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EVALUATION OF δD AND $\delta^{18}O$ AS NATURAL MARKERS OF INVERTEBRATE SOURCE ENVIRONMENT AND DISPERSAL IN THE MIDDLE MISSISSIPPI RIVER-FLOODPLAIN ECOSYSTEM

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ABSTRACT

Movement of invertebrates among large rivers, tributaries, and floodplain lakes or dispersal of adult aquatic insects from riverine or floodplain habitats may provide important subsidies to food webs in receiving habitats. Intensive sampling at habitat interfaces and artificial labeling are two approaches to assess freshwater invertebrate dispersal, but these are difficult to implement at a landscape scale. Natural chemical tracers have been used to track dispersal of fishes and marine invertebrates, but the potential applicability of stable isotope ratios as natural tracers of invertebrate dispersal in freshwater environments has not been assessed. We evaluated stable hydrogen and oxygen isotopes (δD and $\delta^{18}O$) as natural markers of source environment and dispersal of macroinvertebrates in the middle Mississippi River, tributaries, and floodplain wetlands. Water and invertebrates were collected from 12 sites during 2007-2008. Water δD and $\delta^{18}O$ differed among the river, its tributaries, and floodplain wetlands and were strongly correlated with invertebrate δD and $\delta^{18}O$. Variability in invertebrate $\delta^{18}O$ rendered it ineffective as an indicator of invertebrate source environment. Mean δD of Mississippi River invertebrates differed from δD of invertebrates from floodplain wetlands; δD distinguished invertebrates from these two environments with > 80% accuracy. Neither δD nor $\delta^{18}O$ of aquatic insects changed following emergence from their natal site. Preservation method (ethanol or freezing) did not affect invertebrate δD or $\delta^{18}O$. Invertebrate δD may be a useful natural tracer of natal environment and dispersal in the Mississippi River-floodplain ecosystem and other freshwater systems where spatial variation in water δD is present.

INTRODUCTION

Understanding how dispersal affects metapopulation dynamics of species is essential for conservation of critical habitats (Hanski and Simberloff, 1997), particularly in highly fragmented landscapes such as many of the world's temperate, large river-floodplain ecosystems. Aquatic macroinvertebrate community structure in floodplain habitats along large rivers is influenced by river-floodplain connectivity (Kohler et al., 1999; Leigh and Sheldon, 2009). Macroinvertebrates are also known to drift into streams from floodplain habitats and tributaries (Bonetto, 1975; Smock, 1994), but their overall influence on riverine invertebrate populations and food webs is unclear. Dispersal of aquatic macroinvertebrates from tributary or floodplain lakes or wetlands may potentially provide important subsidies (e.g., Polis et al., 1997) to food webs in large rivers or adjacent riparian habitats. Emerging aquatic insects are known to subsidize terrestrial food webs along small streams (Baxter et al., 2005), but the extent to which aquatic invertebrates subsidize terrestrial food webs within the floodplains of large rivers is uncertain. Knowledge of the extent to which aquatic invertebrates disperse among large river, tributary, and floodplain lake and wetland habitats (including restored habitats) would be valuable for maintenance and restoration of river-floodplain ecosystems.

Approaches for measuring population and food web subsidies provided by drifting immature aquatic invertebrates or dispersal of adult stages of aquatic insects include intensive sampling at habitat interfaces or application of artificial markers to trace invertebrate dispersal. However, both of these methods are difficult to implement at a landscape scale. Intensive sampling at habitat interfaces is labor intensive, limiting the number of locations that can be sampled, and does not enable identification of individuals that disperse well beyond the boundaries of their source environment. Dispersal of immature and emerging adult aquatic insects has also been investigated by artificially marking immature aquatic invertebrates with chemical labels such as rubidium (Payne and Dunley, 2002) or compounds enriched in the heavy stable isotope of nitrogen (^{15}N) (Hershey et al., 1993; Caudill, 2003). However, artificial labeling of aquatic invertebrates is more appropriate for relatively small streams and ponds and would not be practical for a landscape-scale investigation of aquatic invertebrate dispersal in large river-floodplain ecosystems.

Natural chemical markers such as stable isotope ratios that are specific to particular habitats or environments represent a potential means to quantify population and food web subsidies provided by dispersing aquatic invertebrates in large river-floodplain ecosystems. Recent studies have demonstrated that some large rivers (including the middle Mississippi River bordering Illinois and Missouri), possess stable isotopic and trace elemental "fingerprints" that are distinct from those of tributaries and floodplain lakes (Crook and Gillanders, 2006; Whitledge et al., 2007; Zeigler, 2009; Zeigler and Whitledge, 2010). These natural environmental signatures can be used to identify source environment (river, tributary, or floodplain lake) of fishes in these large rivers (Crook and Gillanders, 2006; Whitledge et al., 2007; Zeigler, 2009; Zeigler and Whitledge, 2010). Stable hydrogen isotopes have been used to identify natal origins of migratory monarch butterflies in North America (Hobson et al., 1999) and natural trace elemental signatures have been used to track dispersal of marine invertebrate larvae (DiBacco and Levin, 2000), but this approach has not yet been applied to freshwater invertebrates.

