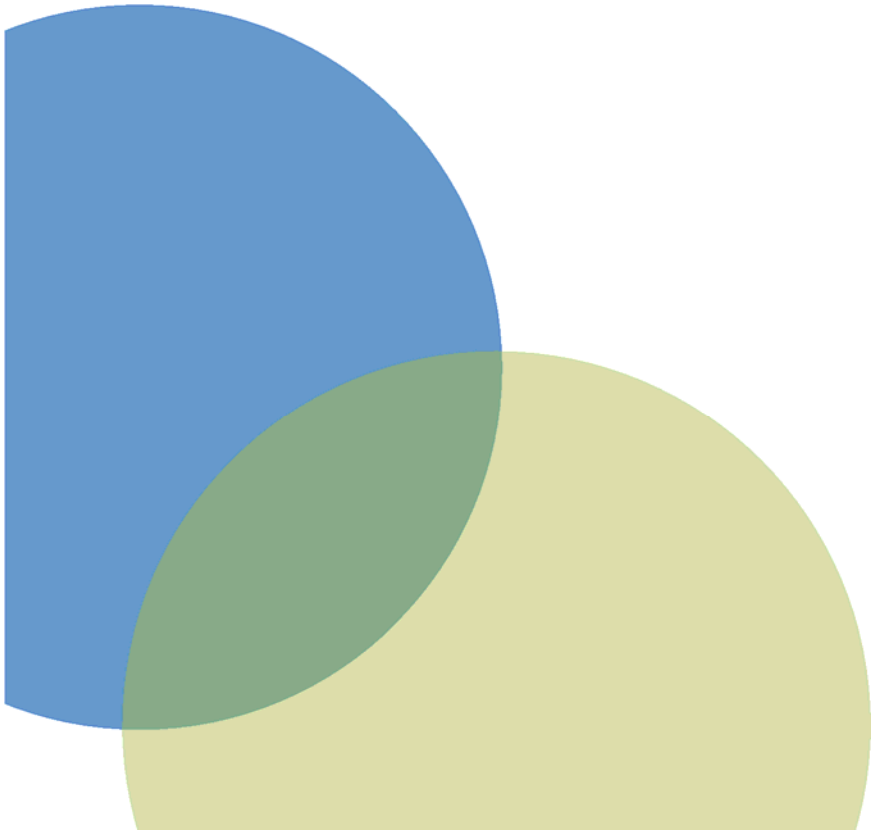
THE DEVELOPMENT OF A STRESSOR-RESPONSE MODEL FOR THE RED RIVER OF THE NORTH

Topical Report RSI-2611

prepared for

International Red River Board
US Section
2000 L Street, NW
Suite #615
Washington, DC 20440

June 2016



RESPEC

THE DEVELOPMENT OF A STRESSOR-RESPONSE MODEL FOR THE RED RIVER OF THE NORTH

Topical Report RSI-2611

by

Tony Miller
Bruce Wilson
Julie Blackburn
Erich Weber

RESPEC
1935 County Road B2, Suite 230
Roseville, Minnesota 55113

prepared for

International Red River Board
US Section
2000 L Street, NW
Suite #615
Washington, DC 20440

June 2016

CREDITS AND ACKNOWLEDGMENTS

GOVERNMENTAL AGENCIES

Environment and Climate Change Canada
International Joint Commission
International Red River Board
International Red River Board Water Quality Committee
Minnesota Pollution Control Agency
North Dakota Department of Health
Province of Manitoba, Conservation and Water Stewardship

INDIVIDUAL CONTRIBUTORS

Pam Anderson, Minnesota Pollution Control Agency
Nicole Armstrong, Manitoba Sustainable Development
Glenn Benoy, International Joint Commission
Stephen Brown, North Dakota Department of Health
Joel Chirhart, Minnesota Pollution Control Agency
Mike Ell, North Dakota Department of Health
Kristina Farmer, Environment and Climate Change Canada
Diana Fred, Environment and Climate Change Canada
Mark Gabriel, International Joint Commission
Iris Griffin, Environment and Climate Change Canada
David Hay, Manitoba Conservation and Water Stewardship
Steve Heiskary, Minnesota Pollution Control Agency
Daniel Helwig, Minnesota Pollution Control Agency
Joy Kennedy, Manitoba Conservation and Water Stewardship
Aaron Larsen, North Dakota Department of Health
Jeff Lewis, Red River Basin Commission
Ben Lundeen, Minnesota Pollution Control Agency
Joe Nett, North Dakota Department of Health
Scott Niemela, Minnesota Pollution Control Agency
James Noren, United States Army Corps of Engineers
Rochelle Nustad, United States Geologic Survey
Paul Olson, North Dakota Department of Health
Elaine Page, Manitoba Conservation and Water Stewardship
Sharon Reedyk, Agriculture and Agri-Food Canada
Victor Serveiss, International Joint Commission
Rob Sip, Minnesota Department of Agriculture
Eric Steinhaus, United States Environmental Protection Agency
Jason Vanrobaeys, Agriculture and Agri-Food Canada
Mike Vavricka, Minnesota Pollution Control Agency
Keith Weston, Red River Retention Authority
Jim Ziegler, Minnesota Pollution Control Agency

EXECUTIVE SUMMARY

The International Red River Board (IRRB), a board of the International Joint Commission (IJC), identified excess nutrients as one of the greatest issues in the Red River of the North because of its impact on the ecological conditions in the Red River itself and its significant contribution to hyper-eutrophic conditions in downstream Lake Winnipeg. Excessive nutrient contributions to the lake have led to excessive algal blooms that impact drinking water, recreation, and commercial fisheries through significant accumulations of algal biomass—the Red River has been identified as the most significant contribution of the nutrients. This excessive algal buildup disrupts recreation and fisheries through its physical presence but also by indirectly attributing to dissolved oxygen depletion at night when photosynthesis shuts down and bacterial decomposition of the dead algae utilize large amounts of oxygen. To initiate determining solutions to these issues, the IRRB developed a proposed approach for a basin-wide, nutrient-management strategy for the international Red River Watershed. One component of this approach involves developing nitrogen and phosphorus concentration targets within the mainstem Red River as a first step in restoring the ecology of the Red River and reducing nutrient contributions to Lake Winnipeg. To determine an approach for establishing nutrient targets, the IRRB contracted with RESPEC to conduct a literature review and provide recommendations on the most appropriate method for developing nitrogen and phosphorus targets in the Red River [Plevan and Blackburn, 2013][1]. Based on these findings, the IRRB determined that a collaborative project to determine a biological stressor response in the Red River to nutrients was necessary. The subsequent project was developed by a team that consisted of agency professionals from Manitoba, Minnesota, North Dakota, and RESPEC. The project results are described in this report and demonstrates the development of a stressor-response model that includes the identification of biologically based nutrient targets in the Red River.

Initially, to consider all possible interactions of environmental conditions, a conceptual stressor-response model that characterized potential pathways for nutrient effects was developed with input and recommendations from experts. Because of their direct and documented responses to nutrient increases, algae were determined to be the ideal biological group to measure a stressor response in the Red River. However, data gaps that prevented developing a stressor-response model were identified that led to supplemental periphyton (attached algae), phytoplankton (sestonic algae), and water quality data-collection effort during the summer of 2015. This data resulted in developing an effective nutrient-stressor-response model for the Red River by using a combination of algae, water quality, and land-use information. Because of consistently elevated suspended sediment in the river, the initial goal of this project was to determine if a stressor response to elevated nutrients was discernable on the chosen biological community (because of the light limiting control on algal growth). This project was not concerned with seasonal variation of algal growth/nutrient loading or specific aspects of the downstream Lake Winnipeg. The function of the study was to determine a measurable response of the algal community to elevated nutrients and subsequently whether or not a discernable nutrient gradient could be aligned with either algal community quality or quantity. If successful, this project would therefore allow nutrient targets to be established for the Red River based on growing-season conditions and initiate local interest

[1] **Plevan, A. B. and J. A. Blackburn, 2013.** *Approaches to Setting Nutrient Targets in the Red River of the North*, RSI-2328, prepared by RESPEC, Roseville, MN, for the International Joint Commission, US Section, Washington, DC.

in reducing nutrient inputs. This approach is based on the Minnesota Pollution Control Agency's (MPCA's) methods for using biological criteria for establishing nutrient limits.

To accomplish the goals outlined above, the project team focused on ideal growth conditions for the algae (i.e., summer growing season during normal flow) and collected periphyton (using floating colonization samplers) and phytoplankton (collected from filtered surface water). Using this biological data combined with water chemistry, several analytical steps were performed to establish a statistically and ecologically valid relationship between the algae community variance between sites and the measured environmental variables, which in part included total and constituents of nitrogen and phosphorus, water temperature, total suspended solids (TSS), conductivity, pH, and various land-use measures. Overall, taxonomic response (i.e., community diversity) to nutrient stressors was not suppressed by TSS concentrations (i.e., available light); however, the effect of light limitation was seen through reduced algal biomass.

Varying concentrations of nutrients was pivotal to observing a stressor effect. The first goal of this project was to determine a nutrient gradient between the river sampling sites. Using water quality data provided by the MPCA and Manitoba Conservation and Water Stewardship, a nutrient gradient was observed that appeared to be associated with both municipal and agricultural inputs. This gradient analysis, in conjunction with the algal quantity and quality results, was subsequently used to establish the nutrient target recommendations.

Determining a response in the algal community to the observed nutrient gradient was documented in the measured quantity and the quality of the community. A response in *quantity* was apparent in both the phytoplankton and periphyton abundance, although the growth of the latter was significantly repressed by TSS concentrations. However, the response of periphyton *quality*, as determined by pertinent diatom metrics, was not suppressed by TSS concentrations because a significant negative quality response was seen with increasing nutrients starting at the first peak in nutrients adjacent to the Fargo/Moorhead urban area. Overall, the periphyton was found to reach nuisance levels toward the mouth of the river that coincided with the highest concentration of nutrients. Phytoplankton was found to reach nuisance concentrations in close proximity to highly developed urban areas with an occasional abundance of blue-green algae.

Multivariate analyses were used to determine that both periphyton and phytoplankton responded significantly to varying nutrient concentrations. Using the information from these analyses, nutrient targets were determined by using the results from sites least influenced by high total phosphorus (TP) and total nitrogen (TN) effects. Substantial consideration was also given to results from sites meeting regional regulatory limits on primary productivity measures and higher quality diatom-based metric results. This process resulted in delineating nutrient targets of 0.15 milligrams per liter (mg/L) for TP and 1.15 mg/L for TN.

The final step in the stressor-response determination sought to define other stressor effects in the Red River Watershed. Algal-stressor influence related to low dissolved oxygen (DO) and elevated biochemical oxygen demand (BOD) had a high probability of influence because of significant land-use associations. Overall, land use related to anthropogenic disturbance was found to have higher explanatory power than in situ water chemistry parameters in determining the algae variance. These results implied a positive

relationship between adjacent wetlands and increases in BOD/decreases in DO possibly related to direct input of decaying algal biomass and low DO concentrations. Conversely, another land-use-dominated analysis suggested that the total abundance of lentic waters (lakes, ponds, and wetlands) in the upper watershed appeared to have a controlling effect on total periphytic primary production potentially through the retention of nutrient/sediment laden runoff and other nutrient dynamics.

This project provided ample evidence for a strong stressor response of the algal community to nutrients in the Red River and identifies the targeted nutrient levels to be achieved in the Red River to begin restoring the ecological health of the river and reducing its downstream impacts. Additionally, the models provide insight into nutrient-related, landscape-feature influences on algal growth. From this collective information, additional studies can be developed to aid management decisions in facilitating reducing phosphorus and nitrogen concentrations related to excessive algal growth, which will subsequently reduce impacts to biological communities (i.e., fish, macroinvertebrates, and algae) and drinking water (i.e., toxins and taste/odor). These nutrient reductions will also facilitate the reduction of similar, yet exacerbated, issues in the downstream Lake Winnipeg.

TABLE OF CONTENTS

TABLE OF CONTENTS (continued)

LIST OF TABLES

LIST OF FIGURES

FIGURE PAGE

LIST OF FIGURES (continued)

FIGURE PAGE

1.0 INTRODUCTION

Clean water is a pivotal concern and necessary for the health, welfare, and economies of society as well as the natural environment. Degrading conditions in the Red River and Lake Winnipeg have mobilized local, regional, and international jurisdiction to direct their attention to addressing the condition of the river and its watershed. Of primary concern is the result of excessive nutrient runoff and its effects on recreation, fisheries, and drinking water.

The International Joint Commission (IJC) established the International Red River Board (IRRB) in 2001 "to assist the Commission in preventing and resolving transboundary disputes regarding the waters and aquatic ecosystem of the Red River and its tributaries and aquifers."[2] One of the IRRB's duties, as outlined in the directive, includes recommending to the IJC appropriate strategies to address water quality and aquatic system health.

The IRRB Water Quality Committee has been tasked by the IRRB with establishing a path forward for developing a nutrient-management strategy for the Red River [International Red River Board, 2011]. The mission statement established for this strategy is "To develop a collaborative, science and watershed-based approach to managing nutrients in the Red River and its watershed with the goal of restoring and protecting aquatic ecosystem health and water uses in the Red River Watershed and Lake Winnipeg" [International Red River Board, 2011].

One component of this approach involves developing nitrogen and phosphorus targets along the Red River. To determine the approach for establishing those targets, the IRRB contracted with RESPEC to conduct a literature review and provide recommendations on the most appropriate method for developing nitrogen and phosphorus targets in the Red River [Plevan and Blackburn, 2013]. Two integrated approaches to developing water quality targets were recommended to the IRRB Water Quality Committee to address the goals of restoring and protecting the Red River and Lake Winnipeg. The recommendation of the 2013 report was to (1) develop a stressor-response model that identified nutrient targets for the Red River and (2) that downstream water quality targets for Lake Winnipeg should be considered in parallel to the stressor-response-model-derived nutrient targets for setting overall water quality targets. The IJC, on behalf of the IRRB, contracted with RESPEC to develop the stressor-response model and recommend nutrient targets.

To develop an appropriate biological stressor-response model, the IRRB Water Quality Committee, RESPEC, and the larger project team quickly determined that supplemental data were needed; specifically, reach-wide algal community assessment and associated nutrient concentration data. The summer growing season was also determined the most pertinent period to discern an algal response to nutrients. Subsequently, an interagency collaborative sampling approach was quickly designed and implemented to provide the needed model inputs. This effort included agency personnel from the province of Manitoba, Minnesota, and North Dakota as well as water quality professionals from RESPEC.

[2] From directive assigned to the IRRB from the IJC on February 7, 2001.

This report describes the development of a conceptual stressor-response model, evaluation of available data, data acquisition and analysis methods, stressor-response modeling, and the resulting identification of recommended biological thresholds in the Red River. The results of this effort will be used to facilitate further study of the nutrient issue in the Red River, which will ultimately lead to management implementation goals for the greater Lake Winnipeg Basin.

This document describes the project efforts in detail within the following specific components:

- Overview of the Red River Basin
- Procedure used for determining available data for the Red River main stem
- Collective data gathering effort
- Stressor-response modeling exercise
- Conclusions that include gaps in understanding and future recommendations.

2.0 OVERVIEW OF THE RED RIVER OF THE NORTH MAINSTEM

The Red River of the North (Red River) flows a distance of approximately 547 miles (880 kilometers [km]) from its beginnings at the confluence of the Bois de Sioux and Otter Tail Rivers near Wahpeton (North Dakota)/Breckenridge (Minnesota) until it empties into Lake Winnipeg, Manitoba, Canada. Along its course, the Red River and its tributaries, including the Assiniboine River, drain a cumulative total of approximately 81,894 square miles (212,105 square km) of very flat terrain. The average slope is approximately ½ foot per mile with a total elevation drop of approximately 250 feet (ft) (76 meters [m]). The Red River's mainstem channel width varies from approximately 200 to 500 feet (ft) (61 m to 152 m) with average depth at bankfull stage ranging from 10 to 30 ft (approximately 3 m to 9 m) [Krenz and Leitch, 1993]. Renowned for its soils' fertility, the basin has been referred to as a major "Bread Basket," with crops that include sugar beets, wheat, barley, soybeans, dry edible beans, corn, potatoes, sunflowers, alfalfa, and other specialty crops [US Department of Agriculture, 2015].

2.1 WATERSHED

Over its entirety, the Red River mainstem has numerous corresponding monitoring sites operated by partnering agencies. This IJC-sponsored study's partnering agencies included the Manitoba Sustainable Development, North Dakota Department of Health (NDDH), and the Minnesota Pollution Control Agency (MPCA) with 30 monitoring locations established along the mainstem. Corresponding mainstem river-mile identifications going from the Headwaters to the Mouth along with latitude and longitude locations are defined in Table 2-1. For the purposes of this report, the Red River has been sectioned into three primary reaches or zones: Headwaters (from River Mile [RM] 547 to 441 – corresponding with the urban area of Fargo/Moorhead), Middle (RM 437 to 155), and Mouth (RM 101 to 0 – corresponding with the confluence of the Assiniboine River).

Table 2-1. Red River of the North Mainstem Study Sites[(a)] (Page 1 of 2)

Site Code	River Mile	Latitude	Longitude
84RD008	547	46.282182	–96.599521
84RD011	535	46.374685	–96.665914
15RD069	502	46.5705	–96.745089
15RD068	471	46.752375	–96.785755
15RD067	450	46.88312	–96.76716
15RD066	447	46.906605	–96.770698
84RD022	441	46.927044	–96.78137
15RD065	437	46.95403	–96.80033
05RD030	421	47.058675	–96.821581
84RD027	389	47.258584	–96.844802

Table 2-1. Red River of the North Mainstem River Study Sites by River Mile and Latitude/Longitude Location[a] (Page 2 of 2)

Site Code	River Mile	Latitude	Longitude
05RD047	375	47.355174	–96.837316
94RD018	357	47.466643	–96.858697
15RD059	308	47.6499	–96.88244
15RD058	297	47.926933	–97.028514
84RD037	294	47.961368	–97.057859
15RD057	290	48.019863	–97.072327
15RD056	274	48.162394	–97.144266
15RD055	245	48.340198	–97.125709
15RD054	236	48.41647	–97.13805
84RD042	226	48.46276	–97.140075
15RD052	189	48.702087	–97.120497
06RD008	158	48.964599	–97.233177
84RD047	157	48.978046	–97.23812
MB050CS007	155	49.0033	–97.221117
MB05OCS033	101	49.3536	–97.350833
MB05OCS004	61	49.7506	–97.133333
MB05OJS057	39	49.9156	–97.126117
MB05OJS004	34	49.9686	–97.065
MB05OJS074	16	50.1411	–96.868617
MB05OJS128	0	50.346117	–96.839717

(a) Green shading = Headwaters
Blue shading = Middle
Red shading = Mouth

Watershed characteristics and land cover as summarized for SPARROW modeling of the basin [Jenkinson and Benoy, 2015] have been incorporated into this study's database and briefly summarized in Table 2-2. The mainstem drainage areas include all contributing tributaries and rivers. Collectively, the Red River Watershed's Headwater, Middle, and Mouth zones comprise 7.9 percent, 38.4 percent, and 56.8 percent of the total watershed area, respectively. The Assiniboine River enters the Red River near RM 42 and nearly doubles the cumulative drainage area (Appendix A provides a complete tabulation of drainage areas by mainstem Red River location).

Table 2-2. Select Red River Watershed Characteristics and Land-Cover Summary

Basin Characteristic	Red River Zones			Entire Basin
	Headwaters	Middle	Mouth[(a)]	
Length (River Miles)	97	292	101	547
Length (River Kilometers)	156	470	163	880
Watershed Area (Square Miles)	6,453	28,902	46,539	81,894
Watershed Area (Square Kilometers)/ Percent of Total	16,714 7.9	74,855 38.4	120,536 56.8	212,105
Point Sources	31	90	223	344
Land-Cover Cumulative Averages by Reach				
Urban/Impervious (%)	5.2	4.5	3.4	3.4
Grassland (%)	4.6	5.2	11.0	11.0
Agricultural (%)	61.1	60.8	58.2	58.2
Pasture (%)	7.8	7.8	8.5	8.5
Wetlands (%)	5.7	10.5	7.0	7.0
Open Water (%)	6.7	4.1	2.9	2.9
Forest (%)	9.0	7.1	8.9	8.9

(a) Mouth Zone includes Assiniboine River.

While at first glance the basin's land covers listed in Table 2-2 look relatively similar, subtle differences are noted that can strongly influence water quality because of their cumulative effects from large geographic areas discharging to each of the mainstem zones. In general, agricultural land cover is homogenous within the three river zones and dominates the basin, varying from 58.2 percent to 61.1 percent, and similarly for pasture land cover with a narrow range of 7.8 percent to 8.5 percent. Wetlands, water, and forest covers comprise approximately 20 percent of the entire basin with notable category shifts occurring among the river zones. The percentages of open water in the watershed decline substantially, moving downstream from 6.7 percent (Headwaters), to 4.1 percent (Middle), to 2.9 percent (Mouth). Forests cover a range of 7.1 percent (Middle) to approximately 9 percent for the other two river zones. Wetland area increases going from the Headwaters (5.7 percent), to the Middle (10.5 percent), and then decline in the Mouth's drainage areas (7.0 percent). Urban/impervious land covers are highest in the Headwaters Zone (5.2 percent) and decline going downstream to values slightly above 3 percent by the time the Red River enters Lake Winnipeg. Larger cities located along the mainstem include Wahpeton (North Dakota), Fargo and West Fargo (North Dakota)/Moorhead (Minnesota), Grand Forks (North Dakota), Drayton (North Dakota), Pembina/St. Vincent (Manitoba, Canada), Emerson (Manitoba, Canada), Saint Jean Baptiste (Manitoba, Canada), Morris (Manitoba, Canada), Saint Adolphe (Manitoba, Canada), Winnipeg (Manitoba, Canada), Saint Andrews (Manitoba, Canada), and Selkirk (Manitoba, Canada). Point sources within the watershed tabulated by SPARROW were distributed by river zone as follows: Headwater (31), Middle (90), Mouth (223), for a total of 344 point sources. On the US side, no phosphorus effluent limits were identified for mainstem Red River community wastewater treatment plants. However, Manitoba effluent regulations for phosphorus came into effect on January 1, 2016. Dam locations noted along the

Red River as identified by the SPARROW dataset included Fargo (Dam 3 and 12th Avenue), Riverside, Drayton, Pembina, and St. Andrews.

2.2 PREVALENT SOILS

As illustrated in Figure 2-1, a prevalence of Hydrologic Soil Group (HSG) Type C (sandy clay loams) and Type D (clay-dominated) soils are found along the core of the river valley [Homer et al., 2015]. Approximately 63 percent of the lower Red River Basin is covered by HSG Types C, D, and mixed C and D soils. Soils in upland areas include a wider range of HSG soil types. Types A (sand-dominated) and B (silt loams) soils are characterized by having higher infiltration rates than Type C and D soils [Natural Resources Conservation Service, 1986]. As such, the heavier soils along the mainstem strongly influence runoff rates, volumes, and the amount of suspended soil particles in the runoff. Available Red River particle-size distribution (PSD) data were limited to one US Geological Survey (USGS) study that reported over 90 percent of particles assessed during the 2010 high flow sampling were less than 62 micrograms (µm) [Blanchard et al., 2011]. Even with wide river channels and low slope, fine-sized particle sedimentation requires extensive periods (e.g., days) of no flow to be removed from the water column. Hence, the Red River has elevated turbidities owing to these small particle-size distributions.

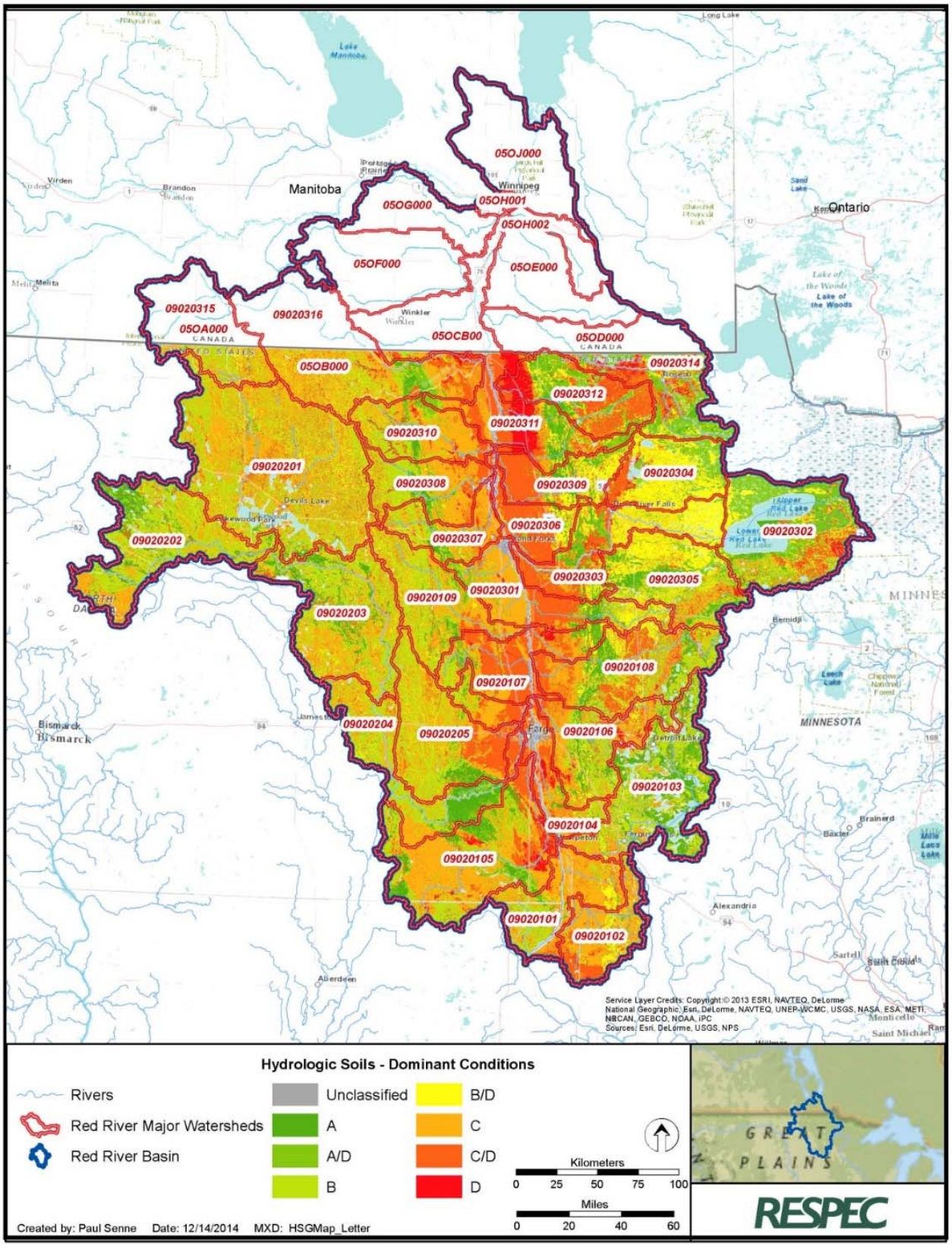


Figure 2-1. Hydrologic Soil Groups of the United States Portion of the Red River Valley.

2.3 CLIMATE

Climate for the Red River area is influenced by its location in the geologically flat Great Plains with distinct seasons and major variation in temperatures over short periods of time because of the collisions of weather systems from the Gulf of Mexico and the Canadian Plains. Winters are long with moderate snowfall and summers are warm and humid. Of particular note is the transition from winter to spring that begins in the Headwaters area with the melting winter snowpack and proceeds downstream (north) through yet-frozen areas often accompanied by ice dams and widespread flooding. Climate normals for Grand Forks (representative of the upper one-third watershed) and Winnipeg (representative of the lower one-third of the watershed) are plotted in Figures 2-2 and 2-3, respectively, and show remarkably similar temperature and precipitation patterns by month.

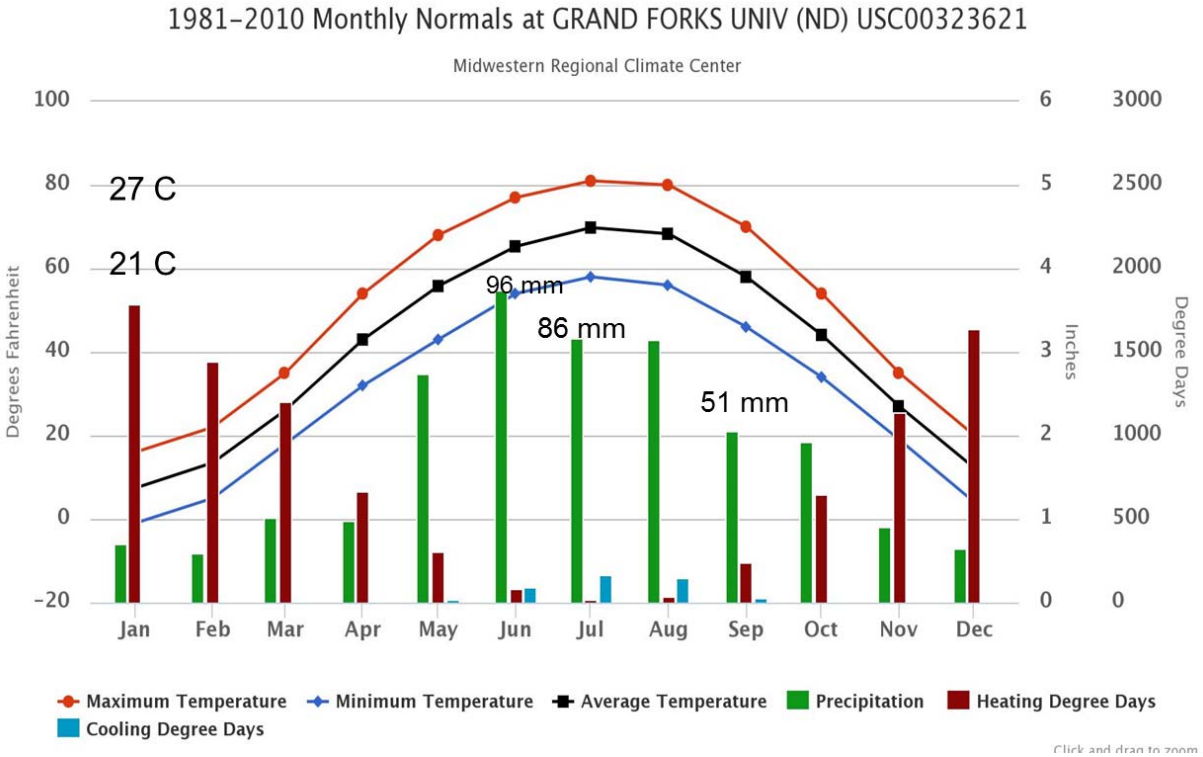


Figure 2-2. Climate Normals at Grand Forks, North Dakota [Midwestern Regional Climate Center, 2015].

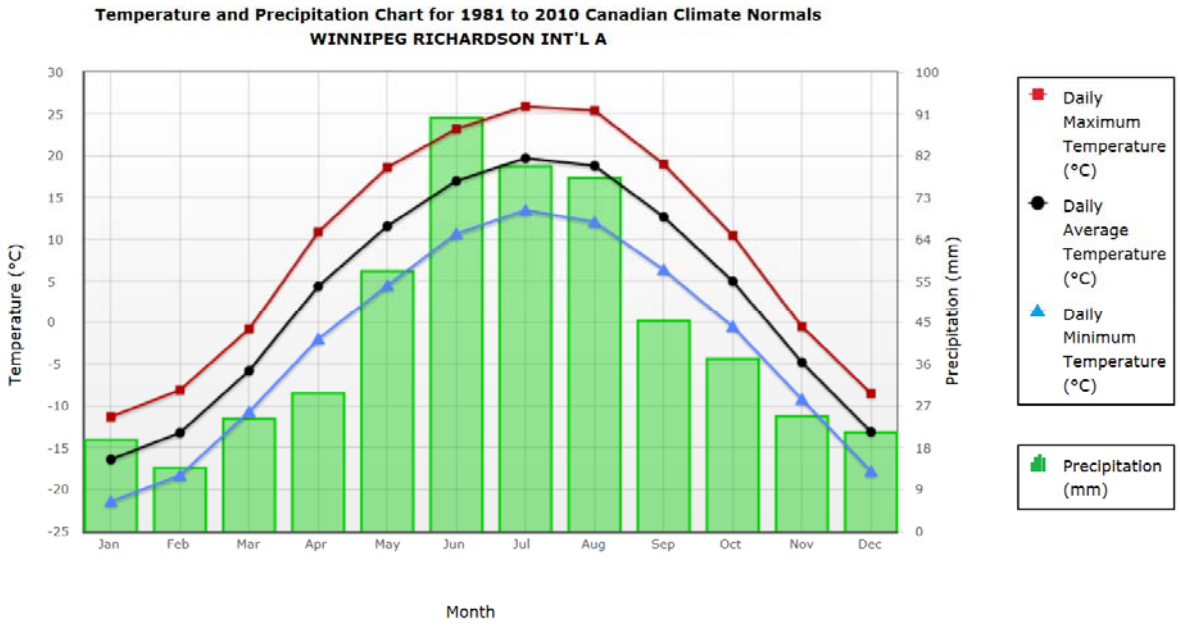


Figure 2-3. Climate Normals at Winnipeg, Manitoba, Canada [Midwestern Regional Climate Center, 2015].

Of concern, particularly to agricultural producers of the region, are climate aspects that define the length of the growing season, growing-season temperatures, and precipitation patterns. The maximum growing season is a portion of the frost-free period, or the number of days between the last freezing date in spring and the first freezing day of autumn. Frost-free data obtained from the National Oceanic and Atmospheric Administration's (NOAA's) Midwest Regional Climate Center were retrieved and plotted for Grand Forks, North Dakota, in Figure 2-4. As may be observed, Grand Fork's frost-free period has generally expanded in length and with increased peak (longer) periods in recent decades.

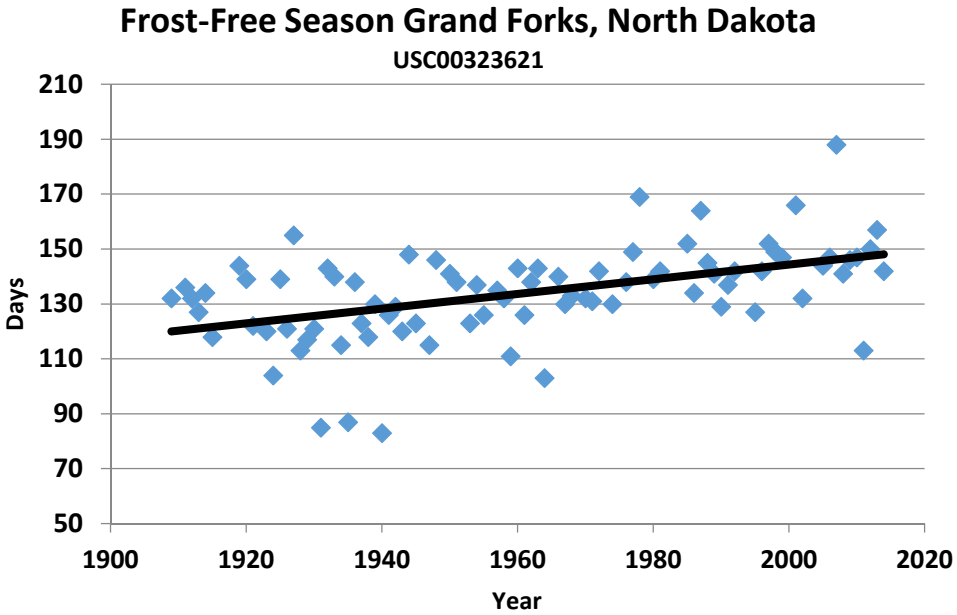


Figure 2-4. Frost-Free Period Length (Days) for Grand Forks, North Dakota [Midwestern Regional Climate Center, 2015].

Further examination of growing-season characteristics included assessing average temperatures and precipitation patterns observed for June through October (1970–2015) using NOAA data for Minnesota's Climate Division 1, which covers the northwest corner of the state. NOAA summary plots are depicted in Figures 2-5 and 2-6. Inter-year variability is considerable over this time, with broader patterns more discernable using smoothed time-series data represented by the green binomial filter line. Recent years show increases in average June through October temperatures with declines in precipitation amounts.

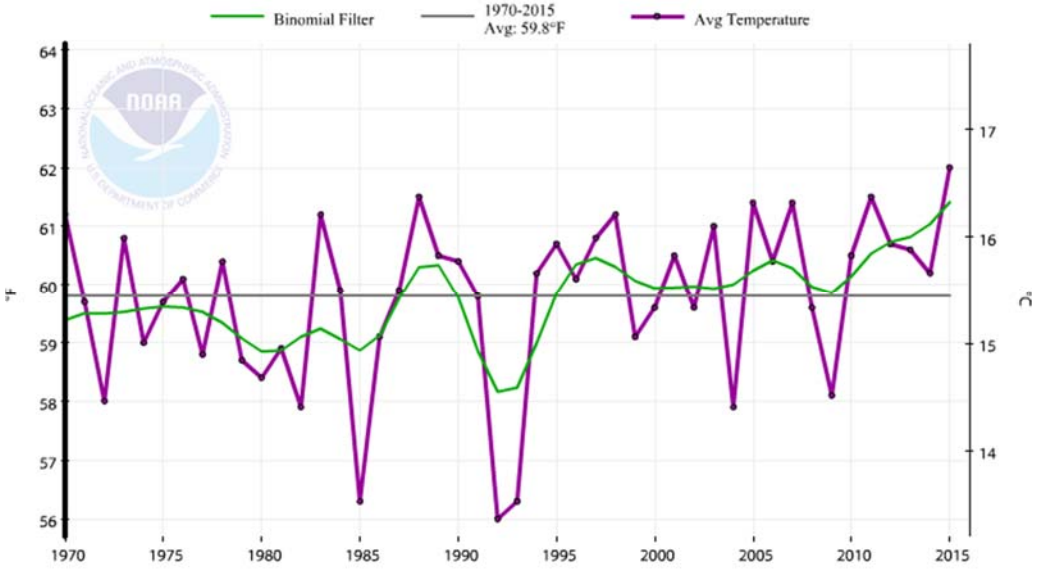


Figure 2-5. Northwest Minnesota Climate Division 1 Average Temperatures From June Through October (1970–2015) [NOAA, 2015].

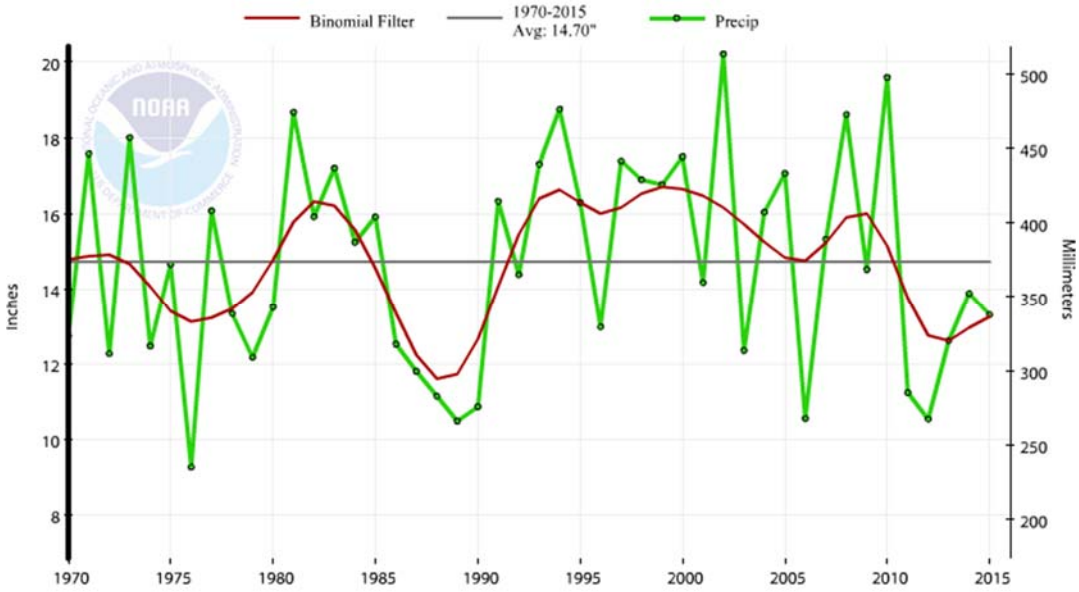


Figure 2-6. Northwest Minnesota Climate Division 1 Average Precipitation From June Through October (1970–2015) [NOAA, 2015].

