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FATTY ACID PROFILES DISTINGUISH CHANNEL CATFISH FROM THREE
REACHES OF THE LOWER KASKASKIA RIVER AND ITS FLOODPLAIN
LAKES

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Running Title: Fatty acid biomarkers in riverine channel catfish

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ABSTRACT

Despite the increasing use of fatty acids (FAs) as biomarkers in aquatic food web analysis, little information is available regarding differences in FA profiles of fish among habitat types in river-floodplain ecosystems. The objectives of this study were to 1) test whether the FA profiles of channel catfish (*Ictalurus punctatus*) differed among three reaches of the lower Kaskaskia River and its floodplain lakes, and 2) to compare FA profiles among muscle, liver, and adipose fin tissues collected from these fish. Profiles differed significantly among sites, especially between upper and lower river sites, and between river channel and oxbow lake sites, suggesting differences in FA availability for channel catfish occupying different habitats and river reaches in the Kaskaskia River system. Specifically, the essential FAs 18:2n-6 and 18:3n-3 increased in catfish tissues from upstream to downstream reaches, which could reflect increased floodplain connectivity and decreasing impoundment effects downstream. Ratios of n-3 to n-6 FAs were higher in fish from oxbow lakes, perhaps suggesting increased use of autochthonous production in the floodplain relative to the main river channel. Muscle and adipose fin FA profiles exhibited similar location-related trends, whereas liver FA profiles were markedly different from the other tissue types. These results suggest that adipose fin tissue samples may be a viable, less-invasive alternative to muscle tissue for analysis of FA profiles in channel catfish. Our study supports the use of tissue FA profiles in identifying habitat utilization by channel catfish, and perhaps habitat-specific energy contributions to riverine consumers. Furthermore, our work highlights floodplain habitat as a potential source of essential n-3 FA and the associated importance of maintaining river-floodplain connectivity to support aquatic food webs.

INTRODUCTION

Food web studies using fatty acid (FA) biomarker methods are becoming increasingly common in aquatic ecology (e.g., Reuss & Poulsen, 2002; Dalsgaard & John, 2004; Alfaro *et al.*, 2006; Budge *et al.*, 2007), but very little research has been conducted using FAs as trophic tracers in large river systems. Rivers typically have diverse energy sources that are influenced by a variety of environmental factors including flow regime, floodplain connectivity, and channel alteration, all of which make discerning primary energy sources challenging (Thorp *et al.*, 2006). One of the key assumptions for using FAs to track trophic transfer is that FA profiles of prey vary spatially (e.g., among habitat types), and therefore can be used to identify habitat use and diet history of consumers (Elsdon, 2010). Although fishes are capable, to a greater or lesser extent, of transforming consumed FA in response to physiological demand and changes in water temperature, dietary intake is known to have an overriding influence on the composition of fish tissues (Reiser *et al.*, 1963). Czesny *et al.* (2011) and Lau *et al.* (2012) both demonstrated that pelagic and benthic fishes in lakes can exhibit distinct FA acid profiles, reflecting differences in the FA compositions of pelagic and benthic prey. These studies and others (Goedkoop *et al.*, 2000; Hessen & Leu, 2006; Ravet *et al.*, 2010) demonstrate the use of FA techniques to differentiate food webs in lentic systems, but the application of these techniques to large river systems is rare.

Two recent studies have indicated that FA profiles of consumers can differ spatially within large river-floodplain ecosystems (Dayhuff, 2004; Rude, 2012). Fatty acid profiles of bluegill (*Lepomis macrochirus*) differed among fish collected in the Illinois River, connected floodplain lakes, and disconnected floodplain lakes, supporting the potential use of FAs to distinguish floodplain lake and riverine energy sources for consumers in the Illinois River food web (Rude, 2012). Dayhuff (2004) was able to distinguish two sub-populations of sauger (*Sander canadensis*) and white bass (*Morone chrysops*) between the lower and upper portions of the Ohio River based on their FA profiles, supporting the use of FAs to contrast habitats along a river continuum. However, whether differences in FA profiles among habitats in the Illinois and Ohio Rivers are also present in other large river-floodplain systems, or whether equivalent habitat-specific differences in FA profiles would also be present in other fish species that more commonly use both river channel and floodplain lake habitats, such as channel catfish, are unknown.

Optimal application of FA biomarker techniques also demands identification of the preferred tissues to use. Some tissues, like muscle, may only reflect food consumed during periods of growth, and therefore reflect long-term diet history from the most recent growing season. In contrast, tissues that are more metabolically active like the liver generally have a more continuous turnover, reflecting changes in diet year round, and on a shorter time scale (Perga & Gerdeaux, 2005). Studies that have observed faster turnover time in liver FAs include Dave *et al.* (1975), Regost *et al.* (2003), Mourente & Bell (2006), and Zamal & Ollevier (1995). In addition to a faster metabolism, different tissue types have been shown to have preferential metabolism of

specific FAs depending on the tissue's initial FA levels relative to the diet (Trushenski *et al.*, 2008; Budge *et al.*, 2011).

