Hydrology and Nutrient Flux in the Shiawassee Flats

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A thesis submitted

in partial fulfillment of the requirements

for the degree of Master of Science

(Natural Resources and Environment)

at the University of Michigan

April 2014

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Abstract

The Saginaw River system is a principal source of nutrient loads to Saginaw Bay, an area of Lake Huron experiencing multiple symptoms of eutrophication , yet the Shiawassee Flats wetland/floodplain region is poorly understood in terms of impacts on hydrology and nutrient fluxes. This study analyzed water, nitrogen, and phosphorus inputs and outputs to the Shiawassee Flats and evaluated the current role that the connected wetlands and floodplain play in water and nutrients to the Saginaw River and the downstream Bay. Discharge measurements and water samples were taken at all of the major inputs to, and output from, the Flats, throughout the spring and summer of 2012-2013, and chemical analyses were conducted for nitrogen and phosphorus. When hydrologic storage was occurring, the output flow was reduced by over 20%; conversely, when combined upstream inputs were very low, at times 80% of the output flow was wetland water. This indicates that the Shiawassee Flats is critical both to flood storage and maintaining base flows. In terms of inorganic nitrogen, the Shiawassee Flats seem to provide a minor decrement in load and a major decrease in concentrations downstream. In terms of total reactive phosphorus, the Flats area contributed to the load in the Saginaw River but reactive phosphorus concentration downstream changed little although there was a trend towards reduction during low flows, particularly in 2013 following large-scale spring flooding. Total phosphorus loads and concentrations increased in the Saginaw River due to contributions (likely organic) by the Flats wetlands.

Acknowledgments

I would like to thank my advisor, Mike Wiley, for his support and continued guidance throughout this process and initially sparking my interest in this topic. Without his initial direction in working with the master's project group at the Refuge, I would not have been prepared to make an informed decision on choosing my thesis topic. I would also like to thank Catherine Riseng for serving on my committee and providing insight and direction, and Kurt Kowalski for acting as a reader and providing valuable edits. Thank you to Eric Dunton and Steve Kahl of the U.S. Fish and Wildlife Service and Kurt Kowalski of the U.S. Geological Survey for facilitating and accommodating my research at the Refuge, and the University of Michigan School of Natural Resources and the Environment for providing funding. Thank you to Alek Kreiger, Genevieve Leet, and Will Argiroff for making our research at the Refuge this past summer an adventure (despite a few long days), and Andrea Dominguez and Brianna Knoppow (and the rest of the 2012 Shiawassee Master's Project) for letting me be a part of their summer field season. Finally, a huge personal thank you to my husband, Brandon Scott, for doing the brunt of the manual labor required for my sampling events, accompanying me on all of my sampling trips, and for his support throughout this long process.

Table of Contents

Preface

In 2011, the U.S. Fish and Wildlife Service and Ducks Unlimited received a $1.5 million grant for the first phase of a wetland restoration project at the Shiawassee National Wildlife Refuge (SNWR) near Saginaw, Michigan. The goal of this project is to reconnect a total of 2,260 acres of farm fields and wetlands to the river system surrounding the refuge. The project seeks to provide fish, birds, and insects with access to a large wetland complex, similar to what would naturally be in the area, through hydrologic reconnection and wetland restoration. This restoration project is also believed to have the potential to contribute to the delisting of at least three of the Beneficial Use Impairments in the Saginaw River/Bay Area of Concern within, and downstream of, the Shiawassee National Wildlife Refuge.

The initial phase of the restoration project aims to convert 940 acres of former farm land to restored wetland, by hydrologically reconnecting this land to the Shiawassee and Flint Rivers. Although the grant received by the refuge included funds for the design and implementation of the restoration project, no funds were allocated to pre- or post-construction monitoring. The SNWR recognized the importance of monitoring to measure the success of the restoration project once it is completed, and in 2012 I worked with a master's project group from the University of Michigan School of Natural Resources and Environment to gather these data in the field. A large part of this data involved hydrological monitoring and water quality sampling in the various rivers entering and exiting the refuge.

To continue to collect these baseline ecological data for the restoration project, the SNWR funded several master's theses to be conducted at the refuge. My work at the refuge involved collecting hydrologic data and water samples from all of the inputs to and outputs from the Shiawassee Flats, and chemically analyzing these samples in the lab. For my thesis, I evaluated total phosphorus, total reactive phosphorus, and total inorganic nitrogen loads into and out of the Refuge. Phosphorus is the main limiting nutrient in freshwaters, and the waters flowing from the SNWR empty into Saginaw Bay, which is noted for having harmful algal blooms. High nitrogen concentrations can also contribute to eutrophic conditions locally. My goal was to put together water and nutrient input-output analyses for the Shiawassee Flats, and determine how much phosphorus and nitrogen is sequestered in wetlands within the SNWR, and evaluate the effects that the restoration project can have on downstream phosphorus and nitrogen loading, and potentially Saginaw Bay itself.

Introduction

Cultural eutrophication became severe in the Great Lakes, most notably in Lake Erie's Western Basin, by the late 1960s. At this time, hypoxia, loss of benthic organisms, and toxic algal blooms were all evident in Lake Erie (Rosa and Burns, 1987). The effects of cultural eutrophication were also evident in other parts of the Great Lakes, and included algal blooms and beach fouling in Saginaw Bay (Bierman et al. 1980). The excessive algal production across the Great Lakes led to debate through the 1970s about the primary cause of the eutrophication, and this was resolved in the mid-1970s with the common acceptance that phosphorus was the limiting nutrient in freshwater systems (Schindler and Vallentyne 2008). The Great Lakes Water Quality Agreement between the United States and Canada subsequently established target phosphorus loads for the Great Lakes, including a 440 tonne/year goal for Saginaw Bay (International Joint Commission, 1978), and efforts to control phosphorus inputs to the lakes were implemented. Early assessments indicated that eutrophication symptoms were disappearing in response to phosphorus load reductions in Saginaw Bay (Bierman et al. 1984). Recently, however, eutrophication symptoms in the form of harmful algal blooms and nuisance algal beach deposits have returned to Saginaw Bay (Bierman et al. 2005), indicating the need for continued remediation projects in the Saginaw Bay Watershed to reduce phosphorus loading.

Point source phosphorus loading to the Saginaw Bay Watershed, which includes the five major tributaries that converge at the SNWR, is attributed to sewage treatment plants and industrial effluent

(Michigan DEQ, 2008). Since the 1972 implementation of the National Pollutant Discharge Elimination System (NPDES) program, point source phosphorus loading to Saginaw Bay has been drastically reduced (Michigan DEQ, 2008). Immediately following the implementation of this program, conditions in the bay seemed to be improving with reduced point source loading (Hinderer and Murray, 2011), but eutrophication problems returned in the mid-90s and recent estimates show that 80-90% of phosphorus inputs in the watershed come from non-point sources (Michigan DEQ 2008; Vanderploeg et al. 2001). Non-point source phosphorus loading to the Saginaw Bay Watershed is primarily attributed to agricultural fertilizer runoff, urban runoff, soil erosion, animal waste, and leaking residential septic tanks (Michigan DEQ, 2008; Public Sector Consultants, 2000).

Because of the use of phosphorus-based fertilizers, the agricultural industry is the largest contributor to increased phosphorus inputs to the Saginaw Bay Watershed (SBCI Phosphorus Committee, 2009). Agriculture accounts for about 45% of land use in the watershed and over 90% of the total phosphorus inputs to Saginaw Bay (SBCI Phosphorus Committee, 2009). Since the mid-1990s, agricultural practices in the Great Lakes region have significantly changed, leading to larger runoff of soluble reactive phosphorus, the biologically available form of phosphorus (Hinderer and Murray, 2011). A study on total phosphorous loading to the Saginaw Bay basin concluded that ten percent of the loading is absorbed by the Shiawassee National Wildlife Refuge near Saginaw, MI, which is a significant amount of TP deposition onto the Refuge (DeMarchi et al. 2010, Cha et al. 2010).

Wetland loss is also a major contributor to the eutrophication of Saginaw Bay. Before European colonization, the Saginaw Bay Watershed contained one of the largest wetlands in the Great Lakes region, encompassing over 700,000 acres (Public Sector Consultants, 2012). Wetlands surrounded the shores of Saginaw Bay and were found along the shores of the Saginaw and Quanicassee Rivers (Public Sector Consultants, 2012). Most of these wetlands were drained to control mosquito populations and increase land available for farming and housing; today, only 20-30% of wetlands remain in the watershed (Public Sector Consultants, 2012). Wetlands provide significant buffering capacities and absorb nutrients before they enter local waterways (Public Sector Consultants, 2000). The draining and destruction of wetlands within the Saginaw Bay Watershed has significantly reduced the natural buffering capacity that was once present (Hinderer and Murray, 2011).

There has been recent renewed interest in the use of wetlands for agricultural and urban nonpoint source pollutant retention (Baker, 1992; Mitsch, 1992). However, coastal wetlands in the Great Lakes region are not usually restored in order to improve water quality in the Great Lakes, despite the fact that there is a growing need to minimize phosphorus emptying into the lakes (Mitsch and Wang, 2000). Hydrologic aspects of these wetlands are a key factor in the ability of natural and created wetlands to improve water quality; however, in most prior studies of water quality improvement processes in wetlands, water chemistry data has been the main focus, with little attention given to hydrologic data (Nairn and Mitsch, 2000). In a situation similar to that of the SNWR, in which natural (but manipulated) wetlands received agricultural drainage waters, the wetlands effectively removed phosphorus but retention was dependent on hydraulic loading rates (Chescheir et al., 1992).

Multiple studies have demonstrated the role of the inundation of floodplains as a natural sink for sediments and nutrients in rivers, including phosphorus (Cooper et al., 1987; Kronvang et al., 1998). The waterways of many river systems, including those of the SNWR, are regulated for the drainage of agricultural lands and other purposes, and the inundation of floodplains is often prevented. This prevents the natural hydrology that links rivers and floodplains, allowing sediments and nutrients to flow downstream without any being captured in the floodplain system (Kronvang et al., 2007). Restored, inundated floodplains can help store sediment, phosphorus, and contaminants through deposition on the floodplain. River restoration has been shown in the past to reduce phosphorus loading to downstream waterways (Kronvang et al., 2007; Woltemade 2000). This indicates that the restoration of wetlands within the SNWR, including the original floodplain, may reduce phosphorus loading to downstream Saginaw Bay.

The effectiveness of these restored wetlands at removing phosphorus is also related to the fertilization history, the form of phosphorus entering the wetland, and biogeochemical cycles, meaning that retention capacity is dependent on past and current site-specific conditions (Carlyle and Hill, 2001). Many drained wetland and floodplain areas have been fertilized in the past for agricultural purposes, resulting in phosphorus accumulation in the soils (Mitsch et al., 1995). This can result in decreased phosphorus retention capacity, or even may result in the release of phosphorus when re-flooding events connect the wetland to the river system (Rupp et al., 2004). A portion of the drained area being restored at the SNWR was cultivated in the past, and this may affect the ability of the restored floodplain as a whole to remove phosphorus from the waterway.

There are a lot of unknowns surrounding the role of the wetlands within the SNWR in terms of nutrient concentrations and loads, especially concerning phosphorus. This is due primarily to the lack of direct monitoring at the input and output tributaries to and from the Shiawassee Flats. Although the Saginaw Bay Multiple Stressors Summary Report published by NOAA-GLERL reported that the wetlands within the SNWR partially mitigate the phosphorus loads originating upstream with an average reduction of 13%, this estimate was based on differences in average loads of the Tittabawassee, Shiawassee, Flint, and Cass Rivers with the Saginaw River, and loads were estimated using regression models of available total phosphorus values from 1998 to 2008 and observed flows (Stow and Hook, 2013). Little direct monitoring of all of the input and output tributaries to the Shiawassee Flats has been conducted in more recent years to develop complete nutrient and water input-output analyses for the SNWR area wetlands.

The goal of my research was to analyze the water and material input-output analyses for the Flats area within the Shiawassee National Wildlife Refuge, and analyze the current role of wetlands and the impact that the proposed restoration project may have on hydrology, water quality, and nutrient loading. An ancillary goal was to provide gage calibration data for the newly-installed gage on the Shiawassee River within the Flats for the Refuge. My research objectives focused on the development of a water input-output analysis for the Flats, based on hydrology measurements collected for the input and output tributaries; the compilation of water quality data; and the development of nutrient input-output analyses, in the form of nitrogen and phosphorus loads.

Materials and Methods

Study Site

The Shiawassee Flats is a floodplain and freshwater wetland area within the Saginaw Bay Watershed. It is located where the Shiawassee, Bad, Cass, Flint, and Tittabawassee Rivers converge (Figure 1). The Shiawassee National Wildlife Refuge (SNWR) partially contains this huge confluence, and protects 9,260 acres of wetlands, floodplain forest, and agricultural land. There are about 38 miles of rivers, streams, ditches, drains, and other flow paths that either cross or are adjacent to the SNWR (Newman, 2011). The single output from the refuge, the Saginaw River, flows through the City of Saginaw and into Saginaw Bay.

Although the hydrology of the Flats, located at the center of the SNWR, is critical to both the management of the refuge and the biology and ecology of Saginaw Bay, the hydrology of this area and the portion of the Saginaw River leading into the Bay are poorly studied. Fluctuations in water levels due to seiches from Saginaw Bay cause water within the Flats to back up, causing backwater effects upstream into the town of St. Charles (Newman, 2011). This causes problems with standard river gaging practices, and a gage directly on the Flats was only installed by the SNWR in June 2013, meaning that flow rates are not well known. There are various USGS gages upstream and downstream of the river confluences within the refuge.

Hydrology

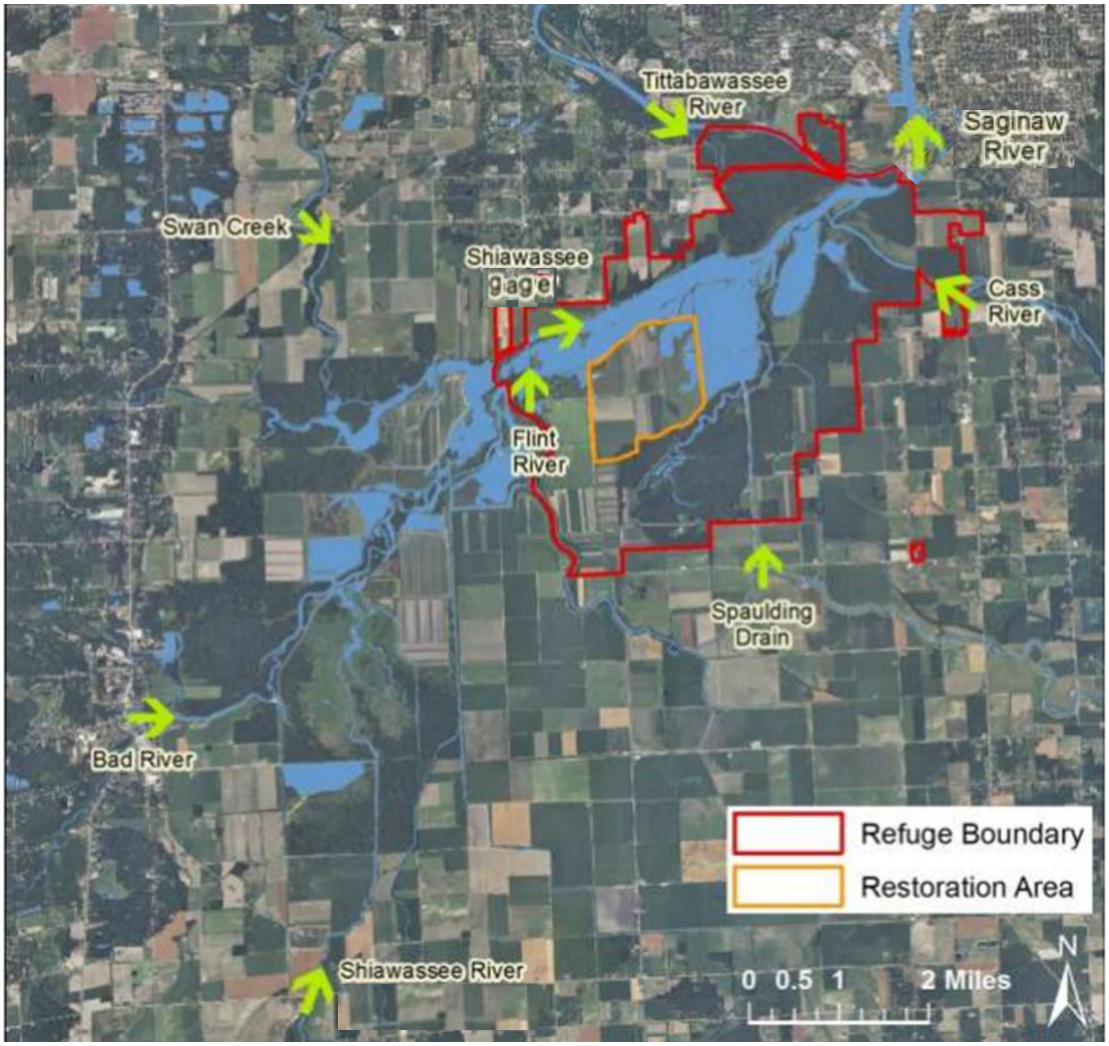
Hydrologic flow measurements were taken on each of the main tributaries, drains, and rivers leading into and out of the SNWR. These included the Shiawassee River at Fergus Road, the Bad River in St. Charles, Swan Creek off of Miller Road, the Tittabawassee River at Center Road, the Saginaw River at Nautical Reserve Road, the Cass River at East Road, and the Flint River, Spaulding Drain, and Ferguson Bayou within the SNWR (Figure 1; Table 1). Discharge measurements were also taken at a newly installed gage located on the Shiawassee River (43°21' 38" N, 84°02' 18" W) within the SNWR in order to provide calibration data (Heitmeyer et al. 2013). During 2012 I measured flows once monthly in July, August, and November. The August 12 sampling followed storm events, with peak flows on the Saginaw River of 219.5 cms. In 2013 I sampled flows every two weeks starting on May 7th and continuing through September. The early May event (132.09 cms on the Saginaw River) and the June 5th (161.96 cms on the Saginaw River) events took place immediately following severe flooding events.

Measurements of flow velocity were primarily taken using a 1.5 MHz SonTek Pulse Coherent Acoustic Doppler Profiler (ADP), and occasionally a 300 KHz Sontek ADP was also used due to availability and calibration needs. The ADP was mounted on a kayak, along with a GPS unit and a Panasonic rugged laptop. Sampling sites on the river were chosen to reflect the most natural channel shape possible, avoiding any anthropogenic structures or altered channel-shape. Data were recorded using the SonTek RiverSurveyor program. At each site, a total of four transects were recorded (two back-and-forth transects). Hydrologic procedures were taken from the USGS handbook for hydrology procedures (Mueller and Wagner 2009; available at http://pubs.usgs.gov/tm/3a22/), although slightly modified to account for the possibility of backward and zero flow due to seiching and backwater events. Data were also used from the online current monitoring program (http://waterdata.usgs.gov/mi/nwis/current/?type=flow), with gages on the Flint, Tittabawassee, Shiawassee, and Saginaw Rivers. Each sampling event took a single day, and each is considered here for analysis purposes to represent the same sampling date. USGS gaging data available for the downstream-most gaging sites on the Cass, Tittabawassee, Shiawassee, Flint, and Saginaw Rivers was analyzed using the program HEC DSS-VUE, and hydrographs were created for the periods of record, along with duration analyses (Appendix 1). Additional measurements of flow velocity were made using either a Rickly Hydrological Company AA Price current meter and bridgeboard or a Marsh-McBirney Flo-Mate 2000 porTable flow meter with standard top-setting rod, depending on the depth and velocity of water at each site at time of sampling.

Water Chemistry

Water samples were taken on each sampling date at each of the sites on the major rivers and tributaries (Figure 1). Samples were taken at the center of each site, avoiding any outside contamination of sediments and water from entering the site. Water was collected in one liter Nalgene bottles, previously triple acid-washed in the lab and then triple rinsed at the sample location. The samples were stored in a cooler with dry ice, keeping them frozen until returning to the lab to place them in the freezer until analysis, which was conducted within one week of each sampling event. Analyses were conducted in the lab for total reactive phosphorus, total phosphorus, total inorganic nitrogen, ammonia, and alkalinity.

Figure 1: Above: Inflows and outflow (Saginaw) measurement sites included in hydrology measurements, including SNWR and restoration project boundaries (Source: Buchanen et al. 2013). Below: Schematic of inflow and outflow measurements: Sum of inputs = Bad+Shiawassee+Swan+Flint+Spaulding Drain+Cass+Tittabawassee, Output=Saginaw (Source: Buchanen et al. 2013)



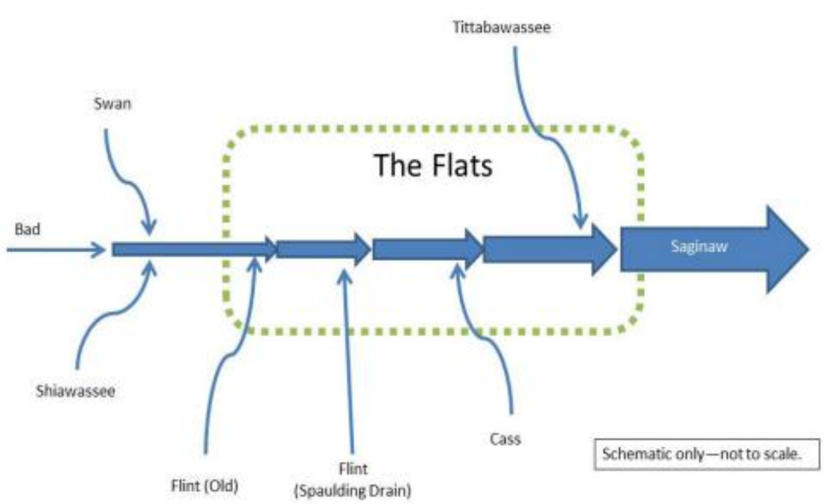


Table 1: Locations of discharge measurements and methods of measuring discharge at each site.

Waterbody	Site Name	Location	Flow Sampling Method
Inputs			
Flint River	Spaulding Drain (Spulding)	West Curtis Road, east of Ambrose Road	Kayak-mounted 1.5 MHz Sontek PC-ADP; Price AA meter and sounding reel
Shiawassee River	Shiawassee (Shi)	Fergus Road, east of Sharon Road	Kayak-mounted 1.5 MHz Sontek PC-ADP; waded cross section with Marsh-McBirney current meter and top-setting rod
Bad River	Bad	Water Street, park in St. Charles	Kayak-mounted 1.5 MHz Sontek PC-ADP
Swan Creek	Swan	Swan Creek Road and Benkert Road	Kayak-mounted 1.5 MHz Sontek PC-ADP; waded cross section with Marsh-McBirney current meter and top-setting rod
Flint River	Flint	Within Refuge	Kayak-mounted 1.5 MHz Sontek PC-ADP
Cass River	Cass	East Road, north of Evon Road	Kayak-mounted 1.5 MHz Sontek PC-ADP
Tittabawassee River	Tittabawassee (Ttb)	Center Street and West Michigan Park	Kayak-mounted 1.5 MHz Sontek PC-ADP
Output			
Saginaw River	Saginaw (Sag)	Wickes Park at Nautical Reserve Road	Kayak-mounted 1.5 MHz Sontek PC-ADP
Within Refuge/Flats (and not included in input-output calculations)			
SNWR	Ferguson Bayou (FB)	Within Refuge	Kayak-mounted 1.5 MHz Sontek PC-ADP
Shiawassee River Flats	Gage	Within Refuge	Kayak-mounted 1.5 MHz Sontek PC-ADP

Total reactive phosphorus (unfiltered sample) was determined for 25 ml subsamples using a modified Ascorbic Acid method. Commercial reagent tabs (PhosVer 3, Hach© method 8048, accepted by the USEPA as equivalent to USEPA and Standard Method 4500-P-E), were used in place of standard ascorbic acid reagent, calibrated in-house to a standard curve, and read on a ThermoSpectronic© UV1 spectrophotometer with 1 cm flowthrough cell. Acid Persulfate Digestion was used to pre-treat samples utilized for total phosphorus analysis (equivalent to USEPA Standard Method 4500-P B & E). Both total reactive phosphorus and total phosphorus concentrations are reported here as elemental phosphorus (P).

The Cadmium Reduction method using Hach© reagents was used in nitrate-N analysis: (NitraVer 3; product # 1406599) and NitraVer 6 (product # 1411999). The Salicylate Method (reagents for Hach© method 8155) were used for ammonia-N analysis. Standard curves were developed for the spectrophotometer using standard solutions to determine nutrient concentrations in each of the above analyses. A digital titrator was used to measure alkalinity in 100 ml samples. Turbidity, conductivity, temperature, and salinity measurements were performed on site using a LaMotte© 2020e turbidimeter and a YSI© 30 salinity/conductivity/temperature meter (model #30-25FT).

Data Analysis

The statistical program R© version 3.0.2, DataDesk© version 6.3, and Microsoft Excel© were used to perform various statistical analyses (ANOVA, ANCOVA, Pearson Correlations, regressions, T-tests) and create accompanying Tables and Figures. Input –output water and mass balance analyses were based on a conceptual model of the Flats complex in which the major tributary inputs were compared to simultaneous (same date) output to the Saginaw River (see Figure 1). Change in storage values (= sum of tributary inputs – Saginaw River output) are interpreted as Flats floodplain wetland and channel storage flux. In this analysis system transience is ignored as are timing differences between samples; obviously mass balance interpretations under these circumstances can only provide rough approximations of system dynamics but are nonetheless informative (Figure 1; Table 1). Annual mass balance estimates were made by graphical integration of the annual flux graph using ImageJ© to calculate the area under the input and output curves of different nutrient loads and flow.

