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INFLUENCE OF RIVER DISCHARGE, DISSOLVED NUTRIENTS, AND ASIAN CARP ON SUSPENDED PARTICLE DYNAMICS IN NAVIGATION POOLS 19 AND 20 OF THE UPPER MISSISSIPPI RIVER

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INFLUENCE OF RIVER DISCHARGE, DISSOLVED NUTRIENTS, AND ASIAN CARP ON SUSPENDED PARTICLE DYNAMICS IN NAVIGATION POOLS 19 AND 20 OF THE UPPER MISSISSIPPI RIVER

By Amanda S. Milde

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ABSTRACT

Milde, A.M. Influence of river discharge, dissolved nutrients, and Asian carp on suspended particle dynamics in Navigation Pools 19 and 20 of the Upper Mississippi River. MS in Biology – Aquatic Science, May 2016, 89pp. (S. Strauss)

Milde, A.M. Chapter I: Spatial and temporal dynamics of suspended particle characteristics and composition in Navigation Pool 19 of the Upper Mississippi River. MS in Biology – Aquatic Science, May 2016, 35pp. (S. Strauss)

Suspended particles are an essential component of large rivers, for example in shaping channel geomorphology, biogeochemical cycling of nutrients, and are primary food web resources. The Upper Mississippi River (UMR) is a large floodplain river that exhibits pronounced spatiotemporal variation in environmental conditions and biota, providing an idea environment for investigating suspended particle dynamics of large river ecosystems by addressing two questions: (1) How do suspended particle characteristics (e.g., size and morphology) vary temporally and spatially? and (2) What environmental variables have the strongest association with particle characteristics? Water sampling was conducted in June, August, and September 2013 and 2014 in Pool 19 of the UMR. Water variables were analyzed according to standard methods. A FlowCAM particle imaging system was used to enumerate and measure particles 53–300 µm in diameter for size and shape characteristics (e.g., volume and elongation). Particle characteristics varied considerably over space and time and were strongly associated with discharge and concentrations of nitrate + nitrite (NO_3^-) and soluble reactive phosphorous (SRP). Particle characteristics from backwaters were distinct from those from other habitats likely due to reduced hydrologic connectivity and higher biotic production in backwaters. Relatively greater proportions of phytoplankton and zooplankton were observed during low discharge and concurrently with higher relative chlorophyll, volatile suspended solids, and total phosphorous concentrations in all habitats. Examining the dynamics of suspended particle characteristics in the UMR provides insight into the complex interactions among river flow, geomorphology, and nutrients as drivers of suspended particle dynamics in large floodplain rivers.

***Keywords*:** suspended particle characteristics, discharge, nutrients, plankton, Mississippi River

Milde, A.M. Chapter II: Potential effects of filter-feeding Asian carp on suspended particle dynamics in the Upper Mississippi River. MS in Biology – Aquatic Science, May 2016, 29pp. (S. Strauss)

The invasive silver carp (*Hypophthalmichthys molitrix*) and bighead carp (*H. nobilis*), collectively referred to as Asian carp (AC), have had adverse impacts on non-native aquatic ecosystems in which they are now established, leading to heightened concerns about their spread throughout the United States and into the Laurentian Great Lakes. These voracious filter-feeders have been previously documented to reduce total plankton abundances and shift community size structure in non-native systems. My objective was to detect changes in suspended particles due to AC feeding by examining differences in particle characteristics (e.g., size and morphology) between areas with different AC abundances, in Pools 19 and 20 of the Upper Mississippi River. Sampling was conducted in June, August, and September 2014. A FlowCAM particle imaging system was used to enumerate and measure particles 53–300 µm in diameter for size and shape characteristics (e.g., volume and elongation). In August and September, characteristics of suspended particles in channel habitats were significantly different between Pools 19 and 20, suggesting the relatively higher AC abundance in Pool 20 versus 19 may have been a factor affecting suspended particles. However, particle characteristics in backwaters did not differ between Pools 19 and 20 nor showed any seasonal pattern, suggesting AC presence had no effect on suspended particles. Variations in discharge made it difficult to interpret the pool differences in particle characteristics as effects of AC abundance. A better understanding of the changes in suspended particles related to AC abundance is crucial for the development of effective AC control measures.

***Keywords*:** Asian carp, suspended particle characteristics, discharge, Mississippi River

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CHAPTER I

SPATIAL AND TEMPORAL DYNAMICS OF SUSPENDED PARTICLE CHARACTERISTICS AND COMPOSITION IN NAVIGATION POOL 19 OF THE UPPER MISSISSIPPI RIVER

INTRODUCTION

Suspended particles are an essential component of aquatic systems and play a major role in the functioning of rivers (Allan and Castillo, 2007). For example, suspended particles (i.e., living, non-living, organic, and inorganic) influence channel geomorphology, biogeochemical cycling of nutrients, and food and habitat for biota (Ongley, 1982; Allan and Castillo, 2007). As primary food resources for filter-feeding organisms, the quantity, quality, and size of suspended particles are important. Suspended particles have not been well studied in most rivers because enumerating, measuring, and identifying particles is difficult and time consuming. Moreover, the ecology of suspended particles in large river ecosystems has received much less attention than those in lentic and marine systems (Sprules and Munawar, 1986; Gaedke, 1992), because large rivers are complex, typically possessing a high diversity of aquatic habitats and biota. Longitudinal (Vannote *et al.*, 1980; Houser *et al.*, 2010) and lateral gradients (Junk *et al.*, 1989; Pongruktham and Ochs, 2015) in structure and function, as well as temporal variation, increase their complexity. For example, the River Wave Concept (RWC) hypothesizes that the production, storage, transformation, and transport of suspended particles are largely a function of river flow (Humphries *et al.*, 2014). They suggest three primary riverine models are important at different stages of river flow (i.e., the river continuum concept [RCC; Vannote *et al.*, 1980] during ascending and descending limbs of the hydrograph, the flood pulse concept [FPC; Junk *et al.*, 1989] at crests or peak flow,

and the riverine productivity model [RPM; Thorp and Delong, 1994] during troughs or base flow). Investigating the spatiotemporal patterns in suspended particle dynamics (e.g., size and morphological characteristics and quantity) can improve our understanding of how discharge and geomorphology interact to affect this vital component of large floodplain rivers.

Research on suspended particles has primarily focused on the total amount of material in suspension and on living components (e.g., Gilvear and Petts, 1985; Burdis and Hoxmeier, 2011). For example, many sediment studies have focused on concentration, load, mineralogy, and the mechanisms for suspension, transport, and deposition of only inorganic particles, such as gravel, sand, and silt (Ongley, 1982; Walling *et al*., 2000; Hicks and Gomez, 2003). Studies on organic and living particles have focused on plankton biomass, chlorophyll *a* concentrations as a surrogate for phytoplankton abundance, taxonomic composition, and environmental variables that influence compartmental groups (e.g., seston and detritus, bacteria, phytoplankton, and zooplankton; Chetelat *et al*., 2006; Houser *et al.*, 2010; Burdis and Hoxmeier, 2011). Few studies have explored suspended particle characteristics, such as particle size and shape parameters (e.g., diameter, elongation, surface roughness, etc.). As a primary resource at the base of the food web, suspended particles are key to our conceptual understanding of large river ecology.

Major factors affecting suspended particles in large rivers include discharge, geomorphology, allochthonous inputs, and autochthonous production. For example, high flow conditions often connect channel and backwater habitats and transport terrestrially derived (allochthonous) materials into the river from upstream and from the floodplain.

Variation in these materials depends on river size and depth (Allan and Castillo, 2007), water discharge (Gilvear and Petts, 1985), and the degree of hydrologic connectivity of aquatic habitats (Pongruktham and Ochs, 2015). Autochthonous production typically increases during low discharge, as long water residence times allow plankton community development (Delong and Thorp, 2006; Houser *et al.*, 2015). Water temperature (Baker and Baker, 1979), light attenuation, and nutrient availability (Basu and Pick, 1996; Houser *et al.*, 2015; Decker *et al*., in press) also act together to drive autochthonous production of biotic particles. Collectively, these mechanisms interact to influence the overall quantity, characteristics (i.e., size and morphological parameters), and quality (i.e., as food) of suspended particles in large rivers.

Faster and higher resolution technologies that combine flow cytometry, microscopy, and digital imagery have recently been developed to more efficiently characterize suspended particles (Alvarez *et al.*, 2011). This new technology, called a FlowCAM particle imaging system, was used to address two questions about suspended particle characteristics (i.e., concentration, size and shape parameters) in a large river system, the Upper Mississippi River (UMR). (1) How do suspended particle characteristics differ spatially and temporally? and (2) What environmental variables, such as river discharge and dissolved nutrients, have the strongest association with suspended particle characteristics? Particles 53–300 µm in diameter were examined because this range reflects size classes that are important to primary consumers, such as filter-feeding insects, crustaceans (e.g., macrozooplankton; Thorp and Covich, 2010), and fishes (e.g., gizzard shad *Dorosoma cepedianum,* and Asian carp; Drenner *et al.*, 1984; Kolar *et al.*, 2007). Particle characteristics data (i.e., area, diameter, elongation,

roughness, symmetry, volume, width, and concentration) and images produced by the FlowCAM may be useful for inferring the relative composition of suspended particles or potentially the quality of particles as food.

I hypothesized that river discharge, flow velocity, aquatic habitat (e.g., main channel or backwater), and nutrient availability would affect suspended particle characteristics by influencing the source (i.e., allochthonous and autochthonous), storage, transformation, and transport of particles. More specifically, I hypothesized that during spring and high discharge, habitats would be more homogenous in particle characteristics due to increased hydrological connectivity facilitating the transport and mixing of materials among aquatic habitats. In contrast, during summer and low discharge particle characteristics would become more divergent among habitats, mainly in backwaters, due to reduced hydrologic connectivity, increased water residence times, and different nutrient concentrations that would promote autochthonous production, affect plankton community characteristics, and therefore increase the proportion of higher quality particles as food for filter-feeding organisms.

METHODS

Study area and field sites

The UMR extends from St. Paul, Minnesota to its confluence with the Ohio River near Cairo, Illinois. This section of the Mississippi River is divided into a series of reaches by 29 locks and dams. The reaches of river between consecutive locks and dams are called navigation pools. Each pool typically has four basic aquatic habitat types: main channel, side channel, backwater, and impounded areas (Wilcox, 1993). The main channel includes channel borders and the navigation channel, which is maintained at 2.75 m (9 feet) for navigation. Side channels are secondary or tertiary channels flowing from the main channel with variable depths and water velocities. In contrast, backwaters are off-channel areas that are typically shallow (< 2 m) and vary in connectivity to channels. Some backwater areas maintain connection to flowing channels at all times, whereas others become isolated during low flow. Impounded areas are large, open areas directly upstream of locks and dams that vary in depth and water velocity. Backwaters and impounded areas are typically characterized by lower water velocities than channel habitats.

Navigation Pool 19 of the UMR extends 74.5 river km from Lock and Dam 18 in Gladstone, Illinois to Lock and Dam 19 in Keokuk, Iowa (Figure 1). Pool 19 consists of islands, side channels, and backwaters in the upper half of the pool while the lower impounded portion is lake-like with few islands or backwater areas (Bhowmik and Adams, 1986). Sampling was conducted at thirteen or fourteen sites and eleven sites in

June, August, and September of 2013 and 2014, respectively, to coincide with spring high flows and late summer or early fall low flows (Figure 1 and 2). Sites were selected *a priori* in a stratified random design from the four aquatic habitat types as designated by the Long Term Resource Monitoring Program (Wilcox, 1993).

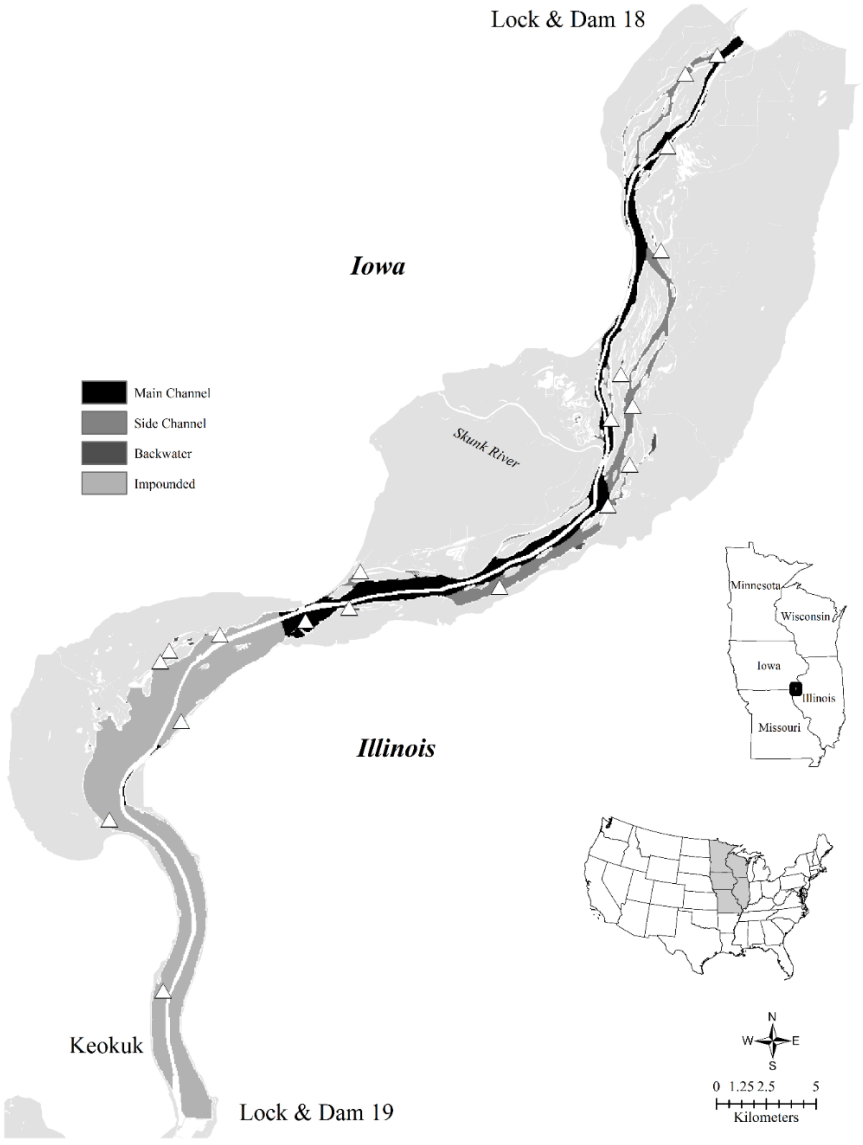

Figure 1. Map of Navigation Pool 19 of the Upper Mississippi River. Triangles indicate sample sites selected by stratified random sampling among four aquatic habitat types including main channel, side channel, backwater, and impounded. The white line is the Navigation channel.

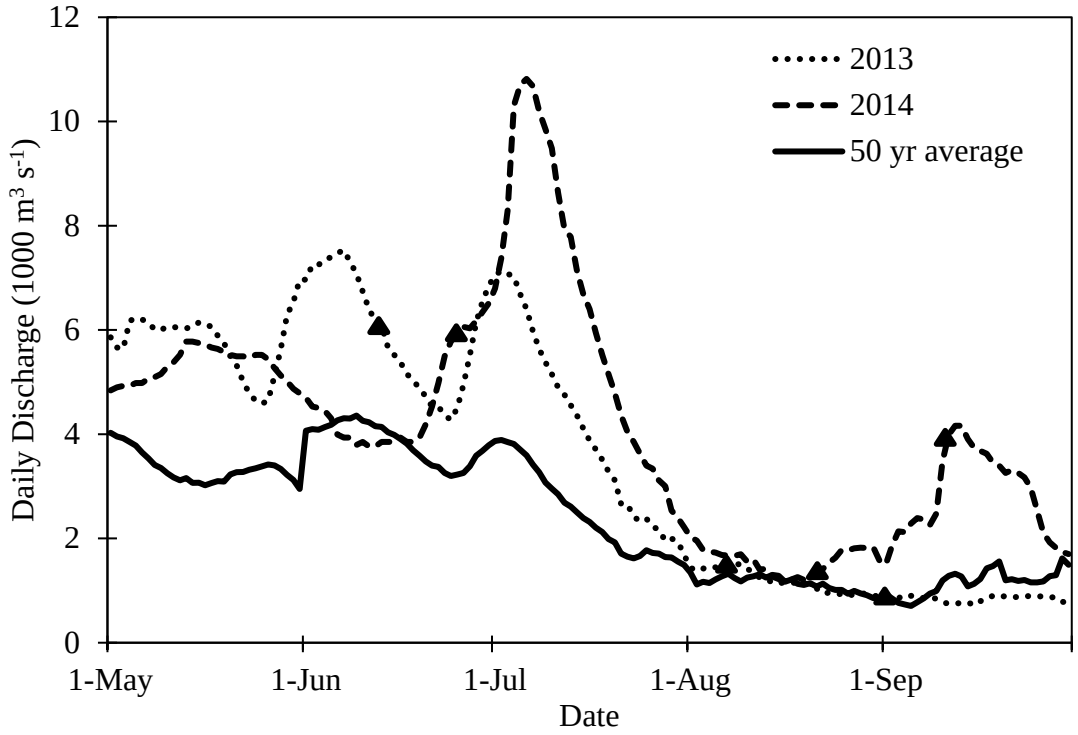

Figure 2. Hydrograph showing mean daily discharge at Lock and Dam 19 of the Upper Mississippi River from May through September of 2013, 2014, and the 50 year average. Triangles indicate sampling events. Data were obtained from the USGS National Water Information System (waterdata.usgs.gov).

Particle analysis

Particle samples were collected at mid-water depth by pumping 15-60 liters of water with a bilge pump (~5678 L hr^{-1}) through 1000-µm and 53-µm Nitex mesh sieves. Dense algae in late summer caused the sieves to clog quickly, so the amount of water filtered varied. Materials collected on the 53-µm sieve (size range of ~50–1000 µm) were rinsed into sample bottles and preserved with 70% ethanol (Black and Dodson, 2003). Duplicates for 10% of the field samples were collected and analyzed for quality assurance purposes.

Immediately prior to particle analysis, preserved samples were filtered once more through 300-µm Nitex mesh. As a result, all particle analyses reported here are within the

53-300 µm size range. Samples were analyzed for particle concentration (# mL^{-1}), size, and shape parameters using a Flow Cytometer and Microscope (FlowCAM® by Fluid Imaging Technologies, Inc., Scarborough, ME, U.S.A.). FlowCAM is a continuous imaging flow cytometer that captures 2-dimensional digital images of individual particles moving through a glass flow cell in front of a microscope. Particles (53 – 300 µm) were photographed using a 300-µm flow cell and 4X objective in auto-image mode, in which photographs were taken 20 frames per second at a constant flow rate of 1.75 mL per min. In a single run, between 8,000 and 15,000 particles were enumerated, measured, and summarized using Visual Spreadsheet Software included with the instrument. Parameters used to characterize suspended particles included particle area, diameter, elongation, roughness, symmetry, volume, width, and particle concentration (Table S1).

Quality assurance procedures for FlowCAM analysis included monthly particle size and count verification to ensure consistent and accurate size and concentration measurements. COUNT-CAL Particle Size and Count Precision Standards (Thermo Scientific, Fremont, CA, U.S.A.) consisted of 50-µm ESD diameter (National Institute of Standards and Technology traceable) beads at 3000 particles/mL. Mean diameters were within the certified range of 50.2 µm ± 1.0 µm and counts were within 20% of the listed concentration, as recommended by the FlowCAM calibration verification procedure (FlowCAM® Manual, 2013). To test instrument precision, the coefficient of variation for the median from triplicate analyses of samples was calculated. The average coefficient of variation for each particle parameter was low (< 3.5%; Table S1). Alvarez *et al.* (2011) showed that the average coefficient of variation for counts from triplicate analyses to be about 12%. They also found that FlowCAM tended to underestimate counts relative to

traditional microscopy, but resulted in an average percentage error, calculated as the absolute difference between FlowCAM and microscopy counts relative to microscopy counts, of 15% between these two methods for preserved samples.