Both liver and muscle tissue involve invasive sampling. Recent research in the field of stable isotope ecology has shown that fin tissues have similar turnover rates to muscle tissues, allowing for less-invasive sampling to evaluate diet history in fish (McCarthy *et al.*, 2000; Suzuki *et al.*, 2005). It is reasonable to assume that fin and muscle tissue might also yield similar information with respect to changing FA profiles. Fin tissue sampling could be especially useful for field sampling, in that it would make collecting and analyzing a large number of samples considerably easier (Suzuki *et al.*, 2005). However, it is unclear whether different tissues would yield the same information in large river fishes with respect to FA biomarkers and associated inferences regarding habitat use. We specifically chose to use the adipose fin for this project because of its non-lethal impact when removed, its ease of collection, and its high lipid content.

The objectives of this study were to 1) determine if FA profiles of channel catfish (*Ictalurus punctatus*) differ among upper, middle, and lower sections of the lower Kaskaskia River between Carlyle and Evansville, Illinois and the river's connected and disconnected oxbow lakes; and 2) to compare the FA profiles of liver and muscle tissue samples (invasive sampling) and adipose fin tissues (less-invasive sampling). Addressing these objectives will help to fill the aforementioned data gaps and will provide useful insights into using FA as biomarkers for distinguishing fish occupying different habitats in large river-floodplain systems.

MATERIALS AND METHODS

Study areas and field sampling

Channel catfish were collected from the Kaskaskia River and its floodplain lakes between Carlyle Lake (N 38° 37' 8", W 89° 21' 12") and the confluence of the Kaskaskia River and Mississippi River (N 37° 58' 30", W 89° 56' 17") in southwestern Illinois (Figure 1). The Kaskaskia River is channelized for navigation for the first 48.3 km upstream from the Mississippi River confluence, so sites were selected to include channelized and non-channelized river reaches. Sites also included two floodplain lakes that were connected to the main river channel, and a floodplain lake that was disconnected from the river. The channelized river sites were near Evansville and Baldwin river accesses. The unchannelized river sites were near Fayetteville and Carlyle river accesses. The connected oxbow lake sites were at the northern end of oxbow #18 near Baldwin, Illinois and at the northern end of oxbow #9 near New Athens, Illinois. The disconnected oxbow site was near the corner of Risdon School Road and Pete Junk Road in Baldwin, Illinois.

Field sampling was performed from 13 July through 15 September 2011. Ten to sixteen channel catfish were collected at eight sites using two sizes of hoopnets and DC electrofishing (8 amps, 60 pulses/s) and immediately placed on ice (Table I). Hoopnets measured 0.9 m in diameter with 4 cm mesh or 1.2 m in diameter with 5 cm mesh, and were set for 48 hours before fish collection. All nets were baited with commercial cheese bait contained in soft mesh webbing. The mean \pm standard error total length for collected fish was 392.0 ± 4.1 mm, and the

mean body weight was 511.5 ± 17.1 g ($N = 112$). This same restricted size range of fish was targeted for both electrofishing and hoopnetting methods in an attempt to include fish that likely had similar diets. Captured fish were immediately placed on ice. Muscle, liver, and adipose fin tissues were harvested from each fish approximately 4 hours after capture and stored in a -80°C freezer for subsequent FA analysis.

Fatty acid analysis

Muscle tissue was removed from the -80°C freezer and freeze dried for 48 hours (Freezone 6, Labconco Corporation, Kansas City, Missouri). The freeze-dried muscle tissue was then pulverized with a coffee grinder. Lipids were extracted from these muscle samples, and fatty acid methyl esters (FAMES) were prepared according to the methods adapted from Folch *et al.* (1957) and Christie (1982) as described by Lane *et al.* (2006). Lipid extraction and FAME preparation followed the same methods for liver and adipose fins, except that these tissues were not freeze dried and were homogenized directly in the chloroform:methanol extraction medium with a Fisher Scientific PowerGen Model 1000 Homogenizer (Thermo Scientific, Barrington, Illinois). The two pulverization/extraction methods provide equivalent results, but freeze-drying/grinding is preferred for creating homogenous samples of larger volumes of tissue (i.e., as is typical for fillet samples). Resultant FAMES were identified and quantified by gas chromatography by reference to external standards (Supelco 37 Component FAME Mix, PUFA-1, and PUFA-3, Supelco, Bellefonte, PA, USA) as described by Trushenski *et al.* (2008). Fatty acid methyl esters were quantified in terms of relative percent area and are reported as g/100g FAME. Lipid content was determined gravimetrically, following chloroform/methanol (2:1) modified from Folch *et al.* (1957).

Statistical analysis

Relative weight (W_r) was calculated for each fish using the standard weight equation for channel catfish reported by Brown *et al.* (1995). One-way analysis of variance (ANOVA) was used to assess whether there were significant differences in mean W_r among sites. Relationships between W_r and essential fatty acid content ([18:2n-6 + 18:3n-3], 20:5n-3, and 22:6n-3) were assessed using linear regression analysis. Differences in total lipid content among sites for each tissue type were evaluated using one-way ANOVAs.

