

THE IMPACTS OF ALGAL SUBSIDIES FROM A RUN-OF-RIVER RESERVOIR ON
DOWNSTREAM FOOD WEBS AND NUTRIENT AVAILABILITY, AND THE
IMPLICATIONS OF A DILUTED BITUMEN SPILL, ON THE KALAMAZOO RIVER

By

Micaleila D. Desotelle

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

Integrative Biology – Doctor of Philosophy
Ecology, Evolutionary Biology and Behavior – Dual Major

2017

ABSTRACT

THE IMPACTS OF ALGAL SUBSIDIES FROM A RUN-OF-RIVER RESERVOIR ON DOWNSTREAM FOOD WEBS AND NUTRIENT AVAILABILITY, AND THE IMPLICATIONS OF A DILUTED BITUMEN SPILL, ON THE KALAMAZOO RIVER

By

Micaleila D. Desotelle

Rivers are important systems for the delivery of nutrients and materials into receiving bodies and provide important ecosystem services. The Kalamazoo River is an ideal system for researching basic river ecosystem processes as well as to understand past contamination, restoration potential, and future risks. In this study, I examined the impacts of a run-of-river reservoir, which alters hydrologic retention time but does not change discharge. The increased water residence time from the reservoir led to an increase of algae downstream. Nutrients upstream were in a bioavailable form but were converted into particulate forms, likely mainly as algal biomass, immediately downstream of the reservoir. Phosphorus appeared to follow suspended chlorophyll-*a* patterns closely. However, the fate of suspended chlorophyll-*a* and nutrients from the reservoir was complex. The suspended chlorophyll-*a* produced in the reservoir provided a subsidy that was evidently consumed by benthic macroinvertebrates. Using natural abundance stable carbon isotopes to indicate longitudinal patterns in macroinvertebrate diets and artificial substrata to quantify macroinvertebrate densities, I was able to trace which taxa responded to the algal food subsidy. Filter feeding caddisflies from the family Hydropsychidae appeared to respond to the subsidy most clearly, although they were also abundant above the reservoir. More work is needed to understand food web dynamics in rivers the size of the Kalamazoo River. Lastly, a major oil sands spill occurred on July 25th, 2010. This work quantified the responses of river macroinvertebrates to the oil and its cleanup. In addition, an in-

situ bioassay using *Hyalella azteca* was conducted, and I collected *Orconectes* spp. crayfish to measure metal bioaccumulation, to understand the impacts of submerged oil. This study found that macroinvertebrates were severely reduced in two sections of river, but showed recovery one year later. Hydropsychidae caddisflies seemed particularly impacted by the oil spill. The in-situ bioassay showed the possibility of sub-lethal impacts. This study from the Kalamazoo River oil spill will be useful for understanding the risks to other rivers, and in particular the impacts of diluted bitumen spills on benthic macroinvertebrates. More work is needed to understand if bitumen is more or less toxic than conventional crude, and the risk of submerged oil in freshwater ecosystems.

Copyright by
MICAILEILA D. DESOTELLE
2017

ACKNOWLEDGEMENTS

I would like to thank my advisor, Dr. Stephen Hamilton for his support and guidance over the years. I would like to thank him for his creativity and dedication to the Kalamazoo River. I would like to thank my committee—Dr. Elena Litchman, Dr. Nathaniel Ostrom, and Dr. R. Jan Stevenson—for their support and expertise over the years. I would like to thank Dr. Catherine Lindell for her support for finishing my Ph.D. and Dr. Brendan O’Neill for helping me through the final stretch.

I am grateful for financial support from Michigan State University through the Environmental Science and Policy Program, the Ecology, Evolutionary Biology and Behavior Program, the College of Natural Science, the Graduate School, the Kellogg Biological Station, the Department of Integrative Biology, and the Biogeochemistry Environmental Research Initiative. I would also like to thank the National Science Foundation’s Long Term Ecological Research program for funding.

I am grateful to all of those who helped me in the lab and field including: Rebecca Blundell, Matt Chansler, Taylor Clements, Rob Davis, Matt Dillon, Brandon Kovnat, Jason Mathis, Melissa Kjolvik, Valerie Morel, Jen Pham, Laura Podzikowski, Ravi Srinivasan, Paige Howell, Kevin Li, Megan Lowenberg, and Pam Mosley. Their work was appreciated especially during challenging conditions on the river. I would like to thank Dr. Jon O’Brien for his help with early stages of this work. I would like to thank the late Suzanne Sippel for her help with GIS maps and on the river.

I would like to thank Dr. Allen Burton and his lab for the generous support and for assistance with the in-situ bioassays. I would like to Dr. David Costello and Anna Harrison for their invaluable advice with the methods. I would like to thank the generous support of the

Exova Corporation for their ICP-MS analyses. I would like to thank USFWS and EPA who facilitated access to field sites on the Kalamazoo River during the oil spill clean-up.

I would especially like to thank Dave Weed, who helped extensively during the research on the oil spill and with laboratory analyses of nutrients. We worked into winter, and then in high river conditions the next year. His help cannot be appreciated enough.

I would like to thank my lab mates Amy Burgin, Jorge Celi, Dustin Kincaid, Lauren Kinsman-Costello, Jason Martina and Bonnie McGill.

I would like to thank Ravi Srinivasan for his support, and for his help in the field. I would like thank all of my extended family for their support over the years, and especially to my grandmothers.

TABLE OF CONTENTS

LIST OF TABLES	x
LIST OF FIGURES.....	xii
INTRODUCTION	1
REFERENCES	7
CHAPTER 1: EFFECTS OF A RUN-OF-RIVER IMPOUNDMENT WITH SHORT WATER RESIDENCE TIMES ON DOWNSTREAM NUTRIENTS AND ALGAE	
ABSTRACT.....	12
INTRODUCTION	13
METHODS	16
Study site.....	16
Sample collection.....	16
Hydrochemical measurements	18
Data analysis	20
RESULTS	20
Discharge and residence time	20
Algal production and loss along a longitudinal transect.....	22
Phosphorus concentrations.....	24
Nitrogen and suspended sediments.....	26
Dissolved oxygen and temperature	27
DISCUSSION	28
Production of algae in Morrow Lake and effects on water quality.....	29
The downstream fate of chlorophyll and nutrients	30
Longitudinal patterns of nutrients along the Kalamazoo River	33
Possible mechanism of downstream losses.....	35
The importance of benthic versus suspended algae in the Kalamazoo River.....	36
CONCLUSIONS.....	39
APPENDIX.....	41
REFERENCES	61
CHAPTER 2: SUBSIDY OF A RIVER FOOD WEB BY ALGAL GROWTH IN A RUN-OF-RIVER IMPOUNDMENT UNDER VARYING LEVELS OF HYDRAULIC FLUSHING.....	
ABSTRACT.....	74
INTRODUCTION	75
OBJECTIVES	78
METHODS	80
Study site.....	80
Discharge	82
Water and algal sampling and analysis.....	82

Macroinvertebrate sampling and isotopic analysis	84
Dissolved CO ₂ partial pressure	86
Data analysis	87
RESULTS	90
Discharge	90
Algal biomass.....	91
Macroinvertebrate densities.....	91
Stable isotope ratios of consumers.....	92
Individual comparisons.....	92
Functional feeding groups.....	93
Comparisons between high and low discharge years	94
Baseline comparisons.....	95
Influence of variable lipid content on consumer $\delta^{13}\text{C}$	96
Dissolved carbon dioxide.....	97
DISCUSSION.....	98
Isotopic evidence	98
Macroinvertebrate densities.....	101
Consumer diet subsidy inferred from $\delta^{13}\text{C}$	102
The role of dissolved CO ₂	105
Other alternative explanations for longitudinal variation in consumer $\delta^{13}\text{C}$	107
Caveats for using primary consumers as estimates of the basal food source $\delta^{13}\text{C}$	108
The role of flow variability in controlling phytoplankton production.....	111
Transfer of energy to higher trophic levels and the terrestrial ecosystem	113
CONCLUSIONS.....	115
APPENDIX.....	118
REFERENCES	140

CHAPTER 3: IMPACTS OF A MAJOR OIL SANDS PIPELINE SPILL ON BENTHIC MACROINVERTEBRATES IN THE KALAMAZOO RIVER (MICHIGAN)	156
ABSTRACT.....	156
INTRODUCTION	157
METHODS	161
Description of the 2010 Kalamazoo River oil spill	162
Artificial substrata.....	164
<i>In situ</i> bioassays	167
Crayfish bioaccumulation of oil sands metal.....	169
Water clarity and nutrients.....	169
Benthic algae.....	170
Data analysis	170
RESULTS	171
Benthic macroinvertebrates on Hester Dendy samplers	171
Benthic macroinvertebrates on leaf pack samplers.....	174
Rapid Bioassessment data analysis.....	175
<i>In situ</i> bioassays	176
Crayfish metal bioaccumulation	177
Water quality and nutrients.....	178

Benthic algae.....	179
DISCUSSION.....	179
Short-term impacts on benthic macroinvertebrates	179
Possible long-term toxicity	184
Metal uptake.....	188
Possible impacts on fish and other higher trophic levels.....	190
Other ecosystem impacts	194
CONCLUSIONS.....	195
APPENDIX.....	199
REFERENCES	221

LIST OF TABLES

Table 1.1 Discharge (m^3/s) of the Kalamazoo River from the US Geological Survey gauging station at Comstock, MI (4106000) (First set of columns) and water residence time at Morrow Lake (second set of columns). Annual means calculated for each water year for both discharge and water residence time. Summer means were calculated from May 1 st through September 30 th . Long term means were calculated from the daily data from 1933-2007. Long term Mean Discharge: 26.20; Summer Mean: 22.48. Crop rotations sampled on the KBS Biodiversity Gradient Experiment.....	42
Table 1.2. Results of paired t-tests comparing sites downstream of Morrow Lake dam to the upstream reference site for water quality variables. Seasonal comparisons were paired t-tests comparing July 2012 and July 2009, or comparing July 2012 and October 2012.....	43
Table 1.3 Mean values of temperature, dissolved oxygen (DO; % saturation) transparency measured with a turbidity tube (Turbidity), selected major ions, and conductivity (Cond) in the Kalamazoo River. Sites are arranged by distance from Morrow Lake in km, with the upstream site being negative.	45
Table 1.4 Distance or reservoir derived plankton travels before reducing at least 50%.	46
Table 2.1 Current velocities of sites along the Kalamazoo River where artificial substrata were deployed (bricks or Hester Dendy samplers).	119
Table 2.2 C:N and $\delta^{13}\text{C}$ corrections for taxa from the Kalamazoo River models used for C:N correction were based on linear models from Post et al. (2007) and Logan et al. (2008) with number of individuals. Included are samples with macroinvertebrates allowed to clear their guts versus those with gut content present.....	120
Table 2. 3 Statistical comparisons of d^{13}C of common taxa along the Kalamazoo River. Comparisons were made by pooling taxa from Fort Custer (the site above Morrow) to taxa pooled downstream of Morrow by site. All taxa were made by comparing common taxa via Paired t-test. Functional feeding group comparisons were made using Two-way t-test. Taxa were pooled by section and compared from above (Fort Custer and Galesburg, MI to below (below Morrow and Parchment, MI at Mosel Ave.), Downstream (D-avenue and Plainwell) and Semi-impounded (Ostego Township Dam and the Township Dam and Trowbridge.....	121
Table 3.1 Freshwater oil spills that were comparable to Kalamazoo River including size in gallons and year.....	200

Table 3.2 Field collection from the-situ bioassay experiment. Stream velocity measurements in flowing habitats and depositional habitats and dissolved oxygen measurements in the Kalamazoo River.	201
Table 3.3 Results of the Two Tailed T-tests performed to compare oil-impacted sites with the upstream reference site (Marshall, MI) for Hester Dendy samplers collected from the Kalamazoo River (sites correspond with Figure 3.1).	202
Table 3.4 Individuals were arranged by functional feeding group in each site in 2010. Results of the Two Tailed T-tests performed for each functional feeding group to compare oil-impacted sites with the upstream reference site (Marshall, MI). Macroinvertebrates sampled from Hester Dendy samplers collected from the Kalamazoo River in 2010 (sites correspond with Figure 1).	203
Table 3.5 Results of the Two Tailed T-tests performed to compare oil-impacted sites with the upstream reference site (Marshall, MI) for leaf pack samplers collected from the Kalamazoo River (sites correspond with Figure 3.1).	204
Table 3.6 Results of the Two Tailed T-tests performed to compare oil-impacted sites with the upstream reference site (Marshall, MI) for a <i>Hyalella azteca</i> in-situ bioassay conducted in the Kalamazoo River (sites correspond with Figure 3.1).	205
Table 3.7 Results of in-situ bioassay lab and field controls. Field controls were taken into the field and then left in beakers over the length of the experiment. Lab controls were selected on day of experiment and kept in aerated beakers through the experiment.	206

LIST OF FIGURES

Figure 1.1 Study reach along the Kalamazoo River in southern Michigan. Morrow Lake and Lake Allegan are the two largest reservoirs on the river system. An additional small dam is just upstream of Lake Allegan in Allegan, MI. Orange squares represent partially removed dams in the reach referred to as “semi-impounded”	47
Figure 1.2 Discharge by water year (1 Oct-30 Sep) from the US Geological Survey for the Comstock, MI gauging station, located below Morrow Lake (downloaded from the National Water Information System). Top: Discharge for the relatively low-discharge summers in which I sampled. Bottom: Discharge for the relatively high- and average-discharge summers..	48
Figure 1.3 Suspended chlorophyll- <i>a</i> (Chl- <i>a</i>) measured biweekly in 2008 from the Kalamazoo River, including two sites above Morrow Lake (vertical lack bar) and three sites below it, extending to 30 km downstream.....	49
Figure 1.4 Suspended chlorophyll- <i>a</i> measured in 2004, 2008 and 2010 along longitudinal transects in the Kalamazoo River from above the Morrow Lake reservoir (negative distances) and below it for up to 66 km downstream. Panels show data from years of relatively low (top) and higher discharges (bottom). Black bar represents the dam at Morrow Lake, while the gray bars represent partially removed dams in the semi-impounded reach.....	50
Figure 1.5 Suspended chlorophyll- <i>a</i> measured at multiple times during 2009-12 along longitudinal transects in the Kalamazoo River from above the Morrow Lake reservoir (negative distances) and below it for up to 66 km downstream. Black bar represents the dam at Morrow Lake, while the gray bars represent partially removed dams in the semi-impounded reach. Asterisks show significant differences between the upstream site at -15 km and sites downstream across all samplings (Paired t-test <0.05).	51
Figure 1.6 Total phosphorus (TP) concentrations measured along longitudinal transects in the Kalamazoo River from above the Morrow Lake reservoir (negative distances) and below it for up to 66 km downstream. Black bar represents the dam at Morrow Lake, while the gray bars represent partially removed dams in the semi-impounded reach. Asterisks show significant differences between the upstream site at -15 km and sites downstream across all samplings (Paired t-test <0.05).....	52
Figure 1.7 Fractions of total phosphorus measured on five dates along longitudinal transects in the Kalamazoo River from above the Morrow Lake reservoir (negative distances) and below it for up to 66 km downstream. Longitudinal transects occurred on July 2012 (a), October 2012 (b), July 2009 (c),	

September 2009 (d) and July 2010 (e) PP = particulate P; Dissolved organic P=DOP, DOP=TDP-SRP; SRP = soluble reactive P; TDP = total dissolved P, which only applies to July 2009 where SRP was not measured. DOP could not be estimated from TDP and SRP concentrations.	53
Figure 1.8 Nitrate concentrations measured along longitudinal transects in the Kalamazoo River from above the Morrow Lake reservoir (negative distances) and below it for up to 66 km downstream. Black bar represents the dam at Morrow Lake, while the gray bars represent partially removed dams in the semi-impounded reach. Asterisks show significant differences between the upstream site at -15 km and sites downstream across all samplings (Paired t-test <0.05).	56
Figure 1.9 Ammonium concentrations measured along longitudinal transects in the Kalamazoo River from above the Morrow Lake reservoir (negative distances) and below it for up to 66 km downstream. Black bar represents the dam at Morrow Lake, while the gray bars represent partially removed dams in the semi-impounded reach.	57
Figure 1.10 Total suspended solids concentrations measured along longitudinal transects in the Kalamazoo River from above the Morrow Lake reservoir (negative distances) and below it for up to 66 km downstream. Black bar represents the dam at Morrow Lake, while the gray bars represent partially removed dams in the semi-impounded reach.	58
Figure 1.11 Relationships between chlorophyll-a ($\mu\text{g/L}$) or TSS (mg/L) (both on x axis) to Particulate-P ($\mu\text{g/L}$) in all samples from this study.	59
Figure 1.12 Dissolved oxygen as percent saturation measured along longitudinal transects in the Kalamazoo River from above the Morrow Lake reservoir (negative distances) and below it for up to 65 km downstream. Black bar represents the dam at Morrow Lake, while the gray bars represent partially removed dams in the semi-impounded reach. Asterisks show significant differences between the upstream site at -15 km and sites downstream across all samplings (Paired t-test <0.05).	60
Figure 2.1 Study reach along the Kalamazoo River in Southern Michigan. Morrow Lake and Lake Allegan are the two largest reservoirs on the river system. Orange squares represent partially removed dams in the reach referred to as “semi-impounded”.	123
Figure 2.2 Discharge of the Kalamazoo River. Showing years of higher and lower summer flows as well as the longer term average. From the National Water Information System.	124

Figure 2.3 Suspended chlorophyll- <i>a</i> concentrations. (a) Chlorophyll- <i>a</i> measurements for 2008 collected biweekly. (b) Comparisons of chlorophyll between suspended algae collected in July 2008, through July 2010 and in the first week of July in 2012. Black bar represents the location of Morrow Lake, and gray bars represent the locations of the partially removed dams. The final dam represents Allegan City, the last small dam before Lake Allegan.....	125
Figure 2.4 Benthic chlorophyll- <i>a</i> densities on clay bricks incubated in the Kalamazoo River during summers of 2010 and 2012. Black bar represents the location of Morrow Lake, and gray bars represent the locations of the partially removed dams. The final dam represents Allegan City, the last small dam before Lake Allegan.	126
Figure 2.5 Macroinvertebrate colonization of artificial substrata (Hester Dendy samplers) placed in the Kalamazoo River. Tall black bar represents that location of Morrow Lake. Sampling sites are grouped into above Morrow Lake (Upstream), immediately below the Morrow Lake dam (Below), further downstream, and in the semi-impounded reach with +/-1 standard error.	127
Figure 2.6 Macroinvertebrate $\delta^{13}\text{C}$ values along the Kalamazoo River from above Morrow Lake through the semi-impounded reach. A. $\delta^{13}\text{C}$ of Hydropsychidae. Gray symbols are from 2010 and black are from 2012. B. $\delta^{13}\text{C}$ values of Heptageniidae. Gray symbols are from 2010 and black is from 2012. C. $\delta^{13}\text{C}$ of Tetragnathidae spiders. Gray is from spiders collected in 2010 and black is from 2012. Black bar represents the location of Morrow Lake, and gray bars represent partially removed dams. Asterix represents $p < 0.05$ of a t-test.	128
Figure 2.7 Average $\delta^{13}\text{C}$ values of (a) Hydropsychidae, Heptageniidae and Tetragnathidae spiders and Amphipoda collected from 2010 and 2012.	130
Figure 2.8 Temporal variation of $\delta^{13}\text{C}$ values of Hydropsychidae and Heptageniidae along the Kalamazoo River in 2008. Black bar represents Morrow Lake. Asterisk represents $p < 0.05$ of a t-test.	131
Figure 2.9 Functional feeding group macroinvertebrates $\delta^{13}\text{C}$ values along the Kalamazoo River from above Morrow Lake through the semi-impounded reach. Gray symbols are from 2010 and black is from 2012. A. $\delta^{13}\text{C}$ of taxa in grazing and collector gatherer functional feeding groups. B. $\delta^{13}\text{C}$ values of taxa from the filterer functional feeding group. Black bar represents the location of Morrow Lake, and gray bars represent partially removed dams.	132

Figure 2.10 Macroinvertebrate $\delta^{15}\text{N}$ values along the Kalamazoo River from above Morrow Lake through the semi-impounded reach in 2010 and 2012. Taxa grouped by (a) benthic feeders and (b) filter feeders. Black bar represents the location of Morrow Lake, and gray bars represent partially removed dams.	133
Figure 2.11 Baseline comparisons of Hydropsychidae and Heptageniidae $\delta^{13}\text{C}$ values between Pleuroceridae snail $\delta^{13}\text{C}$ values and <i>Corbicula</i> $\delta^{13}\text{C}$ values. Sampled averages of snail and <i>Corbicula</i> were included as a longitudinal baseline for 2010 (a) and 2012 (b). <i>Macrostemum</i> average was also included in 2010. Other taxa were included for comparison.	134
Figure 2.12 Differences between Hydropsychidae caddisflies and Pleuroceridae snails in 2010 (blue), Hydropsychidae and Pleuroceridae in 2012 (orange) and Hydropsychidae and <i>Corbicula</i> mussels in 2012 (gray). Black Bar represents the dam at Morrow Lake.	136
Figure 2.13 Dissolved CO_2 (aq) from multiple measurements in 2008 and 2012 (a) and a longitudinal survey of CO_2 (aq) from October 2012 and Tetragnathidae spiders collected in July 2012. Black bars represent two reservoirs located well upstream of Morrow Lake (Ceresco and the Battle Creek Mill Ponds) as well as Morrow Lake (0 km) along the Kalamazoo River.	137
Figure 2.14 Comparisons of $\delta^{13}\text{C}$ of Hydropsychidae collected along the river and CO_2 (aq) (μM) measured near time of macroinvertebrate collection.	138
Figure 2.15 Results of chlorophyll- <i>a</i> freezer storage tests.	139
Figure 3.1 Map of the Kalamazoo River in southwest Michigan (inset shows the watershed). Black stars represent sampling sites, with the reference site above Talmadge Creek where the pipeline rupture occurred.	207
Figure 3.2 Results of in-situ Hester-Dendy artificial substratum samplers deployed in autumn 2010 and 2011. Bars represent total number of invertebrates on the samplers at the sites, which are identified by mile points downstream of where the oil entered the river. Two tailed T-tests were performed to compare oil-impacted sites with the upstream reference site (* $p < 0.05$ in 2010 and * $p < 0.05$ in 2011).	208
Figure 3.3 Taxa arranged by functional feeding groups in 2010. Taxa were sampled by Hester Dendy samplers and deployed in September 2010. Bars represent total number of invertebrates as arranged by functional feeding groups. Heptageniidae (scraper) and Hydroptilidae were added to the Collector Gatherer functional feeding group. Sites are denoted by mile points downstream of where the oil entered Talmadge Creek, a tributary of the Kalamazoo River. Two tailed T-tests were performed to compare oil-impacted sites with the upstream reference site (* $p < 0.05$).	210

Figure 3.4 Results of in-situ leaf-pack samplers deployed in late Fall 2010 (a.) and 2011 (b.). Bars represent macroinvertebrate abundances on the samplers at the sites, which are identified by mile points downstream of where the oil entered the river. Two tailed T-tests were performed to compare oil-impacted sites with the upstream reference site (asterisks represent significant differences at $p < 0.05$). Top figure represents (a.) 2010 and bottom is (b.) 2011.	211
Figure 3.5 Numbers of macroinvertebrate taxa observed in the Rapid Bioassessment Procedure conducted by the Michigan Department of Natural Resources (Matousek and Walterhouse 2013). Previous sampling conducted by the Department of Natural Resources occurred in 2004 Marshall, MI upstream reference site, no previous sampling occurred at the 2.25 mile site, in 2008, multiple samples were collected but only one is presented here at the 9.5 mile site and 2009 at the 21.25 mile site. No replicates were collected during the Rapid Bioassessment Procedure.	212
Figure 3.6 Results of in-situ bioassays using the amphipod <i>Hyaella azteca</i> , showing percent survival (a) and growth rate (b) in flowing water and depositional habitats. Two tailed T-tests were performed to compare oil-impacted sites with the most upstream reference site (Kalamazoo River at Marshall) (* $p < 0.05$.).....	213
Figure 3.7 Metal concentrations in tissues of crayfish (<i>Orconectes</i> sp.) at an oil-impacted site (mile 9.5) compared to an upstream reference site on the Kalamazoo River at Marshall. Tissues samples include muscle (a), hepatopancreas (b), and carapace (c). Samples are composites of ~10 individuals.	214
Figure 3.8 Total suspended solids concentrations from sites above and below the point of oil entry into the river at the confluence with Talmadge Creek (mile points 2.5 to 34.5) sampled post-spill in August 2010 and comparison of historical measurements of total suspended solids at 30 and 34 miles downstream to measurements made during the years 2008 and July 2010.....	216
Figure 3.9 Water clarity as measured by turbidity tube from sites at 2.5 to 34.5 miles downstream on the Kalamazoo River post oil spill (0 is the confluence at Talmadge Creek). Previous measurements (July 2010) include the most downstream sites at 30 and 34.5 miles downstream. Sites above the spill include Marshall, MI and just upstream of the confluence at Talmadge Creek.....	217

Figure 3.10 Phosphorus fractions along the Kalamazoo, showing (a) samples collected before the spill in July 2010; (b) samples collected on July 27 th , just after the spill reached the most downstream sections; (c) samples collected on August 4 th and (d) samples collected at the end of August 2010. Note that in this figure 0 km represents Morrow Lake, and negative distances reflect sites upstream of Morrow Lake and positive distances are sites downstream towards Lake Michigan.	218
Figure 3.11 Benthic algal chlorophyll-a accrual on artificial substrata at sites above and below the point of oil entry into the river at the confluence with Talmadge Creek (mile points 2.5 to 34.5). Artificial substrata were collected late October 2010 after the oil spill on the Kalamazoo River and again in October 2011.	220

INTRODUCTION

Rivers are heavily impacted systems used for waste disposal, navigation and water supplies, and our use and modification of rivers has led to loss of biodiversity and some ecosystem services (Stanford et al. 1996). Dams alter hydrology so that high flows are reduced but low flow periods have larger discharge than found historically (Carlisle et al. 2010). Altered hydrology has led to a mismatch of timing between biota and flows, and improved flow management is necessary for river management (Richter et al. 2003). Some of the world's large rivers have been heavily channelized and impounded, reducing connectivity with floodplains. Restoration of connectivity to channel habitat and floodplains has enhanced nutrient uptake and other biological processes on the Danube River (Hein et al. 1999, Tockner and Schiemer 1997). The modifications to the Missouri River have completely altered the hydrograph and impacted biota, but controlled flood releases to mimic the natural hydrograph have been discussed (Galat et al. 1998). Structural modifications such as wing dams, levees, and lock and dam systems were built on the Mississippi River to maintain a 2.7 m depth for navigation, but this has led to negative impacts on the river (Sparks 2010). A major flood release from the Glen Canyon Dam on the Colorado River led to food web changes, but did not restore the natural river food web completely (Cross et al. 2011). The restoration and management of the Columbia River strives to restore more natural food webs and fisheries (Naiman et al. 2012). The recent removal of the two dams on the Elwha River in Olympic National Park will lead to ecosystem recovery (Morley et al. 2008). Many of these dams that were built over 100 years ago are aging dams with the possibility of being removed (Stanford et al 1996), and there is interest in developing run-of-river dams worldwide (Francois et al. 2016, Kumar and Katoch 2014, Winemiller et al. 2016). These examples highlight past degradation, but also ecological restoration. River management is ongoing and

challenging, which makes them ideal systems for coupled human-natural system studies (Stevenson and Sabater 2010, sensu Liu et al. 2007).

Rivers are important for the transport of material, and the uptake of nutrients before they reach estuaries (Wollheim et al. 2006, Tank et al. 2008, Alexander et al. 2000). Excessive nutrients (phosphorus and nitrogen) have led to eutrophication in downstream water bodies (Rabalais 2002, Paerl 2009). Phosphorus (P) is particularly important in freshwater systems where increasing P has led to algal blooms in lakes and rivers (Carpenter 1998, Mallin et al. 2006, Smith 2015). Rivers represent a sizable area of water bodies in the United States (Downing et al. 2012), and U.S. rivers often have multiple dams on them (Stanley et al. 2002).

Dams on rivers in humid regions often are of the run-of-river design. Run-of-river dams do not alter the hydrology but increase water retention time in rivers, which can lead to algal growth and eutrophication (Reid et al. 2007, Hilton et al. 2006). Interestingly, both variable release (storage) and run-of-river dams can be found in series along a river (i.e. Oliver et al. 2014), which has important cumulative effects.

The Kalamazoo River of southwest Michigan is interesting because it one of the original Superfund sites, and the EPA has been in process of removing polychlorinated biphenyls contaminated sediment for the last thirty years (Wesley 2005). Additionally, a total maximum daily load (TMDL) for phosphorus has been implemented to reduce algal blooms in the most downstream reservoir (Lake Allegan). The sections of river in this study represent one of the rivers studied in the original research on the River Continuum Concept rivers, making it a high quality and well protected section of river (Vannote et al. 1980, Cummins et al. 1981).

The Kalamazoo River is a typical example of a working river in the Midwest, and is an ideal river to consider in context of past impacts and future restoration. Like many U.S. rivers,

dams were built in the early part of the 1900s, in this case mainly for hydroelectricity and for industrial cooling water. The Kalamazoo River drains a 5,230 km² watershed in southwestern Michigan. The river is 260 km long with a mean discharge of 38 m³/s at Comstock (USGS gauge 4106000). The Kalamazoo River is groundwater fed and meanders through glacial deposits (Wesley 2005). As of 2005 the main-stem river had 15 dams (two of which have since been removed), while the entire watershed had 110 registered dams (Wesley 2005).

Morrow Lake, on the Kalamazoo River main-stem near Comstock, MI, is a run-of-river reservoir that is the focus of this dissertation. Run-of-river dams increase water retention time in proportion to the volume of the reservoir, but do not alter downstream river hydrology as much as variable-storage dams do (Csiki and Rhoads 2015). Decommissioned hydroelectric dams or dam remnants also exist in the Kalamazoo River downstream of Plainwell, including at Plainwell (now breached), Otsego City, Otsego Township (now being removed), Trowbridge Township, and Allegan City. Downstream of those dams is Lake Allegan, a reservoir maintained by the functioning Calkins Dam hydroelectric facility. Additionally, channel straightening of meander bends occurred on the river, though there are many reaches of river that remain well connected to the floodplain (Rachol et al. 2005). Another important aspect of the Kalamazoo River is that several paper-making and carbonless paper recycling plants once operated along the river. Wastes from the latter process, which were contaminated with polycyclic chlorinated biphenyls (PCBs), were discharged into the river and floodplain, creating a legacy of contaminated sediments.

Currently, the remaining old decommissioned dams on the mainstem between Morrow Lake and Lake Allegan are partially removed and the remnants are decrepit, but the PCB-contaminated sediment has prevented full removal of these dams. These dams create “semi-

impounded” reaches that have water residence times of less than one day (Reid and Hamilton 2007), with important ecosystem impacts (see Chapters 1 and 2). As of 2017, these dams are seriously deteriorating and are in either in the process of full removal or in the planning stages. A dam downstream of Plainwell, MI was removed in 2009, and the Otsego Township dam is in the process of being removed. The Otsego City dam (built in 1836) is also in stages of collapse but is still in the planning stages of removal. Negotiations between the EPA and the responsible parties have been ongoing. The remaining PCBs have been found to have negative impacts to wildlife such as mink (Beckett et al. 2005) and carp (Fisher et al. 2006). Studies have looked at the PCBs in macroinvertebrates and the possible impacts of emerging insects on swallows (Blankenship et al. 2005).

Despite the lingering problem of contaminated sediments, the water quality of the Kalamazoo River has improved considerably in recent decades. Local community members remember times when the river was heavily polluted, creating massive fish kills leading to national attention. However, non-point source pollution continues to be a problem on the river. Lake Allegan has impaired water quality because of excessive algal growth, low dissolved oxygen and a fish community composed of common carp and channel catfish. The largest upstream reservoir, Morrow Lake, near Comstock, MI, has clear water and has better fish diversity. For Lake Allegan, the US EPA and Michigan Department of Environmental Quality determined a target total phosphorus concentration of 60 µg/L to devise a total maximum daily load (TMDL) to improve water quality by reducing algal production. That concentration was based on observations of Morrow Lake, which was considered similar in size but with fewer impairments. In Morrow Lake, however, water retention time may be particularly important for limiting the production of algae and hence reduced water quality downstream of Morrow Lake

(Reid and Hamilton 2007). Concentrations of nutrients and particularly P tend to explain much of the variation in chlorophyll standing stocks, but that relationship appears to be nonlinear (Stevenson et al. 2008).

Eutrophication of estuaries and lakes from non-point source pollution is increasingly a challenge for protecting water resources (Rabalais 2002, Carpenter 1998). Rivers themselves can become eutrophic (Hilton et al. 2006). Increased water residence time from reservoirs allows for the production of suspended algae (phytoplankton), which frequently increase downstream of lake outflows. Reid and Hamilton (2007) and Bass (2009) conducted studies of nutrient cycling and algal production in Morrow Lake. Chapter 1 builds on the work by Reid and Hamilton (2007). More longitudinal sampling and additional water quality variables were collected along with chlorophyll-*a* to understand upstream and downstream patterns of suspended algae and water quality variables. Chapter 1 uses a semi-Lagrangian sampling approach to understand patterns of suspended chlorophyll-*a* and nutrients both upstream and downstream of Morrow Lake. Nearly ten years of chlorophyll-*a* and phosphorus data show that while TP is decreasing, water retention time is high enough that algae are produced, which is important for the Lake Allegan TMDL.

Algae produced in Morrow Lake could be transported downstream to provide a food web subsidy for river macroinvertebrates. The link between the base of the food web (i.e., primary producers and detritus) and river macroinvertebrates is not well known, but both phytoplankton and benthic algae are potentially important (DeLong and Thorp 2006). Chapter 2 looks at the downstream subsidy from Morrow Lake to understand how macroinvertebrate communities respond to suspended algae exported from the reservoir. This is important for understanding how reservoirs affect secondary production in rivers, and also how PCBs enter the food web and are

biomagnified through higher trophic levels. Lastly, Chapter 3 examines the impacts of a massive oil spill that occurred on the Kalamazoo River on July 25th, 2010 on the macroinvertebrate community. This study looked at the direct toxicity and possible indirect impacts of submerged oil on macroinvertebrate community composition and abundance.

REFERENCES

REFERENCES

- Alexander, R. B., R. A. Smith, G. E. Schwarz. 2000. Effect of stream channel size on the delivery of nitrogen to the Gulf of Mexico. *Nature* 403: 758-761.
- Baas, D. G. 2009. Inferring dissolved phosphorus cycling in a TMDL watershed using biogeochemistry and mixed linear models. Dissertation, Michigan State University, East Lansing, Michigan, USA.
- Beckett, K. J., Millsap, S.D., A. L. Blankenship, M. J. Zwiernik, J. P. Giesy, S. J. Bursian. 2005. Squamous epithelial lesion of the mandibles and maxillae of wild mink (*Mustela vison*) naturally exposed to polychlorinated biphenyls. *Environmental Toxicology and Chemistry* 24: 674-677.
- Blankenship, A. L., M. J. Zwiernik, K. K. Coady, D. P. Kay, J. L. Newsted, K. Strause, C. Park P.W. Bradley, A. M. Neigh, S. D. Millsap, P. D. Jones, J. P. Giesy. 2005. Differential Accumulation of Polychlorinated Biphenyl Congeners in the Terrestrial Food Web of the Kalamazoo River Superfund Site, Michigan. *Environmental Science and Technology*. 39: 5954-5963
- Carlisle, D. M., D. M. Wolock, M. R. Meador. 2010. Alteration of streamflow magnitudes and potential ecological consequences: a multiregional assessment. *Frontiers in Ecology and Environment* 9: 264-270.
- Carpenter, S. R. 2008. Phosphorus control is critical to mitigating eutrophication. *Proceedings of the National Academy of Science* 105: 11039-11040.
- Cummins, K. W., M.J. Klug, G.M. Ward, G. L. Spengler, R. W. Speaker, R. W. Ovink, D. C. Mahan, R. C. Peterson. 1981. Trends in particulate organic matter fluxes, community processes and macroinvertebrate functional feeding groups along a Great Lakes drainage basin river continuum. *Verhandlungen der Internationalen Vereinigung fur theoretische und angewandte Limnologie* 21:841-849.
- Delong, M. D. and J. H. Thorp. 2006. Significance of instream autotrophs in trophic dynamics of the Upper Mississippi River. *Oecologia* 147: 76-85.
- Downing, J.A., J.J. Cole, C.M. Duarte, J.J. Middelburg, J.M. Melack, Y.T. Prairie, P. Kortelainen, R. G. Striegl, W. H. McDowell, L. J. Tranvik. 2012. Global abundance and size distribution of streams and rivers. *Inland Waters* 2: 229-236.
- Francois, B., B. Hingray, D. Raynaud, M. Borga, J.D. Creutin. 2016. Increasing climate-related-energy penetration by integrating run-of-the-river hydropower to wind/solar mix. *Renewable Energy* 87: 686-696.

Fischer, M. A., R. Eversole, C. Mehne, J. C. Means, C. Delong, D. Mihako, C. F. Ide. 2008. Liver cyp1A protein expression and pigmented macrophage aggregates as indicators of polychlorinated biphenyl exposure in carp *Cyprinus carpio* L. from the Kalamazoo River Superfund Site, Michigan. *Journal of Fish Biology* 72: 1960-1975.

Galat, D. L., L. H. Fredrickson, D. D. Humburg, K. J. Bataille; J. R. Bodie, J. Dohrenwend; G. T. Gelwicks, J. E. Havel, D L. Helmers, J. B. Hooker; J. R. Jones, M. F. Knowlton, J. Kubisiak, J. Mazourek, A. C. McColpin, R. B. Renken, Raymond D. Semlitsch. 1998. Flooding to restore connectivity of regulated, large river wetlands. *Bioscience*. 48: 721-733.

Hamilton, S.K., W.M. Lewis, Jr., and S.J. Sippel. 1992. Energy sources for aquatic animals on the Orinoco River floodplain: Evidence from stable isotopes. *Oecologia* 89: 324-330.

Hamilton, S. K., S. J. Sippel, S. E. Bunn. 2005. Separation of algae from detritus for stable isotope for ecological stoichiometry studies using density fractionation in colloidal silica. *Limnology and Oceanography: Methods* 3: 149-157.

Hein, T., G. Hailer, D. Pennetzdorfer, P. Riedler, M. Schagerl, F. Schiemer. 1999. The Danube Restoration Project: functional aspects and planktonic productivity in the floodplain system. *Regulated Rivers and Management* 15: 259-270.

Hilton, J., M. O'Hare, M. J. Bowes, J. I. Jones. 2006. How green is my river? A new paradigm of eutrophication in rivers. *Science of the Total Environment* 365: 66-83.

Mallin, M. A., V. L. Johnson, S. H. Ensign, T. A. MacPherson. 2006. Factors contributing to hypoxia in rivers, lakes and streams. *Limnology and Oceanography* 51: 690-701.

Kumar, D., S.S. Katoch. 2015. Sustainability suspense of small hydropower projects: a study from western Himalayan region of India. *Renewable Energy* 76: 220-233.

Liu, J., T. Dietz, S. R. Carpenter, C. Folke, M. Alberti, C. L. Redman, S. H. Schneider, E. Ostrom, A. N. Pell, J. Lubchenco, W. W. Taylor, Z. Ouyang, P. Deadman, T. Kratz, W. Provencher S. H. Schneider & W. W. Taylor. 2007. Coupled Human and Natural Systems. *Ambio* 36: 639-649.

Paerl, H. W. 2009. Controlling eutrophication along the freshwater-marine continuum: dual nutrient (N and P) reductions are essential *Estuaries and Coasts* 32: 593-601.

Rabalais, N.N. 2002. Nitrogen in aquatic ecosystems. *Ambio* 21: 102-112.

Rachol, C.M., Fitzpatrick, F.A., and Rossi, T., 2005, Historical and simulated changes in channel characteristics of the Kalamazoo River, Plainwell to Otsego, Michigan: U.S. Geological Survey Scientific Investigations Report 2005-5044.

- Reid, N. J., S. K. Hamilton. 2007. Controls on algal abundances in a eutrophic river with varying degrees of impoundment (Kalamazoo River, Michigan, USA). *Lake and Reservoir Management* 23: 219-230.
- Richter, B. D., R. Mathews, D. L. Harrison, R. Wigington. 2003. Ecologically sustainable water management: managing river flows for ecological integrity. *Ecological Applications* 13: 206-224.
- Stanford, J. A., J. V. Ward, W. J. Liss, C. A. Frissell, R. N. Williams, J. A. Lichatowich and C. C. Coutant. 1996. A general protocol for restoration of regulated rivers. *Regulated River Research and Management* 12: 391-413.
- Stanley, E. H., M. A. Luebke, M.W. Doyle, D.W. Marshall. 2002. Short-term changes in channel form and macroinvertebrate communities following low-head dam removal. *Journal of North American Benthological Society* 21: 172-187.
- Stevenson, R. J., S. Sabater. 2010. Understanding effects of global change on river ecosystems: science to support policy in a changing world. *Hydrobiologia* 657: 3-18.
- Stevenson, R. J., B. H. Hill, A. T. Herlihy, L. L. Yuan, S. B. Norton. 2008. Algae-P relationships, thresholds, and frequency distributions guide nutrient criterion development. *Journal of North American Benthological Society* 27: 783-799.
- Sparks, R. E. 2010. Forty years of science and management on the Upper Mississippi River: an analysis of the past and a view of the future. *Hydrobiologia* 640: 3-15.
- Naiman, R. J., J. R. Alldredge, D. A. Beauchamp, P. A. Bisson, J. Congleton, C. J. Henny, N. Huntly, R. Lamberson, C. Levings, E. N. Merrill, W. G. Pearcy, B. E. Reidma, G. T. Ruggersone, D. Scarnecchia, P. E. Smouse, C. C. Wood. 2012. Developing a broader scientific foundation for river restoration: Columbia River food webs. *Proceedings of the National Academy of Sciences*. 109: 21201-21207.
- Tank, J. L., Rosi-Marshall, E. J., Baker, M. A., and Hall, R. O. 2008. Are rivers just big streams? A pulse method to quantify nitrogen demand in a large river. *Ecology* 89: 2935–2945.
- Tockner, K., F. Schiemer 1997. Ecological aspects of the restoration strategy for a river-floodplain system on the Danube River in Austria. *Global Ecology and Biogeography Letters*. 6: 321-329.
- Thorp, J.H., M.D. Delong, K.S. Greenwood, A.F. Casper. 1998. Isotopic analysis of three food web theories in constricted and floodplain regions of a large river. *Oecologia* 117:551-563.
- Morley, S.A., J. J. Duda, H. J. Coe, K. K. Kloehn, M. L. McHenry. 2008. Benthic invertebrates and periphyton in the Elwha River Basin: Current conditions and predicted response to dam removal. *Northwest Science* 82: 179-196.

Walters, D. M., K. M. Fritz, R. R. Otter. 2008. The dark side of subsidies: adult stream insects export organic contaminants to riparian predators. *Ecological Applications* 18: 1835-1841.

Winemiller, K.O., P. B. McIntyre, L. Castello, E. Fluent-Chouinard and 36 other authors. 2016. Balancing hydropower and biodiversity in the Amazon, Congo and Mekong. *Science* 351: 128-129.

Wollheim, W. M., C.J. Vorosmarty, B. J. Peterson, S. P. Seitzinger, C.S. Hopkins. 2006. Relationship between river size and nutrient removal. *Geophysical Research Letters* 33: *L06410*.

CHAPTER 1: EFFECTS OF A RUN-OF-RIVER IMPOUNDMENT WITH SHORT WATER RESIDENCE TIMES ON DOWNSTREAM NUTRIENTS AND ALGAE

ABSTRACT

Impoundment of rivers often creates lacustrine conditions that favor the growth of suspended algae (phytoplankton), and when algal biomass is transported downstream in outflowing water, it can act as a subsidy for river food webs. Morrow Lake, a run-of-river reservoir on the Kalamazoo River (Michigan), has short water residence times but can produce high concentrations of suspended algae at lower river discharges. In this chapter, I examine how Morrow Lake affects downstream patterns of nutrients and algal biomass (as indicated by chlorophyll) over a range of discharges. This study used a semi-Lagrangian method, where water samples were collected along a longitudinal transect as if to track a parcel of water and estimate changes to nutrients. I measured suspended chlorophyll-*a*, total suspended solids, and nutrients (phosphorus, nitrate, ammonium,) to estimate the fate of algae and nutrients downstream of Morrow Lake. It appears that high discharge reduces water retention time to the point where the abundance of algae in water leaving from Morrow Lake is reduced. During low discharge summers, algae decreased downstream of Morrow Lake, with consequent transformation of particulate phosphorus to dissolved inorganic phosphorus. Nitrogen did not closely follow chlorophyll-*a*. Morrow Lake thus produces algal biomass at lower discharges (longer water residence times), and export of this biomass extends variable distances downstream depending on discharge. Phosphorus may be temporarily sequestered in algal biomass but as the algae disappear the phosphorus is released in soluble forms, thereby maintaining relatively constant total phosphorus concentrations along the study transect.

INTRODUCTION

Rivers transport nutrients and organic material from watersheds to receiving water bodies, which can cause eutrophication and sometimes the development of hypoxic zones (Howarth et al. 1996, Vitousek et al. 1997, Rabalais 2002, Smith 2003). Dissolved inorganic nitrogen is frequently cited as the proximate driver for eutrophication in estuaries (Rabalais 2002, Carpenter 1998, Paerl 2009). Phosphorus is considered more likely to be the limiting nutrient for eutrophication in freshwaters (Baker et al. 2014, Lewis and Wurtsbaugh 2008, Carpenter 2008, Basu and Pick 1996, Sondergaard et al. 1992, Istvanovics et al. 2004), though both N and P can limit primary production (Paerl 2009, Lewis and Wurtsbaugh 2008). Rivers transport N and P to receiving bodies, but uptake, retention and removal of nutrients in rivers can prevent nutrients from entering estuaries (Hall et al. 2013, Tank et al. 2008, Ensign and Doyle 2006, Wollheim et al. 2006, Webster 2007, Alexander et al. 2000, Alexander et al. 2008). Rivers themselves suffer from eutrophication, especially when they pass through impounded reaches or broaden into estuaries (Mallin et al. 2006, Reid et al. 2007, Hilton et al. 2006, Lucas et al. 1999, Bowes et al. 2012, Dodds 2006, Smith and Schindler 2009). Phytoplankton or suspended algae increase predictably with total phosphorus in rivers like in lakes (Smith 2016). However, the actual longitudinal pattern of chlorophyll-*a* and nutrients is complex and chlorophyll-*a* can increase (Minaudo et al. 2015, Basu and Pick 1997) or decrease (Bukaveckas et al. 2011, Houser et al. 2010) in the downstream direction.

Dams, especially on medium-sized rivers, are important to consider because they can degrade water quality by increasing water residence times in their reservoirs to the point where high concentrations of phytoplanktonic algae are produced (Bowes et al. 2012). The effects of dams and their impounded waters (reservoirs) on biogeochemistry and water quality have been

amply documented (Powers et al. 2015, Oliver et al. 2014). Reservoirs are important for biogeochemical processing by reducing downstream transport of sediment, N and P (Powers et al. 2015, Oliver et al. 2014, Vorosmarty et al. 2003, Harrison et al. 2009, Wohl et al. 2015), and biological metabolism within reservoirs produces carbon dioxide and methane emissions (Deemer et al. 2016). Reservoirs can increase or decrease organic carbon transport (Ulseth and Hall 2015, Angradi 1993). This is especially true for larger reservoirs that commonly have seasonally variable water storage volumes and long water residence times. Midwestern impoundments in particular can lead to the burial of a large percentage of organic carbon inputs (Downing et al. 2008).

Within reservoirs, water retention time has been found to be an important predictor for suspended algal production (as indicated by chlorophyll-*a* concentrations) and nutrient transformations (Bowes et al. 2012, Neal et al. 2006, Lucas et al. 2009, Soballe and Kimmel 1987, Honti and Istvanovics 2010). Rivers without impoundments were thought to be too deep and turbid, and water residence time too short, to allow for the extensive production of river phytoplankton (Vannote et al. 1980, Lewis 1988, Cole et al. 1992). While numerous phytoplanktonic species are found in rivers (Kohler et al. 2002, Reynolds 2000, Descy et al. 2012, Reynolds and Descy 1996), phytoplankton in rivers was thought to originate from lakes rather than representing growth of an extant population (Reynolds and Descy 1996). The development of phytoplankton in free-flowing reaches of rivers has been difficult to evaluate because dams are ubiquitous along most larger rivers (Welker and Waltz 1998, Wehr and Thorp 1997). Reservoirs are important for understanding P cycling and algal dynamics within and downstream of reservoirs because the contribution of suspended chlorophyll-*a* to particulate organic matter and nutrients in rivers varies from small amounts to a significant proportion

(Bukaveckas et al. 2011, Houser et al. 2010, Dolph et al. 2017). The role of suspended algal production in nutrient uptake in rivers is known to be important (Hall et al. 2016, Reisinger et al. 2015). Short water retention times prevent algal production and reduces nutrient uptake (Smith 2016, Ameziane et al. 2003).

Downstream of dams, the abundance of suspended algae often diminishes; loss mechanisms such as deposition, filter feeders and macrophytes are thought to be important (Strayer et al. 2008, see also Monaghan et al. 2001 for smaller streams). A rich literature exists on the ability of filter-feeders to reduce chlorophyll-*a* concentrations, and this has been studied extensively in the case of invasive zebra mussels and their ability to transform food webs (Alpine and Cloern 1992, Caraco et al. 1997, Descy et al. 2003, Descy 2012, Strayer et al. 2004). As reservoir-derived phytoplankton decreases, inorganic nutrients such as SRP can increase (Welker and Walz 1998). Documentation of chlorophyll-*a* and nutrient patterns downstream of a run-of-river reservoir under a range of discharges can help improve our understanding of production of suspended algae and its downstream fate.

The Kalamazoo River of southwest Michigan has multiple impoundments, like many rivers in the Midwest U.S., with several shallow run-of-river reservoirs along the main channel. Chlorophyll-*a* has been observed to decrease in river waters downstream of Morrow Lake (Reid and Hamilton 2007). Algal production in medium sized rivers such as the Kalamazoo River has not been well studied. Medium-sized rivers are transitional between the better studied small streams and large rivers. Generally, the amount of phytoplankton biomass carried in medium sized rivers is unknown, and such rivers can be shallow and transparent enough to have high benthic algal production. This work examines how a run-of-river impoundment on the Kalamazoo River which is characterized by a short water residence times relative to other

impoundments affects the concentrations of nutrients and algae within the reservoir, and how these effects propagate downstream of the reservoir. Data were collected over various discharges to examine patterns of chlorophyll-*a*, nutrients, total suspended solids, and water clarity.

METHODS

Study site

The Kalamazoo River is located in Southwest Michigan (Figure 1). The watershed area is 5230 km² with a mean discharge of 38 m³/s. The entire river runs 260 km. The study area in the Kalamazoo River has side channels, backwaters and floodplains, some of which have been lost in the vicinity of the urban areas of Battle Creek and Kalamazoo (Rachol et al. 2005). The two largest reservoirs are Morrow Lake and Lake Allegan. Morrow Lake is on average 1.6 m deep and has a water residence time ranging from 2-7 days. Lake Allegan, about 69 km downstream of Morrow Lake, is a larger reservoir, on average 3 m deep, and has a longer water residence time of 3-11 days (Reid and Hamilton 2007). Five smaller dams were built between Lake Allegan and Morrow Lake around the turn of the century. These dams were decommissioned and partially removed, leaving a section of river that is semi-impounded. These reservoirs have short water retention times of less than one day each (Reid and Hamilton 2007). A dam downstream of Plainwell was removed in 2009, another one downstream of Otsego is in the process of removal at the time of this writing, and several others are failing and slated to be removed.

Sample collection

Our approach was to conduct frequent longitudinal sampling under periods of stable flow between storms. Sampling baseflow conditions became challenging because of unexpected and

frequent storms beginning in 2008 and becoming more frequent in 2011. The year 2012 had a particularly warm spring and almost no large rain events, and discharge was more stable. In the summer of 2008, samples were collected nearly biweekly. In 2009, samples were collected monthly. In 2010, water samples were collected over a course of one month, and then monthly. Sampling was monthly in 2011 from May to October. In 2012, water samples were only taken in the first weeks of July and October.

I used a semi-Lagrangian approach to sample chlorophyll-*a* and nutrients along a longitudinal transect during periods of a few days of relatively stable flow. I assumed that the samples across the transect reflect changes experienced by a parcel of water moving through the system (Doyle and Ensign 2009). Lagrangian sampling can identify hotspots or provide insights into the spatial distribution of ecosystem processing (Hensley et al. 2014, Volkmar et al. 2014, Zhang et al. 2013, Houser et al. 2010). Lagrangian sampling designs have also been employed in flow path studies through the hyporheic zone to reveal nutrient transformations (Briggs et al. 2014, Zarnetske et al. 2011, O'Brien et al. 2012). Lagrangian approaches have been used to understand the role of filter feeders in reducing phytoplankton in rivers (Welker and Walz 1998, Basu and Pick 1997, Scherwass et al. 2010). Studies using a semi-Lagrangian approach have been performed to track changes in nutrients and suspended chlorophyll-*a* along a longitudinal transect over time and help to understand the role of dams in affecting river water quality (Oliver et al. 2014, Zhang et al. 2013). Baker et al. (2014) used this approach to understand nutrient dynamics during storm events.

The river was sampled on average every 6 km, with up to three sites upstream of Morrow Lake and twelve downstream, between Morrow Lake and Lake Allegan. Samples were collected by section, so sites above the reservoir, downstream of it, or in the semi-impounded reaches were

collected on the same day. I sampled when flows were stable and avoided high runoff events following storms, except in June 2008. Sampling occurred on the rising limb of the flood pulse in early June 2008. I did not calculate the transit time of water flow along the river because detailed geomorphology was not available, and therefore I was not necessarily following the same parcel of water. By sampling during periods of stable flow, I may be able to obtain an “average” of river processes.

As described in Chapter 3, a major oil spill occurred in late July 2010, followed by aggressive clean-up measures in the river system at and above Morrow Lake. I did not include any nutrient measurements from after July 2010 to October 2010 in this chapter because this was soon after the oil spill. Nutrient concentrations were different in samples collected in the days after the spill, but nutrient patterns appeared to quickly become comparable to previous measurements (Chapter 3). Data from May and early July 2011 were included while clean-up of an oil spill was ongoing because water chemistry appeared comparable to previous years. Intense river clean-up was particularly evident in the August 2011 total suspended solids data (Bejarano et al. 2012, Chapter 3). Additionally, the summer of 2011 was distinctive in that frequent storms decreased water residence times relative to average. The river was at a high flow period most of the summer, which likely reduced reservoir water residence times, and thus concentrations of chlorophyll-*a*. Data from 2012 suggest that nutrient and chlorophyll concentrations in the river had mostly recovered from the 2010 oil spill.

Hydrochemical measurements

Water samples were collected using a dip sampler (2008-2009) and filtered in the field (2010-2012) or put on ice and filtered the day of collection. Samples were filtered through a 0.45 micron Supor filter. Total suspended solids (TSS) concentrations were measured on pre-weighed

filters; samples were dried at 60 °C and reweighed within one month. Ammonium was measured on filtered water using the phenylhypochlorite method (Aminot et al. 1997). Nitrate (filtered water) was measured by membrane suppression ion chromatography. Soluble reactive P (SRP; filtered water) was analyzed using the acid molybdate method (Wetzel and Likens 2000). Total phosphorus (unfiltered water) and total dissolved phosphorus (filtered water) was determined by persulfate digestion (Valderrama 1981, Langner and Hendrix 1982) followed by SRP analysis. Samples were run analyzed five days of collection except for total P. Particulate P was calculated as the difference between total P and total dissolved P. Soluble organic P was calculated as the difference between SRP and total dissolved P.

Chlorophyll *a* was measured on particulate matter collected by filtration onto an ashed A/E glass fiber filter (450°C for at least one hour) that was frozen until analysis. Chlorophyll-*a* analysis was performed by cutting a subsample of the filter and extracting pigments in 95% ethanol for 24 hours at 4°C (Welschmeyer 1994). Storage tests suggested that there were not significant reductions in chlorophyll-*a* for up to 3 months. Samples were analyzed within one month except for 2010 and 2011, when samples were measured after one year of frozen storage. Storage tests suggest that chlorophyll *a* may have been reduced by 50% after six months and remained stable for two years. The reduction is possibly due to automatic defrosting of freezers (Chapter 2, Appendix 1). Suspended chlorophyll-*a* measurements from 2010 and 2011 could be reduced by up to 50%, but also were comparable to measurements made in 2004, 2008 and 2009, however, so whether such large changes really occurred is uncertain. No corrections were made to 2010 or 2011 to suspended chlorophyll-*a*.

Data analysis

Each site downstream of Morrow Lake was compared using paired t-tests to compare the upstream reference site to each site downstream after grouping measurements on all dates for a particular site (Orr et al. 2008). My upstream reference site was at the Fort Custer State Recreation Area, which was the second site upstream of Morrow Lake. Note that this site is different from nearby sites sampled by Reid and Hamilton (2007) and Baas (2009). There are slight differences between the Fort Custer Recreation Area and Galesburg, MI sampling sites in water chemistry variables (Chlorophyll-*a*, TSS, DIN and P fractions), but they were not statistically significant differences for any of these variables. Paired t-tests were also conducted to compare water quality variables between a high (2010 or 2009) vs. low flow summer (2012). Lastly, seasonal variation in hydrochemistry was compared between July 2012 and October 2012 and July 2009 and October 2009.

I acknowledge that groundwater inputs are important across my longitudinal transect: discharge data from days with hydrochemical measurements for the Battle Creek (4105500) (mean 19.8 m³/s) and Comstock (4106000) (mean 28.5 m³/s) gauging stations show the river always gains discharge in that reach (DF=20, -4.59, $p < 0.05$). While not necessarily an indicator of groundwater inputs, conductivity decreases downstream of Morrow Lake compared to the upstream site.

RESULTS

Discharge and residence time

Discharge of the Kalamazoo River is typical of rivers of the temperate Midwest U.S., tending to be lowest during the summer and fall, with the highest flows corresponding with

spring rains and snow melt (Figure 1.2, Table 1.1). River discharge in 2008, 2009, 2010 and 2011 was higher than the annual average discharge. 2003 had the lowest annual average discharge in this study. The annual discharge in 2007 was overall higher than average, while overall discharge in the 2012 summer was close to average.

High summer flows occurred in 2008-2011 as a result of frequent strong storms (Figure 1.2). Summer discharge in 2004, 2008 and 2009 were statistically above average compared to the long-term summer mean. Discharge in the summers of 2003, 2007 and 2012 were years with summer daily discharge below the mean. In 2008, early May discharge was close to average ($30 \text{ m}^3/\text{s}$) but increased due to a storm in early June and July (to $56 \text{ m}^3/\text{s}$). Discharge decreased to $13.5 \text{ m}^3/\text{s}$ by August 2009, which was comparable to the lowest discharges in the summers of 2003 and 2012. Discharge in 2009 was above $50 \text{ m}^3/\text{s}$ through June 2009, but decreased to $17 \text{ m}^3/\text{s}$ in late July followed by an early August storm that increased discharge again. Summer discharge in 2010 and 2011 was higher than the long-term mean because heavy rains occurred through the summer. Discharge was particularly high (above $50 \text{ m}^3/\text{s}$) through spring into early July in 2010. Discharge decreased to $20 \text{ m}^3/\text{s}$ through July until a large flood occurred at the end of July, and discharge increased to $90 \text{ m}^3/\text{s}$. The summer of 2012 was distinct because discharge was $30 \text{ m}^3/\text{s}$ by early May, decreased to $20 \text{ m}^3/\text{s}$ by the end of May, and decreased to $8.5 \text{ m}^3/\text{s}$ in July, which was nearly half of the 2010 discharge during the summer.

Water residence times in Morrow Lake varied inversely with discharge since the reservoir volume is approximately constant. Water years in which discharge was above average had a mean water residence time of ~ 3 days for both annual and summer means (Table 1.1). Summers with particularly high discharges (2010 and 2011) had water residence times below 3 days, though water residence time increased to up to 5 days by the end of July 2011. Years with

relatively low summer discharges had water residence times ~5 days. Low flow summers had water residence times averaging ~5 days (2007) to ~8 days in July (2003 and 2012). The summer 2012 had the lowest discharge with water residence times of over 6 days. The average annual water residence time at Morrow Lake was ~3-4 days for years with average to above average annual discharge years (2004-2012) and 5 days for the low-discharge year 2003.

Algal production and loss along a longitudinal transect

Chlorophyll-*a* had distinct longitudinal patterns along the river (Figures 1.3 and 1.4). Chlorophyll-*a* concentrations were similar above the Morrow Lake reservoir among years. Above the reservoir, chlorophyll-*a* was usually ~3 µg/L even under different discharges (Figure 1.5). Suspended chlorophyll-*a* usually increased markedly at the first sampling site downstream of the Morrow Lake reservoir, often by 15-25 µg /L, but this varied depending on the water residence time in the reservoir (Figure 1.4, Table 1.1). The chlorophyll-*a* increase, from ~3 to ~20 µg /L, started in early May and peaked around late July through August (Figure 1.3). By the most downstream sampling site, chlorophyll-*a* never returned to concentrations comparable to above Morrow Lake in summers with above average discharge (Figure 1.4). Chlorophyll-*a* did not decrease in late July even when discharge decreased to that observed in low flow summers (2008, 2009). In summers with below average discharge, chlorophyll-*a* concentrations decreased by 16 km downstream of the dam to concentrations as low as or lower than those above the reservoir (Figure 1.4). Further downstream, algal concentrations were low, though they tended to become higher in the semi-impounded reaches in 2003. Under high flow periods or years (2004, 2008-10), when chlorophyll-*a* did not decrease markedly downstream of Morrow Lake, chlorophyll-*a* remained high through semi-impounded reaches. However statistically, chlorophyll-*a* was only greater to 30 km downstream.

Suspended chlorophyll-*a* patterns in June and July 2009 were comparable to chlorophyll-*a* patterns observed in the same months in 2008. Chlorophyll-*a* remained high downstream of Morrow Lake and through the semi-impounded reaches (Figure 1.5). The summer 2009 discharge was similar to 2008 with a large rain event in early August. By the end of August, chlorophyll-*a* in the river was low with little increase in algae as water moved through Morrow Lake, unlike what was observed in August 2008. Chlorophyll-*a* concentrations in July 2010 were similar to those sampled in July 2008 and 2009 (Figure 1.5). Monthly measurements of chlorophyll-*a* in 2011 were never as high as 2008, 2009 or 2010, likely because water residence times were particularly short (Table 1.1). However, considering that chlorophyll-*a* in samples was measured after one year in freezer storage, it is possible that chlorophyll-*a* was degraded and hence measured concentrations are likely lower than expected (Chapter 2). Additionally, submerged oil was still present in Morrow Lake and sediment disturbance associated with oil recovery may have had an impact on chlorophyll-*a* (Chapter 3). Concentrations in July 2012 were comparable to measurements made in 2003 and 2007. Chlorophyll-*a* increased to 30 µg/L and decreased to ~5 µg/L by 16 km downstream. Chlorophyll-*a* concentrations remained low downstream to a distance of 60 km.

Suspended chlorophyll-*a* concentrations in all years combined were statistically greater below Morrow Lake until a distance of 30 km downstream (Figure 1.5, Table 1.2). Downstream of Morrow Lake chlorophyll-*a* concentrations usually diminished markedly. Chlorophyll-*a* was statistically higher in 2009 (a high discharge year) than in July 2012 (a low discharge year). Chlorophyll-*a* remained higher below Morrow through early October in 2012. This was not the case at the end of September 2009, where chlorophyll *a* was low and 1 µg /L through the river (Figure 1.5, Table 1.2).

Phosphorus concentrations

Total phosphorus (TP) concentrations along the river averaged 49 $\mu\text{g/L}$ with all dates and sites combined (Figure 1.6). TP was higher in the lower discharge years of 2012 and 2003 compared to higher discharge years (Figure 1.6, Table 1.2). Early in the season, TP was 50-60 $\mu\text{g/L}$ with occasional TP measurements of ~ 100 $\mu\text{g/L}$. Overall, TP appeared to be decreasing in concentration in the river system from 2003-12, with mean concentrations declining from ~ 80 to ~ 50 $\mu\text{g/L}$. Compared to the reference site above Morrow Lake, differences in TP were not statistically significant immediately downstream of the reservoir, but were greater between 8 to 30 km downstream, although not further downstream in the semi-impounded reaches. The City of Kalamazoo's wastewater effluent enters the river at 6 km downstream of the dam and could augment TP concentrations in the river.

Even though TP increased along the river, TDP and its components (soluble reactive and dissolved organic P) did not statistically increase compared to above Morrow in the downstream reaches (Figure 1.7, Table 1.2). These fractions of P had high variance. However, there was a distinct longitudinal pattern that was related to high and low discharge summers (Figure 1.7, Table 1.2). TDP concentrations were higher in July 2012 (below average discharge) than summer 2009 (above average discharge). During the summers of 2010 and 2009, TDP was less than 50% of TP, while TDP was a large percentage of TP in the summer of 2012. By the end of September, TDP was up to 75% of TP for both 2012 and 2009, and TDP was higher in July 2012 compared to October 2012.

Like TDP, SRP did not significantly differ above and downstream of Morrow Lake (Figure 1.7, Table 1.2). Instead seasonal patterns were important. SRP comprised much of the TDP, especially in Summer 2012 where there was more SRP in July 2012 than the summer of

2009. SRP in the summer of 2012 was almost two times higher than 2010, but I have no measurements of SRP in 2009. In 2012, SRP composed almost all of TDP and composed almost 75% of TP in both July and October while SRP was less than 25% of TDP in 2010 and 2009. SRP composed a large percentage of TDP and TP in early October of all years measured. I have fewer measurements of dissolved soluble organic P (DOP), but the fraction of TDP that was organic was small. DOP was 5- 10 $\mu\text{g/L}$ and did not statistically increase or decrease along the river. DOP composed 20-50% of TDP, and SRP composed a greater proportion of TDP than DOP. DOP only composed a large proportion of TDP in late fall of 2009. Statistically DOP was higher in summer 2012 than 2010, but the difference was only a few $\mu\text{g/L}$, DOP did not differ between July and Oct 2012. Among the P fractions, DOP seemed least related to discharge in summer.

Particulate-P (PP) was $\sim 20 \mu\text{g/L}$ averaging all data. While PP appeared to increase downstream of Morrow Lake, there were no significant differences between above and below Morrow Lake (Figure 1.7, Table 1.2). Sites at 4 km and 16 km were different, but at 30 km, PP was not different from the reference site. More importantly, PP concentrations were higher in the summer of 2009 and 2010 than 2012. The difference in PP was smaller than the difference in TDP between a high (2009) and low (2012) discharge summer. PP was only $10 \mu\text{g/L}$ greater in 2009 compared to July 2012. PP composed a small proportion of TP by early October in both high and low flow years. There were larger differences between summer and fall PP concentrations. PP was $20 \mu\text{g/L}$ in July compared to $5 \mu\text{g/L}$ in early October.

Phosphorus patterns appeared to follow chlorophyll-*a* patterns, and these patterns were related to the production and loss of algae. Chlorophyll *a* and SRP followed a distinct pattern, and the fraction of TP that was SRP appeared to be related to discharge. TP, TDP and PP

concentrations were approximately constant with distance along the river when all years are averaged. However, higher- and lower-discharge summers showed clearer patterns along the river. Summers with low discharge had lower chlorophyll-*a* and slightly lower PP, but higher SRP and TDP. In fall, there was less PP and more SRP or DOP. In 2012, TDP increased as chlorophyll-*a* decreased, and TDP followed the chlorophyll-*a* loss pattern. By the end of September and into October, suspended chlorophyll-*a* decreased and TDP increased, marking the end of the algal growing season.