Our goal was to assess the potential of stable hydrogen and oxygen isotopes (δD and $\delta^{18}O$) as natural tracers of source environment and dispersal for aquatic invertebrates in the middle Mississippi River-floodplain ecosystem. Specific objectives were to: 1) determine whether water δD and $\delta^{18}O$ differed among the middle Mississippi River, tributaries, and floodplain wetlands, consistent with findings of Zeigler (2009), 2) characterize the relationship between water and invertebrate δD and $\delta^{18}O$ and determine whether differences in water δD and $\delta^{18}O$ among the middle Mississippi River, its tributaries, and floodplain wetlands were reflected in δD and $\delta^{18}O$ of aquatic invertebrates captured from these environments, 3) determine whether invertebrate δD and $\delta^{18}O$ signatures within environments differed among individuals representing different functional groups, 4) determine whether adult stages of aquatic insects maintain the isotopic fingerprint of their home water body after emergence, and 5) determine the accuracy with which invertebrates could be classified to their environment of capture (Mississippi River, tributary, or floodplain wetland) based on their δD and $\delta^{18}O$ signatures. We also investigated whether preservation method (ethanol vs. freezing) affected invertebrate stable hydrogen and oxygen isotopic signatures.

METHODS

Invertebrate and water sample collection and processing

Invertebrate and water samples were collected from the middle Mississippi River (between the confluence of the Mississippi and Missouri Rivers and the mouth of the Ohio River), three of its tributaries, three intermittent floodplain wetlands, and five permanent floodplain wetlands during spring, summer, and fall 2007 and 2008. Water samples for stable hydrogen and oxygen isotope analysis were collected and stored in scintillation vials containing minimal air space and sealed to curtail evaporative loss and fractionation (Kendall and Caldwell, 1998). Multiple habitats were sampled within each site to obtain a variety of benthic macroinvertebrate taxa for stable isotope analysis. Invertebrates were collected using a 0.3 m x 0.5 m, 500-μm mesh dip net or by picking individual invertebrates from coarse woody debris. Large invertebrates were picked directly from the dip net. Smaller invertebrates, sediment, and organic debris remaining in the net were transferred to a 19-L bucket and water from the site was added. The solution was agitated and poured through stacked 5-mm and 1-mm sieves to separate invertebrates from fine sediments. Invertebrates picked from the dip net, sieves, or organic debris were placed in collection jars containing water from the sampling site and separated by functional group and size to avoid consumption of or damage to smaller specimens by larger taxa. Collection jars were placed in a cool water bath for 24 h to allow for gut evacuation and to slow metabolism for easier handling and identification of invertebrates in the laboratory.

Adult insects were captured during or immediately following emergence to assess whether invertebrates retained the stable hydrogen and oxygen isotopic signatures of their natal environment after metamorphosis. Adult Hydropsychidae from the middle Mississippi River and Coenagrionidae from Mary's River in Randolph County, IL were collected using emergence traps. Libellulidae that were captured as nymphs from a floodplain wetland in Jackson County, IL and raised in the laboratory were also collected following emergence. All adult insects had an immature counterpart of the same taxa captured from the same water body for comparison of stable hydrogen and oxygen isotopic signatures of adult and immature insects.

Invertebrates except crayfish (Cambaridae) were identified to family using Merritt *et al.* (2008). Most invertebrates were then frozen prior to preparation for stable isotope analysis.

However, a subsample of immature invertebrates (n=20) from three collection sites was preserved in ethanol to evaluate the effect of preservation method (ethanol vs. freezing) on invertebrate stable hydrogen and oxygen isotopic signatures. All samples preserved in ethanol had a counterpart of the same taxa captured from the same water body that was frozen. Invertebrate samples were dried at 60°C for 24-48 h, ground with a mortar and pestle, weighed, and placed into silver capsules for stable isotope analysis. Most invertebrates were large enough to be analyzed individually. However, 2-3 individuals had to be combined for a few smaller taxa (e.g., some chironomids and smaller-bodied caddisfly larvae) to obtain sufficient material for stable isotope analysis.

Stable isotope analysis

Water and invertebrate samples were analyzed for stable hydrogen and oxygen isotopic compositions using a high-temperature conversion elemental analyzer (TC/EA) interfaced with a Thermo Finnigan Delta Plus XL® isotope ratio mass spectrometer at the Alaska Stable Isotope Facility, University of Alaska-Fairbanks (ASIF). Stable isotope ratios were expressed in standard δ notation, defined as the parts per thousand deviation between the isotope ratio of a sample and standard material (Vienna Standard Mean Ocean Water):

$$\delta D \text{ or } \delta^{18}O\ (‰) = [(R_{sample} / R_{standard}) - 1] \times 1000$$

where R represents D/H ($^{2}H/^{1}H$) or $^{18}O/^{16}O$. All invertebrate samples were held in the ASIF laboratory for at least two weeks prior to analysis to allow exchangeable hydrogen and oxygen to equilibrate with the lab environment (Wassenaar and Hobson, 2003).

Data analysis

One-way analyses of variance (ANOVAs) followed by Tukey's HSD tests were used to assess whether mean water δD and $\delta^{18}O$ differed among individual sampling sites and among site types (the Mississippi River, tributaries, and floodplain wetlands). Least squares linear regressions were used to relate mean invertebrate δD and $\delta^{18}O$ to corresponding mean water

isotopic values. Differences in mean δD and $\delta^{18}O$ values of invertebrates among individual collection sites and site types were assessed using one-way ANOVAs followed by Tukey's HSD tests for separation of site or site type means.

Differences in δD and $\delta^{18}O$ among immature invertebrates from different functional groups within sites were assessed using one-way ANOVAs followed by Tukey's HSD tests for separation of group means. Two-sample t-tests were conducted to evaluate potential effects of preservation method (ethanol vs. freezing) on invertebrate δD and $\delta^{18}O$. Two-sample t-tests were also used to test for differences in δD and $\delta^{18}O$ between adult and immature insects of the same family collected from the same locations.

