Freshwater Mollusk Biology and Conservation 21:28–35, 2018

REGULAR ARTICLE

STABLE ISOTOPE COMPARISON BETWEEN MANTLE AND FOOT TISSUES OF TWO FRESHWATER UNIONIDS: IMPLICATIONS FOR FOOD WEB STUDIES

Toben Lafrançois[1], Andrea Fritts[2]*, Brent Knights[2], and Byron Karns[3]

[1] *Northland College, Ashland, WI 54806 USA*
[2] *U.S. Geological Survey, Upper Midwest Environmental Sciences Center, La Crosse, WI 54603 USA*
[3] *National Park Service, St. Croix National Scenic Riverway, St. Croix Falls, WI 54024 USA*

ABSTRACT

Unionid mussels are a key taxon for stable isotope studies of aquatic food webs, often serving as the primary integrator of the pelagic baseline. Past isotope studies with mussels have commonly used either foot tissue or mantle tissue, but no study has yet to quantify the relation of both carbon and nitrogen isotopes between these two tissue sources. This makes it difficult to justify cross-study comparisons when different tissue compartments and different species were used as the basis of food web models. Therefore, we collected foot and mantle tissues from two common mussel species, *Amblema plicata* and *Fusconaia flava*, from lotic and lentic sites in the Upper Mississippi and St. Croix rivers (Minnesota/Wisconsin). Paired tissue samples from each individual were analyzed for stable isotopes of nitrogen and carbon. There were strong relations between tissue types for both isotopes between species ($r^2 > 0.93$). Paired *t*-tests indicated that there were statistically significant differences between the tissue sources in some instances, but the difference (0.04–0.21‰) was less than the analytical precision of the mass spectrometer (circa 0.2–0.3‰). We conclude that the isotopic values from these two tissue sources are biologically comparable and recommend that researchers use the tissue source and extraction technique that minimizes stress to the mussels. We also tested for significant differences between species within a site for either isotope or tissue type and found no statistically significant difference between species with the exception of carbon in foot tissue at two sites. The highly correlated isotopic response supports the interchangeable use of both tissue compartments and both species. These findings support comparisons between studies whether the results were based on either of these tissues or the two species studied. Comparability will also simplify sampling designs, save time, and save money for processing samples without diminishing the usefulness of the data.

KEY WORDS: Mississippi River, St. Croix River, tissue comparison, freshwater mussel, threeridge, Wabash pigtoe

INTRODUCTION

Stable isotope analysis of food webs can be a powerful tool for understanding the effects of large-scale ecological changes, such as the introduction of invasive species and eutrophication (Thorp et al. 1998; Herwig et al. 2007; Delong 2010). Unionid bivalves are cornerstones of aquatic food web condition in stable isotope studies because they integrate the food web base over time, have slow turnover rates, and are not as sensitive to seasonal variability as other measurements of the pelagic food web base (Cabana and Rasmussen 1996; Post 2002). A measure of $\delta^{15}N$ from long-lived primary consumers, such as native mussels, allows calibration of site-specific background conditions that allow calculation of cross-site food chain length and other comparisons. Carbon isotope values from freshwater mussels can be used to identify sources of organic matter and to track the flow of carbon into primary consumers (Rounick et al. 1982; Finlay 2001; Brett et al. 2017), and nitrogen isotopes can be used as an indicator of nutrients entering the watershed (Lefebvre et al. 2009; Atkinson et al. 2014). Stable carbon and nitrogen isotopes can be used to

*Corresponding Author: afritts@usgs.gov

calculate trophic niche space and to explore trophic relationships (Layman et al. 2007). In these and other ways, unionid mussels are a critical component for aquatic community stable isotope analysis to delineate food web dynamics. However, freshwater mussels as a group are sensitive to environmental degradation, with declining populations worldwide. Consequently, scientists increasingly employ nonlethal sampling methods for mussels, but this has resulted in the use of a number of different tissue-sampling protocols for isotope studies.

Stable isotope analysis requires sampling animal tissue or hemolymph and circa 1–2 mg (dry weight) of material is needed for mass spectrometer analysis. Given the conservation status of freshwater mussels, nonlethal and minimally invasive sampling methods for tissue collection are highly desirable. Three main tissue types are currently used for isotope studies of mussels: mantle tissue, foot tissue, and hemolymph from adductor muscles (McKinney et al. 1999; Gustafson et al. 2007; Weber et al. 2017). Mantle tissue biopsies have been shown to have no impact on long-term mussel survival (Berg et al. 1995). Foot biopsies and hemolymph extraction had no adverse effects on long-term survival of larger-bodied mussels (i.e., *Amblema plicata*, *Elliptio complanata*, *E. crassidens*; Naimo et al. 1998; Gustafson et al. 2005; Fritts et al. 2015), but the survival of a smaller-bodied species (*Villosa vibex*) was adversely affected by both methods (Fritts et al. 2015).

