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

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ABSTRACT

Suspended particles are an essential component of large rivers influencing channel geomorphology, biogeochemical cycling of nutrients, and food web resources. The Upper Mississippi River is a large floodplain river that exhibits pronounced spatiotemporal variation in environmental conditions and biota, providing an ideal environment for investigating dynamics of suspended particles in large river ecosystems. Here we investigated two questions: (i) How do suspended particle characteristics (e.g. size and morphology) vary temporally and spatially? and (ii) What environmental variables have the strongest association with particle characteristics? Water sampling was conducted in June, August, and September of 2013 and 2014 in Navigation Pool 19 of the Upper Mississippi River. A FlowCAM® (Flow Cytometer and Microscope) particle imaging system was used to enumerate and measure particles 53–300 µm in diameter for size and shape characteristics (e.g. volume, elongation, and symmetry). Suspended particle characteristics varied considerably over space and time and were strongly associated with discharge and concentrations of nitrate + nitrite (NO_3^-) and soluble reactive phosphorus. Particle characteristics in backwaters were distinct from those in other habitats for most of the study period, likely due to reduced hydrologic connectivity and higher biotic production in backwaters. During low discharge, phytoplankton and zooplankton made up relatively greater proportions of the observed particles. Concurrently during low discharge, concentrations of chlorophyll, volatile suspended solids, and total phosphorus were higher. Our results suggest that there are complex interactions among space, time, discharge, and other environmental variables (e.g. water nutrients), which drive suspended particle dynamics in large rivers. Copyright © 2017 John Wiley & Sons, Ltd.

KEY WORDS: suspended particle characteristics; discharge; nutrients; plankton; Mississippi River

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INTRODUCTION

Suspended particles are essential components in aquatic systems and play major roles in the ecological and geomorphic functioning of rivers (Allan and Castillo, 2007). 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). Suspended particles are a primary food source for filter-feeding organisms, so the quantity, quality, and size of available particles are important to meet their energetic needs. Many characteristics of suspended particles have not been well studied in rivers because enumerating, measuring, and identifying particles are difficult and time consuming. Moreover, the ecology of suspended particles in large rivers has received much less attention than 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 (Houser *et al.*, 2010) and lateral gradients (Pongruktham and Ochs, 2015) in structure and function, as well as temporal variation, increase river complexity. For example, the River Wave Concept hypothesizes that the production, storage, transformation, and transport of suspended particles are largely a function of river flow (Humphries *et al.*, 2014), which varies seasonally and inter-annually. Investigating 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 inorganic and organic components separately. For example, studies on the inorganic component have focused on concentration, load, mineralogy, and mechanisms for suspension, transport, and deposition of only inorganic particles, such as gravel, sand, and silt (Ongley, 1982; Gilvear and Petts, 1985; Walling *et al.*, 2000; Hicks and Gomez, 2003). Studies on the organic component have focused on plankton biomass, chlorophyll *a* concentrations as a

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surrogate for phytoplankton abundance, taxonomic composition, and environmental variables that influence compartmental groups (e.g. seston and detritus, bacteria, phytoplankton, and zooplankton; Houser *et al.*, 2010; Burdis and Hoxmeier, 2011). Few studies have explored size and shape characteristics (e.g. diameter, elongation, and symmetry) of both components of suspended particles. These size and shape characteristics can help us examine river particle dynamics and perhaps predict particle composition and quality.

Major factors affecting suspended particle characteristics 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 the floodplain. Variation in these materials depends on river size and depth (Allan and Castillo, 2007), water discharge (Gilvear and Petts, 1985), and hydrologic connectivity of aquatic habitats (Pongruktham and Ochs, 2015). Conversely, autochthonous production typically increases during low discharge, as long water residence time (WRT) allows 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; Decker *et al.*, 2015; Houser *et al.*, 2015) act together to drive autochthonous production of biotic particles. Collectively, these mechanisms interact to influence the overall quantity, characteristics, and quality of suspended particles in rivers.

Faster and higher resolution technologies that combine flow cytometry, microscopy, and digital imagery have been developed to more efficiently characterize suspended particles (Alvarez *et al.*, 2011). A FlowCAM® was used to address two questions about suspended particle characteristics in a large river system, the Upper Mississippi River (UMR): (i) How do suspended particle characteristics differ spatially and temporally? and (ii) What environmental variables have the strongest association with suspended particle characteristics? The time and effort required to analyse the entire particle spectrum is extensive; therefore, a narrower range of particles was examined. Particles 53–300 μm in diameter reflect 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. *Dorosoma cepedianum* and bigheaded carp; Drenner *et al.*, 1984; Kolar *et al.*, 2007).

We hypothesized that river discharge, flow velocity, aquatic habitat (e.g. main channel or backwater), and nutrient availability would affect particle characteristics by influencing the source (i.e. allochthonous and autochthonous), storage, transformation, and transport of particles. More specifically, we hypothesized during spring and high discharge, habitats would be more homogenous in particle characteristics due to increased hydrologic 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 due to reduced hydrologic connectivity, increased WRT, and nutrient concentrations that would promote autochthonous production, affect plankton community characteristics, and therefore increase the proportion of living particles.

