Influence of lateral gradients of hydrologic connectivity on trophic positions of fishes in the Upper Mississippi River

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SUMMARY
1. Riverscapes consist of the main channel and lateral slackwater habitats along a gradient of hydrological connectivity from maximum connection in main channel habitats to minimum connection in backwaters. Spatiotemporal differences in water currents along this gradient produce dynamic habitat conditions that influence species diversity, population densities and trophic interactions of fishes.

2. We examined the importance of lateral connectivity gradients for food web dynamics in the Upper Mississippi River during spring (high flow, moderately low temperatures) and summer (low flow, higher temperatures). We used literature information and gut contents analyses to determine feeding guilds and stable isotope analysis to estimate mean trophic position of local fish assemblages. During June and August 2006, we collected over 1000 tissue samples from four habitats (main channel, secondary channels, tertiary channels and backwaters) distributed within four hydrologic connectivity gradients.

3. Mean trophic position differed among feeding guilds and seasons, with highest values in spring. Mean trophic position of fish assemblages, variability in trophic position and food chain length (maximum trophic position) of the two dominant piscivore species (Micropterus salmoides and M. dolomieu) in both seasons were significantly associated with habitat along the lateral connectivity gradient. Food chain length peaked in tertiary channels in both seasons, probably due to higher species diversity of prey at these habitats. We infer that food chain length and trophic position of fish assemblages were lower in backwater habitats in the summer mainly because of the use of alternative food sources in these habitats.

4. A greater number of conspecifics exhibited significant among-habitat variation in trophic position during the summer, indicating that low river stages can constrain fish movements in the Upper Mississippi River.

5. Results of this study should provide a better understanding of the fundamental structure of large river ecosystems and an improved basis for river rehabilitation and management through knowledge of the importance of lateral complexity in rivers.

Keywords: fishes, food chain length, food web, stable isotopes, trophic position

Introduction
Hydrological connectivity in riverine landscape ecology refers to the spatiotemporal exchange pathways of water, resources and organisms along lateral, vertical and longitudinal (upstream-downstream) dimensions. Lateral river-floodplain gradients (via the floodscape) and longitudinal gradients via surface
and groundwater flow pathways have received the most scientific attention (Junk, Bayley & Sparks, 1989; Ward & Stanford, 1995), but the importance of lateral connectivity gradients in riverine areas below the flood level (the riverscape) is largely unknown. For example, seasonal connectivity between the main channel of large rivers and lateral components of the riverscape influence fish dispersal and subsequent food web structure (Fisher, Brown & Willis, 2001) as well as species assemblages (e.g. Winemiller et al., 2000).

Rivers are sometimes discussed as if they consisted only of a main channel and a somewhat isolated floodplain; however, the riverine landscape in structurally complex rivers actually consists of an intricate riverscape (main channel, numerous slackwater habitats varying in hydrological connectivity) and a periodically connected floodspace (terrestrial floodplains, wetlands and lakes; Thorp, Thoms & Delong, 2008). A dynamic spatial mosaic of large habitat patches forms laterally within the riverscape from the main channel to backwaters as stage height varies with flow and flood pulses. These habitat patches differ in current velocity, temperature, organic and inorganic turbidity, solar radiation, concentrations of oxygen and nutrients, substrate size and type, and other physicochemical and biotic features of the aquatic and riparian zones. Habitat conditions can dramatically alter over time from, for example, a nutrient-rich, oxygenated backwater with abundant eukaryotic phytoplankton to a nutrient-limited, weed-choked system dominated by cyanobacterial plankton. Such seasonal shifts in physicochemical and biotic factors throughout the riverscape create a range of environmental conditions favouring one metazoan species over another, resulting in seasonal changes in the composition and diversity of biota and thus trophic interactions.

The Upper Mississippi River is a structurally complex, floodplain river which retains large areas of the floodplain and a reasonably normal flooding pattern, despite the presence of numerous low-head navigation dams (Delong, 2005). Extensive lateral complexity within the riverscape creates strong gradients in hydrologic connectivity from the main channel through the many secondary channels that pass around wooded islands to the more isolated backwaters. These last habitats have minimal connectivity within the wetted riverscape and experience directional currents only at high river stages, which result from relatively strong flow pulses or even higher flood pulses.

In the present study, we evaluated the trophic position of fish species in four feeding guilds (piscivores, invertivores, planktivores and omnivores/detrivores) during two hydrologic-thermal seasons: in *late spring* when river flow is high and temperatures are moderately low, and in *late summer* when river flow is low and temperatures are high. Fish were collected from main channel, secondary and tertiary channel, and backwater habitats along four lateral hydrologic connectivity gradients in the Upper Mississippi River. Our primary objective was to determine how lateral connectivity gradients altered mean trophic position of fish assemblages and maximum trophic position (=food chain length) in this floodplain river. We originally hypothesized that mean trophic position of fish assemblages and food chain length would peak in channels with intermediate levels of connectivity in relation to some combination of the *ecotonal* distribution patterns of species and the presence of *optimal* environmental conditions.