Environmental variables

Water samples were collected at each site using a one meter vertical integrating tube sampler. Nutrients analyzed included nitrate + nitrite ($NO_3^-N + NO_2^-N$, hereafter referred to as NO_3^-), ammonia + ammonium ($NH_3^-N + NH_4^+-N$, hereafter referred to as NH_4^+), soluble reactive phosphorous (SRP), and total phosphorous (TP). Water samples for nutrient analyses were filtered (0.45-µm Whatman), preserved, and analyzed on a Lachat Flow Injection Auto-Analyzer (Hach Company, Loveland, CO, U.S.A.) according to standard methods (APHA, 2005). TP samples were digested according to the U.S. Geological Survey's (USGS) alkaline persulfate digestion method (Patton and Kryskalla, 2003). NO_3^- concentrations were determined with the automated cadmium reduction method, TP and SRP with the ascorbic acid reduction method, and NH_4^+ with the automated phenate method. Dissolved inorganic nitrogen (DIN) was calculated as the sum of NO_3^- and NH_4^+. Total suspended solids (TSS) and volatile suspended solids (VSS) were analyzed gravimetrically according to standard methods (APHA, 2005).

Algal chlorophyll (CHL) was measured *in situ* using a Turner Designs fluorescence sensor on an YSI Water Quality Sonde (YSI Incorporated, Yellow Springs, OH, U.S.A.) in 2013 and a Hydrolab Water Quality Multiprobe (Hach Company, Loveland, CO, U.S.A.) in 2014. The fluorescence sensor was calibrated daily with a calibration standard, but not related to actual chlorophyll levels with laboratory analysis. Thus, CHL values reported should be only considered as a measure of relative

phytoplankton abundance. Daily river discharge data at Lock and Dam 19 were obtained from the USGS National Water Information System (waterdata.usgs.gov; Figure 2). Surface flow velocity (m s^{-1}) was measured only in 2014 on site with a Marsh-McBirney water velocity meter (water depth ~0.5 m).

Quality assurance procedures were followed according to standard methods (APHA, 2005). Percent relative standard deviations for duplicate and standard reference material analyses were less than 10%. Spike recoveries were between 80 and 120%.

Statistical analyses

Multivariate statistics were used to assess temporal and spatial patterns in suspended particle characteristics and their relationship with select environmental variables. The particle concentration (# mL^{-1}) and median value for each particle parameter (e.g., diameter, etc.) were estimated from each sample. Many of the particle parameters measured by the FlowCAM are mathematically related and thus highly correlated. A draftsman plot (Pearson correlation) was conducted to selectively eliminate highly correlated ($r \geq 0.95$) parameters (Table S1). Prior to multivariate analyses, all data were 4^{th} root transformed to reduce skewness. Missing data were estimated according to PRIMER's EM routine when < 5% of values for a variable were not measured (Clarke and Gorley, 2015). Pearson correlation coefficients were calculated among variables to assess collinearity of environmental variables and relationships among whole water particle measures, particle characteristics (53-300 µm), and nutrients (Table S2). Data were then normalized to a zero mean and unit standard deviation. All multivariate analyses were conducted on Euclidean distance similarity measures with PRIMER statistical software (v7 Primer-E Ltd., Plymouth, U.K.).

Temporal and spatial patterns in suspended particle characteristics (53-300 µm) were examined using principal components analysis (PCA; Clarke *et al*., 2014). Permutational multivariate analysis of variance (PERMANOVA routine in Permanova+ for Primer) was used to test for differences in particle characteristics between years and among months and aquatic habitats. Described in detail elsewhere (Anderson *et al*., 2008), PERMANOVA is analogous to traditional univariate analysis of variance (ANOVA) in that it partitions variability among more than one factor and tests for interactions. As PERMANOVA uses a permutation procedure to test for significance, normality is not required (Anderson *et al*., 2008). I used 9,999 permutations and an α of 0.05 as an indication of statistical significance. Post hoc pairwise comparisons with PERMANOVA however, do not provide enough power when conducted on small sample sizes and unbalanced data sets to determine differences within factor levels (Anderson *et al*., 2008), so trends in particle characteristics (53-300 µm) were more closely examined by comparing the mean and standard error of the principal component (PC) scores for each sample (Figure 3).

Relationships between particle characteristics (53-300 µm) and environmental variables (i.e., discharge, flow velocity, NO_3^-, NH_4^+, DIN, and SRP) were assessed with the Bio-Env test (in Permanova+ for Primer; Anderson *et al*., 2008). Bio-Env is a multivariate non-parametric analogue to univariate multiple linear regression. It measures the extent to which the environmental data is related to or best explains the particle characteristics by calculating the Spearman correlation coefficients for all possible combinations of environmental variable matrices with the particle characteristic matrix. Because of the significant annual variation in hydrographs (Figure 2) and particle

characteristics (Table 3), the Bio-Env was conducted as a constrained analysis within each year, thereby constraining the variable matching within each year and averaging the largest Spearman correlation coefficient (ρ) from both years. This approach, as opposed to a one-way analysis grouping both years together, resulted in higher Spearman correlation coefficients (ρ) overall suggesting there was substantial inter-annual variation. CHL, VSS, and TP were not included in the Bio-Env test as either particle or environmental variables because they were whole water particle measures (not necessarily representative of 53–300 µm size range) and considered more as indicators of particle food quality and relative composition. Relationships between these and other variables were considered by examining Pearson correlations (Table S2).

RESULTS

How do suspended particle characteristics differ temporally and spatially?

Suspended particles varied widely in size and shape characteristics (Table 1 and S3). In the PCA, PC1 (52.4% of variance) revealed considerable inter-annual variation from 2013 to 2014 in particle characteristics strongly represented by particle shape parameters (elongation and width), size parameters (diameter and volume), and particle concentration (Table 2; Figure 3a and S1). PC2 (25.2% of variance) was heavily loaded by size parameters (area, volume, and diameter) and shape parameter (symmetry; Table 2; Figure 3b and S1). Cumulatively, the first two PCs explained approximately 78% of the total variation (Table 2). Main channel, side channel, and impounded habitats grouped relatively close together in most months, whereas backwater particle characteristics often differed from other habitats (Figure 3 and S1). For example in June 2013, backwater particles were not considerably different from other habitats (~0-2 PC1 units; Figure 3a), but by September those differences had increased (~3-4 PC1 units).

Table 1. Summary of particle characteristics for particles 53-300 µm in size collected in June, August, and September of 2013 and 2014 in Pool 19 of the Upper Mississippi River (N=74); including the overall mean, standard deviation (SD), and range of median values of particle parameter distributions and mean particle density. For definitions and abbreviations of particle parameters see Table S1.

Parameter	Mean	SD	Min	Max
Area ABD (µm^2)	2998.95	740.31	1559.26	4891.76
Diameter ESD (µm)	96.65	14.08	74.29	129.49
Elongation	10.36	4.97	2.86	18.61
Roughness	1.27	0.05	1.17	1.39
Symmetry	0.54	0.08	0.33	0.68
Volume ESD (µm^3)	5.04 e^5	2.27 e^5	2.15 e^5	1.14 e^6
Width (µm)	43.30	15.78	19.67	70.78
Particle concentration (# mL^{-1})	44.12	63.3	0.58	394.67

Table 2. Results of the principal components analysis (PCA) on particles 53-300 µm in size, including median particle characteristics and mean particle concentrations, among Upper Mississippi River Pool 19 aquatic habitats in June, August, and September of 2013 and 2014 (N=74).

Principal component	PC1	PC2	PC3
Eigenvalue	4.19	2.01	1.27
% Variation	52.4	25.2	15.9
Cumulative % variation	52.4	77.6	93.5
Parameter			
Area ABD	0.243	-0.589	0.112
Diameter ESD	-0.414	-0.344	0.003
Elongation	-0.459	0.182	0.119
Roughness	-0.142	-0.329	0.678
Symmetry	0.255	-0.408	-0.519
Volume ESD	-0.411	-0.351	-0.006
Width	0.439	-0.200	0.260
Particle concentration (# mL^{-1})	-0.335	-0.254	-0.420

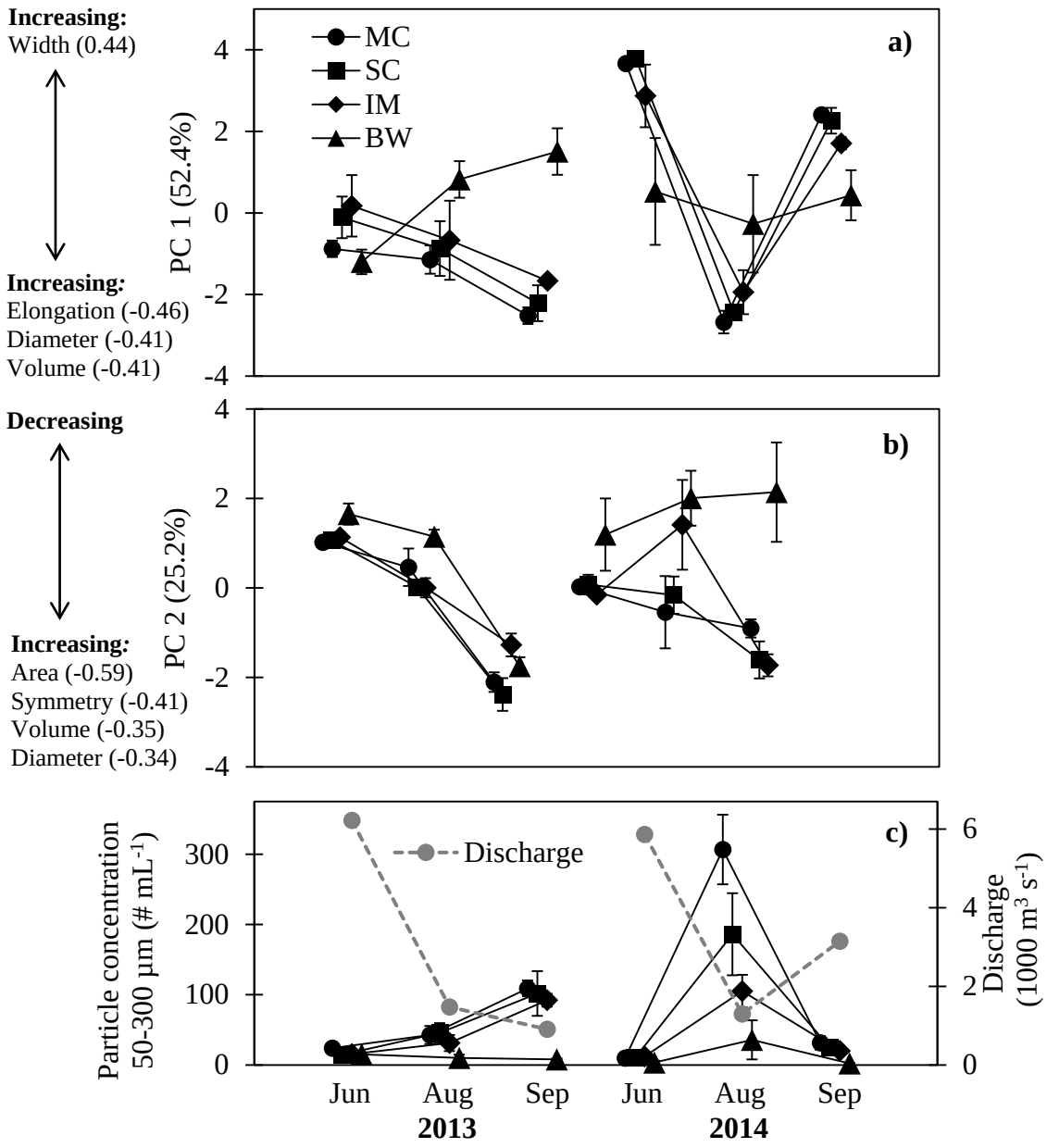

Figure 3. Particle characteristics and concentration of particles 53-300 µm in diameter from Pool 19, Upper Mississippi River in 2013 and 2014 shown across months and aquatic habitats as follows: (a) principal component (PC) 1 scores; (b) PC 2 scores; y-axis title shows percent of variation in parentheses and particle parameters listed in order from largest to smallest coefficient (highest four), their direction of change indicated by the arrow; and (c) particle concentration (# mL^{-1}) and discharge on secondary y-axis. Aquatic habitat type designated in legend as follows: main channel (MC), side channel (SC), impounded (IM), backwater (BW). Error bars indicate standard error.

Particle characteristics in channel and impounded habitats were on similar trajectories over time, whereas backwaters followed a different trajectory. For example, particles in channel and impounded habitats increased in median size (diameter and volume), elongation, and concentration from June to September 2013 (Figure 3). Visual inspection of FlowCAM images suggested particles in June were mostly sediment and detritus, with some phytoplankton and a few zooplankton (i.e., rotifers; Figure S2a). By September 2013, particles in channel and impounded habitats were mostly large filamentous algae and large detrital aggregates and/or colonial algae (Figure S2b).

In contrast, particles in backwaters by September 2013 increased in width and decreased in size and concentration (Figure 3). Although backwater particles appeared to be similarly dominated by sediment and detritus in June 2013, by September backwater particles included many more phytoplankton, smaller rotifers, and copepod nauplii than in other habitats (Figure S3b). Larger particles in backwaters in September 2013 included larger zooplankton (e.g., cladocerans, rotifers, and nauplii), colonial phytoplankton (e.g., *Microcystis*), and vegetation fragments (Figure S3c).

In 2014, channel and impounded habitats showed a different pattern from 2013 in that particle characteristics in September were more similar to those in June (Figure 3a and 3c). From June to August, particles increased in concentration, median size and elongation. Based on the FlowCAM images, this shift in characteristics appeared to be from a greater proportion of suspended sediment in June (Figure S2c) to more filamentous algae in August (Figure S2e and S4a). Then in September, particles in channel and impounded habitats decreased in concentration and shifted back to a smaller median size with higher symmetry (Figure 3a), which likely reflected re-suspension of

sediment due to the rise in discharge (Figure 2 and S4c). However, more living particles (e.g., diatoms, rotifers, and *Microcystis*) were photographed by FlowCAM in September than in June (Figure S2c, S2d, and S4c).

Particle characteristics in backwaters were similar among months in 2014. However, the particle characteristics in backwaters were on a different trajectory over time than those of the other habitats (Figure 3). Despite small changes in median particle characteristics, there were considerable changes in particle assemblages. For example, particles in backwaters in June exhibited higher elongation values than other habitats, likely reflecting a high proportion of filamentous algae. In August, an abundance of zooplankton eggs were seen in backwaters (Figure S4b). Then in September, although sharing similar characteristics with June, particles in backwaters included far more zooplankton (e.g., rotifers and nauplii; Figure S4d). Additionally, a noticeable increase in the frequency of particles 100-135 µm in diameter at a backwater site in September was due to a dinoflagellate bloom (i.e., *Ceratium*), which was not seen in other months. This shift in particle assemblage likely contributed to the higher elongation values recorded in backwaters compared to other habitats in September and suggests secondary production can also at times influence particle characteristics.

Whole water particle measurements, including TSS, VSS, and relative CHL concentrations, were less sensitive in identifying changes in particle dynamics. TSS and VSS concentrations varied little from June to September 2013 despite major changes in (53-300 µm) particle characteristics (Figure 4a and 4b). In 2014, TSS and VSS concentrations were more variable over time and among channel and impounded habitats (Figure 4a) and showed some association with the particle characteristics (Table S2).

Relative CHL concentrations among all habitats increased during both years, except for a small decline in backwaters relative to other habitats in September 2013 (Figure 4c).

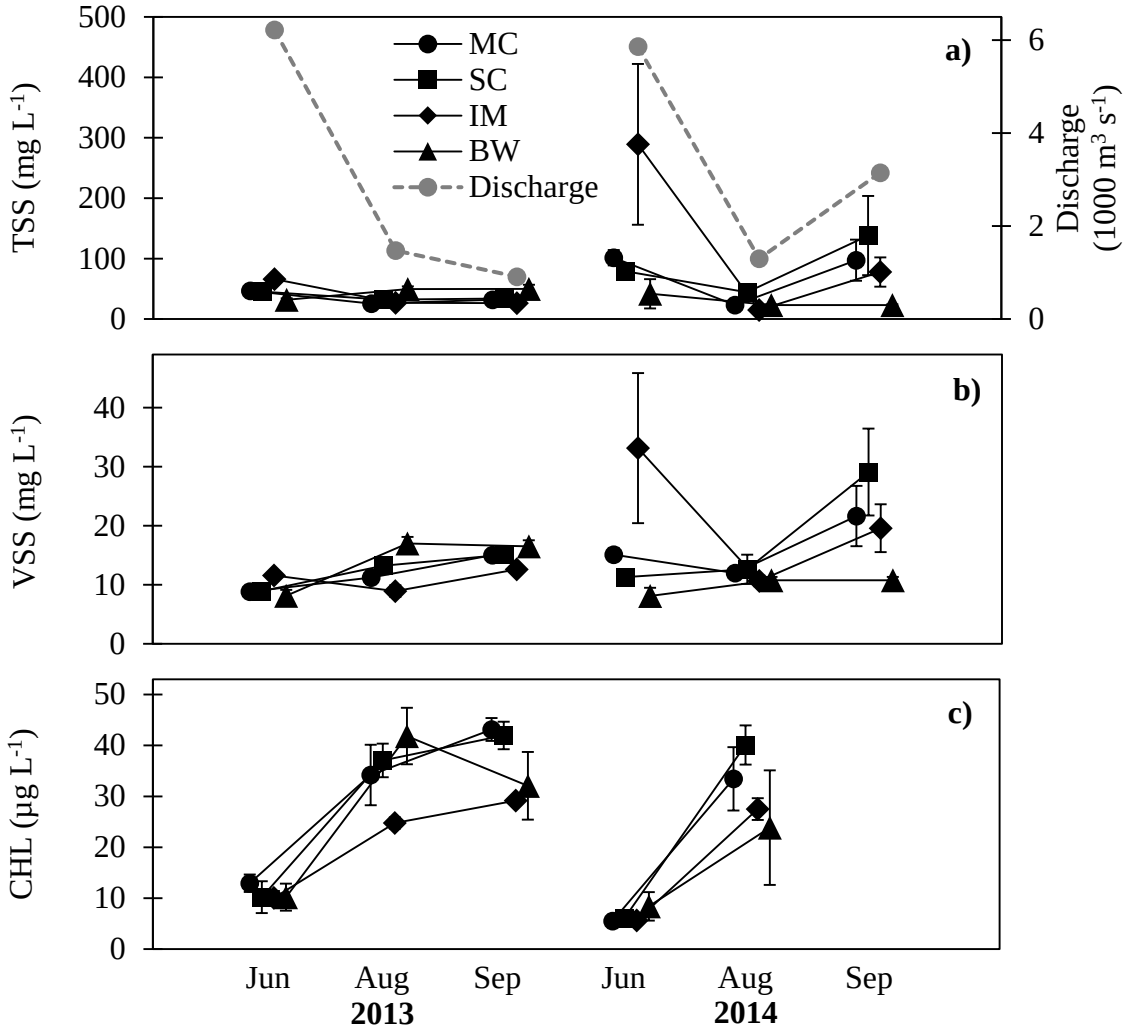

Figure 4. Total suspended solids and relative chlorophyll from Pool 19, Upper Mississippi River in 2013 and 2014 shown across months and aquatic habitats as follows: (a) mean total suspended solids (TSS) and discharge on secondary y-axis; (b) mean volatile suspended solids (VSS); and (c) mean algal chlorophyll concentrations (CHL). Aquatic habitat type designated in legend as follows: main channel (MC), side channel (SC), impounded (IM), backwater (BW). Error bars indicate standard error.