Only FAMES that accounted for $> 1\%$ of total FAME content were used in comparing FA profiles of channel catfish: 14:0, 16:0, 16:1n-7, 18:0, 18:1n-9, 18:1n-7, 18:2n-6, 18:3n-3, 20:4n-6, 20:5n-3, 22:5n-3, and 22:6n-3. Percentage data were arcsine square root transformed prior to analysis in order to meet assumptions of normality. Two groupings of the field sites were used to analyze the data (Table I); the first test grouping consisted of “lower river”, which included the Evansville site, “middle river”, which included the Baldwin and Fayetteville sites, “upper river” which included the Carlyle site, and “oxbow”, which included all the oxbow lakes. The second test grouping consisted of “connected oxbow”, which included both Oxbow #9 at New Athens and Oxbow #18 at Baldwin, “disconnected oxbow”, which included the cut-off oxbow at

Baldwin, and “channelized river”, which included the Evansville and Baldwin river sites. Multivariate analysis of variance (MANOVA) with a tissue type*site interaction was used to determine whether spatial patterns of channel catfish FA profiles differed among tissue types. Pillai’s trace statistic was used to assess significance of differences in multivariate FA profiles. Next, a MANOVA with a canonical discriminant analysis (CANDISC procedure in SAS) was applied separately for each tissue type to assess differences in FA profiles among site groups. This procedure was coupled with linear discriminant function analysis (LDFA) with a leave-one-out jackknife procedure (DISCRIM procedure in SAS) to determine the accuracy with which individual fish could be reassigned back to their collection location based on their tissue-specific FA profiles. Lastly, one-way ANOVAs with Tukey’s HSD test for multiple comparisons were used to determine which individual FA differed significantly among site groups for each tissue type.

All statistical analyses were performed using SAS 9.2 (SAS Institute, Inc. Cary, NC). P-values ≤ 0.05 were considered significant for all analyses.

RESULTS

Comparison of FA profiles among tissues and relationships between Wr and FA levels

Tissue type had a significant effect on channel catfish FA profiles (Pillai’s trace = 1.614, $F_{24,550} = 95.81$, $P < 0.0001$), and the tissue type*site interaction was also significant (Pillai’s trace = 1.17, $F_{144,3420} = 2.57$, $P < 0.0001$). Liver tissue FA profiles were significantly different than muscle and adipose fin FA profiles (Pillai’s trace = 0.92, $F_{12,274} = 262.31$, $P < 0.0001$). The FAs that accounted for the most inter-tissue variation were 22:6n-3, 18:0 (liver > muscle > fin), and 16:1n-7 (fin > muscle > liver) ($F_{2,330} \geq 320.98$, $P < 0.0001$). Muscle and adipose fin FA profiles had similar slopes across sites (Pillai’s trace = 0.37, $F_{72,1104} = 1.01$, $P = 0.4672$), but significantly different intercepts (Pillai’s trace = 0.818, $F_{12,179} = 67.07$, $P < 0.0001$). The FAs 22:6n-3, 20:4n-6, and 18:0 accounted for the most variation between fin and muscle profiles ($t_{220} \geq 14.22$, $P < 0.0001$); relative abundances of these FA’s were higher in muscle than in fin tissue.

Mean relative weight of channel catfish did not differ significantly among sites ($F_{7,103} = 2.1$, $P = 0.05$). Relative weight was not correlated with essential fatty acid ([18:2n-6 + 18:3n-3], 20:5n-3, or 22:6n-3) content ($t_{108} < 1.600$, $P > 0.100$) for individual fish.

Total percentage lipid content was significantly different among all tissue types (adipose fin > muscle > liver) ($F_{2,324} = 675.53$, $P < 0.0001$).

Comparison of channel catfish FA profiles among connected and disconnected oxbow lake and channelized river sites

Muscle total lipid content did not vary among connected and disconnected oxbows and channelized river sites these sites ($F_{79,2} = 2.7$, $P = 0.075$). Muscle FA profiles were significantly different among these sites (Pillai’s trace = 0.624, $F_{24, 142} = 2.68$, $P = 0.0002$) (Figure 2). The muscle FA profiles of connected oxbow fish were significantly different than those of

channelized river fish (Pillai's trace = 0.496, $F_{12,55} = 4.51$, $P < 0.0001$), but muscle FA profiles of disconnected oxbow fish were not significantly different from the other site types (Pillai's trace = 0.184, $F_{12,70} = 1.32$, $P = 0.2288$). The muscle FAs that accounted for the most variation among these site groupings were 18:2n-6 (higher in channelized river than connected oxbow sites) ($F_{2,81} = 12.58$, $P < 0.0001$), 18:1n-9 (higher in connected oxbow sites) ($F_{2,81} = 6.67$, $P = 0.0021$), and 18:1n-7 (higher in oxbows) ($F_{2,81} = 5.47$, $P = 0.0059$) (Table II). Using LDFA, individual channel catfish were successfully classified back to their site type of capture with a 53% mean success rate based on their muscle FA profiles (Table III).