Multivariate analysis of variance (MANOVA) with Pillai's trace statistic and a discriminant analysis (CANDISC procedure in SAS®) were used to assess differences in multivariate isotopic signatures (both δD and $\delta^{18}O$) of invertebrates among site types (Mississippi River, tributaries, and floodplain wetlands); a plot of the first two canonical variates was used to visually depict differences in invertebrate isotopic signatures among site types. Linear discriminant function analysis with a leave-one-out jackknife procedure was used to determine the accuracy with which invertebrates could be classified back to their environment of capture (the Mississippi River, tributaries, or wetlands) based on invertebrate δD and $\delta^{18}O$ values. P-values ≤ 0.05 were considered significant for all statistical tests.

RESULTS

Mean water δD and $\delta^{18}O$ signatures were significantly different among sampling sites ($F_{11, 26} = 13.68$, $p < 0.0001$ for δD; $F_{11, 26} = 7.46$, $p < 0.0001$ for $\delta^{18}O$) (Figure 1). Water δD and $\delta^{18}O$ signatures were highly correlated ($r^2 = 0.90$, $p < 0.0001$). Mean hydrogen and oxygen isotopic signatures also differed among water body types (the Mississippi River, its tributaries, and permanent and intermittent wetlands) ($F_{3, 34} = 91.82$, $p < 0.0001$ for δD; $F_{3, 34} = 20.59$, $p < 0.01$ for $\delta^{18}O$). The Mississippi River had the lowest water δD and $\delta^{18}O$ signatures and floodplain wetlands exhibited the highest water δD and $\delta^{18}O$ values; tributary streams to the Mississippi River had intermediate water δD and $\delta^{18}O$ signatures ($p < 0.05$) (Figure 1). Intermittent and

permanent wetlands generally exhibited more variation in water δD and $\delta^{18}O$ within sites compared to the Mississippi River or its tributaries. High rainfall and flooding during 2008 resulted in some temporal variation in water δD and $\delta^{18}O$ within individual sites, particularly some of the intermittent and permanent wetlands. However, temporal variation in water δD and $\delta^{18}O$ within sites was low in comparison to differences in water hydrogen and oxygen isotopic signatures among site types.

The mean δD signature of invertebrates was highly correlated with the δD signature of the environment of capture ($r^2 = 0.69$, $p < 0.0001$) (Figure 2A). Mean δD signature of invertebrates was significantly different among site types ($F_{3, 199} = 79.18$, $p < 0.0001$), generally reflecting the trend observed for water δD (Figure 2A). Mean δD of invertebrates collected from the Mississippi River was significantly lower than that of invertebrates collected from permanent or intermittent floodplain wetlands ($p < 0.05$). Invertebrates from tributary sites also had lower mean δD values than invertebrates collected from floodplain wetlands ($p < 0.05$). However, mean δD values of invertebrates from the Mississippi River and its tributaries were not significantly different ($p > 0.05$). Invertebrate $\delta^{18}O$ and water $\delta^{18}O$ signature of the environment of capture were correlated ($r^2 = 0.05$, $p = 0.001$), but this relationship was much weaker than the correlation between water and invertebrate δD (Figure 2B). Invertebrate $\delta^{18}O$ was more variable among individual water bodies within site types compared to invertebrate δD. Mean invertebrate $\delta^{18}O$ differed among some individual collection sites ($F_{11, 191} = 3.60$, $p < 0.0001$), but did not differ among site types ($p > 0.05$).

Collection of invertebrates representing five functional groups of aquatic insects (shredders, collector-gatherers, scrapers, collector-filterers, and predators) and crayfish from a Mississippi River tributary (Clear Creek) enabled evaluation of differences in δD and $\delta^{18}O$ among invertebrates from different trophic groups at this site. Mean stable hydrogen and oxygen isotopic signatures of invertebrates representing different functional groups collected from Clear Creek were significantly different ($F_{5, 34} = 22.93$, $p < 0.0001$ for δD; $F_{5, 34} = 8.38$, $p < 0.0001$ for $\delta^{18}O$) (Figure 3). Shredders and collector-gatherers had significantly higher δD values than crayfish and all other functional groups ($p<0.05$). Crayfish had a higher mean $\delta^{18}O$ than all insect functional groups ($p<0.05$). More variation in $\delta^{18}O$ among invertebrates collected from Clear Creek was observed in comparison to variability in δD among invertebrates captured from

this site. Isotopic analysis of basal energy sources (stream-conditioned leaf litter and epilithic algae) showed about a 60 ‰ difference in δD between leaf litter and algae. The δD values of most invertebrates from Clear Creek more closely resembled the leaf litter signature than the algal signature. Epilithic algal δD values were depleted by approximately 150‰ compared to water from Clear Creek.

Preservation method (freezing vs. ethanol preservation) did not significantly affect invertebrate δD ($t=0.47$, $DF=38$, $p=0.64$) or $\delta^{18}O$ ($t=1.76$, $DF=38$, $p=0.10$). Mean δD and $\delta^{18}O$ signatures of adult insects captured soon after emergence were not significantly different from mean δD and $\delta^{18}O$ signatures of immature insects ($t=0.43$, $DF=38$, $p=0.97$ for δD; $t=-1.11$, $DF=38$, $p=0.28$ for $\delta^{18}O$).

Identification of the particular site in which an invertebrate was captured was not possible due to similarity of invertebrate δD and $\delta^{18}O$ signatures among different floodplain wetlands and tributaries, but classifying individuals to their site type of capture (Mississippi River, tributary, or floodplain wetland) was much more successful, as significant differences in invertebrate isotopic signatures were present among site types (Pillai's trace statistic; $F_{4, 400} = 30.91$, $p < 0.0001$) (Figure 4). Linear discriminant function analysis indicated that 86 percent of individuals captured in the Mississippi River were correctly classified as having come from the river, and 82 percent of invertebrates collected from floodplain wetlands were correctly classified back to that site type (Table I). The intermediate and variable stable isotopic signatures of invertebrates captured in Mississippi River tributaries proved problematic, and most invertebrates collected in tributaries were not correctly classified back to their environment of capture (Table I).

