



Zooplankton Community Differences Among Four Main Pools of Lake St. Croix (Minnesota–Wisconsin)

2010–2011

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ON THE COVER

V. Vang, student intern from the St. Croix Watershed Research Station, samples zooplankton from Lake St. Croix (St. Croix National Scenic Riverway) in July of 2010. Photo by T. Lafrancois.

Zooplankton Community Differences Among Four Main Pools of Lake St. Croix (Minnesota– Wisconsin)

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Contents

	Page
Figures.....	v
Tables.....	vii
Appendices.....	ix
Abstract.....	xi
Acknowledgments.....	xiii
Introduction.....	1
The St. Croix National Scenic Riverway	1
Characteristics of Zooplankton Communities in Large Rivers	4
Feeding Preferences and Impacts of Bighead and Silver Carp on Plankton	5
Objectives	6
Methods.....	9
Sampling Locations and Site Characteristics	9
Zooplankton Sampling and Identification	9
Statistical Analysis	10
Results.....	11
Site Characteristics	11
Zooplankton Counts and Community Analyses.....	15
Discussion.....	35
The Spatial and Temporal Context of Zooplankton Communities in Lake St. Croix	35
Patterns of Zooplankton Biomass and Community Structure in Lake St. Croix.....	36
Zooplankton Community Structure in Lake St. Croix and Other Reservoir Systems.....	40
Conclusions.....	45
Literature Cited	47

Figures

Page

Figure 1. Map of the St. Croix watershed with zooplankton sampling sites corresponding to long term monitoring stations of the Great Lakes Inventory and Monitoring Network of the National Park Service. SACN stands for the St. Croix National Scenic Riverway, followed by site number (5, 7, 9 and 11 were used in this study).....	2
Figure 2. Detailed map of zooplankton sampling sites on the St. Croix River, indicating position relative to pool boundaries	3
Figure 3. Streamflow (in cubic feet per second, or cfs) as measured by a gage at St. Croix Falls, Wisconsin, in 2010 and 2011, with reference to 112-year mean	15
Figure 4. Zooplankton sampled from Lake St. Croix and the reservoir above St. Croix Falls, Wisconsin, ranged in size from testate protista (<i>Centropyxis aculeata</i> , inset) to the relatively enormous <i>Leptodora kindtii</i> , a cladoceran predator that can grow up to three centimeters long.	20
Figure 5. Mean zooplankton biomass (micrograms per liter) from May to September of 2010 and 2011 sorted by site and major taxonomic group	21
Figure 6. Number of zooplankton taxa from each of the four major taxonomic groups collected from Lake St. Croix (Minnesota/Wisconsin) and above the dam at St. Croix Falls, Wisconsin, May to September, 2010 and 2011.....	22
Figure 7. Monthly total zooplankton biomass at three sites in Lake St. Croix (Minnesota/Wisconsin) and above St. Croix Falls, Wisconsin, in 2010 and 2011.....	23
Figure 8. Monthly copepod biomass at three sites in Lake St. Croix (Minnesota/Wisconsin) and above St. Croix Falls, Wisconsin, in 2010 and 2011.....	24
Figure 9. Monthly cladoceran biomass at three sites in Lake St. Croix (Minnesota/Wisconsin) and above St. Croix Falls, Wisconsin, in 2010 and 2011.....	25
Figure 10. Monthly rotifer biomass at three sites in Lake St. Croix (Minnesota/Wisconsin) and above St. Croix Falls, Wisconsin, in 2010 and 2011.....	26
Figure 11. Monthly testate protist biomass at three sites in Lake St. Croix (Minnesota/Wisconsin) and above St. Croix Falls, Wisconsin, in 2010 and 2011.....	27
Figure 12. Principal coordinates plot of zooplankton communities by site and date at four sites on the St. Croix River (Minnesota/Wisconsin) sampled from May to September 2010.....	31
Figure 13. Canonical analysis of principal components plot for zooplankton communities sampled on the St. Croix River (Minnesota/Wisconsin) from May to September 2010	32
Figure 14. Principal coordinates plot of zooplankton communities by site and date at four sites on the St. Croix River (Minnesota/Wisconsin) sampled from April to October 2011	33
Figure 15. Canonical analysis of principal components plot for zooplankton communities sampled on the St. Croix River (Minnesota/Wisconsin) from April to October 2011.	34

Tables

	Page
Table 1. Basic features of primary sampling sites (modified from Robertson and Lenz 2002)	4
Table 2. Water quality results from May to September, 2010, taken at four St. Croix River sites (National Park Service, Great Lakes Inventory and Monitoring site designations) and used in PERMANOVA analyses	11
Table 3. Water quality results from April to November, 2011, taken at four St. Croix River sites (National Park Service, Great Lakes Inventory and Monitoring site designations) and used in PERMANOVA analyses	13
Table 4. Correlation matrix between water quality results from 2010 and 2011 (r^2).....	15
Table 5. Zooplankton taxa (35 μm + tow net) sampled May to September, 2010 and 2011, from Lake St. Croix and above the St. Croix Falls dam	16
Table 6. Common diversity indices for zooplankton samples from the St. Croix River (Minnesota / Wisconsin), 2010 and 2011, calculated in PRIMER 7	28

Appendices

	Page
Appendix 1. Implications of Lake St. Croix zooplankton community structure for silver and bighead carp establishment	55
Appendix 2. Sampling zooplankton in large rivers: Lessons from the St. Croix.....	59

Abstract

Lake St. Croix is a natural impoundment of the St. Croix National Scenic Riverway (Minnesota and Wisconsin) under imminent threat of silver and bighead carp (*Hypophthalmichthys molitrix* and *H. nobilis*) establishment, opportunistic omnivores that have dramatically altered zooplankton communities in similar systems. The purpose of this project was to characterize zooplankton at four long term water quality monitoring sites established by the National Park Service–Great Lakes Inventory and Monitoring Network. Three of the sites are located in Lake St. Croix at, in descending order, Stillwater, Minnesota, Hudson, Wisconsin, and Prescott, Wisconsin. The fourth site is in a reservoir above a hydroelectric dam in St. Croix Falls, Wisconsin, a barrier to migration of carp into upstream areas of the Riverway. Quantitative zooplankton samples were collected monthly from May to September of 2010 and 2011. PERMANOVA analysis of zooplankton community composition identified significant differences among sites ($p = 0.005$), over sampling month ($p = 0.002$), and between years ($p = 0.002$). There were no significant interactions between site, month, and year. Ordinations show that the upstream reservoir was uniformly dominated by Rotifera and total zooplankton biomass was three to five orders of magnitude lower than that found at the three pool sites. The upper and lower Lake St. Croix pool sites had similar total zooplankton biomass but were characterized by different dominant crustacean taxa in productive months. In 2010 two sites in Lake St. Croix were dominated by rotifers, correlating to periods of low retention, while the central site near Hudson, Wisconsin, was dominated by rotifers all season long. However, in 2011 this site was crustacean-dominated and supported the highest biomass over all other sites, due primarily to a large bloom of *Mesocyclops* spp. Comparison of communities and environmental conditions between 2010 and 2011 suggests crustacean dominance of the zooplankton community was most likely related to water retention time. We conclude 1) Lake St. Croix is a mosaic of connected but distinct zooplankton communities, with taxa differing among pools in ecologically significant ways; 2) Lake St. Croix supports preferred zooplankton prey species for both silver and bighead carp; and 3) the reservoir above the dam will not act as a recolonization source of preferred zooplankton prey species for downstream areas of the river or Lake St. Croix pools.

Acknowledgments

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Introduction

The St. Croix National Scenic Riverway

The St. Croix National Scenic Riverway (Riverway), a unit of the National Park Service (NPS) established as one of the eight components of the National Wild and Scenic Riverway Act in 1968, covers the 247-km-long St. Croix and Namekagon rivers (Figure 1). Beginning in Stillwater, Minnesota, and ending in Prescott, Wisconsin, at the confluence with the Mississippi River, Lake St. Croix is a 37.7-km-long natural flow-through reservoir consisting of a series of four separate deep pools (up to 20 m) with relatively high water retention times (Figure 2). Basic water quality features of the pools are summarized in Table 1. The Riverway is prized for its recreational value, and Lake St. Croix's proximity to a major metropolitan area guarantees high visitation and economic impact. Small commercial fisheries operating in lower Lake St. Croix are also important for the local economy.

The Riverway is ecologically important, supporting what are considered original species assemblages despite human impacts to the landscape and waterway (Robertson and Lenz 2002). There are over 130 fish species, including the threatened paddlefish (*Polyodon spathula*) and more than forty native unionid mussel species, with several state-listed (Minnesota and/or Wisconsin) threatened and five federally listed endangered species of mussels. In spite of its good condition relative to nearby rivers, invasive species and large scale changes to the landscape have altered the water quality and biota (Kraft et al. 2015). Lake St. Croix is currently under strain from cultural eutrophication and was declared impaired from nutrients (phosphorus) by the Environmental Protection Agency in 2008 (Edlund et al. 2009, Johnson et al. 2010, MPCA and WNDR 2011). A TMDL (total maximum daily load) plan for phosphorus was recently completed as required by the Clean Water Act, targeting reduction of phosphorous (MPCA and WNDR 2011). Furthermore, invasive zebra mussels (*Dreissena polymorpha*) have colonized the lower-most 37.7 km; boating upstream is restricted to control further spread while the NPS and other agencies monitor their impact (for impact of invasive bivalves on plankton see Pace et al. 1998, David et al. 2009).

In addition to nutrient loading and zebra mussels, the lower St. Croix River—below the hydro-dam in St. Croix Falls—is now on the threshold of colonization by the invasive silver and bighead carp (*Hypophthalmichthys molitrix* and *H. nobilis*). Environmental DNA (eDNA) tests in 2011 detected the presence of silver carp below the hydro-dam (Hickox et al. 2011). Two bighead carp were harvested by commercial fisheries near Prescott in 2011 and 2012 (Minnesota DNR press release, 21 November 2012), with six additional bighead carp caught by recreational and Minnesota Department of Natural Resources anglers near Stillwater in 2015 (Minnesota DNR press release, 2 June 2015; N. Frohnauer, Minnesota DNR, personal communication). The presence of these species this soon is surprising since the nearest known breeding populations are located in the Mississippi River at Pool 17, roughly 378 river miles from the St. Croix confluence (Chick and Pegg 2001, G. Jones, Iowa DNR, personal communication). Models show that Minnesota and Wisconsin both offer very suitable habitat for Asian carp (Herborg et al. 2007) and that these carp grow faster and mature earlier in the lower Mississippi than in their native habitats (Williamson and Garvey 2005).

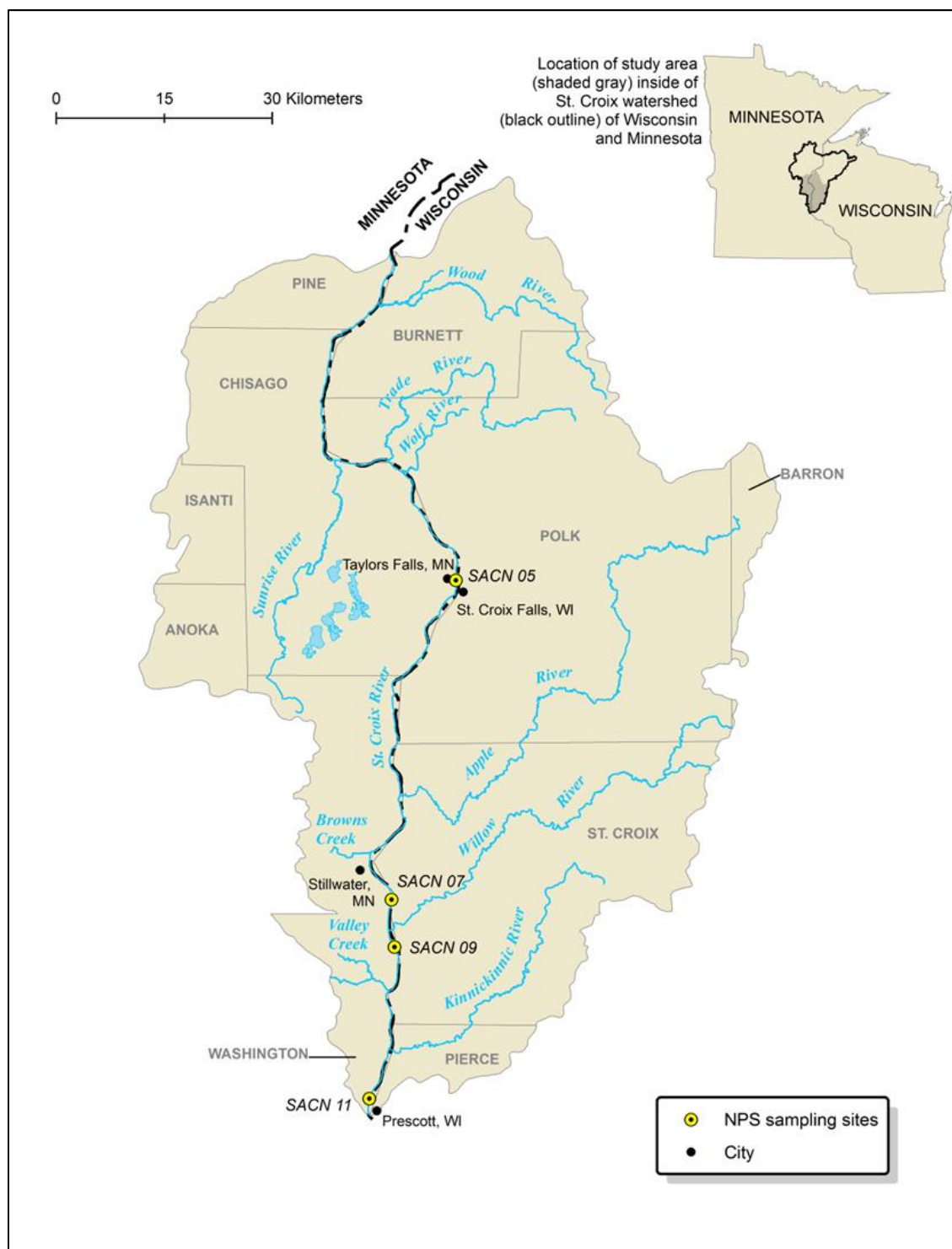


Figure 1. Map of the St. Croix watershed with zooplankton sampling sites corresponding to long term monitoring stations of the Great Lakes Inventory and Monitoring Network of the National Park Service. SACN stands for the St. Croix National Scenic Riverway, followed by site number (5, 7, 9 and 11 were used in this study).

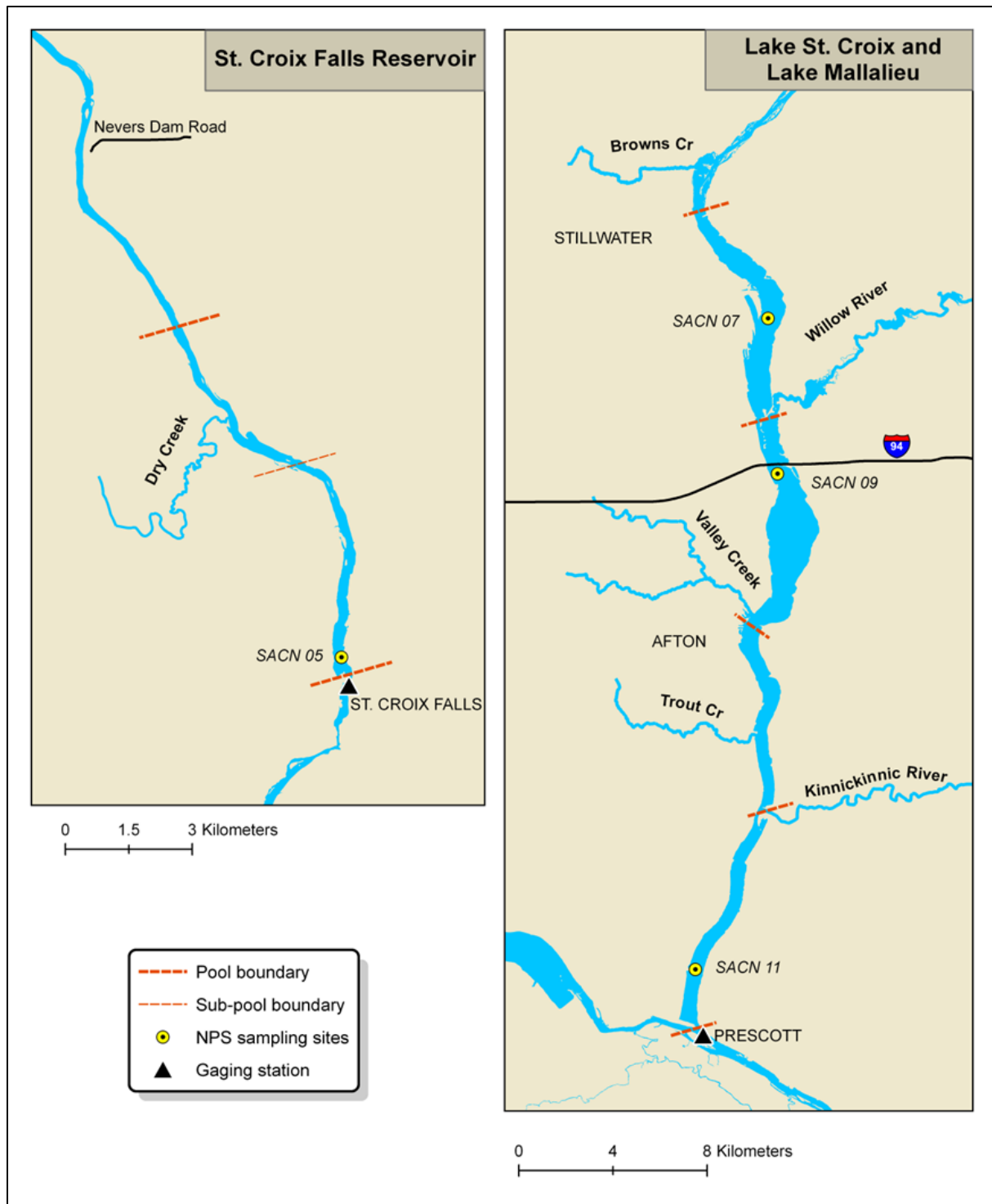


Figure 2. Detailed map of zooplankton sampling sites on the St. Croix River, indicating position relative to pool boundaries. Site SACN05 is above the dam at St. Croix Falls, Wisconsin. Sites SACN07, SACN09, and SACN11 are all within different pools of Lake St. Croix. Site SACN09 is located in a low retention area upstream of Pool 3, while sites SACN07 and SACN09 are close to the bathymetric center of their respective pools.