The widespread use of different tissues for isotopic studies of freshwater mussels has raised some concern that different tissues may produce different isotopic signatures and therefore limit the ability to compare isotope values across studies that have used different tissues (Weber et al. 2017). The published literature lacks a comprehensive comparison of variability between tissue sources for both carbon and nitrogen isotopes. Past studies that have conducted isotopic tissue comparisons between a combination of foot, mantle, and hemolymph have quantified the relation only among tissue sources for nitrogen isotopes (McKinney et al. 1999; Gustafson et al. 2007). However, carbon isotopes also provide valuable information about organic matter sources, including whether or not the sources are allochthonous or autochthonous, benthic or pelagic, and littoral or pelagic (Vander Zanden et al. 1999). Evaluating the relation of these isotopes among species can also provide insight into shared resource use, diet, and comparability of signatures among species at a site (Nichols and Garling 2000; Raikow and Hamilton 2001; Christian et al. 2004; Novais et al. 2016; Weber et al. 2017). Most food web models use both carbon and nitrogen isotopes in concert, which establishes the need for a more comprehensive assessment of tissue compartment comparisons for both isotopes.

The first objective of our study was to quantify the relation of carbon and nitrogen isotopes between foot and mantle tissue of two common freshwater mussel species (*Amblema plicata* and *Fusconaia flava*) in the Mississippi River Basin and to establish if these two tissue compartments produced comparable isotope results. The second objective of our study was to test for significant differences between these two species within a site for each isotope and tissue source. If the isotopic responses are statistically similar between the two species, this could allow for simplified sampling designs, particularly when a species is not able to be sampled consistently over the spatial gradient of interest.

METHODS

Study Location and Field Sampling

Freshwater mussels were collected from the Upper Mississippi and St. Croix rivers. Three main channel locations were sampled from Mississippi River Pool 2 (near St. Paul, MN), three sites from Lake St. Croix, three main channel sites between Lake St. Croix and the St. Croix Falls Dam, and one site above the St. Croix Falls Dam at Norway Point (Fig. 1). Each site was a stretch of relatively uniform habitat 1.5 km long. Scuba divers performed timed searches along each stretch in 2013 and 2014. *Amblema plicata* and *F. flava* were selectively sampled until a maximum of 25 individuals were collected for each species. We then selected five individuals per species per year from each site for tissue sampling and chose specimens as close as possible to the average size range of each species at each site. Sample sizes of five individuals have been reported to be sufficient and have low coefficients of variation for $\delta^{15}N$ (i.e., 5%; Gustafson et al. 2007). Given time constraints and field condition variability between years, some sites produced fewer than five individuals of a given species, and one site (i.e., M2MC2) contained specimens of only one of the target species. Two of the three sites within the Mississippi River had low mussel abundances, and therefore we chose to sample up to 10 individuals per species per year at the site with relatively high mussel abundances to increase our sample size in the Mississippi.

Tissue Sample Collection and Processing

Mantle samples were taken by gently prying open the mussel with a dull, flat-tipped sterile steel diving knife. Mantle tissue was held with a sterile duck billed forceps, and a 1 cm^2 section was snipped with sterile surgical scissors. Foot tissue was sampled in a similar way or with a biopsy needle (Bard Biopsy, Tempe, AZ). A subset of individuals had a duplicate sample collected from the same tissue source to evaluate variability within a tissue compartment. Tissue samples were immediately put on ice and transported to a –20°C freezer within 6 h. Frozen samples were transported to the Aquatic Resources Ecology Laboratory at Northland College (Ashland, Wisconsin) where they were dried at 60°C for 72 h and homogenized with mortar and pestle. Samples were then weighed, rolled into tin capsules, and shipped to Cornell University Stable Isotope Laboratory, Ithaca, New York. Stable isotope ratios of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) were determined with a Thermo Delta V isotope ratio mass spectrometer interfaced with a NC2500 elemental