2.4 HYDROLOGY

Considerable attention has been given to the Red River's flow dynamics because of its history of flooding events. As a result, a wide network of water-level and flow-gaging sites has been developed. For the purposes of this study, USGS gaged flows at nine continuously gaging stations along the Minnesota/North Dakota border and by the Environment and Climate Change Canada's Hydrometric Program at seven sites in Manitoba were obtained for the summer (2015). A subset of long-term flow records from select USGS stations was used to examine annual patterns by use of median monthly flows for six sites plotted in Figure 2-7. Monthly median peak flows from spring runoff typically occur in April and quickly decline through the growing season (June through September) with low values by August through the remainder of the year. As a result, growing-season flows may be expected to quickly decline from June and July levels to lower flows by August and September.

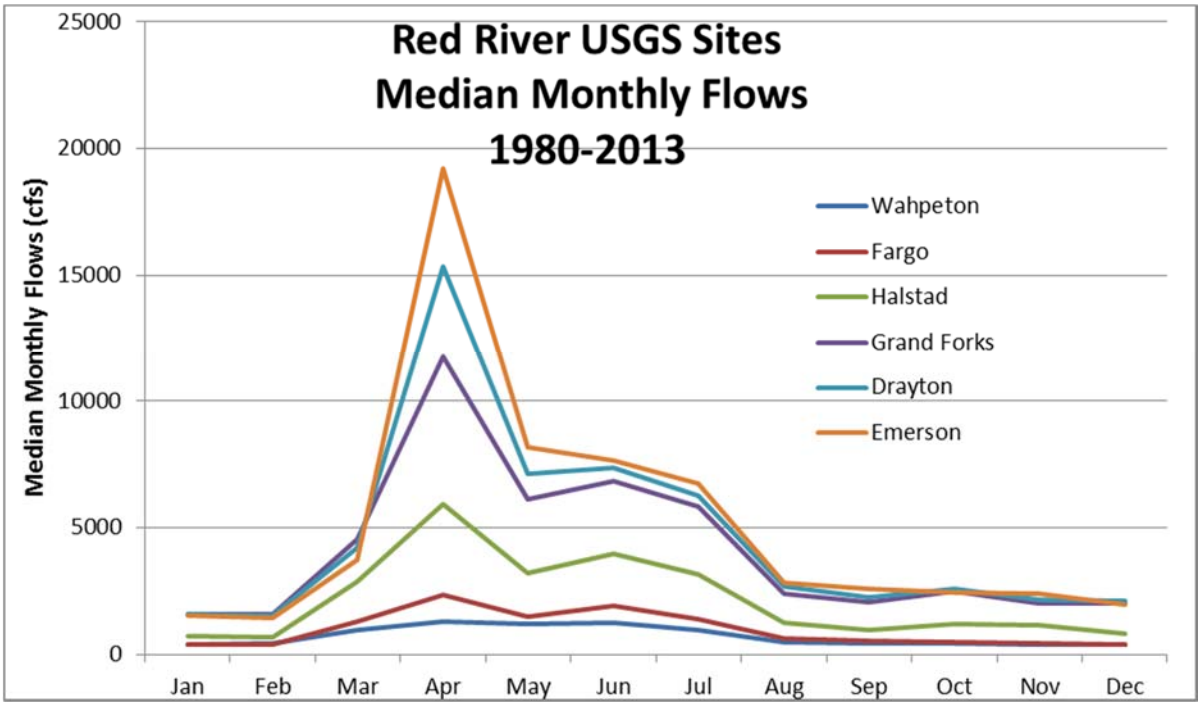


Figure 2-7. Select Red River of the North US Geological Survey Site Median Monthly Flows (1980–2013).

Continuous flows measured at these 16 flow-gaging stations during the periphyton deployment period (approximately July 22 to August 28, 2015) were tabulated by partnering agencies with daily mean flows depicted in Figures 2-8 and Figure 2-9 for sites in Minnesota/North Dakota and Manitoba, respectively. Consistent with previously defined growing-season flow dynamics, peak flows occurred at the beginning of the periphyton monitoring period with overall declining patterns noted at all stations. Flows at the end of the periphyton period were approximately one-half of flows noted at the start of the deployment period. The influence of the Assiniboine River's flows upon Manitoba Red River's flows is quite evident beginning at St. Norbert with flows nearly doubling from those observed at the Emerson site.

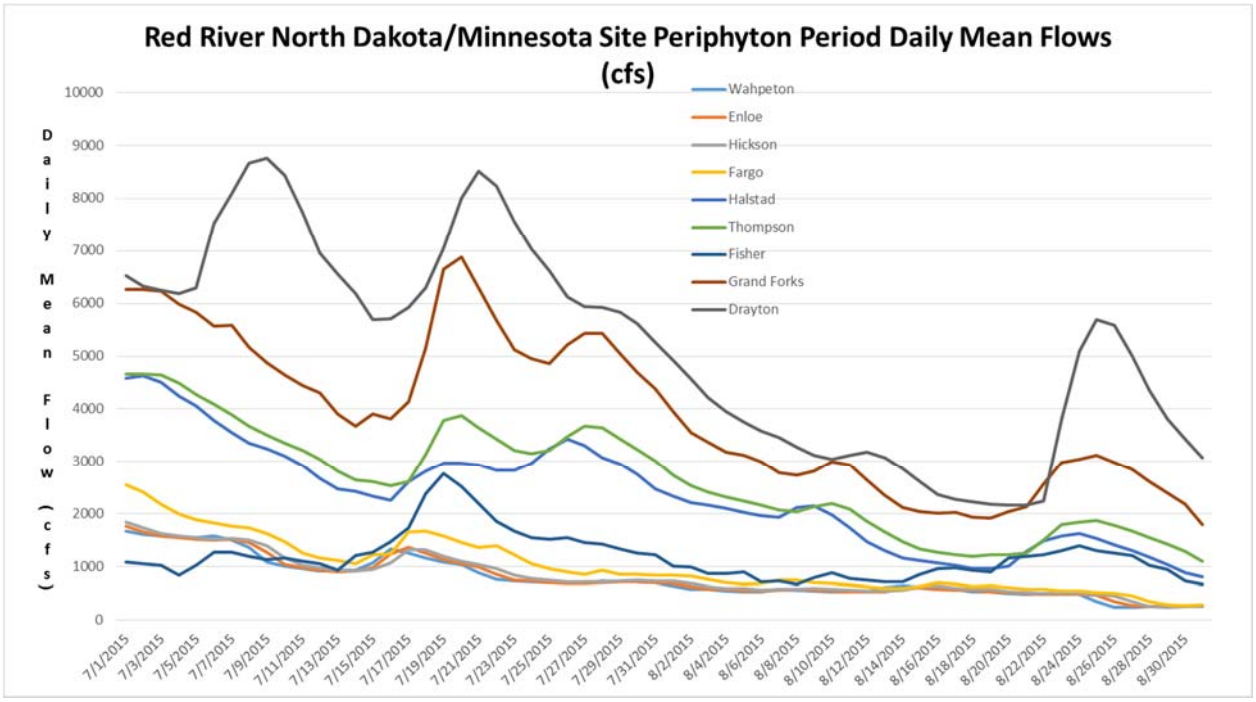


Figure 2-8. Daily Mean Flows (Cubic Feet per Second) for Red River of the North Sites in North Dakota/Minnesota.

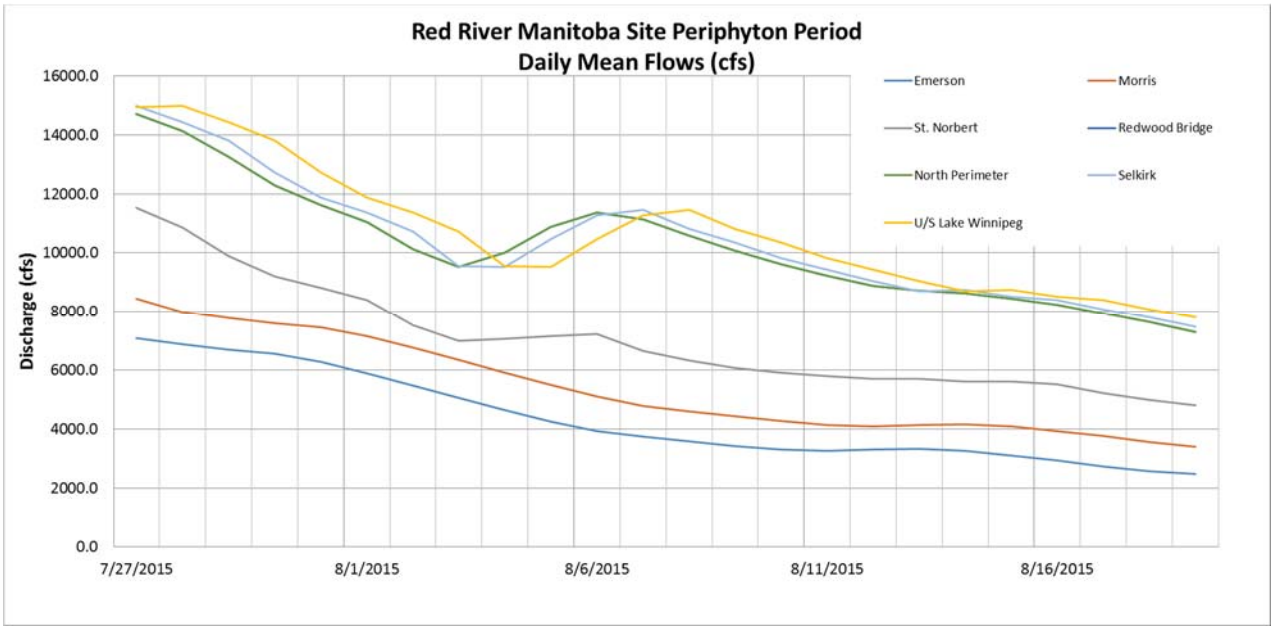


Figure 2-9. Daily Mean Flows (Cubic Feet per Second) for Red River of the North Sites in Manitoba.

2.5 WATER QUALITY

Water quality data for the periphyton deployment period were averaged by river reach/zone for traditional parameters and are listed in Table 2-3 along with minimum and maximum values noted within respective reaches. Of first note is the substantial increase of TSS from the Headwater to the Middle zones of the river. Values increased from 81.5 mg/L to 254.3 mg/L, respectively, and then declined to 108.8 mg/L at the Mouth. Average inorganic suspended solids comprised over 85 percent of the TSS in all zones. TN values also increased downgradient from 1.30 mg/L (Headwaters) to 1.80 mg/L (Middle) and then declining to 1.58 mg/L (Mouth). TP increased from 0.14 mg/L (Headwaters) to 0.31 mg/L (Middle) and remained at that level. Ortho-phosphorus (OP) likewise increased and remained high into the Manitoba reach.

Table 2-3. Averaged Water Quality Data by River Reach/Zone During Periphyton Sampling Period

River Zone	TSS[a] (mg/L)	VSS[b] (mg/L)	Periphyton Chlorophyll *a* mg/m^2	Phytoplankton Chlorophyll *a* (mg/L)	Pheophytin-*a* (mg/L)	TP[c] (mg/L)	Ortho P[d] (mg/L)	TN[e] (mg/L)	NH3-N[f] (mg/L)	NO3+NO2-N[g] (mg/L)	TKN[h] (mg/L)	DO[i] (mg/L)	pH (n/a)	SPC[j] (µS/cm)	Temperature (C)
Headwaters Average	**81.50**	**12.02**	**26.62**	**17.12**	**12.01**	**0.14**	**0.08**	**1.30**	**0.05**	**0.35**	**0.94**	**6.97**	**8.22**	**672.40**	**25.35**
Minimum	50.33	9.07	17.90	16.57	6.32	0.07	0.03	0.78	0.05	0.12	0.66	6.82	8.20	489.25	24.81
Maximum	113.33	15.67	34.83	17.90	17.93	0.18	0.10	1.67	0.05	0.54	1.12	7.15	8.23	741.33	26.11
Middle Average	**254.26**	**33.33**	**31.80**	**16.34**	**26.98**	**0.31**	**0.16**	**1.80**	**0.05**	**0.49**	**1.31**	**6.69**	**8.16**	**925.74**	**25.42**
Minimum	130.00	15.67	11.70	9.35	4.66	0.23	0.12	1.47	0.03	0.32	1.07	6.25	7.97	743.67	24.78
Maximum	336.67	45.33	80.85	25.83	38.67	0.40	0.21	2.11	0.06	0.88	1.55	6.99	8.38	1153.00	25.97
Mouth Average	**108.83**	**14.50**	**142.81**	**12.94**	**6.25**	**0.31**	**0.17**	**1.58**	**0.05**	**0.31**	**1.27**	**6.43**	**8.31**	**787.75**	**24.33**
Minimum	53.00	10.00	67.47	4.46	3.34	0.27	0.14	1.42	0.02	0.26	1.16	5.80	8.24	716.50	23.45
Maximum	192.00	19.50	216.33	18.20	8.39	0.34	0.19	1.68	0.08	0.36	1.38	7.20	8.42	822.33	25.74

(a) TSS = Total Suspended Solids
(b) VSS = Volatile Suspended Solids
(c) TP = Total Phosphorus
(d) Ortho P = Orthophosphate Phosphorus
(e) TN = Total Nitrogen
(f) NH3-N = Ammonia Nitrogen
(g) NO3+NO2-N = Nitrate-Nitrite
(h) TKN = Total Kjehldahl Nitrogen
(i) DO = Dissolved Oxygen
(j) SPC = Specific Conductivity.

The algal response variables (periphyton chlorophyll *a* and phytoplankton/seston chlorophyll *a*) were monitored at the deployment sites. Periphyton chlorophyll *a* values progressively increased downstream and ranged from 26.6 mg/m^2 (Headwaters) to 31.8 mg/m^2 (Middle) and 142.8 mg/m^2 (Mouth). The opposite pattern was noted, however, for phytoplankton chlorophyll *a* values; the zone average declined from 17.1 µg/L (Headwaters) to 16.34 µg/L (Middle) and 12.9 µg/L (Mouth). Monitored phaeophytin chlorophyll *a* (a breakdown product) was noted to range from 12.0 µg/L (Headwaters) to 26.98 µg/L (Middle) and then sharply declined to 6.25 µg/L (Mouth).

For purposes of historical comparison, Figure 2-10 shows the nutrient concentrations observed during the stressor-response study in light of values collected from 1994–2014 at sites samples during the stressor response study (Headwater–84RD011, Mid river–15RD058, and Mouth–MB05OJS004). The boxes represent the upper and lower quartile of the historical data with the protruding vertical lines representing the variability outside the upper and lower quartiles. The red lines indicate the averages from the stressor-response study. The graph indicates that the TP results from the 2015 sampling season fell within the "normal" variance of results.

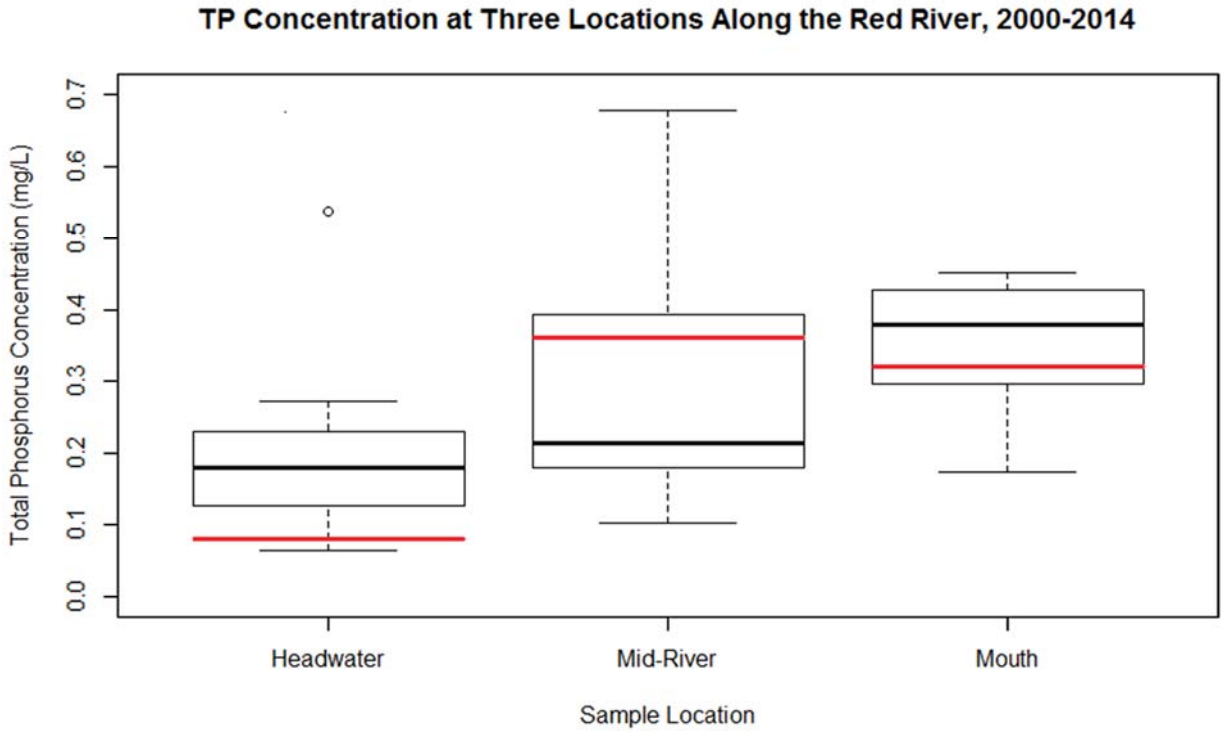


Figure 2-10. Boxplot Graph of Historical Total Phosphorus Concentrations in Comparison to the Stressor-Response Study Total Phosphorus Concentrations at Three Commonly Sampled Sites Within the Three River Zones. The black horizontal line is the historical total phosphorus average, and the red is the stressor-response total phosphorus average.

Based on state level monitoring and reporting requirements of the US Clean Water Act Section 303(d), a summary of Minnesota's impairments for the Red River mainstem is listed in Table 2-4 with a predominance of impairments caused by turbidity (13), dissolved oxygen (3), and bacteria (1). From this summary, a large portion of the US Red River mainstem is listed as impaired for designated aquatic-life

uses. Minnesota water quality rules have recently shifted from turbidity to TSS parameters, with a regulatory standard of 100 mg/L for the mainstem Red River. North Dakota's 2012 303(d) report lists the entire Red River as impaired for fish consumption because of methyl mercury pollution with one midreach section implicated for recreational contact impairment from excessive *E. coli* pollution [NDDH, 2012].

Table 2-4. Minnesota's 303(d) Impaired Red River of the North Mainstem by Reach, Year of Listing, Affected Designated Uses and Stressors [Minnesota Pollution Control Agency, 2015]

Reach Description	Year Added To List	River Assessment Unit Identification Number (AUID)	Affected Designated Use	Pollutant or Stressor	Environmental Protection Agency Category
Breckenridge Dam to Whisky Creek	1996	09020104-503	Aquatic Life	Turbidity	5
Buffalo River to Elm River (ND)	2010	09020107-501	Aquatic Life	Oxygen, Dissolved	5
Buffalo River to Elm River (ND)	1996	09020107-501	Aquatic Life	Turbidity	5
Buffalo River to Elm River (ND)	1994	09020107-501	Aquatic Recreation	Fecal Coliform	5
Cole Creek (ND) to Red Lake River	2010	09020301-501	Aquatic Life	Oxygen, Dissolved	5
Cole Creek (ND) to Red Lake River	1996	09020301-501	Aquatic Life	Turbidity	5
Fargo/Moorhead Dam 1 to Fargo/Moorhead Dam A	1996	09020104-504	Aquatic Life	Turbidity	5
Fargo/Moorhead Dam A to Sheyenne River (ND)	2006	09020104-502	Aquatic Life	Turbidity	5
Pembina River (ND) to MN/Canada Border	1996	09020311-501	Aquatic Life	Turbidity	5
Red Lake River to Grand Forks Dam	2008	09020301-504	Aquatic Life	Turbidity	5
Sandhill River to Buffalo Coulee (ND)	2008	09020301-507	Aquatic Life	Turbidity	5
Tamarac River to Drayton Dam	2008	09020311-502	Aquatic Life	Turbidity	5
Two River to Pembina River (ND)	2010	09020311-504	Aquatic Life	Oxygen, Dissolved	5
Two River to Pembina River (ND)	2008	09020311-504	Aquatic Life	Turbidity	5
Wild Rice River (ND) to Dam 2	2008	09020104-508	Aquatic Life	Turbidity	5
Wild Rice River to Goose River (ND)	1996	09020107-502	Aquatic Life	Turbidity	5
Wolverton Creek to Wild Rice River (ND)	2006	09020104-510	Aquatic Life	Turbidity	5

3.0 EXPERTS PANEL REVIEW: WORKSHOP AND WEBINARS

Experts comprising of local, regional, and federal water quality professionals and academics convened in December 2014 in Grand Fork, North Dakota, to discuss the stressor-response project details. The list of participants is provided in Appendix C. The workshop resulted in information and ideas to develop a stressor-response model in the Red River. The group collectively explored the water quality situation in the Red River with specific emphasis given to its unique challenges. Overall, the group determined that the Red River is a poorly understood system and that much remains to be learned. In an attempt to resolve this disparity, our volunteers provided valuable information on available data, effects of altered hydrology, ecological considerations, complications of excessive turbidity, potential model components to consider, insight into nutrient sources, and ideas on public involvement.

As specifically discussed in the workshop, the influence of excessive turbidity on light attenuation was expected to complicate developing a stressor-response model because of a potential "masking" effect on the biological response to elevated nutrients (i.e., effects of excessive nutrients on biomass production masked by the counter effects of limited light). However, exceptions can possibly occur based on the NDDH evidence of observed DO dips following flooding, which would suggest sources of excessive algal production. This observation is believed to be a result of organic matter inputs from adjacent oxbow wetlands, which would be nutrient related. Additionally, area phycologist's information indicates that the Red River has an observable periphyton response to nutrients, as evidenced from preliminary data collection. This collective information gave indication that DO occasionally fluctuates as a result of excessive algal biomass decay and could impact macroinvertebrate and fish taxa. Evidence is available that the algal community could be used as a model biological group to indicate a direct response to nutrients.

In lieu of adequate algal data, the initial group consensus was that other biological taxa could be used even if they might not show a direct response to elevated nutrients. The primary taxa discussed were fish and macroinvertebrates. Proponents agreed that these datasets (existing and proposed for collection) should be analyzed in an exploratory manner to identify correlated stressors. The identified stressor's correlation with nutrient sources would then be explored (e.g., TSS and nutrients and percent of impervious area and nutrients) with the expectations that potential management strategies could target both.

Subsequent to this meeting, two webinars were held to discuss the project with individuals who did not attend the experts' workshop. The list of participants is provided in Appendix C. Consensus agreement was reached that algae were the correct group to use for the stressor model. After reviewing the expert's comments, the existing data gaps for algae were too significant to derive a direct, measured biological response to variation in nutrient concentration in the Red River. Analysis of other taxa groups (i.e., fish and benthic macroinvertebrates) could provide very meaningful information with regard to watershed impacts, but given the primary interest of direct nutrient influence on biota, periphyton and phytoplankton species data were deemed necessary to develop a true stressor-response indicator and subsequent nutrient thresholds.

For adequate analysis, RESPEC proposed that periphyton collection (from artificial substrate) occur at multiple stations along the mainstem during the summer of 2015. Periphyton was chosen as the primary group of importance for nutrient criteria development because of the prevalence of diatoms in this group. Diatoms are one of the most researched algal taxa groups because of their noted response to nutrient additions. Sampling locations would be constrained by hydrologic sections of the Red River. Collected algal samples would require processing to the lowest feasible taxonomic group. In addition to the algal collection, water quality and phytoplankton (seston) samples collected simultaneously would be required. These samples would need analysis for pertinent, associated parameters and lowest feasible taxonomic group. Ideally, land-use parameters would also be included in this exploratory significance analysis, to better understand the holistic interaction of the biological communities and environmental stressors in the watershed. This information would provide broader management guidance that could facilitate effective nutrient management.

4.0 ASSEMBLY AND ASSESSMENT OF AVAILABLE DATA AND GAPS

Requests for available data occurred on several occasions through expert workshops and webinars (described in Chapter 3.0) along with an email request to all potential known data sources within the region. The results of the data acquisition are described in the following text.

4.1 AVAILABLE BIOLOGICAL DATA (FISHERIES AND MACROINVERTEBRATES)

As was provided to RESPEC, fish survey data were the most abundant biological survey result available. The NDDH provided results from 1994 to 2012 from various sites within the Red River, and the MPCA provided results from 1983 to 2008 surveys. The Minnesota Department of Natural Resources (MNDNR) also indicated that additional fish survey results were available from the Red River; however, these results were never obtained. Macroinvertebrate data were also consistently collected by the MPCA and NDDH over several seasons but this information also was not obtained. Macroinvertebrate surveys conducted by the MPCA during the summer of 2015 have not been made available because of processing time constraints. Mussel survey results were available (as provided by the MNDNR) but were found to be geographically limited.

4.2 PHYSICAL CHEMICAL

The most consistently available data on the Red River were flow (USGS gages), land-use summary information (SPARROW water quality model), and water quality (nutrients, DO, temperature, and biochemical oxygen demand [BOD]) over a long-term period. Flow data from the USGS gages were available in real-time and historically from six gage stations along the river reach (from Wahpeton near the Headwaters to Emerson near the international border). Manitoba had similar information available. Land-use information was most readily available as summarized from the SPARROW model [Jenkinson and Benoy, 2015]. Water chemistry results, with an emphasis on nutrients, were available sporadically from all state and provincial organizations within the region.

4.3 GAP IDENTIFICATION

Because algae are the biological group most responsive to nutrient inputs, the project team and associated experts collectively decided that the sparseness of both phytoplankton and periphyton was the most significant gap in the available data. Water chemistry specifically associated with the period of algal collection was also a distinct gap in the dataset.

4.4 QUALITY ASSURANCE REVIEW PROCESS

Historical data were reviewed for its holistic consistency in collection and its potential relevance in the stressor-response project. Because multiple agencies and two countries were associated with the various data-collection efforts, data consistency was not observed (nor expected). However, the knowledge gleaned from previous collection efforts did provide guidance in developing the 2015 data-collection efforts. Without this information, housed by each respective agency, the stressor-response project could

not have proceeded efficiently. Table 4-1 details the available information as of February 2015, its availability, quality, and plans for collection at the project initiation.

Table 4-1. Available Data as of February 2015 From the Red River of the North Mainstem

Data Needed	Availability	Quality	Collection Plans
Fish	Multiple years from North Dakota, Minnesota, and Manitoba	Good	Unknown
Macroinvertebrates	Exists but full extent not known	Good but not comprehensive	2015 by MPCA
Algae	Qualitative only	Subjective	2015 by project team
Water Chemistry	Exists but full extent not known	Good but not comprehensive	2015 by MPCA
Land Use	Land Cover Institute (LCI) data layers, SPARROW model	Good but dated	2015 land-cover update
Hydrology	USGS Stations + Minnesota Tributaries + Environment and Climate Change Canada (ECCC) Hydrometric Program	Good	Continuous flows

5.0 CONCEPTUAL MODELS

The first steps in evaluating stressor-response relationships are to define the case and develop a conceptual model. A conceptual model is a visual representation of the assumed relationships of the biological communities and their stressors (such as excessive nutrient or sediment inputs). The conceptual model illustrates the understanding of the system, and it guides development of the stressor-response models. The model can identify confounding factors (e.g., sediment interfering with light penetration [Figure 5-1]) and covarying factors (e.g., nutrients with suspended sediment while controlling for sediment). Variables that quantify suspended sediment and other factors along the pathway between suspended sediment and biological integrity should be used in the analysis. Including these variables will increase the accuracy of the stressor-response relationships [Plevan and Blackburn, 2013].

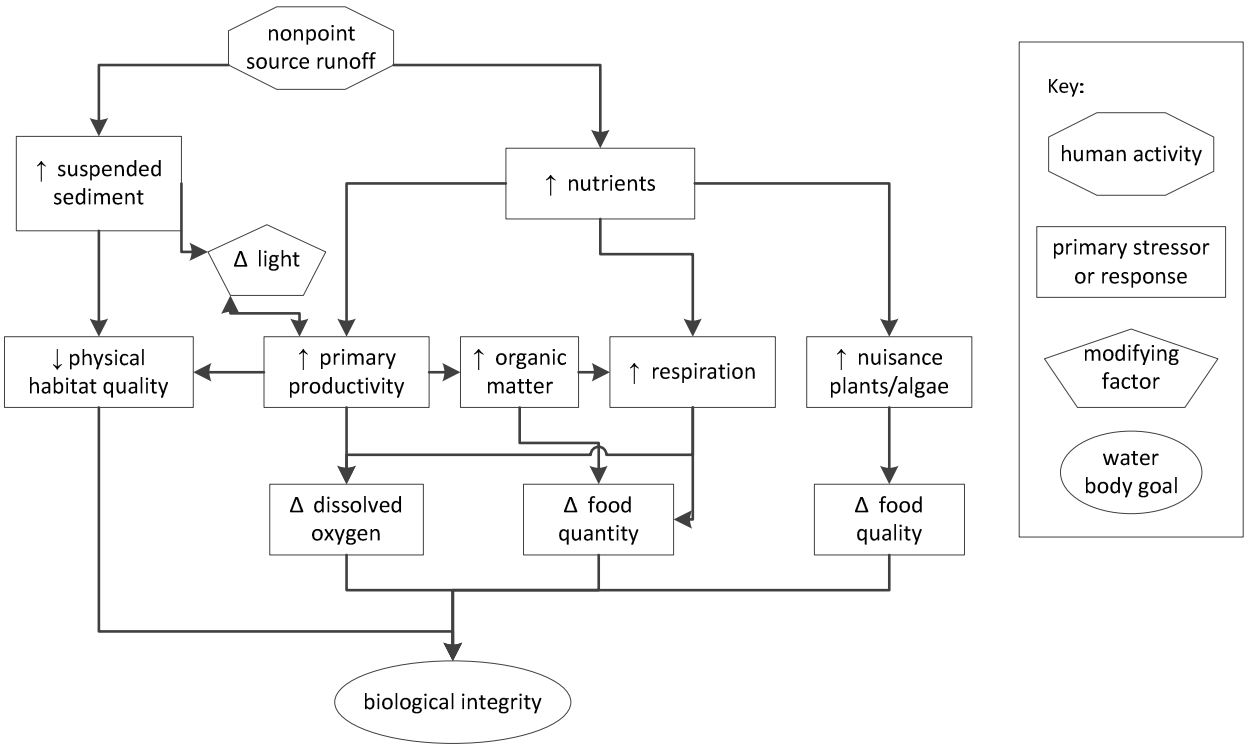


Figure 5-1. Example of a Simplified Conceptual Model for Streams (All Potential Variables Are Not Included) (Modified From US Environmental Protection Agency [2010]).

Initial efforts in developing draft conceptual models depicted cumulative impacts from (1) altered flows and habitat and (2) sediments with an emphasis on fine-sediment burdens carried by the Red River and its effects upon biological responses (conceptual diagrams are included in the Appendix B). A modification of the Heiskary et al. [2013] conceptual model was ultimately chosen by the experts panel for assessing the Red River (Figure 5-2) to explicitly incorporate the effects of small particle-induced turbidities that limit light and, therefore, influence eutrophication responses along the Red River mainstem sites.

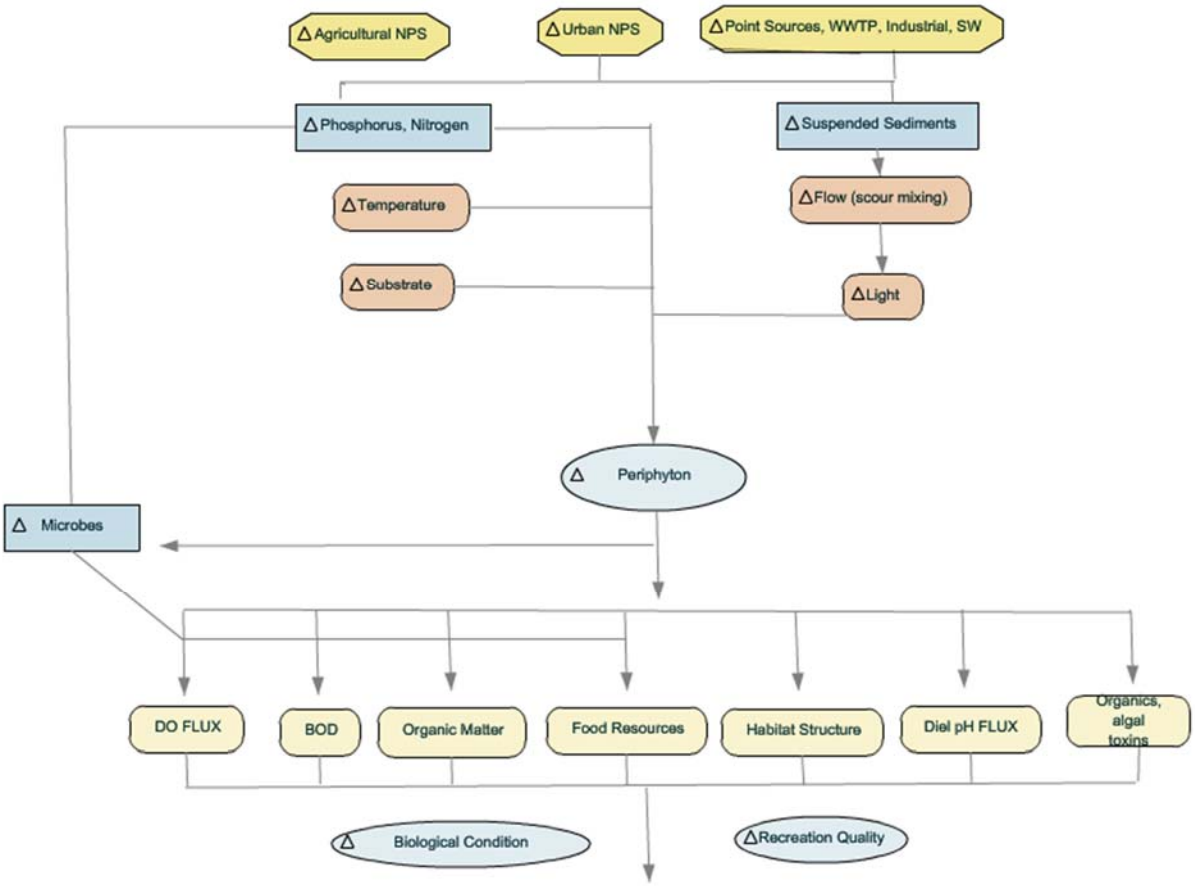


Figure 5-2. Modified Conceptual Model for the Red River of the North (From Heiskary et al. [2013]).

6.0 FILLING GAPS: SUMMER 2015 DATA COLLECTION

A true international/interagency cooperative effort facilitated collecting information to complete the stressor-response model as described in Section 7. Site selection for algae (periphytic and sestonic) and water chemistry sample collection was determined collectively by the MPCA and Manitoba Sustainable Development using existing river monitoring stations. Although identifying existing in situ substrate (e.g., wood, rocks, or mud) would have provided a more natural estimate of periphytic algal growth in the river, the project team determined that floating periphytometers were necessary to accurately survey the attached algae along the river reach (see Section 6.1). This decision was based on several factors, including logistical constraints (i.e., limited time to find potential sampling sites) and consistency of sampling conditions (e.g., depth of sample, possible shading, and available colonization area) to facilitate statistical comparisons. Other issues informing the group's decision for artificial substrate use was based upon the unknowns of periphyton growth given the extreme turbidity of the river. Surface-mounted samplers were expected to provide the greatest opportunity for periphyton growth (i.e., least light limitation), which was important given the general doubt of algal abundance in the river. Although not a natural condition, artificial substrate in biomonitoring is useful because sampling natural substrates in large rivers is often difficult. Artificial periphyton collection substrates have also been shown to provide analogous results to natural substrate in large rivers [Raunio and Soininen, 2007; Lowe and Gale, 1980]. Because the basis for our stressor-response study was to determine a quality and quantity response of the algal community to nutrients, using artificial substrates would not be expected to preclude the validity of the measured response of the biological group in reference to the measured water chemistry and adjacent land use.

The periphytometers were deployed by the MPCA and Manitoba Sustainable Development/ECCC personnel over the course of 3 days (July 20–22, 2015) and routinely checked to determine optimum colonization by various personnel. Samplers were collected approximately 4 weeks after deployment by the NDDH and Manitoba Sustainable Development personnel. Phytoplankton was collected from grab samples during the periphytometer collection event as well as water quality samples. Preserved algae samples were shipped to RESPEC in Helena, Montana, for taxonomic processing while water samples were processed by the Manitoba Sustainable Development and MPCA. The sampling methodology is described fully in Section 6.1. Quality-control measures employed by state staff ensured high-quality and robust datasets for the subsequent analyses.

6.1 METHODS

6.1.1 Periphytometer Deployment and Processing

Artificial substrates (periphytometers) that consist of float-mounted racks with glass microscope slides, as demonstrated in Figure 6-1, were employed to collect periphyton (attached algae) samples from the Red River during the summer of 2015 [Raunio and Soininen, 2007]. Before deployment, the glass slides were cleaned with denatured ethyl alcohol. The slides were then placed into the samplers consistently by using the same orientation of the frost and unfrosted sides. The floating samplers were tethered to surface buoys with nylon cord with the plastic shield facing upstream.

Figure 6-1. Floating Periphytometer Used at 30 Stations Within the Red River of the North During the Summer of 2015.

Periphytometers (samplers) were deployed at predetermined monitoring sites on the Red River (as described in Table 4-1) that were selected after thoroughly reviewing of existing monitoring networks and data from previous studies. Colonization slides floated just below the surface (approximately 1 inch). Three replicate samplers were deployed during the week of July 20, 2015, at each of the 30 sites including 23 US sites (deployed by the MPCA) and 7 Manitoba sites (deployed by the Manitoba Sustainable Development). Site locations in proximity to dams within the river are depicted in Figure 6-2.

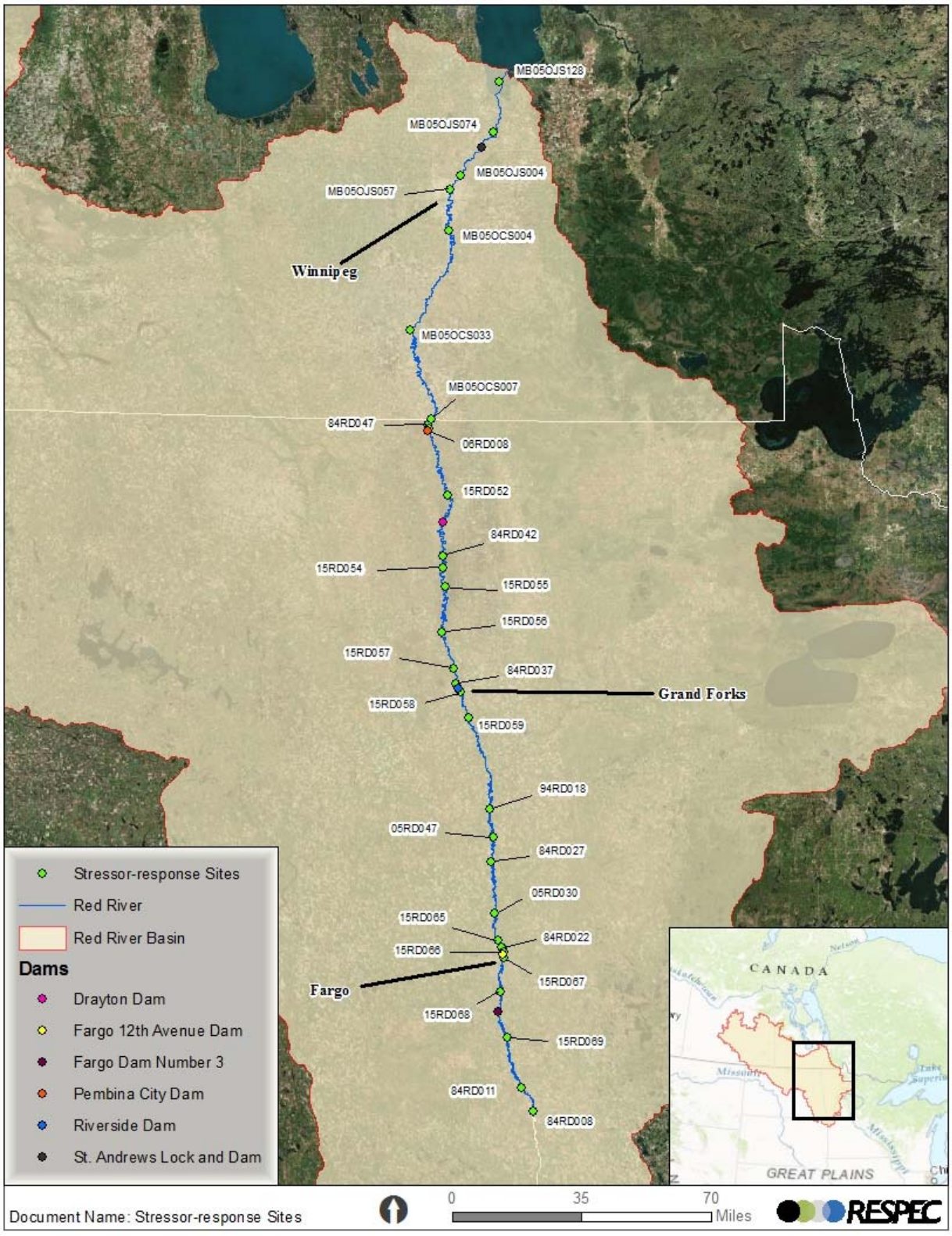


Figure 6-2. Algae and Water Quality Sampling Sites and Dams on the Red River of the North With the Minnesota Pollution Control Agency and Manitoba Site Labels.