The PERMANOVA results supported these spatial and temporal patterns in suspended particle characteristics, suggesting the particle characteristics were

significantly different between years and among months and habitats ($p = 0.0001$ for all three tests). However, all the interactions, including the 3-way (year x month x habitat), were significant ($p = 0.0006$ for the 3-way interaction, $p = 0.0001$ for the 2-way interactions; Table 3) and apparent in the PCA where variations were seen within year, month, and habitat combinations (Figure 3a, 3b and S1).

Table 3. Results from permutational analysis of variance (PERMANOVA) testing for differences in suspended particle characteristics (53-300 µm) sampled in Pool 19, Upper Mississippi River in 2013 and 2014 as an effect of year, month, and aquatic habitat type (N=74). Significance level of $\alpha \leq 0.05$.

Source	*df*	Pseudo-*F*	*P* (perm)
Year	1	29.954	0.0001
Month	2	60.367	0.0001
Habitat	3	8.361	0.0001
Year x month	2	23.234	0.0001
Year x habitat	3	4.690	0.0001
Month x habitat	6	2.937	0.0001
Year x month x habitat	6	1.9895	0.0006
Residual	49		
Total	72		

What environmental variables have the strongest association with particle characteristics?

Using both years of data, the Bio-Env test results showed that the range of the highest Spearman correlations (ρ) for each number of variable models was relatively small ($\rho = 0.730 – 0.855$; Table 4). This suggested that additional variables in 1-2 variable models made minimal improvements to the correlation coefficient (ρ). NO_3^- and SRP were the best two variable model ($\rho = 0.806$) and discharge was the best one

variable model (ρ = 0.730; Table 4) for explaining the patterns in the particle characteristics. These three variables were selected in the five best overall results, however the range of ρ for the 10 best overall results was also small (ρ = 0.809 – 0.855; Table S4). Therefore, the most parsimonious models included NO_3^- and SRP (ρ = 0.806) or NO_3^-, SRP, and discharge (ρ = 0.840; Table 4 and S4).

Table 4. Bio-Env test results giving the average within year Spearman rank correlation coefficient (ρ) between the particle parameter similarity (Euclidean distance) matrix and all possible combinations of environmental variables to find the best fitting explanatory variables. Environmental variables available for selection in the model included: nitrate + nitrite (NO_3^-), ammonia + ammonium (NH_4^-), soluble reactive phosphorous (SRP), dissolved inorganic nitrogen to SRP ratio (DIN:SRP), discharge ($m^3\ s^{-1}$), and 2014 flow velocity ($m\ s^{-1}$) measured at each site.

Number of Variables	Spearman coefficient ρ	Selected variables
Highest correlations for each number of variables		
2013-2014 data with discharge		
1	0.730	Discharge
2	0.806	NO_3^-, SRP
3	0.840	NO_3^-, SRP, Discharge
4	0.848	NO_3^-, SRP, DIN:SRP, Discharge
5	0.855	NO_3^-, NH_4^-, SRP, DIN:SRP, Discharge
2014 data with flow velocity		
1	0.492	SRP
2	0.625	SRP, Flow velocity
3	0.624	NH_4^-, SRP, Flow velocity
4	0.550	NH_4^-, SRP, DIN:SRP, Flow velocity
5	0.500	NO_3^-, NH_4^-, SRP, DIN:SRP, Flow velocity

Using 2014 data with flow velocity ($m\ s^{-1}$) instead of discharge, the ρ for the best two variable model, SRP and flow velocity (ρ = 0.625), was somewhat higher than that

for the one variable model, SRP ($\rho = 0.492$; Table 4). However, additional variables did not improve ρ values (Table 4) and the range of ρ for the overall best results was again small ($\rho = 0.492 – 0.625$; Table S4). As a result, NO_3^-, SRP, and discharge or flow velocity were considered the best variables associated with the particle characteristics (Table 4 and S4). However, NO_3^- and SRP concentrations were also strongly related to discharge and flow velocity (Figure 5a and 5b). Thus, the effects of discharge and nutrients (NO_3^- and SRP) on the particle characteristics are confounded and difficult to separate.

TP concentrations remained relatively stable throughout the 2013 study period, except in backwaters where TP gradually increased from June to September (Figure 5c). In 2014, TP concentrations were associated with discharge (Table S2), decreasing from June to August and increasing with the rise in discharge in September (Figure 5c).

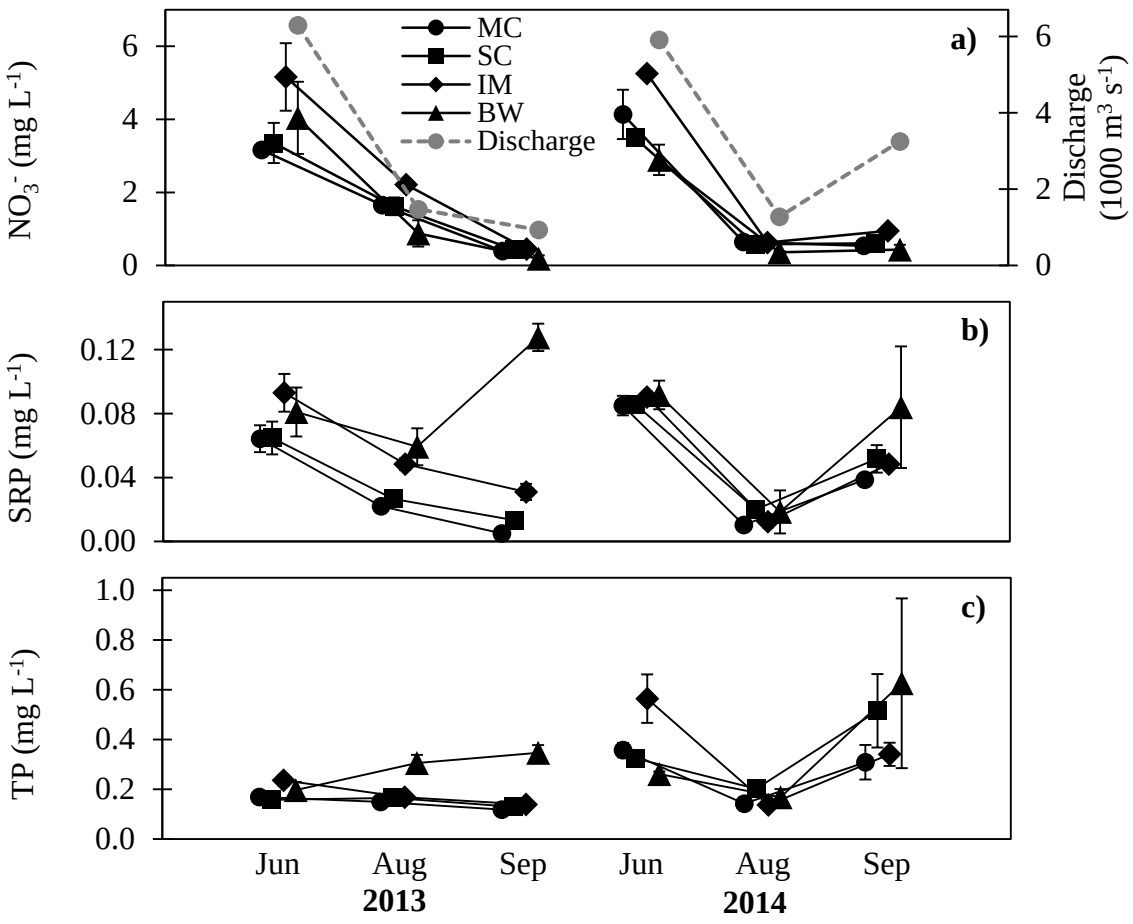

Figure 5. Nutrients measured in June, August, September of 2013 and 2014 among aquatic habitats in Pool 19, Upper Mississippi River as follows: (a) mean nitrate + nitrite concentrations (NO_3^-) and discharge on secondary y-axis; (b) mean soluble reactive phosphorous concentrations (SRP); and (c) mean total phosphorous concentrations (TP). Aquatic habitat type designated in legend as follows: main channel (MC), side channel (SC), impounded (IM), backwater (BW). Error bars indicate standard error.

DISCUSSION

How do suspended particle characteristics differ temporally and spatially?

Suspended particle characteristics exhibited significant temporal and spatial patterns strongly affected by variations in discharge. I predicted that during high discharge in spring, habitats would be more homogenous in particle characteristics because of increased hydrological connectivity and lateral mixing of materials among habitats. Particle characteristics were more similar among habitats during high discharge. For example, backwaters in June 2013 were more similar to other habitats than in other months, likely owing to increased hydrologic connection and mixing caused by high flow velocities as well as wind advection (Rohweder *et al*., 2008). Pongruktham and Ochs (2015) also found that during high river stages Lower Mississippi River backwater sites closely resembled the main channel in terms of turbidity, dissolved nutrient concentrations, and phytoplankton biomass and production.

However, in some instances comparable discharge did not result in similar particle characteristics among aquatic habitats. For example, in June and August 2013 particle characteristics in channel and impounded habitats were similar despite the large difference in discharge (~6300 $m^3 s^{-1}$ and ~1470 $m^3 s^{-1}$, respectively). In contrast, discharge in August and September 2013 were similar (~1470 $m^3 s^{-1}$ and ~930 $m^3 s^{-1}$, respectively), but particle characteristics differed significantly. Therefore, particle characteristics and composition can differ between the rising and falling limb of the hydrograph (Ongley, 1982). As June and August 2013 were both sampled on falling

limbs, their similarities in particle characteristics may be explained by similar preceding flow conditions regardless of large differences in discharge.

In contrast to 2013, June 2014 was sampled on a rising limb. During increasing discharge, erosion of soil surfaces is the greatest contributor to suspended particles (Ongley, 1982). Rising discharge flushes the system of previously deposited materials on the river banks and floodplains, which then limits the supply for transport during subsequent rises and falls (Walling *et al.*, 2000; Hicks & Gomez, 2003). Consequently, peak sediment concentrations typically occur during the rising limb before peak discharge and concentrations are significantly lower after peak discharge (Allan and Castillo, 2007). This was not the exact pattern in our study as the concentration of particles (53–300 µm) was slightly higher on the falling limb (June 2013) than on the rising limb (June 2014) at comparable discharges (~6300 m^3 s^-1 and 5900 m^3 s^{-1}, respectively). Initial spring floods in May possibly restricted the supply of materials (53–300 µm) in suspension during both June sampling events (Ongley, 1982).

Overall, concentrations of particles 53-300 µm were lowest during high discharge and highest during low discharge. However, TSS concentrations in channel and impounded habitats were higher on the rising limb (June 2014) than on the falling limb (June 2013) and showed a positive association with discharge, which has also been documented in other studies on the UMR (Houser *et al.*, 2010). The decrease in particle concentrations (53-300 µm) during high discharge may be explained by preceding conditions limiting particle supply or a dilution effect of this size range. Suspended particle concentrations vary greatly depending on land use, tributary inputs, locations of dams, and the processes dominating at different stages of discharge (Houser *et al.*, 2010).

However, these data suggest that suspended particle concentrations also vary greatly by size fraction.

Images of suspended sediment in June 2014 suggested a higher frequency of inorganic particles on the rising limb than on the falling limb (June 2013). The composition of suspended particles during high discharge is largely driven by soil mineralogy in the drainage basin, tributary contributions, allochthonous inputs, as well as the degree of hydrologic connectivity among aquatic habitats (Ongley, 1982; Walling *et al.*, 2000; Houser *et al*., 2010; Pongruktham and Ochs, 2015). Further, images from the falling limb (June 2013) suggested a higher proportion of small phytoplankton and rotifers than on the rising limb (June 2014). As coarser materials (e.g., sand) settle out of suspension during declining discharge, the proportion of organic materials in suspension increases (Ongley, 1982). CHL concentrations were also slightly higher during the falling limb (June 2013) than the rising limb (June 2014), providing evidence for a higher proportion of phytoplankton than sediment still in suspension.

Another high discharge period occurred in September 2014 during typical base flow conditions, which caused a decrease in median particle size and increase in width and symmetry, suggesting sediment re-suspension. However, plankton particles persisted during this high flow in September, as suggested by the number of plankton in the FlowCAM images. During high flows, scouring can suspend benthic algae into the water column and carry backwater plankton to the channels (Allan and Castillo, 2007; Pongruktham and Ochs, 2015). These were likely sources of plankton in channel and impounded habitats during this late-season rise in discharge. TP and VSS concentrations

were also higher in all habitats during this time, suggesting a large portion of suspended particles were organic as opposed to inorganic sediment.

The transition in characteristics during declining discharge from June to September 2013 in channel and impounded habitats (i.e., smaller sediment and detritus to larger plankton and detritus), suggests an increase in biotic production in the water column as summer progressed. Increases in CHL concentrations from June to September 2013 in these habitats and declines in NO_3^- and SRP throughout 2013 provide evidence for increased phytoplankton production and concomitant nutrient uptake. These results support the RWC, suggesting that declining discharge initiates a transition in suspended particle assemblages from a greater contribution of allochthonous inputs during rising discharge to autochthonous production at lower discharge (i.e., plankton development; Humphries *et al*., 2014).

As discharge and hydrologic connections decreased, backwaters developed distinct particle characteristics on a different trajectory from the other habitats. Low discharge and limited connectivity increases water residence time, which promotes sedimentation, decreases inorganic turbidity, increases light availability, and stimulates primary and secondary production (Basu and Pick, 1996; Burdis and Hoxmeier, 2011; Houser *et al.*, 2015; Pongruktham and Ochs, 2015). Particle images taken from backwaters during declining discharge (August 2013 and 2014), showed an increase in particle concentrations (50-300 µm), and suggested a shift from a larger proportion of sediment and filamentous algae to an increased frequency and diversity of small organisms (e.g., zooplankton eggs, rotifers, nauplii, and phytoplankton). High production

in backwaters is also evidenced by increasing TP concentrations throughout 2013, which would be associated with more organic particles (e.g., plankton and detritus).

Pongruktham and Ochs (2015) also reported that as the degree of connection between the Lower Mississippi River channel and backwaters declined, so did turbidity in backwaters; and chlorophyll and gross primary production increased exponentially. On the Danube River in Austria, Hein *et al.* (2003) found plankton-derived organic matter dominated in connected and isolated backwater habitats during most of their study and suggested that allochthonous inputs were important during high hydrologic connectivity. However, with decreasing connectivity the major source of organic matter shifted to autochthonous production.

Individual backwater sites can differ greatly from each other in environmental variables and particle dynamics owing to their varying degrees of connection with the channel (Strauss *et al.*, 2004; Burdis and Hoxmeier, 2011; Houser *et al.*, 2015; Pongruktham and Ochs, 2015). The highest variation in particle characteristics among sites, especially backwaters, occurred during low discharge (September 2013 and August 2014). During an extended period of low discharge (September 2013), larger zooplankton (e.g., cladocerans and copepods) were more frequent in FlowCAM images, as was a greater diversity of smaller organisms (e.g., diatoms, green algae, and rotifers). Decker *et al.* (in press) also found that phytoplankton species composition differed the greatest between the main channel and a backwater of the UMR during summer low flows and suggested this was due to less hydrologic connectivity and other environmental conditions that influence variation in biotic production.

These results supported my hypothesis that during summer months and low discharge, backwater habitats diverge in particle characteristics from other habitats owing to (1) reduced hydrologic connectivity, (2) increased water residence time, and (3) nutrient dynamics that promote biotic production. The increase in CHL concentrations over both study periods also supports the increase in the frequency of phytoplankton seen in FlowCAM images during summer months as discharge declined and suggests primary production is a driver of suspended particle characteristics during low discharge conditions throughout the river floodplain.

What environmental variables have the strongest association with particle characteristics?

Discharge and flow velocity had the strongest effects on particle characteristics in this study, indicating the importance of discharge in regulating suspended particles in large rivers. However, other environmental variables, such as dissolved nutrients, may affect particle characteristics and compositions during low discharge conditions by influencing the development of the plankton community (Pongruktham and Ochs, 2015; Decker *et al.*, in press).

Pongruktham and Ochs (2015) showed NO_3^- and SRP concentrations significantly declined in less connected backwater habitats due to increases in primary production. Similarly, the transition of particle characteristics from June to September and increase in proportions of planktonic organisms generally coincided with declining NO_3^- and SRP concentrations, presumably owing to biotic assimilation in addition to discharge and denitrification (Houser and Richardson, 2010). NO_3^- concentrations in September 2014 remained low in all habitats despite the increase in discharge, possibly indicating a high

demand for nitrogen due to a high abundance of primary producers in late summer. NO_3^- concentrations were lower in backwaters, where more primary production is presumed to occur, than in the other habitats (Houser *et al.*, 2015; Pongruktham and Ochs, 2015; Decker *et al.*, in press). SRP concentrations increased considerably in backwaters in September 2013, likely due to its release from sediments facilitated by high water temperatures via internal phosphorous loading (Houser and Richardson, 2010). These nutrient patterns and concomitant changes in particle characteristics and assemblages are similar to other UMR studies, suggesting primary production and plankton development are important drivers of suspended particle dynamics as discharge declines and summer progresses (Burdis and Hoxmeier, 2011; Houser *et al.*, 2015; Decker *et al.*, in press).

In conclusion, our results suggest there are complex interactions between space, time, discharge, and other environmental variables which drive suspended particle dynamics in rivers. Suspended particle characteristics in backwaters followed different trajectories from other aquatic habitats over the growing season, which were likely related to hydrologic connectivity and development of plankton communities. Discharge was the strongest driver of suspended particle dynamics, providing support for the River Wave Concept (RWC) suggesting that different river flow stages drive the major processes which act on suspended particles. NO_3^- and SRP were also strongly associated with particle characteristics as well as with discharge. The proportion of plankton organisms in FlowCAM images and concentrations of CHL and VSS increased as discharge declined and summer progressed, therefore I hypothesize that nutrients are a stronger driver of biotic production during lower discharge conditions.

The spatial and temporal variation in whole water particle measures (i.e., TSS, VSS, and TP) were subtle compared to the significant differences in 53-300 µm particle characteristics. There were few consistent patterns between the whole water particle measures, which were used as indicators of particle quality (i.e., VSS, CHL, and TP) and the particle characteristics, suggesting particle characteristics (e.g., volume, symmetry, etc.) may not be the best for predicting particle assemblage composition or quality alone. However, examining suspended particle characteristics can provide information about the structure of particles at different river flow stages. Examining suspended particle dynamics in the UMR provided insight into the complex interactions among river flow, geomorphology, and nutrients as primary drivers of the quantity and quality of suspended particles in large floodplain rivers.

REFERENCES

Allan JD, Castillo MM. 2007. *Stream Ecology: Structure and function of running waters*, 2nd edn. Springer, Netherlands.

Álvarez E, López-Urrutia Á, Nogueira E, Fraga S. 2011. How to effectively sample the plankton size spectrum? A case study using FlowCAM. *Journal of Plankton Research* **33**: 1119–33. DOI: 10.1093/plankt/fbr012.

American Public Health Association (APHA), American Water Works Association, Water Environment Association. 2005. *Standard Methods for the Examination of Water & Wastewater*, 21st edn. Washington, D.C.

Anderson MJ, Gorley RN, Clarke KR. 2008. *PERMANOVA+ for PRIMER: Guide to software and statistical methods*, 1st edn. PRIMER-E Ltd: Plymouth, U.K.

Baker AL, Baker KK. 1979. Effects of temperature and current discharge on the concentration and photosynthetic activity of the phytoplankton in the Upper Mississippi River. *Freshwater Biology* **9**: 191–98. DOI: 10.1111/j.1365-2427.1979.tb01502.x.

Basu BK, Pick FR. 1996. Factors regulating phytoplankton and zooplankton biomass in temperate rivers. *Limnology and Oceanography* **41**: 1572–77. DOI: 10.4319/lo.1996.41.7.1572.

Bhowmik NG, Adams JR. 1986. The hydrologic environment of Pool 19 of the Mississippi River. *Hydrobiologia* **136**: 21–30. DOI: 10.1007/BF00051501.

