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Fatty Acid Profiles are Biomarkers of Fish Habitat Use in a River-Floodplain Ecosystem

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Fatty acid profiles are biomarkers of fish habitat use in a river-floodplain ecosystem

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Abstract

Fatty acid (FA) analyses of fish tissues offer the potential to gain new knowledge of habitat- or forage-specific energy inputs to fishes in river-floodplain ecosystems, although limited information exists regarding among-habitat differences in FA biomarkers. The goal of this study was to determine if differences in fish FA profiles among main channel and connected and disconnected floodplain lakes exist in large river-floodplain systems. Bluegill *Lepomis macrochirus* FA profiles were generated to assess differences among two reaches of the Illinois River, USA and its connected and disconnected floodplain lakes and determine whether FA signatures could be used to reclassify fish to their source habitat. Bluegill FA profiles differed among habitats and river reaches, including differences in levels of individual FAs (e.g., 18:2n-6, an indicator of allochthonous inputs, was higher among main channel fish) and FA groupings (e.g., n-3:n-6 FA ratio, an indicator of aquatic primary productivity, was higher among floodplain lake fish), which enabled >87.5% reclassification accuracy of fish to their source environment. We demonstrated that bluegill FA profiles differed among reaches and laterally among river channel and floodplain habitats, suggesting that FA profiles can be used to infer recent habitat use and habitat-specific foraging of fishes in large river-floodplain ecosystems.

Keywords: Fatty acids, Biomarkers, Large River, Floodplain lakes, Fish

Introduction

Knowledge of habitats and energy sources used by aquatic organisms is important for management and conservation of species in lentic and lotic environments (Schlosser, 1991; Hamilton et al., 1992; Guegan et al., 1998; Fausch et al., 2002). In large river ecosystems, many fishes rely on the connectivity of the main channel with floodplain lakes for spawning, refuge, and larval nursery habitat (Turner et al., 1994; King et al., 2003; King, 2004; Nunn et al., 2007; Schultz et al., 2007; Zeigler & Whitledge, 2010). Fishes may also use these habitats for energy acquisition; however, discerning relative use and importance of habitats within complex river-floodplain lake ecosystems can be difficult due to their energetic complexities (Vannote et al., 1980; Junk et al., 1989; Thorp & Delong, 1994; Thorp et al., 2006). Fatty acid (FA) analyses of fish tissues may offer the potential to gain new knowledge of habitat- or forage-specific energy inputs to fishes in these river-floodplain lake ecosystems.

Use of FAs as dietary biomarkers has become an increasingly common practice in aquatic food web studies (e.g., Napolitano et al., 1996; Rossi et al., 2006; Perga et al., 2009; Ravet et al., 2010). Aquatic primary producers are often distinguishable from one another based on the levels and ratios of FAs in their tissues; similarly, allochthonous energy sources tend to have different FA levels and ratios than aquatic primary producers (Pohl & Zurheide, 1979; Dalsgaard et al., 2003; Torres-Ruiz et al., 2007; Ravet et al., 2010). Fatty acid biomarkers can be used to identify energy sources of consumers such as fishes because fish lack the ability to transform and synthesize certain FAs, i.e., long-chain polyunsaturated fatty acids (LC-PUFAs); therefore, LC-PUFAs must be acquired from dietary sources (Sargent et al., 1987; Reuss & Poulsen, 2002; Tocher, 2003; Ravet et al., 2010). Thus, differences in consumption of forage items of fishes can be detected if their forage bases differ in FA composition. However, to detect

47 spatial differences, distinct FA levels and ratios of energy sources must exist among habitats,
48 either as a result of difference in the forage base and/or differences in the composition of the
49 forage among habitats. For example, Czesny et al. (2011) found that fish and invertebrate FA
50 profiles differed spatially among pelagic and benthic zones, which was attributed to FA
51 compositions of pelagic and benthic prey.
52 Many studies have used FA biomarkers to elucidate food webs in lentic systems (Perga et
53 al., 2009; Ravet et al., 2010; Czesny et al., 2011; Lau et al., 2012), but application of these
54 methods to large river ecosystems is limited. Dayhuff (2004) reported that the FA profiles of
55 white bass *Morone chrysops* (Rafinesque) differed among navigation pools of the Ohio River,
56 USA. Similarly, Young et al. (2015) found that channel catfish *Ictalurus punctatus* (Rafinesque)
57 FA profiles differed between channelized and unchannelized reaches of the Kaskaskia River,
58 Illinois, USA; some differences in FA composition of fish from the river and two connected
59 oxbow sloughs were also detected. While these studies indicate that consumer FA profiles can
60 differ longitudinally along rivers, whether fish FA profiles also differ laterally between river
61 channel and floodplain habitats in large rivers that have extensive floodplains has not been
62 assessed. In addition, whether floodplain lake habitats (including those that differ in
63 connectivity to the river channel) may impart distinct FA profiles to fishes based on differences
64 in producer and fish prey assemblages among floodplain habitats has also not been investigated.
65 Therefore, the goal of this study was determine whether differences in fish FA profiles among
66 main channel and connected and disconnected floodplain lakes occur in large river-floodplain
67 systems. Addressing this objective will provide insight regarding the applicability of FA
68 biomarkers for distinguishing fish use of river channel and floodplain habitats and habitat-
69 specific nutritional histories of consumers in large river-floodplain ecosystems.

Methods

Fish were collected from eight sites along the Illinois River, Illinois, USA during spring and summer 2009 and 2010. These sampling locations included two reaches of the Illinois River (near Grafton, Illinois, USA; 38°58′21″N, 90°33′01″W, and Havana, Illinois, USA; 40°27′53″N, 89°53′53″W), three connected floodplain lakes near Grafton (Swan Lake, Lower Stump Lake, and Upper Stump Lake), and three disconnected floodplain lakes near Havana (Powerton Lake, South Spring Lake, and Banner Marsh; Fig. 1). The Illinois River at these reaches characterized as a low gradient river (2 cm/km), moderate flow river (~639 m^3/s) with a broad floodplain (2.5-5 km wide) that was historically dominated by floodplain forests and backwater areas and floodplain lakes (Starrett, 1971; Koel & Sparks, 2002; US Geological Survey, 2013). These backwater areas and floodplain lakes near Havana have been disconnected via levee system, whereas many of these areas remain near Grafton due to a reduced levee system (Starrett, 1971; Koel & Sparks, 2002). Disconnected floodplain lakes near Havana, IL are primarily shallow (< 1.5 m, however deep areas > 3 m exist in all three lakes), low turbidity (clarity > maximum depth), and aquatic vegetation is present (Stafford et al., 2012). Connected floodplain lakes near Grafton are shallow (mean depth of < 1 m), windswept, highly turbid (clarity < maximum depth), and vegetation is mostly absent (Schultz et al., 2007). These connected floodplain lakes of the Illinois River included representatives with permanent and intermittent (during flooding) connections to the Illinois River and varied in the type of connection to the river (natural channel, ditch or water control structure; Starrett, 1971; Schultz et al., 2007; Zeigler & Whitledge, 2010).