METHODS

Study area and field sites

The UMR extends from St. Paul, Minnesota to its confluence with the Ohio River near Cairo, Illinois. The UMR is divided into navigation pools by 29 locks and dams. Each pool typically possesses 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 for navigation. Side channels are secondary channels flowing from the main channel with variable depths and water velocities. Backwaters are off-channel areas that are typically shallow (<2 m) and vary in connectivity to the main channel. Some backwater areas maintain connection to flowing channels at all times, whereas others become isolated during low flow. Impounded areas are 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 (Wilcox, 1993).

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. 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 in June, August, and September at 13 or 14 sites in 2013 and 11 sites in 2014 (Figure 1). These months coincided with spring high flows and late summer or early fall low flows (Figure 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).

Particle analysis

Particles were collected by pumping water at mid-depth with a bilge pump (~5678 L hr^{-1}) through 1000-μm and 53-μm Nitex mesh sieves. The amount of water filtered varied widely (15–60 L) among sites and sample events, because occasionally dense algae caused sieves to clog

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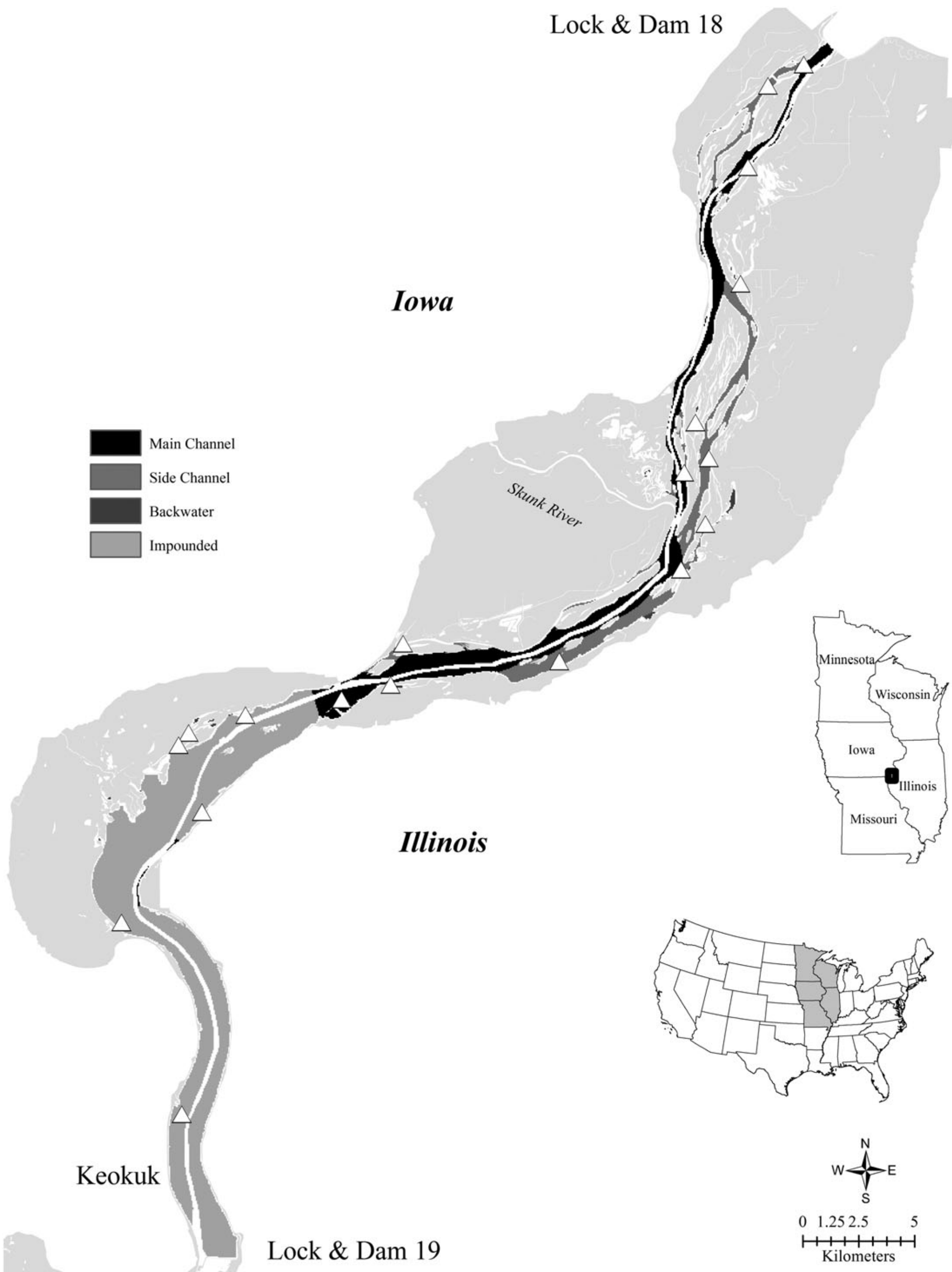

Figure 1. Map of Navigation Pool 19 of the Upper Mississippi River. Triangles indicate sample sites selected by stratified random sampling among four aquatic habitat types including main channel, side channel, backwater, and impounded. Sampling was conducted in June, August, and September at 13 or 14 sites in 2013 and 11 sites in 2014. The white line is the Navigation channel

rapidly. Materials collected on the 53-μm sieve (size range 53–1000 μm) were rinsed into sample bottles and preserved with 70% ethanol (Black and Dodson, 2003). Duplicates for 10% of field samples were collected and analysed for quality control.

