). In this study, an open system approach was utilized, which involves monitoring changes in oxygen directly from the river in a non-closed system. Using this design, one is able to account for contributions from all processes within the ecosystem including; the water column, benthic and hyporheic components (Hall, Jr. and Hotchkiss 2017). This process also accounts for spatial heterogeneity and habitat complexity within the system, something that is difficult to capture using a closed-system design (Bott 1996, Hall, Jr. and Hotchkiss 2017). An open system method also provides a more holistic view of the energetics within the reach vs a closed system approach.

In this study, our objective was to gather information about the energetics of the Grand River along various segments of its mainstem from headwaters to mouth. This can then further be used to better understand the basis of each food web and how each area functions metabolically. Specifically, we wanted to; determine various metabolic parameters (NPP, ER, GPP, NDM, and P/R) at several longitudinal distance reaches in the Grand River, which will provide additional information and context to the insect and water chemistry data that a group of Grand Valley students and GVSU professors collected during the summer of 2021 through the

Student Summer Scholars program. This study helps to provide another piece of information to compare and use when assessing the quality of our study reaches and the Grand River as a whole.

Methods

Site Selection

During the summer of 2021, a total of 7 sites were selected for the study. These sites were the same used for the macroinvertebrate rapid bioassessment project described on page 6 (Figure 1). Biological assessments sites were selected based on the historical locations of assessments conducted by the DEQ, with an attempt to encompass the entire Grand River from headwaters to mouth, yet still, be feasible to sample in our given time frame.

Data Collection

Data collection occurred from late May to mid-September 2021 following a method similar to that of McTammany et al. (2003). At each reach, a data YSI 600QS water chemistry sonde was remotely deployed. Before deployment, the percent dissolved oxygen (%DO) sensor was calibrated, and the sonde set to record; time, temperature, specific conductivity, salinity, %DO, DO mg/L, and pH every 15 minutes once deployed. The sonde was positioned into the stream within a wadeable distance from the bank, but also in an area with minimal canopy cover, good water flow, and few macrophytes/detritus. To secure, the sonde was tied to either a large log in the river or a rebar stake, positioned so that the sensors were near the bottom of the river bed, but would not become covered in sediment. Data was collected for a minimum of two days (48 hours) and a maximum of 8 days, depending on when the site could next be accessed. Once

retrieved, the data was downloaded onto a computer using Ecowatch lite and transferred into an excel spreadsheet for analysis.

Analysis

All data was compiled into an Excel spreadsheet for analysis and evaluation. To calculate river metabolism, a one-station open system analysis method was used. Calculations were followed according to section 31.3.1.5 from Hall, Jr. and Hotchkiss (2017) with slight modifications. In lieu of calculating a K value through a nighttime regression, our gas exchange coefficient during the day was estimated based on river slope, velocity, and a constant as detailed in equation 28.10 in Bott (1996). This value was then temperature adjusted using equation 28.11 to achieve our final K value. Equation 34.14 from Hall & Hotchkiss (2017), was used to calculate metabolism at each interval, and daytime and nighttime intervals summed to achieve NPP and ER respectively. GPP was calculated by adding daytime ER to recorded NPP during the day. NDM was determined by adding GPP and 24hr ER and P/R calculated by dividing GPP by 24hr ER.

NPP, 24hr ER, and GPP data were tested for normality using a Shapiro Wilks test. Further testing was conducted using a one-way ANOVA (analysis of variance) with LSD post hoc pairwise comparisons to test for significant differences between sites. These metrics were also tested using a Kruskal-Wallis test since not all sites had three full days of data, and therefore could not be tested using the Shapiro Wilks test. All statistical analyses were conducted using SPSS (v. 27.0.1.0); figures were generated using Excel (v. 16.58).

Table 3: Physical river characteristics and additional details of each study site. Mean width, velocity and depth were either directly measured (Jackson and Portland) or estimated using USGS surface water , stream data and field measurements (South Lansing, Lyons, Saranac, Grand Rapids, and Grandville).

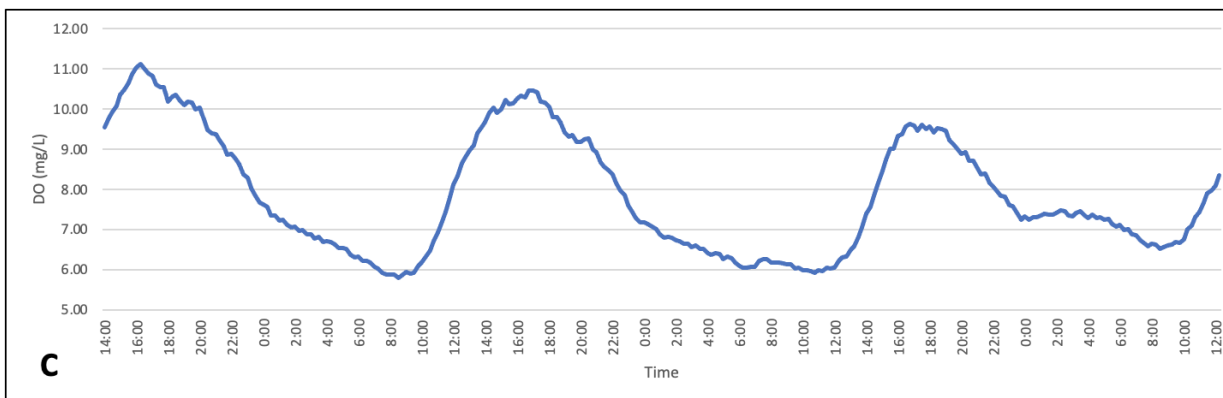
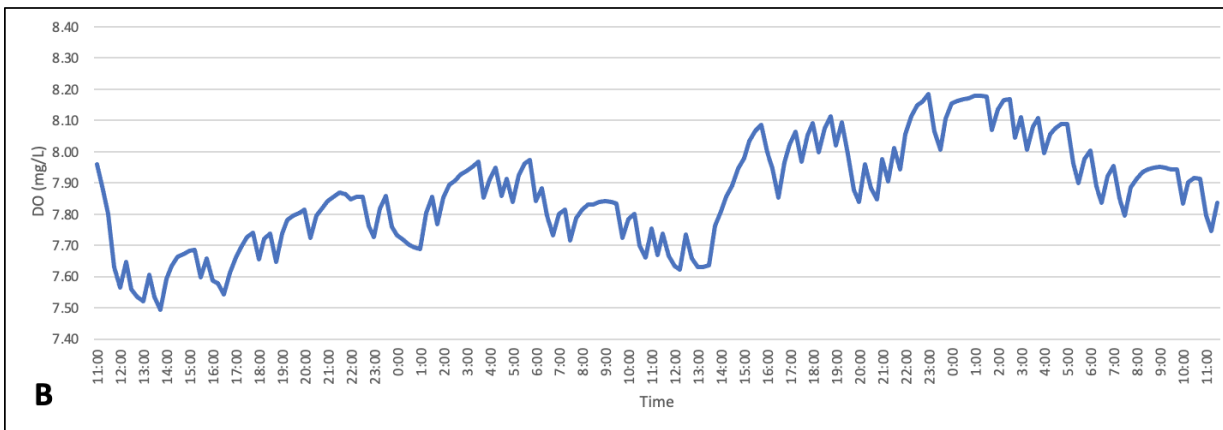
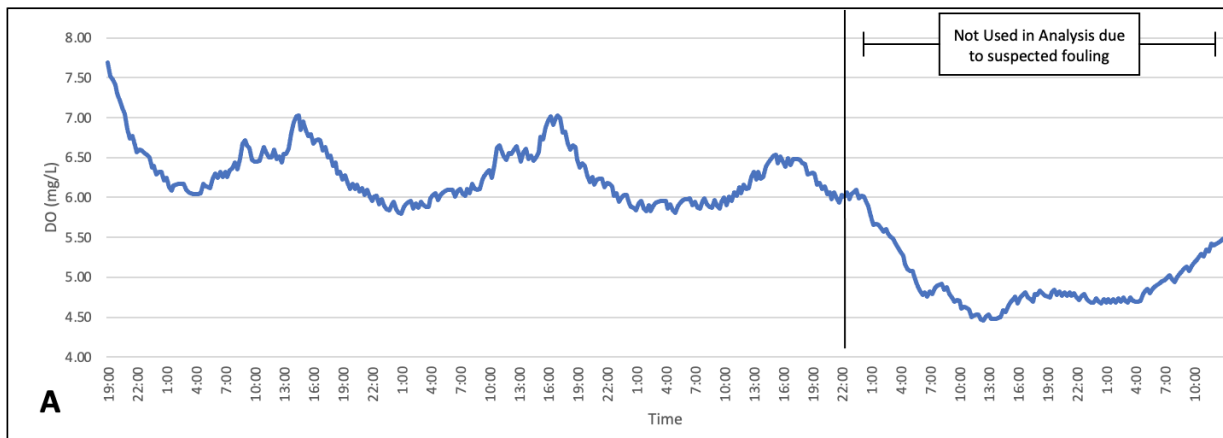
	Jackson	South Lansing	Portland	Lyons	Saranac	Grand Rapids	Grandville
Latitude	42.13862	42.60212	42.81493	42.99106	42.56108	42.96263	42.91337
Longitude	84.35298	84.5935	84.93776	84.94236	85.13351	85.67739	85.78751
Mean Temperature (°C)	22.06	21.08	23.15	24.02	23.19	24.36	21.59
Mean Width (m)	7.45	33	37.3	42.1	75	107	98
Velocity (m/s)	0.256	0.548	0.220	1.356	0.346	0.905	0.365
Depth (m)	0.618	0.821	0.630	1.151	0.900	0.722	1.740
Slope (cm/m)	0.107	0.019	0.052	0.070	0.086	0.015	0.034
k₂(20°C) (/d)	4.203	1.613	1.764	14.476	4.559	2.065	1.914
Date Sampled	6/17	7/12	5/24	7/14	6/3	7/31	9/2