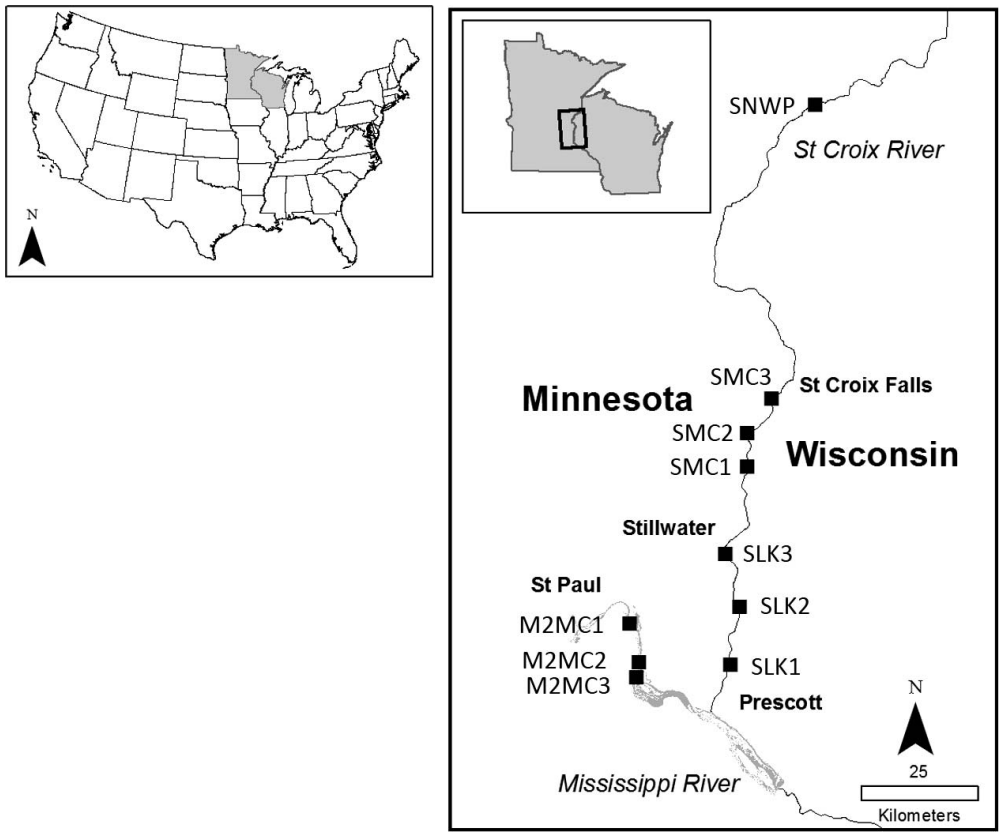

Figure 1. Sampling locations in the Upper Mississippi River basin, including seven sites in the St. Croix River and three sites in Pool 2 of the Mississippi River.

analyzer. In-house standards (mink animal material and methionine chemical standards) were run every 10 samples. In-house standards were routinely calibrated against international reference materials provided by the International Atomic Energy Association. Isotope data were expressed relative to Vienna PeeDee Belemnite for $\delta^{13}C$ and atmospheric air for $\delta^{15}N$. By convention, C and N isotope ratios are expressed as δ, the deviation from standards in parts per thousand (‰), according to the following equation:

$$\delta X(‰) = \left[(R_{\text{sample}}/R_{\text{standard}}) - 1\right] \times 1,000,$$

where X is ^{13}C or ^{15}N and R is the corresponding ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$. Instrument precision for calibrating isotope ratios was 0.10–0.33‰ for $\delta^{13}C$ and 0.12–0.19‰ for $\delta^{15}N$.

Data Analysis

We used simple linear regression models to express relations between mantle and foot tissue results, in addition to performing paired *t*-tests (Infostat, Córdoba, Argentina). All data were assessed for normality. Residuals were normally distributed for both isotopes for the regression analysis, and the isotopic difference between tissues was normally distributed for both isotopes and for both species. We analyzed each species separately and combined them into a single dataset. We also tested the relation between duplicate tissue samples collected from the same tissue source within an individual. To evaluate the isotopic relationship between *A. plicata* and *F. flava*, we tested for differences between the species among locations for each isotope-tissue pairing using two-sample *t*-tests with Satterthwaite's approximation (Satterthwaite 1946; Oulhote et al. 2011). We also used a Bonferroni correction to account for multiple comparisons among locations (i.e., $\alpha = 0.05/9 = 0.0056$). Data from both years were combined for this analysis.