Nitrogen and suspended sediments

Nitrate concentrations show clear longitudinal patterns (Figure 1.8, Table 1.2). Nitrate-N concentrations were on average ~1 mg/L upstream of the reservoir, and decreased to ~0.8 mg/L downstream, and reached as low as 0.4 mg/L during a period of low discharge in 2012. Nitrate always decreased as water passed through the Morrow Lake reservoir (Figure 1.8, Table 1.2). Nitrate reached the lowest concentrations immediately downstream of Morrow Lake, and returned to concentrations comparable to the upstream site by 30 km downstream. Nitrate decreased along the river and this was observed in all seasons where measured and was lowest by the end of July and August. Nitrate was significantly lower in July 2012 (Table 1.2), and nitrate was not reduced in October 2012. Through the semi-impounded reaches, nitrate did not increase or decrease, and concentrations were comparable to upstream.

In contrast to nitrate, ammonium concentrations did not decrease downstream of Morrow, and concentrations were variable along the river. Ammonium appeared to increase downstream of the reservoir during late summer in mid-August 2009, July 2011 and July 2012, and then appeared to return to concentrations comparable to above Morrow Lake by 30 km downstream. However, despite these apparent patterns, ammonium concentrations at the downstream sites

were not statistically different from the upstream reference site for any site downstream.

Ammonium did not change through the semi-impounded reaches (Figure 1.9, Table 1.2).

Ammonium concentrations were higher in July 2012 than 2009, and ammonium was higher in July 2012 than October 2012.

Total suspended solids (TSS) concentrations were usually < 20 mg/L (Figure 1.10, Table 1.2). TSS ranged in each site from 2-15 mg/L. Changes in TSS along the river were not statistically significant overall even though TSS sometimes appeared to increase within the semi-impounded reaches. Total suspended solids were higher under high flow conditions in July 2009 than in July 2012. Both chlorophyll-*a* and TSS are positively correlated with particulate P (Figure 1.11); algal biomass may be a significant component of the TSS below Morrow Lake.

Transparency as measured by turbidity tube was typically > 80 cm (Table 1.2). Above the reservoir, turbidity tube measurements were often greater than 120 cm (the length of the tube). Downstream of the reservoir, turbidity tube transparency was reduced to ~ 90 cm (Table 1.3, Table 1.2). Transparency increased further downstream, then decreased in the semi-impounded reaches.

Concentrations of sodium, sulfate and chloride ranged from 30 to 40 mg/L. Calcium was higher and ranged from 70 to 80 mg/L (Table 1.3). Conductivity was higher in summer 2012 by $50 \mu\text{S}/\text{cm}$ (Table 1.2).

Dissolved oxygen and temperature

Dissolved oxygen in the river ranged both above and below atmospheric equilibrium (Figure 1.12, Table 1.2). The upstream reference site was often undersaturated, but oxygen concentrations increased significantly immediately downstream of the Morrow Lake reservoir,

often showing considerable supersaturation. Dissolved oxygen measurements also were above saturation in the sites at 16- and 30-km downstream, and many measurements were above saturation in the semi-impounded reaches. Dissolved oxygen was significantly greater than above Morrow Lake in almost all sites downstream of Morrow Lake. Temperature significantly increased by 1 °C downstream of Morrow to 16 km (Table 1.3); this extended downstream to 16 km, and the river was warmer in July 2012 than 2010 based on our temperature measurements.

DISCUSSION

The Morrow Lake reservoir on the Kalamazoo River is a run-of-river design with short water residence times that cause the conditions to be more lotic at higher discharges but more lentic at the lowest discharges. Low discharges tend to occur in the summer and fall but do not occur every year. The role of run-of-river reservoirs in altering suspended material in the river was variable in this study. While TP appeared to increase downstream of Morrow (Figure 1.6), the net P balance (i.e., retention vs. release) as water passes through the reservoir work did not support a consistent directional pattern. Longitudinal trends of TDP, PP, SRP, DOP, TSS, and ammonium showed high variance throughout the year (Figures 1.7, 1.9, 1.10). There was no significant difference for these nutrients between upstream and downstream of Morrow Lake when using a paired-t-test. Some nutrients such as phosphorus and its fractions appeared coupled with phytoplankton biomass (as measured by chlorophyll-*a*) from Morrow Lake, while other nutrients such as ammonium did not. At longer water residence times during below average discharge summers, I observed the greatest increases in chlorophyll-*a* and nutrient changes that were propagated for a limited distance downstream. TP and conductivity increased along the river downstream of 8 km, which was observed previously (Reid and Hamilton 2007 and Baas

2009). Only nitrate showed a consistent longitudinal pattern regardless of flow (Figure 1.8), where it decreased below the reservoir and then increased downstream, and its loss and regain will be discussed (needs discussion).

Production of algae in Morrow Lake and effects on water quality

This study highlights that increased water residence times allow the production of algae in nutrient-rich reservoirs. Algal production from Morrow Lake may continue to develop even when nutrient levels decrease, delaying water quality improvements in downstream Lake Allegan downstream, as has been observed in other river systems (Bukaveckas and Crain 2002, Honti and Istvanovics 2010, Bowes et al. 2012). The observation that most samples above Morrow contained substantial concentrations of P in the bioavailable form of SRP could indicate that short water retention times were limiting production in the Kalamazoo River (see Smith 2016). Reservoirs, or in the case of the Kalamazoo River, multiple reservoirs in series, appear to be important sources of algae in suspension (Reid and Hamilton 2007). High nutrient concentrations on the Fox River in Illinois led to high chlorophyll-*a* in multiple reservoirs (sensu Santucci et al. 2005). As in my study, water residence time was particularly important in explaining variation in algal production rivers in addition to nutrients (Soballe and Kimmel 1987, Koch et al. 2004, Neal et al. 2006, Lucas et al. 1999, Sellers and Bukaveckas 2003, Ameziane et al. 2003), light limitations (Philips et al. 2000, Koch et al. 2004, Knowlton and Jones 2000), and temperature (Wehr and Thorp 1997, Knowlton and Jones 2000, De Jager and Houser 2012).

Reservoirs on rivers can also improve water quality downstream by reducing TP and TN concentrations (Powers et al. 2015, Oliver et al. 2014), though under certain conditions, reservoir sediments have also been observed to release P (Powers et al. 2015, Harrison et al. 2009, Baas et al. 2009, Zhang et al. 2013). Sediment P release from Morrow Lake has been ascertained but is

variable from year to year (Reid and Hamilton 2007, Baas 2009). A natural lake on the Upper Mississippi River trapped P and suspended solids, though sediments could also be a source of SRP leading to eutrophication (Engstrom et al. 2009, Houser et al. 2010, James and Barko 2004). The Conowingo Dam on the Susquehanna River traps sediment and P, although its storage capacity is decreasing more quickly than anticipated and high flow events have released sediments and associated P (Zhang et al. 2013).

Nitrogen transformations can also be important in reservoirs. This study found that nitrate decreased during the growing season in Morrow Lake, which like other reservoirs could be important for N reduction and denitrification in the river system (Wall et al. 2005, Finlay et al. 2013). A longitudinal study of nutrients on the Klamath River found that dams reduced nitrate (Oliver et al. 2014). Thus, the role of reservoirs on nutrient transport and algae production in rivers is variable and not necessarily predictable. The Kalamazoo River highlights that reservoirs in series are very common and have important implications for nutrient transport where chlorophyll-*a* remained high (Santucci et al. 2005, Zhang et al. 2013, Oliver et al. 2014).

The downstream fate of chlorophyll and nutrients

Year to year variation of discharge and water residence time was important for the effects of Morrow Lake on nutrients, which appear related to algal growth and also the downstream fate of algae from Morrow Lake. When summer discharge was average (2008) to above average (2004, 2009, 2010, 2009), water residence time was low, and suspended chlorophyll-*a* concentrations did not peak as high downstream of Morrow. In these high discharge summers, suspended chlorophyll-*a*, TSS and PP did not decrease back to concentrations comparable to above Morrow even when discharge decreased in late July.

Sediment resuspension events could partly explain the high chlorophyll-*a* downstream of Morrow Lake. Suspended algae may originate from scoured benthic algae during high discharge events in addition to the growth of phytoplanktonic algae (Istvanovics and Honti 2011, Paul and Hall 1996, Newbold et al. 2005, Knowlton and Jones 2000). Summers with below average discharge (2003, 2007 and 2012) had higher chlorophyll-*a* peaks downstream but chlorophyll-*a* decreased to resemble upstream concentrations by 16 km, as was also observed by Reid and Hamilton (2007). TP concentrations were particularly low in 2012, which may reflect the lack of resuspension events that summer.

SRP composed a large percentage of TP upstream of Morrow while PP was a greater percentage downstream of Morrow. SRP increased as chlorophyll-*a* decreased in a low flow summer (Figure 1.7). Like our study, SRP increased longitudinally when chlorophyll-*a* decreased downstream from the lake in the River Spree (Welker and Waltz 1998). Like this study, SRP was a large component of TP as has been found in other river studies (James and Larson 2008, Baker et al. 2014a, 2014b). Chlorophyll-*a* can increase during low flow periods, drawing down SRP when chlorophyll-*a* was highest (James and Larson 2008). This indicates the importance of suspended algae in P uptake (Welker and Waltz 1998, Basu and Pick 1997, James and Larson 2008). Chlorophyll-*a*, PP and TSS are related, suggesting that algal biomass was an important contributor to both PP and TSS in the Kalamazoo River at times of high chlorophyll concentrations.

However, not all rivers show such strong patterns between SRP and chlorophyll-*a*. Chlorophyll-*a* in rivers exhibits a large range and high chlorophyll-*a* may reflect input from locations upstream (Knowlton and Jones 2000) or chlorophyll-*a* could peak at more intermediate distances (Basu and Pick, 1997, Bukaveckas et al. 2011, Houser et al. 2010, Minaudo et al. 2015,

Oliver et al. 2014, Dolph et al. 2017). Above Morrow Lake, chlorophyll-*a* did not exhibit large increases during high or low flows highlighting the complex patterns of suspended algae in rivers. Increased water residence time by Morrow Lake was important for increasing chlorophyll-*a* in the Kalamazoo River.

The differences in PP and TSS between high and low discharge summers was small, and there was no statistical difference between above and below Morrow Lake for PP and TSS. This suggests that riverine particulate P was composed of both inorganic and organic fractions (Russell et al. 2001, James and Larson 2008, Withers and Jarvie 2008, Findlay et al. 1996, Howarth et al. 1996, Knowlton and Jones 2000). Suspended solids collected from the Kalamazoo River were primarily in the silt size range (Fitzpatrick et al. 2015), and the river contains a high amount of fine particulate organic matter (Cummins et al. 1981). Algae in FPOM vary with the contribution of terrestrial organic matter, and macrophytes, as inferred from natural abundances of stable carbon isotopes (Kendall et al. 2001, Angradi 1993, Bianchi et al. 2007, Wissel et al. 2005, Cloern et al. 2002). The contribution of suspended algae and phytoplankton to particulate organic matter varies from a small percentage to 50%, in larger rivers (Bukaveckas et al. 2011, Admiraal et al. 1992, Knowlton and Jones 2000, Sellers and Bukaveckas 2003, Neal et al. 2006, James and Larson 2008, Dolph et al. 2017, Houser et al. 2010). While PP can be bound in algal biomass, PP in rivers can also be bound with minerals like Fe (James and Larson 2008, Baker et al. 2014a, 2014b). The other components of PP besides algae in the Kalamazoo River are not known, but the contribution of algae to suspended material likely increased downstream of Morrow. These observations are also supported by Bass (2009), who used mixed-models to identify periods when TP transport in the Kalamazoo River was either under hydrologic or biological control; biological control was considered to include sediment P release.

Longitudinal patterns of nutrients along the Kalamazoo River

Summers with low discharge had the lowest nitrate, slightly higher TP, and higher TDP, SRP and ammonium concentrations. Nitrate concentrations were reduced by nearly half in the lowest flow summer. While nitrate decreased, SRP and ammonium were surprisingly not more reduced by the reservoir nor downstream in the river. While SRP longitudinal patterns could possibly be explained by chlorophyll-*a* patterns, ammonium cannot. Rivers are known to be important for nutrient uptake, and uptake lengths of nutrients in river could be longer for nitrate than ammonium (Tank et al. 2008, Hall et al. 2013, Ensign and Doyle 2006, Dodds et al. 2008) and SRP (Hall et al. 2013, Ensign and Doyle 2006). Lagrangian sampling with higher frequencies than this study have found sections of river with possible “hotspots” for nitrate uptake and denitrification (Powers et al. 2012, Hensley et al. 2014). Observed decreases in nitrate could possibly be explained by denitrification along the river. The Kalamazoo River has abundant side channels and slackwater habitats, especially above Morrow Lake that could have low or no nitrate (Hamilton unpublished data). Side channels and floodplains could reduce flow and be sites for denitrification (Welti et al. 2012, Pfeiffer et al. 2006, Gergel et al. 2005, Forshay and Stanley 2005, Houser et al. 2010, Knowlton and Jones 1997). However, sediment release in backwater habitats could be a source for SRP (Schonbrunner et al. 2012, Houser 2016, De Jager and Houser 2012, Pongruktham and Ochs 2015). A flow-through wetland experiment found that nitrate was reduced, but ammonium was released by decomposition (O’Brien et al. 2012). This might explain our higher than expected ammonium concentrations along the river, and the increases in nitrate downstream of Morrow Lake.

A second source for nutrients is from the discharge of treated wastewater from the City of Kalamazoo wastewater treatment facility (WWTF), which enters the river at about 6 km

downstream of Morrow Lake. The 4 km site is upstream of the wastewater treatment facility of the city of Kalamazoo, and the 8 km site is below the Kalamazoo WWTF, with additional facilities downstream (Reid and Hamilton 2007). In our study, the impact of the WWTF was evident in the lowest flows in 2012, as other studies have found (Bowes and et al. 2003, James and Larson 2008). I only observed large increase of SRP and NH_4^+ between these two sites in October 2012 when discharge was lowest. The effect of the WWTF on TP loading in the river has been determined since 2012 based on monitoring of the discharge; data compiled on kalamazooriver.net (maintained by Keiser & Associates) indicate 9,282 lbs per year. TP were discharged over Apr-Sep 2015. During that same period, the outflow from Morrow Lake was monitored more frequently by Keiser & Associates, who estimated a TP load above the WWTF of 91,306 lbs. TP. Thus, on average the WWTF only augments the river TP load by about 10% during Apr-Sep. Nonetheless, it is possible that short-term variation in effluent discharge produces pulses of greater importance. The effect of the WWTF effluent on other variables I measured is unknown, and nitrate, ammonium and SRP sources could be from sediments or WWTFs (Russell et al. 1998, Bowes et al. 2003). Currently, my study found the nitrate reduction downstream of Morrow returned to concentrations comparable to Morrow by 16 km. Our semi-Lagrangian approach cannot determine if denitrification was important in Morrow Lake (Wall et al. 2005), and then new nitrate, ammonium and SRP sources were important further downstream. Alternatively, algal uptake and release was important for observed patterns in nitrate (sensu Finlay et al. 2013). Algal growth in Morrow Lake appears important for driving much of the longitudinal patterns in the Kalamazoo River, but nutrient release from sediments and WWTF are likely contributing to the patterns.

Possible mechanisms of downstream losses

River size appears to be important for the distance that suspended algae travel downstream. In this study, chlorophyll-*a* concentrations were elevated for 16 km (below average discharge) or beyond the transect (above average discharge) (Figure 1.4). In small streams, plankton quickly disappears downstream of lake outfalls but may travel kilometers downstream in rivers with greater discharge (Table 1.4, Vadeboncoeur 1994, Monaghan et al. 2001, Palmer and O’Keefe 1990). Rivers the size of the Kalamazoo River show comparable results where chlorophyll-*a* from reservoirs could persist 20 km downstream (River Spree-Welker and Walz 1998). Lagrangian sampling of the largest US rivers (Mississippi River, Missouri River and Ohio River) found chlorophyll-*a* was high and did not decrease along the river (Bukaveckas et al. 2011). Reservoirs often occur in series (Palmer and O’Keefe 1990, Zhang et al. 2013, Oliver et al. 2014, Santucci et al. 2005), and multiple reservoirs that occur in series likely have an important role in river water quality increasing algal blooms by maintaining phytoplankton in the intervening reaches like I observed in the Kalamazoo River.

Discharge was important for explaining patterns downstream of Morrow Lake. Downstream losses are thought to be explained either by grazing or sedimentation (Reynolds and Descy 1996, Kohler et al. 2002, Admiraal et al. 1992). Discharge has been shown to be particularly important for determining how filter feeding grazers impact plankton (Descy et al. 2012, Lucas and Thompson 2012, Strayer et al. 2008). The Kalamazoo River has hydrogeomorphic characteristics where it the river gains shallow bathymetry and decreased flow becomes wide and shallow (Rachol et al. 2005). Sedimentation could increase in these areas as increased residence times, shallow depth and bed structure could be important for explaining variation of the downstream losses of algae and the fate of nutrients (Doyle et al. 2003, Powers et

al. 2013, Hensley et al. 2015, Hein et al. 2005, Kohler et al. 2002). A thick mat of macrophytes (*Potamogeton* spp.) grew in the Kalamazoo River between 8 and 16 km, which likely increased sedimentation of the algae exported from Morrow Lake (sensu Chandler 1937, Hamilton et al. 1990).

Grazers were also likely important for explaining downstream losses of reservoir derived algae. Hydropsychidae were very abundant on artificial substrata (Chapter 2). However, Monaghan et al. (2001) was able to remove filter feeders from a river reach showing such a decline, and their study concluded that other factors besides filter feeders were important for losses. Additionally, Benke (2015) and Rosi-Marshall and Wallace (2002) did not believe caddisflies (filter-feeders) could account for a large proportion of seston loss, though filter feeders are important for the transformation of seston (Hershey et al. 1996). I was not able to quantify downstream losses by *Corbicula fluminea* even though those exotic mussels appeared to be abundant. A high discharge summer (2008) had high chlorophyll-*a* downstream of Morrow. Several high discharge events could reduce the ability of filter feeders to consume algae. This was preceded by a large *Corbicula* die off which would have reduced grazing pressure (Hamilton *personal observation*, McDowell et al. 2016). Filter feeders are important for the transformation organic matter and nutrients (Atkinson et al. 2014), and such studies have explained chlorophyll-*a* standing stocks (Descy et al 2011, Minaudo et al.2015). The Kalamazoo River highlights complicated effects of high discharge events, which can resuspend benthic algae and reduce the impacts of filter feeding (Strayer et al. 2008).

The importance of benthic versus suspended algae in the Kalamazoo River

The algae in the Kalamazoo River has yet to be identified, but the high water retention times within Morrow Lake allowed for phytoplankton production, which impacted nutrients

(Neal et al. 2006, Smith 2016, Admiraal et al. 1992). SRP appeared to be impacted by suspended algae, but other nutrient and dissolved oxygen measurements from this study also showed a large range driven by a combination of suspended algae, benthic algae, and aquatic macrophytes that was unique to each site (see Hall et al. 2016). Short water retention time and advection could limit phytoplankton production in the Kalamazoo River (Cole et al. 1992, Reynolds and Descy 1996, Lewis 1988, Ameziane et al. 2003), but low flow habitats such as side channels and backwaters could allow for some phytoplankton production (sensu Rachol et al. 2005, Reynolds 2000, Cole et al. 1992, Dzialowski et al. 2013, Hein et al. 2005), and meroplankton or benthic algae could be important (Istvanovics and Honti 2011). Based on a few samples, suspended algae above Morrow Lake were identified as largely benthic in origin but were more phytoplanktonic downstream (Reid and Hamilton 2007). This is also reflected in dissolved oxygen, which was generally below saturation above Morrow Lake, although dissolved oxygen increased to above saturation in late August (Figure 1.12). Turbidity ranged between 60 and over 120 cm, which is likely greater than the average depth of the river. Light limitation could limit algal production in many rivers, and is particularly important in the largest rivers (Houser et al. 2010, Dodds et al. 2013, Knowlton and Jones 2000), but benthic algae were abundant along the river (Chapter 2).

The site immediately downstream of Morrow Lake was supersaturated with oxygen, even at dawn, presumably driven by phytoplankton production, though also could be wind driven. A thick layer of epipelagic algae grew in Morrow Lake by mid-summer, composed of *Oscillatoria* and various diatoms (personal observation). These mats could cause oxygen depletion and consequent sediment P release beneath them, even when the overlying waters were oxygenated (Kraal et al. 2013, Carlton and Wetzel 1988). Wind resuspension (Sondergaard et al. 1992,

Istvanovics et al. 2004) could dislodge and disaggregate these mats and become a source of chlorophyll and P (Baas 2009).

Epipellic algae could also be important in the semi-impounded reaches, where oxygen measurements were at or above saturation (Reid and Hamilton 2007, Figure 1.12). Oxygen suggest net algal production in this section of river (Figure 1.12). Patterns like Reid and Hamilton (2007) reported only appeared to occur in the semi-impounded reaches during below average summers. Even though chlorophyll-*a* remained high through these reaches, no nutrient changes were observed compared to upstream. The water residence times may be too short to produce substantial numbers of phytoplanktonic algae, but benthic algae could important in these reaches (Hamilton *personal observation*). This was also observed in a small estuary with low water residence times where nutrient uptake was observed to be in the benthos rather than in phytoplankton production (Tobias et al. 2003).

Oxygen at sites at 16 and 30 km was above saturation though this was likely driven by thick beds of macrophytes and associated filamentous algae (personal observation) (Figure 1.12). Phosphorus fractions in late October may be explained by the seasonal decomposition of benthic algae and macrophytes. Algal production from Morrow Lake continued into early October in 2012, and the pattern between SRP and chlorophyll-*a* continued. Based on my results, I hypothesize that suspended algae play a role in explaining the observed longitudinal patterns in P fractions, but benthic algae and macrophytes appear to explain larger seasonal patterns for nutrient uptake and release. The identification of the source of suspended algae is important in the Kalamazoo River as the semi-impounded dams are removed because suspended algae influence nutrient dynamics and river food webs (Chapter 2).

CONCLUSIONS

Water retention times within Morrow Lake reservoir affect suspended algae and nutrients, and the strength and downstream persistence are related to river discharge. Suspended algae (as indicated by chlorophyll-a) were important for explaining nutrient patterns, though clearly other processes were also important. It is possible that phytoplankton that is produced from Morrow Lake is transported all the way to Lake Allegan, however isotopic evidence from benthic consumers suggests that it is more complicated. Isotopic evidence for filter feeding caddisflies did not show consistent dependence on suspended algae downstream of Morrow during high flow summers (Chapter 2). The distance downstream of Morrow Lake that suspended algae were transported depended on discharge, and algae persisted further downstream at higher discharge.

This study examined Lagrangian processes at stable flow, but frequent storms were an important factor in the multiple years of study. Early in rising limbs of flows, material can be resuspended and stored nutrients increase following a storm (Baker et al. 2014, Bowes et al. 2014 and 2015, Jarvie et al. 2005, Hamilton et al. 2011). Higher discharges may increase scour and suspend bottom materials, but they also reduce water retention time, creating more lotic conditions in reservoirs. Lower discharges increase water retention time and create more lentic conditions for phytoplankton production, but also increase the opportunity for grazers to affect algal concentrations and associated nutrients in the water column. High-resolution longitudinal surveys using sensors (Istvanovics and Honti 2011, Bowes et al. 2015, Tinka et al. 2016) could be helpful to understand the complex patterns of production, suspension, and deposition of algae and associated nutrients. Also, more frequent Lagrangian surveys may capture changes (Hensley et al. 2014). Climate change will likely change patterns with more intense rainfalls and flooding

events (Pryor et al. 2014), and so more research is needed to understand the role of these large flow events on the Kalamazoo River.

The fate of the algae produced in the reservoir and the associated nutrients that? depends on hydrology. Phosphorus cycling appears to be more tightly likely linked with suspended algae than benthic algae, which were abundant throughout the river including in the reservoir. This work highlights that increased water retention times by dams can hamper nutrient reduction efforts by producing algae even when nutrients are reduced (Baker et al. 2014, Bowes et al. 2012, Smith 2015). Additionally, in many regulated rivers, dams reduce high flow events and increase low flow events (Carlisle et al. 2010). Using Lagrangian techniques, this work highlights the biogeochemical patterns occurring along the river and the role of Morrow Lake, a run-of-river reservoir with relatively short water residence times with a series of dams downstream. Dams on rivers are ubiquitous in developed regions of the world, and their roles in the production of algae and subsidies for downstream food webs is of broad interest.

APPENDIX

Table 1.1. Discharge (m^3/s) of the Kalamazoo River from the US Geological Survey gauging station at Comstock, MI (4106000) (First set of columns) and water residence time at Morrow Lake (second set of columns). Annual means calculated for each water year for both discharge and water residence time. Summer means were calculated from May 1st through September 30th. Long term means were calculated from the daily data from 1933-2007. Long term Mean Discharge: 26.20; Summer Mean: 22.48.

Year	Mean discharge (m^3/s)		Mean water residence time at Morrow Lake (days)	
	Annual	Summer	Annual	Summer
2003	18.87	17.55	5.22	5.75
2004	26.56	29.12	3.82	3.67
2007	29.46	19.66	3.63	5.03
2008	36.04	34.48	3.12	3.45
2009	39.81	33.01	2.59	3.04
2010	33.38	36.90	2.87	2.76
2011	32.62	36.60	3.22	2.89
2012	29.74	15.38	4.00	6.34

Table 1.2. Results of paired t-tests comparing sites downstream of Morrow Lake dam to the upstream reference site for water quality variables. Seasonal comparisons were paired t-tests comparing July 2012 and July 2009, or comparing July 2012 and October 2012.

Distance (km)	0	4	8	16	20	30	35	40	48	55	65
Chl-a (µg/L)	DF=16 t-stat - 7.0 p<0.05	NS	DF=7 t-stat - 2.54 p=0.05	DF=16 t-stat - 4.7 p<0.05	DF=5 t-stat - 1.2 p<0.05	DF=15 t-stat - 3.14 p>0.05	NS	NS	NS	NS	NS
TSS (mg/L)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
TP (µg/L)	NS	NS	DF=7 t-stat -2.70 p<0.05	DF=13 t-stat -4.02 p<0.05	DF=5 t-stat -2.22 p<0.07	DF=13 t-stat 4.80 p>0.05	NS	NS	NS	NS	NS
PP (µg/L)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
SRP (µg/L)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
DP (µg/L)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Org-DP (µg/L)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
N-NO ₃ (mg/L)	DF=9 t-stat 4.93 p<0.05	DF=2 t-stat 5.57 p<0.05	DF=3 t-stat 3.70 p<0.05	DF=3 t-stat 2.72 p<0.05	NS	NS	NS	NS	NS	NS	NS
N-NH ₄ (mg/L)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
DO (%)	DF=15 t-stat -6.1 p<0.05	DF=7 t-stat -2.5 p<0.05	NS	DF=15 t-stat -3.0 p<0.05	NS	DF=14 t-stat -3.8 p<0.05	NS	DF=6 t-stat -2.9 p<0.05	DF=7 t-stat -3.1 p<0.05	DF=7 t-stat -3.1 p<0.05	DF=7 t-stat -4.3 p<0.05
Cond (µS/cm)	DF=16 t-stat 3.8 p<0.05	NS	DF=7 t-stat -2.73 p<0.05	DF=16 t-stat -4.7 p<0.05	NS t-stat -2.6 p<0.05	NS	DF=4 t-stat -2.73 p<0.05	DF=6 t-stat -4.7 p<0.05	NS	NS	NS
Temp (°C)	DF=16 t-stat -3.1 p<0.05	DF=6 t-stat -4.9 p<0.05	NS	NS	NS	NS	NS	NS	NS	NS	NS
Turbidity (cm)	DF=9 t-stat 7.3 p<0.05	NS	NS	NS	DF=16 t-stat 3.4 p<0.05	NS	NS	NS	NS	NS	NS

Table 1.2. (cont'd)

Year	Chl-a (µg/L)	TSS (mg/L)	TP (µg/L)	DP (µg/L)	SRP (µg/L)	PP (µg/L)	Org-TDP (µg/L)	N-NO ₃ (mg/L)	N-NH ₄ (mg/L)	Temp (°C)
Jul. 2012	t-stat=-2.7	t-stat=-2.3	t-stat=3.5	t-stat=5.7	t-stat=3.1	t-stat = 2.6	t-stat=3.1	t-stat=-6.1	t-stat=5.0	t-stat=13.3
Vs. 2009	DF=13	DF=13	DF=13	DF=13	DF=13	DF=13	DF=13	DF=13	DF=13	DF=13
	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05
Season										
Jul.	t-stat= 1.6	NA	t-stat= 7.3	t-stat= 4.5	t-stat= 3.1	t-stat= -6.6	NS	t-stat= 8.5	t-stat= 4.9	t-stat= 32.8
Vs. Oct. 2012	DF=11		DF=11	DF=11	DF=11	DF=11		DF=11	DF=11	DF=11
	p<0.05		p<0.05	p<0.05	p<0.05	p<0.05		p<0.05	p<0.05	p<0.05

Table 1.3 Mean values of temperature, dissolved oxygen (DO; % saturation) transparency measured with a turbidity tube (Turbidity), selected major ions, and conductivity (Cond) in the Kalamazoo River. Sites are arranged by distance from Morrow Lake in km, with the upstream site being negative.

Distance (km)	-15	0	4	8	16	20	30	36	40	48	55	65
Temp (°C)	19.9	21.2	20.8	20.9	20.9	21.5	20.6	21.5	20.9	21.0	21.0	20.4
DO (%)	92.5	111.0	98.1	96.8	109.7	90.0	97.0	96.3	100.8	100.7	103.1	96.1
Turbidity (cm)	107.5	95.7	98.3	99.3	102.7	105.5	98.3	91.4	92.9	82.6	87.4	90.7
Cl ⁻ (mg/L)	46.2	41.5	37.7	43.6	49.4	46.8	48.4	35.8	43.8	43.3	36.6	40.7
SO ₄ ²⁻ (mg/L)	40.3	38.2	34.4	36.4	38.3	34.2	37.7	37.0	39.3	38.9	36.4	37.5
Ca ²⁺ (mg/L)	85.1	79.6	77.2	81.0	80.2	80.8	79.2	81.7	81.1	80.9	79.0	75.5
Na ⁺ (mg/L)	19.7	21.7	21.3	42.2	32.8	36.0	34.7	41.5	38.2	38.5	39.7	36.2
Cond (μS/cm)	620.0	597.0	602.8	645.0	664.5	650.5	661.0	654.5	649.1	641.1	634.0	626.3

Table 1.4 Distance or reservoir derived plankton travels before reducing at least 50%.

River name	River size	Length	Reference
Owl Creek lake outlet, Montana	0.34-2.2 m ³ /s	100 m	Vadeboncoeur 1994
Hiji River, Japan	4th order	2 km	Doi et al. 2008
River Spree, Germany	1 to 26 m ³ /s	20 km	Welker and Waltz 1998
Deep Creek, Idaho	2.7 to 4.4 L	20 m	Monaghan et al. 2001
Buffalo River, South Africa	0.05 m ³ /s	4-8 km	Palmer and O'Keefe 1990
Kalamazoo River, MI	26.2 m ³ /s	30 km	This Study

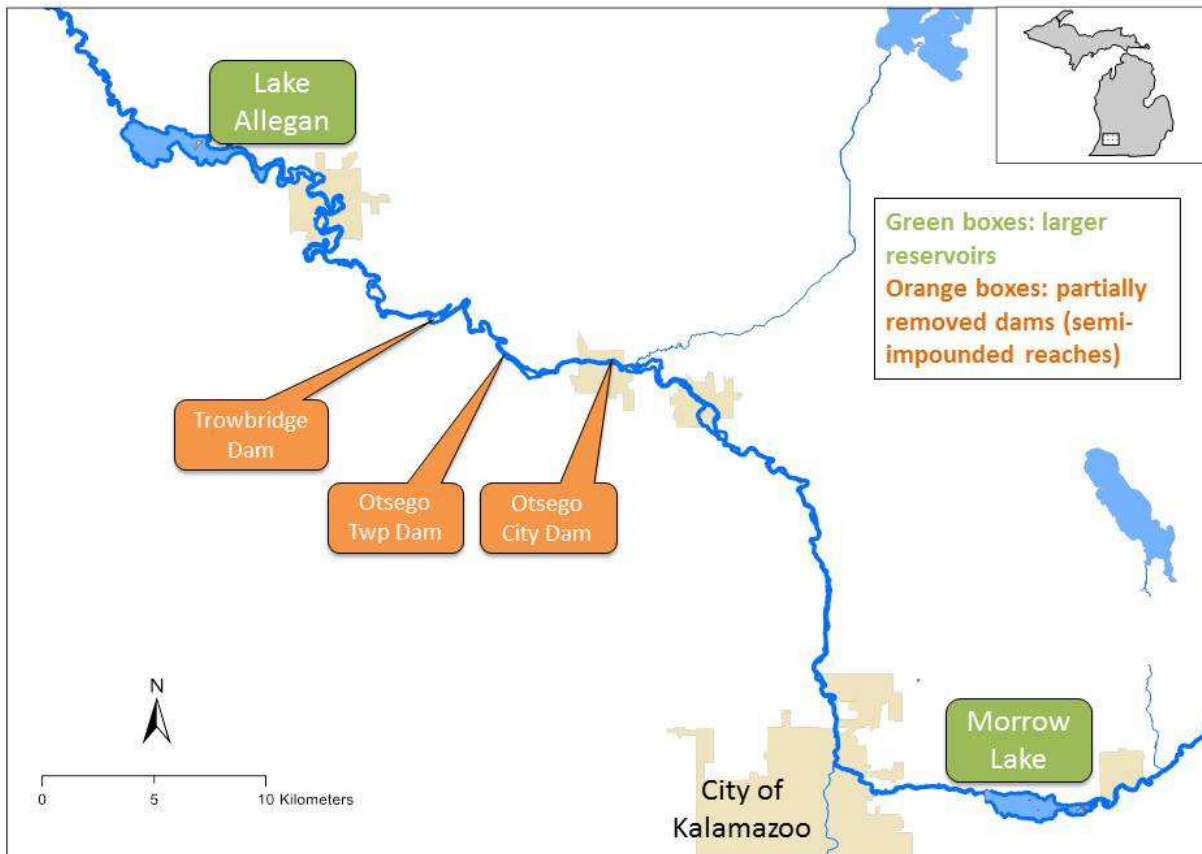


Figure 1.1 Study reach along the Kalamazoo River in southern Michigan. Morrow Lake and Lake Allegan are the two largest reservoirs on the river system. An additional small dam is just upstream of Lake Allegan in Allegan, MI. Orange squares represent partially removed dams in the reach referred to as “semi-impounded”.

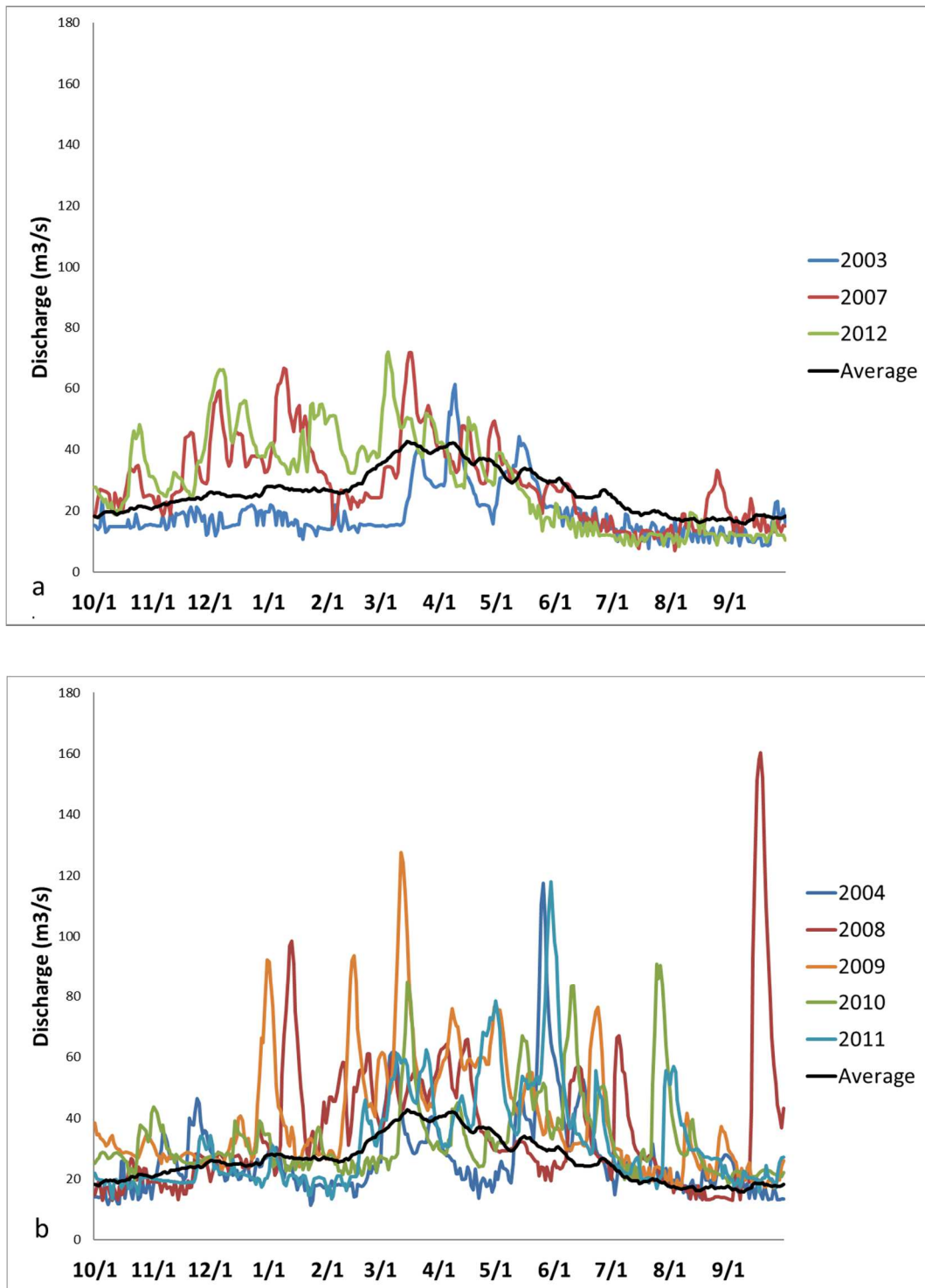


Figure 1.2 Discharge by water year (1 Oct-30 Sep) from the US Geological Survey for the Comstock, MI gauging station, located below Morrow Lake (downloaded from the National Water Information System). Top: Discharge for the relatively low-discharge summers in which I sampled. Bottom: Discharge for the relatively high- and average-discharge summers.

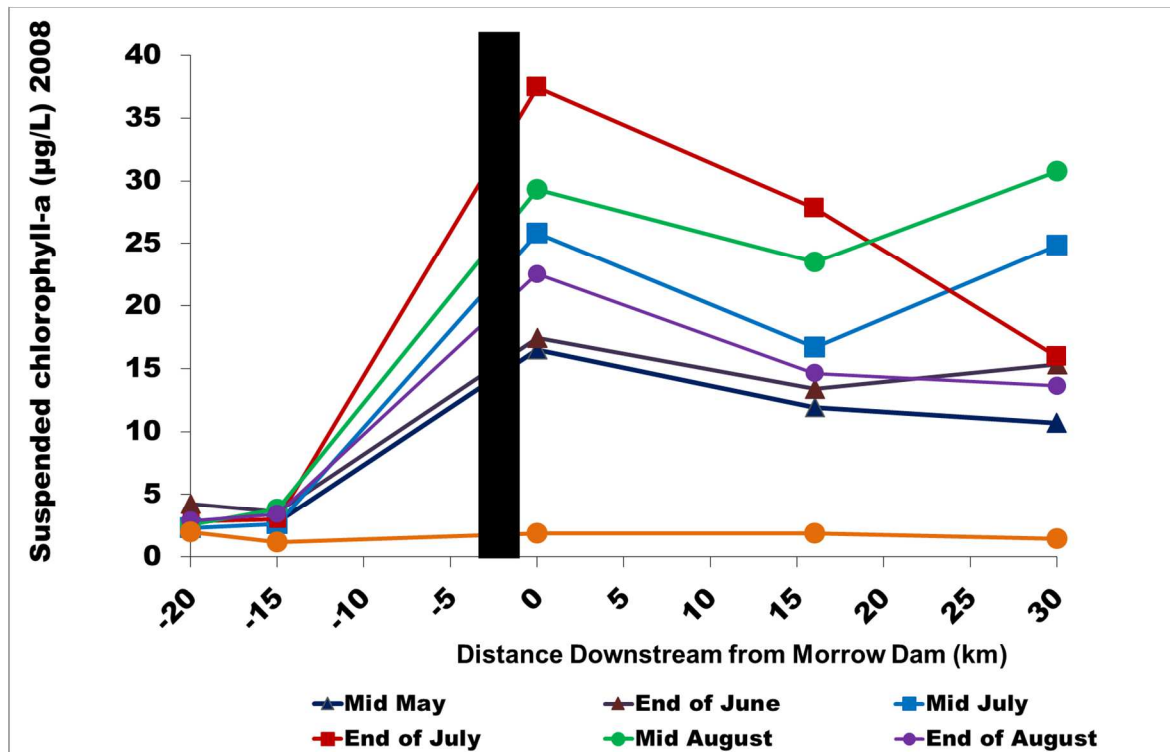


Figure 1.3 Suspended chlorophyll-*a* (Chl-*a*) measured biweekly in 2008 from the Kalamazoo River, including two sites above Morrow Lake (vertical lack bar) and three sites below it, extending to 30 km downstream.

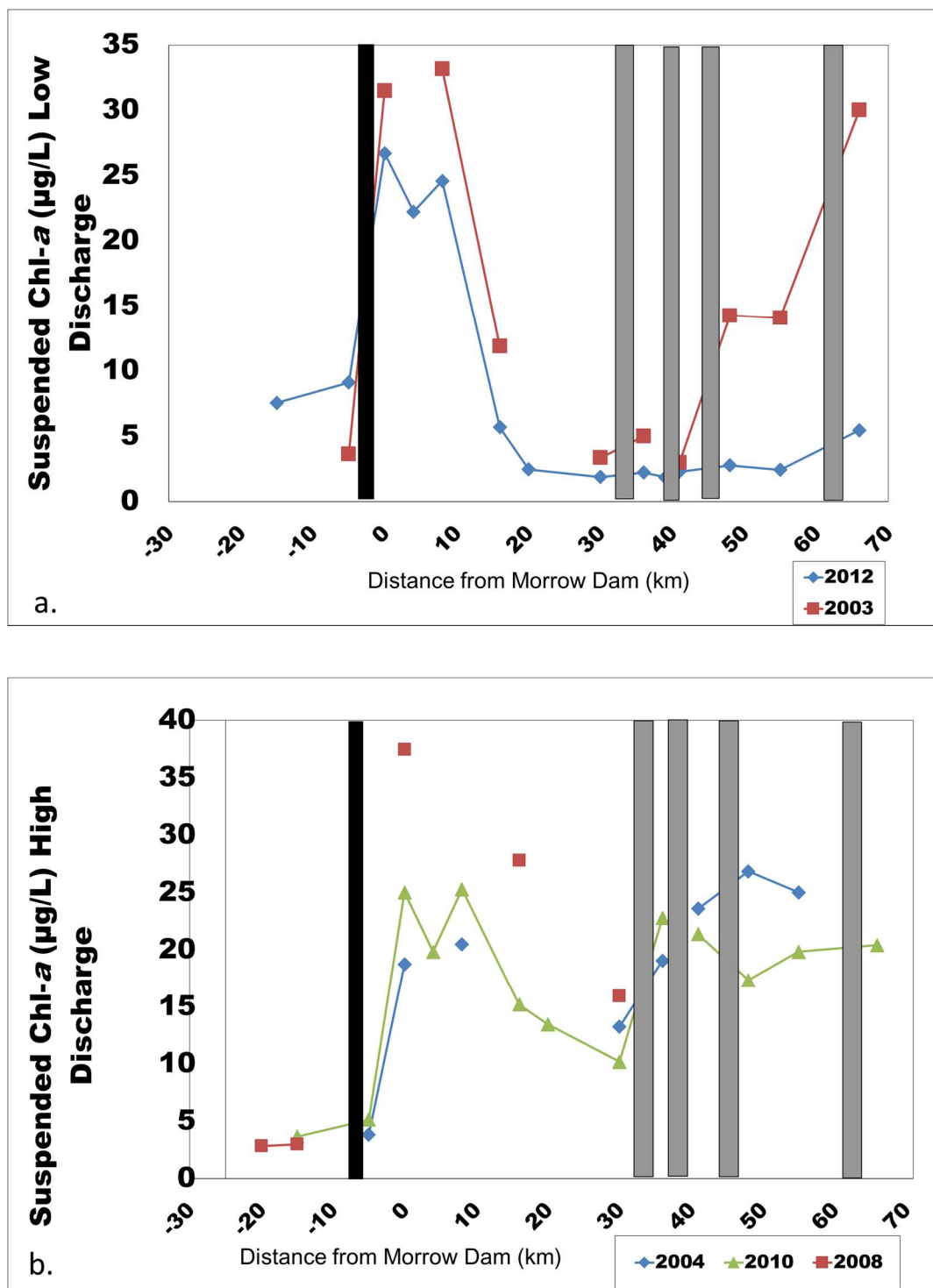


Figure 1.4 Suspended chlorophyll-a measured in 2004, 2008 and 2010 along longitudinal transects in the Kalamazoo River from above the Morrow Lake reservoir (negative distances) and below it for up to 66 km downstream. Panels show data from years of relatively low (top) and higher discharges (bottom). Black bar represents the dam at Morrow Lake, while the gray bars represent partially removed dams in the semi-impounded reach.

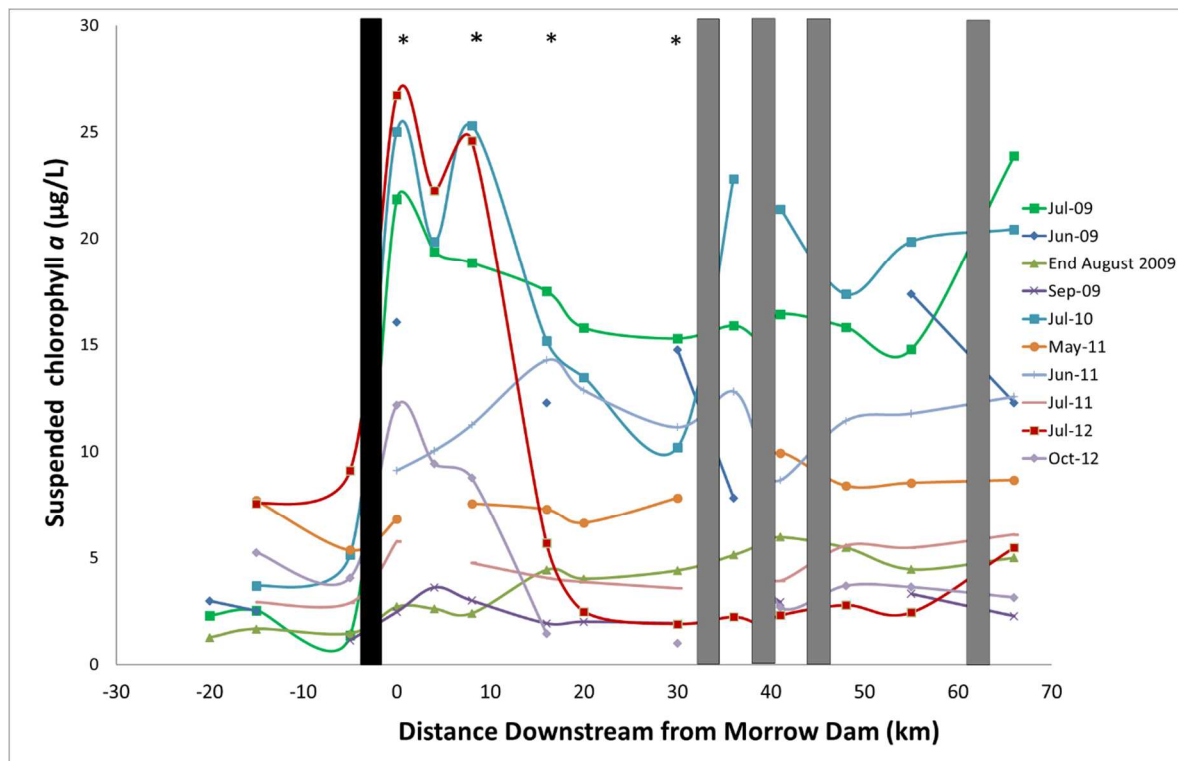


Figure 1.5 Suspended chlorophyll-a measured at multiple times during 2009-12 along longitudinal transects in the Kalamazoo River from above the Morrow Lake reservoir (negative distances) and below it for up to 66 km downstream. Black bar represents the dam at Morrow Lake, while the gray bars represent partially removed dams in the semi-impounded reach. Asterisks show significant differences between the upstream site at -15 km and sites downstream across all samplings (Paired t-test <0.05).

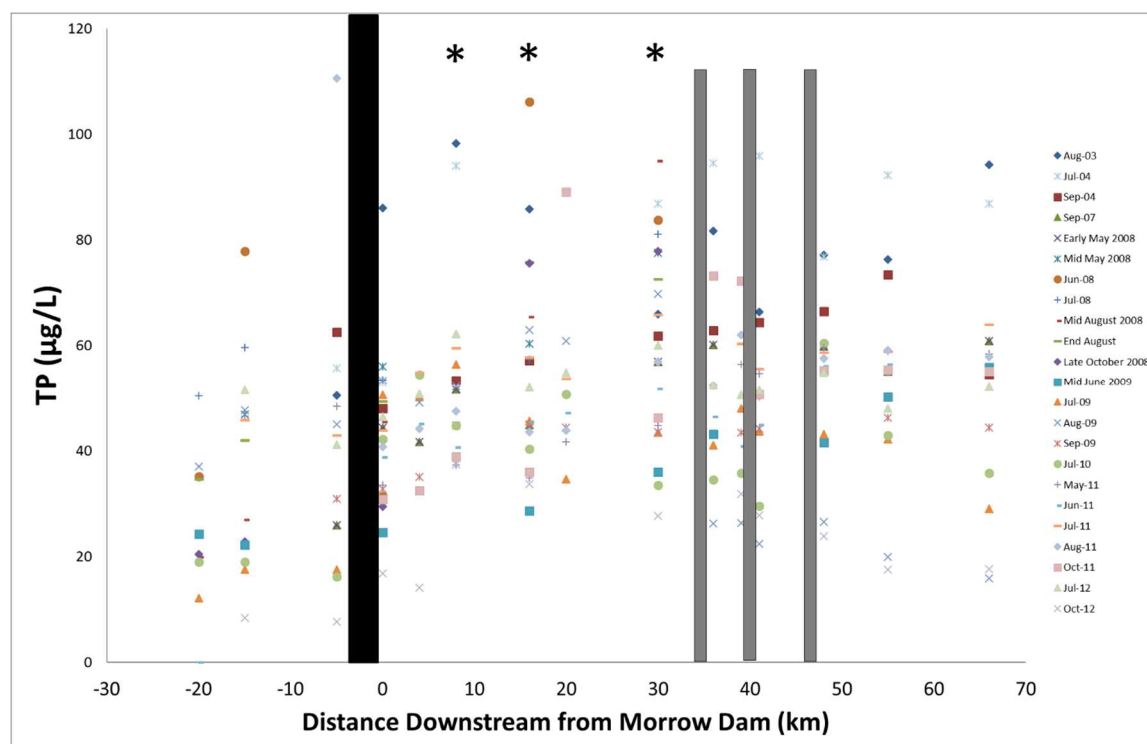


Figure 1.6 Total phosphorus (TP) concentrations measured along longitudinal transects in the Kalamazoo River from above the Morrow Lake reservoir (negative distances) and below it for up to 66 km downstream. Black bar represents the dam at Morrow Lake, while the gray bars represent partially removed dams in the semi-impounded reach. Asterisks show significant differences between the upstream site at -15 km and sites downstream across all samplings (Paired t-test <0.05).

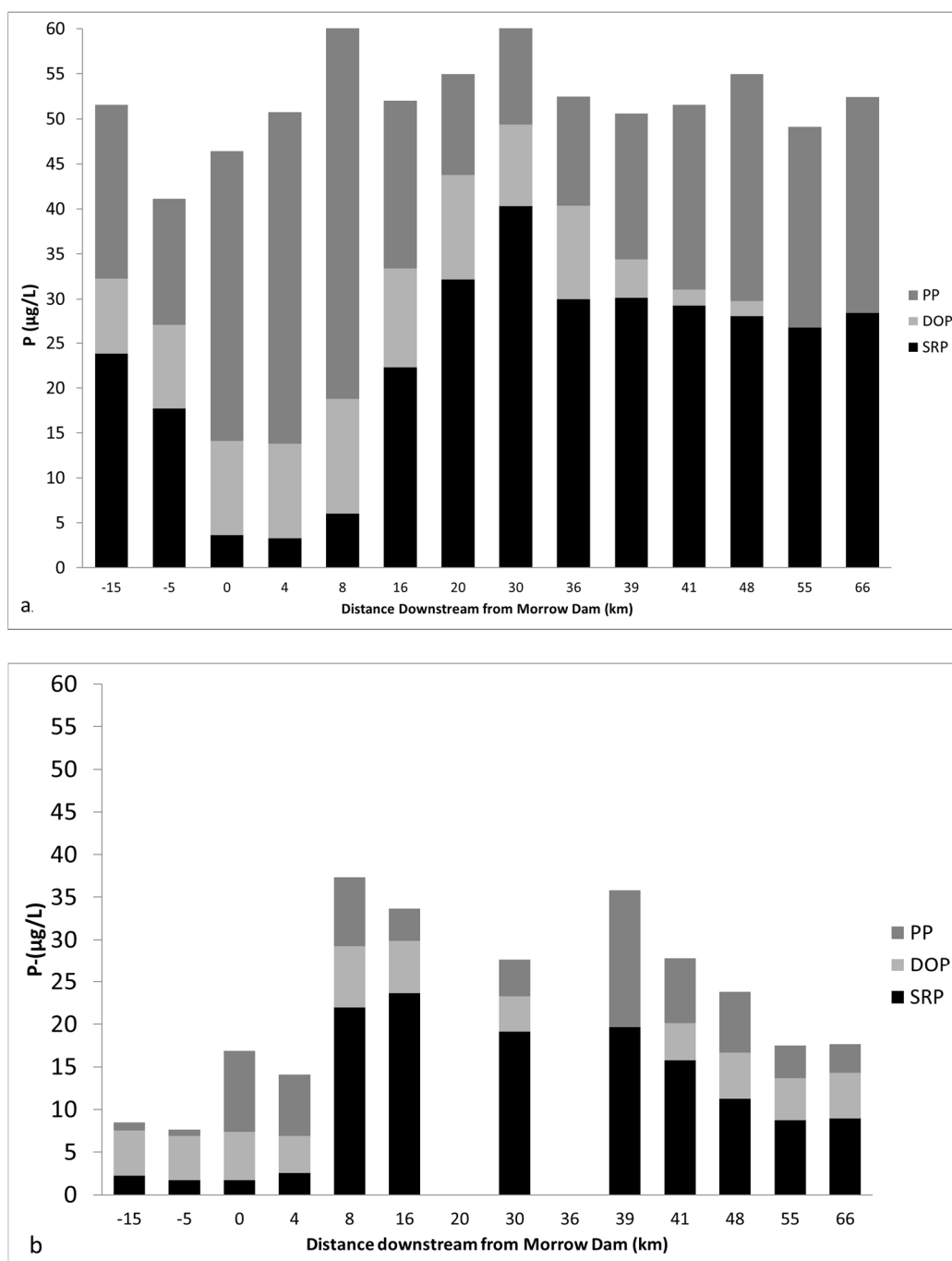


Figure 1.7 Fractions of total phosphorus measured on five dates along longitudinal transects in the Kalamazoo River from above the Morrow Lake reservoir (negative distances) and below it for up to 66 km downstream. Longitudinal transects occurred on July 2012 (a), October 2012 (b), July 2009 (c), September 2009 (d) and July 2010 (e) PP = particulate P; Dissolved organic P=DOP, DOP=TDP-SRP; SRP = soluble reactive P; TDP = total dissolved P, which only applies to July 2009 where SRP was not measured. DOP could not be estimated from TDP and SRP concentrations.

Figure 1.7 (cont'd)

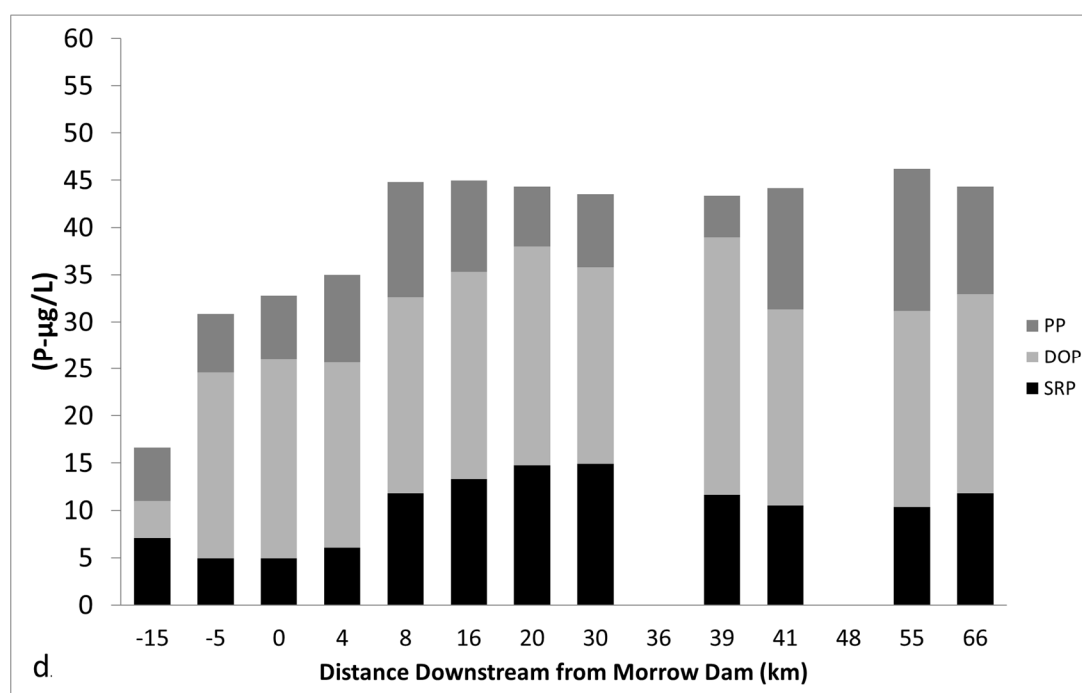
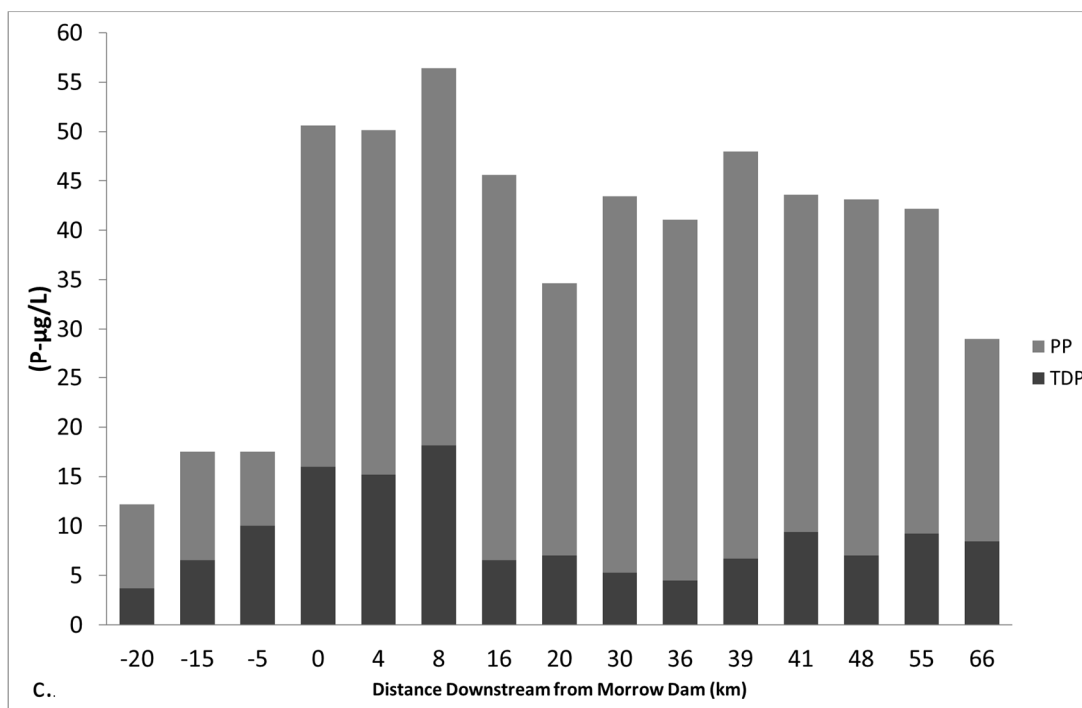
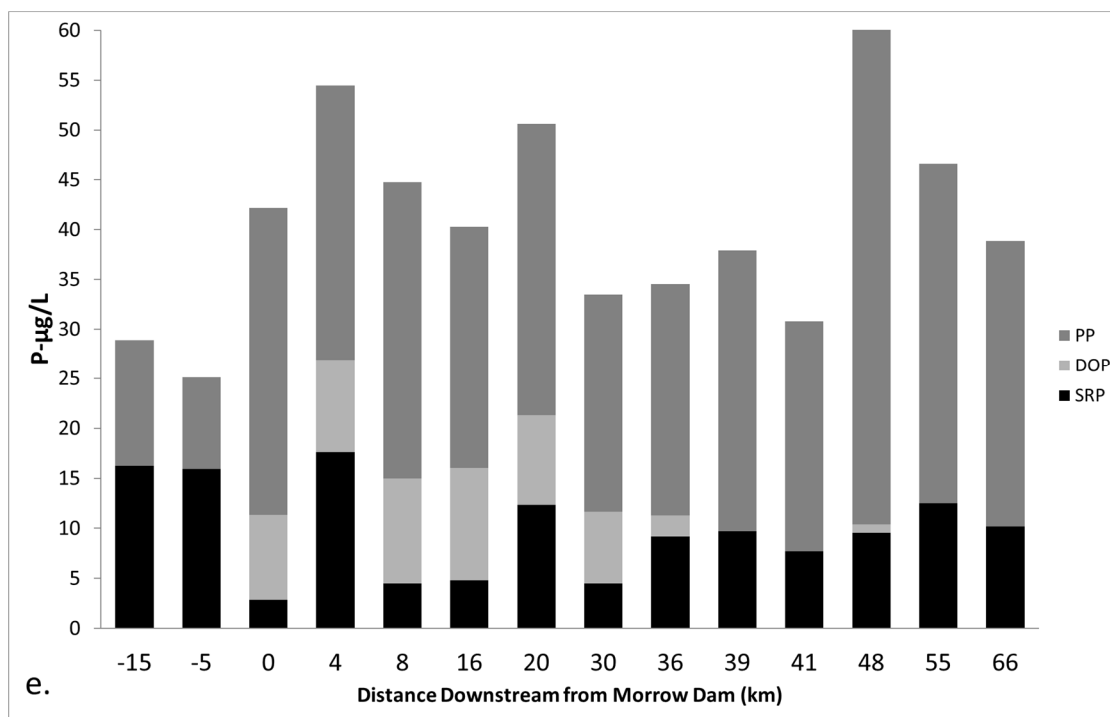


Figure 1.7 (cont'd)



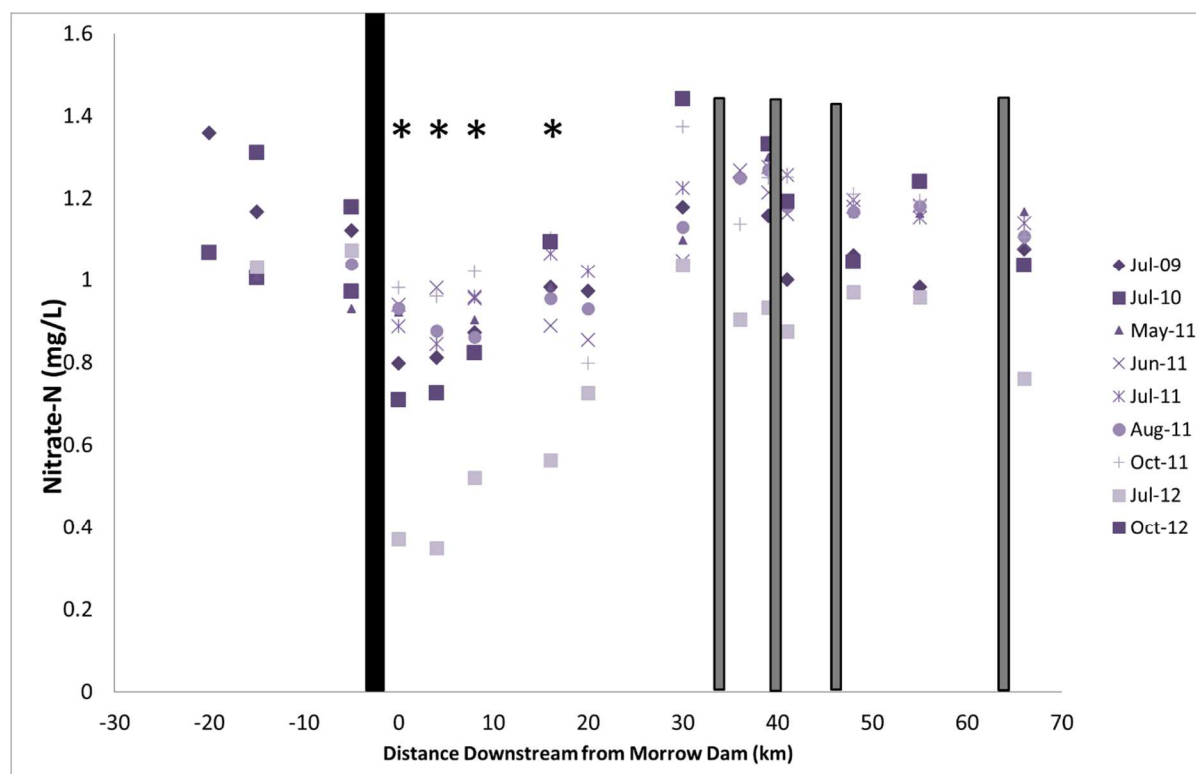


Figure 1.8 Nitrate concentrations measured along longitudinal transects in the Kalamazoo River from above the Morrow Lake reservoir (negative distances) and below it for up to 66 km downstream. Black bar represents the dam at Morrow Lake, while the gray bars represent partially removed dams in the semi-impounded reach. Asterisks show significant differences between the upstream site at -15 km and sites downstream across all samplings (Paired t-test < 0.05).