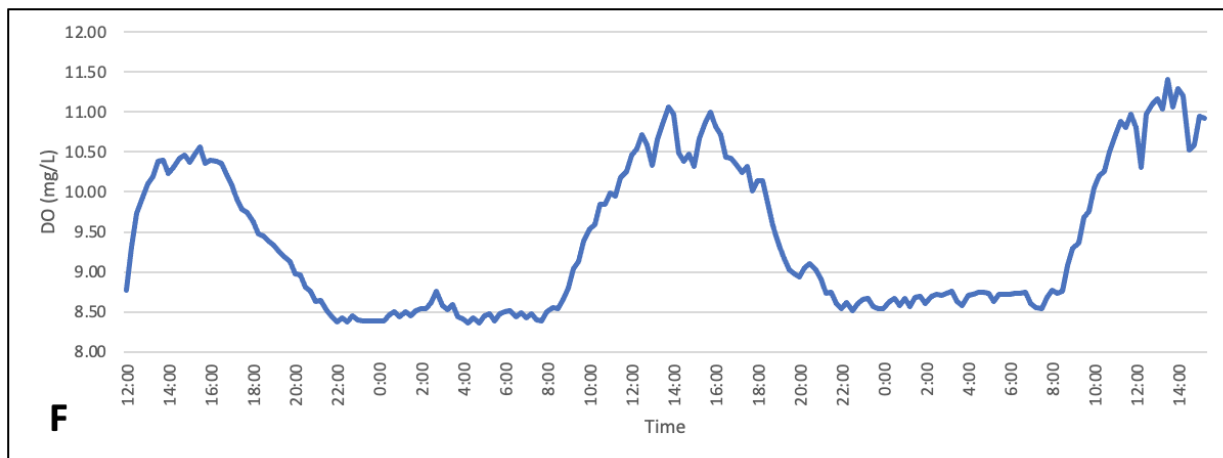
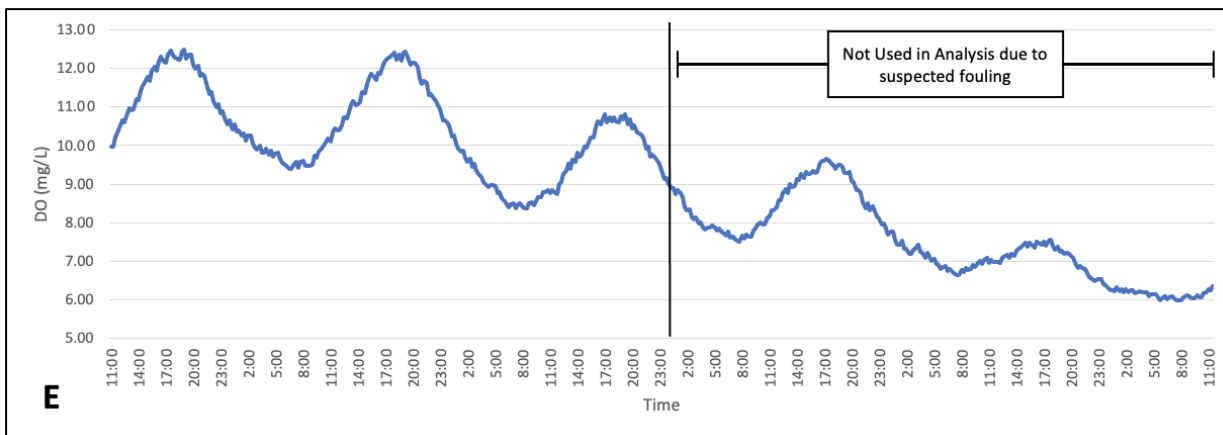
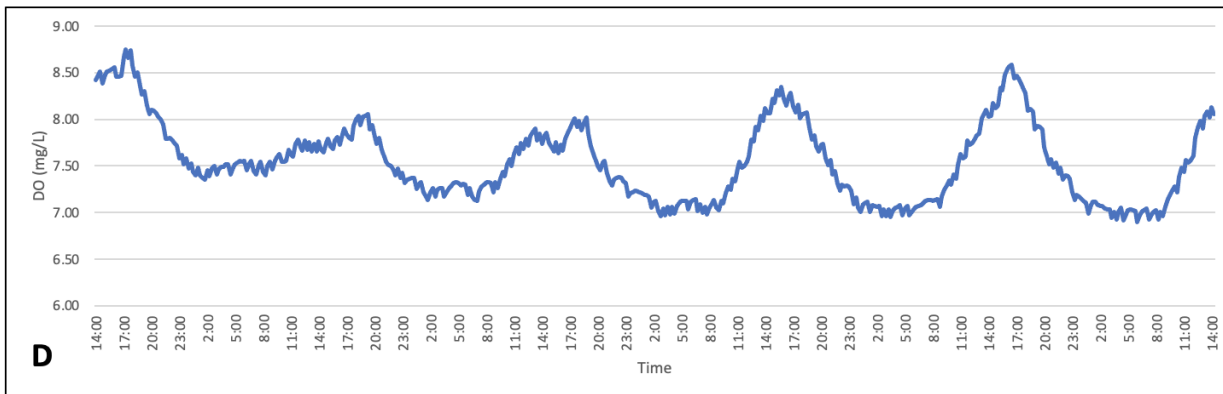
Chlorophyll measurements were collected using procedures outlined by Hall, Jr. and Hotchkiss (2017) and analyzed using spectroscopy (See pg.8 *Algae Collection & Chlorophyll-a Quantification*) . Site distance from Lake Michigan along the river was determined using Google Earth.