RESULTS

We sampled both foot and mantle tissue from 73 *A. plicata* and 87 *F. flava* (Table 1), with 56 replicates of the same tissue source within an individual to evaluate variability within a tissue compartment. All data are publicly available through ScienceBase (doi.org/10.5066/P9G92506). Among sampling locations, $\delta^{15}N$ values ranged from 5.76 to 12.63‰, and $\delta^{13}C$ ranged from –29.26 to –33.96‰. Mantle and foot tissue sources were positively correlated with regard to $\delta^{15}N$ and $\delta^{13}C$ for both species (Figs. 2, 3). Linear regression of $\delta^{15}N$ and $\delta^{13}C$ data resulted in respective r^2 values of 0.96 and 0.93 for *A. plicata* and 0.97 and 0.93 for *F. flava* (Table 2). Combining the data from both species resulted in r^2 values of 0.96 and 0.93 for $\delta^{15}N$ and $\delta^{13}C$ (Table 2).

Paired *t*-tests indicated that there was not a statistically

Table 1. *Amblema plicata* and *Fusconaia flava* sample size (*N*) and size parameters (mean ± SD) of specimens collected from the Mississippi and St. Croix rivers for this study.

Species	*N*	Length (mm)	Height (mm)	Width (mm)
A. plicata	73	84.9 ± 12.1	63.6 ± 5.3	43.9 ± 8.4
F. flava	87	60.1 ± 12.3	52.3 ± 7.2	38.2 ± 10.6

significant difference in $\delta^{13}C$ between tissues in *A. plicata* but $\delta^{15}N$ did differ, while both isotopes were significantly different in *F. flava* (Table 3). Foot tissue was slightly enriched over mantle tissue for both species for $\delta^{15}N$ (0.10–0.21‰). Mantle tissue was slightly enriched in $\delta^{13}C$ relative to foot tissue for *F. flava*, and there was no statistically significant difference between tissue sources for $\delta^{13}C$ in *A. plicata*. When analyzing both species combined, $\delta^{15}N$ was enriched in foot relative to mantle, and there was no difference between the tissue sources for $\delta^{13}C$. However, the overall difference between the tissues was only 0.04–0.21‰ for both isotopes, which is less than the analytical precision of the mass spectrometer (i.e., circa 0.2–0.3‰). There was no statistically significant difference between duplicate samples taken from the same tissue source (Table 4), and the regression relationships were very strong for both species, individually and combined ($r^2 = 0.92$–0.99; Table 5).

The comparison of isotopes between species across sites indicated that $\delta^{15}N$ was not significantly different between the species across all sites for both foot tissue and mantle tissue (Table 6, Fig. 4A, C). Carbon isotopes from foot tissue were significantly different between the species at two locations, one in the Mississippi River and one in Lake St. Croix, but not at any of the remaining locations, and $\delta^{13}C$ from mantle tissue was not significantly different between the species at any location (Table 6, Fig. 4B, D). Only one species (*F. flava*) was

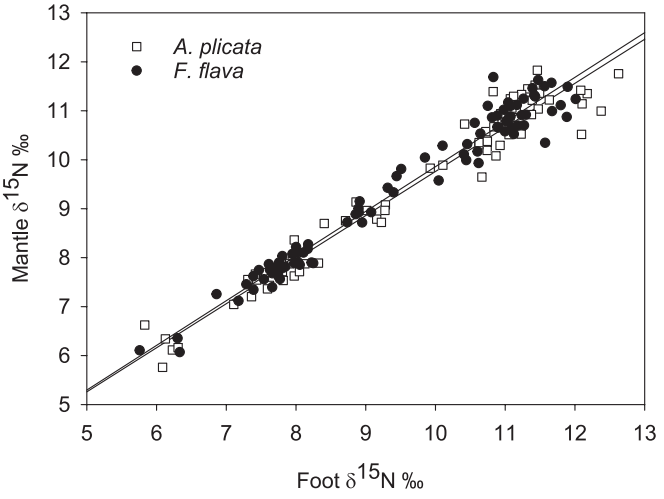

Figure 2. Simple linear regression of foot and mantle $\delta^{15}N$ per individual, with r^2 values of 0.955 for *Amblema plicata* and 0.967 for *Fusconaia flava*.