As illustrated in Figure 603, samplers were placed in-stream in a manner that maximized their chance of survival through the deployment period. Efforts were also employed to minimize significant variability between sites of factors independent of water quality. If multiple site visits were required, early access to sampler installations would also be an important consideration. Locations nearer to the edges of the main channel, but beyond that easily reached by individuals onshore, were preferred. Locations visible from bridges, roads, and streamside trails were deemed less preferable, especially in urban areas and near public access sites. Riparian vegetation, well-anchored downed trees, structures along the stream bank, channel meanders, and other natural and man-made objects were used when applicable to screen the samplers from view. Locations beneath large, living trees or other objects that would significantly shade the samplers were avoided. Samplers were not placed in areas of strong currents that would likely transport large floating debris or in backwater areas likely to accumulate flotsam. Fluctuations in river stage, particularly major decreases that would leave samplers stranded out of the water, were anticipated, and samplers were placed in water of adequate depth.

Figure 6-3. Location of One Periphytometer in Relation to the Bank.

Samplers were suspended above anchors placed on the stream bottom in approximately 2 to 3 m of water depth. The three replicate samplers at each site were placed in similar current velocities and aspects, spaced 5 to 10 m apart, and staggered so as to not interfere with, or "shadow," one another, as illustrated in Figure 6-4. Again, the primary goal was to select suitable locations for each sampler "cluster" that maximized comparability between replicates and sites while minimizing the likelihood of all replicates befalling the same fate to vandals or the elements.

Figure 6-4. Grouping of Periphytometers Shortly After Deployment by the Minnesota Pollution Control Agency Staff.

Retrieving the substrates as near as possible to the peak of periphyton growth, but before the material became decadent or began sloughing off, was an important consideration (see Figure 6-5). An exposure period of approximately 4 weeks was used, although the exact exposure times were determined on a site-specific basis by field assessments of the periphyton growth rate on the sampler substrates. The length of exposure was standardized between all of the sites to the greatest degree possible with coordination between the MPCA, NDDH, and Manitoba Sustainable Development. Algal growth was monitored as follows:

- **Minnesota:** The MPCA routine river monitoring crew checked US samplers every 2 weeks, took photographs, and forwarded the results to the project team.
- **Manitoba:** The Manitoba Sustainable Development personnel checked the Winnipeg sites weekly and information was forwarded to the project team.
- **North Dakota:** The NDDH staff checked sites near Fargo weekly and forwarded information to the project team.

Figure 6-5. Fully Colonized Periphytometer.

Each sampler contained 16 slides and upon collection, the center two slides from each periphytometer were retained for taxonomic processing. Provided all three periphytometers were accounted for, the resulting six composited slides were submerged in tap water, preserved with Lugols iodine, packed on ice, and shipped to Mr. Erich Weber (RESPEC, Helena, Montana) for identification. Some periphytometers were lost during the deployment period but all sites retained at least one sampler, which provided an adequate and comparable sample of periphyton community composition as the purpose was to obtain average values for each sample reach.

The remaining 14 slides from each sampler (maximum of 42) were retained for chlorophyll *a* and ash-free dry-weight analysis. These slides were immediately wrapped in aluminum foil, placed on dry ice, and kept frozen until delivered to Energy Laboratories in Helena, Montana.

6.1.2 Phytoplankton Collection and Processing

A single phytoplankton sample was collected while retrieving the periphytometers at each site. The samples were collected in the main river channel in proximity to the artificial substrates using a horizontal van Dorn sampler and then placed into an 8-liter churn splitter. Using the churn splitter, a known volume of water was filtered through a 20-µm plankton net and placed into a Nalgene bottle (250–500 milliliters [mL]). The samples were labeled (including filtered sample volume), preserved by Lugols IKI, and sent to Mr. Erich Weber (RESPEC) for analyses. Phytoplankton algae were counted to compare algal densities among sites. Literature algal biovolume values were used for most diatoms and soft algae to estimate algal biovolumes. All taxonomic references are included in Chapter 9.0.

6.1.3 Algae Identification

6.1.3.1 *Periphyton*

Periphyton was collected from artificial substrates (glass microscope slides) deployed at 30 sites on the Red River in the US and Canada. A subsample of glass slides from each site was analyzed in the laboratory for nondiatom (soft-bodied) algae and diatom-algae taxonomy. The periphyton material was scraped from the microscope slides into a porcelain dish and returned to the original sample container. Each periphyton sample was thoroughly homogenized by vigorous shaking, and an aliquot of the suspended material was pipetted into a welled microscope slide and covered with a glass cover slip. The prepared wet mount was scanned using an Olympus BHT microscope, under 100X, 200X and/or 400X magnification as necessary to identify nondiatom algae present along a predetermined pattern of vertical, horizontal, and diagonal transects. All nondiatom algae encountered were identified to the lowest taxonomic level practicable (usually genus). Relative abundance and rank by biovolume of each taxon present in the sample were estimated and recorded. The sample material was then returned to the original container for inclusion in diatom-algae analysis.

A subsample of each periphyton sample was placed into a labeled Pyrex beaker, concentrated sulfuric acid and potassium dichromate added, and heated to boiling to oxidize all organic material present. Following cooling and settling, the diatom material remaining in the beakers was washed at least six times to remove the chemicals by decanting the supernatant, refilling with deionized water, resettling, and again decanting. A subsample of the cleaned diatom material was pipetted onto a glass coverslip and thoroughly

dried, and a permanent mount of each sample was prepared on glass microscope slides using Naphrax medium. For each sample, the mounted material was scanned along a single vertical transect (located precisely across the center of the coverslip) and all diatom taxa present were identified to at least the species level. A count of 600 diatom valves (300 frustules or cells) was performed along the same transect. The percent abundance of each diatom taxon and a suite of diatom metrics specific to prairie streams were calculated for each sample.

6.1.3.2 *Phytoplankton*

Phytoplankton samples were collected from the 30 sites on the Red River at the time of retrieval of artificial substrates. A 5-liter grab sample of river water was collected from within the top 0.5 meter of depth at each site. Phytoplankton organisms were collected by filtering the sample through a plankton net with a mesh size of 20 µm. The concentrated phytoplankton sample was rinsed into a wide-mouthed plastic bottle, using a minimum amount of tap water. The sample was preserved with Lugols IKI solution and placed on ice until delivered to the laboratory.

In the laboratory, the volume of each concentrated phytoplankton sample was determined by using precision laboratory glassware. Additional concentrating of phytoplankton organisms generally was required and accomplished the removing a known volume of supernatant from the settled sample. A final concentration factor for each sample was calculated from the measured volumes. An aliquot of the concentrated sample was pipetted into a Palmer-Maloney counting cell for taxonomic analysis and enumeration. Phytoplankton organisms were identified under the microscope at 400X to the lowest taxonomic level practicable (usually genus) and a count of 150 Natural Algal Units (NAU) was performed. The number of microscope fields counted was recorded and the total volume of the counted sample was calculated by using the known volume of sample represented by each microscope field (in this case, 7.1×10^{-5} mL per field of view at 400X).

The number of cells comprising representative specimens of NAU for filamentous and colonial algae was counted and recorded, and the average number of cells per NAU for each algal taxon identified in each sample was calculated. Dimensions of the cells comprising representative specimens of NAU were determined in micrometers (µm), using a calibrated ocular micrometer, and recorded. Using these values, the average cellular volume (in μm^3) of individual cells of each algal taxon was calculated using specific formulas based on geometric shape. For each sample, the total biovolume of each taxon in μm^3 was determined as the product of the average number of cells per NAU, times the average cellular volume, times the number of NAU counted in the sample. The total algal biovolume (in μm^3) of all phytoplankton algae in each sample was calculated by summing the calculated biovolume values of each taxon present. Finally, the number of NAU of each algal taxon present per liter of river water, the biovolume of each taxon (in milliliters per liter of river water), and the total algal biovolume (in milliliters per liter of river water) were calculated for each sample, by applying the final concentration factor calculated in the initial analysis step.

6.1.4 Water Quality Collection and Processing

All results of stressor analyses pertaining to water chemistry, which includes algal taxa analysis with respect to water chemistry (or environmental parameters in general), are based on a subset of the original

30 periphytometer sites (US 23 sites). The differences in analytical methods between the laboratories that processed the water samples from the 23 US sites compared to the 7 Manitoba sites resulted in noncomparable results because of complications with bias associated with high TSS. As a fortunate substitution, the MPCA had collected two water samples during the approximate 2-week periphytometer deployment period from 16 of the 23 US periphytometer sites as part of their state water quality assessments. These samples were processed using the same standard methods as the Manitoba laboratory (who also collected two samples on similar dates during the deployment). The loss of seven sites was deemed acceptable by the project team to allow for more accurate analyses; therefore, the MPCA dataset was used for the US sites going forward. Standard water sample collection methods were employed by both groups that included wearing gloves while collecting a grab sample followed by immediate icing, and preservation if applicable, before delivery to either the MPCA or the Manitoba Sustainable Development laboratory. Samples for dissolved constituents were field filtered (0.45 µm membrane filter). Between the two laboratories, the following parameters were analyzed consistently and the average of the two samples were used in all of the analyses:

- Total Phosphorus (TP)
- Total Nitrogen (TN)
- Total Suspended Solids (TSS)
- Ammonia
- Dissolved Oxygen (DO)
- Nitrate + Nitrite
- Total Kjeldahl Nitrogen (TKN)
- Orthophosphate Phosphorus (OP)
- Specific Conductivity (SPC)
- Temperature
- pH
- Volatile suspended solids (VSS).

Note that soluble reactive phosphorus (SRP), an ecologically meaningful constituent of total phosphorus, was not sampled during this study. Given the very large geographic extent of this monitoring effort, the project team did not believe that SRP holding times could be met by all of the partnering agencies. The focus for phosphorus shifted to TP to be the consistent parameter (as much as possible) with other studies and nutrient standards/criteria. All of the sample results are included in Appendix E.

6.1.5 Land-Use Analysis

Land-use attributes were measured on a site-specific basis within ArcGIS Version 10.3.1. Each site's specific drainage was delineated and each land-use category within that drainage area was determined. The specific methods for each pertinent attribute are described below. All land-use types were delineated from a recently completed (pending publication [Jenkinson and Benoy, 2015] SPARROW model data layer. Per USGS (*water.usgs.gov/snawqa/sparrow*), "SPARROW is a modeling tool for the regional interpretation of water-quality monitoring data (it) empirically estimates the origin and fate of the contaminants in river

networks and quantifies uncertainties in model prediction." SPARROW relies on land-use information for accurately modeling water quality results, which provided our model with subsequent high-quality information for the specifics steps outlined below.

6.1.5.1 *Watershed Delineation*

Watersheds were delineated to each Red River sampling site by creating a geometric network using ArcGIS network analyst tools. This method was chosen to successfully use the data provided by the SPARROW model [Jenkinson and Benoy, 2015] using data from the 2001 National Land Cover Database (NLCD) for the US and 2000 land-cover data for Canada. The line work used to build the network was "StreamsV8.shp," which is a harmonized US and Canada stream network for the Red River Basin provided by the SPARROW model. Once the geometric network was created, the next step was to create a flow direction for the network topology. The Set Flow Direction for Geometric Networks tool was applied with digitized direction as the flow parameter. This flow direction allows for tracing upstream accumulation when given a point within the network. The upstream accumulation operation was performed using the 30 sampling points on the Red River. The upstream accumulation stream datasets were then used to perform a spatial query on the "catchments.shp" also provided by the SPARROW model. The query was designed to select all of the catchments that have an intersection with the upstream accumulation datasets. The result is a selection of catchments that combine to be the upstream watershed of each MPCA sampling point.

6.1.5.2 *SPARROW Land-Use Calculations*

Each catchment provided by the SPARROW model possesses a "SPARROWID" that is a unique identifier field for all of the catchments. This "SPARROWID" was used to create an inner join on the "landusepercentages.shp" attribute table. The land-use information was appended to the watershed layer for only the catchments that were in the MPCA site watershed. Percentage calculations were then performed using Python within the field calculator of ArcGIS. This operation was then applied to all sampling sites that resulted in a land-use percentage for all of the sampling-point watersheds. Data gleaned from the SPARROW land-use coverage included acreage calculations of the following:

- Watershed area
- Open water (ponds, oxbows, rivers)
- Urban/impervious area
- "Barren" land
- Forest
- Grassland
- Agriculture (row crops)
- Wetlands
- Pasture (animal graze land)
- High-nutrient intensity crops (crop-specific need for fertilizer grouping)
- Mass total estimates of nitrogen and phosphorus applications (to row cropland)
- Point sources (quantity).

6.1.5.3 *Point Sources*

The point-source, comma-separated values provided by the SPARROW model was converted to a point shapefile. A spatial query was performed to select all of the point-source locations within the site watershed layer. The process was repeated for each site watershed and resulted in the total number of point sources within each watershed.

6.1.5.4 *High-Intensity Crops*

High-Nutrient Intensity Crops (HNIC) was provided as a ratio of all crops that meet the high-nutrient criteria to the total area of agricultural land use. A ratio was provided for every catchment in the watershed. To obtain the HNIC information for the sampling-point watersheds, an inner join was performed. Once the join had appended the HNIC information to the existing catchments table, Python and the field calculator was used to determine the total acreage of the HNIC parameter and calculate a percentage of the total land area of the watershed. This process was repeated for both HNIC1 and HNIC2.

6.1.5.5 *Fertilizer Mass Total Nitrogen and Phosphorus*

To obtain the Fertilizer Mass Totals, "fertilizer_mass_totals_by_catchments.dbf" was inner joined by "SPARROWID" to the catchments of the sampling-point watersheds. The "mass_n_kg" and "mass_p_kg" fields were then summed together by using the statistics tool within the attribute table. The final result was the total kilograms (kg) of Nitrogen Fertilizer and Phosphorous Fertilizer that were used in each sampling-point watershed.

6.1.5.6 *Distance to Upstream Dam*

To calculate the distance between a sampling point and an upstream dam, a network dataset had to be created from the stream shapefile. Once the network topology had been built, several different analyses could be performed. In this case, a closest facility analysis was performed. A dam shapefile obtained through Open Street Map and the USGS was created and used as the facility features. The sampling points were used as an incident layer. When all of the components were compiled, the algorithm was performed and the shortest path to an upstream dam was selected and measured for each of the 30 sampling sites.

6.1.5.7 *Riparian Wetlands*

Step one in calculating riparian wetlands was to form a buffer around the streams in the watershed. A large buffer of 500 m was chosen to encompass the large oxbow wetlands located along many of the larger tributaries (their physiographic placement was expected to have an interactive influence on the river). Crop data layers provided by the US Department of Agriculture (USDA), which included specific wetland areas, were downloaded and converted to a vector layer to obtain an area measurement. The polygons were then clipped to the boundary of the Red River Watershed. An intersect operation was then performed to allow the crop data layer to isolate the extent of the 500-m buffer on each side of the stream. The next step was to use summary statistics to sum the area of wetlands within the intersected dataset to provide a total area of wetlands within the buffer. The wetlands measurement was then used to calculate the percentage of riparian wetlands to the total land area of the sampling-point watershed. The same operation was repeated for the Canadian sites, but the Agriculture and Agri-Food Canado (AAFC) Annual Crop Inventory 2013 was used to obtain the wetlands area measurement.

7.0 STRESSOR-RESPONSE MODELING

The stressor-response modeling approach employed for this project followed the guidance provided by the US Environmental Protection Agency (EPA) [2010] and included the following:

1. Development of a Conceptual Model (described within Chapter 5.0)
2. Exploratory Analysis
3. Estimation of Stress-Response Relationships
4. Determination of Accuracy and Precision.

The following subsections detail steps 2 through 4 of this process with the inclusion of an additional step of stressor identification. As described in Section 7.3, this step was performed to ascertain the influence of stressors other than nutrients and sediment on the algal community in an attempt to further describe community variance, thereby adding significance to our nutrient-stressor model.

7.1 STATISTICS

Multiple analyses methods were performed on various components of the data collected for this project. Beyond the simple plots of data (e.g., site-level trends of nutrients, TSS, and chlorophyll *a*), the majority of the analyses were multivariate in nature. Multivariate analyses were chosen because of the abundance of data generated through the various processes described previously.

The crux of this study relied on site-level variance both in the response variables (periphyton and phytoplankton) and the predictor variables (water chemistry and land-use attributes). To determine the significance of algal community variance at the site level, the indirect ordination technique Nonmetric Multidimensional Scaling (NMS) was initially used within the statistical software PC-ORD (Version 6.19) [McCune, 2011]. This analysis allowed for the extent of the sampling site differences related to periphyton species/metrics and phytoplankton species to be visualized graphically. "Ordination such as NMS provides views into a high-dimensional space by seeking and displaying the strongest structure" [McCune and Grace, 2002]. NMS is far superior to traditional ordination techniques such as Principal Components Analysis because mathematically, it can "see" a much wider range of structures through its iterative optimization methodology. NMS also allows for analyzing data that are non-normal or arbitrary, discontinuous, or otherwise questionable scales and is one of the best techniques for ecological community studies [McCune and Grace, 2002]. This technique produces a graphical output for interpreting similarities or differences of sites because of variation within the chosen measured response variables (e.g., algae taxa) based on the distance sites that are located from one another on the diagram.

Redundancy Analysis (RDA) is a constrained ordination procedure that was used to compare the algal community response in light of environmental parameters. Within the software CANOCO (Version 5) [ter Braak and Smilauer, 2012], the RDA analyses sought to understand how the site-level algae data varied in light of the influence of nutrients, TSS, and various land-use parameters. The RDA procedure is similar to a well-known procedure called Canonical Correspondence Analysis (CCA) yet is more robust for data that exhibits a linear response (as determined with the collected algae data). These types of analyses "constrain" an ordination of one matrix (e.g., algal taxa by site) by a multiple linear regression on variables

in a second matrix (e.g., nutrients, TSS, and land use) [McCune and Grace, 2002]. More generally, significant correlations between relevant environmental variables and site-level algae communities can be determined to understand the strongest influences on the communities (pending ecological meaningfulness). Using a procedure within RDA called forward selection, each parameter was tested for significance and chosen for a full ordination so that ecological implications could be interpreted. This method provided a needed "culling" of the environmental matrix so that the number of variables were always less than the number of sites (to retain the "constrained" effect of the direct ordination procedure) and to ensure that only variables with statistically significant associations with the algal community variance were retained for interpretation. One additional subset of RDA was performed, called partial RDA (pRDA), which allowed for including a covariable. This method allowed for variance attributed to a particular variable (TSS in our case) to be "partialled-out" and extracted so that subtler trends could be discerned (e.g., the effect of nutrients) [Jongman et al., 1995].

Finally, a Nonparametric Multiplicative Regression (NPMR) was performed on the periphyton chlorophyll *a* data in conjunction with TP, TN, and TSS to find the most significantly correlated variables with periphyton abundance. This NPMR test was performed using Hyperniche 2.0 [McCune and Mefford, 2009]. The most useful aspect of this analysis, besides the additional absence of data normality, is that NPMR within the Hyperniche program is specifically designed for predictive habitat modeling and species-response functions in particular [McCune, 2011]. Models within NPMR are constructed from multiple explanatory variables in a multiplicative (rather than additive) manner that more closely demonstrates the effect of potentially interacting environmental variables [Potapova and Winter, 2006]. The NPMR test was performed with default settings that include using the local mean (Gaussian) model form, medium settings for overfitting controls, automatic settings for "minimum average neighborhood size for acceptable model," step size of 5 percent of range, a maximum allowable missing estimate of 10 percent, and minimal backtracking for the free search. The "delete all but best fit for N predictors" screen option was then subsequently selected to provide the best models.

Before multivariate analysis, all data were relativized by maximum to reduce the overemphasis the analysis would have placed on common taxa and to eliminate the bias from environmental parameters with different units of measure. Rare algae taxa were removed to further eliminate noise in the analysis; at least three occurrences of taxa were required for inclusion in the analysis because three points were needed to form a trend.

Not all variables were included in the analysis as was described in the forward selection description above. Many variables were culled before any type of analysis and some were culled after identifying autocorrelation. Finally, forward selection was used to cull variables statistically. Only the variables significantly associated with algal community variance were retained, and then using only this subset, a full ordination was performed and the significance of the full model was ascertained. As a result, environmental variables smaller than the number of stations. A key point to note, however, is that even if this was not the case, when the quantity of environmental variables approached the number of sampling sites, the "constraining" effect that the enviro variables have on the algal community at the site level is only reduced. In this extreme case example, the constrained ordination becomes similar to an unconstrained ordination (PCA, NMS) that has the environmental variables overlain. These "indirect/unconstrained" ordination procedures are still meaningful but do not provide as strong of an

interpretable model as a "direct/constrained" ordination as we have performed. The final number of environmental variables used on all of the constrained ordinations for our model development was always less than the number of sites (six environmental variables were the most ever used as compared against 23 sampling sites). Therefore, our stressor-response models were always fully constrained and interpreted accordingly.

These techniques are considered exploratory methods in that the models are allowed to determine the statistically relevant associations that are subsequently interpreted with respect to ecological relevance. All variables that were measured and assessed before any data were culled are included in the appendix. Only those variables shown in the ordination diagrams within the Section 7.2 were used in the final models.

7.2 RESULTS

Periphytometers were retrieved after approximately 4 weeks of repeated visits to ensure maximum colonization yet no biomass sloughing. Flows within the Red River were low during the time of deployment, which created ideal growth conditions.

Algal taxonomy resulted in 98 periphyton species and 87 phytoplankton species (Figure 7-1). All land-use, water quality, and taxonomic information used in the stressor-response analysis is included in Appendices A, E, and F, respectively, and statistical output not included within the report is included in Appendix G.

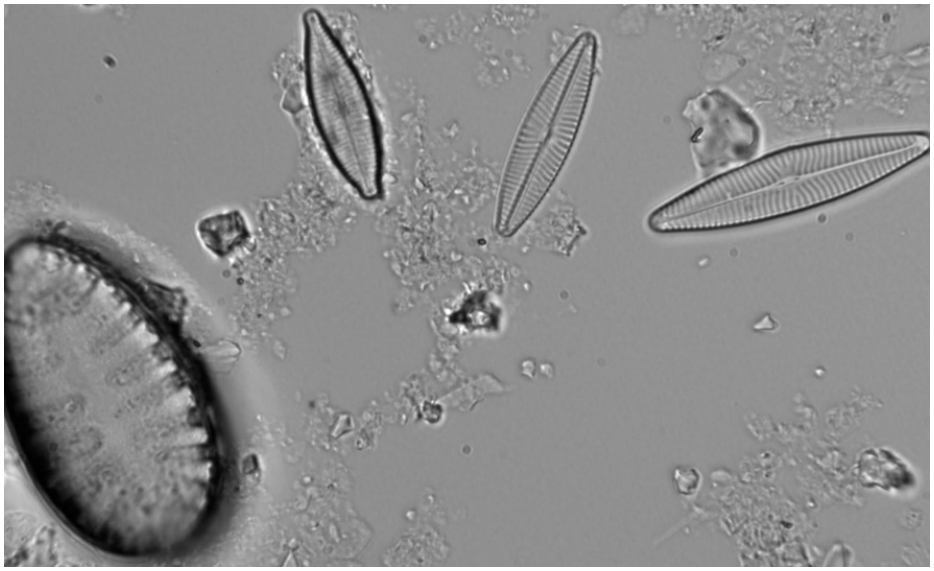


Figure 7-1. Various Periphytic Diatoms From the Stressor-Response Study Samples.

Using this collective algae, water quality, and land-use information, distinct patterns within and between the datasets were immediately discernable. With regard to periphyton, the effects of excessive nutrients were evident on the algae growth observed from the measures of quantity (e.g., periphyton chlorophyll *a*) and quality (i.e., taxonomic response to pollution/nutrient tolerance from various periphyton metrics). However, even though periphytometers rested just below the surface, the effects of light limitation (as

proposed by TSS concentration) appeared strong as inverse correlations between growth and TSS varied consistently along the reach.

The analyses also highlighted information regarding the source of nutrient inputs and the significance of land use associated with algal population dynamics. Phytoplankton was abundant with an occasional proliferation of blue-green taxa. In general, sestonic taxa showed significant correlations with specific water-quality-associated land-use attributes as well as direct measures of water quality.

7.2.1 Visual Trends

With regard to periphyton, patterns of nutrient and algal abundance were immediately apparent, as demonstrated in Figures 7-2 and 7-3. Patterns of TP, TSS, and algal abundance by river mile are also evident in Figures 7-2 and 7-3.

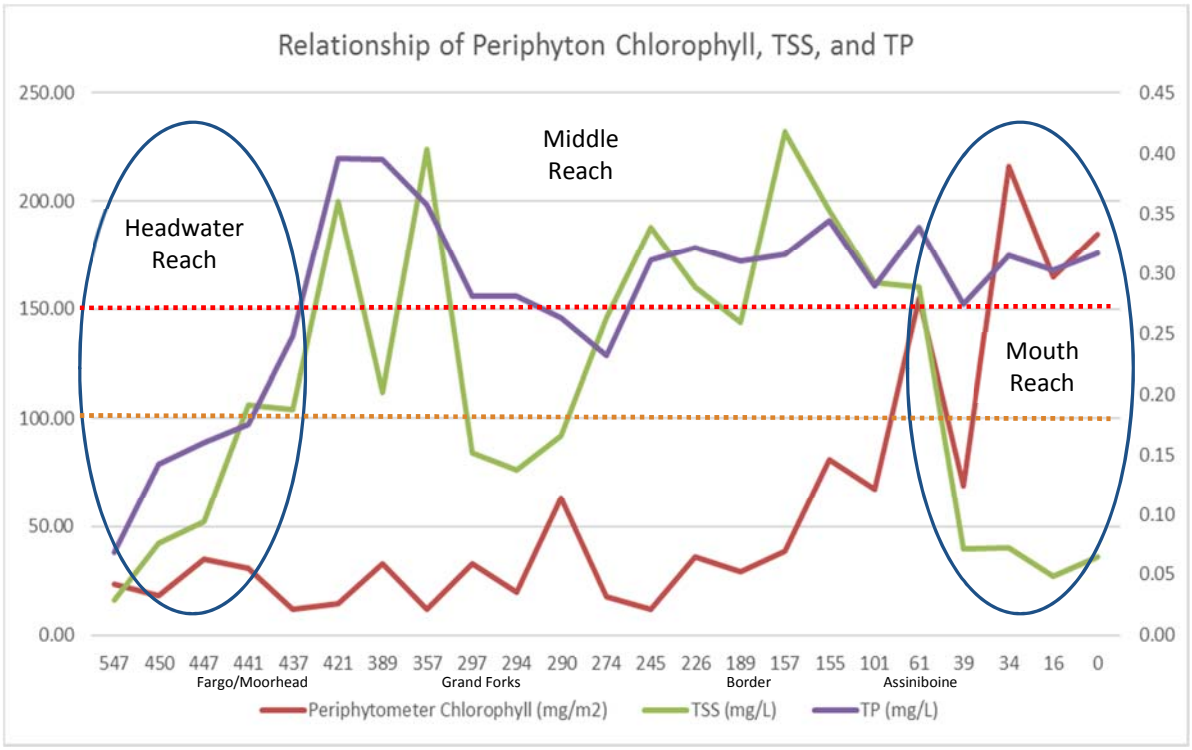


Figure 7-2. Plot of Periphyton Chlorophyll *a* (mg/m^2), Total Suspended Solids (TSS in mg/L), and Total Phosphorus (TP in mg/L). Orange and red dotted lines indicate low and high range of perceived nuisance levels of periphyton. Sites (horizontal axis) are oriented from left to right by river mile in a downstream manner ("0" is the river mouth at Lake Winnipeg). The left vertical axis conveys the concentration of TSS and abundance of periphytic chlorophyll *a*. The right vertical axis conveys the average concentration of TP over the course of the periphytometer deployment.

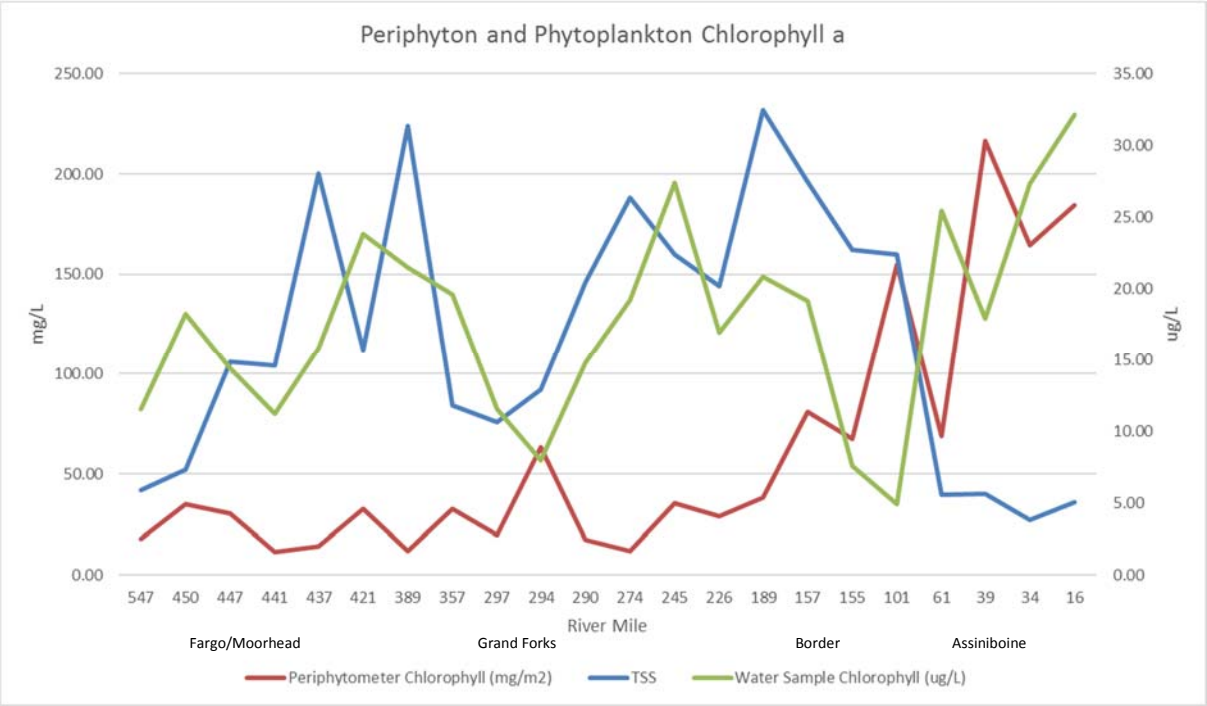


Figure 7-3. Comparison of Periphyton and Phytoplankton Chlorophyll *a* Abundance With Respect to Total Suspended Solids. Left vertical axis represents total suspended solids and periphyton while the right axis represents phytoplankton quantities. Sites (horizontal axis) are oriented from left to right by river mile in a downstream manner.

An attempt was made to represent the combined chlorophyll *a* response (periphyton and phytoplankton), in light of their different units of measure, to a normalized effect of TP by dividing TP by TSS. The underlying thought of this comparison is that the ratio of TP:TSS could portray the effect of TP as "influence-weighted" by the abundance of TSS and that the ratio could be applied as a correction factor to eliminate the observed inhibitory effect of TSS on algal growth. To effectively convey the combined measures of chlorophyll *a* abundance, the differences in units were retained because they currently are interpretable with regard to nuisance level. To put the results on equal basis, each concentration was relativized to maximum and then added. We believe this conveys the relative response of the algal primary productivity. See Figure 7-4 for the resultant relationship of the two artificial variables.

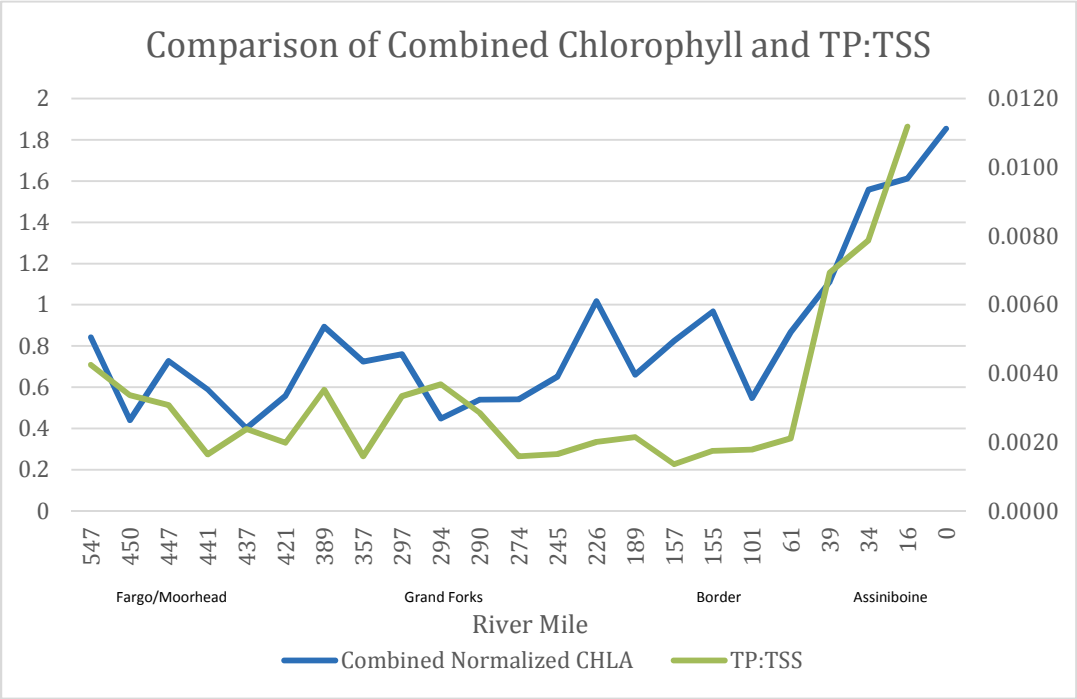


Figure 7-4. Comparison of Combined Chlorophyll *a* Measures From Phytoplankton and Periphyton and TP:TSS Ratio by River Mile.

The TP (as well as TN, following the same trend as TP excluded solely because of scale differences) and TSS concentrations gave an initial indication of zonation along the river sampling reach into distinct Headwater, Middle, and Mouth regions based on regional criteria (Figure 7-2). Within the area indicated as Headwater Reach in Figure 7-2, TSS appeared to be low enough to allow for less impeded periphytic algal growth, yet chlorophyll *a* measures of periphyton did not indicate nuisance levels. TP and TN concentrations within the Headwater zone (RM 547–441) averaged 0.14 and 0.82 mg/L, respectively, while TSS averaged 50 mg/L for this region. Within the Middle Reach (RM 437–155), nutrients reached concentrations normally associated with excessive nuisance algal growth, yet this was not observed. Although nutrient concentrations remain high for the remainder of the river's reach, the Mouth Reach (RM 101-0) of the river was designated as a distinct zone because of the changes in TSS (substantial reduction) and periphytic chlorophyll *a* (substantial increase). Table 7-1 provides averages of nutrients, TSS, and periphytic chlorophyll *a* concentration between each zone.

Table 7-1. Comparison of Red River Zones Based on Averages of Periphyton Chlorophyll *a*, Total Phosphorus, Total Nitrogen, and Total Suspended Solids

Region	Periphyton Chlorophyll *a* (mg/m^2)	Phytoplankton Chlorophyll *a* (µg/L)	TP (mg/L)	TN (mg/L)	TSS (mg/L)
Headwater	26.62	17.12	0.14	1.30	81.50
Middle	31.80	16.34	0.31	1.80	254.26
Mouth	142.81	12.94	0.31	1.58	108.83

Note: Periphyton and total suspended solids data taken from all 30 sites; Phytoplankton, total phosphorus, and total nitrogen taken from the subset of 23 sites

As illustrated in Figure 7-3, the trend of phytoplankton growth appeared to respond to changes in TSS concentration, although the effect was delayed and not always proportional to TSS changes.

To further confirm identifying river water quality and response zones described above, two unconstrained ordination analyses were subsequently performed on pertinent water chemistry and periphyton metric results. These analyses determined the validity of the differences discerned in Figure 7-3 and determined the applicability of the periphyton metrics in relation to the observed trends in the water quality results. NMS was the specific analysis used for this determination. Figure 7-5 is the graphical result of the first NMS on TN, TP, ammonia, and nitrate + nitrite from the 23 stations. Figure 7-6 is an NMS ordination graph specifically on the periphyton metrics of *nitrogen uptake metabolism* and *saprobity* [Van Dam et al., 1996] from all 30 sampling stations. Both tests were verified with Monte Carlo Permutation randomization and were found to be significant on all axes ($P < 0.05$), which indicates that the validity of these analyses accurately portrays the site-based periphytic community differences. Both NMS tests were performed in three dimensions using the Sorensen distance measure. Note that measures of stress for the two tests were 12 and 17, respectively, which indicate that some caution should be used for interpreting ordination values at the upper end, but overall, the imagery provides a usable picture of site variance [McCune and Grace, 2002]. Appendix G contains all of the additional output information related to these analyses.

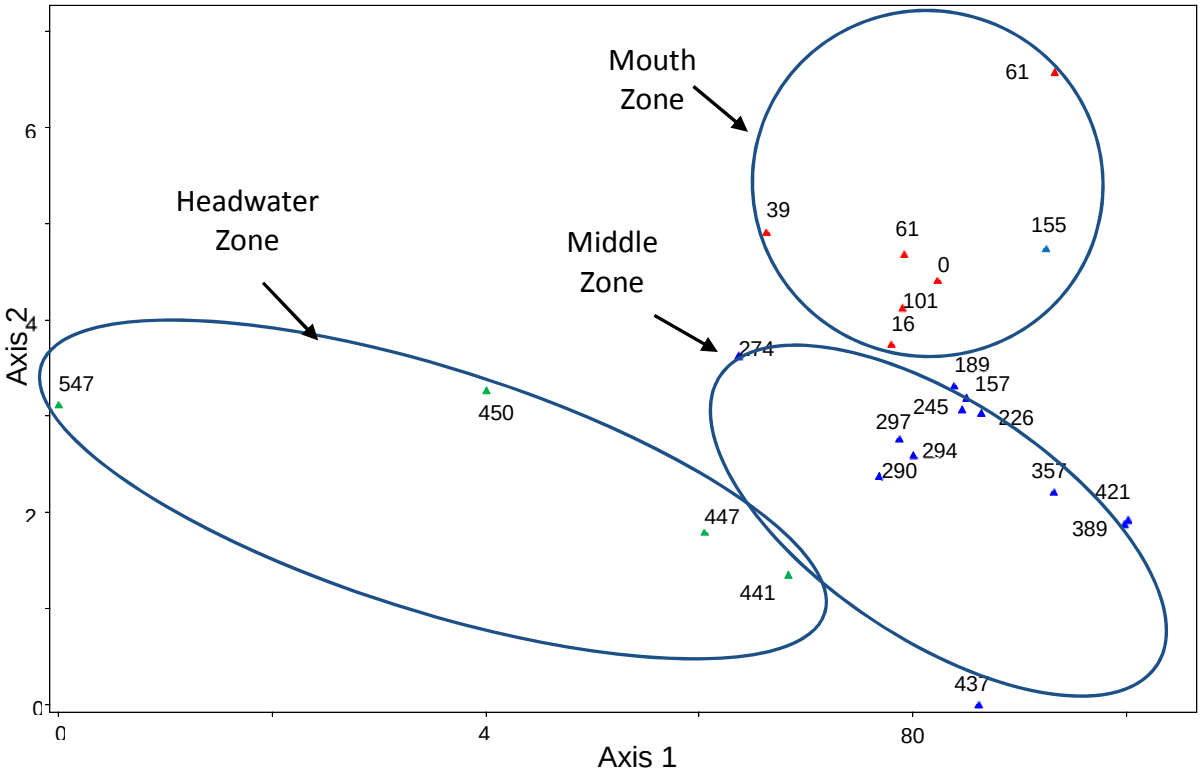


Figure 7-5. Zones of the River as Described by a Nonmetric Multidimensional Scaling Multivariate Analysis of Total Nitrogen, Total Phosphorus, Ammonia, and Nitrate+Nitrite Results From the 23 Stressor-Response Sampling Stations. Symbols represent sampling stations and are labeled by approximate river mile. The proximity of the stations to one another indicate their similarity or difference based on a combined value of all sampled results. Ellipses have been manually added to group stations by perceived zonation. Green symbols are the Headwater zone, blue symbols are the Middle zone, and the red symbols are the Mouth zone.