In the first instance, fish communities in the Upper Mississippi River exhibit considerable differences in lateral species distribution, with different species inhabiting backwater and main channel habitats (Chick et al., 2005). Fluvial specialists (i.e. those preferring at least moderate water currents) often reach peak abundance in main channel habitats and then decline somewhat in secondary channel habitats with their moderate to low flow conditions before falling drastically in low to zero-flow slackwaters. In the opposite trend, slackwater specialists often do best in non-advective habitats and decline in abundance as current velocity increases above low to moderate levels. The combination of these ecotonal patterns, along with the possible presence of mid-current specialists, may produce greater species diversity at secondary and tertiary channels. In the second instance, the presence of optimal environmental conditions, related to lateral changes in current velocity, may be present at intermediate sites on the gradient (and in backwaters during late spring). For example, increased flow is beneficial to organisms in that it transports food, oxygen, nutrients, and particulate and dissolved organic matter while simultaneously carrying away wastes. Yet disadvantages may occur through increased physical stress on an organism.
from higher current velocities, turbulence, turbidity and bed movement. As a consequence, for example, intermediate-flow slackwaters have richer zooplankton fauna (Thorp et al., 1994), and typically support greater invertebrate and fish densities (Angermeier & Karr, 1984; Benke et al., 1985). Food chain length is constrained by predator to prey size ratios (e.g. Pimm & Kitching, 1987), thus trophic position of fishes often increases with body size (Woodward & Hildrew, 2002; Jennings & Mackinson, 2003; Akin & Winemiller, 2008). We reasoned that in habitats with a greater diversity of prey and optimal environmental conditions, fishes would reduce trophic omnivory and forage on larger prey species of higher trophic position, thereby increasing trophic structure (mean trophic position of fish assemblages and food chain length).

Methods

Sample sites and sampling procedures

Our study was conducted between Mississippi River mile 711 and 728 in navigation reaches (or pools) six and seven, just north (upstream) of LaCrosse, Wisconsin. Navigation ‘pools’ (as designated by the U.S. Army Corps of Engineers) are areas of flowing water between adjacent lowhead, navigation dams (<15 m high) and do not represent the typical pools of standard lotic terminology. Each of these pools is highly braided with numerous secondary and tertiary channels and connected backwaters formed by wooded islands (Fig. 1). Although the Upper Mississippi has been altered from its natural state within the last 150 years by some channelization, dredging and construction of flood control and navigation structures, greater than 95% of the floodplain is still connected to the main channel in our study area (Delong, 2005). As a consequence, the Upper Mississippi is much more natural than the middle and lower reaches of the Mississippi.

The annual flow regime of the Upper Mississippi is somewhat comparable to pre-navigation dam periods (Delong, 2005). Although dams cause a pooling effect, especially during low flow periods, the river passes through or over the dams in high-flow periods, thereby allowing some river-floodplain interactions during floods. The mean annual discharge in this region is 840 m$^3$ s$^{-1}$ (1928–2006 data from the U.S. Geological Survey gauging station at Winona, Minnesota). Discharge is typically highest in the spring (April and May) in response to rain and snowmelt, and peak flows remain at flood stage for 6–8 weeks (Delong, 2005). Evapotranspiration and reduced precipitation normally cause discharge to decline throughout the summer. The extent of flooding during the several years immediately prior to our sample periods was slightly diminished from the long-term mean. During our 2006 sample year, however, flows averaged 1770 m$^3$ s$^{-1}$ during the flow/flood pulse period and 510 m$^3$ s$^{-1}$ during the period of low annual discharge.

We collected biotic samples and made physicochemical measurements from four habitat sites (main channel; SC, secondary channel; TC, tertiary channel; BW, backwater) along four transects (i.e. connectivity gradients). Each connectivity gradient intersected all four habitat types (Fig. 1). In all four connectivity gradients, flow decreased progressively from the main channel to backwater sites, and current velocity in all backwater sites was zero. Organism and water quality measurements were made in late spring (23 May–16 June 2006) and mid to late summer (3–10 August 2006), hereafter designated as spring and summer, respectively. Water quality parameters of temperature (°C), dissolved
oxygen concentration (mg L\(^{-1}\)), conductivity (\(\mu\)S cm\(^{-1}\)), and pH were measured the final day of sampling at each site. Other than flow rates, water quality conditions were similar at all sites.

The assemblage in the Upper Mississippi is rich, with approximately 89 fish species (Barko, Palmer & Herzog, 2004). All fish species were \(a\ priori\) divided into trophic feeding guilds following dietary descriptions in texts covering several nearby states and other published information (Table 1). Four traditional feeding guilds (piscivore, invertivore, planktivore and omnivore/detritivore) were used based on texts by Cross & Collins (1995), Pflieger (1997), and Becker (2001). Trophic guilds are approximate because many fishes feed opportunistically and undergo ontogenetic shifts in diet. The principal food of the adult listed in each text was used as a determination of trophic guild. We found some conflict in diets between invertivores and piscivores; therefore, we used information from gut contents analyses to clarify feeding guilds for species in these two feeding categories.