Table 1. Basic features of primary sampling sites (modified from Robertson and Lenz 2002). Site labels follow the National Park Service Great Lakes Inventory and Monitoring Network designations. Site SACN09 was chosen by randomization and is located above pool 2 in a low retention zone just downstream of the I-94 bridge. Consequently retention times shown for SACN09 are not characteristic of the sampling site.

Location	Associated NPS Site	Length (km)	Area (km ²)	Volume (m ³ × 10 ³)	Mean Depth (m)	Water Residence Time (Days)	
						Wet	Dry
St. Croix Falls Reservoir	SACN05	10.1	2.4	9.6	4.0	0.4	0.9
Lake St. Croix (pool 1)	SACN07	9.7	11.3	77.1	6.8	5	11
Lake St. Croix (pool 2)	(SACN09*)	9.7	12.7	133.0	10.5	8	19
Lake St. Croix (pool 4)	SACN11	8.1	5.8	57.1	9.8	3	8

The suitability of the lower Mississippi River to carp establishment and the speed at which the carp are spreading are a cause for concern. The primary motivation of the study presented here was to rapidly acquire information on the available zooplankton food stock for bighead and silver carp since they feed on both algae and zooplankton alike, and because establishment of bighead and silver carp in the lower Riverway may be imminent.

Characteristics of Zooplankton Communities in Large Rivers

Zooplankton include protozoans, rotifers, small planktonic crustaceans, and some insects (Chaoboridae) that live in the water column. Many are size selective omnivores or grazers that eat algae and in turn provide food for larger invertebrates and fish. Zooplankton are not strong swimmers, so it is no surprise that flow regime is a key factor structuring zooplankton communities in lotic systems. Zooplankton biomass in rivers and rheolimnic (flow-through) reservoirs is generally low relative to lakes with similar chlorophyll concentrations primarily because of nutritional constraints or flow (Basu et al. 1996, Acharya et al. 2005, Acharya et al. 2006). While zooplankton diversity and abundance vary wildly over time, communities are generally structured in flowing systems by a weak relationship to chlorophyll concentration and strong association with water residence time (Bledzki and Ellison 2000, Basu and Pick 2006, Obertegger et al. 2007, Claps et al. 2009, Rossetti et al. 2009). These factors create a mosaic of species patterns in river systems. Main channel and other lower water retention areas tend to support smaller rotifers and protozoa, while backwaters and pools with high retention can contain populations of larger crustacean plankton (Basu and Pick 1996, Bergfeld et al. 2009, Dickerson et al. 2009, Vang et al. 2010, Burdis and Hoxmeier 2011).

The recreational value of Lake St. Croix mainly centers on two factors: fishing and water quality/clarity, and zooplankton are important to understanding mechanisms that connect them. Zooplankton grazing has been shown to reduce algal blooms in rivers under conditions of high water retention and temperature (Gosselain et al. 1998). Sellers and Bukaveckas (2003) argue that zooplankton grazing explains why models of low flow conditions consistently overestimate algal production. Recent experimental work using Lake St. Croix zooplankton show they have significant

impact on algae biomass (Magdalene et al. 2013). River zooplankton are also important to phosphorus sedimentation and internal cycling, with different species contributing to these processes in different ways (Bledzki et al. 2000). In relation to higher trophic levels, zooplankton are direct food sources for native riverine fish species such as bigmouth buffalo (*Ictiobus cyprinellus*), paddlefish, shad (*Alosa* spp.), minnows, and the fry of many game species. Zooplankton indirectly support predators that feed on planktivorous insects or fish, and mussel species are known to selectively filter rotifers and other small plankton (Wong et al. 2003, Wong and Levinton 2006). In short, zooplankton are a mechanistic link between top-down (predation) and bottom-up (water quality) processes, and they are important for two critical features of aquatic habitats that are most valued by people—fish production and water clarity.

The spatial structure of a riverine reservoir system is also an important factor for zooplankton and planktivorous fish populations. In river systems zooplankton are carried downstream, so autochthonous production in high retention zones like reservoirs is the chief source of zooplankton biomass downstream (Guelda et al. 2005). In systems with reservoirs, reservoir drift out of high retention areas is often the primary source of plankton to lower retention areas (Havel et al. 2009). Consequently, fish consumption of zooplankton can be heaviest just below dams or reservoirs, tapering downstream exponentially (Cowell, 1967, Chang et al. 2008). In relation to silver and bighead carp introductions, physical structures are important because they provide both barriers to upstream migration and create refugia for zooplankton. Reservoirs then serve as continuous sources of food for downstream native species during invasion stress and could quickly seed populations for downstream waters should carp mitigation efforts succeed. In the St. Croix River, a hydro-dam at St. Croix Falls provides a barrier to upstream fish movement and creates a small reservoir with potential to serve as a zooplankton refuge and repopulation source for the lower river (see Figure 2).

Feeding Preferences and Impacts of Bighead and Silver Carp on Plankton

Silver and bighead carp are highly visible problem species due to their size and the propensity of the silver carp to leap out of the water in large numbers. Both species were brought to the United States in order to control algae in aquaculture ponds, but quickly escaped and found their way into the Mississippi River system by the late 1980s (Hinterthuer 2012). Studies of these species in ponds indicate they feed on algae and can reduce algal biomass, as the intended purpose of their introduction to aquaculture (Cremer and Smitherman 1980). However, both species also eat zooplankton, leading to overall reduction in algal grazing and most often to increased algal blooms in both natural and experimental systems (Opuszynski 1979, Lu et al. 2002). The mechanism behind this pattern is that both species filter food at size levels that overlap both zooplankton and algae (Allan 1976). If secretions of the labyrinthine organ are taken into account, these carp can effectively filter down to the level of bacteria (Kuznetsov 1977, Xie 1999). In short, both *Hypophthalmichthys* species are versatile omnivores whose feeding mechanisms result in both competition with and predation on zooplankton (Burke et al. 1986, Lu et al. 2002, Sampson et al. 2009).

Despite an abundance of experimental studies in ponds, predicting the impact of silver and bighead carp in natural systems is difficult. In their native habitat, bighead and silver carp have been found to be the primary factor structuring the zooplankton community (Xie and Yang 2000). Experimental

studies confirm an inverse relationship between carp biomass and crustacean zooplankton biomass including the elimination of zooplankton with long generation times (Lu et al. 2002) and a general reduction of all zooplankton abundance by as much as an order of magnitude (Burke et al. 1986). Exact results of size selection in dietary studies vary. Opuszynski (1981) reports that silver carp prefer smaller cladocerans and rotifers, while bighead prefer larger cladocera and copepods. Others have found that silver carp prefer algae, while bighead prefer zooplankton (Spaturu et al. 1983, Williamson and Garvey 2005). Dietary studies are confused by the fact that both fish species seem to be versatile omnivores and may switch food sources opportunistically. Compounding this variation, gut content studies are often biased against the rapidly digested crustacean zooplankton making it easy to confuse differential digestion with differential feeding (Bitterlich and Gnaiger 1984, Voros et al. 1997). One common theme to all work on *Hypophthalmichthys* diet is that they are size selective, and the taxonomic groups eliminated in impacted systems correspond to size classes (Sampson et al. 2009).

In natural systems similar to the Riverway, invasive *Hypophthalmichthys* species are known to reduce or eliminate zooplankton (Sellers Bukaveckas 2003, Cooke et al. 2009). The most recent work in the region shows that zooplankton have been more or less eliminated by silver and bighead carp in the Illinois River or, at best, reduced well below levels thought best for sportfish recruitment (DeBoer et al. 2016). While experimental manipulation is difficult in large ecosystems, removing carp from backwaters in the Illinois River resulted in higher copepod abundance with a less clear but positive impact on cladoceran abundance (Zalay and Casper 2016). Elimination of large zooplankton can have top-down and bottom-up effects. Many zooplankton are algal grazers, and in river and reservoir systems they are responsible for significant mitigation of algal blooms (Gosselain et al. 1998). Reduction or elimination of the larger crustacean zooplankton, particularly in the presence of cultural eutrophication, is likely to increase algal blooms (Cottingham et al. 2004).

Zooplankton are also food for native fish including threatened and endangered species (e.g. paddlefish), other ecologically important planktivores, and game fish (directly for some fry and indirectly for key prey species). Competition with *Hypophthalmichthys* species has been shown to reduce biomass of gizzard shad and bigmouth buffalo (Irons et al. 2007, Sampson et al. 2009) and may harm threatened or endangered native species like paddlefish and filter feeding unionid mussels (Wong and Levinton 2006). Less is known about impact on game fish, but carp can reduce zooplankton below levels generally thought to be needed to support game fish in rivers (Zalay and Casper 2016). Establishment of either *Hypophthalmichthys* species has the potential interfere with, compound, or otherwise interact with impacts from other stressors (e.g., cultural eutrophication and zebra mussels), dampening or eliminating the success of related management efforts.

Objectives

Little is known about the zooplankton community in the Riverway despite their importance as the mechanistic link between top-down and bottom-up ecological processes. Monitoring zooplankton represents a direct way to index changes to the Lake St. Croix ecosystem should bighead and/or silver carp become established. As suggested by Hintertheuer (2012), a better understanding of basic ecosystem structure that includes zooplankton is necessary to predict post-invasion impacts of and

remediation efforts for silver and bighead carp. Here we report results from a two-year assessment of zooplankton in Lake St. Croix and an upstream reservoir above a hydro-dam. This study was designed as a first step in understanding the possible impacts of bighead and silver carp in the context of an ongoing water quality monitoring program in the Riverway. Our primary objectives were to

- 1) Determine baseline information on zooplankton composition and distribution in Lake St. Croix;
- 2) Assess whether the reservoir above the hydro-dam at St. Croix Falls, Wisconsin, could serve as a refugium and source population for different types of zooplankton;
- 3) Determine whether zooplankton species are present that are preferentially grazed by silver and bighead carp; and
- 4) Develop a zooplankton monitoring protocol suitable for integration into existing monitoring programs in the Riverway.

Methods

Sampling Locations and Site Characteristics

In 2007 the NPS Great Lakes Inventory and Monitoring Network (GLKN) implemented a long-term water quality monitoring effort on the St. Croix River. This program was established to assess long-term trends in water quality and correlate observed trends to ecosystem factors such as invasive species, landscape dynamics, or weather and climate (Magdalene et al. 2008). Network and Riverway staff conduct monthly water quality monitoring at ten sites throughout the Riverway during ice-free months, typically April through November (Magdalene et al. 2008, VanderMeulen and Elias 2008). The four zooplankton sampling sites used in this study are a subset of GLKN sites and were chosen to provide a large area of coverage along Lake St. Croix (see Figure 1), including one site from three out of the four pools (covering upper, middle, and lower Lake St. Croix) and one site in the reservoir above the hydro-dam at St. Croix Falls (see Figure 2).

Water quality parameters were sampled in accordance with GLKN standard protocol (Magdalene et al. 2008), with the exception that for profiles of temperature, pH, specific conductivity, and dissolved oxygen, measurements were recorded at depths of 1 m, 4 m, and 1 m above the bottom (z-1) using a standard YSI sonde rather than at 1-m intervals from the top to the bottom of the water column.

Zooplankton Sampling and Identification

Samples were collected monthly from May through September in 2010 and from April to November in 2011. Only May to September samples from the two years were used for analysis, though results from April 2011 and October and November 2010 are reported for context. Zooplankton were sampled by taking a composite of three vertical tows at each site with a 35 μ m mesh tow net, 1-m-long with an aperture diameter of 30 cm. Each of the three tows were taken 3 m apart and individually rinsed into a condensing net between tows. The condensing net was 35 μ m mesh Nitex glued in a 10 cm diameter PVC pipe with a stoppered draining outlet. The condenser concentrated the individual tows into one sample which was rinsed off into a 50 mL Falcon tube. Samples were preserved in an approximately 65% solution of ethyl alcohol (ETOH) in the field, which was increased to 90% ETOH and refrigerated within 24 hours. In July of 2010 this process was repeated at three sub-sites, one on the GPS point for the GLKN site, one 10 meters towards the eastern shore, and another 10 meters towards the western shore. This design was intended for a standard ANOVA analysis for the July 2010 period.

Organisms were counted and identified on an Olympus BX50F4 microscope at magnification ranging from 40 $\times$ to 400 $\times$. Standard identification keys were used (Williams 1966, DeMelo and Hebert 1994, Lee et al. 2000, Taylor et al. 2002, Thorp and Covich 2010). Each Falcon tube sample was brought to 40 mL, rigorously agitated, sub-sampled with a 1 mL Hensen-Stempel pipette, and transferred to a 1 mL Sedgwick Rafter counting slide. This process was repeated until stable variance of the cumulative mean was achieved for smaller (<0.8 mm length) organisms (Colwell and Coddington 1994). Direct comparison was made against a full sample count (all organisms in the Falcon tube) for organisms >0.8 mm in length (Cladocera, Copepoda, and *Chaoborus* spp.) These larger organisms were counted by placing the entire 40 mL into a petri dish to compare to rafter

subsamples. After testing six subsamples in this manner, it was determined that two rafter cell counts per sample were sufficient to meet the variance criteria for smaller organisms and produce results within one standard deviation of the full count for larger organisms.

The first ten randomly selected organisms of each taxa present in a sub-sample were measured for biometric variables used to estimate biomass (Doohan 1973, Dumont et al. 1975, Bottrell et al. 1976, Pace and Orcutt 1981, McCauley 1984, Basu and Pick 1996). Stable variance of biometric values was achieved with as few as five organisms per taxa. However, this did not qualitatively appear to account for life cycle stage or sexual dimorphism in some crustacean species, so ten measurements were made per organism per sub-sample as a margin of safety (resulting in up to 20 measurements per taxa per sample). In this manner, biomass was estimated using site- and date-specific measurements rather than mean values or literature values. Biomass estimates are reported in micrograms per liter ($\mu\text{g/L}$) to preserve units used in analysis, but there is a 1:1 conversion to the mg/m^3 units used in other similar work. Graphical representations in figures show biomass converted to $\mu\text{g/m}^3$ for easier visualization.

Statistical Analysis

Two primary statistical approaches to analyses were conducted. First, a traditional ANOVA test using within-site replicates from July 2010 was conducted as a proof-of-concept measure early in the study. Separate one-way ANOVA tests were conducted on total biomass and taxa richness for July 2010 samples, not corrected for multiple comparisons (two). Tests were performed in SYSTAT and SigmaPlot (Systat Software, San Jose, California). For tests where equal variance on transformed data failed, Kruskal-Wallis one-way analysis on ranks was used with pair-wise multiple comparisons determined using Dunn's method at $p < 0.05$. For tests with normal distribution and equal variance, the Holmes-Sidak method for multiple comparisons was used.

Second, the entire data set was analyzed in PRIMER 7 using a combination of ordination and PERMANOVA (Clarke 1993, Clarke and Gorley. 2015). PERMANOVA is a permutational MANOVA technique used for complex community data that fail the requirements of traditional F-statistic assumptions. This method was chosen due to 1) high costs of analyzing enough replicates for a solid ANOVA design, 2) unequal variance of many samples, and 3) the strength of PERMANOVA to handle complex biological community data with patchy distributions of species (Clarke 1993, Clarke and Gorley. 2015). Site, year, and month were used as factors in the analysis, with temperature, chlorophyll-*a* concentration, and specific conductivity (all factors at depths of both 1 and 4 m) tested as environmental factors. Site-specific flow was not analyzed directly by this design because variations in flow regime are the same for each site on each date, making discharge indistinguishable from sample period. Principal components analysis (PCA) plotted biomass-weighted taxa associations, with major taxonomic group, sample site, and sample period independently plotted against the same axes. Canonical analysis of principal components (CAP) was used to clarify groupings shown in the ordination.

Results

Site Characteristics

Water quality results for 2010 are summarized in Table 2; results from 2011 have been reported in VanderMeulen (2012) and are summarized in Table 3. Water quality measurements were not taken with a sonde in June 2010 due to equipment failure. Complete water quality results are regularly published by the GLKN, while results relevant to site differences for this study are summarized in Lenz (2004), VanderMeulen and Elias (2008), and VanderMeulen (2011).

Table 2. Water quality results from May to September, 2010, taken at four St. Croix River sites (National Park Service, Great Lakes Inventory and Monitoring site designations) and used in PERMANOVA analyses. Sonde measurements were made each meter down to z+1 m, but analyses for this study were restricted to values taken at depths of 1 m and 4 m below the surface. Full results are reported in VanderMeulen (2012). These results were used in PERMANOVA analysis.