Results

Hydrology

Two years of stage records are available for the new SNWR gage located on the Shiawassee River, at the center of the Flats area. Based on current calibrations (see Appendix 2) the average flow at this site over this two year period (2012-2013) was 77.62 cms, while the minimum flow was 27.1 cms (October 6, 2012) and the maximum flow was 982.82 cms (April 21, 2013). A duration analysis was completed for the two years of stage data available for the SNWR gage on the Shiawassee. The lowest percent exceedance (0.169%) occurred on the April 21 event. The highest percent exceedance (99.831%) occurred on the October 6 event. While there was no significant correlation between Saginaw Bay level and discharge at the Gage site (R^2=-0.1947, p=0.8869), there was a significant relationship between the discharge in the Saginaw River (Holland Ave. gage) and the stage at the gage on the Shiawassee River within the Refuge (R^2=.97, $F(1,408)$=1.324, p<2.2E-16, Figure 2). There was also a significant relationship between the SNWR gage (on the Shiawassee Flats) stage and the combined discharge of the Flint, Shiawassee, Bad, and Swan Creek (R^2=.9892, $F(4,2)$=137.9, p=.007214, Figure 3). Since the fall of 2012 the USGS has maintained a Doppler gage on the Saginaw River downstream of the SNWR. The Saginaw River at Holland Avenue had a maximum flow in 2013 of 1390.36 cms on April 21 and a minimum flow of 111.09 cms on August 2, 2013. I found no significant correlation between the lake level in Saginaw Bay and the flow in the Saginaw River, although this is approaching significance (R^2=0.3107, p=0.054).

During this study the Tittabawassee River had a maximum flow of 623 cms on April 13, 2013 and a minimum flow of 8.21 cms on September 30, 2012. The Cass River had a maximum flow of 287.3 cms on April 14, 2013 and a minimum flow of 1.07 cms on July 17, 2012. The Shiawassee River had a maximum flow of 90 cms on April 19, 2013 and a minimum flow of 1.64 cms on July 4, 2012. The Flint River had a maximum flow of 193 cms on April 13, 2013 and a minimum flow of 2.32 cms on September 14, 2013. To compare my collected hydrologic data to historical flow frequencies, a duration analysis was completed for each river with USGS gaging data for the period of record (Appendix 1; Table 2). The lowest flows I sampled had a median exceedance frequency of 77% (September 2, 2013) while the highest corresponded to a median exceedance frequency of14% (June 30, 2013).

Flow measurements generally exhibited a seasonal pattern, with higher flows recorded in early spring and summer and lower flows in mid to late summer (Figure 4; Appendix 3). Flow measurements during the 2012 study period were highest in mid-summer, and lowest in early and late summer; this can be attributed to drought conditions and a large rain event during the mid-summer sampling. Flows were significantly different by date ($F=5.4$ on 9 df, $p=0.0001$). Flows varied greatly by site, with the highest flows on the Saginaw and Tittabawassee Rivers (Table 3; Figure 5). Flow was significantly different between sites ($F=39.96$ on 10 df, $p=0.0001$), and tended to be higher on the Saginaw and Tittabawassee Rivers.

Hydrologic Input-Output Estimates

The area where the major tributaries converge within the Refuge at the Flats was treated as a single storage area, and the Saginaw River was treated as its single output (see Figure 1). The difference between tributary inputs (to the Flats) and the Saginaw output (from the Flats) constitutes an estimate of the change in hydrologic storage occurring at that time. Differences between hydrologic inputs and outputs varied substantially by date and year (Table 4, Figure 6), ranging from negative values and implying water losses to storage in the Flats to very large and positive values, implying release of stored water to the downstream Saginaw system (Table 4). Since the majority of my sampling was during low flow months most of the sampling dates had storage fluxes to the Saginaw from the Flats; these were especially pronounced in the late summer of 2013.

During the 2013 high-flow measurement on May 7th (average exceedance frequency = 16.5%) discharge leaving the Flats via the Saginaw River was 20% lower than the sum of measured inputs, implying that substantial water storage was taking place at this time (Figure 6). This was the only sampling event in which I observed significant water storage was taking place, reducing the flow to the Saginaw River. The August 12, 2012 sampling event immediately followed local rain and flooding events in what was otherwise considered a drought year. For that sample date total inputs (215.24 cms) were relatively balanced with the flow in the Saginaw River (219.5 cms), meaning that little to no water was going in or coming out of storage at this time (Table 3). I estimate that based on input-output differences previously stored water accounted for 6.48% of the total flow on May 19, 2013, and only 2.11% on June 5, 2013 (Table 3), thus inputs and outputs were mostly balanced, and water was supplied downstream of the Flats with little storage delay. The May 19 and June 5 (2013) measurements both showed somewhat higher flows in the Saginaw River than could be accounted for by the sum of the inputs (Figure 6), suggesting that the Flats area wetlands were supplying previously stored water to the Saginaw River. June 30 and July 15 (2013) measurements both showed significantly higher flows in the Saginaw River than could be accounted for by summed inputs (Figure 6), suggesting that in mid-summer the Flats area wetlands were supplying a significant amount of stored water to the Saginaw River (18.56% of total flow on June 30, and 37.15% on July 15 ; Table 3). During a comparable seasonal sample the previous year (July 19, 2012) total inputs (25.9 cms) were also significantly lower than the observed output in the Saginaw River (84 cms) and accounted for only 30.83% of its flow. On November 10, 2012, total inputs (34.17 cms) were significantly lower than the flow in the Saginaw River (60.427),

Figure 2: Refuge stage (ft) versus Saginaw discharge (cfs) for period of record (Oct. 1, 2012 – Dec. 30 2013) of USGS Holland Ave. gage (R^2=.97, F(1,408)=1.324, RSE=.3867 on 408 df, p<2.2e-16)

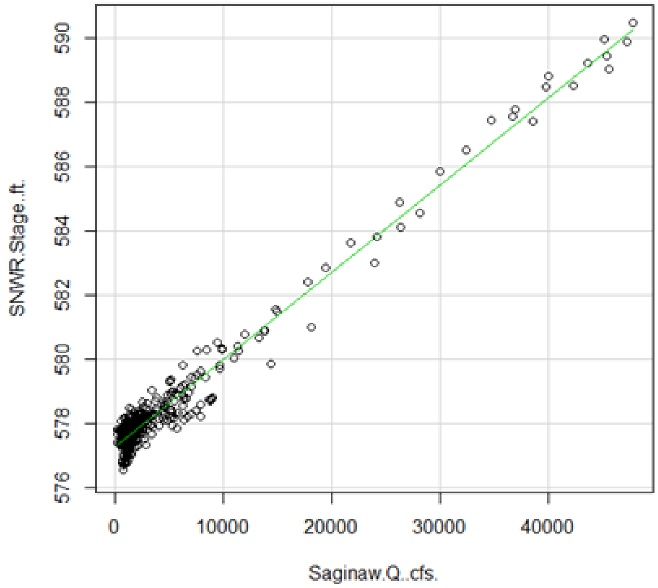


Figure 3: Refuge gage measured discharge (cms) versus sum of inputs discharge (cms) for study period with statistical output (R^2=.9892, F(4,2)=137.9, p=0.0072).

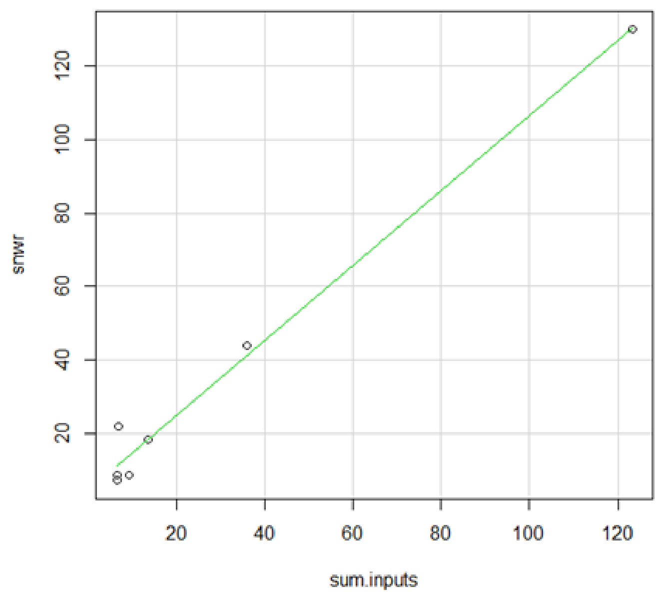


Table 2: Sampling date exceedance frequencies for closest upstream USGS or NOAA gages.

	Shiawassee		**Cass**		**Tittabawassee**		**Flint**		**Median**	**Mean**
Date	*Flow (cfs)*	*% Exceedance*	*Flow (cfs)*	*% Exceedance*	*Flow (cfs)*	*%Exceedance*	*Flow (cfs)*	*%Exceedance*	*Exceedance*	*Exceedance*
7/19/2012	193.52	53.66	122.19	63.31	412.12	72.95	71.95	82.60	72.95	71.95
8/12/2012	928.77	8.08	476.75	27.25	1970.55	20.05	18.86	15.70	20.05	18.85
11/10/2012	143.73	65.12	119.71	64.11	126.07	64.61	66.43	36.55	64.61	66.43
5/7/2013	629.66	15.56	709.11	19.20	2894.04	15.77	16.52	15.98	15.77	16.51
5/19/2013	290.63	38.80	303.35	37.90	1554.19	35.84	35.72	33.78	35.84	35.72
6/5/2013	586.92	17.37	862.00	15.85	2842.05	15.76	15.91	14.74	15.76	15.90
6/30/2013	548.79	19.13	1281.75	9.84	2153.13	14.49	13.78	4.14	14.49	13.78
7/15/2013	240.14	46.05	183.63	51.89	719.71	49.09	52.26	46.30	49.09	52.26
7/28/2013	163.86	60.03	140.90	59.20	444.96	59.62	65.67	57.92	59.61	65.67
9/2/2013	420.24	26.89	75.92	77.99	550.90	77.34	67.95	90.20	77.36	67.95

Figure 4: Boxplot of discharge (cms) by date, over all sites. Numbers correspond to months, letters to multiple samplings in a month (5a=May 7, 5b=May 19, 6a=June 5, 6b=June 30, 7a=Ju y 15, 7b=July 28). Blanks represent months without sampling events. Discharge wassignificantly different between sampling dates (F=5.4 on 9 df, p=0.0001).

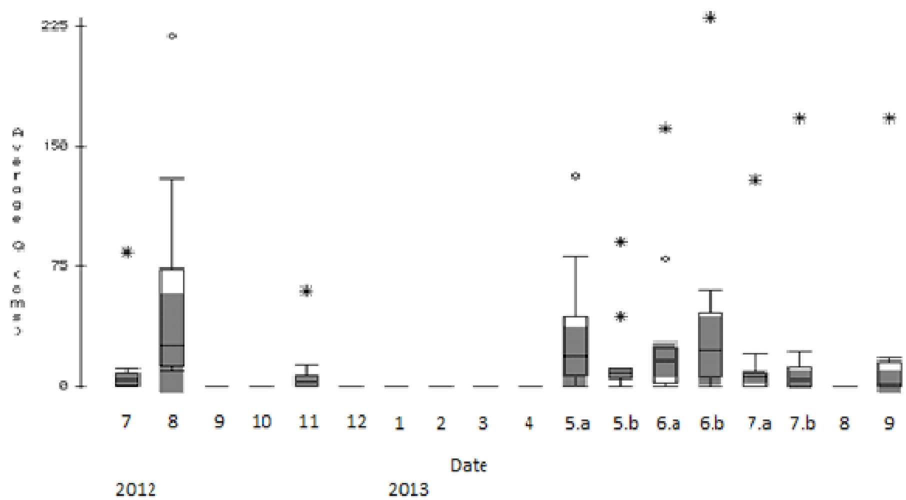


Table 3: Statistical summaries for flow and water quality by station, over all dates. * Indicates significantly different between sites, ** between dates

ANC (mg/l CaCO3) Mean 145.7	Bad	Cass	Flint	Gage	Saginaw	Shiawassee	Spaulding	Swan	Tittabawassee
Count	10.0	10.0	9.0	6.0	10.0	10.0	10.0	10.0	10.0
Mean	116	213	129	132.5	185.9	143.5	189.9	145.8	127.2
StdDev	54.9	237	42.9	16.1	145.3	36.4	136.9	31.7	31.2
Min	59.0	79.0	88.0	116.0	91.0	99.0	109.0	105.0	81.0
Max	207.0	875	226	159.0	579.0	230.0	562.0	218.0	169.0
Cond. (µS) ** Mean 600	**Bad**	**Cass**	**Flint**	**Gage**	**Saginaw**	**Shiawassee**	**Spaulding**	**Swan**	**Tittabawassee**
Mean	509	596	628	583.9	607.8	588.6	591.5	624.7	645.5
StdDev	151	105	122.9	142.8	67.3	110.9	114.2	149.6	197.4
Min	153	438	399	385.0	483.0	348.0	319.0	490.0	457.8
Max	718	811	829	802.0	683.0	694.0	686.0	977.0	953.0
Q (cms) *, ** Mean 28.2	**Bad**	**Cass**	**Flint**	**Gage**	**Saginaw**	**Shiawassee**	**Spaulding**	**Swan**	**Tittabawassee**
Mean	10.1	12.1	6.3	34.1	144.5	11.8	16.2	1.7	38.7
StdDev	22.6	11.4	7.3	44.2	56.3	7.3	12.9	4.1	29.7
Min	0.0	2.2	0.0	7.1	60.4	4.1	2.7	0.1	3.6
Max	73.9	36.3	22.6	130.0	230.7	26.3	46.3	13.3	82.0
TIN (mg/l) ** Mean 0.32	**Bad**	**Cass**	**Flint**	**Gage**	**Saginaw**	**Shiawassee**	**Spaulding**	**Swan**	**Tittabawassee**
Mean	0.46	0.46	0.42	0.30	0.27	0.33	0.50	0.39	0.30
StdDev	0.26	0.29	0.31	0.31	0.11	0.24	0.24	0.42	0.24
Min	0.11	0.11	0.13	0.08	0.03	0.09	0.20	0.03	0.13
Max	0.80	0.96	1.20	0.97	0.41	0.80	1.01	1.15	0.95
TRP (mg/l) *, ** Mean 0.051	**Bad**	**Cass**	**Flint**	**Gage**	**Saginaw**	**Shiawassee**	**Spaulding**	**Swan**	**Tittabawassee**
Mean	0.05	0.03	0.05	0.05	0.06	0.04	0.06	0.09	0.05
StdDev	0.03	0.02	0.03	0.03	0.05	0.02	0.04	0.06	0.03
Min	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.01
Max	0.12	0.08	0.09	0.12	0.17	0.08	0.14	0.21	0.12
TP (mg/l) Mean 0.117	**Bad**	**Cass**	**Flint**	**Gage**	**Saginaw**	**Shiawassee**	**Spaulding**	**Swan**	**Tittabawassee**
Count	7.00	7.00	7.00	4.00	7.00	7.00	7.00	7.00	7.00
Mean	0.08	0.08	0.12	0.11	0.20	0.10	0.09	0.11	0.09
StdDev	0.05	0.04	0.07	0.03	0.22	0.07	0.07	0.05	0.02
Min	0.03	0.04	0.06	0.07	0.01	0.03	0.04	0.04	0.06
Max	0.18	0.13	0.26	0.14	0.62	0.19	0.24	0.19	0.12

Figure 5: Boxplot of discharge (cms) by site, over all dates. Discharge was significantly different between sites (F=39.96 on 10 df, p=0.0001). FB=Ferguson Bayou, Shw=Shiawassee, SD=Spaulding Drain, Ttb=Tittabawassee

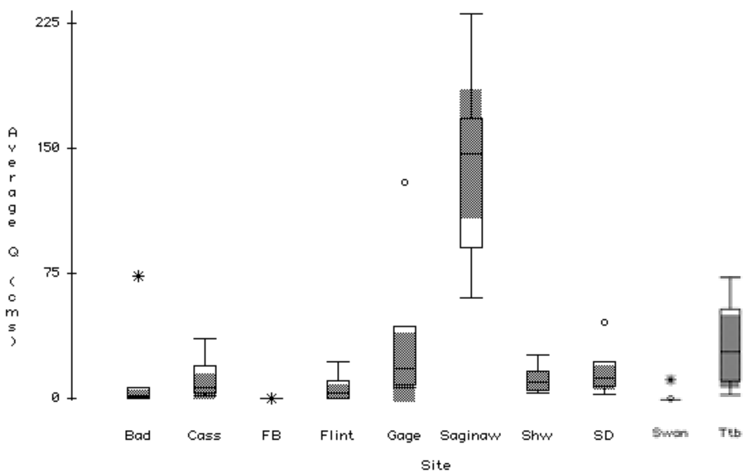


Figure 6: Sum of input and output discharges to and from the Flats area over the study period. No data was collected between the 11/10/12 point and the 5/7/13 point.

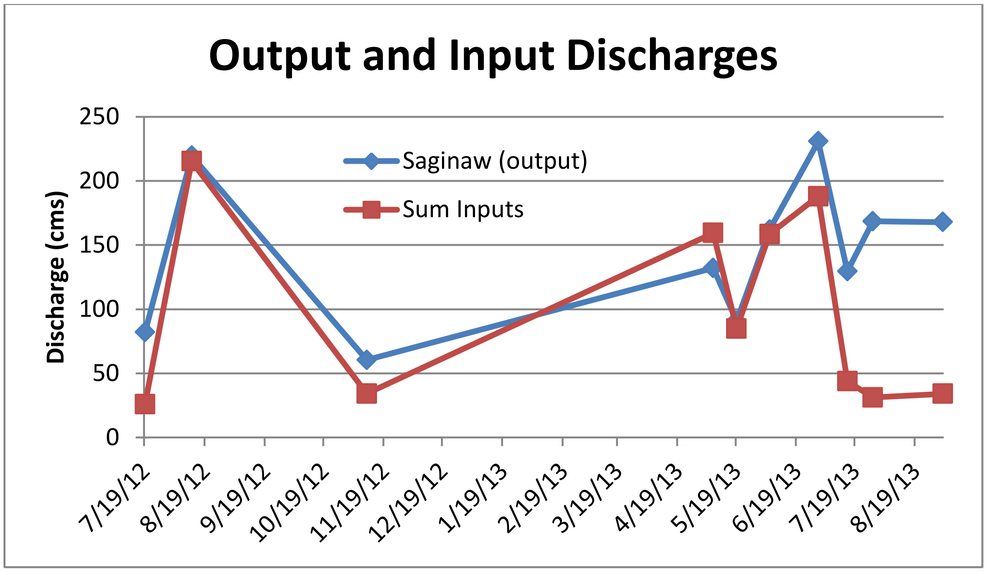


Table 4: Flow measurements (cms) by sampling date and site. The percent of output represents what portion of the output (Saginaw River) the sum of inputs represents.

Date	*Site*	*Flow (cms)*	*% Output*
19-Jul-12	Sum of Inputs	25.90	30.83%
	Saginaw (output)	84.02	
	dStorage	58.12	69.17%
12-Aug-12	Sum Inputs	215.3	98.06%
	Saginaw (output)	219.5	
	dStorage	4.255	1.94%
10-Nov-12	Sum Inputs	34.17	56.55%
	Saginaw (output)	60.43	
	dStorage	26.26	43.45%
7-May-13	Sum Inputs	159.4	120.68%
	Saginaw (output)	132.1	
	dStorage	-27.32	-20.68%
19-May-13	Sum Inputs	84.92	93.55%
	Saginaw (output)	90.78	
	dStorage	5.851	6.45%
5-Jun-13	Sum Inputs	158.5	97.86%
	Saginaw (output)	161.9	
	dStorage	3.464	2.14%
30-Jun-13	Sum Inputs	187.9	81.44%
	Saginaw (output)	230.7	
	dStorage	42.81	18.56%
15-Jul-13	Sum Inputs	43.79	33.81%
	Saginaw (output)	129.5	
	dStorage	85.72	66.19%
28-Jul-13	Sum Inputs	31.19	18.51%
	Saginaw (output)	168.4	
	Storage	137.3	81.49%
2-Sep-13	Sum Inputs	34.13	20.32%
	Saginaw (output)	167.9	
	dStorage	133.8	79.68%

indicating that 43.45% (26.25 cms) of the Saginaw's flow was coming from previously stored water from the Flats area (Table 3).

A linear regression of water storage flux as percent of the Saginaw flow against catchment-wide exceedance frequency indicated a significantly positive relationship, in which low flows result in larger changes in storage (Figure 7; R^2=76.8%, s=0.1813 with 8 df, t= 5.54,p=0.0005). ANCOVA of storage flux against catchment-wide exceedance frequency indicated a similar positive relationship, with a somewhat higher increase in storage flux with change in exceedance frequency for the 2013 study period versus 2012 (F=12.86, p=0.0116; Figure 7).

Water Quality

Water quality varied significantly between rivers and by date (Table 3, Appendices 4 and 5) although a number of parameters were significantly correlated across the data set (Table 3, Figure 8). Alkalinity in the Flats (SNWR gaging site) ranged from 105 mg/l to 203mg/l, the former occurring after an intense period of summer rain in 2012. Inorganic nitrogen concentrations at the SNWR gage site were also highly variable; ranging from 0.077 to 0.973 mg/l. Phosphorus concentrations were variable to a similar degree (Table 3, Figures 9, 10, 11) . The Saginaw River, carrying the collected output of the Flats area, generally had higher alkalinity and nutrients concentrations than the SNWR gage site but also often lower than incoming rivers (Table 3; Figures 9, 10, 11).

Total inorganic nitrogen concentrations were highest at the Flint River/Spaulding Drain (0.470 mg/l), followed by the Cass (0.422 mg/l) and Flint Rivers (0.417 mg/l, Figure 9); again sites in SNWR were typically much lower, for example in 2013 Ferguson Bayou (0.110 mg/l), and the SNWR Gage on the Shiawassee (0.158 mg/l, Figure 9). Although the Bad and Cass Rivers had somewhat higher inorganic nitrogen concentrations than the other sites, ANOVA indicated that there was no significant difference in total inorganic nitrogen concentration between sampling sites across all dates (F=1.84, df=9, p=.07,

Figure 7: Linear regression of water storage flux (as percent of the Saginaw flow) against catchment-wide exceedance frequency (left), with separate regression lines for 2012-red and 2013-blue (F=12.86, p=0.0116). Percent change in storage against catchment-wide exceedance frequency, right (R^2=76.8%, s=0.1813 with 8 df, t= 5.54,p=0.0005)

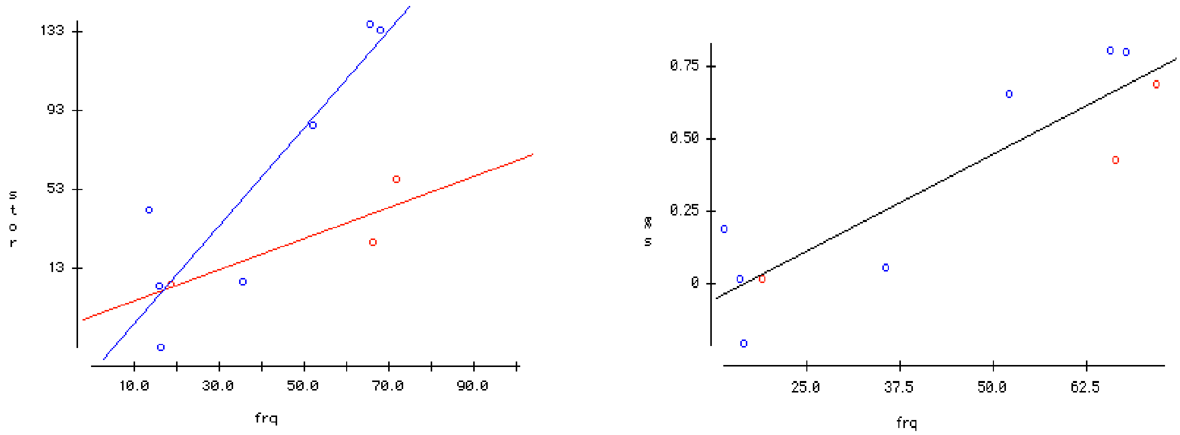


Figure 8: Correlation matrix for alkalinity, discharge, carbonate load, conductivity, temperature, and conductivity for all sites and dates.