Results

Initial Data Collection

Initial DO readings were collected and plotted against time (Figure 7) to observe if any sonde fouling occurred and to ensure the data was appropriately collected. DO was recorded every 15 minutes, therefore data points exist every 15 minutes along the time axis. Fouling is believed to have occurred in the Jackson and Saranac sites and is indicated accordingly on Figures 7A & 7E.





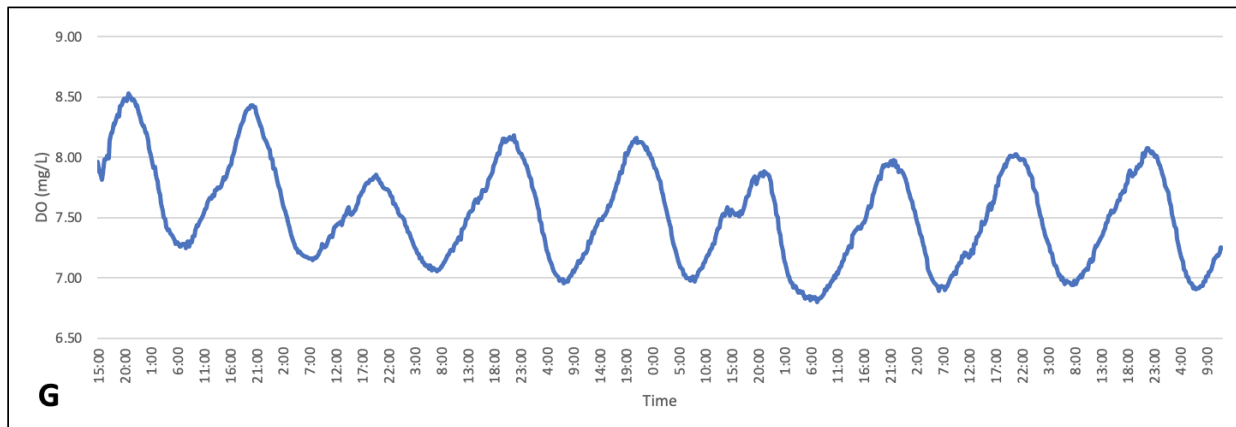
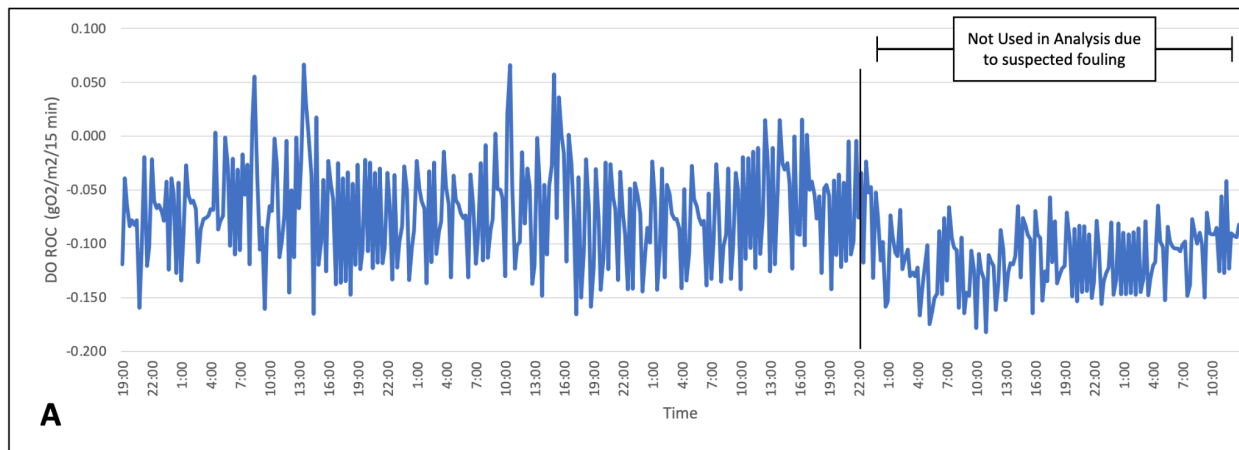
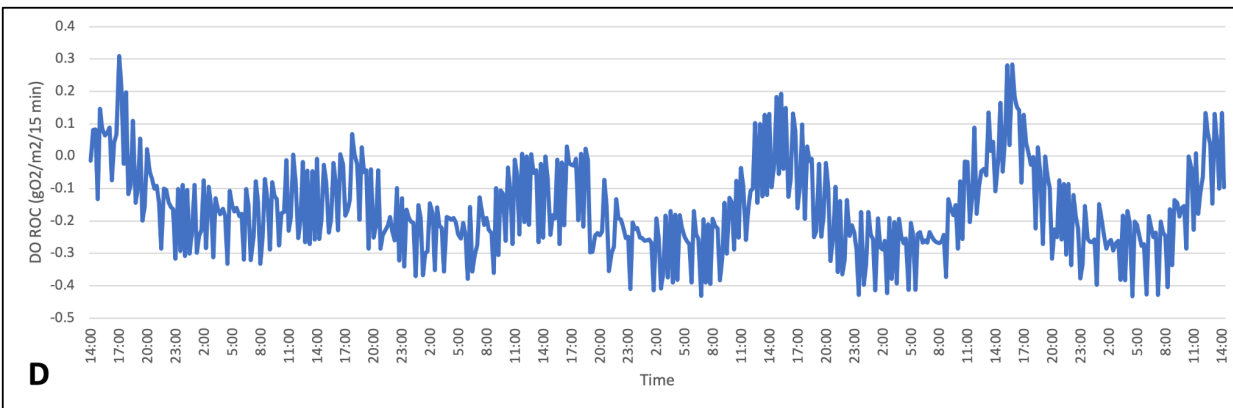
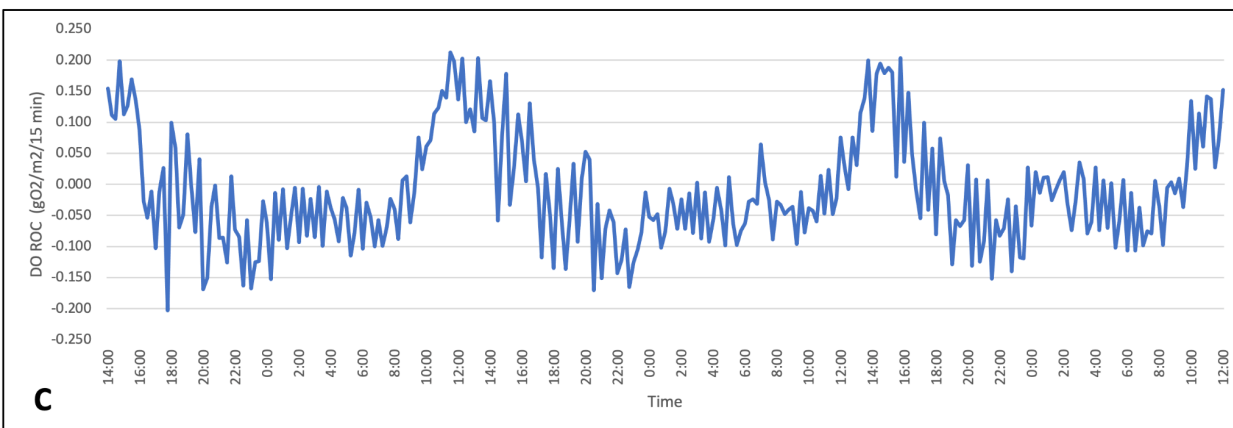
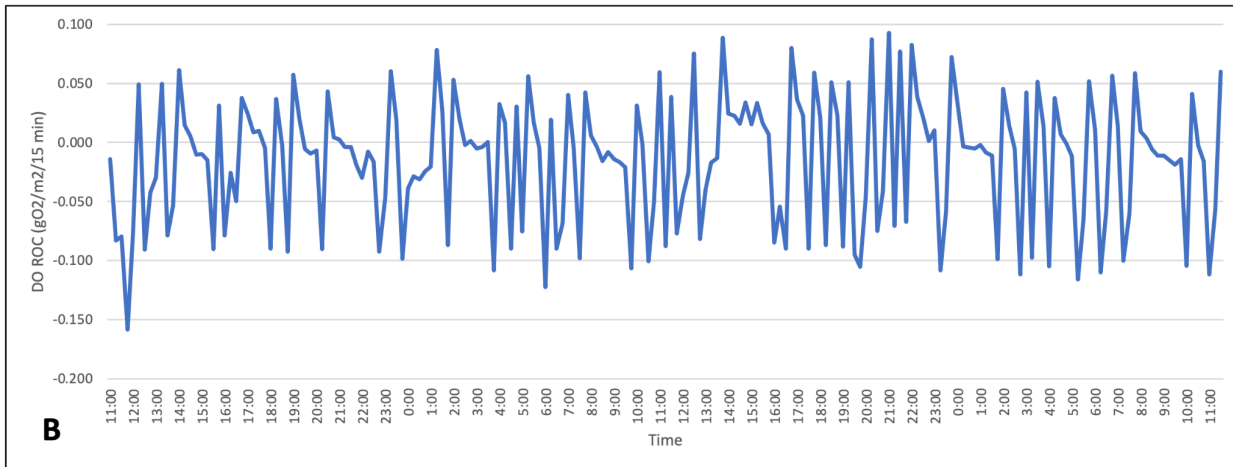