Liver total lipid content was significantly different among connected and disconnected oxbows and channelized river sites ($F_{74,2} = 6.0$, $P = 0.004$). Liver FA profiles were also significantly different among these sites. (Pillai's trace = 0.939, $F_{24,144} = 5.31$, $P < 0.0001$) (Figure 2). Fish from the disconnected oxbow had liver FA profiles that were significantly different from those of connected oxbow fish (Pillai's trace = 0.632, $F_{12,30} = 4.3$, $P = 0.0006$), and the channelized river fish (Pillai's trace = 0.72, $F_{12,45} = 9.66$, $P < 0.0001$). The connected oxbow fish also had FA profiles that were significantly different from those of channelized river fish (Pillai's trace = 0.564, $F_{12,56} = 6.03$, $P < 0.0001$). The liver FAs that accounted for the most variation among these site groupings were 18:1n-7 (higher in oxbows) ($F_{2,82} = 23.24$, $P < 0.0001$), 18:2n-6 (higher in channelized river sites) ($F_{2,82} = 13.06$, $P < 0.0001$), and 22:6n-3 (higher in oxbows) ($F_{2,82} = 10.89$, $P < 0.0001$) (Table II). Using LDFA, fish were successfully classified back to their site type of capture with a 66% mean success rate based on their liver FA profiles (Table III).

Adipose fin total lipid content was significantly different among connected and disconnected oxbows and channelized river sites ($F_{79,2} = 3.9$, $P = 0.023$). Adipose fin FA profiles were also significantly different among these sites. (Pillai's trace = 0.676, $F_{24,142} = 3.02$, $P < 0.0001$) (Figure 2). Adipose fin FA profiles of disconnected oxbow fish were significantly different from those of channelized river fish (Pillai's trace = 0.541, $F_{12,45} = 4.42$, $P = 0.0001$), but not significantly different from the connected oxbow lake fish (Pillai's trace = 0.387, $F_{12,29} = 1.52$, $P = 0.1716$). Adipose fin FA profiles of connected oxbow fish were significantly different from those of channelized river fish (Pillai's trace = 0.418, $F_{12,55} = 3.3$, $P = 0.0012$). The FAs that accounted for the most variation among these site groupings were 18:2n-6 (higher in channelized river) ($F_{2,81} = 11.67$, $P < 0.0001$), 18:0 (higher in oxbows) ($F_{2,81} = 8.06$, $P = 0.0006$), and 20:5n-3 (higher in oxbows) ($F_{2,81} = 3.49$, $P = 0.0353$) (Table II). Using LDFA, fish were successfully classified back to their site type of capture with a 63% mean success rate based on their adipose fin FA profiles (Table III).

Comparison of channel catfish FA profiles among all river reaches and oxbow lakes

Muscle total lipid content was significantly different among lower, middle, and upper river sites, and oxbow sites ($F_{104,3} = 5.04$, $P = 0.003$). Muscle FA profiles were also significantly different among these sites (Pillai's trace = 1.606, $F_{36,300} = 9.6$, $P < 0.0001$) (Figure 3). Also, all site groupings were significantly different from each other individually (Pillai's trace ≥ 0.403 , P

< 0.0001). The FAs that accounted for the most variation in the site groupings were 18:2n-6 (increasing abundance downstream) ($F_{3,109} = 62.94$, $P < 0.0001$), 18:3n-3 (also increasing abundance downstream) ($F_{3,109} = 12.85$, $P < 0.0001$), and 18:1n-7 (decreasing downstream) ($F_{3,109} = 8.38$, $P < 0.0001$) (Table IV). Using LDFA, fish were successfully classified back to their site type of capture with a 79% mean success rate based on their muscle FA profiles (Table V).

Liver total lipid content was significantly different among lower, middle, and upper river sites, and oxbow sites ($F_{99,3} = 5.65$, $P = 0.001$). Liver FA profiles were also significantly different among these sites (Pillai's trace = 1.661, $F_{36,303} = 10.43$, $P < 0.0001$) (Figure 3). Also, all site groupings were significantly different from each other individually (Pillai's trace ≥ 0.493 , $P < 0.0001$). The FAs that accounted for the most variation were 18:2n-6 (increasing relative abundance downstream) ($F_{3,110} = 32.5$, $P < 0.0001$), 18:1n-7 (higher in oxbows than river sites) ($F_{3,110} = 21.24$, $P < 0.0001$), and 16:0 (increasing relative abundance downstream to Baldwin, then decreasing downstream) ($F_{3,110} = 15.28$, $P < 0.0001$) (Table IV). Using LDFA on channel catfish liver FA profile data, fish were successfully classified back to their site type of capture with a 79% mean success rate (Table V).