Immediately prior to particle analysis, preserved samples were filtered once more through 300-μm Nitex mesh, resulting in 53–300 μm size range. Samples were analysed for particle concentration, size, and shape parameters using a Flow Cytometer and Microscope (FlowCAM®, Fluid

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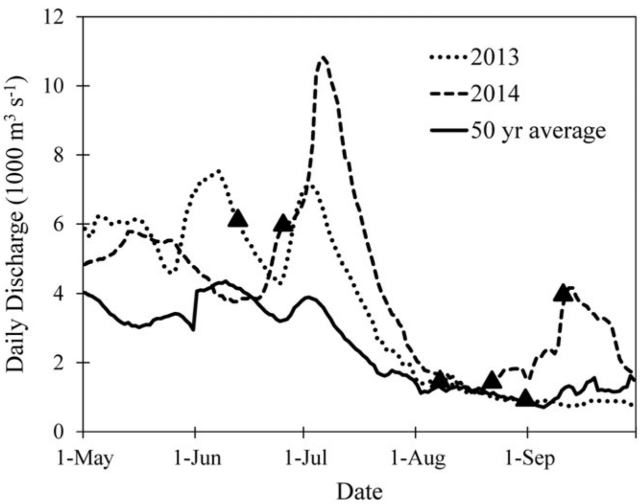

Figure 2. Hydrograph showing mean daily discharge at Lock and Dam 19 of the Upper Mississippi River from May through September of 2013, 2014, and the 50-year average. Triangles indicate sampling events. Data were obtained from the USGS National Water Information System (waterdata.usgs.gov)

Imaging Technologies, Inc., Scarborough, ME, USA). FlowCAM is a continuous imaging flow cytometer that captures two-dimensional digital images of individual particles moving through a glass flow cell in front of a microscope. Particle images were taken in auto-image mode, in which photographs were taken 20 frames per second at a constant flow rate of 1.75 mL/min. Visual Spreadsheet Software® (Fluid Imaging Technologies, Inc.) was used to enumerate, measure, and summarize parameters for 8000 to 15 000 particles per sample to yield a representative distribution. Parameters reported here include particle area, diameter, elongation, roughness, symmetry, volume, width, and concentration (# mL^{-1}; Table A1).

Quality control 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, USA) consisted of 50-µm diameter (NIST traceable) beads at 3000 particles/mL to verify the 4× objective and 300-µm flow cell as recommended in the FlowCAM manual (FlowCAM® Manual, 2013). Mean diameters were within the certified range (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). The average coefficient of variation for each particle parameter was low (<3.5%; Table A1). Alvarez *et al.* (2011) showed the average coefficient of variation for counts from triplicate analyses to be about 12% and that FlowCAM tended to underestimate counts relative to traditional microscopy but had an average percentage error of 15% between the two methods for preserved samples.

Environmental variables

Nutrient samples were collected using a 1-m vertical integrating tube sampler. Nutrients analysed 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 phosphorus (SRP), and total phosphorus (TP). Water samples for nutrient analyses were filtered (0.45-µm Whatman), preserved with sulfuric acid, and analysed on a Lachat Flow Injection Auto-Analyser (Hach Company, Loveland, CO, USA) according to standard methods (APHA, 2005). Total phosphorus samples were digested according to the US Geological Survey (USGS) alkaline persulfate digestion method (Patton and Kryskalla, 2003). NO_3^- concentrations were determined using 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 analysed gravimetrically according to standard methods (APHA, 2005).

Quality control 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%.

Chlorophyll (CHL) was measured *in situ* using a Turner Designs fluorescence sensor on an YSI Water Quality Sonde (YSI Incorporated, Yellow Springs, OH, USA) in 2013 and a Hydrolab Water Quality Multiprobe (Hach Company) in 2014. The fluorescence sensor was calibrated daily with a calibration standard, but not related to actual chlorophyll levels with laboratory analysis. Daily river discharge data at Dam 19 were obtained from the USGS National Water Information System (waterdata.usgs.gov; Figure 2). Surface flow velocity (m s^{-1}) was measured on site with a Marsh-McBirney water velocity meter (2014 only; water depth ~ 0.5 m).

Statistical analyses

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 FlowCAM are mathematically related and thus highly correlated. A draftsman plot (Pearson correlations) was conducted to selectively eliminate highly correlated ($r \geq 0.95$) parameters (Table A1). Prior to multivariate analyses, all data were fourth root transformed to reduce skewness (Clarke *et al.*, 2014). Missing data were estimated according

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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 between whole water particle measures, 53–300 μm particle characteristics, and nutrients (Table A2). 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, UK).

Temporal and spatial patterns in suspended particle characteristics 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 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). We used 9999 permutations and an α of 0.05 to indicate statistical significance. Post hoc pairwise comparisons with PERMANOVA 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 were examined by comparing the mean and standard error of the principal component (PC) scores for each sample (Figure 3).

Relationships between particle characteristics 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 are related to or best explain the particle characteristics by calculating Spearman correlation coefficients for all combinations of environmental variable matrices with the particle characteristic matrix. Because of the significant annual variation in hydrographs and particle characteristics (Table III), 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 (Figure 2). This approach, as opposed to a one-way analysis grouping both years together, resulted in higher ρ overall, suggesting there was substantial inter-annual variation in discharge. Another Bio-Env test was run using the 2014 data and flow velocity instead of discharge to determine if local flow velocity affected the particle characteristics. Chlorophyll, VSS, and TP were not included in the Bio-Env tests 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 the other variables were considered by examining the Pearson correlations (Table A2).