Juvenile bluegill *Lepomis macrochirus* (Rafinesque) (n = 6-19 per site) ranging from 50-105 mm were collected at each site using three-phase alternating current (AC) electrofishing

93 (250 volts, and 7-10 amperes) at near-shore locations within each site. Fish were euthanized
94 with MS-222, placed on ice for transport to the laboratory, and stored frozen (-80º C) until
95 subsequent analyses. Bluegill were used as the study species because they are readily found
96 throughout the Illinois River-floodplain system (Zeigler & Whitledge, 2010), exhibit small home
97 ranges, and are not particularly mobile (Gunning & Schoop, 1963; Paukert et al., 2004). Thus,
98 bluegill FA profiles are likely representative of the sites in which fish were collected.
99 Additionally, juvenile bluegill were chosen because of dietary consistency and stomach contents
100 data indicated that individuals were consuming similar prey items (zooplankton and aquatic
101 insects) regardless of location (Mittelbach, 1984; Werner & Hall, 1988; Rude, 2012).
102 Fatty acid profiles were generated from crude lipid samples extracted from individually
103 freeze-dried, pulverized bluegill according to the methods described by Laporte et al. (2011).
104 Briefly, crude lipids were extracted according to the procedures described by Folch et al. (1957)
105 and processed to yield fatty acid methyl esters (FAME) according to the acid-catalyzed
106 transmethylation methods described by Christie (1982). The resultant FAME were separated
107 using a Shimadzu GC-17A gas chromatograph (Shimadzu Scientific Instruments, Kyoto, Japan).
108 Individual FAME were identified by reference to external standards (Supelco 37 Component
109 FAME Mix, PUFA-1, and PUFA-3; Supelco, Bellefonte, PA, USA).
110 Both univariate and multivariate analyses were used to test the null hypotheses that there
111 were no significant differences in the FA profiles of bluegill from the Illinois River and its
112 connected and disconnected floodplain lakes. One way analyses of variance (ANOVAs)
113 followed by Tukey's HSD tests for multiple comparisons were used to assess differences in
114 individual FA abundance in fish among the Illinois River at Grafton and its connected floodplain
115 lakes, along with the ratio of n-3:n-6 FAs. Individual FA levels that differed significantly among

site types were used in a multivariate analysis of variance (MANOVA) and a discriminant analysis (CANDISC procedure in SAS®) to characterize the multivariate FA profiles of fish from the different site types. A plot of the first two canonical variates was used to visually depict the differences in FA profiles of fish among site types. Pillai's trace statistic was used to assess significance of multivariate FA profiles of fish among the sites. Spearman rank correlations were used to assess the relationship between individual FAs and canonical axes 1 and 2 to determine which individual FAs had the greatest influence on the separation found in the discriminant analysis. In addition, linear discriminant function analysis with a leave-one-out jackknife procedure was used to determine the accuracy in which fish could be classified back to the environment in which they were captured based on their FA profiles. Statistical methods described above were repeated using data from the Illinois River at Havana and its disconnected floodplain lakes only as an independent dataset. Additionally, ANOVAs and a MANOVA along with CANDISC procedure in SAS was used to determine differences in FA profiles of fish among each river reach, and also visually depict differences in FA profiles of fish from the Illinois River (both reaches) and connected and disconnected floodplain lakes. All FA data used for statistical analyses were arcsine square root transformed to help meet the assumptions of normality, except for the n-3:n-6 FA ratio. *P* values were Bonferroni-corrected from $\alpha = 0.05$ by dividing by the number of FAs used in the one-way ANOVAs (26) to account for occurrence of *P* values < 0.05 due to chance alone. Thus *P* values ≤ 0.0019 were considered significant for all one-way ANOVAs. Pillai's Trace statistic was considered significant at $\alpha \leq 0.05$. Spearman rank correlations between canonical axes values and FA levels were also Bonferroni-corrected; thus, *P* values for the canonical axes values and FA level were considered significant at $\alpha = 0.0035$ for both datasets. A *P* value of ≤ 0.05 was considered significant for all statistical tests

(except where noted), and all statistical analyses were performed using SAS 9.2 (SAS Institute, Inc. Cary, NC).

Results

Levels of many individual saturated fatty acids (SFAs) in bluegill tissues were significantly different among the Illinois River and its disconnected floodplain lakes. Levels of even-chain SFAs (14:0, 16:0, and 18:0) were significantly different among sites (ANOVA, $F = 60.38$, df = 3, 27, $P < 0.0001$, ANOVA, $F = 21.28$, df = 3, 27, $P < 0.0001$, and ANOVA, $F = 22.10$, df = 3, 27, $P < 0.0001$, respectively), with SFA levels often lower within fish from the Illinois River compared to its disconnected floodplain lakes (Table 1). Levels of two mono-unsaturated fatty acids (MUFAs; 16:1n-7, and 18:1n-9) in bluegill tissues were significantly different between the Illinois River and its disconnected floodplain lakes (ANOVA, $F = 28.97$, df = 3, 27, $P < 0.0001$, and ANOVA, $F = 8.24$, df = 3, 27, $P = 0.0005$, respectively), with generally higher concentrations found in the Illinois River (Table 1). Levels of medium chain poly-unsaturated fatty acids (MC-PUFAs; 16:2n-4, 18:2n-6, 18:3n-3, and 18:4n-3) were significantly different between the Illinois River and disconnected floodplain lakes (ANOVA, $F = 22.04$, df = 3, 27, $P < 0.0001$, ANOVA, $F = 7.47$, df = 3, 27, $P = 0.0009$, ANOVA, $F = 10.46$, df = 3, 27, $P < 0.0001$, and ANOVA, $F = 37.39$, df = 3, 27, $P < 0.0001$, respectively). Higher levels of 16:2n-4 and 18:2n-6 were found in bluegill from the Illinois River compared to disconnected floodplain lakes and lower levels of 18:3n-3 and 18:4n-3 were found in the Illinois River compared to disconnected lakes (Table 1). Three long chain polyunsaturated fatty acids (LC-PUFAs; 20:4n-6, 20:5n-3, and 22:6n-3) exhibited levels that were significantly different among sites (ANOVA, $F = 68.26$, df = 3, 27, $P < 0.0001$, ANOVA, $F = 6.30$, df = 3, 27, $P = 0.0018$, and ANOVA, $F =$

15.29, df = 3, 27, $P < 0.0001$, respectively). Levels of LC-PUFAs in bluegill were generally lower within fish from the Illinois River compared to its disconnected floodplain lakes (Table 1). The ratio of n-3:n-6 FAs was significantly different among sites (ANOVA, $F = 11.87$, df = 3, 27, $P < 0.0001$), with significantly higher values observed in bluegill from disconnected floodplain lakes compared to the Illinois River (Table 1).