Adipose fin total lipid content was not significantly different among lower, middle, and upper river sites, and oxbow sites ($F_{103,3} = 1.36$, $P = 0.261$). However, adipose fin FA profiles were significantly different among these sites (Pillai's trace = 1.559, $F_{36,300} = 9.01$, $P < 0.0001$) (Figure 3). Also, all site groupings were significantly different from each other individually (Pillai's Trace ≥ 0.363 , $P < 0.0001$). The FAs that accounted for the most variation among these site groupings were 14:0 (decreasing abundance downstream) ($F_{3,109} = 20.38$, $P < 0.0001$), 18:3n-3 (increasing abundance downstream) ($F_{3,109} = 17.36$, $P < 0.0001$), and 16:1n-7 (decreasing abundance downstream) ($F_{3,109} = 15.23$, $P < 0.0001$) (Table IV). Using LDFA, fish were successfully classified back to their site type of capture with a 76% mean success rate based on their adipose fin tissue FA profiles (Table V).

DISCUSSION

Comparison of fatty acid profiles among tissue types

Channel catfish liver tissue had a different FA signature than muscle or adipose fin tissue. This was expected because tissue-specific FA metabolism is known to occur in fishes (Zamal & Ollevier, 1995; Budge *et al.*, 2011). The liver is the main organ for FA metabolism in fish, thereby making a change in dietary FA more quickly apparent in this tissue. Faster apparent turnover time with diet change in liver could also be due to increased triacylglycerol content, which is primarily used for energy storage (Mourente & Bell, 2006) and tends to reflect dietary intake more directly than other lipid types (Trushenski *et al.*, 2008). Conversely, muscle tissue is generally higher in phospholipids (Budge *et al.*, 2011) (an essential component of structural membranes), which preferentially incorporate certain FA (Trushenski *et al.*, 2008), causing them to be generally less responsive to dietary FA than triacylglycerols. Differences in responsiveness to dietary FA and in turnover rates suggests that liver tissue might be more relevant for

investigating recent habitat switches or changes in diet (e.g., on the scale of weeks), whereas muscle or fin tissue might be expected to reflect shifts in habitat use and diet on a longer time scale (e.g., on the scale of months). Gause & Trushenski (2013) noted similar patterns in the response of rainbow trout tissues to changes in dietary fatty acid intake.

Our finding that adipose fin and muscle tissue samples from channel catfish had similar FA profiles is consistent with prior studies that reported similar stable isotope ratios between these tissue types (Sanderson *et al.*, 2009; Hanisch *et al.*, 2010). Collectively, these results suggest that sampling fin tissue represents a less invasive alternative to muscle tissue samples for determining habitat use by channel catfish. Less-invasive tissue sampling techniques would be especially relevant in research involving populations that are sensitive to removal of small numbers of individuals, including threatened or endangered fish species, where sample sizes or more invasive tissue sampling are typically restricted.

Fatty acid profiles compared among fish from oxbow lakes and the river channel

Channel catfish FA profiles were distinguishable between main channel and oxbow lake sites regardless of the tissue sampled, indicating differences in FA availability in these systems perhaps as a result of different basal energy sources for channel catfish between river channel and oxbow lake habitats. The FA 18:2n-6 was a key contributor to differences among these site groupings, and was consistently higher in fish from the main river channel than fish from oxbows across all tissue types. 18:2n-6 is found in aquatic and terrestrial plants, but found in increasing concentrations with a more terrestrially-derived diet (Brett *et al.*, 2009), possibly suggesting greater use of allochthonous carbon by fish in the river channel (Maazouzi *et al.*, 2007; Perga *et al.*, 2009). Greater levels of 18:2n-6 in fish from the river channel compared to floodplain lakes was also observed in bluegill (*Lepomis macrochirus*) from the Illinois River system (Rude 2012). Additionally, higher levels of 20:5n-3 and 22:6n-3 (adipose fin and liver tissue only) in channel catfish from oxbow lakes along the Kaskaskia River compared to fish from the river channel may suggest increased use of autochthonous energy sources by channel catfish in the floodplain (Perga *et al.*, 2009; Galloway *et al.*, 2012). Rude (2012) also observed an increase in 22:6n-3 in bluegill from Illinois River floodplain lakes compared to fish collected in the river channel.

The FAs 20:5n-3, eicosapentaenoic acid (EPA), and 22:6n-3, docosahexaenoic acid (DHA) not only help trace primary energy sources to fish, but are closely linked to food quality and fish health, and are an important part of respiratory, cardiovascular, and nervous system physiology, as well as growth, reproductive, and chemotactic processes (Brett & Muller-Navarra, 1997). Additionally, Dayhuff (2004) observed that W_r was positively correlated with levels of 20:5n-3 in both white bass (*Morone chrysops*) and sauger (*Sander canadensis*) in the Ohio River. Thus, differences in FA profiles of channel catfish between the Kaskaskia River and its floodplain lakes indicate differences in the availability of certain FAs between these habitat types and suggest that oxbow lakes may represent an important source of essential FAs such as EPA and DHA for riverine fish. Although there was no significant correlation between 20:5n-3 and

22:6n-3 levels and channel catfish W_r in our study, the physiological, reproductive, and growth benefits of LC-PUFA to fishes may not necessarily be reflected in W_r .