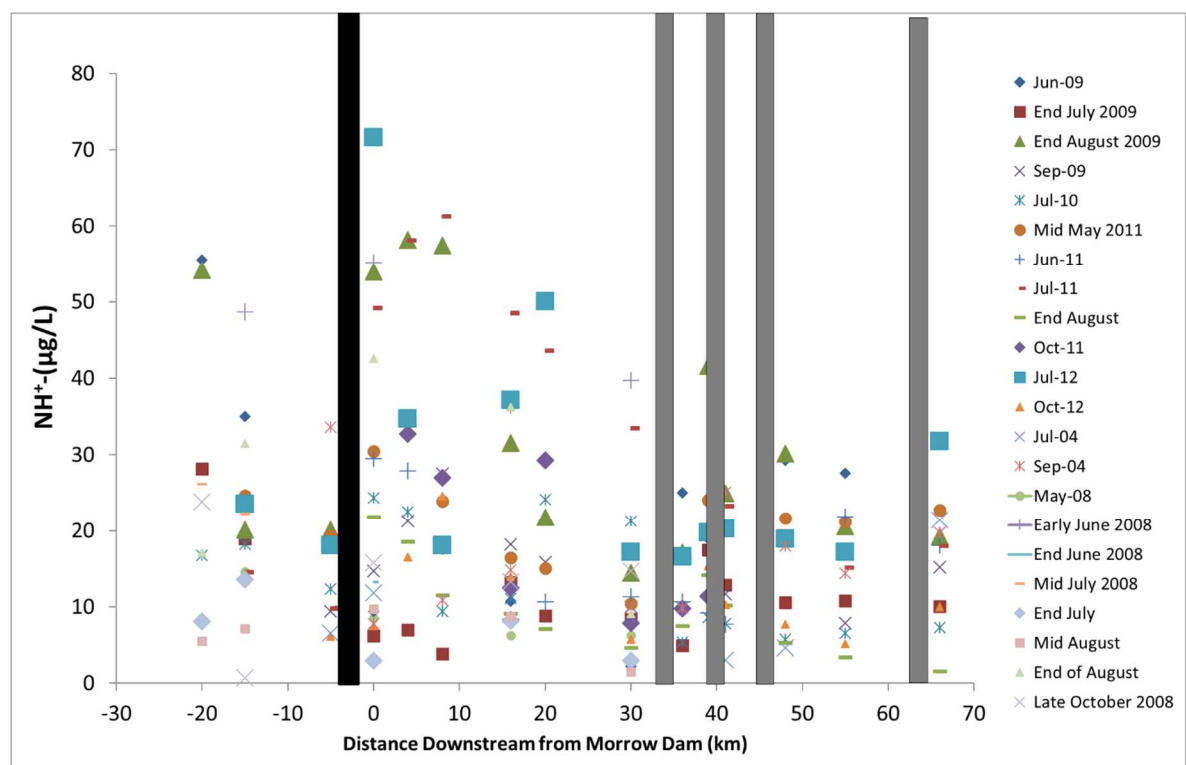


Figure 1.9 Ammonium concentrations measured along longitudinal transects in the Kalamazoo River from above the Morrow Lake reservoir (negative distances) and below it for up to 66 km downstream. Black bar represents the dam at Morrow Lake, while the gray bars represent partially removed dams in the semi-impounded reach.

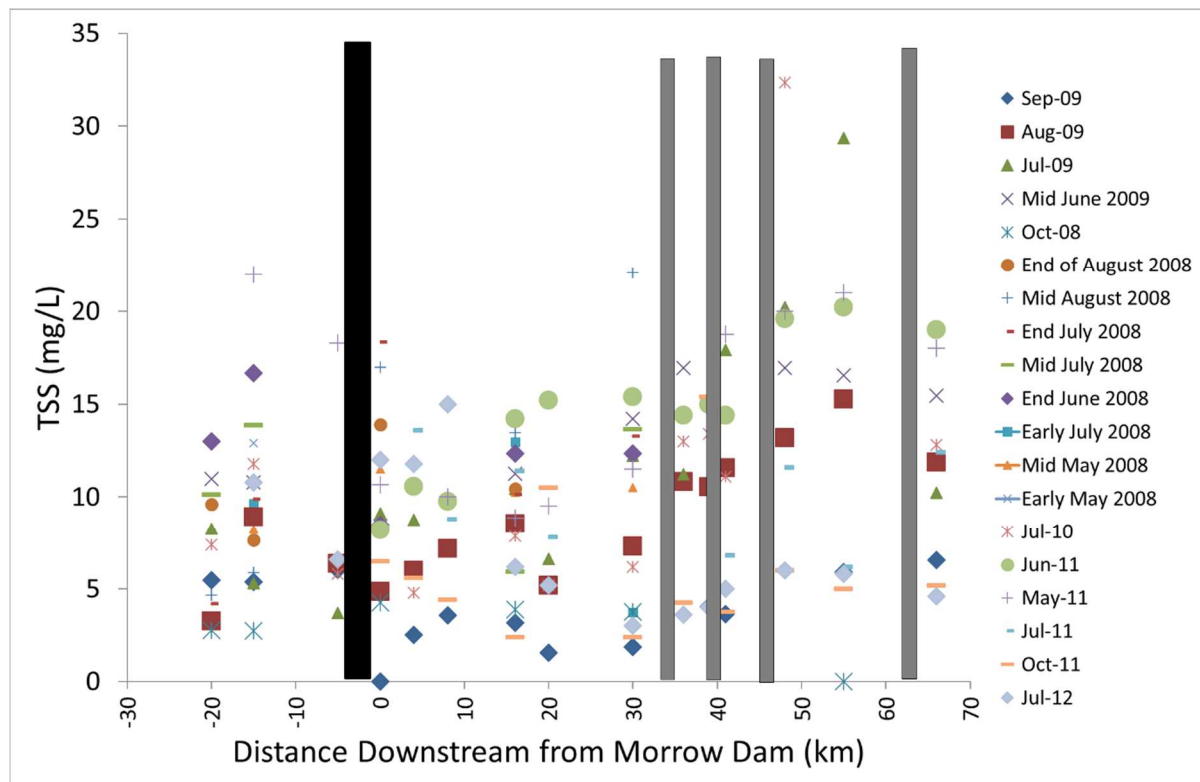


Figure 1.10 Total suspended solids concentrations measured along longitudinal transects in the Kalamazoo River from above the Morrow Lake reservoir (negative distances) and below it for up to 66 km downstream. Black bar represents the dam at Morrow Lake, while the gray bars represent partially removed dams in the semi-impounded reach.

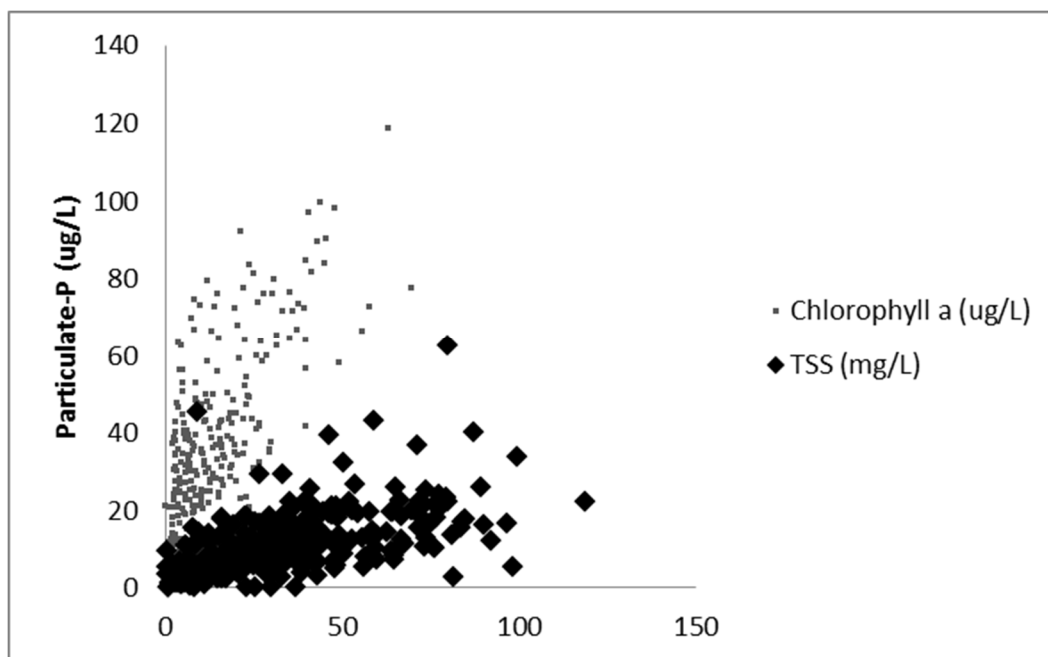


Figure 1.11 Relationships between chlorophyll-a ($\mu\text{g/L}$) or TSS (mg/L) (both on x axis) to Particulate-P ($\mu\text{g/L}$) in all samples from this study.

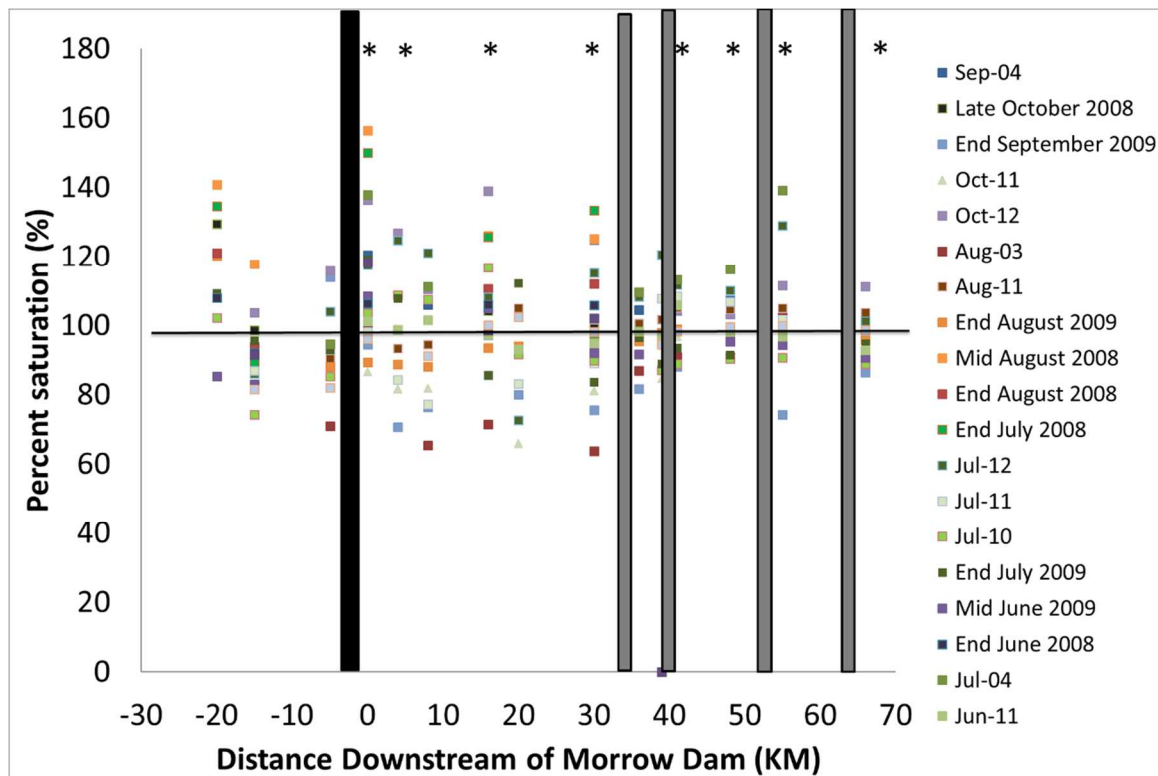


Figure 1.12 Dissolved oxygen as percent saturation measured along longitudinal transects in the Kalamazoo River from above the Morrow Lake reservoir (negative distances) and below it for up to 65 km downstream. Black bar represents the dam at Morrow Lake, while the gray bars represent partially removed dams in the semi-impounded reach. Asterisks show significant differences between the upstream site at -15 km and sites downstream across all samplings (Paired t-test <0.05).

REFERENCES

REFERENCES

- Admiraal, W., A. Jacobs, P. Bruegem, E.D. de Ruyter van Steveninck. 1992. Effects of phytoplankton on the elemental composition (C, N, P) of suspended particulate material in the lower river Rhine. *Hydrobiologia* 235: 479-489.
- Alexander, R. B., R. A. Smith, G. E. Schwarz. 2000. Effect of stream channel size on the delivery of nitrogen to the Gulf of Mexico. *Nature* 403: 758-761.
- Alexander, R. B., R. A. Smith, G. E. Schwarz, E. W. Boyer, J. V. Nolan, J. W. Brakebill. 2008. Differences in phosphorus and nitrogen delivery to the Gulf of Mexico from the Mississippi River basin. *Environmental Science and Technology* 42: 822-830.
- Alpine, A. E. And J. E. Cloern. 1992. Trophic interactions and direct physical effects control phytoplankton biomass and production in an estuary. *Limnology and Oceanography*. 37: 946-955.
- Ameziane T, Dauta A, Le Cohu L. 2003. Origin and transport of phytoplankton in a large river: the Garonne. France. *Archive fur Hydrobiologie* 156:385–404.
- Aminot, A., D.S. Kirkwood and R. Kerouel. 1997. Determination of ammonia in seawater by the indophenol-blue method: evaluation of the ICES NUTS I/C 5 questionnaire. *Mar. Chem.* 56:59-75.
- Anderson Ostergaard, F., H. Skovgaard Jensen. 1992. Regeneration of inorganic phosphorus and nitrogen from decomposition of seston in a freshwater sediment. *Hydrobiologia* 228: 71-81.
- Angradi, T. R. 1993. Stable carbon and nitrogen isotope analysis of seston in a regulated rocky mountain river, USA. *Regulated Rivers: Research and Management* 8: 251-270.
- Baas, D. G. 2009. Inferring dissolved phosphorus cycling in a TMDL watershed using biogeochemistry and mixed linear models. Dissertation, Michigan State University, East Lansing, Michigan, USA.
- Baker, D. B., D. E. Ewing, L. T. Johnson, J. W. Kramer, B. J. Merryfield, R. B. Confesor, Jr. R. P. Richards, A.A. Roerdink. 2014a. Lagrangian analysis of the transport and processing of agricultural runoff in the lower Maumee River and Maumee Bay. *Journal of Great Lakes Research* 40: 479-495.
- Baker, D.B., R. Confesor, D. E. Ewing, L. T. Johnson, J. W. Kramer, B. J. Merryfield. 2014b. Phosphorus loading to Lake Erie from the Maumee, Sandusky and Cuyahoga rivers: the importance of bioavailability. *Journal of Great Lakes Research* 40: 502-517.

- Basu, B.K., F. R. Pick. 1997. Phytoplankton and zooplankton development in a lowland temperate river. *Journal of Plankton Research* 19: 237-253.
- Bejarano, A.C., J. Michel, F. Fitzpatrick, M. DeGraeve, L. Williams, M. Alexander, S.K. Hamilton, and J. Chapman. 2012. Bridging Oil Spill Response and Scientifically Supported Information: A Net Environmental Benefit Analysis for the Kalamazoo River Oil Spill. Society for Environmental Toxicology & Chemistry, Long Beach, CA.
- Benke, A. C., J. B. Wallace. 2015. High secondary production in a Coastal Plain river is dominated by snag invertebrates and fueled mainly by amorphous detritus. *Freshwater Biology* 6: 236-255.
- Bianchi, T. S., L. A. Wysocki, M. Stewart, T. R. Filley, B. A. McKee. 2007. Temporal variability in terrestrially-derived sources of particulate organic carbon in the lower Mississippi River and its upper tributaries. *Geochimica et Cosmochimica Acta* 71: 4425-4437.
- Bowes, M. J., E. Gozzard, A. C. Johnson, P. M. Scarlett, C. Roberts, D. S. Read, L. K. Armstrong, S.A. Harman, H.D. Wickham. 2012. Spatial and temporal changes in chlorophyll-a concentrations in the River Thames basin, UK: Are phosphorus concentrations beginning to limit phytoplankton biomass? *Science of the Total Environment* 426: 45-55.
- Bowes, M. J., W. A. House, R. A. Hodgkinson. 2003. Phosphorus dynamics along a river continuum. *The Science of the Total Environment* 313: 199-212.
- Briggs, M. A., L. K. Lautz, D. K. Hare. 2014. Residence time control on hot moments of net nitrate production and uptake in the hyporheic zone. *Hydrological Processes* 28: 3741-3751.
- Bukaveckas, P. A., A. S. Crain. 2002. Inter-annual, seasonal and spatial variability in nutrient limitation of phytoplankton production in a river impoundment. *Hydrobiologia* 481: 19-31.
- Bukaveckas, P.A., A. MacDonald, A. Aufdenkampe, J.H. Chick, J. E. Havel, R. Schultz, T.R. Angradi, D. W. Bolgrien, T. M. Jicha, D. Taylor. 2011. Phytoplankton abundance and contributions to suspended particulate matter in the Ohio, Upper Mississippi and Missouri Rivers. *Aquatic Sciences* 73: 419-436.
- Caraco, N. F., J. J. Cole, P. A. Raymond, D. L. Strayer, M. L. Pace, S. E. G. Findlay, D.T. Fischer. 1997. Zebra mussel invasion in a large, turbid river: phytoplankton response to increased grazing 78: 588-602.
- Caraco, N., J.E. Bauer, J.J. Cole, S. Petsch, P. Raymond. 2010. Millennial-aged organic carbon subsidies to a modern river food web. *Ecology* 91: 2385-2393.
- Carlton, R. G., R. G. Wetzel. 1988. Phosphorus flux from lake sediments: effect of epipelagic algal oxygen production. *Limnology and Oceanography* 33: 562-570.

- Carpenter, S.R. N.F. Caraco, D.L. Correll, R. W. Howarth, A. N. Sharples, V. H. Smith. 1998. Nonpoint pollution of surface waters with phosphorus and nitrogen. *Ecological Applications* 8: 559-568.
- Carpenter, S. R. 2008. Phosphorus control is critical to mitigating eutrophication. *Proceedings of the National Academy of Science* 105: 11039-11040.
- Chandler, D. C. 1939. Plankton entering the Huron River from Portage and Base Line Lakes, Michigan. *Transactions of the American Microscopical Society* 58: 24-41.
- Church, M. 1995. Geomorphic responses to river flow regulation: case studies and timescales. *Regulated Rivers: Research and Management* 11: 3-22.
- Cifuentes, L.A., Sharp, J.H., Fogel, M.L. 1988. Stable carbon and nitrogen isotope biogeochemistry in the Delaware estuary. *Limnology Oceanography* 33: 1102–1115.
- Cloern, J. E., E. A. Canuel, D. Harris. 2002. Stable carbon and nitrogen isotope composition of aquatic and terrestrial plants of the San Francisco Bay estuarine system. *Limnology and Oceanography* 47: 713-729.
- Cloern, J. E., C. Grenz, L. Videgar-Lucas. 1995. An empirical model of phytoplankton chlorophyll: carbon ratio-the conversion factor between productivity and growth rate. *Limnology and Oceanography* 40: 1313-1321.
- Cohen, R. R. H., P. V. Dresler, E. J. P. Phillips, R. L. Cory. 1984. The effect of the Asiatic clam, *Corbicula fluminea*, on phytoplankton of the Potomac River, Maryland. *Limnology and Oceanography* 29: 170-180.
- Cole, J. J., N. F. Caraco, B. L. Peierls. 1992. Can phytoplankton maintain a positive carbon balance in a turbid, freshwater, tidal estuary. *Limnology and Oceanography* 37: 1608-1617.
- De Jager, N.R., J. N. Houser. 2012. Variation in water-mediated connectivity influences patch distributions of total N, total P and TN:TP ratios in the Upper Mississippi River, USA. *Freshwater Science* 31: 1254-1272.
- Descy, J. P., E. Everbecq, V. Gosselain, L. Viroux, J.S. Smits. 2003. Modelling the impact of benthic filter-feeders on the composition and biomass of the river plankton. *Freshwater Biology* 48: 404-417.
- Descy, J-P., M. Leitao, E. Everbecq, J.S. Smits, J.F. Francois Deliege. 2012. Phytoplankton of the River Loire, France: a biodiversity and modelling study. *Journal of Plankton Research* 34: 120-135.
- Dodds, W. K. 2006. Eutrophication and trophic state in rivers and stream. *Limnology and Oceanography* 51: 671-680.

- Dodds, W.K., J.J. Beaulieu, J.J. Eichmiller, J. R. Fischer, N.R. Franssen, D. A. Gudder, A. S. Makinster, M.J. McCarthy, J. N. Murdock, J.M. O'Brien, J.L. Tank, R. W. Scheibley. 2008. Nitrogen cycling and metabolism in the thalweg of a prairie river. *Journal of Geophysical Research* 113: 2156-2202.
- Dodds, W. K., A. M. Veach, C. M. Ruffing, D. M. Larson, J. L. Fischer, K. H. Costigan. 2013. Abiotic controls and temporal variability of river metabolism: multiyear analysis of Mississippi and Chattahoochee River data. *Freshwater Science* 32: 1073-1087.
- Doi, H., K-H. Chang, T. Ando, H. Imai, S-I Nakano, A. Kajimoto, I. Katano. 2008. Drifting plankton from a reservoir subsidize downstream food webs and alter community structure. *Oecologia* 156: 363-371.
- Dolph, C.L., A. T. Hansen, J. C. Finlay. 2017. Flow-related dynamics in suspended algal biomass and its contribution to suspended particulate matter in an agricultural river network of the Minnesota River Basin, USA. *Hydrobiologia* 785: 127-147.
- Doyle, M.W., E. H. Stanley, J. M. Harbor. 2003. Hydrogeomorphic controls on phosphorus retention in streams. *Water Resources Research* 39: 1147.
- Doyle, M.W., S. H. Ensign. 2009. Alternative reference frames in river system science. *Bioscience* 59: 499-510.
- Dzialowski, A. R., J. L. Bonneau, T. R. Gemeinhardt. 2013. Comparisons of zooplankton and phytoplankton in created shallow water habitats of the lower Missouri River: implications for native fish. *Aquatic Ecology* 47: 13-24.
- Engstrom, D. R., J. E. Almendinger, J. A. Wolin. 2009. Historical changes in sediment and phosphorus loading to the upper Mississippi River: mass-balance reconstruction from the sediments of Lake Pepin. *J. Paleolimnology* 41: 563-588.
- Ensign, S. H., M. W. Doyle. 2006. Nutrient spiraling in streams and river networks. *Journal of Geophysical Research* 111: G04009.
- Findlay, S., M. Pace, D. Fischer. 1996. Spatial and temporal variability in the lower food web of the tidal freshwater Hudson River. *Estuaries* 19: 866-873.
- Forshay, K. J., E. H. Stanley. 2005. Rapid nitrate loss and denitrification in a temperate river floodplain. *Biogeochemistry* 75: 43-64.
- Gergel, S. E., S. R. Carpenter, E. H. Stanley. 2005. Do dams and levees impact nitrogen cycling? Simulating the effects of flood alterations of floodplain denitrification. *Global Change Biology* 11:1352-1367.

- Gu, B., A. D. Chapman, C. L. Schelske. 2006. Factors controlling seasonal variations in stable isotope composition of particulate organic matter in a soft water eutrophic lake. *Limnology and Oceanography* 51: 2837-2848.
- Hall, R. O., M. A. Baker, E. J. Rosi-Marshall, J. L. Tank, J.D. Newbold. 2013. Solute specific scaling of inorganic nitrogen and phosphorus uptake in stream. *Biogeosciences* 10: 7323-7331.
- Hall, R. O., J.L. Tank, M.A. Baker, E. J. Rosi-Marshall, E. R. Hotchkiss. 2016. Metabolism, gas exchange, and carbon spiraling in rivers. *Ecosystems* 19: 73-86.
- Hamilton, S.K., S. J. Sippel, W. M. Lewis, J. F. Saunders. 1990. Zooplankton abundance and evidence for its reduction by macrophyte mats in two Orinoco floodplain lakes. *Journal of Plankton Research* 12: 345-363.
- Hamilton, S.K., W. M. Lewis, Jr. 1992. Stable carbon and nitrogen isotopes in algae and detritus from the Orinoco River floodplain, Venezuela. *Geochimica et Cosmochimica Acta* 56: 4237-4246.
- Hamilton, S. K., S. J. Sippel, S. E. Bunn. 2005. Separation of algae from detritus for stable isotope for ecological stoichiometry studies using density fractionation in colloidal silica. *Limnology and Oceanography: Methods* 3: 149-157.
- Hardenbicker, P., M. Weitere, S. Ritz, F. Scholl, H. Fischer. 2016. Longitudinal plankton dynamics in the rivers Rhine and Elbe. *River Research and Applications* 32: 1265-1278.
- Harrison, J. A., R. J. Maranger, R. B. Alexander, A. E. Gibline, P-A. Jacinthe, E. Mayorga, S. P. Seitzinger, D. J. Sobota, W. M. Wollheim, 2009. The regional and global significance of nitrogen removal in lakes and reservoirs. *Biogeochemistry* 93: 143-157.
- Hein, T., W. Reckendorfer, J. H. Thorp, F. Schiemer. 2005. The role of slackwater areas for biogeochemical processes in rehabilitated river corridors: examples for the Daube. *Archiv fur Hydrobiologie Supplement* 155: 425-442.
- Hensley, R. T., M. J. Cohen, L. V. Korhnak. 2014. Inferring nitrogen removal in large rivers from high-resolution longitudinal profiling. *Limnology and Oceanography* 59: 1152-1170.
- Hilton, J., M. O'Hare, M. J. Bowes, J. I. Jones. 2006. How green is my river? A new paradigm of eutrophication in rivers. 365: 66-83.
- Honti, M., V. Istvanovics, A. S. Kovacs. 2010. Balancing between retention and flushing in river networks-optimizing nutrient management to improve trophic state. *Science of the Total Environment*. 408: 4712-4721.
- Houser, J. N., D. W. Bierman, R. M. Burdis, L. A. Soeken-Gittinger. 2010. Longitudinal trends and discontinuities in nutrients, chlorophyll, and suspended solids in the Upper Mississippi

River: implications for transport, processing, and export by large rivers. *Hydrobiologia* 651: 127-144.

Houser, J. N. 2016. Contrasts between channels and backwaters in a large, floodplain river: testing our understanding of nutrient cycling, phytoplankton abundance, and suspended solid dynamics. *Freshwater Science* 35: 457-473.

Howarth, R.W., G. Billen, D. Swaney, A. Townsend, N. Jaworski, K. Lajtha, J. A. Downing, R. Elmgren, N. Caraco, T. Jordan, F. Berendse, J. Freney, V. Kudeyarov, P. Murdoch, Z.-L. Zhu. 1996. Regional nitrogen budgets and riverine N and P fluxes for the drainages to the Northern Atlantic Ocean: Natural and human influences. *Biogeochemistry* 35: 75-139.

Howarth, R. W., R. Schneider, D. Swaney. 1996. Metabolism and organic carbon fluxes in the tidal freshwater Hudson River. *Estuaries* 19: 848-865.

Istvanovics, V., A. Osztóics, M. Honti. 2004. Dynamics and ecological significance of daily internal load of phosphorus in shallow Lake Balaton, Hungary. *Freshwater Biology* 49: 232-252.

Istvanovics, V., M. Honti, L. Voros, Z. Kozma. 2010. Phytoplankton dynamics in relation to connectivity, flow dynamics and resource availability- the case of a large lowland river, the Hungarian Tisza. *Hydrobiologia* 637: 121-141.

Istvanovics V. & Honti M. 2011. Phytoplankton growth in three rivers: the role of meroplankton and the benthic retention hypothesis. *Limnology and Oceanography* 56:1439–1452.

James, W. F., C. E. Larson. 2008. Phosphorus dynamics and loading in the turbid Minnesota River (USA): controls and recycling potential *Biogeochemistry* 90: 75-92.

Kendall, C., S. R. Silva, V. J. Kelly. 2001. Carbon and nitrogen isotopic compositions of particulate organic matter in four large river systems across the United States. *Hydrological Processes* 15: 1301-1346.

Kinsman-Costello, L. E., S. K. Hamilton, J. M. O'Brien, J. T. Lennon. 2016. Phosphorus release from the drying and reflooding of diverse shallow sediments. *Biogeochemistry* 130: 159-176.

Knowlton, M. F., J. R. Jones. 1997. Trophic status of Missouri River floodplain lakes in relation to basin type and connectivity. *Wetlands* 4: 468-475.

Knowlton, M. F., J. R. Jones. 2000. Seston, light, nutrients and chlorophyll in the Lower Missouri River, 1994-1998. *Journal of Freshwater Ecology* 15: 283-297.

Koch, R. W., D. L. Guelda, P.A. Bukaveckas. 2004. Phytoplankton growth in the Ohio, Cumberland and Tennessee Rivers, USA: inter-site differences in light and nutrient limitation. 38: 17-26.

- Kohler, J., M. Bahnwart, K. Ockenfeld. 2002. Growth and loss processes of riverine phytoplankton in relation to water depth. *International Review of Hydrobiology* 87: 241-254.
- Langner, C.L. and P.F. Hendrix. 1982. Evaluation of a persulfate digestion method for particulate nitrogen and phosphorus. *Water Res.* 16:1451-1454.
- Lewis, W. M., 1988. Primary production in the Orinoco River. *Ecology* 69: 679-692.
- Lewis, W. M., W. A. Wurtsbaugh. 2008. Control of lacustrine phytoplankton by nutrients: erosion of the phosphorus paradigm. *International Review Hydrobiology* 93: 446-465.
- Lucas, L. V., J. E. Cloern, J. K. Thompson, N. E. Monsen. 2002. Functional variability of habitats within the Sacramento-San Joaquin Delta: restoration implications. *Ecological Applications* 12: 1528-1547.
- Lucas, L. V., J. K. Thompson, L. R. Brown. 2009. Why are diverse relationships observed between phytoplankton biomass and transport time? *Limnology and Oceanography* 54: 381-390.
- Lucas, L. V., J. K. Thompson. 2012. Changing restoration rules: exotic bivalves interact with residence time and depth to control phytoplankton productivity. *Ecosphere* 3: 1-25.
- Mackay, R. J. and T. F. Waters. 1986. Effects of small impoundments on Hydropsychid caddisfly production in Valley Creek, Minnesota. *Ecology* 67: 1680-1686.
- Mallin, M. A., V. L. Johnson, S. H. Ensign, T. A. MacPherson. 2006. Factors contributing to hypoxia in rivers, lakes and streams. *Limnology and Oceanography* 51: 690-701.
- McDowell, W.G., W.H. McDowell, J.E. Byers. 2017. Mass mortality of a dominant invasive species in response to an extreme climate event: implications for ecosystem function. *Limnology and Oceanography* 62: 177-188.
- Minaudo, C., M. Meybeck, F. Moatar, N. Gassama, F. Curie. 2015. Eutrophication mitigation in rivers: 30 years of trends in spatial and seasonal patterns of biogeochemistry of the Loire River (1980-2012). *Biogeosciences* 12: 2549-2563.
- Monaghan, M. T., S.A. Thomas, G. W. Minshall, J. D. Newbold, C.E. Cushing, 2001. The influence of filter feeding benthic macroinvertebrates on the transport and deposition of particulate organic matter and diatoms in two streams. *Limnology and Oceanography* 46: 1091-1099.
- Neal, C., J. Hilton, A. J. Wade, M. Neal, H. Wickham. 2006. Chlorophyll-a in the rivers of eastern England. *Science of the Total Environment* 365: 84-104.
- Newbold, J. D., S. A. Thomas, G. W. Minshall, C. E. Cushing, T. Georgian. 2005. Deposition, benthic residence, and resuspension of fine organic particles in a mountain stream. *Limnology and Oceanography* 50: 1571-1580.

Nichols, S., R. Norris, W. Maher, M. Thoms. 2006. Ecological effects of serial impoundment on the Cotter River, Australia. *Hydrobiologia* 572: 255-273.

O'Brien, J.M., S. K. Hamilton, L. E. Kinsman-Costello, J. T. Lennon, N. E. Ostrom. 2012. Nitrogen transformations in a through-flow wetland revealed using whole-ecosystem pulsed ¹⁵N additions. *Limnology and Oceanography* 57: 221-234.

Ochs, C. A., O. Pongruktham & P. V. Zimba, 2013. Darkness at the break of noon: phytoplankton production in the Lower Mississippi River. *Limnology and Oceanography* 58: 555–568.

Oliver, A. A., R. A. Dahlgren, M. L. Deas. 2014. The upside-down river: Reservoirs, algae blooms, and tributaries affect temporal and spatial patterns in nitrogen and phosphorus in the Klamath River, USA. *Journal of Hydrology* 519: 164-176.

Paerl, H. W. 2009. Controlling eutrophication along the freshwater-marine continuum: dual nutrient (N and P) reductions are essential *Estuaries and Coasts* 32: 593-601.

Palmer, R., J. O'Keeffe. 1990. Transported material in a small river with multiple impoundments. *Freshwater Biology* 24: 563-575.

Paul, M. J., R. O. Hall. 2002. Particle transport and transient storage along a stream-size gradient in the Hubbard Brook Experimental. *Journal of the North American Benthological Society* 21: 195-205.

Petts, G. E., A. M. Gurnell. 2005. Dams and geomorphology: research progress and future directions. *Geomorphology* 71: 27-47.

Pfeiffer, S. M., J. M. Bahr, R. D. Beilfuss. 2006. Identification of groundwater flowpaths and denitrification zones in a dynamic floodplain aquifer. *Journal of Hydrology* 325: 262-272.

Pongruktham, O., C. Ochs. 2015. The rise and fall of the Lower Mississippi: effects of hydrological connection on floodplain backwaters. *Hydrobiologia* 742: 169-183.

Powers, S.M., J. P. Julian, M. W. Doyle, E. H. Stanley. 2013. Retention and transport of nutrients in a mature agricultural impoundment. *Journal of Geophysical Research; Biogeosciences* 118: 91-113.

Powers, S. M., J. L. Tank, D. M. Robertson. 2015. Control of nitrogen and phosphorus transport by reservoirs. *Biogeochemistry* 124: 417-439.

Rabalais, N.N. 2002. Nitrogen in aquatic ecosystems. *Ambio* 21: 102-112.

Rachol, C.M., F.A. Fitzpatrick, and T. Rossi. 2005. Historical and simulated changes in channel characteristics of the Kalamazoo River, Plainwell to Otsego, Michigan: U.S. Geological Survey Scientific Investigations Report 2005-5044.

Reid, N. J., S. K. Hamilton. 2007. Controls on algal abundances in a eutrophic river with varying degrees of impoundment (Kalamazoo River, Michigan, USA). *Lake and Reservoir Management* 23: 219-230.

Reisinger, A.J., J. L. Tank, E. J. Rosi-Marshall, R.O. Hall, M.A. Baker. 2015. The varying role of water column nutrient uptake along river continua in contrasting landscapes. *Biogeochemistry* 125: 115-131.

Reisinger, A. J., J. Tank, M. M. Dee. 2016. Regional and seasonal variation in nutrient limitation of river biofilms. *Freshwater Science* 35: 474-489.

Reynolds, C. S. and J. P. Descy. 1996. The production, biomass and structure of phytoplankton in large rivers. *Archiv fur Hydrobiologie: Large rivers* 10: 161-187.

Reynolds, C.S. 2000. Hydroecology of river plankton: the role of variability in channel flow. *Hydrological processes* 14: 3119-3132.

Rosi-Marshall, E. J. and J. B. Wallace. 2002. Invertebrate food webs along a stream resource gradient. *Freshwater Biology* 47: 129-141.

Russell, M. A., D.E. Walling, R.A. Hodgkinson. 2001. Suspended sediment sources in two small lowland agricultural catchments in the UK. *Journal of Hydrology* 252: 1-24.

Russell, M.A., D. E. Walling, B. W. Webb, R. Bearne. 1998. The composition of nutrient fluxes from contrasting UK river basins. *Hydrological Processes* 12: 1461-1482.

Santucci, V. J., S. R. Gephard, S. M. Pescitelli. 2005. Effects of multiple low-head dams on fish, macroinvertebrates, habitat, and water quality in the Fox River, Illinois. *North American Journal of Fisheries Management* 25: 975-992.

Scherwass, A. T. Bergfeld, Andreas Schol, M. Weitere, H. Arndt. 2010. Changes in the plankton community along the length of the River Rhine: Lagrangian sampling during a spring situation. *Journal of Plankton Research* 32: 491-502.

Schonbrunner, I.M., S. Preiner, T. Hein. 2012. Impacts of drying and re-flooding of sediment on phosphorus dynamics of river-floodplain systems. *Science of the Total Environment* 432: 329-337.

Sellers, T., P.A. Bukaveckas. 2003. Phytoplankton production in a large, regulated river: a modeling and mass balance assessment. *Limnology and Oceanography* 48: 1476-1487.

Smith, V. H. 2003. Eutrophication of freshwater and marine ecosystems: a global problem. *Environmental Sciences Pollution Research* 10: 126-139.

Smith. 2016. Effects of eutrophication on maximum algal biomass in lake and river ecosystems. *Inland Waters* 6: 147-154.

- Smith, V. H., D. W. Schindler. 2009. Eutrophication science: where do we go from here? *Trends in Ecology and Evolution* 24: 201-207.
- Soballe, D. M., B. L. Kimmel. 1987. A large scale comparison of factors influencing phytoplankton abundance in rivers, lakes and impoundments. *Ecology* 68: 1943-1954.
- Sondergergaard, M., P. Kristensen, E. Jeppesen, 1992. Phosphorus release from resuspended sediment in the shallow and wind-exposed Lake Arreso, Denmark. *Hydrobiologia*: 228: 91-99.
- Stanley, E. H., M. A. Luebke, M.W. Doyle, D.W. Marshall. 2002. Short-term changes in channel form and macroinvertebrate communities following low-head dam removal. *Journal of North American Benthological Society* 21: 172-187.
- Strayer, D. L., K. A. Hattala, A. W. Kahnle. 1994. Effects of an invasive bivalve (*Dreissena polymorpha*) on fish in the Hudson River estuary. *Canadian Journal of Fisheries and Aquatic Science* 61: 924-941.
- Strayer, D. L., M. L. Pace, N. F. Caraco, J.J. Cole, S. E. G. Findlay. 2008. Hydrology and grazing jointly control a large-river food web. *Ecology* 89: 12-18.
- Tank, J. L., Rosi-Marshall, E. J., Baker, M. A., and Hall, R. O. 2008. Are rivers just big streams? A pulse method to quantify nitrogen demand in a large river. *Ecology* 89: 2935–2945.
- Thorp, J.H., M.D. Delong, K.S. Greenwood, A.F. Casper. 1998. Isotopic analysis of three food web theories in constricted and floodplain regions of a large river. *Oecologia* 117:551-563.
- Thorp, J. H., M. C. Thoms, and M. D. Delong. 2006. The riverine ecosystem synthesis: Biocomplexity in river networks across space and time. *River Research and Application*. 22: 123–147.
- Tinka, A., Q. Wu, K. Weekly, C. A. Oroza, J. Beard, A. M. Bayan. 2016. Heterogeneous fleets of active and passive floating sensors for river studies. *Journal of Field Robotics* 33: 618-638.
- Tobias, C.R., M. Cieri, B. J. Peterson, L. A. Deegan, J. Vallino, J. Hughes. 2003. Processing watershed -derived nitrogen in a well-flushed New England estuary. *Limnology and Oceanography* 48: 1766-1778.
- Ulseth, A. J., R. O. Hall. 2015. Dam tailwaters compound the effects of reservoirs on the longitudinal transport of organic carbon in an arid river. *Biogeosciences* 12: 6081-6114.
- Vadeboncoeur, Y. 1994. Longitudinal dynamics of seston concentration and composition in a lake outlet stream. *Journal of North American Benthological Society* 13: 181-189.
- Valderrama, J.C. 1981. The simultaneous analysis of total nitrogen and total phosphorus in natural waters. *Mar. Chem.* 10:109-122.

- Vannote, R. L., G. W. Minshall, K. W. Cummins, J. R. Sedell, C. E. Cushing. 1980. The river continuum concept. *Canadian Journal of Fisheries and Aquatic Science* 37: 130-137.
- Vitousek, P.M., J.D. Aber, R.W. Howarth, G.E. Likens, P. A. Matson, D. W. Schindler, W. H. Schlesinger, D.G. Tilman. 1997. Human alteration of the global nitrogen cycle: sources and consequences. *Ecological Application* 7: 737-750.
- Volkmar, E. C., R. A. Dahlgren, W. T. Stringfellow, S. S. Henson, S. E. Borglin, C. Kendall, E. E. Van Nieuwenhuysen. 2011. Using Lagrangian sampling to study water quality during downstream transport in the San Luis Drain, California, USA. *Chemical Geology* 283: 68-77.
- Vorosmarty, C. J., M. Maybeck, B. Fekete, V. Sharma, P. Green, J. P.M. Syvitski. 2003. Anthropogenic sediment retention: major global impact from registered river impoundments. *Global and Planetary Change* 39: 169-190.
- Wall, L. G., J. L. Tank, T. V. Royer, M. J. Bernot. 2005. Spatial and temporal variability in sediment denitrification within an agriculturally influenced reservoir. *Biogeochemistry* 76: 85-111.
- Walling, D. E., A. L. Collins, R. W. Stroud. 2008. Tracing suspended sediment and particulate phosphorus sources in catchments. *Journal of Hydrology* 350: 274-289.
- Webster, J. R. 2007. Spiraling down the river continuum: stream ecology and the U-shaped curve. *Journal of North American Benthological Society* 26: 375-389.
- Wehr, J.D., J. H. Thorp. 1997. Effects of navigation dams, tributaries, and littoral zones on phytoplankton communities in the Ohio River. *Canadian Journal of Fisheries and Aquatic Sciences* 54: 378-395.
- Welker, M. and N. Walz. 1998. Can mussels control plankton in rivers? A planktological approach applying a Lagrangian sampling strategy. *Limnology and Oceanography* 43: 753-762.
- Welschmeyer, N.A. 1994. Fluorometric analysis of chlorophyll *a* in the presence of chlorophyll *b* and pheopigments. *Limnol. Oceanogr.* 39:1985-1992.
- Wetzel, R. G., G. E. Likens. 1991: *Limnological analyses*. 2nd Ed. Springer-Verlag, New York.
- Welti, N., E. Bondar-Kunze, M Mair, P. Bonin, W. Wanek, G. Pinay, T. Hein. 2012. Mimicking floodplain reconnection and disconnection using ¹⁵N mesocosm incubation. *Biogeosciences* 9: 4263-4278.
- Whiles, M. R. and W. K. Dodds. 2002. Relationships between streams size, suspended particles, and filter feeding macroinvertebrates in a Great Plains Drainage Network. *Journal of Environmental Quality* 31: 1589-1600.

Wissel, B., A. Gace, B. Fry. 2005. Tracing river influences on phytoplankton dynamics in two Louisiana estuaries. *Ecology* 86: 2751-2762.

Withers, P. J.A., H.P. Jarvie. 2008. Delivery and cycling of phosphorus in rivers: a review. *Science of the Total Environment* 400: 379-395.

Wollheim, W. M., C.J. Vorosmarty, B. J. Peterson, S. P. Seitzinger, C.S. Hopkins. 2006. Relationship between river size and nutrient removal. *Geophysical Research Letters* 33: *L06410*.

Zhang, Q., D.C. Brady, W. P. Ball. 2014. Long term seasonal trends of nitrogen, phosphorus and suspended sediment load from the non-tidal Susquehanna River Basin to Chesapeake Bay. *Science of the Total Environment*. 452-453- 208-221.

CHAPTER 2: SUBSIDY OF A RIVER FOOD WEB BY ALGAL GROWTH IN A RUN-OF-RIVER IMPOUNDMENT UNDER VARYING LEVELS OF HYDRAULIC FLUSHING

ABSTRACT

River food webs can be based on locally produced benthic algae and on imports of material from the watershed and, in many systems, from upstream lakes and reservoirs as well. Dams are important because they can transform seston, trapping sedimented material and often producing suspended algae (phytoplankton), and algal export from reservoirs can provide subsidies to downstream food webs. This study uses natural abundances of stable carbon isotopes to quantify possible food sources along the river and track the subsidy from a run-of-river reservoir (Morrow Lake on the Kalamazoo River, southwest Michigan). I examined the hypothesis that phytoplankton were more ^{13}C depleted than benthic algae and that this would be reflected in the seston as well as in macroinvertebrates that feed directly or indirectly on seston. This study considered certain consumers as indicators of basal food isotope ratios (Pleuroceridae snails and *Corbicula fluminea*) as well as previous studies to estimate consumer food sources. One taxon in particular, the filter-feeding caddisflies of the family Hydropsychidae, showed the greatest response to the subsidy strongly as indicated by both carbon isotope ratios and abundances, while other taxa responded weakly to the subsidy. This study highlights that seston is important to food webs in rivers the size of the Kalamazoo. Reservoirs produce suspended algae, a food subsidy that is propagated downstream in consumers. The subsidy extended downstream to ~8 km on average, possibly the longest food web subsidy documented downstream from a reservoir.

INTRODUCTION

Dams are ubiquitous features on our nation's rivers (Benke 1990), and have major impacts on flow regimes and river biota (Carlisle et al. 2010, Dynesius and Nilsson 1994, Stanford et al. 1996, Poff et al. 2007, Lehner et al. 2011). Dams on rivers can be generally distinguished as either variable storage or run-of-river designs. Due to increased interest in hydropower worldwide, run-of-river hydroelectric dams are an attractive renewable energy source both in the United States and abroad (Francois et al. 2016, Kumar and Katoch 2014, Winemiller et al. 2016). Run-of-river dams in particular have been portrayed as less environmentally damaging and more carbon neutral, though this is increasingly being challenged (Deemer et al. 2016).

Run-of-river reservoir operation has important differences compared to variable-storage reservoir operation. Water inflows are approximately balanced by outflows in a run-of-river operation, and there is no large seasonal change in storage volume. Many run-of-river reservoirs are relatively shallow and thus tend not to develop persistent thermal stratification. In comparison to hypolimnetic release dams, run-of-river dams often release water from the surface (or the entire water column if it is shallow). They may have little effect on discharge, but can increase water residence time in the impoundment long enough to produce a lacustrine environment in the reservoir that facilitates the growth of suspended algae and subsequent export of algae in outflowing water (Reid and Hamilton 2007).

The role of dams in affecting downstream river food webs and ecosystems has been studied mainly in larger impoundments, and particularly in the western United States, where most are of the variable storage design. Hypolimnetic (or deep release) storage reservoirs are particularly known for altering ecosystem processes in rivers downstream of them, and can

decrease summer water temperatures (Ward and Stanford 1983, Angradi 1994, Vinson 2001, Cross et al. 2011, Kelly et al. 2013). Dams can affect downstream aquatic food webs both because they capture upstream organic material transported by the river, and if the water residence time is long enough, they can export algae produced within the reservoir (Angradi 1993, Angradi 1994, Palmer and O’Keefe 1990, Voshell and Parker 1985, Vinson 2001, Holt 2015, Doi et al. 2008).

Previous research on smaller dams has focused on how dams and their removal affect stream macroinvertebrate communities (Grubbs and Taylor 2004, Hansen and Hayes 2012, Stanley et al. 2002), mussels (Singer and Gangloff et al. 2011, Stanley 2002, Gangloff et al. 2011) and fishes (Santucci et al. 2005, Cumming 2004, Wang et al. 2011, Benstead et al. 1999, Catalano et al. 2016, Puolos et al. 2014). Run-of-river dams also may be important for trapping and altering the composition of seston (Singer 2011) as has been observed in hypolimnetic release dams (Ulseth and Hall 2015, Angradi 1994). This could have important food web consequences (Doi et al. 2008). Filter-feeding caddisflies have been observed to increase in abundance downstream of reservoirs and lake outlets (i.e. Oswood 1979). However, studies on whether and how run-of-river dams impact food webs are just emerging (Singer and Gangloff 2011, Hornbach et al. 2014, Mackay and Waters 1986, Doi et al. 2008).

Run-of-river dams are defined loosely, however, and these dams can vary greatly in height and water residence time. Particularly small ones can be privately owned and may not be registered (Csiki and Rhoads 2015, Orr et al. 2004). While the individual impact of a small run-of-river dam may be less than a variable storage dam in the same location, run-of-river dams may have significant cumulative effects on downstream water quality, particularly if closely

spaced (Santucci et al. 2005, Nichols et al. 2006, Kibler and Tullos 2013, Zhang et al. 2013, Oliver et al. 2014).

Compared to headwater streams, food webs in free-flowing, medium-sized rivers are thought to be based on benthic algal growth given the open canopy and shallow depth that permits light to reach the bottom (Vannote et al. 1980). Seston, and specifically small particles of transported organic matter (i.e., fine particulate organic matter - FPOM), is an important food source for stream invertebrates. It is composed of variable proportions of algae and/or detrital material derived from terrestrial vascular plants, and may also include detritus from aquatic macrophytes, invertebrate fecal pellets, and flocculated DOM (Hershey et al. 1996, Cummins et al. 1981, Grubaugh et al. 1997, Minshall et al. 1983, Wallace et al. 1997, Naiman 1983). Studies using natural abundances of stable carbon and nitrogen isotopes increasingly have suggested that algal production is important in food webs of diverse river and stream ecosystems (Hamilton et al. 1992, Thorp et al. 1998, Lewis et al. 2001, Jardine et al. 2012, McCutchan and Lewis 2002, Rounick et al. 1982, reviewed by Roach 2013), and this conclusion has sometimes been supported by simultaneous analyses of consumer gut contents (McNeely et al. 2006, Collins et al. 2015). It is important to note that terrestrial organic matter is also significant (Zeug and Winemiller 2008, Benke 2015, Caraco et al. 2010). Given the variable composition of river seston, its importance to food webs is still not well understood.

Benthic macroinvertebrate communities often show marked differences below dams compared to otherwise similar upstream reaches, suggesting differences in the base of the food web (sensu Doi et al 2008, Mackay and Waters 1986). Changes in seston could have important impacts on macroinvertebrates, especially filter feeders because they consume suspended FPOM (Grubaugh et al. 1997, Benke 2015). Changes in food sources can extend to emerging adult

insects, which provide food web subsidies (Polis et al. 1997, Nakano et al. 1999) to the riparian terrestrial ecosystem by transferring organic matter (energy) from the aquatic environment as food for spiders, birds and bats (Baxter et al. 2005, Marczak and Richardson 2007, Paetzold et al. 2006, Sanzone et al. 2003, Sabo and Power 2002, Walters 2008, Marcarelli et al. 2011).

The water residence time in run-of-river reservoirs varies temporally as a function of river discharge and may fluctuate between more lentic and more lotic conditions (Reid and Hamilton 2007). In low flow years, water residence time will be longer, which may produce more algae that subsidize the downstream food web (Reid and Hamilton 2007). Higher flow reduces the water residence time in the reservoir and can inhibit phytoplankton production, but the higher flow can also transport the phytoplankton subsidy further downstream, and entrain benthic algae or particulate organic matter into the water column along the river (Newbold et al. 2005, Strayer et al. 2008, Lucas et al. 2012). Food webs of rivers have often been considered to be subsidized by organic matter inputs from upstream terrestrial sources (Vannote et al. 1980) and, in larger rivers, by organic matter inputs from lateral floodplains (Junk et al. 1989, Jardine et al. 2015).

OBJECTIVES

In this chapter, I examine the role of a run-of-river reservoir in providing organic matter subsidies to downstream aquatic and riparian food webs. Natural abundances of stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotopes are examined along with macroinvertebrate densities. I sampled during three years of varying flow conditions that created water residence times in the reservoir ranging from a few days to over a week. Comparison of river food webs above and below the reservoir provides a “natural experiment” to examine the effect of an algal subsidy on

food webs. I used stable isotopes to estimate how far downstream the subsidy extended under high and low flow conditions. I quantified benthic macroinvertebrates by measuring changes in densities of filter feeders and collector-gatherers vs. scrapers in reaches with varying amounts of phytoplankton and benthic algae. Riparian spiders (Tetragnathidae) were used to indicate the isotopic composition of emerging aquatic insects that serve as food sources to the terrestrial food web.

My hypotheses are based on the common observation that phytoplankton tend to have the most depleted ^{13}C compared to other major organic matter sources in many lakes. Vascular-plant detrital $\delta^{13}\text{C}$ should be dominated by C-3 plant sources in the Kalamazoo River, which is lined by a broad and largely forested floodplain. I predicted that stable carbon isotope ratios of invertebrates in reaches receiving phytoplankton subsidies would be closer to phytoplankton $\delta^{13}\text{C}$ and distinct from the $\delta^{13}\text{C}$ of terrestrial organic detritus and benthic algae (periphyton) that support consumers upstream. Filter-feeder $\delta^{13}\text{C}$ would be similar to periphyton upstream of the reservoir, being supported by dislodged benthic algae that are enriched in ^{13}C , but would be ^{13}C -depleted downstream of the reservoir where suspended algae are a more important component of the seston. I further predicted that this shift in food sources would result in altered community structure of invertebrates, with higher densities of filter feeders immediately downstream of the reservoir, while further downstream reaches with lower concentrations of suspended algal biomass should also have lower densities of filter feeders.

METHODS

Study site

The Kalamazoo River drains a 5230 km² watershed in southwestern Michigan (Figure 2.1). The river is 260 km long with a mean discharge of 38 m³/s at the Comstock (4106000) USGS gauge. I sampled in the middle section of the river, which has two larger reservoirs. The upper reservoir (Morrow Lake), which is the primary focus of this chapter, has a mean depth of 1.6 m whereas the lower reservoir (Lake Allegan) is larger and has a mean depth of 3 m. Morrow Lake is 70 km upstream of Lake Allegan.

The Kalamazoo River was one of the original River Continuum Concept study sites, distinguished by its abundance of fine particulate organic matter and filter feeding insects, although the River Continuum study reach was located upstream of my study reach to avoid reservoirs and pollution from the Kalamazoo area (Cummins et al. 1981). While the water quality of the river has since improved, anthropogenic enrichment with nitrogen and phosphorus remains significant, and the lowermost reservoir (Lake Allegan) has a Total Maximum Daily Load for phosphorus due to a history of algal blooms.

Morrow Lake has water residence times of a few days to over a week during the summer (Reid and Hamilton 2007). Past work has found that suspended algae, as measured by chlorophyll-*a*, are considerably more abundant in the reach immediately below Morrow Lake than above it. The source of suspended algae downstream of Morrow Lake is not necessarily limited to phytoplankton growth in the reservoir because the reservoir is shallow and the bottom has benthic algal mats, which can become dislodged and suspended (Reid and Hamilton 2007, Baas 2009). Suspended algae (measured as chlorophyll-*a*) have been observed to decrease

substantially in the free-flowing reach downstream of Morrow Lake, although this is most readily apparent at low flows (Reid and Hamilton 2007).

Beginning 30 km downstream of Morrow Lake, there are several additional former hydropower dams that have been partially dismantled but still have some impounded waters above them (Figure 2.1). PCB-contaminated sediment behind these reservoirs has so far precluded their full removal, although one (Plainwell Dam) was removed in 2009 and another (Otsego Township Dam) is in the process of being removed. Residence times of water behind the three decommissioned dams is short, probably totaling around one day at lower flows (Reid and Hamilton 2007). These partially removed dams create a section of river that I will refer to as semi-impounded.

Downstream in the semi-impounded reaches, an increase in chlorophyll-a concentrations has been observed at summer low flows. It is not understood how this algal production can occur when residence times are less than 1-2 days in the semi-impounded reaches; benthic algal production and dislodgment may explain it (Reid and Hamilton 2007).

Thus, the impoundments created by these larger and smaller dams span a range of water residence times, fostering the growth of phytoplankton to varying degrees. This series of impoundments provides an ideal setting to study the relationship between dams, water residence time, and the importance of algae exported from reservoirs to downstream food webs (Fig. 1). The Kalamazoo River is also an ideal setting for studying the influence of impoundments on phytoplankton production because there are few tributaries in the study reach, and the water residence times of the run-of-river reservoirs are directly and inversely proportional to the main stem river discharge.

Discharge

Discharge data for US Geological Survey's Comstock gauging station (4106000) were obtained from the National Water Information System. Discharge was averaged by water year (1 Oct-30 Sep). The mean discharge for each water year (2008, 2010, 2012) was compared against a long-term mean (1937-2016) by a paired T-test. This was also done for daily discharge during summer (1 May-30 Sep).

Water and algal sampling and analysis

Water samples for analysis of suspended particulate matter were collected biweekly in 2008, over the month of July 2010, and in early July 2012. Samples were first filtered through a 250-micron sieve to remove coarse particulate matter and larger invertebrates. Particulate matter was then collected on Pall A/E glass fiber filters that were pre-combusted at 450°C for at least one hour. Water samples were filtered in the field in 2008, wrapped in foil, and transported to the lab to be frozen before analysis. In 2010 and 2012, samples were collected in 250 mL bottles and stored in a cooler with ice. These were filtered in the lab on the day of collection. Samples collected in 2008 showed the most consistent downstream increase in chlorophyll-*a* over the month of July, so in subsequent years, food-web samples were collected during July.

Benthic algal production was measured as the accumulation of chlorophyll-*a* on clay bricks, which served as a standard substratum resembling the natural cobbles in the river. Bricks were placed in similar flow velocities in each site, and they were placed among cobbles to the extent possible to minimize variation in turbulence that could impact benthic algal $\delta^{13}\text{C}$ (Finlay et al. 1999). Some site to site variation existed; flow varied from 0.20 to 0.50 m s⁻¹ (Table 2.1). Bricks were left in the river for at least 6 weeks, then carefully removed and placed in aluminum baking pans, covered with foil and stored on ice. Bricks were processed on the day of collection

or in a few cases within 24 hours of collection. Bricks were removed in 2010 when discharge reached $35 \text{ m}^3 \text{ s}^{-1}$ and all were removed in a two-week period in July for both years.

Benthic algae on the bricks were removed by scrubbing the upper surface with a nail brush and rinsing the dislodged material into an enamel pan. The mixed algal slurry was subsampled into a 100-mL graduate cylinder. In 2012, the method was modified where the slurry was mixed in a one-liter jar, allowing the coarsest particulate matter to settle. The upper fraction was subsampled in a 30-mL syringe. This sample was poured through a 250-micron sieve to strain out filamentous algae and macroinvertebrates, and the particulate material that passed the sieve was collected onto precombusted Pall A/E glass fiber filters. Comparisons of methods found that allowing heavier material to settle led to a decrease in the calculated chlorophyll-*a* density of up to 10 mg m^{-2} (mean, 5 mg m^{-2}) and that difference was significant (paired t-test, $DF=3$, $t\text{-stat}=2.33$). Samples were wrapped in foil and frozen to be processed later.

Chlorophyll-*a* samples were kept frozen until they could be extracted in 95% ethanol for 12-24 hours at 4°C . Chlorophyll-*a* was measured with a Turner Fluorometer using the method of Welschmeyer (1994). Suspended chlorophyll-*a* was measured within one month in 2008 and 2012, but in 2010, samples were measured after two years of frozen storage. All benthic algal samples were analyzed after two years. Subsequent storage tests for benthic and algal samples revealed that there was no change in concentrations up to three months (Figure 2.15), but there was a 50% decrease after 6 months, after which measurements were consistent over two years. Suspended chlorophyll-*a* from 2010 may have decreased by up to 50% but the values from 2010 are within the range of values that would be expected given the nutrient and flow condition (Figure 2.15).

Macroinvertebrate sampling and isotopic analysis

To quantify macroinvertebrate communities along the river, I deployed 14-plate Hester Dendy artificial substrata in eight sites. These were placed in the river in July 2010 and 2012 and removed in late September of those years. Samplers were scraped, and the dislodged material passed through a 1 mm sieve and preserved with 95% ethanol. This method was selected over kick sampling (Stanley et al. 2002, Santucci et al. 2012) or Surber samplers (Doi et al. 2008), as neither of these was appropriate given the wide range of substrata in the Kalamazoo River. Hester Dendy samplers served as a standard substratum targeting macroinvertebrates that use hard substrata. Taxa like mussels and Chironomidae that dwell in soft substrata are not sampled correctly with this method (Benke 2015). Samplers were placed in areas of comparable flow, and where surrounding substrata were a mix of cobble, gravel and sand. Bricks and Hester Dendy samplers were placed in the same locations. One Hester Dendy sampler per site was subsampled and at least 300 individuals were counted per sample. Paired t-tests were performed after pooling upstream sites—denoted as Fort Custer (2010 and 2012) and Galesburg (2010 and 2012)—as four replicates and comparing these to pooled samplers downstream of Morrow Lake: Below (below Morrow and Mosel Ave); Downstream (D-avenue and Plainwell); and Semi-impounded (Otsego Township Dam and the Township Dam near Allegan, MI).

For measurements of the stable isotope ratios in consumers, macroinvertebrates dwelling in natural cobble substrata were collected in flowing water habitats in early July and August of 2008 and in July 2010 and 2012. The macroinvertebrates were placed in centrifuge tubes containing river water at ambient temperature and transported to the lab in 2008, thereby allowing their guts to clear for 24 hours. Macroinvertebrate gut contents were not allowed to clear in 2010. In July 2010 and 2012, I collected benthic macroinvertebrates soon after the river

levels dropped to typical summer discharge, and macroinvertebrates were only sampled from bricks. Material scrubbed from clay bricks was put directly into zip lock bags and transported to the lab and frozen. In 2012, additional macroinvertebrates were collected from cobble. Some taxa were allowed to clear their guts in unfiltered water from a nearby lake for 24 hours. Hydropsychidae with gut contents removed were 0.45‰ more depleted than samples with gut contents (Paired t test, DF=3, t stat 1.59). Heptageniidae taxa (n=2) were 0.46 ‰ enriched after guts were removed, while Amphipoda (n=1) was 0.24‰ depleted and *Macrostemum* (n=1) was 0.79 ‰ enriched after guts were removed. In this study, I am more interested in general patterns along the river. In making comparisons between 2010 and 2012 samples, care was taken to use individuals with guts contents not cleared (Table 2.2).

Spiders from the family Tetragnathidae were sampled because they are obligate riparian predators feeding on emerging riverine insects (Sanzone et al. 2003, reviewed by Baxter et al. 2005). Their webs were full of emerged aquatic insects including Chironomidae and Trichoptera (personal observation). In early August 2010 and late July 2012, Tetragnathidae were collected from overhanging vegetation along the river in Ziploc bags and transported back to the lab on ice and frozen for up to 3 years. Spiders were collected from three sites above Morrow Lake to 10 sites downstream into the semi-impounded reaches. In 2012, spiders were collected from 4 sites 30 km downstream of Morrow. Spiders were collected from over Morrow Lake, two sites above Fort Custer Recreational Area, and over three smaller reservoirs upstream of Battle Creek, MI.

Multiple individuals of spiders and macroinvertebrates were pooled for stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotopic analysis, rinsed in DI water and dried at 50°C for five hours. Subsamples of 0.7 – 6 mg were weighed to the nearest 0.001 mg into tin capsules. Samples were ground in a mortar and pestle, weighed into tin capsules, and sent to the University of California,

Davis stable isotope facility for analysis, where they were run on a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer. Bovine liver, USGS-41 Glutamic Acid, Nylon 5 and Glutamic Acid were run as reference values. On occasion isotope samples were run with replicates.

Isotopic measurements focused on Hydropsychidae and Heptageniidae because they were the most abundant taxa in the river. Chironomidae were also abundant but represent a diverse trophic group, and therefore were not investigated (except as indicated by the spiders). I analyzed *Macrostemum* separately from other Hydropsychidae because their diets may be distinct given mesh size differences in their nets (Benke 2015).

Dissolved CO₂ partial pressure

The $\delta^{13}\text{C}$ of dissolved inorganic carbon (DIC) is an important driver of variation in the $\delta^{13}\text{C}$ of lake plankton (Bade et al. 2006, Gu et al. 2011, Smyntek et al. 2012, Zohary et al. 2001) and stream benthic algae (Finlay et al. 2004). For example, algae tend to be relatively ^{13}C depleted when dissolved CO_2 used by aquatic photosynthesis occurs at high concentrations in water bodies due to an excess of aquatic respiratory production over photosynthetic uptake (Finlay et al. 2004). This is more likely in lakes and wetlands than in well-aerated streams. Finlay et al. (2004) described changes in the $\delta^{13}\text{C}$ of benthic algae from headwater streams to downstream river reaches. They observed that changes in DIC and pCO_2 caused ^{13}C enrichment of benthic algae in downstream reaches, which has also been observed elsewhere (Rasmussen 2010, Kobayashi et al. 2011, Walters et al. 2007, Sullivan 2013). Aquatic primary production in a reservoir may reduce dissolved free CO_2 (Crawford et al. 2016). Some food-web studies include natural abundance $\delta^{13}\text{C}$ measurements of DIC (e.g. Junger and Planas 1994, Rounick et

al. 1982, Caraco et al. 2010, Jepsen and Winemiller 2007) though such studies in rivers are fewer.

The dissolved CO₂ partial pressure (pCO₂) has been found to be important for understanding the $\delta^{13}\text{C}$ of aquatic primary producers in lakes (Bade et al. 2006), although it has only occasionally been considered in river studies (Caraco et al. 2010, Van den Meersche 2009, Jepsen and Winemiller 2007). In this study, I measured temperature, conductivity and pH in the field. Total alkalinity was measured using the Gran titration on unfiltered water (Wetzel and Likens 1991). Partial pressure of CO₂ was calculated from pH, alkalinity, temperature and major ions (Kempe 1982, Hamilton et al. 1995, Calheiros and Hamilton 1998). Ionic strength was ~ 0.01 and pH was ~8.10 with a few pH measurements ~ 8.6.

Data analysis

Previous measurements from a nearby tributary have found high overlap between terrestrial organic matter sources and benthic algae (Hamilton, unpublished data), and seston in the river is likely a mix of terrestrial and algal organic matter (Hamilton et al. 2005). While natural abundance carbon isotopes have been used effectively in river food web studies (Bunn et al. 2003), variation and overlap in the $\delta^{13}\text{C}$ of basal food sources can present challenges (Bade et al. 2006, Middleburg 2014). Because of this, I expected poor separation and significant overlap of end members (Peterson and Fry 1987). Since algal organic matter is difficult to separate from detrital organic matter in mixtures of fine particulate material, I did not measure algal isotope ratios directly in this study.

I focused on the longitudinal and seasonal patterns in $\delta^{13}\text{C}$ by frequently sampling consumers, rather than separating allochthonous and autochthonous fine particulate matter. To infer the possible $\delta^{13}\text{C}$ of suspended algae and benthic algae I used as indicators snails (most

likely in the family Pleuroceridae) and Asiatic clams (*Corbicula fluminea*), both of which are long-lived consumers known to specialize on benthic or suspended algae, respectively (Cabana and Rasmussen 1996, Post 2002, Vander Zanden 1996, Fry et al. 2008, Jardine et al. 2012, Vander Zanden et al. 2011).

Lipid synthesis produces tissue that is more depleted than protein or carbohydrates (DeNiro and Epstein 1977), which reduces the ability to estimate food sources (Post et al. 2007, Logan et al. 2008). Models for lipid corrections based on measured C:N in consumers are not as effective as direct lipid extractions, and two samples must be run because lipid extraction alters $\delta^{15}\text{N}$ (de Lecea and de Charmony 2015). Data in this study are reported as not lipid corrected because variation of consumer- $\delta^{13}\text{C}$ is likely greater than variation related to lipids. However, to have some estimate of possible response of consumer $\delta^{13}\text{C}$, I used equations from Post et al. (2007) developed for aquatic animals, and Logan et al. (2008) developed for Heptageniidae to calculate corrections (Table 2.2). Linear regression corrections for a whole invertebrate body samples are different than for muscle tissue (Logan et al. 2008, Post et al. 2007). I used uncorrected $\delta^{13}\text{C}$ data for comparisons with previous studies (DeLong and Thorp 2006, Kautza and Sullivan 2016, Herwig 2007, Leigh et al. 2010), and because lipid correction models are currently better developed for fish organs than for the whole macroinvertebrates I measured (Logan et al. 2008, Post et al. 2007). The high C:N in Heptageniidae relative to other taxa could be an indication that lipids are relatively important (Logan et al. 2008). Given that general patterns were similar with and without corrections, I decided that presenting and analyzing data without lipid corrections was preferable in my study (Leigh et al. 2010). However, variable lipid contents in macroinvertebrate tissues could lead to ^{13}C depletion compared to food sources, and that possibility should not be ignored.

Statistics were performed using two-way T-tests comparing the upstream Fort Custer site with each site downstream of Morrow Lake. An additional comparison separated two functional feeding groups as in Rasmussen (2010): Filter feeders (Hydropsychidae and *Macrostemum* caddisflies, *Corbicula* mussels, and *Isonychia* mayflies) and grazers (Amphipoda sideswimmers, Heptageniidae mayflies, Pleuroceridae snails, and *Orconectes* spp. crayfish) by site. I also did this with the common taxa Amphipoda, Hydropsychidae and Heptageniidae, which I compared between upstream and downstream in 2008 and 2012.

A final test individually examined diets of the most abundant macroinvertebrates (Hydropsychidae, Heptageniidae, Amphipoda) by comparing the $\delta^{13}\text{C}$ of each macroinvertebrate taxon. For above and below comparisons in 2010, replicates were pooled by site: upstream sites to sites below the reservoir (below and 8 km), downstream (16 and 30 km) and in the semi-impounded reach. In 2012, taxa with guts cleared and not cleared were used as replicates. I compared the Fort Custer with each downstream site (below Morrow Lake, 8km, 16 km, 30 km, and the two semi-impounded reaches). In 2008, replicates were collected at each site, and Fort Custer was compared with 16 km and 30 km downstream of Morrow Lake data collected in late July 2008. These comparisons were used to determine how far downstream the reservoir algal subsidy extended.