Multivariate analysis of bluegill FA profiles indicated that fish from the Illinois River and its disconnected floodplain lakes possessed significantly different FA profiles (MANOVA, Pillai's trace, $F = 12.48$, df = 36, 54, $P < 0.0001$). A plot of the first two canonical variates from the CANDISC procedure in SAS illustrated the distinct FA profiles of bluegill among the Illinois River and disconnected floodplain lakes (Fig. 2a). The first discriminant function (axis 1) from this model accounted for 69.9% of the total dispersion in the dataset. Many of the SFAs were positively correlated with axis 1, whereas many of the MC-PUFAs were both positively (18:3n-3 and 18:4n-3) and negatively (18:2n-6) correlated with axis 1 (Table 2). The second discriminant function (axis 2) from this model accounted for 22.3% of the total dispersion in the dataset. Many of the SFAs and LC-PUFAs were negatively correlated with axis 2, and MUFAs were positively correlated with axis 2 (Table 2). Linear discriminant function analysis with a leave-one-out jackknife procedure indicated that individual bluegill could be classified back to their site type of capture with 87.5% overall accuracy based on their FA profiles (Table 3).

Bluegill from the Illinois River and three of its connected floodplain lakes had significant differences in levels of four SFAs (14:0, 15:0, 17:0, and 18:0) (ANOVA, $F = 16.90$, df = 3, 47, $P < 0.0001$, ANOVA, $F = 46.18$, df = 3, 47, $P < 0.0001$, ANOVA, $F = 28.08$, df = 3, 47, $P = 0.0011$ and ANOVA, $F = 11.67$, df = 3, 47, $P < 0.0001$, respectively), with a general trend of lower levels of both odd- and even-chain SFAs in bluegill from the Illinois River in comparison

to its connected floodplain lakes (Table 4). Levels of two MUFAs (16:1n-7 and 18:1n-7) in bluegill tissues were significantly different between fish from the Illinois River and the connected floodplain lakes (ANOVA, $F = 14.15$, df = 3, 47, $P < 0.0001$ and ANOVA, $F = 38.97$, df = 3, 47, $P < 0.0001$, respectively), with a general trend of lower levels of these MUFAs in bluegill from the Illinois River in comparison to the connected floodplain lakes (Table 4). Levels of three MC-PUFAs (16:2n-4, 18:3n-3 and 18:4n-3) were significantly different between fish from the Illinois River and its connected floodplain lakes (ANOVA, $F = 53.23$, df = 3, 47, $P < 0.0001$, ANOVA, $F = 52.90$, df = 3, 47, $P < 0.0001$, and ANOVA, $F = 78.61$, df = 3, 47, $P < 0.0001$, respectively). Levels of these FAs in tissues of bluegill from the Illinois River and its connected floodplain lakes were highly variable among sites. However, bluegill from the Illinois River generally had lower levels of these FAs than most of fish from the connected lakes (Table 4). Levels of three LC-PUFAs (20:4n-6, 20:5n-3, and 22:6n-3) also differed significantly between fish from the Illinois River and its connected floodplain lakes (ANOVA, $F = 15.45$, df = 3, 47, $P < 0.0001$, ANOVA, $F = 6.28$, df = 3, 47, $P = 0.0011$, and ANOVA, $F = 11.89$, df = 3, 47, $P < 0.0001$, respectively). Levels of these LC-PUFAs in bluegill from the Illinois River sites were highly variable; however, bluegill from one of the connected floodplain lakes (Swan Lake) exhibited significantly lower levels of these FAs than fish from the other sites (Table 4). The ratio of n-3:n-6 FAs was significantly different between bluegill from the Illinois River and its connected floodplain lakes (ANOVA, $F = 7.22$, df = 3, 47, $P = 0.0004$), with significantly higher values observed in bluegill from some of the connected floodplain lakes in comparison to the Illinois River (Table 4).

Fatty acid profiles of bluegill from the Illinois River and its connected floodplain lakes were significantly different (MANOVA, Pillai's Trace Statistic: $F = 16.33$, df = 36, 114, $P <$

208 0.0001). A plot of the first two canonical variates from the CANDISC procedure in SAS
209 illustrated the distinct FA profiles of bluegill between sites (Fig. 2b). The first discriminant
210 function (axis 1) from this model accounted for 64.19% of the total dispersion in the dataset. All
211 SFAs were negatively correlated with axis 1 except for 14:0. Many of the 16 and 18 carbon
212 MUFAs and PUFAs were positively correlated with axis 1, whereas 20:4n-6 and 22:6n-3 were
213 negatively correlated with axis 1 (Table 5). The second discriminant function (axis 2) from this
214 model accounted for 18.37% of the total dispersion in the dataset. Only 17:0 was positively
215 correlated with axis 2 and 16:1n-7 was negatively correlated with axis 2 (Table 5). Linear
216 discriminant function analysis with a leave-one-out jackknife procedure indicated that individual
217 fish could be classified back to their environment of capture with 88% accuracy based on their
218 FA profiles (Table 6).
219 Bluegill from both reaches of the Illinois River had significant differences in levels of
220 four SFAs (15:0, 16:0, 17:0, and 18:0) (ANOVA, $F = 5.87$, df = 1, 16, $P = 0.0277$, ANOVA, $F =$
221 83.40, df = 1, 16, $P < 0.0001$, ANOVA, $F = 19.14$, df = 1, 16, $P = 0.0005$ and ANOVA, $F =$
222 7.37, df = 1, 16, $P = 0.0153$, respectively), with a higher levels of SFAs in bluegill from the
223 Illinois River at Grafton (Table 7). Levels of three MC-PUFAs (18:2n-6, 18:3n-3 and 18:4n-3)
224 were significantly different between reaches of the Illinois River (ANOVA, $F =8.57$, df = 1, 16,
225 $P = 0.0099$, ANOVA, $F = 15.32$, df = 1, 16, $P = 0.0012$, and ANOVA, $F = 18.75$, df = 1, 16, $P =$
226 0.0005, respectively). Bluegill from the Illinois River at Grafton had higher levels on n-3 MC-
227 PUFAs and lower levels of n-6 MC-PUFAS in comparison to the Illinois River at Havana (Table
228 7). The ratio of n-3:n-6 FAs in bluegill tissues was significantly different between reaches of the
229 Illinois River (ANOVA, $F = 10.66$, df = 1, 16, $P = 0.0048$), with significantly higher values
230 observed in bluegill from the Illinois River at Grafton (Table 7).