While FA profiles of channel catfish were distinguishable between river channel and oxbow habitats, fish from connected and disconnected oxbow lakes could not be consistently distinguished based on their FA profiles, suggesting the diet of fish in these habitats was broadly similar. Muscle and adipose fin tissue FA profiles showed very little distinction between connected and disconnected oxbow habitats; however, liver tissue FA profiles were able to distinguish channel catfish from connected and disconnected oxbow lakes with moderate success. This is most likely due to the faster turnover of liver tissue showing more recent diet changes, coupled with the record spring rainfall in Illinois during 2011, which caused the Kaskaskia River to flood into the normally disconnected oxbows through the middle of July (Illinois State Water Survey 2011; Matthew Young, personal observation). Consequently, channel catfish were sampled from the disconnected oxbow only one month after the river had receded and a connection to the river was no longer present. Increased river-floodplain connectivity would allow fish to more readily move and feed among different habitat types and allow upstream energy sources like suspended particulates, terrestrial plant matter, and riverine plankton to wash into the oxbow lakes (Junk *et al.*, 1989). Flooding would likely result in food webs being less distinct among habitats, making it more difficult to distinguish among fish from different oxbow lakes than during prolonged periods of lower river flows, which could potentially allow distinct food webs to develop in connected and disconnected oxbow lakes. The FA 18:1n7 accounted for the most variance in liver FA profiles between the connected and disconnected oxbows and was highest in the disconnected oxbow; 18:1n-7 can be a biomarker for bacteria (Alfaro *et al.*, 2006). Bacterial energy in aquatic systems is usually derived from the microbial processing of terrestrial C-3 plants (Roach, 2013), and is especially prevalent in rivers with high levels of dissolved organic matter that shades out aquatic primary producers (Walker, 1985; Wallace *et al.*, 1987). The Kaskaskia is a turbid river (Larimore *et al.*, 1973; personal observation), even within the oxbow lakes. Abundance of 18:1n-7 is consistent with greater contributions of microbes to energy processing (Roach, 2013).

Fatty acid profiles compared among river reaches

Changes in channel catfish FA profiles from upstream to downstream were observed in the Kaskaskia River, with fish from the furthestmost upstream and downstream sites being the most distinct, suggesting longitudinal differences in FA availability within the river channel. This finding is consistent with results of Dayhuff (2004), who observed an increase in the FAs 14:0, 16:1n-7, and 18:3n-3 with increasing distance downstream in both sauger and white bass in the Ohio River. Sub-populations of sauger and white bass in the lower and upper portions of the Ohio River could be distinguished based on their FA profiles (Dayhuff, 2004), and our results indicate that distinguishing channel catfish from different river reaches of the lower Kaskaskia River is also possible based on their FA profiles.

Changes in FA profiles of channel catfish from upstream to downstream within the lower Kaskaskia River are likely due to shifts in the taxonomic composition of primary producers and the influence of Carlyle Lake, a riverine impoundment located upstream of our study area. For muscle and liver tissues, 18:2n-6 accounted for the most variation in the FA profiles of channel catfish among river reaches. The FA 18:3n-3 was also a large contributor to profile variance in muscle and adipose fin tissue, and was markedly lower in the upper river. 18:3n-3 is an essential FA like 18:2n-6, with increasing concentrations of 18:3n-3 in the FA profile indicating increasing levels of autochthonous energy sources in the diet. (Henderson & Tocher, 1987; Alhgren *et al.*, 1994). This may suggest a lesser reliance of channel catfish on autochthonous production in the upper portion of the lower Kaskaskia River. However, the observation that both essential FAs 18:2n-6 (typically a biomarker of terrestrial energy sources) (Koussoroplis, 2008) and 18:3n-3 were both significantly lower in fish from the upper river may suggest a longitudinal difference in the taxonomic composition of primary producers within our study area. This shift in producer composition could stem from the presence of Carlyle Lake upstream of our sampling sites. Impoundments are known to decrease dissolved and particulate organic matter in their outflows, thereby disrupting nutrient spiraling and altering invertebrate community composition and the riverine food web (Ward & Stanford, 1983). Angradi (1994) and Hoeninghaus *et al.* (2007) studied the effects of impoundment on sediment laden rivers (Colorado; Parana and Paranapanema; respectively) and found a decrease in terrestrial C3 plant energy and an increase in algal and phytoplankton energy sources post-impoundment (Roach, 2013). Additionally, Carlyle Lake has a surface release dam, which can introduce lentic plankton to river food webs immediately downstream and could enhance filter-feeding stream invertebrates below the dam (Ward & Stanford, 1983). However, these lentic influences should rapidly dissipate downstream, returning the food web to a more riverine state, which may be causing the observed increase in 18:2n-6 and 18:3n-3 in channel catfish further downstream.