Pearson Product-Moment Correlation

	Carbonate load (kg/day)	Q (cms)	Alkalinity (mg/l CaCO3)	Turbidity (NTU)	Conductivity (µS)	Temp. C
Carbonate load (kg/day)	1					
Q (cms)	0.993	1				
Alkalinity (mg/l CaCO3)	-0.092	-0.099	1			
Turbidity (NTU)	0.064	0.019	-0.345	1		
Conductivity (µS)	-0.014	-0.081	-0.008	0.266	1	
Temperature C	-0.016	-0.046	-0.382	0.55	0.333	1

Figure 9: Total inorganic nitrogen concentration by site for entire study period, across all dates. There was no significant difference between sites (F=1.8, p=0.07) FB=Ferguson Bayou, Shw=Shiawassee, SD=Spaulding Drain, Ttb=Tittabawassee

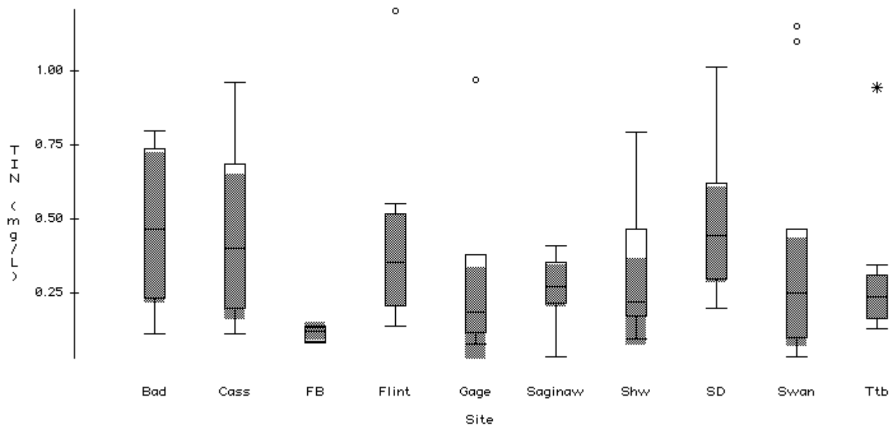


Figure 10: Reactive phosphorus concentrations by site for entire study period, across all dates. There was a significant difference between sites (F=3.73, p=0.0007). FB=Ferguson Bayou, Shw=Shiawassee, SD=Spaulding Drain, Ttb=Tittabawassee

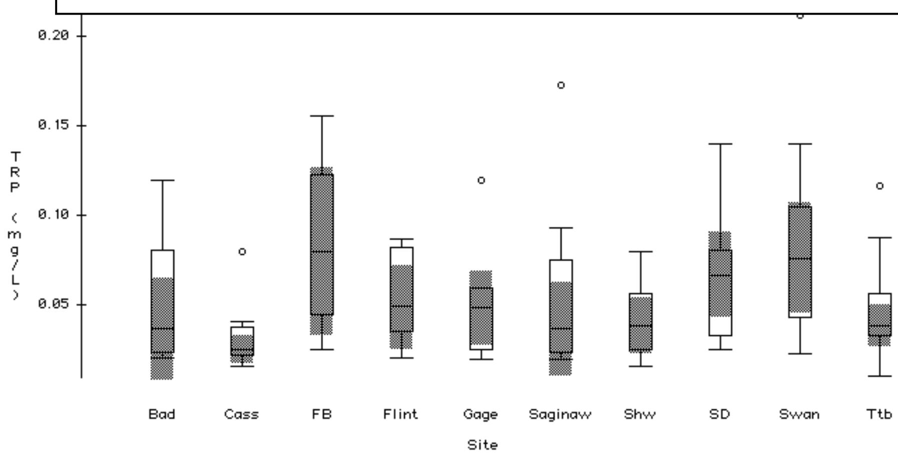


Figure 11: Total phosphorus concentration by site for entire study period, across all dates. There was no significant difference in concentration between sites (F=1.9, p=0.072). FB=Ferguson Bayou, Shw=Shiawassee, SD=Spaulding Drain, Ttb=Tittabawassee

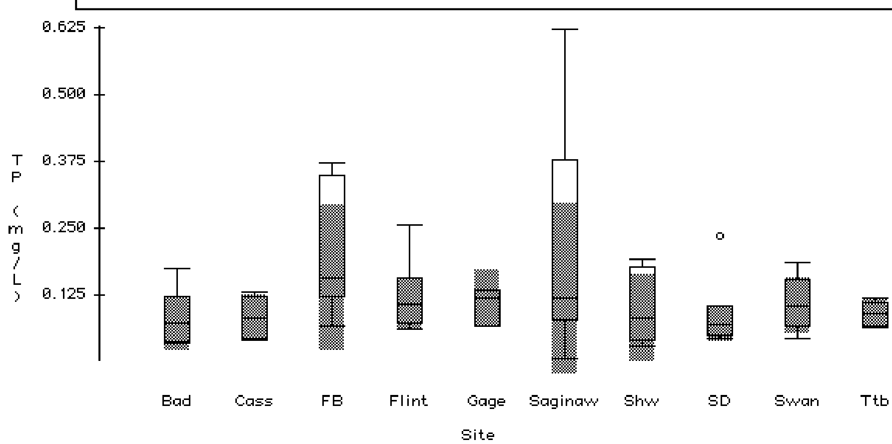
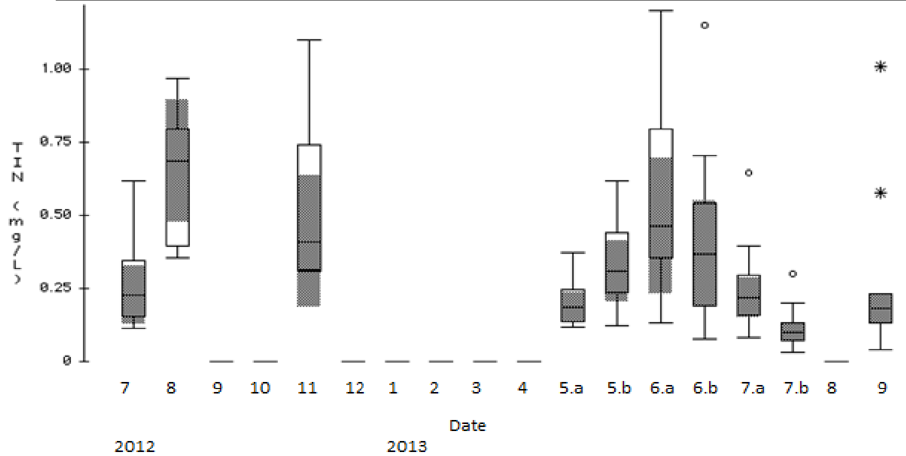
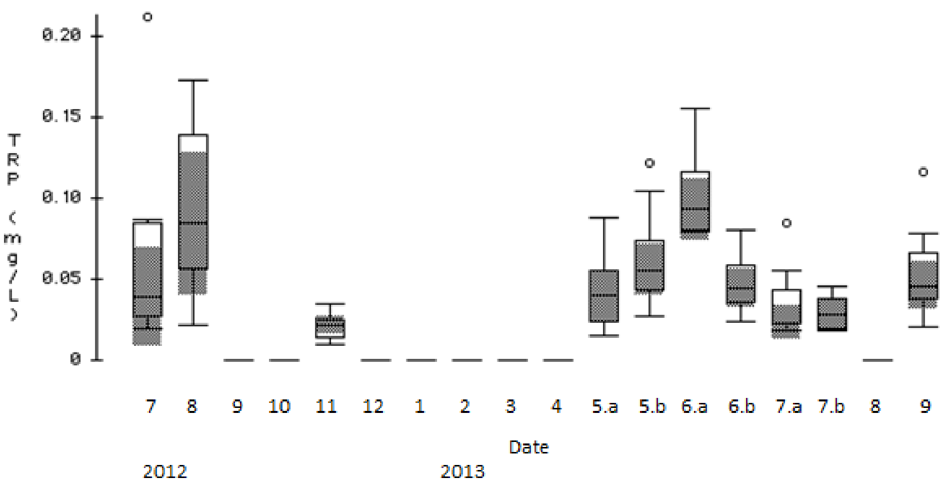


Figure 12: Total inorganic nitrogen (top), total reactive phosphorus (middle) and total phosphorus (bottom) concentrations , by date over all sites for the entire study period. Numbers represent the month, letters correspond to months with multiple samplings (5a=May 7, 5b=May 19, 6a=June 5, 6b=June 30, 7a=July 15, 7b=July 28). Blanks represent months without sampling events. There was a significant difference TIN and TRP between dates (TIN: F=4.9, p=0.0001; TRP: F=8.3, p=0.0001; TP: F=1.7, p=0.14.





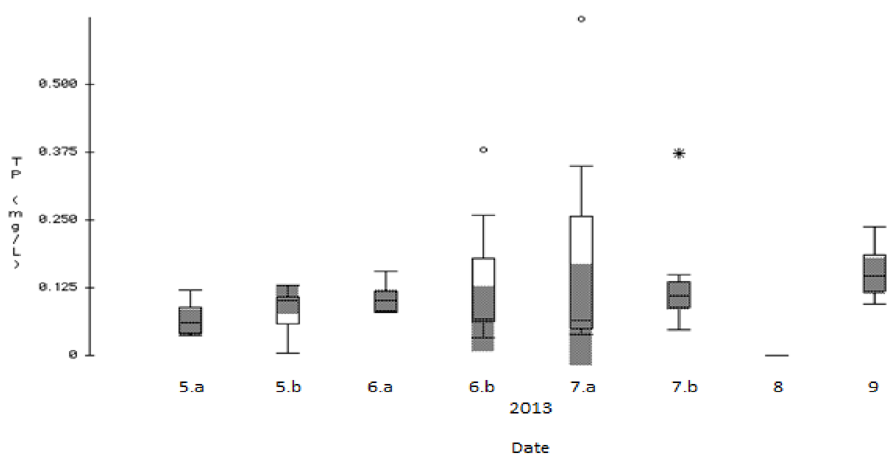


Figure 9). There was a seasonal pattern in nitrogen concentrations, with higher concentrations in mid-summer, and ANOVA indicated a significant difference in inorganic nitrogen concentration between sampling dates (F=4.96, df=9, p=0.0001; Figure 12).

In contrast ANOVA indicated that there was a highly significant difference in total reactive phosphorus concentrations between the different sampling dates across all sites (F=8.31, df=9, p=0.0001, Figure 12) during the entire study period and between the different sampling sites across all dates (F=3.73, df=9, p=.0007, Figure 10). Total reactive phosphorus concentrations were highest at Swan Creek (0.069 mg/l), followed by the Tittabawassee River (0.057 mg/l, Figure 10). Reactive phosphorus concentrations were lowest at the Cass River (0.034 mg/l), and the SNWR gage in the Flats (0.040 mg/l, Figure 10). There was no statistically significant difference in total phosphorus concentrations between sites (F=1.9, df=9, p=.07, Figure 11), and there was no evidence of significant seasonal changes in total phosphorus concentration (F=1.7, df=9, p=0.14; Figure 12). Total phosphorus concentrations were highest at Ferguson Bayou (0.207 mg/l), followed by the Saginaw River (0.205 mg/l, Figure 11). Total phosphorus concentrations were lowest on the Bad River (0.0829 mg/l), followed by the Cass River (0.0835 mg/l, Figure 11).

Estimated Nutrient Loads

Nutrient loads were highly variable across sites (Table 5; appendix 7). Carbonate loads ranged from 1800 kg/day to 3.32E6 kg/day, while total dissolved solid loads ranged from 3009 kg/day to 5.04E6 kg/day. Inorganic nitrogen loads ranged from 0.7 kg/day to 10923 kg/day. Reactive phosphorus loads ranged from 0.93 kg/day to 3287 kg/day, while total phosphorus loads ranged from 1 kg/day to 7577 kg/day.

In terms of contributing inorganic nitrogen load, the input rivers are all highly variable and dependent on variations in flow and surrounding precipitation conditions (see appendix 9), although the

Tittabawasse, Cass, and Spaulding Drain tended to contribute the highest nitrogen loads. Sites were significantly different in terms of inorganic nitrogen load (F=6.8, p=0.0001), as were sampling dates (F=6.8, p=0.0001). Total inorganic nitrogen load was highest on the Saginaw River during the August 2012 sampling event (12971 kg/day), and this date also exhibited the highest inorganic nitrogen input loadings (13712.29 kg/day, Figure 13). Inorganic nitrogen loading on the Saginaw River was similar during the July and November 2012 events (2069 kg/day and 2154 kg/day, respectively). Input loading of nitrogen was lowest during 2012 on the July event (725.03 kg/day), while input loading during the November 2012 event was somewhat higher (1358.99 kg/day). Total inorganic nitrogen was lowest on the Saginaw River during the July 28, 2013 sampling event (480 kg/day), and this event exhibited some of the lower flows over the study period (Figure 6). Input nitrogen loadings during 2013 were lowest during the July 28 and September 2 events (444.36 kg/day and 790.66 kg/day, respectively; Figure 13). A paired t-test revealed that there was no significant difference in total inorganic nitrogen load between the sum of inputs to the Flats and the outputs to the Saginaw River (mean of differences=1467.34, t=-0.7992, df=9, p=0.4448; Figure 13).

In terms of overall reactive phosphorus load, the Tittabawassee River tended to have the highest loads throughout the study, while the Cass River and Spaulding Drain also had higher levels of both these nutrients (Figure16). As with inorganic nitrogen load, the main contributors for each date tended to vary with varying flows (see appendix 10). ANOVA indicated a significant difference in reactive phosphorus load by date (F=3.53, p=0.0011; Figure 17) and sampling site (F=5.5, p=0.0001;Figure 17).

During the 2012 study period, total reactive phosphorus load was highest on the Saginaw River during the August sampling event (1934 kg/day), and this date also exhibited the highest input loadings

Table 5: Statistical summaries for load by station, over all dates. * Indicates statistically significant difference between sites, ** indicates significant difference between dates.

TIN (kg/day) *, **	Mean 984.7								
	Bad	**Cass**	**Flint**	**Gage**	**Saginaw**	**Shiawassee**	**Spaulding**	**Swan**	**Tittabawassee**
Count	9	10	9	7	10	10	10	10	10
Mean	653.461	547.039	327.009	1775.25	3213.34	441.99	550.865	56.5588	1077.84
StdDev	1628.02	634.969	447.819	4036.88	1888.84	564.904	296.966	126.448	1365.2
Min	14.4111	20.1238	4.90555	84.3566	480.877	36.9075	198.246	0.719621	96.2847
Max	4988.96	2030.65	1169.11	10923.1	6985.37	1811.89	958.589	411.536	4562.37
TRP (kg/day) *, **	Mean 199.5								
	Bad	**Cass**	**Flint**	**Gage**	**Saginaw**	**Shiawassee**	**Spaulding**	**Swan**	**Tittabawassee**
Mean	74.3092	40.6698	36.2906	253.802	789.651	45.0164	97.9149	18.5568	215.172
StdDev	176.242	55.4655	37.9937	487.158	948.227	44.8443	92.6339	50.1924	276.651
Min	1.51252	4.38709	0.997208	15.2906	117.47	5.27292	18.6471	0.933091	3.08111
Max	542.762	168.307	93.6159	1347.84	3287.23	128.785	273.392	161.161	814.593
TP (kg/day) *	Mean 581.1								
	Bad	**Cass**	**Flint**	**Gage**	**Saginaw**	**Shiawassee**	**Spaulding**	**Swan**	**Tittabawassee**
Mean	23.2439	81.617	61.8107	235.09	2926.1	104.863	106.049	3.41037	360.427
StdDev	10.0116	56.9034	53.4955	169.656	3046	85.4308	86.7925	3.19325	286.477
Min	13.4379	19.0939	5.28519	48.4012	39.6309	20.3262	31.5983	1.04968	70.3128
Max	39.6071	171.227	139.544	460.366	7577.38	240.988	275.056	10.27	824.216
Carbonate Load (kg/day) *		Mean 452482							
	Bad	**Cass**	**Flint**	**Gage**	**Saginaw**	**Shiawassee**	**Spaulding**	**Swan**	**Tittabawassee**
Mean	89099.9	197464	124664	689862	1.22E+06	351441	205561	134912	470444
StdDev	138250	187366	123675	1.17E+06	811044	681559	142962	385522	389555
Min	7406.89	17477.7	4078.77	85872.2	11471.5	52562.2	27861	1858.6	84870.6
Max	444949	498613	412182	3.32E+06	2.20E+06	2.28E+06	436317	1.16E+06	1.18E+06
TDS Load (kg/day) *		Mean 775687							
	Bad	**Cass**	**Flint**	**Gage**	**Saginaw**	**Shiawassee**	**Spaulding**	**Swan**	**Tittabawassee**
Mean	111128	303427	192707	841332	2.65E+06	442714	394484	137710	846457

StdDev	135317	265596	181309	1.12E+06	1.88E+06	658483	346717	384495	504869
Min	19009.6	20317.8	10130.7	172498	11471.5	68997	65099.7	3009.31	84870.6
Max	444949	769729	559916	3.32E+06	5.04E+06	2.28E+06	1.14E+06	1.16E+06	1.50E+06

Figure 13: Total inorganic nitrogen inputs and outputs flow weighted mean concentration (left) and load (right).

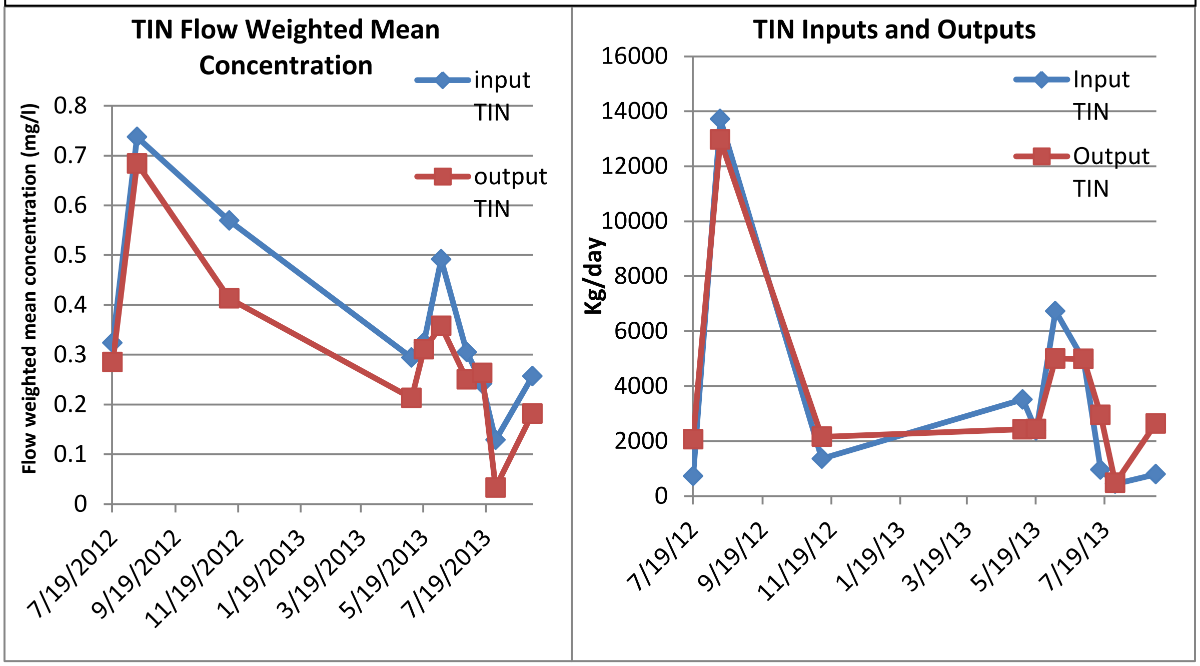


Figure 14: Total reactive phosphorus input and output flow weighted mean concentrations (left) and loads (right)

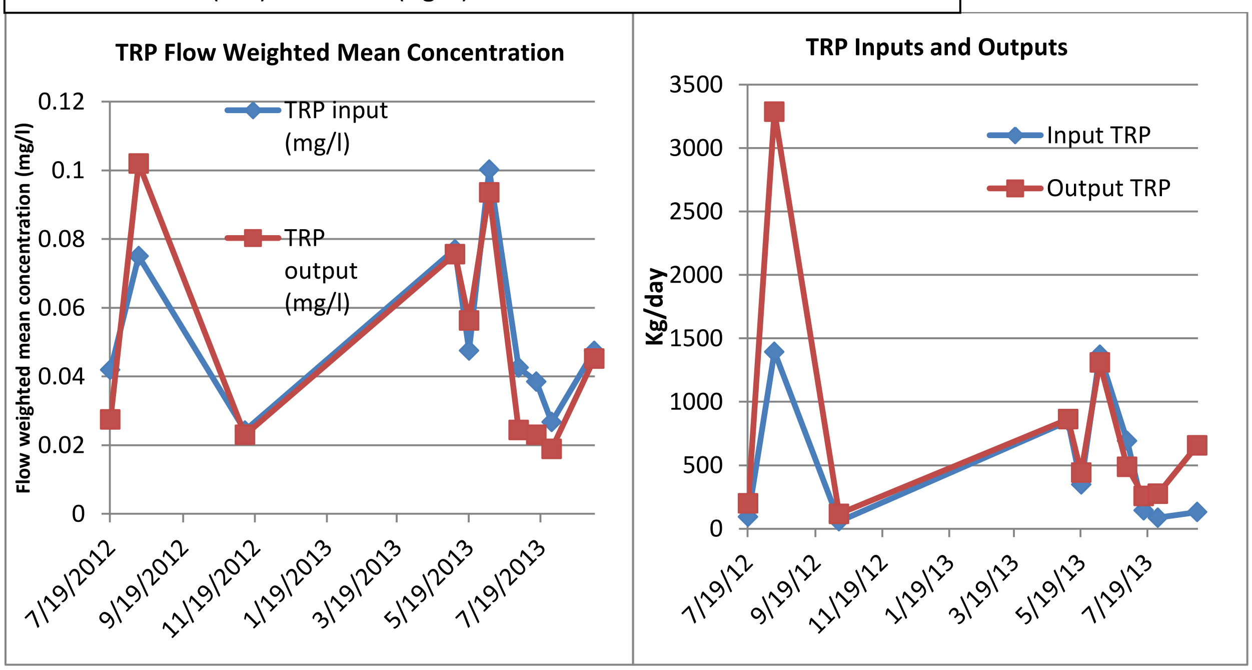
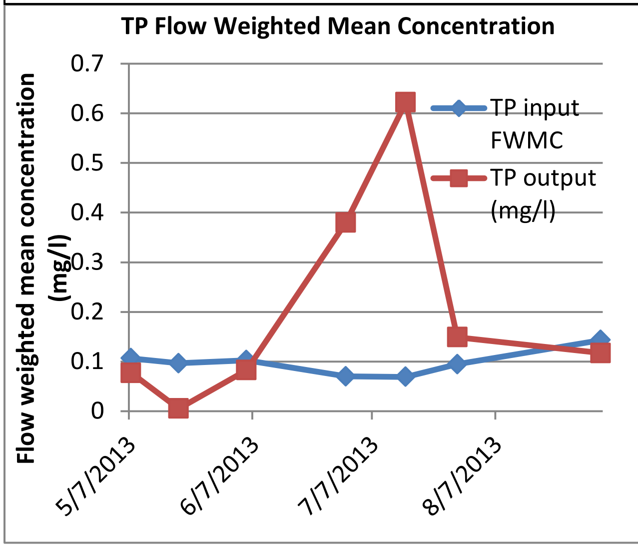


Figure 15: Total phosphorus flow weighted mean concentration (left) and total phosphorus load (right) for the only study period it was measured.



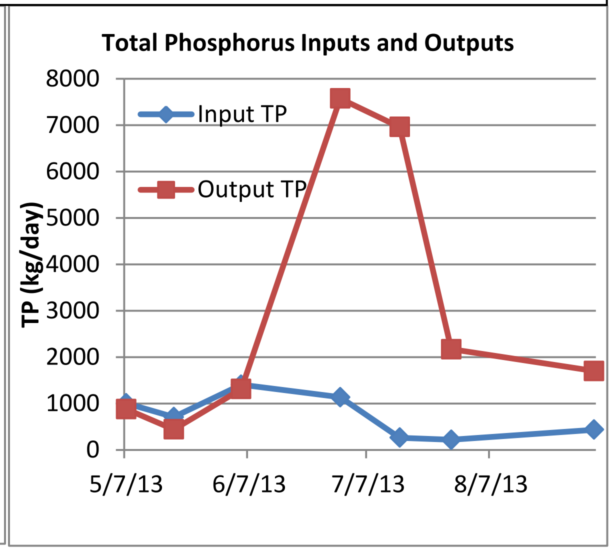
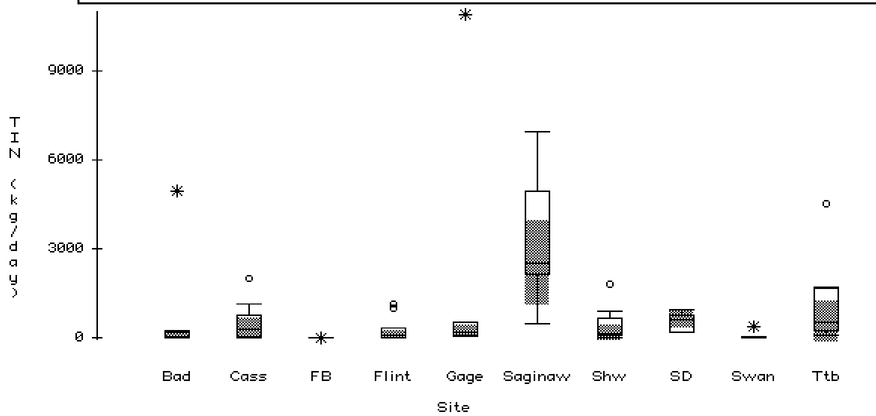


Figure 16: Total inorganic nitrogen load by site (top) and date (bottom) for entire study period, across all dates. FB=Ferguson Bayou, Shw=Shiawassee, SD=Spaulding Drain, Ttb=Tittabawassee Numbers correspond to months, letters to multiple samplings in a month (5a=May 7, 5b=May 19, 6a=June 5, 6b=June 30, 7a=July 15, 7b= July 28), blanks represent months without sampling events. There was a significant difference in total inorganic nitrogen load between sites (F=6.8, p=0.0001) and between sampling dates (F=6.8, p=0.0001).



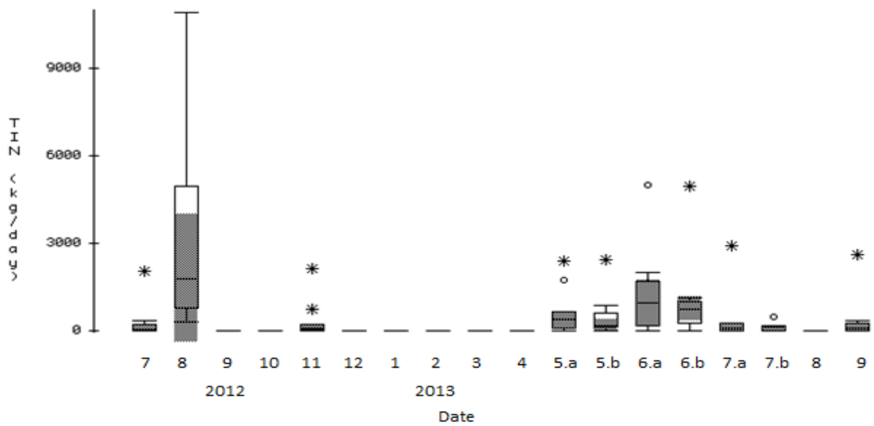
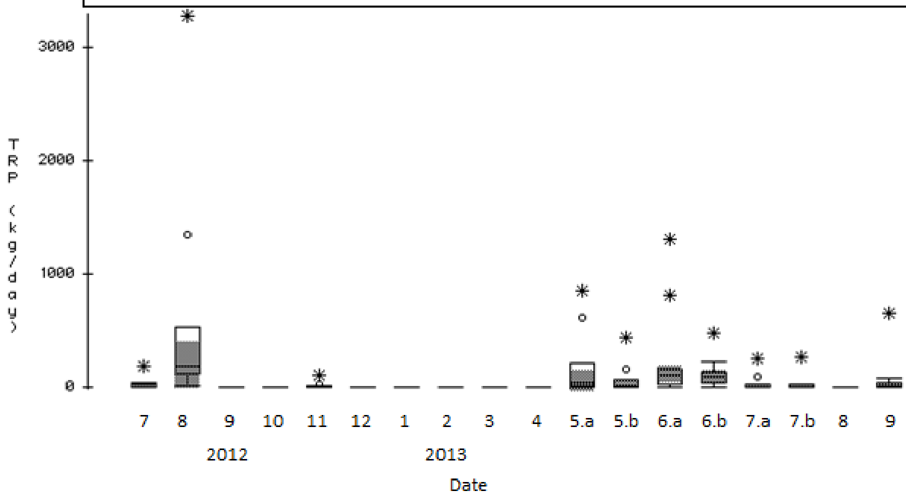


Figure 17: Top: total reactive phosphorus load by date for the entire study period, across all sites. Numbers represent months, letters represent multiple sampling events in a single month (5a=May 7, 5b=May 19, 6a=June 5, 6b=June 30, 7a=July 15, 7b=July 28), and blank bars represent months without sampling events. Bottom: total reactive phosphorus load by site, across all dates. FB=Ferguson Bayou, Shw=Shiawassee, SD=Spaulding Drain, Ttb=Tittabawassee There was a significant difference in reactive phosphorus load between dates (F=3.53, p=0.0011) and sites (F=5.5, p=0.0001).