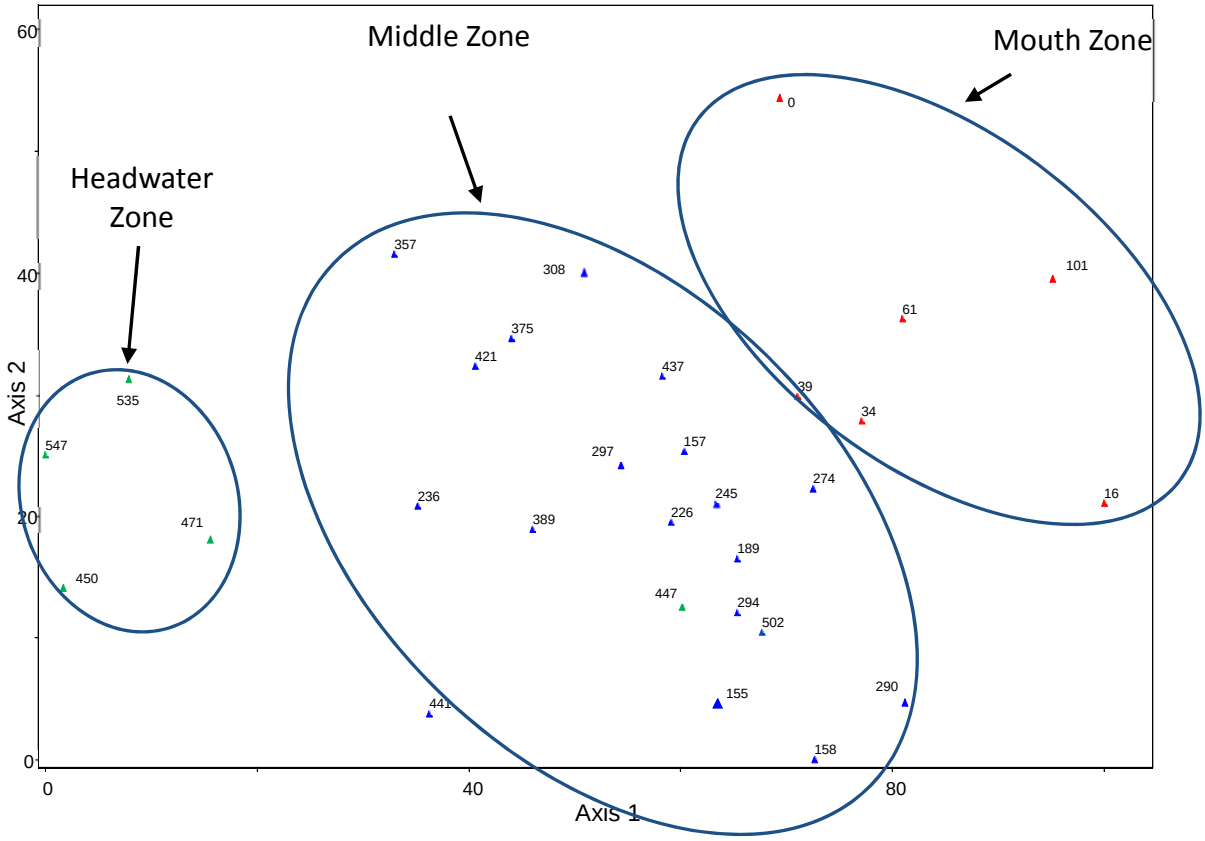


Figure 7-6. Zones of the Red River Watershed as Illustrated by a Nonmetric Multidimensional Scaling Ordination of Periphyton Nutrient and Saprobity Metrics. Green triangles are sites designated as Headwater, blue are Middle zone, and red are Mouth zone.

Overall, a trend of distinction between the previously identified zones was observed from the two NMS ordinations. Regarding the second analysis, an NMS ordination was initially attempted with all measured periphyton metrics and another ordination on all periphyton taxa. The results of these all-inclusive NMS ordinations did not reveal patterns as distinct as the response seen in Figure 7-6, although both analyses distinguished the Headwater sites from the rest of the downstream sites.

The changes in the periphytic algal community in regard to the saprobity and nutrient uptake metabolism metric designations exhibit interesting variation within the identified zones. Using all periphytometer sites, Figure 7-7 indicates a significant reduction in the algal group that has a preference for lower BOD and higher DO occurs between the Headwater and the Middle/Mouth zones. A dominance of the β-mesosaprobous group in the Headwater zone, which have preferences for BOD ranges from 2–4 mg/L^{-1} and DO from 70–85 percent saturation, is replaced by a dominance of the α-meso-polysaprobous groups that have much higher tolerance for increased BOD (13–22 mg/L^{-1}) and reduced DO (10–25 percent) [Van Dam et al., 1996] from an upstream to downstream perspective as listed in Table 7-2.

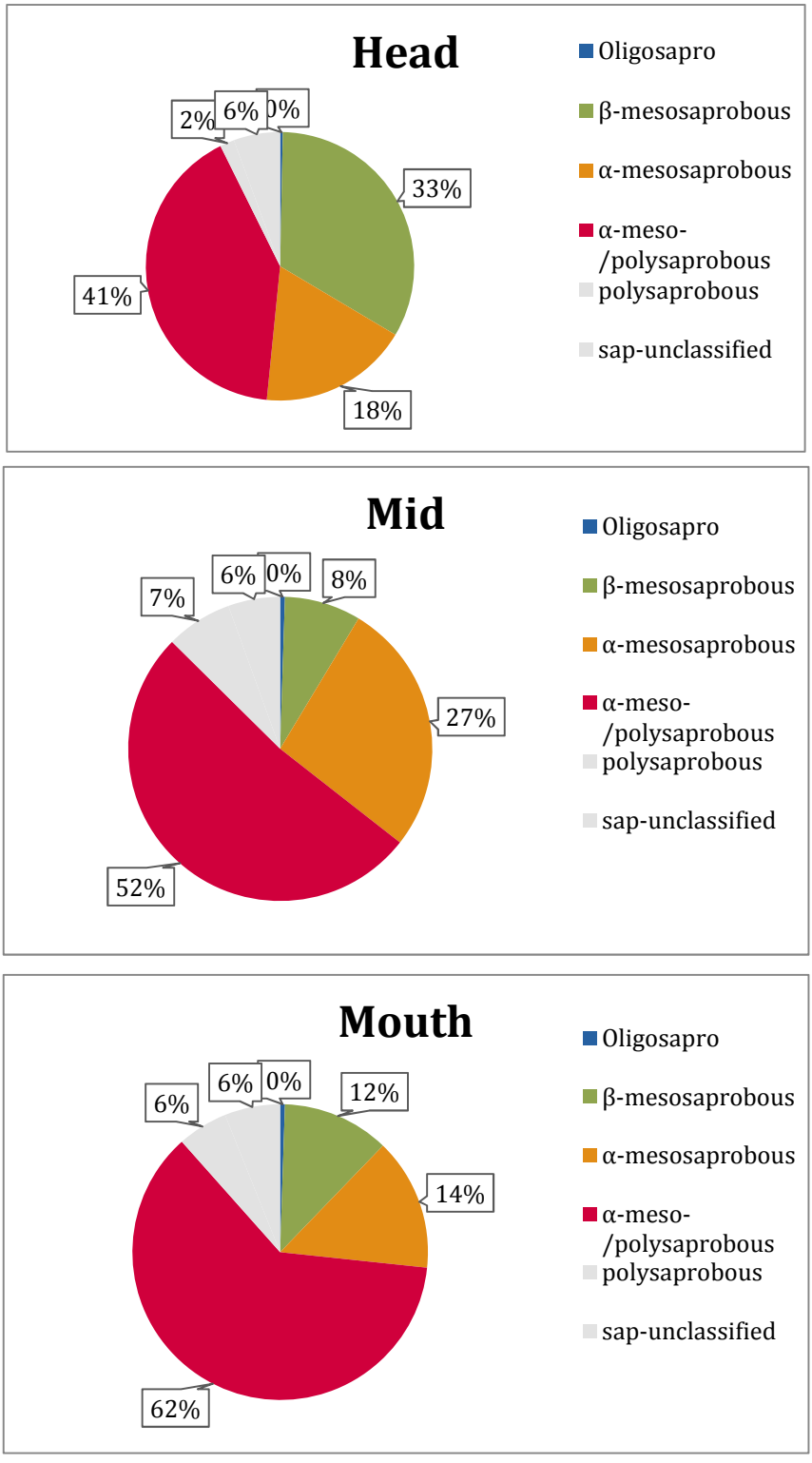


Figure 7-7. Percentages of Saprobity Groupings Within the Three Zones of the Red River of the North. With reference to the legend, increasing saprobity (a measure of response to organic loading) increases from the top down. Based on several analyses, the Headwater reach was designated as the first six sites, the Middle reach comprised the next 19 sites, and the Mouth reach was grouped from the last six sites.

Table 7-2. Periphyton Saprobity Descriptors and Corresponding Water Quality Class, Oxygen Saturation, and Biochemical Oxygen Demand Designations per Van Dam et al. [1996]

	Oxygen Saturation (%)	BOD (mg/L^{-1})
Oligosaprobous	> 85	< 2
β-mesosaprobous	70–85	2–4
α-mesosaprobous	25–70	4–13
α-meso-/polysaprobous	10–25	13–22
Polysaprobous	< 10	> 22

A similar trend to that demonstrated in Figure 7-8 was observed within the nitrogen uptake metabolism metric for periphyton. Again, using the previously described zonation, an upstream to downstream dominance shift was observed within the taxa with regard to their tolerance to excessive nitrogen. Within the Headwater zone, a dominance of the groups *somewhat tolerant* of excessive nitrogen was observed; whereas, by the Middle and Mouth zones, a shift occurred to a dominance of groups *dependent* on excessive nitrogen. A visualization of this trend is illustrated in Figure 7-8 and a description of the nitrogen uptake metabolism groups is provided in Table 7-3.

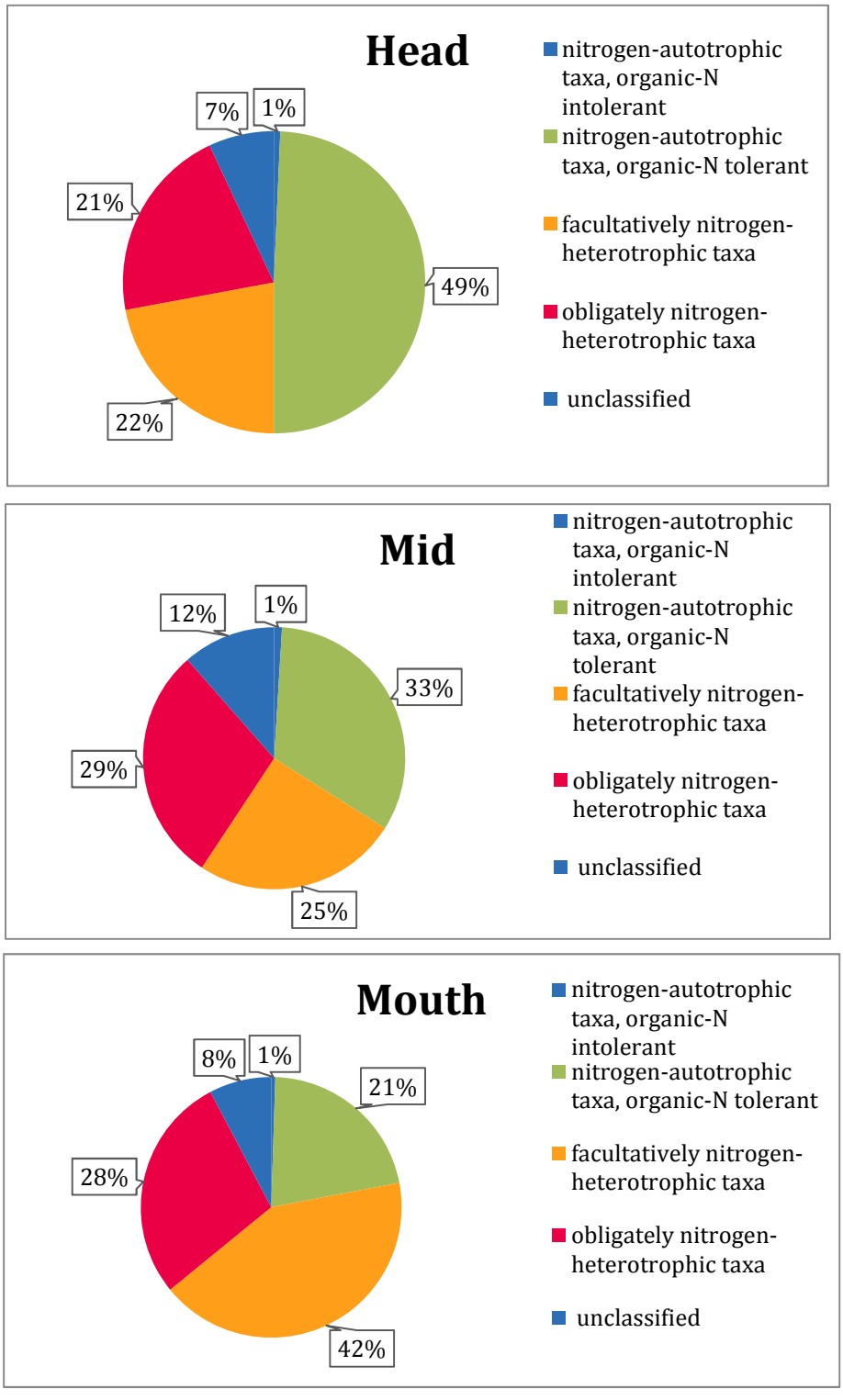


Figure 7-8. Percentages of Nitrogen Uptake Metabolism Metric Groupings by the Red River of the North Zones.

Table 7-3. Categories of Periphytic Nitrogen Uptake Metabolism Groupings and Their Associated Organic Nitrogen Preferences

Nitrogen Uptake Metabolism Group	Organic-N Preference
Nitrogen-autotrophic	Organic-N intolerant
Nitrogen-autotrophic	Organic-N tolerant
Facultatively nitrogen-herteotrophic	Needs periodic elevated organic-N
Obligately nitrogen-heterotrophic	Needs continuous organic-N
Unclassified	N/A

This same zonation trend was even further illustrated through the metric of "Percent Nutrient Tolerance" [Van Dam et al., 1996]. As illustrated in Figure 7-9, significant shifts in an increased percentage of nutrient tolerance was found using the same site groupings as the previous figures.

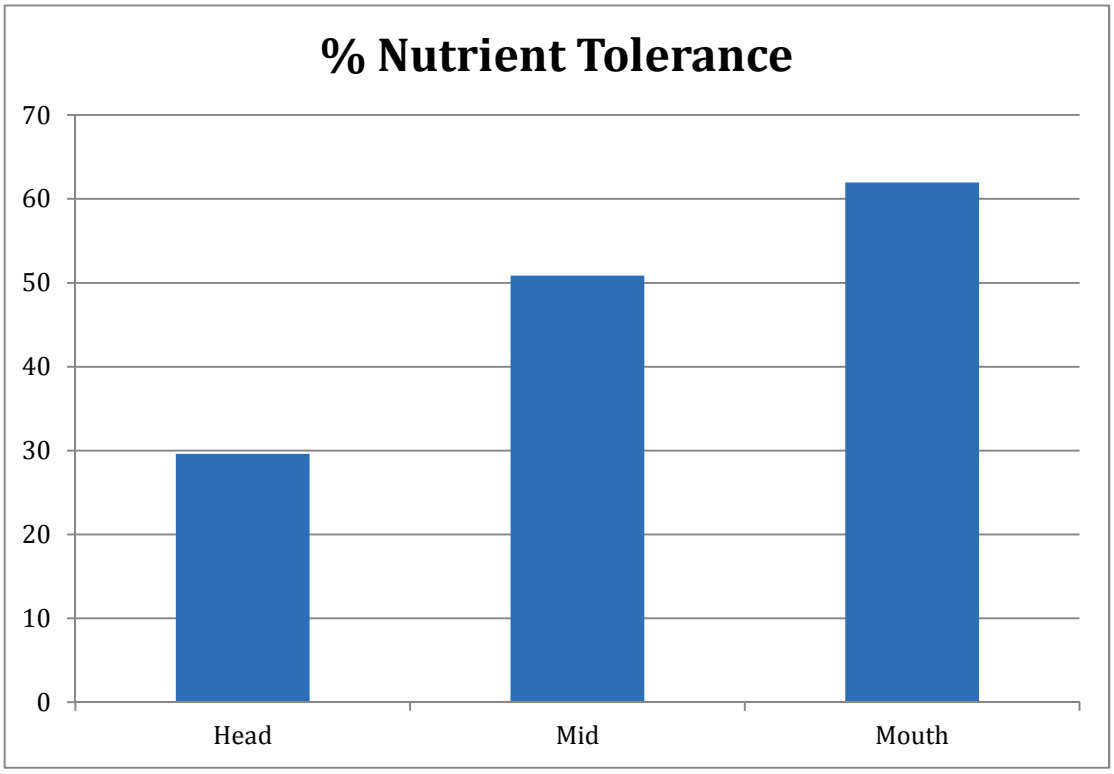


Figure 7-9. Zone Comparison of "Percentage of Nutrient Tolerance" Metric on Periphyton Taxa.

Phytoplankton and periphyton abundance exhibited various peaks along the length of the river, however, the two different methods of abundance measures obtained during the study did not always align (Figure 7-10). This would be expected being that the biomass estimate is such a small snapshot of the sample, yet trends within this result would suggest an estimate of relative changes. Figure 7-10 shows the comparison of chlorophyll *a* from MPCA routine sampling events as compared to the phytoplankton biovolume derived from the phytoplankton taxonomy sample. Most dramatically, a peak of chlorophyll *a*

concentration was found to occur toward the mouth of the river when TSS dropped, yet it also shows evidence of chlorophyll *a* peaks lagging behind declines in TSS concentrations.

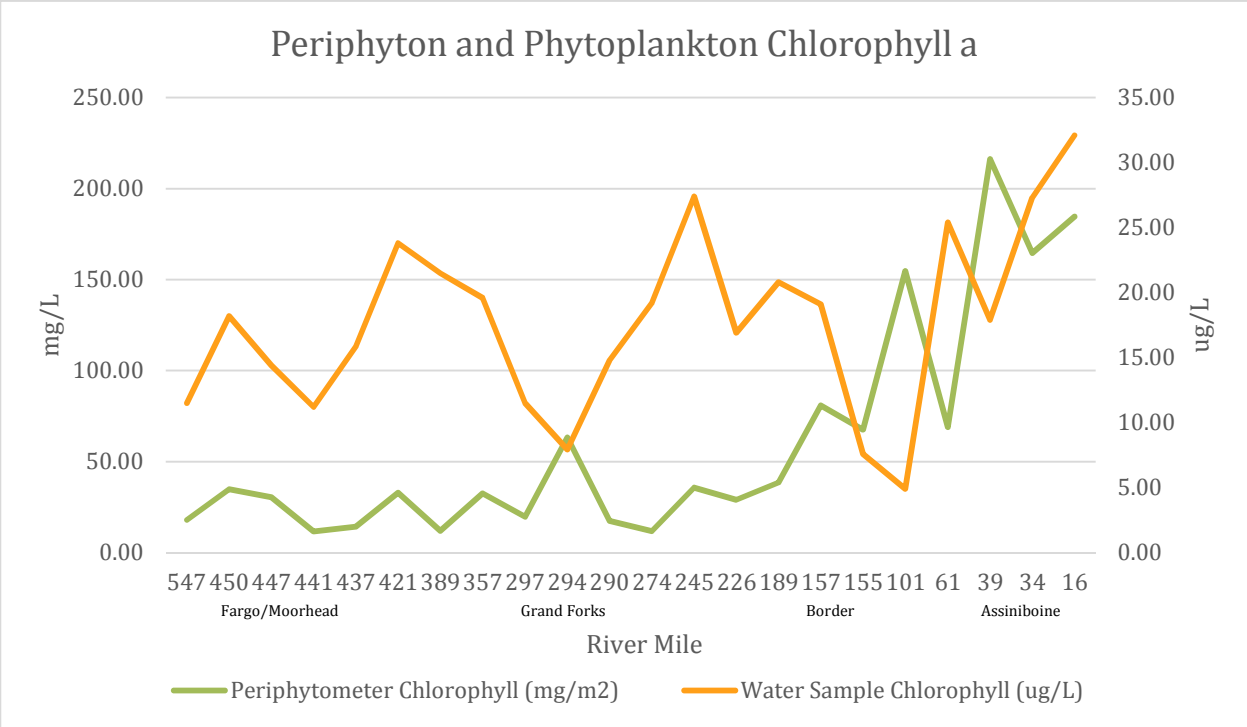


Figure 7-10. Comparison of Normalized Periphyton Chlorophyll *a* Concentrations (mg/m^2) and Phytoplankton Chlorophyll *a* (µg/L) Along the River Gradient. From left to right, the *x*-axis lists sites in an upstream to downstream direction by river mile from the Mouth reach.

The quantity of phytoplankton measured by chlorophyll *a* concentration routinely reached 20 µg/L and occasionally 30 µg/L along the river length. Figure 7-11 indicates that the community of sestonic algae was commonly populated with cyanobacteria species; at times, the percentage of the community was 40 percent and up to 50 percent of the total population, especially within the Headwater and the Mouth regions.

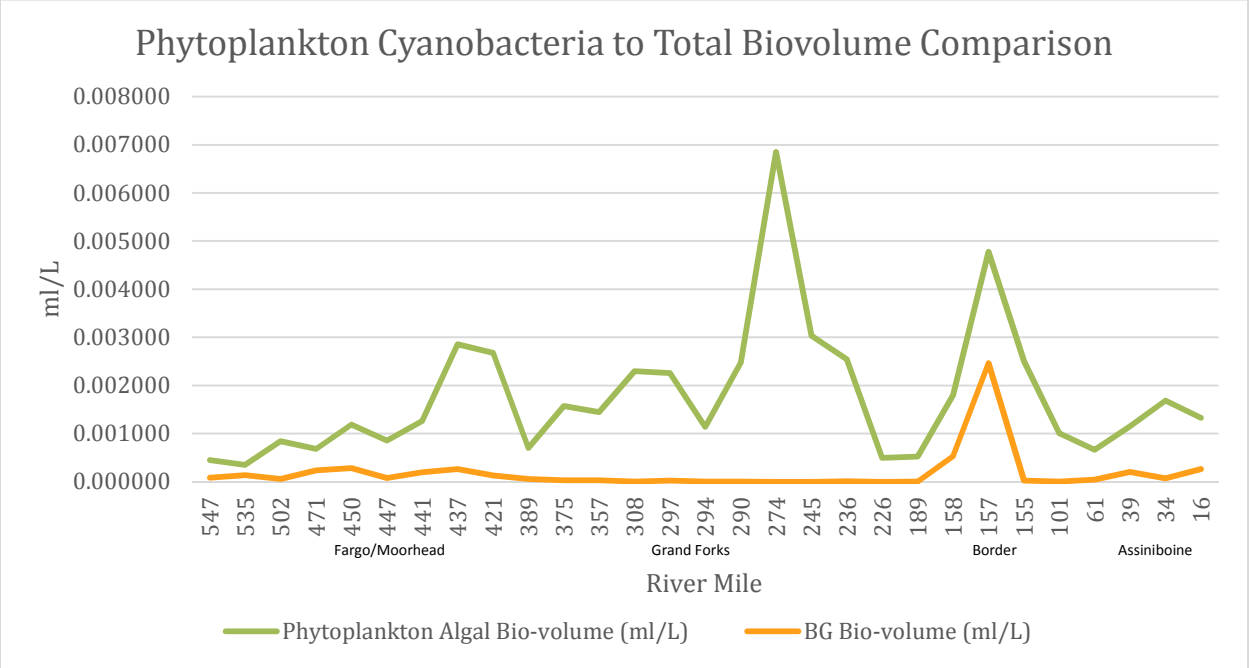


Figure 7-11. Proportional Abundance of Blue-Green Algae as a Function of Total Phytoplankton Algal Biovolume.

Although not measured quantifiably, an estimate of relative abundance was determined for periphytic (nondiatom) cyanobacteria during the taxonomic identification. Sixteen blue-green algae were found commonly within each site's sample. The most common taxa between sites (*Leptolyngbya* sp.) is a known producer of cyanotoxins. Appendix E details all of the observed cyanobacteria collected from the periphytometers and their estimated biovolume rank (1 is most abundant, 16 is least abundant).

7.2.2 Constrained Ordination Analyses

As described in Section 7.1, an attempt to further demonstrate the correlations seen in the above "zone" summaries was made to discern statistically significant correlations between algal taxa variance and measured environmental parameters (i.e., water chemistry and land-use measures).

The collection was accomplished through direct, constrained ordination procedures. The confirmation of visible trends was necessary before determining true algal response to nutrients, primarily because of concerns associated with complications of limited light within the river. Using RDA within CANOCO statistical software [ter Braak and Smilauer, 2012], the forward selection procedures found ecologically meaningful and significant correlations between the water chemistry and land use for both the periphyton and phytoplankton communities. The RDA test was used consistently for the direct ordination procedures because of the consistently short gradient lengths found within the response data (standard deviation < 3) which indicates a linear (not unimodal) data distribution. All RDA tests (including pRDA) were performed using unrestricted permutations on the datasets (499 total).

Controlling for TSS using partial RDA (pRDA; TSS as a covariable) allowed for ascertaining the additional subtle responses by algae to nutrients. Using these techniques, nutrient concentrations from sites having algal communities that were most significantly correlated with the phosphorus and nitrogen (either positively or negatively) were chosen as the basis for nutrient-stressor limits.

The following analysis results present a unique methodology for discerning nutrient effects on algal abundance and diversity in light of varying TSS concentrations in the river.

7.2.2.1 *Phytoplankton*

A pRDA model was used on the phytoplankton taxa and water chemistry to account for the variance specifically associated with TSS, yet to extract it from the model so that subtler influences (i.e., nutrients) could be more fully described. For the predictor variables (water chemistry), autocorrelated nutrient constituents were removed before the analysis to reduce noise in the dataset. Within the response variables, only taxa with at least three occurrences were used because at least three points are needed to define a trend. This data culling resulted in 49 taxa being used in the analysis after eliminating rare species. All data (species and chemistry) were subsequently relativized by maximum to minimize the dominance of large numbers resulting from dominant taxa or various units of measure [McCune and Grace, 2002].

The results of this pRDA yielded a significant model (First axes, P = 0.05; Full model, P = 0.002) when constrained by TP and TN. These chosen chemical parameters were significantly correlated with (or "explained") 16 percent of the community variance between the 23 sampling sites (Table 7-4). The relationship of the site-specific taxa response to the significant parameters is illustrated in Figure 7-12.

Table 7-4. Summary Statistics for Partial Redundancy Analysis (Total Suspended Solids as Covariable) of Phytoplankton Taxa and Water Chemistry

Statistic	Axis 1	Axis 2	Axis 3	Axis 4
Eigenvalues	0.0899	0.0638	0.1380	0.0951
Explained variation (cumulative)	9.16	16.30	31.75	42.39
Pseudo-canonical correlation	0.8519	0.7610	0	0
Explained fitted variation (cumulative)	56.2	100		

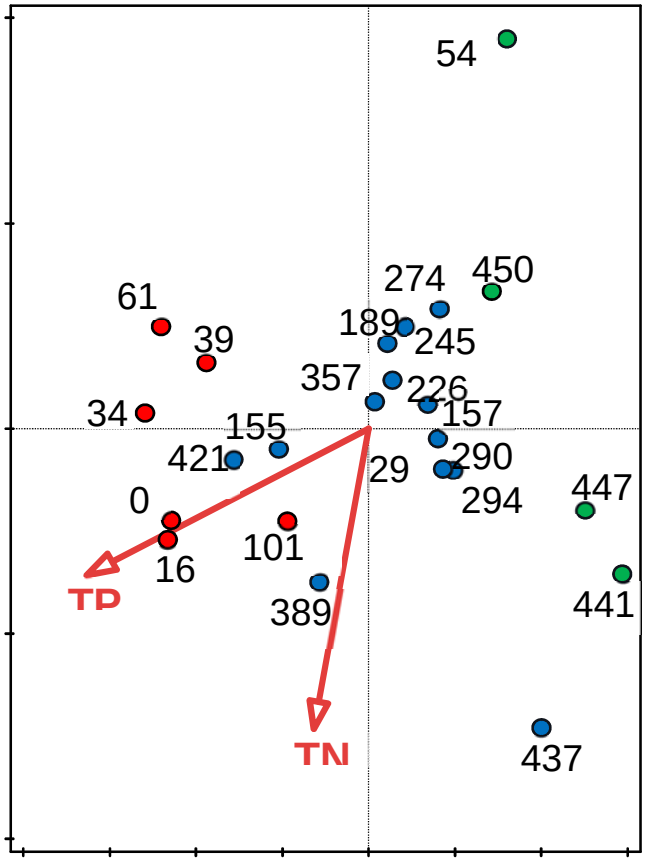


Figure 7-12. Partial Redundancy Analysis of Site-Specific Phytoplankton Taxa Against Water Chemistry (Total Suspended Solids as Covariable). Nutrient metrics (arrows) point in the direction of their highest concentration (and vice versa). The location of site symbols with respect to these vectors indicate the influence of the nutrients on the taxa at a site (and the site's overall relative nutrient concentration). Green dots are Headwater sites, blue dots are Middle sites, and red dots are Mouth sites. Site labels are river miles from the Mouth reach.

As seen in Figure 7-12, the algal communities from sites at RM 0, 16, and 34 had the highest positive correlation with TP while the sites at RM 389, 437, and 441 had the highest positive correlation with TN. Algal communities from sites at RM 547, 450, and 274 had the highest negative correlation with both TP and TN. Table 7-5 details the average nutrient values for these sites.

Table 7-5. Average Nutrient Concentrations From Sites (Algal Communities) Having Most-Significant Positive and Negative Correlations to Total Phosphorus and Total Nitrogen as Exhibited in the Figure 7-12 Partial Redundancy Analysis

Site Nutrient Association	TP Average (mg/L)	TN Average (mg/L)
Positive	0.31	1.91
Negative	0.15	1.15

The results of the phytoplankton taxa/land-use RDA resulted in even more explained variance within the communities. The site-specific land-use parameters of percent riparian wetlands, percent high-nutrient crops, water residence time, percent forest, and the application of N and P fertilizers were found to have a significant correlation (P = 0.002) with 41 percent of the phytoplankton community variance as indicated in Table 7-6. The associations are shown in Figures 7-13 through 7-18.

Table 7-6. Statistical Summary of Phytoplankton/Land-Use Redundancy Analysis

Statistic	Axis 1	Axis 2	Axis 3	Axis 4
Eigenvalues	0.1523	0.1153	0.0839	0.0589
Explained variation (cumulative)	15.23	26.76	35.15	41.05
Pseudo-canonical correlation	0.9393	0.9381	0.9355	0.9287
Explained fitted variation (cumulative)	32.02	56.25	73.88	86.27

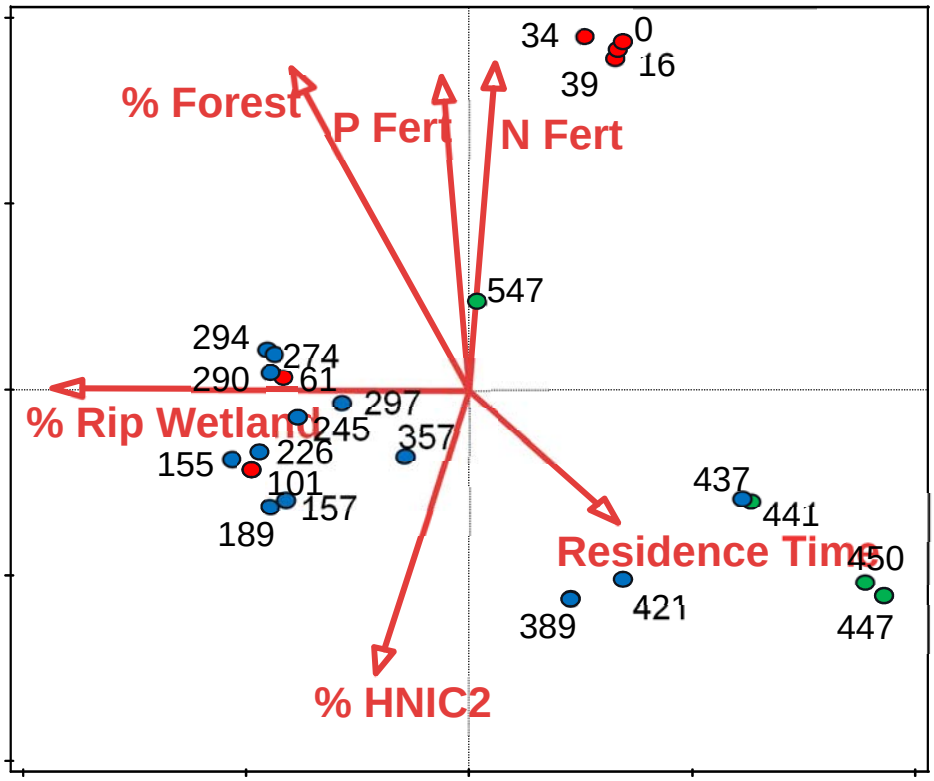


Figure 7-13. Diagram of Phytoplankton/Land-Use Redundancy Analysis. Land-use variable abbreviations are percentage of forest within site-specific drainage (% Forest), quantity of applied phosphorus or nitrogen fertilizer in the site-specific drainage (P Fert and N Fert, respectively), site-specific percentage of wetland within 500 meters of the each side of the river (% Rip Wetland), the estimated time of water residence within each sampling reach (Residence Time), and the site-specific percentage of High-Nutrient Intensity Crops (2) per the SPARROW model designation (% HNIC2). Green symbol color = Headwater zone, blue = Middle zone, red = Mouth zone.

7.2.2.2 *Periphyton*

The results of the periphyton taxa/chemistry pRDA (TSS as covariable) also yielded a significant explanatory model. The parameters used were TP and TN. Seventy-two taxa remained after removing rare taxa. The pRDA model explained 15 percent of the site-specific species variance and was deemed significant at P = 0.014 (first axes) and P = 0.004 (full model) (Table 7-7 and Figure 7-14).

Table 7-7. Results of Periphyton Taxa/Chemistry Partial Redundancy Analysis

Statistic	Axis 1	Axis 2	Axis 3	Axis 4
Eigenvalues	0.0868	0.0563	0.1237	0.0836
Explained variation (cumulative)	9.35	15.41	28.73	37.73
Pseudo-canonical correlation	0.8398	0.8794	0	0
Explained fitted variation (cumulative)	60.66	100	0	0

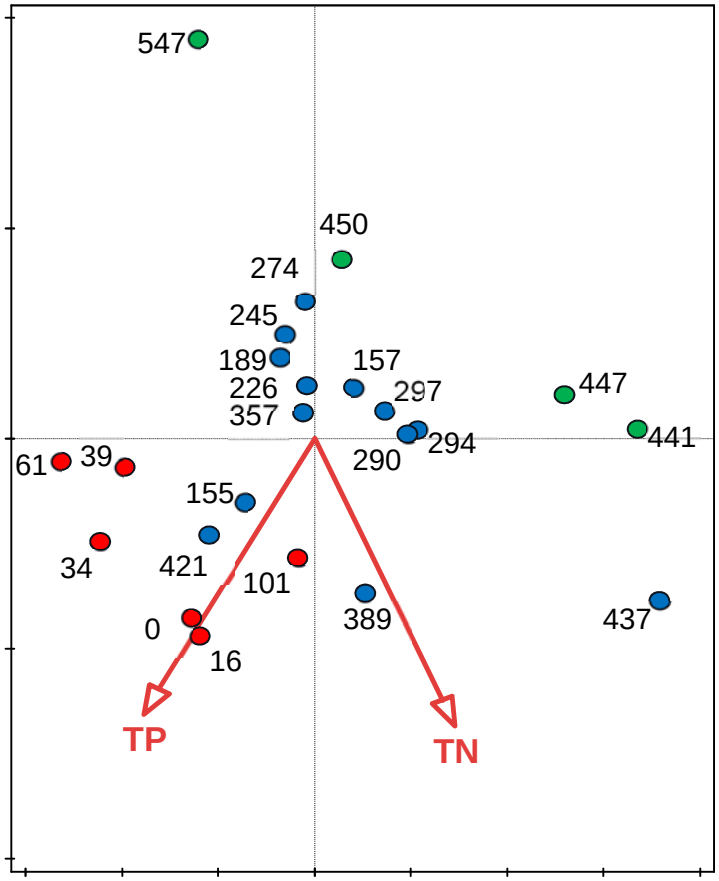


Figure 7-14. Ordination Diagram of Periphyton Taxa/Chemistry Partial Redundancy Analysis.

The location of the periphytometers at the water surface should have ensured adequate surface light, although the possibility of limited light appeared to remain because no other explanations were apparent to describe the limited algal growth in the presence of abundant nutrients. This observation possibly occurred through the attenuation of subsurface light and reduction in water column light scattering. Because of the complications of discerning algal response to nutrient concentrations with respect to

TSS/light limitation in the Red River as previously described, the results of this analysis are a direct indication as to which site's taxa were responding directly to nutrients (both positively and negatively). Subsequently, the physiological responses of those site-specific taxa associated with the nutrient concentrations can be verified by reviewing at the metrics between the sites chosen to be most highly correlated with high nutrients versus the metrics at low nutrient sites (from the ordination layout). If the metric descriptions regarding preference to nutrients agree with the phosphorus and nitrogen correlations, then the nutrient concentrations found at these sites can be assumed to represent true influences of growth and further assumptions regarding these nutrient concentrations can be made. Tables 7-8 and 7-9 summarize this information.