DISCUSSION

Results suggested that δD may be a useful tool for tracing the origins and movements of invertebrates among water bodies that differ substantially in δD, such as the middle Mississippi River and its floodplain wetlands. Invertebrate δD values reflected water δD of an invertebrate's environment of capture and reliably distinguished individuals collected from the Mississippi River and its floodplain wetlands. Similarly, Schimmelmann and DeNiro (1986) found a strong positive relationship between δD of terrestrial beetle chitin and δD of local rainfall. Stable oxygen isotopic composition of invertebrates collected in the Mississippi River, its tributaries,

and floodplain wetlands also reflected water $\delta^{18}O$, but to a much lesser extent than observed for δD.

For stable isotopic signatures to be consistently useful as tracers of food web subsidies or invertebrate dispersal, water δD and $\delta^{18}O$ should remain distinct among environments over time. Mean water δD for the middle Mississippi River during this study was within the range of δD values reported by Coplen and Kendall (2000) for the middle Mississippi River during November 1984-August 1987, suggesting that the middle Mississippi River's δD signature is relatively stable across years. Despite some temporal variation in δD and $\delta^{18}O$ within habitat types, differences in water δD and $\delta^{18}O$ among the middle Mississippi River, tributaries, and floodplain wetlands persisted over the course of this study. Temporal variation in water δD and $\delta^{18}O$ within environments was likely due to a combination of evaporative fractionation, particularly in wetlands (Hoefs, 2004), and relatively high rainfall and flooding during 2008. Greater evaporative fractionation of H and O isotopes in floodplain wetlands with relatively small volumes and longer water residence times also likely explains the more positive water δD and $\delta^{18}O$ values in floodplain wetlands compared to lotic environments (Hoefs, 2004). Differences in water δD between the middle Mississippi River and its floodplain wetlands are consistent with results of prior studies which have demonstrated that water δD and $\delta^{18}O$ can differ between large rivers and their floodplain lakes (Whitledge et al, 2007; Zeigler, 2009; Zeigler and Whitledge, 2010). The strong correlation between mean water δD and $\delta^{18}O$ signatures for our sampling sites was not surprising given that water δD and $\delta^{18}O$ are subjected to the same fractionation processes during the hydrologic cycle (Hoefs, 2004).

Although water δD differed among environments and invertebrate δD differed between wetlands and the Mississippi River, sources of variation in invertebrate δD other than water δD precluded effectively distinguishing invertebrates collected from tributary habitats from invertebrates captured in the other two habitat types. One possible explanation for the misidentification of capture location for two invertebrates collected in the Mississippi River is that these individuals may have drifted into the river from tributaries. Additional sources of variation in invertebrate δD within sites that may have contributed to our inability to reliably distinguish tributary invertebrates from their counterparts collected in the Mississippi River or floodplain wetlands include variation in contributions of allochthonous and autochthonous

energy sources to aquatic invertebrates, variable contributions of water and food to invertebrate hydrogen, and differences in δD among individuals with different proximate compositions.

Differences in invertebrate δD among functional groups in Clear Creek, particularly shredders and other functional groups, suggest that differences in the trophic basis of invertebrate production (allochthonous vs. autochthonous organic matter sources) likely contributed to variation in δD among invertebrates at Clear Creek and perhaps other sites. Epilithic algal and stream-conditioned leaf litter samples collected from Clear Creek exhibited distinct δD values, consistent with recent studies in streams and rivers in other regions of North America (Doucett et al., 2007; Jardine et al., 2009). Thus, δD may also be potentially useful as a tracer of terrestrial energy subsidies to stream food webs.

For application of δD as a natural marker of invertebrate dispersal, development of classification models for particular functional groups or taxa may improve classification accuracy when attempting to identify an invertebrate's home water body. There does not appear to be a trophic shift for δD (Solomon et al., 2009), so it is unlikely that trophic level directly contributed to variation in δD among invertebrates within sites. Variation in environmental water and food as hydrogen sources for invertebrates may have contributed to variation in invertebrate δD within sites. Prior studies have determined that 61-86% of hydrogen in aquatic invertebrates is derived from their diet, with 14-39% of hydrogen derived directly from environmental water (Solomon et al., 2009; Wang et al., 2009). Differences in proximate composition of invertebrates may also have contributed to variation in δD among individuals, as lipids have more negative δD values than proteins and carbohydrates (Sessions et al., 1999). Although the sources of variation in invertebrate δD described above may limit the applicability of δD as a natural marker of invertebrate source environment and dispersal when an individual moves between water bodies with similar δD signatures, results of this study indicate that δD can potentially serve as a natural tracer of aquatic invertebrate dispersal when δD signatures of source and receiving environments (e.g., the middle Mississippi River and its floodplain wetlands) are sufficiently distinct. In contrast to use of δD for quantifying contributions of different energy sources to a consumer, application of δD as a natural marker of source environment and dispersal does not require quantitative understanding of sources and isotopic fractionation of hydrogen in pathways that lead from water to consumers. Invertebrates from

different environments of interest must simply possess consistently different δD signatures and retain the source environmental signature for some period of time following dispersal.