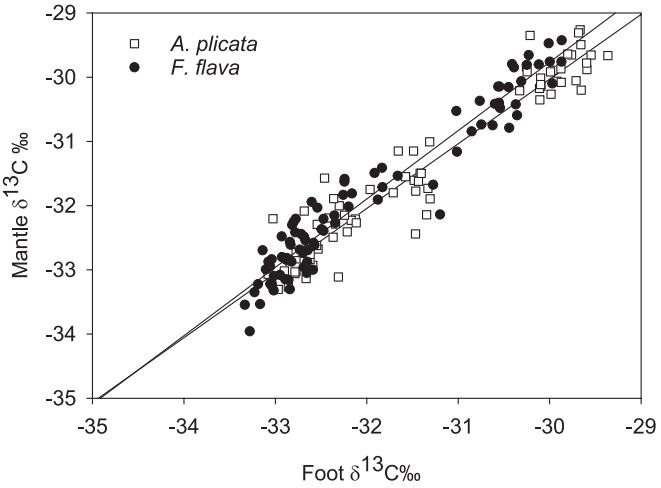

Figure 3. Simple linear regression of foot and mantle $\delta^{13}C$ per individual, with r^2 values of 0.925 for *Amblema plicata* and 0.934 for *Fusconaia flava*.

able to be collected at M2MC2, therefore species comparisons could not be conducted for this site.

DISCUSSION

This is the first study to compare both carbon and nitrogen isotopes between mantle and foot tissue compartments in freshwater mussels. For these two common and widely distributed species in the Upper Mississippi and St. Croix river systems, foot and mantle tissue sources were very similar with regard to $\delta^{15}N$ and $\delta^{13}C$. For the isotope/species combinations that had statistically significant per-mil differences in isotope composition between paired tissue compartments, the differences (i.e., 0.10–0.21‰) were within the range of instrumentation error (i.e., circa 0.2–0.3‰) and suggest that these differences would be unmeaningful in regard to food web analyses. We conclude that the tissue compartments are effectively interchangeable for carbon- and

Table 2. Simple linear regression results between the stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) of foot tissue versus mantle tissue for *Amblema plicata*, *Fusconaia flava*, and both species combined.

				Regression Coefficients	
Isotope	r^2	*F*	*P* Value	Intercept	Slope
A. plicata					
$\delta^{15}N$	0.955	1,490.34	<0.0001	0.757	0.900
$\delta^{13}C$	0.925	877.15	<0.0001	0.186	1.007
F. flava					
$\delta^{15}N$	0.967	2,472.81	<0.0001	0.733	0.913
$\delta^{13}C$	0.934	1,197.42	<0.0001	2.177	1.065
Combined					
$\delta^{15}N$	0.960	3,781.09	<0.0001	0.765	0.905
$\delta^{13}C$	0.927	2,007.00	<0.0001	0.642	1.019

Table 3. Stable isotope results of paired *t*-tests for foot versus mantle tissue for *Amblema plicata* ($N = 73$), *Fusconaia flava* ($N = 87$), and both species combined ($N = 160$). Difference (‰) = average difference between tissue types, and SD (dif) = standard deviation of the difference. Statistically significant differences are denoted in bold.

Isotope	Difference (‰)	SD (dif)	*t* Value	*P* Value
A. plicata				
$\delta^{15}N$	0.21	0.41	4.49	**<0.001**
$\delta^{13}C$	0.04	0.35	0.92	0.359
F. flava				
$\delta^{15}N$	0.10	0.32	2.83	**0.006**
$\delta^{13}C$	−0.11	0.32	−3.06	**0.003**
Combined				
$\delta^{15}N$	0.15	0.37	5.20	**<0.001**
$\delta^{13}C$	−0.04	0.34	−1.48	0.140

Table 4. Stable isotope results of paired *t*-tests for duplicate samples taken from the same tissue source from *Amblema plicata* ($N = 22$), *Fusconaia flava* ($N = 34$), and both species combined ($N = 56$). Difference (‰) = average difference between duplicate samples, and SD (dif) = standard deviation of the difference.

Isotope	Difference (‰)	SD (dif)	*t* Value	*P* Value
A. plicata				
$\delta^{15}N$	−0.01	0.36	−0.16	0.875
$\delta^{13}C$	−0.12	0.37	−1.51	0.147
F. flava				
$\delta^{15}N$	0.00	0.24	−0.06	0.950
$\delta^{13}C$	−0.01	0.12	−0.67	0.505
Combined				
$\delta^{15}N$	−0.01	0.29	−0.16	0.870
$\delta^{13}C$	−0.05	0.25	−1.63	0.108

nitrogen-stable isotopes for these two species within this study system.