Site	Month	1 m below surface			4 m below surface		
		DO ($\mu\text{g/L}$)	Con ($\mu\text{s/cm}^3$)	Temp ($^{\circ}\text{C}$)	DO ($\mu\text{g/L}$)	Con ($\mu\text{s/cm}^3$)	Temp ($^{\circ}\text{C}$)
SACN05	May	10.1	155.1	10.5	10	155.6	10.5
	July	6.1	184	26.7	6.5	184.1	26.5
	Aug	6.4	172.5	26	6.6	175.6	25.9
	Sept	8.1	169.8	18.3	8	171	18.3
SACN07	May	10	210.3	13.3	10	209.9	13.3
	July	7.4	188.7	27	5.7	192.1	26.1
	Aug	7.2	188.4	27.1	6.1	186.1	26.5
	Sept	7	183.6	20.8	6.6	184.6	20.1
SACN09	May	10.2	220.5	13.1	10.2	220.5	13.1
	July	8.3	182.8	26.4	5.8	173.2	24.2
	Aug	6.3	139.8	22.4	6.3	140.2	22.4
	Sept	5.9	174.7	20.9	6.1	179.3	20.4
SACN11	May	9.3	194.7	12.6	9.3	194.9	12.6
	July	9.7	180.2	30	6.4	182	24.7
	Aug	6.8	205.2	26.6	5.2	204.4	26.1
	Sept	5.3	173.9	22.4	4.9	172.2	21.4

Suspended sediment and nutrient loads from Riverway tributaries reported in Lenz et al. (2003) explain some of the differences between Lake St. Croix pools and upstream reservoirs based on tributary input. Because Pool 1 is fed directly by the upstream waters of the St. Croix River, its

physical, chemical, and biological conditions are more heavily influenced by river conditions. Within-pool processes become progressively more important to river conditions from Pool 1 to Pool 4, explaining many of the spatial patterns in water quality parameters for Lake St. Croix (VanderMeulen 2011).

Basic physical features of the sites chosen for this study are listed in Table 1, modified from Robertson and Lenz (2002). Pools are similar in length, an important factor for zooplankton drift, but retention time is much shorter in the reservoir above the hydro-dam than at other sites. Our sampling sites can be ranked in order of retention time, with SACN11 and SACN07 the highest, SACN09 intermediate, and SACN05 the lowest (Lenz et al. 2003, VanderMeulen and Elias 2008,

Table 3. Water quality results from April to November, 2011, taken at four St. Croix River sites (National Park Service, Great Lakes Inventory and Monitoring site designations) and used in PERMANOVA analyses. Sonde measurements were made at each meter down to z+1 m, but analyses were restricted to values taken at depths of 1 m and 4 m below the surface. Chlorophyll was taken via an integrated sample of surface-to-2 m. Full results are reported in VanderMeulen (2012).

Site	Month	Chl-a ($\mu\text{g/L}$)	1 m below surface			4 m below surface		
			DO ($\mu\text{g/L}$)	Con ($\mu\text{s/cm}^3$)	Temp ($^{\circ}\text{C}$)	DO ($\mu\text{g/L}$)	Con ($\mu\text{s/cm}^3$)	Temp ($^{\circ}\text{C}$)
SACN05	April	2.98	9.91	83.00	9.56	9.84	83.80	9.54
	May	4.92	9.06	117.30	15.76	9.01	118.50	15.77
	June	4.33	6.96	152.70	24.58	6.81	148.30	24.23
	July	5.96	6.93	175.10	25.87	6.64	175.40	25.68
	Aug	3.67	6.22	125.70	21.71	6.16	126.60	21.71
	Sept	2.34	8.97	191.20	19.94	8.47	191.80	18.74
SACN07	April	2.72	11.28	91.30	8.42	11.25	91.50	8.26
	May	9.50	9.63	123.90	14.36	9.30	123.70	12.98
	June	6.39	7.49	147.20	25.15	6.42	144.80	21.02
	July	4.31	5.87	170.30	27.00	5.78	170.10	27.01
	Aug	4.17	6.03	113.80	25.09	5.59	116.40	24.17
	Sept	7.24	7.05	206.90	22.19	6.82	207.20	22.13
	Nov	8.25	11.06	227.90	17.42	10.20	227.10	16.48
SACN09	April	2.73	11.50	98.30	7.88	11.43	99.50	7.64
	May	8.43	9.87	127.30	12.93	9.73	127.50	12.63
	June	10.94	7.46	149.90	22.46	6.84	149.10	21.26
	July	3.85	6.04	177.30	26.43	5.49	176.40	26.21
	Aug	4.22	5.06	125.40	24.32	5.02	124.30	24.30
	Sept	8.41	6.51	203.90	22.67	6.36	211.80	21.97
	Nov	11.39	11.68	244.30	8.50	6.36	211.80	21.97
SACN11	April	2.45	10.86	145.40	5.29	10.77	144.00	5.00
	May	9.76	10.46	148.30	11.15	9.89	146.60	9.90
	June	6.36	7.67	149.50	21.55	6.98	146.70	19.88
	July	2.46	6.18	178.20	25.97	6.02	177.40	25.88
	Aug	4.76	5.22	193.60	25.06	5.08	193.40	25.04
	Sept	1.92	4.78	197.80	23.36	4.42	196.90	22.84

VanderMeulen 2011). Site SACN05 is above the hydro-dam at St. Croix Falls, an area with a deceptively reservoir-like appearance, but with a retention time lower than all the major pools in Lake St. Croix by an order of magnitude. This site was selected because the dam is a physical barrier to upstream migration of silver and bighead carp, and in other rivers above-dam sites act as feeder systems for zooplankton (Havel et al. 2009). Site SACN09 was chosen by GLKN using randomized design to fall somewhere in pool 2 of Lake St. Croix, but ended up at the very upstream point of pool 2 in the Hudson Narrows (see Figure 2). Retention time data from pool 2 (see Table 1) is not characteristic of conditions at site SACN09, which has a very low retention time as a constriction between pools. The sandy bottom here is in constant fluctuation, and flows tend to be highly variable and sensitive to rainfall events (personal observation of all three authors during work requiring SCUBA).

In addition, in contrast to SACN07 and SACN11, SACN09 is well mixed and does not stratify with respect to temperature or dissolved oxygen (VanderMeulen and Elias 2008, VanderMeulen 2011). Aside from the features that decrease retention time, total flow is similar at all sample sites on any given day but can vary within and between years (Figure 3). Standing algal stock as indexed by chlorophyll-*a* concentration is generally lowest at SACN05 and increases with each site downstream; however in some years productivity is highest at Stillwater, Minnesota, (SACN07) and Prescott, Wisconsin, (SACN11), and lower at Hudson, Wisconsin (SACN09). Recent work on algal densities showed strong year-to-year variation from 2011 to 2012, but in general algal densities are higher at the downstream pools (Magdalene et al. 2013).

The primary differences among measured water quality parameters of the four sites are the result of stratification in late summer. Lake St. Croix is known to regularly stratify thermally and chemically (VanderMeulen and Elias 2008, VanderMeulen 2011). The 2010 results show similar patterns to other years, with deoxygenated waters at depth for Lake St. Croix sites SACN07 and SACN11 in August, but not in the reservoir above St. Croix Falls (site SACN05) or the well mixed, low retention Lake St. Croix (site SACN09) (see Table 2). PERMANOVA detected no site-to-site differences in water quality parameters including chlorophyll-*a* concentrations, nor were there interactions among water quality variables and site. Simple correlations ran independently of the ANOVA and PERMANOVA tests show that dissolved oxygen, temperature, and chlorophyll-*a* correlate to month but not site (Table 4).

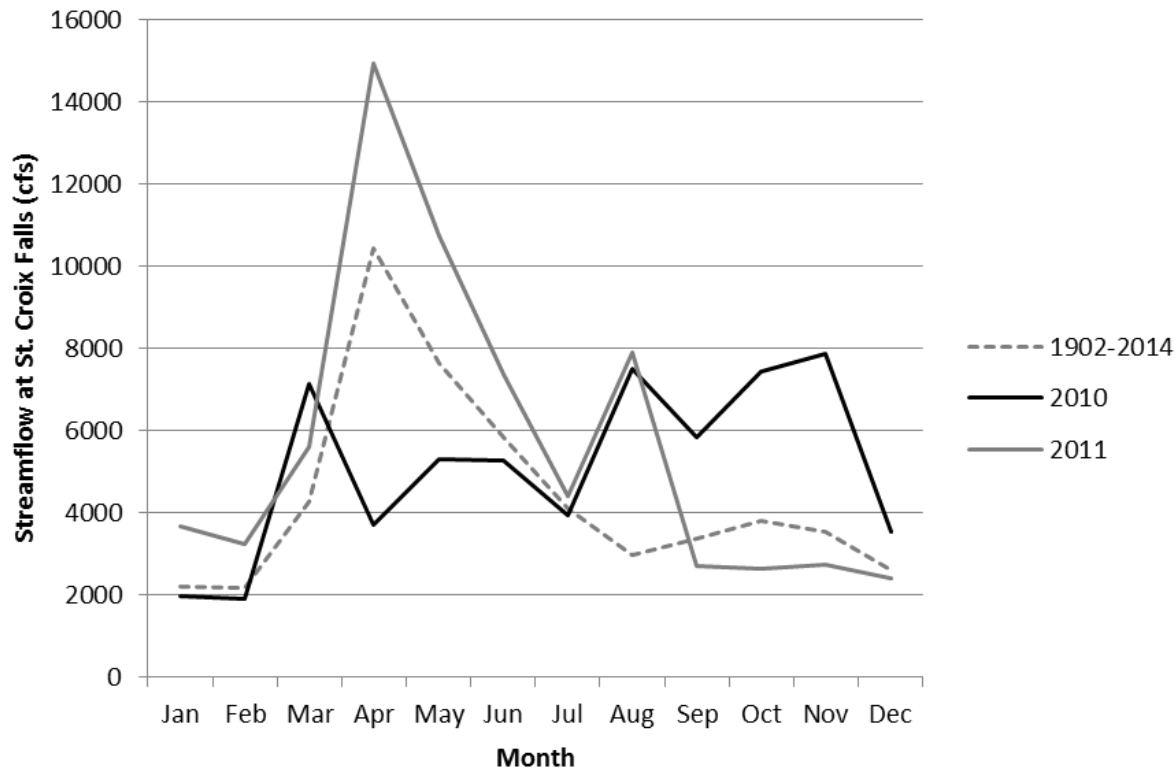


Figure 3. Streamflow (in cubic feet per second, or cfs) as measured by a gage at St. Croix Falls, Wisconsin, in 2010 and 2011, with reference to 112-year mean. This gage is operated and maintained by the U.S. Geological Survey's Northwest Wisconsin field office, in cooperation with Excel Energy and the Wisconsin Department of Natural Resources.

Table 4. Correlation matrix between water quality results from 2010 and 2011 (r^2). Values over |0.5| are highlighted.

Parameter	DO1	Con1	Temp1	Site	Month	Chl-a
DO1	1	--	--	--	--	--
Con1	-0.13306574	1	--	--	--	--
Temp1	-0.789837192	0.243501945	1	--	--	--
Site	-0.070503999	0.25399047	-0.019242224	1	--	--
Month	-0.534234154	0.607194615	0.501546639	0.052175423	1	--
Chl-a	0.25451516	0.255607292	0.203439729	0.027624029	0.020553821	1

Zooplankton Counts and Community Analyses

A total of 96 taxa from 53 genera of pelagic zooplankton were identified during the 2010 and 2011 sampling periods (Table 5). Size of plankton ranged with taxa from testate protists (mean 80 μm diameter) to the largest crustacean plankton *Leptodora kindtii* (up to 3 cm long) (Figure 4). Yearly mean number of taxa per site by taxonomic group are shown in Figure 5. Mean biodiversity per site by taxonomic group are shown in Figure 6. Biomass estimates, total and broken down by major

taxonomic group, are shown over time in Figures 7 through 11. Common diversity indices are summarized in Table 6; though not analyzed, these results are presented for future comparison.

We determined with standard ANOVA for the July 2010 period that taxa richness was significantly higher at sites SACN07, SACN09, and SACN11 than at SACN05, ($F(3,10) = 14.44$, $p < 0.001$). Calculations of total biomass for the Riverway in July 2010 were skewed by a single *Holopedium gibberum*, a larger crustacean with a gelatinous matrix. Removing this point resulted in a normally distributed total zooplankton biomass ($\log x+1$ transformed) with equal variance. A one-way ANOVA with Holmes-Sidak for multiple pair-wise comparisons confirmed the test on ranks, showing biomass at sites SACN11 and SACN07 was significantly higher than both SACN09 and SACN05 ($F(3,9) = 10.834$, $p = 0.002$). Leaving in the *Holopedium* from SACN05, running the test without equal variance produced similar but less significant results, where biomass was significantly higher at SACN07 and SACN11 than at SACN09 and SACN05 ($H = 11.00$ with 3 df, $p = 0.012$).

Table 5. Zooplankton taxa (35 μm + tow net) sampled May to September, 2010 and 2011, from Lake St. Croix and above the St. Croix Falls dam. Taxa in parentheses are morphologically strong but not genetically confirmed identifications. Taxa in brackets are questionable, supported by provisional evidence, but were not keyed out or individuals were not whole or complete. Taxa identified using genetic analyses are indicated with an asterisk (Boyer and Lafrancois 2014).

Taxonomic Group	Taxonomic Sub-group	Scientific Name
Subclass Copepoda	Order Cyclopoida	<i>Cyclops scutifer</i>
	Order Cyclopoida	<i>Diacyclops</i> spp.
	Order Cyclopoida	<i>Diacyclops nanus</i>
	Order Cyclopoida	<i>Megacyclops (viridis)</i>
	Order Cyclopoida	<i>Mesocyclops americanus</i>
	Order Cyclopoida	<i>Mesocyclops edax</i>
	Order Cyclopoida	<i>Microcyclops</i> sp.
	Order Cyclopoida	<i>Paracyclops</i> sp.
	Order Calanoida	<i>Leptodiaptomus (minutus)</i>
	Order Calanoida	<i>Skistodiaptomus pallidus</i>
	Order Calanoida	<i>Skistodiaptomus oregonensis</i>
	Suborder Cladocera	<i>Alona</i> sp.
	Suborder Cladocera	<i>Bosmina Eubosmina freyi</i> *
	Suborder Cladocera	<i>Bosmina Sinobosmina leideri</i> *
	Suborder Cladocera	<i>Bosmina Eubosmina [maritima]</i>
	Suborder Cladocera	<i>Bosmina Eubosmina coregoni</i>
	Suborder Cladocera	<i>Ceriodaphnia</i> sp.

Table 5 (continued). Zooplankton taxa (35 µm + tow net) sampled May to September, 2010 and 2011, from Lake St. Croix and above the St. Croix Falls dam. Taxa in parentheses are morphologically strong but not genetically confirmed identifications. Taxa in brackets are questionable, supported by provisional evidence, but were not keyed out or individuals were not whole or complete. Taxa identified using genetic analyses are indicated with an asterisk (Boyer and Lafrancois 2014).

Taxonomic Group	Taxonomic Sub-group	Scientific Name
Subclass Copepoda (continued)	Suborder Cladocera	<i>Chydorus piger</i>
	Suborder Cladocera	<i>Chydorus sphaericus</i>
	Suborder Cladocera	<i>Daphnia ambigua</i>
	Suborder Cladocera	<i>Daphnia longiremus</i>
	Suborder Cladocera	<i>Daphnia mendotae</i>
	Suborder Cladocera	<i>Daphnia retrocurva</i>
	Suborder Cladocera	<i>Diaphanosoma</i> sp.
	Suborder Cladocera	<i>Graptoleberis</i> sp.
	Suborder Cladocera	<i>Holopedium gibberum</i>
	Suborder Cladocera	<i>Leptodora kindtii</i>
	Suborder Cladocera	<i>Polyphemus</i> sp.
Phylum Rotifera	--	<i>Adineta</i> sp.
	--	<i>Ascomorpha saltans</i>
	--	<i>Anuraeopsis</i> sp. (incl. <i>A. fissa</i>)
	--	<i>Asplanchna priodonta</i>
	--	<i>Asplanchna herricki</i>
	--	<i>Brachionus calyciflorus</i>
	--	<i>Cephalodella</i> sp.
	--	<i>Collotheca</i> sp.
	--	<i>Conochiloides</i> spp.
	--	<i>Conochilus unicornis</i>
	--	<i>Colurella</i> sp.
	--	<i>Dicranophorus</i> sp.
	--	<i>Enteroplea</i> sp.
	--	<i>Euchlanis alata</i>
	--	<i>Euchlanis dilatata</i>
	--	<i>Filinia longiseta</i>

Table 5 (continued). Zooplankton taxa (35 µm + tow net) sampled May to September, 2010 and 2011, from Lake St. Croix and above the St. Croix Falls dam. Taxa in parentheses are morphologically strong but not genetically confirmed identifications. Taxa in brackets are questionable, supported by provisional evidence, but were not keyed out or individuals were not whole or complete. Taxa identified using genetic analyses are indicated with an asterisk (Boyer and Lafrancois 2014).

Taxonomic Group	Taxonomic Sub-group	Scientific Name
Phylum Rotifera (continued)	--	<i>Filinia terminalis</i>
	--	<i>Gastropus</i> sp.
	--	<i>Kellicottia bostoniensis</i>
	--	<i>Keratella cochlearis</i> (incl. <i>hispida</i> , <i>robusta</i> , and <i>cochlearis</i> varieties)
	--	<i>Keratella crassa</i>
	--	<i>Keratella earlinae</i>
	--	<i>Keratella hiemalis</i>
	--	<i>Keratella serrulata</i>
	--	<i>Keratella tecta</i>
	--	<i>Keratella testudo</i>
	--	<i>Lecane inermis</i>
	--	<i>Lecane mira</i>
	--	<i>Lecane tenuiseta</i>
	--	<i>Lepadella acuminata</i>
	--	<i>Lepadella patella</i>
	--	<i>Lepadella ovalus</i>
	--	<i>Monostyla bulla</i>
	--	<i>Monostyla copeis</i>
	--	<i>Monstyla lunaris</i>
	--	<i>Mytilina</i> sp.
	--	<i>Polyarthra</i> sp.
	--	<i>Polyarthra dolichoptera</i>
	--	<i>Polyarthra euryptera</i>
	--	<i>Polyarthra major</i>
	--	<i>Polyarthra remata</i>
	--	<i>Polyarthra vulgaris</i>
	--	<i>Pompholyx sulcata</i>

Table 5 (continued). Zooplankton taxa (35 µm + tow net) sampled May to September, 2010 and 2011, from Lake St. Croix and above the St. Croix Falls dam. Taxa in parentheses are morphologically strong but not genetically confirmed identifications. Taxa in brackets are questionable, supported by provisional evidence, but were not keyed out or individuals were not whole or complete. Taxa identified using genetic analyses are indicated with an asterisk (Boyer and Lafrancois 2014).