231 Fatty acid profiles of bluegill from both reaches of the Illinois River and connected and
232 disconnected floodplain lakes were significantly different (MANOVA, Pillai's Trace Statistic: F
233 = 8.08, df = 36, 207, $P < 0.0001$). A plot of the first two canonical variates from the CANDISC
234 procedure in SAS illustrated the distinct FA profiles of bluegill among the sites (Fig. 2c). The
235 first discriminant function (axis 1) from this model accounted for 73.1% of the total dispersion in
236 the dataset, and the second discriminant function (axis 2) from this model accounted for 26.3%
237 of the total dispersion in the dataset. Linear discriminant function analysis with a leave-one-out
238 jackknife procedure indicated that individual fish could be classified back to their environment
239 of capture with 91.4% accuracy based on their FA profiles, and 88.8% accuracy with fish from
240 only the Illinois River reaches.
241
242 **Discussion**
243 Results indicated that bluegill from the Illinois River and its connected and disconnected
244 floodplain lakes could be distinguished with a high degree of accuracy based on their FA
245 profiles. Spatial differences in FA profiles of bluegill among environments can be attributed to
246 differences in FA availability within these environments, which is likely due to differences in
247 basal energy sources among environments (Vannote et al., 1980; Twombly & Lewis, Jr, 1987;
248 Junk et al., 1989; Thorp & Delong, 1994; Garcia de Emiliani, 1997; Thomaz et al., 2007;
249 Lehman et al., 2008), as FA compositions of both basal energy sources and invertebrate prey can
250 influence the FA profiles within fish tissues (Sargent et al., 1987; Reuss & Poulsen, 2002;
251 Tocher et al., 2003; Ravet et al., 2010; Burns et al., 2011). These differences in FA profiles are
252 not likely due to major diet differences, as bluegill stomach contents were similar across sites
253 and were comprised of primarily of cladocerans, and aquatic insects (chironomidae, corixidae,

ephemeroptera, odonota; *see*, Rude, 2012), and bluegill tend to feed similarly in different habitats (Mittelbach, 1984; Werner & Hall, 1988). Our results are similar to other studies investigating spatial differences in FA profiles of fish in both lentic (Czesny et al., 2011; Lau et al., 2012), and longitudinal differences in lotic systems (Dayhuff, 2004; Young et al., 2015). Although studies of spatial differences in FA profiles of organisms in lotic environments are limited, our classification success rates for individual fish to environment of capture in this study were greater than one published study using FA profiles of channel catfish to distinguish location of capture in the Kaskaskia River and its connected oxbow lakes (Young et al., 2015).

Differences in bluegill FA profiles from the Illinois River and its disconnected floodplain lakes were detected, which are resultant from differential energy sources and FA availability. Many FAs contributed to differences among these environments, however, certain FA and ratios were key contributors to these differences. The n-3:n-6 ratio was significantly lower in fish from the river compared to disconnected lake fish. This ratio is built on the fact that aquatic primary producers synthesize large amounts of n-3 FAs (e.g., 18:3n-3, 20:5n-3, and 22:6n-3; Ahlgren, 2009), and terrestrial primary producers contain elevated levels of n-6 FAs (e.g., 18:2n-6 and 20:4n-6; Napolitano, 1999; Ahlgren, 2009). Thus, a low n-3:n-6 ratio is a putative marker of a diet more based on terrestrial inputs (Torres-Ruiz et al., 2007; Ahlgren, 2009), suggesting that fish from the river received an increased degree of allochthonous production in comparison to its disconnected floodplain lakes. Further supporting increased allochthonous energy sources to fish in the river was significantly higher 18:2n-6 levels in river fish, as this FA is associated with terrestrially derived diet (Maazouzi et al., 2007; Koussoroplis et al., 2008; Brett et al., 2009; Perga et al., 2009; Young et al., 2015). Similarly, Young et al. (2015) observed elevated levels of 18:2n-6 in channel catfish from the Kaskaskia River main channel compared to fish from its

277 oxbow lakes. We also detected increased levels of MUFAs 16:1n-7 and 18:1n-9 in fish from the
278 river, which have been shown to be related to increased microbial and detritus energetic
279 contributions (Gonzalzez-Baro & Pollero, 1988; Wakeham & Canuel, 1990; Scholz & Boon,
280 1993; Boon et al., 1996; Torres-Ruiz et al., 2007), which are major constituents of allochthonous
281 energy pathways (*see*, Cummins, 1974; Vannote et al., 1980; Roach, 2013). Similarly, the
282 riverine ecosystem synthesis suggests a link between microbial and fish production (Thorp et al.,
283 2006). In contrast to fish from the river, bluegill from disconnected lakes exhibited increased n-
284 3:n-6 ratio, suggestive of more aquatic-origin energy sources, but also the LC-PUFA 22:6n-3
285 was significantly higher in fish from disconnected lakes compared to the river. High levels of
286 22:6n-3 are often associated with autochthonous energy pathways via aquatic primary production
287 (Perga et al., 2009; Ravet et al., 2010), and is an important component for overall fish health,
288 reproduction, growth, and many physiological processes (Brett & Muller-Navarra, 1997;
289 Ahlgren et al., 2009). Young et al. (2015) also observed elevated 22:6n-3 levels in channel
290 catfish from oxbow lakes compared to main channel fish. This study and Young et al. (2015)
291 suggest these floodplain lake environments may be a potential source of important FAs (e.g.,
292 22:6n-3) for fish in large river-floodplain systems, however, the lack of connectivity between the
293 Illinois River at Havana and its floodplain lakes limits potential transfer of energy and potentially
294 important FAs.