While no studies have compared carbon isotopes between tissue sources, two studies have compared nitrogen isotopes among different tissues. *Elliptio* sp. foot tissue had slightly less spatial variability for nitrogen isotope signatures across small ponds as compared to mantle and adductor muscle tissue compartments (McKinney et al. 1999). Adductor muscle was enriched by 1.06‰ relative to mantle and foot tissues, and mantle tissue was enriched by 0.13‰ relative to foot tissue. Our findings for *A. plicata* and *F. flava* were the opposite, with foot tissue being more enriched in $\delta^{15}N$ relative to mantle tissue (i.e., 0.10–0.21‰). Like our study, the differences between foot and mantle tissues in the McKinney et al. (1999) study were less than the instrumental error and thus unmeaningful for the purposes of the food web analyses typically used.

Gustafson et al. (2007) found a strong positive relation (r^2 of 0.792) between nitrogen isotopes of foot tissue and hemolymph from *Elliptio complanata*. Foot tissue was generally enriched relative to hemolymph, but the actual difference in $\delta^{15}N$ between the tissues was not reported (Gustafson et al. 2007). We found even stronger correlations for $\delta^{15}N$ ($r^2 > 0.96$) and a difference of only 0.10–0.21‰ between mantle and foot tissue for *A. plicata* and *F. flava*. The tighter relation in our study compared to Gustafson et al. (2007) suggests that cross-study comparisons where foot or mantle tissues only were used might be more robust than comparisons that included hemolymph and other tissues.

The remarkably low variability in isotopic signatures among freshwater mussels within a location is one of the hallmarks that has made them an ideal taxon for isotopic baseline adjustment in food web studies (Cabana and Rasmussen 1996; Post 2002). Studying the isotopic relationship between/among species within a location can further enhance our understanding of the utility of freshwater mussels in isotope studies. A number of studies have indicated that $\delta^{15}N$ does not differ substantially among unionid species within a location (Nichols and Garling 2000; Raikow and Hamilton 2001; Christian et al. 2004; but see Weber et al. 2017), while the relationship of $\delta^{13}C$ between/among species has been more mixed (Nichols and Garling 2000; Christian et al. 2004; Weber et al. 2017). Unionid mussels have also been documented to differ in their carbon and nitrogen isotopic ratios relative to the invasive *Corbicula fluminea* (Atkinson et al. 2010).

Our data indicated that there was not a statistically significant difference for either isotope or tissue type between the species at nearly any site in this study. The equivalent isotopic signatures from these two unionid species by site justifies using one, rather than both, species for isotope studies in the Upper Mississippi and St. Croix rivers. These results also lay the foundation for using these two species interchangeably if one is not able to be sampled consistently over a spatial gradient in these Midwestern rivers. In our study system, this opens up more options for comparing food webs in the Mississippi and St. Croix Rivers over larger longitudinal (up and down the river and tributaries) and lateral (on-channel

Table 5. Simple linear regression results between the stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) of duplicate samples taken from the same tissue within an individual for *Amblema plicata*, *Fusconaia flava*, and both species combined.

				Regression Coefficients	
Isotope	r^2	*F*	*P* Value	Intercept	Slope
A. plicata					
$\delta^{15}N$	0.946	348.94	<0.0001	0.022	0.997
$\delta^{13}C$	0.924	243.96	<0.0001	2.741	1.092
F. flava					
$\delta^{15}N$	0.980	1,588.70	<0.0001	−0.297	1.031
$\delta^{13}C$	0.992	4,186.73	<0.0001	1.244	1.039
Combined					
$\delta^{15}N$	0.969	1,712.25	<0.0001	−0.174	1.017
$\delta^{13}C$	0.963	1,405.79	<0.0001	1.220	1.040

Table 6. Two sample *t*-test comparison of isotopes between species across sites. Statistically significant values after Bonferroni correction are denoted in bold. Sites that begin with M2 are from the Mississippi River Pool 2, and sites that begin with S are locations in the St. Croix River, listed from most downstream to most upstream. *N* = number of individuals per species per site, SD = standard deviation. Only one species was able to be collected at M2MC2, therefore species comparisons could not be conducted for this site.