Taxonomic Group	Taxonomic Sub-group	Scientific Name
Phylum Rotifera (continued)	--	<i>Rotaria</i> sp.
	--	<i>Synchaeta</i> sp.
	--	<i>Synchaeta oblonga</i>
	--	<i>Synchaeta kitina</i>
	--	<i>Testudinella</i> sp.
	--	<i>Trichocerca cylindrica</i>
	--	<i>Trichocerca multicroinis</i>
	--	<i>Trichocerca pusilla</i>
	--	<i>Trichocerca similis</i>
Protozoa (Testate)	--	<i>Codonella</i> sp.
	--	<i>Arcella</i> sp.
	--	<i>Arcella discoides</i>
	--	<i>Arcella (hemisphaera, intermedia, gibbosa complex)</i>
	--	<i>Arcella megastoma</i>
	--	<i>Arcella vulgaris</i>
	--	<i>Centropyxis aculeata</i>
	--	<i>Cyclopyxis</i> spp.
	--	Cyphoderiidae
	--	<i>Diffugia</i> spp.
	--	<i>Diffugia corona</i>
	--	<i>Diffugia globosa</i>
	--	<i>Diffugia lobostoma</i>
	--	<i>Diffugia viscidula</i>
	--	[<i>Geopyxella</i> sp.]
	--	<i>Euglypha</i> sp.
	--	<i>Trinema</i> sp.



Figure 4. Zooplankton sampled from Lake St. Croix and the reservoir above St. Croix Falls, Wisconsin, ranged in size from testate protista (*Centropyxis aculeata*, inset) to the relatively enormous *Leptodora kindtii*, a cladoceran predator that can grow up to three centimeters long.

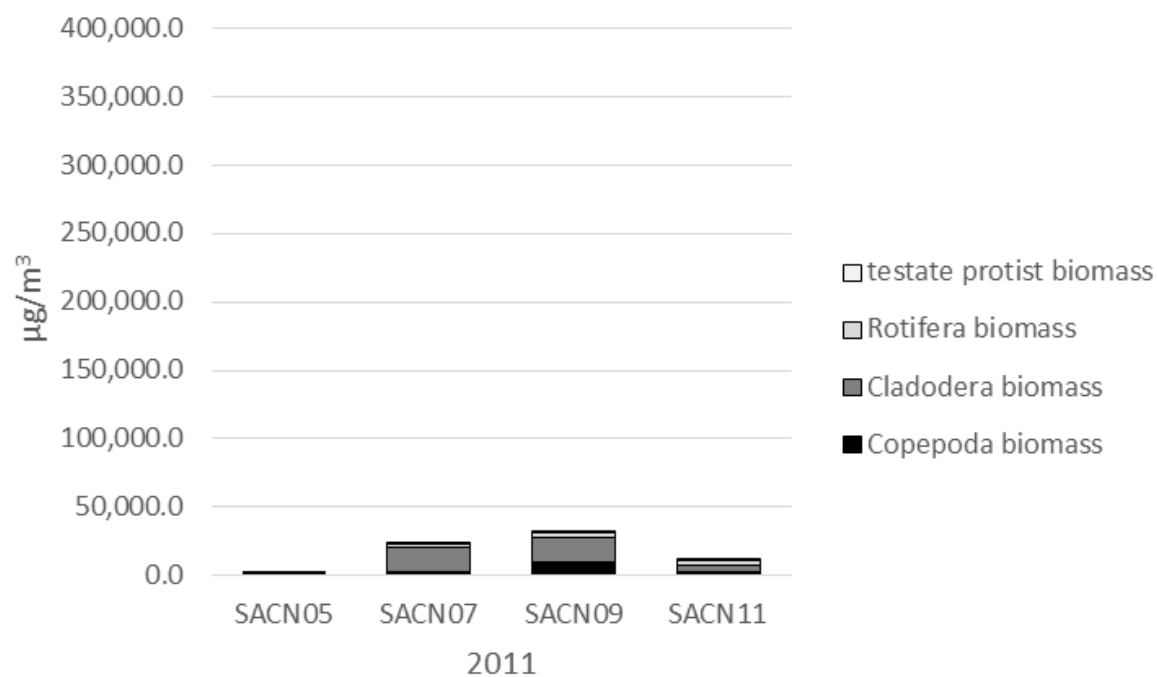
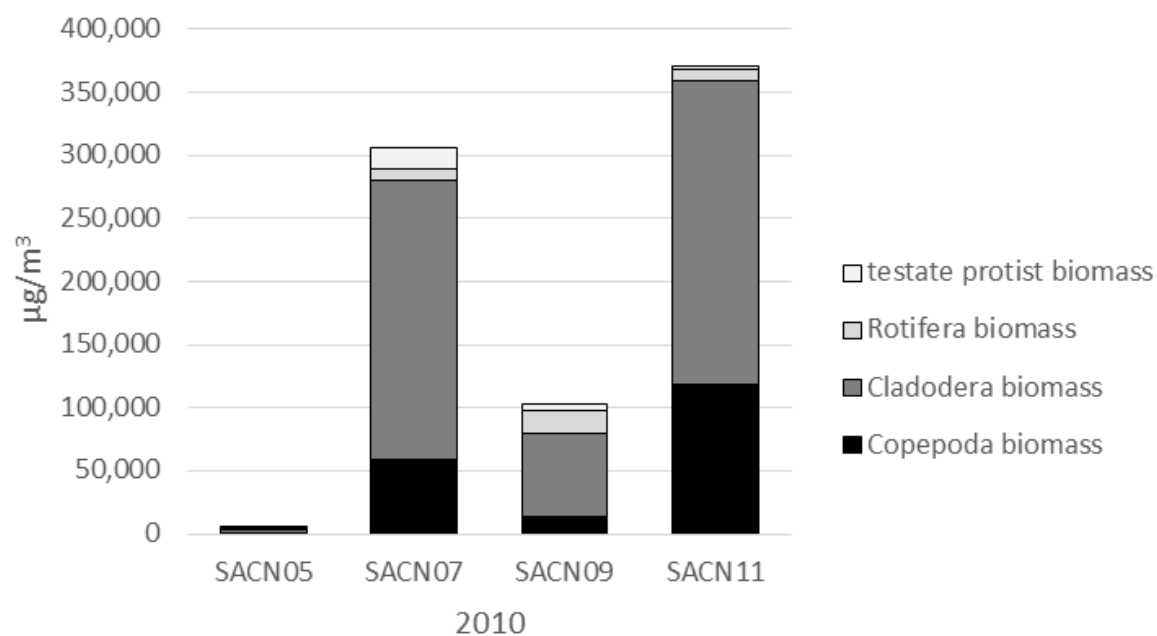


Figure 5. Mean zooplankton biomass (micrograms per liter) from May to September of 2010 and 2011 sorted by site and major taxonomic group. Samples are from Lake St. Croix (Minnesota/Wisconsin) and above the reservoir at St. Croix Falls, Wisconsin.

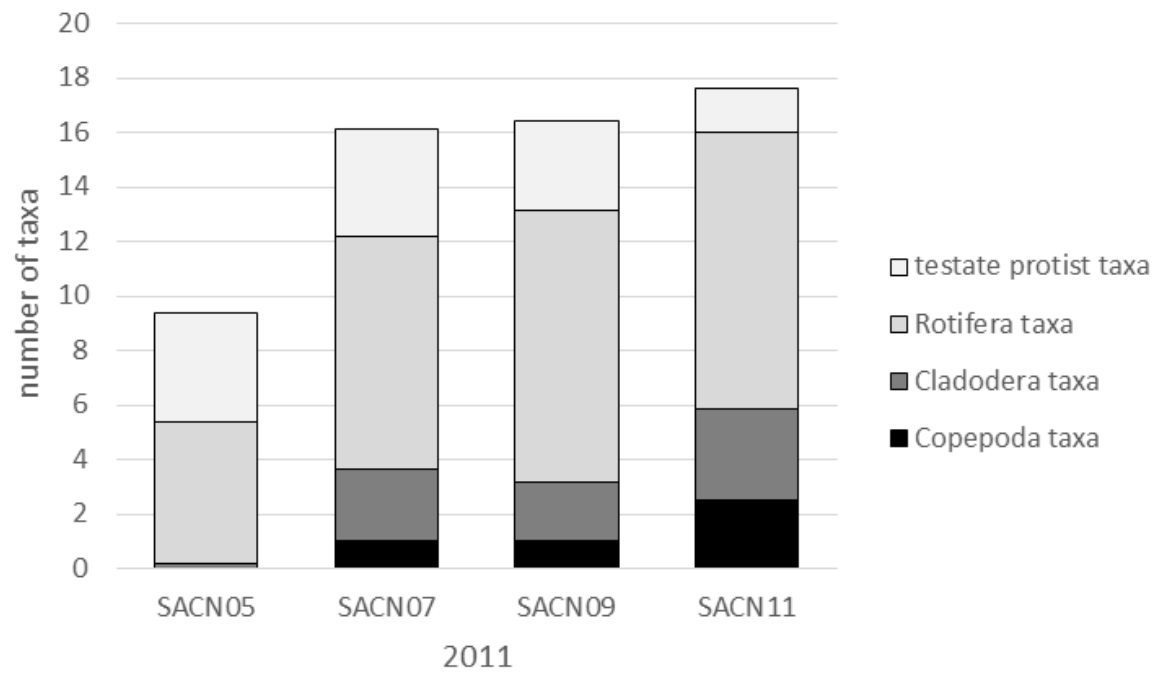
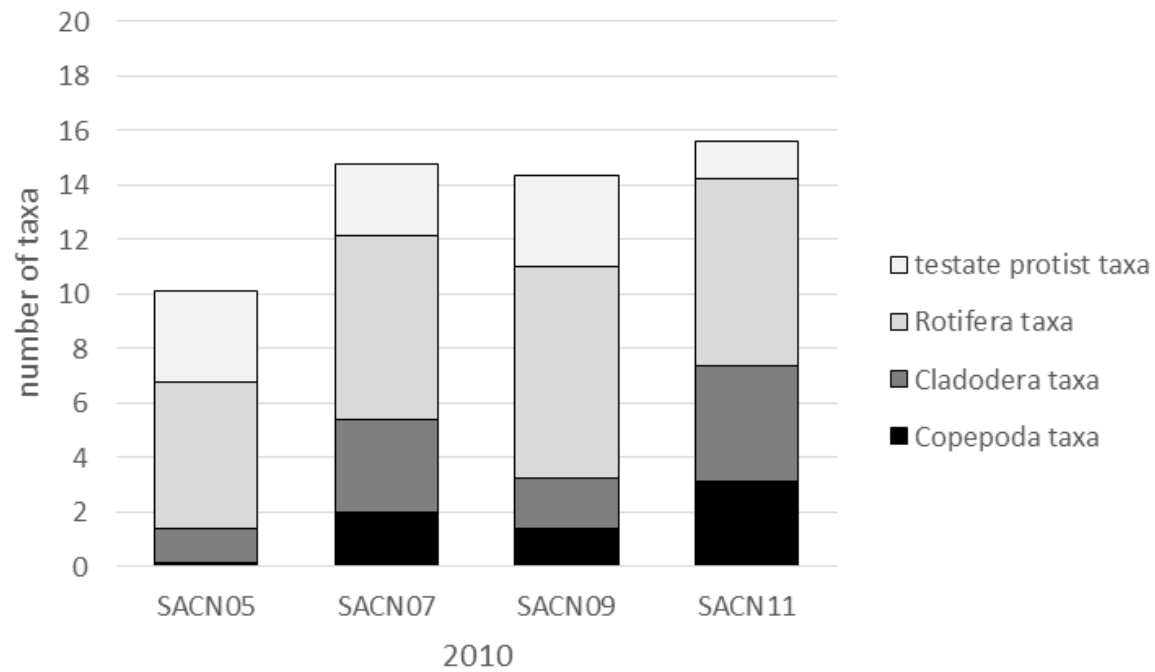


Figure 6. Number of zooplankton taxa from each of the four major taxonomic groups collected from Lake St. Croix (Minnesota/Wisconsin) and above the dam at St. Croix Falls, Wisconsin, May to September, 2010 and 2011.

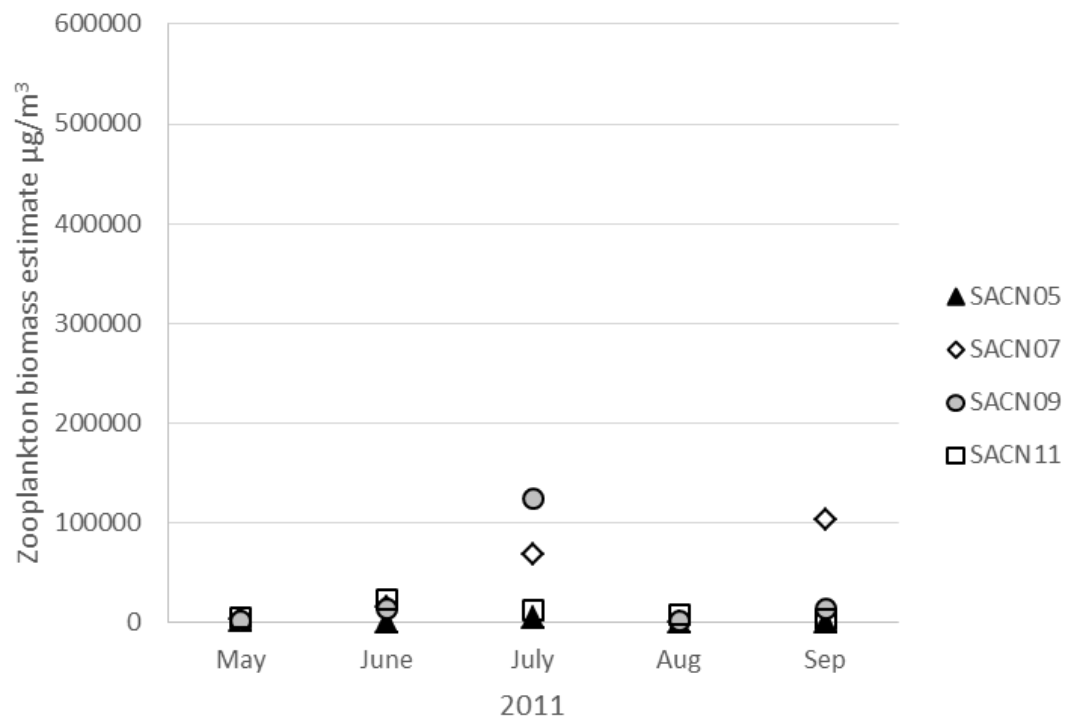
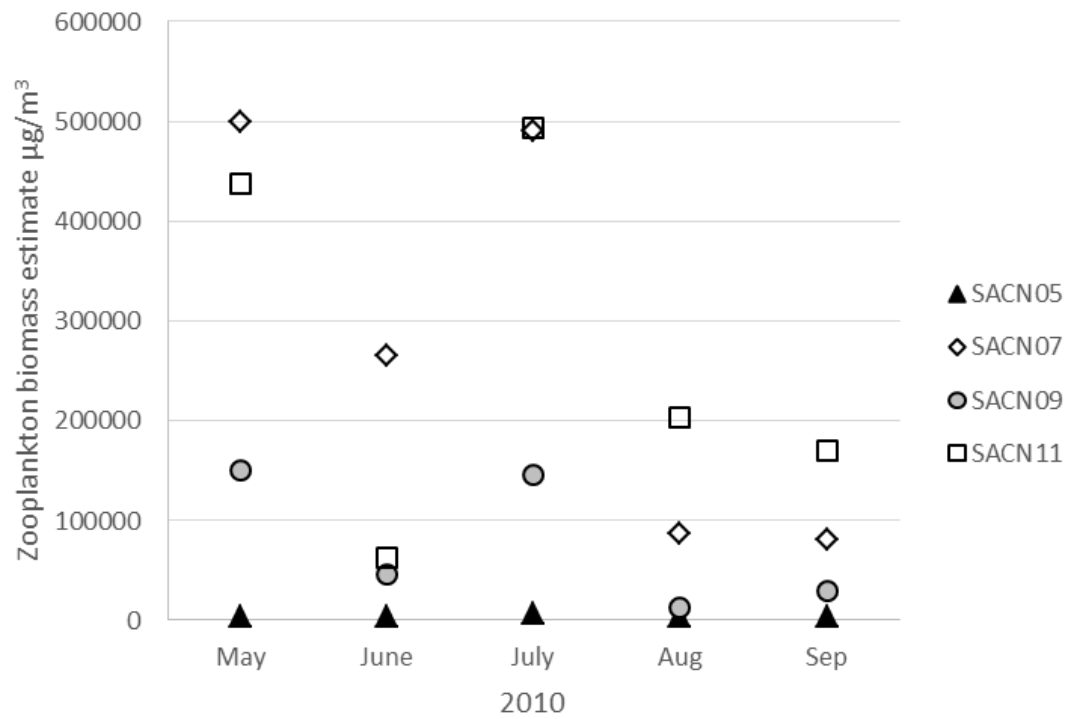


Figure 7. Monthly total zooplankton biomass at three sites in Lake St. Croix (Minnesota/Wisconsin) and above St. Croix Falls, Wisconsin, in 2010 and 2011.