At each of our 16 habitat sites, we collected fish by electrofishing for trophic analyses. Our goal was to collect five individuals from each of two fish species in four trophic guilds at each site. Because of the vagaries of fish distribution and sampling, however, we sometimes collected fewer individuals but from more species within each trophic guild. After identifying all fish to species in the field using taxonomic keys in Pflieger (1997), we extracted a small plug of muscle from near the base of the dorsal fin, and then returned the fish to the river. All but the smallest fish were generally released alive, except for two individuals from the two most common species each of piscivores and invertivores at each site; those were sacrificed for gut contents analyses to aid determination of trophic guilds. The muscle plug and gut samples were then frozen, with the former later analysed for stable isotopes. Gut contents were later identified to genus where possible, following taxonomic keys in Thorp & Covich (2001) and McCafferty (1981).

We collected herbivorous mussels and snails by hand at each site to establish base levels of pelagic and benthic resources for determination of trophic position (Post, 2002b). Of the bivalve molluscs large enough for tissue sampling, zebra mussels (\(Dreissena polymorpha\) Pallis) were the numerical dominants and were collected from their rock attachment sites in both seasons as representative suspension feeders. The most abundant gastropods, \(Pleuroceras\) spp., were collected from rocks or the softer sediment littoral zone of each site as representative benthic grazers. In some sites, we used snails in the family Physidae or Lymnaeidae. Molluscs were frozen and later analysed for stable isotopes. To eliminate individual variation in isotopic signature, we determined \(\delta^{15}N\) and \(\delta^{13}C\) of the soft tissue of five snail and five mussel individuals and used the mean value as a measure of the base level for each site. Primary consumers often provide the best baseline because they can integrate multiple basal resources supporting the secondary consumer and take into account temporal and spatial changes in the food base (Cabana & Rasmussen, 1996; Vander Zanden & Rasmussen, 1999; Post, 2002b). Molluscs in particular effectively integrate temporal and spatial variation of \(\delta^{13}N\) and \(\delta^{13}C\) at the base of aquatic food webs (Post, 2002b).

**Trophic position and stable isotope analysis**

Trophic position was calculated using stable isotope measures for all species at our 16 sample sites. Trophic position estimates have been used to describe food webs in many large rivers (e.g. Thorp et al., 1998; Delong & Thorp, 2006; Zeug & Winemiller, 2008) and are especially useful for determining the primary food source for the plentiful omnivorous species whose diet preferences are otherwise difficult to delineate. Trophic position can be estimated using stable isotopes because the ratio (\(\delta^{15}N\)) of the heavy isotope of nitrogen (\(^{15}N\)) to the much more common light isotope (\(^{14}N\)) is enriched with each trophic transfer – a process termed fractionation. An enrichment of 3.4\(^{\circ}\) per trophic level has been widely accepted (e.g. Peterson & Fry, 1987) and was used as the trophic fractionation value. The stable isotope ratio (\(\delta^{13}C\)) of \(^{13}C\) to the more common \(^{12}C\) tends to exhibit an average enrichment of <1\(^{\circ}\) per trophic level and helps indicate the diet source of the organism as a baseline for estimating trophic position (Peterson & Fry, 1987; Post, 2002b). The combined use of carbon and nitrogen isotopes increases the ability to detect relationships between food sources and consumers (Post, 2002b).

Tissue samples from fish and molluscs were processed for stable isotope analysis at the University of Kansas. Samples were dried at 60 \(^\circ\)C for 48 h, ground
to a fine power with a Wig-L-Bug (Rinn Corporation, Elgin, IL, U.S.A.), and sealed in a tin capsule for later analysis. Isotope levels were measured on a Finnigan 253 continuous-flow, gas-source mass spectrometer (Thermo Electron Corporation, Waltham, MA, U.S.A.) coupled to an elemental analyser. Isotope ratios from fish with high amount of lipids (e.g. carp) were normalized following procedures in Post et al. (2007) to correct for errors of -3 to -4% in $\delta^{13}C$ using the equation: $\delta^{13}C_{\text{normalized}} = \delta^{13}C_{\text{untreated}} - 3.32 + 0.99 \times (C/N)$. Post et al. (2007) recommended using this equation only for fish with C : N < 7.0 because it has not been tested on fish with higher ratios, and they advised removing the lipids and remeasuring the

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<table>
<thead>
<tr>
<th>Family</th>
<th>Genus species-authority</th>
<th>Common name</th>
<th>Feeding guild</th>
<th>n</th>
<th>Mean TP</th>
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</table>