RESULTS

Suspended particles varied widely in size and shape characteristics (Table I; Table A3; Figure A1). In the PCA, PC1 (52.4% of variance) revealed considerable inter-annual variation in particle characteristics from 2013 to 2014. PC1 was strongly represented by particle shape parameters (elongation and width), size parameters (diameter and volume), and particle concentration (Table II; Figure 3a). PC2 (25.2% of variance) was heavily loaded by size (area, volume, and diameter) and shape parameters (symmetry; Table II; Figure 3b). Cumulatively, the first two PCs explained approximately 78% of the total variation (Table II). 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 A1). For example, in June 2013, backwater particles were somewhat different than other habitats (~0–2 PC1 units; Figure 3a), and by September those differences had increased (~3–4 PC1 units).

Particle characteristics in channel and impounded habitats were on similar trajectories over time, whereas backwaters followed a different trajectory (Figure 3). For example, particles in channel and impounded habitats increased in median size (diameter and volume), elongation, and concentration from June to September 2013. FlowCAM images showed these particles were mostly sediment and detritus in June, with some phytoplankton and a few zooplankton (i.e. rotifers; Figure A2a). By September 2013, particles in channel and impounded habitats were mostly large filamentous algae and large detrital aggregates or colonial algae (Figure A2b).

In contrast, particles in backwaters increased in width and decreased in size and concentration from June to September 2013 (Figure 3). Although backwater particles were also dominated by sediment and detritus in June, by September backwater particles included many more phytoplankton, rotifers, and nauplii than in other habitats (Figure A3b). 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 A3c).

In 2014, channel and impounded habitats showed a different pattern from 2013 in that particle characteristics in

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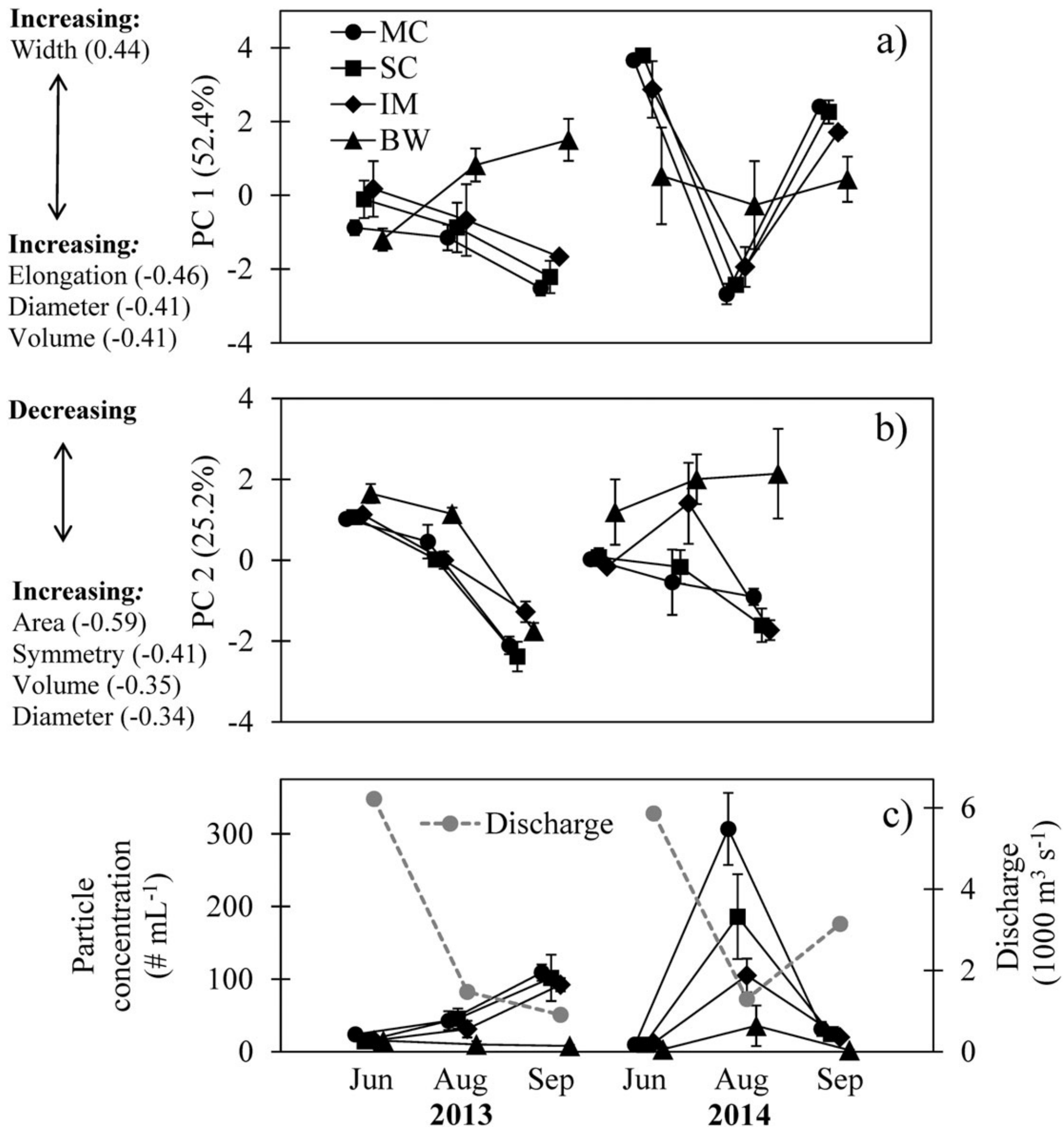