295 Increased river-floodplain connectivity allows organisms to actively (or passively) move
296 and feed among habitats, and allow exchange of energy sources, which may create a more
297 homogeneous system in terms of energetic contributions to fish and other organisms (Junk et al.,
298 1989; Tockner et al., 2000; Amoros & Bornette, 2002). Despite the potential for increased
299 homogeneity in energy sources, bluegill FA levels and ratios differed among connected

environments of the Illinois River and floodplain lake system near Grafton. Similar to the river and its disconnected floodplain lakes, bluegill from the river had lower n-3:n-6 ratios compared to fish from connected floodplain lakes, indicating an increased influence of terrestrial dietary inputs (Torres-Ruiz et al., 2007; Ahlgren, 2009). However, other FAs indicative of a more terrestrial-origin diet (e.g., 18:2n-6, 18:1n-9, and 16:1n-7; Gonzalzez-Baro & Pollero, 1988; Wakeham & Canuel, 1990; Scholz & Boon, 1993; Boon et al., 1996; Maazouzi et al., 2007; Torres-Ruiz et al., 2007; Koussoroplis et al., 2008; Brett et al., 2009; Perga et al., 2009) were not substantially different between the river and its connected floodplain lakes, and FAs indicative of increased autochthonous energy sources (e.g., 22:6n-3; Perga et al., 2009; Ravet et al., 2010) were not substantially higher in fish from floodplain lakes compared to fish from the main channel. Much of the multivariate differences in bluegill FA levels and ratios between the main channel and its connected floodplain lakes occurred within floodplain lake habitats. The FA biomarkers commonly associated with primary producers (n-3 FAs such as 18:3n-3, 20:5n-3, and 22:6n-3; Perga et al., 2009; Ravet et al., 2010) differed between floodplain lakes, and can be attributed to site-specific differences in primary producer assemblages. Lateral habitats of large river-floodplain lake systems differ in depth, fluvial geomorphology, and connectivity resulting in different energy production dynamics among sites (Thorp et al., 2006). These physical differences among habitats result in site-specific succession of primary producer communities (Garcia de Emiliani, 1993; Huszar & Reynolds, 1997; Miranda, 2005), leading to different FA availability in each lake, resulting in different FA levels and ratios in fish tissues among habitats (Zenebe et al., 1998; Dayhuff, 2004; Czesny et al., 2011; Young et al., 2015).

We observed longitudinal differences in FA profiles of bluegill across both Illinois River reaches. This finding is consistent with the results of Young et al. (2015) and Dayhuff (2004) as

they documented distinct FA profiles of fish within channelized and unchannelized reaches of the Kaskaskia River, and among pools of the Ohio River, respectively. These differences can be attributed to changes in taxonomic composition of primary producers and prey items among different river reaches (Dayhuff, 2004; Young et al., 2015). More specifically in this study, bluegill from the Illinois River at Havana had a lower n-3:n-6 FA ratio in comparison to the Illinois River at Grafton, which is indicative of increased allochthonous energy production available to bluegill at this site. These observed differences in bluegill FAs can be attributed to differences in river-floodplain connectivity at each reach, as productivity in lateral habitats influence productivity in main channel habitats, resulting in longitudinal differences in food web structure among reaches (Thorp et al., 2006). Our results suggest that floodplain energy inputs are important for bluegill in river reaches with extensive connectivity to floodplain lakes, further highlighting the potential importance of maintaining river-floodplain connectivity to support production of riverine consumers such as bluegill.

This study demonstrates that the FA profiles of fish differed longitudinally among reaches, and laterally among floodplain habitats in a large river-floodplain system. Consistent with the riverine ecosystem synthesis view of rivers as a set of linked hydrogeomorphic patches that can result in both longitudinal and lateral differences in food web structure and function (Thorp et al., 2006). Our data suggest FA profiles of fish tissues can potentially be used to identify recent habitat use of fishes in large river-floodplain systems, similar to use of FA profiles to distinguish energy sources of fish in lentic systems (Czesny et al., 2011). Furthermore, these methods may potentially be used to assess spatially explicit energy sources of fish (or other organisms) in large river-floodplain systems. However, further research is needed to assess inter-annual variability in habitat or river reach FA profiles of fishes, along with

differences in primary producer and consumer taxa within these distinct habitats in river-floodplain systems to determine whether these differences persist among river reaches (Dayhuff, 2004; Young et al., 2015), and river floodplain lakes. Furthermore, research is needed to facilitate efforts to quantify energy subsidies and lipid allocation to fish in large river-floodplain systems, particularly in areas with high connectivity among habitats, and of fishes that utilize both main channel and floodplain lake environments (Polis et al., 1997).

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549 Table 1. Fatty acid levels (percentages relative to total fatty acids, mean ± SE) and ratios of
550 bluegill collected from the Illinois River (at Havana, IL) and its disconnected floodplain lakes.
551 Means that are marked with different letters are significantly different (ANOVA followed by
552 Tukey's HSD test, $P < 0.05$).

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	Illinois River and Disconnected Floodplain Lakes			
Fatty Acid	Illinois River	Banner Marsh	Powerton Lake	S. Spring Lake
n	10	10	6	5
SFA	24.4 ± 0.3	28.5 ± 0.5	30.5 ± 0.5	28.5 ± 0.7
14:0	2.7 ± 0.1*b*	3.4 ± 0.2*b*	7.2 ± 0.3*a*	2.9 ± 0.3*b*
15:0	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.9 ± 0.1
16:0	15.9 ± 0.2*b*	18.7 ± 0.2*a*	18.8 ± 0.4*a*	19.0 ± 0.8*a*
17:0	0.5 ± 0.1	0.6 ± 0.1	0.7 ± 0.1	0.7 ± 0.1
18:0	4.6 ± 0.2*a*	5.2 ± 0.2*a*	3.2 ± 0.1*b*	5.1 ± 0.3*a*
MUFA	35.9 ± 1.7	23.0 ± 0.7	34.3 ± 1.0	24.9 ± 1.6
16:1n-7	12.6 ± 0.7*a*	7.9 ± 0.3*b*	13.8 ± 0.5*a*	7.7 ± 0.9*b*
18:1n-7	6.1 ± 0.2	4.8 ± 0.3	4.9 ± 0.1	5.5 ± 0.4
18:1n-9	17.2 ± 1.9*a*	10.4 ± 0.4*c*	15.6 ± 0.8*a*	11.7 ± 1.1*b*
MC-PUFA	15.3 ± 0.5	15.4 ± 0.6	15.5 ± 0.3	13.1 ± 1.1
16:2n-4	1.2 ± 0.1*a*	0.5 ± 0.1*b*	1.0 ± 0.1*a*	0.5 ± 0.1*b*
18:2n-6	9.9 ± 0.5*a*	8.2 ± 0.3*ab*	7.1 ± 0.1*b*	9.2 ± 0.6*a*
18:3n-3	3.5 ± 0.3*b*	5.0 ± 0.4*a*	5.3 ± 0.2*a*	2.9 ± 0.4*b*
18:4n-3	0.6 ± 0.1*b*	1.8 ± 0.2*a*	2.1 ± 0.1*a*	0.6 ± 0.1*b*
LC-PUFA	19.9 ± 1.6	28.7 ± 1.0	15.2 ± 1.1	29.5 ± 2.1
20:4n-6	3.8 ± 0.3*c*	6.3 ± 0.2*b*	2.0 ± 0.2*d*	8.8 ± 0.8*a*
20:4n-3	0.4 ± 0.1	0.9 ± 0.1	0.8 ± 0.1	0.5 ± 0.1
20:5n-3	4.8 ± 0.4*ab*	6.0 ± 0.2*a*	4.4 ± 0.4*b*	4.0 ± 0.3*b*
22:5n-3	3.6 ± 0.3	4.5 ± 0.3	2.9 ± 0.2	4.7 ± 0.5
22:6n-3	7.4 ± 0.7*b*	11.0 ± 0.7*a*	5.1 ± 0.4*b*	11.4 ± 0.8*a*
n-3:n-6	1.5 ± 0.1*b*	2.0 ± 0.1*a*	2.3 ± 0.1*a*	1.3 ± 0.1*b*