PI, piscivore; IN, invertivore; PK, planktivore; O/D, omnivore/detritivore; n, sample size; TP, trophic position.
$\delta^{13}C$ before recalculating the equation for fish with high C : N ratios. Therefore, lipids were extracted from some fishes with C : N ratios significantly over 7.0 following the methods of Folch, Lees & Stanley (1957) as revised by Post et al. (2007). The muscle tissue, less its lipids, was then redried and the corrected isotope ratio determined by reanalysing the tissues in the mass spectrometer. For samples from fish with high C : N ratios but with an insufficient mass of tissue for lipid extraction and reanalysis, the original isotope ratios were corrected using a derived linear equation. The equation was based on results from the five fishes for which lipid extractions were possible. $\delta^{13}C$ results for fishes with C : N over 7.5 were normalized for the effect of lipids using the equation: 
$$\delta^{13}C_{\text{normalized}} = \delta^{13}C_{\text{untreated}} + 3.5713 + 0.9923 \times C:N.$$

Trophic position was determined using a standard two-source mixing model (Post, 2002b) based on the equation: Trophic position $= \lambda + (\delta^{15}N_{sc} - [\delta^{15}N_{\text{base}} \times \alpha + \delta^{15}N_{\text{base}} \times (1-\alpha)]) / 3.4$, where $\lambda$ was the trophic level of food bases (e.g. 2 for primary consumers); $\delta^{15}N_{sc}$ was the $\delta^{15}N$ for the secondary consumer and 3.4%o was the average nitrogen isotope fractionation per trophic level. Trophic position calculations used a baseline based on zebra mussels, which mostly reflect the isotopic signature of seston that forms the base of pelagic food webs, and snails, which tend to reflect the isotopic signature of detritus and periphyton from the littoral food web (Post, 2002b). During both seasons, zebra mussels exhibited low among-habitat variability in $\delta^{15}N$ (ANOVA, $F_{3,9} = 2.516$ in spring, $P = 0.124$ in spring) and $\delta^{13}C$ (ANOVA, $F_{3,9} = 0.408$ in spring, $P = 0.751$ in spring, $F_{3,9} = 1.144$ in summer, $P = 0.383$ in summer). When snails (Pleuracereidae, Physidae and Lymnaeidae) were separated by genera, $\delta^{15}N$ differed in both spring (ANOVA, $F_{2,12} = 20.347, P < 0.001$) and summer (ANOVA, $F_{2,9} = 12.597, P < 0.001$). In spring, $\delta^{15}N$ of Physidae and Lymnaeidae was similar [Tukey’s Honestly Significant Differences (HSD) test: $P = 0.998$], but $\delta^{15}N$ of Pleuroceridae was greater than $\delta^{15}N$ of Physidae (Tukey’s HSD test: $P < 0.001$) and Lymnaeidae (Tukey’s HSD test: $P < 0.001$). However, in the summer, $\delta^{15}N$ of Pleuroceridae and Physidae was similar (Tukey’s HSD test: $P = 0.994$) and more enriched in $\delta^{13}N$ than Lymnaeidae (Tukey’s HSD test: $P < 0.05$ for Pleuroceridae and Lymnaeidae). $\delta^{13}C$ of the genera also differed in spring (ANOVA, $F_{2,12} = 16.562, P < 0.001$) and summer (ANOVA, $F_{2,9} = 5.851, P < 0.05$). In both seasons, Pleuroceridae was more enriched in $\delta^{13}C$ relative to Physidae (Tukey’s HSD test: $P < 0.001$ in spring, $P < 0.05$ in summer) and Lymnaeidae (Tukey’s HSD test: $P < 0.01$ in spring, $P < 0.05$ in summer). However, when snails were pooled into a single value for each habitat, among-habitat variation was non-significant during both seasons for $\delta^{15}N$ (ANOVA, $F_{3,11} = 0.661$ in spring, $P = 0.593$ in spring, $F_{3,11} = 0.957$ in summer, $P = 0.447$ in summer) and $\delta^{13}C$ (ANOVA, $F_{3,8} = 0.580$ in spring, $P = 0.645$ in spring, $F_{3,8} = 1.795$ in summer, $P = 0.226$ in summer).

We used the mean trophic position for all feeding guilds combined in each habitat as a measure of mean trophic position of the fish assemblage. Maximum trophic position, or food chain length, can be defined in several ways, all of which tend to correlate to varying degrees with mean trophic position. These can include, for example: (i) the highest mean trophic position for a species of the highest trophic group (e.g. Post, 2002b) or (ii) some measure of the range or variability of mean trophic position within one or more species (e.g. Cabana & Rasmussen, 1996). In order to compare food chain length among habitats within the Upper Mississippi River, we wanted to minimize the effects of species identity on food chain length as much as possible. Therefore, for food chain length, we used the mean trophic position of large-mouth bass (*Micropterus salmoides* [Lacepede]) and smallmouth bass (*Micropterus dolomieu* Lacepede) in each habitat because these taxa are the most widespread and numerically dominant piscivores in that portion of the ecosystem (K. A. ROACH, J. H. THORP, M. D. DELONG, pers. obs.) and are historically important in this ecosystem (Javrin, 2005).