Figure 3. Particle characteristics and concentrations of particles 53–300 μm in diameter from Pool 19, Upper Mississippi River in 2013 and 2014 shown across months and aquatic habitats as follows: (a) PC1 scores; (b) PC2 scores; *y*-axis title shows percent of variation in parentheses and particle parameters listed in order from largest to smallest coefficient (highest four), their direction of change indicated by the arrow; and (c) particle concentration with discharge on secondary *y*-axis. Aquatic habitat type designated in legend as follows: main channel (MC), side channel (SC), impounded (IM), and backwater (BW). Error bars indicate standard error

September were more similar to those in June (Figure 3a and c). From June to August 2014, particles increased in concentration, median size, and elongation. Based on FlowCAM images, these changes were from a greater proportion of suspended sediment in June (Figure A2c) to more filamentous algae in August (Figure A2e and A4a). Then, by 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 A4c). However, more living particles (e.g. diatoms, rotifers, and *Microcystis*) were in suspension in September than in June (Figure A2d, A4c, and A2c, respectively).

As in 2013, particle characteristics in backwaters in 2014 followed a different trajectory than the other habitats (Figure 3). Despite small changes in median particle characteristics over time, there were considerable changes in particle assemblages. For example, particles in backwaters in June 2014 exhibited higher elongation values than other habitats, likely reflecting a high proportion of filamentous algae. In August, an abundance of zooplankton eggs was seen in backwaters (Figure A4b). Then, in September, although sharing similar particle characteristics with June, backwaters

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Table I. Summary of particle characteristics (53–300 μm) collected in June, August, and September 2013 and 2014 in Pool 19, Upper Mississippi River (N = 74), including the overall mean, standard deviation (SD), and range of median values of parameter distributions and mean particle concentrations. For definitions and abbreviations of particle parameters, see Table A1

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

included far more zooplankton (e.g. rotifers and nauplii; Figure A4d). Additionally, a noticeable increase in the frequency of particles 100–135 μm in diameter at backwater sites in September was due to a dinoflagellate bloom (i.e. *Ceratium*; Figure A4e), which was not seen in other months. These shifts in particle assemblage suggest secondary production can at times influence particle characteristics and the divergence of habitats (e.g. backwaters from other habitats).

Whole water particle measurements, including TSS, VSS, and CHL concentrations, were less sensitive in identifying changes in particle dynamics. Total suspended solids and VSS concentrations varied little from June to September 2013 despite major changes in 53–300 μm particle characteristics (Figure 4a and b). In 2014, TSS and VSS concentrations were more variable over time and among channel and impounded habitats but showed association with some of the particle characteristics (Table A2). Chlorophyll concentrations among all habitats increased during both years, except for a slight decline in backwaters in September 2013 relative to the other habitats (Figure 4c).

Table II. Principal components analysis (PCA) on 53–300 μm particles, including median particle characteristics and mean particle concentrations, among Upper Mississippi River Pool 19 aquatic habitats in June, August, and September 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

The PERMANOVA results supported these spatial and temporal patterns, suggesting the particle characteristics were significantly different between years, among months, and among habitats (p = 0.0001 for all three tests; Table III). However, all interactions, including the three-way (year × month × habitat), were significant (p = 0.0006 for three-way interaction, p = 0.0001 for two-way interactions) and obvious in the PCA, where significant variation was seen within year, month, and habitat combinations (Figure 3 and A1).

The Bio-Env test results showed the range of the highest Spearman correlations (ρ) for each number of variable models was relatively small (ρ = 0.730–0.855; Table IV). This suggested that adding variables (e.g. DIN:SRP and NH_4^+) to simpler models made minimal improvements to ρ and therefore are not further discussed. NO_3^- and SRP were the best two variable model (ρ = 0.806), and discharge was the best one variable model (ρ = 0.730). These three variables were also selected in the five best overall results; however, the range of ρ for the 10 best overall results was small (ρ = 0.809–0.855; Table A4). Therefore, the most parsimonious models included NO_3^- and SRP (ρ = 0.806) or NO_3^-, SRP, and discharge (ρ = 0.840; Table IV).

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 higher than that for the one variable model, SRP (ρ = 0.492; Table IV). However, additional variables did not improve ρ values, and the range of ρ for the overall best results was small (ρ = 0.492–0.625; Table A4). As a result, NO_3^-, SRP, and discharge (or flow velocity in 2014) were considered the best variables associated with the particle characteristics (Table IV and A4). However, NO_3^- and SRP concentrations were also strongly related to discharge and flow velocity (Figure 5a and b; Table A2). Thus, the effects of discharge and nutrients (NO_3^- and SRP) on the particle characteristics are confounded and difficult to separate.

Total phosphorus 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 A2), decreasing from June to August and increasing with discharge in September (Figure 5c).