Figure 7: Measured dissolved oxygen (mg/L) every 15 minutes at each site along the Grand River, MI. [A] Jackson, [B] South Lansing, [C] Portland, [D] Lyons, [E] Saranac, [F] Grand Rapids, [G] Grandville.

DO readings were then used to calculate the rate of change in dissolved oxygen at each 15 minute interval, which was then plotted against its given time of record (Figure 8).





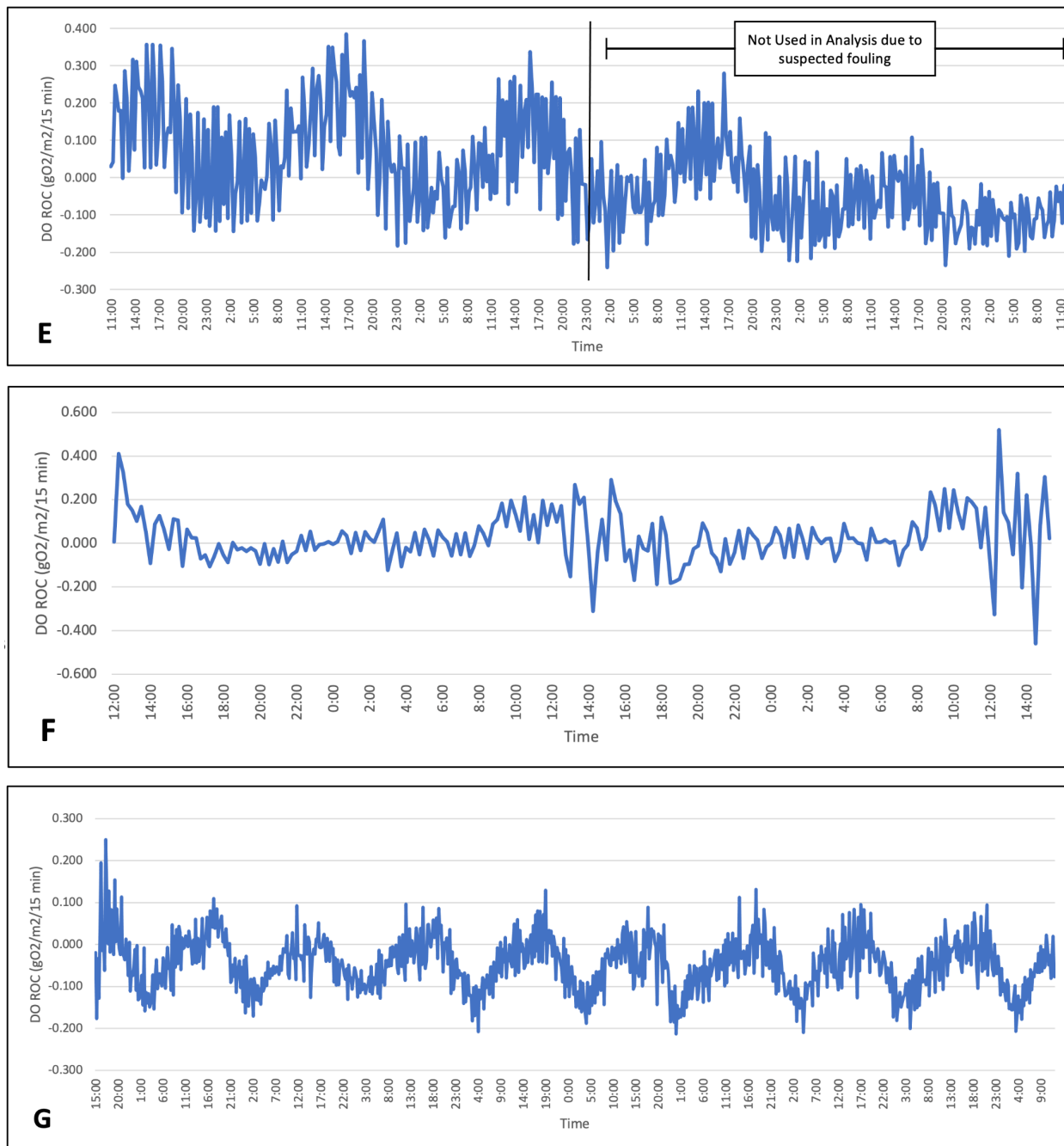
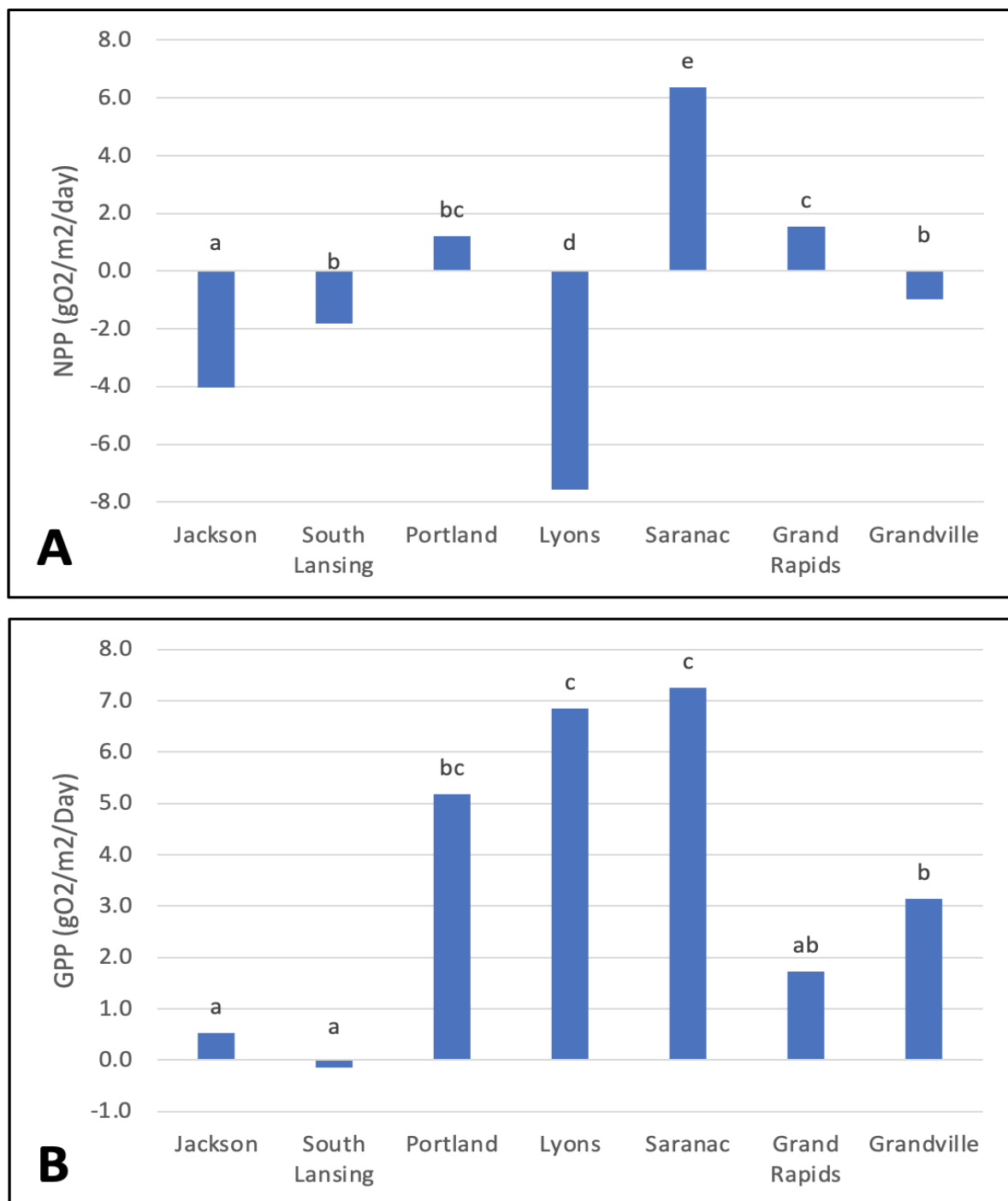
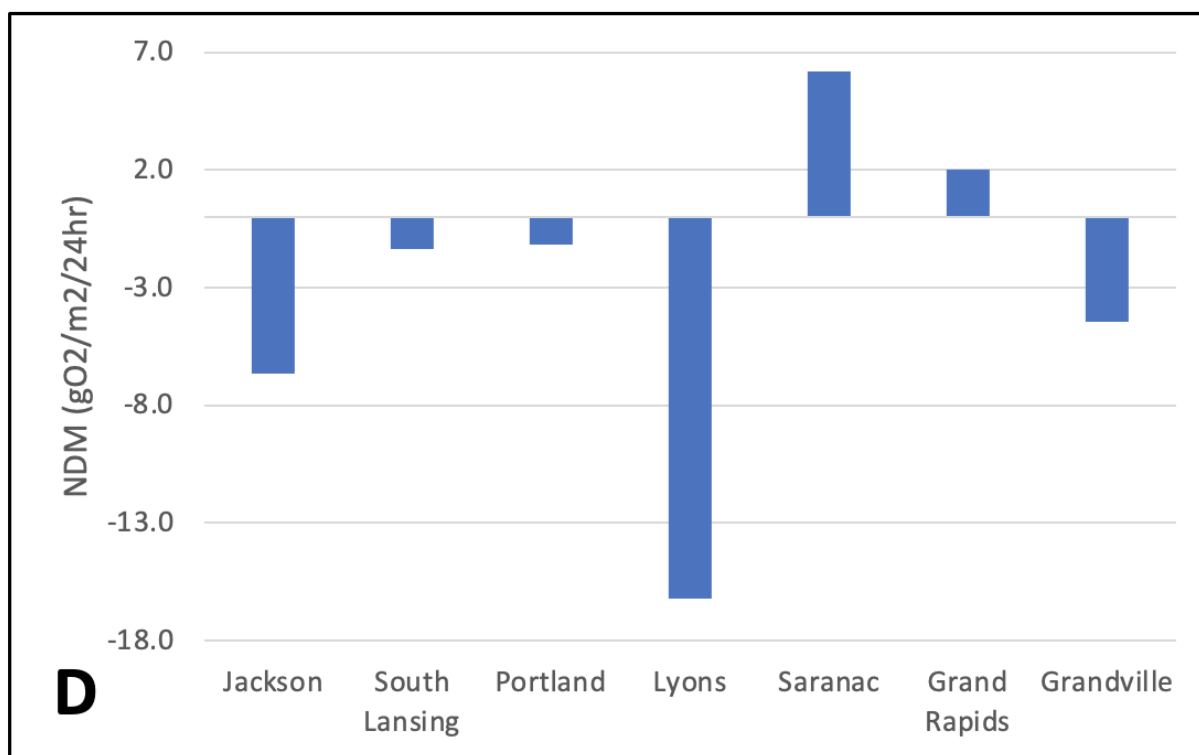
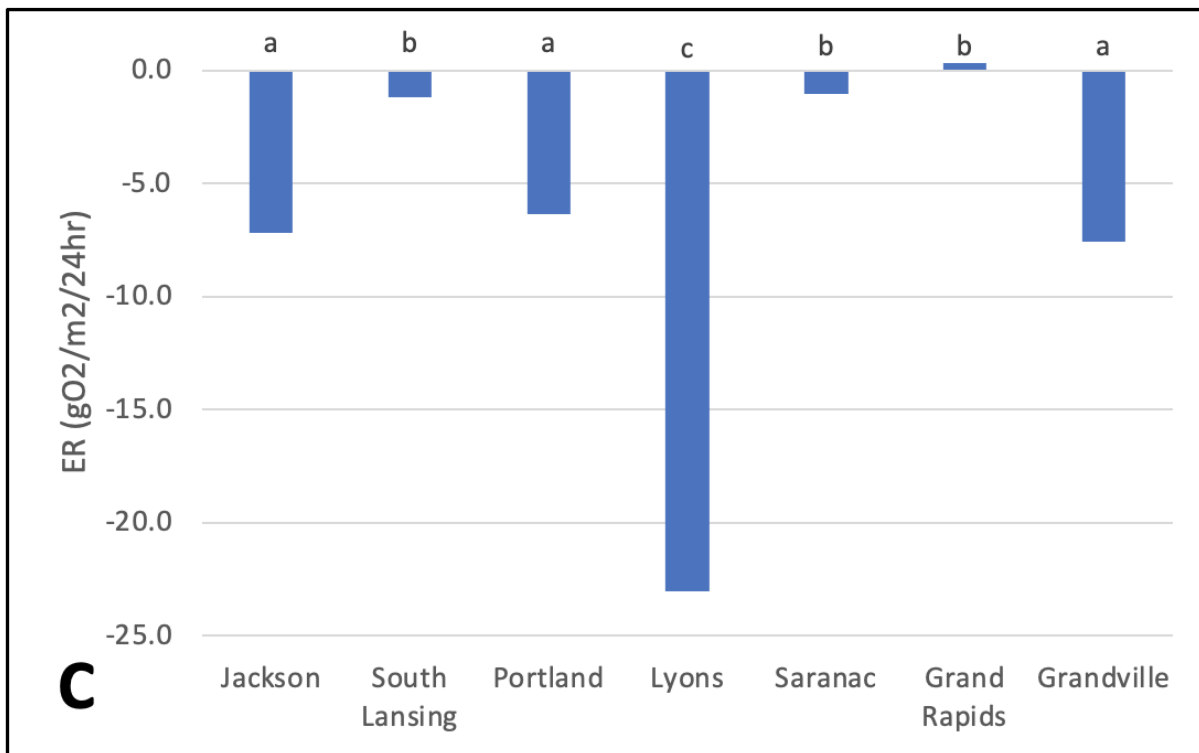