Black AR, Dodson SI. 2003. Ethanol: a better preservation technique for *Daphnia*. *Limnology and Oceanography: Methods* **1**: 45–50. DOI: 10.4319/lom.2011.1.45.

Burdis RM, Hoxmeier RJH. 2011. Seasonal zooplankton dynamics in main channel and backwater habitats of the Upper Mississippi River. *Hydrobiologia* **667**: 69–87. DOI: 10.1007/s10750-011-0639-y.

Chételat J, Pick FR, Hamilton PB. 2006. Potamoplankton size structure and taxonomic composition: influence of river size and nutrient concentrations. *Limnology and Oceanography* **51**: 681–89. DOI: 10.4319/lo.2006.51.1_part_2.0681.

Clarke KR, Gorley RN. 2015. *PRIMER v7: User Manual/Tutorial*, 1st edn. PRIMER-E Ltd: Plymouth, U.K.

Clarke KR, Gorley RN, Somerfield PJ, Warwick RM. 2014. *Change in marine communities: an approach to statistical analysis and interpretation*, 3rd edn. PRIMER-E Ltd: Plymouth, U.K.

Decker JK, Wehr JD, Houser JN, Richardson WB. 2015. Spatiotemporal phytoplankton patterns in the Upper Mississippi River in response to seasonal variation in discharge and other environmental factors. *River Systems*: in press. DOI: 10.1127/rs/2015/0103.

Delong MD, Thorp JH. 2006. Significance of instream autotrophs in trophic dynamics of the Upper Mississippi River. *Oecologia* **147**: 76–85. DOI: 10.1007/s00442-005-0241-y.

Drenner RW, Mummert JR, deNoyelles Jr. F, Kettle D. 1984. Selective particle ingestion by a filter-feeding fish and its impact on phytoplankton community structure. *Limnology and Oceanography* **29**(5): 941–48. DOI: 10.4319/lo.1984.29.5.0941.

Fluid Imaging Technologies, Inc. 2013. *FlowCAM® Manual*, version 3.4. Scarborough, ME.

Gaedke U. 1992. The size distribution of plankton biomass in a large lake and its seasonal variability. *Limnology and Oceanography* **37**: 1202–20. DOI: 10.4319/lo.1992.37.6.1202.

Gilvear DJ, Petts GE. 1985. Turbidity and suspended solids variations downstream of a regulating reservoir. *Earth Surface Processes and Landforms* **10**: 363–73. DOI: 10.1002/esp.3290100408.

Hicks DM, Gomez B. 2005. Sediment transport. In *Tools in Fluvial Geomorphology*, Kondolf GM, Piegay H (eds). John Wiley & Sons, Ltd: Chichester, U.K.; 425–61. DOI: 10.1002/0470868333.ch15.

Houser JN, Bartsch LA, Richardson WB, Rogala JT, Sullivan JF. 2015. Ecosystem metabolism and nutrient dynamics in the main channel and backwaters of the Upper Mississippi River. *Freshwater Biology* **60**: 1863–79. DOI: 10.1111/fwb.12617.

Houser JN, Bierman DW, Burdis RM, Soeken-Gittinger LA. 2010. Longitudinal trends and discontinuities in nutrients, chlorophyll, and suspended solids in the Upper Mississippi River: implications for transport, processing, and export by large rivers. *Hydrobiologia* **651**: 127–44. DOI: 10.1007/s10750-010-0282-z.

Houser JN, Richardson WB. 2010. Nitrogen and phosphorus in the Upper Mississippi River: transport, processing, and effects on the river ecosystem. *Hydrobiologia* **640**: 71–88. DOI: 10.1007/s10750-009-0067-4.

Humphries P, Keckeis H, Finlayson B. 2014. The river wave concept: integrating river ecosystem models. *BioScience* **64**(10): 870–82. DOI: 10.1093/biosci/biu130.

Junk WJ, Bayley PB, Sparks RE. 1989. The flood pulse concept in river-floodplain systems. *Canadian special publication of fisheries and aquatic sciences* **106**: 110–27. DOI: 10.1371/journal.pone.0028909.

Kolar CS, Chapman DC, Courtenay WR, Housel CM, Williams JD, Jennings DP. 2007. Bigheaded carps: a biological synopsis and environmental risk assessment. In *American Fisheries Society, Special Publication*. Vol. 33. Bethesda, Maryland: American Fisheries Society. DOI: 10.1643/OT-09-041.

Ongley ED. 1982. Influence of season, source and distance on physical and chemical properties of suspended sediment. In *Recent Developments in the Explanation and Prediction of Erosion and Sediment Yield (Proceedings of the Exeter Symposium)*. International Association of Hydrological Sciences (IAHS) Press: Wallingford, U.K.; 371–83.

Patton CJ, Kryskalla JR. 2003. U.S. Geological Survey national water quality laboratory: evaluation of alkaline persulfate digestion as an alternative to Kjeldahl digestion for determination of total and dissolved nitrogen and phosphorous in water. *Water Resources Investigations Report 03-4174*. Denver, CO.

Pongruktham O, Ochs C. 2015. The rise and fall of the Lower Mississippi: effects of hydrologic connection on floodplain backwaters. *Hydrobiologia* **742**(1): 169–83. DOI: 10.1007/s10750-014-1983-5.

Rohweder J, Rogala JT, Johnson BL, Anderson D, Clark S, Chamberlin F, Runyon K. 2008. Application of wind fetch and wave models for habitat rehabilitation and enhancement projects. *U.S. Geological Survey Open-File Report 2008-1200*, 43 pp.

Sprules WG, Munawar M. 1986. Plankton size spectra in relation to ecosystem productivity, size, and perturbation. *Canadian Journal of Fisheries and Aquatic Sciences* **43**: 1789–94. DOI: 10.1139/f86-222.

Strauss EA, Richardson WB, Bartsch LA, Cavanaugh JC, Bruesewitz DA, Imker H, Heinz JA, Soballe DM. 2004. Nitrification in the Upper Mississippi River: patterns, controls, and contribution to the NO_3^- budget. *Journal of the North American Benthological Society* **23**: 1–14. DOI: 10.1899/0887-3593(2004)023<0001:NITUMR>2.0.CO;2.

Thorp JH, Covich AP. 2010. *Ecology and classification of North American freshwater invertebrates*, 3rd edn. Elsevier Inc.: Burlington, MA.

Thorp JH, Delong MD. 1994. The riverine productivity model: an heuristic view of carbon sources and organic processing in large river systems. *Oikos* **70**: 305–08. DOI: 10.2307/3545642.

U.S. Department of Interior, U.S. Geological Survey. *National Water Information System*. http://www.waterdata.usgs.gov/. Date of access: 2015-08-03.

Vannote RL, Minshall GW, Cummins KW, Sedell JR, Cushing CE. 1980. The River Continuum Concept. *Canadian Journal of Fisheries and Aquatic Sciences* **37**: 130–37. DOI: 10.1139/f80-017.

Walling DE, Owens PN, Waterfall BD, Leeks GJL, Wass PD. 2000. The particle size characteristics of fluvial suspended sediment in the Humber and Tweed catchments, UK. *Science of the Total Environment* **251/252**: 205–22. DOI: 10.1016/S0048-9697(00)00384-3.

Wilcox DB. 1993. An aquatic habitat classification system for the Upper Mississippi River system. U.S. Fish and Wildlife Service, Environmental Management Technical Center, Onalaska, WI. *EMTC 93-T003*, 9 pp.

CHAPTER II

POTENTIAL EFFECTS OF FILTER-FEEDING ASIAN CARP ON SUSPENDED PARTICLE DYNAMICS IN THE UPPER MISSISSIPPI RIVER

INTRODUCTION

Invasive species threaten newly invaded ecosystems by homogenizing global biodiversity, disturbing local habitats, affecting native fauna through competition and predation, modifying food webs through trophic cascades, and ultimately altering ecosystem functions and services (Kolar and Lodge, 2002). The invasive silver carp (*Hypophthalmichthys molitrix*) and bighead carp (*H. nobilis*), collectively referred to as Asian carp (AC), have had strong, negative impacts on aquatic ecosystems in which they are now established (Chick and Pegg, 2001), leading to heightened concerns about their spread throughout the United States (U.S.) and into the Great Lakes.

AC are indigenous to large rivers of central and south Asia and eastern China (e.g., the Amur and Yangtze Rivers; Kolar *et al.*, 2007) and were brought to the U.S. in the 1970s and 1980s for water quality and algae control in aquaculture (Freeze and Henderson, 1982). In the 1980s, flooding facilitated their escape into the Mississippi River and its tributaries. Subsequently, the abundance of AC has increased exponentially throughout the 2000s in certain areas of the Mississippi River Basin (Chick and Pegg, 2001). Populations are currently established in the Illinois, Ohio, Wabash, Missouri, Arkansas, Tennessee, and the middle and lower Mississippi Rivers (Kolar *et al.*, 2007). Due to their rapid growth and dispersal, high fecundities, and feeding abilities, the Great Lakes and the Upper Mississippi River (UMR) are at imminent risk for detrimental impacts (Kolar *et al.*, 2007; DeGrandchamp *et al.*, 2008).

Large rivers provide adequate flow, temperatures, and abundant plankton for the reproduction, feeding, and optimal growth of AC (Kolar *et al.*, 2007). However, relatively little is known about AC feeding habits in North American rivers compared to those in their native range (*but see* Williamson and Garvey, 2005; Sampson *et al.*, 2009, Pongruktham *et al*., 2010; Calkins *et al.*, 2012; Sass *et al.*, 2014). Most research on the diet and feeding ecology of AC has been done in their native range of China and in lentic systems (e.g., ponds, reservoirs, lake mesocosms, and aquaculture). AC are voracious planktivorous filter-feeders that consume a wide and variable range of particles, including phytoplankton, zooplankton, detritus, bacteria, and inorganic particles (e.g., silt and sand; Adamek and Spittler, 1984; Opuszynski *et al*., 1991; Costa-Pierce, 1992; Xu and Xie, 2004; Calkins *et al*., 2012). For example, silver carp are capable of consuming particles as small as one micrometer (Adamek and Spittler, 1984) and bighead carp have been documented to consume particles up to several millimeters in size, as well as benthic organisms when plankton is limited (Opuszynski *et al.*, 1991). Studies have indicated AC possess feeding preferences, mainly particle size selection (Dong and Li, 1994; Lu et al., 2002; Ma et al., 2009), and can alter zooplankton and phytoplankton communities.

AC have been observed to reduce total zooplankton and phytoplankton abundances and biomass (Wu *et al.*, 1997; Tang *et al*., 2002; Ma *et al*., 2009). Shifts in plankton community size structure from large to small organisms has also been well documented (Opuszynski *et al*., 1991; Voros *et al*., 1997; Wang *et al.*, 2008). AC can filter larger particles more efficiently than small particles, therefore shifting the overall composition to small forms. Various interactions contribute to this pattern, primarily their selective consumption of large-bodied zooplankton (e.g., cladocerans and copepods)

which reduces grazing pressure on the phytoplankton community causing a competitive release for phytoplankton and small-bodied zooplankton (Sampson *et al*., 2008; Cooke *et al.*, 2009; Sass *et al.*, 2014). Zooplankton may also be indirectly reduced by competition with AC for phytoplankton (Lu *et al*., 2002; Cooke *et al.*, 2009). Enhanced growth of small phytoplankton and increased nanoplankton abundance due to AC presence has also been associated with increases in chlorophyll *a* concentrations, primary production, and reduced water clarity (Wu *et al*., 1997; Tang *et al*., 2002; Wang *et al*., 2008).

The wide ranges and variation in particle size selection by AC can also be attributable to differences in their feeding morphology from other planktivores. Bighead carp gill rakers are long and comb-like with clear separation, whereas silver carp gill rakers are modified to form a dense, porous net-like filtration system (Costa-Pierce, 1992; Kolar *et al*., 2007; Walleser *et al.*, 2014). A suprabranchial organ in the buccal cavity of both species produces mucus that facilitates retention of a wide size range of particles. Their specialized feeding morphologies provide AC a competitive advantage over native filter-feeding fishes.

Direct competition between native filter-feeders and AC for food has been observed in the Illinois and Mississippi Rivers. Significantly reduced body conditions of gizzard shad and bigmouth buffalo have been reported since the AC invasion in the Illinois River (Irons *et al*., 2007). In a mesocosm experiment, a reduction in the relative growth of age-0 paddlefish was observed when bighead carp were present (Shrank *et al*., 2003). A diet study by Sampson *et al*. (2009) found substantial diet overlap between AC and gizzard shad, bigmouth buffalo, and to a lesser extent adult paddlefish in the Illinois and Mississippi Rivers.

Examining ecological changes in complex ecosystems is challenging, however a thorough understanding of the potential effects of AC on suspended particles as primary food web resources is crucial for ecosystem management and for developing effective control measures for AC. Our objective was to detect changes in suspended particles due to AC feeding by examining the temporal and spatial patterns of suspended particle characteristics, such as particle size and shape parameters (e.g., diameter, elongation, surface roughness, etc.), in regard to the relative abundance of AC. A FlowCAM (Flow Camera and Microscope) particle imaging system was used to compare suspended particle characteristics between two UMR reaches with varying AC abundance. In Pool 20, AC are extremely abundant and accumulate below Lock and Dam 19 because upstream movement is hindered by the 11.6 m tall dam serving as a bottleneck for AC. Above the dam in Pool 19, AC are relatively less abundant than in Pool 20. This area is considered the AC invasion front on the UMR and provides a unique situation to examine ecosystem changes related to AC abundance by evaluating if there are differences in suspended particle characteristics between Pools 19 and 20. I hypothesized the most prominent effects of AC feeding on suspended particles would be in late summer during low flow and in less hydrologically connected backwater habitats. In areas with higher relative AC abundance (i.e., Pool 20 and backwater habitats), I predicted a reduction in particle sizes due to selective feeding of large particles by AC. Therefore, I hypothesized that relatively higher abundances of AC would cause a detectable shift in suspended particle size distributions towards an increased frequency of small particles.

METHODS

Study area and field sites

The UMR is divided into river reaches called navigation pools by a series of 29 locks and dams extending from St. Paul, Minnesota to its confluence with the Ohio River in Illinois. Lock and Dam 19 in Keokuk, Iowa was the first dam built on the UMR in 1913 to allow navigation through the shallow Des Moines rapids (Jahn & Anderson, 1986). Lock and Dam 19 is the oldest and tallest (11.6 m) dam on the Mississippi River separating Navigation Pools 19 and 20. Pool 19 extends 74.5 river km from Lock and Dam 18 in Gladstone, Illinois to Lock and Dam 19. Conversely, Pool 20 is less than half the length of Pool 19 extending only about 34 river km from Lock and Dam 19 to Lock and Dam 20 in Canton, Missouri (Jahn & Anderson, 1986).

Pool 19 is characterized by islands, side channels, and backwaters in the upper half of the pool while the lower impounded portion is lake-like with few islands and backwater areas (Bhowmik and Adams, 1986). Channel habitats, including the main and side channels, are constantly flowing with relatively higher water velocities than backwater areas. Backwater habitats are off-channel areas that vary in the degree of hydrological connectivity to flowing channels; some always maintain hydrologic connection and others become isolated during low flow. Backwaters are typically shallow (< 2 m) and have relatively lower water velocities than channels, providing important habitat for biota. Backwaters and other pooled areas (e.g., behind wing dams and

impounded habitats) provide flow refuge and nursery habitat for AC (DeGrandchamp *et al.*, 2008; Calkins *et al.*, 2012).

Pool 20 is geomorphologically very different from Pool 19 in that it has a steep gradient making it chute-like with few side channel and backwater areas (Figure 6; Jahn & Anderson, 1986). Because of the differing pool geomorphology, only three aquatic habitat types were present: main channel, side channel, and backwater habitats (Wilcox, 1993). Main channel and side channel sites were selected *a priori* in a stratified random design as designated by the Long Term Resource Monitoring Program (LTRMP; Wilcox, 1993) and included six sites in Pool 19 (3 main channel, 3 side channel) and six in Pool 20 (2 main channel, 4 side channel; Figure 6). Little variation in geomorphology and limnological conditions among main channel sites (Jahn & Anderson, 1986) allowed for reduced sampling efforts in Pool 20 for that habitat type. In contrast, three backwater sites in each pool (Figure 6) were selected as areas with relatively higher AC abundances based on monitoring information from this reach (Jim Lamer, Western Illinois University, pers. comm.); and thus, backwater sites were analyzed separately from channel sites. Sampling was conducted in June, August, and September of 2014 to coincide with high flows in spring and low flows in late summer and early fall (Figure 2). Daily river discharge data at Lock and Dam 19 were obtained from the USGS National Water Information System (waterdata.usgs.gov).

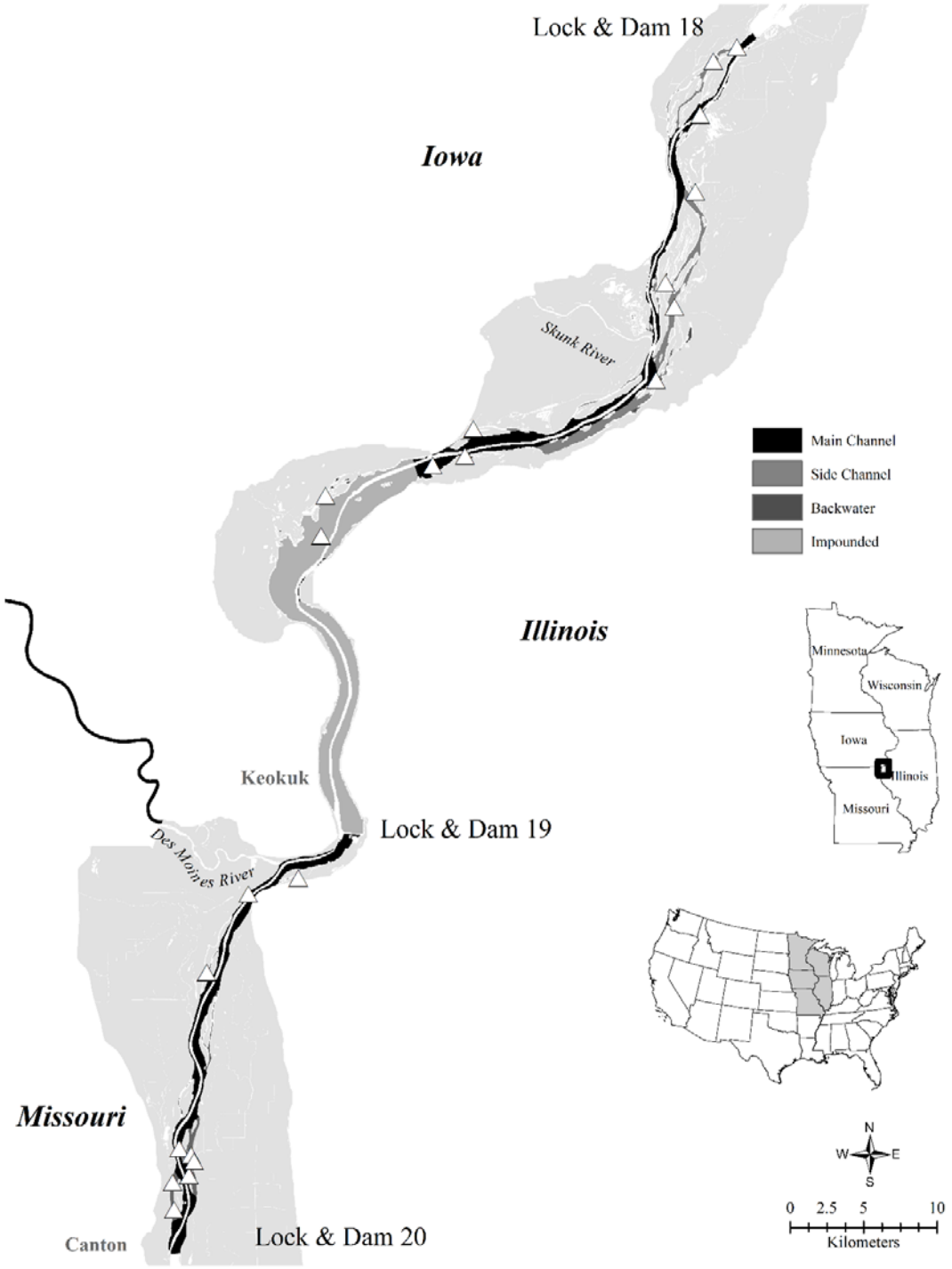

Figure 6. Map of Navigation Pools 19 and 20 of the Upper Mississippi River. Triangles indicate sample sites selected by stratified random sampling among aquatic three habitat types including main channel, side channel, and backwater. The white line is the Navigation channel. Pool 19 is characterized by relatively lower Asian carp abundance compared to Pool 20 with a relatively higher Asian carp abundance.