Variation in invertebrate $\delta^{18}O$ within sites precluded its use as an effective natural marker of an invertebrate's source environment. There are several potential sources of variation in invertebrate $\delta^{18}O$ within environments, including those mentioned previously for δD. In contrast to hydrogen, aquatic invertebrates derive approximately 70% of the oxygen in their tissues directly from environmental water (Wang et al., 2009), so $\delta^{18}O$ of immature aquatic invertebrates may be more sensitive to fine-scale fluctuation in water $\delta^{18}O$ compared to δD (Myers, 2010). Variation in $\delta^{18}O$ among invertebrates collected from Clear Creek despite the similar $\delta^{18}O$ signatures of algae and leaf litter suggests that differences in the relative importance of aquatic and terrestrial energy sources among invertebrate consumers are not likely responsible for observed variation in invertebrate $\delta^{18}O$. The behavior of $\delta^{18}O$ in food webs is poorly understood, and further basic research is needed. Organically bound oxygen, particularly in carbonyl and carboxyl functional groups, is readily exchangeable with environmental water (Hoefs, 2004), and oxygen exchange likely represents a major source of variability in invertebrate $\delta^{18}O$ values. Thus, $\delta^{18}O$ appears to be much less effective than δD as a natural marker of aquatic invertebrate source environment and dispersal.

Despite some variation in invertebrate δD within sites, we were able to distinguish invertebrates from the middle Mississippi River and its floodplain wetlands with a high degree of accuracy. This is consistent with recent studies that have used stable isotopes to distinguish fishes from large rivers (including the Mississippi River) and their floodplain lakes (Whitledge et al., 2007; Zeigler, 2009; Zeigler and Whitledge, 2010). Our results suggest that δD may be a useful natural marker of invertebrate origin and dispersal in the middle Mississippi River-floodplain ecosystem and in other connected river and lake systems where spatial variation in water δD is present. Results of this study also indicate that preservation of invertebrate samples in ethanol or by freezing does not significantly affect invertebrate δD, enabling the convenience of using specimens freshly preserved in ethanol in applications of δD as a natural tracer. Other natural chemical markers such as strontium:calcium (Sr:Ca) and barium:calcium (Ba:Ca) ratios and strontium isotope ratios ($^{87}Sr/^{86}Sr$) have been successfully used to identify natal origin and track dispersal of fishes (Kennedy et al., 2002; Wells et al, 2003; Brazner et al, 2004) and marine invertebrates (DiBacco and Levin, 2000) and may also be effective as natural tracers of

freshwater invertebrate dispersal. Water Sr:Ca differs between the middle Mississippi River and its tributaries, enabling fishes from the Mississippi River, its tributaries, and floodplain lakes to be distinguished using a combination of otolith Sr:Ca and stable isotope ratios (Zeigler, 2009). Inclusion of additional natural chemical tracers such as Sr:Ca might improve classification accuracy of invertebrates collected in the Mississippi River-floodplain ecosystem to their water body of origin (river, tributary, or floodplain wetland) over use of δD alone.

There are several potential applications of environmental δD signatures as a natural tracer of aquatic invertebrate dispersal. Stable hydrogen isotopic signatures could be used to identify source environment for drifting immature invertebrates. Application of δD for this purpose would require knowledge of the time span over which the δD signature of the invertebrate's source environment persists in invertebrate tissues following movement into a new environment with a different δD signature. The degree to which an invertebrate would retain its source environmental signature over time will depend on the rate at which the δD signature of the source environment is replaced in the invertebrate's tissues by the new environmental signature through growth dilution and elemental turnover (Fry, 2006). Further research should assess persistence of environmental δD signatures in aquatic invertebrates that move among water bodies that differ in δD. Application of δD and other natural tracers of aquatic invertebrate dispersal may also facilitate assessment of the extent to which invertebrates that drift into the Mississippi River from floodplain lakes, wetlands, or tributaries subsidize riverine invertebrate populations and food webs.

Stable hydrogen isotopic analysis may also be useful for identifying natal environment of dispersing adult aquatic insects. Hobson et al. (1999) used δD of metabolically inert wing keratin to identify natal origin of migratory monarch butterflies in North America. Our results showed no change in δD or $\delta^{18}O$ signatures of aquatic insects following emergence, indicating that natal environmental signatures are retained in recently emerged adult aquatic insects and that δD will likely be applicable as a natural tracer of dispersal for aquatic as well as terrestrial insects. Many adult aquatic insects do not feed, but the natal environment δD signature may be replaced over time in aquatic insect taxa that feed as adults. Further research should assess the effect of feeding on changes in δD signatures of adult aquatic insects over time. In contrast to artificial labeling methods for tracking dispersal of adults of aquatic insect taxa (Payne and Dunley, 2002; Caudill, 2003), use of δD and other natural tracers of invertebrate dispersal is not

limited to relatively discrete locations in which application of an artificial label is practical. Thus, natural tracers such as δD will facilitate assessment of population and food web subsidies provided by adult stages of aquatic insects at a landscape scale (e.g., Polis et al., 1997).

We anticipate that δD may be a useful natural marker of origin and dispersal for both immature and adult stages of aquatic invertebrates not only in the middle Mississippi River-floodplain ecosystem, but also in other aquatic environments where spatial variation in water δD is present. Use of natural markers such as δD can further our understanding of the complex foodwebs and energy flow pathways of large river-floodplain ecosystems and thereby enhance management and restoration efforts.

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Table I. Results of linear discriminant function analysis showing classification accuracy (determined by jackknife procedure) for individual invertebrates to the environment in which they were collected (Mississippi River, tributary, or floodplain wetland) based on invertebrate $\delta^{18}O$ and δD. n=number of invertebrates collected from each environment.