Another possible reason for the variation in FA profiles of channel catfish from upstream to downstream in the lower Kaskaskia River could be the increased floodplain connectivity maintained by the U.S. Army Corps of Engineers by dredging select oxbow connections from 2010 to 2011 between New Athens and Baldwin, Illinois (Dredging News Online, 2010). This oxbow lake dredging may also be influencing the observed increase in 18:3n-3 and 18:2n-6 moving downstream, which may suggest that the oxbow lakes influence the river food web by providing unique energy sources for riverine consumers such as channel catfish. Channel catfish are known to use floodplain habitat under elevated flows and forage on a wide variety of items, including those of terrestrial origin (Flotemersch, 1996). Flotemersch *et al.* (1997) found that channel catfish moved from disconnected river sections into river sections connected to the floodplain during high flow. This may suggest that the floodplain is more important for channel catfish foraging than are upstream energy contributions. Our study highlights floodplain habitat as a potentially important source of essential n-3 and n-6 FA to channel catfish in the lower Kaskaskia River and the associated importance of maintaining river-floodplain lake connectivity to support riverine consumers such as channel catfish.

Conclusions

Differences in FA profiles among channel catfish from oxbow lakes and different reaches of the lower Kaskaskia River suggest that FA profiles of fish tissues can potentially be used to identify recent habitat use of riverine fishes, analogous to use of FA profiles to distinguish fish use of benthic and pelagic prey in lakes (Czesny *et al.*, 2011; Lau *et al.*, 2012). Additional studies should assess potential inter-annual variability in habitat- and river reach-specific FA profiles of channel catfish observed in this study, differences in FA profiles among consumer taxa within habitats or river reaches, and whether differences in consumer FA profiles among river reaches (Dayhuff, 2004; this study) or between rivers and their floodplain lakes (Rude, 2012; this study) are common features of river-floodplain ecosystems.

This study also demonstrated that FA profiles of channel catfish differed among tissue types, although differences in FA profiles among river reaches and between river and oxbow lake sites were present for all tissue types and muscle and adipose fin FA profiles exhibited similar location-related trends. Thus, FA profiles of liver, muscle, or adipose fin tissue could potentially be used to assess spatially-explicit energy sources of channel catfish in the Kaskaskia River. Our results also suggest that sampling adipose fin tissue is a viable, less-invasive alternative to muscle tissue for analysis of FA profiles for fishes that possess adipose fins, though further research is needed to facilitate extrapolation of these results to fishes that exhibit different patterns of lipid allocation to the tissues.

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Table I. Sample sites used on the Kaskaskia River during the summer of 2011 listed by statistical grouping and number of fish captured.

Sample Site	1st Statistical Grouping	2nd Statistical Grouping	Number of Fish Captured
Carlyle	Upper River	<i>Not Included</i>	13
Fayetteville	Middle River	<i>Not Included</i>	16
New Athens Oxbow Lake (connected)	Oxbow	Connected Oxbow	12
Baldwin Oxbow Lake (disconnected)	Oxbow	Disconnected Oxbow	16
Baldwin Oxbow Lake (connected)	Oxbow	Connected Oxbow	15
Baldwin Sample 1	Middle River	Channelized River	15
Baldwin Sample 2	Middle River	Channelized River	10
Evansville	Lower River	Channelized River	15
		<i>total</i>	112

Table II. Mean fatty acid composition (% FAME) of channel catfish compared among channelized river, connected oxbow lake, and disconnected oxbow lake sites in the Kaskaskia River.

Fatty Acid (s)	Channelized River	Connected Oxbows	Disconnected Oxbow	Pooled Standard Error
<u>Muscle FA Profile</u>				
18:2n-6	11.7 <i>a</i>	6.9 <i>b</i>	8.0 <i>b</i>	1.1 - 1.4
18:1n-9	33.7 <i>b</i>	37.8 <i>a</i>	34.6 <i>b</i>	1.1 - 1.5
18:1n-7	4.5 <i>b</i>	4.9 <i>a</i>	4.9 <i>a</i>	0.1 - 0.2
<u>Liver FA Profile</u>				
18:1n-7	4.9 <i>c</i>	5.7 <i>b</i>	7.4 <i>a</i>	0.2 - 0.3
18:2n-6	5.7 <i>a</i>	5.1 <i>b</i>	7.7 <i>b</i>	0.8 - 1.0
22:6n-3	7.4 <i>b</i>	5.1 <i>b</i>	10.4 <i>a</i>	0.5 - 0.7
<u>Adipose Fin FA Profile</u>				
18:2n-6	12.8 <i>a</i>	7.9 <i>b</i>	9.1 <i>b</i>	1.1 - 1.4
18:0	4.0 <i>a</i>	4.1 <i>a</i>	3.5 <i>b</i>	0.1
20:5n-3	1.5 <i>b</i>	1.8 <i>ab</i>	1.8 <i>a</i>	0.1 - 0.2

Note: Only the top 3 FA that accounted for the most variation among sites in the FA profile are listed in this table. Means with the same letter are not statistically different.