Lastly, I compared taxa from the highest discharge year (2010) to the lowest discharge year (2012) to understand the overall influence of discharge. Paired t-tests were conducted for common taxa (e.g., between Hydropsychidae in 2010 vs. 2012. This test was performed for spiders, Amphipoda and Heptageniidae. Statistics were performed in Microsoft Excel.

RESULTS

Discharge

The average discharge regime of the Kalamazoo River typically shows highest discharges in the spring after rains and snowmelt and lowest discharges in summer and fall, though with substantial year to year variation in snowmelt and precipitation events (Figure 2.2). However recent years have brought a noticeable increase in summer flood pulses as a result of intensifying precipitation (personal observations), as has been observed throughout the Midwest and Northeast U.S. (Pryor et al. 2014).

The summers of 2008 and 2010 had several strong precipitation events, and river discharge was above average for both years (paired T-test, t stat=5.62 and 15.63, DF=152). In 2008, a spring flood reaching 65 m³/s occurred in mid-April, decreasing to ~30 m³/s by 1 May. Two storm events in early June and early July 2008 doubled the discharge in 24 hours to 56 m³/s, decreasing to ~30 m³/s after each event. By August, discharge had fallen to around 18 m³/s, closer to the long-term average. In 2010, spring high flow occurred in mid-March at 85 m³/s and often remained above 50 m³/s through to July. Discharge increased to ~90 m³/s again on 26 July and returned back to more normal discharge for the rest of the water year.

Overall, the discharge during the water year 2012 was close to the long-term average (paired T-test, t stat=0.300, DF=364), whereas discharge was above average in 2008 and 2010 (paired T-test, t stat=8.80 and 5.62, DF=364). In contrast, summer 2012 was below average for that season (paired T-test, t stat=-20.87, DF=152). In 2012, a spring discharge peak occurred in early March at 72 m³/s and decreased to ~30 m³/s by May and was around 12 m³/s through the summer with no large precipitation events. Summer rain events in 2008 and 2010 resulted in

very high summer discharges, while 2012, though low and close to a record low, was not the lowest on record, which occurred in 1934.

Algal biomass

Suspended chlorophyll-*a* (i.e., suspended algal biomass) below the Morrow Lake dam varied both longitudinally and temporally (Figure 2.3). Multiple samplings in 2008 (Figure 2.3a) showed that chlorophyll-*a* concentrations were consistently relatively low ($\sim 3 \mu\text{g/L}$) above the Morrow Lake reservoir, increasing downstream of the dam to $\sim 20 \mu\text{g/L}$ by mid-May, and peaking at $\sim 37 \mu\text{g/L}$ in mid-July. Chlorophyll-*a* below the dam remained high through July and decreased in August and October. Transect sampling further downstream of the reservoir showed that chlorophyll-*a* concentrations decreased, although in 2008 and 2010, concentrations in the reaches at 16 and 30 km did not return to levels comparable to upstream (Figure 2.3a and 2.3b). In 2012, chlorophyll-*a* returned to levels comparable to upstream by 16 km below the dam.

Benthic chlorophyll-*a* densities, which indicate attached algal biomass on rock surfaces, averaged 49 mg/m^2 in July 2010 and 27 mg/m^2 in July 2012 (Figure 2.4). Benthic chlorophyll-*a* densities were not significantly different between years (paired t-test, $t \text{ stat}=1.64$, $DF=6$), nor did they show consistent longitudinal patterns in relation to Morrow Lake.

Macroinvertebrate densities

Macroinvertebrate colonization on Hester Dendy samplers was dominated by Hydropsychidae, Heptageniidae and Chironomidae (Figure 2.5a). Hydropsychidae were present along the river just above Morrow Lake, but became extremely abundant immediately below the Morrow Lake dam (Table 2.3). They decreased downstream and in the semi-impounded sites, although still occurring at statistically greater densities than upstream. Heptageniidae and

Chironomidae varied spatially along the river, but there was no clear pattern in relation to Morrow Lake, nor was there a statistical difference among the sites. Philopotamidae were common in the downstream reaches. Other taxa such as Amphipoda, Leptohiphidae, *Isonychia*, and Baetidae were present but not abundant (Figure 2.5b). Taxa mentioned above represent 90-99% of the individuals found on each Hester Dendy sampler.

Stable isotope ratios of consumers

Individual comparisons

Comparing the $\delta^{13}\text{C}$ among taxa, Hydropsychidae and Heptageniidae were more ^{13}C -enriched compared to other filter feeders such as *Corbicula*, *Isonychia*, and *Macrostemum*, but more ^{13}C -depleted compared to Pleuroceridae snails, crayfish and Amphipoda (Figures 2.6 and 2.7). Above the reservoir, Hydropsychidae were -31 ‰ in 2010 and -29 ‰ in 2012, and Heptageniidae were -31 ‰ and -30.6 ‰ in 2010 and 2012 (Figure 2.6a and 2.6b, Table 2.3). Immediately downstream of Morrow Lake, both Hydropsychidae and Heptageniidae were ^{13}C -depleted by up to 3 ‰. Further downstream of the dam, they became as ^{13}C -enriched as at upstream sites (Figure 2.7a, Table 2.3). Only Hydropsychidae was significantly ^{13}C -depleted downstream of Morrow Lake in 2008 and 2010, and only marginally significantly so in 2012 (Table 2.3). The $\delta^{13}\text{C}$ values of Hydropsychidae and Heptageniidae in 2008 were comparable, though intermediate to 2010 and 2012 (Figure 2.8).

The distance downstream of Morrow Lake where consumer $\delta^{13}\text{C}$ returned to being comparable to upstream depended on the year. In 2008, Hydropsychidae were ^{13}C -depleted downstream though 16 km to 30 km in 2008, which was statistically significant. I was not able to detect how far the subsidy traveled in 2010 and 2012 in Hydropsychidae and Heptageniidae. Hydropsychidae $\delta^{13}\text{C}$ appeared to be comparable to upstream by 16 km downstream in 2010 and

2012, but not statistically. Hydropsychidae and Heptageniidae $\delta^{13}\text{C}$ values were slightly ^{13}C -depleted above the reservoir, and therefore, did not show significant differences in $\delta^{13}\text{C}$ among the sites in 2010.

Tetragnathidae spider $\delta^{13}\text{C}$ (Figures 2.6c, 2.7b) followed a similar pattern to Hydropsychidae and Heptageniidae $\delta^{13}\text{C}$. The $\delta^{13}\text{C}$ values of these spiders were comparable to Amphipoda, and ^{13}C -enriched compared to Hydropsychidae and Heptageniidae. In both years, spiders were ^{13}C -depleted downstream of Morrow Lake compared to upstream, at $\sim -32\text{‰}$ in 2010 and -30‰ in 2012. Spider $\delta^{13}\text{C}$ was statistically depleted downstream when pooling spiders from downstream (below Morrow and Mosel Avenue (8 km)), where spiders were -30‰ in 2012 and -31.2‰ in 2010. Spiders were still 2‰ depleted in the downstream (16 km and 30 km) reaches in 2010, and remained relatively ^{13}C -depleted within the semi-impounded reaches, a difference that is significant statistically. Compared to immediately below the dam, spiders were relatively enriched in ^{13}C by 16 km downstream in 2012.

Besides Hydropsychidae and spiders few other taxa were found to be significantly ^{13}C -depleted downstream of Morrow Lake compared to upstream. *Corbicula* in 2012 were significantly ^{13}C -depleted both below Morrow Lake and in the downstream reaches, but not in the semi-impounded reaches (Table 2.3). *Macrostemum* and *Isonychia* did not differ in $\delta^{13}\text{C}$, whereas Amphipoda were ^{13}C -depleted in the downstream reaches.

Functional feeding groups

Taxa can also be compared by pooling them into functional feeding groups (Figure 2.9a and 2.9b, Table 2.3). Filter-feeding macroinvertebrates (Hydropsychidae, *Isonychia* spp., *Macrostemum* spp. and *Corbicula fluminea*) were more ^{13}C -depleted than the other functional feeding groups (grazers and collector gatherers). The caddisflies (*Macrostemum* and

Philopotamidae) were even more ^{13}C -depleted at -35 ‰ and -36 ‰ in 2010 and -31 ‰ to -33 ‰ in 2012. *Corbicula* ranged from -30 ‰ above Morrow Lake to -34 ‰ downstream of the lake, then became more ^{13}C -enriched downstream. Filter feeders were significantly ^{13}C -depleted downstream of the reservoir compared to above it, but at 16 to 30 km and in the semi-impounded reaches they were statistically similar in $\delta^{13}\text{C}$ of filter feeders in the upstream reaches (Table 2.3). Benthic feeders can be considered another functional feeding group; they would include grazers, scrapers and collector gatherers (Heptageniidae, Pleuroceridae snails, Amphipoda and crayfish) (Figure 2.9d). The $\delta^{13}\text{C}$ values of this group were -30 ‰ below the reservoir and -28.8 ‰ above the reservoir (Table 2.3). While many benthic feeders were ^{13}C -depleted downstream of the reservoir relative to above it, the differences were generally not statistically significant, except at the Mosel Avenue site (8 km below Morrow Lake).

Comparisons between high and low discharge years

To understand the role of river discharge, comparisons were made between the samples collected at higher flows in 2010 and those collected at lower flows in 2012 (Figure 2.6, Figure 2.7, Table 2.3). Paired t-tests comparing sites across these two years indicate that Hydropsychidae and Heptageniidae were much more ^{13}C -depleted (by up to 2‰) in 2010 compared to 2012. Both Amphipoda and Pleuroceridae snails were not significantly different between 2010 and 2012. Tetragnathidae spiders were different between 2010 and 2012 by 1 ‰. This can be explained by the fact that spiders were likely similar above the reservoir and more ^{13}C -depleted by 2 ‰ downstream.

Baseline comparisons

I did not use a mixing model to estimate dietary contributions in this study. To provide an indication of likely food sources, Pleuroceridae snails and *Corbicula* were chosen to reflect grazing and filter-feeding diets, respectively, for 2010 and 2012 (Figure 2.11). The average $\delta^{13}\text{C}$ of snails, *Corbicula* and *Macrostemum* were also plotted to provide some estimate of range in $\delta^{13}\text{C}$ of food sources (Figure 2.11). However, *Corbicula* $\delta^{13}\text{C}$ follows patterns like other taxa, being enriched above the reservoir, though still depleted compared to other taxa. It is important to note that the $\delta^{13}\text{C}$ values of *Corbicula* were 3 ‰ depleted compared to snails in 2012.

Macrostemum was slightly more ^{13}C -depleted than *Corbicula*. Comparisons between *Corbicula* and Hydropsychidae highlight changing food sources along the river. Above Morrow Lake, *Corbicula* were enriched compared to this taxon collected below Morrow Lake, though they were depleted compared to Hydropsychidae. *Corbicula* were very ^{13}C -depleted downstream of Morrow Lake compared to most other taxa, with a gradual return to values similar to upstream of Morrow Lake. This suggests that *Corbicula* is consuming variable, and sometimes ^{13}C -depleted, food sources depending on location in the river system. Snails were ^{13}C -enriched, though less so downstream of Morrow.

Differences between Hydropsychidae and snails appeared greater in 2010 than 2012 where measured (Figure 2.12). Hydropsychidae and Heptageniidae were intermediate between the two baselines (Figures 2.11, 2.12). Compared to snails, the $\delta^{13}\text{C}$ values of Hydropsychidae and Heptageniidae were 3 ‰ more depleted in 2010 and 1 ‰ depleted in 2012. The $\delta^{13}\text{C}$ values of Hydropsychidae and Heptageniidae were 1.6 ‰ enriched compared to *Corbicula* in 2012. Both taxa were similar to snails above the reservoir where measured. Hydropsychidae and Heptageniidae were depleted in 2010, but because I lack snails for upstream reaches and

Corbicula in 2010, I am less able to explain patterns. Also, *Isonychia* closely followed patterns of Hydropsychidae when collected in both 2010 and 2012, while *Macrostemum* was more ^{13}C -depleted. Amphipoda and crayfish were ^{13}C -enriched compared to other taxa.

Influence of variable lipid content on consumer $\delta^{13}\text{C}$

The consumer $\delta^{13}\text{C}$ values reported in this study were not lipid corrected to facilitate comparison with most previous studies, and because lipid corrections present problems (see Methods). The C:N mass ratios of most macroinvertebrates were just outside ranges recommended for not correcting for lipid content, ranging between 4- 6 (with some up to 8) (Table 2.2).

Corrections for lipids were calculated to estimate the possible difference for consumer $\delta^{13}\text{C}$ if lipids had been extracted. This study used both the Post et al. (2007) model and the Logan et al. (2008) model to estimate corrections. Post et al. (2007) led to corrections of +1-3 ‰ while the Logan et al. (2008) correction was 1.2-1.7 ‰. The C:N ratio of Hydropsychidae averaged 5.5, which results in a correction of +2 ‰ using the Post et al. (2007) and +1.5 ‰ using Model 3 of Logan et al. (2008). The linear equation method using the $\delta^{13}\text{C}$ values of whole macroinvertebrates such as Heptageniidae, *Macrostemum*, Amphipoda and *Isonychia* had larger C:N ratios (~6), which led to greater lipid corrections of up to 2 ‰. Samples of muscle tissues (Snail) had lower C:N and thus smaller lipid corrections.

Most taxa had lipid corrections for $\delta^{13}\text{C}$ of +1.5-3 ‰, which was expected (Table 2.2). An important caveat is that Heptageniidae C:N mass ratios would lead to larger corrections than Hydropsychidae of up to +2.90 ‰ using the Post et al. (2007) model or about +1.5 ‰ using Logan et al. (2008). The larger enrichment corrections using the Post et al. (2007) model cause Heptageniidae ^{13}C to be more enriched than Hydropsychidae, and could shift interpretation. The

Logan et al. (2008) Model 3 specifically parameterizes for Heptageniidae, a taxon which has a higher C:N, and accounts for chitin. Model 3 of Logan et al. (2008) resulted in a lower correction of 1.8 ‰. Corrections for this model do not alter $\delta^{13}\text{C}$ values as much and would not change my interpretation. However, in this study, spatial patterns are likely greater than variation related to lipids, data are presented as lipid uncorrected for comparison with previous studies.

Dissolved carbon dioxide

The concentration of dissolved CO_2 in the Kalamazoo River ranged from 26 to 145 μM , well above values expected for equilibration with atmospheric CO_2 (Figure 2.13). There was a marked decrease in dissolved CO_2 immediately downstream of the reservoir (Paired test, t stat=4.06, $\text{DF}=6$), and then concentrations rose further downstream but remaining below concentrations measured above Morrow Lake by 16 km (Paired t test, t stat=3.92, $\text{DF}=9$), only returning to comparable concentrations by 30 km downstream (Paired t -stat=1.41, $\text{DF}=4$ $p>0.05$). This observed pattern was not consistent season to season. The dissolved CO_2 concentrations I frequently measured in 2008 were higher in spring and fall and decreased through the summer with the lowest observed in August at 25 μM . Comparing dissolved CO_2 between summers, July 2012 was slightly higher at 69 μM than July 2008. Dissolved CO_2 was higher in October 2008 than July 2008 (Paired T -test, t stat=5.48 $\text{DF}=4$). I did not measure dissolved CO_2 in 2010. While dissolved CO_2 was greater in the autumn in 2008, this was not the case in 2012 where dissolved CO_2 was marginally significantly higher in July 2012 at 61 μM than October 2012 which was 43 μM (Paired T -test, t stat=-3.52, $\text{DF}=11$).

It appears that for Hydropsychidae, $\delta^{13}\text{C}$ values were more depleted even when dissolved CO_2 was lower (Figure 2.14). Collected over a longer transect, spiders were enriched up to -27

‰, which was surprising (Figure 2.14). Spider $\delta^{13}\text{C}$ values became lower downstream. Spiders collected along the shorelines of Morrow Lake had a mean $\delta^{13}\text{C}$ of -29.5 ‰. Dissolved CO_2 from October 2012, which was collected over the same longitudinal transect, varied along the river.

DISCUSSION

In this study, I consistently found low suspended chlorophyll-*a* concentrations above the reservoir, a post-dam peak in chlorophyll-*a* concentrations (especially at lower flows), and decreasing concentrations downstream of the post-dam peak. The progressive loss of chlorophyll-*a* with distance downstream of Morrow dam appears to show a rate of decrease inversely proportional to discharge during the summer (Figure 2.3). The reservoir algal subsidy became apparent in early May and lasted through September (Figure 2.3a, see also Reid and Hamilton 2007). Benthic algae were abundant all along the river, and chlorophyll densities found on artificial substrata suggest a eutrophic river (Dodds 2006). I found that the passage of the Kalamazoo River water through the Morrow Lake reservoir changed the downstream food web from one being based on benthic algae, seston and terrestrial organic matter to one supported more by suspended algae produced within the reservoir.

Isotopic evidence

The distance downstream that the subsidy extended in consumers was unclear from the $\delta^{13}\text{C}$ data alone. I observed a large increase in chlorophyll-*a* downstream of Morrow Lake, which would suggest a switch in algal food availability from benthic to suspended algae. Many consumers become ^{13}C -depleted downstream as expected for a transition to predominantly phytoplankton as a food source. Hydropsychidae, Heptageniidae and Tetragnathidae (spiders)

exhibited the clearest response in all three years where taxa were ^{13}C -depleted immediately below the dam compared to upstream samples (Table 2.3). Hydropsychidae and Tetragnathidae ^{13}C were statistically depleted in ^{13}C all years studied, while Heptageniidae was significantly depleted only in 2008.

This study did not find strong evidence that there was enhanced uptake of transported phytoplankton during high flows. Chlorophyll-*a* patterns suggest that material was transported downstream but it was not necessarily assimilated subsidy was transported further downstream during high flows (2008 and 2010) compared to low flows (2012), even though chlorophyll-*a* concentrations suggested that algal biomass showed that pattern. Based on my observations for chlorophyll-*a*, I hypothesized that Hydropsychidae would remain ^{13}C -depleted for longer during high flows. Hydropsychidae collected in 2008 were intermediate between 2010 and 2012, but showed the clearest response to the reservoir-derived phytoplankton. Hydropsychidae was significantly ^{13}C -depleted to 30 km in 2008 (Figure 2.6). In 2010 and 2012, Hydropsychidae ^{13}C was statistically enriched by 8 km in both low and high flow summers. It appeared that consumers were ^{13}C -depleted to 16-20 km and slowly became enriched, but this was not statistically detected (Figure 2.6a and 2.6b). Tetragnathidae spider $\delta^{13}\text{C}$ did support this hypothesis, with spider $\delta^{13}\text{C}$ following the chlorophyll-*a* pattern. Spider $\delta^{13}\text{C}$ was statistically depleted a greater distance downstream (30 km) in 2010 and only just downstream of Morrow in 2012. Field observations also suggest that POM exported in water from Morrow Lake may settle on the bottom and within submersed macrophytes. Benthic consumers may feed on this settled material, which may explain why I was unable to detect depleted ^{13}C in consumers at 16 km and downstream.

Chlorophyll-*a* remained high from the Morrow Lake outflow through to Lake Allegan during high flow summers, suggesting that the semi-impounded dams with low water retention times may keep water column algae suspended longer (Chapter 1). Chlorophyll-*a* increased slightly in high and low flow summers (Chapter 1), but production of phytoplankton from the semi-impounded reaches may be minimal. It is not certain if t phytoplankton has been produced in the reservoirs themselves or if resuspended benthic algae produced within the semi-impounded reaches are important (Reid and Hamilton 2007). C isotope on tetragnathidae spiders from 2010 may support this hypothesis because they were more ^{13}C -depleted through the semi-impounded reaches, suggesting that some aquatic insects may consume a greater proportion of phytoplankton there (Figure 2.6c). Also, in transects sampled in later years, neither chlorophyll-*a* nor consumer $\delta^{13}\text{C}$ appear to support that phytoplankton is being produced from the semi-impounded reaches. Hydropsychidae and Heptageniidae slowly became ^{13}C -enriched in 2010 and were highly ^{13}C -enriched in the semi-impounded reaches in 2012 (a low flow summer). This suggests benthic food sources could be important (Figures 2.6a and 2.6b).

I did not observe a large change in macroinvertebrate $\delta^{13}\text{C}$ below the Morrow Lake dam, and therefore I did not find evidence for a strong switch from benthic algal dependence upstream of Morrow Lake to suspended algal dependence below, in contrast to what I expected. Hydropsychidae $\delta^{13}\text{C}$ did not remain depleted though the river, even as chlorophyll-*a* remained high, suggesting that phytoplankton from Morrow was not necessarily important for consumers through the river. The reservoir algal subsidy extended only a limited distance downstream, and the $\delta^{13}\text{C}$ values of most consumers gradually approached a baseline comparable to that above the reservoir, even as chlorophyll-*a* remained elevated. Consumer $\delta^{13}\text{C}$ suggests that the transport of material in the Kalamazoo River is complicated. While the actual nature of the food sources is

less clear, Hydropsychidae and Heptageniidae were abundant throughout the river, including the semi-impounded reaches, suggesting an abundant food source. Even though I cannot be certain how far downstream the Morrow Lake subsidy extended, most studies downstream of reservoirs have found that reservoir plankton decreased within ~1 km downstream (Doi et al. 2008), and I was able to detect transport of phytoplankton material up to 8 km, making this study one of the longest documented downstream subsidies of reservoir-produced algae.

Macroinvertebrate densities

In contrast to the isotopic evidence, the patterns in macroinvertebrate densities and functional feeding groups supported my hypothesis of a reservoir subsidy, with Hydropsychidae increasing greatly in density immediately downstream of the reservoir (Figure 2.5a). The patterns in Hydropsychidae densities reflect chlorophyll-a pattern patterns. Other filter feeders such as Brachycentridae and Isonychiidae were not abundant overall and were found at similar abundances both upstream and downstream of the reservoir. This work supports other studies which have also found that Hydropsychidae densities increase downstream of reservoirs and lakes (Doi et al. 2008, Mackay and Waters 1986, Oswood 1979, Parker and Voshell 1983, Richardson 1984, Robinson and Minshall 1990). Our work on the Kalamazoo River is also comparable to smaller streams such as those studied by Mackay and Waters (1986) and Doi et al. (2008), who found that only filter feeders responded to the subsidy. Macroinvertebrate composition and densities in the Kalamazoo River are similar to other rivers of comparable size, even without dams (Grubaugh et al. 1997, Benke 2015). Densities are also comparable to those reported in free-flowing and recovering reaches in dam removal studies (Stanley et al. 2002, Hansen and Hayes 2012, Orr et al. 2008, Grubbs and Taylor 2004).

Consumer diet subsidy inferred from $\delta^{13}\text{C}$

Hydropsychidae and Heptageniidae are of particular interest in this study given their high abundances in the Kalamazoo River, and their known variation in diets across a range of river sizes. Gut contents of macroinvertebrates along the river continuum have shown the importance of amorphous detritus, with a decreasing contribution of terrestrial organic matter and increasing contribution of algae or animals as rivers grow in size (Rosi-Marshall and Wallace 2002, McNeely et al. 2007). In this study, Hydropsychidae above the reservoir were likely supported by suspended detritus and dislodged benthic algae, whereas suspended algae may have been more important to their diets downstream of the reservoir. Additionally, Hydropsychidae $\delta^{13}\text{C}$ and densities consistently showed statistically significant responses to the reservoir subsidy, and the $\delta^{13}\text{C}$ values of Hydropsychidae immediately downstream of Morrow were comparable to typical $\delta^{13}\text{C}$ values of lake and reservoir phytoplankton (Doi et al. 2008, Junger and Planas 1994). Longitudinal patterns in this study are comparable to those found by Doi et al. (2008) in another 4th order river. However, the $\delta^{13}\text{C}$ values of consumers in the Kalamazoo River were comparable to taxa in studies of larger rivers (Delong and Thorp 2006, Kautza and Sullivan 2016). Casper et al. (2006) reported isotopic changes following a dam removal for *Hydropsyche* (Hydropsychidae) and *Stenonema* (Heptageniidae), and attributed the changes to a diet switch from predominantly reservoir-produced phytoplankton to benthic algae. Longitudinal patterns are not always observed, however, and Hydropsychidae diets as inferred from $\delta^{13}\text{C}$ values and gut contents may not be impacted by reservoirs (Growth et al. 2014).

Heptageniidae densities below the reservoir did not respond to the subsidy, but $\delta^{13}\text{C}$ values were surprisingly ^{13}C -depleted and similar to Hydropsychidae (Figures 2.5a, 2.6b). Heptageniidae are classified as scrapers like gastropods, and yet isotopically they were

comparable to Hydropsychidae. Importantly, Heptageniidae were ^{13}C -depleted upstream of Morrow Lake (Figure 2.7a), though this is based on one data point. Heptageniidae diets become more dependent on algae as river size increases (Finlay et al. 2001, McNeely et al. 2006 and 2007, Collins et al. 2015). In the Kalamazoo River below Morrow Lake, Heptageniidae may be feeding on deposited seston, which may explain why these taxa were ^{13}C -depleted compared to grazing snails. This suggests that Heptageniidae have a flexible feeding strategy that can include sedimented particulate organic matter (McShaffrey and McCafferty 1986, McNeely et al. 2006 and 2007, Huryn et al. 2001).

Compared to other functional feeding groups, filter feeders such as *Corbicula*, *Macrostemum*, *Isonychia* and Philopotamidae were ^{13}C -depleted throughout the river relative to other macroinvertebrates (Figure 2.9). *Isonychia* $\delta^{13}\text{C}$ values closely followed Hydropsychidae $\delta^{13}\text{C}$ where both were collected, while Philopotamidae and *Macrostemum* were especially ^{13}C -depleted. Philopotamidae and *Macrostemum* nets have especially small mesh sizes, and other studies have shown that their gut contents were dominated by small particles and amorphous detritus (Cundey and Wallace 1980, Wallace 1975, Parker and Voshell 1983, Benke and Wallace 1997 and 1980, Benke 2015). It is possible that they are selectively feeding on small phytoplankton in the Kalamazoo River below Morrow Lake.

Tetragnathidae, Amphipods, and crayfish were more similar in $\delta^{13}\text{C}$ to snails. These taxa did not respond strongly to the subsidy, like grazers in another study by Doi et al. (2008). These taxa were relatively enriched in ^{13}C and only slightly depleted downstream of the reservoir, suggesting that they consumed some settled algae from the reservoir (Figure 2.9). Amphipoda and crayfish are known to have versatile diets (Hamilton et al. 2004, Evans White et al. 2003).

Given their $\delta^{13}\text{C}$ in relation to baseline taxa, Hydropsychidae diets are likely a mix between seston (as indicated by *Corbicula*) and dislodged benthic algae above Morrow (as indicated by Pleuroceridae snails) (Figure 2.11). At Galesburg in 2012, the $\delta^{13}\text{C}$ values of Hydropsychidae, Heptageniidae and Isonychiidae were close to those of snails suggesting a benthic diet. Hydropsychidae were more ^{13}C -depleted in 2010, but in that year, I was unable to collect snails above Morrow Lake, and I did not collect *Corbicula*. I expect that Hydropsychidae would be ^{13}C -depleted compared to snails if they had been collected above Morrow Lake. Other taxa such as Amphipoda show $\delta^{13}\text{C}$ values that are similar along the river, which suggests that using an average for snails as a proxy for snail $\delta^{13}\text{C}$ along the river is appropriate. Additionally, *Macrostemum* where measured were more ^{13}C -depleted in 2010. These proxies for food sources suggest that Hydropsychidae consumed a depleted food source both above and below Morrow in 2010. This could also suggest that the base of the food web is more complex and variable than simply a mixture between benthic and suspended algae.

Many food-web studies group taxa by order or family, as I have done here, but subtle differences in diets may exist even within a family such as Hydropsychidae (Rasmussen 2010, Jardine et al. 2012, Sullivan 2013). One study on a southeastern U.S. river showed that Hydropsychidae species with larger mesh sizes were more ^{13}C -enriched, while those with smaller mesh sizes were ^{13}C -depleted, suggesting dietary differences (Benke 2015). Amorphous detritus is an important component in gut contents of macroinvertebrates (Rosi-Marshall and Wallace 2002, Benke 2015), and this often originates from upstream processing of detrital vascular-plant organic matter (Vannote et al. 1980) but can also be of algal origin. Isotopic studies suggest the importance of algae in macroinvertebrate diets given the greater nutritional

content and digestibility of algal organic matter even if gut contents are high in detritus (McNeely et al. 2006 and 2007, Collins et al. 2016, Kelly et al. 2013).

The role of dissolved CO₂

If the $\delta^{13}\text{C}$ of DIC varies mainly because of variable free CO₂, it would likely influence the $\delta^{13}\text{C}$ of algal biomass. One would expect that at higher CO₂ concentrations, the $\delta^{13}\text{C}$ of DIC would be more ¹³C-depleted. This would propagate up the food web. However, years when Hydropsychidae $\delta^{13}\text{C}$ was more depleted did not necessarily correspond to years with lower dissolved CO₂. In 2012, Hydropsychidae were more ¹³C-enriched but dissolved CO₂ was not lower. Consumer $\delta^{13}\text{C}$ values were 1-2‰ depleted in 2010 compared to 2012, and the $\delta^{13}\text{C}$ values of taxa I measured were unexpectedly similar to taxa found in some of the largest impounded rivers in the Upper Mississippi River lock and dam system (DeLong et al. 2001, Delong and Thorp 2006, Herwig et al. 2007), where dissolved CO₂ has been recently measured (Crawford et al. 2016). Studies using natural abundances of carbon isotopes from the largest rivers also found depleted consumers depleted in ¹³C within or downstream of reservoirs, where whole food webs were more ¹³C-enriched than those in the Upper Mississippi River (Casper et al. 2006, Freedman et al. 2014). Given that taxa were more ¹³C-enriched in 2012, the role of DIC and pCO₂ could explain these patterns. Factors related to algal growth are better studied in lake and stream systems than in large river studies where flow and dissolved CO₂ conditions are different. Broadly, increases of dissolved CO₂ (Bade et al. 2006, Finlay et al. 2004) or dissolved organic carbon concentrations (Lennon et al. 2006, Jepsen and Winemiller 2007) could decrease $\delta^{13}\text{C}$ of DIC and lead to depleted $\delta^{13}\text{C}$ values of algae (Quay et al. 1986, Smyntek et al. 2012, Zohary et al. 1994, Yoshika et al. 1994, Lehman et al. 2004, Yoshii et al. 1999, Hodell and Schelske 1998 Gu et al. 2011).

In the Kalamazoo River system, it does not appear that the depletion ^{13}C of consumers below Morrow Lake can be explained by changes in the $\delta^{13}\text{C}$ of dissolved CO_2 as the river water passes through the reservoir. The observed decrease in dissolved CO_2 concentrations below Morrow Lake could possibly lead to enriched algae, which was not observed in this study. The pCO_2 and DIC of the Kalamazoo River appear to be comparable to other rivers (Cole and Caraco 2001, Van den Meersche et al. 2009, Caraco et al. 2010, Jepsen and Winemiller 2007 and Junger and Planas 1994). A previous study found that the $\delta^{13}\text{C}$ of DIC in the Kalamazoo River downstream of Morrow Lake ranged from -8.7 to -11.4 ‰ (Atekwana and Krishnamurthy 1998). Based on other studies, ^{13}C enrichment of the dissolved CO_2 would seem more likely as water moved through Morrow Lake; in freshwaters, the $\delta^{13}\text{C}$ of dissolved CO_2 normally becomes more ^{13}C -enriched as photosynthetic assimilation draws the dissolved CO_2 concentrations down (Finlay and Kendall 2007). However, my observation that pCO_2 actually increases in Morrow Lake suggests that respiratory CO_2 reaches higher concentrations in the reservoir than upriver.

The $\delta^{13}\text{C}$ values of consumers in 2012 were ^{13}C -enriched, but in mid-July, dissolved CO_2 was higher in 2012 than 2008 (I lack data for 2010). It has been shown in lakes that dissolved CO_2 influences algal photosynthetic fractionation at concentrations around 10 to 70 μM , and that lower concentrations can result in ^{13}C -enriched phytoplankton (Smyntek et al. 2012, Bade et al. 2006, Gu et al. 2011, Lennon et al. 2006, Yoshika et al. 1994, Karlsson 2014, Finlay et al. 2004). In our study, dissolved CO_2 decreased through the season to $\sim 56 \mu\text{M}$ in 2012 and reached $\sim 70 \mu\text{M}$ in July and August 2008. This seasonal pattern is similar to that reported by Smyntek et al. (2012) where pCO_2 was lowest in July. However, in my data I did not find that dissolved CO_2 explained the variation in consumer $\delta^{13}\text{C}$ (Figure 2.13 and 2.14). Amphipoda and Gastropoda ^{13}C values were enriched along the river, and did not vary much. These patterns suggest the

consumer $\delta^{13}\text{C}$ is more likely a result of a food source change as related to suspended algae rather than dissolved CO_2 .

Other alternative explanations for longitudinal variation in consumer $\delta^{13}\text{C}$

I assert that ^{13}C -depletion in consumer taxa is an indicator of the algal subsidy downstream of the reservoir because growth of benthic algae produces boundary layers that generally cause ^{13}C -enrichment compared to phytoplankton (Hecky and Hesslein 1995). However, other possible explanations in addition to a change in dissolved CO_2 deserve consideration. While lake studies suggest that zooplankton with low $\delta^{13}\text{C}$ are supported by ^{13}C -depleted phytoplankton (Kling et al. 1992, France 1995, Hecky and Hesslein 1995), studies of lake phytoplankton do show considerable $\delta^{13}\text{C}$ variability (Bade et al. 2006, Smyntek et al. 2012). Besides dissolved CO_2 limitations, growth rates are also important drivers of variation in the $\delta^{13}\text{C}$ of phytoplankton in lakes, causing enrichment. (Gu et al. 2011, Bade et al. 2006, Smyntek et al. 2012) and algal taxa may respond differently to the $\delta^{13}\text{C}$ of DIC and CO_2 (Zohary et al. 1994, Lehman et al. 2004, Yoshii et al. 1999, Yoshioka et al. 1994, Cifuentes et al. 1988). I have no information on algal taxa or growth rates to evaluate this possibility, however.

Alternatively, benthic algal $\delta^{13}\text{C}$ can vary because flow variation in velocity and turbulence, creating a variable boundary-layer limitation of CO_2 assimilation (Singer et al. 2005, Trudeau and Rasmussen 2007, Finlay et al. 1999, Sullivan 2013). Benthic algal $\delta^{13}\text{C}$ can be more enriched in areas of slower flow (Finlay et al. 2004, Sullivan 2013). Benthic algal $\delta^{13}\text{C}$ in streams can shift from more ^{13}C -depleted in the headwaters, where most DIC originates from groundwater sources, to more ^{13}C -enriched downstream as a greater fraction of the DIC is derived from the atmosphere (Rasmussen 2010, Kobayashi et al. 2011, Finlay et al. 2004). I did not observe this pattern in Tetragnathidae spiders collected over a longitudinal gradient.

Temporal variability matters if the time scales of isotopic turnover vary among consumers and/or sampling sites (Woodland et al. 2012). Seasonable changes in benthic algal biomass and composition exist, but may not be not predictable (reviewed by Ishikawa et al. 2012). Spatial rather than temporal variability of macroinvertebrate $\delta^{13}\text{C}$ was likely greater in this study (Figure 2.8). I tried to control for flow velocity by choosing similar conditions for my sampling sites, but nonetheless velocities varied (Table 2.1) and could possibly contribute to variation in benthic algal $\delta^{13}\text{C}$. There is no evidence to indicate for these alternative explanations of the longitudinal patterns that I observed within particular taxa on particular sampling dates. Hence, the algal subsidy downstream of the reservoir seems the most likely explanation.

Caveats for using primary consumers as estimates of basal food source $\delta^{13}\text{C}$

Estimation of $\delta^{13}\text{C}$ of POM has been an ongoing challenge and the use of $\delta^{13}\text{C}$ as a tracer has received criticism because of challenges of overlap in $\delta^{13}\text{C}$ values of detritus from transported terrestrial organic matter and autochthonous algal carbon (Hamilton et al. 2005, Taipale et al. 2015, Marty and Planas 2008, Bade et al. 2006, Middelburg 2014). Stable carbon isotope ratios in seston show that POM is often a diverse mixture that may or may not include a high proportion of algal biomass (Kendall et al. 2001). Hamilton et al. (2005) used density gradient centrifugation in colloidal silica to separate algae from detritus in samples from various aquatic environments and showed that the bulk seston does not always represent the algal $\delta^{13}\text{C}$.

Instead of measuring baseline $\delta^{13}\text{C}$ of seston or epilithon directly to indicate the $\delta^{13}\text{C}$ of basal food resources, I used primary consumers (*Corbicula* and snails) as indicators of food source variation (Vander Zanden et al. 2011, Post 2002, Fry et al. 2008). I expected that $\delta^{13}\text{C}$ of snails along the Kalamazoo River to closely track variability in benthic $\delta^{13}\text{C}$ along a river gradient, which has been observed in other studies (Rasmussen 2010, Sullivan 2013, Finlay et al.

1999, Jardine et al. 2012). *Corbicula*, also a baseline in this study, was one of the most ^{13}C -depleted taxa throughout the river (Figure 2.9). Using primary consumers as indicators of the $\delta^{13}\text{C}$ of basal food resources has its own challenges (Taipale et al. 2015, Marty and Planas 2008, Middelburg 2014, Hamilton et al 2005). While *Corbicula* are known to consume phytoplankton (Cohen et al. 1984, Atkinson et al. 2011), they also pedal feed on benthic organic matter (Bullard and Hershey 2013). Snails may consume sedimented phytoplankton as well as benthic algae. Consuming variable diets is especially evident in this study as *Corbicula* and snails $\delta^{13}\text{C}$ varied above and below the reservoir (Figure 2.9).

Additionally, Hamilton (unpublished) found little difference in $\delta^{13}\text{C}$ between stream benthic algae and terrestrial organic matter in a small tributary stream of the Kalamazoo River, and gastropods were comparable to his result (~ -29 to -30 ‰ or -27 to -29 ‰ after calculated lipid correction). This suggests that I would be unlikely to decipher dietary differences from direct measurements of the $\delta^{13}\text{C}$ values of basal resources. Given these caveats, I decided not to use an isotopic mixing model to infer dietary proportions (i.e. Fry et al. 2008, Vander Zanden et al. 2011). Issues with $\delta^{13}\text{C}$ overlap among sources continue to confound data interpretation and limit our ability to quantify dietary sources in stable isotope studies (Peterson and Fry 1987, Jepsen and Winemiller 2007, Herwig et al. 2007), and this is important for inferring trophic position (Kristensen et al. 2016).

Many aquatic studies have observed that consumers are slightly ^{13}C -depleted compared to food sources (Bunn and Boon 1993, Hamilton et al. 1992, Karlsson et al. 2003, reviewed by Brett 2014). Hydropsychidae were more ^{13}C -depleted than sampled food sources in the Mississippi River (Herwig 2007). In the upper Mississippi River system, where there are impoundments for navigation that create extensive backwater flooding, FPOM of algal origin

was shown to be a food source for both Hydropsychidae and Heptageniidae (Delong and Thorp 2006). Seston collected from a nearby river would be more enriched than filter feeders in this study (Marko et al. 2013), and I hypothesized that seston would be enriched compared to Hydropsychidae in the Kalamazoo River. Use of algal separation techniques (e.g., Hamilton et al. 2005) in this study may have shown patterns related to dissolved carbon dioxide that were not detected in bulk seston. Such techniques have shown variability in seston composition (Junker and Cross 2014), and the importance of algal components in the POM to consumer diets (Delong and Thorp 2006).

Another caveat is that I am reporting $\delta^{13}\text{C}$ of consumers without correcting for variation in lipid content. Data in this study are reported as lipid-uncorrected to compare with previous studies (Zeug et al. 2008, Hoeinghaus et al. 2007, Kautza and Sullivan 2016, Delong and Thorp 2006). Lipids are relatively ^{13}C -depleted leading to variable lipid content in consumers (Logan et al. 2008, Post et al. 2007). Post et al. (2007) model was developed more broadly for aquatic organisms but appears to overcorrect for lipids in macroinvertebrates in this study. The consumer C:N values in this study are comparable to those reported by Logan et al. (2008), whose model considers chitin in whole macroinvertebrates (Logan et al. 2008). Based on model corrections, all taxa would be enriched by 1 to 2 ‰, which would shift taxa to 27 ‰. Heptageniidae and Hydropsychidae would also be perhaps more enriched than phytoplankton estimates. I did not use lipid extraction because it can lead to alteration of $\delta^{15}\text{N}$, though not always (Logan et al. 2008), and model estimates should be used with caution (de Lecea and de Charmony 2015). Lipid extraction is recommended when using mixing models, especially for Heptageniidae and Hydropsychidae, given the limited number of studies that do so on macroinvertebrates (i.e. Logan et al. 2008).

Lastly, samples of consumers collected in 2010 and 2012 were processed with gut contents. The differences in $\delta^{13}\text{C}$ between specimens with guts cleared or not is less than that the difference between years, suggesting that the ^{13}C -depleted taxa observed in 2010 indicate a change in food source rather than a gut content effect (Jardine et al. 2005). Gut content removal can be important. Jardine et al. (2005) found that invertebrate samples with gut contents will be perhaps 0.5 ‰ depleted in ^{13}C . My study was more interested in longitudinal patterns and temporal changes, which were likely greater than additional variation related to lipids in tissues and gut contents. Improve techniques for estimating baselines (i.e. Hamilton et al. 2005, Middelburg 2014), gut content removal, and lipid removal or corrections should be conducted to improve mixing model results in future studies (sensu Junker and Cross 2014).

The role of flow variability in controlling phytoplankton production

This study focuses on the role of seston in river food webs, and the food web response to the changes in seston attributable to impoundment. Rivers of this size often carry seston that represents an important high-quality food source for filter feeders (Naiman 1983, Wilkinson et al. 2013, Van Den Meersche et al. 2009, Caraco et al. 2010, Hoeninghaus et al. 2007, Benke 2015, Rosi-Marshall and Meyer 2004), although we do not understand what is driving food quality. The base of the food web of a river of this size is hypothesized to be a mix of local benthic algae and transported organic material from upstream (Vannote et al. 1980, Cummins et al. 1981, McCutchan and Lewis 2002, Hamilton et al. 2004). The changes in the relative proportions of organic matter sources as rivers increase in size has been embodied in the River Continuum Concept (Vannote et al. 1980). Phytoplankton production in large river systems is better understood (Cole et al. 1992, Lewis 1988, Descy and Reynolds 1996, Ochs et al. 2013). Stable isotope tracer studies of food webs in larger rivers have noted that phytoplanktonic algal

production can be important to food webs particularly those where there are natural floodplain lakes or artificial impoundments (Hamilton et al. 1992, Bunn and Boon 1993, Hoeinghaus et al. 2007, Caraco et al. 2010, Leigh et al. 2010, Thorp et al. 1998). Phytoplankton production in smaller streams is less important to food webs, likely because of residence time limitations (e.g., McCutchan and Lewis 2002). However, the River Continuum Concept was underpinned by just a few case studies (Vannote et al 1980), and it is not known when the transition from small stream to large river occurs (Reisinger et al. 2015).

The literature suggests that downstream of dams, macroinvertebrate responses to reservoirs is related to river size. In smaller streams, Hydropsychidae and Heptageniidae can be isotopically similar (Walters et al. 2007) and both benthic detritus and algae are thought to be important to their diets (McCutchan and Lewis 2002, Doi et al. 2007, Collins et al. 2015, Ishikawa et al. 2014, Hamilton et al. 2004, McNeely et al. 2006). As river size increases, the $\delta^{13}\text{C}$ values of Hydropsychidae become depleted compared to Heptageniidae (Herwig et al. 2004 and 2007, Delong and Thorp 2006, Kautza and Sullivan 2016). The macroinvertebrate response below a dam was likely intermediate between a smaller stream and the largest rivers, though Hydropsychidae were comparable to larger river studies. A synoptic examination of algae in seston upstream of Morrow Lake showed that they were mostly benthic diatoms with some green algae, while downstream the seston contained more phytoplanktonic diatoms and green algae (Reid and Hamilton 2007). Filter-feeder gut contents often contain amorphous detritus of uncertain origin, whereas isotopic evidence can support assimilation of a mix of vascular-plant detritus and benthic algae (e.g., McNeely et al. 2007) and possibly phytoplankton.

The depleted $\delta^{13}\text{C}$ of Hydropsychidae upstream of Morrow Lake relative to C-3 plants could indicate a ^{13}C -depleted food source in the absence of artificial impoundment, while

considering the above caveats. Production of ^{13}C -depleted algae could occur in slack water habitats along the floodplain of the Kalamazoo River (sensu Thorp et al. 2006, Hein et al. 2003, Hamilton et al. 1992), but quantifying this is challenging (Herwig et al. 2007). High flows could be important for the transport of organic material including algae to the river food web (Jardine et al. 2015, Atkinson et al. 2009), and dislodged benthic algae could be an important source of suspended algal biomass (Reynolds 2000, Delong and Thorp 2006, Istvanovics et al. 2011, Gu and Schelske 1996).

Overall, in the low-discharge year 2012 macroinvertebrates were ^{13}C -enriched compared to macroinvertebrates in 2010 and may be mostly reliant on benthic algae, even with long water retention times that could produce more phytoplankton, and few resuspension events. During low flows in summer, the river may have been less connected to the floodplain, but warm temperatures and slower flows would increase in-stream algal production (Jardine et al. 2015, Winemiller et al. 2015). Connectivity to flood plains is important for food web dynamics (Junk et al. 1988, Benke 2015, Roach et al 2013, Bellmore et al. 2013). The relative importance of benthic algae to phytoplankton in large rivers is less known (Thorp et al. 1998). This means that river channel modifications and the role of run-of-river dams in altering seston concentrations are important to understand. This study was conducted in the face of large interannual variability, and detecting food web differences was thus difficult. However, Hydropsychidae in particular were more ^{13}C -depleted below Morrow Lake, suggesting that phytoplankton could be important, at least in high flow years.

Transfer of energy to higher trophic levels and the terrestrial ecosystem

Macroinvertebrates represent important intermediate consumers for the flow of energy from the base of the food web to higher trophic levels including fishes. Like in other reservoir

studies, we have observed a change from benthic algae to suspended algae as potential food sources for invertebrate consumers when comparing the river above and immediately below Morrow Lake. Longitudinal variation along the river in the diets of primary consumers influences energy transfer to higher trophic levels. Aquatic insects with flying adult stages represent a potentially important food source for birds, bats and spiders. While Tetragnathidae spiders show similar longitudinal patterns to Hydropsychidae and Heptageniidae, they were more ^{13}C enriched, possibly due in part to an expected trophic enrichment of $\sim 1\text{ ‰}$ for a predator relative to its prey. Even with a longitudinal sampling of Tetragnathidae over a 100 km section of river, I did not see a pattern related to the downstream direction as predicted by the River Continuum Concept. Past longitudinal sampling along rivers may or may not have detected the role of reservoir-produced phytoplankton in diets of filter feeders, as filter feeders become ^{13}C -depleted along the river continuum (Kobayashi et al. 2011). I found that Tetragnathidae spiders were isotopically more comparable to snails than *Corbicula*, suggesting that benthic production was important to subsidizing the terrestrial food web via emerging insects. Tetragnathidae spiders are an important indicator of the subsidy from aquatic to terrestrial systems (Marczak and Richardson 2007, Paetzold et al. 2006, Sanzone et al. 2003 Baxter et al. 2005, Polis et al. 1997, Bellmore et al. 2013).

Even along the banks of the reservoir, the spiders were more enriched in ^{13}C than I would have predicted compared to the ^{13}C -depleted filter feeders below the dam. Based on $\delta^{13}\text{C}$ values, Kautza and Sullivan (2016) found that Tetragnathidae were supported by prey feeding on a mixture of phytoplankton, benthic algae and terrestrial organic matter. Tagwireyi and Sullivan (2016) found that Chironomidae, Hydropsychidae and Heptageniidae were assumed to be important to Tetragnathidae and in their study, the emerging adult insect composition was

comparable to macroinvertebrates found on Hester Dendy samples in the Kalamazoo River. Many macroinvertebrates were ^{13}C -enriched and possibly supported mainly by benthic algae. Hydropsychidae were depleted. The algal subsidy transported downstream from Morrow Lake may have been important to downstream filter-feeding insect larvae, which upon emergence effectively transferred this subsidy to the terrestrial food web.

CONCLUSIONS

An increasing global interest in the development of run-of-river dams may lead to the increase of dam building in many parts of the world, though not in the coterminous United States (Francois et al. 2016, Kumar and Katoch 2014, Winemiller et al. 2015). Some of the best studies of their impacts come from such dams on Midwestern US streams, but these have still been less studied from a river ecosystem perspective (Stanley et al. 2002). My research suggests that Morrow Lake, a run-of-river reservoir, produced an algal subsidy in outflowing water, but only one taxon, filter-feeding Hydropsychidae, clearly responded, as has been observed in other studies (Mackay and Walters 1986, Doi et al. 2008). Collecting data across a broad range of discharges, I observed food source differences among years that could be related to food source changes but evidently not to dissolved CO_2 . Downstream of Morrow Lake, chlorophyll-*a* quickly decreased in low flow summers but could possibly be transported all the way to the next large downstream reservoir (Lake Allegan) in high flow years. The isotopic evidence from caddisflies suggest that the impacts of the dam on seston composition are complex. Run-of-river dams may impact macroinvertebrates, organic matter processing, and food webs in subtler ways than deep storage reservoirs with longer water residence times (Kibler and Tullos 2013). Like our study, reservoir plankton can be produced if water residence times are long enough and export of that

plankton subsidizes downstream food webs and can alter macroinvertebrate communities (Stanley et al. 2002, Grubbs and Taylor 2004, Hansen and Hayes 2012). Tools like natural abundances of stable carbon isotopes can be used to better understand what is driving production in food webs in rivers with run-of-river dams, but the differences may not be as marked as in the case of reservoirs with long water residence times.

Large hypolimnetic release dams, which have been better studied, can alter sediment (Wohl 2015), trapping transported material and seston (Angradi 1993, Ulseth and Hall 2015), and may degrade macroinvertebrate and fish communities downstream (Cross et al. 2011, Kennedy et al. 2016, Holt et al. 2015). Run-of-river dams have not been studied as extensively. Like all dams, run-of-river reservoirs can be a barrier to upstream fish movement (Wang et al. 2011, Cumming 2004, Santucci et al. 2005). Studies have suggested leaving dams in place so they could provide a food source, especially for endangered Unionidae mussels (Singer and Gangloff 2011). However, the base of food webs in rivers is still being explored. Determining when phytoplankton production becomes important, especially under changing climate and variable flow regime, remains an important challenge.

Many small run-of-river dams were built in the early 1900s and were critical for hydroelectricity and the development of the local economy, but have remained long after their hydropower facilities or other uses were decommissioned (Csiki and Rhoads 2015, Stanley and Doyle 2002, Stanford et al. 1996, Benstead et al. 1999). The cumulative impacts of run-of-river dams provide lessons regarding degradation of water quality and fisheries (Doyle et al. 2005). Run-of-river reservoirs span a range of sizes and water residence times, and their impacts would be expected to vary as a result (Csiki and Rhodes 2015). The Kalamazoo River represents an abundant type of river system (Downing et al. 2012) and many such systems have run-of-river

reservoirs like those on the Kalamazoo River (Stanley 2002, Poff et al. 2007). While aging infrastructure in the U.S. and other industrialized nations has made it imperative that dams be removed before they could fail (Stanford et al. 1996), further development of run-of-river schemes for hydroelectricity will continue to be an attractive energy source and such dams are increasingly being constructed worldwide (i.e. Winemiller et al. 2016). Although the impacts of a single run-of-river dam can be subtle, the cumulative impacts of such reservoirs along the river system are important.

APPENDIX

Table 2.1 Current velocities of sites along the Kalamazoo River where artificial substrata were deployed (bricks or Hester Dendy samplers).

Site	Flow (m/s)	
	2010	2012
Battle Creek	0.25	
Fort Custer	0.50	0.54
Galesburg	0.33	0.38
Downstream of Morrow		0.32
Parchment, MI	0.33	0.26
D-avenue	0.50	
Plainwell	0.50	0.39
Township Dam	0.57	0.30
Trowbridge	0.37	0.45

Table 2.2 C:N and $\delta^{13}\text{C}$ corrections for taxa from the Kalamazoo River models used for C:N correction were based on linear models from Post et al. (2007) and Logan et al. (2008) with number of individuals. Included are samples with macroinvertebrates allowed to clear their guts versus those with gut content present.

	<u>Hydropsychidae</u>	<u>Heptageniidae</u>	<u>Amphipoda</u>	<u>Snail</u>	<u>Coricula</u>	<u>Macrostemum</u>	<u>Isonychia</u>	<u>Crayfish</u>
C:N	5.47 (34)	6.26 (28)	6.29 (19)	4.67 (13)	5.41 (9)	5.99 (11)	6.64 (13)	5.55 (9)
Post et al. (2007)								
Lipid correction	2.09 (34)	2.88 (28)	2.90 (19)	1.30 (13)	2.03 (9)	2.61 (11)	3.26 (13)	2.18 (9)
Logan et al. (2008)								
Lipid correction	1.51 (32)	1.77 (28)	1.77 (19)	1.21 (13)	1.48 (9)	1.69 (11)	1.88 (13)	1.54 (9)
Model 3								
Gut content	-0.24 (5)	-0.43(4)	-0.02(3)			-0.92(1)	0.25(3)	

Table 2.3 Statistical comparisons of $\delta^{13}\text{C}$ of common taxa along the Kalamazoo River. Comparisons were made by pooling taxa from Fort Custer (the site above Morrow) to taxa pooled downstream of Morrow by site. All taxa were made by comparing common taxa via Paired t-test. Functional feeding group comparisons were made using Two-way t-test. Taxa were pooled by section and compared from above (Fort Custer and Galesburg, MI to below (below Morrow and Parchment, MI at Mosel Ave.), Downstream (D-avenue and Plainwell) and Semi-impounded (Ostego Township Dam and the Township Dam and Trowbridge).

Distance from Morrow (km)	Fort Custer	Galesburg	Morrow 0	Mosel 8	D-avenue 16	Plainwell 30	Otsego 36	Trowbridge 48
Filter feeder								
2010	-31.61		-35.59	-35.26	-32.31	-32.39	-33.28	-33.69
2010			DF=2, T=13.16, p<0.05	NS	NA	NS	NS	NS
2012	-30.82		-33.02	-32.44	-31.58	-31.94	-30.22	-30.24
2012			DF=5, T=4.31, p<0.05	NS	NS	NS	NS	NS
Grazers								
2010	-28.75		-31.24	-31.85	-30.62	-30.06	-30.18	-31.41
2010			NS	DF=5, T=2.78, p<0.07	NS	NS	NS	NA
2012	-28.78		-30.6	-30.16	-29.17	-28.76	-26.59	-28.37
2012			NS	NS	NS	NS	NS	NS
		Morrow	Mosel	D-avenue	Plainwell	Otsego	Trowbridge	
Hydropsychidae								
2008	DF=3, T=6.74, p<0.05	NS	DF=3, T=4.47, P=0.06	DF=3, T=3.28, P=0.05		NS		NS
2012	DF=2, T=4.06, p=0.06	NS	NS	DF=2, T=3.28, P=0.05		NS		DF=4, T=-6.32, p<0.05
Heptageniidae								
2008	DF=3, T=9.34, p=0.05		NS	NS		NS		NS
2012	NA	NS	NA	NS		DF=4, T=7.90, p=0.05		DF=3, T=-3.48, p<0.05

Table 2.3 (cont'd)

Data and stats				
pooled by site		Below	Downstream	Semi-impounded
Spiders	2010	DF=3, T=4.51, p<0.05	DF=3, T=2.97 p=0.06	DF=4, T=3.87 p<0.05
	2012	DF=2, T=4.77, p<0.05	NS	NA
	Year to Year	Difference=1.00 ‰	DF=5, t stat=-2.54, p=0.05	
Hydropsychidae	2010	DF=3, T=3.80, p<0.05	NS	NS
	2012	DF=6, T=3.83, p<0.05	DF=3, T=3.45, p<0.05	DF=8, T=-4.44, p<0.05
	Year to Year	Difference=2.41 ‰	DF=14, t stat=3.38 p<0.05	
Heptageniidae	2010	NS	NS	NS
	2012	NS	NS	DF=7, T=-3.74, p<0.05
	Year to Year	Difference=1.79 ‰	DF=14, t stat=2.51 p<0.05	
Amphipoda	2010	NS	DF=3, t stat=-3.02, p=0.06	NS
	2012	NA	DF=4, t stat=-3.51, p=0.05	DF=2, t stat=7.50, p=0.05
	Year to Year	Difference=1.00 ‰	NS	
Corbicula	2012	DF=2, T=24.7, p<0.05	DF=3, T=-2.63, p=0.08	NS
Isonychia	2012	NS	NS	NS
Comparisons of				
Hester Dendy Samplers		Below	Downstream	Semi-impounded
Hydropsychidae		DF=6, T=-6.203	DF=4, T=-3.00	DF=6, T=-3.67
Heptageniidae		NS	NS	NS
Chironomidae		NS	NS	NS

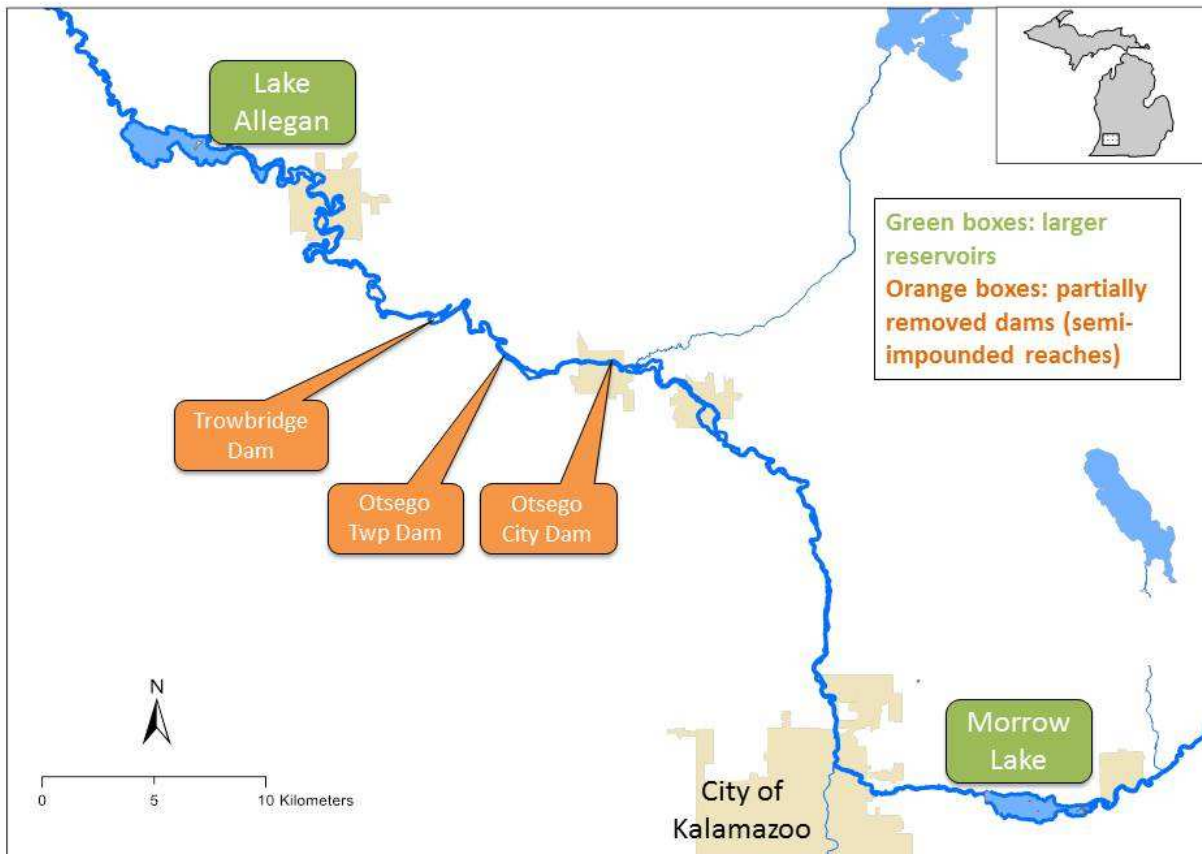


Figure 2.1 Study reach along the Kalamazoo River in Southern Michigan. Morrow Lake and Lake Allegan are the two largest reservoirs on the river system. Orange squares represent partially removed dams in the reach referred to as “semi-impounded.”

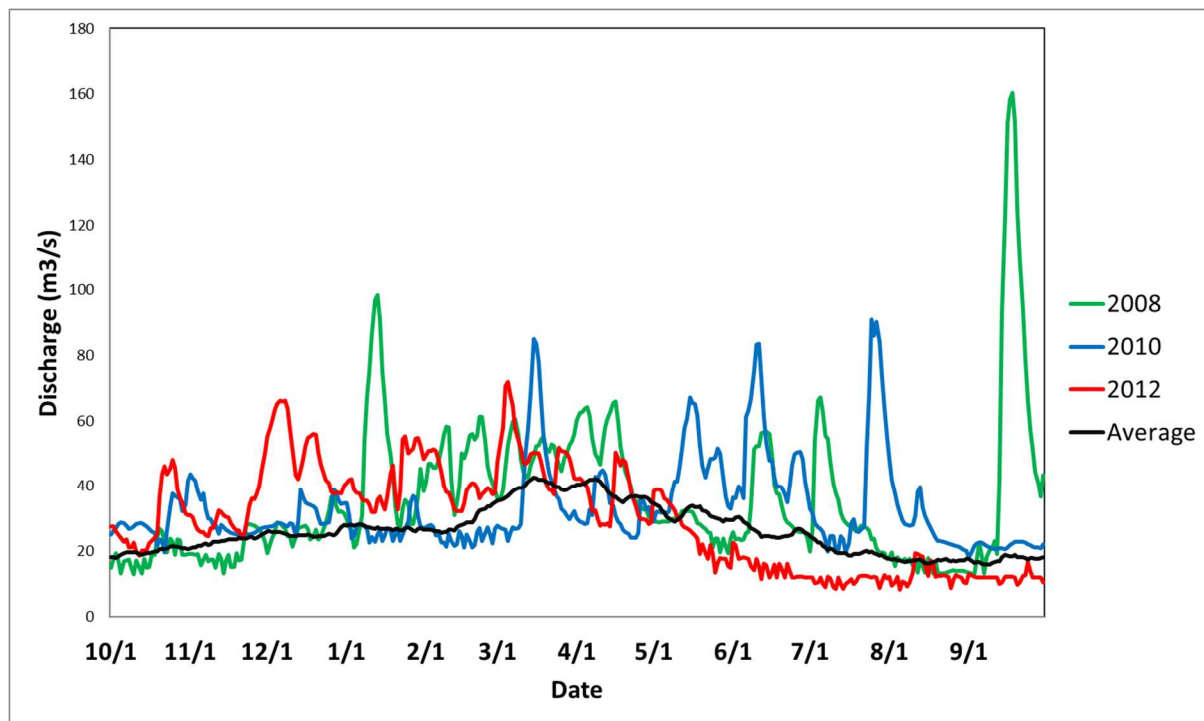


Figure 2.2 Discharge of the Kalamazoo River. Showing years of higher and lower summer flows as well as the longer term average. From the National Water Information System.

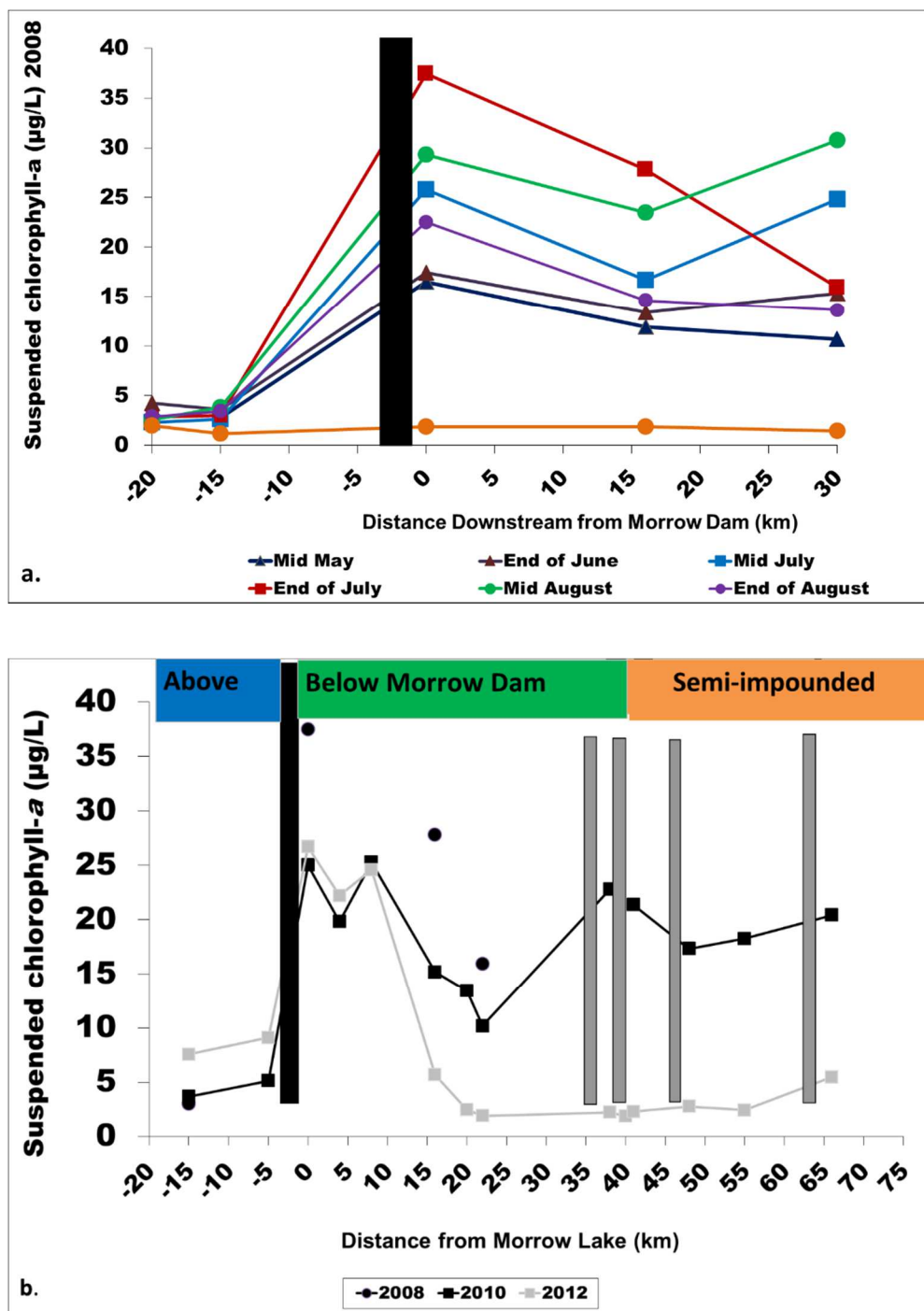


Figure 2.3 Suspended chlorophyll-*a* concentrations. (a) Chlorophyll-*a* measurements for 2008 collected biweekly. (b) Comparisons of chlorophyll between suspended algae collected in July 2008, through July 2010 and in the first week of July in 2012. Black bar represents the location of Morrow Lake, and gray bars represent the locations of the partially removed dams. The final dam represents Allegan City, the last small dam before Lake Allegan.