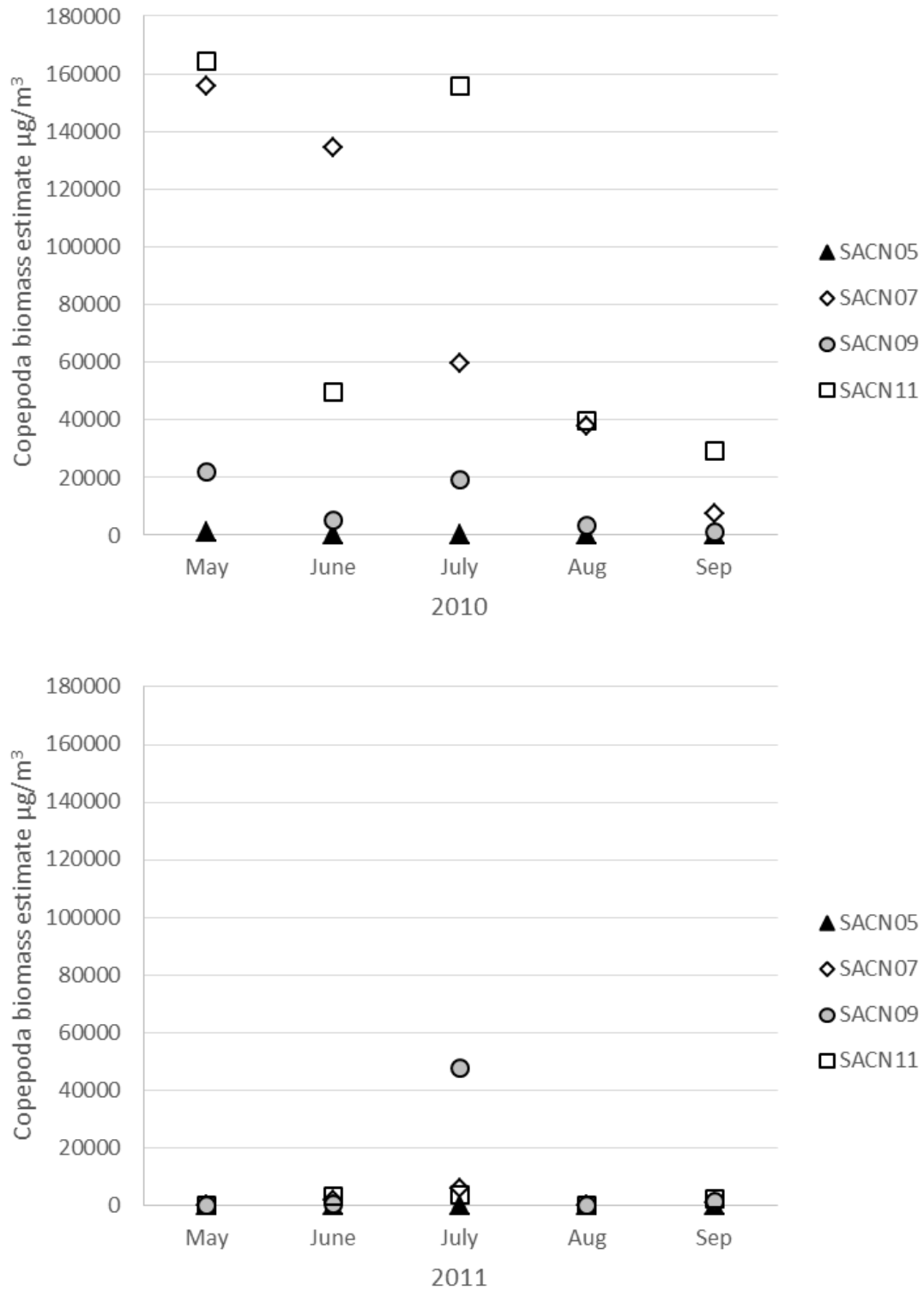


Figure 8. Monthly copepod biomass at three sites in Lake St. Croix (Minnesota/Wisconsin) and above St. Croix Falls, Wisconsin, in 2010 and 2011.

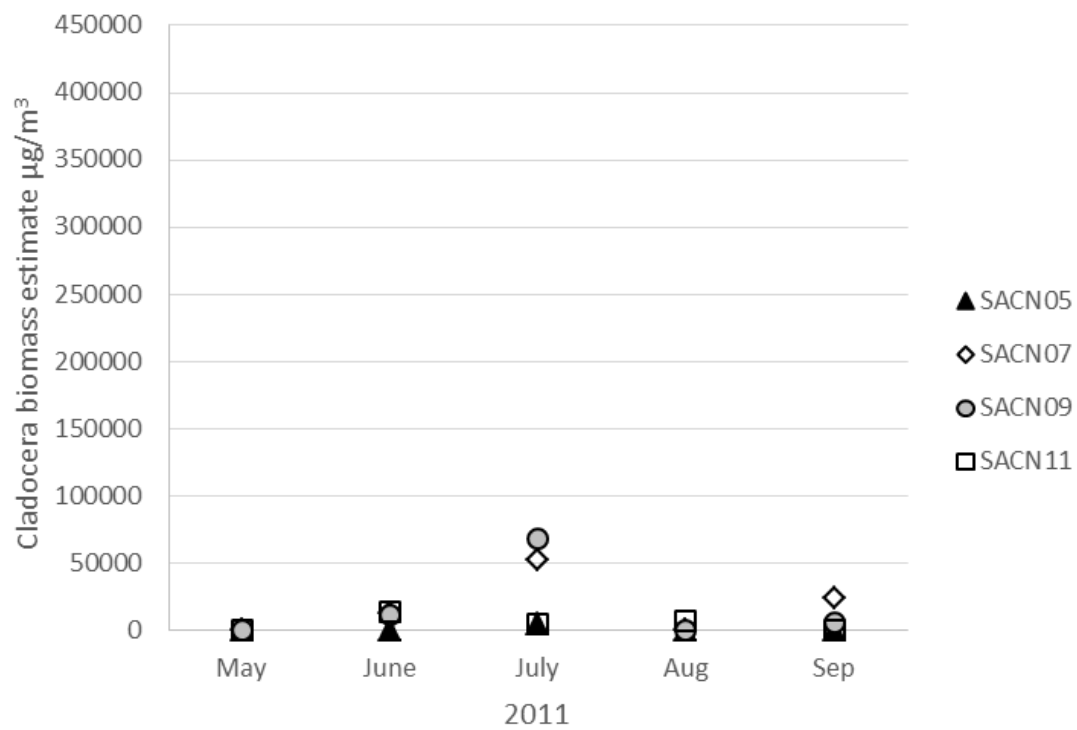
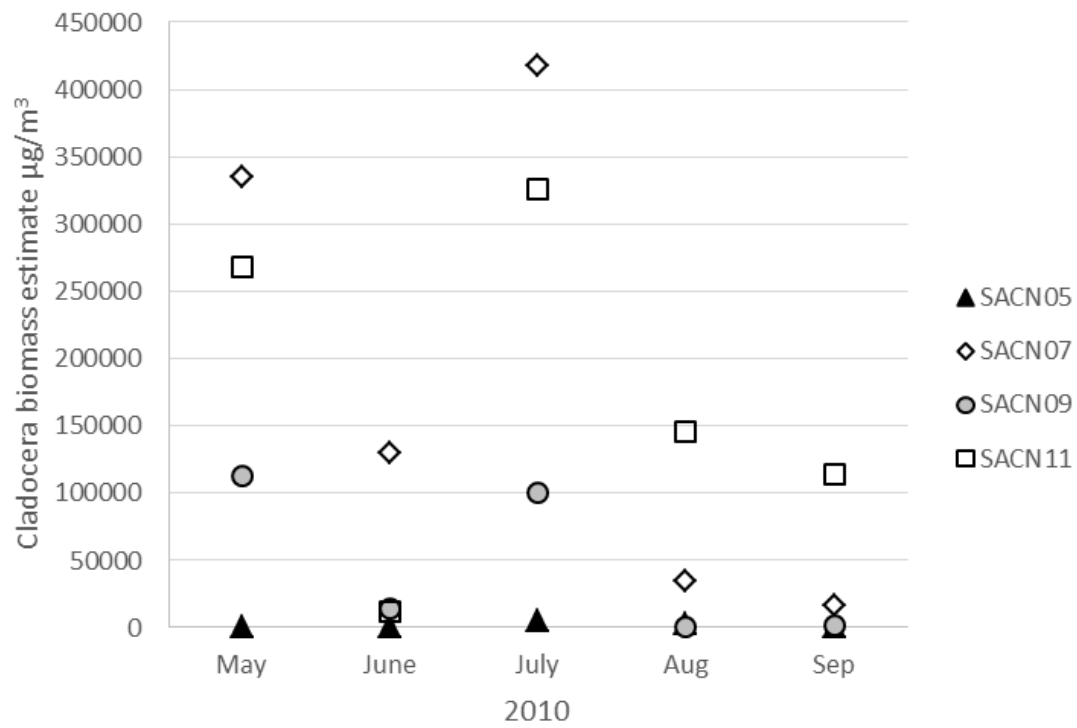


Figure 9. Monthly cladoceran biomass at three sites in Lake St. Croix (Minnesota/Wisconsin) and above St. Croix Falls, Wisconsin, in 2010 and 2011.

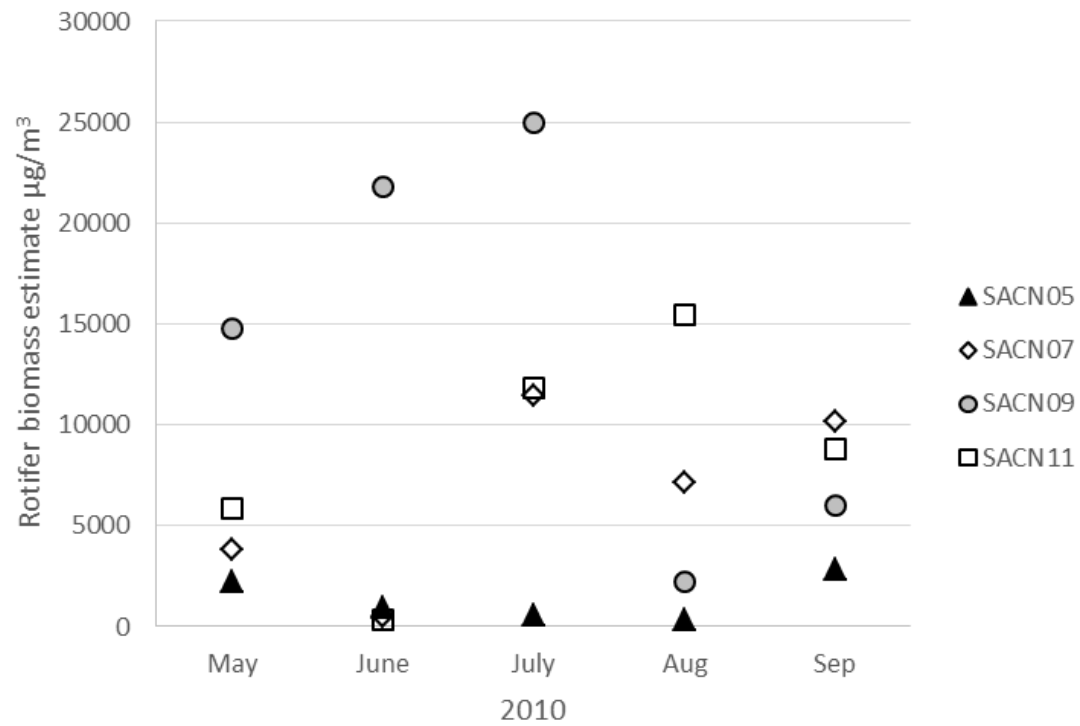


Figure 10. Monthly rotifer biomass at three sites in Lake St. Croix (Minnesota/Wisconsin) and above St. Croix Falls, Wisconsin, in 2010 and 2011.

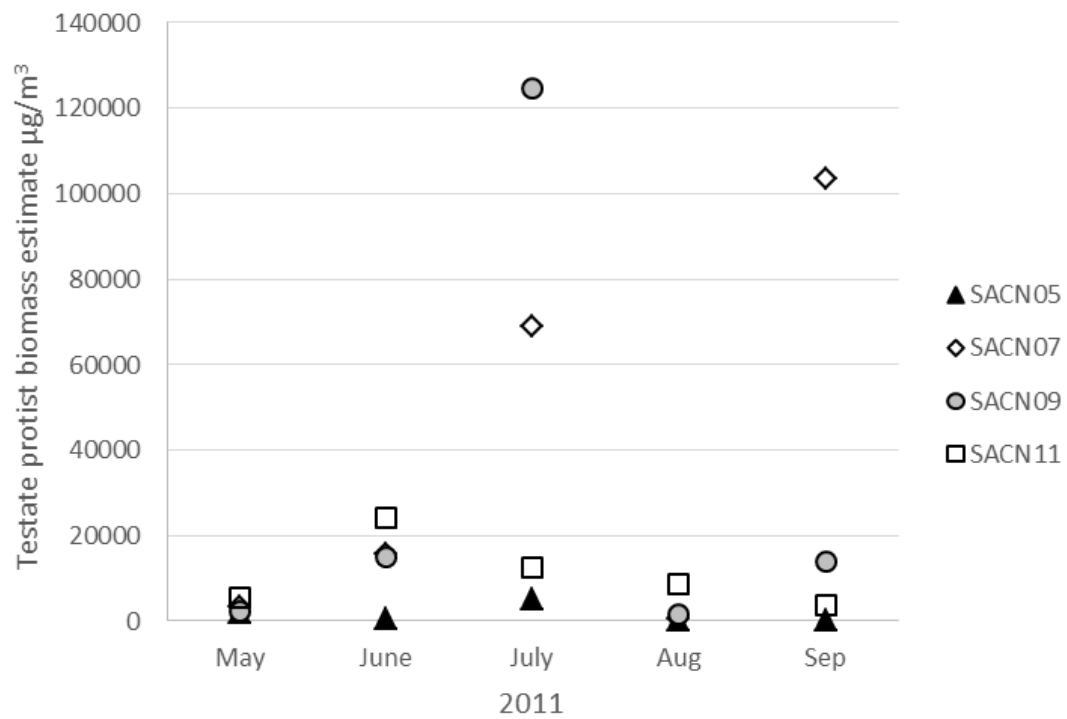
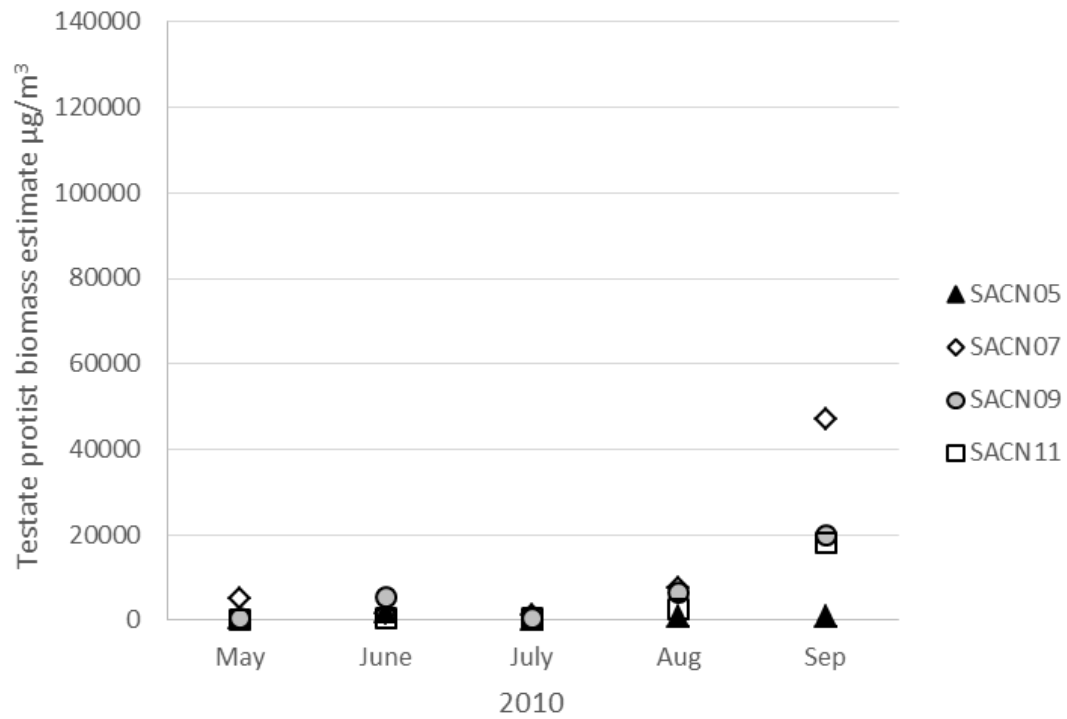


Figure 11. Monthly testate protist biomass at three sites in Lake St. Croix (Minnesota/Wisconsin) and above St. Croix Falls, Wisconsin, in 2010 and 2011.

Table 6. Common diversity indices for zooplankton samples from the St. Croix River (Minnesota / Wisconsin), 2010 and 2011, calculated in PRIMER 7. Results include number of taxa (lowest identifiable resolution), Margalef's diversity index (d), species evenness (J'), Brillouin diversity, Shannon diversity (H') formulated using natural log, and Simpson's diversity (1-λ'). Asterisks indicate samples with taxa distributions incompatible with assumptions of that index.