Figure 8: Dissolved oxygen rate of change (ROC) every 15 minutes at each site. [A] Jackson, [B] South Lansing, [C] Portland, [D] Lyons, [E] Saranac, [F] Grand Rapids, [G] Grandville.

Metabolism Metrics

All given metabolism metrics were averaged per day and plotted together for comparison amongst each site (Figure 9). Additionally Post hoc analysis was performed on NPP, 24hr ER, and GPP measurements as indicated.





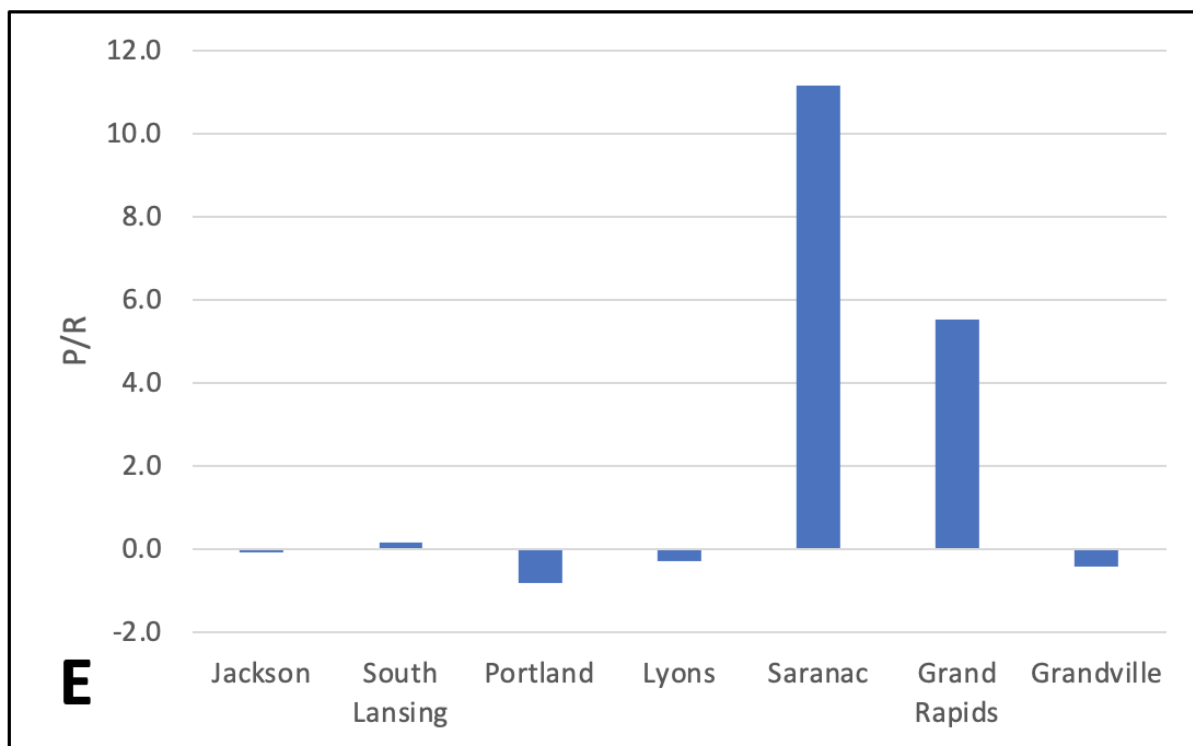


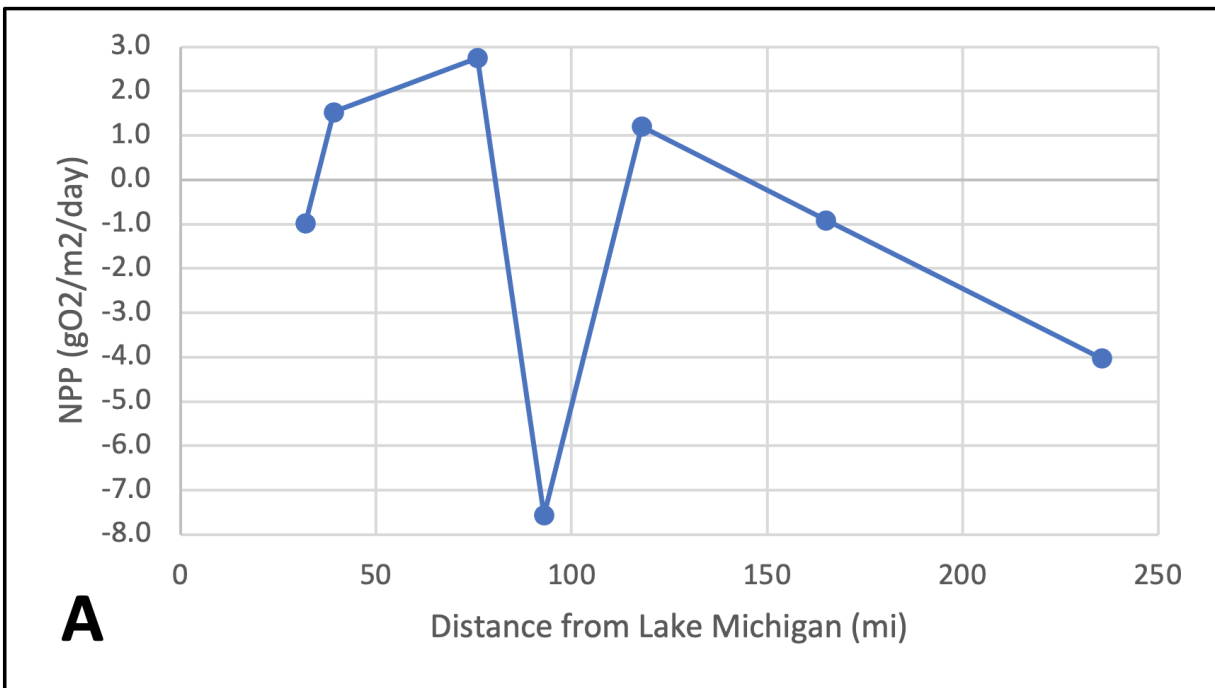
Figure 9: Various metabolism parameters at each site along the Grand River. Jackson (n=3), South Lansing (n=2), Portland (n=2), Lyons (n=4), Saranac (n=2), Grand Rapids (n=2), and Grandville (n=8), n indicates number of days and nights used for mean parameter calculations. In graphs A-C, sites with the same letter are not statistically significantly different according to LSD post hoc tests.

Selected metabolism parameters (NPP, 24hr ER and GPP) were analyzed using both a Shapiro Wilks test with LSD as well as Kruskal Wallis test (Table 4). Significance values from the Shapiro Wilks test are given by site, while results from the Kruskal-Wallis test are presented by each metabolism parameter.