Table 7-8. Summary of Phosphorus-Specific Periphyton Metrics and Water Quality Results Associated With Positive and Negative Correlations Determined From Partial Redundancy Analysis Shown in Figure 7-14

RMI	Response to TP	TP (avg mg/L)	TSS (mg/L)	Periphyton Chlorophyll *a* (mg/m^3)	Pollution Index	General Nutrient Metrics		Saprobity Metrics						Nitrogen Uptake Metabolism Metric				
						Nutrient Increase	% NUTTOL	Oligo-saprobous	β-meso-saprobous	α-meso-saprobous	α-meso-/poly-saprobous	Poly-saprobous	Sap-unclassified	Nitrogen-autotrophic taxa, organic-N intolerant	Nitrogen-autotrophic taxa, organic-N tolerant	Facultatively nitrogen-hetero-trophic taxa	Obligately nitrogen-hetero-trophic taxa	Unclassified
34	Positve	0.34	192	154.75	1.45	28.83	82	0.5	3.5	6.67	83.67	1.33	4.33	0.5	6	30.33	57.17	6
0	Positve	0.32	88.67	184.67	1.59	39	43	1.17	6.83	45	39	5.33	2.67	1.17	35.67	51.67	7.17	4.33
16	Positve	0.30	53	164.67	1.59	54.5	59.5	0	11.33	8.5	64.17	8.67	7.33	0.33	25.5	51.17	15.33	7.67
Averages		**0.32**	**111.22**	**168.03**	**1.54**	**40.78**	**61.5**	**0.56**	**7.22**	**20.06**	**62.28**	**5.11**	**4.78**	**0.67**	**22.39**	**44.39**	**25.56**	**6**
547	Negative	0.07	50.33	23.33	2.43	18.5	13.33	0	49.67	21.33	13.83	2	13.17	0.5	50.67	24.5	6.17	18.17
450	Negative	0.14	75.67	17.90	2.21	20.33	12.17	0.33	49.67	14.83	30.83	1.17	3.17	0.5	72.33	20.83	2.5	3.83
274	Negative	0.23	193.33	17.37	1.36	29.83	67.33	0	2	28	50.17	18.67	1.17	0	23.17	35.5	39.33	2
Averages		**0.15**	**106.44**	**19.53**	**2.00**	**22.89**	**30.94**	**0.11**	**33.78**	**21.39**	**31.61**	**7.28**	**5.84**	**0.33**	**48.72**	**26.94**	**16.00**	**8.00**

Table 7-9. Summary of Nitrogen-Specific Periphyton Metrics and Water Quality Results Associated With Positive and Negative Correlations Determined From Partial Redundancy Analysis Shown in Figure 7-14

Site	Response to TN (and NO3+NO2)	TN (mg/L)	NO3+NO2 (mg/L)	TSS (mg/L)	Periphyton Chlorophyll *a* (mg/m3)	Pollution Index	General Nutrient Metrics		Saprobity Metrics						Nitrogen Uptake Metabolism Metric				
							Nutrient Increase	% NUTTOL	Oligo-saprobous	β-meso-saprobous	α-meso-saprobous	α-meso-/poly-saprobous	Poly-saprobous	Sap-unclassified	Nnitrogen-autotrophic taxa, organic-N intolerant	Nitrogen-autotrophic taxa,organic-N tolerant	Facultatively nitrogen-hetero-trophic taxa	Obligately nitrogen-hetero-trophic taxa	Unclassified
389	Positive	2.11	0.56	300	32.90	1.37	32.5	33.17	0.33	7	12.67	71	2	7	0.33	52.83	28.83	10.83	7.17
437	Positive	1.95	0.88	130	*11.53*	1.42	48.83	46.83	0.5	12.83	13.83	62	6.33	4.5	0.5	39.5	43.83	11.33	101.33
16	Positive	1.61	0.36	53	164.67	1.46	54.5	59.5	0	11.33	8.5	64.17	8.67	7.33	0.33	25.5	51.17	15.33	7.67
0	Positive	1.65	0.35	88.67	184.67	1.59	39	43	1.17	6.83	45	39	5.33	2.67	1.17	35.67	51.67	7.17	4.33
Averages		**1.83**	**0.54**	**142.92**	**98.44**	**1.46**	**43.71**	**45.63**	**0.50**	**9.50**	**20.00**	**59.04**	**5.58**	**5.38**	**0.58**	**38.38**	**43.88**	**11.17**	**30.13**
547	Negative	0.78	0.12	50.33	23.33	2.43	18.5	13.33	0	49.67	21.33	13.83	2	13.17	0.5	50.67	24.5	6.17	18.17
274	Negative	1.47	0.32	193.33	17.37	1.36	29.83	67.33	0	2	28	50.17	18.67	1.17	0	23.17	35.5	39.33	2
450	Negative	1.21	0.27	75.67	17.9	2.21	20.33	12.17	0.33	49.67	14.83	30.83	1.17	3.17	0.5	72.33	20.83	2.5	3.83
Averages		**1.15**	**0.24**	**106.44**	**58.6**	**2.0**	**22.89**	**30.94**	**0.11**	**33.78**	**21.39**	**31.61**	**7.28**	**5.84**	**0.33**	**26.94**	**26.94**	**16**	**8**

The nutrient concentrations from the sites included on Table 7-8 and 7-9 represent the most-significant negative and positive correlations with the nutrient vectors shown in Figure 7-14. As with the phytoplankton, the nutrient results shown in the tables for each site were averaged from two collection dates from samples collected by the MPCA and the province of Manitoba during the deployment period of the periphytometers. Although not available with the phytoplankton because of an overall absence of developed metrics, quality metrics were calculated from the periphyton species data based on published criteria [Van Dam et al., 1996; Potapova et al., 2004]. Using the stations selected from the ordination as having the most significant correlations with nutrients, Figures 7-15 through 7-17 show the response of selected metrics to the averaged nutrients from the sites. The "positive" and "negative" categories refer to the grouping of sites (and their respective taxa metrics) that responded either positively or negatively to the referenced nutrient.

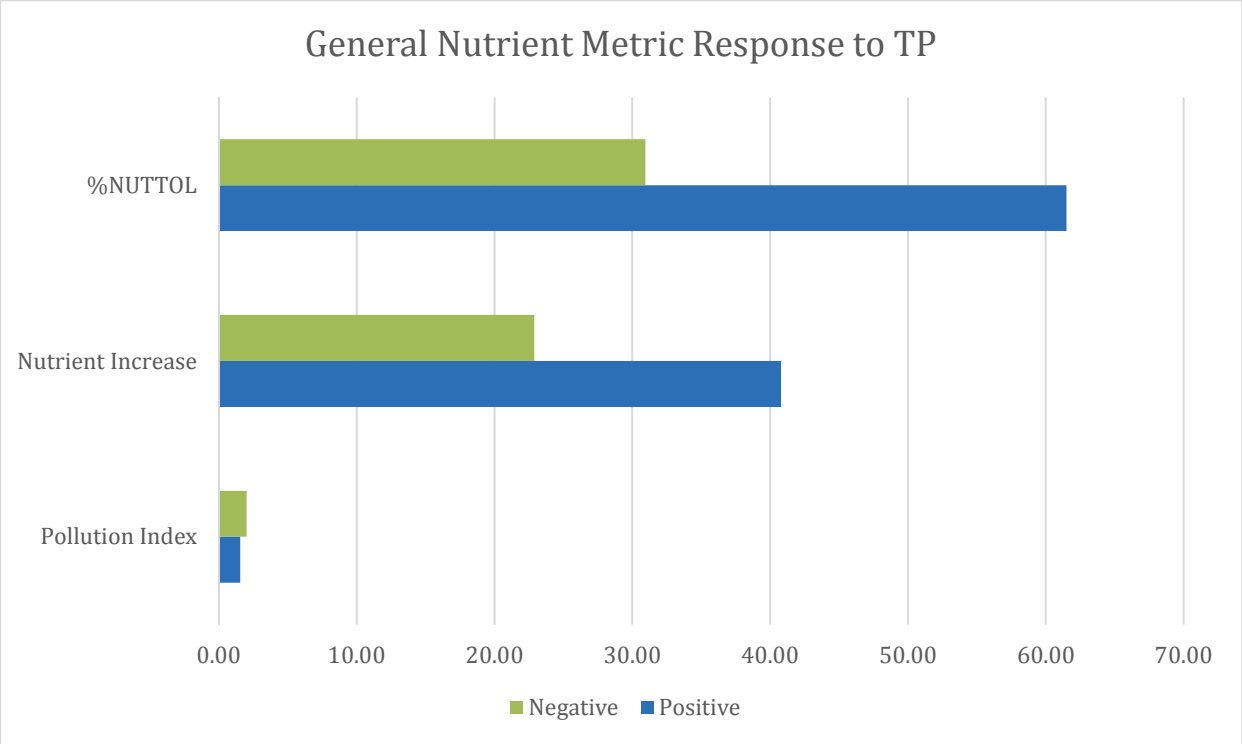


Figure 7-15. The Response of %NUTTOL (Percent Nutrient Tolerant Organisms) and Nutrient Increase (Metric Related to Preference to Increasing Nutrient) Algal Metrics Within Sites Positively and Negatively Correlated With Increasing Phosphorus Concentrations. Data taken from Table 7-8.

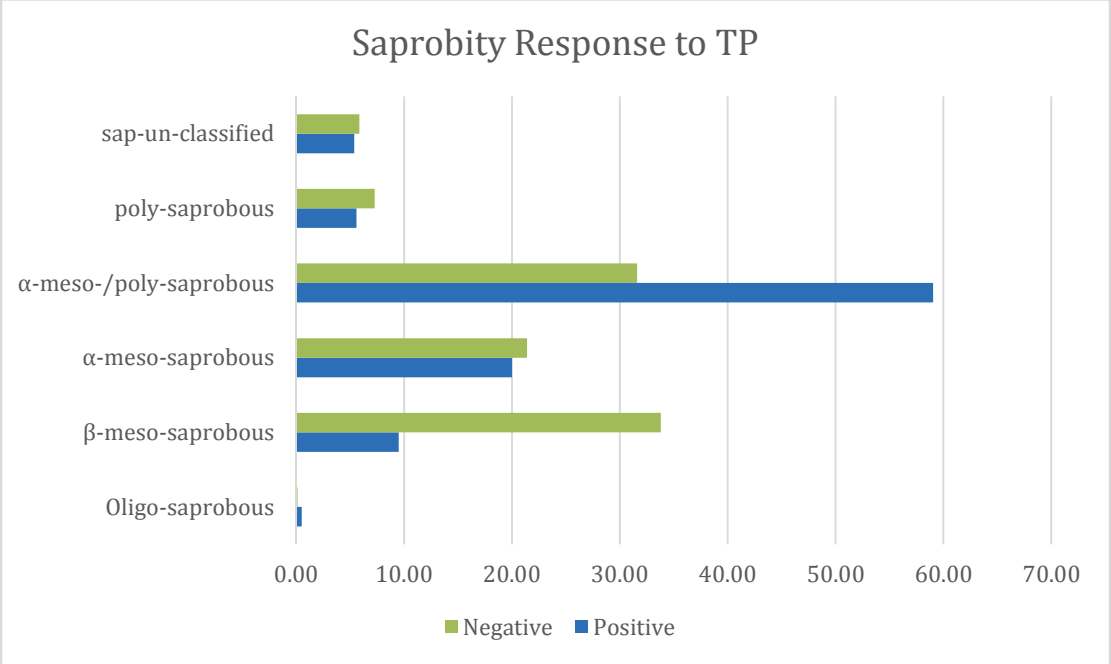


Figure 7-16. The Saprobity Response (Measure of Response to Organic Loading) of Algae From Selected Sites Both Positively and Negatively Correlated With Increasing Total Phosphorus Concentrations. Data taken from Table 7-8.

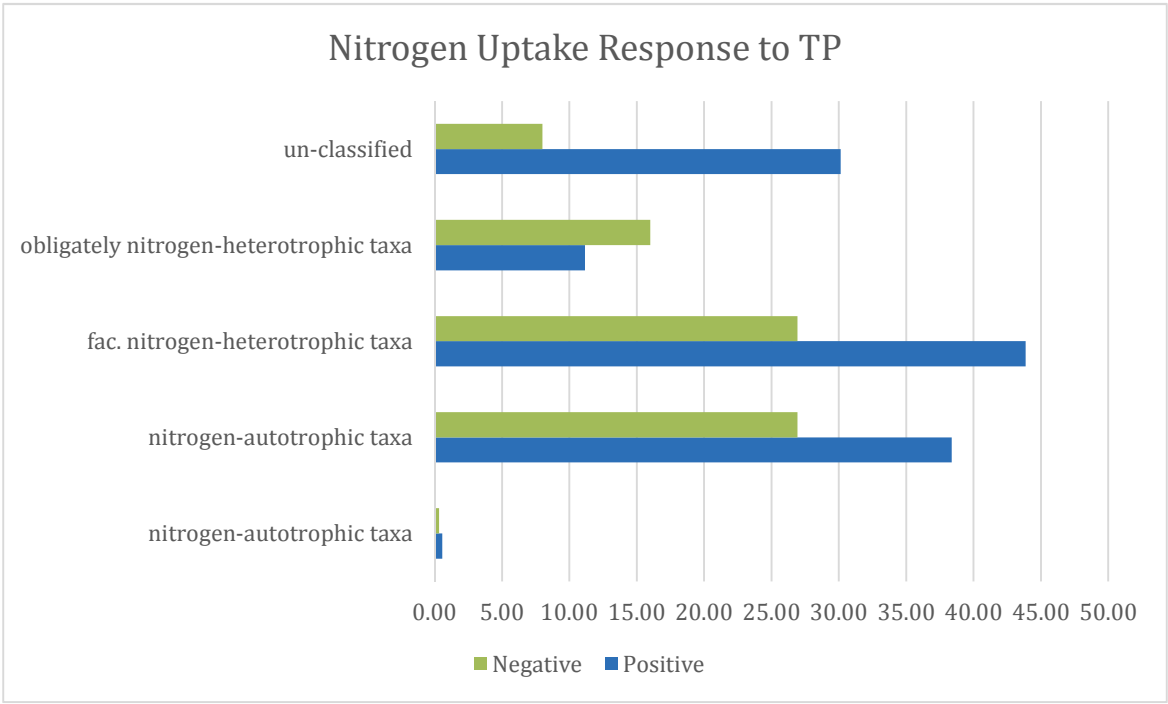


Figure 7-17. Nitrogen Uptake Metabolism Metric Relationships Between Sites Having Positive and Negative Correlations With Increasing Total Phosphorus Concentrations. Information taken from Table 7-9.

As with phytoplankton, an attempt to discern additional variance in the periphyton taxa between sites was made using forward selection in RDA on land-use variables from the SPARROW model dataset (Appendix A). The selection process chose the following five site-specific land-use attributes as significant:

- Percentage of urban/impervious area in watershed
- Percentage of high-nutrient intensity crop
- Percent of forest
- Percentage of grassland
- Percentage of barren land.

These parameters were significantly associated with 35 percent of the variance in the periphyton community between sites (Table 7-10). The site-level associations with the significant parameters are shown in Figure 7-18.

Table 7-10. Statistical Summary of Land Use-Based Redundancy Analysis on Periphyton Taxa

Statistic	Axis 1	Axis 2	Axis 3	Axis 4
Eigenvalues	0.1516	0.079	0.064	0.0558
Explained variation (cumulative)	15.16	23.06	29.45	35.03
Pseudo-canonical correlation	0.9563	0.9503	0.9045	0.8846
Explained fitted variation (cumulative)	39.56	60.17	76.86	91.42

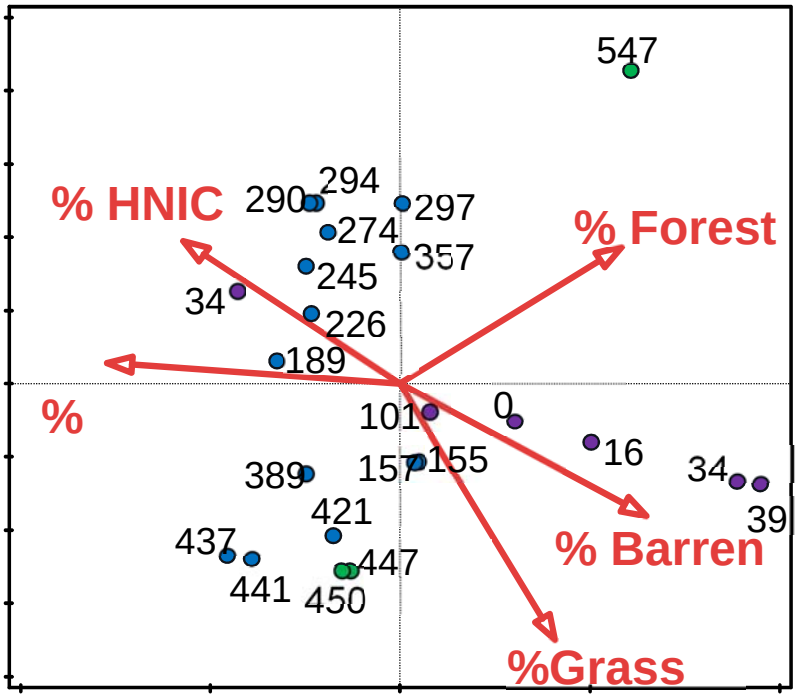


Figure 7-18. Redundancy Analysis on Periphyton Taxa Constrained by Significant Land-Use Parameters. Land-use parameters are site-specific percentage of high-nutrient intensity crops (% HNIC), site-specific percentage of forest (% Forest), urban/imperviousness (% Urban), grassland (% Grass), and barren land (% Barren) per SPARROW model designation.

Finally, to further illustrate the effect of TSS and nutrients on periphyton growth (abundance), an NPMR was performed on the periphyton chlorophyll *a* abundance (mg/m^2) and all site-specific water chemistry and land-use parameters. Using the free model search function within NPMR, the best fit R^2 (0.67) was selected for a model associated with the percentage of open water in the site-specific drainage and total nitrogen (mg/L). Figure 7-19 is a three-dimensional graph of the association.

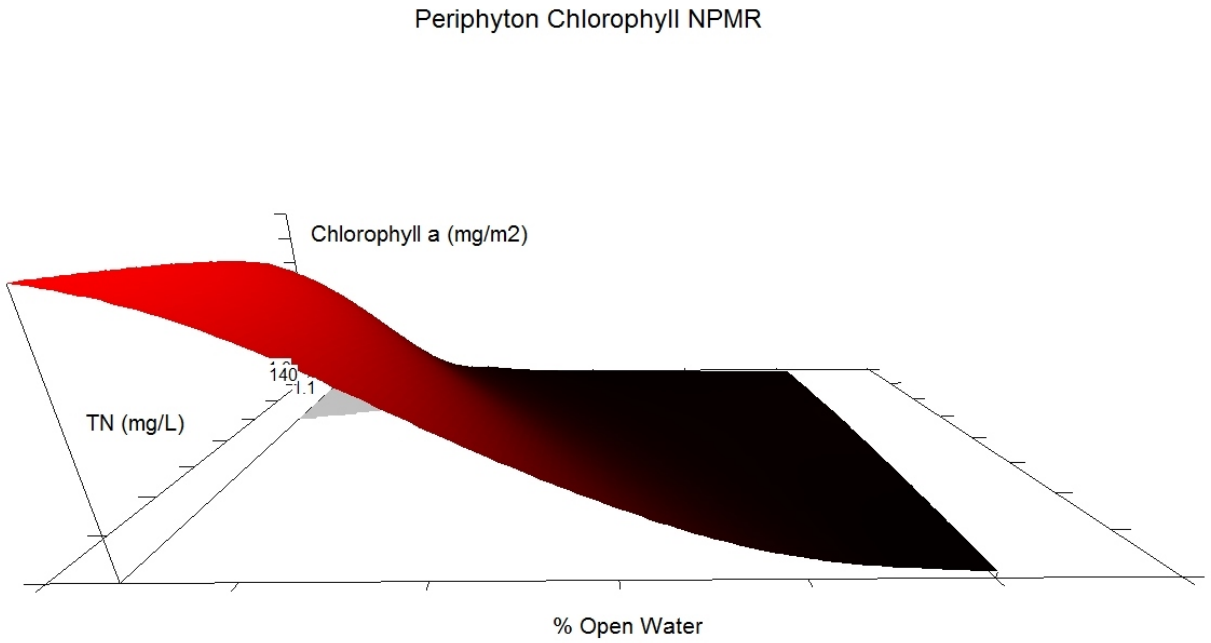


Figure 7-19. Graph of Nonparametric Multiplicative Regression Model of Periphyton Chlorophyll *a* Abundance (mg/m^2) With Percentage of Open Water (% Open Water) and Total Nitrogen (TN mg/L).

7.3 DISCUSSION

The innate complications of fully describing a biological stressor response because of excessive nutrients in a large-order river (>6) are significant. Large rivers seemingly defy typical smaller-order river stressor responses to nutrient targets and, traditionally, nutrient limits have been more commonly dictated by loading to a downstream lake. Even in more traditional lotic environments, the effects of eutrophication are difficult to ascertain on the biological communities because the responses are indirect and cascading within the food chain and are significantly influenced by flow—with regard both to physical disruption and reduction in nutrient uptake. Within the Red River, the additional complexity because of excessive turbidity and resulting light limitation makes the task of discerning a nutrient stressor much more difficult.

7.3.1.1 *Concerns of Excessive Algal Growth*

Typically, in aquatic systems with excessive algal growth, the most measureable negative effects are associated with wide diurnal oscillations in DO and potential alteration of food resources and habitat structure. Regarding DO fluctuations, an increase in primary production from the algal community and associated increases in daytime photosynthetic oxygen production is accompanied by subsequent increases in bacterial respiration of the decaying algal biomasses, which leads to excessive oxygen

consumption (and associated BOD elevation). During the night when photosynthesis ceases, oxygen levels can drop to concentrations that are stressful to most truly aquatic life (< 5 mg/L) [USEPA, 1986]. These factors combine to shift endemic biological communities toward a dominance of taxa tolerant of low DO, which are typically less desirable.

Another issue of concern with excessive nutrient inputs and subsequent algal growth is related to the growth of cyanobacteria that can produce algal toxins that are harmful to aquatic life and humans [Dodds and Welch, 2000]. The USEPA [2015] issued a recent recommendation that health advisories be issued for drinking water with microcystin-LR concentrations at or above 0.3 µg/L. Although these issues are uncommon for large rivers, values noted in Lake Erie during the early August 2012 Toledo, Ohio, municipal water ban briefly reached 2.5 µg/L of microcystin-LR. Cyanobacteria blooms specifically in rivers have been documented in the St. Johns River in Florida (*www.sjrwmd.com/algae*) and by numerous other water quality managers (summarized by Hilton and Irons [1998]). With regard to the concern of the human exposure of cyanotoxin-producing cyanobacteria, the USEPA states that "effects including gastroenteritis and liver and kidney damage have been reported in humans following short-term exposure to cyanotoxins in drinking water. Recreational exposure to cyanobacterial blooms has been reported to lead to allergic reactions, including hay fever-like symptoms; skin rashes; and gastrointestinal distress. Animal studies have shown that long-term adverse effects from cyanotoxins include liver and kidney damage. However, more research is needed to quantify these effects."

Within lentic environments, algae blooms are a well-documented effect of eutrophication; however, in rivers, complications arise because of constant water flow/mixing and the limitations of algae to capture the moving resource [Dodds et al., 1998]. Within rivers like the Red River, the further complication of excessive limited light that result from high levels of suspended sediment significantly increases the complexity of the eutrophication impact. Until this study, both attached (periphyton) and sestonic (phytoplankton) forms of algal growth and subsequent issues with BOD/DO were thought to be minimal because of this significant characteristic of the Red River. The results from the 2015–2016 stressor-response study data and a survey of previous water quality results indicate that this is not the case. Significant quantities of algae with varying community quality were found that exhibited direct response to nutrient concentrations with an occasional dominance of cyanobacteria. In conjunction (although not measured in this study) the results from both the NDDH and the MPCA indicate elevated BOD (organics) and low DO are occasionally observed (discussed below). The Minnesota 303(d) listing of three DO-impaired reaches would seem to reinforce this conclusion.

7.3.1.2 *Stressor Response Demonstration*

Using the guidelines provided by the USEPA for deriving numeric nutrient criteria using stressor-response relationships [USEPA, 2010], all four steps described below were used to ensure that the model was properly developed:

1. Development of a conceptual model
2. Exploratory analysis
3. Estimation of stress-response relationships
4. Determination of accuracy and precision.

After developing the conceptual model as described in Chapter 4.0, the exploratory analysis was performed. This aspect of the model development was divided into several steps. First, simple graphing techniques were used to understand the river's nutrient, sediment, and algae trends using the collected data. Demonstrated in Figure 7-2, this step was necessary to establish the existence of gradients; the absence of which would prevent establishing correlations between the response group (algae) and the stressor (nutrients).

With regard to nutrients, suspended sediment, and periphytic algal response, gradients were readily apparent because the river appeared to divide into three distinct zones, as illustrated in Figure 7-2. The distinct combination of the lowest nutrient and TSS concentrations with low periphyton abundance distinguished the Headwater zone (from the Headwater to RM 441) from the rest of the river (see Table 7-1). The Middle zone (RM 437–155) was unique in its consistently high nutrients and TSS concentrations with low periphyton densities, while the Mouth zone (RM 101–0) distinguished itself from the upstream zones by having considerably lower TSS concentrations and marked increased periphyton abundance. The location and source of this zonation influence is clearly distinguished by the successive introduction of known point and scattered nonpoint sources within the Middle reach combined with a significant inflow of lowered TSS water in Winnipeg from the Assiniboine River.

With reference to the river zonation, Figure 7-2 gives indication that, pending available substrate for colonization, periphytic algae can and does reach nuisance concentration toward the Mouth of the river (100–150 mg/m^2). At the Manitoba sampling site near RM 61, periphyton density reached 154 mg/m^2 and eventually peaked at 216 mg/m^2 near RM 34 before reaching Lake Winnipeg. These periphyton densities significantly exceed levels of 100 mg/m^2 [Dodds, 2006] and 150 mg/m^2 [Heiskary and Parson, 2013] previously described as regional nuisance levels. Previous studies indicate that these and many other site results are indicative of eutrophic conditions (>55 mg/m^2) [Porter et al., 2008] and values consistent with the results at the Mouth exceed the 90th percentile for Midwest agriculture streams and rivers [USEPA, 2000]. Even at the river's surface, TSS still heavily constrained the algae growth on the periphytometers, which is apparent in Figure 7-2. Although nutrient levels were consistently found at levels typically associated with nuisance growth (TP > 0.3 mg/L) just before Fargo/Moorhead and the rest of the way downstream, excessive periphyton abundance on the periphytometers did not occur until TSS dropped significantly downstream of the confluence of the Assiniboine River in Manitoba at RM 39.

By comparison to Minnesota's recent numeric river eutrophication standards [Heiskary et al., 2013], monitored phytoplankton chlorophyll *a* concentrations (as seen in Figure 7-3) were below the growing season average standard of 35 µg/L for southern rivers, yet values approach that limit toward the lower reach of the Red River. Although below the MPCA standard, concentrations above 20 µg/L exceed the 50th percentile as compared to Midwest agriculture streams and rivers described in the USEPA Nutrient Criteria Technical Guidance Manual [USEPA, 2000]. An additional potential concern was the representation of cyanobacteria therein. As illustrated in Figure 7-11, from the samples taken for phytoplankton taxonomy, the percentage of cyanobacteria within the total algal biovolume estimates was occasionally sizable. The percentage of cyanobacteria within the estimates appeared to spike within the Headwater and Mouth regions—in close proximity to areas with the least amount of TSS—reaching 35 percent of the total biovolume at RM 450, 24 percent at RM 447, and up to 52 percent at RM 155. As mentioned in Section 7.2, the concern of cyanobacterial dominance is because of the potential for

cyanotoxin production, which can have significant effects on aquatic and terrestrial species as well as introducing toxins and taste/odor-treatment issues for drinking water supplies. Within the biovolume estimates, the most dominant cyanobacteria species found in the samples is a known producer of cyanotoxins (*Leptolyngbya* sp.). The reason for the blue-green algal-dominance trend is potentially associated with the competitive advantages of nitrogen-fixing cyanobacteria in the presence of adequate phosphorus, especially given that ratios of N:P from the 2015 survey results were consistently less than 6:1 within the river, which indicates freshwater nitrogen limitation [Smith, 1983; Havens et al., 2003; Schindler et al., 2012; USEPA, 2013; Heiskary and Wilson, 2005]. The percentages of the blue-green taxa found within the Red River samples as a part of the total biovolume mirrored that found in Lake Okeechobee (Florida) with similar N:P ratios [Havens et al., 2003]. The nitrogen-fixing properties of the cyanobacteria combined with its mobility adaptations [Herrero and Flores, 2008] would allow for cyanobacteria to successfully outcompete other taxa, even in situations where light was somewhat limiting [Havens et al., 2003]. This trend appears apparent in Figure 7-11.

Although the indications of a stressor-response interaction were indicated in the initial assessment described above, establishing a true relationship per Step 3 of the USEPA methodology [USEPA, 2010] was accomplished through several more specific methods. Beyond the stressor-response trends seen within the abundance measures, periphyton taxa variation between sites showed strong stressor responses with respect to nutrient concentrations, even in light of high turbidity/limited light infiltration. Using descriptive metric groupings, distinct dominance shifts were seen in the communities between the Headwater, Middle, and Mouth zones of the river that seemingly related to organic loading and nutrients. As seen in Figure 7-7 and Table 7-2, the saprobity metric indicative of algal response to organic loading showed a significant dominance shift from algal taxa that require high DO/low BOD concentrations to a dominance of those tolerant of very low DO/high BOD. This community shift is supported by DO and BOD results from the MPCA from RM 308 within the Middle zone. From 130 daytime results ranging from 1995–2010, DO fell below 5 mg/L on 25 occasions (19 percent of measured values). Because DO would be expected to fall to its lowest concentration at night when photosynthesis has ceased, these daylight results would be considered conservative and possibly indicate a more significant problem than is currently documented. The limited measures of BOD from MPCA collections ranging from the late 1960s to 2010 indicate a dominance of values above 3 mg/L (42 percent of all measured values) associated with four other sites within the Middle reach, which is higher than the ≤3 mg/L recommendation for southern rivers by MPCA [Heiskary and Bouchard, 2015]. The effect of low DO on biological communities has been documented in the past on the Red River. Goldstein et al. [1996] determined through multivariate habitat analysis that low DO was one of the primary water quality indicators associated with fish community structure. Emmons & Oliver Resources, Inc. [2009] compiled Red River data from the MPCA results, which indicated 24 impairment listings for low DO over the entire reach of the river and discussed its effect on all biological communities, especially in relation to areas having excessive BOD. Additionally, MPCA's 2014 303(d) list [MPCA, 2014] implicates DO impairment as the cause of aquatic-life impairment within three sections of the Red River: Cole Creek to Red Lake River, Buffalo River to Elm River, and Two River to Pembina River. All of these sections are located within the Middle zone of the Red River.

A stressor-associated shift was also seen between the river zones with the nitrogen uptake metabolism metric shown in Figure 7-8 and was accomplished through direct, constrained ordination procedures. The confirmation of visible trends was necessary before determining algal response to nutrients, primarily

because of concerns associated with complications of limited light within the river. Using RDA within CANOCO statistical software [ter Braak and Smilauer, 2012], the forward selection procedures found ecologically meaningful and significant correlation patterns between the stressor matrices (water chemistry and land use) and the response matrices (periphyton and the phytoplankton communities). The RDA tests were used consistently for the direct ordination procedures because of the short gradient lengths found within the response data (SD < 3), which indicates a linear (not unimodal) data distribution. All RDA tests (including pRDA) were performed using unrestricted permutations on the datasets (499 total).

The nitrogen-influenced metric shifted between the zones from taxonomic groups that were *tolerant of* excessive nitrogen to those *dependent upon it,* which matches the dramatic increase in total nitrogen and its constituents between the Headwater and Middle/Mouth zones (Table 7-1). Finally, the periphytic species comprising the metric of "percent nutrient tolerance" exhibited the same zonation shift of increasing downstream tolerance to excessive nutrients (Figure 7-9) with indifference to TSS concentrations. The general location of these initial metric-associated community shifts coincides with the urban area of Fargo/Moorhead and continues with Grand Forks and other smaller municipalities, as would be expected in response to wastewater treatment plants (WWTP) and other National Point-Source Discharge Elimination System (NPDES) discharges.

The perceived zonation of the river seen in Figure 7-2 was further validated by the two NMS analyses from the perspective of water chemistry (Figure 7-5) and algal tolerance metric shifts (Figure 7-6) through observations of the proximity of site symbols to one another. Nutrients were found to vary significantly between the zones even in lieu of TSS (NMS was initially performed with TSS and resulted in more distinct zones). When grouped by the saprobity and nitrogen uptake metabolism metrics, the taxa demonstrated significant and consistent shifts between each region, as was determined visually from the diagram by the distance of symbols from one another (i.e., closer = more similar, farther apart = more dissimilar). The changes in these pertinent metrics were further illustrated in Figures 7-7 and 7-8 as distinct proportional changes were found downstream of Fargo/Moorhead. The significance of nutrient and TSS changes between these zones was supported through the constrained ordinations shown in Figures 7-12 and 7-14. Covariables in the partial RDA allowed for the effect of nutrients on the algal community to be more fully discerned without the influence of TSS. Coincidentally, this step satisfies the final step (Step 4) of the USEPA stressor-response guidance [USEPA, 2010] of evaluating the stressor with regard to a confounding variable. Although significant in its association, the explained algal variance attributed to nutrients was relatively small as determined by these ordinations (15 percent of variance for periphyton and 16 percent for phytoplankton). As described previously for rivers, this observation could be the result of the delayed response of algae in its ability to use resources [Dodds et al., 1998]. Unlike lakes where algae can bathe continuously in available light and nutrients, river algae are exposed to ever-changing nutrient concentrations in addition to physical disruptions from suspended sediments and mixing (flow), both of which would be expected to reduce potential nutrient uptake.

Although the methodology presented above satisfies the general published guidance for developing a stressor-response model, this methodology is based on using a preconceived stressor. Preconceived notions of ecological interaction can result in a limited understanding of an ecosystem. To increase our understanding of all stressors in the Red River, an additional assessment to discern the effect of land use

on the algal community was performed. Constrained ordinations with measures of land-use attributes from the SPARROW model and from subsequent GIS manipulations of the summarized data (Figure 7-13 and Figure 7-18) were included in an attempt to quantify the persistent influence of stressors based on general knowledge of the effect of anthropogenic disturbance drivers in subwatersheds on aquatic communities. Efforts were made to view these stressors in light of potential nutrient sources. Although phytoplankton's explained variance with the land-use ordination was higher than the chemistry analysis (23 percent versus 16 percent), the explained variance in the periphyton communities was appreciatively higher (35 percent versus 15 percent), and both analyses revealed ecologically meaningful correlations. Shown in Figure 7-13 (phytoplankton/land-use RDA diagram), the strong association of the percentage of riparian wetlands with the x-axis indicates that the variance in the phytoplankton data between sites was very strongly correlated with this land-use parameter. The sites with the strongest association with the wetland vector are those earlier described as having a dominance of the saprobity group tolerant of very low DO and high BOD. Before the initiation of the algae sampling, team members expressed interest in the effect of riparian oxbow wetlands specifically with regard to their potential to affect DO concentrations in the adjacent river.

Although the methodology presented above satisfies the general published guidance for developing a stressor-response model, this methodology is based on using a preconceived stressor. Preconceived notions of ecological interaction can result in a limited understanding of an ecosystem. To increase our understanding of all stressors in the Red River, an additional assessment to discern the effect of land use on the algal community was performed. Constrained ordinations with measures of land-use attributes from the SPARROW model and from subsequent GIS manipulations of the summarized data (Figure 7-13 and Figure 7-18) were included in an attempt to quantify the persistent influence of stressors based on general knowledge of the effect of anthropogenic disturbance drivers in subwatersheds on aquatic communities. Efforts were made to view these stressors in light of potential nutrient sources. Although phytoplankton's explained variance with the land-use ordination was higher than the chemistry analysis (23 percent versus 16 percent), the explained variance in the periphyton communities was appreciatively higher (35 percent versus 15 percent) and both analyses revealed ecologically meaningful correlations. Shown in Figure 7-13 (phytoplankton/land-use RDA diagram), the strong association of the percentage of riparian wetlands with the *x*-axis indicates that the variance in the phytoplankton data between sites was very strongly correlated with this land-use parameter. The sites with the strongest association with the wetland vector are those earlier described as having a dominance of the saprobity group tolerant of very low DO and high BOD. Before the initiation of the algae sampling, team members expressed interest in the effect of riparian oxbow wetlands specifically with regard to their potential to affect DO concentrations in the adjacent river.

Previous surveys by NDDH personnel indicated significant dips in DO adjacent to these wetlands following minor flooding events. Personnel hypothesized a potential relationship between the wetlands and DO because of potentially high BOD being introduced from the wetlands. As is commonly observed, dense algal blooms occur in these oxbow wetlands, which result from their retention and uptake of nutrients and the subsequent excessive bacterial respiration associated with the dead algae decomposition. The MPCA DO data discussed above (consistently measured below 5 mg/L) were collected from an area of the river within the stressor-response study algal sample sites that were seen to be closely associated with the percentage of riparian wetlands, as shown in Figure 7-13. Coincidentally, just upstream of this

sampling site is a small tributary that exhibits excessive algal growth, as seen from somewhat dated (ca. 1991) aerial imagery (Figure 7-20) from Google Earth. These same aerial views of the areas immediately adjacent to the algae clogged streams indicated abundant agricultural practices in the area with a high potential for nutrient runoff. This interesting pattern is not proof of causation but it definitely warrants additional investigation into the potential source of high BOD/low DO and subsequent stressor influence on the biological communities.

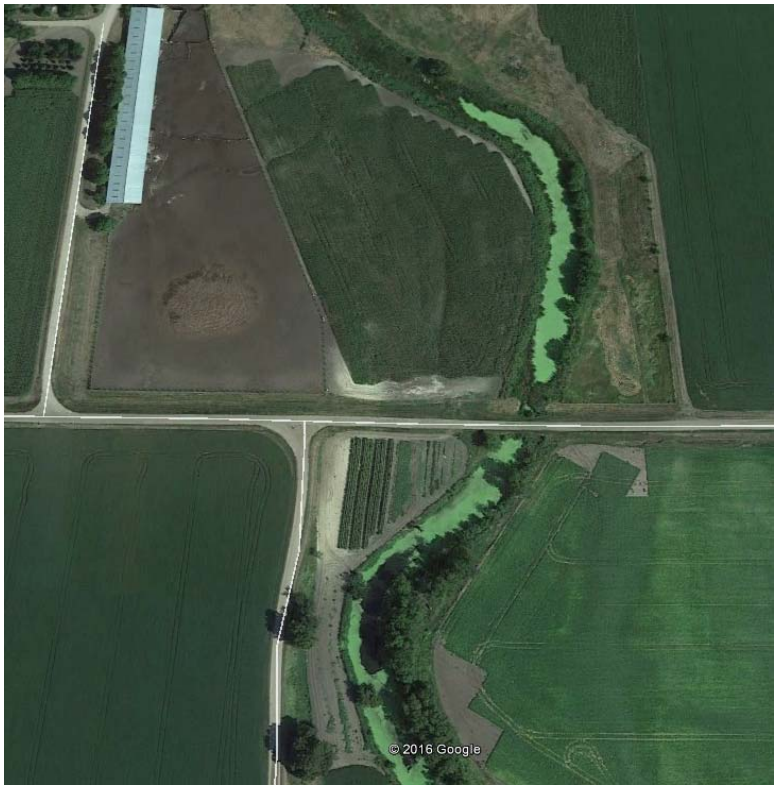


Figure 7-20. Tributary to the Red River of the North With Excessive Algal Growth Near RM 308.

Other associated land-use attributes had meaningful ecological relevance as assessed via the phytoplankton ordination. The amount of applied phosphorus and nitrogen fertilizer had significant associations with the algal communities from the Manitoba sites, which indicated a potential effect of agricultural practices. In a seemingly unrelated association, these sites also exhibited a strong correlation with the highest percentage of forested area in the watershed. An association with the percentage of the SPARROW designation of "high-nutrient intensity croplands" was also significant, although not associated with the fertilizer application sites. Also of note is the relevance of water residence time in explaining species variance between sites. This parameter, calculated for the sampling period, was included in an attempt to convey varying growth conditions affected by water movement. The high correlation of this association gives measurable evidence that the floating algae respond to slower moving water, as would be expected. Longer residence times would give the opportunistic organisms time to use the nutrient resources.

As previously mentioned, relationships of land-use and periphyton variance were explored. As illustrated in Figure 7-18, five land-use attributes were found to have statistical significance to periphyton taxa site

variance. Of note was the percentage of urban/impervious area and its strong association with the Axis 1 (greatest variance explained in model). This association could be an indirect measure of the increased runoff and potential nutrient contribution from the developed areas of the Red River Watershed. In a similar fashion, the abundance of high-nutrient intensity crops and percentage of forest cover were found to be associated with periphyton variability as were the percentage of grassland and barren land covers. As would be expected, the high-nutrient intensity crops were associated with degraded algal communities; whereas, those associated with forest cover, grassland, and barren land were more diverse and less dependent on high-nutrient concentrations (per periphyton metrics). Two other parameters (percentage of grassland and barren land) were also found to have strong correlations with periphyton. These later land covers could have positive implications on water quality because they would be expected to have higher infiltration rates and less runoff, thereby conveying fewer nutrients to the river. This association is confirmed by the percent grassland vector's association with Headwater sites although the correlation between the percent barren vector and the Manitoba sites (where the algal growth was most abundant) does not follow this pattern.

In an attempt to further define the ecological implications of land-use attributes upon water chemistry and the abundance of periphytic chlorophyll *a*, an NPMR assessment of these environmental matrices was performed (Figure 7-19). Interestingly, the primary strength of the model's high R^2 values was directly attributed to the percentage of open water in the subwatersheds with a small increase attributed to TN. The results of this analysis imply a negative correlation between periphytic chlorophyll *a* abundance and the percentage of open water in each site's subwatershed. Although the abundance of riparian open water (percent riparian wetlands within the 500-m buffer) appeared to negatively influence the quality of phytoplankton communities, the abundance of lentic waters outside of the riparian zone appears to have a controlling effect on total periphytic primary production, potentially through shallow water nutrient- (N and P) and TSS-processing dynamics.