As an estimate of AC relative abundance in Pools 19 and 20, I used silver carp electrofishing catch per unit effort (CPUE, no. hr^{-1}) collected in 2013 and 2014 following LTRMP protocols (Figure 7; Gutreuter *et al.,* 1995). Electrofishing however, is not an effective method for capturing bighead carp. According to the fisheries monitoring programs on the Illinois River, bighead carp abundances have increased along with silver carp abundances (Irons *et al.,* 2007), therefore I used silver carp relative abundance estimates as a measure of the degree of both bighead and silver carp establishment (Sass *et al.,* 2014).

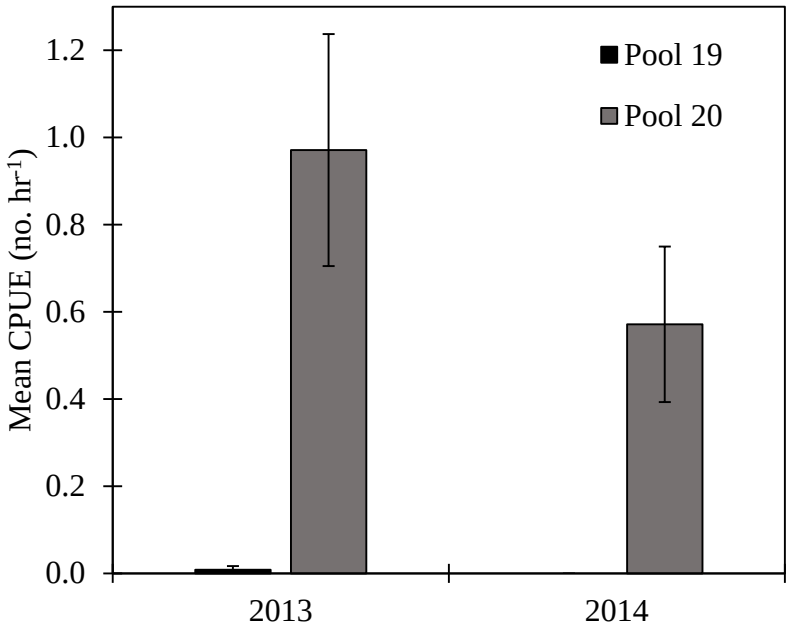

Figure 7. Mean (± S.E.) pulsed-DC electrofishing relative abundances (weighted catch-per-unit-effort, CPUE, no. hr^{-1}) of silver carp, *Hypophthalmichthys molitrix*, collected in Pools 19 and 20 of the Upper Mississippi River in 2013 and 2014 according to Long Term Resource Monitoring Program protocols. One fish was collected in Pool 19 in 2013 and 2014.

Particle analysis

Water samples were collected for particle analysis at mid-depth by pumping 15-60 liters of water with a bilge pump (~5678 L hr^{-1}) and filtering through 1000-µm and 53-µm Nitex mesh sieves. Materials collected on the 53-µm mesh sieve were rinsed into sample bottles and preserved with 70% ethanol to yield a concentrated sample of particles and plankton approximately 53–1000 µm in diameter.

Prior to particle analysis, preserved samples were filtered again through 300-µm Nitex mesh yielding the targeted 53-300 µm size range. A FlowCAM® (Flow Cytometer and Microscope by Fluid Imaging Technologies, Inc., Scarborough, ME, U.S.A.) was used to analyze filtered particle samples for size and shape distributions. FlowCAM is a continuous imaging flow cytometer that digitally photographs individual particles flowing in front of a microscope (Alvarez *et al.* 2011). Photos of particles and plankton ranging 53–300 µm in diameter were captured in auto-image mode, in which photographs were taken 20 frames per second at a constant flow rate of 1.75 mL per min through a 300-µm flow cell and with a 4X objective. Between 8,000 and 15,000 particles per sample were enumerated and measured for size and shape parameters from 2D images and then summarized using Visual Spreadsheet Software (Fluid Imaging Technologies, Inc.). The following parameters were used to describe suspended particle characteristics: area, diameter, elongation, roughness, symmetry, volume, width, and particle concentration (Table S1).

To ensure consistent and accurate FlowCAM particle size and concentration measurements, monthly size and count verification procedures were conducted using 50-µm diameter (National Institute of Standards and Technology [NIST] traceable) beads at

a concentration of 3000 particles per mL (COUNT-CAL Particle Size and Count Precision Standards, Thermo Scientific, Fremont, CA, U.S.A.). As recommended by the FlowCAM calibration verification procedure (FlowCAM® Manual, 2013), particle counts were within 20% of the listed concentration and mean particle diameters were within the NIST certified range of 50.2 ± 1.0 µm. Alvarez *et al.* (2011) found that FlowCAM tended to underestimate counts relative to traditional microscopy on preserved samples, but resulted in an average percentage error between the two methods of about 15%.

Statistical analyses

Multivariate analyses were used to examine differences in suspended particle characteristics between Pool 19 with relatively low AC abundance and Pool 20 with relatively high AC abundance (Figure 7). The median value for each particle parameter (e.g., elongation, etc.) and particle concentration (# mL^{-1}) were estimated for each sample. Particle parameters measured by the FlowCAM were first examined by draftsman correlation plots to assess collinearity of mathematically related parameters. I selectively eliminated particle parameters which were highly correlated ($r \geq 0.95$) for ease of interpretation (Table S1). Data were 4^{th} root transformed to reduce skewness and normalized to a zero mean and unit standard deviation to generate comparable, dimensionless scales. Euclidean distance similarity measure was used in all multivariate analyses using PRIMER statistical software (v7 Primer-E Ltd., Plymouth, U.K.).

Principal components analysis (PCA; Clarke *et al*., 2014) was used to examine the temporal patterns and differences between Pools 19 and 20 in suspended particle characteristics. Differences in suspended particle characteristics between pools and among months were tested using an analysis of similarity (ANOSIM; Clarke *et al*., 2014)

and an α of 0.05 as an indication of statistical significance. ANOSIM, similar to univariate ANOVA, is a non-parametric test for differences in rank similarities between samples and does not test for interactions among factors. Seasonal variation of particle characteristics, however, can be significant and is evaluated in prior analyses (Chapter 1). Spatial variation in particles among aquatic habitats (e.g., main channel vs. backwaters) can also be significant (Chapter 1), thus two separate analyses were conducted. One analysis on main and side channel habitats, because those are typically very similar in environmental conditions, and a separate analysis on backwater habitats, as those sites were not selected randomly.

RESULTS

Suspended particles varied greatly between Pools 19 and 20 in some characteristics (Table 5). For example, particles in Pool 19 ranged in area from about 2000 – 4500 μm^2, while in Pool 20 ranged from 1,600 – 3,900 μm^2. Concentrations of particles (53-300 μm) over the entire study period averaged about 69 particles per mL in Pool 19 and 34 particles per mL in Pool 20. However, some shape characteristics, such as roughness and symmetry, did not differ considerably between pools (Table 5 and S3).

The PCA on main and side channel habitats revealed an interesting pattern in particle characteristics that was well explained by the first two principal components (PC; ~91% cumulative variation; Table 6; Figure 8). Seasonal changes in both Pools 19 and 20 were distinguishable along PC1 (78.6% of variance) with the largest and longest particles, in terms of median diameter, volume, and elongation, occurring in August during low flow (Figure 2; Figure 8). However, suspended particles in both pools in June and September during high flow had a smaller median diameter and larger median width compared to August, likely owing to small sediment in suspension during spring flooding in June and re-suspension of sediment during the late-season rise in September.

Table 5. Summary of particle characteristics for particles 53-300 µm in size collected in Navigation Pools 19 and 20, Upper Mississippi River in June, August, and September 2014; including the overall mean, standard deviation (SD), and range of median values of particle parameter distributions and mean particle density. Pool 19 has relatively lower Asian carp abundance (n=26), while Pool 20 has relatively higher Asian carp abundance (n=18). For definitions and abbreviations of particle parameters see Table S1.

Parameter	Mean	SD	Min	Max
Pool 19				
Area ABD (μm^2)	3119.88	760.40	1993.89	4496.27
Diameter ESD (µm)	94.47	13.39	76.25	128.05
Elongation	8.88	4.91	3.14	16.05
Roughness	1.26	0.05	1.19	1.39
Symmetry	0.55	0.07	0.38	0.66
Volume ESD (μm^3)	4.68 e^5	2.10 e^5	2.32 e^5	1.10 e^6
Width (µm)	47.51	17.32	22.17	69.46
Particle concentration (# mL^{-1})	69.36	110.74	0.58	394.67
Pool 20				
Area ABD (μm^2)	3106.14	710.13	1566.35	3850.13
Diameter ESD (µm)	89.47	14.58	74.29	118.40
Elongation	7.91	5.61	2.86	18.59
Roughness	1.25	0.06	1.17	1.34
Symmetry	0.58	0.10	0.33	0.68
Volume ESD (μm^3)	4.05 e^5	2.19 e^5	2.15 e^5	8.69 e^5
Width (µm)	48.32	16.13	20.31	63.24
Particle concentration (# mL^{-1})	34.28	25.44	0.80	99.00

Table 6. Results of principal components analysis (PCA) on suspended particles (53-300 µm in diameter) from main channel and side channel habitats in Pools 19 and 20, Upper Mississippi River sampled in June, August, and September 2014 (n=26); including median particle characteristics and mean particle concentrations.

Principal component	PC1	PC2	PC3
Eigenvalue	5.960	0.933	0.494
% Variation	78.6	12.3	6.5
Cumulative % variation	78.6	90.9	97.5
Parameter			
Area ABD	-0.212	0.344	-0.258
Diameter ESD	0.466	0.088	0.027
Elongation	0.429	-0.031	0.173
Roughness	0.275	0.756	-0.284
Symmetry	-0.198	-0.108	-0.019
Volume ESD	0.470	0.077	0.030
Width	-0.399	0.275	-0.312
Particle concentration (# mL^{-1})	0.242	-0.456	-0.850

PC2 (12.3% of variance) separated Pools 19 and 20 into distinct groups in August and September, suggesting dissimilarities between the pools in suspended particle characteristics these months (Figure 8). In August, Pool 19 showed higher mean concentrations of particles (53-300 µm), lower median roughness, and larger particles in median area and width than in Pool 20. In September, however, this pattern was reversed (Figure 8). The two-way ANOSIM on main and side channel habitats confirmed significant differences in particle characteristics between Pools 19 and 20, as well as among months (Table 8). These results suggest that the relatively higher abundance of AC in Pool 20 may have affected suspended particle characteristics. However, PC2 only explained 12.3% of the total variation, thus the distinct pool groups in August and September may not indicate strong variations in particle characteristics between pools because the majority of the variation in the data was represented in PC1 (Table 6).

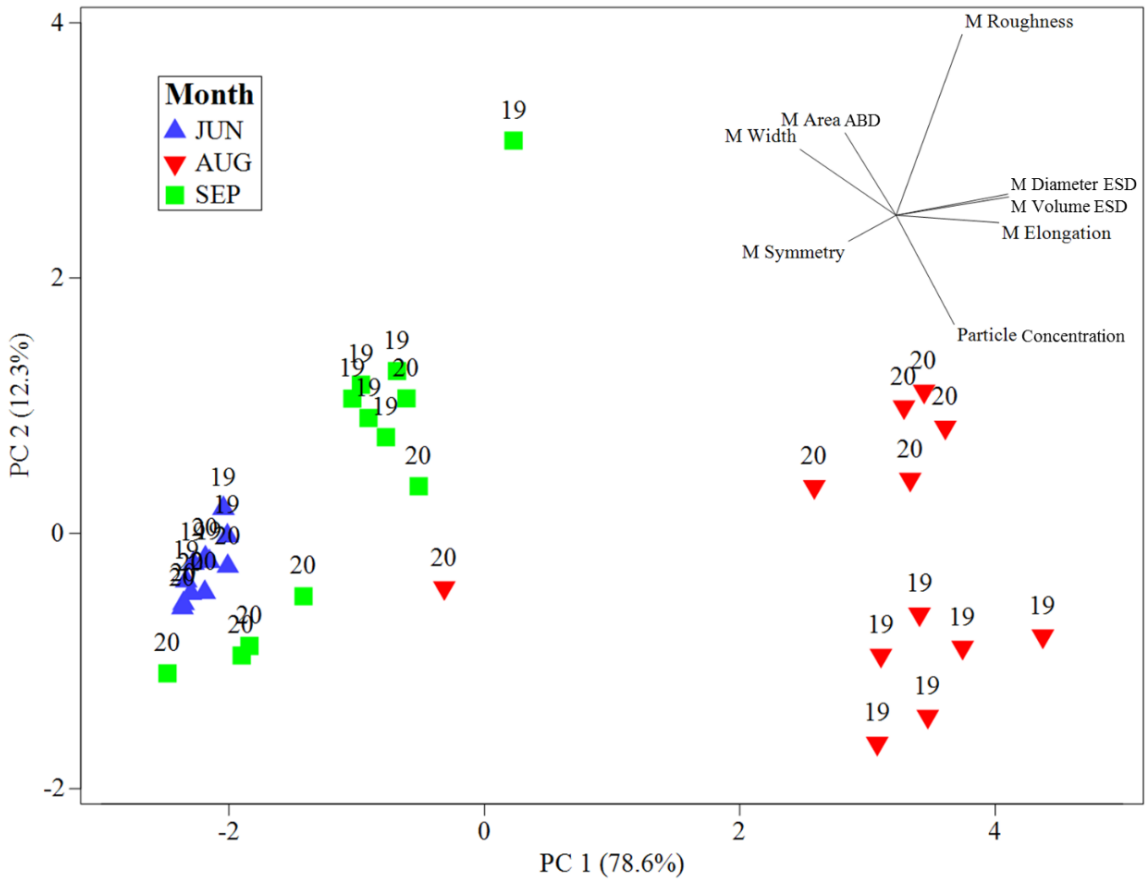

Figure 8. Principal components analysis (PCA) on median particle characteristics (53-300 µm) and mean particle concentrations (# mL^{-1}) in main and side channel habitats of Pools 19 and 20, Upper Mississippi River (indicated by point labels). The percent of variation for the principal components are shown in parentheses (90.9% cumulative variation). A biplot in the upper right corner represents the direction of influence for each particle parameter in relation to the axes and its importance is proportional to the length of the line. Pool 19 has a relatively lower Asian carp abundance than Pool 20, which has a relatively high abundance of Asian carp.

The PCA on backwater habitats showed no clear pattern in suspended particle characteristics between pools or among months (Figure 9). The first two PCs explained ~79% cumulative variation in the data (Table 7), however the ANOSIM on backwater habitats indicated no differences between pools or among months in particle characteristics (Table 8). Homogenous particle characteristics across pools suggests that the relatively higher abundance of AC in Pool 20 had no effect on particle characteristics

in backwaters, although one would expect to see significant seasonal variation in backwaters regardless of the abundance of fish.

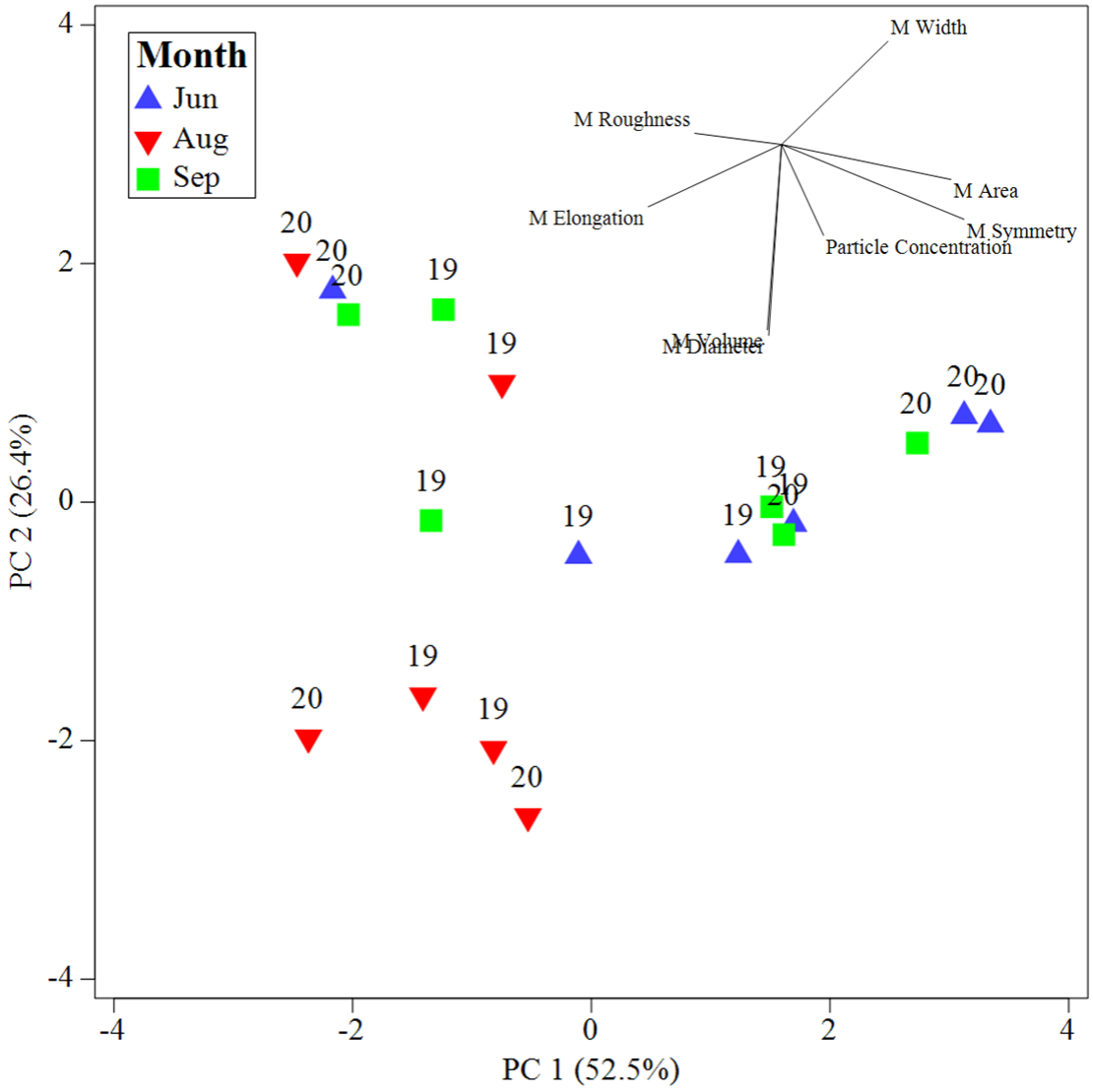

Figure 9. Principal components analysis (PCA) on median particle characteristics (53-300 µm) and mean particle concentrations (# mL^{-1}) in backwater habitats of Pools 19 and 20, Upper Mississippi River (indicated by point labels). The percent of variation for the principal components are shown in parentheses (78.9% cumulative variation). A biplot in the upper right corner represents the direction of influence for each parameter in relation to the axes and its importance is proportional to the length of the line. Pool 19 has a relatively lower Asian carp abundance than Pool 20, which has a relatively high abundance of Asian carp.