Site	Month	Year	# taxa	Margalef d	J'	Brillouin	H'(loge)	1- λ'
SACN05	May	2010	10	6.3	0.8	0.8	1.9	1.1
	June	2010	14	11.6	0.8	0.6	2.2	1.3
	July	2010	11	3.3	0.2	0.3	0.4	0.2
	July	2010	12	13.5	0.8	0.3	1.9	1.4
	Aug	2010	15	9.8	0.5	0.3	1.2	0.6
	Sep	2010	9	6.3	0.6	0.6	1.4	0.9
SACN07	May	2010	12	1.8	0.8	1.9	1.9	0.8
	June	2010	14	2.3	0.6	1.6	1.6	0.7
	July	2010	18	2.7	0.5	1.4	1.5	0.7
	July	2010	19	2.8	0.5	1.4	1.4	0.6
	July	2010	19	3.1	0.5	1.4	1.4	0.7
	Aug	2010	23	4.9	0.7	1.9	2.1	0.9
	Sep	2010	17	3.6	0.6	1.4	1.6	0.7
	Sep	2010	15	3.2	0.5	1.1	1.3	0.5
SACN09	May	2010	16	3.0	0.4	1.1	1.2	0.5
	June	2010	15	3.6	0.7	1.6	2.0	0.8
	July	2010	16	4.3	0.7	1.4	1.8	0.8
	July	2010	16	3.1	0.7	1.8	1.9	0.8
	July	2010	21	3.6	0.5	1.4	1.5	0.7
	Aug	2010	12	4.4	0.8	1.3	2.0	0.9
	Sep	2010	14	3.9	0.5	1.0	1.3	0.6
SACN11	May	2010	16	2.5	0.5	1.3	1.4	0.6
	June	2010	13	2.9	0.7	1.5	1.7	0.8
	July	2010	17	2.4	0.6	1.6	1.6	0.7
	July	2010	15	2.4	0.6	1.5	1.5	0.7
	July	2010	16	2.5	0.6	1.7	1.7	0.8
	July	2010	17	2.5	0.6	1.6	1.7	0.8

Table 6 (continued). Common diversity indices for zooplankton samples from the St. Croix River (Minnesota / Wisconsin), 2010 and 2011, calculated in PRIMER 7. Results include number of taxa (lowest identifiable resolution), Margalef's diversity index (d), species evenness (J'), Brillouin diversity, Shannon diversity (H') formulated using natural log, and Simpson's diversity (1-λ'). Asterisks indicate samples with taxa distributions incompatible with assumptions of that index.

Site	Month	Year	# taxa	Margalef d	J'	Brillouin	H'(loge)	1- λ'
SACN11	Aug	2010	24	4.3	0.6	1.6	1.8	0.7
	Sep	2010	22	4.1	0.6	1.8	2.0	0.8
SACN05	May	2011	9	13.4	0.6	0.3	1.3	1.2
	July	2011	14	8.0	0.1	0.0	0.3	0.1
	Aug	2011	7	****	0.9	****	1.8	****
	Sept	2011	10	****	0.8	****	1.9	****
SACN07	April	2011	16	****	0.8	0.0	2.2	****
	May	2011	15	11.4	0.2	0.0	0.6	0.3
	June	2011	12	4.0	0.7	1.2	1.6	0.8
	July	2011	22	5.0	0.4	1.0	1.1	0.5
	Aug	2011	22	****	0.7	****	2.2	****
	Sept	2011	18	3.7	0.3	0.8	0.9	0.4
	Nov	2011	20	15.1	0.7	0.8	2.1	1.1
SACN09	April	2011	17	****	0.9	0.0	2.4	****
	May	2011	12	14.6	0.3	0.0	0.8	0.6
	June	2011	18	6.3	0.6	1.0	1.6	0.7
	July	2011	22	4.4	0.5	1.5	1.7	0.8
	Aug	2011	19	36.5	0.8	0.3	2.3	2.2
	Sept	2011	19	6.8	0.6	1.2	1.9	0.8
	Nov	2011	20	5.2	0.6	1.4	1.8	0.8
SACN11	April	2011	15	****	0.8	0.0	2.0	****
	May	2011	11	5.8	0.4	0.6	0.9	0.5
	June	2011	21	6.3	0.6	1.4	1.9	0.8
	July	2011	26	9.9	0.6	1.3	2.1	0.9
	Aug	2011	27	12.0	0.4	1.0	1.5	0.8
	Sept	2011	19	13.4	0.8	0.8	2.4	1.2
	Oct	2011	26	44.7	0.7	0.3	2.3	1.9

PERMANOVA (permutational MANOVA) was run on Bray-Curtis similarity using site specific biomass estimates. The analysis was performed on genera rather than species to avoid taxonomic bias of better understood groups, and data were square root transformed to decrease the influence of outliers. Factors tested were sample month, year, and site. Zooplankton communities were significantly different among sites ($p = 0.005$, pseudo- $F = 4.69$), month ($p = 0.001$, pseudo- $F = 2.86$) and year ($p = 0.002$, pseudo- $F = 10.23$). There were no significant ($p < 0.05$) interactions among site, month, and year. Month interactions with year showed weak evidence of interaction ($p = 0.061$, pseudo- $F = 1.17$). Environmental factors used in the PERMANOVA and ordination analyses were tested for trends with a simple correlation analysis (see Table 4). Dissolved oxygen, conductivity, and temperature correlated with month ($r^2 = -0.54$, 6.1 , and 0.50 , respectively). Site and year did not correlate strongly with any of the environmental variables ($|r^2| < 0.25$).

Principal coordinates analyses were based on Bray-Curtis similarity of square root transformed biomass of genera present at each site per month. Genera indicated are associated with site community composition at correlations > 0.2 , while points plotted indicate site and month (e.g., SACN05 from July 2010 is labelled SACN05July). Vectors associated with environmental variables were not strong enough to appear on the plot and were removed for clarity, indicating that the zooplankton community differences among our sites were not strongly structured by these factors relative to other site differences. PCA results for 2010 are shown in Figure 12. Canonical analysis of principal coordinates (CAP) was used to further distinguish patterns seen in the PCA (Anderson 2003). CAP for 2010 is shown in Figure 13. Site SACN05 groups very tightly with itself throughout the year in 2010 (indistinguishable on the PCA, but seen more clearly in Figure 13). Community composition groupings are very clearly associated with site, with SACN05 distinct from the Lake St. Croix sites on CAP1 axis, and site SACN09 distinct from SACN07 and 11 on CAP2. The PCA for 2011 is less clear due to vastly different community composition of SACN09, the result of *Mesocyclops* spp. biomass that was 6.3 times greater than the next highest value at any other sampling period in 2011 (Figure 14). The CAP plot clarifies other groupings, showing site SACN05 distinct from other sites on the CAP1 axis and site SACN11 distinct from SACN07 and SACN09 on the CAP2 axis (Figure 15).

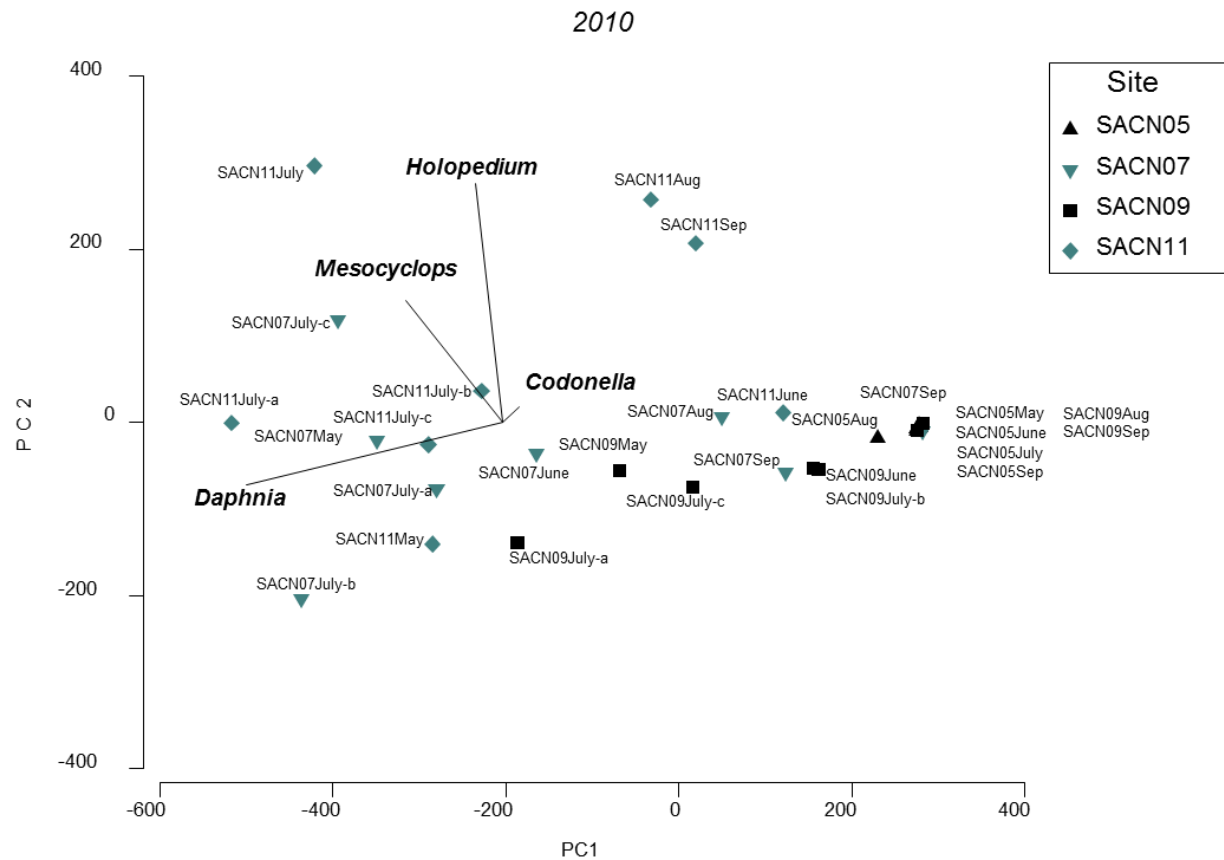
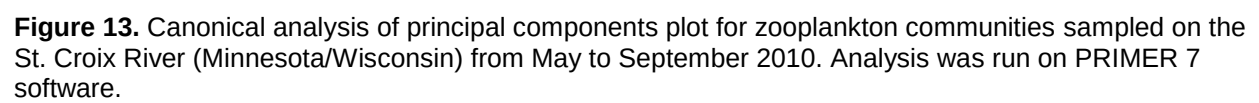


Figure 12. Principal coordinates plot of zooplankton communities by site and date at four sites on the St. Croix River (Minnesota/Wisconsin) sampled from May to September 2010. Field duplicates taken in July 2010, 10 m away from the central site, are labelled with letters (a = GPS of sampling site, b = 10 m west of a, c = 10 m east of a). PCA was run in PRIMER 7 software.



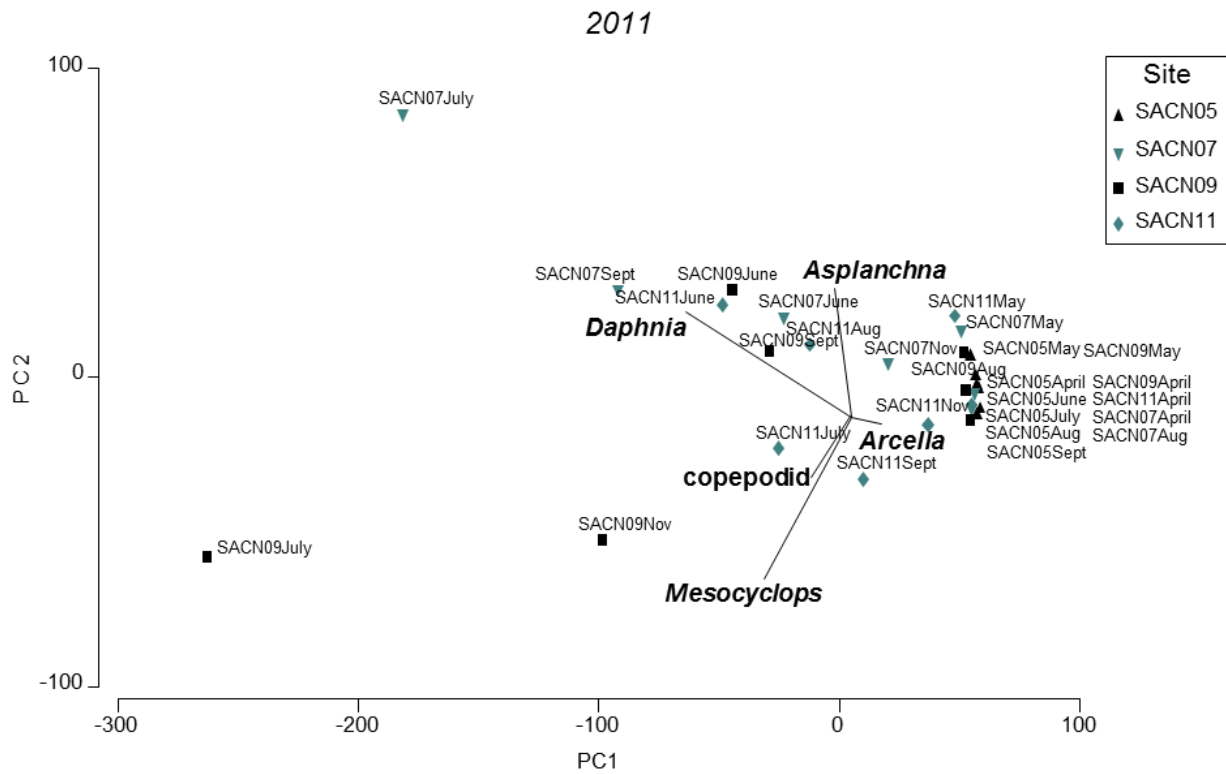


Figure 14. Principal coordinates plot of zooplankton communities by site and date at four sites on the St. Croix River (Minnesota/Wisconsin) sampled from April to October 2011. November samples were taken on the first of the month and grouped with the final week of October samples for the rest of the sites. PCA was run in PRIMER 7 software.

2011

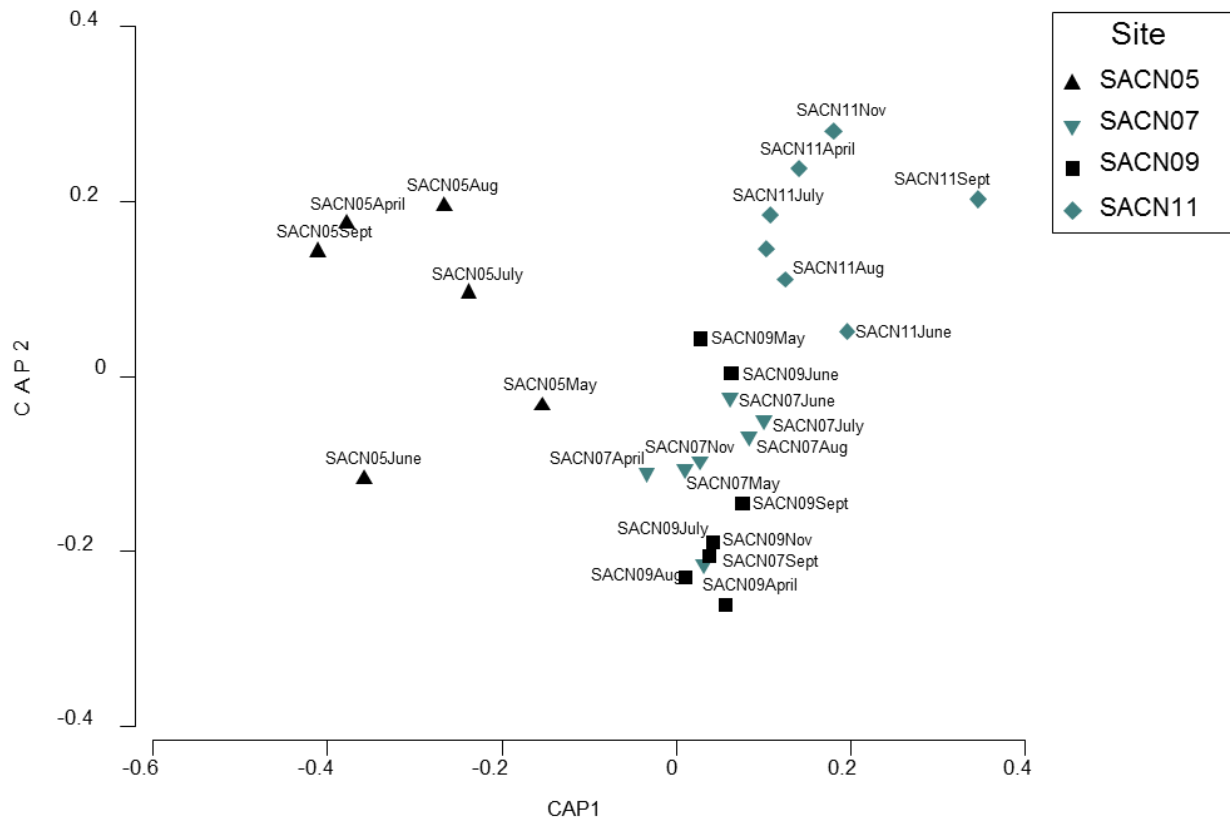


Figure 15. Canonical analysis of principal components plot for zooplankton communities sampled on the St. Croix River (Minnesota/Wisconsin) from April to October 2011. November samples were taken on the first of the month and grouped with the final week of October samples for the rest of the sites. Analysis was run on PRIMER 7 software.

Discussion

In this study we show that Lake St. Croix supports a rich, diverse, and abundant assemblage of lake-like zooplankton. We also found that zooplankton form at least three distinct communities throughout Lake St. Croix despite their proximity and physical connectivity. Through use of PERMANOVA, ANOVA, and ordinations we determined that 1) community composition differs significantly among pools, over a season, and between years, and 2) the zooplankton communities of Lake St. Croix are uniquely structured compared to similar reservoirs. These findings have implications for bighead and silver carp management and expected environmental impacts from the arrival of those non-native species.

The Spatial and Temporal Context of Zooplankton Communities in Lake St. Croix

The scale of this study focuses on long-term monitoring sites on Lake St. Croix to determine zooplankton patterns within the lake at a coarse scale (upper, middle, and lower pools) compared to a reservoir farther upstream. Our inferences are limited to spatial distribution at this scale and are not intended to analyze environmental drivers over the mosaic of different habitats in the St. Croix system (although that effort is underway on the St. Croix River and Mississippi River Pools 2 and 19, T. Lafrancois personal communication with B. Knights).