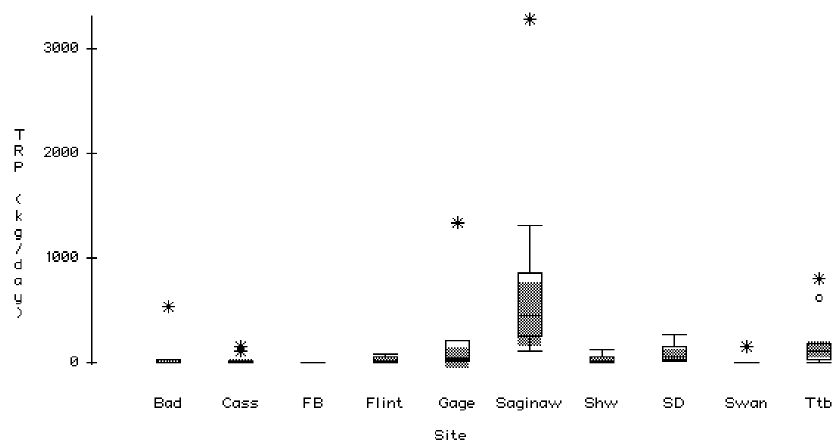
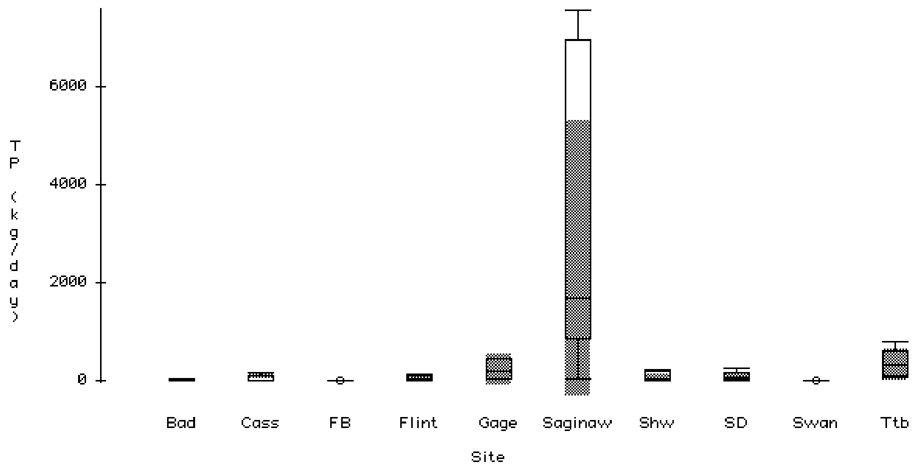
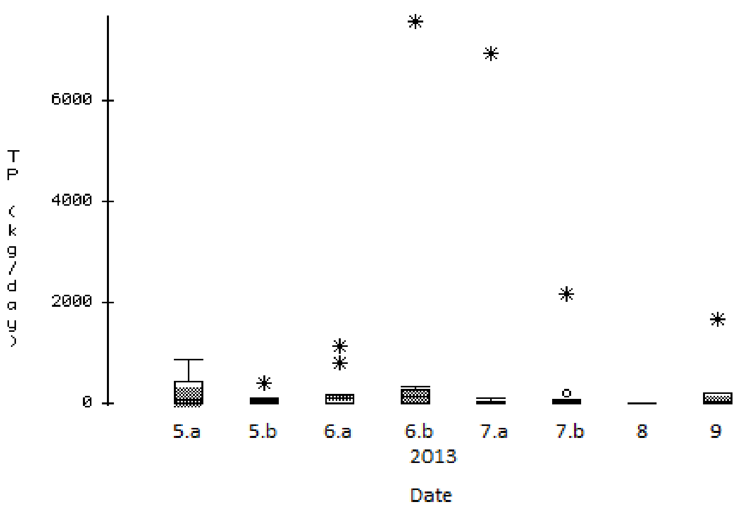


Figure 18: Above: Total phosphorus load by site, across all dates FB=Ferguson Bayou, Shw=Shiawassee, SD=Spaulding Drain, Ttb=Tittabawassee (F=5.7, p=0.0001). Below: Total phosphorus load by date across all sites, along with statistical output. Numbers represent months, letters represent multiple sampling events in a month (5a=May 7, 5b=May 19, 6a=June 5, 6b=June 30, 7a=July 15, 7b=July 28), blank bar represents lack of sampling event in August. There was no significant difference in total phosphorus load between sampling dates (F=0.96, p=0.46).





(1394 kg/day, Figure 14). Reactive phosphorus loading was lowest on the Saginaw River during the November 2012 event (117 kg/day), and input loadings were considerably lower on this date (57.89 kg/day, Figure 14). During the July 2012 sampling event loadings in the Saginaw River (200 kg/day) were again higher than those of the inputs (93.83 kg/day). Total reactive phosphorus was highest in 2013 on the Saginaw River during the June 5 event (1310.12 kg/day), and this date also exhibited the highest reactive phosphorus input loadings (1372.12 kg/day, Figure 14). Total reactive phosphorus was lowest on the Saginaw River during the July 15 and July 28 (2013) events (257.856 kg/day and 275 kg/day, respectively), and the July 28 event also exhibited the lowest input loading (86.8 kg/day; Figure 14). Paired t-tests revealed that there was no significant difference in total reactive phosphorus load between the sum of inputs to the Flats and the output to the Saginaw River over the entire study period (mean of differences =132.068, t=1.758, df=9, p=0.1126; Figure 14).

The only major contributor of total phosphorus load to the Saginaw River during the 2013 study period was the Tittabawassee River, with some minor contributions from the Cass, Spaulding Drain, and the Shiawassee later in the year (Figure 18; appendix 11). ANOVA indicated a significant difference in total phosphorus load between sites (F=5.7, p=0.0001; Figure 18), but no significant difference between sampling dates (F=0.95, p=0.46; Figure 18). During the 2013 study period, total phosphorus load was highest on the Saginaw River during the June 30 event (7577.38 kg/day), while the highest input loadings occurred during the June 5 event (1404.5 kg/day, Figure 15). Total phosphorus loading was lowest on the Saginaw River during the May 19 event (441.24 kg/day), while the lowest input loadings occurred during the July 28 event (216.86 kg/day, Figure 17). Inputs of total phosphorus tended to exceed the outputs at the beginning of the study period, up until the June 5 event, after which output always exceeded input (Figure 15) . A paired t-test revealed that there was no significant difference in total phosphorus load between the sum of inputs to the Flats and the output to the Saginaw River (mean of differences=2188.0047, t=1.849, df=6, p=0.1140; Figure 15). Total phosphorus was not measured during the 2012 study period. There was a significant relationship between total phosphorus load and discharge (R^2=93.4%, p≤0.0001), and there was also a significant correlation between total phosphorus load and reactive phosphorus load (R^2=86.6%, p≤0.0001); as reactive phosphorus load increases, so does total phosphorus load.

A linear regression of inorganic nitrogen load against discharge for each sampling site indicated little variability between the input sites, and revealed that there was little change in load from the inputs to the Saginaw River overall (R^2=0.7057, F=9.328, Figure 19). A similar linear regression involving reactive phosphorus and discharge revealed even less variability in input tributary reactive phosphorus load, and a lower slope value in the Saginaw River (R^2=0.7376, F=3.334e-8, Figure 20). The same linear regression between total phosphorus load and discharge revealed virtually no variability between the input tributaries, and a significant increase in total phosphorus load in the Saginaw River (R^2=0.6398, F=4.393, p=1.841e-5, Figure 21).

Nutrient Input-Output Analysis

As was the case in the hydrologic input-output analysis, there were substantial differences between inflow concentrations (Table6) and loads (Table 7), and outflow concentrations and loads from the Flats implying substantial storage flux throughout the study period. Nitrogen concentrations generally declined, while reactive phosphorus concentrations changed little and total phosphorus concentrations increased in the output of the Saginaw River. However, only one of the differences (inorganic nitrogen concentration) could be shown to be statistically significant in a paired T-Test. There was large seasonal variability in the differences with the larger changes occurring during later in the summer and fall, especially in 2013 (Figures 12, 16-18). Storage flux for N and P

Table 6: Flow-weighted mean concentration for Flats Input-Output study sampling dates. TIN = total inorganic nitrogen (as N), TRP represents total reactive phosphorus-P, TP represents total phosphorus-P.

	TP input (mg/l)	*TP output (mg/l)*	*TRP input (mg/l)*	*TRP output (mg/l)*	*TIN input (mg/l)*	*TIN output (mg/l)*
7/19/2012	-	-	0.042	0.027	0.324	0.285
8/12/2012	-	-	0.075	0.173	0.737	0.684
11/10/2012	-	-	0.024	0.023	0.569	0.413
5/7/2013	0.106	0.077	0.077	0.075	0.293	0.212
5/19/2013	0.096	0.005	0.047	0.056	0.324	0.311
6/5/2013	0.102	0.082	0.100	0.094	0.491	0.358
6/30/2013	0.070	0.380	0.043	0.024	0.305	0.250
7/15/2013	0.069	0.622	0.039	0.023	0.242	0.263
7/28/2013	0.094	0.149	0.027	0.019	0.129	0.033
9/2/2013	0.143	0.117	0.047	0.045	0.257	0.182
MEAN	0.10	0.20	0.05	0.06	0.37	0.30

Table 7: Nutrient loads for Flats input-output study sampling dates. TIN = total inorganic nitrogen (as N), TRP represents total reactive phosphorus-P, TP represents total phosphorus-P.

	Input TIN (kg/day)	*Output TIN (kg/day)*	*Input TRP (kg/day)*	*Output TRP (kg/day)*	*Input TP (kg/day)*	*Output TP (kg/day)*
7/19/12	725.03	2069	93.829	199.64		
8/12/12	13712.29	6985.368	1394.9	3287.23		
10/10/12	1358.99	2153.62	57.89	117.47		
5/7/13	3513.2	2429	847.33472	863.19	1005.138	878.9851
5/19/13	2378.8	2436.1	348.49111	441.238	708.086	441.24
6/5/13	6729.8	5006.18	1372.1164	1310.123	1404.51	1310.13
6/30/13	4954.7	4987.6	691.39913	486.9805	1134.447	7577.382
7/15/13	958.9	2947	144.88476	257.856	263.1354	6963.185
7/28/13	444.36	480.87	86.8	275	216.8564	2168.683
9/2/13	790.66	2637.76	131.0464	657.7733	434.5151	1699.658

Figure 19: Linear regression of inorganic nitrogen load against discharge for each sampling site across all dates, along with statistical output. Saginaw River load remains relatively unchanged compared to the input rivers. RSE=566 on 47 df, R^2=0.71, F=9.3, p=2.9e-10

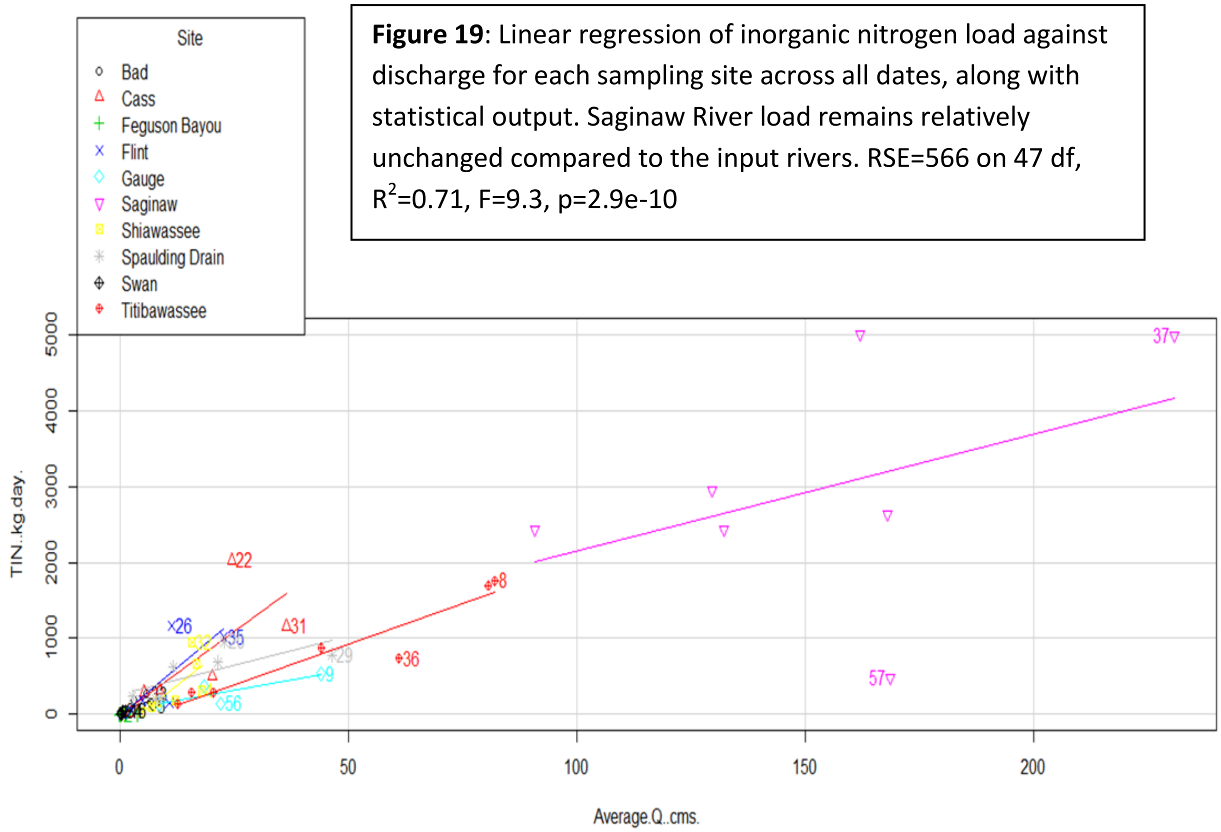
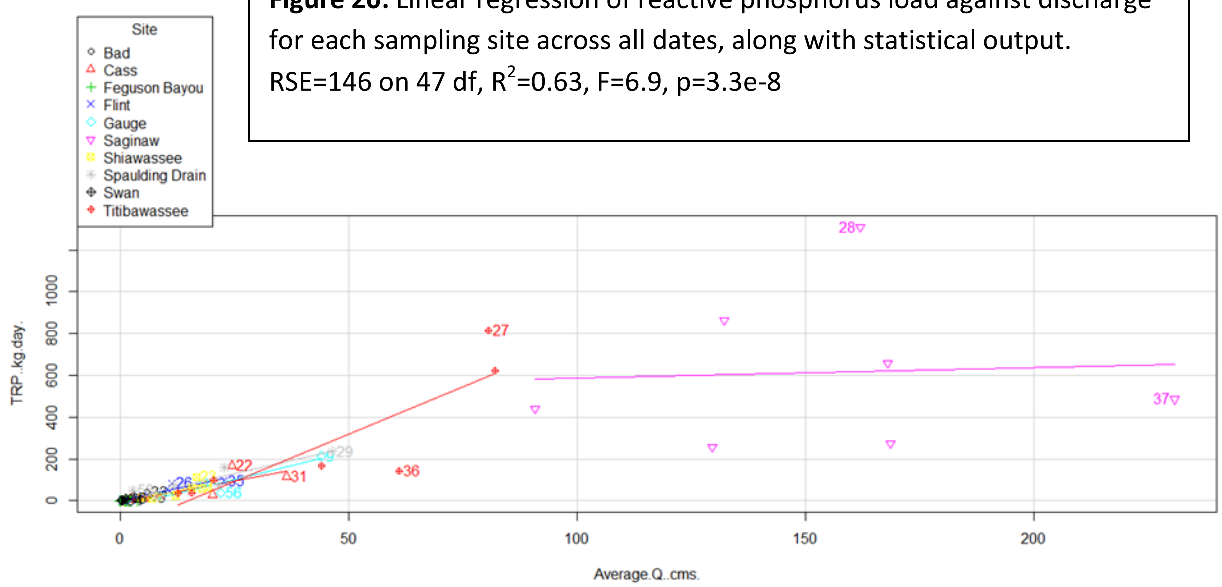


Figure 20: Linear regression of reactive phosphorus load against discharge for each sampling site across all dates, along with statistical output. RSE=146 on 47 df, R^2=0.63, F=6.9, p=3.3e-8



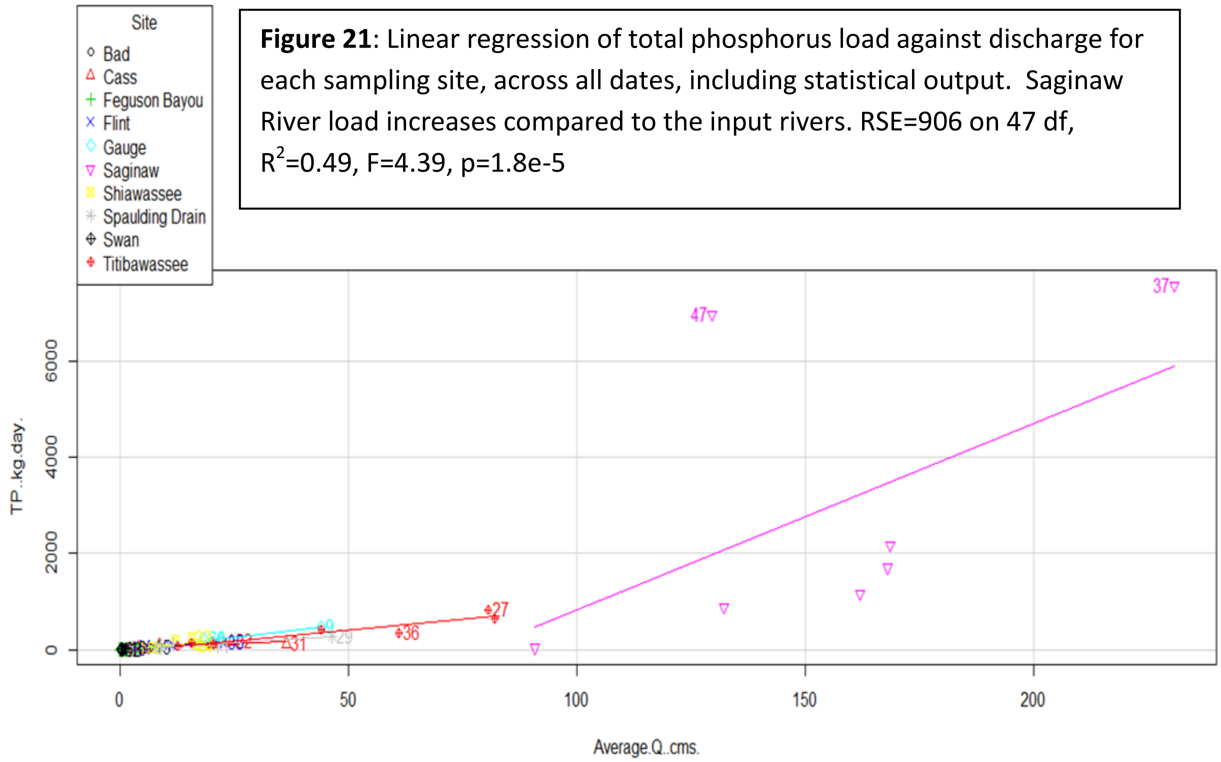
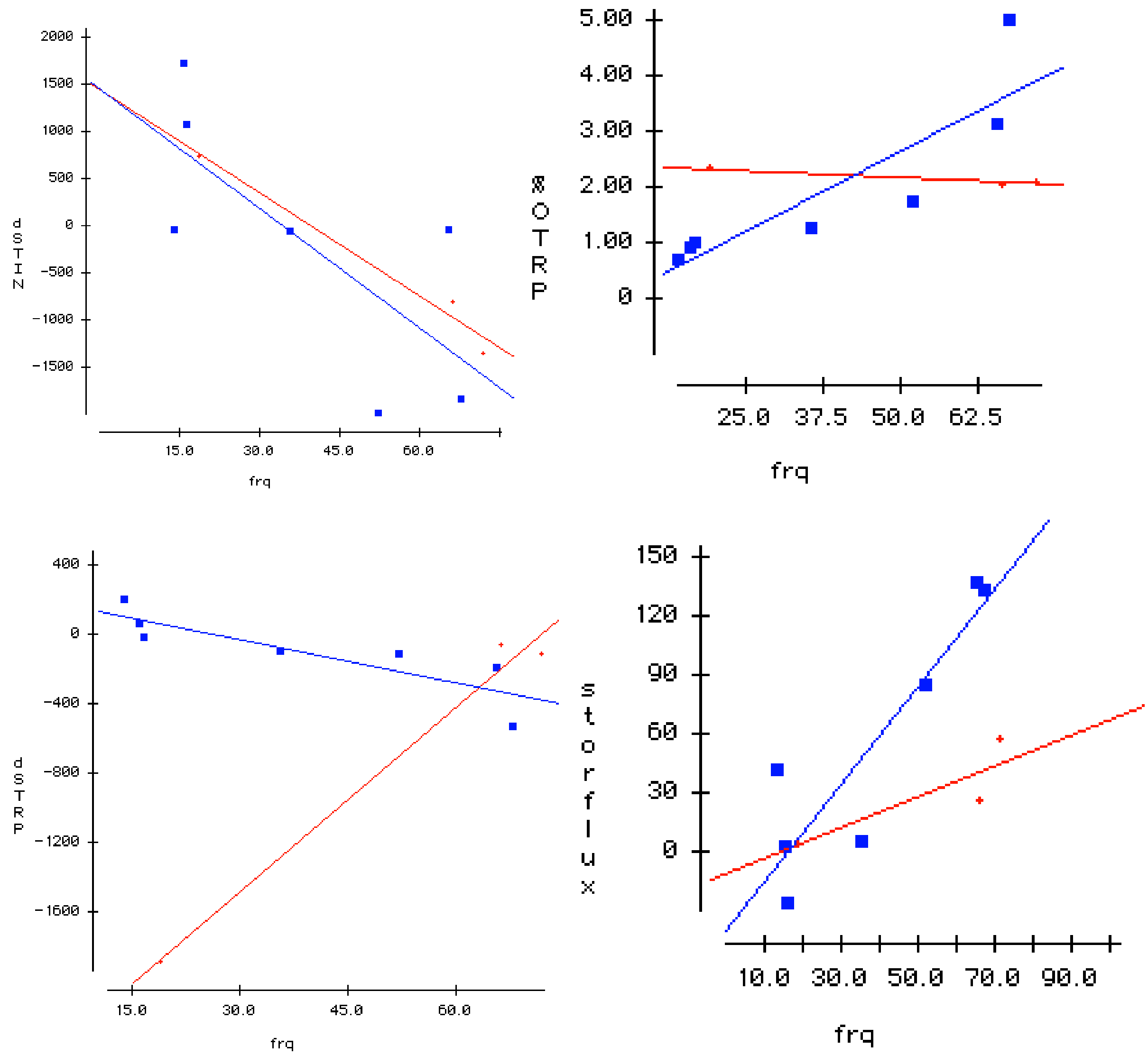


Figure 21: Linear regression of total phosphorus load against discharge for each sampling site, across all dates, including statistical output. Saginaw River load increases compared to the input rivers. RSE=906 on 47 df, R^2=0.49, F=4.39, p=1.8e-5

Figure 22: Storage flux for nitrogen, phosphorus and water, related to catchment-wide exceedance frequency. Red represents 2012 study period, Blue represents 2013 . Left: Change in storage TIN (kg/day) against catchment-wide exceedance frequency (R^2=61%, s=822 on 8 df). Right: Percent change in output TRP against catchment-wide exceedance frequency (R^2=45%, s=1 on 8 df). Below,left: Change in storage TRP (kg/day) against exceedance frequency (R^2=1.2%, s=631 on 8 df). Below, right: Water storage flux (cms) against exceedance frequency (R^2=51%, s=41.8 on 8 df).



concentrations were related to system-wide flow exceedance with strong differences related to the different years (Figure 22).

Discussion

Hydrology

The Shiawassee Flats area (including SNWR) is an extremely complicated hydrologic system, affected by lake levels, regional weather patterns, and river geomorphology. This is evidenced by the contrast between the hydrology results for 2012 and the results for 2013. In 2012, the Flats contributed to the flow of the Saginaw River only during low-flow, and inputs and outputs remained relatively balanced during high-flow. There was no significant flow reduction via water storage observed in 2012, while in 2013 I saw very high river levels and the most severe flooding events that have been noted in the Refuge within recent years.