7.3.1.3 *Nutrient Target Identification*

The stressor response of both phytoplankton and periphyton is well documented by the previous analyses. In light of the complications within the Middle zone of the Red River where suspended sediment was found to limit periphytic algal productivity, the analysis shown in Figure 7-14 sought to determine which sites from the study had the strongest taxonomic response to phosphorus and nitrogen so that only nutrient results from these sites would be used for nutrient target averaging. These sites' nutrient and other pertinent information results were summarized in Tables 7-8 and 7-9. The results of this summary give indication as to the average nutrient values associated with periphytic algal communities/low primary production as well as less desirable algal communities/high primary production. As listed in Table 7-8, the TP average most significantly associated with the highest periphyton biomass and least desirable communities was 0.32 mg/L, and the TN average was 1.83 mg/L. On the other end of the spectrum, the TP and TN averages for the three sites having the lowest biomass and most desirable communities was 0.15 mg/L and 1.15 mg/L, respectively. With phytoplankton chlorophyll *a* measures, nutrient values were identical for the low end and were 0.31 and 1.91 for TP and TN on the high end, respectively. As described in Section 7.2, pRDA ordination graphs of both periphyton and phytoplankton were used to choose the sites most strongly oriented in the opposite direction of the of TP and TN gradients. This negative association implied by site location on the ordination diagrams indicates that the

algal communities at these sites have the least influence of excessive TP. These trends in the community/ taxa-based analyses were further validated by subsequent periphyton quality metric comparison (Figures 7-16 and 7-17) because quality metrics indicated substantial increases in nutrient-related tolerance in the sites with higher positive correlations with high nutrients. Because of this analyses agreement, nutrient averages of the three sites having the strongest negative correlation with high nutrients were calculated resulting in 0.15 mg/L for TP and 1.15 mg/L for TN. Coincidentally, the TP target of 0.15 mg/L coincides with the southern-river, nutrient-region maximum value of 0.15 mg/L, as defined by the MPCA eutrophication studies [Heiskary and Bouchard, 2015]. This indicates a convergence of methodology results. With this convergence, evidence is sufficient to recommend that our values of 0.15 mg/L for TP and 1.15 mg/L for TN should be considered nutrient targets to prevent nuisance algal growth in the Red River (Table 7-11). Further, in support of the relationships described above, the Heiskary and Bouchard [2015] study also recommends a maximum BOD of ≤3 mg/L. A search of the limited available BOD data indicates that exceedances of this value occur regularly because the five available sites within our Middle zone of the Red River were found to exceed this value 27–100 percent of the time.

Table 7-11. Nutrient Target Limits Recommended by Stressor-Response Study

Nutrient	Concentration (mg/L)
TN	1.15
TP	0.15

8.0 CONCLUSIONS: COLLECTIVE WEIGHT OF EVIDENCE

A nutrient-stressor-response model was developed for the Red River using a collective weight-of-evidence approach that combined periphyton, phytoplankton, water quality, and land-use information. The steps for the model development are reiterated below. Specific details of each point are described in detail sections 7.2 and 7.3.

8.1 CONCEPTUAL MODEL

A conceptual model is a visual representation of the assumed relationships of the biological communities and their stressors (such as excessive nutrient or sediment inputs) and guides in developing the stressor-response assessments. A range of analyses is required to examine algal eutrophication responses to land-use factors and water quality parameters that may covary. Initial efforts in developing draft conceptual models depicted cumulative impacts from (1) altered flows and habitats and (2) altered sediments with an emphasis on fine-sediment burdens and its effects upon biological responses. A modification of the Heiskary et al. [2013] conceptual model was ultimately chosen by the experts panel for assessment of the Red River (Figure 5-2) to explicitly incorporate the effects of small particle-induced turbidities limiting light and, therefore, influencing periphyton and phytoplankton responses along the Red River of the North mainstem sites.

8.2 STRESSOR-RESPONSE MODEL

After developing the conceptual model to guide the process, the following steps were performed to establish a statistically and ecologically valid relationship between the biological response group and the measured environmental variables:

1. Because having varying concentrations of nutrients was pivotal to observing a stressor effect, step one was to determine a nutrient gradient through simple graphing techniques of the sites and their concentrations arranged in a downstream gradient. The gradient, which increased in a downstream manner, was very apparent (Figure 7-2) and appeared to be directly influenced by the municipalities and agricultural land uses in the Middle reach of the river and then, the effect of which, was further modified by the confluence of the Assiniboine River near the Mouth (decreased turbidity).
2. The second step was determining a response in the algal community to the nutrient gradient both within the quantity and the quality of the community. A response in quantity was apparent in both the phytoplankton (Figures 7-3, 7-4, and 7-10 as well as Table 7-1) and periphyton (Figures 7-2 through 7-4, and 7-10 as well as Table 7-1), although the growth of the latter appeared to be significantly repressed by TSS concentrations (indicated in Figure 7-3 because abundant nutrients do not result in excessive growth until a drop in TSS). The response of periphyton quality, as determined by pertinent diatom metrics, was not suppressed by TSS concentrations because a significant increase in tolerance was apparent with an increase in nutrients (Figures 7-7 and 7-8).

3. The third step involved determining the significance of algal community and nutrient variance correlations. Multivariate analyses determined that both periphyton (Figure 7-14) and phytoplankton taxa (Figure 7-12) responded significantly to varying nutrient concentrations, even with respect to excessive turbidity.
4. Using the information from step three, this step sought to provide nutrient limits associated with acceptable primary productivity measures as well as high-quality metric results. This process resulted in nutrient targets of 0.15 mg/L for TP and 1.15 mg/L for TN specifically for the Red River main stem.
5. Algal-stressor influence related to low DO and elevated BOD was implicated because of significant land-use associations. Overall, land use related to anthropogenic disturbance was found to have higher explanatory power than in situ water chemistry parameters on determining the algae variance. These results implied a positive relationship between adjacent wetlands and increased BOD/decreased DO that possibly related to direct input of decaying algal biomass and low DO concentrations. Conversely, another land-use-dominated analysis suggested that the total abundance of lentic waters in the watershed appears to have a controlling effect on total periphytic primary production potentially through the retention of nutrient runoff from agricultural application.

As outlined above, this analysis provides ample evidence for a strong stressor response of the algal community to nutrients in the Red River. Additionally, the analysis provides insight into nutrient-related, landscape-feature influences on algal growth.

8.3 GAPS IN UNDERSTANDING

Several issues were identified during the stressor-response project that warrant consideration and potential additional study. DO impairments from previous agency datasets strongly suggest that additional examinations of nitrogen, phosphorus, and algal responses coupled with more intensive DO/BOD monitoring are warranted because the results indicate that DO/BOD is affecting biota (periphyton metrics). The exact source of the issue is unclear, although it could be associated with agricultural tributaries and/or the proximity of wetlands to the river (and their retention of nutrients/algal biomass). The collective influence of oxbows and riparian wetlands deserve further attention as does the associated degree of artificial drainage within the basin. Lastly, excessive concentrations of TP and TN have resulted in low N:P ratios which are indicative of conditions that can be preferential to developing river cyanobacteria blooms before entering Lake Winnipeg. Elevated cyanobacterial concentrations suggest that future monitoring include cyanobacteria toxins such as microcystin-LR to protect municipal water supplies.

8.4 RECOMMENDATIONS

This report focused on developing a stressor-response model and recommended nutrient targets for the Red River. This was one of the two integrated approaches recommended in the 2013 report [Plevan and Blackburn, 2013]. The other approach that should be integrated is the consideration of downstream water resources, specifically the N and P loading goals of Lake Winnipeg. As previously noted in Plevan and Blackburn [2013], these approaches may derive at two different candidate targets that will need to be resolved to ensure compatibility and develop management strategies that result in improved ecological

health of both the Red River and its receiving water (Lake Winnipeg). In the event that resolution must be achieved, a comparative evaluation should be completed that addresses the impacts on both the Red River and Lake Winnipeg for each approach, as well as the feasibility of each approach. The management strategies that are adopted collectively and individually by the jurisdictions to meet the adopted targets must be feasible, result in measurable outcomes, and be accepted by stakeholders.

9.0 REFERENCES

Blanchard, R. A., C. A. Ellison, J. M. Galloway, and D. A. Evans, 2011. *Sediment Concentrations, Loads, and Particle-Size Distributions in the Red River of the North and Selected Tributaries Near Fargo, North Dakota, During the 2010 Spring High-Flow Event*, US Geological Survey Scientific Investigations Report 2011–5064, US Geological Survey, Reston, VA.

Dillard, G., 1999. *Common Freshwater Algae of the United States: An Illustrated Key to the Genera (Excluding the Diatoms)*, J. Cramer, Berlin, Stuttgart.

Dodds, W. K., 2006. "Eutrophication and Trophic State in Rivers and Streams," *Limnology and Oceanography*, Vol. 51 No. 1, part 2, pp. 671–680.

Dodds, W. K. and E. B. Welch, 2000. "Establishing Nutrient Criteria in Streams," *Journal of the North American Benthological Society*, Vol. 19, No. 1, pp. 186–196.

Dodds, W. K., J. R. Jones, and E. B. Welch, 1998. "Suggested Classification of Stream Trophic State: Distributions of Temperate Stream Types by Chlorophyll, Total Nitrogen, and Phosphorus," *Water Resources Management*, Vol. 32, No. 5, pp. 1455–1462.

Emmons & Oliver Resources, Inc., 2009. *Red River Biotic Impairment Assessment*, prepared by Emmons & Oliver Resources, Inc., Oakdale, MN, for the Minnesota Pollution Control Agency, St. Paul, MN.

Goldstein, R. M., J. C. Stauffer, P. R. Larson, and D. L. Lorenz, 1996. *Relation of Physical and Chemical Characteristics of Streams to Fish Communities in the Red River of the North Basin, Minnesota and North Dakota, 1993-95*, Water-Resources Investigations Report 96-4227, US Geological Survey, Denver, CO.

Havens, K. E., R. T. James, T. L. East, and V. H. Smith, 2003. "N:P Ratios, Light Limitation and Cyanobacterial Dominance in a Subtropical Lake Impacted by Non-Point Source Nutrient Pollution," *Environmental Pollution 2003*, Vol. 122, No. 3, pp. 379–390.

Heiskary, S. A. and C. B. Wilson, 2005. *Minnesota Lake Water Quality Assessment Report: Developing Nutrient Criteria,* Third Edition, prepared by the Minnesota Pollution Control Agency, St. Paul, MN.

Heiskary, S. A. and R. W. Bouchard, 2015. "Development of Eutrophication Criteria for Minnesota Streams and Rivers Using Multiple Lines of Evidence," *Freshwater Science*, Vol. 34, No. 2, 574–592.

Heiskary, S. A., R. W. Bouchard, and H. Markus, 2013. M*innesota Nutrient Criteria Development for Rivers*, wq-s6-08, prepared for the Minnesota Pollution Control Agency, St. Paul, MN (Draft).

Heiskary, S. and K. Parson, 2013. *Regionalization of Minnesota's Rivers for Application of River Nutrient Criteria*, prepared by the Minnesota Pollution Control Agency Environmental Analysis and Outcomes Division, St. Paul, MN.

Herrero, A. and E. Flores, 2008. *The Cyanobacteria: Molecular Biology, Genomics and Evolution*, Caister Academic Press, Norfolk, United Kingdom.

Hilton, J. and G.P. Irons. 1998. *Determining the Causes of Apparent Eutrophication Effects,* Environmental Agency R&D Technical Report P203, prepared by the Institute of Freshwater Ecology, Great Britain, for the Environment Agency, Rotherham, UK.

Homer, C. G., J. A. Dewitz, L. Yang, S. Jin, P. Danielson, G. Xian, J. Coulston, N. D. Herold, J. D. Wickham, K. Megown, 2015. "Completion of the 2011 National Land Cover Database for the conterminous United States-Representing a Decade of Land Cover Change Information," *Photogrammetric Engineering and Remote Sensing*, Vol. 81, No. 5, pp. 345–354.

International Red River Board, 2011. *A Proposed Approach to Developing a Basin-Wide Nutrient Management Strategy for the International Red River Watershed*, prepared by the International Red River Commission, Fargo, ND, September 7.

Jenkinson, R. W. and G. A. Benoy, 2015. *Red-Assiniboine Basin SPARROW Model Development Technical Document*, prepared for the National Research Council of Canada, Ottawa, ON, Canada.

Jongman, R. H. G., C. J. F. ter Braak, and O. F. R. Van Tongeren, 1995. *Data Analysis in Community and Landscape Ecology*, Cambridge University Press, Cambridge, UK.

Krammer, K., 1997a. "Die cymbelloiden Diatomeen: Eine Monographie der Weltweit Bekannten Taxa," Teil 1, *Allegemeines and Encyonema*, J. Cramer, Berlin, Germany.

Krammer, K., 1997b. "Die Cymbelloiden Diatomeen: Eine Monographie der Weltweit Bekannten Taxa," Teil 2, Encyonema part., *Encyonopsis and Cymbellopsis*, J. Cramer, Berlin, Germany.

Krammer, K., 2001. "Navicula senso stricto. 10 Genera Separated from Navicula senso lato Frustulia," Vol. 2, *Diatoms of Europe*, A.R.G. Gantner Verlag, K. G. Germany.

Krammer, K., 2002. "Cymbella," *Diatoms of Europe*, A.R.G. Gantner Verlag, K. G. Germany.

Krammer, K. and H. Lange-Bertalot, 1986. Bacillariophyceae, Part 2, "Naviculaceae." Vol. 1, *Freshwater Flora of Middle Europe*, Gustav Fischer/Spectrum, New York, NY.

Krammer, K. and H. Lange-Bertalot, 1988. Bacillariophyceae, Part 2, "Bacillariaceae, Epithemiaceae, Surirellaceae" Vol. 2, *Freshwater Flora of Middle Europe*, Gustav Fischer/Spectrum, New York, NY.

Krammer, K. and H. Lange-Bertalot, 1991a. Bacillariophyceae, Part 2, "Centrales, Fragilariaceae, Eunotiaceae." Vol. 3, *Freshwater Flora of Middle Europe*, Gustav Fischer/Spectrum, New York, NY.

Krammer, K. and H. Lange-Bertalot, 1991b. Bacillariophyceae, Part 2, "Achnanthaceae, Critical Supplement to Navicula (Lineolatae) and Gomphonema, Complete List of Literature for Volumes 1-4," Vol. 4, *Freshwater Flora of Middle Europe*,.Gustav Fischer/Spectrum, New York, NY.

Krenz, G. and J. Leitch, 1993. *A River Runs North: Managing an International River,* Red River Water Resources Council, Bismarck, ND.

Lange-Bertalot, H., 1993. "85 New Taxa and Much More Than 100 Taxonomic Clarifications" supplement to *Freshwater Flora of Middle Europe*, Volumes 1-4, J. Cramer, Berlin, Germany.

Lowe, R. L. and W. F. Gale, 1980. "Monitoring River Periphyton with Artificial Benthic Substrates," *Hydrobiologia*, Vol. 69, Vol. 3, pp. 235–244.

McCune, B., 2011. *Nonparametric Multiplicative Regression for Habitat Modeling*, prepared by Oregon State University, Corvalliz, OR.

McCune, B. and J. B. Grace, 2002. *Analysis of Ecological Communities*, MjM Software Design, Gleneden Beach, OR.

McCune, B. and M. J. Mefford, 2009. *HyperNiche. Nonparametric Multiplicative Habitat Modeling*, Version 2.0, MjM Software Design, Gleneden Beach, OR.

McCune, B. and M. J. Medford, 2011. *PC-ORD, Multivariate Analysis of Ecological Data*, MjM Software, Gleneden Beach, OR.

Midwestern Regional Climate Center, 2015. "cli-MATE, the MRCC's Application Tools Environment Database," *illinois.edu*, retrieved November 17, 2015, from *http://mrcc.illinois.edu/CLIMATE*

Minnesota Pollution Control Agency, 2015. *2014 Proposed Impaired Waters List*, wq-iwl-47.xlsx, prepared by the Minnesota Pollution Control Agency, St. Paul, MN.

National Oceanic and Atmospheric Administration, 2015. "Hydrometeorological Design Studies Center, Precipitation Frequency Data Server," *noaa.gov*, retrieved November 3, 2015, from *http://hdsc.nws.noaa.gov/hdsc/pfds/*

Natural Resources Conservation Service, 1986. "Appendix A: Hydrologic Soil Groups," *Urban Hydrology for Small Watersheds*, TR-55, prepared by the Natural Resources Conservation Service, Washington, DC.

Patrick, R. and C. W. Reimer, 1966. *The Diatoms of the United States*, Volume 1, Academy of Natural Sciences, Philadelphia, PA.

Patrick, R. and C. W. Reimer, 1975. *The Diatoms of the United States*, Volume 2, Part 1, Academy of Natural Sciences, Philadelphia, PA.

Plevan, A. B. and J. A. Blackburn, 2013. *Approaches to Setting Nutrient Targets in the Red River of the North*, RSI-2328, prepared by RESPEC, Roseville, MN, for the International Joint Commission, US Section, Washington, DC.

Porter, S. D., D. K. Mueller, N. E. Spahr, M. D. Munn, and N. M. Dubrovsky, 2008. "Efficacy of Algal Metrics for Assessing Nutrient and Organic Enrichment in Flowing Waters," *Freshwater Biology*, Vol. 53, No. 5, pp. 1036–1054.

Potapova, M. G. and D. M. Winter, 2006. "Use of Nonparametric Multiplicative Regression for Modeling Diatom Habitat: a Case Study of Three Geissleria Species from North America," *Advances in Phycological Studies*, Pensoft Publishers & University Publishing House, Moscow, pp. 319–332.

Potapova, M. G., D. F. Charles, K. C. Ponader, and D. M. Winter, 2004. "Quantifying Species Indicator Values for Trophic Diatom Indices: A Comparison of Approaches," *Hydrobiologia*, Vol. 517, No. 1, pp. 25–41.

Prescott, G. W., 1962. *Algae of the Western Great Lakes Area*, Wm. C. Brown Company Publishers, Dubuque, IA.

Prescott, G. W., 1978. *How to Know the Freshwater Algae*, Wm. C. Brown Company Publishers, Dubuque, IA.

Raunio, J. and J. Soininen, 2007. "A Practical and Sensitive Approach to Large River Periphyton Monitoring: Comparative Performance of Methods and Taxonomic Levels," *Boreal Environment Research*, Vol. 12, pp. 55–62.

Reichardt, E., 1997. "Taxonomische Revision des Artenkomplexes um Gomphonema pumilum (Bacillariophyta)," *Nova Hedwigia*, Vol. 65, No. 1-4, pp. 99–129.

Reichardt, E., 1999. *Zur Revision der Gattung Gomphonema,* A.R.G. Gantner Verlag. Konigstein, Germany.

Schindler, D. W., R. E. Hecky, and G. K. McCullough, 2012. "The Rapid Eutrophication of Lake Winnipeg: Greening Under Global Change," *Journal of Great Lakes Research*, Vol. 38, Supplement 3, pp. 6–13.

Smith, V. H., 1983. "Low Nitrogen to Phosphorus Ratios Favor Dominance by Blue-Green Algae in Lake Phytoplankton," *Science*, Vol. 221, No. 4611, pp. 669–670.

ter Braak, C. J. and P. Smilauer, 2012. *Canoco Reference Manual and User's Guide: Software for Ordination*, Version 5.0, Microcomputer Power Ithaca, NY.

US Department of Agriculture, 2015. "2015 State Agriculture Overview, North Dakota," *usda.gov*, retrieved March 28, 2015, from *http://www.nass.usda.gov/Quick_Stats/Ag_Overview/stateOverview.php?state=NORTH%20DAKOTA*

US Environmental Protection Agency, 1986. *Quality Criteria for Water*, prepared by the US Environmental Protection Agency, Office of Water, Washington, DC.

US Environmental Protection Agency, 2000. *Nutrient Criteria Technical Guidance Manual, Rivers and Streams,* EPA-822-B-00-002, prepared by the US Environmental Protection Agency, Office of Water, Washington, DC.

US Environmental Protection Agency, 2010. *Using Stressor-Response Relationships to Derive Numeric Nutrient Criteria*, EPA-820-S-10-001, prepared by the US Environmental Protection Agency, Office of Water, Washington, DC.

US Environmental Protection Agency, 2013. *Nutrient Enrichment Indicators in Streams*, prepared by the US Environmental Protection Agency, Washington, DC.

US Environmental Protection Agency, 2015. *2015 Drinking Water Health Advisories for Two Cyanobacterial Toxins*., 820-F-15-003, prepared by the US Environmental Protection Agency, Office of Water, Washington, DC.

Van Dam, H. A., 1996. "A Coded Checklist and Ecological Indicator Values of Freshwater Diatoms from the Netherlands," *Netherlands Journal of Aquatic Ecology*, Vol. 28, No. 1, pp. 117–133.

Wehr, J. D. and R. S. Sheath, 2003. *Freshwater Algae of North America: Ecology and Classification*, Academic Press, New York, NY.

APPENDIX A
SPARROW MODEL INFORMATION BY ALGAL SAMPLING SITE

Landuse Calculations from SPARROW Model

	Watershed Area (Sq Km)	Residence Time (days)	Cumulative Age (days)	% Open Water	% Urban/ Impervious	% Barren	% Forest	% Grassland	% Agricultural	% Wetlands	% Pasture	% Riparian Wetlands	Point Sources	% High Nutrient Intensity Crops 1	% High Nutrient Intensity Crops 2	Fertilizer Mass Total N (tonnes)	Fertilizer Mass Total P (tonnes)	Distance to Upstream Dams (meters)
84RD008	10,129.32	1.00	1.00	4.36	4.65	0.02	7.05	4.31	60.04	11.25	8.32	3.10	21	23.49	23.22	32,804,070.94	5,764,650.49	1.00
15RD067	28,991.42	4.05	5.05	5.44	4.81	0.03	5.52	9.16	55.87	6.45	12.71	2.29	31	17.24	16.71	101,078,981.11	17,158,798.28	50,622.21
15RD066	28,991.42	4.65	8.69	5.44	4.81	0.03	5.52	9.16	55.87	6.45	12.71	2.29	31	17.24	16.71	101,078,981.11	17,158,798.28	1,591.93
84RD022	29,066.00	0.36	9.06	5.43	4.88	0.03	5.50	9.14	55.88	6.45	12.69	2.30	32	17.23	16.70	101,304,429.83	17,198,669.48	10,454.56
15RD065	29,076.64	0.20	9.26	5.43	4.89	0.03	5.50	9.14	55.87	6.45	12.68	2.30	33	17.22	16.69	101,326,706.58	17,202,391.29	16,768.61
05RD030	34,196.03	0.84	10.10	4.79	5.02	0.03	4.81	8.02	59.91	6.06	11.35	2.21	34	20.50	19.79	127,383,916.43	21,529,026.26	41,372.38
84RD027	37,574.67	1.89	11.98	4.65	5.01	0.03	5.07	7.48	60.84	6.06	10.86	2.52	44	20.88	20.20	139,621,890.87	23,668,791.68	93,755.88
94RD018	46,705.21	1.74	13.72	4.19	4.86	0.03	6.18	6.39	62.24	6.30	9.82	2.77	56	22.60	21.36	175,609,633.50	29,759,138.44	143,412.51
15RD058	64,721.52	2.69	16.41	5.26	4.51	0.03	7.75	5.03	55.81	12.40	9.23	3.84	85	19.11	17.93	208,092,472.55	35,435,833.50	230,591.40
84RD037	65,136.03	0.16	16.56	5.24	4.56	0.03	7.71	5.00	55.97	12.32	9.18	3.82	86	19.03	17.84	210,235,458.06	35,791,822.45	2,942.25
15RD057	65,185.66	0.22	16.78	5.24	4.57	0.03	7.70	4.99	55.99	12.32	9.17	3.82	87	19.03	17.85	210,463,844.63	35,830,510.38	10,982.45
15RD056	66,567.00	0.66	17.44	5.14	4.57	0.03	7.56	4.89	56.74	12.08	8.99	3.76	87	19.07	17.85	216,755,014.36	36,942,955.04	36,198.96
15RD055	68,599.87	1.33	18.78	5.03	4.61	0.02	7.40	4.81	57.42	11.86	8.83	3.73	90	19.10	17.74	227,153,737.09	38,671,131.05	64,478.15
84RD042	72,907.88	1.02	19.80	4.77	4.63	0.02	7.15	4.62	58.58	11.55	8.68	3.75	96	19.30	17.74	246,720,187.36	41,989,731.71	96,930.24
15RD052	77,517.47	1.99	21.79	4.52	4.66	0.02	7.00	4.47	59.62	11.20	8.50	3.77	102	18.95	17.36	267,823,774.87	45,553,426.85	24,188.27
84RD047	91,523.60	1.93	23.73	4.11	4.51	0.02	7.11	5.16	60.77	10.50	7.82	3.74	121	17.14	15.53	324,665,686.14	59,098,397.55	3,148.88
5OCS007	91,568.66	0.13	23.86	4.11	4.51	0.02	7.10	5.15	60.78	10.49	7.82	3.74	121	17.13	15.52	324,979,090.79	59,173,571.67	7,276.34
5OCS033	99,913.57	2.14	26.00	3.81	4.41	0.02	7.66	5.83	59.44	11.25	7.57	4.16	141	16.23	14.52	352,536,754.46	67,659,684.09	80,742.69
5OCS004	108,823.16	2.10	28.10	3.52	4.33	0.04	8.24	6.92	59.20	10.53	7.23	4.16	173	15.38	13.61	396,383,441.78	82,637,302.84	151,688.57
5OJS057	208,070.42	1.31	29.41	2.94	3.36	0.07	8.90	10.66	58.39	7.07	8.61	2.73	321	9.17	7.46	646,230,420.90	164,209,270.77	188,012.12
5OJS004	208,142.97	0.31	29.73	2.94	3.38	0.07	8.90	10.66	58.37	7.07	8.61	2.73	322	9.17	7.45	646,297,998.46	164,232,354.54	196,675.12
5OJS074	209,833.57	1.28	31.01	2.93	3.39	0.07	8.93	10.79	58.31	7.02	8.57	2.71	336	9.11	7.40	654,106,709.76	166,899,738.64	11,521.16
5OJS128	212,105.07	1.26	32.27	2.94	3.39	0.08	8.94	10.99	58.15	6.99	8.53	2.68	344	9.02	7.33	662,075,073.74	169,621,651.26	39,815.89

APPENDIX B
CONCEPTUAL MODELS

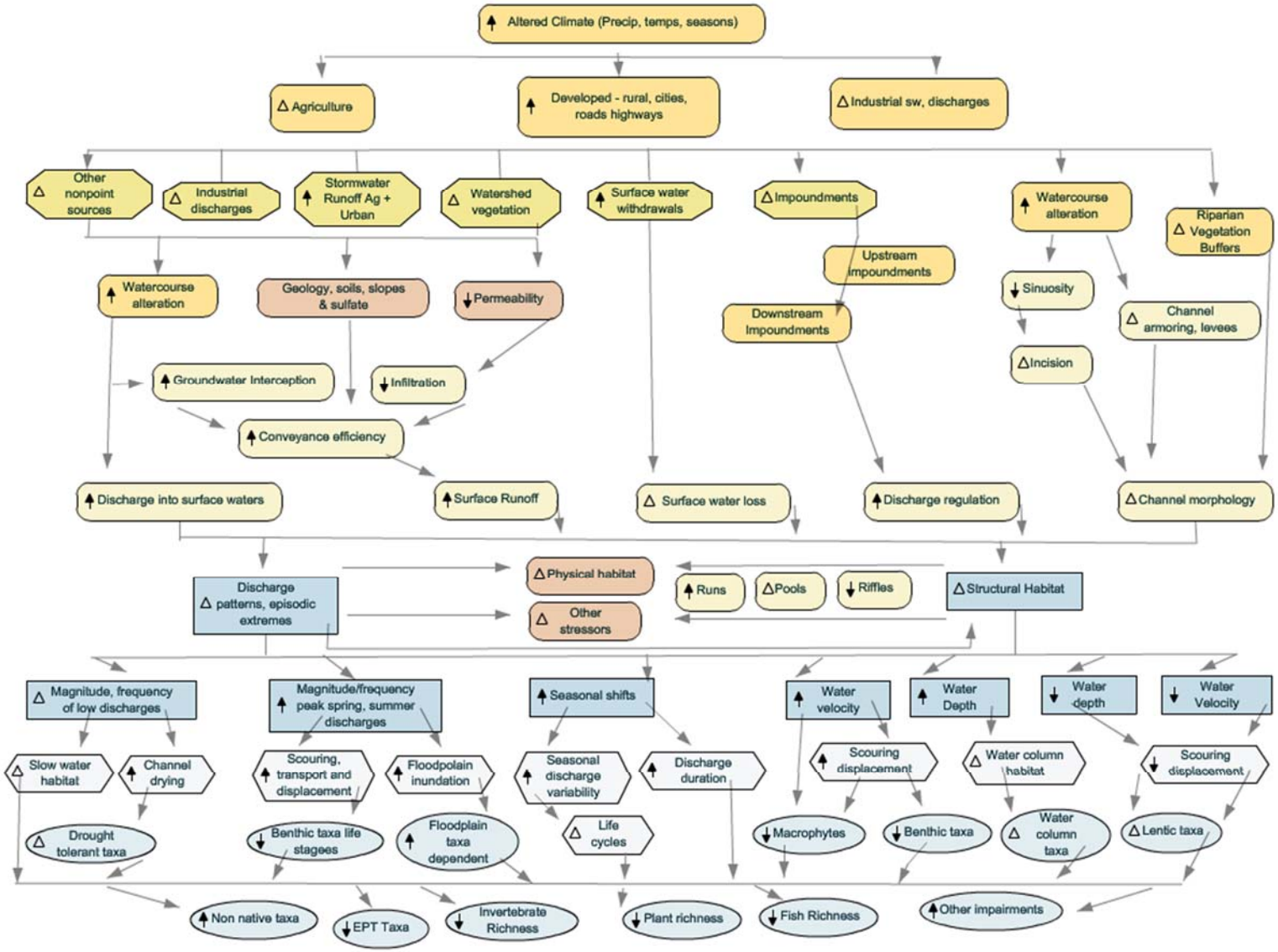


Figure B-1. Altered Flow and Habitat (prepared by RESPEC for the Experts Panel Meeting, December, 2015).

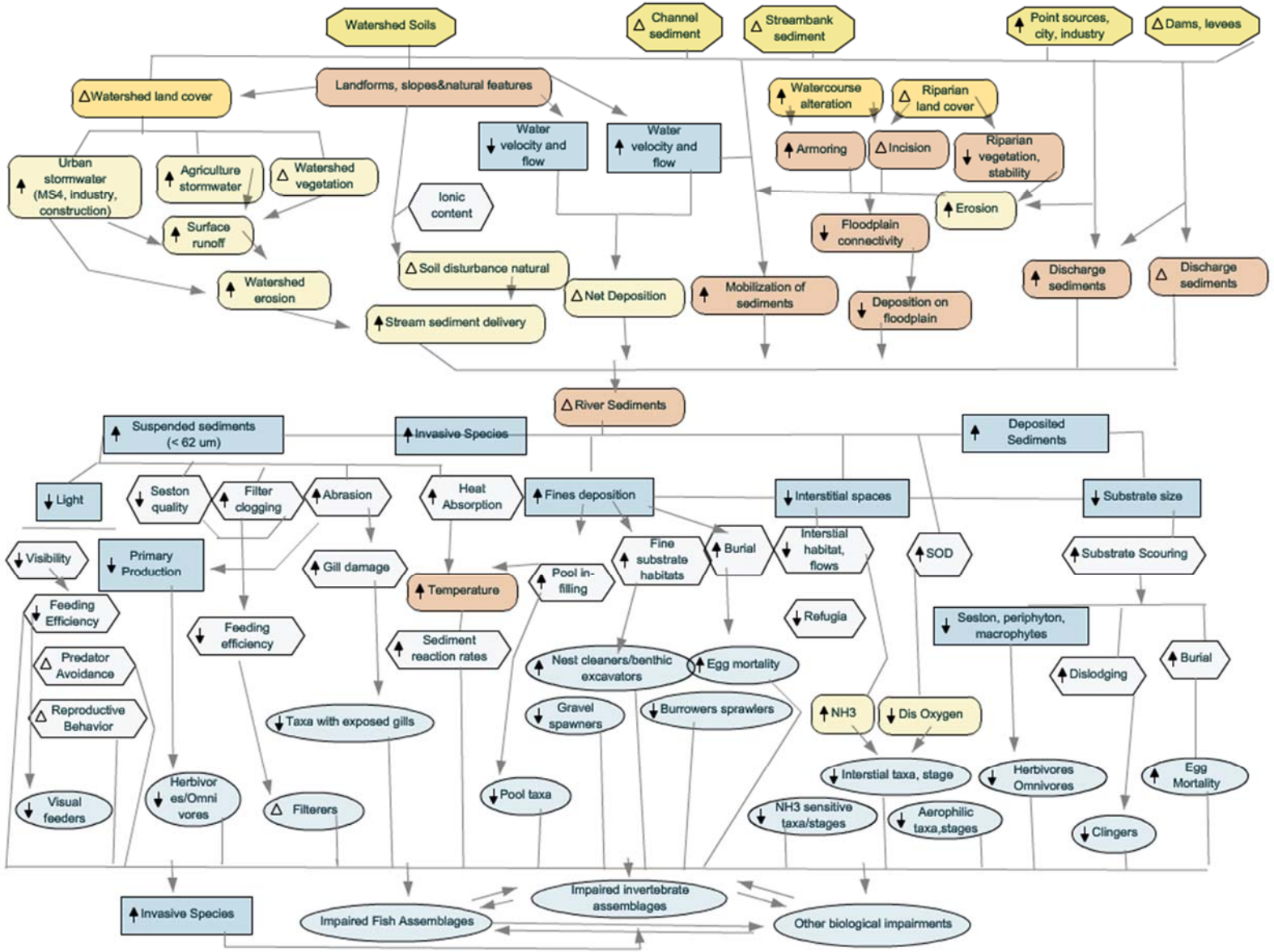


Figure B-2. Sediment Conceptual Diagram (prepared by RESPEC for the Experts Panel Meeting, December, 2015).

APPENDIX C
WORKSHOP AND WEBINAR ATTENDEES

APPENDIX C WORKSHOP AND WEBINAR ATTENDEES

RED RIVER STRESSOR-RESPONSE MODEL EXPERT'S MEETING ATTENDANCE LIST

First	Last	Organization	Title	Email
Nicole	Armstrong	Manitoba Sustainable Development	Director of Water Science and Management	nicole.armstrong@gov.mb.ca
Glenn	Benoy	International Joint Commission	Science Advisor	benoyg@ottawa.ijc.org
Patricia	Chambers	Environment and Climate Change Canada	Research Scientist	patricia.chambers@ec.gc.ca
Mike	Ell	North Dakota Dept of Health	Environmental Scientist	mell@nd.gov
Kristina	Farmer	Environment and Climate Change Canada	Head, Ecosystem Health Assessment	Kristina.Farmer@ec.gc.ca
Mark	Gabriel	International Join Commission	Engineering Advisor	GabrielM@Washington.IJC.org
Steve	Heiskary	Minnesota Pollution Control Agency	Research Scientist	steven.heiskary@state.mn.us
Jeff	Lewis	Red River Basin Commission	Executive Director	jeff@redriverbasincommission.org
Ben	Lundeen	Minnesota Pollution Control Agency	Biological Monitoring - streams, macroinverts	benjamin.lundeen@state.mn.us
Nathan	Mielke	Minnesota Pollution Control Agency	Biological Monitoring - streams, macroinverts	Nathan.Mielke@state.mn.us
Rochelle	Nustad	USGS	Hydrologist	ranustad@usgs.gov
Sharon	Reedyk	Agriculture Canada	Manager, Water Quality Impacts	Sharon.Reedyk@AGR.GC.CA
Les	Rutherford	Environment and Climate Change Canada	Manager, Lake Winnipeg Basin Initiative	Les.Rutherford@ec.gc.ca
John	Sandberg	Minnesota Pollution Control Agency	Environmental Research Scientist	john.sandberg@state.mn.us
Victor	Serveiss	International Joint Commission	Environmental Advisor	serveissv@Washington.IJC.org
Justin	Shead	Manitoba Sustainable Development	Senior Water Quality Specialist - Biologist	justin.shead@gov.mb.ca
Rob	Sip	Minnesota Dept of Agriculture	Agency Policy Specialist	Rob.Sip@state.mn.us
Luke	Stuewe	Minnesota Department of Agriculture	Soils Specialist	luke.stuewe@state.mn.us
Mike	Vavricka	Minnesota Pollution Control Agency	Research Scientist	michael.vavricka@state.mn.us
Jim	Ziegler	Minnesota Pollution Control Agency	Pollution Control Program Administration	jim.ziegler@state.mn.us
RESPEC Attendees				
Julie	Blackburn	RESPEC	Project Manager	julie.blackburn@respec.com
Tony	Miller	RESPEC	Senior Ecologist	tony.miller@respec.com
Bruce	Wilson	RESPEC	Senior Water Quality Scientist	bruce.wilson@respec.com

WEBEX EXPERT'S MEETING ATTENDEES; JANUARY 29 AND FEBRUARY 2 COMBINED

First	Last	Organization
Luther	Aadland	Minnesota Department of Natural Resources
Nicole	Armstrong	Manitoba Sustainable Development
Mike	Ell	North Dakota Department of Health
Kristina	Farmer	Environment and Climate Change Canada
Arthur	Friesen	
Iris	Griffin	Environment and Climate Change Canada
Mike	Hargis	
Steve	Heiskary	Minnesota Pollution Control Agency
Candice		EPA Region 5
Marinus	Otte	North Dakota State University
Ed	Ranking	
Sharon	Reedyk	Agriculture Canada
Chuck	Regan	Minnesota Pollution Control Agency
Justin	Shead	Manitoba Sustainable Development
Chris	Yoder	Midwest Biodiversity Institute
Jason	Vanrobaeys	Manitoba Sustainable Development
Jim	Ziegler	Minnesota Pollution Control Agency

APPENDIX D
ALGAE COLLECTION AND ANALYSIS METHODS

APPENDIX D ALGAE COLLECTION AND ANALYSIS METHODS

Field Procedures for Collection of Periphyton Samples Using Artificial Substrates

Note: A more thorough procedure for collection of algae is described within Section 6.1. These procedures were distributed as an initial memo to the project team.

Revised Draft June 24, 2015

Erich Weber, Staff Scientist
RESPEC

Artificial substrates ("periphytometers"), consisting of float-mounted racks containing glass microscope slides, will be employed to collect periphyton (attached algae) samples from the Red River of the North (RRN) in Minnesota/North Dakota, USA and Manitoba, Canada during the summer of 2015. Periphytometers (samplers) will be deployed at predetermined monitoring sites on the RRN, selected after thorough review of existing monitoring networks and data from previous studies. Three replicate samplers will be deployed at each of 30 sites including 23 U.S. sites (additional site being considered by MPCA in the upper reach with greatest nutrient change) and 7 Manitoba sites.

An exposure period of from 3 to 6 weeks will be utilized, with the length to be determined by field assessments of the rate of periphyton growth on the substrates at selected sites. The length of exposure will be standardized between all sites to the greatest degree possible with coordination between MPCA and Manitoba as algal growth is monitored as follows:

- Minnesota sites. The MPCA routine river monitoring will check every two weeks and will take pictures and forward results to Nicolle Armstrong.
- Manitoba: Check site weekly, at the Winnipeg site and forward to Nicole Armstrong.
- North Dakota: The ND Department of Health can check sites near Grand Forks weekly and forward information to Nicole Armstrong.

Deployment

Deployment by boat/wading will occur in mid-July for all the sites. It is critical that the samplers are placed in-stream in a manner that maximizes their chance of survival through the deployment period, while minimizing significant variability between sites of factors independent of water quality. It is also important to consider the ease of access to sampler installations if multiple site visits are required. Hazards to floating samplers include, but aren't limited to: boat traffic, curious recreationalists, vandals, floating debris and fluctuating water stage. Locations nearer to the edges of the main channel, but beyond

that easily reached by individuals onshore, are preferred. Locations visible from bridges, roads and streamside trails are less desirable. This is a greater concern in urban areas and near public access sites. Monitoring sites may be at bridges, fishing access sites, etc. out of necessity. Unless the time and effort is expended to access secluded locations, it may be difficult to deploy samplers out of the public eye. Riparian vegetation, well-anchored downed trees, structures along the stream bank, channel meanders and other natural and man-made objects can serve to screen the samplers from view. Locations beneath large, living trees or other objects that would significantly shade the samplers should be avoided. Samplers should not be placed in areas of strong current that would likely transport large floating debris or in backwater areas likely to accumulate flotsam. Fluctuations in river stage, particularly major decreases that would leave samplers stranded out of the water, must be anticipated, and samplers placed in water of adequate depth.

Sampler installation, inspection and retrieval of will require the use of boats, because of water depth and the muddy nature of the stream banks and bottom. Samplers will be tethered to buoys, suspended above anchors placed on the stream bottom in about 2-3 meters of water depth. The 3 replicate samplers at each site should be placed in similar current velocities and aspects, spaced from 5-10 meters apart, and staggered as to not interfere with, or "shadow", one another. Again, a primary goal is to select suitable locations for each sampler "cluster" that maximizes comparability between replicates and sites, while minimizing the likelihood of all replicates befalling the same fate to vandals or the elements.

Dry Run Deployment

An early joint deployment (dry run) is proposed at one site by staff from the MPCA/ND/Manitoba for training to help standardize procedures. Logistics will be coordinated by Nicole Armstrong and tentatively include team members: (1) MPCA: Joel Chirhart, Ben Lundeen, Pam Anderson; (2) Manitoba: Nicole Armstrong, David Hay, Joy Kennedy; (3) ND: Mike Ell; and (RESPEC)Erich Weber.