**Data analyses**

Primary and secondary hypotheses were tested with a variety of statistical packages all using a maximal type I error rate of 0.05 unless otherwise noted. We employed a repeated-measures ANOVA using a 4 trophic guild × 4 habitat design. The factors for trophic guild were detritivore, planktivore, invertivore and piscivore with each represented by two or more species. The factors for habitat included main channel, secondary channel, tertiary channels and backwaters. Four replicates were obtained per season per habitat.
Guild, habitat and the interaction between guild and habitat were tested for between-subject effects with mean trophic position and coefficient of variation \([SD \times 100]/\text{mean}\) used as dependent variables. Additionally, season and habitat were tested for effects on mean trophic position and trophic variability for all fish combined. Mean values were compared among treatments for significant ANOVAs with post hoc Tukey’s HSD tests. Because seasons affected both mean trophic position and food chain length, spring and summer results were separated and ranked using a Kruskal–Wallis test (Sokal & Rohlf, 1995). Guild, habitat and the interaction between guild and habitat were then tested for between-subject effects of mean trophic position for all species combined and food chain length using ANOVAs.

Results
Relative abundance of species varied between seasons, with some species collected in only one sample period. In the spring largemouth bass was the numerically dominant piscivore \((n = 62\) and \(16\) for the spring and summer, respectively), but we collected greater numbers of smallmouth bass in the summer \((n = 27\) and \(61\), respectively). Planktivores were predominately spotfin shiners \((Cyprinella spilotera \text{[Cope]})\) in the spring \((n = 37\) and \(5\), respectively), whereas summer planktivores were almost entirely gizzard shad \((Dorosoma cepedianum \text{[Lesueur]})\) \((n = 5\) and \(57\), respectively).

Trophic designations derived from literature surveys were compared with trophic position values based on stable isotope analysis and pooled across lateral gradients. In spring the mean trophic position by species ranged from 2.96 for bigmouth buffalo \((Ictiobus cyprinellus \text{[Valenciennes]})\) to 4.28 for longnose gar \((Lepisosteus osseus \text{[Linnaeus]})\), whereas the summer range was 2.48 for gizzard shad to 4.21 for yellow bass \((Morone mississippiensis \text{[Jordan and Eigenmann]})\) (Table 1). Trophic position analyses (pooled across gradients and seasons) produced some surprising results for two species (Table 1). Highfin carpsuckers \((Carpiodes velifer \text{[Rafinesque]})\) and weed shiners \((Notropis texanus \text{[Girard]})\) were in the feeding guild of omnivore/detritivore, yet yielded mean trophic position values similar to that of piscivores. Only four piscivores had mean trophic position values greater than these two species of omnivore/detritivores: lognose gar and grass pickerel \((Esox americanus \text{[Gmelin]})\) at 4.25, yellow bass at 4.21, and walleye \((Sander vitreus \text{[Mitchill]})\) at 4.12. Sample size of these two species was very small \((n = 6)\), therefore this did not significantly bias our conclusions.

Seasonal effects on trophic position of feeding guilds
Season affected mean trophic position of feeding guilds \((\text{ANOVA}, F_{1,45} = 50.572, P < 0.001; \text{Fig. 2}); however, the coefficient of variation was not influenced by season \((F_{1,39} = 0.091, \ P = 0.765)\). Mean trophic position differed among feeding guilds during both spring \((\text{ANOVA}, \ F_{3,45} = 25.416, \ P < 0.001)\) and summer \((\text{ANOVA}, F_{3,45} = 15.496, P < 0.001)\). Mean trophic positions of piscivores and planktivores were highest and lowest, respectively, and were significantly different from all trophic guilds (Tukey’s HSD test: \(P < 0.05\); Fig. 2). Invertivores and omnivore/detritivores did not differ significantly from each other. No significant guild and gradient interactions were noted for mean trophic position during either season.

The variability of trophic position, as measured by coefficient of variation, differed among feeding guilds \((\text{ANOVA}, F_{3,39} = 5.506, P < 0.01)\), and there was a significant interaction between feeding guild and habitat \((F_{9,39} = 2.180, P = 0.045)\). Thus, the degree of variability in trophic position among feeding guilds depends on habitat type. Variability in trophic position within a feeding guild tended to decline in the order planktivore, omnivore/detritivore, piscivore and invertivore. The level of variation within guilds,
however, was significantly different only for comparisons of planktivores with piscivores and invertivores.