Differences among sites in temperature, dissolved oxygen, conductivity, and depth as drivers of zooplankton community structure were ruled out using several independent tests. First, simple correlations over field values were calculated to determine whether there were any significant site-to-site differences (see Table 4). There were strong correlations between sonde measurements and month ($r^2 > 0.5$), but no strong correlations with site ($r^2 < 0.2$), indicating that dissolved oxygen, temperature, conductivity and chlorophyll—all related to zooplankton community in general—were not different enough among sites to impact zooplankton community structure at the scale of this study. Secondly, water chemistry results were included as factors in the ordinations, but there were no strong associations with any of the axes that explained community variation (in fact, the vectors were too short to show up on an unmodified plot). These results show the impact of such environmental drivers were similar at each site on each date, and consequently differences in community structure can be attributed to other factors. On the other hand, this compounds seasonal phenology and environmental factors with sampling date (month and year). Consequently, we cannot statistically separate seasonal phenology (and relation to temperature and productivity) from retention time (the impact of geomorphology on how overall flow translates into current).

The two years covered by this study varied greatly in seasonal rainfall, resulting in very different flow regimes each year (see Figure 3) that had definite impacts on zooplankton communities (discussed below). While Heraklitus famously said, “one cannot step in the same river twice”, we take seriously the words of his student Kratylus, who went further to say, “one cannot step in the same river once” (Aristotle, *Metaphysics* 4.5 1010a10-15). The years 2010 and 2011 represented a nice range over the 112-year mean flow in the St. Croix system, but caution must be taken in identifying the time scale of what characterizes a “normal” zooplankton community. Taking these two caveats together, our interpretation of the main drivers of zooplankton community can be

cautiously attributed to water retention time differences between the two study years. Future comparisons need to take these conditions into account. We feel that if more habitat types were sampled or comparisons with other systems with similar retention regimes are studied, the influence of other environmental parameters could be stronger. Nonetheless, attributing community differences over sampling period to retention time is supported by other large-scale studies of multiple rivers where residence time is not correlated with productivity yet is the only factor statistically associated with zooplankton abundance (e.g., Basu and Pick 1996).

Patterns of Zooplankton Biomass and Community Structure in Lake St. Croix

We found that Lake St. Croix is characterized by a diverse assemblage of zooplankton, including a surprising number of larger crustaceans—including several species of *Daphnia*, large calanoid copepods, and both small and large cyclopoid copepods (the most common being *Diacyclops* and *Mesocyclops* species). The phylum Rotifera is well represented in the river zooplankton, with highly abundant and common species of relatively small and consistent sizes (Rotifera are eutelic) usually averaging 80 to 120 μm in length but with some large (>1-mm-long) *Asplanchna* spp. The largest crustacean plankton was *Leptodora*, while the smallest zooplankton found were testate protozoa (see Figure 4). Non-testate protozoa were not studied, as they are not well-preserved and rapidly deteriorate in ETOH. The role of testate protozoa in river systems is unclear. We include them as a potential index of total protozoa presence and indicator group, following their use in paleoecology to index allochthonous input, pH, temperature, and productivity including systems (Ellison and Ogden 1987). This taxon could be highly relevant given their use globally as indicators in other systems that contain silver and bighead carp (Qin et al. 2009). In some systems they are useful indicators of allochthonous input (Fulone et al. 2008). For these reasons we cautiously include them for posterity in the event there is a response to bighead and silver carp elimination of other plankton.

Taxa diversity over all sites in Lake St. Croix for 2010–2011 included 96 species from 53 genera. Raw taxa diversity (if not species then taxa at the lowest practical taxonomic resolution) is higher than recent reports of the Mississippi River pool 4 with 39 taxa (Burdig and Hoxmeier 2011) and the Missouri River with 79 taxa (Dickerson et al. 2009). It remains unclear how biologically significant these differences are or whether they are due to differences in taxonomic expertise or sampling protocol (see below). This study focused only on Lake St. Croix and the reservoir above St. Croix Falls, while the work on the Mississippi and Missouri rivers included backwaters and other channel configurations that are often more taxa-rich. It is reasonable to expect more taxa in the St. Croix system as a whole if more habitat types were sampled, as suggested by early work in backwaters done as part of the education/outreach for this project. (Vang et al. [2010] would add at least 12 taxa, making reported raw diversity over 100 taxa for the Riverway.) In addition to diversity, the unique zooplankton composition of Lake St. Croix makes it stand out from other similar river zooplankton studies. Zooplankton communities in the upper Mississippi and Missouri rivers are dominated by rotifers that compose 71.3% to well over 90% of the biomass even in high retention backwaters (Dickerson et al. 2009, Burdis and Hoxmeier 2011).

Evenness-weighted species diversity was calculated using the Shannon index (H'), which provides meaningful cross-system comparisons over both α - and β -diversity (see Table 6). Unfortunately,

similar work to-date on nearby river systems does not report diversity indices, which defeats their purpose as cross-system comparative metrics. We did not find diversity measures helpful for site-to-site comparisons within this study either, primarily due to large numbers of rotifers common to all sites combined with patchy distributions of other taxa. We include them for comparisons over time, particularly if non-native carp should become established.

Total number of taxa was very consistent over the study period (see Figure 5), while biomass changed significantly with time (see Figure 6). This highlights the importance of community composition even using relatively coarse community metrics. For example, shifting from smaller to larger cladoceran species has major impacts on biomass, and large populations of copepods like *Mesocyclops* spp. at some sites usually dominated by rotifers is an important shift in biomass. Community composition of the four primary zooplankton groups was consistent between years and among sites (see Figure 5), with the reservoir site above the hydro-dam differing from Lake St. Croix sites due to higher dominance of rotifers and testate protists, but fewer or no cladocerans and copepods. The biomass-based breakdown of community composition (see Figure 6) further demonstrates the stark differences between Lake St. Croix sites and the reservoir.

The basic patterns of the biomass of primary zooplankton groups between years and among sites were confirmed by the more detailed PERMANOVA results and associated ordinations. While proportions of the major zooplankton groups do not change between the study years, total zooplankton biomass was up to an order of magnitude higher in 2010 compared to 2011 (see Figure 6). Site SACN05 supported dramatically lower biomass than other sites in 2010 and most of 2011, and is much more similar to zooplankton biomass reported for the upper Mississippi River (Burdiss and Hoxmeier 2011) than Lake St. Croix (this study).

Biomass plots of each major zooplankton group over time are shown for both 2010 and 2011 in Figures 7–11. As with total zooplankton biomass, biomass of the individual groups was also much higher in 2010 compared to 2011. However, two other distinct patterns are evident. The first is that SACN09, a low retention site similar to that of SACN05 during most sampling periods, had the highest biomass of all the sites in July of 2010 due to an explosion of the *Mesocyclops edax* and *M. americanus* populations. This is an important exception to the usual dominance of retention time as a key factor driving zooplankton biomass since flow values for July of both 2010 and 2011 were both very close to the 112-year average (see Figure 3).

Secondly, seasonal phenology and its impact on zooplankton biomass was not expressed as would be expected based on previously conducted work. In many upper Midwest lakes, for example, zooplankton are rotifer-dominated in early spring, transitioning to larger crustaceans with warmer water temperatures and increased productivity over the summer months, decreasing again in the fall with lower temperatures and reduced productivity (Emmons and Olivier Resources 2009). This unimodal pattern was evident to some extent in Lake St. Croix, as in observed changes in cladoceran biomass in 2011 (see Figure 9). However, in 2010 cladoceran biomass was bimodal (high in the spring, low in June, high in July, and low in August and September). This pattern closely tracks river discharge (see Figure 3), implying that discharge is driving cladoceran biomass and not lake temperature and productivity. This conclusion is further supported by the low flow periods of 2010

corresponding to higher copepod and cladoceran biomass, a pattern that also appeared to be decoupled from the impacts of temperature and productivity. Temperature and productivity (i.e., chlorophyll-*a*) correlate positively with sample period, but flow was only different between years. Trend analysis on larger long-term data sets would help further demonstrate whether the patterns we are seeing here have mechanistic drivers, but the most likely scenario is that Lake St. Croix zooplankton are largely decoupled from drivers like temperature and productivity and respond primarily to water retention time as has been reported in other systems (Basu and Pick 1996, Dickerson et al. 2010).

To statistically test these patterns and tease out more specific information about zooplankton community structure we used PERMANOVA, a robust test of complex species data that does not require the assumptions of the F distribution (equal variance and normal distributions). The PERMANOVA results were clarified by species ordinations that plot each site-date sampling event based on community composition (biomass of each taxa present) derived from a Bray-Curtis similarity matrix. Taxa that are associated with the primary axes were independently plotted as vectors (see Figures 12 and 14). Canonical analysis of principal components was used to clarify which groups in the plots form true clusters (see Figures 13 and 15).

PERMANOVA confirmed that the zooplankton communities were significantly different over site, year, and month. None of the interactions between these variables were significant, although the significance of month could be an index of seasonal phenology, a response to flow conditions, or both. The absence of significant interaction between month and year in the PERMANOVA suggests that, for the most part, the month variable is more strongly associated with flow differences than seasonal phenology (see Figure 4), but it is difficult to distinguish with our data and without a “control” system to define “normal” phenology (we use nearby lakes in part of this analysis with caution). The impact of month appears on the PCA plots as scatter and is not associated directly with the ordination axes. However, the plots generally show that month within year is related to the large copepod and cladoceran production later in the season for SACN07 and SACN11. This indicates that seasonal phenology follows lake-like patterns at these sites. SACN05 and to some extent SACN09 do not show very much variation over month, with much tighter groupings due to continued dominance of rotifer species. We think that this is most strongly related to the low retention time at these sites being, for the most part, stronger than phenology in structuring those communities.

The zooplankton community structures at our sites in 2010 and 2011 are different enough that it is best to discuss each year separately. In 2010, SACN09 and SACN05 formed one group characterized by rotifers and protist community dominance (see Figure 12). There were no strong characteristic taxa associated with the positive (right) PC1 axis because the rotifers and testate protists were common to all sites—it is more the absence of other groups that truly characterizes SACN09 and SACN05 for 2010 (see Figure 12). Larger crustaceans, particularly *Daphnia* spp., characterize the shift towards SACN07 and SACN11 during most time periods, with scatter between the two based on the presence of the very large cladoceran *Holopedium gibberum* that tended to dominate the biomass of SACN11 on most dates, and the copepod *Mesocyclops* spp. that characterized SACN07 (but also SACN11). The CAP for 2010 clarifies these patterns further, confirming that SACN05

forms a distinct group on CAP1 axis that most strongly corresponds to total biomass (see Figure 13). SACN09 is most often distinct from SACN07 and SACN11 on CAP2, which corresponds to dominance of large cladocerans and mid-sized copepods.

Our test of the three spatially distinct field replicates per site in July 2010 warrants caution. These sites are labelled *a*, *b*, and *c*, respectively, for each July sampling period (site *a* is 10 m east of the main site, site *b* corresponds to the NPS water quality monitoring site, and site *c* is 10 m to the west) (see Figure 12). The replicates from SACN05 are too close to each other to show any difference in the plot, but all the Lake St. Croix sites and associated replicates are spread out across the plot. Statistical tools help sort through the variability due to zooplankton community patchiness. The CAP analysis (see Figure 13) is robust across this variation, with July replicates appearing in the same quadrant for each site. The CAP also shows slight separation between field duplicates (labelled with a number, e.g. SACN05Julyc1 and SACN05Julyc2 indicating repeated sample at SACN05 c in July) that appeared very close if not on top of one another in the PCA (see Figure 12). The ANOVA for this sampling period confirmed the pattern shown by the CAP plot, with significantly higher biomass and richness at the Lake St. Croix sites compared to that at SACN05 above the hydro-dam, and the two higher retention lake sites (SACN07 and SACN11) had significantly higher biomass than the lower retention SACN09 and SACN05.

Nonetheless, scatter of replicates in the ordinations shows that while replicates appear in similar quadrants they can be as dissimilar as the same site at different months. The variation here is a clear indication that finer-scale community understanding will require spatial variability to be addressed, particularly in future efforts to evaluate finer spatial scale changes in this dynamic system (e.g., for identifying impacts of specific tributaries or changes to plankton above specific mussel beds). The notorious patchiness of zooplankton communities presents a logistical challenge for a long-term monitoring program and suggests composite samples taken from a larger area would be more representative (while still avoiding the high costs associated with counting all subsamples—which of course would be ideal).

We also found significant differences in community patterns between 2010 and 2011. Significantly lower zooplankton biomass was present in 2011 compared to 2010, and there were shifts in dominant or characteristic zooplankton species. In addition, while the PCA shows grossly similar patterns for both years, there are differences in community structure. Both years generally show that SACN05 grouped tightly over all months, and SACN09 and SACN011 both showed a general dominance by either *Daphnia* or *Mesocyclops* species (see Figure 14). In 2011, however, SACN09 varies in interesting ways from the overall patterns detected in the rest of the study. Like 2010, the community at SACN09 groups with SACN05, but only in spring months. In the summer months of 2011, SACN09 varies greatly, characterized by a *Daphnia*-dominated community in June and September, while in November and very distinctly in July SACN09 is dominated by *Mesocyclops* species (*M. americanus* and *M. edax*). SACN07 had a distinctly *Daphnia* spp.-dominated July as well (*D. mendotae* being the most dominant followed by *D. retrocurva*, a species found only at SAC11 at other sampling dates).

The CAP for the 2011 PCA sorts these differences out more clearly. Like the 2010 results, SACN05 groups by itself on the CAP1 axis. In 2011, however, SACN07 and SACN09 group much more closely together on CAP2 with SACN11 in its own cluster on CAP2. Unlike the 2010 plot, the reasons for this spread are not as plainly seen in the species associations with the PCA axes. The most likely explanation of the CAP2 axis is that SACN11 produced very large biomass of the relatively gigantic (1.0 to 1.5 mm) rotifer *Asplanchna* spp. in addition to very large cohorts of *Daphnia retrocurva* and very large (up to 3.0 mm) calanoid copepods. SACN07 and 09 communities were generally composed of smaller species of *Daphnia* and mid-range copepods, but in very large numbers (and consequently much higher biomass than other sites).

Zooplankton Community Structure in Lake St. Croix and Other Reservoir Systems

We have shown that the zooplankton communities of Lake St. Croix are different than those of the upper Mississippi River in both total biomass and community composition. Lake St. Croix also contains several distinct community structures in different pools and above its reservoir near St. Croix Falls. The comparison with the upper Mississippi makes biogeographical sense given that the St. Croix River is a tributary to the Mississippi River, and both are susceptible to establishment of bighead and silver carp. Relative to the geomorphology of the St. Croix watershed, however, it also makes sense to compare Lake St. Croix to other reservoir or impounded systems. The main drivers (water retention time and productivity) of the zooplankton community in Lake St. Croix are similar to other reservoirs, but the physical arrangement of an undammed confluence and a large hydro-dam upstream is reverse that of most other similar systems. This arrangement affects the zooplankton community in ways that will have major consequences for how the Riverway responds to bighead and silver carp establishment.

Like other reservoir systems with high retention time, deeper pools, and full or partial impoundment, Lake St. Croix zooplankton communities correspond to retention time both within and among years (Basu and Pick 1996, Bledzki and Ellison 2000, Obertegger et al. 2007, Bergfeld et al. 2009, Claps et al. 2009, Dickerson et al. 2009, Rossetti et al. 2009, Burdis and Hoxmeier 2011). Categorizing the time variable (within a year and between years) as a surrogate for flow conditions is supported by the strong correlations between environmental variables in contrast with the independence of community composition from temperature and productivity. In our results this shows that retention time was the single most important factor in determining how zooplankton communities were structured in Lake St. Croix, although many more years of results will be needed to disentangle retention time from seasonal phenology.

The influence of reservoir structure forming Lake St. Croix is unique. Lake St. Croix is formed by a flow-over natural impoundment at its confluence with the Mississippi River. The hydro-dam is several miles upstream from the Lake St. Croix itself. Consequently we found the above dam site to be more like a main channel, low retention site while the lower Lake St. Croix pools well below the dam are more lake-like with high retention time, and the zooplankton communities reflect this.

It is difficult to discount productivity, a main determinant of zooplankton community structure in some similar systems (e.g., Basu and Pick 1996, Bledski and Ellison 2000). While we found no statistical relationship between temperature and/or chlorophyll and zooplankton biomass, it is likely

that productivity is also influenced by water retention time and our monitoring did not occur at a high enough frequency necessary to disentangle these factors. To broadly address this, our study sites can be ranked according to productivity using long-term monitoring data and other recent studies. In general, sites increase in productivity from upstream pools to downstream pools (Lenz et al. 2003, VanderMeulen and Elias 2008, VanderMeulen 2011), a relationship confirmed by recent experimental and field study of algal productivity and grazing (Magdalene et al. 2013). This makes physical sense in terms of increasing allochthonous (and anthropogenic) inputs of nutrients increasing downstream combined with nutrient spiraling downstream, and because Pool 1 is fed by the relatively less productive upstream river, which has progressively less influence on downstream pools.

Productivity ranking is interesting but not clear-cut when compared to zooplankton community structure. Our 2010 results show that zooplankton productivity follows the pattern of increased downstream (down pool) productivity, with SACN09 showing less productivity than SACN07 upstream. The mismatch with the general pattern at SACN09 is understandable because SACN09 is not located within Pool 2 but is between pools and represents a main-channel, low retention time environment. However, in 2011 SACN09 supported the highest zooplankton biomass in the productive months as well as a large copepod community. Much of this biomass was *Mesocyclops* species, which are usually considered predators. *Mesocyclops edax*, the most common species in Lake St. Croix, is generally thought to be carnivorous but may actively filter phytoplankton. All taxa of this genus can in fact be herbivorous during nauplius to copepodid III life stages for species closely related to those found in Lake St. Croix, and adults may even be facultative detritivores (Gophen 1977, Balcer 1984, Papirńska 1985).