Table 4: Statistical Tests on selected metabolism parameters (NPP, 24hr ER and GPP).

Site	Shapiro Wilk- Significance Values		
	NPP	24hr ER	GPP
Jackson	0.048	0.871	0.654
Lyons	0.609	0.73	0.772
Grandville	0.068	0.512	0.278
Kruskal-Wallis			
	20.057	18.731	18.082
Degrees of Freedom			
	6	6	6
Statistical Significance			
	0.003	0.005	0.006

NPP and NDM at each site was plotted against the sites given distance from the mouth of Lake Michigan as traveled along the Grand River. This helps show longitudinal patterns between the 7 sites.



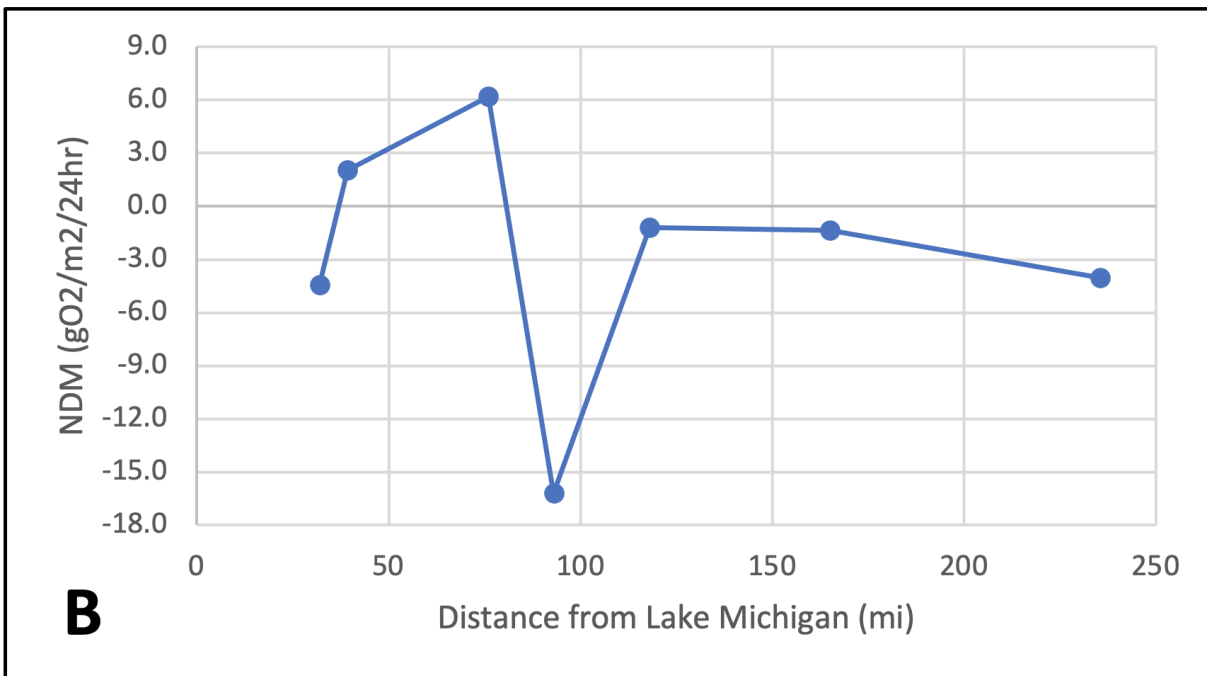


Figure 10: Various metabolism measurements compared longitudinally along the Grand River, MI.

Additional Habitat and Biological Measurements

Mean GPP at each site was divided by mean chlorophyll-*a* concentration as detailed in Figure 5 to determine the amount of production per gram of chlorophyll in each site (Figure 11).