Response of trophic position to habitats along the connectivity gradient

Mean trophic position of fish assemblages did not differ among habitats in spring ($F_{3,45} = 2.436, P = 0.077$; Fig. 3) but varied significantly among habitats in summer ($F_{3,45} = 4.418, P < 0.01$). The only significant comparison of all combined means for feeding guilds in the summer was for mean trophic position of backwaters, which was markedly lower than other sites in the summer (Tukey’s HSD: $P < 0.05$).

Food chain length (maximum trophic position) as represented by the mean trophic position of two dominant species of bass (*Micropterus* spp.), did not differ significantly in spring ($F_{3,12} = 0.45, P = 0.724$), but habitat affected food chain length in the summer ($F_{3,12} = 7.93, P = 0.035$; Fig. 3). There was a significant season × habitat interaction ($F_{3,12} = 4.20, P = 0.030$); however, Tukey’s HSD indicated the only differences between habitats occurred in the summer. The major seasonal difference was a result of the low food chain length observed during the summer, especially in backwater habitats. Comparisons of means using Tukey’s HSD tests indicated that during the high flows of spring, backwater sites had the second highest food chain length next to tertiary channel sites, but the difference was not statistically significant. Food chain length in the summer was significantly higher in tertiary channels than in backwater and main channel habitats, but did not differ from secondary channel habitats.

Among-habitat variability in trophic position of conspecifics

In spring, trophic position exhibited among-habitat variability in two out of six conspecifics collected in all habitats. Trophic position was similar among habitats for freshwater drum (*Aplodinotus grunniens*, ANOVA, $F_{3,32} = 0.531, P = 0.664$), bluegill (*Lepomis macrochirus* [Rafinesque], ANOVA, $F_{3,58} = 0.407, P = 0.749$), silver redhorse (*Moxostoma anisurum* [Rafinesque], ANOVA, $F_{3,27} = 0.607, P = 0.617$), and smallmouth bass (ANOVA, $F_{3,23} = 1.194, P = 0.334$). However, in spring, habitat affected trophic position in shorthead redhorse (*Moxostoma macrolepidotum* [Leseuer], ANOVA, $F_{3,44} = 5.072, P < 0.01$) and largemouth bass (ANOVA, $F_{3,58} = 2.786, P < 0.05$). Shorthead redhorse trophic position was lower in backwater habitats than in secondary channel habitats (Tukey’s HSD test: $P < 0.01$), and trophic position of largemouth bass in main channel habitats was lower than in tertiary channel habitats (Tukey’s HSD test: $P < 0.05$).

Three out of seven conspecifics collected in all habitats exhibited among-habitat variability in summer. Trophic position was similar among habitats for common carp (*Cyprinus carpio* Linnaeus, ANOVA, $F_{3,10} = 2.065, P = 0.169$), bluegill (ANOVA, $F_{3,88} = 0.727, P = 0.538$), shorthead redhorse (ANOVA, $F_{3,26} = 1.734, P = 0.185$), and smallmouth bass (ANOVA, $F_{3,62} = 0.486, P = 0.693$). Habitat affected trophic position in emerald shiner (*Notropis atherinoides* Rafinesque, ANOVA, $F_{3,52} = 77.584, P < 0.001$), gizzard shad (ANOVA, $F_{3,57} = 5.972, P < 0.01$), and freshwater drum (ANOVA, $F_{3,27} = 4.913, P < 0.01$). Trophic position of emerald shiner was higher in backwater habitats compared to all other habitats (Tukey’s HSD test: $P < 0.0001$), and in gizzard shad, trophic position was higher in backwater habitats compared to tertiary and main channel habitats (Tukey’s HSD test: $P < 0.05$). In freshwater drum, trophic position in backwaters was higher than in secondary channel habitats (Tukey’s HSD test: $P < 0.05$).
Discussion

The term connectivity is increasingly employed in fundamental and applied river research from many perspectives, such as species conservation and studies of diversity patterns for floodscape lakes connected at various frequencies and stage heights (e.g. Miranda, 2005; Thorp, Thoms & Delong, 2006). While a few ecological studies have demonstrated effects of hydrological connectivity within the riverscape (Tockner, Schiemer & Ward, 1998), far more research has focused on the periodically flooded terrestrial and aquatic habitats of the floodscape. A pattern of biodiversity peaking at intermediate levels of connectivity in large rivers was noted by Ward & Tockner (2001), but Tockner et al. (1998) accentuated the taxonomic variability of species distribution patterns. In our study, we found that food chain length peaked in tertiary channel habitats in both seasons and was significantly greater than in main channel and backwater habitats during summer. Food chain length was lowest in backwater habitats during the low-flow summer period, but food chain length in backwaters was relatively high (second to nearby tertiary sites) in the spring when many fish species had been using these habitats as refugia or spawning sites.