Another odd thing about the *Mesocyclops* bloom in 2011 was that during that period, chlorophyll was an order of magnitude less at SACN09 than just before and just after the zooplankton bloom. It is unclear why the zooplankton biomass was so high, and without experimental work it is impossible to tell if the low productivity was due to or independent of grazing, let alone why such high numbers of crustacean plankton were found in a low retention area. What this does show is that it is hasty to assert that grazing capacity will always progressively increase from Pool 1 to Pool 4 in Lake St. Croix, and that productivity alone drives the plankton ecology. The ecological function of the zooplankton community is going to change as biomass and community composition respond to a complex of drivers that are most directly related to retention time, which in at least some important cases is decoupled from productivity.

Pool position and reservoir structure can also influence zooplankton communities directly because they are at the mercy of drift. In any series of high retention pools (like Lake St. Croix), it is expected that there should be an increase in zooplankton biomass going downstream since zooplankton produced in an upper pool should eventually drift to a lower pool. Impoundments themselves act as both areas of high retention (fostering crustacean plankton production) but also areas that collect plankton drifting from upstream. Areas downstream from reservoirs are typically more river-like and flush out plankton. Consequently, in most studies of riverine reservoirs, higher zooplankton diversity and biomass is found in pools above an impoundment or dam with a corresponding (and usually

exponential) decline below (Welker and Walz 1998, Havel et al. 2009). Pool order and how that might be important in structuring zooplankton communities may be important in Lake St. Croix but we did not detect it, and in fact it seems counter-indicated. Positioned at the end of the chain of pools, site SACN11 in Pool 4 should be subject not only to the most upstream input but also to backflow from the Mississippi River. However, neither the number of taxa nor total biomass increased or decreased consistently from upstream to downstream (see Figures 5 and 6). SACN11 did have the highest biomass of plankton during most sampling periods, but the relationship was not consistent (see Figure 7) and not statistically distinct from SACN07 in terms of total biomass or diversity. On the other hand, SACN11 did support the largest zooplankton species, but the community was quite different than upstream sites so we interpret this to be related to either productivity or retention characteristics and not drift.

It might be expected that zooplankton drift would impact the smaller, less vagile rotifer populations more than the larger crustacean plankton. Patterns of rotifer distribution in Lake St. Croix, however, correspond primarily with retention time rather than distance from a source pool. This follows patterns reported by Dickerson et al. (2009) in contrast to other systems that report rotifer biomass decreasing exponentially downstream from pools (Welker and Walz 1998). Other studies show that while rotifers can decrease with distance downstream from reservoirs due to direct mortality from mussels, they may also increase as crustaceans decline and the rotifers are released from competition and predation pressures (Welker and Walz 1998).

In this study we show that rotifer biomass was most often highest at site SACN09, the lowest retention site in Lake St. Croix but with much higher productivity than SACN05 above the dam. Given the high density of unionids in the area directly upstream from SACN09 that may be impacting rotifers, it seems reasonable to hypothesize that release from crustacean predation and competition is a likely driver rather than drift (Doolittle 1988). We think this suggests a pattern of rotifer dominance in inter-pool areas, a pattern that runs contrary to results from the Missouri River, where rotifers were found to be common downstream of reservoirs but not between pools (Havel et al. 2009). However, early months of 2011 show a pattern in which the high retention site furthest downstream had the highest rotifer productivity, followed by the upstream pool in July (see Figure 10), suggesting that both processes outlined in Welker and Walz (1998) could be at work.

Finally, the physical structure of Lake St. Croix is unique, since it is only partially impounded at the confluence with the Mississippi River, and site SACN05 above the dam is a low retention area with very low zooplankton biomass and diversity. Reservoirs or locks in rivers often create high retention areas behind dams or impoundments. The St. Croix Falls dam on the other hand creates a shallow reservoir-like area with low retention that is well upstream (20 river-miles) from Lake St. Croix. The reservoir was over 20 m deep at the time of dam construction, but is currently between 5- and 6-m-deep due to sedimentation (Troelstrup et al. 1993, MacGregor et al. 2011).

The above-dam reservoir supports dramatically lower zooplankton biomass than other study sites and is dominated by rotifers and testate protists with very few crustacean plankton found. Our study shows definitively that this community will not act as a feeder or re-colonization source for the lower St. Croix River or Lake St. Croix. Backwater and side channel areas just above Lake St. Croix also

support crustacean plankton but primarily smaller species (Vang et al. 2010) with occasional blooms of very large calanoid copepods in isolated backwaters like those near Osceola, Wisconsin (T. Lafrancois, personal observation, August 2013). Even if these backwaters are highly productive for zooplankton, they are also exposed to bighead and silver carp. Effectively, then, the St. Croix system has a unique structure where the hydro-dam is above the pool system and does not serve as a refugium or as a source population for crustacean zooplankton, which are preferentially grazed by silver and bighead carp.

Conclusions

- Lake St. Croix supports a diverse array of zooplankton including large crustaceans. Diversity and biomass exceed that of nearby lakes, and more than twice as many taxa have been reported than from a study of the Mississippi River in the same region.
- Zooplankton in three pools of Lake St. Croix form distinct communities that appear primarily structured by water retention time.
- Zooplankton communities in Lake St. Croix were significantly different among sites, months, and between 2010 and 2011, with no interactions among these factors.
- Lake St. Croix supports a high biomass of cladoceran grazers that other studies have shown to be responsible for reducing algal blooms.
- The hydro-dam at St. Croix falls is a barrier to silver and bighead carp, but the reservoir above the hydro-dam will not act as a source for zooplankton to recolonize downstream areas in the event that downstream zooplankton communities are reduced by the arrival of non-native carp.
- Zooplankton biomass and preferred species in Lake St. Croix appear adequate if not ideal for silver carp establishment. Biomass is within the range of that required for bighead carp to show positive growth in experimental systems (see Appendix 1).
- Common mesh sizes used in related plankton studies miss a majority of rotifer species that are often the primary components of the zooplankton community in river systems (see Appendix 2).

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Appendix 1. Implications of Lake St. Croix zooplankton community structure for silver and bighead carp establishment

Zooplankton communities of Lake St. Croix and *Hypophthalmichthys* establishment

The zooplankton communities of Lake St. Croix appear driven by similar environmental factors as other large rivers with impoundment or reservoir-like areas, with retention time being a primary factor. If bighead and silver carp populations establish in this system, they will likely become the most important determinant of zooplankton community biomass and species composition, with consequences for nutrient management, water clarity, and harm to native species of fish and mussels (Xie and Yang 2000, Williamson and Garvey 2005, Sampson et al. 2009, Ma et al. 2010).

Our results place Lake St. Croix's peak zooplankton biomass at the top of the range for lakes in the northern hemisphere and well above nearby river systems that have lower diversity and biomass than local lakes (Basu and Pick 1996). In 2010 we found biomass estimates of more than 700 µg/L for planktonic crustaceans in July at SACN07 and SACN11. In 2011 with much higher flows, biomass was lower, but nonetheless total zooplankton biomass was relatively high (over 120 µg/L estimated at SACN09 in July 2011; other Lake St. Croix sites over 100 µg/L). In comparison, a study of North American and European lakes reported average zooplankton biomass peaks of 88 µg/L (range = 10.7–786.0 µg/L) (Hanson and Peters 1984). At the lower end of the scale for large, oligotrophic systems, Lake Superior's total zooplankton biomass average is reported to be 20 µg/L (Yurista et al. 2009).

Experimental mesocosm studies for bighead carp used treatments of 1,200 µg/L and 700 µg/L of zooplankton for a high and low food density treatment (Cooke et al. 2009). Bighead carp showed positive growth in the high treatment but lost weight in the lower treatment, which is at the high end of biomass estimates for Lake St. Croix during the most productive time of our sampling period. If the results of this experiment hold in a natural system, bighead carp may not grow well in Lake St. Croix, but we are cautious about applying this experiment designed to determine the suitability of the Great Lakes for bighead carp.

Experiments with silver carp, on the other hand, show that they can grow at zooplankton densities as low as 512 µg/L (Lu et al. 2002), well within the productivity of Lake St. Croix. In fact, silver carp in particular have been found to grow faster and mature earlier in the Mississippi River than in their native habitats (Williamson and Garvey 2005). In the end, bighead carp may have a harder time establishing in Lake St. Croix than silver carp, but this is based on one experimental pond study, and recent captures of bighead in Lake St. Croix indicate that they are at least spending some time feeding there.

Another concern is that site SACN09 has flow characteristics that may be favorable to silver carp (an island mediated channel area not more than 4 m deep [DeGrandchamp 2006]). In 2010 SACN09 zooplankton were dominated by rotifers, but in 2011 this site produced the highest zooplankton biomass of mid-sized crustaceans exactly in the preferred size range for silver carp (Cremer and

Smitherman 1980, Opuszynski 1981, Sampson et al. 2009, Ma et al. 2010). During a food web study in 2014, we observed that this site attracted extremely large numbers of gizzard shad that feed in the same size range as silver carp (B. Knights and T. Lafrancois, personal observation). Based on zooplankton biomass and field observations of natural competitors for the same foods, we feel that Lake St. Croix is not only likely to be able to support silver and bighead carp, but to be very attractive to them. Even though both species of carp are not typically found below 4 m (DeGrandchamp 2006), they will still be able to inhabit the shallow nearshore areas of the pools in Lake St. Croix.

It is clear from previous studies in similar systems that silver and bighead carp reduce zooplankton by both predation and competition, and the primary expected impact of establishment in the Riverway will be a reduction in zooplankton biomass (Burke et al. 1986, Sampson et al. 2009, DeBoer et al. 2016). Any predictions of how the carp will affect the variety of different zooplankton communities in the Riverway are provisional because both species are highly versatile omnivores (Burke et al. 1986). Impacts on zooplankton could also be different depending on which carp species arrives first and/or how the two species competitively interact with each other. With these challenges in mind, it is still possible at this point to compare reported size-selective feeding preferences with species composition results from our survey.

Size-selective Feeding Preferences of Carp and Zooplankton Species Composition

Silver carp have been reported to prefer smaller foods than bighead carp and in particular seem to favor colonial algae, rotifers, *Bosmina*, and other small cladocerans. In contrast, bighead carp tend to prefer larger Crustacea like *Daphnia* and larger copepods (Cremer and Smitherman 1980, Opuszynski 1981, Sampson et al. 2009, Ma et al. 2010). Bighead carp presence tends to shift zooplankton communities from cladoceran dominance to small copepods (Cremer and Smitherman 1980, Cooke et al. 2009) or to other small crustaceans (Lu et al. 2002) due to direct predation. In contrast, silver carp reduces populations of larger crustaceans, out-competing them for rotifers and smaller cladocerans (Cremer and Smitherman 1980, Opuszynski 1981).

Overlaying carp feeding preferences on our findings, we expect that bighead carp will prefer the lower pools of Lake St. Croix that support the largest crustacean plankton, while silver carp will prefer the upper pools and transitional zones that support smaller crustaceans and rotifers. Site SACN07 in Pool 1 consistently supported larger populations of *Bosmina freyi* and *B. leideri*, known favorites of silver carp since they are often smaller than other bosminid species. These cautious predictions are corroborated by carp environmental DNA work that detected silver carp eDNA in upstream reaches (Hickox et al. 2011), while bighead carp have recently been physically captured in the lower pools of Lake St. Croix (MN DNR 2015).

Finally, the most important finding of our study is the role of the hydro-dam at St. Croix Falls, currently the only barrier to upstream migration of silver and bighead carp. The natural impoundment that forms the downstream end of Lake St. Croix at its confluence with the Mississippi River is relatively deep (6–8 m) and easily allows fish passage upstream. This obvious point has several less obvious repercussions. In other systems, fish will gather and feed below reservoirs, benefiting from a plankton source spilling over that they can feed on but never deplete (Cowell 1967, Chang et al.

2008, Havel et al. 2009). In cases of invasive species, this allows at least some constant food source for native species facing competition from the invasives.

The St. Croix Falls dam is not only far above Lake St. Croix, but the zooplankton above the reservoir are very low biomass with even mid-sized crustaceans being very rare. This translates to a relatively minimal food source coming in from the reservoir above the dam, and competition with native filter-feeding fish and several federally endangered species of unionid mussels directly below the dam will not be mediated by a constant re-supply. This could be devastating for paddlefish, where dams have already blocked movement, restricting them to unfavorable habitat (Sparrowe 1986). If their food supply is then limited below the dam, these endangered icons of the Mississippi River system could be subjected to serious stress (Schmidt and Proulx 2009).

A second consequence of the unique impoundment structure is that if silver and bighead carp deplete the zooplankton of Lake St. Croix, there is no re-colonization source operating at a short time scale. Egg banks from the sediment are the most direct source of recovery, but re-population of zooplankton communities has been shown to take from one to thirty years after fish removal from previously fishless mountain lakes, and recovery takes longer for larger species (Parker et al. 2001). In some cases, the largest species, usually calanoid copepods, do not recolonize at all (Parker et al. 2001, Toro et al. 2006). Larger crustaceans are preferred food for many fish fry, and as mentioned above are important for nutrient cycling and algal mitigation. Tracking these changes on the zooplankton community is a critical step for understanding carp impacts, but also critical for assessing mitigation efforts and recovery, as carp impacts will interact with other critical issues like nutrient reductions, unionid protection, and sport fisheries.

Appendix 2. Sampling zooplankton in large rivers: Lessons from the St. Croix.

One objective of this study was to evaluate sampling and laboratory techniques for purposes of designing a long-term zooplankton monitoring program for reservoir systems. Even in non-flowing waters, zooplankton are notoriously patchy in distribution, requiring a large number of samples to reliably characterize a community (e.g., Avois et al. 2000, Zhang et al. 2006, Viljanen et al. 2009). Single samples have been found to detect roughly half of the zooplankton species actually present in a system (Arnott et al. 1998). Nevertheless it is very common for studies in systems similar to the Riverway to rely on one or two vertical plankton net tows each sampling period (e.g., Geraldles and Boavida 2007). Single samples can be appropriate if the scale is large enough, such as for certain long-term monitoring comparing numerous different lakes or rivers (Stemberger et al. 2001).

On the other hand, sampling enough areas in a traditional randomized design to get high quality population estimates can be logistically challenging and extremely expensive. Standard methods of dividing each pool into quadrants (e.g., Galbraith and Schneider 2000) would be costly and strategically untenable for a long-term monitoring program at current levels of support. In light of these constraints, we feel that taking a composite sample of several tows is a useful way to provide robust results while keeping laboratory work manageable and within budget. Our results show high variation in community composition even 10 m apart, but we are encouraged that we were still able to detect significant differences between sites.

Mesh size on zooplankton nets tends to be either 63 μm or 80 μm for small lakes, while 153 μm is standard for the Great Lakes. The “correct” size is clearly relative to study objectives and system properties. For an experimental zooplankton grazing rate study applicable to the St. Croix River, Magdalene et al. (2013) used a 153- μm mesh-size. This has practical advantages since smaller mesh sizes (10 to 30 μm) can quickly clog with algae or other suspended solids, creating a pocket of water that pushes algae and zooplankton out of the net. However, using larger mesh sizes underestimates smaller zooplankton such as rotifers and protozoans, which are critical taxonomic groups, particularly in river systems (Chick et al. 2010). Particularly in low water retention areas (but in certain instances in high retention areas as well), we found that the majority of taxa were smaller than 100 μm at their longest dimension. Our results show that 153- μm mesh is not adequate for capturing 1) the size of zooplankton that may compose a large portion of silver and bighead carp diet, 2) common and abundant rotifer species found in Lake St. Croix, or 3) early stages of copepod nauplii that would be necessary to evaluate changes in recruitment under conditions of carp establishment.

A common method of counting zooplankton in the lab is based on separate samples for smaller (protist and rotifer) and larger (crustacean) zooplankton (e.g., Basu and Pick 1996). A strapping version of this method has been developed and evaluated by Chick et al. (2006), where smaller zooplankton are sampled by pumping water through a 20- μm -mesh net and larger zooplankton are sampled using a larger mesh plankton tow net. The two size fractions are counted by different methods. Smaller species from the pump sample are counted by subsampling with a 1 mL Hensen-Stempel pipette and then transferred to a 1 mL Sedgwick Rafter counting slide. Larger species are

counted using a dissecting microscope, subsampled with a splitter if there are too many individuals to count. See Chick et al. (2006, 2010) for details. These methods are highly recommended if they are available and within budget. However, we do not agree that grab samples or pump samples commonly used for the smaller size fraction are better than net samples. In a related food web study, we found that grab samples for rotifers underestimate populations by two orders of magnitude if they capture any rotifers at all (Lafrancois and Knights, in development).

The two sample counting method has other drawbacks, particularly when working in high retention areas. First, it complicates the field process while also increasing laboratory costs or forcing a balance between taxonomic reliability and accurate counts. It is also difficult to employ in deep (>20 m) areas like the pools in Lake St. Croix because of the amount of water needed to be pumped to get rotifers from the entire water column. These issues can be daunting if zooplankton monitoring is added to ongoing programs with limited funding.

We worked to modify traditional methods to create a reliable, biologically meaningful data set while keeping fieldwork simple and laboratory costs lower. A single sample strategy outlined in the methods above was found to produce density estimates within a standard deviation of the actual, whole sample count for all size fractions (with similar results from Vang et al. 2010). Larger crustacean counts on the rafter cell can be tedious, but they led to much greater taxonomic resolution (e.g., discovering *Mesocyclops americanus* among *M. edax* would be much more difficult counting on a petri dish under a dissecting scope). Stable variance was achieved for all size fractions with as few as two sub-samples, and results from crustaceans counted with this method were within one standard deviation of whole-sample counts. Laboratory replicates were closely associated on the ordinations, falling so close to each other they were indistinguishable. Any variation introduced by our counting methods, then, was very small relative to differences in abundance and diversity between zooplankton communities.

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