DISCUSSION

Patterns in suspended particle characteristics were strongly influenced by variations in discharge. We predicted that during high discharge in spring, habitats would be more

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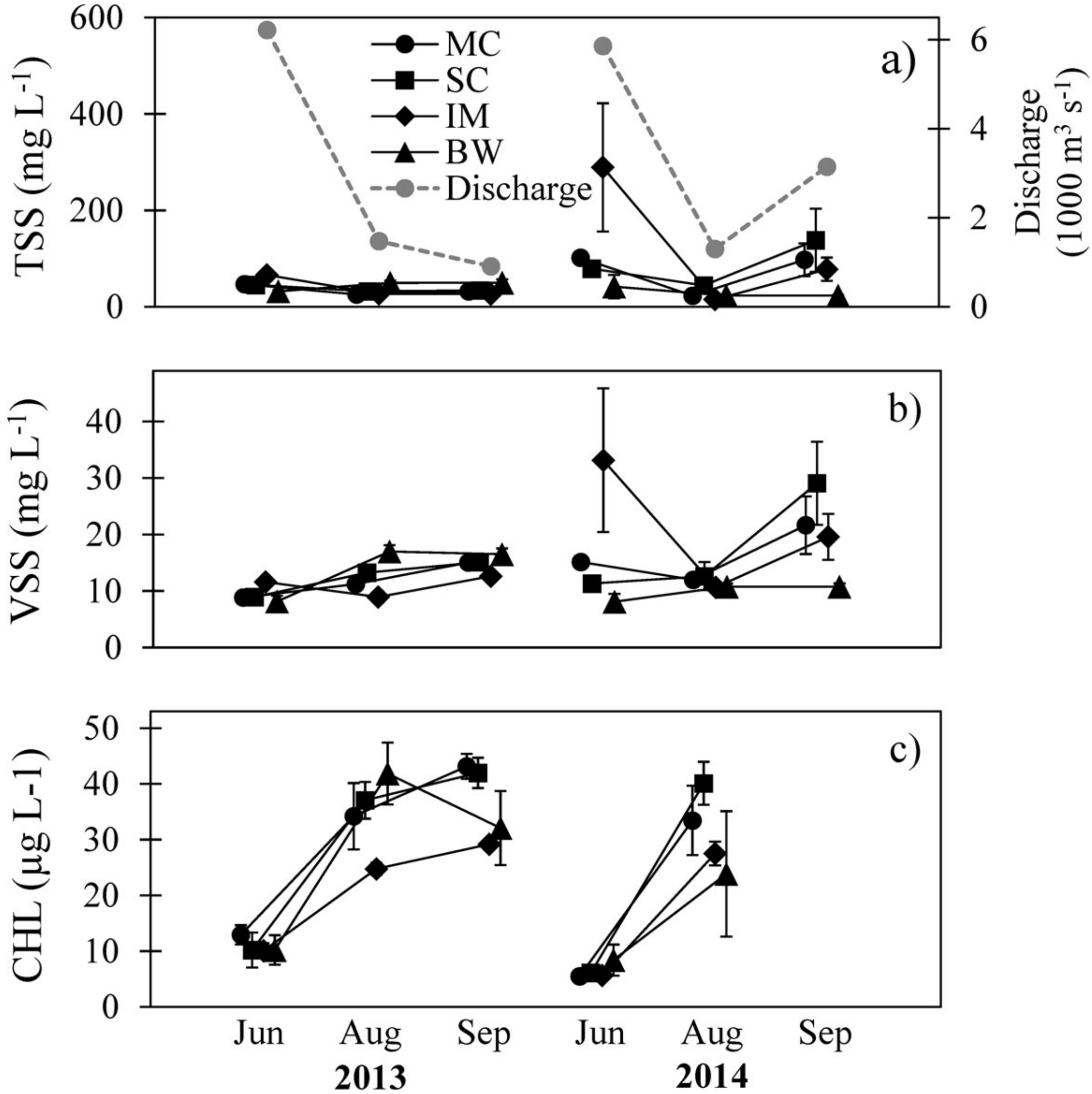

Figure 4. Total suspended solids and chlorophyll from Pool 19, Upper Mississippi River in 2013 and 2014 shown across months and aquatic habitats as follows: (a) mean total suspended solids (TSS) concentrations and discharge on secondary y-axis; (b) mean volatile suspended solids (VSS) concentrations;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

homogenous in particle characteristics because of increased hydrological connectivity and lateral mixing of materials among habitats. Particle characteristics were 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 higher flow velocities as well as wind advection (Rohweder *et al*., 2008). Pongruktham and Ochs (2015) 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, large temporal differences in discharge did not cause particle characteristics to differ. For example, in June and August 2013, particle characteristics in channel and impounded habitats were similar despite the large difference in discharge (~6300 and ~1470 $m^3 s^{-1}$, respectively). Further, discharge in August and September 2013 were similar (~1470 and ~930 $m^3 s^{-1}$, respectively), but particle characteristics differed significantly in channel and impounded habitats. Particle characteristics and composition are known to differ between rising and falling limbs of the hydrograph (Ongley, 1982). Therefore, as June and August 2013 were sampled on falling limbs, their similarities in particle characteristics may be explained by similar

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Table III. Permutational analysis of variance (PERMANOVA) testing for differences in suspended particle characteristics (53–300 μm) sampled from Pool 19, Upper Mississippi River in 2013 and 2014 as an effect of year, month, and aquatic habitat type (N = 74)