The lowest exceedance frequency in 2012 was during the August 12 sampling event, while the lowest exceedance frequency occurred during the June 30 event in 2013. However, when looking at the range of exceedance frequency values for all dates, both the May 7 and June 5 events in 2013 appear to have lower exceedance frequencies across all rivers. These differences in exceedance frequencies demonstrate the extreme differences in hydrology between the two study periods. The higher exceedance frequencies of July 2012 indicate that low flows were occurring at this time, while the lower values in August reflect a relatively higher flow period following a summer rainstorm. In contrast, the 2013 study period was overall a much higher flow year with severe flooding occurring throughout the spring, starting in April. This is reflected in the low exceedance frequencies of the May 7 sampling event, the earliest time at which it was deemed safe to navigate the rivers. There was a large difference in flow between the 2012 and 2013 calendar years, with twice as much overall flow in 2013 (94,270 cms) than in 2012 (53,973 cms; Table 8). The 2012 study period saw prevailing drought conditions, with a rain event in August, while 2013 saw extreme flooding in April and early May, with significant water storage taking place at these times. Overall, flows were higher during the 2013 study period, and it is clear that 2012 saw extremely dry conditions and low flows overall, with no significant flooding of the wetlands on the other side of the dikes within the refuge. In 2013 significant flooding of the diked wetlands and floodplain area occurred. Levees were breached in multiple areas, flooding roads in the city of Saginaw and surrounding farm fields (see pictures, Figure 23), and the area proposed for future hydrologic reconnection actually flooded naturally at this time. For much of the 2013 study period flood waters were observed slowly being released back into the main channel system (June, July, September 2013). Hydrographs of the gaged rivers on and near the Refuge indicate that peak runoff values have increased in the last thirty years, and these hydrographs show larger peaks in the spring and early fall months in more recent years (Newman, 2011). This coincides with an evaluation of Great Lakes water levels, which shows that water inputs to Lake Huron and Lake Michigan are increasing in the fall and occurring earlier in the spring as precipitation patterns change (Argyilan and Forman 2003). These increasing fall water inputs could be a function of climate change, land use or water change (Newman, 2011).

The largest discharge I measured in the Saginaw River was on June 30, 2013 with a flow of 230 cms. The Tittabawassee, Spaulding Drain, previously stored water from the Flats area, and the Cass River contributed the bulk of the flow to the Saginaw River at this time, accounting for 26.4%, 20%, 18.5%, and 17.3%, respectively. Although this sampling event did follow local rainstorms, a large portion of the flow is accounted for by water actually leaving storage within the Flats and entering the Shiawassee/Saginaw channel. The next highest discharge throughout the two study periods occurred on August 12, 2012, with a flow of 219.5 cms on the Saginaw River. This sampling event caught the tail end of a local rain event that saw local flooding in what was otherwise considered a drought year, and the combined inputs to the Flats area made up essentially all (98.06%) of the flow of the Saginaw River and wetland storage made no real contribution. This indicates that immediately following this storm event, water was being flushed from the tributaries to the output of the Saginaw River relatively quickly, with little to no storage taking place. This is reasonable, since water levels in all of the rivers were extremely low due to drought conditions; most riparian wetlands were dry, and the water surface elevation low leaving little opportunity for water to enter storage at this time and there was nothing stopping this water from following its normal path downstream. The next highest total discharge occurred on June 5, 2013 with a flow of 161.96 cms in the Saginaw River. During this event, the Tittabawassee, the Cass, Spaulding Drain, and the Shiawassee River contributed the bulk of the total flow, accounting for 49.7%, 15%, 14%, and 10.3%, respectively. In this case, very little (2.11%) water is leaving storage from the Refuge and entering the Saginaw channel. This is similar to the case of the sampling event on May 19, 2013 during which only 6% of the flow was accounted for by water leaving storage.

On May 7, 2013 with a more moderate total flow in the Saginaw River of 132 cms, I found a significant amount of water being stored within the Refuge. During this sampling event, the Tittabawassee, Spaulding Drain, the Cass, and the Shiawassee accounted for 62%, 16%, 15%, and 13%, respectively, of the total flow in the Saginaw River. Water was stored in the Flats wetlands, removing 21% of the total flow of the Saginaw, meaning that the SNWR actually reduced the flow of the Saginaw River. A similar flow in the Saginaw River (129 cms) was recorded on the July 15, 2013 sampling event, but in this case previously stored water was entering the Saginaw channel and accounted for 66% of the total flow, more than any two tributaries combined. The July 28, 2013 sampling event resulted in a flow of 168.48 cms on the Saginaw River, with 137.29 cms (59.5% of the total output) coming from storage within the Flats area. The Tittabawassee River contributed the most to the flow, with a discharge of 12.605 cms. The September 2, 2013 results were very similar, with a discharge of 167.92 cms on the Saginaw River and 57.99% (133.79 cms) of this coming from storage within the Flats. Lower flows were noted in the Saginaw River during the July and November sampling events of the 2012 drought year (84

Figure 23 : Aerial photographs showing a) exposed mud Flats on the Shiawassee Flats during drought conditions of 2012 and b) extreme flooding within SNWR during spring 2013 (Source: Heitmeyer et al. 2013)

Table 8: Accumulated flow in cms for rivers with gage data for each calendar year of the study period, with overall sum of flow.

	2012	*2013*
Cass	30671.5	54967.2
Flint	5419.8	8760.9
Shiawassee	3053.7	4193.4
Tittabawassee	14828.2	26348.5
Sum	**53973**	**94270**

cms and 60 cms, respectively). During both of these sampling events, previously stored water from the Flats area contributed a significant amount of water to the flow of the Saginaw River; in July, previously stored water made up 69.17% (58.12 cms) of the output flow, while in November stored water made up 43.45% (26.25 cms) of the output flow. These numbers indicate that the Flats area can be extremely important to maintaining the baseflow of the Saginaw River, especially in drought years when the input rivers are contributing very little flow to the system.

It may seem strange that a more moderate flow event would be the only one observed to result in significant water storage, but background information on weather events and flow patterns surrounding this date can explain this phenomenon. The May 7, 2013 sampling event occurred in the middle of an extreme flooding event that started earlier in the month, and was caused by a combination of high rainfall and high river flows. This was the earliest date that sampling could be achieved due to extremely flooded and unsafe conditions. At this time, a significant amount of new flooding was occurring within the refuge, both because of the antecedent high water levels within the river system and already established flow connections to various SNWR wetland units due to high water surfaces and levee failures at the beginning of the season. There were clearly higher flows and even more storage occurring earlier that year, because a significant amount of water was already stored within the wetlands once my sampling began, but there was still room for more water. Even though there were higher flows later that spring and early summer, the storage areas within the wetlands were already full of water, and there had been no new breaching of the dikes within the Refuge and Flats area.

Are there other possible sources of flow besides wetland and channels storage in the Flats that may be unaccounted for in my analysis? Although it is possible that some of the unaccounted for flow in the Saginaw River may come from nearby urban drains or other runoff sources, the sampling site on the Saginaw River was located before the city of Saginaw comes in contact with the river. It is more likely that the majority of all non-tributary flow originates in the upstream Flats area, and represents some combination of channel, bank and off-channel storage contributions. These data indicate that the wetland systems within the SNWR contribute significantly to both the reduction of peak flows and the maintenance of low flow in the Saginaw River. These water balances indicate that the Flats area and the SNWR play an important role in shaping the hydrology of the Saginaw River throughout the year, and this influence varies in direction and magnitude seasonally. This is consistent with the findings of the Water Resource and Assessment Summary Report for the SNWR, which states that hydrographs reflect larger peaks in the spring and early fall months (Newman, 2011). This also suggests the Flats will be important as water inputs to Lake Huron are increasing in the fall and occurring earlier in the spring (Argyilan and Forman, 2003). Specifically, the Flats area acts as a significant storage area during spring flooding events, after which during drier and lower flow conditions the Flats appears to act as a source and contribute water to the flow of the Saginaw River.

Part of the significance of both the Flats' storage and contribution of water may be determined by lake levels within Lake Huron. When lake levels are lower in mid-summer (July) the Flats area may be an important contributor to the Saginaw River. However, when lake levels are higher, the Flats area may not make any significant contribution to the flow, and inputs and outputs to the Flats remain relatively balanced. These findings are consistent with a study on Great Lakes coastal wetlands, which suggested that hydrogeomorphic factors of wetlands are associated with seiches and seasonal lake level change (Keough, 1999). Fluctuations in lake levels can increase or decrease the velocity of lake-ward river flow by changing the base-level of the river (Keough, 1999). Thus, when levels in Lake Huron are low, the base-level of the Saginaw River is flow velocities increase and water will flow out of the wetlands, helping increase the overall flow. When lake levels are high, flow in the Saginaw will be stalled or even move backwards, resulting in water remaining in storage within the Flats area wetlands; or if upstream flows increase, the storage of the water in excess of the Saginaw's conveyance capacity.

During 2013 I did not sample during backwards flow on the Saginaw or Shiawassee Rivers, and found little correlation with Saginaw Bay lake levels for any of the hydrologic parameters measured on these rivers. On the other hand, in 2012 I did note several sampling events with flow reversals on these rivers, indicating that lake levels can have an impact on the system depending on the surrounding hydrologic conditions.

Water Quality

The linear regression of alkalinity against discharge by site (appendix 12) indicates that alkalinity decreased coming out of the Flats area. This is one indicator that water entering the Saginaw River from the Flats area looks more like wetland water than groundwater, because wetland water in this region tends to have lower alkalinities (Tompkins et al. 1997). Because the alkalinity is lower, this is also an indicator of storage of surface precipitation within the Flats area and release of it later after biological processing.

Some interesting trends were revealed in the linear regression plots of nutrient concentrations against discharge (Appendix 13). Total inorganic nitrogen concentration increased with increasing discharge, indicating that no significant amount of dilution was occurring in the case of inorganic nitrogen, or that inorganic nitrogen was being mobilized at times of higher discharge. This may also indicate that the major sources of inorganic nitrogen to the Saginaw River are non-point sources, which corresponds to the high amount of agricultural land cover surrounding the Refuge. Cohn et al. (1992) reported that positive values in this linear regression relationship suggest sediment-related, non-point sources. Flow-weighted mean concentration of total inorganic nitrogen inputs to the Flats area tended to exceed the concentration of the output from the Flats area (see Figure 13). Following significant high flow flood events, such as the May and June events in 2013, the difference in concentration between the inputs and the output appears to be greater. However, the role of the Flats area in terms of nitrogen is not as clear for the rest of the study period.

Although at most times it appears that inorganic nitrogen inputs are greater than the output from the Flats, and there is a statistically significant difference between the input and output concentrations ($t=-2.907$, $df=9$, $p=0.0174$), there are also sampling dates when the inputs and outputs appear to be balanced (August 2012, May 19, 2013 and July 15, 2013, Figure 13). These dates indicate that, at this time, the Flats area is acting as neither a source nor a sink for inorganic nitrogen and instead just releases to the Saginaw River exactly what is put into it. There was no point at which the Flats area acted as a significant additional source of inorganic nitrogen (in terms of concentration) to the Saginaw River. The linear regression of total inorganic nitrogen concentration against discharge by site indicated that the output (the Saginaw River) had a lower slope value than the input rivers, meaning that the Flats area contributed to a significant decrement in total inorganic nitrogen concentration (appendix 14). Overall, this indicates that the Flats area acted as a sink for inorganic nitrogen in terms of concentration during the study period, or significantly contributed storage water in enough quantity to dilute the concentration in the Saginaw River.

It is also clear that there were different sources of nitrogen between the 2012 and 2013 study periods; in 2012 the source of nitrogen was mostly from the watersheds, as evidenced by the higher inorganic nitrogen concentrations and lower reactive and total phosphorus concentrations during this time. This is evidenced in the plots of both inorganic nitrogen and reactive phosphorus flux against catchment-wide exceedance frequency grouped by study year; the lines for the study years have different slope values, indicating differences in the way nutrients were entering the system (Figure 22). However, inorganic nitrogen concentrations were much lower during the 2013 study period, while reactive and total phosphorus concentrations tended to be higher, indicating the source of water in the

Saginaw River at this time was likely from wetlands. Lower concentrations of oxidized forms of nitrogen and higher orthophosphorus concentrations are a common signature of reducing chemical conditions, typically associated with wetlands in this catchment (Tompkins et al. 1997). These conclusions make sense in terms of the two years based on what was happening in terms of hydrology. The 2012 study period was overall a low flow year with little water going entering storage within the Flats area, so there was little water available to come out of storage from within the Flats area and little biologically processed water available to the Saginaw River. However, the 2013 study period saw a significant amount of water enter storage in the Flats area after a large flooding event before the May 7 sampling event, with smaller high flow events following. The rest of the 2013 study period saw various amounts of this stored water augmenting the flow of the Saginaw River, and, thus, biologically processed wetland water entering the Saginaw River. Inorganic forms of these nutrients would have shifted to organic forms after biological processing within the wetlands, resulting in the observed chemical signatures.

Flow-weighted mean concentration of total reactive phosphorus output from the Flats area exceeded the inputs concentration during several sampling events, but overall input and output concentrations were relatively similar (Figure 14). Reactive phosphorus output concentration in the Saginaw River exceeded the input to the Flats during the 2012 summer high flow event (the large difference between the inputs and outputs explained previously) and during the spring high flow events. These events indicate that the Flats area was acting as a source of reactive phosphorus to the Saginaw River in terms of concentration. However, during the summer low flow events reactive phosphorus input concentrations exceeded that of the output, indicating that the Flats area was acting as a sink for reactive phosphorus, or enough storage water was contributed to dilute the concentration. During the remaining sampling dates inputs and outputs were relatively balanced. It is also important to note that there was no statistically significant difference between the input and output concentrations of reactive phosphorus. The linear regression of total reactive phosphorus concentration against discharge by site (appendix 15) indicates that the output (the Saginaw River) has a lower slope value than the input rivers, meaning that the Flats area contributed to a significant decrement in reactive phosphorus concentration in the Saginaw River. Overall, this indicates that the Flats area acted as a sink for reactive phosphorus, or contributed enough storage water to dilute the concentration in the Saginaw River.

It is important to note that the August 2012 date greatly affects the interpretation of my data (i.e. has high leverage). It is suspicious in terms of being extremely high in both inorganic nitrogen and reactive phosphorus. If more data points were available, I would have removed it; however, 2012 was a unique year with drought conditions, and the August date did see a high flow rain event, so it was hard to justify removing the date because it is possible that abnormally high loads were generated during this event. However, without this point there would have been an overall decrease in mean reactive phosphorus concentration in the output in the Saginaw River, and a greater decrease in nitrogen.

Overall, total phosphorus concentrations increased in the output of the Saginaw River, most likely due to organic activity. Flow-weighted mean concentration of total phosphorus inputs remained relatively constant throughout the 2013 study period, while the output concentrations peaked at the July 15, 2013 sampling event (Figure 15). Total phosphorus input concentrations clearly exceeded output concentrations during the May 7 and May 19 (high flow) sampling events, indicating that the Flats area was acting as a sink for total phosphorus at this time. Total phosphorus output concentrations exceeded input concentrations during the June 30 (high flow), July 15 (low flow), and July 28 (low flow) sampling events, indicating that the Flats area was acting as a source of total phosphorus in terms of concentration during these times. There was a much larger gap between the inputs and outputs when the Flats area appeared to be acting as a source of total phosphorus, indicating that overall the Flats is a more significant source of total phosphorus than it is a sink. Inputs and outputs remained relatively balanced in terms of total phosphorus during the June 5 and September 2 events, indicating that no significant phosphorus storage or addition was taking place at these times. The linear regression of total phosphorus concentration against discharge by site (appendix 16) indicates that the output (the Saginaw River) has a higher slope value than the input rivers, meaning that the Flats area contributed to a significant increase in total phosphorus concentration. This indicates that the Flats area acted as a source of total phosphorus to the Saginaw River. However, it is important to note that total phosphorus includes biologically particulate things, and this increase in concentration could really be representing an increase in biological productivity in the form of algae or other organisms that naturally accompanies the change in seasons.

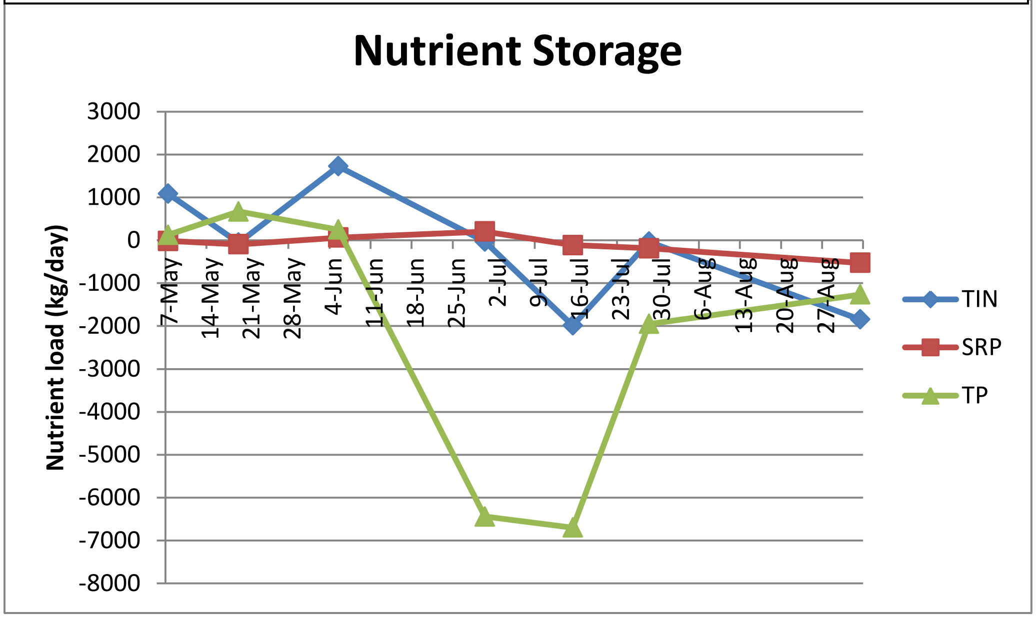
Nutrient Load Processing

Loads unsurprisingly were generally related to flows, and different rivers varied in the relative contributions on different dates depending on specifics of antecedent hydrologic events. I found that at high flows (low exceedance frequencies) nitrogen, phosphorus, and water were stored in the Flats (i.e. change in storage estimates were positive) but at lower flows (higher exceedance frequencies) nitrogen, phosphorus, and water were released from storage and contributed to output loads in the Saginaw River (Figure 22). Loads to the Saginaw River were reduced in the spring by storage within the Flats, corresponding to low exceedance frequencies (high flows); conversely, loads to the Saginaw River increased in the summer due to contribution from the Flats, corresponding to high exceedance frequencies (low flows). Because higher flows tend to occur earlier in the spring, with lower flows more evident in the summer, this resulted in a seasonal pattern of nutrient/water storage and release. The hydrologic and nutrient loading results of this study indicate that the function of wetlands associated with the Flats area within the SNWR can vary greatly throughout the year. In the early spring, during high flow and flooding events, these wetlands can act as a storage area for water, removing water and dissolved materials from the flow of the Saginaw River. This is supported by previous studies that found that wetlands both play a role in flow stability by reducing peak flows (Novitzki 1981) and slowly release stored water during dry periods of the year (Hammer 1992). At this same time, these wetlands also act as a significant sink for both reactive phosphorus and inorganic nitrogen. However, as the season changes and lower flows occur, these wetlands often act as a source of flow to the Saginaw River, and also a source of both reactive phosphorus and inorganic nitrogen. Although numerous studies have concluded on the nutrient storage capabilities of wetlands, there have been many studies that question the ability of some wetlands to serve as both nutrient sinks and sediment traps (Kraus 1987; Richardson 1989), and these reflect some of the findings of this study. Richardson et al. (1978) found that, under certain conditions, nutrients are rapidly lost from wetland ecosystems, showing that they are ineffective as nutrient sinks. The seasonality of nutrient storage, especially that of total phosphorus, reflects the findings of Devito et al. (1991). They found total phosphorus export was highest in the spring when discharge was highest, and total phosphorus retention was highest in the mid to late summer when discharge was lowest (Devito et al. 1991). My data suggests there is no single characterization of the Shiawassee Flats wetlands as either a source or sink throughout the entire year, and instead the characterization must be based upon river flow and seasonality.

The May 7, 2013 event, during which more inorganic nitrogen entered the Flats than left in the Saginaw River, indicates that the Flats area and the Refuge as a whole can act as an important sink for nitrogen (Figure 24). This event corresponds to higher flows and more water entering storage, which reflects the fact that the wetlands within the Refuge sequester inorganic nitrogen after flooding and rain events. However, the June 5th event also showed nitrogen being absorbed by the Refuge (Figure 24), although no water was actually entering storage in the Refuge. Water input to the Flats was almost equal to water output to the Saginaw River, and this indicates that the Flats area was sequestering nitrogen without actually removing water from the system. However, the July 15, 2013 event (and July and November, 2012 events) also indicates that the Refuge can act as a significant source of inorganic nitrogen to the Saginaw River (Figure 24), and this corresponds to water leaving storage at this time. The contribution of nitrogen from the Refuge may also correspond to seasonal changes in nitrogen content within wetlands, along with changing agricultural practices throughout the year. Biological denitrificaton that occurs within wetlands is most likely responsible for the inorganic nitrogen removed from the system during the May 7 and June 5 events of 2013 (Hien et al. 2011). Following flooding events, anaerobic conditions increase and provide more opportunity for nitrates to experience denitrification. This denitrification allows the nitrates, present in inorganic nitrogen, to be reduced to gaseous nitrogen. The above results reflect this idea of denitrification following flooding events, because the largest amount of inorganic nitrogen was removed from the Saginaw River following a major flooding event (the May 7, 2013 event) with high flow.

Sediments, such as those within the Flats area and surrounding wetlands, can act as phosphorus reservoirs and contribute reactive phosphorus to overlying waters (Mayer et al. 2006). This corresponds to the May 7, May 19, July 15, July 28, and September 2 events of 2013, and all of the 2012 events, during which the Flats area contributed reactive phosphorus to the Saginaw River (Figure 24). Anaerobic wetland conditions following large rain events can lead to reduction of sediments, and result in increases in dissolved phosphorus in freshwater systems (Patrick and Khalid, 1974). Because the 2012 events, May 19 and July 15 (2013) events saw the Flats contributing phosphorus to the Saginaw River (Figure 24), it is likely that the sediments of the Flats contributed phosphorus to the water running over them. Also, most of these events saw water leaving storage, meaning that the phosphorus in contact with this stored water also was added to the Saginaw River. It is also likely that the August 2012, May 7th and June 30th events, all of which occurred during high flow events following rain events, contributed more reactive phosphorus partly because there was just more water going through the system, which corresponds with a wetland study that found that phosphorus concentrations were more closely related to precipitation events and river flow than seasonal patterns (Nairn and Mitsch, 2000). Sedimentation is

Figure 24: Nutrient load storage for 2013 study period; negative load signifies coming out of storage and into Saginaw River



an important mechanism for phosphorus storage in wetlands (Fennessy et al. 1994), and decreases in reactive phosphorus in wetlands are usually related to biological activity (Nairn and Mitsch, 2000). Wetlands have been proven to effectively remove phosphorus, but it has also been shown that retention is dependent on hydraulic loading rates (Chescheir et al., 1992). Mitsch et al. (1995) determined that phosphorus retention increases with loading. It is most likely that the Flats area acts as a sink for reactive phosphorus during high flow flooding events, when water is entering the wetlands, because the phosphorus loading accompanying the entering water is taken up by plants and other biological activity within the wetlands. However, during low flow when water leaves storage within the wetlands, the water entering the Saginaw River appears to have high concentrations of reactive phosphorus. This indicates that, although the Flats area successfully sequesters phosphorus during flooding events, this initial uptake of reactive phosphorus by biological activity reaches a maximum. At this point, the plants within the wetlands cannot take up any more reactive phosphorus, and the water within the wetlands accumulates this reactive phosphorus, and releases it with water to the Saginaw River during low flow.

Mass Balance

2012 and 2013 were extremely different in terms of water balance and nutrient loading for the Saginaw system. The 2012 study year was a drought year, with very little rain except for sporadic storms, and typically very low flows. On the other hand, the 2013 study year saw extreme spring flooding right before the first May sampling event, overflowing the dikes within the Refuge and flooding many farms and streets in the area. This is an important contrast because the 2013 study year saw a huge amount of water enter storage early in the spring, along with accompanying nutrients, and then the slow release of this water was also in evidence throughout the study. This contrast can be seen in the water and nutrient loads that came out of storage in 2013, compared to those of 2012 (Table 9). It is also important to note that 2012 was only sampled three times from July 19 to November 11, while the 2013 study year included seven samples from May 7 to September 2, and thus the time frames do not match up exactly.   In both years, however, there was evidence of reactive phosphorus coming out of storage from within the Refuge; in 2012 the average daily load of reactive phosphorus out of the Flats was 547 kg/day, while in 2013 it was 101 kg/day (Table9). Similar results were in evidence for inorganic nitrogen load coming out of the Flats (1131 kg/day 2012, 161 kg/day 2013; Table 9). These are estimates based only for the dates sampled in each year (July-November 2012, May-September 2013) and are extremely conservative estimates of the water and nutrient fluxes occurring within the Flats, as evidenced by an extrapolation based on the accumulated flows presented in Table 8. But we know from USGS gaging data that just the four gaged systems alone actually delivered almost twice the volume that I estimated in Table 9 (both years).

Reactive phosphorus is of particular interest in the case of the Saginaw Bay Watershed because of phosphorus being the primary limiting nutrient in freshwater, and the presence of harmful algal blooms in Saginaw River and Bay downstream of the Refuge. In the case of reactive phosphorus, although the Flats area wetlands appear to act as a source during my study days, closer examination of the hydrological differences between the two years shows something quite different. In 2013, natural flooding of diked floodplain area occurred, and six times the amount of water that came out of storage in 2012 came out of storage in 2013 later in the season and entered the Saginaw River. Yet, only 11,900 kg of reactive phosphorus entered the Saginaw River in 2013, compared to 62,368 kg in 2012. Despite the fact that a significantly larger amount of water came out of storage and into the Saginaw in River in 2013, a much lower amount of reactive phosphorus accompanied this water to the Saginaw River. This is evidence that the reactive phosphorus went into storage within the wetlands in the inundated floodplain at the time of flooding, and phosphorus that otherwise would have immediately been transported downstream was prevented from reaching Saginaw Bay all at one time. Although the reactive phosphorus was still released from these wetlands later in the season, the effect was not as noticeable as a one-time transport of such a large amount could have been, as would have been the case in 2012. The case with inorganic nitrogen does not seem as straightforward, although it appears that in drought years (like that of 2012) the Flats area may act as a sink for inorganic nitrogen, while in flood years (like that of 2013) the Flats area may act as a source of inorganic nitrogen. It may be important to note that a large portion of the flooded land was agricultural in nature, and could have associated nitrogen in the soils, accounting for the high amount of nitrogen contributed to the Saginaw River in 2013.