Periphytometer Preparation and Deployment Procedures

6. Glass microscope slides (frosted on one side, 20 per sampler) must be pre-cleaned prior to deployment; denatured ethyl alcohol or alcohol wipes work well, and the vapors are less-offensive than acetone.
7. Install 20 pre-cleaned slides in the plastic sampler rack, maintaining the same orientation of frosted and unfrosted slide faces across the rack. Install the rack of slides between the floats on the sampler frame. It may be easier to transport the loaded racks of slides into the field in a covered container, and install them in the sampler frames immediately before deployment.
8. Samplers should be tethered to the buoys with nylon parachute cord or similar high-strength water-resistant cordage. Securely tie one end of a 4' (1.25m) length of cord to the upstream eye of the sampler, and the other end to the buoy. The sampler should trail about 1m downstream of the buoy, with the plastic shield facing **upstream**, and move freely with the current without radical oscillations because of turbulence. Hester-Dendy sampler(s) for aquatic macroinverteb-rates will be deployed at the same time as the periphyton samplers, suspended from one or more of the buoys at each site, per MPCA field procedures.

9. Field data collection will follow established MPCA and Manitoba River monitoring protocols (MPCA sampling list follows). As possible, grab samples will be obtained at deployment and retrieval of the periphytometers with requested analytes listed below with a primary focus on N, P and TSS. The MPCA sites will also be sampled by the Surface Water Monitoring Section of the Environmental Analysis and Outcomes Division following their river monitoring program protocol as specified below. Data from this effort will rely upon existing MPCA and Manitoba QA/QC protocols for field duplicates, field blanks and laboratory precision and accuracy.
10. Water Quality Samples. The MPCA is scheduled to collect water chemistry at the established MPCA Red River sites. E. coli collected 3x per month and BOD once per month. The MPCA list does not include SRP, air temperature or turbidity (the MPCA employs a transparency tube). Otherwise the MPCA samples will be collected twice per month June–August as below (with parenthesis indicating laboratory number):

TSVS (4)
TSS (3)
Total P (59)
Ammonia-N (64)
TKN (68)
NO_2+NO_3 (69)
Sulfate (293)
Chloride (297)
Hardness (239)
BOD (96)
Chl-a (451)
Pheophytin-a (452)
E. coli (335)

Periphytometer Retrieval and Handling

At the end of the deployment period, the three replicate samplers (or however many have survived) at each site will be retrieved and the microscope slides preserved for future analysis. The main priority is to carefully retrieve and distribute the slides intended for taxonomic and chlorophyll *a*/AFDW analysis without compromising the attached film of algae.

11. Gently remove each sampler from the water and detach it from the tether line, and place the entire unit in a plastic tote or basin. Carefully remove the slide rack from the float frame and place it in a small pan, and cover with aluminum foil. Slides must be shielded from direct sunlight and processed as quickly as possible to minimize drying and exposure to heat.
12. A composite sample of subsamples from each of the three replicates will be collected for taxonomic analysis. Open the slide racks and remove 3 representative microscope slides from each replicate, selecting one from each of the right, center and left sections of the slide rack, and

place them in a labeled 250 mL wide-mouth HDPE Nalgene bottle. A total of 9 slides per site will be composited in the single sample container.

13. Add tap water to submerge the slides (**do not** use river water or deionized water), and preserve with 5 mL of Lugols IKI solution (sample should be the color of strong tea). As noted by field staff, Lugols preservative will turn regular paper labels black and make them illegible, so protection of labels with clear packing tape is required.
14. Place the taxonomic samples on wet ice and maintain in a chilled state until they are received at the RESPEC office in Helena, MT. For shipping, sample bottles should be sealed in 1L Ziploc-type freezer bags (a single site per bag is preferable), placed between blue gel ice packs in a plastic cooler, and the cooler sealed with duct tape and shipped by expedited means (e.g., UPS or Fed Ex next-day or two day delivery). **Taxonomy samples should not be frozen or allowed to come in contact with dry ice, as freezing will compromise the sample.** Shipping times must be coordinated so as to guarantee that samples arrive in Helena during weekday business hours, and do not sit untended over a weekend. This likely will necessitate shipping early in the week, probably on Monday or Tuesday. Notice of sample shipping must be sent by email to Erich Weber at the RESPEC office in Helena.
15. The remaining microscope slides (17 slides per replicate) will be processed for chlorophyll *a*/AFDW analyses **by the MPCA/Manitoba Sustainable Development or affiliated partner laboratories**. Again, slides must be handled in a location shielded from direct sunlight and not allowed to dry out excessively so as not to degrade the chlorophyll. Place all 17 slides from each replicate into a **separate**, labeled, solvent-proof sample container (e.g. 250 mL wide-mouth HDPE Nalgene bottle). **Do not cover the chlorophyll slides with water.** There will be 3 replicates per site.
16. Tightly cap each sample bottle and wrap completely in aluminum foil to exclude light. Seal the three replicate sample bottles from each site inside a labeled 1L Ziploc-type freezer bag, and **immediately place the samples on dry ice.** Chlorophyll *a*/AFDW samples must be kept well-frozen until they are delivered to the MPCA, Manitoba Sustainable Development or Energy Laboratories (Helena) analytical lab (specific lab to be determined). **Shipped samples must be kept frozen on sufficient quantities of dry ice.** This will likely pose some logistical and regulatory challenges that need to be ironed out before shipping. Hand delivery of samples to the laboratory is the best option, when practical. Samples can be stored in a deep freeze for up to 28 days prior to analysis. Solvents **should not** be added to sample bottles in the field.
17. The analytical laboratory will follow Standard Methods for chlorophyll *a*/AFDW analyses, which are summarized as follows: After receipt by the analytical laboratory, the required solvent for extraction of chlorophyll *a* is measured directly into the Nalgene sample bottle containing the 17 glass slides. Following the measurement of the chlorophyll *a* concentration by fluorimetic or spectrophotometric methods, the entire contents of the sample bottle (glass slides, extractant and any sloughed algal material) is placed into a suitable, high-temperature resistant vessel. The solvent is evaporated, the sample thoroughly dried in a drying oven, and the initial dry weight determined. The sample then is ashed in a Muffle furnace at 500°C, cooled, re-wetted and re-dried to restore the water of hydration mass, and the final weight determined. Mike Ell is coordinating laboratory questions that arise for this parameter.

Seston (Phytoplankton) Samples

Duplicate phytoplankton samples will be collected at the time of installation of each periphytometer at each site and combined into one sample for analysis. Samples will be labeled, Lugol preserved and sent to Erich Weber for analyses at the below address. The sample should be collected in the main river channel in proximity to the artificial substrates. If possible, the sample will be collected and handled as a quantitative sample (i.e., known volume of water passing a defined phytoplankton net (20 micron) tow or peristaltic pump volume to allow for calculation of # cells or algal units per L. If plankton net tows are employed, the sample should include documentation of phytoplankton tow net diameter and length of tows (or the duration and current speed if held) or volume of water pumped.

18. Counts of the phytoplankton algae will be accomplished, to allow comparisons of algal densities between sites. Literature algal biovolume values will be used for most diatoms and soft algae to estimate algal biovolumes. Please forward known RRN studies/references that have quantified phytoplankton biovolumes.

Contact Information:
Mr. Erich Weber – RESPEC (Helena, Montana)
406.439.0563 – cell
406.502.1546 – office
erich.weber@respec.com

Shipping address for taxonomy samples:
RESPEC
820 North Montana Ave., Suite A
Helena, MT 59601

Shipping address for Energy Laboratories in Helena, Montana:
Energy Laboratories
3161 E. Lyndale Ave.
Helena, MT 59601
Phone: 877.472.0711 (toll free)
Business hours: 8 AM to 5 PM Monday-Friday

APPENDIX E
WATER CHEMISTRY RESULTS

Consistently measured analytes between labs

		Periphyton Chlorophyll a	Periphyton Ash Free Dry Weight	Phytoplankton Chlorophyll a	Pheophytin a	Volatile Suspended Solids	Total Suspended Solids	Total Phosphorus	Ortho-phosphate	Dissolved Oxygen	Total Nitrogen	NO_3+NO_2	Total Kjeldahl Nitrogren	Ammonia	pH	Specific Conductivity	TEMP
		mg/m^2	g/m^2	µg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	N/A	$\mu S/cm^{-1}$	°C
Site Name	**River Mile**	A10200 H	A10300 C	10200-H	10200-H	2540-E	2540-D	4500-P-I(F)	4500-P-F	Field	Additive	353.20	351.20	350.10	Field	Field	Field
84RD008	547	23.33	7.17	17.27	6.32	9.07	50.33	0.07	0.03	7.15	0.78	0.12	0.66	0.05	8.23	489.25	26.11
15RD067	450	17.90	3.34	16.73	10.35	10.67	75.67	0.14	0.08	6.93	1.21	0.27	0.94	0.05	8.22	732.67	25.26
15RD066	447	34.83	5.32	16.57	13.43	12.67	86.67	0.16	0.10	6.99	1.53	0.47	1.06	0.05	8.22	741.33	25.21
84RD022	441	30.40	3.62	17.90	17.93	15.67	113.33	0.18	0.10	6.82	1.67	0.54	1.12	0.05	8.20	726.33	24.81
15RD065	437	17.30	3.29	14.23	14.57	15.67	130.00	0.25	0.16	6.68	1.95	0.88	1.07	0.05	8.16	743.67	24.78
05RD030	421	14.13	10.78	22.17	25.17	38.33	290.00	0.40	0.21	6.79	1.93	0.59	1.34	0.06	8.27	1153.00	25.16
84RD027	389	32.90	5.21	25.83	30.43	35.33	300.00	0.40	0.20	6.72	2.11	0.56	1.55	0.05	8.27	1147.25	25.71
94RD018	357	11.97	12.13	21.23	32.60	45.33	336.67	0.36	0.19	6.82	1.90	0.57	1.33	0.05	8.27	1057.75	25.69
15RD058	297	32.53	12.73	16.07	22.63	33.00	230.00	0.28	0.15	6.53	1.74	0.45	1.28	0.05	8.12	787.67	25.26
84RD037	294	19.65	11.03	9.35	38.67	29.67	226.67	0.28	0.16	6.79	1.77	0.47	1.31	0.05	8.18	819.00	25.27
15RD057	290	63.30	9.69	13.41	23.97	25.33	190.00	0.26	0.16	6.56	1.70	0.47	1.23	0.05	8.14	779.00	24.84
15RD056	274	26.05	12.50	15.13	20.10	24.67	193.33	0.23	0.12	6.99	1.47	0.32	1.16	0.06	8.18	867.75	25.97
15RD055	245	11.70	13.10	14.70	31.77	42.67	303.33	0.31	0.16	6.63	1.72	0.45	1.27	0.06	8.14	952.75	25.90
84RD042	226	35.67	9.32	16.77	33.43	37.67	293.33	0.32	0.14	6.64	1.78	0.44	1.34	0.06	8.06	991.00	25.70
15RD052	189	28.93	5.31	14.13	37.53	37.33	283.33	0.31	0.14	6.78	1.71	0.40	1.31	0.06	7.97	944.00	25.43
84RD047	157	38.43	5.20	16.53	35.24	36.33	296.67	0.32	0.15	6.83	1.82	0.41	1.41	0.05	7.97	894.25	25.21
MB05OCS007	155	80.85	14.60	12.89	4.66	32.00	232.00	0.34	0.12	6.25	1.80	0.33	1.47	0.03	8.38	897.50	25.60
MB05OCS033	101	67.47	16.40	5.79	4.35	15.50	116.00	0.29	0.16	6.10	1.68	0.30	1.38	0.08	8.38	814.00	25.74
MB05OCS004	61	154.75	17.90	4.46	3.34	19.50	192.00	0.34	0.14	5.85	1.57	0.31	1.25	0.02	8.24	716.50	24.95
MB05OJS057	39	69.00	22.05	18.03	6.19	14.00	95.33	0.27	0.17	6.83	1.42	0.26	1.16	0.04	8.42	822.33	23.61
MB05OJS004	34	216.33	6.30	13.76	7.10	14.00	108.00	0.32	0.15	5.80	1.54	0.27	1.27	0.07	8.30	802.50	24.40
MB05OJS074	16	164.67	6.00	17.39	8.17	10.00	53.00	0.30	0.19	7.20	1.61	0.36	1.25	0.05	8.29	787.50	23.45
MB05OJS128	0	184.67	5.70	18.20	8.39	14.00	88.67	0.32	0.19	6.80	1.65	0.35	1.30	0.04	8.26	783.67	23.81

All values are an average of two samples taken during periphytometer deployment
Brown denotes processing by Energy Labs (MT)
Blue denotes processing by MPCA lab
Green denotes processing by Manitoba Conservation and Water Stewardship lab

APPENDIX F
ALGAE TAXONOMIC RESULTS

(See attached jump drive for electronic spreadsheets)

APPENDIX G
STATISTICAL OUTPUT

```
********** Nonmetric Multidimensional Scaling **********
PC-ORD, 6.19
 1 Apr 2016, 10:04:09


NMS of stressor-response site nutrients (TN, TP, NH4, NO3+NO2)
Ordination of Sites    in Species  space.         23 Sites           4 Parameters

         The following options were selected:
ANALYSIS OPTIONS
         1.   SORENSEN = Distance measure
         2.          4 = Number of axes (max. = 6)
         3.        200 = Maximum number of iterations
         4.     RANDOM = Starting coordinates (random or from file)
         5.          1 = Reduction in dimensionality at each cycle
         6. NO PENALTY = Tie handling (Strategy 1 does not penalize
                         ties with unequal ordination distance,
                         while strategy 2 does penalize.)
         7.       0.20 = Step length (rate of movement toward minimum stress)
         8.   USE TIME = Random number seeds (use time vs. user-supplied)
         9.         50 = Number of runs with real data
        10.         50 = Number of runs with randomized data
        11.        YES = Autopilot
        12.   0.000010 = Stability criterion, standard deviations in stress
                         over last  10 iterations.
        13.     MEDIUM = Speed vs. thoroughness
OUTPUT OPTIONS
        14.         NO = Write distance matrix?
        15.         NO = Write starting coordinates?
        16.         NO = List stress, etc. for each iteration?
        17.         NO = Plot stress vs. iteration?
        18.         NO = Plot distance vs. dissimilarity?
        19.         NO = Write final configuration?
        20. PRINC.AXES = Write varimax-rotated, principal axes, or unrotated scores
for graph?
        21.        YES = Write run log?
        22.         NO = Write weighted-average scores for Species ?
-------------------------------------------------------------------------------

       911 = Seed for random number generator.

           4 = Number of tie blocks in dissimilarity matrix.
           8 = Number of elements involved in ties.
         253 = Total number of elements in dissimilarity matrix.
       3.162 = Percentage of elements involved in ties.


RUN LOG
-------------------------------------------------------------------------
    Random Start Dimen-    Final  Iter-              Best for   File
Run  data? file?  sions   stress ations Instability*  x axes   saved**
-------------------------------------------------------------------------
  1     0     0      4    2.134   172   0.00000000
  1     0     0      3    2.839    42   0.00000000
  1     0     0      2    9.949    24   0.00000000
  1     0     0      1   20.898    20   0.00000000
  2     0     0      4    2.190   105   0.00000000
  2     0     0      3    2.834    58   0.00000000
  2     0     0      2    8.184    34   0.00000000
  2     0     0      1   20.947    23   0.00000000
  3     0     0      4    2.084   117   0.00000000
```

3	0	0	3	2.815	46	0.00000000		
3	0	0	2	8.189	24	0.00000000		
3	0	0	1	23.145	46	0.00000000		
4	0	0	4	2.143	63	0.00000000		
4	0	0	3	2.868	96	0.00000000		
4	0	0	2	8.198	36	0.00000000		
4	0	0	1	21.187	43	0.00000000		
5	0	0	4	2.056	111	0.00000000		
5	0	0	3	2.803	31	0.00000000	3	CONFIG3.GPH
5	0	0	2	8.199	36	0.00000000		
5	0	0	1	50.891	26	0.00000000		
6	0	0	4	2.194	95	0.00000000		
6	0	0	3	2.816	112	0.00000000		
6	0	0	2	16.857	83	0.00000000		
6	0	0	1	55.035	15	0.00000000		
7	0	0	4	2.052	77	0.00000000		
7	0	0	3	2.820	72	0.00000000		
7	0	0	2	13.521	45	0.00000000		
7	0	0	1	20.924	50	0.00000000		
8	0	0	4	2.054	86	0.00000000		
8	0	0	3	2.846	65	0.00000000		
8	0	0	2	38.277	26	0.00000000		
8	0	0	1	55.117	21	0.00000000		
9	0	0	4	2.115	200	0.00010116*		
9	0	0	3	2.887	99	0.00000000		
9	0	0	2	8.783	34	0.00000000		
9	0	0	1	23.414	37	0.00000000		
10	0	0	4	2.153	99	0.00000000		
10	0	0	3	2.808	48	0.00000000		
10	0	0	2	12.783	45	0.00000000		
10	0	0	1	21.163	44	0.00000000		
11	0	0	4	2.050	113	0.00000000		
11	0	0	3	2.830	106	0.00000000		
11	0	0	2	8.191	30	0.00000000		
11	0	0	1	55.168	21	0.00000000		
12	0	0	4	2.138	73	0.00000000		
12	0	0	3	2.885	81	0.00000000		
12	0	0	2	13.714	54	0.00000000		
12	0	0	1	21.002	41	0.00000000		
13	0	0	4	2.118	87	0.00000000		
13	0	0	3	7.074	72	0.00000000		
13	0	0	2	8.196	42	0.00000000		
13	0	0	1	20.966	36	0.00000000		
14	0	0	4	2.521	81	0.00000000		
14	0	0	3	2.823	59	0.00000000		
14	0	0	2	8.204	49	0.00000000		
14	0	0	1	29.174	37	0.00000000		
15	0	0	4	2.041	104	0.00000000		
15	0	0	3	2.831	112	0.00000000		
15	0	0	2	13.579	45	0.00000000		
15	0	0	1	21.114	46	0.00000000		
16	0	0	4	2.017	46	0.00000000		
16	0	0	3	2.815	27	0.00000000		
16	0	0	2	14.604	48	0.00000000		
16	0	0	1	22.763	32	0.00000000		
17	0	0	4	2.199	99	0.00000000		
17	0	0	3	2.867	90	0.00000000		
17	0	0	2	16.868	52	0.00000000		
17	0	0	1	55.145	15	0.00000000		
18	0	0	4	2.068	106	0.00000000		
18	0	0	3	2.811	86	0.00000000		
18	0	0	2	8.190	54	0.00000000		
18	0	0	1	23.471	46	0.00000000		

19	0	0	4	2.041	90	0.00000000		
19	0	0	3	2.866	75	0.00000000		
19	0	0	2	8.203	53	0.00000000		
19	0	0	1	20.898	39	0.00000000		
20	0	0	4	2.057	68	0.00000000		
20	0	0	3	2.832	135	0.00000000		
20	0	0	2	14.949	69	0.00000000		
20	0	0	1	54.920	33	0.00000000		
21	0	0	4	2.078	97	0.00000000		
21	0	0	3	2.828	69	0.00000000		
21	0	0	2	8.789	68	0.00000000		
21	0	0	1	22.767	52	0.00000000		
22	0	0	4	2.073	98	0.00000000		
22	0	0	3	2.871	77	0.00000000		
22	0	0	2	13.524	53	0.00000000		
22	0	0	1	23.296	47	0.00000000		
23	0	0	4	2.034	159	0.00000000		
23	0	0	3	2.826	74	0.00000000		
23	0	0	2	15.320	54	0.00000000		
23	0	0	1	22.976	38	0.00000000		
24	0	0	4	2.126	77	0.00000000		
24	0	0	3	2.824	54	0.00000000		
24	0	0	2	8.190	56	0.00000000		
24	0	0	1	48.908	33	0.00000000		
25	0	0	4	2.483	75	0.00000000		
25	0	0	3	2.853	135	0.00000000		
25	0	0	2	8.781	73	0.00000000		
25	0	0	1	23.412	39	0.00000000		
26	0	0	4	2.280	79	0.00000000		
26	0	0	3	2.866	69	0.00000000		
26	0	0	2	8.202	58	0.00000000		
26	0	0	1	55.158	15	0.00000000		
27	0	0	4	2.411	158	0.00000000		
27	0	0	3	2.866	83	0.00000000		
27	0	0	2	16.840	41	0.00000000		
27	0	0	1	20.893	72	0.00000000		
28	0	0	4	2.012	94	0.00000000		
28	0	0	3	2.809	34	0.00000000		
28	0	0	2	14.625	60	0.00000000		
28	0	0	1	21.996	32	0.00000000		
29	0	0	4	2.045	89	0.00000000		
29	0	0	3	2.858	57	0.00000000		
29	0	0	2	8.203	44	0.00000000		
29	0	0	1	54.043	19	0.00000000		
30	0	0	4	2.212	132	0.00000000		
30	0	0	3	2.869	74	0.00000000		
30	0	0	2	9.950	30	0.00000000		
30	0	0	1	22.763	38	0.00000000		
31	0	0	4	2.265	100	0.00000000		
31	0	0	3	2.884	98	0.00000000		
31	0	0	2	14.689	64	0.00000000		
31	0	0	1	22.903	47	0.00000000		
32	0	0	4	2.251	83	0.00000000		
32	0	0	3	2.878	112	0.00000000		
32	0	0	2	8.183	74	0.00000000	2	CONFIG2.GPH
32	0	0	1	23.261	48	0.00000000		
33	0	0	4	2.153	95	0.00000000		
33	0	0	3	2.877	65	0.00000000		
33	0	0	2	15.921	45	0.00000000		
33	0	0	1	21.597	38	0.00000000		
34	0	0	4	2.163	179	0.00000000		
34	0	0	3	2.864	85	0.00000000		
34	0	0	2	8.194	28	0.00000000		

34	0	0	1	55.166	12	0.00000000		
35	0	0	4	1.985	51	0.00000000	4	CONFIG4.GPH
35	0	0	3	2.827	73	0.00000000		
35	0	0	2	14.534	32	0.00000000		
35	0	0	1	22.903	37	0.00000000		
36	0	0	4	2.173	114	0.00000000		
36	0	0	3	2.860	75	0.00000000		
36	0	0	2	9.952	77	0.00000000		
36	0	0	1	22.122	44	0.00000000		
37	0	0	4	2.035	70	0.00000000		
37	0	0	3	2.827	94	0.00000000		
37	0	0	2	8.199	55	0.00000000		
37	0	0	1	21.478	59	0.00000000		
38	0	0	4	2.094	107	0.00000000		
38	0	0	3	2.832	50	0.00000000		
38	0	0	2	8.186	48	0.00000000		
38	0	0	1	54.663	16	0.00000000		
39	0	0	4	2.121	115	0.00000000		
39	0	0	3	2.865	122	0.00000000		
39	0	0	2	8.788	44	0.00000000		
39	0	0	1	20.926	40	0.00000000		
40	0	0	4	2.146	73	0.00000000		
40	0	0	3	2.863	95	0.00000000		
40	0	0	2	9.948	81	0.00000000		
40	0	0	1	29.396	28	0.00000000		
41	0	0	4	2.030	128	0.00000000		
41	0	0	3	2.820	98	0.00000000		
41	0	0	2	14.679	41	0.00000000		
41	0	0	1	21.659	46	0.00000000		
42	0	0	4	2.280	88	0.00000000		
42	0	0	3	2.815	82	0.00000000		
42	0	0	2	9.951	65	0.00000000		
42	0	0	1	20.923	47	0.00000000		
43	0	0	4	2.082	154	0.00000000		
43	0	0	3	2.843	114	0.00000000		
43	0	0	2	14.205	54	0.00000000		
43	0	0	1	20.888	45	0.00000000	1	CONFIG1.GPH
44	0	0	4	2.126	169	0.00000000		
44	0	0	3	2.869	124	0.00000000		
44	0	0	2	9.951	122	0.00000000		
44	0	0	1	20.937	44	0.00000000		
45	0	0	4	2.154	154	0.00000000		
45	0	0	3	2.814	101	0.00000000		
45	0	0	2	9.948	48	0.00000000		
45	0	0	1	23.452	48	0.00000000		
46	0	0	4	2.040	69	0.00000000		
46	0	0	3	2.827	56	0.00000000		
46	0	0	2	13.529	46	0.00000000		
46	0	0	1	20.898	39	0.00000000		
47	0	0	4	2.167	92	0.00000000		
47	0	0	3	2.815	94	0.00000000		
47	0	0	2	8.204	59	0.00000000		
47	0	0	1	21.000	39	0.00000000		
48	0	0	4	2.123	87	0.00000000		
48	0	0	3	2.838	55	0.00000000		
48	0	0	2	13.517	50	0.00000000		
48	0	0	1	54.909	12	0.00000000		
49	0	0	4	2.116	90	0.00000000		
49	0	0	3	2.821	46	0.00000000		
49	0	0	2	8.194	44	0.00000000		
49	0	0	1	20.921	38	0.00000000		
50	0	0	4	2.017	86	0.00000000		
50	0	0	3	2.841	79	0.00000000		

50	0	0	2	8.198	33	0.00000000
50	0	0	1	21.083	43	0.00000000

51	1	0	4	4.728	111	0.00000000
51	1	0	3	8.103	103	0.00000000
51	1	0	2	16.568	78	0.00000000
51	1	0	1	55.168	34	0.00000000
52	1	0	4	5.794	140	0.00000000
52	1	0	3	8.567	91	0.00000000
52	1	0	2	26.296	62	0.00000000
52	1	0	1	52.549	20	0.00000000
53	1	0	4	7.832	88	0.00000000
53	1	0	3	10.255	142	0.00000000
53	1	0	2	22.378	47	0.00000000
53	1	0	1	39.380	28	0.00000000
54	1	0	4	5.933	68	0.00000000
54	1	0	3	9.684	182	0.00000000
54	1	0	2	16.432	38	0.00000000
54	1	0	1	54.659	13	0.00000000
55	1	0	4	6.065	53	0.00000000
55	1	0	3	8.345	56	0.00000000
55	1	0	2	22.296	53	0.00000000
55	1	0	1	39.151	41	0.00000000
56	1	0	4	6.843	77	0.00000000
56	1	0	3	8.756	54	0.00000000
56	1	0	2	17.358	56	0.00000000
56	1	0	1	38.205	31	0.00000000
57	1	0	4	4.986	70	0.00000000
57	1	0	3	6.838	74	0.00000000
57	1	0	2	11.003	44	0.00000000
57	1	0	1	14.809	37	0.00000000
58	1	0	4	4.634	78	0.00000000
58	1	0	3	8.177	93	0.00000000
58	1	0	2	14.939	95	0.00000000
58	1	0	1	31.728	44	0.00000000
59	1	0	4	6.147	89	0.00000000
59	1	0	3	8.540	96	0.00000000
59	1	0	2	29.007	76	0.00000000
59	1	0	1	38.667	30	0.00000000
60	1	0	4	6.449	119	0.00000000
60	1	0	3	10.156	46	0.00000000
60	1	0	2	22.088	33	0.00000000
60	1	0	1	36.326	38	0.00000000
61	1	0	4	6.838	48	0.00000000
61	1	0	3	10.141	112	0.00000000
61	1	0	2	17.411	80	0.00000000
61	1	0	1	41.190	36	0.00000000
62	1	0	4	3.592	55	0.00000000
62	1	0	3	5.855	70	0.00000000
62	1	0	2	12.695	43	0.00000000
62	1	0	1	55.002	27	0.00000000
63	1	0	4	4.628	63	0.00000000
63	1	0	3	8.485	72	0.00000000
63	1	0	2	15.356	95	0.00000000
63	1	0	1	33.143	30	0.00000000
64	1	0	4	4.024	123	0.00000000
64	1	0	3	7.245	83	0.00000000
64	1	0	2	13.614	60	0.00000000
64	1	0	1	26.971	45	0.00000000
65	1	0	4	4.399	104	0.00000000
65	1	0	3	8.151	62	0.00000000
65	1	0	2	12.606	38	0.00000000
65	1	0	1	31.473	37	0.00000000

66	1	0	4	4.409	86	0.00000000
66	1	0	3	7.817	62	0.00000000
66	1	0	2	15.476	51	0.00000000
66	1	0	1	30.052	33	0.00000000
67	1	0	4	4.282	79	0.00000000
67	1	0	3	5.626	78	0.00000000
67	1	0	2	11.371	93	0.00000000
67	1	0	1	40.303	30	0.00000000
68	1	0	4	3.087	44	0.00000000
68	1	0	3	6.472	69	0.00000000
68	1	0	2	10.221	42	0.00000000
68	1	0	1	21.995	38	0.00000000
69	1	0	4	4.333	71	0.00000000
69	1	0	3	8.236	89	0.00000000
69	1	0	2	19.234	27	0.00000000
69	1	0	1	36.168	37	0.00000000
70	1	0	4	5.075	84	0.00000000
70	1	0	3	8.337	68	0.00000000
70	1	0	2	19.485	68	0.00000000
70	1	0	1	41.771	29	0.00000000
71	1	0	4	5.783	81	0.00000000
71	1	0	3	8.009	127	0.00000000
71	1	0	2	19.167	64	0.00000000
71	1	0	1	54.483	14	0.00000000
72	1	0	4	5.079	71	0.00000000
72	1	0	3	8.293	195	0.00000000
72	1	0	2	16.950	55	0.00000000
72	1	0	1	40.445	31	0.00000000
73	1	0	4	5.383	136	0.00000000
73	1	0	3	6.975	75	0.00000000
73	1	0	2	13.575	61	0.00000000
73	1	0	1	43.425	30	0.00000000
74	1	0	4	4.975	69	0.00000000
74	1	0	3	8.175	96	0.00000000
74	1	0	2	11.769	55	0.00000000
74	1	0	1	18.265	34	0.00000000
75	1	0	4	4.449	134	0.00000000
75	1	0	3	7.609	43	0.00000000
75	1	0	2	21.733	40	0.00000000
75	1	0	1	54.512	33	0.00000000
76	1	0	4	4.784	64	0.00000000
76	1	0	3	8.461	87	0.00000000
76	1	0	2	18.562	42	0.00000000
76	1	0	1	36.282	30	0.00000000
77	1	0	4	4.824	81	0.00000000
77	1	0	3	8.000	117	0.00000000
77	1	0	2	18.554	43	0.00000000
77	1	0	1	30.115	27	0.00000000
78	1	0	4	4.661	58	0.00000000
78	1	0	3	10.879	60	0.00000000
78	1	0	2	15.926	43	0.00000000
78	1	0	1	28.765	42	0.00000000
79	1	0	4	5.507	125	0.00000000
79	1	0	3	8.780	110	0.00000000
79	1	0	2	18.865	60	0.00000000
79	1	0	1	36.949	41	0.00000000
80	1	0	4	5.162	97	0.00000000
80	1	0	3	8.085	105	0.00000000
80	1	0	2	28.349	34	0.00000000
80	1	0	1	49.764	25	0.00000000
81	1	0	4	4.039	78	0.00000000
81	1	0	3	7.349	90	0.00000000
81	1	0	2	12.215	89	0.00000000

81	1	0	1	21.542	41	0.00000000
82	1	0	4	3.664	123	0.00000000
82	1	0	3	5.300	194	0.00000000
82	1	0	2	9.707	73	0.00000000
82	1	0	1	18.920	68	0.00000000
83	1	0	4	4.593	107	0.00000000
83	1	0	3	10.008	111	0.00000000
83	1	0	2	18.335	126	0.00000000
83	1	0	1	39.680	30	0.00000000
84	1	0	4	3.891	61	0.00000000
84	1	0	3	7.170	46	0.00000000
84	1	0	2	19.152	51	0.00000000
84	1	0	1	32.952	33	0.00000000
85	1	0	4	5.336	60	0.00000000
85	1	0	3	11.292	58	0.00000000
85	1	0	2	18.460	49	0.00000000
85	1	0	1	32.235	86	0.00000000
86	1	0	4	5.887	108	0.00000000
86	1	0	3	8.407	89	0.00000000
86	1	0	2	20.630	123	0.00000000
86	1	0	1	41.281	46	0.00000000
87	1	0	4	4.374	81	0.00000000
87	1	0	3	8.782	62	0.00000000
87	1	0	2	16.592	49	0.00000000
87	1	0	1	29.011	36	0.00000000
88	1	0	4	3.939	83	0.00000000
88	1	0	3	7.308	51	0.00000000
88	1	0	2	15.812	150	0.00000000
88	1	0	1	54.304	13	0.00000000
89	1	0	4	4.238	42	0.00000000
89	1	0	3	7.927	58	0.00000000
89	1	0	2	19.376	73	0.00000000
89	1	0	1	43.272	29	0.00000000
90	1	0	4	4.761	102	0.00000000
90	1	0	3	7.796	60	0.00000000
90	1	0	2	12.564	34	0.00000000
90	1	0	1	30.881	42	0.00000000
91	1	0	4	3.807	102	0.00000000
91	1	0	3	7.162	39	0.00000000
91	1	0	2	14.903	40	0.00000000
91	1	0	1	29.411	34	0.00000000
92	1	0	4	5.762	57	0.00000000
92	1	0	3	8.313	36	0.00000000
92	1	0	2	16.030	41	0.00000000
92	1	0	1	37.116	36	0.00000000
93	1	0	4	5.967	48	0.00000000
93	1	0	3	9.164	103	0.00000000
93	1	0	2	16.899	51	0.00000000
93	1	0	1	32.363	32	0.00000000
94	1	0	4	4.716	159	0.00000000
94	1	0	3	7.402	43	0.00000000
94	1	0	2	16.602	87	0.00000000
94	1	0	1	54.896	12	0.00000000
95	1	0	4	5.326	79	0.00000000
95	1	0	3	8.285	56	0.00000000
95	1	0	2	18.666	66	0.00000000
95	1	0	1	51.679	23	0.00000000
96	1	0	4	4.176	69	0.00000000
96	1	0	3	7.561	48	0.00000000
96	1	0	2	14.514	34	0.00000000
96	1	0	1	35.444	40	0.00000000
97	1	0	4	5.583	65	0.00000000
97	1	0	3	9.045	139	0.00000000

97	1	0	2	15.329	64	0.00000000
97	1	0	1	54.826	12	0.00000000
98	1	0	4	6.223	75	0.00000000
98	1	0	3	8.788	139	0.00000000
98	1	0	2	16.434	84	0.00000000
98	1	0	1	36.570	31	0.00000000
99	1	0	4	4.711	75	0.00000000
99	1	0	3	8.878	58	0.00000000
99	1	0	2	17.158	64	0.00000000
99	1	0	1	54.929	22	0.00000000
100	1	0	4	7.074	147	0.00000000
100	1	0	3	8.817	161	0.00000000
100	1	0	2	20.956	75	0.00000000
100	1	0	1	55.145	17	0.00000000

Random data: 0 = not randomized, 1 = randomized
Start file: 0 = random starting coordinates, 1 = read from file
Seeds: initial seeds for random number generator
* Stability criterion not met.
**To run a single NMS ordination that repeats the best result,
specify this file as the starting configuration,
rather than using a random start. It is best to
save this file under a new name, to avoid it being
overwritten by the next NMS run. To do this, open the
file using File | Open | Graph Row file, then
File | Save as | Graph Row file (then specify new name).

STRESS IN RELATION TO DIMENSIONALITY (Number of Axes)

	Stress in real data 50 run(s)			Stress in randomized data Monte Carlo test, 50 runs			
Axes	Minimum	Mean	Maximum	Minimum	Mean	Maximum	p
1	20.888	29.893	55.168	14.809	38.763	55.168	0.0784
2	8.183	11.541	38.277	9.707	17.192	29.007	0.0196
3	2.803	2.926	7.074	5.300	8.236	11.292	0.0196
4	1.985	2.134	2.521	3.087	5.055	7.832	0.0196

p = proportion of randomized runs with stress < or = observed stress
i.e., p = (1 + no. permutations <= observed)/(1 + no. permutations)

Conclusion: a 3-dimensional solution is recommended.
Now rerunning the best ordination with that dimensionality.

Selected file CONFIG3.GPH for the starting configuration for
the final run.

NMS of phytoplankton
Ordination of Sites in Species space. 23 Sites 4 Species

The following options were selected:
ANALYSIS OPTIONS
1. SORENSEN = Distance measure
2. 3 = Number of axes (max. = 6)
3. 200 = Maximum number of iterations
4. FROM FILE = Starting coordinates (random or from file)
5. 3 = Reduction in dimensionality at each cycle
6. NO PENALTY = Tie handling (Strategy 1 does not penalize
ties with unequal ordination distance,

```
                        while strategy 2 does penalize.)
         7.       0.20 = Step length (rate of movement toward minimum stress)
         8.   USE TIME = Random number seeds (use time vs. user-supplied)
         9.          1 = Number of runs with real data
        10.          0 = Number of runs with randomized data
        11.        YES = Autopilot
        12.   0.000010 = Stability criterion, standard deviations in stress
                        over last  10 iterations.
        13.     MEDIUM = Speed vs. thoroughness
OUTPUT OPTIONS
        14.         NO = Write distance matrix?
        15.         NO = Write starting coordinates?
        16.        YES = List stress, etc. for each iteration?
        17.        YES = Plot stress vs. iteration?
        18.         NO = Plot distance vs. dissimilarity?
        19.        YES = Write final configuration?
        20. PRINC.AXES = Write varimax-rotated, principal axes, or unrotated scores
for graph?
        21.         NO = Write run log?
        22.        YES = Write weighted-average scores for Species ?
-------------------------------------------------------------------------------


File containing starting coordinates:
CONFIG3.GPH


List of stress, step length, and magnitude of the gradient
    vector at each iteration.

  Step    Stress Instability  StepLength    Mag(G)
     1  35.69408                 0.20000   0.004670690
     2  30.85600                 0.20000   0.001729131
     3  27.73200                 0.32263   0.001279066
     4  23.58051                 0.36058   0.003582360
     5  19.47626                 0.43741   0.008720865
     6  25.68679                 0.53314   0.019599637
     7  15.99067                 0.46563   0.015314190
     8  10.72195                 0.21178   0.006479906
     9  10.68316                 0.14742   0.010658556
    10   9.04468  0.08771507     0.12382   0.005037515
    11   8.27160  0.07983496     0.07990   0.003273049
    12   8.00693  0.07273774     0.08515   0.006246402
    13   7.43125  0.06483667     0.10012   0.008957434
    14   6.21147  0.05960229     0.08541   0.005966006
    15   5.77310  0.05687920     0.08362   0.010083983
    16   5.09287  0.03022140     0.07761   0.008196498
    17   4.27514  0.02111088     0.05475   0.004345950
    18   4.15808  0.02042507     0.04736   0.006553387
    19   3.83692  0.01789710     0.04527   0.005467725
    20   3.47566  0.01684419     0.03001   0.001976174
    21   3.45516  0.01550136     0.02730   0.003701134
    22   3.27705  0.01316921     0.02453   0.001862459
    23   3.17166  0.01019469     0.01585   0.000822163
    24   3.14249  0.00834859     0.01505   0.001260448
    25   3.08168  0.00613263     0.01321   0.000674782
    26   3.04115  0.00426649     0.00916   0.000340465
    27   3.01876  0.00356626     0.00854   0.000399902
    28   2.99498  0.00254261     0.00756   0.000271951
    29   2.97589  0.00173851     0.00618   0.000188480
    30   2.96037  0.00148138     0.00544   0.000154628
    31   2.94677  0.00101521     0.00474   0.000118885
```

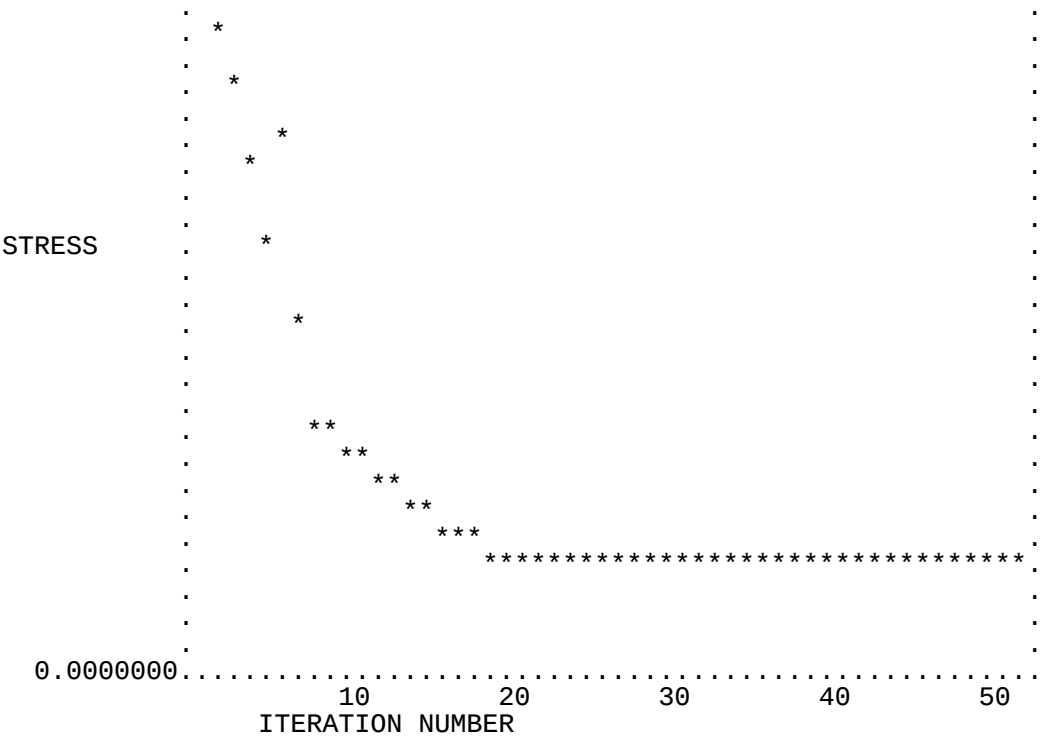
32	2.93503	0.00077854	0.00407	0.000090947
33	2.92480	0.00066316	0.00348	0.000070318
34	2.91576	0.00051599	0.00308	0.000057701
35	2.90694	0.00042898	0.00310	0.000052679
36	2.89810	0.00037668	0.00342	0.000055209
37	2.89058	0.00032817	0.00329	0.000056995
38	2.88421	0.00029166	0.00281	0.000046267
39	2.87862	0.00026262	0.00239	0.000036761
40	2.87327	0.00023807	0.00231	0.000032605
41	2.86745	0.00021837	0.00269	0.000033992
42	2.86220	0.00020179	0.00284	0.000040373
43	2.85795	0.00018619	0.00241	0.000034254
44	2.85424	0.00017042	0.00198	0.000025574
45	2.85089	0.00015542	0.00175	0.000021364
46	2.84716	0.00014375	0.00203	0.000020979
47	2.84323	0.00013487	0.00249	0.000026926
48	2.84031	0.00012636	0.00219	0.000028098
49	2.83770	0.00011738	0.00178	0.000019487
50	2.83546	0.00010794	0.00146	0.000015019
51	2.83325	0.00010005	0.00143	0.000013435
52	2.83060	0.00000000		

2.83060 = final stress for 3-dimensional solution
0.00000 = final instability
52 = number of iterations

Final configuration (ordination scores) for this run

Sites		Axis		
Number	Name	1	2	3
1	547	-0.7202	2.1642	-1.8138
2	450	-0.5085	1.0493	-0.7874
3	447	0.2526	0.5120	-0.5887
4	441	0.5242	0.2997	-0.5130
5	437	1.1716	0.2162	0.2652
6	421	0.7356	-0.5759	0.3785
7	389	0.7626	-0.7141	0.0902
8	357	0.5614	-0.5240	0.0505
9	297	0.1587	-0.0584	-0.0260
10	294	0.2374	-0.0865	-0.0242
11	290	0.2567	0.1018	0.0148
12	274	-0.3625	0.4929	0.0441
13	245	0.1077	-0.0722	0.4609
14	226	0.1490	-0.1792	0.3994
15	189	0.0107	-0.0611	0.4808
16	157	0.0987	-0.2488	0.2166
17	155	-0.3187	-0.8591	0.0867
18	101	-0.3686	0.2113	0.7790
19	61	-0.9234	-1.1780	0.0154
20	39	-0.7404	0.0191	-0.2616
21	34	-0.5486	0.0782	0.7158
22	16	-0.1900	-0.1413	0.0197
23	0	-0.3460	-0.4460	-0.0030

PLOT OF STRESS V. ITERATION NUMBER



Principal axes rotation of 3-dimensional solution.