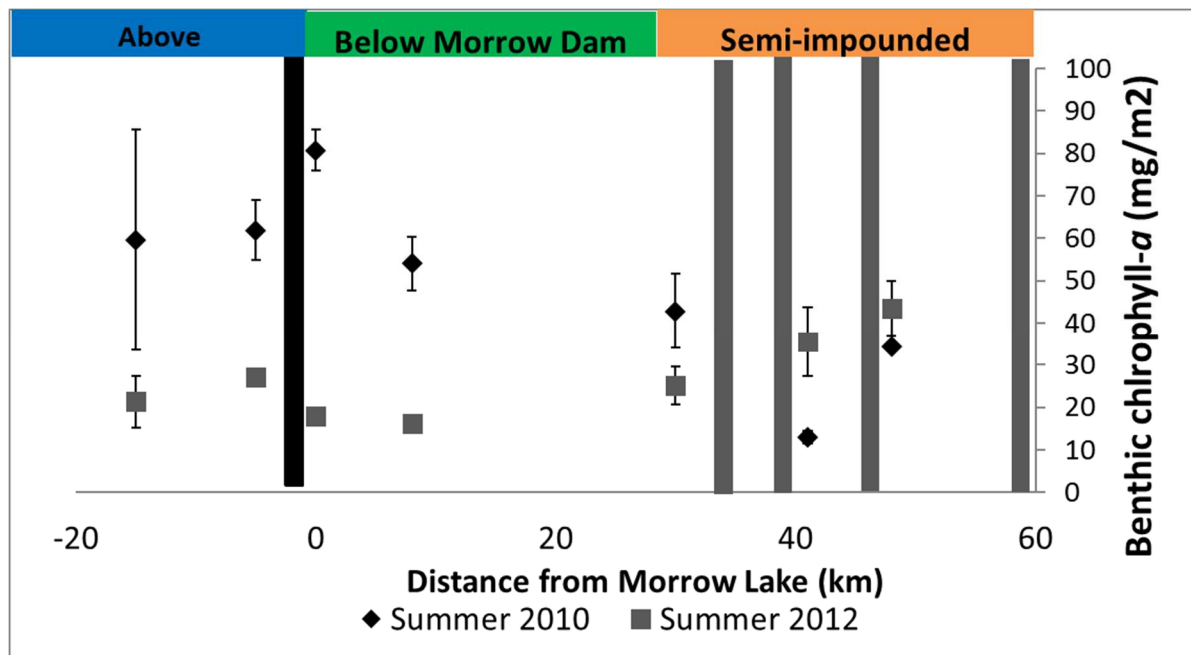


Figure 2.4 Benthic chlorophyll-*a* densities on clay bricks incubated in the Kalamazoo River during summers of 2010 and 2012. Black bar represents the location of Morrow Lake, and gray bars represent the locations of the partially removed dams. The final dam represents Allegan City, the last small dam before Lake Allegan.

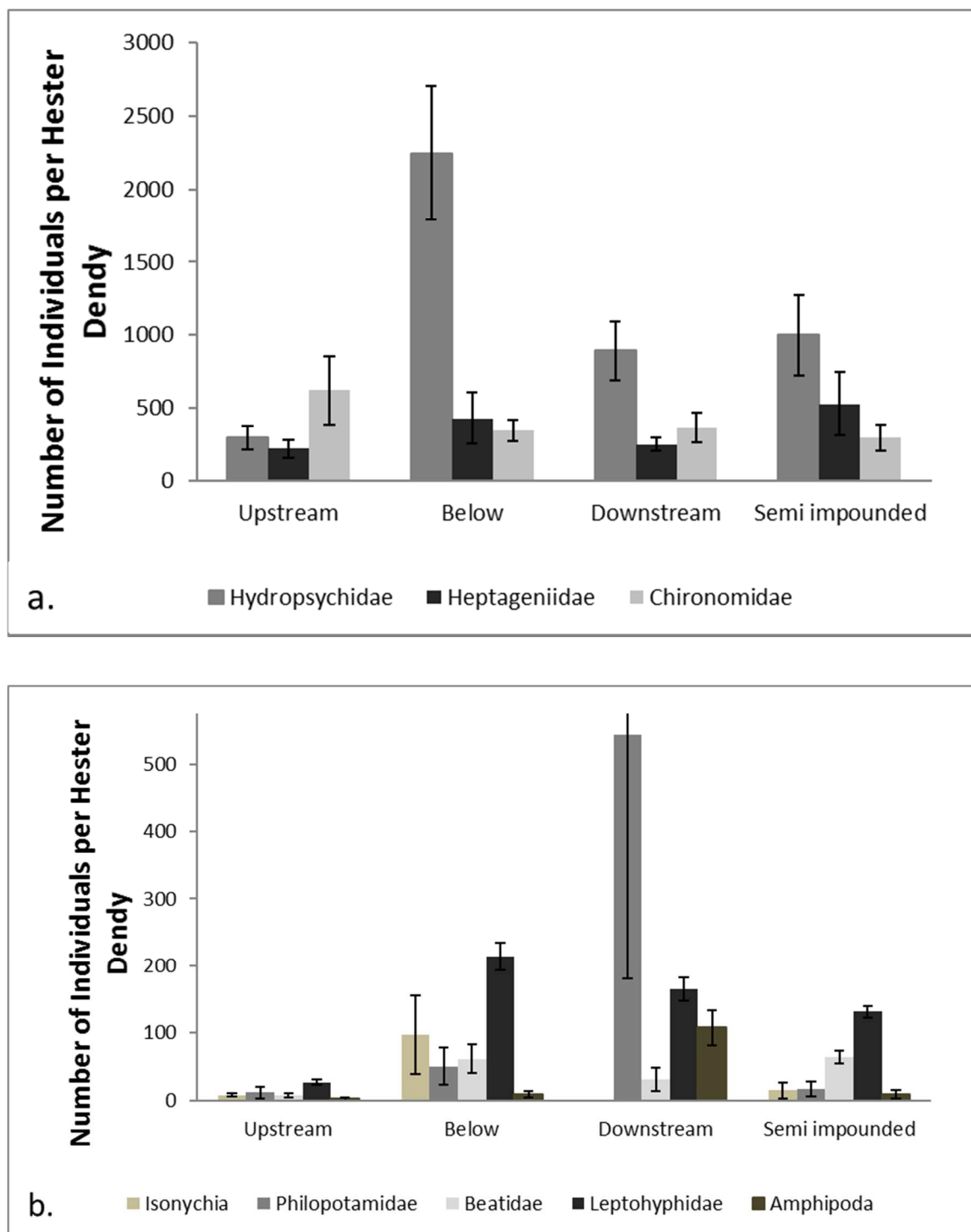


Figure 2.5 Macroinvertebrate colonization of artificial substrata (Hester Dendy samplers) placed in the Kalamazoo River. Tall black bar represents that location of Morrow Lake. Sampling sites are grouped into above Morrow Lake (Upstream), immediately below the Morrow Lake dam (Below), further downstream, and in the semi-impounded reach. with ± 1 standard error.

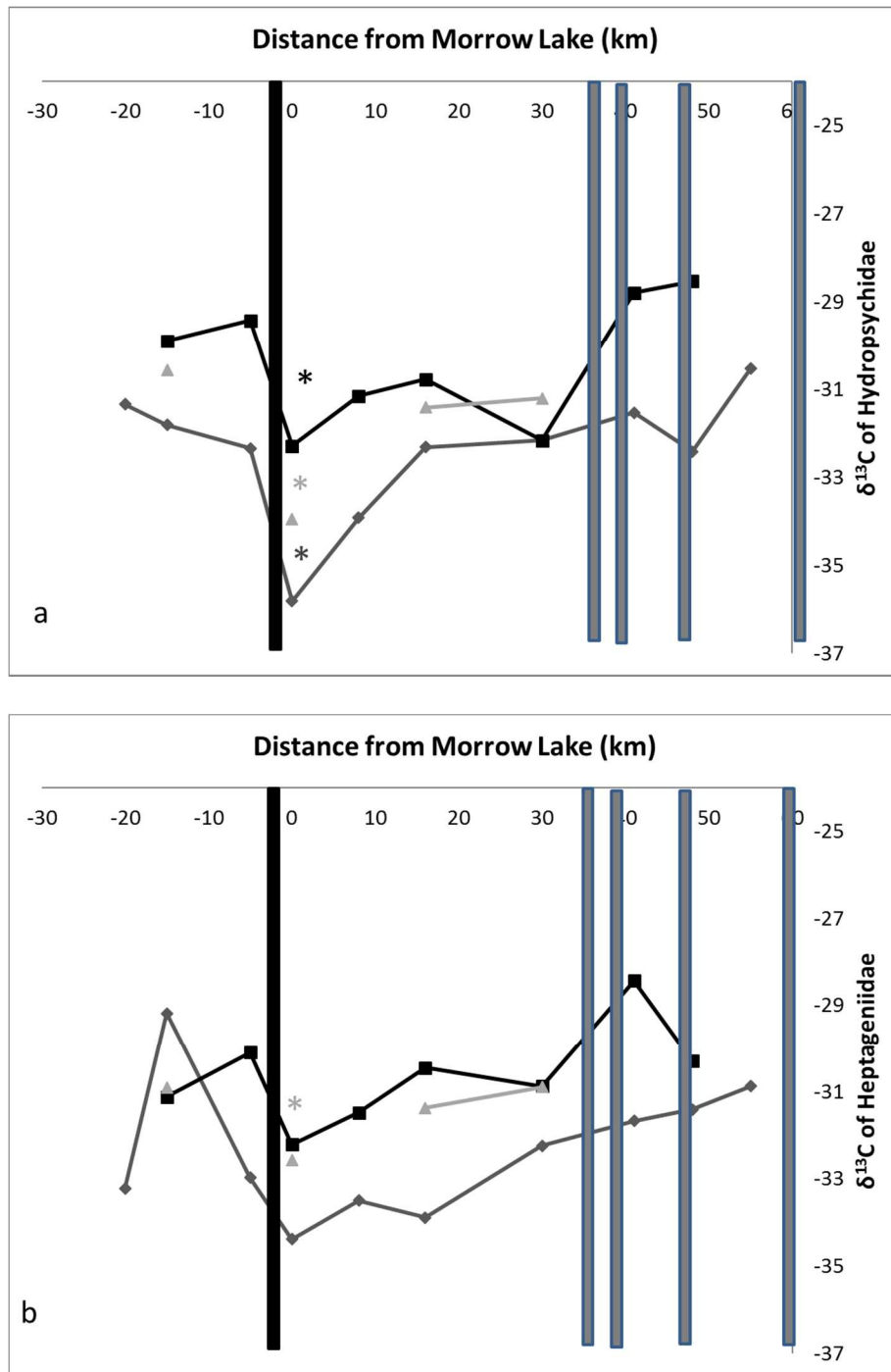
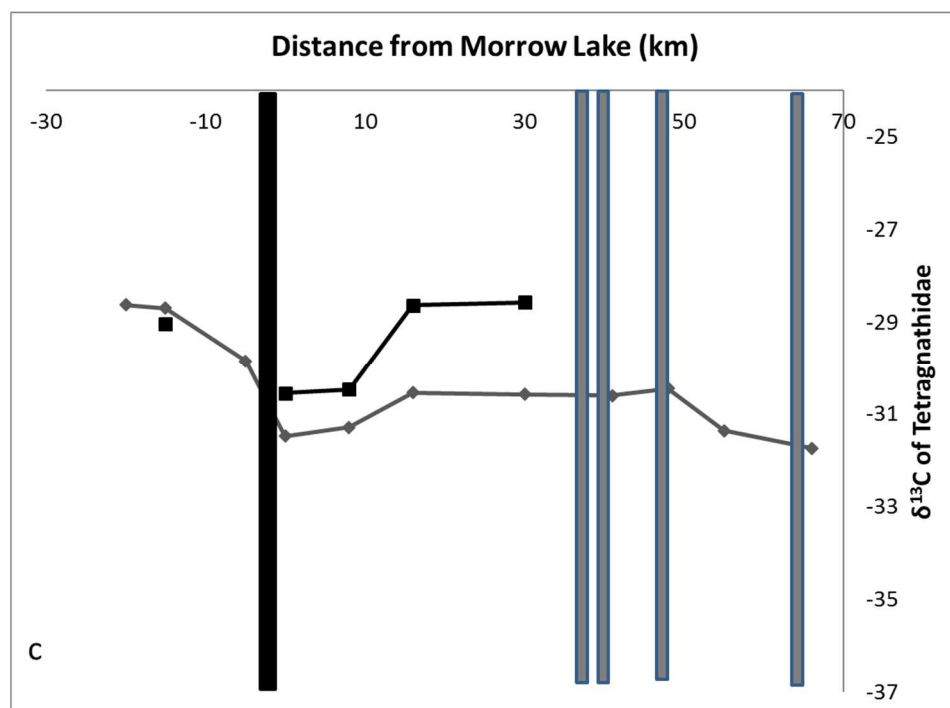


Figure 2.6 Macroinvertebrate $\delta^{13}\text{C}$ values along the Kalamazoo River from above Morrow Lake through the semi-impounded reach. A. $\delta^{13}\text{C}$ of Hydropsychidae. Gray symbols are from 2010 and black are from 2012. B. $\delta^{13}\text{C}$ values of Heptageniidae. Gray symbols are from 2010 and black is from 2012. C. $\delta^{13}\text{C}$ of Tetragnathidae spiders. Gray is from spiders collected in 2010 and black is from 2012. Black bar represents the location of Morrow Lake, and gray bars represent partially removed dams. Asterix represents $p < 0.05$ of a t-test.

Figure 2.6 (cont'd)



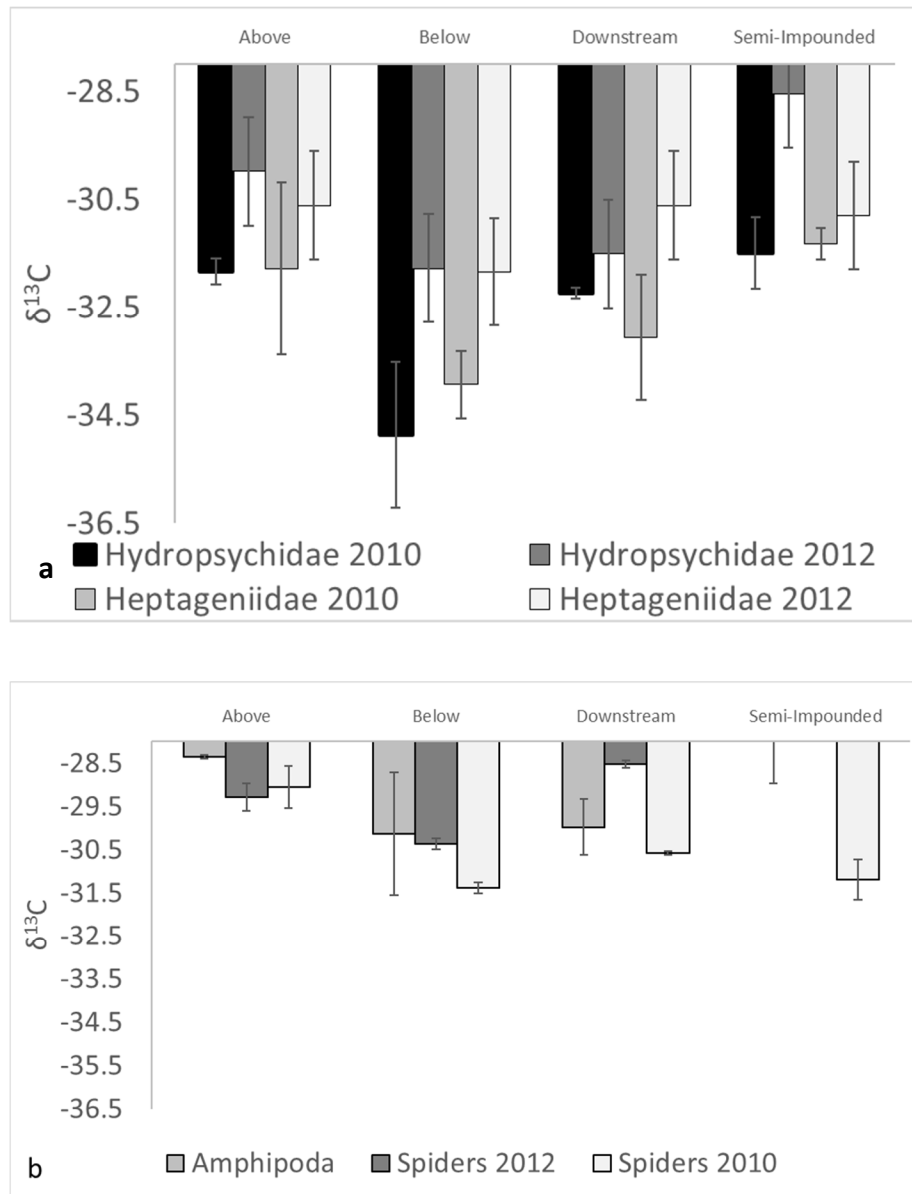


Figure 2.7 Average $\delta^{13}\text{C}$ values of (a) Hydropsychidae, Heptageniidae and Tetragnathidae spiders and Amphipoda collected from 2010 and 2012.

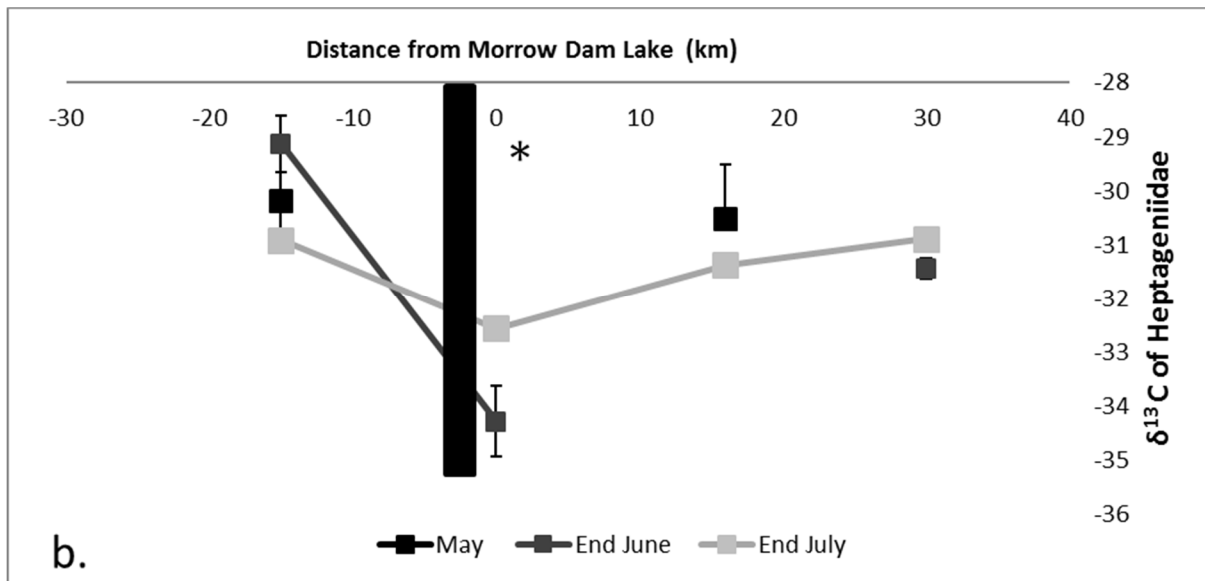
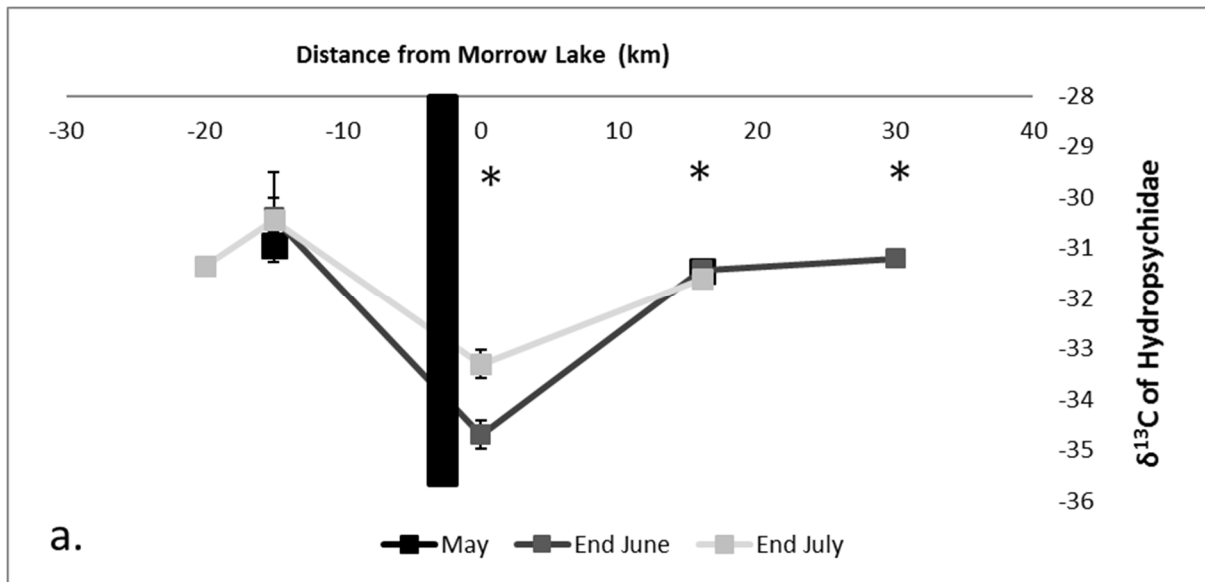


Figure 2.8 Temporal variation of $\delta^{13}\text{C}$ values of Hydropsychidae and Heptageniidae along the Kalamazoo River in 2008. Black bar represents Morrow Lake. Asterisk represents $p < 0.05$ of a t-test.

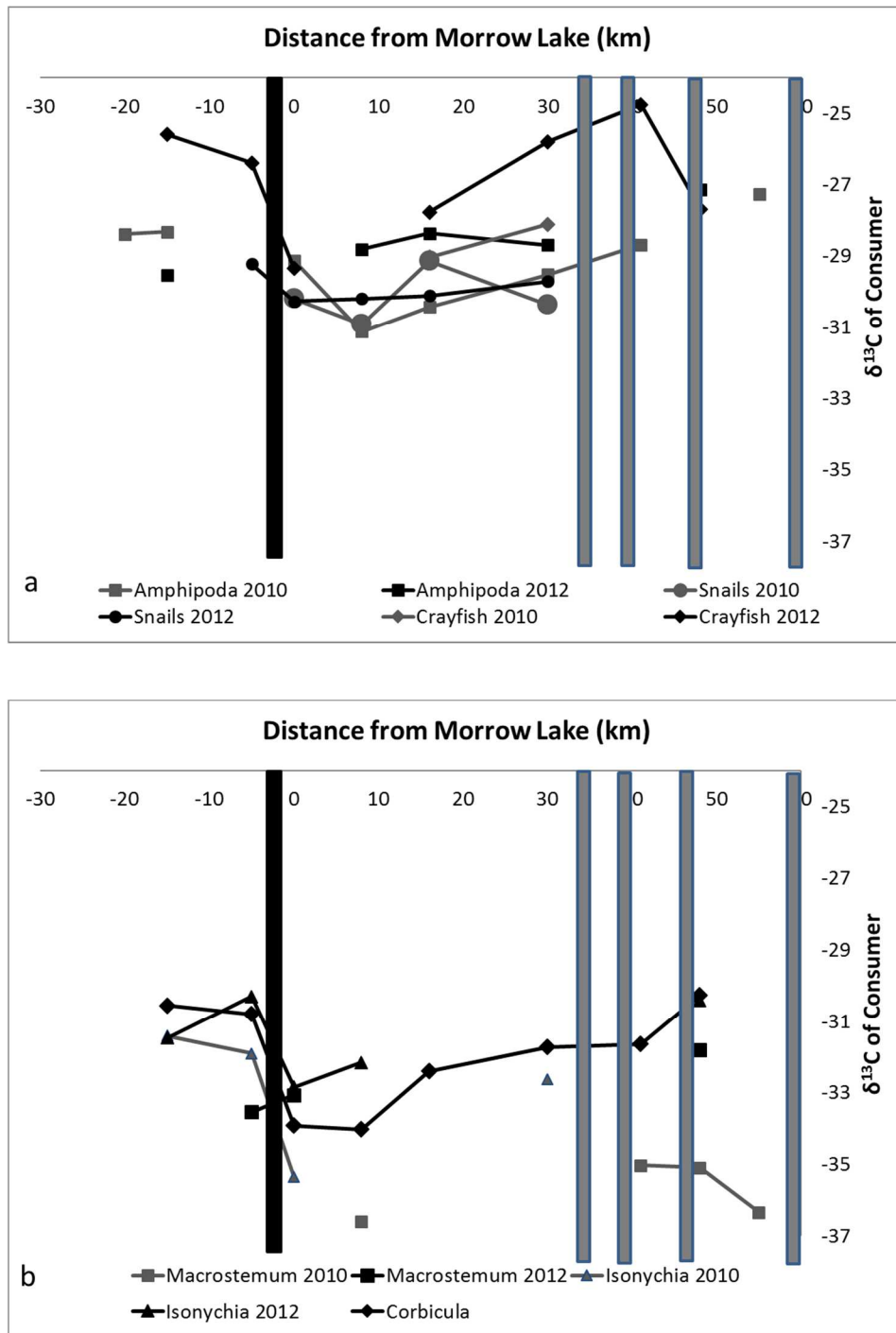


Figure 2.9 Functional feeding group macroinvertebrates $\delta^{13}\text{C}$ values along the Kalamazoo River from above Morrow Lake through the semi-impounded reach. Gray symbols are from 2010 and black is from 2012. A. $\delta^{13}\text{C}$ of taxa in grazing and collector gatherer functional feeding groups. B. $\delta^{13}\text{C}$ values of taxa from the filterer functional feeding group. Black bar represents the location of Morrow Lake, and gray bars represent partially removed dams.

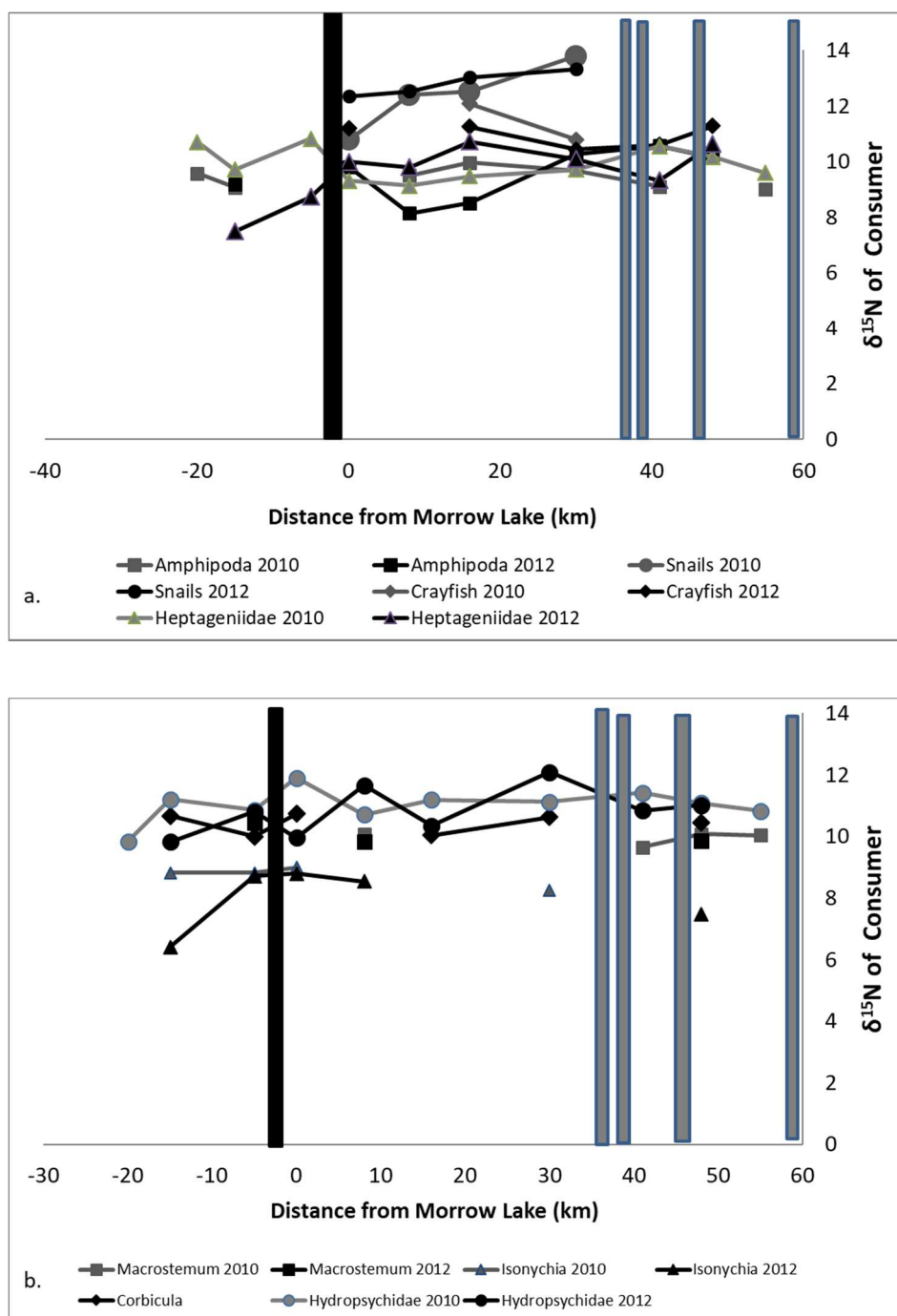


Figure 2.10 Macroinvertebrate $\delta^{15}\text{N}$ values along the Kalamazoo River from above Morrow Lake through the semi-impounded reach in 2010 and 2012. Taxa grouped by (a) benthic feeders and (b) filter feeders. Black bar represents the location of Morrow Lake, and gray bars represent partially removed dams.

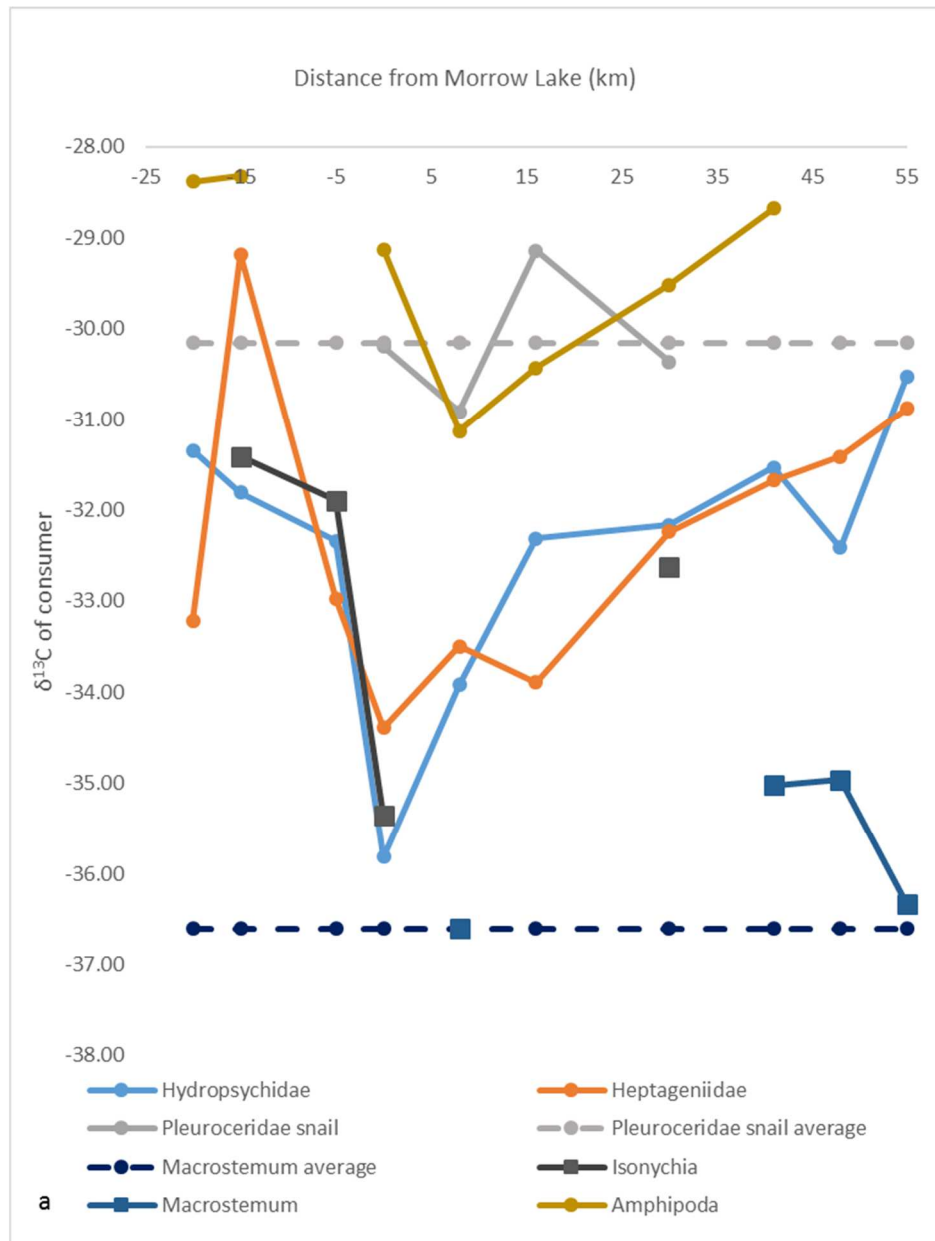
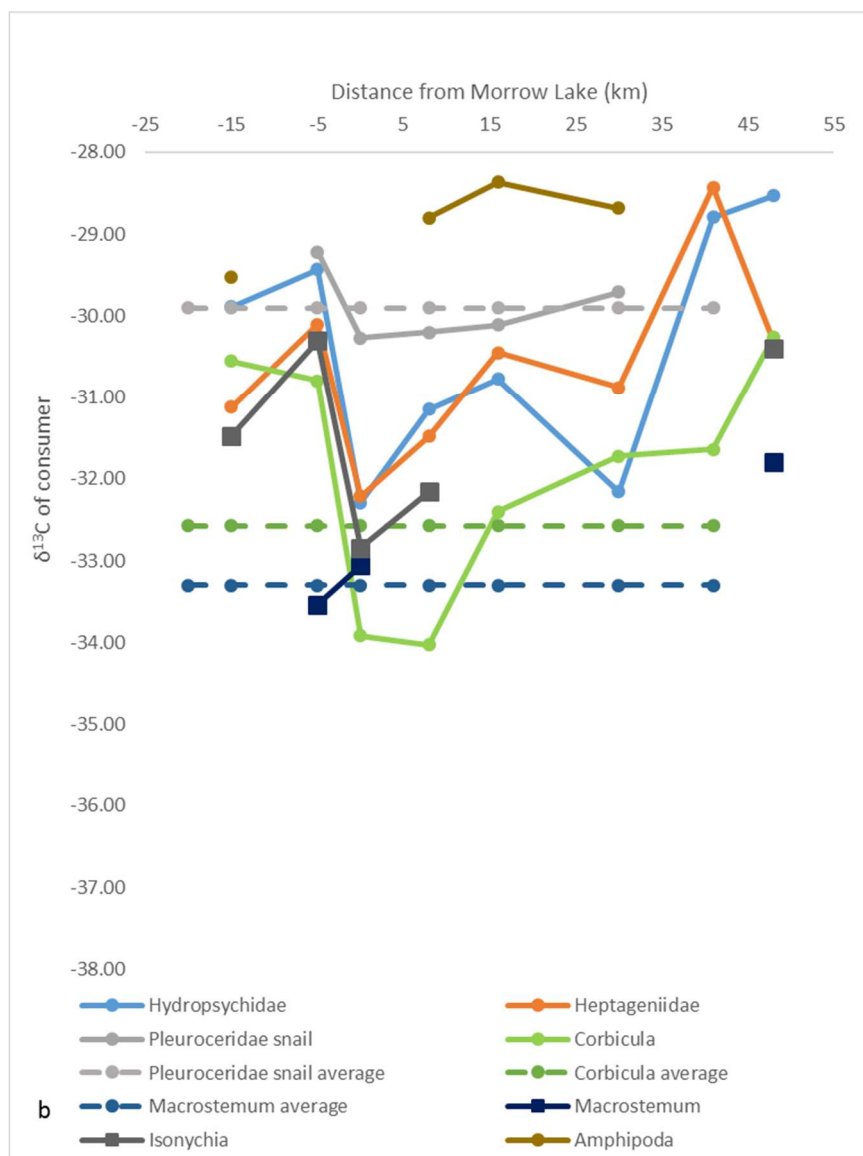


Figure 2.11 Baseline comparisons of Hydropsychidae and Heptageniidae $\delta^{13}\text{C}$ values between Pleuroceridae snail $\delta^{13}\text{C}$ values and *Corbicula* $\delta^{13}\text{C}$ values. Sampled averages of snail and *Corbicula* were included as a longitudinal baseline for 2010 (a) and 2012 (b). *Macrostemum* average was also included in 2010. Other taxa were included for comparison.

Figure 2.11 (cont'd)



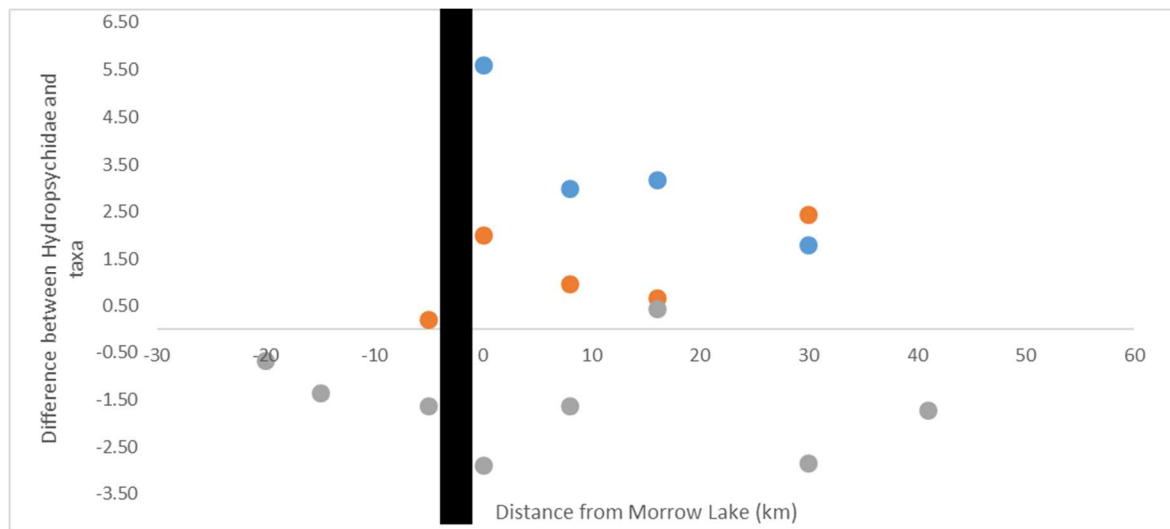


Figure 2.12 Differences between Hydropsychidae caddisflies and Pleuroceridae snails in 2010 (blue), Hydropsychidae and Pleuroceridae in 2012 (orange) and Hydropsychidae and *Corbicula* mussels in 2012 (gray). Black Bar represents the dam at Morrow Lake.

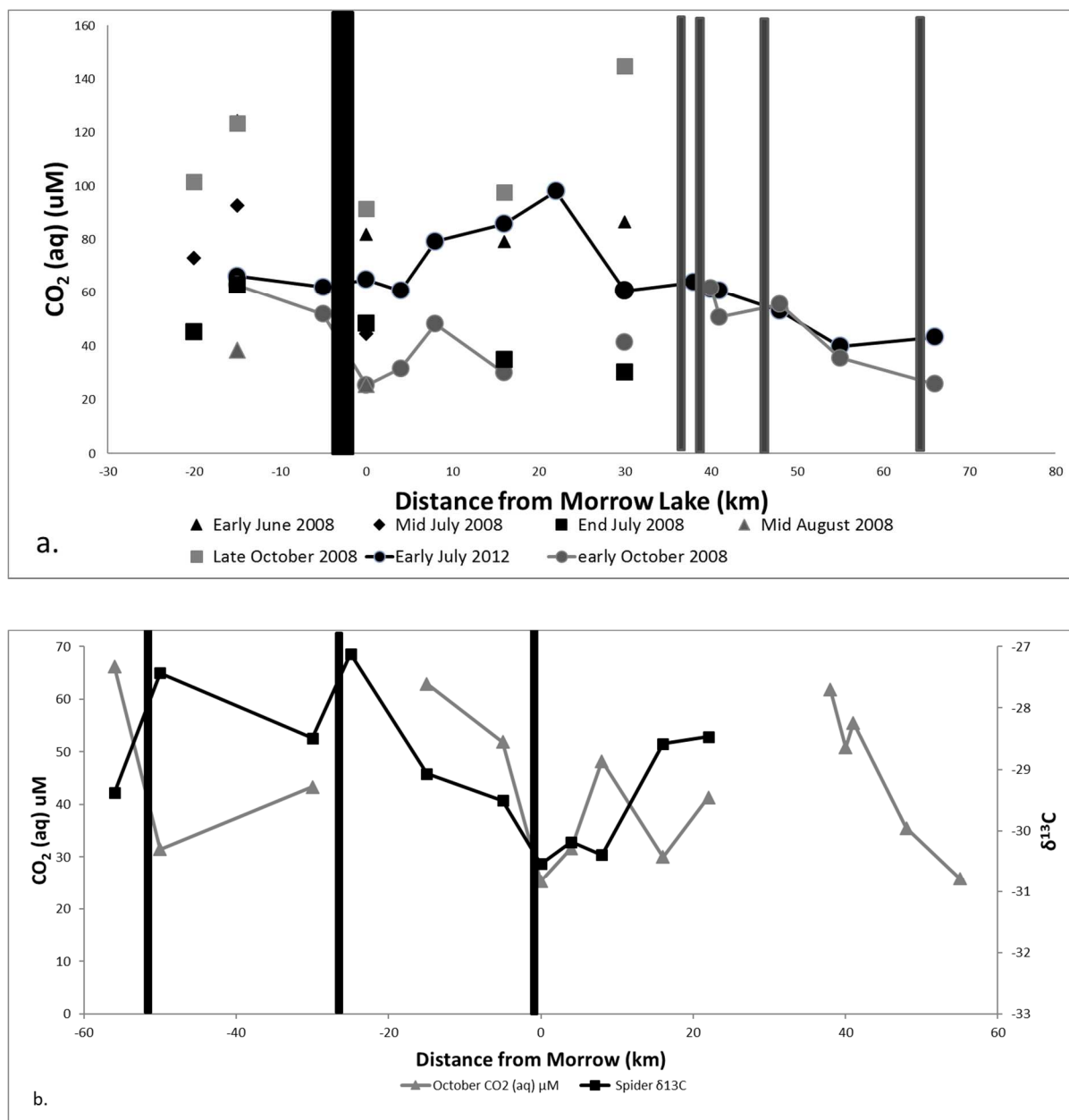


Figure 2.13 Dissolved CO_2 (aq) from multiple measurements in 2008 and 2012 (a) and a longitudinal survey of CO_2 (aq) from October 2012 and Tetragnathidae spiders collected in July 2012. Black bars represent two reservoirs located well upstream of Morrow Lake (Ceresco and the Battle Creek Mill Ponds) as well as Morrow Lake (0 km) along the Kalamazoo River.

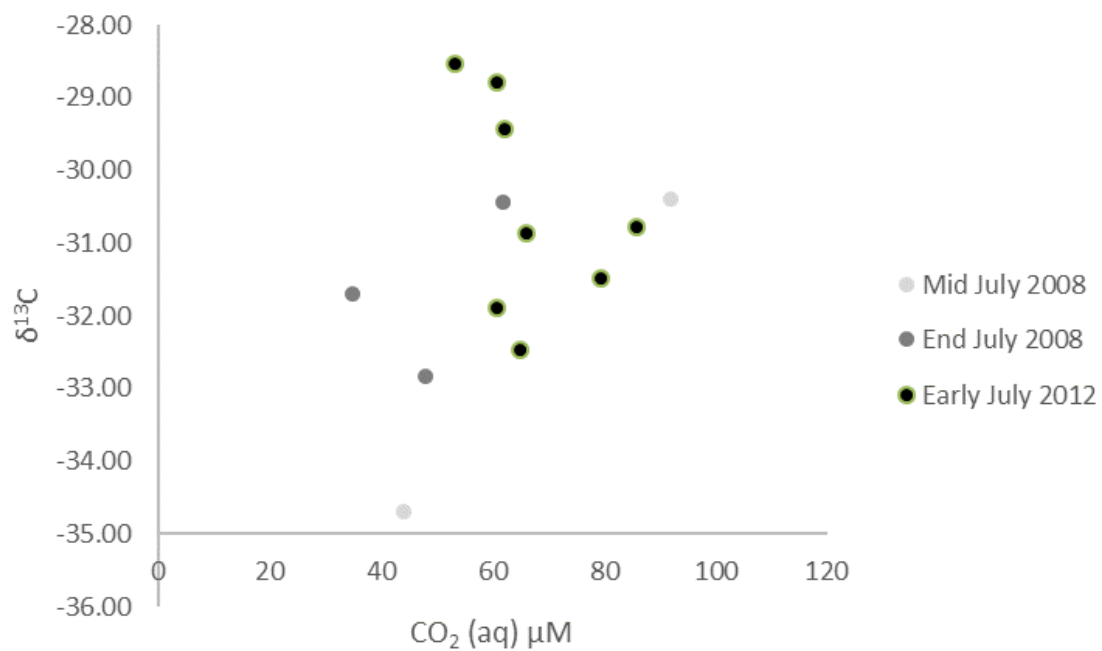


Figure 2.14 Comparisons of $\delta^{13}\text{C}$ of Hydropsychidae collected along the river and $\text{CO}_2(\text{aq})$ (μM) measured near time of macroinvertebrate collection.

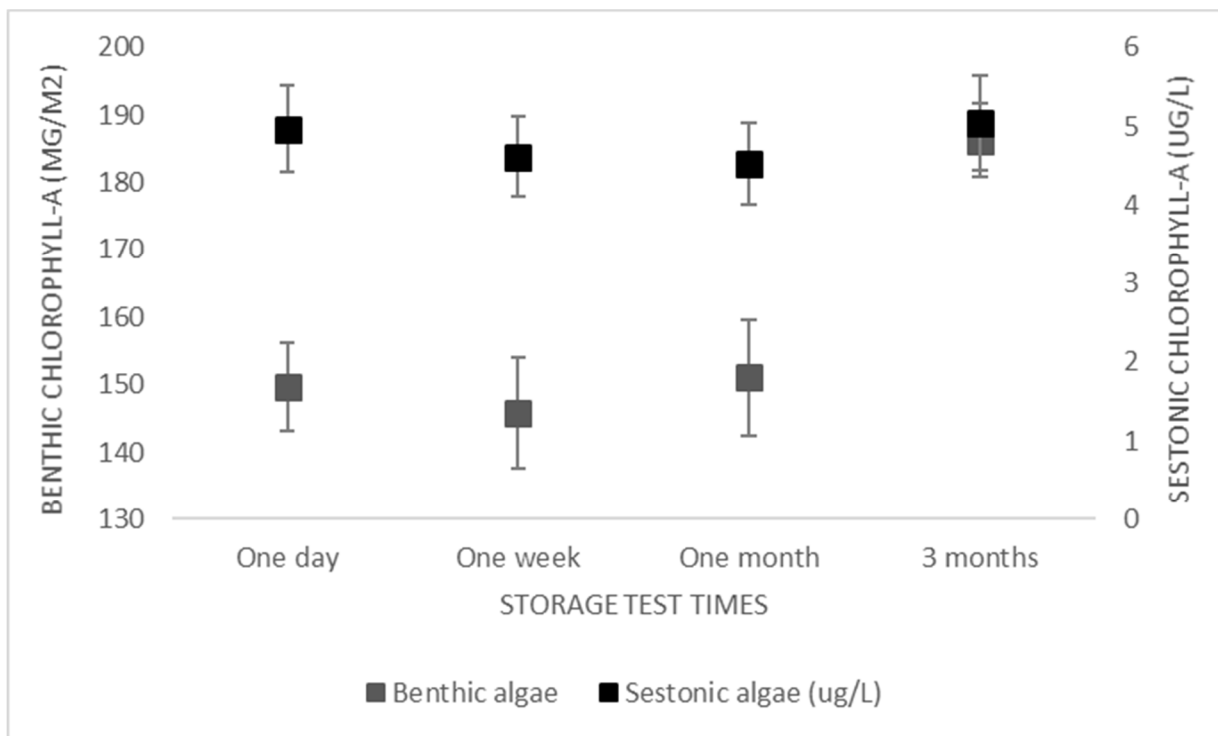
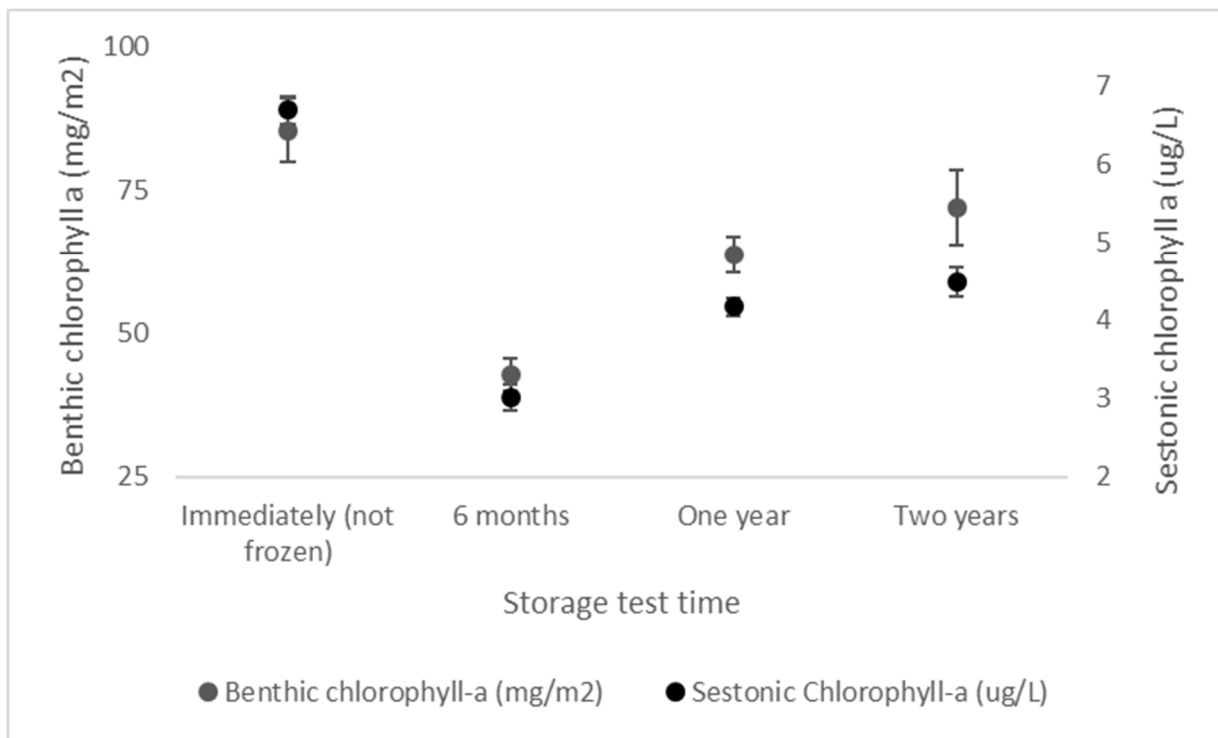


Figure 2.15 Results of chlorophyll-*a* freezer storage tests.

REFERENCES

REFERENCES

- Angradi, T.R. 1993. Stable carbon and nitrogen isotope analysis of seston in a regulated rocky mountain river, USA. *River Research and Application* 8: 251-270.
- Angradi, T. R. 1994. Trophic linkages in the Lower Colorado River: Multiple Stable Isotope Evidence. *Journal of the North American Benthological Society* 13:479-495.
- Atekwana, E. A., R. V. Krishnamurthy. 1998. Seasonal variations of dissolved inorganic carbon and $\delta^{13}\text{C}$ of surface waters: application of a modified gas evolution technique. *Journal of Hydrology* 205: 265-278.
- Atkinson, C.A., S. W. Golladay, S. P. Opsahl, A. P. Covich. 2009. Stream discharge and floodplain connections affect stream quality and stable isotopic signatures in a coastal plain stream. *The Society for Freshwater Science* 28:360-370.
- Bade, D. L., M. L. Pace, J.J. Cole, S.R. Carpenter. 2006. Can algal photosynthetic inorganic carbon isotope fractionation be predicted in lakes using existing models? *Aquatic Science* 68: 142-153.
- Baxter, C.V, K. D Fausch, W. C. Saunders. 2005. Tangled webs: reciprocal flows of invertebrate prey link streams and riparian zones. *Freshwater Biology* 50:201-220.
- Benke, A. C. 1990. A perspective on America's vanishing streams. *Journal of the North American Benthological Society* 9:77-88.
- Benke, A. C., J. B. Wallace. 2015. High secondary production in a Coastal Plain river is dominated by snag invertebrates and fueled mainly by amorphous detritus. *Freshwater Biology* 6: 236-255.
- Benke, A.C., and J.B. Wallace. 1980. Trophic basis of production among net-spinning caddisflies in a southern Appalachian stream. *Ecology* 61: 108-118.
- Benke, A.C. and J. B. Wallace. 1997. Trophic basis of production among riverine caddisflies: implications for food web analysis. *Ecology* 78: 1132-1145.
- Benstead, J.P., J. G. March, C. M. Pringle, F. N. Scatena. 1999. Effects of a low-head dam and water abstraction on migratory tropical stream biota. *Ecological Applications* 9: 656-668.
- Brett, M. T., D. C. Mueller-Navarra, A. P. Ballantyne, J. L. Ravel, C. R. Goldman. 2006. *Daphnia* fatty acid composition reflects that of their diet. *Limnology and Oceanography* 51: 2428-2437.
- Brett, M. T. and D. C. Mueller-Navarra. 1997. The role of highly unsaturated fatty acids in aquatic foodweb processes. *Freshwater Biology* 38: 483-499.

Bullard, A. E., A. E. Hershey. 2013. Impact of *Corbicula fluminea* (Asian clam) on seston in an urban stream receiving wastewater effluent. *Freshwater Science* 32: 976-990.

Bunn, S.E., P. I. Boon. 1993. What sources of organic carbon drive food webs in billabongs? A study based on stable isotope analysis. *Oecologia* 96: 84-94.

Cabana, G., J. B. Rasmussen. 1996. Comparison of aquatic food chains using nitrogen isotopes. *Proceedings of the National Academy of Sciences of the United States of America* 93: 10844-10847.

Calheiros, D. F., S. K. Hamilton. 1998. Limnological conditions associated with natural fish kills in the Pantanal Wetland of Brazil. *Verh. Internat. Verein. Limnol.* 26: 2189-2193.

Caraco, N., J.E. Bauer, J.J. Cole, S. Petsch, P. Raymond. 2010. Millennial-aged organic carbon subsidies to a modern river food web. *Ecology* 91: 2385-2393.

Carlisle, D. M., D. M. Wolock, M. R. Meador. 2010. Alteration of streamflow magnitudes and potential ecological consequences: a multiregional assessment. *Frontiers in Ecology and Environment* 9: 264-270.

Casper, A. F., J. H. Thorp, S. P. Davis, D. L. Courtemanch. 2006. Ecological responses of zooplankton to dam removal on the Kennebec River, Maine, USA. *Archiv fur Hydrobiologie* 158: 541-555.

Catalano, M.J., M.A. Bozek, T. D. Pellett. 2007. Effects of dam removal on fish assemblage structure and spatial distribution in the Baraboo River, Wisconsin. *North American Journal of Fisheries Management* 27: 519-530.

Chester, H. and R. Norris. 2006. Dams and flow in the Cotter River, Australia: effects on instream trophic structure and benthic metabolism. *Hydrobiologia* 572: 275-286.

Cohen, R. R. H., P. V. Dresler, E. J. P. Phillips, R. L. Cory. 1984. The effect of the Asiatic clam, *Corbicula fluminea*, on phytoplankton of the Potomac River, Maryland. *Limnology and Oceanography* 29: 170-180.

Cole, J. J. and N. F. Caraco. 2001. Carbon in catchments: connecting terrestrial carbon losses with aquatic metabolism. *Marine Freshwater Resources*. 52: 101-110.

Cole, J. J., N. F. Caraco, B. L. Peierls. 1992. Can phytoplankton maintain a positive carbon balance in a turbid, freshwater, tidal estuary? *Limnology and Oceanography* 37: 1608-1617.

Collins, S. M., T. J. Kohler, S. A. Thomas, W.W. Fetzer, A. S. Flecker. 2015. The importance of terrestrial subsidies in stream food webs varies along a stream size gradient. *Oikos*: 674-685.

- Collins, S. M., J. P. Sparks, S.A. Thomas, S. A. Wheatley, A. S. Flecker. 2016. Increased light availability reduces the importance of bacterial carbon in headwater stream food webs. *Ecosystems* 19: 396-410.
- Crawford, J. T., L. C. Loken, E. H. Stanley, E. G. Stets, M.M. Dornblaser, R. G. Striegl. 2014. Basin scale controls on CO₂ and CH₄ emissions from the Upper Mississippi River. *Geophysical Research Letters*: 28: 197–210.
- Cross, W. F., C.V. Baxter, E. J. Rosi-Marshall, R. O. Hall, Jr., T. A. Kennedy, K. C. Donner, H. A. Wellard-Kelly, S. E. Z. Seegert, K. E. Behn, M D. Yard. 2013. Food-web dynamics in a large river discontinuum. *Ecological Monographs* 83: 311-337.
- Cross, W. F., C. V. Baxter, K.C. Donner, E.J. Rosi-Marshall, T.A. Kennedy, R. O. Hall, H A. Wellard Kelly, R. S. Rogers. 2011. Ecosystem ecology meets adaptive management: food web response to a controlled flood on the Colorado River, Glen Canyon. *Ecological Applications* 21: 2016-2033.
- Csiki, S., B. L. Rhoads. 2010. Hydraulic and geomorphological effects of run-of-river dams. *Progress in Physical Geography* 34:755-780.
- Cudney, M.D., J. B. Wallace. 1980. Life cycles, microdistribution and production dynamics of six species of net spinning caddisflies in large southeastern (USA) river. *Holarctic Ecology* 3: 169-182.
- Cumming, G. S. 2004. The impact of low-head dams on fish species richness in Wisconsin, USA. *Ecological Applications* 14: 1495-1506.
- Cummins, K. W., M.J. Klug, G.M. Ward, G. L. Spengler, R. W. Speaker, R. W. Ovink, D. C. Mahan, R. C. Peterson. 1981. Trends in particulate organic matter fluxes, community processes and macroinvertebrate functional feeding groups along a Great Lakes drainage basin river continuum. *Verhandlungen der Internationalen Vereinigung fur theoretische und angewandte Limnologie* 21:841-849.
- Deemer, B. R., J. A. Harrison, S. Li, J. J. Beaulieu, T. Delsontro, N. Barros, J. F. Bezerra-Neto, S. M. Powers. M. A. Dos Santos, J. A. Vonk. 2016. Greenhouse gas emissions from reservoir water surfaces: a new global synthesis *Bioscience* 66: 949-964.
- DeNiro. M.J. and Epstein, S. 1977. Mechanisms of carbon isotope fractionation associated with lipid synthesis. *Science* 197: 261-263.
- Delong, M. D. and J. H. Thorp. 2006. Significance of instream autotrophs in trophic dynamics of the Upper Mississippi River. *Oecologia* 147: 76-85.
- Delong, M. D., J. H. Thorp, K. S. Greenwood, M. C. Miller. 2001. Responses of consumers and food resources to a high magnitude, unpredicted flood in the Upper Mississippi River basin. *Regulated Rivers: Research and Management* 17: 217-234.

- Dickman, E. M., J. M. Newell, M. J. Gonzalez, M. J. Vanni. 2008. Light, nutrients and food chain length constrain planktonic energy transfer efficiency across multiple trophic levels. *Proceedings of the National Academy of Sciences* 105: 18408-18412.
- Dodds, W. K. 2006. Eutrophication and trophic state in rivers and stream. *Limnology and Oceanography* 51: 671-680.
- Doi, H., K-H. Chang, T. Ando, H. Imai, S-I Nakano, A. Kajimoto, I. Katano. 2008. Drifting plankton from a reservoir subsidize downstream food webs and alter community structure. *Oecologia* 156: 363-371.
- Doi, H., Y. Takemon, T. Ohta, Y. Ishida, E. Kikuchi. 2007. Effects of reach-scale canopy cover on trophic pathways of caddisfly larvae in a Japanese mountain stream. *Marine and Freshwater Research* 58: 811-817.
- Downing, J.A., J.J. Cole, C.M. Duarte, J.J. Middelburg, J.M. Melack, Y.T. Prairie, P. Kortelainen, R. G. Striegl, W. H. McDowell, L. J. Tranvik. 2012. Global abundance and size distribution of streams and rivers. *Inland Waters* 2: 229-236.
- Doyle, M. W., E. H. Stanley, C. H. Orr, A. R. Selle, S. A. Sethi, J. M. Harbor. 2005. Stream ecosystems response to a small dam removal: lessons from the heartland. *Geomorphology* 71: 227-244.
- Englund, G. 1993. Interactions in a lake outlet community: direct and indirect effects of net-spinning caddis larvae 66: 431-438.
- Environmental Protection Agency. NPL fact sheets for Michigan. Allied Paper, Inc; Portage Creek; Kalamazoo River. <http://www.epa.gov/R5Super/npl/michigan/MID006007306.htm>.
- Fairchild, M. P. and J. R. Holomuzki. 2002. Spatial variability and assemblage structure of stream hydropsychid caddisflies. *Journal of North American Benthological Society* **21**: 576-588.
- Finlay, J.C. 2004. Patterns and controls of lotic algal stable carbon isotope ratios. *Limnology and Oceanography*. 49: 850-861.
- Finlay, J. C., C. Kendall. 2007. Stable isotope tracing of temporal and spatial variability in organic matter sources to freshwater ecosystems. in Michener, R.H. and Lajtha, K. (Eds.) *Stable Isotopes in Ecology and Environmental Science*. 2nd edition, Blackwell Publishing.
- Finlay, J.C., S. Khandwala, M. E. Power. 2002. Spatial scales of carbon flow in a river food web. *Ecology* 83: 1845-1859.
- Finlay, J.C., M. E. Power, G. Cabana. 1999. Effects of water velocity on algal carbon isotope ratios: implications for river food web studies. *Limnology and Oceanography* 44: 1198-1203.

- Fitzgerald, S. A. and J. J. Steuer. 2006. Association of polychlorinated biphenyls (PCBs) with live algae and total lipids in rivers- a field based approach. *Science of the Total Environment* 354: 60-74.
- Francois, B., B. Hingray, D. Raynaud, M. Borga, J.D. Creutin. 2016. Increasing climate-related-energy penetration by integrating run-of-the-river hydropower to wind/solar mix. *Renewable Energy* 87: 686-696.
- Fry, B., M. Cieri, J. Hughes, C. Tobias, L. A. Deegan, B. Peterson. 2008. Stable isotope monitoring of benthic-planktonic coupling using salt marsh fish. *Marine Ecology Progress Series*. 369: 193-204.
- Gangloff, M.M., E. E. Hartfield, D. C. Werneke, J. W. Feminella. 2011. Association between small dams and mollusk assemblages in Alabama streams. *Journal of North American Benthological Society* 30: 1107-1116.
- Growns, I., B. Chessman, S. Mitrovic, D. Westhorpe. 2014. The effects of dams on longitudinal variation in river food webs. *Journal of Freshwater Ecology* 29: 69-83.
- Grubaugh J.W., Wallace J.B. & Houston E.S. 1997. Secondary production along a Southern Appalachian river continuum. *Freshwater Biology* 37: 581-596.
- Grubbs, S.A., J. M. Taylor. 2004. The influence of flow impoundment and river regulation on the distribution of riverine macroinvertebrates at Mammoth Cave National Park, Kentucky, U.S.A. *Hydrobiologia*. 520: 19-28.
- Gu, B., A. D. Chapman, C. L. Scheleske. 2006. Factors controlling seasonal variations in stable isotope composition of particulate organic matter in a soft water eutrophic lake. *Limnology and Oceanography* 51: 2837-2848.
- Hamilton, S.K., W.M. Lewis, Jr., and S.J. Sippel. 1992. Energy sources for aquatic animals on the Orinoco River floodplain: Evidence from stable isotopes. *Oecologia* 89: 324-330.
- Hamilton, S. K., S. J. Sippel, S. E. Bunn. 2005. Separation of algae from detritus for stable isotope for ecological stoichiometry studies using density fractionation in colloidal silica. *Limnology and Oceanography: Methods* 3: 149-157.
- Hamilton, S.K., S. J. Sippel, J.M. Melack. 1995. Oxygen depletion and carbon dioxide and methane production in waters of the Pantanal wetland of Brazil. *Biogeochemistry* 30: 115-141.
- Hamilton, S. K., J. L. Tank, D. F. Raikow, E. R. Siler, N. J. Dorn, N. E. Leonard. 2004. The role of instream vs allochthonous N in stream food webs: modeling the results of an isotope addition experiment. *Journal of the North American Benthological Society* 23: 429-448.

Hansen, J. F., D. B. Hayes. 2012. Long-term implications of dam removal for macroinvertebrate communities in Michigan and Wisconsin Rivers, United States. *River Research and Applications* 28: 1540-1550.

Hecky, R. E., R. H. Hesslein. 1995. Contributions of benthic algae to lake food webs as revealed by stable isotope analysis. *Journal of the North American Benthological Society* 14: 631-653.

Hein, T., C. Baranyi, G. J. Herndl, W. Wanek, F. Schiemer. 2003. Allochthonous and autochthonous particulate organic matter in floodplains of the River Danube: the importance of hydrological connectivity. *Freshwater Biology* 48: 220-232.

Hershey, A. E., R. W. Merritt, M. C. Miller, J. S. McCrea. 1996. Organic matter processing by larval black flies in a temperate woodland stream. *Oikos* 75: 524-532.

Herwig, B. R., D. A. Soluk, J. M. Dettmers, D. H. Wahl. 2004. Trophic structure and energy flow in backwater lakes of two large floodplain rivers assessed using stable isotopes. *Canadian Journal of Fisheries Aquatic Sciences* 61: 12-22.

Herwig, B. R., D. H. Wahl, J. M. Dettmers, D. A. Soluk. 2007. Spatial and temporal patterns in the food web structure of a large floodplain river assessed using stable isotopes. *Canadian Journal of Fisheries and Aquatic Sciences* 64: 495-508.

Hilton, J., M. O'Hare, M. J. Bowes, J. I. Jones. 2006. How green is my river? A new paradigm of eutrophication in rivers. *Science of the Total Environment* 365: 66-83.

Hoeinghaus, D. J., K. O. Winemiller, A. A. Agostinho. 2007. Landscape-scale hydrological characteristics differentiate patterns of carbon flow in large-river food webs. *Ecosystems* 10: 1019-1033.

Holt, C.R., D. Pfitzer, C. Scalley, B.A. Caldwell, P.I. Capece, D.P. Batzer. 2015. Longitudinal variation in macroinvertebrate assemblages below a large-scale hydroelectric dam. *Hydrobiologia* 755: 13-26.

Hornbach, D.J., M.C. Hove, H.T. Liu, F. R. Schenck, D. Rubin, B. J. Sansom. 2014. The influence of two differentially sized dams on mussel assemblages and growth. *Hydrobiologia* 724: 279-291.

Hurn, A. D., R.H. Riley, R. G. Young, C. J. Arbuckle, K. Peacock, G. Lyons. 2001. Temporal shift in contribution of terrestrial organic matter to consumer production in a grassland river. *Freshwater Biology* 46: 213-226.

Ishikawa, N.F., H. Doi, J.C. Finlay. 2012. Global meta-analysis for controlling factors on carbon stable isotope ratios of lotic periphyton. *Oecologia* 170: 541-549.

Istvanovics V. & Honti M. 2011. Phytoplankton growth in three rivers: the role of meroplankton and the benthic retention hypothesis. *Limnology and Oceanography* 56:1439–1452.

Jardine, T.D., R. A. Curry, K. S. Heard, R. A. Cunjak. 2005. High fidelity: isotopic relationship between stream invertebrates and their gut contents. *Journal of North American Benthological Society* 24: 290-299.