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Table 2. Results of Spearman rank correlations of axis 1 and 2 scores vs. individual fatty acid values for linear discriminant function analysis of bluegill from the Illinois River at Havana, IL and its disconnected floodplain lakes. Numbers under axes are correlation coefficients scores for individual fatty acids and bolded *P* values indicate significance after Bonferroni correction ($\alpha = 0.0033$).

Fatty Acid	Axis 1	*P* value	Axis 2	*P* value
14:0	0.748	< **0.0001**	0.084	0.6537
16:0	0.716	< **0.0001**	-0.603	**0.0003**
16:1n-7	-0.069	0.7124	0.821	< **0.0001**
16:2n-4	-0.280	0.1273	0.833	< **0.0001**
18:0	-0.317	0.0819	-0.524	**0.0025**
18:1n-9	-0.206	0.2671	0.711	< **0.0001**
18:2n-6	-0.724	< **0.0001**	-0.052	0.7811
18:3n-3	0.636	**0.0001**	0.058	0.7564
18:4n-3	0.794	< **0.0001**	-0.049	0.7919
20:4n-6	-0.164	0.3777	-0.876	< **0.0001**
20:5n-3	0.085	0.6491	-0.109	0.5584
22:6n-3	-0.098	0.5985	-0.706	< **0.0001**

Table 3. Results of linear discriminant function analysis of bluegill from the Illinois River at Havana and its disconnected floodplain lakes showing reclassification accuracy (determined by jackknife procedure) for individual fish to environment of collection based on bluegill FA profiles.

Source Location	Assigned Location				
	IL River (Havana)	Banner Marsh	Powerton Lake	S. Spring Lake	% Correct
IL River (Havana)	10	0	0	0	100
Banner Marsh	0	9	0	1	90
Powerton Lake	0	0	6	0	100
S. Spring Lake	0	2	0	3	60

593 Table 4. Fatty acid levels (percentages relative to total fatty acids, mean ± SE) of bluegill
594 collected from the Illinois River and connected floodplain lakes. Means that are marked with
595 different letters are significantly different (ANOVA followed by Tukey's HSD test, $P < 0.05$).

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		Illinois River and Connected Floodplain Lakes		
Fatty Acid	Illinois River	L. Stump Lake	U. Stump Lake	Swan Lake
n	8	19	18	6
SFA	29.8 ± 0.2	31.2 ± 0.2	34.2 ± 0.7	33.1 ± 1.2
14:0	3.1 ± 0.2*bc*	3.8 ± 0.1*b*	2.9 ± 0.2*c*	5.1 ± 0.4*a*
15:0	0.8 ± 0.1*b*	0.8 ± 0.1*b*	1.7 ± 0.1*a*	0.7 ± 0.1*b*
16:0	19.6 ± 0.4	19.8 ± 0.2	21.0 ± 0.5	21.0 ± 0.7
17:0	0.9 ± 0.1*bc*	1.0 ± 0.1*b*	1.5 ± 0.1*a*	0.7 ± 0.1*c*
18:0	5.5 ± 0.3*b*	5.8 ± 0.1*b*	7.1 ± 0.3*a*	5.6 ± 0.2*b*
MUFA	30.2 ± 1.3	28.3 ± 0.4	29.5 ± 0.8	35.8 ± 1.8
16:1n-7	10.6 ± 0.8*b*	9.7 ± 0.2*b*	10.8 ± 0.5*b*	15.8 ± 1.4*a*
18:1n-7	6.0 ± 0.2*c*	6.6 ± 0.1*b*	5.5 ± 0.1*c*	7.8 ± 0.3*a*
18:1n-9	13.6 ± 0.8	12 ± 0.3	13.2 ± 0.4	12.2 ± 0.3
MC-PUFA	15.5 ± 0.6	17.5 ± 0.2	11.8 ± 0.5	13.9 ± 0.8
16:2n-4	0.9 ± 0.1*c*	1.1 ± 0.1*b*	0.8 ± 0.1*c*	2.3 ± 0.2*a*
18:2n-6	7.6 ± 0.6	7.1 ± 0.1	7.0 ± 0.3	5.8 ± 0.6
18:3n-3	5.5 ± 0.4*b*	6.9 ± 0.1*a*	3.4 ± 0.2*c*	4.2 ± 0.4*c*
18:4n-3	1.4 ± 0.2*b*	2.4 ± 0.1*a*	0.6 ± 0.1*c*	1.6 ± 0.2*b*
LC-PUFA	21.1 ± 1.0	19.7 ± 0.3	21.4 ± 1.3	12.9 ± 2.0
20:4n-6	4 ± 0.3*a*	3.1 ± 0.1*a*	3.7 ± 0.2*a*	1.7 ± 0.4*b*
20:4n-3	0.6 ± 0.1	0.8 ± 0.1	0.3 ± 0.1	0.6 ± 0.1
20:5n-3	4.6 ± 0.2*a*	4.7 ± 0.2*a*	4.2 ± 0.3*a*	2.8 ± 0.7*b*
22:5n-3	4.2 ± 0.2	4.1 ± 0.1	3.5 ± 0.2	3.2 ± 0.4
22:6n-3	7.7 ± 0.6*ab*	6.9 ± 0.2*b*	9.7 ± 0.7*a*	4.5 ± 0.8*c*
n-3:n-6	2.1 ± 0.1*b*	2.6 ± 0.1*a*	2 ± 0.1*b*	2.3 ± 0.2*ab*

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Table 5. Results of Spearman rank correlations of axis 1 and 2 scores vs. individual fatty acid values for linear discriminant function analysis of bluegill from the Illinois River and its connected floodplain lakes. Numbers under axes are correlation coefficients scores for individual fatty acids and bolded *P* values indicate significance after Bonferroni correction ($\alpha = 0.0035$).