Table III. Percent of individual catfish correctly assigned to the environment in which they were collected (channelized section of the Kaskaskia River, connected or disconnected oxbow lakes) using linear discriminant function analysis of muscle, liver, and adipose fin tissue fatty acid profiles.

Percent of observations correctly reassigned into site group			
Site Group	Muscle %	Liver %	Adipose Fin %
Channelized River	73	71	64
Connected Oxbows	48	63	62
Disconnected Oxbow	38	63	63
Mean	53	66	63

Table IV. Mean fatty acid composition (% FAME) of channel catfish muscle, liver, and adipose fin tissues compared among sites in Kaskaskia River ordered from upstream to downstream.

Fatty Acid (s)	Upper River	Middle River	Oxbow Lakes	Lower River	Pooled Standard Error
<u>Muscle FA Profile</u>					
18:2n-6	5.8 <i>c</i>	7.7 <i>b</i>	7.3 <i>b</i>	17.7 <i>a</i>	0.6 - 1.0
18:3n-3	2.0 <i>b</i>	4.2 <i>a</i>	5.0 <i>a</i>	4.9 <i>a</i>	0.4 - 0.7
18:1n-7	4.6 <i>a</i>	4.3 <i>b</i>	4.9 <i>a</i>	4.2 <i>b</i>	0.1 - 0.2
<u>Liver FA Profile</u>					
18:2n-6	4.9 <i>b</i>	5.7 <i>b</i>	5.1 <i>b</i>	12.0 <i>a</i>	0.5 - 0.9
18:1n-7	5.1 <i>b</i>	4.4 <i>c</i>	6.0 <i>a</i>	4.7 <i>bc</i>	0.2 - 0.3
16:0	18.7 <i>c</i>	22.8 <i>a</i>	20.9 <i>b</i>	19.8 <i>bc</i>	0.5 - 0.8
<u>Adipose Fin FA Profile</u>					
14:0	2.6 <i>b</i>	1.9 <i>b</i>	2.0 <i>b</i>	1.3 <i>c</i>	0.1 -0.2
18:3n-3	2.6 <i>b</i>	5.4 <i>a</i>	6.4 <i>a</i>	5.7 <i>a</i>	0.4 -0.7
16:1n-7	7.2 <i>a</i>	6.3 <i>b</i>	6.4 <i>b</i>	4.6 <i>c</i>	0.2 -0.4

Note: Only the top 3 FA that accounted for the most variation among sites in the FA profile are listed in this table. Means with the same letter are not statistically different.

Table V. Percent of individual catfish correctly assigned to the environment in which they were collected (upper, middle, or lower portion of the Kaskaskia River or its oxbow lakes) using linear discriminant function analysis of muscle, liver, and adipose fin tissue fatty acid profiles.

Percent of observations correctly reassigned into site group			
Site Group	Muscle %	Liver %	Adipose Fin %
Lower River	73	73	93
Middle River	60	65	51
Oxbow Lakes	81	79	81
Upper River	100	100	77
Mean	79	79	76

Figure Captions

Figure 1. Map showing location of the study area on the Kaskaskia River in Illinois, USA.

Figure 2. Ordination plots of canonical axes 1 and 2 for channel catfish muscle (a), liver (b), and adipose fin (c) fatty acid profiles compared among connected and disconnected oxbows, along with channelized river sites. For muscle tissue, the CANDISC 1 axis is most correlated with 18:2n-6, 18:1n-7, and 18:1n-9, and the CANDISC 2 axis was most correlated with 18:1n-9, 20:5n-3, and 22:6n-3. For liver tissue, the CANDISC 1 axis is most correlated with 18:1n-7, 18:2n-6, and 22:6n-3, and the CANDISC 2 axis is most correlated with 18:1n-9, 18:1n-6, and 22:6n-3. For adipose fin tissue, the CANDISC 1 axis is most correlated with 18:2n-6, 20:5n-3, and 14:0, and the CANDISC 2 axis is most correlated with 18:0, 20:4n-6, and 18:1n-9.

Figure 3. Ordination plots of canonical axes 1 and 2 for channel catfish muscle (a), liver (b), and adipose fin (c) fatty acid profiles compared among lower, middle, and upper Kaskaskia River sites, along with oxbow sites. The muscle CANDISC 1 axis is most correlated with 18:2n-6, 14:0, and 16:1n-7, and the CANDISC 2 axis is most correlated with 18:3n-3, 18:2n-6, and 16:0. The liver CANDISC 1 axis is most correlated with 18:2n-6, 20:5n-3, and 14:0, and the CANDISC 2 axis is most correlated with 22:5n-3, 16:0, and 18:0. The adipose fin CANDISC 1 axis is most correlated with 18:2n-6, 14:0, and 16:1n-7, and the CANDISC 2 axis is most correlated with 18:3n-3, 18:0, and 16:0.