Jardine, T.D., N.E. Pettit, D. M. Warfe, B. J. Pusey, D. P. Ward, M. M Douglas, P. M Davis, S.E. Bunn. 2012. Consumer-resource coupling in wet-dry tropical rivers. *Journal of Animal Ecology* 81: 310-322.

Jardine, T.D. R. Woods, J. Marshall, J. Fawcett, J. Lobegeiger, D. Valdez, M. J. Kainz. 2015. Reconciling the role of organic matter pathways in aquatic food webs by measuring multiple tracers in individuals. *Ecology* 96:3257–3269.

Jepsen, D. B., K. O. Winemiller. 2002. Structure of tropical river food webs revealed by stable isotope ratios. *Oikos* 96: 46-55.

Junger, M., D. Planas. 1994. Quantitative use of stable carbon isotope analysis to determine the trophic base of invertebrate communities in a boreal forest lotic system. *Canadian Journal of Fisheries and Aquatic Science* 51: 52-61.

Junk, W.J., P. B. Bayley, R. E. Sparks. 1989. The flood pulse concept in river-floodplain systems. D.P. Dodge [ed] *Proceedings of the International Large River Symposium*. Can. Spec. Publ. Fish. Aquat. Sci. 106: 110-127.

Junker, J. R., W. F. Cross. 2014. Seasonality in the trophic basis of a temperate stream invertebrate assemblage: Importance of temperature and food quality. *Limnology and Oceanography* 59: 507-518.

Karlsson, J., M. Berggren, J. Ask, P. Bystrom, A. Jonsson, H. Laudon, M. Jansson. 2014. Response to comment: Terrestrial support of pelagic consumers in unproductive lakes- Uncertainty and potential in assessments using stable isotopes. *Limnology and Oceanography* 59: 1800-1803.

Kautza, A., S. M. Sullivan. 2016. The energetic contribution of aquatic primary production to terrestrial food webs in a mid-size river system. *Ecology* 97: 694-705.

Kay, D. P., A. L. Blakenship, K. K. Coady, A. M. Neigh, M. J. Zwiernik, S. D. Millsap, K. Stause, C. Park, P. Bradley, J. L. Newsted, P. D. Jones, J. P. Giesy. 2005. Differential accumulation of polychlorinated biphenyl congeners in the aquatic food web at the Kalamazoo River Superfund Site, Michigan. *Environmental Science and Technology* 39: 5964-5974.

Kempe S (1982) Long-term records of CO₂ pressure fluctuations in fresh waters. *Mitt Geol-Palaont Inst, Univ Hamburg, SCOPE/UNEP Sonderband* 52: 91-332.

- Kendall, C., S. R. Silva, V.J. Kelly. 2001. Carbon and nitrogen isotopic compositions of particulate organic matter in four large river systems across the United States. *Hydrological Processes* 15: 1301-1346.
- Kennedy, T. A., J. D. Muehlbauer, C. B. Yackulic, D. A. Lytle, S. M. Miller, K. L. Dibble, E. W. Korenhoeven, A. N. Metcalfe, C. V. Baxter. 2016. Flow management for hydropower extirpated aquatic insects, undermining river food webs. *Bioscience*. 66:561-575.
- Keough, J.R., M. E. Sierszen, C. A. Hagley. 1996. Analysis of a Lake Superior coastal food web with stable isotope techniques. *Limnology and Oceanography* 41: 136-146.
- Kibler, K. M., D. D. Tullos. 2013. Cumulative biophysical impact of small and large hydropower development in Nu River, China. *Water Resources Research* 49: 3104-3118.
- King, R. S. and C. J. Johnson. 2007. Subsidy-stress response of macroinvertebrate community biomass to a phosphorous gradient in an oligotrophic wetland ecosystem. *Journal of North American Benthological Society* 26: 491-508.
- Kobayashi, S., F. Akamatsu, K. Amano, S. Nakanishi, Y. Oshima. 2011. Longitudinal changes in $\delta^{13}\text{C}$ of riffle macroinvertebrates from mountain to lowland sections of a gravel-bed river. *Freshwater Biology* 1434-1446.
- Kumar, D., S.S. Katoch. 2015. Sustainability suspense of small hydropower projects: a study from western Himalayan region of India. *Renewable Energy* 76: 220-233.
- Lehmann, M.F., S. M. Bernasconi, J. A. McKenzie, A. Barbieri, M. Simona, M. Veronesi. 2004. Seasonal variation of the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of particulate and dissolved carbon and nitrogen in Lake Lugano: Constraints on biogeochemical cycling in a eutrophic lake. *Limnology and Oceanography* 49: 415-429.
- Lehner, B., C. R. Liermann, C. Ravenga, C. Vorosmarty, B. Fekete, P. Crouzet, P. Doll, M. Endejan, K. Frenken, J. Magome, C. Nilsson, J.C. Robertson, R. Rodel, N. Sindork, D. Wisser. 2011. High resolution mapping of the world's reservoirs and dams for sustainable river- flow management. *Frontiers in Ecology and the Environment* 9: 494-502.
- Leigh, C., M. A. Burford, F. Sheldon, S. E. Bunn. 2010. Dynamic stability in dry season food webs within tropical floodplain rivers. *Marine and Freshwater Research* 61: 357-358.
- Lennon, J. T., A.M. Faiia, X. Feng, K. L. Cottingham. 2006. Relative importance of CO_2 recycling and CH_4 pathways in lake food webs along a dissolved organic carbon gradient. 51: 1602-1613.
- Lewis, W. M., 1988. Primary production in the Orinoco River. *Ecology* 69: 679-692.

- Lewis, W.M., S. K. Hamilton, M. A. Rodriguez, J. F. Saunders, M. A. Lasi. 2001. Foodweb analysis of the Orinoco floodplain based on production estimates and stable isotope data. *Journal of North American Benthological Society* 20: 241-254.
- Logan, J. M., T. D. Jardine, T. J. Miller, S. E. Bunn, R. A. Cunjak, M. E. Lutcavage. 2008. Lipid correction in carbon and nitrogen stable isotope analyses: comparison of chemical extraction and modelling methods. *Journal of Animal Ecology* 77: 838-846.
- Lucas, L. V., J. K. Thompson. 2012. Changing restoration rules: exotic bivalves interact with residence time and depth to control phytoplankton productivity. *Ecosphere* 3: 1-25.
- Mackay, R. J. and T. F. Waters. 1986. Effects of small impoundments on Hydropsychid caddisfly production in Valley Creek, Minnesota. *Ecology* 67: 1680-1686.
- Marcarelli, A. M., C. V. Baxter, M. M. Mineau, R. O. Hall. 2011. Quantity and quality: unifying food web and ecosystem perspectives on the role of resource subsidies in freshwaters. *Ecology* 92: 1215-1225.
- Marczak, L.B., J.S. Richardson. 2008. Growth and development rates in a riparian spider are altered by asynchrony between the timing and amount of a resource subsidy. *Oecologia* 156:249-258.
- Marty, J., D. Planas. 2008. Comparison of methods to determine algal $\delta^{13}\text{C}$ in freshwater. *Limnology and Oceanography: Methods* 6: 51-63.
- McCutchan, J. H. Jr., W. M. Lewis, Jr. 2002. Relative importance of carbon sources for macroinvertebrates in a Rocky Mountain stream. *Limnology and Oceanography* 47: 742-752.
- McNeely, C., S. M. Clinton, J. M. Erbe. 2006. Landscape variation in C sources of scraping primary consumers in streams. *Journal of North American Benthological Society* 25: 787-799.
- McNeely, C., J. C. Finlay, M. E. Power. 2007. Grazer traits, competition, and carbon sources to a headwater-stream food web. *Ecology* 88: 391-401.
- McShaffry, D., W. P. McCafferty. 1986. Feeding behavior of *Stenacron interpunctatum* (Ephemeroptera: Heptageniidae). *Journal of the North American Benthological Society* 5:200-210.
- Middelburg, J.J. 2014. Stable isotopes dissect aquatic food webs from the top to bottom. *Biogeosciences* 11: 2357-2371.
- Munn, M. D. and M. A. Brusven. 2004. The influence of Dworshak Dam on epilithic community metabolism in the Clearwater River, U.S.A. *Hydrobiologia* 513: 121-127.
- Naiman, R. J. 1983. The influence of stream size on the food quality of seston. *Canadian Journal of Zoology* 61: 1995-2010.

- Nakano, S., H. Miyasaka, N. Kuhara. 1999. Terrestrial-aquatic linkages: riparian arthropod inputs alter trophic cascades in a stream food web. *Ecology* 80: 2435-2441.
- Newbold, J. D., S. A. Thomas, G. W. Minshall, C. E. Cushing, T. Georgian. 2005. Deposition, benthic residence, and resuspension of fine organic particles in a mountain stream. *Limnology and Oceanography* 50: 1571-1580.
- Nichols, S., R. Norris, W. Maher, M. Thoms. 2006. Ecological effects of serial impoundment on the Cotter River, Australia. *Hydrobiologia* 572: 255-273.
- Ochs, C. A., O. Pongruktham & P. V. Zimba, 2013. Darkness at the break of noon: phytoplankton production in the Lower Mississippi River. *Limnology and Oceanography* 58: 555–568.
- Oliver, A. A., R. A. Dahlgren, M. L. Deas. 2014. The upside-down river: Reservoirs, algae blooms, and tributaries affect temporal and spatial patterns in nitrogen and phosphorus in the Klamath River, USA. *Journal of Hydrology* 519: 164-176.
- Orr, C.H., B. M. Roth, K. J. Forshay, J. D. Gonzales, M. M. Papenfus, R. D. G. Wassell. 2004. Examination of physical and regulatory variables leading to small dam removal in Wisconsin. *Environmental Management* 33: 99-109.
- Orr, C. H., S. J. Kroiss, K. L. Rogers. E. H. Stanley. 2008. Downstream benthic responses to small dam removal in a coldwater stream. *River Research and Applications* 24: 804-822.
- Oswood, M. W. 1979. Abundance patterns of filter-feeding caddisflies (Trichoptera: Hydropsychidae) and seston in a Montana (U.S.A.) lake outlet. *Hydrobiologia* 63: 177-183.
- Paetzold, A., C.J. Schubert, K. Tockner. 2005. Aquatic terrestrial linkages along a braided-river: riparian arthropods feeding on aquatic insects. *Ecosystems* 8:748-759.
- Palmer, R., J. O’Keeffe. 1990. Transported material in a small river with multiple impoundments. *Freshwater Biology* 24: 563-575.
- Parker, C. R., J.R. Voshell. 1983. Production of filter-feeding Trichoptera in an impounded and a free-flowing river. *Canadian Journal of Zoology* 61: 70-87.
- Peterson B. J., B. Fry. 1987. Stable isotopes in ecosystem studies. *Annual Review of Ecology and Systematics*. 18: 293-320.
- Phillips, D. L. and J. W. Gregg. 2003. Source partitioning using stable isotopes: coping with too many sources. *Oecologia* 136: 261-269.
- Poff, N. L., J. D. Olden, D. M. Merritt, D. M. Pepin. 2007. Homogenization of regional river dynamics by dams and global biodiversity implications. *Proceedings of the National Academy of Science*. 104: 5732-5737.

- Polis, G. A., W. B. Anderson, R. D. Holt. 1997. Toward an integration of landscape and food web ecology: the dynamics of spatially subsidized food webs. *Annual review of Ecology and Systematics* 28: 289-316.
- Post, D.M. 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology*. 83: 703-718.
- Post, D. M., C. A. Layman, D. A. Arrington, G. Takimoto, J. Quattrochi, C. G. Montaña. 2007. Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analysis. *Oecologia* 152: 179-189.
- Poulos, H. M., K. E. Miller, M. L. Kraczkowski, A. W. Welchel, R. Heineman, B. Chernoff. 2014. Fish assemblage response to a small dam removal in the eight mile river system, Connecticut, U.S.A. *Environmental Management* 54: 1090-1101.
- Rachol, C.M., F.A. Fitzpatrick, and T. Rossi. 2005. Historical and simulated changes in channel characteristics of the Kalamazoo River, Plainwell to Otsego, Michigan: U.S. Geological Survey Scientific Investigations Report 2005-5044: 62 pages.
- Rasmussen, J.B. 2010. Estimating terrestrial contribution to stream invertebrates and periphyton using a gradient-based mixing model for $\delta^{13}\text{C}$. *Journal of Animal Ecology* 79: 393-402.
- Rasmussen, J.B., V. Trudeau. 2007. Influence of velocity and chlorophyll standing stock on periphyton $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in the Ste. Marguerite River system, Quebec. *Canadian Journal of Fisheries and Aquatic Sciences* 64: 1370-1381.
- Reid, N. J. and S. K. Hamilton. 2007. Controls on algal abundance in a eutrophic river with varying degrees of impoundment (Kalamazoo River, Michigan, (USA)). *Lake and Reservoir Management* 23: 219-230.
- Reisinger, A.J., J. L. Tank, E. J. Rosi-Marshall, R.O. Hall, M.A. Baker. 2015. The varying role of water column nutrient uptake along river continua in contrasting landscapes. *Biogeochemistry* 125: 115-131.
- Reynolds, C. S. and J. P. Descy. 1996. The production, biomass and structure of phytoplankton in large rivers. *Archiv fur Hydrobiologie Supplement* 10: 161-187.
- Reynolds, C.S. 2000. Hydroecology of river plankton: the role of variability in channel flow. *Hydrological Processes* 14: 3119-3132.
- Richardson, J. S. and R. J. Mackay. 1991. Lake outlets and the distribution of filter feeders: an assessment of hypotheses. *Oikos* 62: 370-380.
- Richardson, J. S., 1984. Effects of seston quality on the growth of a lake-outlet filter feeder. *Oikos*. 43: 386-390.

- Roach, K. A., 2013. Environmental factors affecting incorporation of terrestrial material into a large river food web. *Freshwater Science* 32: 283-298.
- Robinson, C.T., G. W. Minshall. 1990. Longitudinal development of macroinvertebrate communities below oligotrophic lake outlets. *Great Basin Naturalist* 50: 303-311.
- Rosi-Marshall, E. J. and J. B. Wallace. 2002. Invertebrate food webs along a stream resource gradient. *Freshwater Biology* 47: 129-141.
- Rosi-Marshall, J.L. Meyer. 2004. Quality of suspended fine particulate matter in the Little Tennessee River. *Hydrobiologia* 519: 29-37.
- Rounick, J. S., M. J. Winterbourn, G. L. Lyon. 1982. Differential utilization of allochthonous and autochthonous impacts by aquatic invertebrates in some New Zealand streams: a stable carbon isotope study. *Oikos* 39: 191-198.
- Sabo, J. L., M. E. Power. 2002. Numerical response of lizards to aquatic insects and short-term consequences for terrestrial prey. *Ecology* 83: 3023-3036.
- Santucci, V. J., S. R. Gephard, S. M. Pescitelli. 2005. Effects of multiple low-head dams on fish, macroinvertebrates, habitat, and water quality in the Fox River, Illinois. *North American Journal of Fisheries Management* 25: 975-992.
- Sanzone, D. M., J. L. Meyer, E. Marti, E. P. Gardiner, J. L. Tank, N. B. Grimm. 2003. Carbon and nitrogen transfer from a desert stream to riparian predators. *Oecologia* 134: 238-250.
- Sellers, T. and P. A. Bukaveckas. 2003. Phytoplankton production in a large, regulated river: A modeling and mass balance assessment. *Limnology and Oceanography* 48: 1476-1487.
- Singer, E.E, M.M. Gangloff. 2011. Effects of a small dam on freshwater mussel growth in an Alabama (U.S.A.) stream. *Freshwater Biology* 56: 1904-1915.
- Smyntek, P.M., S.C. Maberly, J. Grey. 2012. Dissolved carbon dioxide concentrations controls baseline stable carbon isotope signatures of a lake food web. *Limnology and Oceanography*. 57: 1292-1302.
- Soballe, D. M. and B. L. Kimmel. 1987. A large scale comparison of factors influencing phytoplankton abundance in rivers, lakes and impoundments. *Ecology* 68: 1943-1954.
- Stanford, J. A., J. V. Ward, W. J. Liss, C. A. Frissell, R. N. Williams, J. A. Lichatowich and C. C. Coutant. 1996. A general protocol for restoration of regulated rivers. *Regulated River Research and Management* 12: 391-413.
- Stanley, E. H, M. W. Doyle. 2002. A geomorphic perspective on nutrient rendition following dam removal. *Bioscience* 52: 693-701.

Stanley, E. H., M. A. Luebke, M.W. Doyle, D.W. Marshall. 2002. Short-term changes in channel form and macroinvertebrate communities following low-head dam removal. *Journal of North American Benthological Society* 21: 172-187.

Stanley, E. H. and M. W. Doyle. 2002. Trading off: the ecological effects of dam removal. *Frontiers in Ecology and Environment* 1: 15-22.

Strayer, D. L., M. L. Pace, N. F. Caraco, J.J. Cole, S. E. G. Findlay. 2008. Hydrology and grazing jointly control a large-river food web. *Ecology* 89: 12-18.

Sullivan, S. M. P. 2013. Stream foodweb $\delta^{13}\text{C}$ and geomorphology are tightly coupled in mountain drainages of northern Idaho. *Freshwater Science* 32: 606-621.

Taipale, S. J., Peltomaa, E., Hiltunen, M., R. I. Jones, M. W. Hahn, C. Biasi, M. Brett. 2015. Inferring phytoplankton, terrestrial plant and bacteria bulk $\delta^{13}\text{C}$ from compound specific analysis of lipids and fatty acids. *PLoS One* 10 (7).

Tagwireyi, P., S. M. P. Sullivan. 2015. Distribution and trophic dynamics of riparian tetragnathid spiders in a large river system. *Marine and Freshwater Research* 67: 30-318.

Thorp, J.H., M.D. DeLong, K.S. Greenwood, A.F. Casper. 1998. Isotopic analysis of three food web theories in constricted and floodplain regions of a large river. *Oecologia* 117:551-563.

Torres-Ruiz, M., J. D. Wehr, A. A. Perrone. 2007. Trophic relations in a stream food web: importance of fatty acids for macroinvertebrate consumers. *Journal of North American Benthological Society*. 26: 509-522.

Ulseth, A. J., R. O. Hall. 2015. Dam tailwaters compound the effects of reservoirs on the longitudinal transport of organic carbon in an arid river. *Biogeosciences* 12: 6081-6114.

Van den Meersche, K., P. Van Rijwijk, K. Soetaert, J. J. Middelburg. 2009. Autochthonous and allochthonous contributions to mesozooplankton diet in a tidal river and estuary: Integrating carbon isotope and fatty acid constraints. *Limnology and Oceanography* 54: 62-74.

Vander Zanden, M. J., J. B. Rasmussen. 1996. A trophic position model of pelagic food webs: impact on contaminant bioaccumulation in lake trout 66: 451-477.

Vander Zanden, M. J., Y. Vadeboncoeur, S. Chandra. 2011. Fish reliance on littoral-benthic resources and the distribution of primary production in lakes. *Ecosystems* 14: 894-903.

Vannote, R. L., G. W. Minshall, G., K. W. Cummins, J. R. Sedell, and C. E. Cushing. 1980. The river continuum concept. *Canadian Journal of Fisheries and Aquatic Science* 37: 130-137.

Vinson, M.R. 2001. Long term dynamics of an invertebrate assemblage downstream from a large dam. *Ecological Applications* 11: 711-730.

- Voshell, J.R., C. R. Parker. 1985. Quantity and quality of seston in an impounded and free-flowing river in Virginia, USA. *Hydrobiologia* 122: 271-280.
- Vuorio, K., M. Meili, J. Sarvala. 2006. Taxon-specific variation in the stable isotopic signatures $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of lake phytoplankton. *Freshwater Biology* 51: 807-822.
- Wallace, J. B. 1975. Food partitioning in net-spinning Trichoptera larvae: *Hydropsyche venularis*, *Cheumatopsyche etrona*, and *Macronema zebratum*. - *Ann. Ent. Soc. Am.* 68: 463-72.
- Wallace, J. B., S. L. Eggert, J. L. Meyer, and J. R. Webster. 1997. Multiple trophic levels of a forested stream linked to terrestrial litter inputs. *Science* 277: 102-104.
- Walters, D.M., K. M. Fritz, D. L. Phillips. 2007. Reach-scale geomorphology affects organic matter and consumers $\delta^{13}\text{C}$ in a forested Piedmont stream. *Freshwater Biology* 52: 1105-1119.
- Walters, D. M., K. M. Fritz, R. R. Otter. 2008. The dark side of subsidies: adult stream insects export organic contaminant to riparian predators. *Ecological Applications* 18: 1835-1841.
- Wang, L., D. Infante, J. Lyons, J. Stewart, A. Cooper. 2011. Effects of dams in river networks on fish assemblages in non-impoundment sections of rivers in Michigan and Wisconsin, USA. *River Research and Applications*. 27: 473-487.
- Ward, J. V., J. A. Stanford. 1983. The serial discontinuity concept of lotic ecosystems. in Fontaine, T. D. and Bartell, S. M. (Eds) *Dynamics of Lotic Ecosystems*. Ann Arbor Science, Ann Arbor pp. 29-42.
- Wellard Kelly, H.A., E. J. Rosi-Marshall, T. A. Kennedy, R. O. Hall, Jr., W.F. Cross, C. V. Baxter. 2013. Macroinvertebrate diets reflect tributary inputs and turbidity-drive changes in food availability in the Colorado River downstream of Glen Canyon Dam. *Freshwater Science* 32: 397-410.
- Welschmeyer, N.A. 1994. Fluorometric analysis of chlorophyll *a* in the presence of chlorophyll *b* and pheopigments. *Limnol. Oceanogr.* 39:1985-1992.
- Wesley, J.K. 2005. Kalamazoo River assessment. Michigan Department of Natural Resources, Fisheries Division, Special Report 35, Ann Arbor.
- Wetzel, R. G., G. E. Likens. 1991: *Limnological analyses*. 2nd Ed. Springer-Verlag, New York.
- Whiles, M. R. and W. K. Dodds. 2002. Relationships between stream size, suspended particles, and filter feeding macroinvertebrates in a Great Plains Drainage Network. *Journal of Environmental Quality* 31: 1589-1600.
- Wilkinson, G. M., M.L. Pace, J.J. Cole. 2013. Terrestrial dominance of organic matter in north temperate lakes. *Global Biogeochemical Cycles* 27: 43-51.

- Winemiller, K.O., P. B. McIntyre, L. Castello, E. Fluent-Chouinard and 36 other authors. 2016. Balancing hydropower and biodiversity in the Amazon, Congo and Mekong. *Science* 351: 128-129.
- Wohl, E., B. P. Bledsoe, R. B. Jacobson, N. L. Poff, S. L. Rathburn, D. M. Walters., A. C. Wilcox. 2015. The natural sediment regime in rivers: broadening the foundation for ecosystem management. *Bioscience* 65: 358-371.
- Woodland, R. J., M. A. Rodriguez, P. Magnan, H. Glemet, G. Cabana. 2012. Incorporating temporally dynamic baselines in isotopic mixing models. *Ecology* 93: 131-144.
- Yoshii, K., N. G. Melnik, O. A. Timoshikin, N. A. Bondarkenko, P. N Anoshko, T. Yoshioka. E. Wada. 1999. Stable isotope analyses of the pelagic food web in Lake Baikal. *Limnology and Oceanography* 44: 501-511.
- Yoshioka, T., E. Wada, H. Hayashi. 1994. A stable isotope study on seasonal food web dynamics in a eutrophic lake. *Ecology* 75: 835-846.
- Zah, R., P. Burgherr, S. M. Bernasconi, U. Uehlinger. 2001. Stable isotope analysis of macroinvertebrates and their food sources in a glacier stream. *Freshwater Biology* 46: 871-882.
- Zeug, S. and K. O. Winemiller. 2008. Evidence supporting the importance of terrestrial carbon in a large river food web. *Ecology* 89: 1733-1743.
- Zhang, Q., D.C. Brady, W. P. Ball. 2014. Long term seasonal trends of nitrogen, phosphorus and suspended sediment load from the non-tidal Susquehanna River Basin to Chesapeake Bay. *Science of the Total Environment*. 452-453- 208-221.
- Zohary, T., J. Erez, M. Gophen, I. Berman-Frank, M. Stiller. 1994. Seasonality of stable carbon isotopes within the pelagic food web of Lake Kinneret. *Limnology and Oceanography* 39: 1030-1043.

CHAPTER 3: IMPACTS OF A MAJOR OIL SANDS PIPELINE SPILL ON BENTHIC MACROINVERTEBRATES IN THE KALAMAZOO RIVER (MICHIGAN)

ABSTRACT

The Kalamazoo River experienced one of the largest freshwater oil spills in North America and one of the first major releases of oil sands crude. On 25 July 2010, a pipeline break released an estimated 843,000 gallons of diluted bitumen oil. Submerged oil became particularly important in the Kalamazoo River spill because of the heavy nature of the bitumen, and clean up continued for four years after the spill. Hester Dendy multi-plate artificial substrata were used to quantify the effects on macroinvertebrates that live on hard substrata in mid fall, three months after the spill. Leaf pack bags were deployed in late fall to quantify effects on macroinvertebrates that consume fallen leaves over winter. An in-situ bioassay using *Hyaletta azteca* amphipods was conducted to assess lethal and sublethal toxicity one year after the spill. This study found that macroinvertebrate densities on Hester Dendy and leaf pack samplers were reduced immediately after the oil spill in two sections of river, but partially recovered one year later and fully recovered two years after the spill. Densities of shredders, filter feeders, and collector gatherers were all reduced in two sections of river. Filter-feeding macroinvertebrates were reduced by 90%. The results of the in-situ bioassay showed that survival was not reduced, but growth was reduced in some sections of river. This work supports conclusions from other studies of conventional crude oil spills into rivers where macroinvertebrates were reduced but recovered after about one year. Studies of other oil spills using in-situ bioassays with invertebrates have shown sublethal impacts as observed in this study, and studies of fishes have also shown sublethal impacts. Hydropsychidae in particular appeared to have been reduced in the most impacted reaches of the Kalamazoo River during the first year after the spill, which has also been observed in other studies.

INTRODUCTION

An extensive network of pipelines currently exists throughout North America transporting both conventional crude oils and diluted oil-sands bitumen. In addition, several new pipelines have been in development to distribute diluted oil sands bitumen or its products, and existing pipelines have been replaced with larger capacity pipelines (Crosby et al. 2013). Transportation of crude oil via pipelines, rail and barge each brings different risks (Crosby et al. 2013, State Department 2013). However, diluted bitumen does not appear to be more corrosive to pipelines than conventional crudes (Barteau et al. 2013). Oil sands development and production have been increasing steadily in the recent decades with improvements of technology and until recently, high prices of petroleum (Giesy et al. 2010, Schindler 2010).

The Kalamazoo River oil spill in 2010 was one of the largest inland spills in U.S. history, and the largest diluted bitumen spill to reach surface waters. Several conventional crude oil spills of the magnitude of the Kalamazoo River occurred in the late 1980s including the Gasconade River (Poulton et al. 1997), Chariton River (Poulton et al. 1998), Asher Creek (Crunkilton and Duchrow 1990), and the Monongahela and Ohio River spill (Cronk et al. 1990). Since then, such spills have been less frequent in North America with small spills occurring with greater frequency (i.e. Lytle and Peckarsky 2001). More recently, several comparably large spills have occurred in Canada, the Yellowstone River, and in Arkansas, along with train derailments (Crosby et al. 2013, Table 3.1). The increased development of oil exploration and transportation puts freshwater systems at increasing risk of spills.

Studies from conventional crude oil spills into streams have shown nearly 90% reduction of macroinvertebrate densities in the months after the spill, and recovery usually occurs within one year (McCauley 1966, Crunkilton and Duchrow 1990, Poulton et al. 1997, Poulton et al.

1998, Lytle and Peckarsky 2001). Studies following these large river spills have been limited to studying ecological impacts in the Gasconade River (Poulton et al. 1997), Chariton River (Ort et al. 1995, Poulton et al. 1998), Patuxent River (Michel et al. 2009), and Delaware River (Aquatic Technical Working Group 2007). Macroinvertebrate communities have been known to be impacted from river surveys on the Athabasca River, which contains bitumen in sediments from natural sources (Barton and Lock 1979 a and b). Based on these studies, macroinvertebrates were expected to be reduced in abundance following the spill on the Kalamazoo River.

The nature of the diluted bitumen makes it more prone to submergence than most conventional crude oils. The viscous bitumen must be diluted with lighter hydrocarbons (often natural gas condensate) to be transported, and the diluted bitumen carried in pipelines is a mixture with a density comparable to conventional crude (Masliya 2004, Crosby et al. 2013, McKnight et al. 2016). However, the diluent quickly evaporates after a spill leaving the heavier components to attach to particles and sink (Crosby et al. 2013, King et al. 2014, Fitzpatrick et al. 2015, Perez et al. 2016, McKnight et al. 2016). These oil-particle aggregates are transported downstream and may accumulate in side channels and backwaters (Crosby et al. 2013, Fitzpatrick et al. 2015). Submerged oil became particularly important following the Kalamazoo River spill (McKnight et al. 2016). Given the unique nature of diluted bitumen, understanding the immediate impacts is important for managing spill clean-up and response (Crosby et al. 2013, Fitzpatrick et al. 2015, McKnight et al. 2016).

Studies from conventional oil spills have found sublethal impacts from residual hydrocarbons lasting from a few weeks to several years after the spill (Ort et al. 1995, Michel et al. 2009). Long after these spills occur, remnant oil can be measured in sediments (Wang et al. 1998, Uhler et al. 2005, Michel et al. 2009). Additionally, more diffuse petroleum hydrocarbon

contamination originates from multiple sources in freshwater systems (Yunker et al. 2002, Uhler et al. 2005, Forsberg et al. 2014). To test for toxicity and sublethal impacts, in-situ bioassays using amphipods (*Hyaletella azteca*) have found that river sediment with historical polycyclic aromatic hydrocarbon contamination exhibited toxicity ranging from low to high (Ingersoll et al. 2001, Michel et al. 2009, Tao et al. 2010, Harding et al. 2011). Studies conducted before the spill called Athos that occurred on the Delaware River found that longer term oil refinery discharge had resulted in biological impacts from hydrocarbon contamination (Alexander et al. 2005, Salazar et al. 2005, Uhler et al. 2005).

Direct toxic effects of the most volatile fractions (i.e., the diluent, which was mostly benzene in the case of the Kalamazoo River spill) were expected, but the toxicity of the bitumen from a spill into a river had not been studied much. Toxicological studies from the Athabasca oil sands mining area have found lethal and sublethal impacts from diluted bitumen, unprocessed oil sands, or oil sands process water on fish, macroinvertebrates and other organisms (Philibert et al. 2016, Lari et al. 2016 Alharbi et al. 2016, Colavecchia et al. 2004, Madison et al. 2015, Morandi et al. 2015, Schwalb et al. 2015). Laboratory bioassays using Chironomidae and oil sands bitumen from the Athabaskan region have found sublethal impacts (Anderson et al. 2012, Wiseman et al. 2013 a). This would suggest that toxic and sublethal impacts could result from the submerged bitumen in the case of the Kalamazoo River oil spill.

Oil spills into marine systems have been studied for longer periods following a spill, and cover a larger range of responses. Such examples are useful for estimating future impacts and recovery on the Kalamazoo River. For example, effects from the Exxon Valdez have been observed, but lack of baseline (pre-spill) information as well as perturbations created by clean-up actions confounded the identification of impacts from the spill (Paine 1996). Polycyclic aromatic

hydrocarbons were present in sediments but gradually weathered in beach sediments following the Exxon Valdez spill (Carls et al. 2016). Impacts on oiled mussel beds (Boehm et al. 2004, Carls et al. 2004a), otters (Bowyer et al 2003), fish (Carls et al. 2004b, Incardona et al. 2015), birds (Seiser et al 2000) and orcas (Paine et al. 1996) have been documented to last for decades after the Exxon Valdez spill. These impacts extend into higher trophic levels (Peterson et al. 2003). Similarly, the impacts of the 2010 Deepwater Horizon spill and clean-up on wetland habitats were found to be significant albeit variable (Mendelssohn et al. 2011). While massive oil spills appear to occur from time to time in marine systems, large conventional oil spills in freshwater systems are less common, and their ecological effects have been studied less frequently (Green and Trent 1989). This makes predicting impacts from the submerged bitumen oil challenging.

Hydrocarbons in general contain metals, but Canadian oil sands crude is particularly high in certain metals including nickel and vanadium (Zuliani et al. 2016, Jensen-Fontaine et al. 2014, Millson et al. 1966). Vanadium has been found to bioaccumulate in animals following marine spills known as Erika (Amiard et al. 2008 and Chiffoleau et al. 2004), Prestige (Laville et al. 2006), and the Gulf War (Sadiq and McCain 1993). In freshwater systems, vanadium has been found to bioaccumulate in proportion to concentration when using vanadium test solutions in lab studies (Jensen-Fontaine et al. 2014) and some taxa bioaccumulated metals in wetlands receiving oil sands process water (Baker et al. 2012). Vanadium was found to bioaccumulate in *Sphagnum* moss collected from bogs impacted by atmospheric contamination in the Athabaskan oil sands mining area (Shotyk et al. 2014). In freshwater studies, crayfish have been found to bioaccumulate heavy metals into tissues including vanadium and nickel (e.g., Kouba et al. 2010, Martin-Diaz et al. 2006, Lawrence 1992) and they bioaccumulate polycyclic aromatic hydrocarbons.

(Forsberg et al. 2014). For these reasons, I selected crayfish as an indicator of bioaccumulation of metals following the Kalamazoo River oil spill.

This study examines the impacts of the 2010 Kalamazoo River oil spill, which was the largest diluted bitumen spill ever to occur in an aquatic environment. The spill occurred in the midst of my dissertation work on the river, and the oiled reaches overlapped with the upper end of my study reach. My research thus had to be reorganized to examine the effects of the oil spill. Here I summarize the immediate impacts and examine the longer-term effects of the spill on benthic macroinvertebrates. I hypothesized that while macroinvertebrate communities would be reduced following the spill, they would recover after a year or so, as had been found in spills of conventional crude oils. I employed several approaches to examine the impacts of the oil spill.

METHODS

As soon as I was granted access to the river, I sampled benthic macroinvertebrates by deploying artificial substrata six weeks deployed following the spill (Hester Dendy multi-plate samplers) to determine immediate toxicity to the oil. Five months after the spill, I placed “leaf pack” samplers, which were leaves in mesh bags, in the river to measure colonization by macroinvertebrates that are important decomposers of leaf litter. Later, I conducted in-situ bioassays using juvenile *Hyalella azteca* (Amphipoda) (Burton et al. 2005) to look for possible longer term, sub-lethal impacts of submerged oil after one year following the spill. I hypothesized that due to the likelihood of submerged oil, I would see negative impacts in the assays, especially in depositional habitats where submerged oil may be more likely to occur rather than in flowing water habitats. In addition, I collected crayfish (*Orconectes* spp.) to measure possible metal uptake, hypothesizing that crayfish collected from areas with the greatest

macroinvertebrate impacts would also have increased metal bioaccumulation, including particularly vanadium and nickel found in the oil, 1.5 years after the spill. Lastly, I present data related to other ecosystem impacts including algal production and phosphorus concentrations that were collected as ancillary information.

Description of the 2010 Kalamazoo River oil spill

On 25 July 2010, the Kalamazoo River experienced one of the largest freshwater spills and the first major oil sands spill in the United States. The rupture occurred on Line 6B, a pipeline owned by Enbridge Corporation, at a segment buried under a wetland adjacent to Talmadge Creek, a small tributary of the river. Investigation found that the weakness of the aging pipeline caused the failure, and human error contributed to the magnitude of the spill. Some discrepancy exists between estimates of how much oil was released from the pipeline, how much was trapped within the wetland around Talmadge, and how much was transported into the Kalamazoo River (McKnight et al. 2015, Crosby et al. 2013). It has been estimated that 843,000 gallons were released into Talmadge Creek and then transported through over 30 miles of river including the upper end of Morrow Lake (Figure 1). It is also possible that complete mixing did not occur between Talmadge and the Kalamazoo River in the first few kilometers based on observation of river mixing and deposition of oil along bridges and trees. The river discharge was at $85 \text{ m}^3 \text{ s}^{-1}$ at the time of the spill, which was at the 4% exceedance of long term flow records (Hoard et al. 2010). The high water levels deposited oil along floodplain habitats. Downstream, much of the spill was contained upstream and within a medium-sized reservoir (Morrow Lake).

In autumn 2010, significant remnants of submerged oil were found in depositional habitats, oxbows and reservoirs, where it readily generated sheen upon disturbance. Removal of

submerged oil involved intense agitation of soft sediments in depositional habitats to suspend and capture oil (McKnight et al. 2016). Expensive and innovative fingerprinting techniques were necessary to distinguish the diluted bitumen crude from historical polycyclic aromatic hydrocarbon contamination methods (Newfields 2013). The Kalamazoo River had low turbulence and low total suspended solids, but suspended material was thought to be important for the transport and submergence of bitumen (Fitzpatrick et al. 2015). Early in the response, thousands of turtles were recovered, cleaned and ultimately returned. Hundreds of oiled birds and less than 50 mammals were also recovered and rehabilitated. There were no major fish kills, but mussels were crushed by air boat traffic (Williams et al. 2015). The unique nature of this spill, and particularly the tendency of the bitumen to submerge, created one of the most expensive cleanups in US history costing over \$1 billion. During the response, thousands of people and hundreds of boats were on this river and its floodplain, disturbing habitats and increasing turbidity (Bejarano et al. 2012, Williams et al. 2015).

The Kalamazoo River watershed drains 5,230 km² in Southwestern Michigan (Figure 1). The entire river is 260 km long with a mean discharge of 38 m³/s at the Comstock (4106000) USGS gauge, which is 35 river miles downstream of Marshall, MI. The mean annual river discharge at the Marshall, MI (04103500) USGS gauge is 9 m³/s. The Kalamazoo River is a low gradient river that meanders through glacial deposits. The substratum is sand and gravel with some cobble (Wesley 2005).

The section impacted by the spilled oil is upstream of reach studied in my other chapters, but sites just upstream of Morrow had been sampled before the spill (sites at 30 and 34.5 miles) (Chapter 2), which provides insight into spill impacts on macroinvertebrates. Several low-head dams produce small reservoirs in this section of river: 1) the USGS gauge site at Marshall is just

downstream of a small reservoir; 2) the former Ceresco reservoir, which was just upstream of the 9.5 mile site and is now removed; and 3) the Millpond at Battle Creek, which is just downstream of the 13.8 mile site. The oil-impacted reach ends at a medium-sized reservoir called Morrow Lake.

Artificial substrata

Due to spatial variation in the benthic environment, I used artificial substrata to examine longitudinal patterns of macroinvertebrate abundance and composition in the oil-impacted reach and in reference sites. Hester-Dendy multiple plate samplers simulated hard substrata, and leaf packs in mesh bags simulated freshly fallen tree leaves. Study sites were constrained to road crossings because access to the river was limited by cleanup operations (Figure 1). A reference site was established near Marshall, MI in the Kalamazoo River above the confluence of the small tributary (Talmadge Creek) that carried the oil into the river. The locations of the Hester Dendy samplers coincided well with a rapid macroinvertebrate bioassessment known as Procedure 51 conducted by the Michigan Department of Natural Resources. These sites include: A reference site in the Kalamazoo River at Marshall MI, as well as a second reference site above the spill in Talmadge Creek, which was included only in the leaf pack sampling and the amphipod in-situ bioassay studies. Sites downstream of the pipeline break include: a site just below the inflow at Talmadge where 15 Mile Road crosses the Kalamazoo River (2.25 miles); a site 9.5 miles downstream where 11 mile road crosses the Kalamazoo River (9.5 mile site); a site 13.8 miles downstream just upstream of Battle Creek, MI (13.8 mile site); a site at 30 miles downstream near the Fort Custer Recreation Area boat launch (30 mile site); and a site 34.5 miles downstream in Galesburg, MI (34.5 mile site). The in-situ bioassay included an additional site at the inflow of Morrow Lake. The two sites in the most downstream reaches (30 and 34.5 miles

sites) were sampled before the spill occurred, allowing for comparisons of pre- vs. post-spill changes.

14-plate Hester Dendy samplers (0.16 m²) were selected as a standard substratum rather than using kick sampling (e.g., Stanley et al. 2002) or Surber samplers (e.g., Doi et al 2008). The substrata of the Kalamazoo River contained variable proportions of sand, gravel and cobble. Using Hester Dendy samplers provided consistent substrata in each site that would target macroinvertebrates that use hard substrates. However, taxa found in soft substrata like mussels and Chironomidae would not be collected (Benke 2015). Hester Dendy samplers were placed in the Kalamazoo River two months following the spill, affixed with zip ties on top of either clay (2 x 8 x 4 inches) or cement bricks (slightly smaller). Samplers were deployed in the river for six weeks in mid-September, 7 weeks after the spill occurred, and removed in early November. Hester Dendy samplers were removed by gently lifting the sampler (without the brick) into a 30 cm D-net with 500-micron mesh. Upon retrieval, any upstream debris such as leaves or filamentous algae was carefully removed. Extra leaves were removed from the face of the Hester Dendy sampler if they formed a thick pack, and debris was removed without disturbing the Hester Dendy sampler. Samplers were heavily covered with leaves only in the most downstream reaches (30 and 34.5 mile sites). Hester Dendy samplers were placed in double Ziploc bags on ice and taken to the lab where they were processed on the day of collection. Samplers were disassembled and scraped with plastic putty knives to remove invertebrates. Samples of dislodged material were sieved through 1 mm and 250 micron sieves and preserved with 95% ethanol. Samples were then moved into 75% ethanol at a later date. Macroinvertebrates from the samplers were subsampled and identified to family (samplers with fewer than 500 individuals were counted in their entirety).

The disturbance of the river bottom by boats led to three sampler sites being compromised. Two samplers at the 9.5 mile site were tipped on their sides in 2010 though this did not happen in 2011. All Hester Dendy samplers from the 13.8 mile site were on their sides in 2010 and similarly moved extensively in 2011, which affected comparisons between years for the 13.8 mile site. Hester Dendy samplers were also disturbed and on their side at the 30 mile site, and one sampler was missing in 2010. None of the other sites' samplers were disturbed. Disturbed Hester Dendy samplers were attributed to the clean-up activities.

To examine impacts of the oil spill on invertebrates colonizing freshly fallen leaves in the autumn, leaf packs were added following methods similar to those of Poulton et al. (1998). Briefly, leaf packs were enclosed in 20-cm pouches made of plastic screen (mesh openings of 5 mm) and stapled or sewn closed with nylon string. Freshly fallen leaves of silver maple (*Acer saccharinum*), a common riparian tree on the river, were collected and air dried before deployment. Leaves were soaked for 30 minutes and 10 grams wet weight of leaves were inserted so that leaves were arranged in the same orientation. Leaf packs were created the day before deployment. Leaf packs were secured to bricks or zinc coated (galvanized) or iron metal spikes in equal proportion per site. Leaf pack samplers were installed in mid-December and removed in early January after three weeks in the river.

Upon retrieval, leaf pack samplers were gently lifted into a 30 cm D-net with 500 micron mesh using a hooked pole, though a few leaf packs were unexpectedly challenging to remove at the 9.5 and 13.8 mile sites, possibly due to more turbulent flow in those sites, or higher flows in 2011. About half of the samplers were quickly removed, and the other half took more time. A few samplers at the 9.5 and 13.8 mile sites possibly had reduced total numbers of individuals as a result of the difficult retrieval. Samplers were placed into double Ziploc bags and taken to the lab

and frozen. Later, leaves were gently rinsed in water and material was sieved through 1 mm and 250 micron sieves and preserved with 95% ethanol. Samples were then transferred into 75% ethanol at a later date. All taxa were identified to family.

In addition to artificial substrata, pre-spill data on macroinvertebrates were selected from the Rapid Bioassessment Procedure 51 surveys conducted by the Michigan Department of Natural Resources (Matousek and Walterhouse 2013). Data were selected from the most recent sampling before the oil spill. Marshall, MI was sampled in 2004, whereas the 9.5 mile site (Eleven Mile road) was sampled in both 2004 and 2008, but only 2008 data were selected. A site just upstream of the 30 mile site (Fort Custer State Recreation Area) was sampled in 2004 and 2009; the 2009 data were selected. To compare with pre- vs. post-spill communities, I used data from the 2010, 2011 and 2012 samplings of these three sites, respectively (Matousek and Walterhouse 2013).

In situ bioassays

In addition to documenting spatial patterns in macroinvertebrate communities, field in-situ bioassays were conducted 12 months after the oil spill using the test organism *Hyaella azteca*, an amphipod that is commonly employed for toxicity bioassays, though most often in the lab. Bioassays in this study were conducted in the field. Animals were caged in screened chambers using methods developed by Burton et al. (2005). Chambers had small openings covered with 250-micron mesh and glued with waterproof silicon adhesive. Chambers were acid washed and soaked in deionized water before deployment.

The in-situ bioassays were conducted in two types of habitats at each site. Flowing habitats were defined as having flow >15 cm/s, with substrata consisting of sand, gravel and cobble. Depositional habitats were areas along the river with little or no flow and fine sediment

(silt and/or fine particulate organic matter). The amphipods were obtained from the laboratory culture of Dr. G. Allen Burton Jr. at the University of Michigan when they were 12-19 days old. Amphipods were acclimatized to filtered local stream water (Augusta Creek, MI). Ten amphipods were transported to sites in centrifuge tubes filled with filtered water from a local creek. Amphipods were transported in a cooler with small amounts of ice. Once at the field site, amphipods in vials were gradually acclimatized to the local water temperature ($\sim 24^{\circ}\text{C}$) over a period of 1-2 hours. Once acclimatized, amphipods were placed into chambers. These chambers were placed between shelving units. Metal spikes were used to hold the units against the bottom in flowing habitats and poles were used to gently hold units above soft sediment in depositional habitats. Chambers were left in the river for nine days.

Field controls of amphipods were placed into tubes, taken into the field, and brought back to be kept in aerated beakers through the experiment. Filtered stream water was replaced through the experiment. No food was added. Day zero amphipods were selected and dried on the day of deployment to calculate growth rates per day. Chambers were retrieved after 9 days. The wave action from boat traffic raised the shelving units from the bottom at the Morrow inflow site (depositional and flowing habitats) and in the depositional habitat at the 13.8 mile site. All others remained fixed against the bottom. Dissolved oxygen was measured close to the bottom of the river, and by checking dissolved oxygen from a dummy chamber when units were carefully retrieved (Table 3.2). Samples were poured into an enamel pan into the field, then poured through a 125-mm sieve and rinsed with water to find all amphipods. Chambers were 30% filled with sediment, and a sheen developed while sieving. Amphipods were recorded as live or dead, and surviving individuals were dried and weighed on a microbalance.

Crayfish bioaccumulation of oil sands metals

To measure the possible bioaccumulation of metals from the spilled bitumen, I collected crayfish 1.5 years after the spill in January 2012 using a large net. Crayfish are a useful organism to bioassay metal uptake (Kouba et al. 2010). Crayfish (11 per site) were collected from the reference site in the Kalamazoo River at Marshall and at the oil-impacted 9.5 mile site. Individuals were rinsed with distilled water once collected and put into Whirl Pak bags and frozen. The hepatopancreas (digestive gland) and muscle (removed from tail and claw) were dissected. The remaining exoskeleton was included as an additional sample. Tissues from individuals were dried and then ground in mortar and pestle, pooled to make one composite sample per site. Equipment was acid rinsed between samples. Samples were sent to Exova Corporation where they were analyzed for metal content by inductively-coupled plasma mass spectrometry (ICP-MS).

Water clarity and nutrients

Water clarity was measured in two ways during installation and removal of experiments. Transparency was measured using a 120-cm long turbidity tube (any measurement >120 cm indicates water clarity greater than 120 cm). Water samples for nutrient analysis were collected and filtered in the lab on the day of collection. Total suspended solids were measured concurrently on filters by gravimetry. Samples were filtered the day of collection onto tared Supor filters (0.45 micron), and dried to a constant weight at 40°C.

Phosphorus fractions were analyzed as follows. Soluble reactive phosphorus (SRP; filtered water) was measured by the acid molybdate method (Wetzel and Likens 2001). Total phosphorus (unfiltered water) and total dissolved phosphorus (filtered water) was measured by persulfate digestion (Valderrama 1981, Langner and Hendrix 1982). Particulate P was calculated

by the difference between total P and total dissolved P. Dissolved organic P was calculated as the difference between total dissolved P and SRP.

Benthic algae

Clay bricks were added to measure benthic algal growth. Bricks were deployed and removed alongside the Hester Dendy samplers. Upon retrieval the bricks were placed into aluminum trays and covered with foil and processed in the lab on the day of collection. Epilithon from the top of bricks was carefully removed using a nail brush into an enamel pan. The slurry was mixed and measured in a graduated cylinder. Typically, subsample of <50 mL was poured through a 250-micron sieve onto a pre-combusted Pall A/E glass fiber filter. Samples were frozen for up to two years before processing. Chlorophyll-a was extracted at 4°C for 24 hours and measured on a Turner fluorometer (Welschmeyer 1994). Because samples were run after considerable time in storage, some chlorophyll-a (up to 50%) could have been lost to degradation based on storage tests on samples of benthic algae (Chapter 2).

Data analysis

A two-way t-test was employed to compare the reference site with each downstream site impacted by the oil spill (Orr et al 2008). Data on the total number of individuals from both the Hester Dendy samplers and leaf pack samplers were analyzed by making these pairwise comparisons. In addition, Hydropsychidae, Heptageniidae, and Chironomidae were selected for comparisons because these groups represented at least 75 % of individuals found on artificial substrata, and are the groups most often impacted by oil spills (Poulton et al 1997). I pooled Taeniopterygidae and Capniidae stoneflies together and analyzed them as the order Plecoptera. Taxa were also pooled into functional feeding groups. Filter feeders including Hydropsychidae,

Philopotamidae, Isonychiidae, Brachycentridae, Polycentropodidae, and Simuliidae. Collector-gatherers include Chironomidae, Amphipoda, Isopoda, Leptoceridae, Psychomyiidae, Baetidae, Caenidae, Ephemerellidae, Leptohyphidae, Leptophlebiidae, Heptageniidae and Hydroptilidae; some of these taxa are classified as scrapers but were pooled with collector-gatherers. Shredders that were pooled included Taeniopterygidae and Capniidae, Pteronarcyidae, Lepidostomatidae, and predators include Athericidae and Perlidae.

Amphipod in-situ bioassays were analyzed similarly, comparing the reference site with each downstream each site (9.5, 13.8, 34.5 and inflow to Morrow Lake). Analyses were also separately performed for flowing sites and depositional sites. If variances were found to be unequal, data were log transformed. Amphipod survival data (proportions) were arcsine transformed. Statistics were performed in Microsoft Excel.

RESULTS

Benthic macroinvertebrates on Hester Dendy samplers

In the first sampling two months after the oil spill (fall 2010), the total number of individuals was reduced by ~80% from 1000 - 2000 individuals to 100-300 individuals per sampler. Total numbers of macroinvertebrates that colonized the Hester-Dendy artificial substrata at the 9.5 mile and 13.8 mile sites were reduced compared to the reference site (Figure 3.2, Table 3.3). The macroinvertebrate community was dominated by Chironomidae (nonbiting midges), Hydropsychidae (filter feeding caddisflies), and Heptageniidae (mayflies) (Figure 3.2, Table 3.3). However, neither the total number of individuals nor individual taxa were reduced at the first site directly below Talmadge Creek (2.25 miles).

Samplers were disturbed at the 13.8 mile site and the 30 mile site, which could have impacted total number of individuals. Two samplers were on their sides at the 9.5 mile site, where they had 200-400 individuals per sampler. One sampler was on its side at the 30 mile site but both samplers had 1000-2000 individuals. If total number of individuals were reduced because they were on their side, the effect appears less than the difference between the reference site and sites with reduced number of individuals. Total numbers of individuals were abundant at the most downstream sites; there was no difference in the total number of individuals between the reference and the 30 and 34.5 mile sites.

One year later (summer 2011), macroinvertebrates were still reduced in abundance compared to the reference site in the most oil-impacted sites (Figure 3.2, Table 3.3). Total numbers of individuals were still reduced at 9.5 mile and 13.8 mile sites in 2011, but some recovery was evident because samplers from these sites had close to 1000 individuals at the 9.5 mile site. Total numbers of individuals were still reduced at 13.8 mile site at ~500 individuals. The total numbers of macroinvertebrates in the Hester Dendy samplers were unexpectedly reduced compared to the reference site by 50% at the 30 mile site, and ~600 individuals were found at the 34.5 mile site (Figure 3.2, Table 3.3).

Two months after the spill (Fall 2010), Hydropsychidae were found to be either eliminated or only a few individuals were found on each sampler at the 9.5 and 13.8 mile sites. Heptageniidae and Chironomidae were reduced compared to the reference site by over 60% in the 9.5 mile site and over 75% in the 13.8 mile site. There was no statistical difference between densities above Morrow and in the site at 2.25 miles downstream from the spill and in the most downstream sites (30 and 34.5 miles) for any of those taxa (Figure 3.2, Table 3.3).

The longitudinal patterns observed in Fall 2011 were comparable to patterns observed in 2010 (Figure 3.2, Table 3.3). However, Hydropsychidae and Heptageniidae were reduced by ~50% at the site below the Talmadge Creek inflow (2.25) compared to the reference site, but Chironomidae were nearly double in densities compared to upstream. Hydropsychidae and Heptageniidae were still significantly reduced in the two middle sites in 2011 by ~ 70% at 9.5 mile site and 90% at the 13.8 mile site. Chironomidae were not statistically reduced in the 9.5 mile site but were reduced by 65% at the 13.8 mile site. Unexpectedly, Hydropsychidae, Heptageniidae and Chironomidae were reduced in 2011 at the most downstream site (34.5 mile) and only Chironomidae were reduced at the 30 mile site. While numbers were still reduced along the river, Hydropsychidae were evidently recovering in the most impacted sections (9.5 and 13.8 mile sites), with individuals increasing from low densities (none to two individuals per sampler) to densities of ~100 per sampler. No samplers were disturbed at 9.5 miles, but all were disturbed at 13.8 miles in 2011, and numbers of individuals could be reduced because of some compromised samplers.

Plecoptera including Taeniopterygidae were moderately abundant along the river, with especially high abundances in the most downstream reaches (Table 3.3). They were present on Hester Dendy samplers in 2010 but at low numbers. Plecoptera densities were not different between the reference and 2.25 mile sites, and they were not abundant at the 9.5 mile site in 2010 with only one sampler having a few Taeniopterygidae and other replicates having none. Hester Dendy samplers had half the Plecoptera at the 13.8 mile site compared to the reference site. In the most downstream sites (30 and 34.5 miles), Plecoptera were even more abundant than the upstream reference site with ~200 individuals per sampler. Plecoptera on Hester Dendy samplers were mostly very early instars from the family Taeniopterygidae, Capniidae and Perlidae

compared to the more mature larvae found in leaf packs (see later). Capniidae were abundant only at the 13.8 mile site.

The most impacted sites downstream of the oil spill (9.5 and 13.8 mile sites) had reduced numbers of individuals within functional feeding groups (Figure 3.3, Table 3.4). Hydropsychidae composed 70-90% of the filter feeding macroinvertebrates, and Chironomidae and Heptageniidae composed 70-95% of the collector gatherer group. Filter feeding macroinvertebrates were reduced from hundreds of individuals to less than 50 individuals at the 9.5 mile site and only a few individuals at the 13.5 mile site. Filter feeders were not reduced at the most downstream sites compared to the reference site (30 mile and 34.5 mile sites). Collector gathers were reduced by 70% in the impacted sites (9.5 and 13.8 mile sites). Collector gatherers were not reduced in the most downstream reaches. Shredders were reduced at the most impacted sites from ~50 individuals to a few individuals. Hester Dendy samplers had hundreds of individuals of shredders at the 30 and 34.5 km sites. Lastly, predators were not abundant on leaf pack samplers and were not reduced on samplers downstream of the oil spill.

Benthic macroinvertebrates on leaf pack samplers

Leaf pack samplers suggest a similar pattern though there were no significant differences in the total number of individuals among the sites (Figure 3.4, Table 3.5). Plecoptera composed nearly 50% of individuals found on leaf packs. There were no differences in total numbers of individuals among the sites because of the large numbers of the Plecoptera in leaf packs, especially at the 13.8 mile site. Total number of individuals on leaf packs were reduced only at 9.5 mile site, which had a 30% reduction of individuals compared to leaf packs from the reference site (Table 3.4). This experiment included an additional reference site above the spill in Talmadge Creek. This second reference site had comparable abundances of Chironomidae, and

greater abundances of Heptageniidae and Hydropsychidae. The reference site used for statistical comparisons in this study was the Kalamazoo River at Marshall, which was the same used for analysis of data from the Hester Dendy samplers and the Rapid Bioassessment Procedure.

Samplers from the site directly below Talmadge (2.25 miles), like the Hester Dendy samplers, and had comparable abundances of Hydropsychidae though greater numbers of Heptageniidae and Chironomidae compared to the reference site. Hydropsychidae and Heptageniidae were not abundant on any of the leaf pack samplers. Like Hester Dendy samplers, only one or two Hydropsychidae were present on leaf pack samplers at the 9.5 and 13.8 mile sites, compared to 2-5 individuals at the reference site. Heptageniidae were reduced from 8 to 2-3 individuals. Chironomidae were reduced by 30% at the 9.5 mile site (to 150 individuals per sampler) and 50% (100 individuals) at the 13.8 mile site.

Like Hester Dendy samplers, leaf pack samplers from 2011 had greater numbers of Hydropsychidae at 9.5 and 13.8 mile sites, indicating recovery of macroinvertebrates (Figure 4, Table 3.5). Hydropsychidae were still significantly reduced at 9.5 mile site compared to the reference site, but abundance on samplers increased to 2-7 individuals per sampler at the 9.5 mile site and 7-11 individuals at the 13.8 mile site. Heptageniidae were reduced from 25 at the reference site to 4 individuals at the 9.5 and 13.8 mile sites. Chironomidae were also reduced significantly in both sites. Winter stoneflies (Taeniopterygidae) and early instars of Capniidae were abundant throughout the river with no significant differences among sites.

Rapid Bioassessment data analysis

Rapid bioassessment data from the Michigan Department of Natural Resources were comparable to the Hester Dendy results (Figure 5), although the assessment included taxa that were less abundant whereas I only enumerated dominant taxa in the Hester Dendy samples. The

number of taxa sampled at the 9.5-mile site was 44-48 when collected in 2008. The number of taxa decreased to 31 in 2010 but increased to 36 one year later in 2011 and recovered to 42 in 2012. The Rapid Bioassessment procedure also sampled a few miles upstream of the 30 mile site. In 2009, the procedure counted 33 taxa, which decreased to 20 taxa in 2010, increased to 27 in 2011 and increased to 28 in 2012.

In situ bioassays

Amphipod survival in the in-situ bioassays was not statistically lower than the reference site for any of the downstream oil-impacted sites in the depositional and flowing habitats (Figure 3.6, Table 3.6). Survival in the depositional habitats at the Kalamazoo River reference site was 38% and 50% in flowing habitat. Survival was 80% in the second reference site at Talmadge Creek in both habitats. Mean survival was ~60% in both depositional and flowing habitats from chambers installed at 9.5 and 13.8 mile sites. Survival in chambers in depositional habitats ranged from 20-100% for 9.5 mile site, and 30-80% in the 13.8 mile site. Survival in flowing habitats at the 34.5 mile site ranged from 60-80% while chambers from the depositional habitat had high survival although one set had no survival. Survival was 100% for the inflow of Morrow Lake. The inflow to Morrow Lake in the flowing habitat had 100% survival. There was also no difference in survival when comparing in-situ assays in depositional habitats at the 34.5 mile site and the inflow to Morrow Lake.

Mean growth from in situ bioassays was reduced by 50% in the oil-impacted sites compared to the Kalamazoo River reference site (Figure 3.6, Table 3.6). Growth rates in the two upstream reference sites were 0.006 mg/day for both flowing and depositional habitats, whereas rates were 0.0028-0.004 mg/day at the oil-impacted sites. However, reductions in amphipod growth rates were statistically significant only at the 13.8 mile site in the depositional habitat

compared to the reference site (Table 3.6). There were no statistical differences observed in depositional habitats in the other sections of river. Amphipod growth rates were also reduced both at 9.5 mile and 13.8 mile flowing habitats (Table 3.6). Growth was not reduced in flowing habitats in the most downstream sites (30 and 34.5 mile sites).

Upon retrieval, dissolved oxygen in dummy chambers was ~90% of atmospheric equilibrium in chambers from flowing habitat and depositional habitats for most sites (Table 3.2). Dissolved oxygen was 60-77% in chambers from the depositional habitats at the 13.8 mile site, the 34.5 mile site, and the Morrow Lake inflow site. Survival of field controls was low through the experiment; amphipods had high survival (90%) on the day of experiment, but only half survived through the experiment in the lab suggesting poor laboratory conditions such as feeding rather than handling from the experiment (Table 3.6).

Crayfish metal bioaccumulation

Crayfish tissues were examined for metals at an oil-impacted site (9.5 miles) and the upstream reference site on the Kalamazoo River (Figure 7). No differences between the two sites were found in the crayfish for the metals known to be in bitumen. Vanadium in particular was between 0.5 to 1 ppm for both sites and tissues analyzed, and there was no statistical difference between samples from above the spill and at the 9.5 mile site. Muscle contained on average 0.41 ppm at the 9.5 mile site and 0.44 ppm at the reference site. Vanadium concentrations were greater in the hepatopancreas with 1.4 ppm the downstream site and ~1 ppm at the reference site. Carapace concentrations were comparable with means of 1.5 and 0.88 ppm at the downstream and reference sites, respectively. Nickel was 50 ppm (Muscle) and 100 ppm (hepatopancreas). Nickel in the carapace was higher in the reference site than the oil impacted site. In general metal concentrations were highest in the (hepatopancreas) and lowest in the carapace, especially nickel.

Water quality and nutrients

The cleanup activities and particularly the attempts to recover sunken oil increased turbidity and total suspended solids in the river (Figure 8). Total suspended solids were measured as experiments were deployed or removed. Concentrations of total suspended solids ranged from 5 to 40 mg/L. TSS ranged from 1-10 mg/L for the two reference sites and the 2.25 mile site. Statistically, there was no difference in TSS comparing the Kalamazoo River and Talmadge Creek reference sites with the first site downstream of Talmadge (2.25 miles) ($DF=3$, $t\text{-stat}=-1.15$ and -0.49 , $p>0.05$). TSS appeared to be greater at the 9.5 mile site at 9.8 mg/L and ranged from 1-10 mg/L. I had fewer measurements at the 30 and 34.5 mile sites, but the mean was 11 mg/L and 27 mg/L, and high measurements of TSS were recorded (20 to 60 mg/L). It appeared that TSS at the 9.5 mile site, the 30 mile site, and 34.5 mile site would be statistically greater than the reference site, but this was not the case ($DF=7,4,4$, $t\text{-Stat}=-2.20$, -2.50 , -1.7 , $p>0.05$). Total suspended solids were statistically higher in concentration at the 13.8 mile site than the Kalamazoo River reference site, which ranged from 2 to 40 mg/L ($DF=7$, $t\text{ Stat}=-3.96$, $p<0.05$). Besides TSS, water clarity was measured using a turbidity tube. Water clarity was generally around 100 cm, but appeared reduced in August 2011 to ~60-70 cm (Figure 9).

Phosphorus is of particular interest in the Kalamazoo River system because there is a Total Maximum Daily Load for P in Lake Allegan, a downstream reservoir. Much of the total phosphorus was in particulate form as measured in July 2010 (Figure 10) except for a few days following the spill (27 July 2010), when the SRP fraction dominated. However, by 4 August 2010, TP was equally partitioned between SRP and the particulate P fraction. TP was still lower in concentration than before the spill. By August, the concentrations and partitioning of P forms were more comparable to the samples collected before the spill in July.

Benthic algae

Benthic chlorophyll-*a* concentrations were measured three months and one year after the spill. Benthic chlorophyll-*a* densities ranged from 40 to 80 mg m⁻² (Figure 11). Three months after the spill, benthic chlorophyll-*a* densities were typically ~50 mg m⁻² for most sites (Figure 10). Benthic chlorophyll-*a* densities were higher at the 9.5 mile site (~100 mg m⁻²) and lower at the 13.8 mile site (20 mg/m²). These results were comparable in 2011, though chlorophyll-*a* densities were closer to 70 mg m⁻² at the 13.8 mile site. Based on a paired t-test between 2010 and 2011, there was no difference between years (DF=10, T-stat = -1.04).

DISCUSSION

This study is the first field investigation of the ecological effects of a major diluted bitumen spill on a freshwater system. Although the type of crude oil makes this spill distinctive, the magnitude of the spill on the Kalamazoo River was comparable to other crude oil spills that have occurred on similar sized rivers. The results regarding the responses of the benthic macroinvertebrate communities to the Kalamazoo River oil spill are broadly similar to what has been reported for other oil spills in rivers, including both lighter (conventional) crude oils and heavier oil that is more prone to submergence, as I detail below. This work further suggests that sublethal toxicity may have persisted for at least a year even though there was partial recovery in abundances of major macroinvertebrate taxa.

Short-term impacts on benthic macroinvertebrates

This study found that within three months of the oil spill there had been a 90% reduction of filter feeders and shredders and a near elimination of Hydropsychidae in flowing habitats. Collector-gatherers were reduced by 75% compared to samplers collected at the upstream

reference site. This finding is consistent with studies that have shown that following freshwater oil spills in rivers, benthic macroinvertebrates appear to be strongly affected but then recover, though not fully, after about one year. Samples from the Steepbank River of the Athabasca region, which has natural deposits of bitumen, had particularly low Hydropsychidae and Philopotamidae densities, possibly attributable to suspended bitumen could impact these filter feeders and their nets (Barton and Lock 1979 a and b). Studies of oil spills in other rivers have shown nearly 90% reductions of macroinvertebrate densities in the months after the spill (McCauley 1966, Harrell 1985, Crunkilton and Duchrow 1990, Poulton et al. 1997, Poulton et al. 1998, Lytle and Peckarsky 2001). In one study, riffle communities appeared to recover quickly (i.e., within one year of the spill) though impacts on backwater areas appeared to be more protracted (Poulton et al. 1997). Recovery of macroinvertebrates took nearly two years following a spill in a small stream (Harrell 1985). Immediately after an oil spill, studies have shown that densities of sensitive taxa such as Trichoptera, Ephemeroptera and Plecoptera were severely reduced, whereas Chironomidae, other Diptera and oligochaetes were generally less affected or even increased (Barton and Wallace 1979 a and b, Woodward and Riley 1983, Lock et al. 1981 a and b, Crunkilton and Duchrow 1990, Poulton et al. 1998). I sampled only in flowing habitats. Direct toxicity from diluted bitumen to macroinvertebrates has been little studied, and depositional habitats were not sampled in this study.

Filter feeders and collector-gatherers were reduced in abundance, though Chironomidae were not as reduced as Hydropsychidae. The results of the the MDNR's rapid bioassessment sampling (Figure 4) are consistent with my sampling in showing strong reductions followed by some recovery in the year following the spill (Figure 2 and 3). Other taxa including Amphipoda, Isonychia, Hydroptilidae, Elmidae, Polycentropodidae, Brachycentridae, Philopotamidae,

Leptohyphidae, Empididae, Ephemerellidae, Baetidae, Perlidae, Odonata, and Simuliidae were found along the river in my Hester Dendy and leaf pack samplers as well as in the Rapid Bioassessment sampling (Walterhouse 2011, Matousek and Walterhouse 2013). However, these taxa were not abundant in either Hester Dendy samplers or leaf pack samplers. These bioassessment data show that these taxa were not eliminated by the spill.

The rapid bioassessment showed that the total number of taxa were reduced in the 9.5 and the 30 mile sites. They were unable to collect 300 individuals from the site near 30 miles (Walterhouse 2011). In the rapid bioassessment conducted in 2010, 300 individuals were collected from their sites near the 2.25 and 9.5 mile sites. Total numbers of taxa were reduced in the oil-impacted reaches but some recovery was observed in number of individuals and number of taxa in 2011 and 2012 (Matousek and Walterhouse 2013).