Fatty Acid	Axis 1	*P* value	Axis 2	*P* value
14:0	0.679	< **0.0001**	0.099	0.4912
15:0	-0.823	< **0.0001**	0.185	0.1928
16:1n-7	0.003	0.9859	-0.491	**0.0003**
16:2n-4	0.803	< **0.0001**	0.031	0.8321
17:0	-0.693	< **0.0001**	0.402	**0.0035**
18:0	-0.536	< **0.0001**	0.252	0.0734
18:1n-7	0.865	< **0.0001**	0.092	0.5321
18:3n-3	0.654	< **0.0001**	0.366	0.0083
18:4n-3	0.814	< **0.0001**	0.381	0.0059
20:4n-6	-0.548	< **0.0001**	-0.089	0.5339
20:5n-3	0.013	0.9247	0.222	0.1173
22:6n-3	-0.729	< **0.0001**	0.052	0.7178

Table 6. Results of linear discriminant function analysis of bluegill from the Illinois River at Grafton and its connected floodplain lakes showing reclassification accuracy (determined by jackknife procedure) for individual fish to environment of collection based on bluegill FA profiles.

Source Location	Assigned Location				
	IL River (Grafton)	L. Stump Lake	U. Stump Lake	Swan Lake	% Correct
IL River (Grafton)	6	1	1	0	75
L. Stump Lake	0	19	0	0	100
U. Stump Lake	1	0	17	0	94.4
Swan Lake	0	1	0	5	83.3

637 Table 7. Fatty acid levels (percentages relative to total fatty acids, mean ± SE) of bluegill
638 collected from the Illinois River at Grafton and the Illinois River at Havana. Means that are
639 marked with different letters are significantly different (ANOVA followed by Tukey's HSD test,
640 $P < 0.05$).

641

Fatty Acid	Illinois River Grafton	Illinois River Havana
n	8	10
SFA	29.8 ± 0.2	24.4 ± 0.3
14:0	3.1 ± 0.2	2.7 ± 0.1
15:0	0.8 ± 0*a*	0.6 ± 0.1*b*
16:0	19.6 ± 0.4*a*	15.9 ± 0.2*b*
17:0	0.9 ± 0.1*a*	0.5 ± 0*b*
18:0	5.5 ± 0.3*a*	4.6 ± 0.2*b*
MUFA	30.2 ± 1.3	35.9 ± 1.7
16:1n-7	10.6 ± 0.8	12.6 ± 0.7
18:1n-7	6 ± 0.2	6.1 ± 0.2
18:1n-9	13.6 ± 0.8	17.2 ± 1.9
MC-PUFA	15.5 ± 0.6	15.3 ± 0.5
16:2n-4	0.9 ± 0.1	1.2 ± 0.1
18:2n-6	7.6 ± 0.6*b*	9.9 ± 0.5*a*
18:3n-3	5.5 ± 0.4*a*	3.5 ± 0.3*b*
18:4n-3	1.4 ± 0.2*a*	0.6 ± 0.1*b*
LC-PUFA	21.1 ± 1	19.9 ± 1.6
20:4n-6	4 ± 0.3	3.8 ± 0.3
20:4n-3	0.6 ± 0.1	0.4 ± 0
20:5n-3	4.6 ± 0.2	4.8 ± 0.4
22:5n-3	4.2 ± 0.2	3.6 ± 0.3
22:6n-3	7.7 ± 0.6	7.4 ± 0.7
n-3: n-6	2.1 ± 0.1*a*	1.5 ± 0.1*b*

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644 Fig. 1. Map showing sites where bluegill were collected for this study. Filled triangles
645 represent disconnected floodplain lake sites, open triangles represent connected floodplain lake
646 sites, and diamonds represent sampling sites on the Illinois River at Havana (filled diamond) and
647 the Illinois River at Grafton (open diamond), respectively.

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650 Fig. 2. Plot of the first two canonical variates obtained through linear discriminant function
651 analysis including all the FAs that were significantly different among the Illinois River at
652 Havana and its disconnected floodplain lakes (a), the Illinois River at Grafton and its connected
653 floodplain lakes (b), and both reaches of the Illinois River and disconnected and connected
654 floodplain lakes (c).