Table 7. Results of principal components analysis (PCA) on suspended particles (53-300 µm in diameter) from backwater habitats in Pools 19 and 20, Upper Mississippi River sampled in June, August, and September 2014 (n=18); including median particle characteristics and mean particle concentrations.

Principal component	PC1	PC2	PC3
Eigenvalue	3.83	1.93	1.03
% Variation	52.5	26.4	14.1
Cumulative % variation	52.5	78.9	93.0
Parameter			
Area ABD	0.534	-0.110	0.418
Diameter ESD	-0.040	-0.602	0.215
Elongation	-0.420	-0.197	-0.009
Roughness	-0.273	0.035	0.648
Symmetry	0.575	-0.236	-0.155
Volume ESD	-0.045	-0.585	0.200
Width	0.335	0.325	0.338
Particle concentration (# mL^{-1})	0.132	-0.287	-0.425

Table 8. Results from analysis of similarities (ANOSIM) testing for differences in suspended particle characteristics (53-300 µm) among months and between Upper Mississippi River Pools 19 and 20 (areas of relatively low and high Asian carp abundance, respectively) within habitat types: channel habitats (n=26) and backwaters (n=18). Significance level of $\alpha \leq 0.05$.

ANOSIM Test	Global R	P Value	Pairwise comparisons
Main and side channels			
Pools	0.461	P = 0.0001	
Months	0.812	P = 0.0001	Jun – Aug*; Jun – Sep*; Aug – Sep*
Backwater habitats			
Pools	-0.012	P = 0.490	
Months	0.267	P = 0.066	Jun – Aug; Jun – Sep; Aug – Sep

*Pairwise significance, P = 0.0001

DISCUSSION

The size range of suspended particles examined here represent only a small fraction of suspended particles in the river (particles <63-µm are typically most abundant; Ongley, 1982). However, the 53-300 µm size range reflects size classes of plankton and particles that are consumed by AC (Costa-Pierce, 1992; Kolar et al., 2007). For example, larger zooplankton, which may be selectively filtered by AC, are found at the upper end of this range and >300-µm (Sass *et al*., 2014). Small rotifers, of which abundance has been associated with AC abundance in the Illinois River, are found within this size range (Sass *et al*., 2014). Therefore, even though this range represents only a portion of the total suspended particles, a decrease in the median value of these size distributions may indicate a shift in particle sizes to an increased frequency of smaller particles potentially associated with AC abundance.

Suspended particle characteristics in main and side channel habitats were significantly different between Pools 19 and 20 in August and September 2014. This suggests that the relatively higher abundance of AC in Pool 20 may be a factor affecting suspended particles. However, the divergences between pools along PC2 are only represented by about 12% of the total variation in the data, and thus may not indicate strong or major alterations in the particle characteristics between pools. Regardless of the percent of variation accounted for in PC2, the ANOSIM test did indicate there were significant differences ($p = 0.0001$) in particle characteristics between Pools 19 and 20. I

hypothesized that if changes in suspended particle characteristics with respect to AC abundance were detectable, the most prominent effects would appear in late summer during low flow conditions. The largest divergences in particle characteristics between pools occurred later in the growing season in August and September. August sampling occurred during low flow, however September experienced an uncommon rise in discharge (Figure 2).

In August, Pool 19 showed higher concentrations of particles (53-300 µm) than Pool 20. Lower particle concentrations in Pool 20, where AC are relatively more abundant, suggests an effect of feeding by AC on particles in this size range. Total zooplankton and phytoplankton abundance and biomass has been observed to decrease in lentic systems with AC presence and increasing AC abundance (Wu *et al*., 1997**;** Lu *et al.*, 2002; Tang *et al*., 2002; Ma et al., 2009). However in contrast to August, particles in September exhibited the opposite pattern. Pool 20 showed higher particle concentrations (53-300 µm) than Pool 19. Higher concentrations of particles in Pool 20, where AC are relatively more abundant, may suggest an increase in the abundance of small phytoplankton, which are common in this size range later in the growing season. Wang *et al*. (2008) saw significantly higher chlorophyll *a* concentrations in lakes of the Yangtze River that had higher AC abundance and suggested that feeding and excretion by AC may accelerate nutrient cycling and promote primary production of smaller-celled phytoplankton. Again, the size range of particles examined (53-300 µm) does not represent the total plankton abundance in the water column. Therefore, effects of AC abundance on particle characteristics are difficult to discern from these data.

Another possible and more likely mechanism for higher particle concentrations in September in Pool 20 may be the disturbance of bottom sediments by fish, creating more suspended sediment where AC are abundant. Median particle surface roughness was lower in Pool 20 than Pool 19 in September, suggesting a higher abundance of suspended sediment, which would exhibit smoother surface roughness values than plankton particles. However, discharge is a major factor regulating suspended particle dynamics in large rivers (Allan and Castillo, 2007; Chapter 1). Therefore, the changes in particle concentrations and median roughness values from August to September in both pools were likely more driven by the rise in discharge in September, which occurred during a period normally with low flow, rather than an effect of AC abundance.

Particle size (i.e., area and width) also contributed to the distinction between pools in August and September. I hypothesized that in areas with higher AC abundance there would be a shift in particle size distributions towards an increased frequency of smaller particles due to AC selectively feeding on larger particles and plankton. The pattern in particle characteristics seen in September supports this hypothesis, exhibiting a higher abundance of smaller particles (in area and width) in Pool 20 where AC were relatively more abundant. Various studies have documented shifts in particle size distributions related to AC presence and abundance. For example, several researchers found that the growth, abundance, and biomass of small phytoplankton, especially nanoplankton (< 20 µm), were significantly enhanced in the presence of AC, likely because of size selectivity for large zooplankton and phytoplankton (Opuszynski *et al*., 1991; Wu *et al*., 1997; Voros *et al*., 1997; Tang *et al*., 2002; Wang *et al*., 2008; Cooke *et al*., 2009).

More recently, Sass *et al*. (2014) documented broad-scale changes in zooplankton communities in the Illinois River since AC establishment. Rotifer abundance and biomass was positively associated with AC relative abundance, which has not been previously documented in lentic systems (lotic studies have documented reductions in rotifer and total zooplankton abundances; Lieberman, 1996; Wu *et al*., 1997; Fukushima *et al.*, 1999). This suggests AC establishment and population growth in native riverine ecosystems are associated with changes in particle size distributions, phytoplankton community size structure, as well as shifts in zooplankton community composition to smaller species (i.e., rotifers). The results of Sass *et al*. (2014) also suggests that lotic systems may respond differently to AC establishment than lentic systems. Moreover, our results suggest that river discharge is undoubtedly confounding the changes in suspended particle characteristics between pools, making it difficult to assess any potential direct effects to suspended particles in regard to AC abundance and potentially offsetting negative AC effects on planktonic food-webs.

In contrast to September, suspended particle characteristics during low flow in August conflicted with our hypothesis and showed an opposite pattern between pools. For example, during low flow Pool 20 exhibited larger sized particles (in median area and width) than Pool 19 when I expected to see a shift to small particle sizes due to preferential filtering of large particles by a greater abundance of AC in Pool 20. The larger particles in Pool 20 in September were also associated with higher median roughness values, possibly suggesting increased autochthonous production of organic particles (e.g., plankton and detritus, which would exhibit higher surface roughness) as opposed to suspended inorganic particles (e.g., sediment and sand, which would yield

lower roughness values). Autochthonous production in Pool 20 during late season low flow conditions could have been promoted by rapid biological cycling due to high AC abundance and excretion (Wang *et al*., 2008).

Contrary to channel habitats, suspended particle characteristics in backwater habitats did not show any distinct differences between pools nor clear seasonal patterns. We hypothesized that changes in suspended particle characteristics with respect to AC abundance would be more evident in backwaters, because these habitats typically become less hydrologically connected over the growing season which reduces particle and nutrient resupply. These results infer that the presence of AC had no real effects on suspended particles. However, there are AC present in backwater habitats of Pool 19 (Brent Knights and Jon Vallazza, USGS, La Crosse, WI, unpublished data) as backwaters are common refuge and nursery habitat for AC (DeGrandchamp *et al.,* 2008; Calkins *et al.,* 2012). Therefore, AC abundance in Pool 19 backwaters may have already passed a threshold where they could be triggering similar changes in suspended particle characteristics as in Pool 20 backwaters. Furthermore, discharge and seasonal variation are strong drivers of suspended particle dynamics, even in backwater habitats which characteristically show a different seasonal trajectory than channel and impounded habitats (Chapter 1). Therefore, it is intriguing as to why backwaters in both Pools 19 and 20 showed an unclear temporal pattern from June to September. However, backwaters can show considerably high variation among sites in environmental characteristics and suspended particle dynamics, because of varying hydrological connectivity throughout the growing season (Strauss *et al.,* 2004; Burdis and Hoxmeier, 2011; Houser *et al.,* 2015; Pongruktham and Ochs, 2015).

From these analyses, there were not clear patterns from which to infer that the differences in suspended particle characteristics between Pools 19 and 20 were related to AC abundance. The September rise in discharge and inconsistencies in particle characteristics between August and September suggests discharge was a major factor affecting suspended particle characteristics and made it difficult to interpret the pool differences as potential effects of AC filter-feeding. However, these results should be investigated further, perhaps linking the particle characteristics to the relative composition of particle and plankton communities through examination of the FlowCAM images.

AC have had direct and indirect impacts on ecosystems in which they are already established through habitat change, water quality degradation, trophic alteration, and competition (Kolar *et al.*, 2007). Thus, AC are an imminent threat to newly invaded systems, such as the UMR and Great Lakes. The wide variation in AC diets across systems and diet overlap with natives indicate AC are opportunistic and easily adaptable feeders to changing food availability and environmental conditions. The complexity of large river ecosystems and co-varying factors, such as discharge, geomorphology, nutrients, and other biota, confound potential effects and make assessing ecological changes due to AC even more difficult. A thorough understanding of changes in suspended particles as primary basal food web resources related to AC invasion is crucial for the development and implementation of effective control measures for AC and the management of aquatic systems.

REFERENCES

Adamek Z, Spittler P. 1984. Particle size selection in the food of silver carp, H*ypophthalmichthys molitrix*. *Folia Zoologica* **33**: 363–70.

Allan JD, Castillo MM. 2007. *Stream Ecology: Structure and function of running waters*, 2nd edn. Springer, Netherlands.

Álvarez E, López-Urrutia Á, Nogueira E, Fraga S. 2011. How to effectively sample the plankton size spectrum? A case study using FlowCAM. *Journal of Plankton Research* **33**: 1119–33. DOI: 10.1093/plankt/fbr012.

Bhowmik NG, Adams JR. 1986. The hydrologic environment of Pool 19 of the Mississippi River. *Hydrobiologia* **136**: 21–30. DOI: 10.1007/BF00051501.

Burdis RM, Hoxmeier RJH. 2011. Seasonal zooplankton dynamics in main channel and backwater habitats of the Upper Mississippi River. *Hydrobiologia* **667**: 69–87. DOI: 10.1007/s10750-011-0639-y.

Calkins HA, Tripp SJ, Garvey JE. 2012. Linking silver carp habitat selection to flow and phytoplankton in the Mississippi River. *Biological Invasions* **14**: 949–58. DOI: 10.1007/s10530-011-0128-2.

Chick JH, Pegg MA. 2001. Invasive carp in the Mississippi River Basin. *Science* **292**: 2250–51. DOI: 10.1126/science.292.5525.2250.

Clarke KR, Gorley RN, Somerfield PJ, Warwick RM. 2014. *Change in marine communities: an approach to statistical analysis and interpretation*, 3rd edn. PRIMER-E Ltd: Plymouth, U.K.

Cooke SL, Hill WR, Meyer KP. 2009. Feeding at different plankton densities alters invasive bighead carp (*Hypophthalmichthys nobilis*) growth and zooplankton species composition. *Hydrobiologia* **625**: 185–93. DOI: 10.1007/s10750-009-9707-y.

Costa-Pierce BA. 1992. Review of spawning requirements and feeding ecology of the silver carp (*Hypophthalmichthys molitrix*). *Reviews in Aquatic Sciences* **6**: 257–73.

DeGrandchamp KL, Garvey JE, Colombo RE. 2008. Movement and habitat selection by invasive Asian carps in a large river. *Transactions of the American Fisheries Society* **137**: 45–56. DOI: 10.1577/T06-116.1.

Dong S, Li D. 1994. Comparative studies on the feeding selectivity of silver carp *Hypophthalmichthys molitrix* and bighead carp *Aristichthys nobilis*. *Journal of Fish Biology* **44**: 621–26. DOI: 10.1111/j.1095-8649.1994.tb01238.x.

Fluid Imaging Technologies, Inc. 2013. *FlowCAM® Manual*, version 3.4. Scarborough, ME.

Freeze M, Henderson S. 1982. Distribution and status of the bighead carp and silver carp in Arkansas. *North American Journal of Fisheries Management* **2**: 197–200. DOI: 10.1577/1548-8659(1982)2<197:DASOTB>2.0.CO;2.

Fukushima M, Takamura N, Sun L, Nakagawa M, Matsushige K, Xie P. 1999. Changes in the plankton community following introduction of filter-feeding planktivorous fish. *Freshwater Biology* **42**: 719–35. DOI:10.1046/j.1365-2427.1999.00507.x.

Gutreuter S, Burkhardt R, Lubinski K. 1995. Long term resource monitoring program procedures: fish monitoring. National Biological Service, Environmental Management Technical Center, Onalaska, WI. *LTRMP 95-P002-1*, 42 pp.

Houser JN, Bartsch LA, Richardson WB, Rogala JT, Sullivan JF. 2015. Ecosystem metabolism and nutrient dynamics in the main channel and backwaters of the Upper Mississippi River. *Freshwater Biology* **60**: 1863–79. DOI: 10.1111/fwb.12617.

Irons KS, Sass GG, McClelland MA, Stafford JD. 2007. Reduced condition factor of two native fish species coincident with invasion of non-native Asian carps in the Illinois River, U.S.A. Is this evidence for competition and reduced fitness? *Journal of Fish Biology* **71**: 258–73. DOI: 10.1111/j.1095-8649.2007.01670.x.

Jahn LA, Anderson RV. 1986. The ecology of Pools 19 and 20, of the Upper Mississippi River: a community profile. *U.S. Fish and Wildlife Service Biological Report* **85** (7.6): 142 pp.

Kolar CS, Chapman DC, Courtenay WR, Housel CM, Williams JD, Jennings DP. 2007. Bigheaded carps: a biological synopsis and environmental risk assessment. In *American Fisheries Society, Special Publication*. Vol. 33. Bethesda, Maryland: American Fisheries Society. DOI: 10.1643/OT-09-041.

Lieberman DM. 1996. Use of silver carp (*Hypophthalmichthys molitrix*) and bighead carp (*Aristichthys nobilis*) for algae control in a small pond: changes in water quality. *Journal of Freshwater Ecology* **11**: 391–97. DOI: 10.1080/02705060.1996.9664466.

Lu M, Xie P, Tang H, Shao Z, Xie L. 2002. Experimental study of trophic cascade effect of silver carp (*Hypophthalmichthys molitrix*) in a subtropical lake, Lake Donghu: on plankton community and underlying mechanisms of changes of crustacean community. *Hydrobiologia* **487**: 19–31. DOI: 10.1023/A:1022940716736.

Ma H, Cui F, Liu Z, Fan Z. 2009. Pilot study on control of phytoplankton by zooplankton coupling with filter-feeding fish in surface water. *Water Science and Technology* **60**: 737–43. DOI: 10.2166/wst.2009.437.

Ongley ED. 1982. Influence of season, source and distance on physical and chemical properties of suspended sediment. In *Recent Developments in the Explanation and Prediction of Erosion and Sediment Yield (Proceedings of the Exeter Symposium)*. International Association of Hydrological Sciences (IAHS) Press: Wallingford, U.K.; 371–83.

Opuszynski K, Shireman JV, Cichra CE. 1991. Food assimilation and filtering rate of bighead carp kept in cages. *Hydrobiologia* **220**: 49–56. DOI: 10.1007/BF00017491.

Pongruktham O, Ochs C. 2015. The rise and fall of the Lower Mississippi: effects of hydrologic connection on floodplain backwaters. *Hydrobiologia* **742**(1): 169–83. DOI: 10.1007/s10750-014-1983-5.

Pongruktham O, Ochs C, Hoover JJ. 2010. Observations of silver carp (*Hypophthalmichthys molitrix*) planktivory in a floodplain lake of the lower Mississippi River Basin. *Journal of Freshwater Ecology* **25**: 85–93. DOI: 10.1080/02705060.2010.9664361.

Sampson SJ, Chick JH, Pegg MA. 2009. Diet overlap among two Asian carp and three native fishes in backwater lakes on the Illinois and Mississippi Rivers. *Biological Invasions* **11**: 483–96. DOI: 10.1007/s10530-008-9265-7.

Sass GG, Hinz C, Erickson AC, McClelland NN, McClelland MA, Epifanio JM. 2014. Invasive bighead and silver carp effects on zooplankton communities in the Illinois River, Illinois, USA. *Journal of Great Lakes Research* **40**: 911–21. DOI: 10.1016/j.jglr.2014.08.010.

Schrank SJ, Guy CS, Fairchild JF. 2003. Competitive interactions between age-0 bighead carp and paddlefish. *Transactions of the American Fisheries Society* **132**: 1222–28. DOI: 10.1577/T02-071.

Strauss EA, Richardson WB, Bartsch LA, Cavanaugh JC, Bruesewitz DA, Imker H, Heinz JA, Soballe DM. 2004. Nitrification in the Upper Mississippi River: patterns, controls, and contribution to the NO_3^- budget. *Journal of the North American Benthological Society* **23**: 1–14. DOI: 10.1899/0887-3593(2004)023<0001:NITUMR>2.0.CO;2.

Tang H, Xie P, Lu M, Xie L, Wang J. 2002. Studies on the effects of silver carp (*Hypophthalmichthys molitrix*) on the phytoplankton in a shallow hypereutrophic lake through an enclosure experiment. *International Review of Hydrobiology* **87**: 107–19. DOI: 10.1002/1522-2632(200201)87:1<107::AID-IROH107>3.0.CO;2-J.

U.S. Department of Interior, U.S. Geological Survey. *National Water Information System*. http://www.waterdata.usgs.gov/. Date of access: 2015-08-03.

Vörös L, Oldal I, Présing M, Balogh KV. 1997. Size-selective filtration and taxon-specific digestion of plankton algae by silver carp (*Hypophthalmichthys molitrix* Val.). *Hydrobiologia* **342/343**: 223–28. DOI: 10.1023/A:1017039423485.

Walleser LR, Sandheinrich MB, Howard DR, Gaikowski MP, Amberg JJ. 2014. Spatial and temporal variation of the gill rakers of gizzard shad and silver carp in three midwestern rivers. *North American Journal of Fisheries Management* **34**: 875–84. DOI: 10.1080/02755947.2014.920740.

Wang HJ, Liang XM, Jiang PH, Wang J, Wu SK, Wang HZ. 2008. TN : TP ratio and planktivorous fish do not affect nutrient-chlorophyll relationships in shallow lakes. *Freshwater Biology* **53**: 935–44. DOI: 10.1111/j.1365-2427.2007.01950.x.

Wilcox DB. 1993. An aquatic habitat classification system for the Upper Mississippi River system. U.S. Fish and Wildlife Service, Environmental Management Technical Center, Onalaska, WI. *EMTC 93-T003*, 9 pp.

Williamson CJ, Garvey JE. 2005. Growth, fecundity, and diets of newly established silver carp in the middle Mississippi River. *Transactions of the American Fisheries Society* **134**: 1423–30. DOI: 10.1577/T04-106.1.

Wu L, Xie P, Dai M, Wang J. 1997. Effects of silver carp density on zooplankton and water quality: Implications for eutrophic lakes in China. *Journal of Freshwater Ecology* **12**: 437–44. DOI: 10.1080/02705060.1997.9663554.