The only dominant taxon that was nearly eliminated in the most oil-impacted reaches was the Hydropsychidae caddisflies, as I observed in both the Hester Dendy and leaf pack samplers. Based on previous work, Hydropsychidae are expected to be abundant throughout the Kalamazoo River mainstem (Cummins et al. 1981, Chapter 2). Three months after the spill, Hydropsychidae in the two most impacted reaches were virtually or entirely absent in the Hester Dendy samplers, while upstream reference site had higher abundances (Figure 2). Hydropsychidae abundances were also very low in all of the leaf pack samplers including the upstream reference site (Figure 3), although this is not surprising because they are unlikely to be active during the period when leaf packs were deployed and are unlikely to use leaf packs as habitat (Rutherford and Mackay 1986). Additionally, at the 13.8 mile site, the Hester Dendy samplers were always disturbed, confounding our ability to determine if the reduction of macroinvertebrates was caused by the oil or by clean-up actions. However, even with

disturbance of the Hester Dendy samplers, the virtual or complete absence of Hydropsychidae seems likely to be attributable to the spill.

In this study, faster growing taxa such as midges of the family Chironomidae, while reduced at the 9.5 and 13.8 miles compared to upstream in 2010 and 2011, may have started to some recover soon after the spill, as has been observed in other oil spills (Poulton et al. 1997 and 1998). Incomplete mixing may have prevented the most toxic components from reaching Hester Dendy samplers at the 2.25 mile site, which may explain the higher than expected densities on Hester Dendy samplers in the 2.25 site. Hydropsychidae recovery may have taken longer (Rutherford and Mackay 1986). Procedure 51 rapid bioassessment data conducted by the Michigan Department of Natural Resources also support the Hester Dendy sampler data (Walterhouse 2011, Matousek and Walterhouse 2013). Hydropsychidae likely recovered in the 9.5 mile site and 13.8 mile site by 2012, and the total numbers of individuals were likely recovered two years after the spill.

Plecoptera (stoneflies) on Hester Dendy samplers, which were removed in early November, were early instars and were not particularly abundant. Plecoptera were abundant on leaf pack samplers and represented a large percentage of the total individuals. Plecoptera, including Taeniopterygidae and Perlidae, were very abundant in the most downstream sites of the river on Hester Dendy samplers, whereas no stoneflies were found in the most downstream reach during the rapid bioassessment procedure (Walterhouse 2011, Matousek and Walterhouse 2013). Winter stoneflies in the families Capniidae and Taeniopterygidae were common in leaf pack samplers, and taxa from these families were abundant on leaf pack samplers from the sites that were heavily impacted by the spill (9.5 and 13.8 mile sites). Leaf pack samplers were installed by mid-December and removed early January in 2010 and earlier in 2011. The larvae of

these stonefly taxa mature during this time as shown in Ontario rivers (Harper et al. 1989), and hence it is possible that these taxa were not active during and immediately after the spill and so avoided the negative impacts.

The clean-up activities and particularly the recovery of sunken oil were likely as important as the spill itself for macroinvertebrate impacts, particularly in the most downstream sites (30 miles and 34.5 miles) in 2011 where macroinvertebrates were unexpectedly reduced. Total suspended solids concentrations were statistically greater at the 13.8 mile site, but unusually high concentrations were measured at the 9.5 mile and the 34.5 mile sites. Measurements using the turbidity tube showed some of the lowest clarities at the 34.5 mile site. Water clarity was reduced to 20 cm, when water clarity could be expected to be 80 to 100 cm at that time of year (Figure 9, Chapter1). It is possible that the clean-up increased suspended solids, which was important in these sites for reducing macroinvertebrate numbers. Filter feeding macroinvertebrates appeared to be particularly impacted, and the clean-up likely delayed recovery in the most downstream reaches where numbers of individuals were reduced.

Cleanup activities also compromised some of my samplers. Half of the Hester Dendy samplers were on their sides in the 9.5 mile and 30 mile sites, and Hester Dendy samplers were disturbed at the 13.8 mile site. The intense clean-up involving a large number of boats led to disturbance of the river bottom in many locations (Williams et al. 2015). Macroinvertebrate numbers on disturbed samplers were comparable at the 9.5 and 30 mile site compared to Hester Dendy samplers that were not disturbed. Hester Dendy samplers from 13.8 mile site had fewer number of individuals. Disturbance could lead to reduced numbers of individuals, but Hester Dendy samplers with none or a few Hydropsychidae are more likely due to toxic effects from diluted bitumen rather than clean-up. Leaf pack samplers were not disturbed like the Hester

Dendy samplers were data are comparable. Some samplers were more difficult to remove, but these were flagged.

Possible long-term toxicity

I conducted in-situ bioassays to look for possible sublethal toxicity from the submerged oil remaining one year after the spill (Figure 5). My bioassay work was complemented by a later laboratory bioassay study conducted for Enbridge by the Great Lakes Environmental Center (GLEC) (DeGraeve 2012, Bejarano 2012). Their sediment samples were collected 19 months after the spill mostly from backwater sites and some flowing sites. Results from the laboratory toxicity tests showed that *Chironomus dilutus* was more sensitive than *Hyalella azteca* to contaminated sediment. *Chironomus dilutus* survival was particularly impacted by sediment composition (silt versus sand and organic matter) and sediment hydrocarbons. *Hyalella azteca* survival was impacted by total extractable hydrocarbons. Their study found that many sites had acceptable survival, but two heavily oiled and one lightly oil site had reduced survival. Additionally, their study noted that 9 days may not have been long enough to detect sublethal impacts (DeGraeve et al. 2012, Bejarano 2012).

The results of my in-situ bioassays are comparable to the results of the GLEC lab study in the case of *Hyalella*. In my study, amphipod survival did not differ upstream and downstream, except at the inflow of Morrow Lake. The in-situ bioassay conducted one year after the spill found reduced growth rates especially in the 13.8 mile site and somewhat in the 9.5 mile site (Figure 3.5, Table 3.5). The reference site selected for statistical comparisons in this study was the same Kalamazoo River site at Marshall that was used in the artificial substrata studies and the rapid bioassessment procedure. In-situ bioassays at the Kalamazoo River reference site had lower survival than expected, but similar growth. The Talmadge Creek reference site had high

(80%) survival in both flow and depositional habitats. Leaf pack samplers from this site had greater abundances of individuals than at the Kalamazoo River reference site.

While I had hypothesized that I would see greater impacts in depositional habitats, only the 13.8 mile site showed reduced growth rates in depositional habitats compared to the upstream reference site. (Figure 3.5, Table 3.5). Amphipod growth rates were not reduced in the depositional habitat in the most downstream reaches. Reduced growth rates were observed in flowing habitats at the 9.5 mile and 13.8 mile sites, which was surprising, but may be a result of clean-up actions; the chambers were up to a third full of fine sediment, while chambers at the upstream reference site had little material. The clean-up may have suspended fine sediments, which became deposited into chambers. Sheen was observed while sieving amphipods from this sediment (personal observation). Notably, reduced growth of *H. azteca* occurred at the same sites where macroinvertebrates densities on artificial substrata were reduced the most. Other factors besides sunken bitumen oil can impact amphipod survival including dissolved oxygen, ammonia, high sulfide and other contaminants (Burton et al. 2005, Tao et al. 2010, Harding et al. 2011, Kinsman-Costello et al. 2015).

Previous studies in the oil sands mining region have suggested the potential for long-term toxicity when bitumen is present in aquatic environments. Athabasca River sediment naturally contains bitumen-derived hydrocarbons (Headley and Akre 2001), which negatively impact macroinvertebrates (Barton and Wallace 1979, Lock 1981). Exposure to oil sands and oil sands process water led to little change in survival but did impact growth and altered emergence of *Chironomus* (Anderson et al. 2012, Wiseman et al. 2013 a). Exposure to oil sands process water impacted *Daphnia* feeding (Lari et al. 2016). Sand coke leachates from oil sands processing contained components and metals such as vanadium and nickel that led to toxicity in *Daphnia*

(Puttaswamy and Liber 2011, Puttaswamy et al. 2010). Bioassessment of macroinvertebrates in lakes within the Athabasca oil sands region showed impaired macroinvertebrate communities, which were affected by atmospheric deposition from the mining operations (Parsons et al 2010). These studies suggest that there are sublethal impacts to macroinvertebrates from the oils sands bitumen and oil sands process water.

Toxicity has been examined in other oil spills, albeit not using the in-situ bioassay methodology. The results of this study are comparable to previous lab based bioassays testing hydrocarbons (Michel et al. 2009, Anderson et al. 2012). Lethal toxicity was low in sediments from the Gasconade River spill though there was an increase in toxicity after three weeks (Ort et al. 1995). Seven years after a spill into the Patuxent River, degraded hydrocarbons were present (Michel et al. 2009). Lab based amphipod assays in that study showed the sediments were toxic in 25% of samples, and 38% of samples could negatively affect sensitive organisms. Survival of our controls appears to be comparable to reference stream survival in other studies, and the results of this study are comparable to field and lab bioassays for non-point source pollution (Burton et al. 2005, Custer and Burton 2008, Tao et al. 2010, Tucker and Burton 1999). In-situ bioassays of *Chironomus riparius* in rivers with mining and industrial contamination showed reduced growth but no changes to survival (Faria et al. 2006). In general, weathering of hydrocarbons is important to toxicity (Haritash and Kaushik 2009) and weathered oil can be less (Alexander 2000) or more toxic (Carls and Meador 2009). One of the largest oil spills in Canada was the Nipisi spill (60,000 barrels), where hydrocarbons were still present after 25 years but were highly weathered (Wang et al. 1998). This study found that survival was not reduced by hydrocarbons one year after a diluted bitumen spill, but growth was reduced in the same sites where macroinvertebrates were reduced.

It is important to note that this in-situ bioassay study was conducted after the most toxic components of the diluent, such as benzene, would have been lost to the atmosphere or degraded. Nevertheless, my assays detected sublethal toxicity one year following the Kalamazoo River spill, and the submerged bitumen is likely to remain in the sediments indefinitely. Assessment of the toxicity of spilled oil is complicated by the fact that historical and ongoing petroleum contamination unrelated to oil spills is known to exist in rivers. In the Kalamazoo River, hydrocarbon fingerprinting revealed significant petroleum contamination from other sources, mainly of pyrogenic origin (i.e., fossil fuel combustion), as detailed in a consultant's report to the US EPA (Newfields 2013). This seems to be the case in other rivers. Hydrocarbon fingerprinting and toxicological studies that were conducted on the Delaware River before the Athos spill occurred found that nonpoint urban sources and discharge from an oil refinery were important (Uhler et al. 2005, Alexander et al. 2005, Hall and Burton 2005). Fish collected in the Delaware Bay showed exposure to hydrocarbon toxicity and cytochrome P4501A induction (McCoy et al. 2002, Pickney and Harshbarger 2006, Pena 2015), and bivalves were enriched with hydrocarbons, especially close to the refinery (Salazar et al. 2005). The relative toxicity of petroleum compounds from other sources vs. from the Enbridge pipeline release is unknown, but the best indication comes from comparison of oil-impacted sites to the upstream reference site.

Studies of the long-term impacts from marine oil spills such as the Exxon Valdez have observed impacts to the highest trophic levels. The lack of baseline (pre-spill) data along with intensive clean-up actions have often confounded the ability to detect toxic effects of the oil itself (Paine et al. 1996). Hydrocarbon biomarkers from the *Exxon Valdez* spill have been detected 25 years later though the oil is weathering (Carls et al. 2016, Nixon et al. 2013, Irvine et al. 2006). Hydrocarbons in mussels from oiled beds (Boehm et al. 2004), and pink salmon

habitat (Carls et al. 2004b) are returning to background concentrations. However, pacific herring and pink salmon exposed to even low levels of hydrocarbons show heart deformities and reduced swimming speed (Incardona et al. 2016). Impacts and recovery have been documented in sea otters (Bodkin et al. 2002), river otters (Bowyer et al. 2003), salmon (Murphy 1999) and birds such as the Pigeon Guillemot eight years following the spill (Seiser et al. 2000). Harlequin ducks showed population impacts and cytochrome P- 450-1-A induction 10 years after spill (Esler et al. 2002). Food web risks to higher trophic levels were thought to be low (Boehm et al. 2004). Herring populations and some orca pods were likely heavily impacted, and these populations may never recover though other causes could be important (Paine et al. 1996, Peterson et al. 2003). Recovery from impacts to the food web and higher trophic levels can be protracted (Peterson et al. 2003). Studies following the Deepwater Horizon oil spill showed that clean-up actions negatively affected coastal marshes (Mendelssohn et al. 2011). Long term studies following spills on rivers appear to few (i.e. Gasconade River, Delaware River). Research from marine spills can last decades and provide insight into future impacts on wildlife in the Kalamazoo River.

Metal uptake

I hypothesized that metal uptake could occur if the submerged oil contained these metals, and that I would observe increased metal uptake in crayfish collected from areas where macroinvertebrates were the most impacted by the oil spill. My hypothesis was not supported because I did not observe elevated concentrations of Ni or V in particular in my limited sampling of crayfish (Figure 6). Elevated metals were expected, especially in the crayfish hepatopancreas, and perhaps elevated Ni in the exoskeleton (Kouba et al. 2010). Nickel appears to be high

compared to some previous studies (Kouba et al. 2010, Lawrence 1992), but an increase attributable to the oil spill could not be detected.

Hydrocarbons in general contain metals, but Athabaskan bitumen is particularly high in certain metals including nickel (Ni) and vanadium (V) (Zuliani et al. 2016, Jensen Fontaine et al. 2014, Millson et al. 1966). Vanadium was taken up by *Hyaella azteca* in proportion to exposure concentrations, although these were lab solutions (Jensen-Fontaine et al. 2014). Vanadium metal uptake has been studied following marine oil spills (Morelles-Caselles 2008, Lavilla et al. 2006, Sadiq and McCain 1993). Chironomidae were found to bioaccumulate V and Ni in ponds (Baker et al. 2012). Livers from white suckers collected from oil sands tailings ponds had metal concentrations of 0.4 mg/kg for V and Ni 0.8 to 0.21 mg/kg (Arens et al. 2015). Trout hepatocytes were found to express metallothionein when exposed to river water and oil sands process water (Gagne et al. 2012). Metals including nickel and vanadium were found in clams in the Gulf near Kuwait, and tissue concentrations appeared to increase with seawater concentrations (Sadiq and McCain 1993). Caged marine crabs and clams show biomarker responses to marine oil spills (Morales-Caselles et al. 2008). Oil sands process water and water exposed to oil sands showed toxicity to algae, which was attributed to vanadium (Debenest et al. 2012). Vanadium was detected in fish otoliths in marshes following the Deepwater Horizon spill, but concentrations were variable and did not differ from reference samples (Lopez Duarte et al. 2016). Vanadium and Ni uptake was found in mussels and whelks following a marine oil spill (Amiard et al. 2004). Metal concentrations were spatially variable but greater following the spill, reaching up to 4 µg/kg five months after the spill (Chiffolleau et al. 2004). However, increased metal concentrations were not found in tissues of seals, otters and small dolphins (Ridoux et al. 2004). Vanadium is more soluble under alkaline conditions, and its toxicity is based on oxidative

state (Puttaswamy and Liber 2012, Puttaswamy and Liber 2011). Ni is more toxic with increasing alkalinity (Puttaswamy and Liber 2012).

Field collections of crayfish were collected in January 2012, which was the winter after peak clean-up of submerged oil occurred (Fitzpatrick et al. 2015). The Kalamazoo River is a typical urban contaminated river (Baas 2009), and the results may not be surprising because metal contamination from other sources is present in urban streams (Lawrence 1992, Kouba et al. 2010). Additionally, vanadium in bitumen may not be bioavailable because it is bound in organic porphyrin rings in bitumen (Zuliani et al. 2016, Chiffaleau et al. 2004). Also, while bitumen is rich in V, the processing itself extracts the metals and some may have been lost in the leachate (Puttaswamy and Liber 2011, Giesy et al. 2010). Crayfish collected from a site impacted by the oil spill did not show elevated metals based on our limited sampling. However, V and Ni in crayfish collected from the Kalamazoo River reflect metals collected in crayfish elsewhere and from urban impacted streams.

Possible impacts on fish and other higher trophic levels

This study did not directly assess the impacts of the oil spill on higher trophic levels, although the macroinvertebrates studied supported food webs of aquatic (e.g., fishes, turtles) and terrestrial consumers (e.g., spiders, birds). One study did show that fishes were directly impacted by the oil spill; fish collected from oil impacted reaches three weeks after the spill showed EROD and cytochrome P450 1A responses and changes to immune function. Gills and livers showed lesions and abnormalities (Papoulias et al. 2014). Although this was the only fish toxicity study conducted for the Kalamazoo river oil spill, exposure to and impacts of petroleum hydrocarbons to fishes have been extensively studied elsewhere (Van der Oost et al. 2003,

Stegeman et al. 1991). Exposure of fish embryos to marine oil spills is known to produce deformities and heart arrhythmia (Irie et al. 2011, Jefferies et al. 2015, Incardona et al. 2012).

The toxicity of bitumen in freshwater may not resemble that of conventional crude oil because of the unique chemistry of the bitumen, which is already a highly weathered crude (Madison et al. 2015, Philibert et al. 2016, Gagne et al. 2012). Blood plasma from wild fish collected from the Athabasca River had some indication of contaminants (Simmons and Sherry 2016). Naphthenic acids were found in fish tissue if exposed to naphthenic acid in the laboratory, but was not observed in wild fish caught in the Athabasca River (Young et al. 2011). Mining and processing of oil sands crude as well as other factors could have effects on fish densities and condition in the Athabasca River (Schwalb et al. 2015, Timoney and Lee 2011, Headley and Akre 2001).

Direct exposure to oil sands bitumen has been shown to produce heart deformities, cytochrome P450 1A expression, and reproductive problems in fish (Philibert et al. 2016, Madison et al. 2015, Colavecchia et al. 2004). Studies that have looked at the impacts of unprocessed bitumen have demonstrated larval mortality, spinal deformities, and pericardial edema arrhythmia in fathead minnows (Colavecchia et al. 2004 and 2006). Trout hepatocytes exposed to Athabasca River sediment showed changes in gene expression (Gagne et al. 2012). The slimy sculpin showed EROD expression when exposed to oil sands sediments from the Steep Bank River in the Athabaskan area (Tetreault et al. 2003). Exposure of zebrafish to diluted bitumen versus sweet crude and sour crude showed fish exposed to diluted bitumen had pericardial edema, which affected mortality and behavior. This was dependent on the amount of polycyclic aromatic hydrocarbons in the water, and diluted bitumen could be more or less toxic than conventional crude oils (Philibert et al. 2016). Deformities, pericardial edema, and oxidative

stress were measured on Japanese Medaka fish embryos after exposure to diluted bitumen in water as well if water was chemically treated with the dispersant Corexit® was added (Madison et al. 2015).

Studies from oil sands processing ponds in the Athabasca region are informative for predicting impacts on the Kalamazoo River. An interesting aspect of bitumen processing is that the extraction process leaves metals and naphthenic acids in the waste process water stored in tailings ponds (Giesy et al. 2010). Oil sands process water containing naphthenic acid is particularly toxic to fishes (Morandi et al. 2015). Naphthenic acids are a component of oil sands that is water-soluble and toxic (Headley and Martin 2004, Headley et al. 2016, Kavanagh et al. 2009, Scott et al 2005, Ross et al. 2012, Gagne et al. 2012, Gagne et al. 2013). Fathead minnows had poorer reproductive success when exposed to process water ponds (Kavanagh et al. 2011, Kavanagh et al. 2012). Studies using naphthenic acids from oil sands process water and commercial extract water in particular found deformities in yellow perch and Japanese medaka embryos (Peters et al. 2007), and in young yellow perch (Nero et al. 2006). Exposure using extracts led to only short-term and not long-term immune responses (MacDonald et al. 2013). Exposure to untreated oil sands process water led to growth deformities and pericardial arrhythmia and gene expression in fathead minnows (He et al. 2012, Wiseman et al. 2013), and in Japanese medaka fish (Alharbi et al. 2016). Adult white suckers (fish) from tailing ponds had increased cytochrome P450 1A and EROD expression (Arens et al. 2015). Caged trout in tailing ponds showed altered immune responses (McNeill et al. 2012). Adult yellow perch collected from ponds had lesions and gill deformities (Van den Heuvel et al. 2000). Currently, studies do not show if diluted bitumen is more or less toxic than conventional crude. Bitumen found in natural deposits, diluted bitumen and oil sands process water contain components of bitumen are

potentially toxic to fishes. These studies suggest that certain components such as naphthenic acids are particularly toxic. These studies are also informative for estimating future toxic and sublethal impacts to wildlife exposed to the submerged oil.

Oil sands mining in the Athabasca region has caused impacts on mammals and birds (Kelly et al. 2009 and 2010, Kurek et al. 2013, Hodson 2013), and studies conducted there are useful for understanding impacts on the Kalamazoo River. Atmospheric contamination from oil sands development can impact local lakes (Hawwinkler et al. 2008, Ross et al. 2012, Evans and Talbot 2012, Parsons et al. 2010) though PAHs from forest fires could also contribute (Ahad et al. 2015). Petroleum hydrocarbons have been detected in scat of larger mammals such as moose and wolves (Ludin et al. 2015). Macroinvertebrates from tailing ponds had reduced diversity with increased Chironomidae abundance (Bendell Young 2000), and macroinvertebrates showed hydrocarbon bioaccumulation (Wayland et al. 2008). Petroleum compounds in invertebrates could be transferred to higher trophic levels and could extend to the terrestrial food webs by emergent insects that are consumed by birds such as swallows (Fleeger et al. 2003, Kolvalenko et al. 2013). Constructed wetlands from tailings water were able to support swallow populations that did not seem negatively impacted (Gentes 2007, Harms et al. 2010), though immune function was impacted (Cruz Martinez et al. 2015). Metals such as vanadium were found in aquatic macroinvertebrates from ponds, and emerging insects were consumed by swallows. Metal concentrations appear that concentrations may be below that which is toxic (Goodwin et al. 2016, Baker et al. 2012). Food web impacts in the case of the Kalamazoo River oil spill could be important but are currently unknown (McKnight et al. 2015).

Other ecosystem impacts

This study was also able to examine short-term impacts to nutrients and primary production after the Kalamazoo River oil spill. Phosphorus concentrations in the Kalamazoo River appeared to quickly increase in the soluble reactive phosphorus (SRP) component shortly after spill, but even a few days after the spill and within one month, most P was in the particulate fraction as is typically observed in the river (Chapter 1; also Reid and Hamilton 2007), suggesting a quick recovery. One hypothesis is that P contained in algae was released, increasing SRP, which was soon taken up as algae recovered. Studies from the Athabaskan region support these results. Exposure to bitumen on sediment reduced biofilm chlorophyll *a* and bacteria (Yergeau et al. 2013) and benthic algal communities (Burton and Locke 1981, Lavoie et al. 2011). Exposure to oil sands process water negatively influenced phytoplankton communities in ponds (Leung et al. 2001, Leung et al. 2003).

Benthic algal biomass appeared to recover quickly based on chlorophyll-*a* measurements on artificial substrata in this study (Figure 8), although this conclusion may not apply to natural substrata that became covered with fine sediment during the protracted clean-up actions. Brick artificial substrata were buried in the 13.8 mile site, with one 30% buried and three others completely buried and not analyzed for benthic algae. Additionally, cobble appeared to be free of benthic algae (Walterhouse 2010). Benthic algal biomass measured as chlorophyll-*a* showed similar patterns in November 2010 and in 2011, and appear similar to benthic chlorophyll-*a* measurements made in 2010 before the spill or sites downstream of Morrow Lake (Chapter 2). Benthic chlorophyll-*a* concentrations were found to increase when exposed to oil on limestone bricks (Locke et al. 1981a), and algae increased when bricks were dipped in oil (Locke et al. 1981b). Phytoplankton were impacted negatively by the Deepwater Horizon spill, but the oil

spill also stimulated growth (Ozhan et al 2014). Algal production appears to be impacted differently when toxic impacts can reduce growth and alter communities, but reduced grazing by macroinvertebrates can lead to an increase in biomass.

It is likely impossible separate the effects of the oil from the clean-up in the case of the Kalamazoo River. Hundreds of boats worked on the river, and agitation and dredging of sediments increased the turbidity and total suspended solids of the river water (Figure 7), causing visible siltation of benthic habitats. Macroinvertebrate densities could have been reduced in the oil-impacted reaches as much because of the intensive clean-up actions as the direct toxicity of the spilled oil, which may explain the reduced macroinvertebrate densities at the 34.5 mile site in 2011. A consulting firm conducted a more thorough analysis of sediment resuspension and potential negative effects on fishes caused by agitation actions and found some impacts (Bejarano et al. 2012).

CONCLUSIONS

Benthic macroinvertebrates in the Kalamazoo River, though highly impacted by the 2010 oil spill and its clean-up actions, have shown recovery. The river experienced one of the largest inland oil spills and the released bitumen led to large amounts of submerged and sunken oil. Like previous studies that examined massive freshwater oil spills, macroinvertebrates were reduced but partially recovered within one year (based on data from my Hester Dendy and Leaf Pack samplers and the rapid bioassessments of Matousek and Walterhouse 2013) and evidently fully recovered within two years (Matousek and Walterhouse 2013). This spill was different from conventional crude oil spills because sunken bitumen was found in depositional and floodplain habitats along the river. The clean-up was costly and destructive, and oil likely remains in

depositional zones. One year after the spill, some sub-lethal impacts were measured using in-situ bioassays, which suggests some impacts to benthic animals. Oil-derived metal bioaccumulation in crayfish was not detected. The Kalamazoo River has a legacy of contamination that makes separating the impacts of the spill from preexisting impacts difficult.

Macroinvertebrates were markedly reduced in the months after the oil spill. Hydropsychidae caddisflies—one of the most dominant taxa in the river—were virtually eliminated. Nutrient data suggest that there was a possible quick loss of algal production because of the change from particulate to dissolved forms of phosphorus, but this was apparently followed by rapid recovery. Benthic algal biomass accrual on artificial substrata appeared to be comparable to the upstream reference site within a few months, and there were no differences between 2010 and 2011, although benthic algal biomass may have been reduced on natural substrata that were more subject to siltation during clean-up actions.

The clean-up of the Kalamazoo River was one of the longest and costliest in history, involving four years of recovery of sunken oil from depositional habitats using highly invasive procedures (agitation and dredging). Nevertheless, the observations in this study indicate that the clean-up was successful and that the benthic macroinvertebrate communities in the river appear to have largely recovered within 1-2 years. However, studies from the Athabaskan oil sands region and the documented ecological legacies of marine oil spills suggest that longer-term risks of sublethal toxicity from residual bitumen in the river sediments may still exist.

The Kalamazoo River is a river that has shown high resilience to historical contamination from paper making, dam building and other structural modifications. Many dams in the United States are failing and being removed. Studies have shown that macroinvertebrate communities appear to recover after dams are removed. However, run-of-river dams are becoming an

attractive option for renewable energy worldwide. This work showed that increased water retention time in reservoirs along river systems with excess nutrients like the Kalamazoo River are likely to increase the production of algae and degrade downstream water quality. Chapter 1 showed patterns between suspended algae and nutrients under either high or low summer discharge. Climate change in Michigan could lead to changes in the timing and intensity of precipitation events, and this work provides some information related to biological responses to extremes of discharge. The total maximum daily load (TMDL) on the Kalamazoo should take into account patterns downstream of Morrow Lake. Using a semi-Lagrangian approach, Chapter 1 also found that certain nutrients were transported in particulate forms during high flow and transported in inorganic forms during low flow. Longitudinal studies can show important spatial patterns related to algae and nutrients.

Chapter 2 shows potential food web implications of algal subsidies exported from Morrow Lake, a run-of-river reservoir, under a range of discharges. Hydropsychidae and Heptageniidae appeared to isotopically follow the subsidy from Morrow, though only a limited downstream. This study found that this is still one of the longest subsidies of suspended algae downstream from a reservoir. Other taxa in the Kalamazoo River had $\delta^{13}\text{C}$ comparable to snail $\delta^{13}\text{C}$, and snails $\delta^{13}\text{C}$ often follow epilithon $\delta^{13}\text{C}$. This work highlights that food webs in rivers like the Kalamazoo are complex. Floodplain connectivity and locally produced benthic algae are important. More work is needed to understand the role of seston in food webs and the impacts of dams like Morrow Lake on seston.

Chapter 3 described the largest bitumen spill in the United States, which occurred on the Kalamazoo River. This study found that macroinvertebrates were reduced and moderately recovered after one year and likely fully recovered after two years. Results from the in-situ

bioassays indicate that there were some sub-lethal impacts in the river one year later. Historical and other sources of polycyclic aromatic hydrocarbons are important. Metals commonly found in bitumen did not appear to bioaccumulate in stream organisms like crayfish. Our work on insect densities and food webs from Chapter 2 provide additional insight into the impacts of the Kalamazoo River spill and possible food web impacts. Rivers the size of the Kalamazoo River are abundant and many have reservoirs or multiple reservoirs like the Kalamazoo River. The cumulative impact of these reservoirs is not well understood, but will be important.

APPENDIX

Table 3.1 Freshwater oil spills that were comparable to Kalamazoo River including size in gallons and year.

River	Year	Volume spilled (Millions L)	Source
Delaware	2004	1.0	Aquatic Technical Working Group 2007
Monongahela and Ohio	1988	2.7	Cronk et al. 1990
Peace River (Muskeg)	2011	4.5	www.cbc.ca
Gasconade	1988	3	Poulton et al. 1997
Chariton	1990	4.2	Poulton et al. 1998
Yellowstone	2011	0.24	State and Federal Trustees State of MT and U.S.
Yellowstone	2015	0.11	Scientific America
Arkansas	2013	1.4	Crosby et al. 2013
Saskatchewan	2016	0.25	globalnews.ca

Table 3.2 Field collection from the-situ bioassay experiment. Stream velocity measurements in flowing habitats and depositional habitats and dissolved oxygen measurements in the Kalamazoo River.

Site	Flow (m/s)	Dissolved oxygen (%)
Marshall, MI		
Flow	0.2	89.0
Depositional	0.1	93.1
Above Talmage		
Flow	0.3	109.0
Depositional	0.1	116.0
11-mile road (9.5 mile site)		
Flow	0.3	98.5
Depositional	0.1	100.0
Main St. Battle Creek, MI (13.8 mile site)		
Flow	0.5	89.6
Depositional	0.1	63.1
Galesburg, MI (34.5 mile site)		
Flow	0.4	86.5
Depositional	0.1	71.4
Inflow to Morrow Lake		
Flow	0.1	91.3
Depositional	0.0	77.0

Table 3.3 Results of the Two Tailed T-tests performed to compare oil-impacted sites with the upstream reference site (Marshall, MI) for Hester Dendy samplers collected from the Kalamazoo River (sites correspond with Figure 3.1)

Hester Dendy Sampler		Hydropsychidae	Heptageniidae	Chironomidae	Plecoptera	Total
2.25 miles (15 Mile Road)						
2010	DF=6 T-stat=1.47 p>0.05	DF=6 T-stat=2.77 p<0.05	DF=6 T-stat=-0.22 p>0.05	DF=6 T-stat=0.55 p>0.05	DF=6 T-stat=0.657 p>0.05	
2011	DF=5 T-stat=2.94 p<0.05	DF=5 T-stat=8.69 p<0.05	DF=5 T-stat=-12.21 p<0.05	DF=5 T-stat=3.51 p<0.05	DF=5 T-stat=-4.66 p<0.05	
9.5 miles (11 Mile Road)						
2010	DF=6 T-stat=10.92 p<0.05	DF=6 T-stat=3.66 p<0.05	DF=6 T-stat=4.05 p<0.05	DF=6 T-stat=7.63 p<0.05	DF=6 T-stat=5.28 p<0.05	
2011	DF=5 T-stat=7.28 p<0.05	DF=5 T-stat=10.50 p<0.05	DF=5 T-stat=2.06 p>0.05	DF=5 T-stat=-0.13 p>0.05	DF=5 T-stat=6.14 p<0.05	
13.8 miles (Main St, Battle Creek)						
2010	DF=6 T-stat=22.43 p<0.05	DF=6 T-stat=7.20 p<0.05	DF=6 T-stat=4.80 p<0.05	DF=6 T-stat=3.54 p<0.05	DF=6 T-stat=8.44 p<0.05	
2011	DF=5 T-stat=4.72 p<0.05	DF=5 T-stat=8.71 p<0.05	DF=5 T-stat=3.55 p<0.05	DF=5 T-stat=-0.99 p>0.05	DF=5 T-stat=7.31 p<0.05	
30 miles (Fort Custer Recreational Area)						
2010	DF=4 T-stat=1.60 p>0.05	DF=4 T-stat=2.30 p>0.05	DF=4 T-stat=- 2.14 p>0.05	DF=4 T-stat=-6.96 p<0.05	DF=4 T-stat=-0.29 p>0.05	
2011	DF=5 T-stat=1.60 p>0.05	DF=5 T-stat=1.59 p>0.05	DF=5 T-stat=14.90 p<0.05	DF=5 T-stat=-3.72 p>0.05	DF=5 T-stat=4.83 p<0.05	
34.5 miles (Galesburg, MI)						
2010	DF=5 T-stat=0.51 P>0.05	DF=5 T-stat=1.32 p>0.05	DF=5 T-stat=- 2.75 p>0.05	DF=5 T-stat=-10.5 p<0.05	DF=5 T-stat=-1.3 p>0.05	
2011	DF=5 T-stat=0.50 p<0.05	DF=5 T-stat=2.02 p<0.05	DF=5 T-stat=16.19 p<0.05	DF=5 T-stat=-6.21 p<0.05	DF=5 T-stat=9.05 p<0.05	

Table 3.4 Individuals were arranged by functional feeding group in each site in 2010. Results of the Two Tailed T-tests performed for each functional feeding group to compare oil-impacted sites with the upstream reference site (Marshall, MI). Macroinvertebrates sampled from Hester Dendy samplers collected from the Kalamazoo River in 2010 (sites correspond with Figure 1).

	Shredder	Filter-Collector	Collector-Gatherer/Scraper	Predator
2.25 miles (15 Mile Road)				
2010	DF=6 T-stat=2.74 p<0.05	DF=6 T-stat=1.70 p>0.05	DF=6 T-stat=0.17 p>0.05	DF=6 T-stat=-1.02 p>0.05
9.5 miles (11 Mile Road)				
2010	DF=6 T-stat=7.25 p<0.05	DF=6 T-stat=8.11 p<0.05	DF=6 T-stat=4.46 p<0.05	DF=6 T-stat=1.00 p>0.05
13.8 miles (Main St, Battle Creek)				
2010	DF=6 T-stat=2.88 p<0.05	DF=6 T-stat=25.8 p<0.05	DF=6 T-stat=7.16 p<0.05	DF=6 T-stat=0.80 p>0.05
30 miles (Fort Custer Recreational Area)				
2010	DF=4 T-stat=-6.03 p<0.05	DF=4 T-stat=2.42 p<0.05	DF=4 T-stat=-0.73 p>0.05	DF=4 T-stat=0.11 p>0.05
34.5 miles (Galesburg, MI)				
2010	DF=5 T-stat=-9.04 p<0.05	DF=5 T-stat=0.11 p>0.05	DF=5 T-stat=-1.34 p>0.05	DF=5 T-stat=1.98 p>0.05

Table 3.5 Results of the Two Tailed T-tests performed to compare oil-impacted sites with the upstream reference site (Marshall, MI) for leaf pack samplers collected from the Kalamazoo River (sites correspond with Figure 3.1).

Leaf Pack Samplers

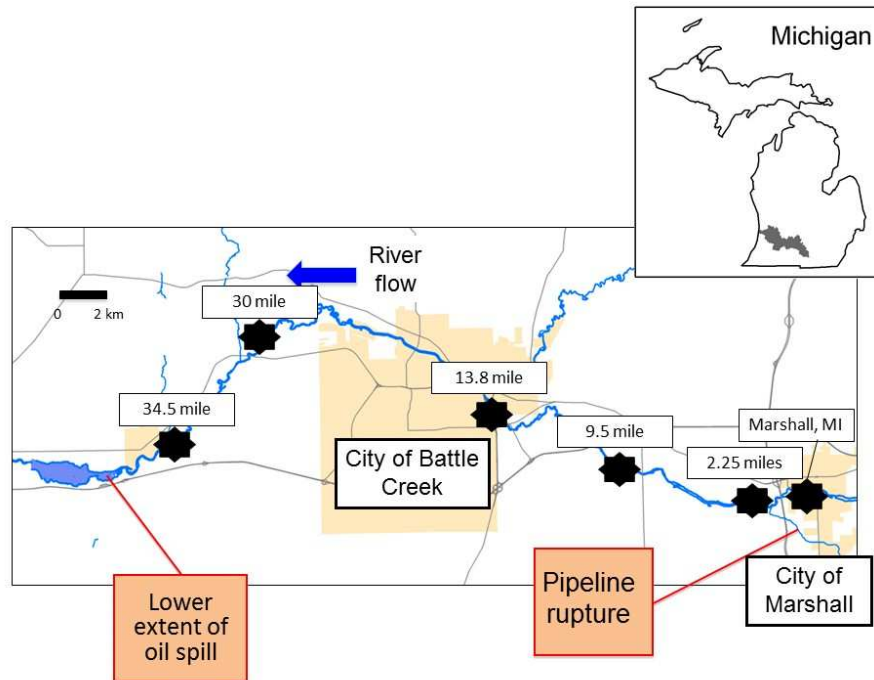
	Hydropsychidae	Heptageniidae	Chironomidae	Total	Plecoptera
Above Talmadge	DF=10, T-stat=-7.04 p<0.05	DF=10, T-stat=-6.48 p<0.05	DF=10, T-stat=-1.93 p>0.05	DF=10, T-stat=-5.50 p<0.05	DF=10, T-stat=-8.51 p<0.05
2.25 miles (15 Mile Road)	DF=13 T-stat=-2.46 p<0.05	DF=13 T-stat=-5.67 p<0.05	DF=13 T-stat=-2.29 p<0.05	DF=13 T-stat=-2.97 p<0.05	DF=13 T-stat=-9.23 p<0.05
9.5 miles (11 Mile Road)					
2010	DF=13 T-stat=3.26 p<0.05	DF=13 T-stat=3.13 p<0.05	DF=12 T-stat=2.29 p<0.05	DF=12 T-stat=1.68 p>0.05	DF=12 T-stat=-2.29 p<0.05
2011	DF=6 T-stat=3.21 p<0.05	DF=6 T-stat=3.23 p<0.05	DF=6 T-stat=10.14 p<0.05	DF=6 T-stat=6.54 p<0.05	DF=6 T-stat=-0.60 p>0.05
13.8 miles (Main St, Battle Creek)					
2010	DF=14 T-stat=2.67 p<0.05	DF=14 T-stat=3.78 p<0.05	DF=14 T-stat=3.94 p<0.05	DF=13 T-stat=-0.024 p>0.05	DF=14 T-stat=-11.9 p<0.05
2011	DF=6 T-stat=1.34 p>0.05	DF=6 T-stat=3.59 p<0.05	DF=6 T-stat=11.30 p<0.05	DF=6 T-stat=5.86 p<0.05	DF=6 T-stat=-1.0 p>0.05

Table 3.6 Results of the Two Tailed T-tests performed to compare oil-impacted sites with the upstream reference site (Marshall, MI) for a *Hyalella azteca* in-situ bioassay conducted in the Kalamazoo River (sites correspond with Figure 3.1).

In-situ bioassays	Survival	Growth
Above Talmadge		
Flow	DF=8, T-stat=-1.93 p>0.05	DF=7, T-stat=0.56 p>0.05
Depositional	DF=8, T-stat=-2.61 p>0.05	DF=6 T-stat=-0.46 p>0.05
11 Mile Road		
Flow	DF=8, T-stat=-1.43 p>0.05	DF=7, T-stat=-2.41 p<0.05
Depositional	DF=8 T-stat=-1.39 p>0.05	DF=6, T-stat 1.05 p>0.05
Main St, Battle Creek		
Flow	DF=8, T-stat=-0.021 p>0.05	DF=7, T-stat=2.35 p=0.05
Depositional	DF=8 T-stat= -1.59 p>0.05	DF=6, T-stat=3.84 p<0.05
Galesburg, MI		
Flow	DF=8, T-stat=-1.98 p>0.05	DF=7, t-stat = 2.12 p>0.05
Depositional	DF=8 T-stat=0.47 p>0.05	DF=3, T-stat=1.98 p>0.05
Inflow to Morrow Lake		
Flow	DF=8, T-stat=-4.25 P<0.05	DF=8, T-stat 1.38 p>0.05
Depositional	DF=8 T-stat=-0.060 p>0.05	DF=4 T-stat=2.33 p>0.05

Table 3.7 Results of in-situ bioassay lab and field controls. Field controls were taken into the field and then left in beakers over the length of the experiment. Lab controls were selected on day of experiment and kept in aerated beakers through the experiment.

Date	Lab Control	Field Control
August 8, 2011 Finished August 17 th 2011	4	Field Control- 9 on day of experiment and one alive Field Control- 6 on day of experiment and 0 at end
August 9, 2011 Finished August 18 th 2011	6	Field Control-9 on day of deployment of experiment and 7 alive Field Control -7 on the day of experiment and 5 alive
August 10, 2011 Finished August 19 th 2011	7	Field control 9 on day of deployment and only one alive Field Control 9 on day of deployment and 8 alive



1

Figure 3.1 Map of the Kalamazoo River in southwest Michigan (inset shows the watershed). Black stars represent sampling sites, with the reference site above Talmadge Creek where the pipeline rupture occurred.

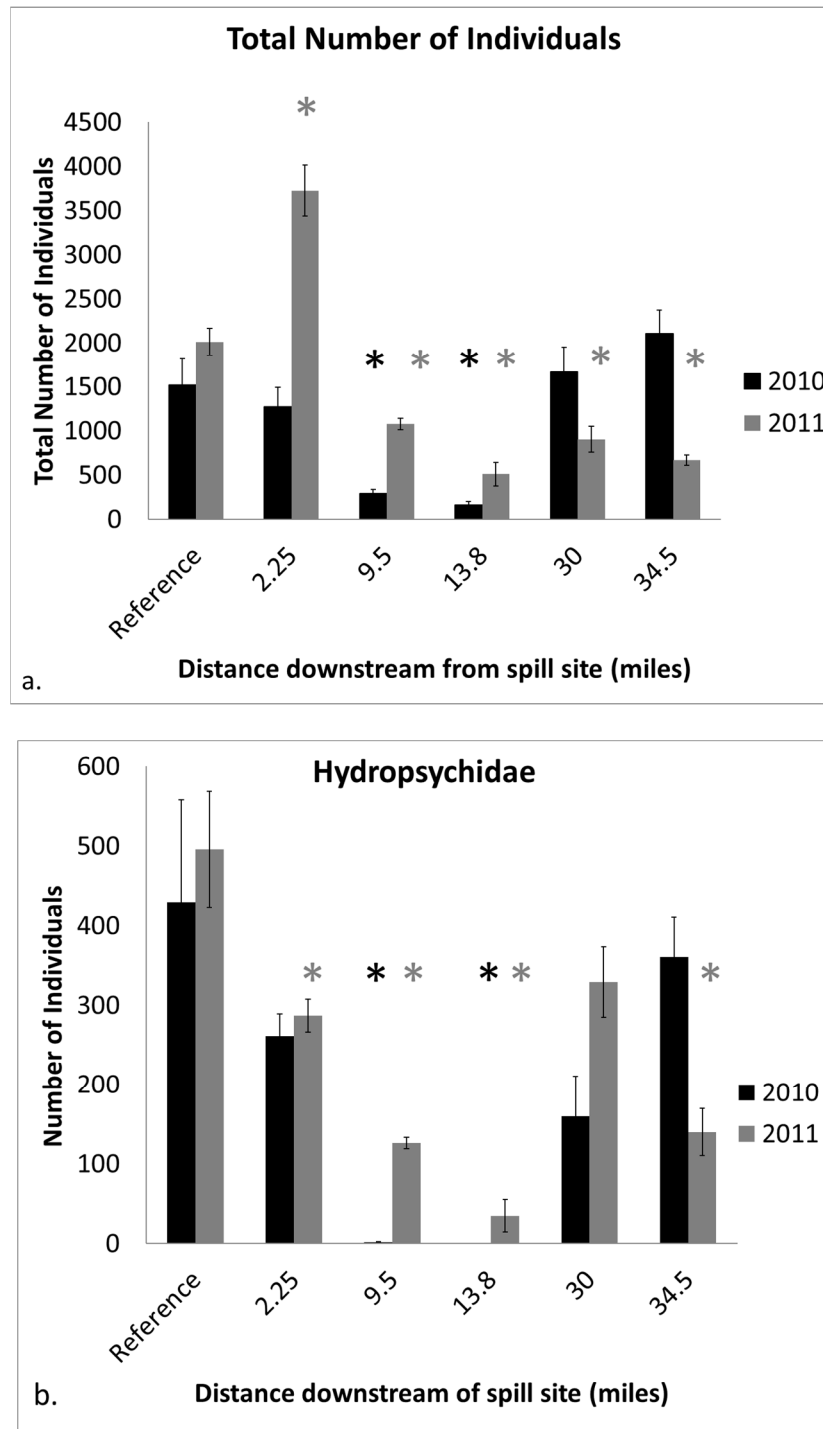
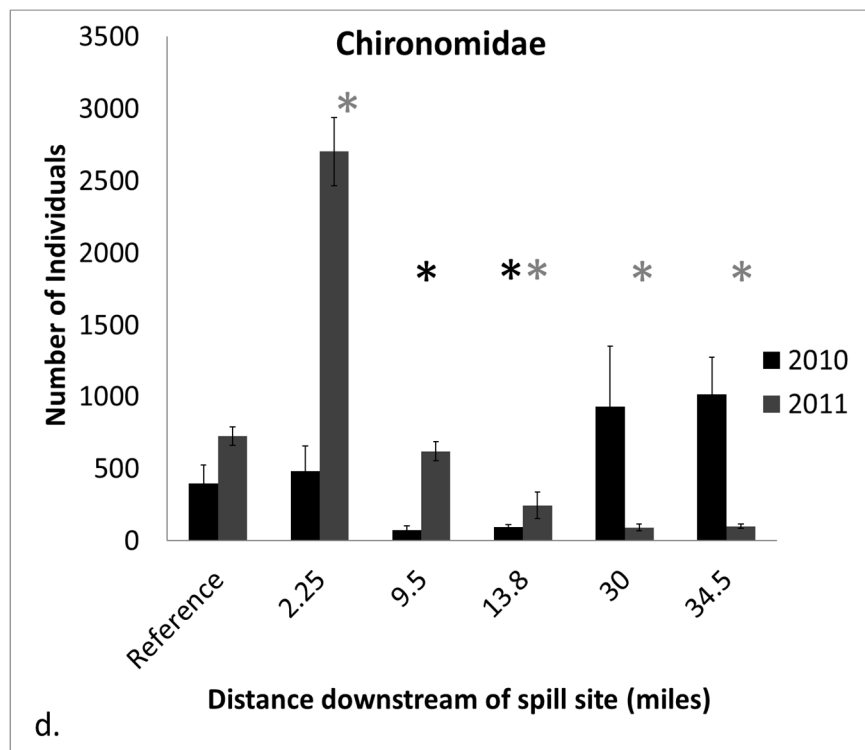
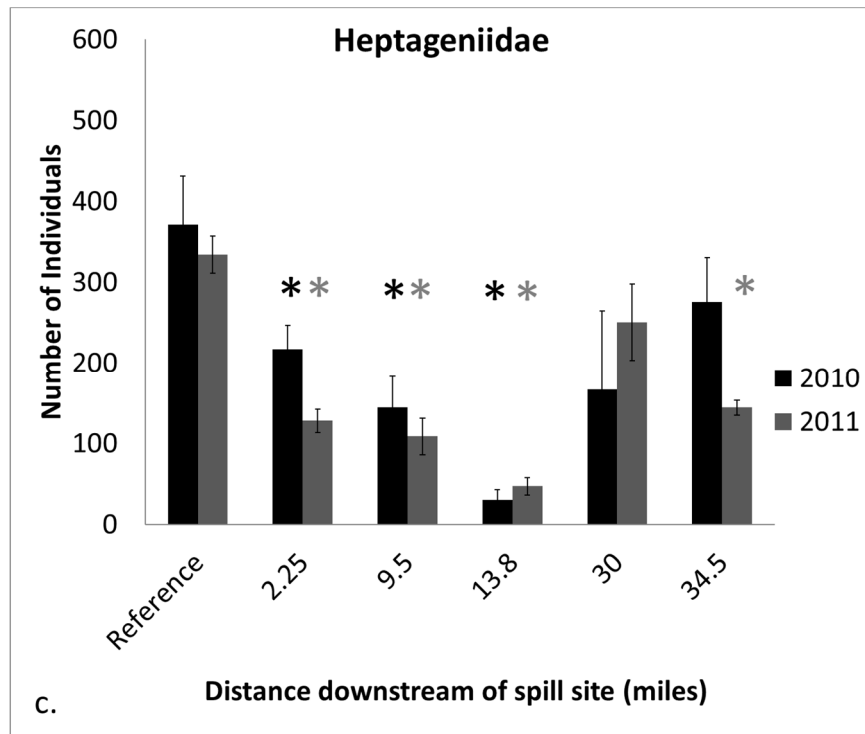


Figure 3.2 Results of in-situ Hester-Dendy artificial substratum samplers deployed in autumn 2010 and 2011. Bars represent total number of invertebrates on the samplers at the sites, which are identified by mile points downstream of where the oil entered the river. Two tailed T-tests were performed to compare oil-impacted sites with the upstream reference site (* $p < 0.05$ in 2010 and * $p < 0.05$ in 2011).

Figure 3.2 (cont'd)



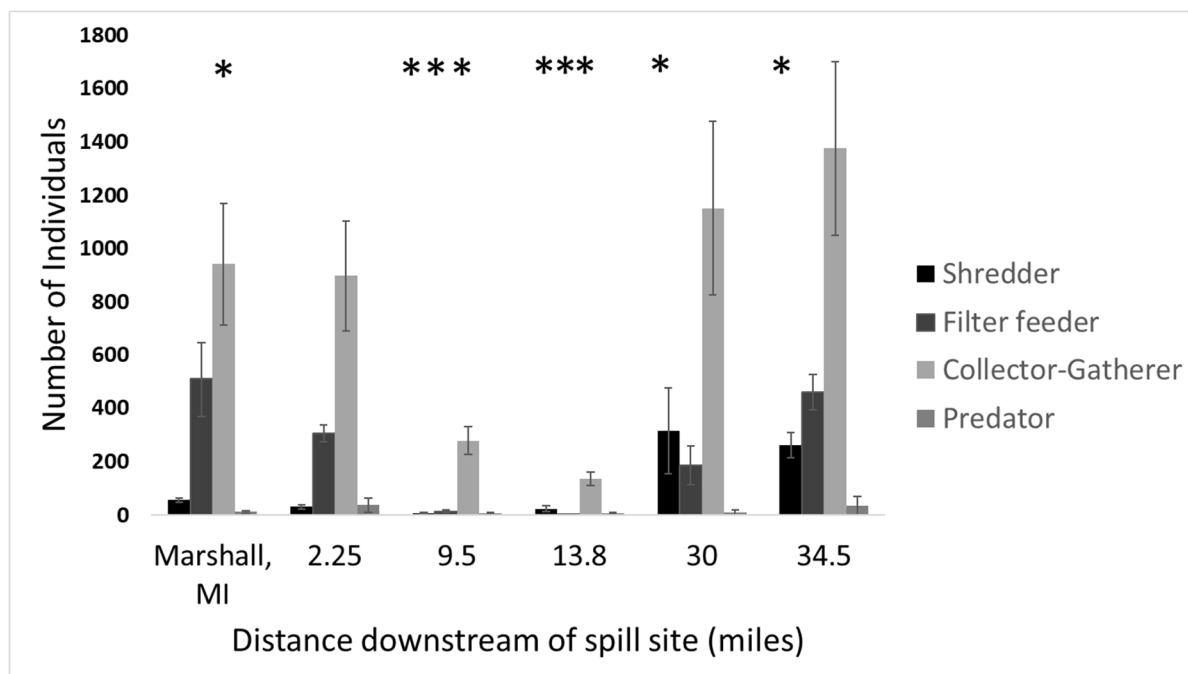


Figure 3.3 Taxa arranged by functional feeding groups in 2010. Taxa were sampled by Hester Dendy samplers and deployed in September 2010. Bars represent total number of invertebrates as arranged by functional feeding groups. Heptageniidae (scraper) and Hydroptilidae were added to the Collector Gatherer functional feeding group. Sites are denoted by mile points downstream of where the oil entered Talmadge Creek, a tributary of the Kalamazoo River. Two tailed T-tests were performed to compare oil-impacted sites with the upstream reference site (* $p < 0.05$).

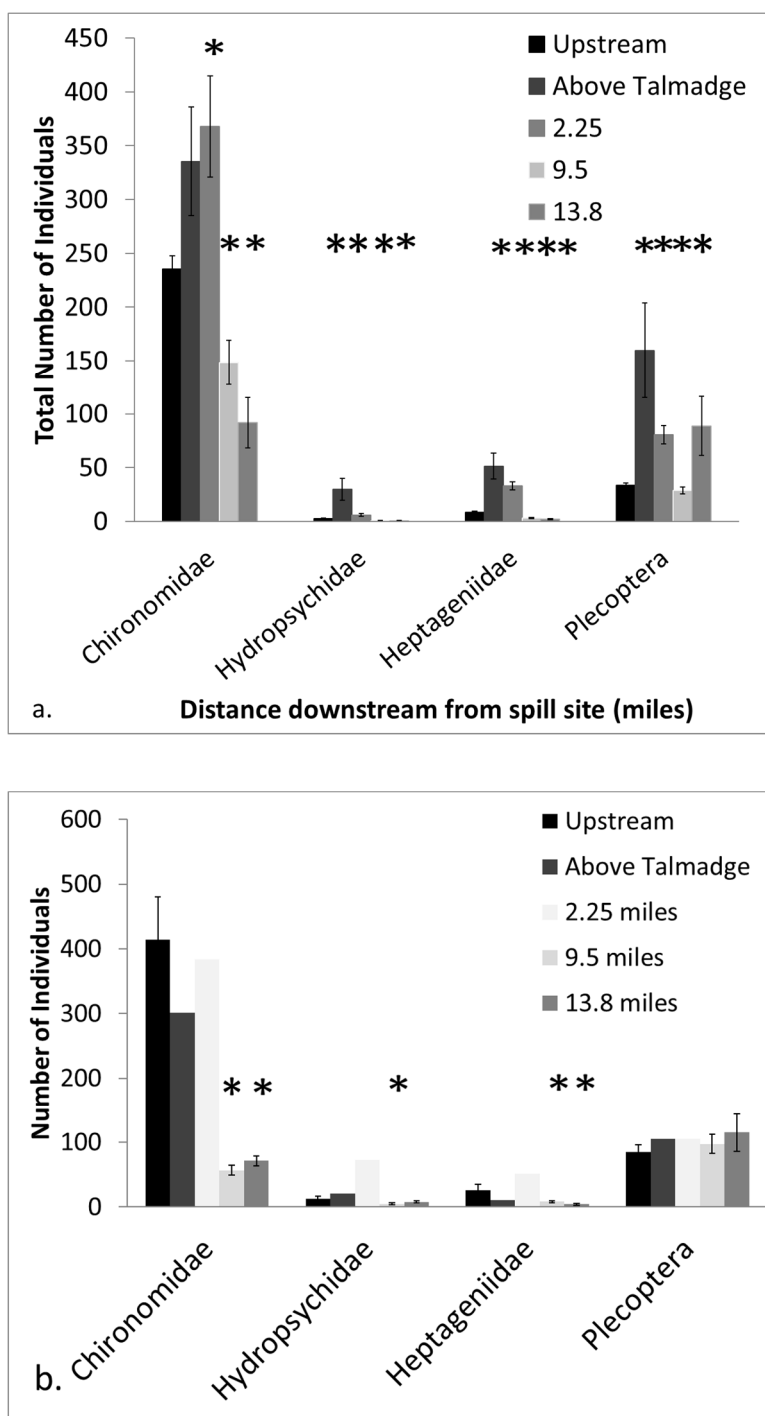


Figure 3.4 Results of in-situ leaf-pack samplers deployed in late Fall 2010 (a.) and 2011 (b.). Bars represent macroinvertebrate abundances on the samplers at the sites, which are identified by mile points downstream of where the oil entered the river. Two tailed T-tests were performed to compare oil-impacted sites with the upstream reference site (asterisks represent significant differences at $p < 0.05$). Top figure represents (a.) 2010 and bottom is (b.) 2011.

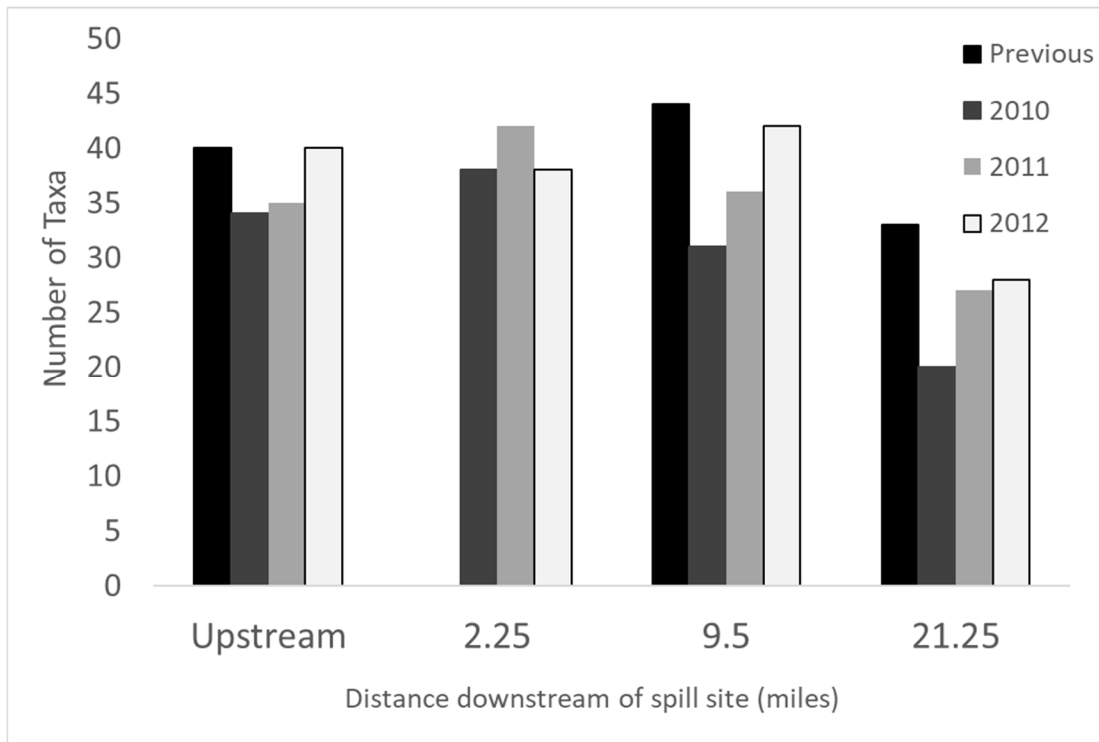


Figure 3.5 Numbers of macroinvertebrate taxa observed in the Rapid Bioassessment Procedure conducted by the Michigan Department of Natural Resources (Matousek and Walterhouse 2013). Previous sampling conducted by the Department of Natural Resources occurred in 2004 Marshall, MI upstream reference site, no previous sampling occurred at the 2.25 mile site, in 2008, multiple samples were collected but only one is presented here at the 9.5 mile site and 2009 at the 21.25 mile site. No replicates were collected during the Rapid Bioassessment Procedure.

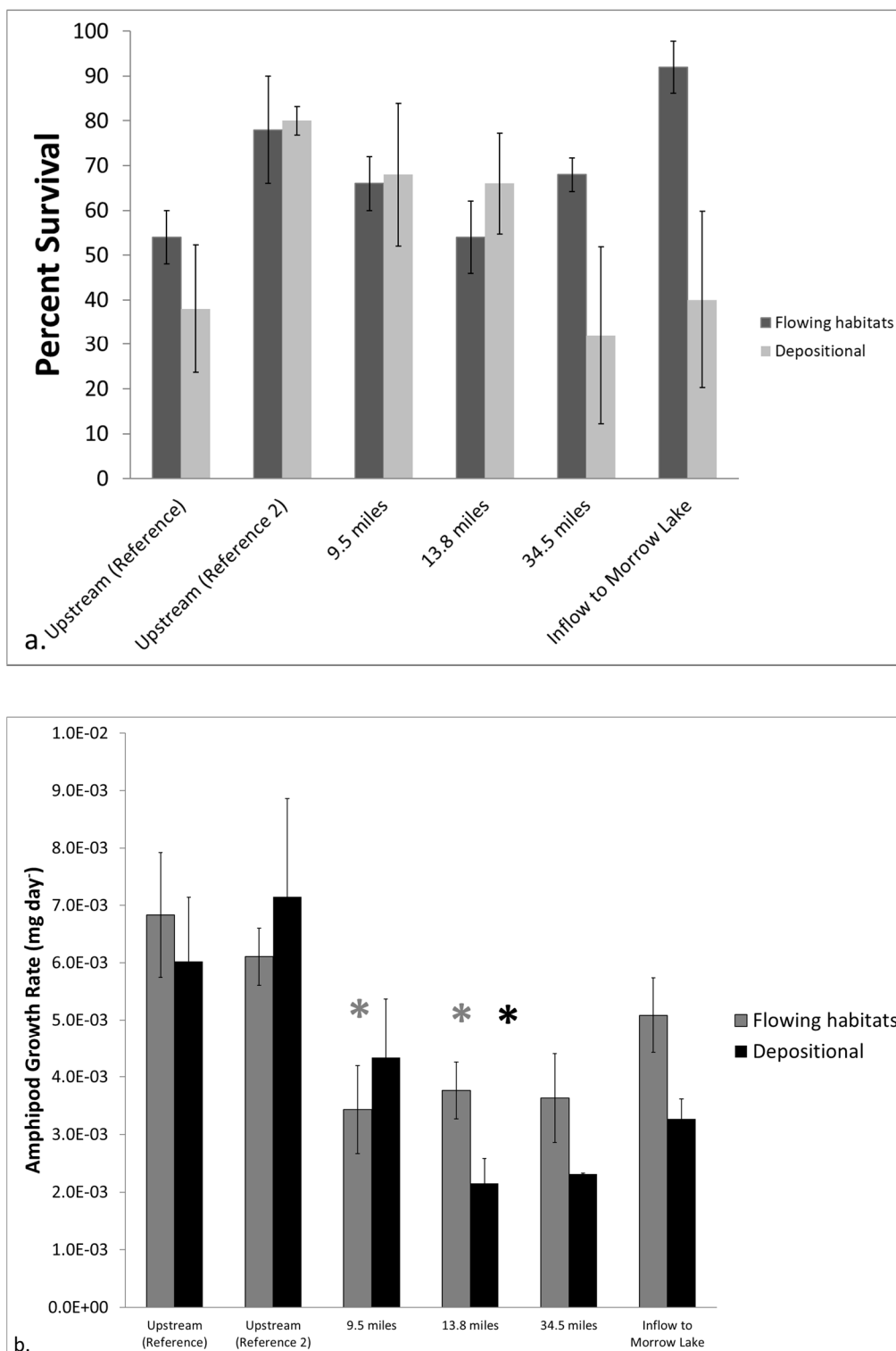


Figure 3.6 Results of in-situ bioassays using the amphipod *Hyalella azteca*, showing percent survival (a) and growth rate (b) in flowing water and depositional habitats. Two tailed T-tests were performed to compare oil-impacted sites with the most upstream reference site (Kalamazoo River at Marshall) (* $p < 0.05$).

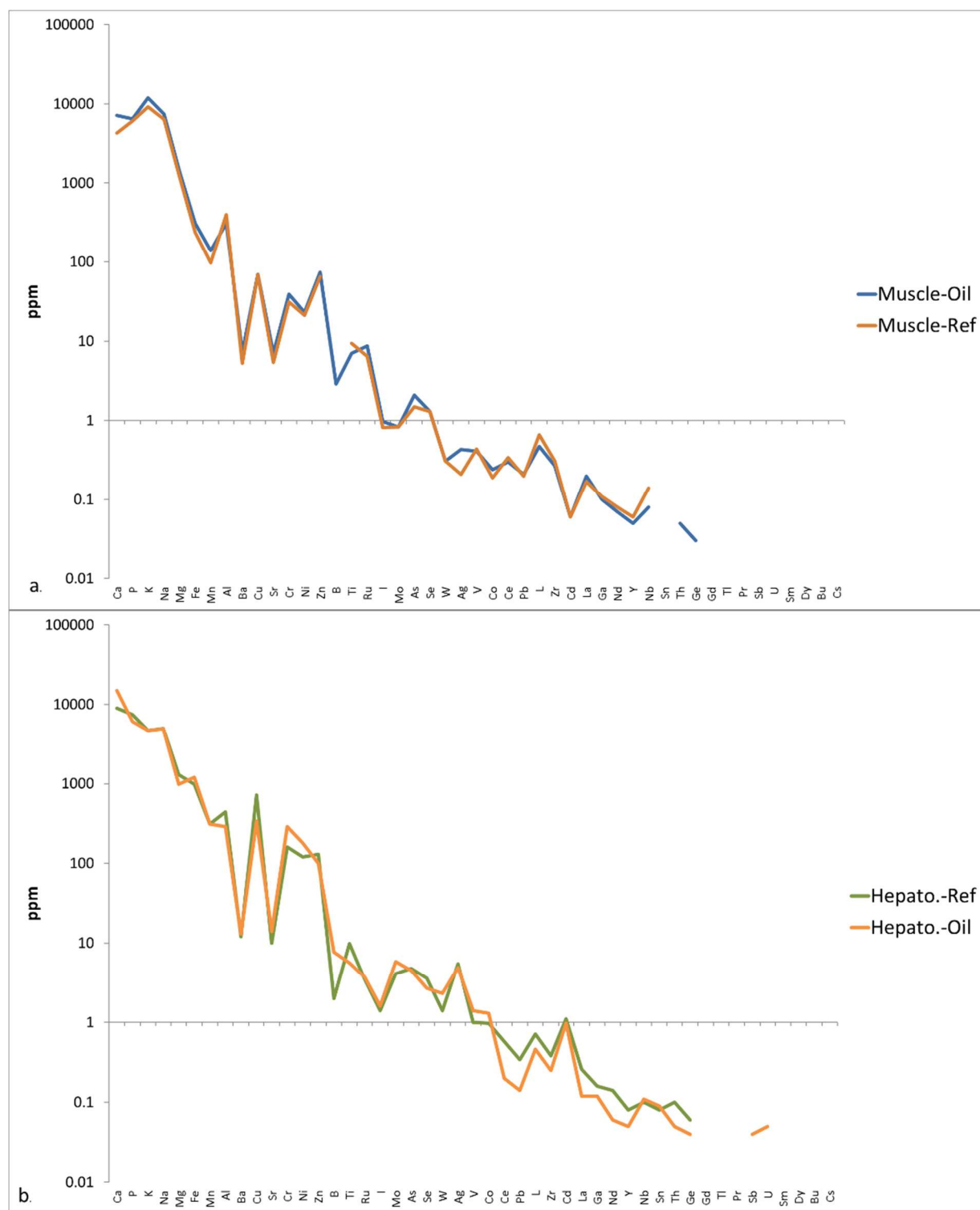
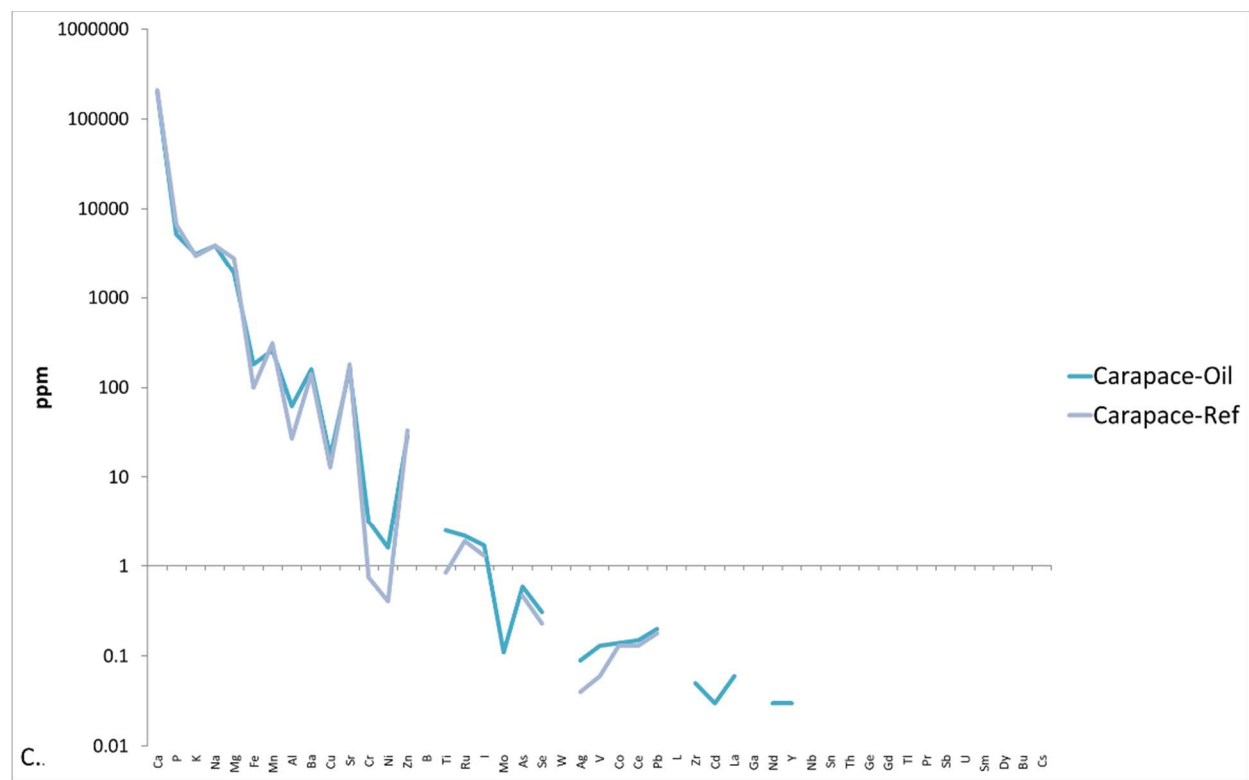


Figure 3.7 Metal concentrations in tissues of crayfish (*Orconectes* sp.) at an oil-impacted site (mile 9.5) compared to an upstream reference site on the Kalamazoo River at Marshall. Tissues samples include muscle (a), hepatopancreas (b), and carapace (c). Samples are composites of ~10 individuals.