The most important factors causing differences in food chain length among ecosystems is still disputed (reviewed in Post, 2002a), but empirical studies have shown a relationship between food chain length and colonization history (Persson et al., 1996), predator–prey size ratios (Pimm & Kitching, 1987) and ecosystem size in lakes (Post, Pace & Hairston, 2000), with the latter theoretically related to habitat heterogeneity and species diversity (Cohen & Newman, 1991; Holt, 1993). The importance of resource availability has been long debated as a limit of food chain length (e.g. Hutchinson, 1959). Somewhat surprisingly, primary productivity has proven a poor predictor of food chain length except in very oligotrophic systems (Post et al., 2000). The relative importance of these factors within ecosystems has not garnered much attention and is unknown within the riverscape of large floodplain rivers.

An increase in species diversity could have been a factor in the increased food chain length observed in tertiary channel habitats compared to main channel habitats. Though we did not measure biodiversity in each habitat, certain tertiary channels supported additional prey species such as bullhead minnow (Pimephales vigilax [Baird and Girard]) and logperch (Percina caprodes [Rafinesque]). Greater prey diversity may have allowed largemouth and smallmouth bass in tertiary channels to forage opportunistically on larger prey of a higher trophic position, thereby increasing food chain length. Thus, the greater number of trophic transfers may have occurred through some combination of reduced trophic omnivory by Micropterus spp. and the insertion of intermediate prey species (Post & Takimoto, 2007).

We infer that the availability of alternative food sources in backwater habitats was the ultimate mechanism driving changes in the low food chain length and mean trophic position of fish assemblages observed in these habitats during summer. Riverscape food bases vary spatially and temporally in response to many factors, including those associated with flow conditions (e.g. nutrient concentrations and advective losses), variations in riparian cover and substrate conditions. Seasonal and lateral variations include differences in allochthonous inputs, autochthonous production and relative abundance of various taxa of aquatic autotrophs (cyanobacteria, species and morphotypes types of vascular macrophytes, and micro- and macro-eukaryotic algae in pelagic and benthic habitats). We observed differences in biomass and diversity of vascular macrophytes along our lateral transects, with peaks in backwater sites. However, the majority of isotope-based and other food web studies in large rivers support conclusions of the riverine productivity model (Thorp & Delong, 1994, 2002) that phytoplankton, periphyton and fine particulate organic matter (FPOM) derived from algae are the primary energy source for metazoan food webs in both temperate and tropical floodplain rivers (Hamilton, Lewis & Sippel, 1992; Thorp et al., 1998; Lewis et al., 2001; Delong & Thorp, 2006), even in floodplains where macrophyte production exceeds algal production (Araujo-Lima et al., 1986; Forsberg et al., 1993; Herwig et al., 2004; Winemiller, 2004). In addition to change, we observed in total algal biomass across the connectivity gradients, seasonally prominent lateral shifts in the relative abundance of cyanobacteria versus more palatable algae, such as diatoms and green algae, have been recorded in the Upper Mississippi River (see the LTRMP program at the Upper Midwest Environmental Sciences Center in LaCrosse,
Wisconsin, U.S.A. at http://www.umesc.usgs.gov). In slackwater habitats with abundant phosphorus, the often unpalatable and less nutritious (for fish) cyanobacterial populations (Bernardi & Guisanni, 1990; Engstrom-Ost, Karjalainen & Viitasalo, 2006) may seasonally out-compete eu-karyotic phytoplankton because of the ability of these prokaryotes to fix atmospheric nitrogen. Dominance by cyanobacteria at certain sites along the gradient would alter not only the autotrophic nature of the food base but also the calculation of trophic position using stable isotopes if fishes were utilizing this food source and the organisms for our baseline (i.e. mussels and snails) were not. The isotopic signature of atmospheric nitrogen dissolved in water is very similar to that of atmospheric nitrogen. Dominance by cyanobacteria at certain sites along the gradient would alter not only the autotrophic nature of the food base but also the calculation of trophic position using stable isotopes if fishes were utilizing this food source and the organisms for our baseline (i.e. mussels and snails) were not. The isotopic signature of atmospheric nitrogen dissolved in water is very similar to that of atmospheric nitrogen (Cline & Kaplan, 1975), and its fixation by cyanobacteria is accompanied by relatively little isotopic fractionation, producing a $\delta^{15}N$ value close to zero (Goericke, Montoya & Fry, 1994). This could contribute to a seasonal shift in the nitrogen isotope signature of the autotrophic base and herbivores in backwater habitats if these cyanobacteria are consumed in abundance and if they relied extensively on the atmosphere as a nitrogen source.

We observed higher trophic positions of planktivores (emerald shiner, gizzard shad and spotted sucker) in backwater habitats relative to conspecifics in other habitats along the connectivity gradient during the summer. Pooled trophic positions of certain omnivore/detritivores (highfin carpsuckers and weed shiners) were also high compared to estimates based on gut content analysis; these species are known to consume mostly detritus and zooplankton and probably assimilated invertebrates and microorganisms, with most of the detritus being excreted. Jennings et al. (2001) attributed high variability in trophic position estimates of fishes that assimilate plankton to the microbial food loop, in which zooplankton can vary by two or more trophic levels (Azam et al., 1983). For example, zooplankton can feed directly on phytoplankton, or can be positioned at a higher trophic position by feeding on microorganisms (e.g. heterotrophic microflagellates and ciliates).