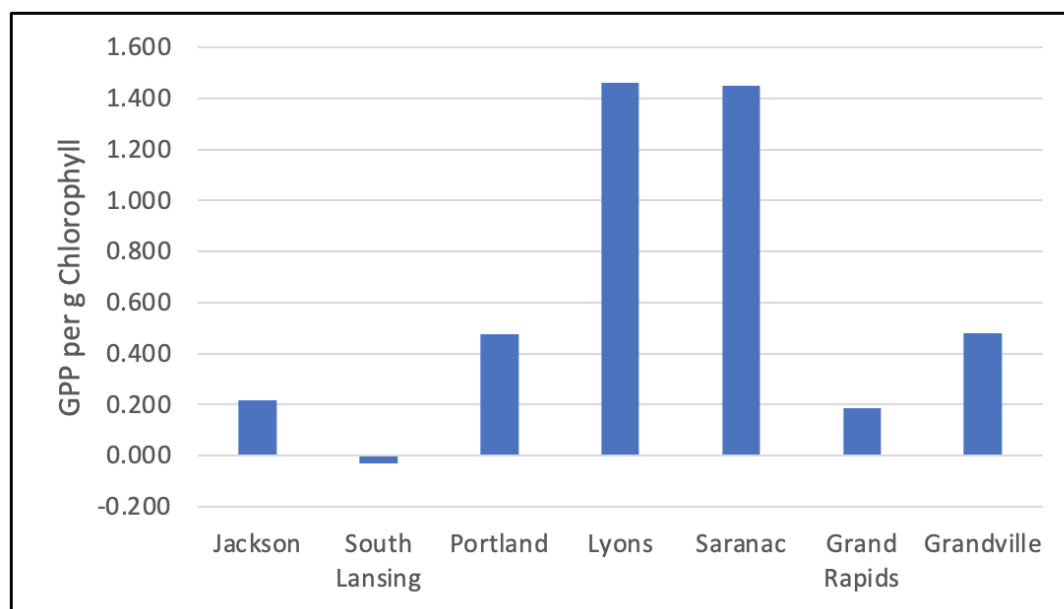


Figure 11: Gross Primary Productivity per gram of chlorophyll (n=5) at each site along the Grand River, MI. Jackson (n=3), South Lansing (n=2), Portland (n=2), Lyons (n=4), Sarnac (n=2), Grand Rapids (n=2), and Grandville (n=8).

Light readings were collected on either the deployment or retrieval of the water chemistry sonde and were attempted to be taken when sunny. In South Lansing it was heavily cloudy on both occasions, thus it has a comparatively lower light intensity.

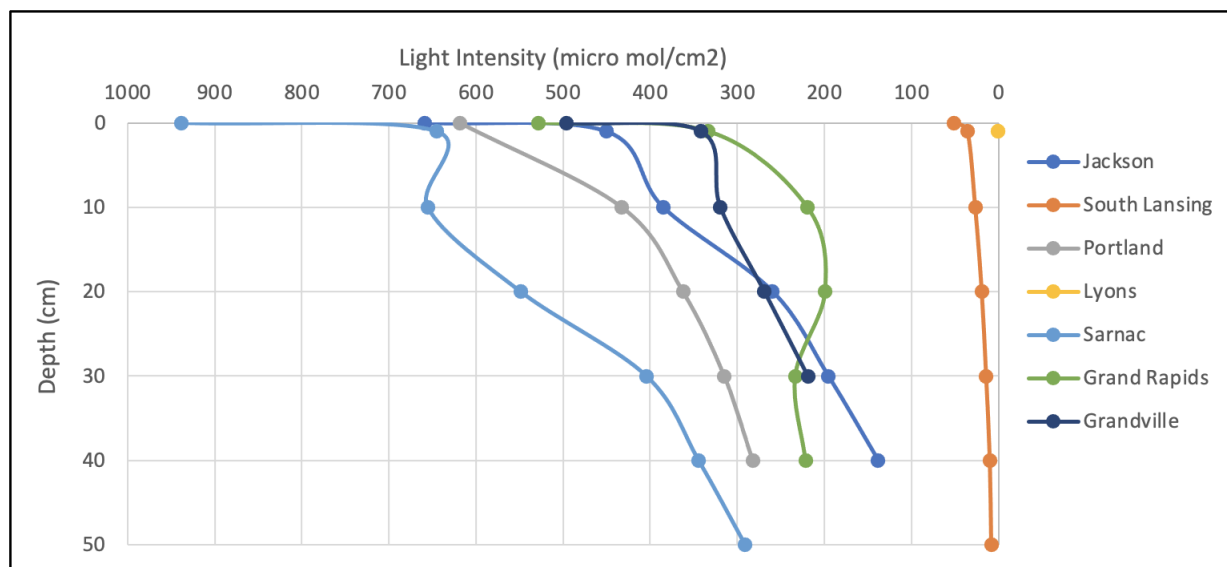


Figure 12: Water column light penetration at each site measured in 10 cm increments (n=5) along the Grand River, MI.

Discussion

Initial diel oxygen curves showed expected oscillations between daytime and nighttime periods in all sites except South Lansing (Figure 7B) which showed no discernible pattern. DO at both the Jackson and Saranac sites appeared to show slow declines over time, especially in later days (Figure 7A & 7E). It is believed that this trend is due to sonde fouling and therefore each respective period was excluded from metabolism analysis. The Portland reach showed the largest change in DO over a 24hr period ranging from 5.80-11.11 mg/L DO (change of 5.31 mg/L). Comparatively, the Jackson site only fluctuated about 1 mg/L between day and nighttime during the first two days. Similar trends are also observed when assessing dissolved oxygen ROC at various time periods with increases during the daytime and decreases at night (Figure 8). This is expected and is actually required for the energy dissipation model to be able to determine respiration rates.

Calculated metabolism indicators show varying effectiveness. NPP, GPP and NDM were highest in the Saranac reach, indicating it is the most autotrophic and has the highest amounts of primary photosynthetic production (Figure 9A, 9B & 9D). This is further supported in comparing the P/R ratios between each site, which shows Saranac having the highest value of 11.1 (Figure 9E). Grand Rapids was the only other site that appeared autotrophic based on NDM calculations, with all other sites classified as heterotrophic (Figure 9D). Portland, Lyons and Grandville sites showed high amounts of GPP (Figure 9C) but also had the highest amounts of respiration, indicating that although there is a high amount of photosynthetic activity, there is more respiration occurring leading to an overall net loss in energy (Figure 9D).

Longitudinally, NPP and NDM appear to mostly fit with the predictions of the river continuum concept, with the exception of the Lyons reach (Vannote et al. 1980). As one travels downstream in the headwaters, both NPP and NDM increase (Figure 10). This aligns with the prediction that as the river increases in size in the early orders its trends to transition from heterotrophy to autotrophy (Vannote et al. 1980). In these middle orders (4-6) the river is wider than the headwaters but is still relatively shallow allowing for lots of vegetation and light penetration leading to more autotrophic production (Vannote et al. 1980). Saranac reach (\approx 75 miles from Lake Michigan) is the peak in NPP and NDM before trending back towards heterotrophy. Once again, this fits with the river continuum concept, since eventually the river becomes too turbulent and deep for light to penetrate the bottom and therefore leads to a decrease in production.

Light intensity aligns closely with these ideas, with Saranac and Portland having the most intense light penetration into the water column and correspondingly higher amounts of NPP. In all sites, light intensity quickly declined between the air and water surface and continued to

decrease with increasing depth. It is important to note that these readings were taken over a period of 4 months during the summer and therefore the sun's intensity also varies during this period.

Conclusions

Metabolism serves as an important method of assessing ecological river health since it encompasses all facets within the system and is sensitive to changes within the watershed. Using the energy dissipation model, it was observed that the Grand River closely follows the predictions and concepts outlined by the river continuum concept. Our headwater (Jackson) and lowest reaches (Grand Rapids & Granville) were largely heterotrophic while the middle order areas were mainly autotrophic, changes concluded through various metabolism metrics. Light penetration and substrate size showed to be important factors influencing production, with clearer water and larger substrate correlating with higher production. Holistically, this information helps to display the complex and connected nature of the river and its watershed. With this, it is important to protect and improve the river as a whole in order to help enhance the Grand River. Furthermore, these measurements can provide a baseline for future assessments within the watershed or can be compared to other large rivers within Michigan.

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