	Amblema plicata			*Fusconaia flava*				
Site	*N*	Mean (‰)	SD (‰)	*N*	Mean (‰)	SD (‰)	*T*	*P* Value
δ^{15}N: Foot tissue								
M2MC1	19	11.15	0.18	19	11.13	0.31	0.27	0.7856
M2MC3	7	10.96	0.45	2	10.96	0.10	0.01	0.9937
SLK1	8	12.00	0.50	9	11.55	0.41	2.03	0.0602
SLK2	8	10.57	0.36	10	10.34	0.47	1.14	0.2707
SLK3	8	8.99	0.31	10	9.05	0.25	−0.43	0.6722
SMC1	5	7.87	0.22	10	7.88	0.31	−0.04	0.9725
SMC2	5	7.36	0.18	10	7.72	0.26	−2.77	0.0160
SMC3	8	7.86	0.27	10	7.67	0.41	1.12	0.2788
SNWP	5	6.11	0.18	3	6.13	0.32	−0.09	0.9335
δ^{15}N: Mantle tissue								
M2MC1	19	11.07	0.36	19	11.02	0.35	0.41	0.6856
M2MC3	7	10.79	0.39	2	10.74	0.10	0.19	0.8578
SLK1	8	11.10	0.42	9	11.00	0.44	0.48	0.6370
SLK2	8	10.10	0.30	10	10.18	0.43	−0.46	0.6543
SLK3	8	8.89	0.17	10	9.07	0.31	−1.51	0.1517
SMC1	5	7.90	0.28	10	7.87	0.29	0.20	0.8426
SMC2	5	7.34	0.22	10	7.73	0.21	−3.29	0.0059
SMC3	8	7.68	0.16	10	7.75	0.34	−0.53	0.6066
SNWP	5	6.20	0.32	3	6.18	0.15	0.12	0.9065
δ^{13}C: Foot tissue								
M2MC1	19	−29.88	0.27	19	−30.35	0.33	4.88	**<0.0001**
M2MC3	7	−29.93	0.22	2	−30.44	0.18	2.91	0.0227
SLK1	8	−32.67	0.32	9	−32.78	0.16	0.89	0.3858
SLK2	8	−32.16	0.23	10	−32.52	0.20	3.52	**0.0028**
SLK3	8	−31.61	0.36	10	−32.06	0.27	3.05	0.0077
SMC1	5	−32.71	0.19	10	−32.93	0.18	2.21	0.0458
SMC2	5	−32.86	0.08	10	−33.05	0.20	2.02	0.0640
SMC3	8	−32.34	0.44	10	−32.84	0.18	3.01	0.0147
SNWP	5	−31.39	0.07	3	−31.16	0.13	−3.28	0.0168
δ^{13}C: Mantle tissue								
M2MC1	19	−29.82	0.33	19	−30.13	0.45	2.49	0.0174
M2MC3	7	−30.03	0.14	2	−30.10	0.06	0.71	0.5029
SLK1	8	−32.50	0.36	9	−32.57	0.17	0.52	0.6138
SLK2	8	−32.03	0.20	10	−32.20	0.25	1.50	0.1521
SLK3	8	−31.41	0.27	10	−31.75	0.29	2.51	0.0232
SMC1	5	−32.97	0.18	10	−33.05	0.41	0.40	0.6922
SMC2	5	−33.14	0.14	10	−33.15	0.26	0.09	0.9317
SMC3	8	−32.61	0.34	10	−32.93	0.29	2.13	0.0488
SNWP	5	−31.84	0.37	3	−31.66	0.49	−0.59	0.5779

to off-channel) gradients, and we suspect this will also be useful for comparisons over time (Hornbach et al. 2018). The isotopic similarity of *A. plicata* and *F. flava* could simplify sampling designs and save time and money on sampling, all without diminishing the usefulness of the data.

This project advances the state of the science for isotopic studies in freshwater mussels by comparing two tissues for two isotopes in two species in two rivers over a large gradient of isotopic values. We argue that the similarity of the isotopic responses of foot and mantle tissue justifies retroactively comparing results between studies that have used these tissue types. However, we urge caution in overextending the implications of these findings outside of this geographic area, these species, or the range of isotopic values encountered in