Xie P. 2001. Gut contents of bighead carp (*Aristichthys nobilis*) and the processing and digestion of algal cells in the alimentary canal. *Aquaculture* **195**: 149–61. DOI: 10.1016/S0044-8486(00)00549-4.

Xie P. 1999. Gut contents of silver carp, *Hypophthalmichthys molitrix*, and the disruption of a centric diatom, *Cyclotella*, on passage through the esophagus and intestine. *Aquaculture* **180**: 295–305. DOI: 10.1016/S0044-8486(99)00205-7.

Xu J, Xie P. 2004. Studies on the food web structure of Lake Donghu using stable carbon and nitrogen isotope ratios. *Journal of Freshwater Ecology* **19**: 645–50. DOI: 10.1080/02705060.2004.9664746.

APPENDIX A

SUPPLEMENTAL TABLES

Table S1. Definitions of particle characteristics (FlowCAM® Manual, 2013). The coefficient of variation (CV) for the median from triplicate analyses of samples was calculated to test instrument precision.

Parameter	CV	Definition
Area ABD[a]	1.6%	Calculated by the number of pixels in the binary image threshold and converted to a measure of area (μm^2)
Diameter ESD[b]	0.8%	Mean value of 36 feret measurements[c] (µm)
Elongation	2.2%	Measured as the ratio of geodesic length[d] to thickness[e]; 1 = circle or square, values > 1 are elongated
Roughness	0.7%	Measure of the unevenness or irregularity of the particle surface by ratio of the particle perimeter to the convex perimeter; 1 = filled with convex perimeter, values > 1 have non-convex perimeter or interior holes
Symmetry	0.9%	Measure of particle symmetry about its center; 0 = asymmetric, 1 = symmetric
Volume ESD[b]	2.5%	Estimated sphere volume calculated from Diameter ESD[b] (μm^3)
Width	1.5%	Minimum of 36 feret measurements[c] (µm)
Particle concentration	3.2%	The number of particles per mL of sample water (# mL^{-1})

[a] ABD: Area Based Diameter
[b] ESD: Equivalent Spherical Diameter
[c] Feret measurement: Distance between opposite parallel tangents of a particle
[d] Geodesic length: Length if particle were modeled as a rectangle using area and perimeter
[e] Geodesic thickness: Width if particle were modeled as a rectangle using area and perimeter

Table S2. Pearson correlation coefficients for environmental variables, whole water and fractionated particle measures, and particle characteristics (not transformed) from Pool 19, Upper Mississippi River in 2013 and 2014, including nitrate + nitrite (NO_3^-), ammonia + ammonium (NH_4^-), soluble reactive phosphorous (SRP), total phosphorus (TP), whole water total suspended solids (TSS), whole water volatile suspended solids (VSS), VSS:TSS ratio, fractionated TSS and VSS in 2014, discharge ($m^3\ s^{-1}$), flow velocity ($m\ s^{-1}$) in 2014, particle concentration ($\#\ mL^{-1}$), and median (M) particle size and shape characteristics.

2013	NO_3^-	NH_4^-	SRP	TP	CHL	TSS	VSS	VSS:TSS	Discharge
NH_4^-	-0.480*								
SRP	0.457*	0.120							
TP	0.077	0.256	0.792*						
CHL	-0.721*	0.110	-0.480*	0.020					
TSS	0.358˜	-0.023	0.583*	0.547*	-0.359˜				
VSS	-0.598*	0.344˜	-0.043	0.441*	0.721*	0.216			
VSS:TSS	-0.616*	0.239	-0.514*	-0.240	0.709*	-0.707*	0.413*		
Discharge	0.826*	-0.511*	0.426*	-0.022	-0.821*	0.432*	-0.651*	-0.744*	
Particle concentration	-0.461*	0.088	-0.679*	-0.609*	0.452*	-0.455*	0.218	0.561*	-0.453*
M Area ABD	-0.545*	0.481*	-0.004	0.111	0.415*	-0.082	0.475*	0.387*	-0.530*
M Diameter ESD	-0.485*	0.233	-0.591*	-0.578*	0.381*	-0.516*	0.194	0.589*	-0.475*
M Elongation	0.231	-0.383*	-0.425*	-0.690*	-0.256	-0.272	-0.431*	-0.052	0.389*
M Roughness	-0.500*	0.338˜	-0.216	-0.083	0.382*	-0.017	0.463*	0.271	-0.445*
M Symmetry	-0.476*	0.458*	-0.016	0.104	0.348˜	-0.237	0.273	0.462*	-0.605*
M Volume ESD	-0.529*	0.463*	0.011	0.124	0.415*	-0.081	0.468*	0.380*	-0.514*
M Width	-0.131	0.326˜	0.571*	0.702*	0.053	0.498*	0.356*	-0.235	-0.109

* indicates $p < 0.02$ level of significance level
˜ indicates $p < 0.05$ level of significance level

Table S2. *Continued...*

2014	NO_3^-	NH_4^-	SRP	TP	CHL	Whole TSS	Whole VSS	Whole VSS:TSS	53-300µm TSS	53-300µm VSS	Discharge
NH_4^-	-0.403*										
SRP	0.680*	-0.054									
TP	0.353˜	0.071	0.595*								
CHL	-0.760*	0.104	-0.883*	-0.607*							
TSS Whole	0.465*	-0.162	0.351˜	0.817*	-0.351						
VSS Whole	0.157	0.081	0.174	0.793*	-0.251	0.888*					
VSS:TSS Whole	-0.377˜	0.440*	-0.296	-0.420*	0.119	-0.404*	-0.100				
TSS 53-300 µm	0.103	-0.079	0.061	0.291	0.178	0.190	0.132	-0.294			
VSS 53-300 µm	-0.175	-0.062	-0.223	0.073	0.485*	0.140	0.129	-0.171	0.438*		
Discharge	0.851*	-0.280	0.889*	0.463*	-0.830*	0.360˜	0.099	-0.432*	0.041	-0.188	
Flow velocity	0.606*	-0.540*	0.264	0.248	-0.458˜	0.435*	0.223	-0.438*	0.260	-0.007	0.493*
Particle concentration	-0.351˜	-0.196	-0.598*	-0.386*	0.677*	-0.185	-0.152	0.105	0.054	0.222	-0.554*
M Area ABD	0.303	-0.203	0.285	0.453*	-0.600*	0.402*	0.374˜	-0.507*	0.144	-0.003	0.391*
M Diameter ESD	-0.455*	0.087	-0.528*	-0.387*	0.668*	-0.350˜	-0.257	0.199	0.002	0.145	-0.592*
M Elongation	-0.495*	0.258*	-0.461*	-0.512*	0.736*	-0.512*	-0.427*	0.469*	-0.126	0.060	-0.581*
M Roughness	-0.474*	0.457*	-0.083	0.168	0.314	-0.144	0.086	0.000	-0.071	-0.028	-0.272
M Symmetry	0.376˜	-0.518*	0.003	0.137	-0.311	0.401*	0.281	-0.386*	0.207	0.127	0.246
M Volume ESD	-0.285	-0.181	0.271	0.446*	-0.616*	-0.392*	0.376˜	-0.487*	0.134	-0.008	0.376˜
M Width	0.436*	-0.156	0.514*	0.567*	-0.758*	0.454*	0.384*	-0.530*	0.098	-0.089	0.599*

* indicates $p < 0.02$ level of significance level
˜ indicates $p < 0.05$ level of significance level

Table S3. Summary of particle characteristics for particles 53-300 µm in size from samples collected in June, August, and September of 2013 and 2014 among four aquatic habitat types in Pools 19 and 20 of the Upper Mississippi River, including the overall mean, standard deviation (SD), and range of median values of particle parameter distributions and mean particle density. Habitat type abbreviated as follows: main channel (MC), side channel (SC), impounded (IM), backwater (BW). For definitions and abbreviations of particle parameters see Table S1.

Date	Habitat	Statistics	Area ABD (μm^2)	Diameter ESD (µm)	Elongation	Roughness	Symmetry	Volume ESD (μm^3)	Width (µm)	Particle concentration (# mL^{-1})
2013	**Pool 19**									
Jun	MC	Mean	2472.54	98.23	16.53	1.29	0.47	4.97 e^5	36.31	23.97
	n = 3	SD	125.42	1.17	1.83	0.03	0.03	1.80 e^4	4.39	10.50
		Min	2353.41	97.47	15.18	1.27	0.43	4.85 e^5	31.60	13.00
		Max	2603.42	99.58	18.61	1.32	0.49	5.17 e^5	40.29	33.92
Jun	SC	Mean	2584.74	95.32	13.91	1.26	0.49	4.57 e^5	38.67	14.71
	n = 4	SD	216.16	4.78	3.59	0.01	0.02	6.53 e^4	7.91	2.18
		Min	2351.71	88.16	8.65	1.25	0.47	3.59 e^5	30.52	12.50
		Max	2842.51	97.98	16.38	1.27	0.52	4.92 e^5	49.17	16.67
Jun	IM	Mean	2510.91	92.63	12.92	1.26	0.51	4.21 e^5	38.14	15.59
	n = 4	SD	243.57	6.68	4.58	0.03	0.04	8.53 e^4	9.52	10.69
		Min	2314.61	83.00	6.20	1.23	0.47	3.00 e^5	29.62	8.63
		Max	2840.00	98.43	16.49	1.29	0.57	4.99 e^5	51.71	31.50
Jun	BW	Mean	2230.88	100.04	16.37	1.25	0.46	5.27 e^5	27.14	15.24
	n = 3	SD	102.61	5.24	1.47	0.02	0.05	8.48 e^4	2.64	4.78
		Min	2112.44	96.37	15.07	1.24	0.41	4.69 e^5	24.50	9.71
		Max	2293.15	106.04	17.96	1.27	0.51	6.24 e^5	29.78	18.00

Aug	MC	Mean	2448.61	104.87	13.69	1.25	0.54	6.14 e^5	26.79	42.78
	n = 3	SD	370.90	9.45	0.76	0.02	0.02	1.68 e^5	5.47	22.37
		Min	2173.37	96.40	12.81	1.24	0.52	4.69 e^5	21.03	22.33
		Max	2870.39	115.07	14.13	1.27	0.56	7.98 e^5	31.91	66.67
Aug	SC	Mean	2660.68	104.67	12.82	1.27	0.54	6.07 e^5	32.71	45.78
	n = 3	SD	276.80	7.61	2.60	0.01	0.01	1.26 e^5	10.50	23.89
		Min	2341.09	96.04	9.93	1.26	0.53	4.64 e^5	23.59	27.00
		Max	2824.58	110.41	14.97	1.28	0.55	7.05 e^5	44.19	72.67
Aug	IM	Mean	2734.80	105.15	12.41	1.27	0.54	6.28 e^5	33.42	31.29
	n = 3	SD	146.16	13.21	3.26	0.02	0.02	2.14 e^5	13.41	20.00
		Min	2628.93	89.99	8.65	1.25	0.52	3.83 e^5	24.35	9.27
		Max	2901.56	114.18	14.42	1.28	0.56	7.80 e^5	48.83	48.33
Aug	BW	Mean	2512.11	88.37	9.94	1.29	0.49	3.66 e^5	44.62	10.19
	n = 4	SD	28.70	6.68	1.68	0.02	0.03	7.73 e^4	2.52	9.13
		Min	2482.72	78.68	7.47	1.27	0.45	2.55 e^5	41.80	3.79
		Max	2551.06	93.42	11.03	1.30	0.52	4.27 e^5	47.85	23.33
Sep	MC	Mean	3578.01	126.06	15.08	1.32	0.52	1.05 e^6	31.03	109.06
	n = 3	SD	246.66	5.47	0.96	0.03	0.02	1.34 e^5	2.28	19.46
		Min	3323.72	119.75	14.05	1.30	0.50	8.99 e^5	28.98	91.00
		Max	2816.25	129.49	15.96	1.36	0.54	1.14 e^6	33.49	129.67
Sep	SC	Mean	3811.26	125.77	14.41	1.32	0.53	1.04 e^6	34.71	101.78
	n = 3	SD	817.98	2.70	2.07	0.02	0.01	6.70 e^4	10.98	55.29
		Min	3211.80	123.14	12.34	1.30	0.51	9.78 e^5	27.09	39.33
		Max	4743.10	128.53	16.48	1.34	0.54	1.11 e^6	47.29	144.50
Sep	IM	Mean	3098.87	113.09	13.04	1.31	0.55	7.62 e^5	28.26	92.25
	n = 4	SD	390.83	5.01	0.48	0.04	0.04	1.02 e^5	3.87	18.34

		Min	2589.67	106.95	12.40	1.28	0.51	6.41 e^5	25.01	76.00
		Max	3509.60	119.08	13.41	1.35	0.60	8.88 e^5	33.48	113.00
Sep	BW	Mean	4143.34	101.51	7.08	1.31	0.58	5.50 e^5	62.60	8.02
	n = 2	SD	682.13	4.16	1.93	0.05	0.03	6.85 e^4	7.14	2.65
		Min	3556.51	98.11	4.99	1.27	0.55	4.94 e^5	57.59	5.92
		Max	4891.76	106.14	8.80	1.36	0.61	6.26 e^5	70.78	11.00
2014	**Pool 19**									
Jun	MC	Mean	3472.20	80.13	3.52	1.21	0.64	2.70 e^5	59.91	9.99
	n = 3	SD	135.45	2.89	0.07	0.00	0.00	2.86 e^4	1.18	3.82
		Min	3327.39	79.80	3.43	1.21	0.63	2.37 e^5	58.75	7.17
		Max	3595.79	81.89	3.56	1.21	0.64	2.88 e^5	61.10	14.33
Jun	SC	Mean	3492.38	80.54	3.33	1.19	0.65	2.74 e^5	59.88	9.86
	n = 2	SD	149.42	0.80	0.27	0.00	0.02	8.20 e^3	1.25	5.38
		Min	3386.72	79.97	3.14	1.19	0.63	2.68 e^5	59.00	6.06
		Max	3598.03	81.10	3.52	1.19	0.66	2.79 e^5	60.77	13.67
Jun	IM	Mean	3554.01	85.26	5.21	1.24	0.59	3.32 e^5	60.42	12.24
	n = 3	SD	51.09	8.78	2.93	0.06	0.09	1.05 e^5	1.13	10.35
		Min	3515.86	78.44	3.26	1.20	0.48	2.53 e^5	59.20	1.39
		Max	3613.11	95.17	8.50	1.31	0.65	4.51 e^5	61.44	22.00
Jun	BW	Mean	2773.39	95.95	11.24	1.25	0.48	4.69 e^5	43.59	3.51
	n = 3	SD	837.61	7.81	5.83	0.02	0.08	1.11 e^5	18.01	4.41
		Min	1858.63	87.43	5.71	1.23	0.40	3.50 e^5	24.10	0.73
		Max	3502.79	102.77	17.34	1.27	0.56	5.69 e^5	59.61	8.59
Aug	MC	Mean	2478.46	114.77	15.73	1.26	0.54	8.08 e^5	23.52	306.67
	n = 3	SD	505.51	11.61	0.28	0.02	0.03	2.54 e^5	1.47	85.92

		Min	2136.30	106.52	15.56	1.24	0.52	6.33 e^5	22.17	223.00
		Max	3059.09	128.05	16.05	1.27	0.58	1.10 e^6	25.10	394.67
Aug	SC	Mean	2398.21	112.25	15.32	1.27	0.52	7.43 e^5	23.91	186.03
	n = 3	SD	189.65	4.14	0.18	0.01	0.02	8.06 e^4	0.54	101.10
		Min	2193.80	107.59	15.13	1.26	0.51	6.52 e^5	23.46	125.67
		Max	2568.45	115.52	15.48	1.28	0.55	8.07 e^5	24.51	302.75
Aug	IM	Mean	1937.29	101.25	15.28	1.25	0.49	5.61 e^5	21.78	105.11
	n = 3	SD	553.03	13.00	2.12	0.07	0.06	2.11 e^5	1.90	40.08
		Min	1559.26	88.24	13.04	1.19	0.44	3.60 e^5	19.67	63.67
		Max	2572.02	114.24	17.25	1.33	0.55	7.81 e^5	23.37	143.67
Aug	BW	Mean	2153.10	91.58	12.13	1.24	0.46	4.20 e^5	33.33	35.86
	n = 2	SD	8.44	15.52	1.62	0.02	0.03	2.05 e^5	13.31	39.32
		Min	2147.14	80.61	10.99	1.22	0.44	2.74 e^5	23.91	8.05
		Max	2159.07	102.56	13.28	1.25	0.48	5.65 e^5	42.74	63.67
Sep	MC	Mean	3672.24	86.53	4.81	1.29	0.59	3.40 e^5	61.87	31.56
	n = 3	SD	283.79	1.65	0.53	0.02	0.02	1.93 e^4	2.11	16.55
		Min	3344.69	84.74	4.38	1.28	0.57	3.19 e^5	59.43	21.67
		Max	3844.14	87.99	5.41	1.31	0.60	3.57 e^5	63.23	50.67
Sep	SC	Mean	4151.59	91.13	4.99	1.31	0.58	3.99 e^5	66.09	24.56
	n = 3	SD	330.16	5.13	0.85	0.06	0.03	6.86 e^4	3.10	8.04
		Min	3838.17	86.99	4.32	1.28	0.55	3.45 e^5	63.37	17.00
		Max	4496.27	96.87	5.94	1.39	0.61	4.76 e^5	69.46	33.00
Sep	IM	Mean	4053.55	96.05	5.78	1.33	0.57	4.67 e^5	63.58	20.11
	n = 3	SD	296.33	5.26	0.54	0.02	0.02	7.60 e^4	1.59	2.04
		Min	3717.04	90.67	5.16	1.31	0.55	3.91 e^5	62.16	18.33
		Max	4275.48	101.19	6.12	1.35	0.58	5.43 e^5	65.30	22.33

Sep	BW	Mean	2394.98	86.99	11.24	1.31	0.40	3.61 e^5	43.88	2.25
	n = 2	SD	567.23	15.19	0.01	0.05	0.03	1.82 e^5	5.65	0.96
		Min	1993.89	76.25	11.23	1.27	0.38	2.32 e^5	39.89	1.58
		Max	2796.07	97.74	11.24	1.35	0.42	4.89 e^5	47.88	2.93
2014	**Pool 20**									
Jun	MC	Mean	3485.13	79.92	3.34	1.20	0.65	2.68 e^5	59.70	17.00
	n = 2	SD	161.29	3.32	0.25	0.00	0.01	3.33 e^4	0.95	2.83
		Min	3371.08	77.57	3.16	1.19	0.65	2.44 e^5	59.03	15.00
		Max	3599.17	82.27	3.51	1.20	0.66	2.92 e^5	60.38	19.00
Jun	SC	Mean	3711.07	80.58	3.11	1.19	0.67	2.74 e^5	62.01	23.00
	n = 4	SD	77.87	1.15	0.12	0.01	0.01	1.17 e^4	0.65	9.57
		Min	3639.29	79.17	2.97	1.18	0.66	2.60 e^5	61.40	14.33
		Max	3811.64	81.66	3.25	1.20	0.68	2.85 e^5	62.81	36.33
Jun	BW	Mean	2987.41	79.42	5.96	1.22	0.57	2.62 e^5	54.04	17.27
	n = 3	SD	1103.59	1.26	5.18	0.08	0.17	1.23 e^4	12.23	15.54
		Min	1713.79	77.99	2.89	1.18	0.38	2.48 e^5	39.92	0.80
		Max	3660.64	80.35	11.94	1.31	0.68	2.72 e^5	61.50	31.67
Aug	MC	Mean	2859.27	101.10	12.19	1.28	0.56	5.73 e^5	33.89	28.00
	n = 2	SD	46.40	20.02	4.97	0.09	0.02	3.24 e^5	12.05	15.56
		Min	2826.46	86.94	8.68	1.22	0.55	3.44 e^5	25.37	17.00
		Max	2892.08	115.26	15.71	1.34	0.58	8.02 e^5	42.40	39.00
Aug	SC	Mean	2752.82	115.05	15.57	1.30	0.53	8.02 e^5	24.95	27.67
	n = 4	SD	225.06	5.54	0.58	0.02	0.01	1.10 e^5	0.79	12.83
		Min	2463.14	106.77	14.91	1.29	0.52	6.38 e^5	24.15	15.00
		Max	3012.79	118.40	16.23	1.32	0.55	8.69 e^5	25.77	43.67

Aug	BW	Mean	1967.41	95.48	15.52	1.29	0.45	4.88 e^5	28.24	25.29
	n = 3	SD	634.16	18.43	2.73	0.02	0.09	2.39 e^5	9.81	22.09
		Min	1566.35	74.29	13.36	1.27	0.36	2.15 e^5	20.31	3.87
		Max	2698.53	107.76	18.59	1.32	0.54	6.55 e^5	39.21	48.00
Sep	MC	Mean	3435.85	80.12	3.37	1.19	0.64	2.70 e^5	59.13	51.75
	n = 2	SD	54.29	3.49	0.72	0.04	0.03	3.52 e^4	0.75	30.76
		Min	3397.46	77.65	2.86	1.17	0.62	2.45 e^5	58.61	30.00
		Max	3474.24	82.59	3.89	1.22	0.66	2.95 e^5	59.66	73.50
Sep	SC	Mean	3607.96	83.60	4.14	1.24	0.62	3.08 e^5	60.61	80.08
	n = 4	SD	166.28	4.68	0.98	0.05	0.03	5.14 e^4	1.98	18.68
		Min	3478.08	79.39	3.29	1.20	0.59	2.62 e^5	58.48	54.33
		Max	3850.13	87.95	5.10	1.30	0.65	3.56 e^5	63.24	99.00
Sep	BW	Mean	3051.17	83.97	6.66	1.27	0.51	3.13 e^5	54.01	27.13
	n = 3	SD	1012.06	5.45	3.71	0.05	0.16	6.21 e^4	11.30	19.12
		Min	1882.62	79.52	3.53	1.21	0.33	2.63 e^5	41.05	5.40
		Max	3646.13	90.05	10.76	1.32	0.63	3.82 e^5	61.83	41.33

Table S4. Bio-Env test results giving the best overall Spearman rank correlation coefficients (ρ) between the particle parameter similarity (Euclidean distance) matrix and all possible combinations of environmental variables to find the best fitting explanatory variables. Environmental variables available for selection in the model included: nitrate/nitrite (NO_3^-), ammonia/ammonium (NH_4^-), soluble reactive phosphorous (SRP), dissolved inorganic nitrogen to SRP ratio (DIN:SRP), discharge ($m^3\ s^{-1}$), and 2014 flow velocity ($m\ s^{-1}$) measured at each site.