		Assigned location			
Source location	n	MS River	Tributary	Wetland	% Correct
MS River	14	12	2	0	86
Tributary	68	29	22	17	32
Wetland	121	0	22	99	82

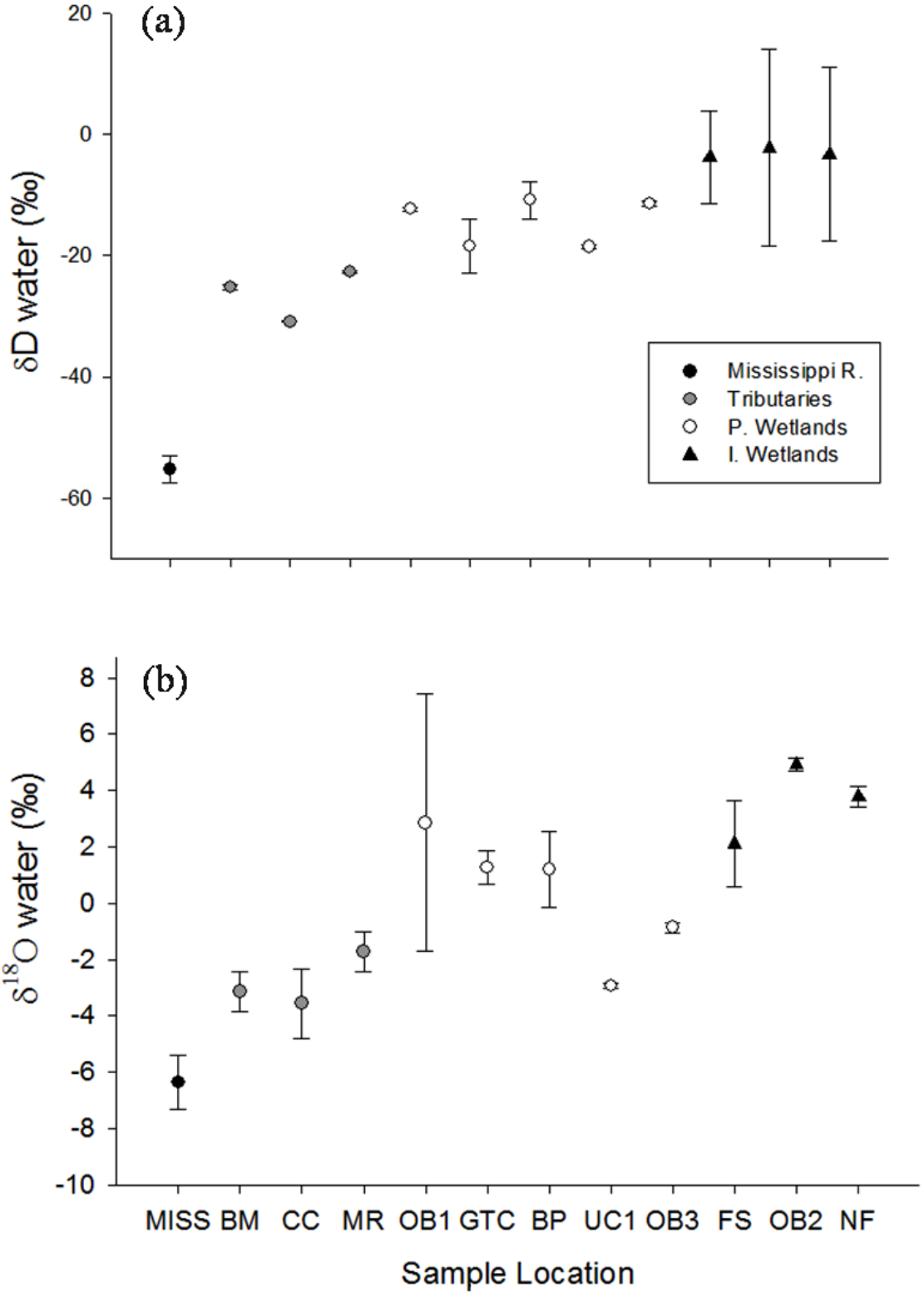
Figure Captions

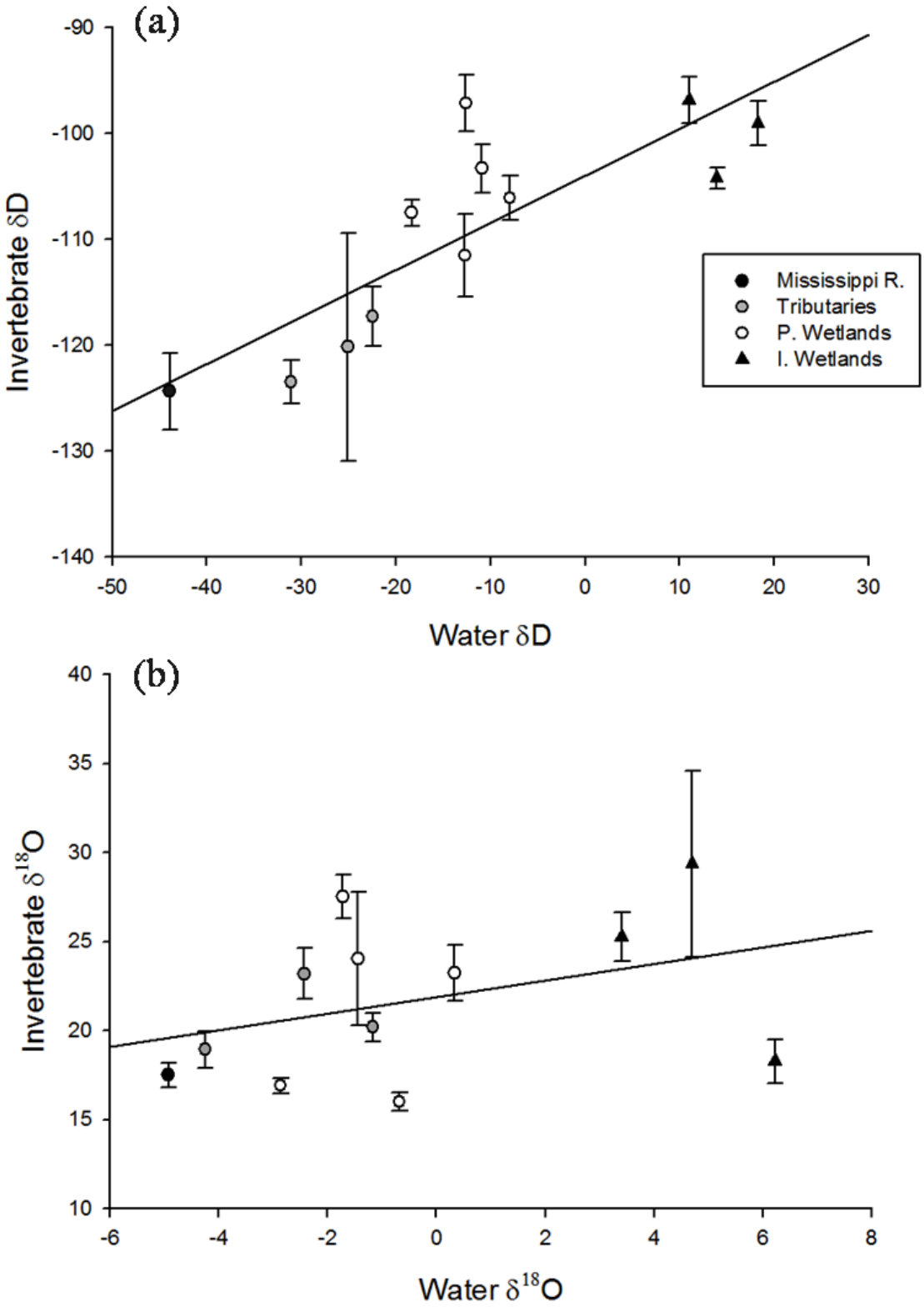
Figure 1. Mean water δD (a) and $\delta^{18}O$ (b) values (±SE) for the middle Mississippi River, tributaries, permanent wetlands (P. wetlands) and intermittent wetlands (I. wetlands) during 2007 and 2008. X-axis sampling location codes: MISS = Mississippi River, BM = Big Muddy River, CC = Clear Creek, MR = Mary's River, OB1, 2, 3 = Oakwood Bottoms wetlands, GTC = Grand Tower Chute, BP = Cape Bend State Fish and Wildlife Area wetland #1, UC1 = Union County Refuge wetland, FS = Cape Bend State Fish and Wildlife Area wetland #2, NF = Shawnee National Forest wetland.

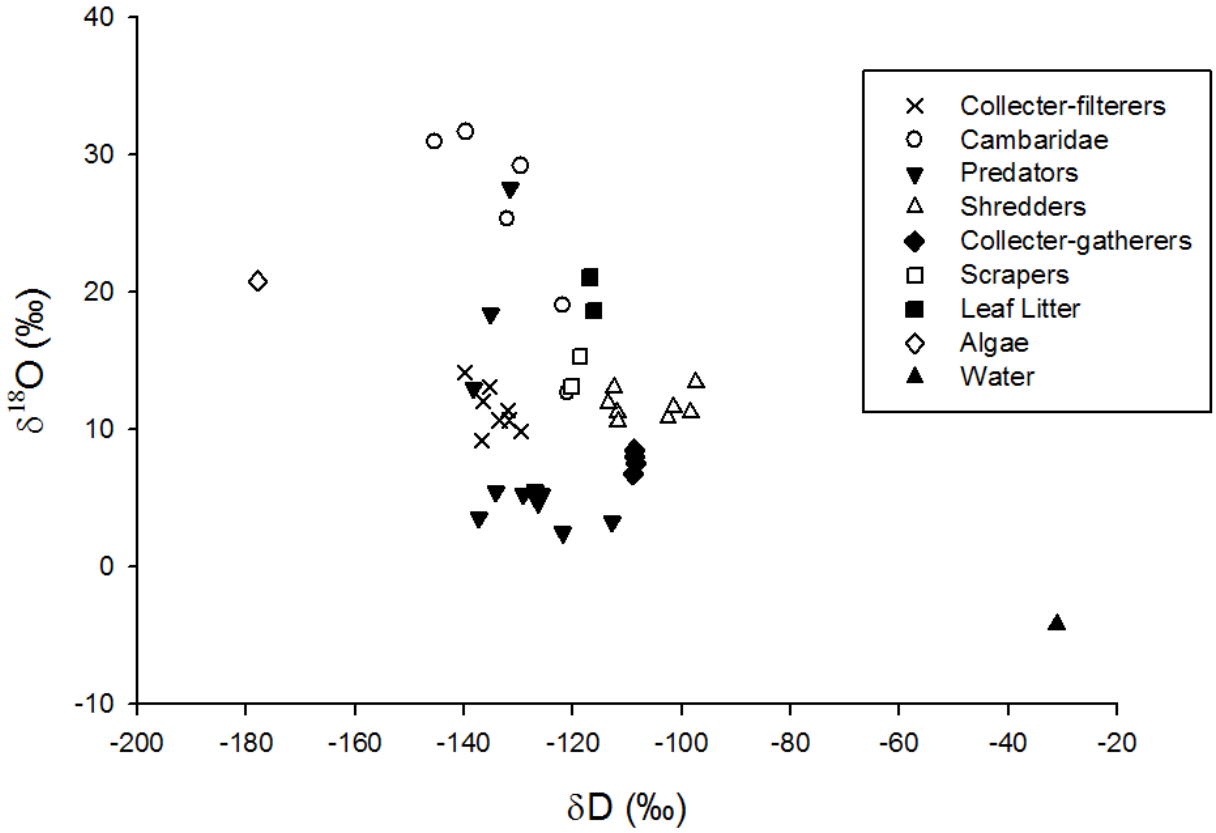
Figure 2. Relationships between (a) mean invertebrate δD (± SE) and water δD and (b) mean invertebrate $\delta^{18}O$ (±SE) and water $\delta^{18}O$ for invertebrates collected from the Mississippi River, tributaries, permanent wetlands ("P. Wetlands") and intermittent wetlands ("I. Wetlands"). Solid lines in each panel depict regression lines fit to the data.