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 × month	2	23.234	0.0001
Year × habitat	3	4.690	0.0001
Month × habitat	6	2.937	0.0001
Year × month × habitat	6	1.9895	0.0006
Residual	49		
Total	72		

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 is the greatest contributor to suspended particles (Ongley, 1982). Rising discharge flushes the system of previously deposited materials on river banks and floodplains, which 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 pattern for concentrations of particles 53–300 μm. Concentrations of particles 53–300 μm were slightly higher on the falling limb (June 2013) than on the rising limb (June 2014) at comparable discharges (~6300 and 5900 m^3 s^{-1}, respectively). However, TSS concentrations were higher on the rising limb than the falling limb, presumably because particles <53 μm make up the largest proportion of suspended particles in rivers (Ongley, 1982). Additionally, the supply of materials 53–300 μm was possibly restricted during both June sampling events by earlier floods in May.

Table IV. Bio-Env test results giving the average within year Spearman correlation coefficient (ρ) between the particle parameter similarity (Euclidean distance) matrix and all combinations of environmental variables. Variables available for model selection included the following: nitrate + nitrite (NO_3^-), ammonia + ammonium (NH_4^-), soluble reactive phosphorus (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

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 positively associated with discharge—higher in June 2013/2014 and September 2014 and lower in August 2013/2014 and September 2013. A positive association between discharge and TSS has also been documented in other UMR studies (Houser *et al.*, 2010). The decrease in 53–300 μm particle concentrations during high discharge may be explained by a dilution effect on 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.

Particle images suggested a higher frequency of inorganic particles on the rising limb than the falling limb. The composition of suspended particles during high discharge is largely driven by soil mineralogy in the drainage basin, tributary contributions, allochthonous inputs, and degree of hydrologic connectivity among 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 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).

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. Larger width and symmetry values showed a greater proportion of sediment, suggesting sediment resuspension. However, plankton persisted during this September high flow. During high flows, scouring can suspend benthic algae into the water column and carry backwater plankton to the channels (Allan and Castillo, 2007;

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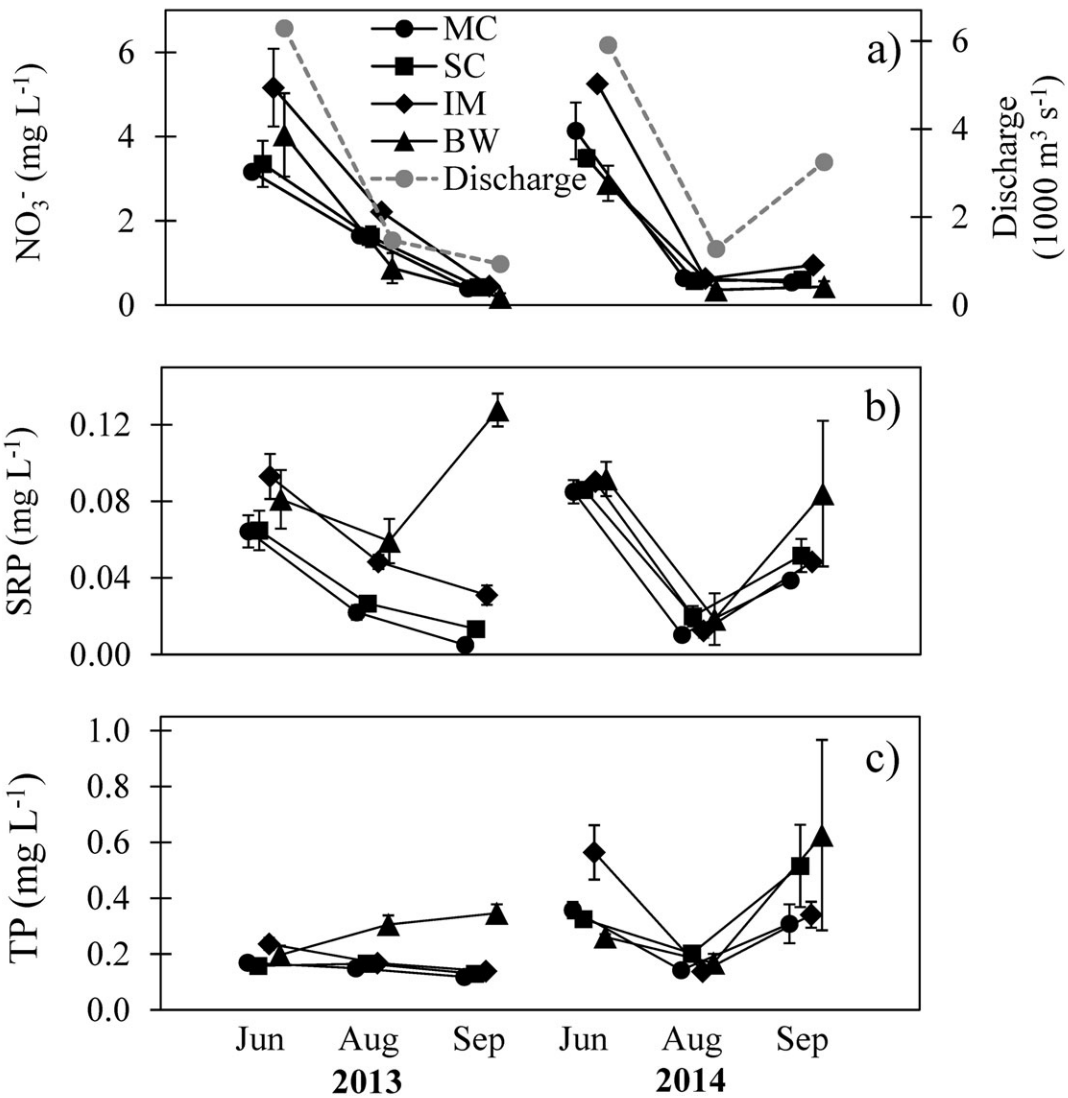