Overall Role of Flats Area Wetlands and Floodplain

The hydrologic input-output analysis for the Flats indicates that stored water is a huge component of flow coming out of the Flats area wetlands, with significant storage occurring in the spring and stored water coming out of these wetlands during low flow periods (i.e. high exceedence flows) later in the year. Nitrogen concentrations (and phosphorus concentrations during low flows) were generally lowered in the output (and therefore in the Saginaw River), and an important question surrounding these lower concentrations is whether they are lower because the Flats wetlands were sequestering nutrients, or are the concentrations lowered because the water from these wetlands is diluting these loads? It can be extremely difficult to determine which of these processes is occurring, and it is more likely that a combination of both is occurring in these wetlands. Such a combination of dilution and nutrient sequestration was documented in a wetland study on the upper Tittabawassee (Tompkins et al. 1997).

Because this study did not have continuous monitoring of storm events and ignored transience, it is possible that sampling at downstream locations could have on each sample date been on the rising end of events, while upstream sampling could have been on the falling end of events. This could be responsible for the reduced concentration effect evident throughout this study, although the hydrology suggests that the wetlands are truly responsible for the concentration reductions. There were multiple cases when there was no recent storm event, during very dry conditions, that huge storage flux events took place (for example, July 15, 2013 66% of flow of Saginaw was coming from storage, despite dry summer conditions, sum of inputs 43 cms).

In terms of inorganic nitrogen, the overall role of the Flats area wetlands and floodplain over the combined 2012 and 2013 study years was to provide a minor decrement in load and a major decrement in concentration in the Saginaw River. Generally, there was an inorganic nitrogen load decrease when storage water from the Flats was added to the Saginaw River. In terms of total reactive phosphorus, the Flats area contributed to a reactive phosphorus load increase in the Saginaw River, and also a reactive phosphorus concentration increase although total reactive phosphorus increments were much smaller than total phosphorus increments. When storage water supplemented the flow of the Saginaw River, there was generally enough accompanying reactive phosphorus load to increase the total output load. Similarly, the Flats area appears to contribute total phosphorus loads to the Saginaw River, most significantly after flooding events following dry periods. Both of the inorganic nitrogen decrements (load and concentration) are indicators of wetland water, as wetland water tends to be lower in nitrogen concentration due to the prevalence of reducing conditions. Increases in reactive phosphorus and decreases in inorganic nitrogen seem to be a common indicator of wetland sources water in this region (Tompkins et al.1997) . These nitrogen decrements in the Saginaw can clearly be seen in the plots of nitrogen loads and concentrations in the individual rivers against discharge, and by comparing the slope of the Saginaw to the input rivers (Figure 19; appendix 14) in the load versus flow plot. The reverse can be seen in the plots of reactive phosphorus load and concentration against discharge (Figure 20; appendix 15).

Implications for Restoration

The results of this study are relevant to future restoration projects within the SNWR. At the highest flows observed during this study, the Flats area was capable of storing 20.68% of the total flow of the Saginaw River (removing 27.32 cms from the output flow). During a flood time, this provides critical storage of water that might otherwise cause significant damage to nearby residential or agricultural areas. More importantly, these wetlands can act as both a source and sink with regards to total reactive phosphorus. During the June 5 and June 30 events of 2013 the Flats area acted as a sink for reactive phosphorus, removing a combined total of 34% of the reactive phosphorus inputs. However, the only significant amount of storage of reactive phosphorus occurred on the June 30, 2013 event, during which a total of 29% of the input reactive phosphorus was stored within the wetlands. In contrast, the Flats area acted as an additional source of reactive phosphorus to the Saginaw River throughout the rest of the study period, although with dilution concentrations in the Saginaw declined.

It is important to note that the observed phenomenon involving reactive phosphorus and inorganic nitrogen may not reflect the eventual results of any restored wetlands within the refuge. The Flats area wetlands that were involved in this study have been associated with the river system for a considerable amount of time. It is possible that these wetland soils are saturated with both phosphorus and inorganic nitrogen, and might help explain why so much of both these nutrients are being released with the water from these wetlands. Similarly, these wetlands may have played an important role in nutrient removal in the past, before reaching saturation point. Because the future restoration project within the Refuge seeks to reconnect floodplain to the river system, it is possible that these future wetlands could sequester a considerable amount of nitrogen and phosphorus. It is also important to note that a portion of the proposed restoration site was previously agricultural land, and it is possible that these areas are already saturated with nitrogen and phosphorus from fertilizer applications. There is the potential for restoration to actually result in the contribution of more of these nutrients to downstream waters once the saturated sediments are in contact with river water.

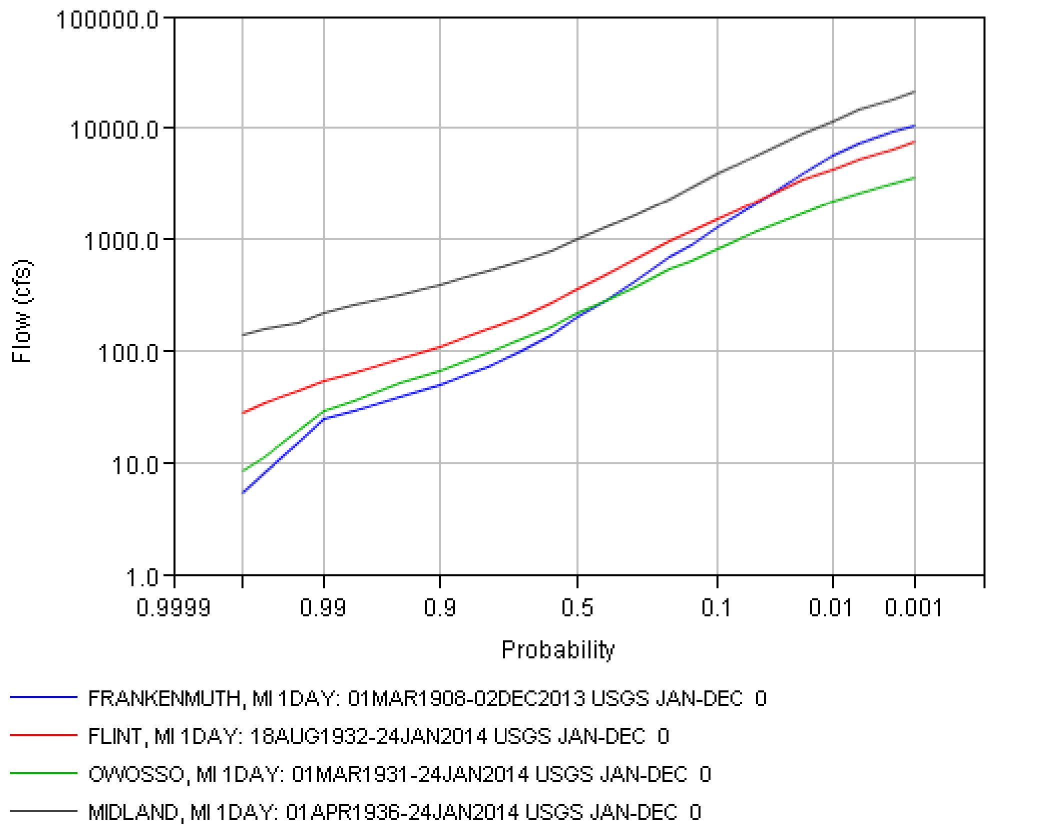
Table 9: Water and nutrient masses for 2012 and 2013 sampling periods, and average daily loads over sampling periods including percent change.

2012	n	Water (cubic meters)	Water (kg)	TIN (kg)	TRP (kg)	
Sum Inputs	3	1.24E+09	1.23673E+12	880160.2	84117.95	
Saginaw (output)	3	1.41E+09	1.41348E+12	1009166	146486	
Storage	3	-1.8E+08	-1.7675E+11	-129006	-62368	
Percent Change		-14.2921	-14.29207	-14.6571	-74.1435	
Average Daily Load (unit/day)		-1.55E+06	-1.55E+09	-1131.64	-547.088	
2013						
Sum Inputs	9	9.8E+08	9.79826E+11	340287.5	58549.19	85245.42
Saginaw (output)	9	1.66E+09	1.65559E+12	359374.8	70487.95	365289.2
Storage	9	-6.8E+08	-6.7576E+11	-19087.4	-11938.7	-280044
Percent Change		-68.9677	-68.9677237	-5.60918	-20.3909	-328.515
Average Daily Load (unit/day)		-5.73E+06	-5.73E+09	-161.757	-101.175	-2373.25

Case studies in Maryland, Illinois, and Iowa found that wetlands can remove up to 68% of nitrate-nitrogen from agricultural drainage waters (Woltemade, 2000). Similarly, a modeling study of Great Lakes wetlands found that the restoration of 31.2 square kilometers of wetlands along Saginaw Bay would retain 53% of the phosphorus flow from upstream (Mitsch and Wang, 2000). Using these numbers and the plan to restore 2,260 acres (9.1459 square kilometers) of wetland in the Refuge, there is the potential to remove 15.54% of the phosphorus flow from upstream. During the highest inputs of reactive and total phosphorus of this study (1372.12 and 1404.5 kg/day, respectively), this would result in the removal of 213.23 kg/day of reactive phosphorus and 218.26 kg/day of total phosphorus. Using the above study's value of 68% nitrate-nitrogen removal, during the highest input of inorganic nitrogen (6729 kg/day) this would result in the removal of 4575.72 kg/day of inorganic nitrogen. These results show that there is great potential for future restoration projects to have significant impacts on downstream water quality, potentially reducing the eutrophication of Saginaw Bay.

Wetlands can provide a number of crucial services in the Great Lakes region, especially in areas experiencing issues with eutrophication and algal blooms, such as Saginaw Bay. Phosphorus loading to the Bay can be a major problem, causing blooms of toxic *Microcystis* during the warmer months, and it is tempting to look at wetland restoration projects strictly in terms of the amount of phosphorus load they could potentially remove from the Saginaw River. However, this can be an extremely difficult to quantify, especially in such a highly agricultural area where farming practices can vary so greatly from year to year and season to season. Recently, spring discharge has been reported as the main driver of these algal blooms (Stumpf et al. 2012), and this study exemplifies how variable discharge can be between just two years. Instead of looking strictly at phosphorus loads removed by these wetlands, it is important to look at the biological processes taking place within the wetlands and the time delay that is occurring with the nutrients. When the severe flooding events occurred during this study, large amounts of phosphorus and nitrogen were being flushed into the Flats area. But when large amounts of this water entered storage within the Flats wetlands, it was prevented from reaching the Saginaw River all at one time, and this means that not all of these nutrients were reaching Saginaw Bay at the same time. Even though these nutrients, or at least the phosphorus, were inevitably slowly released over the summer, loads were not all reaching the Saginaw River during spring flooding events. Similarly, a large proportion of the reactive phosphorus, the more bioavailable portion of phosphorus, was being transformed to total phosphorus within the wetlands and then similarly released. When this total phosphorus is then released into the Saginaw River, then it is at least not so readily available for use by algae in the shallow waters of Saginaw Bay. So, although the Flats area cannot necessarily be characterized as a sink for phosphorus throughout the year, it is performing an extremely necessary service when it comes to delaying and transforming flooding events and nutrients.

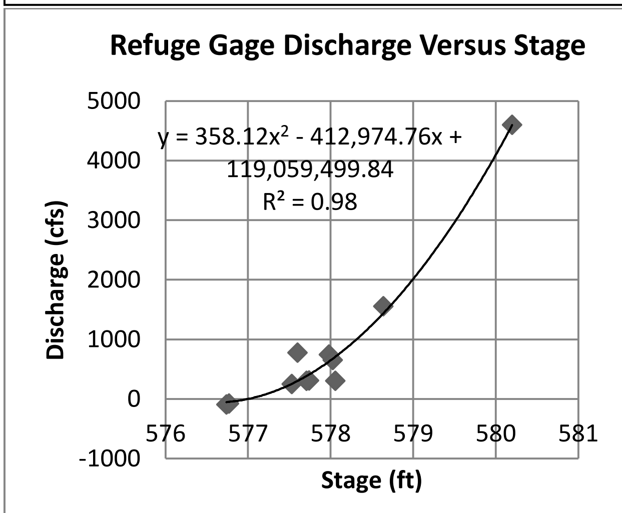
Appendix 1 – Duration analysis for rivers with active gaging. Log flow in cfs. Frankenmuth represents the Cass River, Owosso the Shiawassee, Midland the Tittabawassee, and Flint the Flint River.

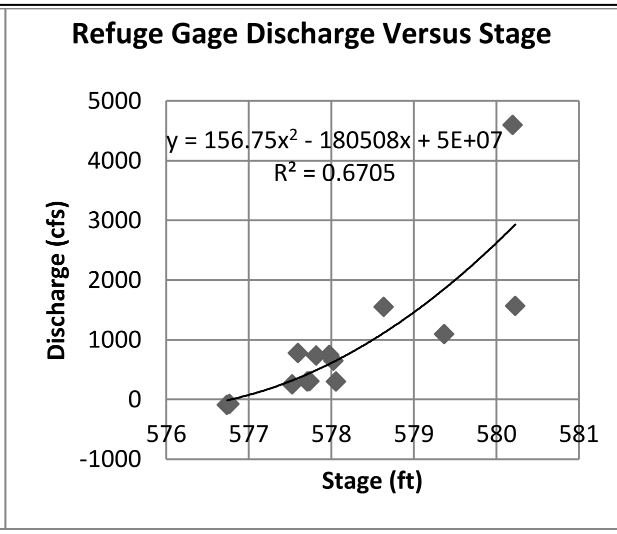


Appendix 2- Gage Calibration

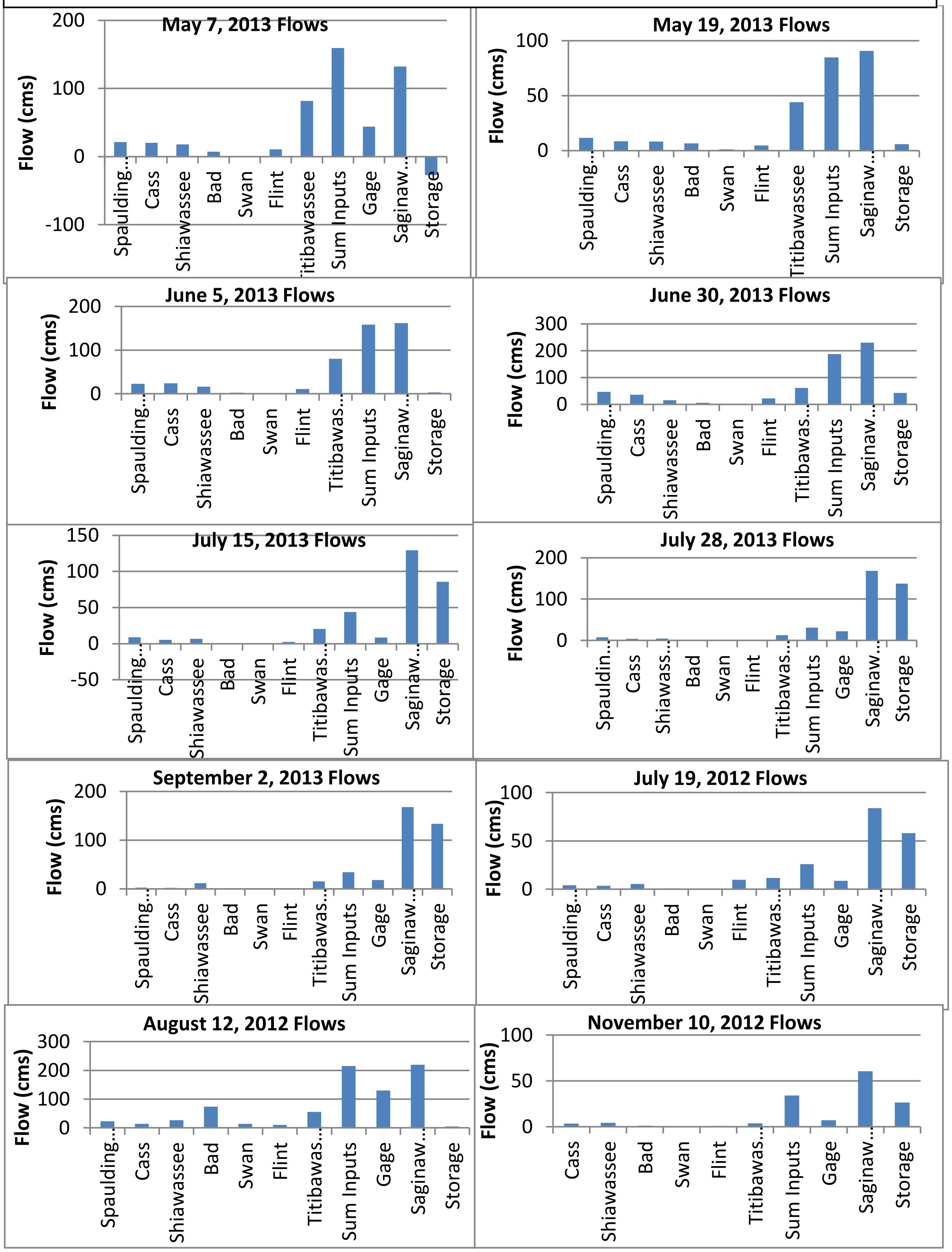
There is a definite predictive relationship between the stage at the gage on the Shiawassee River within the Refuge and discharge at this location. The Refuge gage stage and discharge measurements are summarized in Table 4. This relationship can be seen in the below Figures. As stage increases, river discharge also increases, although not linearly. Including only direct measurements of discharge taken at the gage location in 2012 and 2013, the equation to predict discharge (in cfs) from stage (in feet) is $y=358.12x^2-412975x+1E08$ ($R^2=0.9751$, left Figure). Taking into account all collected data throughout 2012 and 2013, including both direct measurements at the gage using the adp and estimated measurements by combining the discharge measurements taken at Swan Creek and the Flint, Bad, and Shiawassee River, the equation to predict discharge in cfs from stage in feet is $y = 156.75x^2-180508x+5E07$ ($R^2=0.6705$, right Figure).

Calibration curve for Shiawassee refuge gage: discharge against stage including 2012 and 2013 directly measured discharges (left) and including estimated discharges from combined input flows (right).





Appendix 3: Flows for each sampling site, grouped by date, and including the sum of inputs and storage. Negative values indicate water going into storage.



Appendix 4: Temperature, conductivity, turbidity, flow and alkalinity measurements for 2012 and 2013 study periods.

Site	Date	Temp C	Cond. (µS)	Turbidity (NTU)	Alkalinity (mg/L CaCO3)	Q (cms)
Bad	7/19/12	27	553	15	207	0.875
Flint	7/19/12	27	811	30	193	3.457
Spaulding Drain	7/19/12	25	829	12	NA	0
Gage	7/19/12	-	-	-	203	4.127
Cass	7/20/12	26	683	12	190	84.024
Saginaw	7/20/12	27	692	16	139	5.477
Shiawassee	7/20/12	27	802	4	116	8.49
Swan	7/20/12	27	977	35	177	0.298
Tittabawassee	7/20/12	26	517	6	157	11.67
Bad	8/12/12	18	153	15	68	73.906
Spaulding Drain	8/12/12	22	491	11	115	13.542
Shiawassee	8/12/12	19	399	13	88	9.769
Swan	8/12/12	19	319	32	562	22.602
Cass	8/13/12	21	483	12	579	219.5
Flint	8/13/12	20	348	22	99	26.304
Saginaw	8/13/12	20	385	27	-	130
Gage	8/13/12	-	-	-	105	13.323
Tittabawassee	8/13/12	21	538	10	134	55.799
Bad	11/10/12	6	477	3	68	1.109
Flint	11/10/12	7	667	6	875	3.385
Shiawassee	11/10/12	7	497	3	134	0.776

Site	Date	Temp C	Cond. (µS)	Turbidity (NTU)	Alkalinity (mg/L CaCO3)	Q (cms)
Gage	11/10/12	-	-	7	116	14.187
Swan	11/10/12	7	547	11	144	60.427
Cass	11/11/12	8	519	7	168	4.069
Spaulding Drain	11/11/12	9	472	6	159	7.079
Saginaw	11/11/12	8	490	16	124	0.534
Tittabawassee	11/11/12	10	606	3	169	3.566
Spaulding Drain	5/7/13	20	583	7.36	196	21.334
Ferguson Bayou	5/7/13	20.2	472.8	5.05	149	-0.196
Cass	5/7/13	20	641	7.53	148	20.086
Shiawassee	5/7/13	18.4	563	8.25	144	17.835
Bad	5/7/13	18.3	494.2	11.5	150	7.195
Swan	5/7/13	19.9	548	10.86	147	0.296
Flint	5/7/13	20.5	555	6.87	116	10.718
Tittabawassee	5/7/13	19.3	457.8	5.15	102	81.951
Gage	5/7/13	18.7	557	7.95	141	43.925
Saginaw	5/7/13	20.5	608	2.57	153	132.093
Spaulding Drain	5/19/13	21.4	686	8.51	216	11.593
Ferguson Bayou	5/19/13	24.8	688	10.56	260	-0.034
Cass	5/19/13	20.3	661	11.3	236	8.596
Shiawassee	5/19/13	19.5	620	4.56	230	8.232
Bad	5/19/13	16.8	575	34.1	188	6.540
Swan	5/19/13	22.5	630	12.2	218	1.121
Flint	5/19/13	20.6	648	26.6	226	4.843
Tittabawassee	5/19/13	18.5	584	7.28	168	44.001

Site	Date	Temp C	Cond. (µS)	Turbidity (NTU)	Alkalinity (mg/L CaCO3)	Q (cms)
Saginaw	5/19/13	19.8	675	37.8	234	90.776
Spaulding Drain	6/5/13	19.8	654	22.9	135	22.711
Ferguson Bayou	6/5/13	20.1	663	24.1	138	0.045
Cass	6/5/13	19.4	553	17.4	145	24.416
Shiawassee	6/5/13	19.2	667	21.4	158	16.622
Bad	6/5/13	16.2	448	14.1	148	2.886
Swan	6/5/13	16.4	545	14.2	153	0.141
Flint	6/5/13	20.1	660	20.6	168	11.242
Tittabawassee	6/5/13	16.2	475	10.38	140	80.478
Saginaw	6/5/13	19.7	585	14.3	157	161.96
Spaulding Drain	6/30/13	20.2	626	20.5	109	46.33
Ferguson Bayou	6/30/13	20.7	631	21.8	131	0.0023
Cass	6/30/13	23.2	540	13.2	159	36.296
Shiawassee	6/30/13	21.4	607	29.3	121	15.545
Bad	6/30/13	19.7	499	11.6	129	5.745
Swan	6/30/13	19.3	712	27.8	135	0.431
Flint	6/30/13	21.1	631	18.3	116	22.595
Tittabawassee	6/30/13	22.7	507	13	81	60.979
Saginaw	6/30/13	22.7	556	22.3	91	230.733
Spaulding Drain	7/15/13	30.1	611	31.5	118	8.835
Ferguson Bayou	7/15/13	30.3	617	32.8	109	0.0023
Cass	7/15/13	28.2	569	24.2	79	5.210
Shiawassee	7/15/13	29.5	683	40.3	117	6.8

Site	Date	Temp C	Cond. (µS)	Turbidity (NTU)	Alkalinity (mg/L CaCO3)	Q (cms)
Bad	7/15/13	25.3	670	22.6	59	-0.005
Swan	7/15/13	26.1	601	38.8	127	0.213
Flint	7/15/13	29.8	721	29.3	97	2.350
Tittabawassee	7/15/13	27.5	953	24	94	20.385
Gage	7/15/13	30.1	687	33.3	117	8.495
Saginaw	7/15/13	28.2	667	42.5	93	129.51
Spaulding Drain	7/28/13	25.1	631	33.8	123	7.626
Ferguson Bayou	7/28/13	24.3	630	35.1	117	0.003
Cass	7/28/13	22.2	590	26.5	91	3.992
Shiawassee	7/28/13	24.5	694	42.6	131	4.644
Bad	7/28/13	20.7	718	24.9	72	1.505
Swan	7/28/13	21.8	622	41.1	138	0.247
Flint	7/28/13	23.6	729	31.6	108	0.569
Tittabawassee	7/28/13	21.5	931	26.3	111	12.605
Gage	7/28/13	22.4	667	35.6	133	21.964
Saginaw	7/28/13	23.9	675	43.7	107	168.48
Spaulding Drain	9/2/13	24.8	622	34.7	121	2.665
Ferguson Bayou	9/2/13	23.9	615	34.9	121	0.0002
Cass	9/2/13	22.1	438	25.4	94	2.152
Shiawassee	9/2/13	24.3	493	43.1	128	11.950
Bad	9/2/13	21.6	510	23.7	77	1.113
Swan	9/2/13	21.9	497	38.8	134	0.253
Flint	9/2/13	23.2	612	28.4	112	0.421
Tittabawassee	9/2/13	21.3	886	25.6	116	15.574

Site	Date	Temp C	Cond. (µS)	Turbidity (NTU)	Alkalinity (mg/L CaCO3)	Q (cms)
Gage	9/2/13	22.3	517	35.4	129	18.373
Saginaw	9/2/13	23.7	599	43.2	111	167.919

Appendix 5: ANOVA Tables corresponding to water quality parameters (*=significant at alpha=.05)

Cond. µS	*Constant*	*Date*	*Site*	*Error*	*Total*
df	1	9	9	72	90
Sums of Squares	3.27E+07	709946	112211	656075	1.49E+06
Mean Square	3.27E+07	78882.8	12467.9	9112.16	
F-ratio	3584.1	8.6569	1.3683		
Prob	□ 0.0001	□ 0.0001*	0.2186		

Turbidity (NTU)	Constant	Date	Site	Error	Total
df	1	9	9	73	91
Sums of Squares	38863.3	1247.96	8495.8	3357.93	13209
Mean Square	38863.3	138.662	943.977	45.999	
F-ratio	844.87	3.0145	20.522		
Prob	□ 0.0001	0.0041*	□ 0.0001*		

Alkalinity (mg/l CaCO3)	Constant	Date	Site	Error	Total
df	1	9	9	73	91
Sums of Squares	2.19E+06	180676	86798.4	774624	1.05E+06
Mean Square	2.19E+06	20075.1	9644.27	10611.3	
F-ratio	206.81	1.8919	0.90887		
Prob	□ 0.0001	0.0665	0.5224		