Figure 3.7 (cont'd)



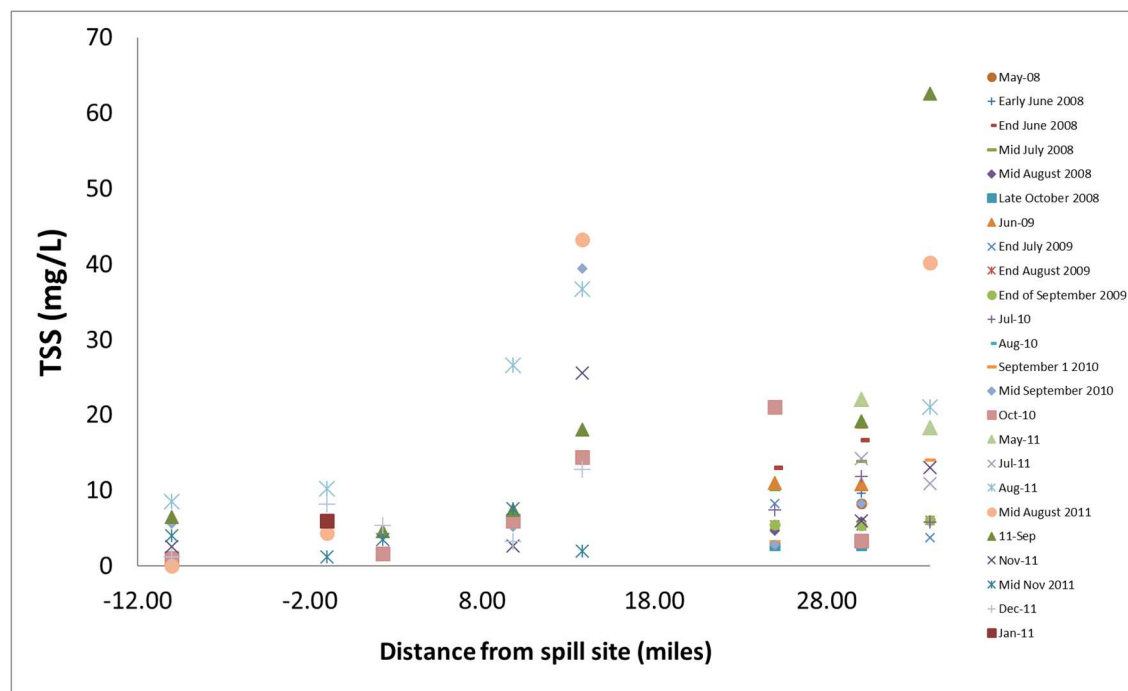


Figure 3.8 Total suspended solids concentrations from sites above and below the point of oil entry into the river at the confluence with Talmadge Creek (mile points 2.5 to 34.5) sampled post-spill in August 2010 and comparison of historical measurements of total suspended solids at 30 and 34 miles downstream to measurements made during the years 2008 and July 2010.

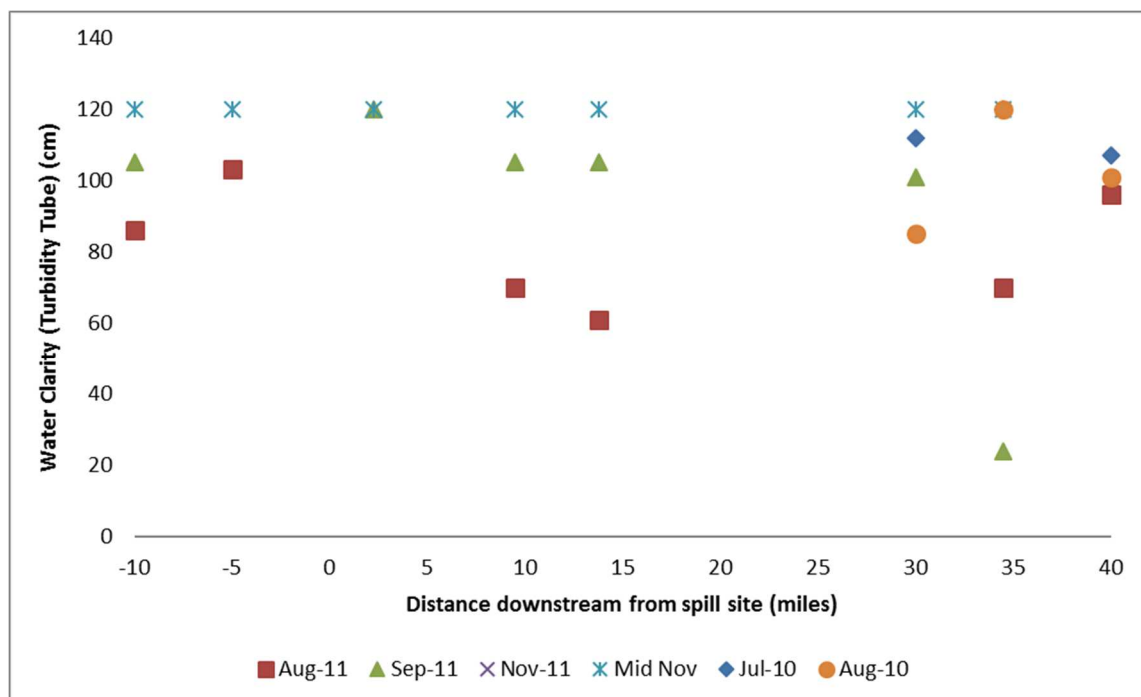


Figure 3.9 Water clarity as measured by turbidity tube from sites at 2.5 to 34.5 miles downstream on the Kalamazoo River post oil spill (0 is the confluence at Talmadge Creek). Previous measurements (July 2010) include the most downstream sites at 30 and 34.5 miles downstream. Sites above the spill include Marshall, MI and just upstream of the confluence at Talmadge Creek.

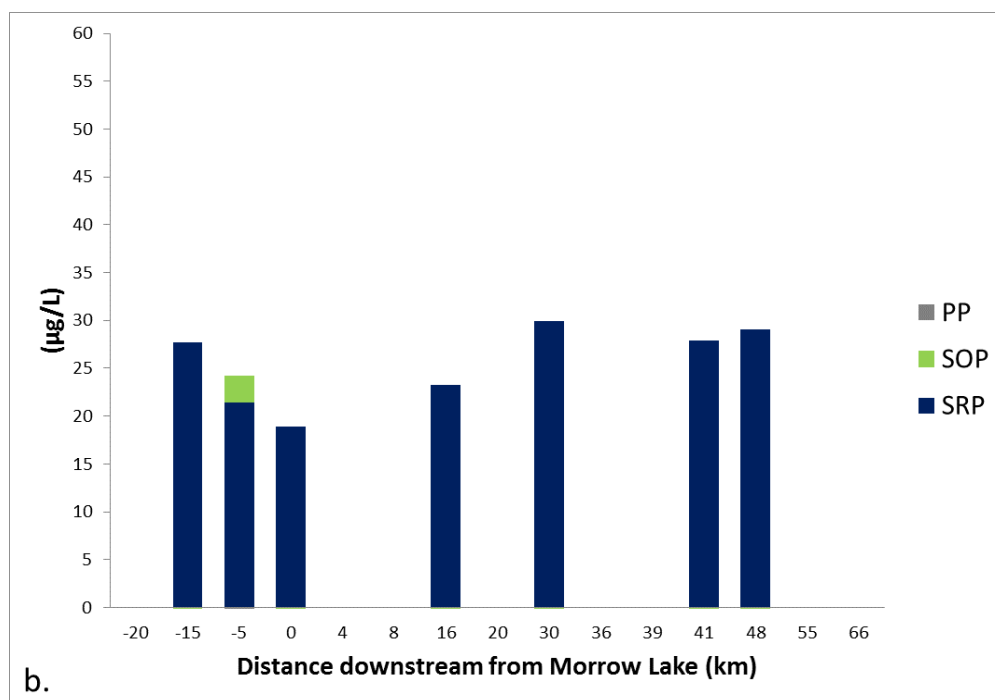
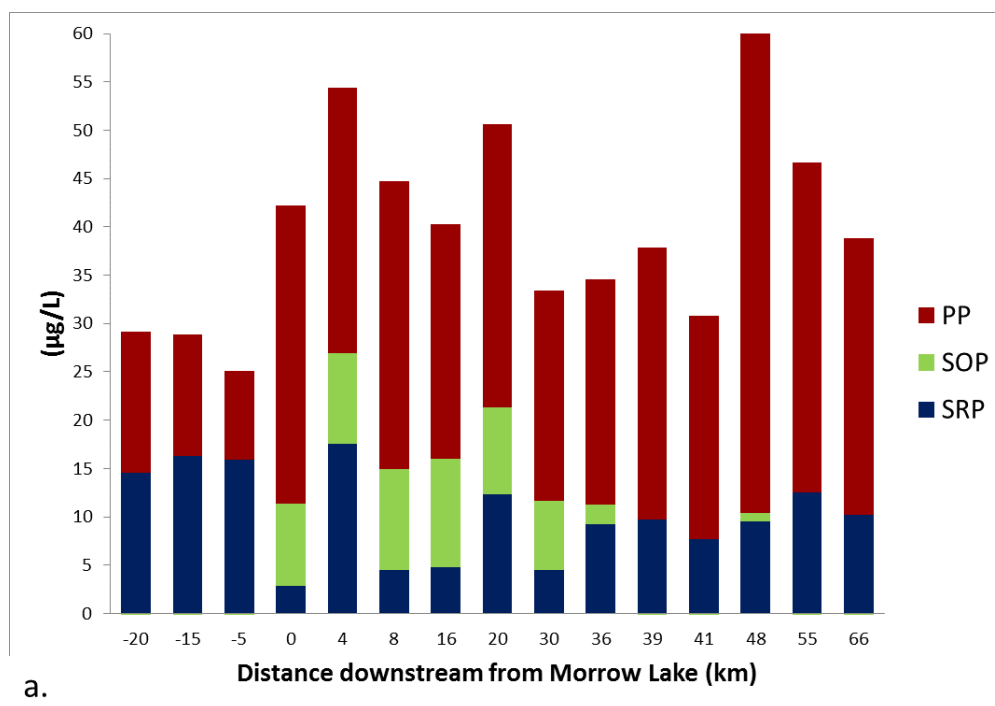
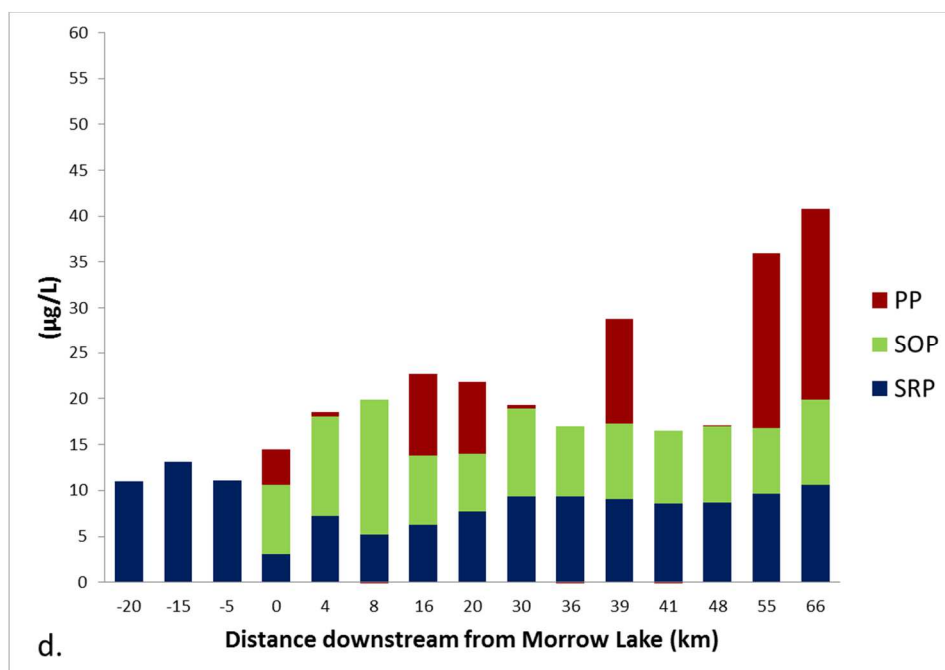
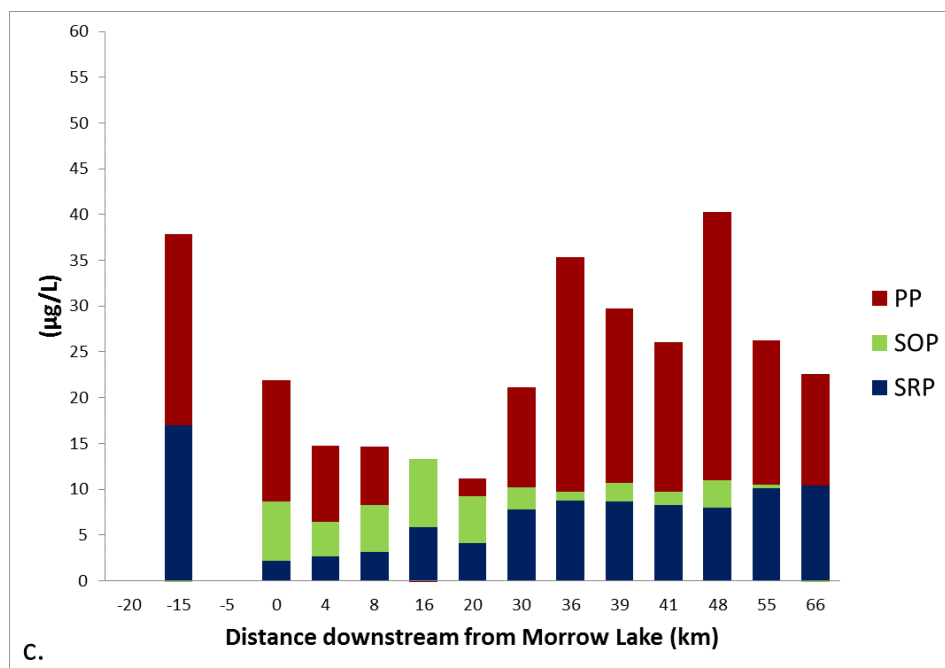


Figure 3.10 Phosphorus fractions along the Kalamazoo, showing (a) samples collected before the spill in July 2010; (b) samples collected on July 27th, just after the spill reached the most downstream sections; (c) samples collected on August 4th and (d) samples collected at the end of August 2010. Note that in this figure 0 km represents Morrow Lake, and negative distances reflect sites upstream of Morrow Lake and positive distances are sites downstream towards Lake Michigan.

Figure 3.10 (cont'd)



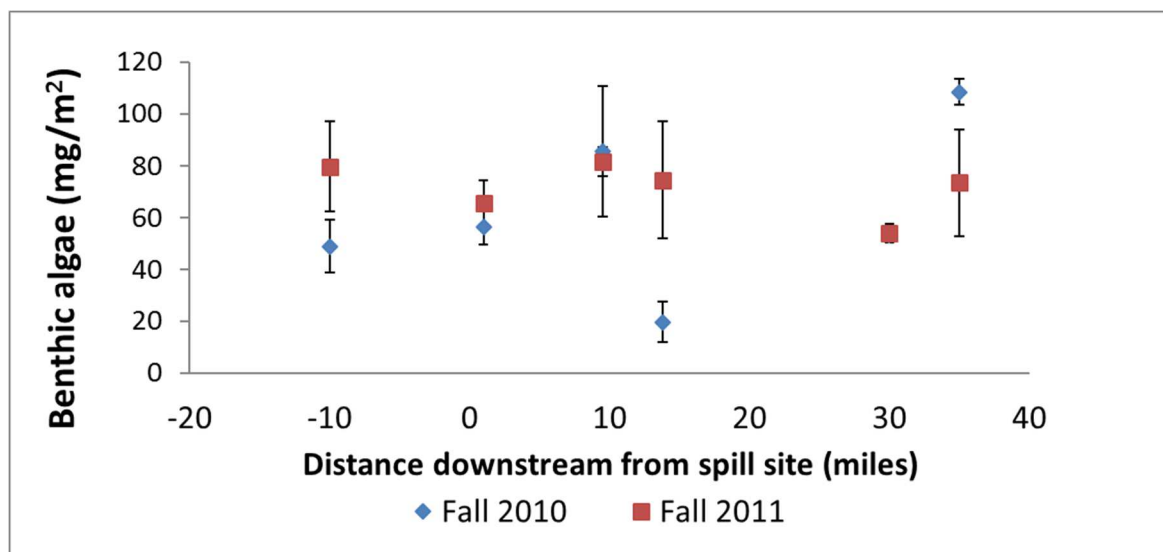


Figure 3.11 Benthic algal chlorophyll-a accrual on artificial substrata at sites above and below the point of oil entry into the river at the confluence with Talmadge Creek (mile points 2.5 to 34.5). Artificial substrata were collected late October 2010 after the oil spill on the Kalamazoo River and again in October 2011.

REFERENCES

REFERENCES

- Ahad, J. M.E., J. J. Jautzy, B. F. Cumming, B. Das, K. R. Laird, H. Sanei. 2015. Sources of polycyclic aromatic hydrocarbons (PAHs) to northwestern Saskatchewan lakes east of the Athabasca oil sands. *Organic Geochemistry* 80L 35-45.
- Alexander, M. 2000. Aging, bioavailability and overestimation of risk from environmental pollutants. *Environmental Science and Technology* 34: 4259- 4264.
- Alexander, C.R., R. F. Lee, D. T. Burton, L. W. Hall., Jr. 2005. An integrated case study for evaluating the impacts of an oil refinery effluent on aquatic biota in the Delaware River: sediment core studies. *Human and ecological risk assessment* 11: 861-877.
- Alharbi, H.A., G. Morandi, J. P. Giesy, S. B. Wiseman. 2016. Effect of oil sands processes-affected water on toxicity of retene to early life-stages of Japanese medaka (*Oryzias latipes*). *Aquatic Toxicology* 176: 1-9.
- Amiard, J.C., H. Bacheley, A.L. Barille, L. Barille, A. Geffard, N. Himery. 2004. Temporal changes in nickel and vanadium concentrations and in condition index and metallothionein levels in three species of molluscs following the “Erika” oil spill. *Aquatic Living Resource* 17: 281-288.
- Aminot, A., D.S. Kirkwood and R. Kerouel. 1997. Determination of ammonia in seawater by the indophenol-blue method: evaluation of the ICES NUTS I/C 5 questionnaire. *Mar. Chem.* 56:59-75.
- Anderson, J., S. B. Wiseman, A. Moustafa, M. Gama El-Din, K. Liber, J. P. Giesy. 2012. Effects of exposure to oil sands process-affected water from experiment reclamation ponds on *Chironomus dilutus*. *Water Research* 46: 1662-1672.
- Arens, C. J., N. S. Hogan, R. J. Kavanagh, A. G. Mercer, G. J. Van Der Kraak, M. R. van den Heuvel. 2015. Sublethal effects of aged oil sands-affected water on white sucker (*Catostomus commersoni*). *Environmental Toxicology and Chemistry* 34: 598-599.
- Baas, D. G. 2009. Inferring dissolved phosphorus cycling in a TMDL watershed using biogeochemistry and mixed linear models. Dissertation, Michigan State University, East Lansing, Michigan, USA.
- Baker, L.F., J.J.H. Ciborowski, M.D. MacKinnon 2012. Petroleum coke and soft tailings sediment in constructed wetlands may contribute to the uptake of trace metals by algae and aquatic invertebrates. *Science of the Total Environment* 414:177-186.
- Barteau and others. .2013. Effects of Diluted Bitumen on crude oil transmissions pipes. National Academy of Science.

Barton, D. R. and R. R. Wallace. 1979. The effects of an experimental spillage of oil sands tailings sludge on benthic invertebrates. *Environmental Pollution* 18: 305-312.

Barton, D. R., and R. R. Wallace. 1979. Effects of eroding oil sand and periodic flooding on benthic macroinvertebrate communities in a brown water stream in Northeastern Alberta, Canada. *Canadian Journal of Zoology* 57: 533-541.

Bejarano, A.C., J. Michel, F. Fitzpatrick, M. DeGraeve, L. Williams, M. Alexander, S.K. Hamilton, and J. Chapman. 2012. Bridging Oil Spill Response and Scientifically Supported Information: A Net Environmental Benefit Analysis for the Kalamazoo River Oil Spill. Society for Environmental Toxicology & Chemistry, Long Beach, CA.

Bejarano, A. C. 2012. Analysis on the association between aquatic toxicity results and sediment characteristics from samples collected within the Kalamazoo River Enbridge Line 6B MP 608 Marshall, MI Pipeline Release. In support of the SSCG's Net Environmental Benefit Analysis Subgroup.

Bendell-Young, L. I., Bennett, K. E., Crowe, A., Kennedy, C. J., Kermode, A. R., Moore, M. M., Plant, A. L., and Wood, A. 2000. Ecological characteristics of wetlands receiving an industrial effluent. *Ecological Applications* 10:310–322.

Benke, A. C. and A. D. Huynh. 2010. Benthic invertebrate production-facilitating answers to ecological riddles in freshwater ecosystems. *Journal of American Benthological Society* 29: 264-285.

Benke, A. C. and J. B. Wallace. 1997. Trophic basis of production among riverine caddisflies: implications for food web analysis. *Ecology* 78: 1132-1145.

Berg, S. A., L. L. Williams. 2015. Wildlife response activities for the July 25-26 2010 Enbridge Line 6B Discharges near Marshall, MI. United States Fish and Wildlife Service, East Lansing Office.

Blaise, C., F. Gagne, N. Chevre, M. Harwood, K. Lee, J. Lappalainen, B. Chial, G. Persoone, K. Doe. 2004. Toxicity assessment of oil-contaminated freshwater sediments. *Environmental Toxicology* 19: 267-273.

Bodkin, J. L., B. E. Ballachey, T. A. Dean., A. K. Fukuyama S. C. Jewett, L. McDonald, D. H. Monson, C.E. O'Clair, G.R. VanBlaricom. 2002. Sea otter populations status and the process of recovery from the 1989 "Exxon Valdez" oil spill. *Marine Ecology Progress Series* 241: 237-253.

Boehm, P. D., D. S. Page, J. S. Brown, J. M. Neff, W. A. Burns. 2004. Polycyclic aromatic hydrocarbon levels in mussels from Prince William Sound, USA, document the return to baseline conditions. *Environmental Toxicology and Chemistry* 23: 2916-2929.

- Bowyer, R. T., G. M. Blundell, M. Ben-David, S. C. Jewett, T. A. Dean. L. K. Duffy. 2003. Effects of the Exxon Valdez oil spill on river otters: injury and recovery of a sentinel species. *The Journal of Wildlife Management* 67: 1-53.
- Burton, G. A., M. S. Greenberg, C. D. Rowland, C.A. Irvine, D. R. Lavoie, J. A. Brooker, L. Moore, D. F. N. Raymer, R. A. McWilliam. 2005. In situ exposures using caged organisms: a multi-compartment approach to detect aquatic toxicity and bioaccumulation. *Environmental Pollution* 134: 133-144.
- Burton, G. A. and E. L. Johnston. 2010. Assessing contaminated sediments in the context of multiple stressors. *Environmental Toxicology and Chemistry* 29: 2625-2643.
- Carls, M. G., L. Holland, G. V. Irvine, D. H. Mann, M. Lindeberg. 2016. Petroleum biomarkers as tracers of Exxon Valdez oil. *Environmental Toxicology and Chemistry* 35: 2683-2690.
- Carls, M. G., P. M. Harris, S. D. Rice. 2004a. Restoration of oiled mussel beds in Prince William Sound, Alaska. *Marine Environmental Research* 57: 359-376.
- Carls, M. G., J. P. Meador. 2009. A perspective on the toxicity of petrogenic PAHs to developing fish embryos related to environmental chemistry. *Human and Ecological Risk Assessment: An International Journal* 15: 1084-1098.
- Carls, M. G., S. D. Rice, G. D. Marty, D. K. Naydan. 2004b. Pink salmon spawning habitat is recovering a decade after the Exxon Valdez oil spill. *Transactions of the American Fisheries Society* 133: 834-844.
- Chiffolleau, J.F., L. Chauvaud, D. Amouroux, A. Barats, A. Dufour, C. Pecheyran, N. Roux. 2004. Nickel and vanadium contamination of benthic invertebrates following the “Erika” wreck. *Aquatic Living Resources* 17: 273-280.
- Chindah, A. C. 1998. The effects of industrial activities on the periphyton community at the upper reaches of the new Calabar River, Niger Delta, Nigeria. *Water Research* 32: 1137-1143.
- Clark, R. M., A. H. Vicory, J. A. Goodrich. 1990. The Ohio River Oil Spill: A Case Study. *Journal of the American Water Works Association*. 82: 39-44.
- Colavecchia, M. V., S. M. Backus, P. V. Hodson, J. L. Parrott. 2004. Toxicity of oil sands to early life stages of fathead minnows (*Pimehales promelas*). *Environmental Toxicology and Chemistry* 23: 1709-1718.
- Colavecchia, M. V., P. V. Hodson J. L. Parrott. 2006. CYP1A induction and blue sac disease in early life stages of white suckers (*Catostomus commersoni*) exposed to oil sands. *Journal of Toxicology and Environmental Health, Part A* 69:967-994.

Cole, J.J., S. R. Carpenter, J. Kitchell, M. L. Pace, C. T. Solomon, B. Weidel. 2011. Strong evidence for terrestrial support of zooplankton in small lakes based on stable isotopes of carbon, nitrogen, and hydrogen. p. 108: 1975-1980.

Committee on Oil in the Sea. 2003. Oil in the sea III: Inputs, fates and effects. National Academies Press. Washington D.C.

Cronk, J. K., W. J. Mitsch, R. M. Sykes. 1990. Effective modelling of a major inland oil spill on the Ohio River. *Ecological Modelling* 51: 161-192.

Crosby, S., R. Fay, C. Groark, A. Kani, J. R. Smith, T. Sullivan, R. Pavia, G. Shigenaka. 2013. Transporting Alberta Oil Sands Products: Defining the issues and assessing the risks. U.S. Department of Commerce, NOAA Technical Memorandum NOS OR&R 43 Seattle, WA: Emergency Response Division, NOAA. 153 pp.

Crunkilton, R. L. and R. M. Duchrow. 1990. Impact of a massive crude oil spill on the invertebrate fauna of a Missouri Ozark stream. *Environmental Pollution* 63: 13-31.

Cruz-Martinez, L., K. J. Fernie, C. Soos, T. Harner, F. Getachew, J. E. G. Smits. 2015. Detoxification, endocrine, and immune responses of tree swallow nestlings naturally exposed to air contaminants from the Alberta oil sands. *Science of the Total Environment* 502: 8-15.

Cummins, K. W., M. J. Klug, G. M. Ward, G. L. Spengler, R. W. Speaker, R. W. Ovink, D. C. Mahan, R.C. Peterson. 1981. Trends in particulate organic matter fluxes, community processes and macroinvertebrate functional feeding groups along a Great Lakes drainage basin river continuum. *Verhandlungen der Internationalen Vereinigung für theoretische und angewandte Limnologie* 21:841-849.

Cushman, R. M. and J. C. Goyert. 1984. Effects of a synthetic crude oil on pond benthic insects. *Environmental Pollution (Series A)* 33: 163-186.

Custer, K. W., G.A. Burton. 2008. *Isonychia* spp. and macroinvertebrate community responses to stressors in streams utilizing the benthic in situ toxicity identification evaluation (BiTIE) method. *Environmental Pollution* 151: 101-109.

Debenest, T., P. Turcotte, F. Gagne, C. Gagnon, C. Blaise. 2012. Ecotoxicological impacts of effluents generated by oil sands bitumen extraction and oil sand lixiviation on *Pseudokirchneriella subcapitata*. *Aquatic Toxicology* 112-113: 83-91.

DeGraeve, G. M. 2012. Final Report: *Chironomus dilutus* and *Hyaella azteca* 10-day whole sediment toxicity testing results, Kalamazoo River sediment sampling line 6B oil spill, Marshall, MI. Report for Enbridge Energy: Great Lake Environmental Center.

Doi, H., K-H. Chang, T. Ando, H. Imai, S-I Nakano, A. Kajimoto, I. Katano. 2008. Drifting plankton from a reservoir subsidize downstream food webs and alter community structure. *Oecologia* 156: 363-371.

Esler, D., T. D. Bowman, K. A. Trust, B. E. Ballachey, T. A. Dean, S. C. Jewett, C. E. O'Clair. 2002. Harlequin duck population recovery following the "Exxon Valdez" oil spill: progress, process and constraints. *Marine Ecology Progress Series*. 241: 271-286.

Evans, M. S., A. Talbot. 1989. Investigations of mercury concentrations in walleye and other fish in the Athabasca River ecosystem with increasing oil sands developments. *Journal of Environmental Monitoring* 14: 1989-2003.

Evans, M., M. Davis, K. Janzen, D. Muir, R. Hazewinkel, J. Kirk, D. de Boer. 2016. PAH distributions in sediments in the oil sands monitoring area and western Lake Athabasca: Concentration, composition and diagnostic ratios. *Environmental Pollution* 213: 671-687.

Faria, M.S., A. Re, J. Malcato, P.C.L.D. Silva, J. Pestana, A. R. Agra, A. J. A. Nogueira, A. M.V.M. Soares. 2006. Biological and functional responses of in situ bioassays with *Chironomus riparius* larvae to assess river water quality and contamination 371: 125-137.

Federle, T. W., J. R. Vestal, G. R. Hater, M. C. Miller. 1979. Effects of Prudhoe bay crude oil on primary production and zooplankton in arctic tundra thaw ponds. *Marine Environmental Research* 2: 3-18.

Final Restoration Plan and Environmental Assessment for the Delaware River. 2009.
http://www.darrp.noaa.gov/northeast/athos/pdf/Athos_Final_RP.pdf

Fitzpatrick, F. A., M. C. Boufadel, R. Johnson, K. Lee, T. P. Graan, A. C. Bejarano, Z. Zhu, D. Waterman, D. M. Capone, E. Hayter, S. K. Hamilton, T. Dekker, M. H. Garcia, J S. Hassan. 2015. Oil-particle interactions and submergence from crude oil spills in marine and freshwater environments: Review of the science and future science needs. Open-file report 2015-1076.

Fleege, J. W., K. R. Carman, R. M. Nisbet. 2003. Indirect effects of contaminants in aquatic ecosystems. *The Science of the Total Environment* 317: 207-233.

Forsberg, N.D., B.W. Smith, G. J. Sower, K. A. Anderson. 2014. Predicting polycyclic aromatic hydrocarbon concentrations in resident aquatic organisms using passive samplers and partial least-squares calibration. *Environmental Science and Technology* 48: 6291-6299.

Gagne, F., M. Douville, C. Andre, T. Debenest, A. Talbot, J. Sherry, L. M. Hewitt, R. A. Frank, M. E. McMaster, J. Parrott, G. Bickerton. 2012. Differential changes in gene expression in rainbow trout hepatocytes exposed to extracts of oil sands process-affected water and the Athabasca River. *Comparative Biochemistry and Physiology* 155: 551-559.

Gagne, F., C. Andre, P. Turcotte, C. Gagnon. 2013. A comparative toxicogenomic investigation of oil sand water and processed water in rainbow trout hepatocytes. *Archives for Environmental Contamination and Toxicology* 65: 309-323.

- Gentes, M. L., C. Waldner, C., Z. Papp, J. E. G. Smits. 2007. Effects of exposure to naphthenic acids in tree swallows (*Tachycineta bicolor*) on the Athabasca Oil Sands, Alberta, Canada. *Journal of Toxicology and Environmental Health, Part A*. 70: 1182-1190.
- Giesy, J. P., J. C. Anderson, S. B. Wiseman. 2010. Alberta oil sands development. *Proceedings of the National Academy of Science* 107:951-952.
- Green, J., M. W. Trett. 1989. *The Fate and Effects of Oil in Freshwater*. Springer Netherlands.
- Goodwin, C.M., J.E.G. Smits, R. M. R. Barclay. 2016. Metals and metalloids in nestling tree swallows and their dietary items near oil sands mine operations in Northern Alberta 562: 714-723.
- Hall, L. W., D. T. Burton. 2005. An integrated case study for evaluating the impacts of an oil refinery effluent on aquatic biota in the Delaware River: Introduction, study approach, and objectives. *Human and Ecological Risk Assessment* 11: 647-656.
- Haring, H.J., K. A. Blocksom, M. E. Smith, T. Angradi, M.C. Wratschko, B. Armstrong, D. Bolgrien, J.M. Lazorchak. 2011. Sediment toxicity in mid-continent great rivers (USA). *Archives for Environmental Contamination and Toxicology* 60: 57-67.
- Haritash, A. K., C.P. Kaushik. 2009. Biodegradation aspects of polycyclic aromatic hydrocarbons (PAHs): A review. *Journal of Hazardous Materials*. 169: 1-15.
- Harms, N. J., G. D. Fairhurst, G. R. Bortolotti, J. E. G. Smits. 2010. Variation in immune function, body condition, and feather corticosterone in nestling Tree Swallows (*Tachycineta bicolor*) on reclaimed wetlands in the Athabasca oil sands, Alberta, Canada. *Environmental Pollution* 158: 841-848.
- Harper, P. P., M. Lauzon, F. Harper. 1991. Life cycles of 12 species of winter stoneflies from Quebec (Plecoptera: Capniidae and Taeniopterygidae). *Canadian Journal of Zoology* 69: 787-796.
- Harrel, R. C. 1985. Effects of a crude oil spill on water quality and macrobenthos of a southeast Texas. stream *Hydrobiologia* 124: 223-228.
- Harwell, M. A. and J. H. Gentile. 2006. Ecological significance of residual exposures and effects from the Exxon Valdez Oil Spill. *Integrated Environmental Assessment and Management* 2: 204-246.
- Hazewinkel, R. R. O., A. P. Wolfe, S. Pla, C. Curtis, K. Hadley. 2008. Have atmospheric emissions from the Athabasca Oil Sands impacted lakes in northeastern Alberta, Canada? *Canadian Journal Fisheries Aquatic Science* 65: 1554-1567.

He, Y., S. Patterson, N. Wang, M. Hecker, J. W. Martin, M. G. El-Din, J. P. Giesy, S. B. Wiseman. 2012. Toxicity of untreated and ozone-treated oil sands process-affected water (OSPW) to early life stages of the fathead minnow (*Pimephales promelas*). *Water Research* 46: 6359-6368.

Headley, J.V., C. Akre. 2001. Preliminary characterization and source assessment of PAHs in tributary sediments of the Athabasca River, Canada. *Environmental Forensics* 2: 335-345.

Headley, J.V., D. W. McMartin. 2004. A review of the occurrence and fate of naphthenic acids in aquatic environments. *Journal of Environmental Science and Health, Part A* 39: 1989-2010.

Headley, J.V., K. M. Peru, M. P. Barrow. 2016. Advances in mass spectrometric characterization of naphthenic acid fraction compounds in oil sands environmental samples and crude oil: a review. *Mass Spectrometry Reviews* 35: 311-328.

Hoard, C.J., K.K. Fowler, M.H. Kim, C.D. Menke, S. E. Morlock, M. C. Peppler, C. M. Rachol, M T. Whitehead. 2010. Flood-inundation maps for a 15-mile reach of the Kalamazoo River from Marshall to Battle Creek, MI. *Scientific Investigations Map* 3135.

Hodson, P. V. 2010. History of environmental contamination by oil sands extraction. *Proceedings of the National Academy of Science*. 110: 1569-1570.

Hossler, R., R. Greene, S. Michels, C. Schirey, D. Bean, T. Baum, M. Boyer, J. Hoff, J. Steinbacher, K. Clark, S. Hahn. Aquatic Technical Working Group. 2007. Aquatic injury assessment M/T Athos 1 Oil spill, Delaware River system.

Incardona, J. P., C. A. Vines, B. F. Anulacion, D. H. Baldwin, H. L. Day, B. L. French, J. S. Lebenia, T. L. Linbo, M. S. Myers, O. Paul Olson, C. A. Sloan, S. Sol, F. J. Giffin, K. Menard, S. G. Morgan, J. E. West, T. K. Collier, G. M. Ylitalo, G. N. Cherr, N. L. Scholz. 2012. Unexpectedly high mortality in Pacific herring embryos exposed to the 2007 *Cosco Busan* oil spill in San Francisco Bay. *Proceedings of the National Academy of Science* 109: E51-E58.

Incardona, J.P., M.G. Carls, L. Holland, T. L. Linbo, D. H. Baldwin, M.S. Myers, K.A. Peck, M. Tagal, S. D. Rice, N.L. Scholz. 2015. Very low embryonic crude oil exposures cause lasting cardiac defects in salmon and herring. *Scientific Reports* 5:1-15.

Ingersoll, C.G., D.D. MacDonald, N. Wang, J.L. Crane, L. J. Field, P.S. Haverland, N.E. Kemble, R. A. Lindskoog, C. Severn, D. E. Smorong. 2001. Predictions of sediment toxicity using consensus based freshwater sediment quality guidelines. *Archives Environmental Contamination Toxicology* 41: 8-21.

Irie, K., M. Kawaguchi, K. Mizuno, J.-Y. Song, K. Nakayama, S. I. Kitamura, Y. Murakami. 2011. Effect of heavy oil on the development of the nervous system of floating and sinking teleost eggs. *Marine Pollution Bulletin* 63: 297-302.

Irvine, G. V., D. H. Mann, J. W. Short. 2006. Persistence of 10-year old Exxon Valdez oil on Gulf of Alaska beaches: the importance of boulder armoring. *Marine Pollution Bulletin* 52: 1011-1022.

Jefferies, M. K. S., A. M. Stultz, A. W. Smith, D. A. Stephens, M. M. Rawlings, S. E. Belanger, J. T. Oris. 2015. The fish embryo toxicity test as a replacement for the larval growth and survival tests: comparison of test sensitivity and identification of alternative endpoints in zebrafish and fathead minnows. *Environmental Toxicology and Chemistry* 34: 1369-1381.

Jensen-Fontaine, M., W. P. Norwood, M. Brown. D. G. Dixon, X. C. Le. 2014. Uptake and speciation of vanadium on it the benthic invertebrate *Hyalella azteca*. *Environmental Science and Technology* 48: 731-738.

Kavanagh, R. J., B. K. Burnison, R. A. Frank, K. R. Solomon, G. Van Der Kraak. 2009. Detecting oil sands process-affected waters in the Alberta oil sands region using synchronous fluorescence spectroscopy. *Chemosphere*. 76: 120-126.

Kavanagh, R. J., R. A. Frank, B. K. Burnison, R. F. Young, P. M. Fedorak, K. R. Solomon, G. Van Der Kraak. 2012. Fathead minnow (*Pimephales promelas*) reproduction is impaired when exposed to a naphthenic acid extract. *Aquatic Toxicology*: 116-177 34-42.

Kavanagh, R. J., R. A. Frank, K. D. Oakes, M. R. Servos, R. F. Young, P. M. Fedorak, M. D. MacKinnon, K. R. Solomon, D. G. Dixon, G. Van Der Kraak. 2011. Fathead minnow (*Pimephales promelas*) reproduction is impaired in aged oil sands process-affected waters. *Aquatic Toxicology* 101: 214-220.

Kelly, L. D., L. R. McGuiness, J. E. Hughes, S. C. Wainright. 1999. Effects of phenanthrene on primary production of phytoplankton in two New Jersey Estuaries. *Bulletin of Environmental Contaminant and Toxicology* 63: 646- 653.

Kelly, E. N., J. W. Short, D. W. Schindler, P. V. Hodson, M. Ma, A. K. Kwan. 2009. Oil sands development contributes polycyclic aromatic compounds to the Athabasca river and its tributaries. *Proceedings of the National Academy of Science*. 106: 22346-22351.

Kelly, E. N., D. W. Schindler, P. V. Hodson, J. W. Short, R. Radmanovich, C.C. Nielsen. 2010. Oil sands development contributes elements toxic at low concentrations to the Athabasca River and its tributaries. *Proceedings of the National Academy of Science*. 107: 16178-16183.

King, T. L., B. Robinson, M. Boufadel, K. Lee. 2014. Flume tank studies to elucidate the fate and behavior of diluted bitumen spilled at sea. *Marine Pollution Bulletin*. 83: 32-37.

Kinsman-Costello, L. E., J. M. O'Brien, S. K. Hamilton. 2015. Natural stressors in uncontaminated sediments of shallow freshwaters: the prevalence of sulfide, ammonia, and reduced iron. *Environmental Toxicology and Chemistry* 34: 467-479.

- Kovalenko, K. E., J.J. H. Ciborowski, C. Daly, D.G. Dixon, A. J. Farwell, A. L. Foote, K. R. Frederick, J.M. Gardner Costa, K. Kennedy, K. Liber, M.C. Roy, C.A. Slama, J. E. G. Smits. 2013. Food web structure in oil sands reclaimed wetlands. *Ecological Applications* 23: 1048-1060.
- Kouba, A., M. Buric, P. Kozak. 2010. Bioaccumulation and effects of heavy metals in crayfish: a review. *Water Air Soil Pollution* 211: 5-16.
- Kurek, J., J. L. Kirk, D. C. G. Muir, X. Wang, M. S. Evans, J. P. Smol. 2013. Legacy of a half century of Athabasca oil sands development recorded by lake ecosystems. *Proceedings of the National Academy of Science*. 110: 1761-1766.
- Langner, C.L. and P.F. Hendrix. 1982. Evaluation of a persulfate digestion method for particulate nitrogen and phosphorus. *Water Res.*16:1451-1454.
- Lari, E., S. Wiseman, E. Mohaddes, G. Morandi, H. Alharbi, G.G. Pyle. 2016. Determining the effect of oil sands process-affected water on grazing behavior of *Daphnia magna*, long term consequences and mechanisms. *Chemosphere* 146: 362-370.
- Lavilla, I., P. Vilas, J. Millos, C. Bendicho. 2006. Development of an ultrasound-assisted extraction method for biomonitoring vanadium and nickel in the coastal environment under the influence of the Prestige fuel spill (North east Atlantic Ocean). *Analytica Chimica Acta* 577: 119-125.
- Lavoie, I., C. Gueguen, C. Fortin. 2011. Benthic diatom communities from rivers in close proximity to the Athabasca tar sands (Alberta, Canada). *Water Quality Research Journal of Canada* 46:355-365.
- Lawrence, S. J. 1992. Trace-element enrichment in streambed sediment and crayfish, Carson and Truckee Rivers, Nevada and California. *Water-Resources Investigations Report* 9-4258.
- Leung, S. S., M. D. MacKinnon, R. E. H. Smith. 2001. Aquatic reclamation in the Athabasca, Canada, oil sands: naphthenate and salt effects on phytoplankton communities. *Environmental Toxicology and Chemistry* 20: 1532-1543.
- Leung, S. S., M. D. MacKinnon, R. E. H. Smith. 2003. The ecological effects of naphthenic acids and salts on phytoplankton from the Athabasca oil sands region. *Aquatic Toxicology* 62: 11-26.
- Lock, M. A., R. R. Wallace, D. R. Barton, S. Charlton. 1981a. The effects of synthetic crude oil on microbial and macroinvertebrate benthic river communities: Part I-Colonization of synthetic crude oil contaminated substrata. *Environmental Pollution (Series A)* 24: 207-217.

- Lock, M. A., R. R. Wallace, D. R. Barton, S. Charlton. 1981b. The effects of synthetic crude oil on microbial and macroinvertebrate benthic river communities: Part II-The response of an established community to contamination by synthetic crude oil. *Environmental Pollution (Series A)* 24: 263-275.
- Lopez-Duarte, P., F. J. Fodrie, O.P. Jensen, A. Whitehead, F. Galvez, B. Dubansky, K. W. Able. Is exposure to Macondo Oil reflected in the otolith chemistry of marsh resident fish? *PLOS-One* 11:1-24.
- Ludin, J. I., J. A. Riffell, S. K. Wasser. 2015. Polycyclic aromatic hydrocarbons in caribou, moose, and wolf scat samples from three areas of the Alberta oil sands. *Environmental Pollution* 206: 527-534.
- Lytle, D. A. and B. L. Peckarsky. 2001. Spatial and temporal impacts of a diesel fuel spill on stream invertebrates. *Freshwater Biology* 46: 693-704.
- MacDonald, G. Z., N. S. Hogan, B. Kollner, K. L. Thorpe, L. J. Phalen, B. D. Wagner, M. R. van den Heuvel. 2013. Immunotoxic effects of oil sands-derived naphthenic acids to rainbow trout. *Aquatic Toxicology* 126: 95-103.
- Mackay, R. J. 1986. Life cycles of *Hydropsyche roila* and *Cheumatopsyche pettiti* (Trichoptera: Hydropsychidae) in a spring fed stream in Minnesota. *American Midland Naturalist* 115: 19-24.
- Mackay, R. J., T. F. Waters. 1986. Effects of small impoundments on hydropsychid caddisfly production in Valley Creek, Minnesota. *Ecology* 67: 1680-1686.
- Madison, B. N., P. V. Hodson, V. S. Langlois. 2015. Diluted bitumen causes deformities and molecular responses indicative of oxidative stress in Japanese medaka embryos. *Aquatic Toxicology* 165: 222-230.
- Martin-Diaz, M.L., S. R. Tuberty, C.L. McKenney, Jr., J. Blasco, C. Sarasquete, T.A. Delvalls. 2006. The use of bioaccumulation, biomarkers and histopathology diseases in *Procambarus clarkii* to establish bioavailability of Cd and Zn after a mining spill. *Environmental Monitoring and Assessment* 116: 169-184.
- Masliyah, J., Z. Zhou, Z. Xu, J. Czarnecki, H. Hamza. 2004. Understanding water-based bitumen extraction from Athabaskan oil sands. *Canadian Journal of Chemical Engineering* 82: 628-654.
- Matousek, J., M. Walterhouse. 2013. A biological survey of sites on the Kalamazoo River and Talmadge Creek near the Enbridge oil spill in Marshall Calhoun County, Michigan. Michigan, September 2012. Department of Environmental Quality. MI/DEQ/WRD-13/011.
- McCauley, R. N. 1966. The biological effects of oil pollution in a river. *Limnology and Oceanography* 11: 475-486.

- McCoy, D. L., J. M. Jones, J. W. Anderson, M. Harmon, I. Hartwell, J. Hameedi. 2002. Distribution of cytochrome P4501A1-inducing chemicals in sediments of the Delaware River-Bay system USA. *Environmental Toxicology and Chemistry* 21: 1618-1627.
- McKnight, D. and others. National Academies of Sciences, Engineering, and Medicine 2016. Spills of diluted bitumen from pipelines: a comparative study of environmental fate, effects, and response. Washington, DC: The National Academies Press.
- McNeill, S. A., C. J. Arens, N. S. Hogan, B. Kollner, M. R. van den Heuvel. 2012. Immunological impacts of oil sands-affected waters on rainbow trout evaluated using an in-situ exposure. *Ecotoxicology and Environmental Safety* 84: 254-261.
- Mendelssohn, I.A., G.L. Anderson, D. M. Baltz, R. H. Caffey, K. R. Carmen, J. W. Fleeger, S. B. Joye, Q. Lin, E. Maltby, E. B. Overton, L. P Rozas. 2012. Oil impacts on coastal wetlands: implications for the Mississippi River Delta ecosystems after the Deepwater Horizon oil spill. *Bioscience* 62: 562-574.
- Michel, J., Z. Nixon, J. Dahlin, D. Betenbaugh, M. White, D. Burton, S. Turley. 2009. Recovery of interior brackish marshes seven years after the chalk point oil spill. *Marine Pollution Bulletin*. 58: 995-1006.
- Miller, M. C., V. Alexander, R. J. Barsdate. 1978. The effects of oil spills on phytoplankton in an arctic lake and ponds. *Arctic*. 31: 192-218.
- Millson, M.F., D.S. Montgomery, S.R. Brown. 1966. An investigation of the vanadyl porphyrin complexes of the Athabasca oil sands. *Geochimica et Cosmochimica Acta*. 30: 207-221.
- Morales-Caselles, C., M. L. Martin-Diaz, I. Riba, C. Saraquete, T. A. DelValls. 2008. Sublethal responses in caged organisms exposed to sediments affected by oil spills. *Chemosphere* 72: 819-825.
- Morandi, G.D., S. B. Wiseman, A. Pereira, R. Mankidy, I. G. M. Gault, J. W. Martin, J. P. Giesy. 2015. Effects-directed analysis of dissolved organic compounds in oil sands process water. *Environmental Science and Technology* 49: 12395-12404.
- Mozley, S. C. and M. G. Butler. 1978. Effects of crude oil on aquatic insects of tundra ponds. *Arctic* 31:229-241.
- Müller, Helga. 1987. Hydrocarbons in the freshwater environment: a literature review. *Advances in Limnology* 24: 1-69.
- Murphy, M. L., R. A. Heintz, J. W. Short, M. L. Larsen, S. D. Rice. 1999. Recovery of pink salmon spawning areas after the Exxon Valdez oil spill. *Transactions of the American Fisheries Society* 128: 909-918.

- Nero, V., A. Farwell, L. E. J. Lee, T. Van Meer, M.D. MacKinnon, D.G. Dixon. 2006. The effects of salinity on naphthenic acid toxicology to yellow perch: gill and liver histopathology. *Ecotoxicology and Environmental Safety* 65: 252-264.
- Newfields, G. D. 2013. Technical Review of Enbridge's Quantification of Submerged Oil Report.
- Nixon, Z., J. Michel, M. O. Hayes, G. V. Irvine, J. Short. 2013. Geomorphic factors related to the persistence of subsurface oil from the Exxon Valdez Oil spill. *Journal of Coastal Research* 69: 115-127.
- Omoigberale, M. O. and A. E. Ogbeibu. 2010. Environmental impacts of oil exploration and production on the macrobenthic invertebrate fauna of Osse River, Southern Nigeria. *Research Journal of Environmental Science* 4: 101-114.
- Ort, M. P., S. E. Finger, J. R. Jones. 1995. Toxicity of crude oil to mayfly, *Hexagenia bilineata* (Ephemeroptera: Ephemerida) *Environmental Pollution* 90: 105-110.
- Ozhan, K., M. L. Parsons, S. Bargu. 2014. How were phytoplankton affected by the Deepwater Horizon Oil Spill. *Bioscience*. 64: 829-836.
- Paine, R. T., J. L. Ruesink, A. Sun, E. L. Soulanille, M. J. Wonham, C.D.G. Harley, D. R. Brumbaugh, D. L. Secord. 1996. Trouble on oiled waters: lessons from the Exxon Valdez oil spill. *Annual review of ecology and systematics* 27: 197-235.
- Papoulias, D. M., V. Velez, D. K. Nicks, D. E. Tillitt. 2014. Health assessment and histopathologic analyses of fish collected from the Kalamazoo River, Michigan, following discharges of diluted bitumen crude oil from Enbridge Line 6B.
- Parsons, T. R., W. K. W. Li, R. Waters. 1976. Some preliminary observations on the enhancement of phytoplankton growth by low levels of mineral hydrocarbons. *Hydrobiologia* 51: 85-89.
- Parsons, B. G., S. A. Watmough, P.J. Dillon, K. M. Somers. 2010. A bioassessment of lakes in the Athabasca Oil Sands Region Alberta, using benthic macroinvertebrates. *Journal of Limnology* 69: 105-117.
- Payne, J. R., W. B. Driskell, J. W. Short, M. L. Larsen. 2008. Long term monitoring for oil in the Exxon Valdez spill region. *Marine Pollution Bulletin*. 56: 2067-2081.
- Pena, E. A., L. M. Ridley, W. R. Murphy, J. R. Sowa, C. S. Bentivegna. 2015. Detection of polycyclic aromatic hydrocarbons (PAHs) in raw menhaden fish oil using fluorescence spectroscopy: method development. *Environmental Toxicology and Chemistry* 34: 1946-1958.

- Perez, S., P. Furlan, S. Ellenberger, P. Banker, 2016. Estimating diluted bitumen entrained by suspended sediments in river rapids using O₂ absorption rate. *International Journal of Environmental Science and Technology* 13: 403-412.
- Peters, L. E., M. MacKinnon, T. Van Meer, M. R. van den Heuvel, D. G. Dixon. 2007. Effects of oil sands process-affected waters and naphthenic acids on yellow perch (*Perca flavescens*) and Japanese medaka (*Oryzias latipes*) embryonic development. *Chemosphere* 67: 2177-2183.
- Peterson, C. H., S. D. Rice, J. W. Short, D. Esler, J. L. Bodkin, B. E. Ballachey, D. B. Irons. 2003. Long-term ecosystem response to the Exxon Valdez oil spill. *Science* 302: 2082-2086.
- Pettigrove, V. and A. Hoffman. 2005. Effects of long-chain hydrocarbon-pollution on freshwater Macroinvertebrates. *Environmental Toxicology and Chemistry* 24: 2500-2508.
- Philibert, D. A., C. P. Philibert, C. Lewis, K. B. Tierney. 2016. Comparison of diluted bitumen (dilbit) and conventional crude oil toxicity to developing zebrafish. *Environmental Science and Technology*. 50: 6091-6098.
- Pinkney, A. E., J.C. Harshbarger. 2006. Tumor prevalence in Mummichogs from the Delaware Estuary Watershed. *Journal of Aquatic Animal Health* 18: 244-451.
- Poulton, B.C., E. V. Callahan, R. D. Hurtubise, B.G. Mueller. 1998. Effects of an oil spill on leaf pack inhabiting macroinvertebrates in the Chariton River, Missouri. *Environmental Pollution* 99: 115-122.
- Poulton, B. C., S. E. Finger, S. A. Humphrey. 1997. Effects of a crude oil spill on the benthic invertebrate community in the Gasconade River, Missouri. *Archive Environmental Contamination and Toxicology* 33: 268-276.
- Pulich, W. M., K. Winters, C. Van Baalen. 1974. The effects of a No.2 fuel oil and two crude oils on the growth and photosynthesis of microalgae. *Marine Biology* 28: 87-94.
- Puttaswamy, N., K. Liber. 2012. Influence of inorganic anions on metals release from oil sands coke and on toxicity of nickel and vanadium to *Ceriodaphnia dubia*. *Chemosphere* 86: 521-529.
- Puttaswamy, N., and K. Liber. 2011. Identifying the causes of oil sands coke leachate toxicity to aquatic insects. *Environmental Toxicology and Chemistry* 30: 2576-585.
- Puttaswamy, N., D. Turcotte, K. Liber 2010. Variation in toxicity response of *Ceriodaphnia dubia* to Athabasca oil sands coke leachates. *Chemosphere* 80: 489-497.
- Reid, N. J. and S. K. Hamilton. 2007. Controls on algal abundance in a eutrophic river with varying degrees of impoundment (Kalamazoo River, Michigan, USA). *Lake and Reservoir Management* 23: 219-230.

- Ridoux, V., L. Lafontaine, P. Bustamante, F. Caurant, W. Dabin. And others 2004. The impact of the “Erika” oil spill on pelagic and coastal marine mammals: Combining demographic, ecological, trace metals and biomarker evidences. *Aquatic Living Resources* 17: 379-387.
- Richardson, J. S. and Mackay, R. J. Mackay. 1991. Lake outlets and the distribution of filter feeders: an assessment of hypotheses. *Oikos* 62: 370-380.
- Richardson, 1984. Effects of seston quality on the growth of a lake-outlet filter feeder. *Oikos* 43: 386-390.
- Rosi-Marshall, E. J., J. B. Wallace. 2002. Invertebrate food webs along a stream resource gradient. *Freshwater Biology* 47: 129-141.
- Ross, M. S., A. Pereira, J. Fennell, M. Davis, J. Johnson, L. Sliva, J. W. Martin. 2012. Quantitative and qualitative analysis of naphthenic acids in natural waters surrounding the Canadian oil sands industry. *Environmental Science and Technology*. 46: 12796-12805.
- Rutherford, J. E., R. J. Mackay. 1986. Variability in the life-history patterns of four species of Hydropsyche (Trichoptera: Hydropsychidae) in Southern Ontario streams. *Holarctic Ecology* 9: 149-163.
- Sadiq, M., H. Al-Mohanna. 1993. Effect of the 1991 Gulf War on metal bioaccumulation by the clam (*Meretrix meretrix*) *Marine Pollution Bulletin* 27: 163-170.
- Salazar-Coria, L., M.A. Amezcua-Allieri, M. Tenorio-Torres, C. Gonzalez-Macias. 2007. Polyaromatic hydrocarbons (PAHs) and metal evaluation after a diesel spill in Oaxaca, Mexico. *Bulletin Environmental Contamination and Toxicology* 79: 462-467.
- Salazar M. H., S. M. Salazar, D. T. Burton, L. W. Hall, Jr. 2005. An integrated case study for evaluating the impacts of an oil refinery effluent on aquatic biota in the Delaware River: bivalve bioavailability studies. *Human and Ecological Risk Assessment* 11: 837-859.
- Schindler, D.W. 2010. Tar sands need solid science. *Nature* 468: 499-501.
- Schlosser, I. J. 1992. Effects of life-history attributes and stream discharge on filter-feeder colonization. *Journal of North American Benthological Society* 11: 366-376.
- Schwalb, A. N., A. C. Alexander, A. J. Paul, K. Cottenie, J. B. Rasmussen. 2015. Changes in migratory fish communities and their health, hydrology, and water chemistry in rivers of the Athabasca oil sands region: a review of historical and current data. *Environmental Reviews* 23: 133-150.
- Scott, A. C., M D. Mackinnon, P. M. Fedorak. 2005. Naphthenic acids in Athabasca oil sands tailing waters are less biodegradable than commercial naphthenic acids. *Environmental Science and Technology* 39: 8388-8394.

- Seiser, P.A., L. K. Duffy, A. D. McGuires, D.D. Roby, G. H. Golet, M.A. Litzow. 2000. Comparison of pigeon guillemot, *Cepphus Columba*, blood parameters from oiled and unoled areas of Alaska eight years after the Exxon Valdez oil spill 40: 152-164.
- Shotyk, W., R. Belland, J. Duke, H Kempter, M. Krachler, T. Noemberg, R. Pelletier, M. A. Vile, K. Wieder, C. Zaccane, S. Zhang. Sphagnum mosses from 21 ombrotrophic bogs in the Athabasca bituminous sands region show no significant atmospheric contamination of “Heavy metals”. Environmental Science and Technology 48: 12603-12611.
- Simmons, D. B.D., J. P. Sherry. 2016. Plasma proteome profiles of White Sucker (*Catostomus commersoni*) from the Athabasca River within the oil sands deposits. Comparative Biochemistry and Physiology 19: 181-189.
- Stanford, J. A., J. V. Ward, W. J. Liss, C. A. Frissell, R. N. Williams, J. A. Lichatowich and C. C. Coutant. 1996. A general protocol for restoration of regulated rivers. Regulated River Research and Management 12: 391-413.
- Stegeman, J.J., J. J. Lech.1991. Cytochrome P-450 Monooxygenase systems in aquatic species: carcinogen metabolism and biomarkers for carcinogen and pollutant exposure. Environmental Health Perspectives 90:101-109.
- Tao, J., C.G. Ingersoll, N.E. Kemble, J. R. Dias, J.B. Murowchick, G. Welker, D. Huggins. 2010. Sediment contamination of residential streams in the metropolitan Kansas City area, USA: Part II. whole-sediment toxicity to the amphipod *Hyalella azteca*. Archives for Environmental Contamination and Toxicology 59: 370-381.
- Tetreault, G. R., M. E. McMaster, D. G. Dixon, J. L. Parrott. 2003. Using reproductive endpoints in small forage fish species to evaluate the effects of Athabasca oil sands activities. Environmental Toxicology and Chemistry 22: 2775-2775.
- Timoney, K. P., P. Lee. 2011. Polycyclic aromatic hydrocarbons increase in Athabasca River Delta Sediment: temporal trends and environmental correlates. Environmental Science and Technology 45: 4278-4284.
- Tucker, K. A., G. A. Burton. 1999. Assessment of nonpoint-source runoff in a stream using in-situ and laboratory approaches. Environmental Toxicology and Chemistry 18: 2797-2803.
- Uhler, A. D., S. Emsbo-Mattingly, B. Liu, L. W. Hall, Jr., D. T. Burton. 2005. An integrated case study for evaluating the impacts of an oil refinery effluent on aquatic biota in the Delaware River: Advances Chemical Fingerprinting of PAHs. Human and Ecological Risk Assessment 11: 771-836.
- U.S. Department of State. 2013. Draft Supplemental Environmental Impact Statement for the Keystone XL project. Washington DC.

- Valderrama, J.C. 1981. The simultaneous analysis of total nitrogen and total phosphorus in natural waters. *Mar. Chem.* 10:109-122.
- Van den Heuvel, M. Power, J. Richards, M. MacKinnon, D. G. Dixon. 2000. Disease and gill lesions in yellow perch (*Perca flavescens*) exposed to oils sands mining-associated waters. *Ecotoxicology and Environmental Safety* 46: 334-341.
- Van der Oost, R., J. Beyer, N.P.E. Vermeulen. 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. *Environmental Toxicology and Pharmacology* 13: 57-149.
- Van Meter, R. J., J. R. Spotila, H. W. Avery. 2006. Polycyclic aromatic hydrocarbon affect survival and development of common snapping turtle (*Chelydra serpentina*) embryos and hatchlings. *Environmental Pollution* 142: 466-475.
- Vannote, R. L., G. W. Minshall, G., K. W. Cummins, J. R. Sedell, and C. E. Cushing. 1980. The River Continuum Concept. *Canadian Journal of Fisheries and Aquatic Science* 37: 130-137.
- Walterhouse, M. 2011. A Biological Survey of Sites on the Kalamazoo River and Talmadge Creek Near the Enbridge Oil Spill in Marshall, Calhoun County, Michigan. MDEQ Staff Report #MI/DEQ/WRD-11/010.
- Walterhouse, M. 2012. A Biological Survey of Sites on the Kalamazoo River and Talmadge Creek Near the Enbridge Oil Spill in Marshall, Calhoun County, Michigan. August 2011. MDEQ Staff Report #MI/DEQ/WRD-12/012.
- Wang, Z., M. Fingas, S. Blenkinsopp, G. Sergy, M. Landriault, L. Sigouin, P. Lambert. 1998. Study of the 25-year-old Nipisi oil spill: persistence of oil residues and comparisons between surface and subsurface sediments. *Environmental Science and Technology* 32: 2222-2232.
- Ward, J. V., J. A. Stanford. 1983. The serial discontinuity concept of lotic ecosystems. in Fontaine, T. D. and Bartell, S. M. (Eds) *Dynamics of Lotic Ecosystems*. Ann Arbor Science, Ann Arbor pp. 29-42.
- Wayland, M., Trudeau, S., Marchant, T., Parker, D., and Hobson, K. A. 1998. The effect of pulp and paper mill effluent on an insectivorous bird, the tree swallow. *Ecotoxicology* 7:237-251.
- Wayland, M., J. V. Headley. 2008. Levels of polycyclic aromatic hydrocarbons and dibenzothiophenes in wetland sediments and aquatic insects in the oil sands area of Northeastern Alberta, Canada. *Environmental Monitoring Assessment* 136: 167-182.
- Welschmeyer, N.A. 1994. Fluorometric analysis of chlorophyll *a* in the presence of chlorophyll *b* and pheopigments. *Limnology Oceanography* 39:1985-1992.

Whiles, M. R. and W. K. Dodds. 2002. Relationships between streams size, suspended particles, and filter feeding macroinvertebrates in a Great Plains drainage network. *Journal of Environmental Quality* 31: 1589-1600.

Willis, L. D., A. C. Hendricks. 1992. Life history, growth, survivorship and production of *Hydropsyche slossonae* in Mill Creek, Virginia. *Journal of North American Benthological Society* 11: 290-303.

Wiseman, S. B., J.C. Anderson, K. Liber, J.P. Giesy. 2013. Endocrine disruption and oxidative stress in larvae of *Chironomus dilutes* following short-term exposure to fresh or aged oil sands process-affected water. *Aquatic Toxicology* 142-143: 414-421.

Wiseman, S. B., Y. He, M. G.-E. Din, J. W. Martin, P. D. Jones, M. Hecker, J. P. Giesy. 2013. B Transcriptional responses of male fathead minnows exposed to oil sands process-affected water. *Comparative Biochemistry and Physiology, Part C* 157: 227-235.

Woodward, D. F. and R. G. Riley. 1983. Petroleum hydrocarbon concentrations in a salmonid stream contaminated by oil field discharge water and effects of macrobenthos. *Archiv fur Environmental Chemistry* 12: 327-334.

Yergeau, E., J. R. Lawrence, S. Sanschagrin, J. L. Roy, G. D. W. Swerhone, D. R. Korber, C. W. Greer. 2013. Aerobic biofilms grown from Athabasca Watershed sediments are inhibited by increasing concentration of bituminous compounds. *Appl. Environ. Microbiol.* 79: 7398-7412.

Young, R. F., L. M. Michel, P. M. Fedorak. 2011. Distribution of naphthenic acids in tissues of laboratory-exposed fish and in wild fish from near the Athabasca oil sands in Alberta, Canada. *Ecotoxicology and Environmental Safety* 74: 889-896.

Yunker, M. B., R. W. Macdonald, R. Vingarzan, R. H. Mitchell, D. Goyette, R. Sylvestre. 2002. PAHs in the Fraser River basin: a critical appraisal of PAH ratios as indicators of PAH source and composition. *Organic Geochemistry* 33; 489-515.

Zuliani, J. E., T. Miyata, T. Mizoguchi, J. Feng, D. W. Kirk, C. Q. Jia. 2016. Characterization of vanadium oil sands fluid petroleum coke using electron microscopy. *Fuel*. 178: 124-128.