Configuration after rotation is listed below.

Final configuration (ordination scores) for this run

Sites		Axis		
Number	Name	1	2	3
1	547	-2.9092	0.0240	0.1683
2	450	-1.3990	-0.1495	-0.0065
3	447	-0.6700	0.4261	0.2049
4	441	-0.3911	0.6258	0.2883
5	437	0.2901	1.1605	-0.2424
6	421	0.8558	0.5257	0.0852
7	389	0.8039	0.5404	0.4015
8	357	0.5814	0.3996	0.3076
9	297	0.0716	0.1397	0.0680
10	294	0.1150	0.2080	0.0893
11	290	-0.0035	0.2721	-0.0492
12	274	-0.4510	-0.2244	-0.3500
13	245	0.3493	0.0456	-0.3243
14	226	0.4077	0.0627	-0.2092
15	189	0.3269	-0.0466	-0.3550
16	157	0.3432	0.0119	-0.0246
17	155	0.6328	-0.5371	0.3978
18	101	0.1887	-0.3661	-0.7860
19	61	0.6816	-1.1955	0.5890
20	39	-0.3581	-0.6850	0.1400
21	34	0.2086	-0.5685	-0.6728
22	16	0.0714	-0.2212	0.0495
23	0	0.2539	-0.4484	0.2305

```
Writing weighted average scores on 3 axes for     4 Species
   into file for graphing.
 1 Apr 2016, 10:04:10
        0.02 minutes elapsed time.

*************************** Calculations finished ****************************
```

********** Nonmetric Multidimensional Scaling **********
PC-ORD, 6.19
1 Apr 2016, 10:16:39

NMS of saprobity and nitrogen-uptake metab. periphyton metrics

Ordination of sites in taxa space. 30 sites 9 taxa

The following options were selected:

ANALYSIS OPTIONS

1.	SORENSEN	= Distance measure
2.	4	= Number of axes (max. = 6)
3.	200	= Maximum number of iterations
4.	RANDOM	= Starting coordinates (random or from file)
5.	1	= Reduction in dimensionality at each cycle
6.	NO PENALTY	= Tie handling (Strategy 1 does not penalize ties with unequal ordination distance, while strategy 2 does penalize.)
7.	0.20	= Step length (rate of movement toward minimum stress)
8.	USE TIME	= Random number seeds (use time vs. user-supplied)
9.	50	= Number of runs with real data
10.	50	= Number of runs with randomized data
11.	YES	= Autopilot
12.	0.000010	= Stability criterion, standard deviations in stress over last 10 iterations.
13.	MEDIUM	= Speed vs. thoroughness

OUTPUT OPTIONS

14.	NO	= Write distance matrix?
15.	NO	= Write starting coordinates?
16.	NO	= List stress, etc. for each iteration?
17.	NO	= Plot stress vs. iteration?
18.	NO	= Plot distance vs. dissimilarity?
19.	NO	= Write final configuration?
20.	PRINC.AXES	= Write varimax-rotated, principal axes, or unrotated scores for graph?
21.	YES	= Write run log?
22.	NO	= Write weighted-average scores for taxa ?

3901 = Seed for random number generator.

0 = Number of tie blocks in dissimilarity matrix.
0 = Number of elements involved in ties.
435 = Total number of elements in dissimilarity matrix.
0.000 = Percentage of elements involved in ties.

RUN LOG

Run	Random data?	Start file?	Dimen-sions	Final stress	Iter-ations	Instability*	Best for x axes	File saved**
1	0	0	4	6.255	158	0.00000000		
1	0	0	3	9.980	70	0.00000000		
1	0	0	2	17.184	36	0.00000000		
1	0	0	1	34.027	20	0.00000000		
2	0	0	4	6.253	130	0.00000000		
2	0	0	3	9.979	29	0.00000000		
2	0	0	2	17.241	19	0.00000000		
2	0	0	1	55.043	27	0.00000000		

3	0	0	4	6.906	64	0.00000000		
3	0	0	3	9.981	76	0.00000000		
3	0	0	2	17.401	63	0.00000000		
3	0	0	1	41.602	28	0.00000000		
4	0	0	4	6.240	95	0.00000000		
4	0	0	3	9.980	69	0.00000000		
4	0	0	2	17.322	80	0.00000000		
4	0	0	1	55.577	76	0.00000000		
5	0	0	4	6.259	88	0.00000000		
5	0	0	3	9.980	91	0.00000000		
5	0	0	2	17.254	43	0.00000000		
5	0	0	1	48.364	44	0.00000000		
6	0	0	4	6.900	64	0.00000000		
6	0	0	3	11.492	90	0.00000000		
6	0	0	2	17.184	39	0.00000000		
6	0	0	1	51.036	42	0.00000000		
7	0	0	4	6.243	95	0.00000000		
7	0	0	3	9.980	63	0.00000000		
7	0	0	2	17.242	46	0.00000000		
7	0	0	1	55.551	16	0.00000000		
8	0	0	4	6.867	69	0.00000000		
8	0	0	3	9.987	63	0.00000000		
8	0	0	2	17.314	70	0.00000000		
8	0	0	1	49.385	45	0.00000000		
9	0	0	4	6.882	46	0.00000000		
9	0	0	3	11.927	41	0.00000000		
9	0	0	2	17.183	33	0.00000000	2	CONFIG2.GPH
9	0	0	1	45.012	19	0.00000000		
10	0	0	4	6.244	65	0.00000000		
10	0	0	3	11.350	45	0.00000000		
10	0	0	2	17.188	56	0.00000000		
10	0	0	1	52.375	29	0.00000000		
11	0	0	4	6.242	85	0.00000000		
11	0	0	3	9.980	57	0.00000000		
11	0	0	2	22.392	104	0.00000000		
11	0	0	1	55.569	12	0.00000000		
12	0	0	4	6.255	82	0.00000000		
12	0	0	3	11.815	49	0.00000000		
12	0	0	2	17.186	46	0.00000000		
12	0	0	1	55.363	12	0.00000000		
13	0	0	4	6.870	64	0.00000000		
13	0	0	3	9.980	60	0.00000000		
13	0	0	2	17.183	48	0.00000000		
13	0	0	1	41.508	23	0.00000000		
14	0	0	4	6.249	80	0.00000000		
14	0	0	3	9.988	57	0.00000000		
14	0	0	2	17.314	54	0.00000000		
14	0	0	1	54.541	30	0.00000000		
15	0	0	4	6.243	75	0.00000000		
15	0	0	3	11.353	88	0.00000000		
15	0	0	2	22.129	74	0.00000000		
15	0	0	1	55.222	37	0.00000000		
16	0	0	4	6.240	58	0.00000000		
16	0	0	3	11.903	28	0.00000000		
16	0	0	2	17.183	69	0.00000000		
16	0	0	1	41.294	51	0.00000000		
17	0	0	4	6.255	158	0.00000000		
17	0	0	3	9.987	91	0.00000000		
17	0	0	2	17.401	92	0.00000000		
17	0	0	1	55.710	12	0.00000000		
18	0	0	4	6.250	74	0.00000000		
18	0	0	3	9.981	96	0.00000000		
18	0	0	2	17.183	42	0.00000000		

18	0	0	1	44.317	33	0.00000000		
19	0	0	4	6.907	59	0.00000000		
19	0	0	3	9.981	83	0.00000000		
19	0	0	2	17.248	78	0.00000000		
19	0	0	1	55.070	24	0.00000000		
20	0	0	4	6.242	69	0.00000000		
20	0	0	3	13.658	63	0.00000000		
20	0	0	2	17.248	34	0.00000000		
20	0	0	1	54.915	23	0.00000000		
21	0	0	4	6.240	61	0.00000000		
21	0	0	3	9.980	79	0.00000000		
21	0	0	2	17.184	61	0.00000000		
21	0	0	1	52.584	23	0.00000000		
22	0	0	4	6.236	52	0.00000000	4	CONFIG4.GPH
22	0	0	3	9.980	22	0.00000000		
22	0	0	2	23.180	50	0.00000000		
22	0	0	1	33.948	72	0.00000000	1	CONFIG1.GPH
23	0	0	4	6.886	59	0.00000000		
23	0	0	3	9.981	74	0.00000000		
23	0	0	2	17.314	32	0.00000000		
23	0	0	1	41.424	36	0.00000000		
24	0	0	4	6.242	82	0.00000000		
24	0	0	3	9.981	59	0.00000000		
24	0	0	2	22.588	58	0.00000000		
24	0	0	1	54.123	22	0.00000000		
25	0	0	4	6.239	128	0.00000000		
25	0	0	3	9.987	42	0.00000000		
25	0	0	2	17.318	60	0.00000000		
25	0	0	1	55.355	22	0.00000000		
26	0	0	4	6.254	47	0.00000000		
26	0	0	3	9.986	47	0.00000000		
26	0	0	2	17.313	50	0.00000000		
26	0	0	1	54.631	28	0.00000000		
27	0	0	4	6.246	85	0.00000000		
27	0	0	3	9.980	110	0.00000000		
27	0	0	2	17.401	44	0.00000000		
27	0	0	1	55.595	12	0.00000000		
28	0	0	4	6.255	157	0.00000000		
28	0	0	3	9.981	98	0.00000000		
28	0	0	2	17.241	63	0.00000000		
28	0	0	1	55.420	38	0.00000000		
29	0	0	4	6.249	90	0.00000000		
29	0	0	3	11.925	79	0.00000000		
29	0	0	2	17.401	56	0.00000000		
29	0	0	1	55.583	28	0.00000000		
30	0	0	4	6.874	97	0.00000000		
30	0	0	3	9.988	72	0.00000000		
30	0	0	2	17.422	85	0.00000000		
30	0	0	1	55.445	13	0.00000000		
31	0	0	4	6.252	94	0.00000000		
31	0	0	3	11.719	163	0.00000000		
31	0	0	2	17.401	65	0.00000000		
31	0	0	1	55.570	20	0.00000000		
32	0	0	4	6.258	93	0.00000000		
32	0	0	3	11.916	80	0.00000000		
32	0	0	2	17.403	77	0.00000000		
32	0	0	1	49.793	38	0.00000000		
33	0	0	4	6.257	54	0.00000000		
33	0	0	3	11.348	51	0.00000000		
33	0	0	2	17.318	58	0.00000000		
33	0	0	1	52.242	33	0.00000000		
34	0	0	4	6.255	119	0.00000000		
34	0	0	3	11.485	42	0.00000000		

34	0	0	2	17.248	35	0.00000000		
34	0	0	1	41.479	47	0.00000000		
35	0	0	4	6.254	119	0.00000000		
35	0	0	3	9.981	107	0.00000000		
35	0	0	2	22.120	90	0.00000000		
35	0	0	1	55.728	92	0.00000000		
36	0	0	4	6.250	70	0.00000000		
36	0	0	3	9.982	58	0.00000000		
36	0	0	2	22.730	67	0.00000000		
36	0	0	1	55.722	17	0.00000000		
37	0	0	4	6.885	52	0.00000000		
37	0	0	3	9.979	51	0.00000000		
37	0	0	2	17.401	43	0.00000000		
37	0	0	1	54.006	38	0.00000000		
38	0	0	4	6.251	90	0.00000000		
38	0	0	3	9.986	63	0.00000000		
38	0	0	2	17.242	48	0.00000000		
38	0	0	1	55.475	12	0.00000000		
39	0	0	4	6.255	121	0.00000000		
39	0	0	3	9.980	76	0.00000000		
39	0	0	2	17.184	60	0.00000000		
39	0	0	1	47.861	26	0.00000000		
40	0	0	4	6.243	47	0.00000000		
40	0	0	3	9.978	65	0.00000000	3	CONFIG3.GPH
40	0	0	2	17.190	34	0.00000000		
40	0	0	1	45.029	52	0.00000000		
41	0	0	4	6.851	48	0.00000000		
41	0	0	3	9.988	43	0.00000000		
41	0	0	2	17.314	54	0.00000000		
41	0	0	1	43.453	38	0.00000000		
42	0	0	4	6.241	63	0.00000000		
42	0	0	3	9.986	77	0.00000000		
42	0	0	2	17.314	57	0.00000000		
42	0	0	1	55.567	19	0.00000000		
43	0	0	4	6.894	100	0.00000000		
43	0	0	3	11.486	36	0.00000000		
43	0	0	2	17.314	67	0.00000000		
43	0	0	1	44.848	32	0.00000000		
44	0	0	4	6.255	55	0.00000000		
44	0	0	3	11.658	57	0.00000000		
44	0	0	2	17.183	94	0.00000000		
44	0	0	1	52.677	25	0.00000000		
45	0	0	4	6.238	57	0.00000000		
45	0	0	3	9.980	52	0.00000000		
45	0	0	2	22.586	55	0.00000000		
45	0	0	1	52.957	21	0.00000000		
46	0	0	4	6.893	72	0.00000000		
46	0	0	3	9.981	48	0.00000000		
46	0	0	2	17.242	51	0.00000000		
46	0	0	1	54.784	17	0.00000000		
47	0	0	4	6.252	60	0.00000000		
47	0	0	3	11.868	72	0.00000000		
47	0	0	2	17.183	43	0.00000000		
47	0	0	1	39.856	54	0.00000000		
48	0	0	4	6.246	94	0.00000000		
48	0	0	3	9.983	71	0.00000000		
48	0	0	2	24.110	71	0.00000000		
48	0	0	1	43.164	101	0.00000000		
49	0	0	4	6.242	78	0.00000000		
49	0	0	3	9.981	115	0.00000000		
49	0	0	2	17.197	81	0.00000000		
49	0	0	1	55.738	16	0.00000000		
50	0	0	4	6.254	153	0.00000000		

50	0	0	3	9.983	37	0.00000000
50	0	0	2	17.314	69	0.00000000
50	0	0	1	55.710	13	0.00000000
51	1	0	4	11.075	112	0.00000000
51	1	0	3	16.434	119	0.00000000
51	1	0	2	27.625	46	0.00000000
51	1	0	1	45.784	32	0.00000000
52	1	0	4	10.464	68	0.00000000
52	1	0	3	15.456	99	0.00000000
52	1	0	2	26.978	85	0.00000000
52	1	0	1	55.176	13	0.00000000
53	1	0	4	10.475	148	0.00000000
53	1	0	3	15.717	78	0.00000000
53	1	0	2	27.408	66	0.00000000
53	1	0	1	55.777	26	0.00000000
54	1	0	4	11.129	73	0.00000000
54	1	0	3	15.618	45	0.00000000
54	1	0	2	21.942	66	0.00000000
54	1	0	1	43.122	21	0.00000000
55	1	0	4	10.311	118	0.00000000
55	1	0	3	16.730	70	0.00000000
55	1	0	2	25.700	80	0.00000000
55	1	0	1	40.255	22	0.00000000
56	1	0	4	10.529	94	0.00000000
56	1	0	3	16.399	84	0.00000000
56	1	0	2	28.005	100	0.00000000
56	1	0	1	52.173	37	0.00000000
57	1	0	4	12.600	92	0.00000000
57	1	0	3	16.357	57	0.00000000
57	1	0	2	24.412	84	0.00000000
57	1	0	1	52.136	30	0.00000000
58	1	0	4	12.838	127	0.00000000
58	1	0	3	28.244	44	0.00000000
58	1	0	2	26.545	156	0.00000000
58	1	0	1	55.516	24	0.00000000
59	1	0	4	11.863	97	0.00000000
59	1	0	3	15.773	93	0.00000000
59	1	0	2	23.564	62	0.00000000
59	1	0	1	55.578	16	0.00000000
60	1	0	4	11.158	140	0.00000000
60	1	0	3	15.904	68	0.00000000
60	1	0	2	28.105	53	0.00000000
60	1	0	1	55.433	24	0.00000000
61	1	0	4	10.728	111	0.00000000
61	1	0	3	16.246	69	0.00000000
61	1	0	2	29.288	47	0.00000000
61	1	0	1	51.220	19	0.00000000
62	1	0	4	10.349	49	0.00000000
62	1	0	3	16.696	49	0.00000000
62	1	0	2	25.129	36	0.00000000
62	1	0	1	42.154	43	0.00000000
63	1	0	4	11.743	105	0.00000000
63	1	0	3	16.298	89	0.00000000
63	1	0	2	26.174	83	0.00000000
63	1	0	1	54.495	32	0.00000000
64	1	0	4	9.759	62	0.00000000
64	1	0	3	14.414	62	0.00000000
64	1	0	2	21.538	72	0.00000000
64	1	0	1	55.473	12	0.00000000
65	1	0	4	12.382	105	0.00000000
65	1	0	3	17.317	102	0.00000000
65	1	0	2	31.743	78	0.00000000

65	1	0	1	55.631	13	0.00000000
66	1	0	4	12.870	89	0.00000000
66	1	0	3	16.168	89	0.00000000
66	1	0	2	29.451	49	0.00000000
66	1	0	1	49.955	34	0.00000000
67	1	0	4	9.553	89	0.00000000
67	1	0	3	14.235	52	0.00000000
67	1	0	2	21.733	57	0.00000000
67	1	0	1	53.168	37	0.00000000
68	1	0	4	10.719	92	0.00000000
68	1	0	3	17.371	136	0.00000000
68	1	0	2	24.724	82	0.00000000
68	1	0	1	51.881	55	0.00000000
69	1	0	4	11.375	52	0.00000000
69	1	0	3	15.119	57	0.00000000
69	1	0	2	23.378	105	0.00000000
69	1	0	1	45.539	28	0.00000000
70	1	0	4	11.352	84	0.00000000
70	1	0	3	16.299	44	0.00000000
70	1	0	2	25.018	40	0.00000000
70	1	0	1	40.635	45	0.00000000
71	1	0	4	11.098	70	0.00000000
71	1	0	3	15.440	96	0.00000000
71	1	0	2	28.434	56	0.00000000
71	1	0	1	54.340	16	0.00000000
72	1	0	4	11.112	94	0.00000000
72	1	0	3	14.840	66	0.00000000
72	1	0	2	24.537	81	0.00000000
72	1	0	1	42.063	28	0.00000000
73	1	0	4	10.641	85	0.00000000
73	1	0	3	14.933	105	0.00000000
73	1	0	2	22.352	100	0.00000000
73	1	0	1	55.584	15	0.00000000
74	1	0	4	10.916	76	0.00000000
74	1	0	3	15.629	68	0.00000000
74	1	0	2	24.314	72	0.00000000
74	1	0	1	55.091	15	0.00000000
75	1	0	4	10.747	119	0.00000000
75	1	0	3	16.868	48	0.00000000
75	1	0	2	27.564	62	0.00000000
75	1	0	1	48.365	31	0.00000000
76	1	0	4	11.534	71	0.00000000
76	1	0	3	15.963	117	0.00000000
76	1	0	2	24.937	45	0.00000000
76	1	0	1	46.842	34	0.00000000
77	1	0	4	9.789	65	0.00000000
77	1	0	3	13.774	49	0.00000000
77	1	0	2	21.448	78	0.00000000
77	1	0	1	55.472	54	0.00000000
78	1	0	4	10.364	61	0.00000000
78	1	0	3	17.627	74	0.00000000
78	1	0	2	25.241	47	0.00000000
78	1	0	1	53.482	17	0.00000000
79	1	0	4	12.646	88	0.00000000
79	1	0	3	18.450	64	0.00000000
79	1	0	2	27.626	57	0.00000000
79	1	0	1	53.324	39	0.00000000
80	1	0	4	10.089	70	0.00000000
80	1	0	3	14.392	59	0.00000000
80	1	0	2	23.299	55	0.00000000
80	1	0	1	46.690	40	0.00000000
81	1	0	4	10.998	150	0.00000000
81	1	0	3	15.466	58	0.00000000

81	1	0	2	23.794	104	0.00000000
81	1	0	1	46.401	31	0.00000000
82	1	0	4	12.033	69	0.00000000
82	1	0	3	17.886	56	0.00000000
82	1	0	2	27.462	64	0.00000000
82	1	0	1	50.737	65	0.00000000
83	1	0	4	11.427	100	0.00000000
83	1	0	3	17.280	76	0.00000000
83	1	0	2	26.832	169	0.00000000
83	1	0	1	53.779	28	0.00000000
84	1	0	4	13.016	103	0.00000000
84	1	0	3	18.395	81	0.00000000
84	1	0	2	28.731	53	0.00000000
84	1	0	1	50.178	27	0.00000000
85	1	0	4	12.060	103	0.00000000
85	1	0	3	17.081	59	0.00000000
85	1	0	2	26.134	166	0.00000000
85	1	0	1	49.380	32	0.00000000
86	1	0	4	13.748	99	0.00000000
86	1	0	3	17.648	104	0.00000000
86	1	0	2	29.557	74	0.00000000
86	1	0	1	55.605	16	0.00000000
87	1	0	4	10.545	91	0.00000000
87	1	0	3	15.401	72	0.00000000
87	1	0	2	23.468	121	0.00000000
87	1	0	1	45.967	35	0.00000000
88	1	0	4	12.010	83	0.00000000
88	1	0	3	18.064	76	0.00000000
88	1	0	2	26.753	59	0.00000000
88	1	0	1	45.982	44	0.00000000
89	1	0	4	11.832	101	0.00000000
89	1	0	3	17.102	98	0.00000000
89	1	0	2	26.731	65	0.00000000
89	1	0	1	55.574	17	0.00000000
90	1	0	4	12.219	64	0.00000000
90	1	0	3	17.575	105	0.00000000
90	1	0	2	25.228	154	0.00000000
90	1	0	1	50.077	25	0.00000000
91	1	0	4	11.002	52	0.00000000
91	1	0	3	17.510	92	0.00000000
91	1	0	2	24.831	129	0.00000000
91	1	0	1	47.610	19	0.00000000
92	1	0	4	10.805	69	0.00000000
92	1	0	3	16.187	105	0.00000000
92	1	0	2	25.502	84	0.00000000
92	1	0	1	54.842	49	0.00000000
93	1	0	4	10.701	84	0.00000000
93	1	0	3	15.349	98	0.00000000
93	1	0	2	22.655	58	0.00000000
93	1	0	1	37.823	34	0.00000000
94	1	0	4	10.012	97	0.00000000
94	1	0	3	15.853	99	0.00000000
94	1	0	2	26.345	94	0.00000000
94	1	0	1	55.647	23	0.00000000
95	1	0	4	10.697	191	0.00000000
95	1	0	3	15.826	82	0.00000000
95	1	0	2	24.831	119	0.00000000
95	1	0	1	53.166	37	0.00000000
96	1	0	4	11.171	77	0.00000000
96	1	0	3	16.852	75	0.00000000
96	1	0	2	28.268	68	0.00000000
96	1	0	1	52.891	46	0.00000000
97	1	0	4	12.105	67	0.00000000

97	1	0	3	16.060	60	0.00000000
97	1	0	2	23.582	54	0.00000000
97	1	0	1	43.761	27	0.00000000
98	1	0	4	11.529	139	0.00000000
98	1	0	3	16.158	103	0.00000000
98	1	0	2	27.388	77	0.00000000
98	1	0	1	50.268	61	0.00000000
99	1	0	4	10.988	135	0.00000000
99	1	0	3	15.193	76	0.00000000
99	1	0	2	38.806	23	0.00000000
99	1	0	1	55.587	15	0.00000000
100	1	0	4	10.362	80	0.00000000
100	1	0	3	17.293	65	0.00000000
100	1	0	2	26.645	131	0.00000000
100	1	0	1	53.577	43	0.00000000

Random data: 0 = not randomized, 1 = randomized
Start file: 0 = random starting coordinates, 1 = read from file
Seeds: initial seeds for random number generator
* Stability criterion not met.
**To run a single NMS ordination that repeats the best result,
  specify this file as the starting configuration,
  rather than using a random start. It is best to
  save this file under a new name, to avoid it being
  overwritten by the next NMS run. To do this, open the
  file using File | Open | Graph Row file, then
  File | Save as | Graph Row file (then specify new name).

STRESS IN RELATION TO DIMENSIONALITY (Number of Axes)

	Stress in real data 50 run(s)			Stress in randomized data Monte Carlo test, 50 runs			
Axes	Minimum	Mean	Maximum	Minimum	Mean	Maximum	p
1	33.948	50.545	55.738	37.823	50.624	55.777	0.0196
2	17.183	18.147	24.110	21.448	26.035	38.806	0.0196
3	9.978	10.526	13.658	13.774	16.498	28.244	0.0196
4	6.236	6.401	6.907	9.553	11.229	13.748	0.0196

p = proportion of randomized runs with stress < or = observed stress
i.e., p = (1 + no. permutations <= observed)/(1 + no. permutations)

Conclusion: a 3-dimensional solution is recommended.
Now rerunning the best ordination with that dimensionality.

Selected file CONFIG3.GPH for the starting configuration for
   the final run.

NMS of significant periphyton metrics
Ordination of sites in taxa space. 30 sites 9 taxa

The following options were selected:
ANALYSIS OPTIONS
1. SORENSEN = Distance measure
2. 3 = Number of axes (max. = 6)
3. 200 = Maximum number of iterations
4. FROM FILE = Starting coordinates (random or from file)
5. 3 = Reduction in dimensionality at each cycle
6. NO PENALTY = Tie handling (Strategy 1 does not penalize

```
                        ties with unequal ordination distance,
                        while strategy 2 does penalize.)
         7.       0.20 = Step length (rate of movement toward minimum stress)
         8.   USE TIME = Random number seeds (use time vs. user-supplied)
         9.          1 = Number of runs with real data
        10.          0 = Number of runs with randomized data
        11.        YES = Autopilot
        12.   0.000010 = Stability criterion, standard deviations in stress
                        over last  10 iterations.
        13.     MEDIUM = Speed vs. thoroughness
OUTPUT OPTIONS
        14.         NO = Write distance matrix?
        15.         NO = Write starting coordinates?
        16.        YES = List stress, etc. for each iteration?
        17.        YES = Plot stress vs. iteration?
        18.         NO = Plot distance vs. dissimilarity?
        19.        YES = Write final configuration?
        20. PRINC.AXES = Write varimax-rotated, principal axes, or unrotated scores
for graph?
        21.         NO = Write run log?
        22.        YES = Write weighted-average scores for taxa    ?
------------------------------------------------------------------------------


File containing starting coordinates:
CONFIG3.GPH



List of stress, step length, and magnitude of the gradient
    vector at each iteration.

  Step    Stress Instability  StepLength    Mag(G)
     1  35.83929                 0.20000   0.003301967
     2  31.41119                 0.20000   0.000873451
     3  29.62747                 0.32716   0.000649981
     4  29.78344                 0.32828   0.001213204
     5  28.47920                 0.27262   0.000827965
     6  27.97922                 0.24339   0.001394376
     7  26.89811                 0.21876   0.001100696
     8  25.72049                 0.18605   0.001094547
     9  24.72532                 0.17448   0.001164682
    10  23.93053  0.03330369     0.18063   0.001571568
    11  23.04516  0.02624589     0.16691   0.001269833
    12  22.17900  0.02590352     0.14503   0.001054378
    13  21.66364  0.02649038     0.14022   0.001247339
    14  20.97441  0.02519868     0.12023   0.000765270
    15  20.52729  0.02409749     0.10535   0.000790188
    16  20.11793  0.02177605     0.09672   0.000681817
    17  19.70814  0.01938492     0.08605   0.000565042
    18  19.37836  0.01728405     0.07938   0.000535289
    19  19.05116  0.01538397     0.07355   0.000475721
    20  18.76090  0.01342194     0.06777   0.000450239
    21  18.43398  0.01185479     0.06278   0.000448244
    22  18.02164  0.01102358     0.05982   0.000482986
    23  17.51590  0.01050962     0.05733   0.000459376
    24  16.94890  0.01083416     0.06221   0.000435338
    25  16.38974  0.01150475     0.07542   0.000538050
    26  16.01625  0.01206336     0.07870   0.000889935
    27  15.66116  0.01250967     0.08111   0.001019823
    28  15.25178  0.01282373     0.07452   0.000805254
    29  14.92192  0.01286276     0.06858   0.000776073
    30  14.61484  0.01252651     0.06382   0.000698089
```

31	14.31573	0.01183980	0.05864	0.000632507
32	14.01805	0.01094639	0.05564	0.000640216
33	13.68610	0.01012084	0.05432	0.000675817
34	13.29438	0.00967059	0.05347	0.000729149
35	12.80828	0.00981364	0.05322	0.000825797
36	12.19708	0.01042220	0.05466	0.000958359
37	11.46238	0.01158679	0.06122	0.000936469
38	10.98621	0.01272639	0.07178	0.001156113
39	11.13140	0.01276895	0.08123	0.002599219
40	10.42695	0.01316031	0.04972	0.000132648
41	10.31557	0.01290554	0.05484	0.000505323
42	10.56650	0.01169261	0.05983	0.001731903
43	10.16744	0.01036808	0.02445	0.000326270
44	10.87379	0.00808723	0.07456	0.003447701
45	10.24790	0.00604950	0.02493	0.001046085
46	10.72295	0.00400881	0.07751	0.002579434
47	10.31856	0.00319042	0.01708	0.001256711
48	10.15183	0.00309750	0.05494	0.000711981
49	10.02456	0.00254410	0.01173	0.000166346
50	10.31736	0.00254581	0.04375	0.001341641
51	10.15376	0.00262584	0.00941	0.000715181
52	10.01689	0.00269868	0.03355	0.000157047
53	9.98360	0.00284124	0.00754	0.000013382
54	10.10192	0.00207012	0.02312	0.000527117
55	10.04371	0.00211686	0.00535	0.000280684
56	9.98427	0.00119270	0.01815	0.000024643
57	9.97868	0.00102083	0.00394	0.000000135
58	9.98001	0.00102276	0.00422	0.000006849
59	9.97847	0.00104711	0.00258	0.000000263
60	9.97845	0.00058668	0.00088	0.000000266
61	9.97841	0.00038954	0.00026	0.000000043
62	9.97851	0.00039231	0.00079	0.000000517
63	9.97845	0.00039458	0.00019	0.000000242
64	9.97840	0.00019105	0.00060	0.000000056
65	9.97840	0.00000000		

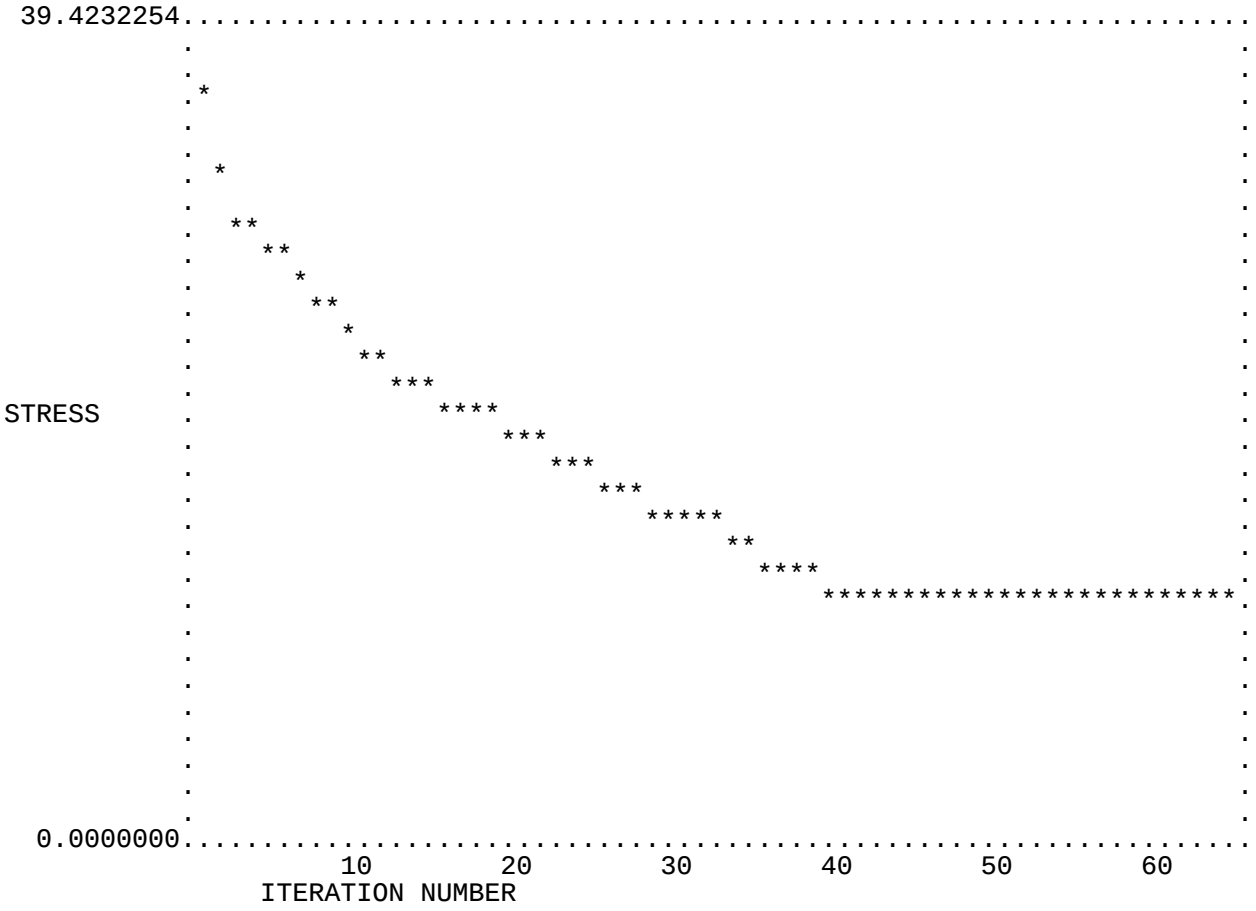
9.97840 = final stress for 3-dimensional solution
0.00000 = final instability
65 = number of iterations

Final configuration (ordination scores) for this run

sites		Axis		
Number	Name	1	2	3
1	547	-0.5165	-1.3628	-0.3700
2	535	-0.6239	-1.0837	-0.0389
3	502	0.6241	0.1673	-0.3962
4	471	-0.1147	-1.0790	-0.4436
5	450	-0.7329	-1.1871	-0.6109
6	447	0.2706	0.2502	-0.5733
7	441	-0.6753	-0.2583	-0.6446
8	437	-0.4970	0.1887	0.0117
9	421	0.0066	-0.3767	0.7125
10	389	-0.5525	0.0106	-0.3954
11	375	-0.4765	-0.7170	0.9886
12	357	0.5322	-0.7096	1.1870
13	308	-0.2011	-0.0315	1.1042
14	297	0.3176	-0.1144	0.5958
15	294	0.5109	0.1382	-0.0269
16	290	0.7108	0.7183	-0.7623
17	274	0.6296	0.6453	0.5763
18	245	0.4578	0.3886	0.2859
19	236	0.5520	-0.6360	0.3415
20	226	0.1339	0.1657	-0.0367

21	189	0.1332	0.5136	-0.7208
22	158	0.8286	0.4082	0.0497
23	157	0.1050	-0.0042	-0.0066
24	155	-0.3259	0.6027	-0.3337
25	101	0.4207	0.1692	-1.1105
26	61	0.3146	1.1023	-0.7322
27	39	-0.2551	0.5983	0.3480
28	34	-0.6516	0.8281	0.0050
29	16	-0.4267	0.7757	0.0569
30	0	-0.4986	-0.1106	0.9395

PLOT OF STRESS V. ITERATION NUMBER
(to prevent wrapping of wide plots when printing, use small font)



Principal axes rotation of 3-dimensional solution.

Configuration after rotation is listed below.

Final configuration (ordination scores) for this run

sites		Axis		
Number	Name	1	2	3

1	547	1.1543	0.9401	0.2115
2	535	1.1015	0.5901	-0.0602
3	502	-0.5253	0.0946	0.5381
4	471	0.7476	0.7824	0.4505
5	450	0.9783	1.1670	-0.0192
6	447	-0.5492	0.3397	0.2179
7	441	0.1695	0.8758	-0.3773
8	437	0.0188	0.0897	-0.5238
9	421	0.6194	-0.5121	0.0607
10	389	0.0106	0.5272	-0.4286
11	375	1.1886	-0.4895	-0.2570
12	357	0.9208	-0.9959	0.5967
13	308	0.5722	-0.9048	-0.3384
14	297	0.2433	-0.5949	0.2362
15	294	-0.3027	-0.1870	0.3927
16	290	-1.1727	0.2120	0.4269
17	274	-0.5060	-0.9263	0.1753
18	245	-0.3582	-0.5291	0.1845
19	236	0.4877	-0.2755	0.7156
20	226	-0.2000	-0.0649	0.0499
21	189	-0.7853	0.4295	0.0028
22	158	-0.6041	-0.4463	0.5400
23	157	-0.0355	-0.0269	0.0954
24	155	-0.5344	0.2059	-0.5028
25	101	-0.7653	0.7943	0.4715
26	61	-1.3434	0.1890	-0.0981
27	39	-0.2608	-0.4209	-0.5468
28	34	-0.4640	-0.0623	-0.9440
29	16	-0.4752	-0.1645	-0.7308
30	0	0.6694	-0.6362	-0.5391

Writing weighted average scores on 3 axes for 9 taxa
into file for graphing.
1 Apr 2016, 10:16:40
0.03 minutes elapsed time.

********** Calculations finished **********