Q (cms)	Constant	Date	Site	Error	Total
df	1	9	9	75	93
Sums of Squares	7.48E+04	22211.8	164514	34311.2	220626
Mean Square	74831.7	2467.97	18279.4	457.482	
F-ratio	163.57	5.3947	39.956		
Prob	□ 0.0001	□ 0.0001*	□ 0.0001*		

TIN (mg/l)	Constant	Date	Site	Error	Total
df	1	9	9	75	93
Sums of Squares	1.24E+01	2.27E+00	8.44E-01	3.81E+00	7.11301
Mean Square	12.4035	2.52E-01	0.093773	0.050838	
F-ratio	243.98	4.9608	1.8445		
Prob	□ 0.0001	□ 0.0001*	0.074		

TRP (mg/l)	Constant	Date	Site	Error	Total
df	1	9	9	75	93
Sums of Squares	0.293147	0.057812	0.025928	0.057906	0.140689

Mean Square	0.293147	0.006424	0.002881	7.72E-04	
F-ratio	379.69	8.3199	3.7313		
Prob	< 0.0001	< 0.0001*	0.0007*		

TP (mg/l)	Constant	Date	Site	Error	Total
df	1	6	9	51	66
Sums of Squares	0.971821	0.083688	0.141538	0.420699	0.644331
Mean Square	0.971821	0.013948	0.015726	0.008249	
F-ratio	117.81	1.6909	1.9065		
Prob	< 0.0001	0.1423	0.072		

Appendix 6: Nutrient concentration measurements for 2012 and 2013 study periods

Site	Date	TP (mg/L)	TRP (mg/L)	TIN (mg/L)	NO3 (mg/L)	NH3 (mg/L)
Bad	7/19/12		0.020	0.230	0.043	0.188
Flint	7/19/12		0.088	0.553	0.450	0.103
Spaulding Drain	7/19/12		0.085	0.623	0.480	0.143
Gage	7/19/12		0.060	0.115	0.045	0.070
Cass	7/20/12		0.040	0.195	0.085	0.110
Saginaw	7/20/12		0.028	0.285	0.155	0.130
Shiawassee	7/20/12		0.025	0.155	0.038	0.118
Swan	7/20/12		0.213	0.145	0.050	0.095
Tittabawassee	7/20/12		0.033	0.348	0.140	0.208
Bad	8/12/12		0.085	0.781	0.656	0.125
Spaulding Drain	8/12/12		0.140	0.408	0.185	0.223
Shiawassee	8/12/12		0.057	0.797	0.655	0.143
Swan	8/12/12		0.140	0.358	0.238	0.120
Cass	8/13/12		0.023	0.688	0.566	0.123
Flint	8/13/12		0.083	0.398	0.213	0.185
Saginaw	8/13/12		0.173	0.684	0.123	0.245
Gage	8/13/12		0.120	0.973	0.850	0.123
Tittabawassee	8/13/12		0.040	0.946	0.819	0.128
Bad	11/10/12		0.020	0.741	0.616	0.125
Flint	11/10/12		0.035	0.315	0.078	0.238
Shiawassee	11/10/12		0.015	0.310	0.243	0.068
Gage	11/10/12		0.025	0.380	0.285	0.095
Swan	11/10/12		0.023	1.102	0.995	0.108
Cass	11/11/12		0.015	0.783	0.708	0.075
Spaulding Drain	11/11/12		0.033	0.638	0.530	0.108
Saginaw	11/11/12		0.023	0.413	0.233	0.180
Tittabawassee	11/11/12		0.010	0.313	0.228	0.085

Spaulding Drain	5/7/13	0.042419	0.0410353	0.3733544	0.265288	0.1080664
Ferguson Bayou	5/7/13	0.064562	0.0244281	0.119692	0.007352	0.11234
Cass	5/7/13	0.059026	0.0161245	0.2881212	0.193944	0.0941772
Shiawassee	5/7/13	0.039651	0.0382674	0.19869	0.0852816	0.1134084
Bad	5/7/13	0.036883	0.0230441	0.1758156	0.0688176	0.106998
Swan	5/7/13	0.041035	0.0396513	0.1275212	0.0205232	0.106998
Flint	5/7/13	0.114384	0.0493389	0.1638004	0.0589392	0.1048612
Tittabawassee	5/7/13	0.089473	0.088089	0.2478244	0.0446704	0.203154
Gage	5/7/13	0.121303	0.0562585	0.1405756	0.029304	0.1112716
Saginaw (output)	5/7/13	0.077018	0.0756336	0.2129004	0.0973552	0.1155452
Spaulding Drain	5/19/13	0.101928	0.0742497	0.6191464	0.468344	0.1508024
Ferguson Bayou	5/19/13	0.124071	0.1226873	0.126774	0.0325968	0.0941772
Cass	5/19/13	0.130991	0.0341156	0.439298	0.3344368	0.1048612
Shiawassee	5/19/13	0.02858	0.0271959	0.240574	0.133576	0.106998
Bad	5/19/13	0.070098	0.0507228	0.2776004	0.1599184	0.117682
Swan	5/19/13	0.10608	0.1046962	0.4680332	0.2499216	0.2181116
Flint	5/19/13	0.059026	0.0576425	0.441464	0.3355344	0.1059296
Tittabawassee	5/19/13	0.108848	0.0438031	0.2300068	0.1379664	0.0920404
Saginaw (output)	5/19/13	0.005053	0.0562585	0.310616	0.1961392	0.1144768
Spaulding Drain	6/5/13	0.082553	0.0811693	0.4885148	0.3772432	0.1112716
Ferguson Bayou	6/5/13	0.157286	0.1559017	0.134854	0.0150352	0.1198188
Cass	6/5/13	0.081169	0.0797854	0.9626196	0.8492112	0.1134084
Shiawassee	6/5/13	0.081169	0.0797854	0.467894	0.3651696	0.1027244
Bad	6/5/13	0.121303	0.1199195	0.7974832	0.665912	0.1315712
Swan	6/5/13	0.103312	0.1019283	0.3736344	0.2356528	0.1379816
Flint	6/5/13	0.101928	0.0867051	1.2037	1.0358032	0.1678968
Tittabawassee	6/5/13	0.118536	0.1171516	0.2439252	0.139064	0.1048612
Saginaw (output)	6/5/13	0.082553	0.0936247	0.3577544	0.2411408	0.1166136
Spaulding Drain	6/30/13	0.068714	0.0590264	0.1953388	0.0797936	0.1155452
Ferguson Bayou	6/30/13	0.259697	0.0797854	0.0808792	-0.0058192	0.0866984
Cass	6/30/13	0.038267	0.0368835	0.3702832	0.2301648	0.1401184
Shiawassee	6/30/13	0.179429	0.0451871	0.7078888	0.5912752	0.1166136
Bad	6/30/13	0.032732	0.0811693	0.5414624	0.4398064	0.101656

Swan	6/30/13	0.065946	0.0424192	1.1529132	1.0544624	0.0984508
Flint	6/30/13	0.071482	0.0479549	0.5189848	0.3980976	0.1208872
Tittabawassee	6/30/13	0.064562	0.0271959	0.1397116	0.0369872	0.1027244
Saginaw	6/30/13	0.380099	0.0244281	0.2501896	0.133576	0.1166136
Spaulding Drain	7/15/13	0.050723	0.0244281	0.2996228	0.2246768	0.074946
Ferguson Bayou	7/15/13	0.349652	0.0438031	0.0842888	0.001864	0.0824248
Cass	7/15/13	0.042419	0.0230441	0.6496696	0.5715184	0.0781512
Shiawassee	7/15/13	0.065946	0.0188923	0.171116	0.0929648	0.0781512
Bad	7/15/13	0.039651	0.0230441	0.397414	0.2861424	0.1112716
Swan	7/15/13	0.155902	0.0853211	0.0960704	0.0029616	0.0931088
Flint	7/15/13	0.258313	0.0202763	0.2568164	0.1818704	0.074946
Tittabawassee	7/15/13	0.063178	0.0562585	0.1609456	0.0721104	0.0888352
Gage	7/15/13	0.065946	0.0396513	0.1821796	0.1072336	0.074946
Saginaw	7/15/13	0.622287	0.0230441	0.2633728	0.1873584	0.0760144
Spaulding Drain	7/28/13	0.047955	0.0299638	0.3008664	0.2312624	0.069604
Ferguson Bayou	7/28/13	0.374563	0.046571	0.0958076	-0.0069168	0.1027244
Cass	7/28/13	0.113	0.025812	0.1360048	0.0984528	0.037552
Shiawassee	7/28/13	0.137911	0.0382674	0.091984	0.0501584	0.0418256
Bad	7/28/13	0.103312	0.0285799	0.1107944	-0.0058192	0.1166136
Swan	7/28/13	0.089473	0.0438031	0.033782	-0.009112	0.042894
Flint	7/28/13	0.107464	0.0202763	0.2040852	0.1676016	0.0364836
Tittabawassee	7/28/13	0.064562	0.0341156	0.12591	0.04028	0.08563
Gage	7/28/13	0.114384	0.0188923	0.0769388	0.0468656	0.0300732
Saginaw	7/28/13	0.148982	0.0188923	0.0330348	0.0029616	0.0300732
Spaulding Drain	9/2/13	0.237554	0.0784015	1.0134304	0.9117744	0.101656
Ferguson Bayou	9/2/13	0.119919	0.1171516	0.133552	0.0062544	0.1272976
Cass	9/2/13	0.119919	0.0216602	0.1082316	0.018328	0.0899036
Shiawassee	9/2/13	0.194652	0.0604103	0.1823428	0.073208	0.1091348
Bad	9/2/13	0.176661	0.0438031	0.5785592	0.5089552	0.069604
Swan	9/2/13	0.186348	0.06733	0.0445828	-0.0047216	0.0493044
Flint	9/2/13	0.15867	0.0382674	0.1347028	0.089672	0.0450308
Tittabawassee	9/2/13	0.096393	0.0354995	0.213584	0.1632112	0.0503728
Gage	9/2/13	0.135143	0.0479549	0.2353608	0.1785776	0.0567832
Saginaw	9/2/13	0.117152	0.0451871	0.181812	0.133576	0.048236

Appendix 7: Calculated nutrient loading and total dissolved solid loading measurements by sampling site and date. Negative values indicate reversed flow direction.

Site	Date	TRP (kg/day)	TIN (kg/day)	TP (kg/day)	TDS load (kg/day)	Carbonate load (kg/day)
Bad	7/19/12	2	17		19000	16000
Flint	7/19/12	-	-		-	-
Spaulding Drain	7/19/12	30	222		134000	-
Gage	7/19/12	44	84		-	149000
Cass	7/20/12	12	58		93000	57000
Saginaw	7/20/12	200	2069		2283000	1009000
Shiawassee	7/20/12	12	73		172000	55000
Swan	7/20/12	5	4		11000	5000
Tittabawassee	7/20/12	33	350		237000	158000
Bad	8/12/12	543	4989		445000	433000
Spaulding Drain	8/12/12	273	796		436000	225000
Shiawassee	8/12/12	129	1812		412000	199000
Swan	8/12/12	161	412		167000	647000
Cass	8/13/12	26	806		257000	678000
Flint	8/13/12	70	336		134000	83000
Saginaw	8/13/12	3287	12971		3315000	-
Gage	8/13/12	1348	10923		-	1174000
Tittabawassee	8/13/12	193	4562		1179000	644000
Bad	11/10/12	2	71		21000	6000
Flint	11/10/12	2	21		20000	59000
Shiawassee	11/10/12	5	109		79000	47000
Gage	11/10/12	15	232		-	71000
Swan	11/10/12	1	51		11000	7000
Cass	11/11/12	4	229		69000	49000
Spaulding Drain	11/11/12	40	782		263000	195000
Saginaw	11/11/12	117	2154		1163000	647000
Tittabawassee	11/11/12	3	96		85000	52000

Site	Date	TRP (kg/day)	TIN (kg/day)	TP (kg/day)	TDS load (kg/day)	Carbonate load (kg/day)
Spaulding Drain	5/7/13	76	688	78	488463.264	361278.4896
Ferguson Bayou	5/7/13	-0.412	-2	-1	-3631.6507	-2517.883056
Cass	5/7/13	28	500	102	505628.725	256837.3056
Shiawassee	5/7/13	59	306	61	394341.578	221895.936
Bad	5/7/13	14	109	23	139635.042	93240.72
Swan	5/7/13	1	3	1	6371.74944	3760.262352
Flint	5/7/13	46	152	106	233602.527	107415.072
Tittabawassee	5/7/13	624	1755	634	1473401.5	722217.7728
Gage	5/7/13	214	534	460	960866.319	535118.0112
Saginaw	5/7/13	863	2430	879	3154080.7	1746157.176
Spaulding Drain	5/19/13	74	620	102	312328.067	216353.2032
Ferguson Bayou	5/19/13	-0.36	-0.37	-0.36	-922.99078	-767.37024
Cass	5/19/13	25	326	97	223137.472	175269.2515
Shiawassee	5/19/13	19	171	20	200430.759	163577.3616
Bad	5/19/13	29	157	40	147677.187	106224.8429
Swan	5/19/13	10	45	10	27723.8877	21105.35957
Flint	5/19/13	24	185	25	123250.731	94568.30784
Tittabawassee	5/19/13	167	874	414	1009174.94	638683.3152
Saginaw	5/19/13	441	2436	441	2406389.24	1835272.858
Spaulding Drain	6/5/13	159	959	162	583324.004	264904.02
Ferguson Bayou	6/5/13	1	1	1	1174.50088	537.825744
Cass	6/5/13	168	2031	171	530251.39	305877.384
Shiawassee	6/5/13	115	672	117	435411.779	226910.2464

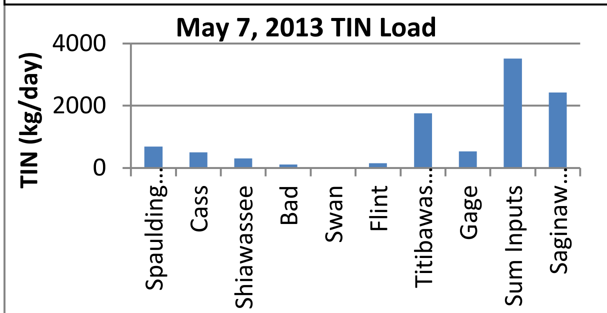
Site	Date	TRP (kg/day)	TIN (kg/day)	TP (kg/day)	TDS load (kg/day)	Carbonate load (kg/day)
Bad	6/5/13	30	199	30	50785.166	36909.93312
Swan	6/5/13	1	5	1	3009.31382	1858.596386
Flint	6/5/13	84	1169	99	291379.68	163172.6208
Tittabawassee	6/5/13	815	1696	824	1501285.17	973464.912
Saginaw	6/5/13	1310	5006	1310	3720957.38	2196955.008
Spaulding Drain	6/30/13	236	782	275	1139010.41	436317.408
Ferguson Bayou	6/30/13	0	0	0	57.24432	26.145504
Cass	6/30/13	116	1161	120	769728.567	498613.0608
Shiawassee	6/30/13	61	951	241	370570.189	162513.648
Bad	6/30/13	40	269	16	112578.085	64027.37599
Swan	6/30/13	2	43	2	12041.9416	5023.113264
Flint	6/30/13	94	1013	140	559916.359	226451.1168
Tittabawassee	6/30/13	143	736	340	1214184.43	426760.6824
Saginaw	6/30/13	487	4988	7577	5038190.97	1814111.208
Spaulding Drain	7/15/13	19	229	39	212002.753	90075.14673
Ferguson Bayou	7/15/13	0	0	0	57.8521636	22.48452
Cass	7/15/13	10	292	19	116418.579	35559.84024
Shiawassee	7/15/13	11	101	39	182398.255	68739.84
Bad	7/15/13	0	0	0	-120.70964	-23.38524
Swan	7/15/13	2	2	3	5025.5314	2336.328576
Flint	7/15/13	4	52	52	66544.577	19695.71808
Tittabawassee	7/15/13	99	283	111	762960.017	165561.5232
Gage	7/15/13	29	134	48	229192.793	85872.23424

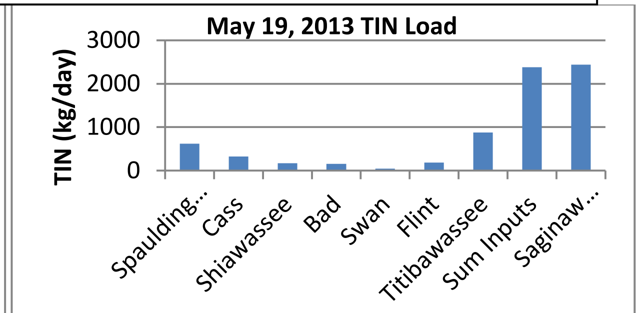
Site	Date	TRP (kg/day)	TIN (kg/day)	TP (kg/day)	TDS load (kg/day)	Carbonate load (kg/day)
Saginaw	7/15/13	258	2947	6963	3392502.68	1040638.752
Spaulding Drain	7/28/13	20	198	32	188989.273	81046.74672
Ferguson Bayou	7/28/13	0	0	0	67.1121818	27.42012
Cass	7/28/13	9	47	39	92513.9181	31392.01084
Shiawassee	7/28/13	15	37	55	126572.474	52562.22905
Bad	7/28/13	4	14	13	42450.4054	9365.10336
Swan	7/28/13	1	1	2	6022.63375	2939.66496
Flint	7/28/13	1	10	5	16296.8083	5311.55232
Tittabawassee	7/28/13	37	137	70	460875.469	120886.992
Gage	7/28/13	36	146	217	575344.983	252392.7168
Saginaw	7/28/13	275	481	2169	4466251.64	1557563.904
Spaulding Drain	9/2/13	52	233	55	65099.6509	27860.976
Ferguson Bayou	9/2/13	0	0	0	5.84496	2.5299648
Cass	9/2/13	7	20	22	37017.5302	17477.6832
Shiawassee	9/2/13	24	188	201	231367.446	132156.3341
Bad	9/2/13	6	56	17	22299.3884	7406.89488
Swan	9/2/13	1	1	4	4934.28829	2926.81728
Flint	9/2/13	2	5	6	10130.7142	4078.7712
Tittabawassee	9/2/13	39	287	130	541907.241	156088.8576
Gage	9/2/13	91	374	215	373040.316	204775.3224
Saginaw	9/2/13	658	2638	1700	3950185.27	1610409.419

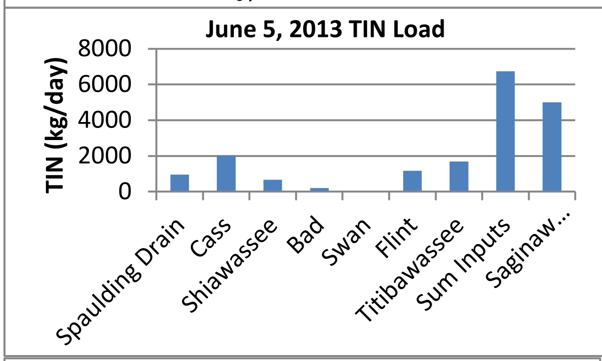
Appendix 8: ANOVA Tables to accompany statistical summary for load by station, over all dates. * Indicates statistically significant at alpha=0.05.

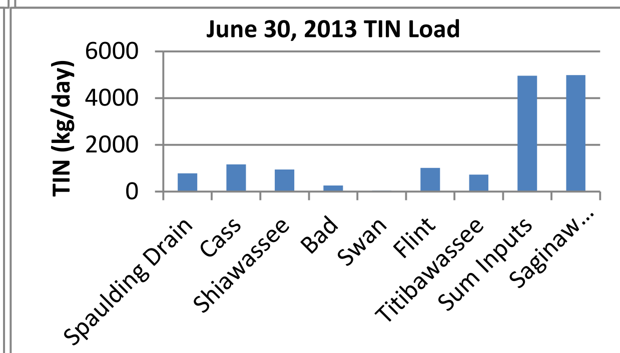
TIN (kg/day)	**Constant**	**Date**	**Site**	**Error**	**Total**
df	1	9	9	75	93
Sums of Squares	6.83E+07	8.01E+07	7.96E+07	9.72E+07	2.58E+08
Mean Square	6.83E+07	8.91E+06	8.84E+06	1.30E+06	
F-ratio	52.677	6.8683	6.8209		
Prob	⍰ 0.0001	⍰ 0.0001*	⍰ 0.0001*		
TRP (kg/day)	Constant	Date	Site	Error	Total
df	1	9	9	75	93
Sums of Squares	2.34E+06	3.16E+06	4.94E+06	7.45E+06	1.56E+07
Mean Square	2.34E+06	351576	549239	99368.9	
F-ratio	23.582	3.5381	5.5273		
Prob	⍰ 0.0001	0.0011*	⍰ 0.0001*		
TP (kg/day)	Constant	Date	Site	Error	Total
df	1	6	9	51	66
Sums of Squares	1.06E+07	5.70E+06	5.07E+07	5.07E+07	1.07E+08
Mean Square	1.06E+07	950784	5.63E+06	993519	
F-ratio	10.621	0.95699	5.6709		
Prob	0.002	0.4635	⍰ 0.0001*		
Carbonate Load (kg/day)	Constant	Date	Site	Error	Total
df	1	9	9	71	89
Sums of Squares	1.13E+07	2.56E+06	1.12E+07	1.90E+07	3.29E+07
Mean Square	1.13E+07	284616	1.25E+06	267246	
F-ratio	42.287	1.065	4.6615		
Prob	⍰ 0.0001	0.3991	⍰ 0.0001*		
TDS (kg/day)	Constant	Date	Site	Error	Total
df	1	9	9	71	89
Sums of Squares	3.40E+07	5.56E+06	5.44E+07	4.32E+07	1.02E+08
Mean Square	3.40E+07	617997	6.05E+06	608328	
F-ratio	55.898	1.0159	9.9425		
Prob	⍰ 0.0001	0.4359	⍰ 0.0001*		

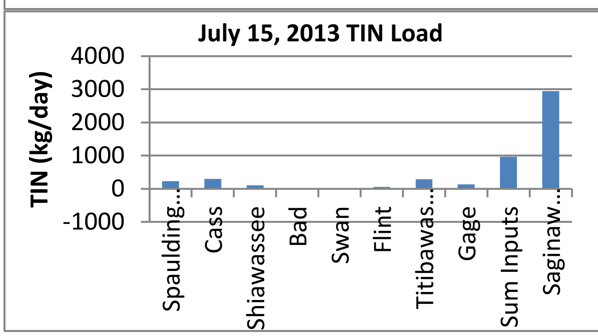
Appendix 9: Total inorganic nitrogen loads for sampling sites by date for study period

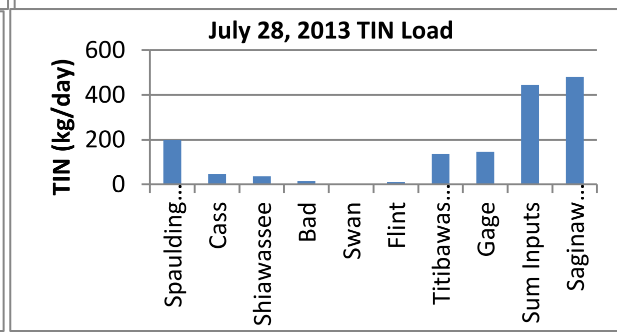


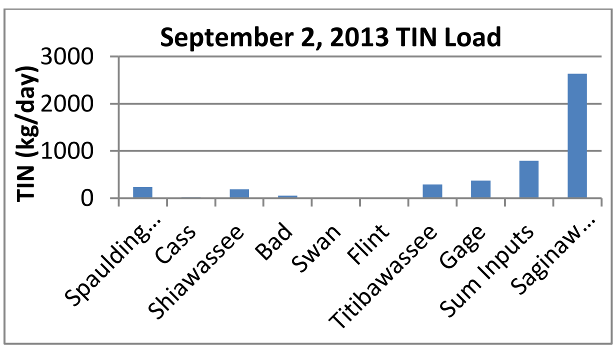


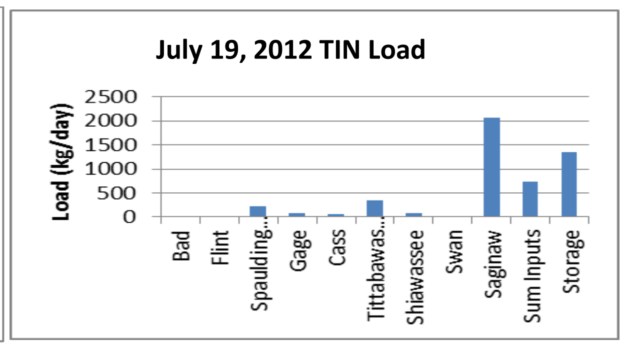


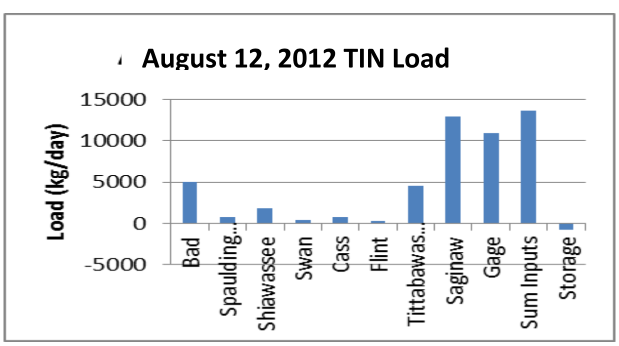


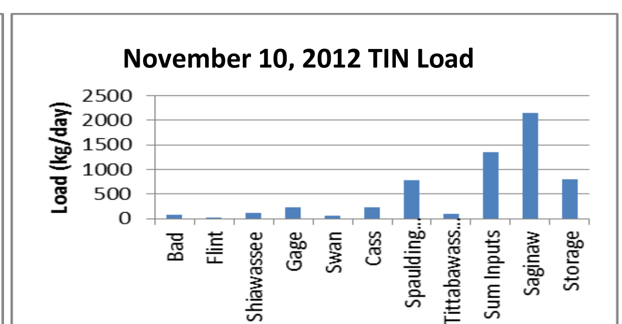




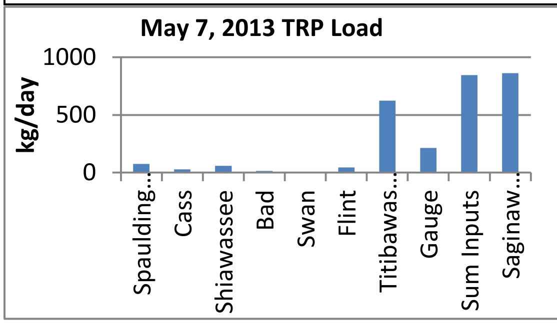


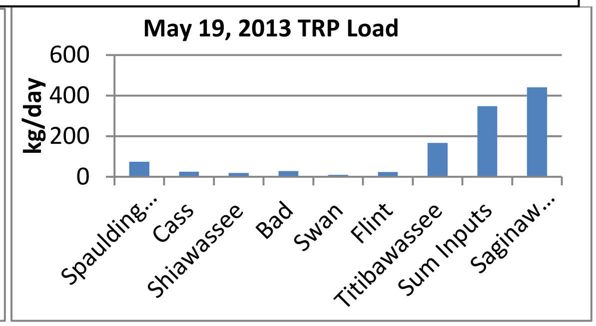


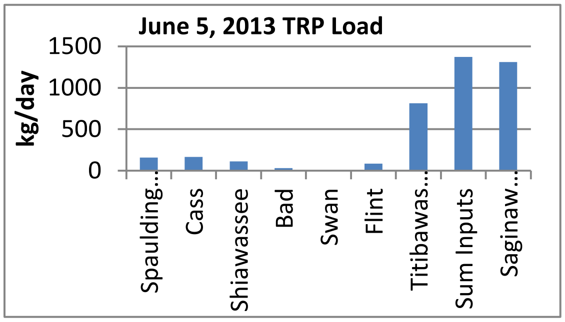


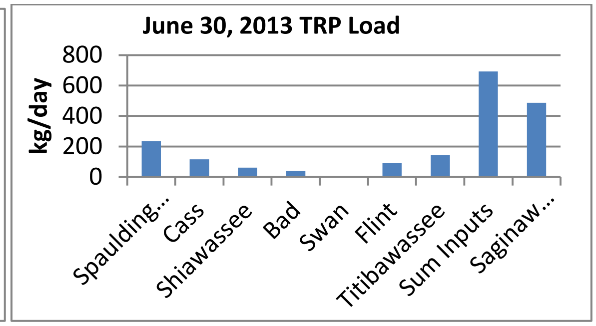


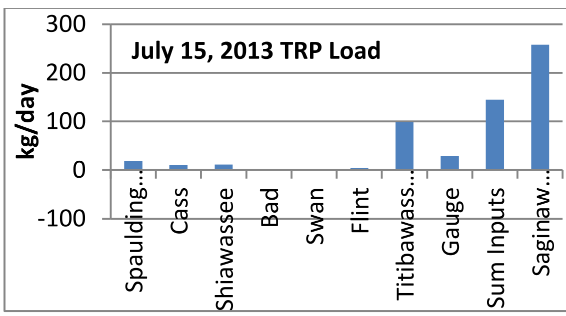
Appendix 10: Total reactive phosphorus loads for each site grouped by date for 2013 study period