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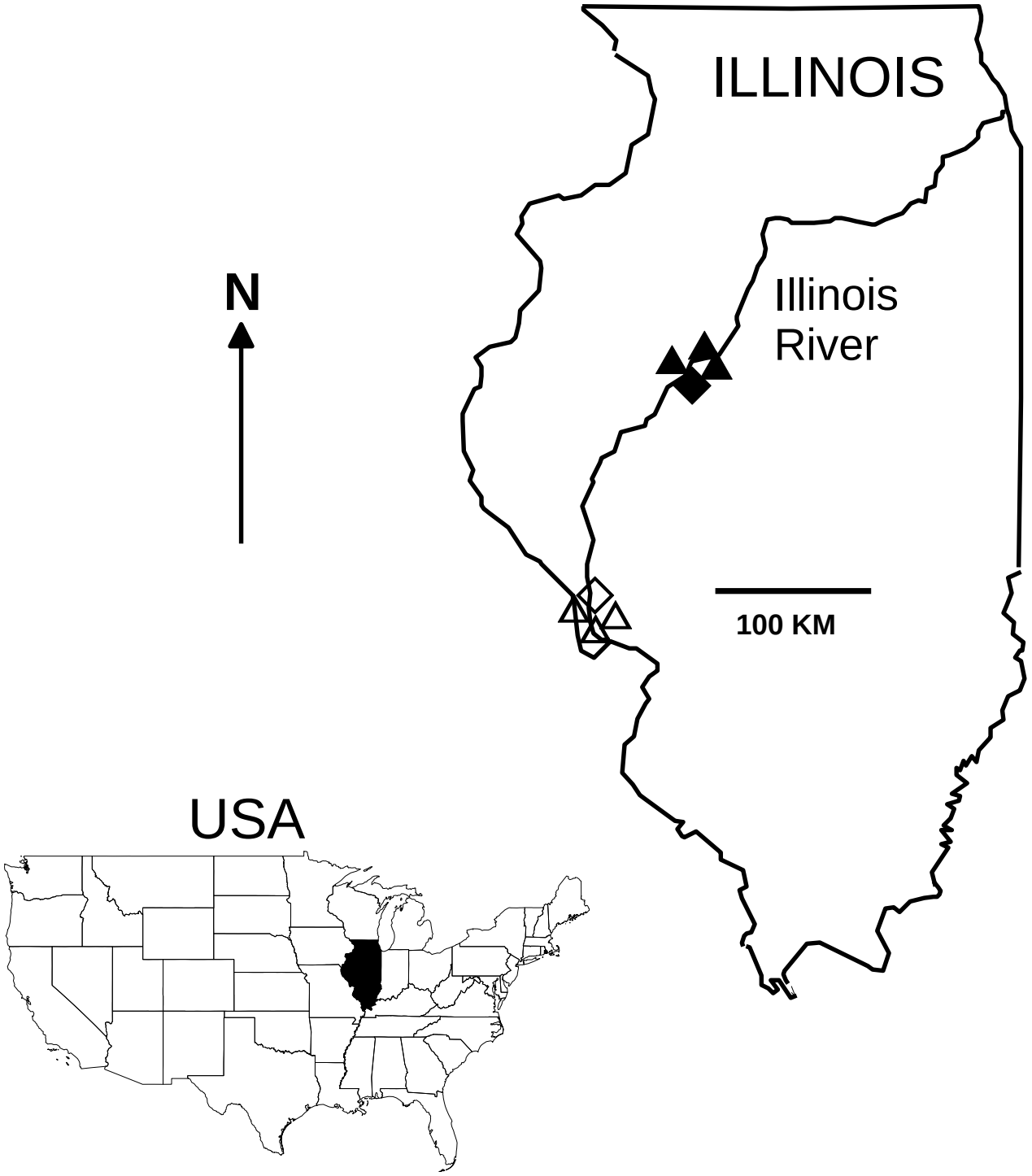

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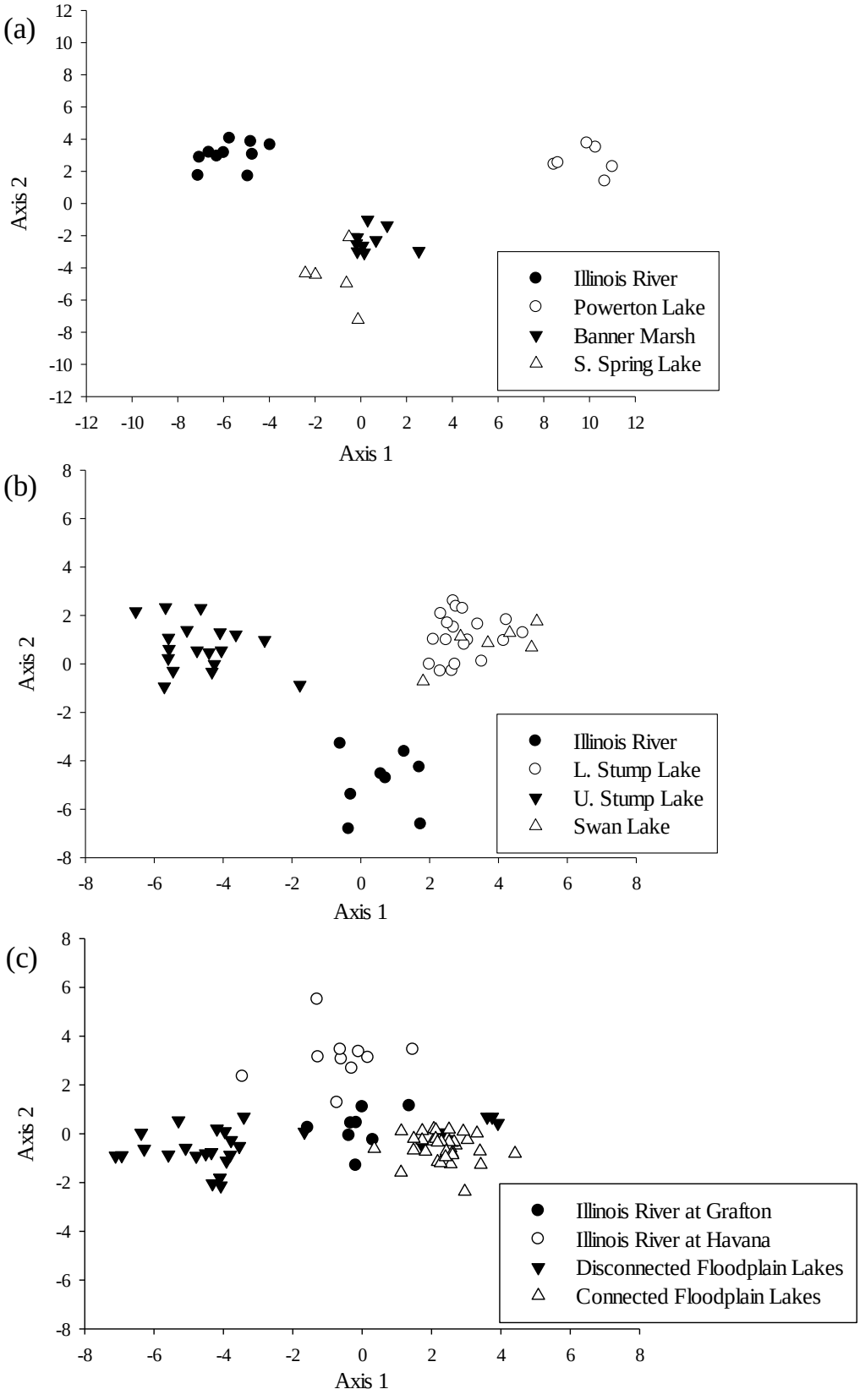
(a)
Axis 2
Axis 1
Illinois River
Powerton Lake
Banner Marsh
S. Spring Lake
(b)
Axis 2
Axis 1
Illinois River
L. Stump Lake
U. Stump Lake
Swan Lake
(c)
Axis 2
Axis 1
Illinois River at Grafton
Illinois River at Havana
Disconnected Floodplain Lakes
Connected Floodplain Lakes

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