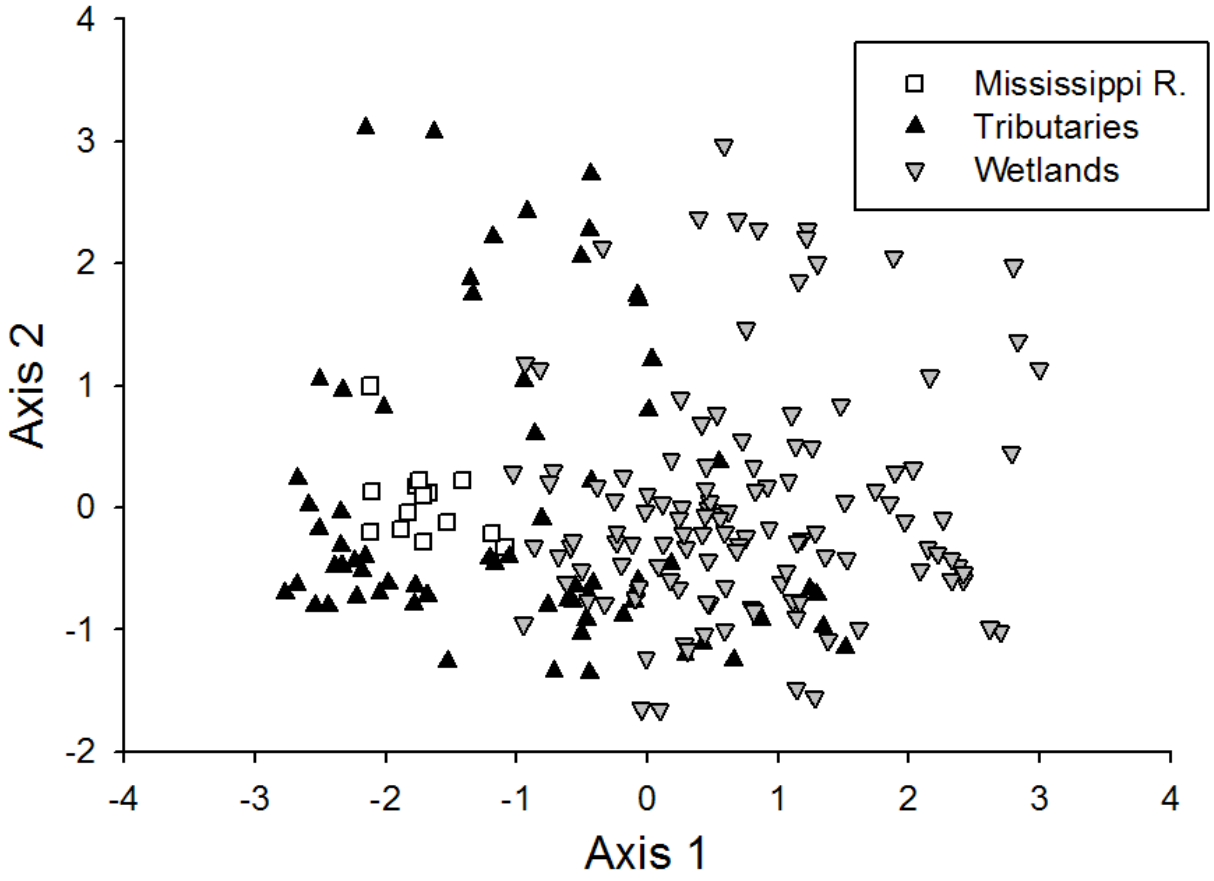
Figure 3. δD and $\delta^{18}O$ signatures of individual invertebrates from different functional groups and δD and $\delta^{18}O$ signatures of water, periphyton, and conditioned leaf litter from Clear Creek, a tributary of the middle Mississippi River in Union County, IL.

Figure 4. Stable isotopic signatures of invertebrates collected from the middle Mississippi River, tributaries, and floodplain wetlands based on the first two canonical variates obtained through linear discriminant function analysis (proc CANDISC in SAS®) on invertebrate δD and $\delta^{18}O$.


(a)
20
0
-20
-40
-60
δD water (‰)
Mississippi R.
Tributaries
P. Wetlands
I. Wetlands
(b)
8
6
4
2
0
-2
-4
-6
-8
-10
δ18O water (‰)
MISS BM CC MR OB1 GTC BP UC1 OB3 FS OB2 NF
Sample Location


(a)
-90
-100
-110
-120
-130
-140
Invertebrate δD
-50
-40
-30
-20
-10
0
10
20
30
Water δD
Mississippi R.
Tributaries
P. Wetlands
I. Wetlands
(b)
40
35
30
25
20
15
10
Invertebrate δ18O
-6
-4
-2
0
2
4
6
8
Water δ18O


40
30
20
10
0
-10
-200
-180
-160
-140
-120
-100
-80
-60
-40
-20
δ18O (‰)
δD (‰)
Collecter-filterers
Cambaridae
Predators
Shredders
Collecter-gatherers
Scrapers
Leaf Litter
Algae
Water


Mississippi R.
Tributaries
Wetlands
Axis 2
Axis 1
4
3
2
1
0
-1
-2
-4
-3
-2
-1
0
1
2
3
4