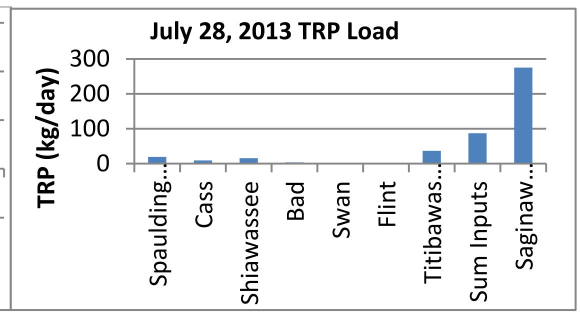


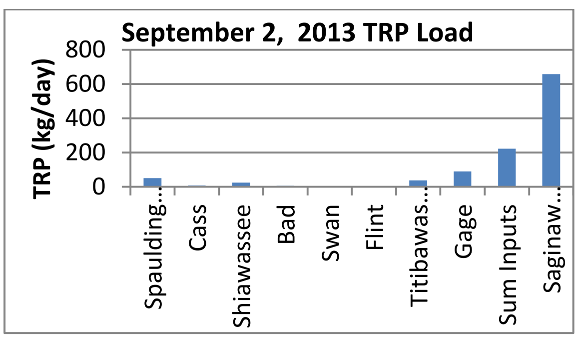


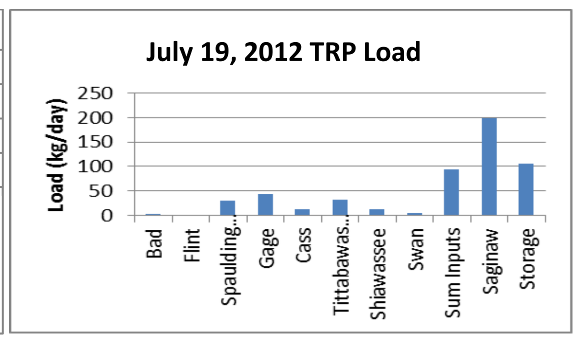


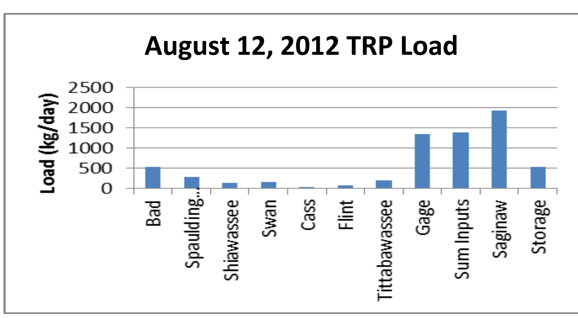


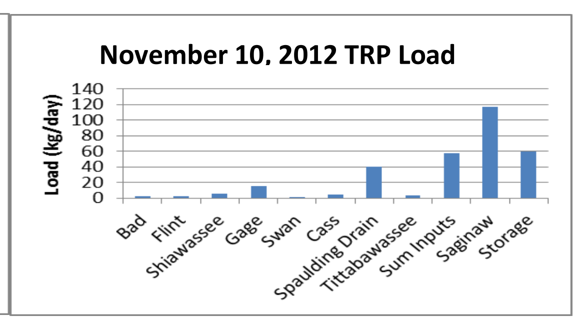




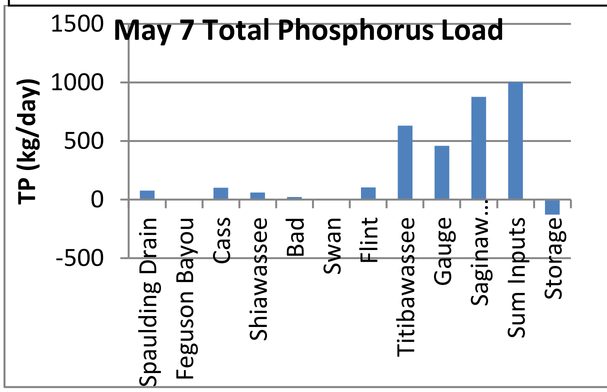


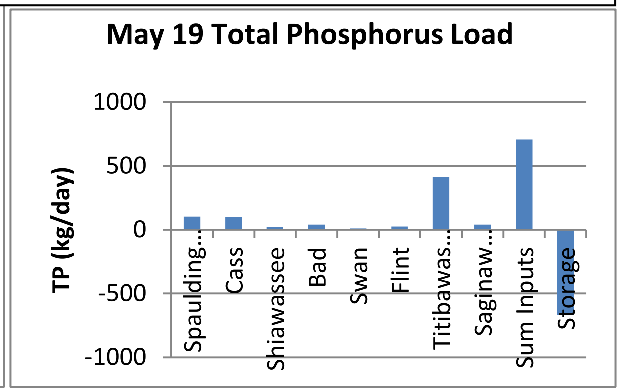


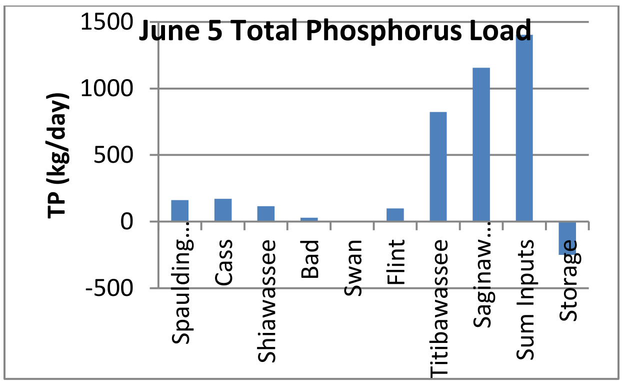


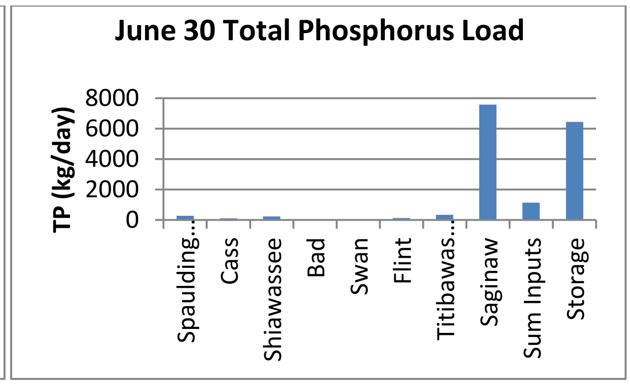


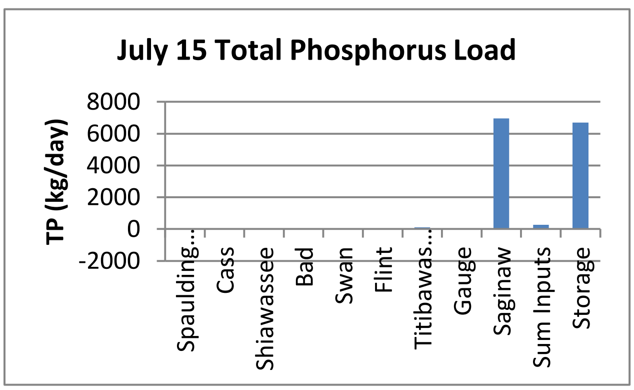
Appendix 11: Total phosphorus loads by site for each sampling date for the 2013 study period. Negative values signify phosphorus going into storage from within the Refuge.

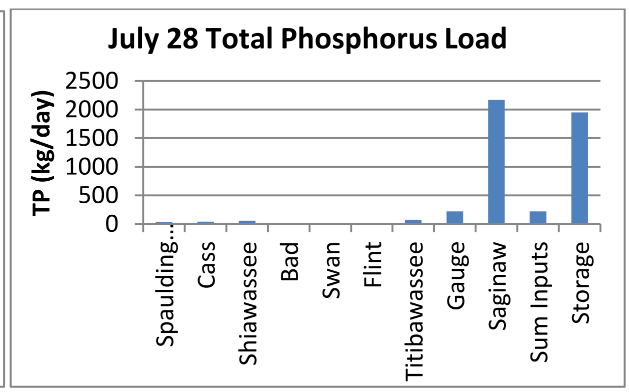


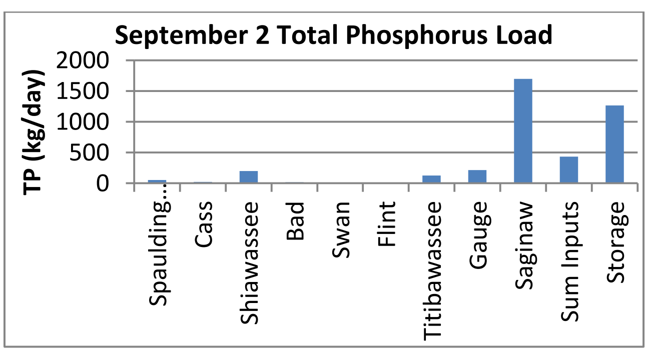




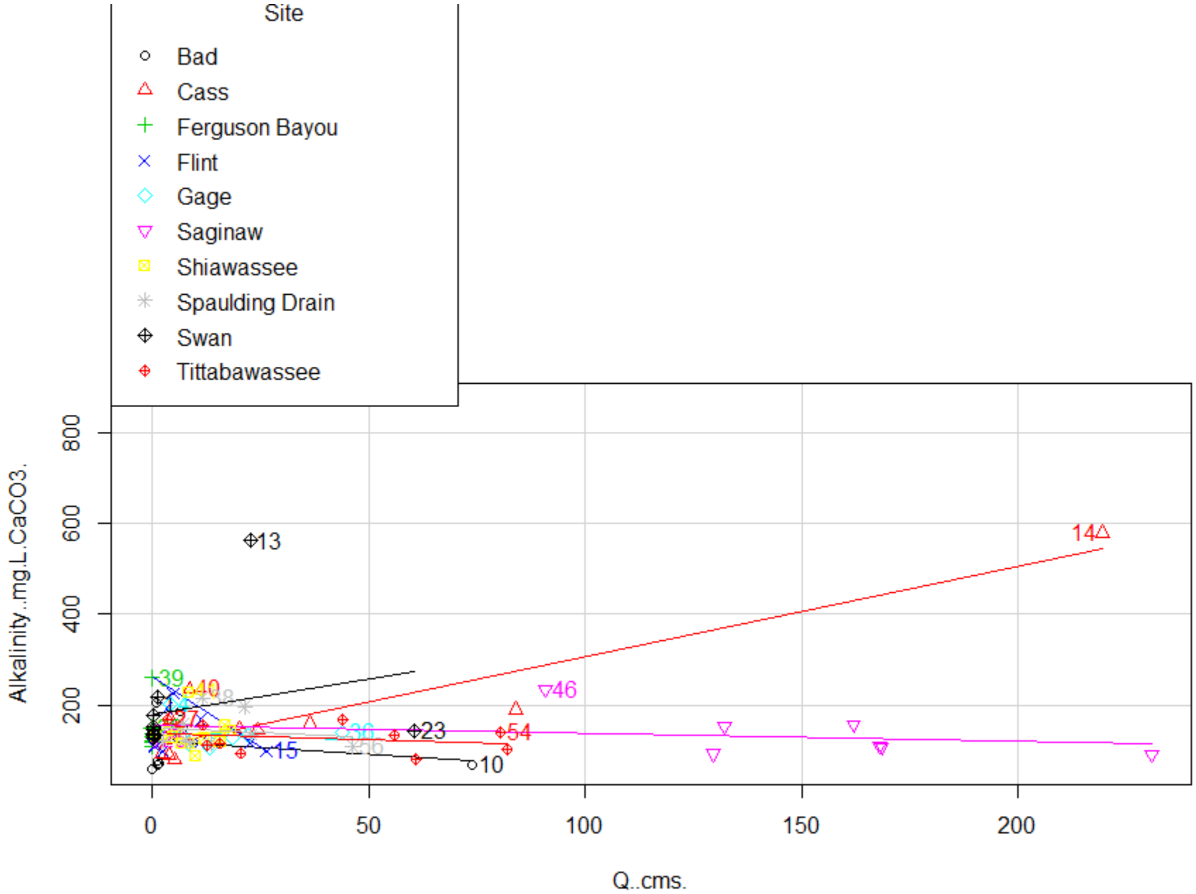






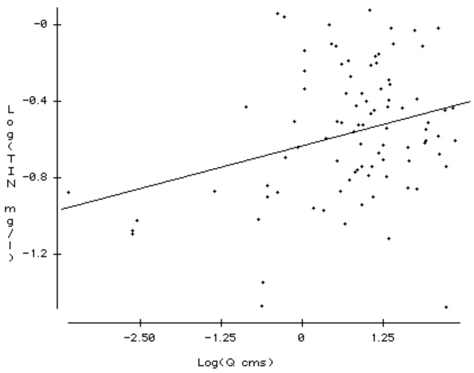


Appendix 12: Linear regression of alkalinity against discharge, grouped by site (RSE=101.9 on 72 df, R^2=0.1, F=1.5, p=0.11)

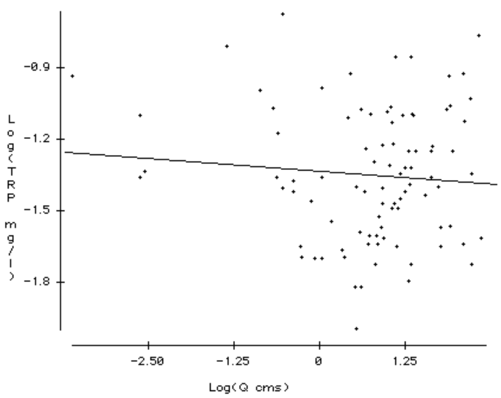


Appendix 13: Nutrient concentrations plotted against discharge for all sampling sites and sampling dates.

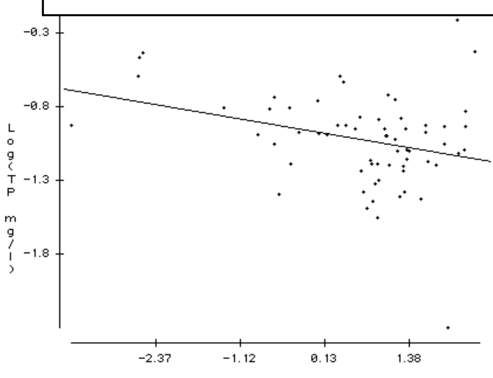
a: Linear regression of inorganic nitrogen concentration (mg/l) against discharge (cms) across all sites and dates, with both axes logged (R^2=8.5%).



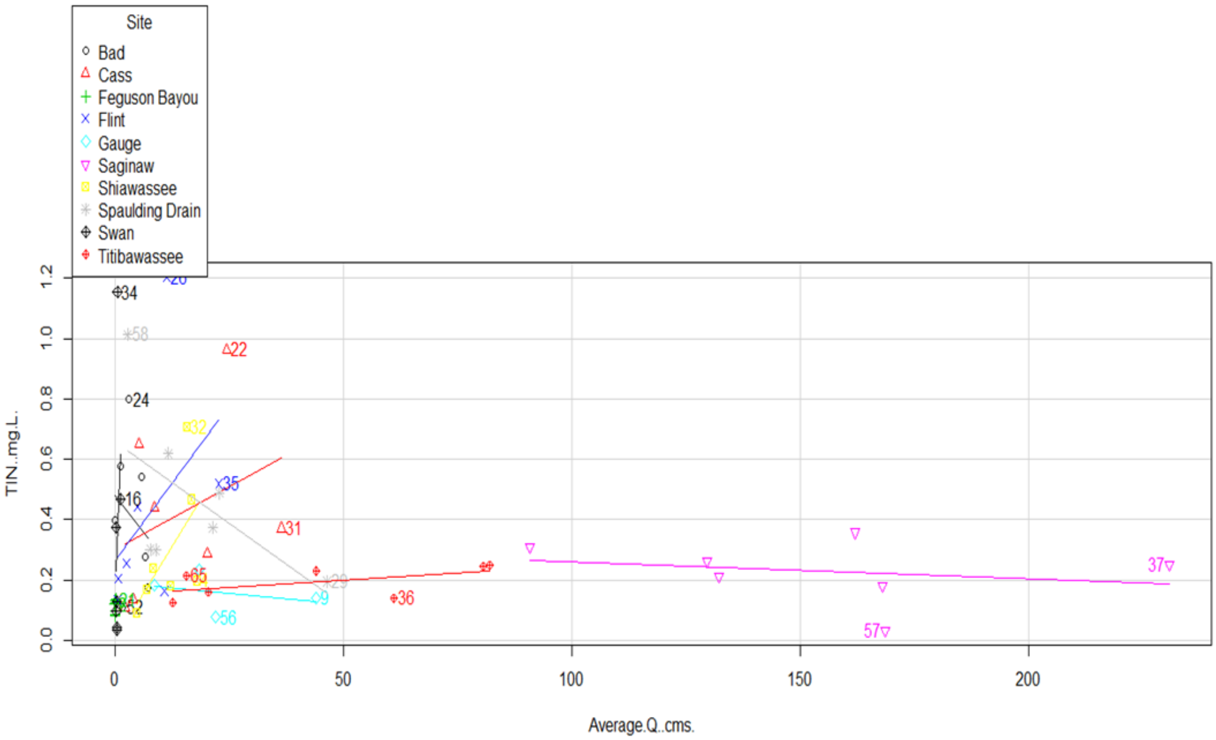
b: Linear regression of reactive phosphorus concentration (mg/l) against discharge (cms) across all sites and dates, with both axes logged (R^2=0.7%).



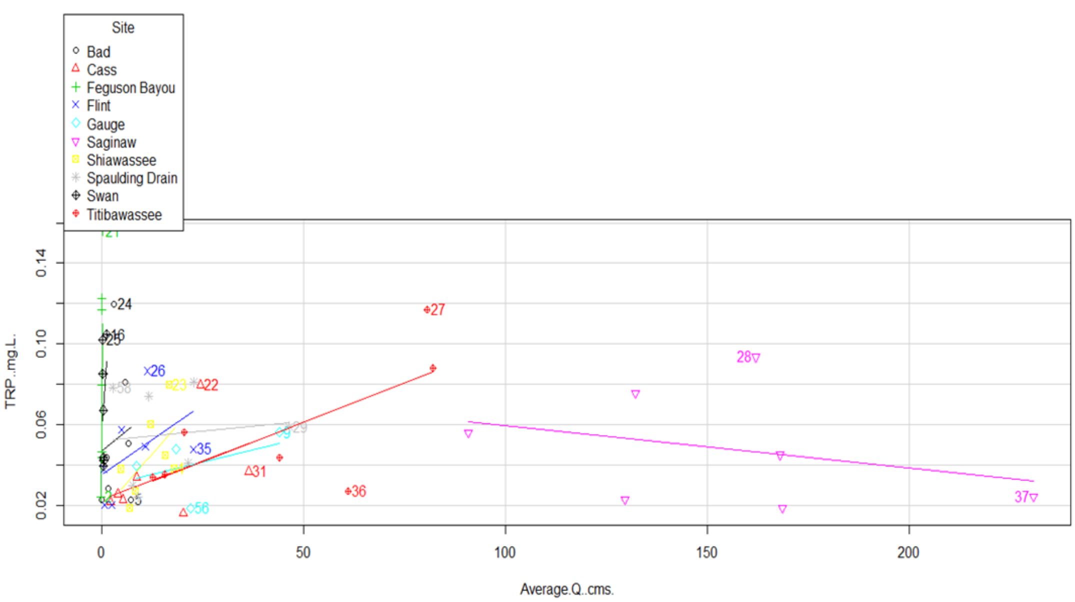
c: Linear regression of total phosphorus concentration (mg/l) against discharge (cms) across all sites and dates, with both axes logged (R^2=9.3%).



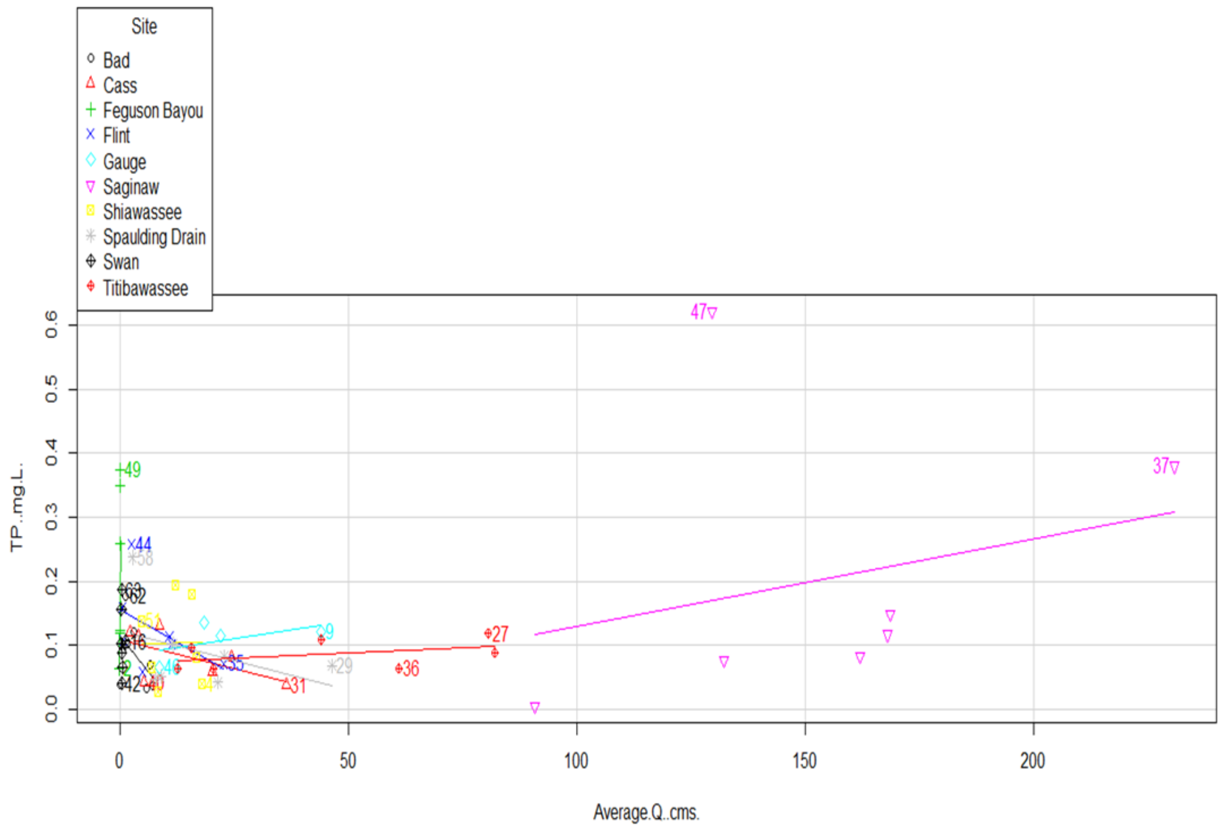
Appendix 14: Total inorganic nitrogen concentration against discharge (cms) by site (RSE=0.25, 47 df, R^2=0.08, F=1.302, p=0.227).



Appendix 15: Total reactive phosphorus concentration against discharge (cms) by site (RSE=0.03, 47 df, R^2=0.4, F=1.56, p=0.11).



Appendix 16: Total phosphorus concentration against discharge (cms) by site (RSE=0.25, 47 df, R^2=0.08, F=1.3, p=0.23).



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