Other riverine studies have found significant between-habitat differences in stable isotope signatures of secondary consumers at the reach scale (Finlay, Power & Cabana, 1999; Finlay, Khandwala & Power, 2002; Walters, Fritz & Phillips, 2007). Furthermore, a recent study in the Brazos River, a floodplain river in Texas, observed significant differences in trophic positions of some conspecifics between the main channel and periodically connected oxbow lakes (Zeug & Winemiller 2008). A previous food web study in the Upper Mississippi River, however, found little variation in stable isotope signatures of secondary consumers between the main channel versus slackwater habitats (Herwig et al., 2007). Whether secondary consumers exhibit significant among-habitat differences in trophic position in floodplain rivers is probably due to the complex relationship between the flow regime and seasonal shifts in lateral hydrologic connectivity, which affect the availability of alternative food sources and movements of secondary consumers. We observed similar trophic positions of most conspecifics among habitats of the riverscape during the spring, yet certain conspecifics experienced significant among-habitat differences in trophic positions during the summer. In the Upper Mississippi River, the spring flow pulse may homogenize basal food sources and restructure fish assemblages.

Interspecific differences in trophic positions among habitats can only occur if movements between the habitats are relatively restricted. Previous research has found that fishes move among riverine habitats as a response to changing river discharge (Winemiller & Jepsen, 1998; David & Closs, 2002) and associated life history patterns (Lytle & Poff, 2004). Movements also can occur in response to changes in resource availability, abiotic conditions, behavioural and life history adaptations and ontogeny (Schlosser, 1991; Galat & Zweimuller, 2001). For example, Dettmers et al. (2001) found that at least 18 species were present in the main channel of the Mississippi River during summer, autumn, and winter but only eight species occurred during the spring high-flow period. However, our results indicate that low river stages can constrain fish movements relative to the time of stable isotope turnover (several months in fishes; Hesslein, Hallard & Ramal, 1993; McIntyre & Flecker, 2006).

During low river stages, fish also become concentrated in smaller areas. This can result in resource limitation for some species, especially those feeding on algae and invertebrates (Winemiller, 2004). In our study, mean trophic position decreased for all feeding guilds during the summer low-water period while variability in trophic position increased at all sites. Increased competition in some habitats could have forced some species to feed at lower trophic levels.
to obtain sufficient food, whereas other species may have become more generalist in their feeding behaviour.

We originally hypothesized that mean trophic position of fish assemblages and food chain length would peak in channels with intermediate levels of connectivity. We reasoned that higher species diversity and the presence of optimal environmental conditions in these habitats would allow fishes to reduce trophic omnivory and forage more consistently on prey species at higher trophic levels. Indeed, our data indicate that some predators in tertiary channels of the Upper Mississippi River were feeding at a higher trophic position during our summer sampling period. While further research is clearly needed to determine absolutely the relative role of several factors controlling food webs in aquatic systems, including along a hydrologic connectivity gradient, we contend that two primary factors are responsible for the isotope patterns we observed. These are relative differences among habitats in fish density and diversity and the importance of food quality. Within tertiary channel habitats, higher food chain length could be due to a combination of: (i) greater use of higher quality, basal resources by lower level consumers and (ii) a greater density and diversity (both species and functional group) of prey in these areas. The later would allow piscivores to concentrate on eating more fish and fewer invertebrates. In contrast, the fish assemblages in the connected backwater habitats, which included more lentic specialists and fewer fluvial specialists, may have had lower trophic positions in general because of relatively more consumption of cyanobacteria (a lower quality resource) by herbivores and a lower density and diversity of fishes. Other scientists (e.g. Post & Takimoto, 2007) have shown that lower community diversity reduces the number of intermediate predators in the food web, thereby lowering trophic position.

Acknowledgments

We appreciate the help of Sarah Schmidt, Brian O’Neill and many undergraduates (Ann Leaser, James Hofmann, Emily Zelenka and Danielle Quist) in collecting fish and molluscs from the Upper Mississippi River. We are grateful to David Post for advice on trophic position calculations, Erin Questad for comments on statistics, Mark Jakubauskas for locating aerial photographs, Aisha Al-Suwaidi and Michael Bruemmer for help with stable isotope analysis and Kirk Winemiller for comments on the manuscript. Collection and processing of samples was supported by an EPA Science to Achieve Results (STAR) grant #R83244201 to MDD and JHT, as managed by the EPA’s Office of Research and Development (ORD), National Center for Environmental Research (NCER). All aspects of this study complied with the current laws of the U.S.A. pertinent to research with freshwater organisms and ecosystems.

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(Manuscript accepted 25 September 2008)