Figure 5. Nutrients measured in June, August, and September of 2013 and 2014 among aquatic habitats in Pool 19, Upper Mississippi River as follows: (a) mean nitrate + nitrite concentrations (NO_3^-) and discharge on secondary *y*-axis; (b) mean soluble reactive phosphorus concentrations (SRP); and (c)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

Pongruktham and Ochs, 2015). These were likely sources of plankton in channel and impounded habitats during this late-season rise in discharge. Volatile suspended solids concentrations were also higher in all habitats during this time, suggesting a large portion of suspended particles were organic.

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. Increasing productivity and phytoplankton densities over summer during low discharge have been documented in the UMR (Decker *et al*., 2015; Houser *et al*., 2015). 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 River Wave Concept, 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 hydrologic connections among habitats likely decreased as a result of decreasing discharge, backwaters developed distinct particle characteristics on a different trajectory from the other habitats. Low discharge and limited connectivity increases WRT, which promotes

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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) reported that as the degree of connection between the Lower Mississippi River channel and backwaters declined, so did turbidity in backwaters, while chlorophyll and gross primary production increased. Particle images from backwaters during declining discharge (August 2013 and 2014) showed an increase in 53–300 μm particle concentrations and suggested a shift from a larger proportion of sediment and filamentous algae to an increased frequency and diversity of zooplankton eggs, rotifers, nauplii, and other phytoplankton. High production in backwaters was also evidenced by increasing VSS concentrations throughout 2013, which would be associated with more organic particles (e.g. plankton and detritus).

On the Danube River in Austria, Hein *et al.* (2003) found that with decreasing hydrologic connectivity, the major source of organic matter in connected and isolated backwaters shifted to autochthonous production, but suggested that allochthonous inputs were important during high hydrologic connectivity. Discharge thus determines the degree of connectivity and mixing of particles among habitats, while WRT (related to discharge), light availability (associated with turbidity), and nutrients influence plankton community development (Decker *et al*., 2015; Pongruktham and Ochs, 2015). Longer WRT not only facilitates sedimentation and light availability for primary production but also allows time for plankton growth, especially secondary production with their longer generation times.

The highest variation in particle characteristics among sites, especially among backwaters, occurred during low discharge (September 2013 and August 2014). Backwater sites can differ greatly from each other in environmental variables owing to their varying degrees of connection with the channel (Hein *et al.*, 2003; Strauss *et al.*, 2004; Burdis and Hoxmeier, 2011; Houser *et al.*, 2015). During an extended period of low discharge (September 2013), larger zooplankton species and life stages (e.g. cladocerans and copepods) were more frequently imaged, as was a greater diversity of smaller organisms (e.g. diatoms, green algae, and rotifers). Decker *et al*. (2015) found that phytoplankton species composition differed the greatest between the UMR main channel and a backwater during summer low flows and suggested this was due to less hydrologic connectivity and other environmental conditions that influence variation in biotic production.

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. This was presumably owing to biotic assimilation, in addition to denitrification and declining discharge (and hydrologic connections) limiting nutrient inputs (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 other habitats (Decker *et al.*, 2015; Houser *et al.*, 2015; Pongruktham and Ochs, 2015). However, it is possible that even with the increase in discharge, river stage did not rise enough to cause substantial connection and mixing among habitats to affect NO_3^- concentrations. Soluble reactive phosphorus concentrations increased considerably in backwaters in September 2013, likely due to its release from sediments facilitated by high water temperatures via internal phosphorus 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; Decker *et al.*, 2015; Houser *et al.*, 2015).

In conclusion, our results suggest there are complex interactions among 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 owing to (i) discharge, related to reduced hydrologic connectivity and increased WRT, and (ii) nutrient dynamics that promoted biotic production. Discharge was the strongest driver of suspended particle dynamics; NO_3^- and SRP were also strongly associated with particle characteristics, although with discharge as well. The proportion of plankton in FlowCAM images increased as discharge declined and summer progressed, as did concentrations of CHL and VSS. Therefore, we hypothesize that nutrients become an important driver of biotic production during lower discharge conditions.

The 53–300 μm size range examined here is only a small fraction of the suspended particles in the river but reflects size classes that are important to many primary consumers (e.g. filter-feeding macrozooplankton and fishes). Variations in whole water particle measures (i.e. TSS, VSS, and TP) were subtle compared to the differences in 53–300 μm particle characteristics. There were few consistent patterns between whole water particle measures (i.e. VSS, CHL, and TP; as indicators of particle quality) and the 53–300 μm particle characteristics, suggesting these particle measures alone may not be the best for predicting particle assemblage composition or quality. However, ecologically important

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changes in the 53–300 μm size class certainly seem sensitive to changes in connectivity and production at different river flow stages.

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