Number of Variables	Spearman coefficient ρ	Selected variables
Best overall results		
2013-2014 data with discharge		
5	0.855	NO_3^-, NH_4^-, SRP, DIN:SRP, Discharge
4	0.848	NO_3^-, SRP, DIN:SRP, Discharge
4	0.842	NO_3^-, NH_4^-, SRP, Discharge
3	0.840	NO_3^-, SRP, Discharge
4	0.832	NH_4^-, SRP, DIN:SRP, Discharge
3	0.830	NO_3^-, NH_4^-, SRP
3	0.825	SRP, DIN:SRP, Discharge
4	0.821	NO_3^-, NH_4^-, DIN:SRP, Discharge
3	0.821	NO_3^-, DIN:SRP, Discharge
4	0.809	NO_3^-, NH_4^-, SRP, DIN:SRP
2014 data with flow velocity		
2	0.625	SRP, Flow velocity
3	0.624	NH_4^-, SRP, Flow velocity
3	0.550	SRP, DIN:SRP, Flow velocity
4	0.550	NH_4^-, SRP, DIN:SRP, Flow velocity
4	0.525	NO_3^-, NH_4^-, SRP, Flow velocity
3	0.524	NO_3^-, SRP, Flow velocity
2	0.513	NH_4^-, SRP
5	0.500	NO_3^-, NH_4^-, SRP, DIN:SRP, Flow velocity
4	0.500	NO_3^-, SRP, DIN:SRP, Flow velocity
1	0.492	SRP

APPENDIX B

SUPPLEMENTAL FIGURES

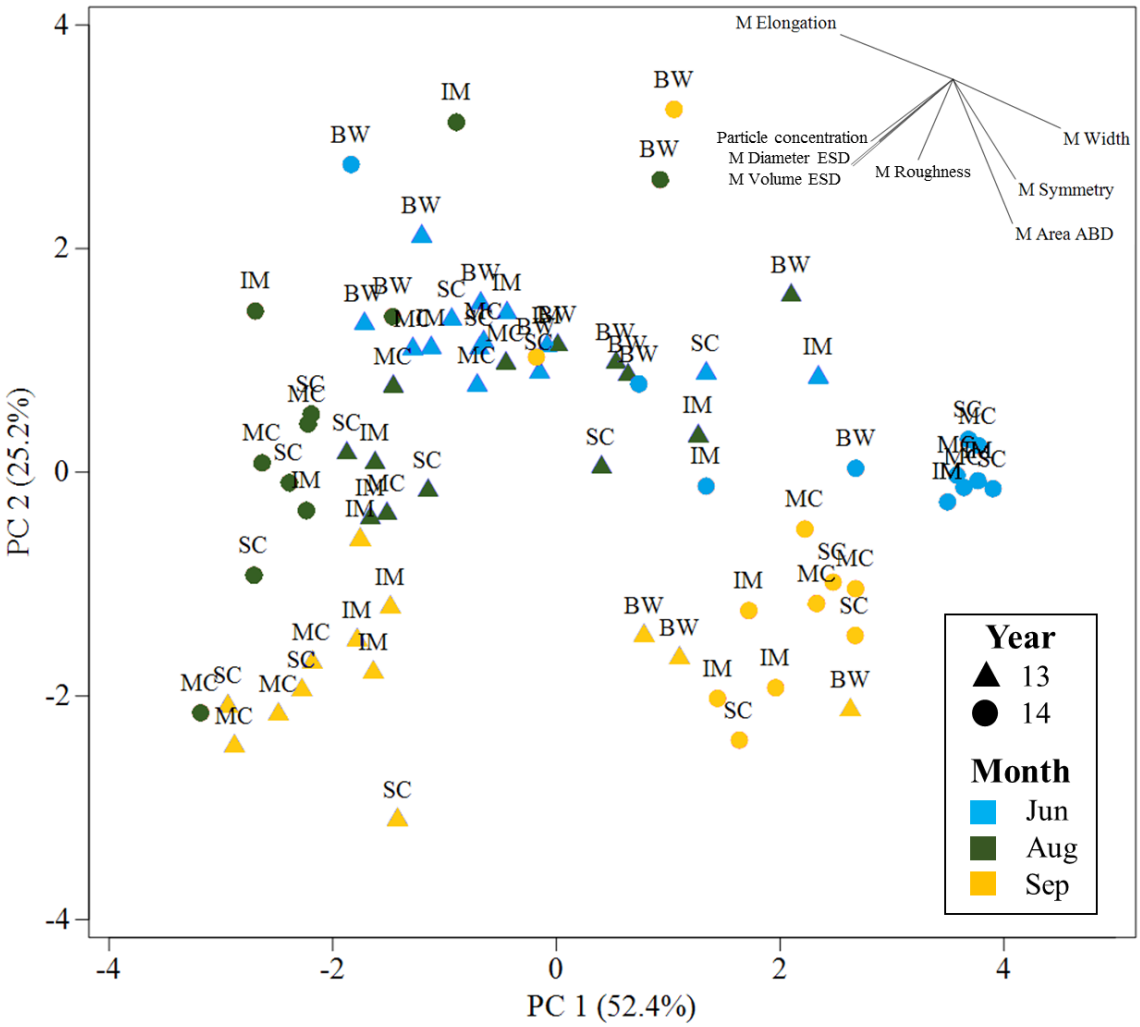

Figure S1. Principal components analysis (PCA) on median particle characteristics (53-300 µm) and mean particle concentrations (# mL^{-1}) among aquatic habitats in Pool 19, Upper Mississippi River in June, August, and September of 2013 and 2014; percent of variation for the first two principal component are shown in parentheses (77.6% cumulative variation). Year and month designated in legend and point labels indicate aquatic habitat type as follows: main channel (MC), side channel (SC), impounded (IM), backwater (BW). A biplot in the upper right corner represents the direction of influence for each particle parameter in relation to the axes and its importance is proportional to the length of the line.

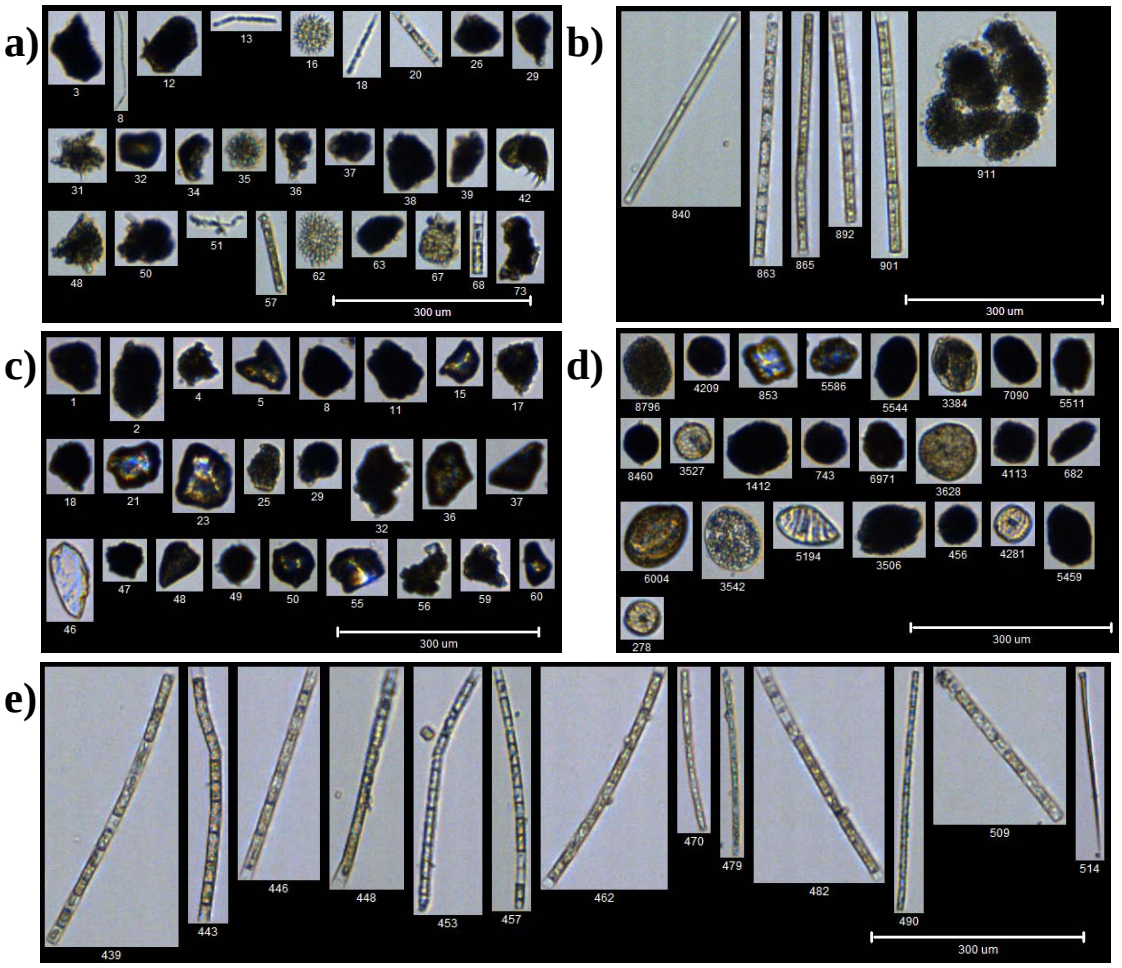

Figure S2. FlowCAM images of suspended particles in Pool 19 of the Upper Mississippi River collected in side channel habitats in 2013 and 2014. Image details are as follows: (a) June 2013 (small diameter and low roughness); (b) September 2013 (large diameter and high roughness); (c) June 2014 (small diameter and low elongation); (d) September 2014 (small diameter, low roughness, and high symmetry); and (e) particles collected in August 2014 (large diameter and elongate). Scale bars at lower right corners are 300 µm.

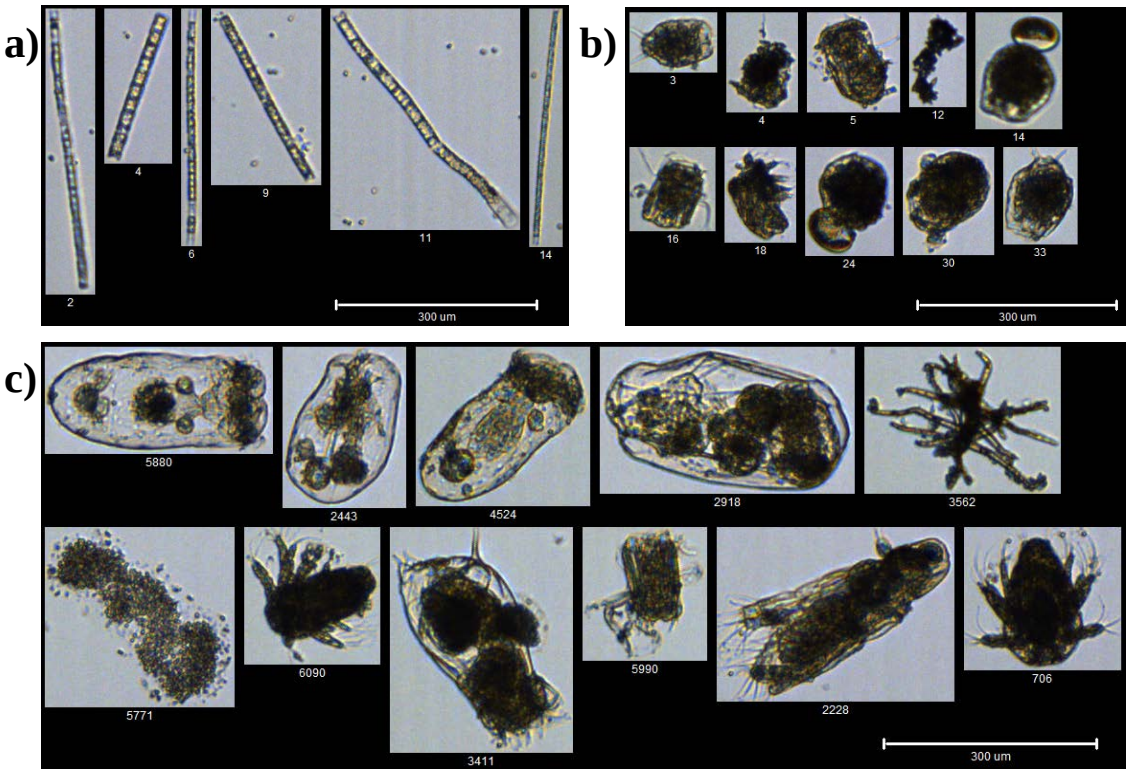

Figure S3. FlowCAM images of suspended particles in Pool 19 of the Upper Mississippi River collected in September 2013. Image details are as follows: (a) collected in a main channel habitat (large diameter and elongate); (b) backwater habitat (small diameter, wide, and high roughness); and (c) backwater habitat (large diameter, wide, and high roughness). Scale bars at lower right corners are 300 µm.

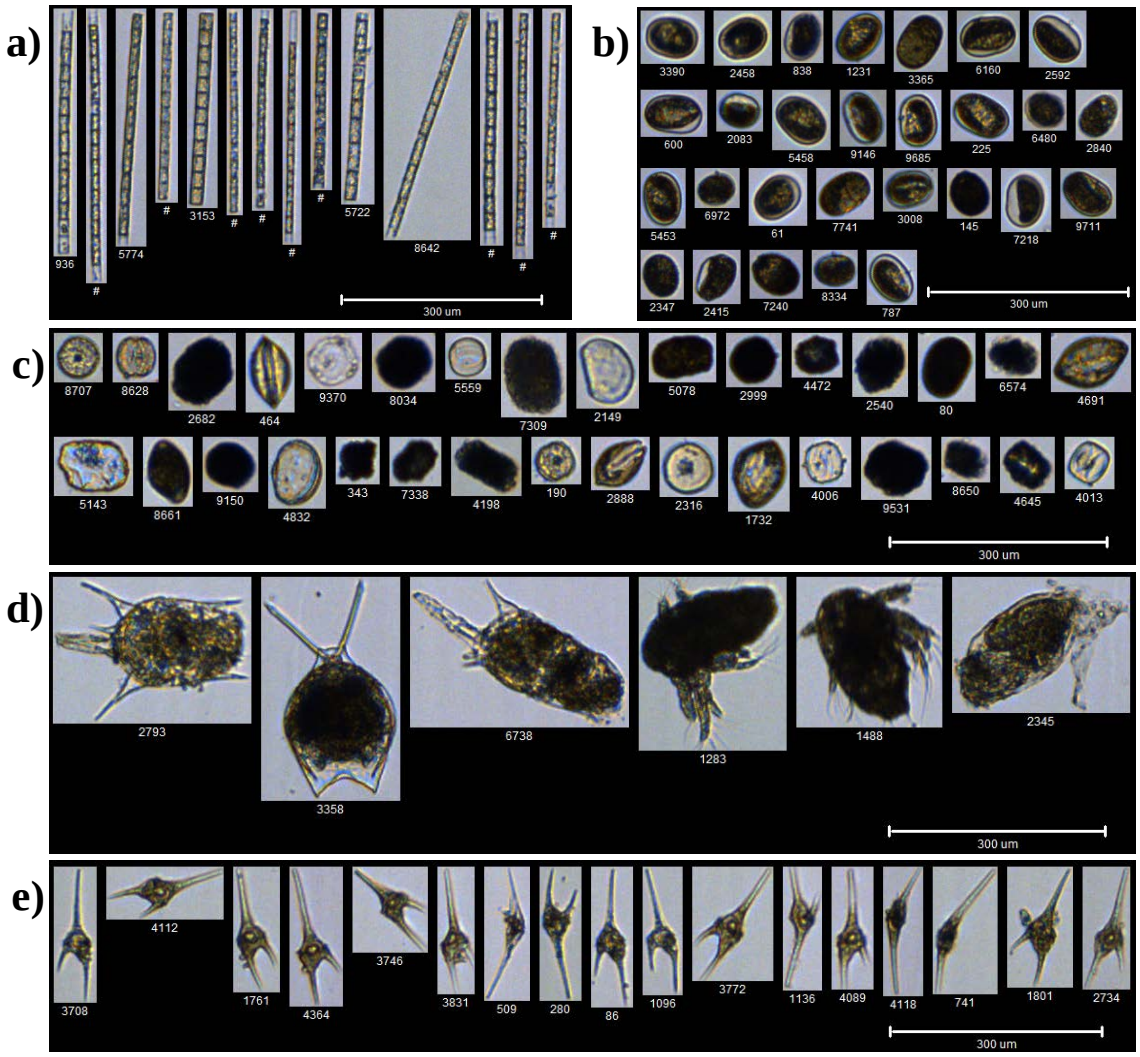

Figure S4. FlowCAM images of suspended particles in Pool 19 of the Upper Mississippi River collected in 2014. Image details are as follows: (a) collected at a main channel habitat in August 2014 (large diameter and elongate); (b) backwater habitat in August 2014 (small diameter and high symmetry); (c) main channel habitat in September 2014 (small diameter and high symmetry); (d) backwater habitat in September 2014 (large area and elongate); and (e) backwater habitat in September 2014 (spike in particle distribution from 100-135 µm diameter). Scale bars at lower right corners are 300 µm.