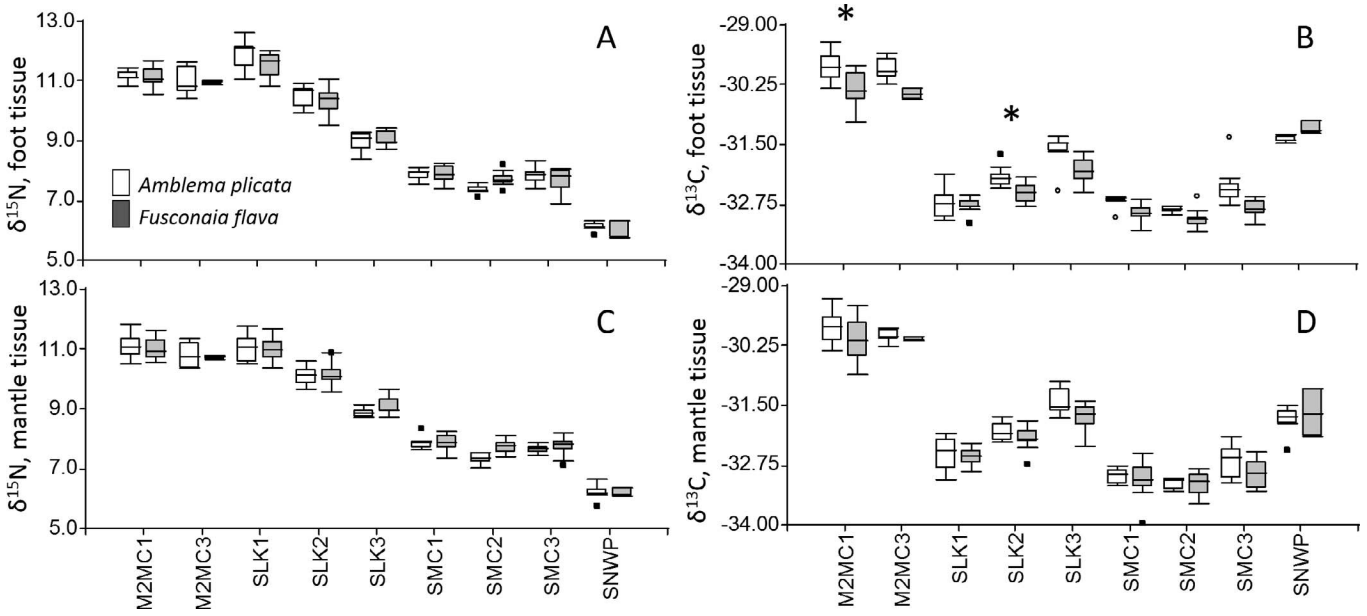

Figure 4. Box plots of a species comparison of isotopic values between *Amblema plicata* (white boxes) and *Fusconaia flava* (grey boxes) among sampling locations. Center horizontal lines are medians, upper and lower margins of the box are 25th and 75th percentiles, and the bars are the 5th and 95th percentile. Outliers are represented as individual points. A = foot tissue $\delta^{15}N$, B = foot tissue $\delta^{13}C$, C = mantle tissue $\delta^{15}N$, D = mantle tissue $\delta^{13}C$. Sites at which the species were significantly different are denoted with an asterisk above the paired boxes.

our study. Future research should evaluate the comparability of isotopic signatures between tissues of additional species from different geographic regions and habitats to evaluate the potential for species specific or location-based factors that may result in different patterns than what we observed in our study.

ACKNOWLEDGEMENTS

We thank staff from the U.S. National Park Service St. Croix National Scenic Riverway and Mississippi National River and Recreation Area for assistance in the field and laboratory. Thanks go to Levi Rhody and Joseph Fitzgerald, our interns from Northland College. J. C. Nelson (USGS) assisted with map creation. The St. Croix National Scenic Riverway Dive Team supported field work. Students from Edgewood High School (Madison, WI) Environmental Field Education course helped with collections on the upper St. Croix. This work was funded by the U.S. National Park Service Natural Resource Preservation Program and the U.S. Geological Survey Ecosystems Mission Area Fisheries Program. Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

LITERATURE CITED

Atkinson, C. L., S. P. Opsahl, A. P. Covich, S. W. Golladay, and L. M. Conner. 2010. Stable isotopic signatures, tissue stoichiometry, and nutrient cycling (C and N) of native and invasive freshwater bivalves. Journal of the North American Benthological Society 29:496–505.

Atkinson, C. L., J. F. Kelly, and C. C. Vaughn. 2014. Tracing consumer-derived nitrogen in riverine food webs. Ecosystems 17:485–496.

Berg, D. J., W. R. Haag, S. I. Guttman, and J. B. Sickel. 1995. Mantle biopsy: a technique for nondestructive tissue-sampling of freshwater mussels. Journal of the North American Benthological Society 14:577–581.

Brett, M. T., S. E. Bunn, S. Chandra, A. W. E. Galloway, F. Guo, M. J. Kainz, P. Kankaala, D. C. P. Lau, T. P. Moulton, M. E. Power, J. B. Rasmussen, S. J. Taipale, J. H. Thorp, and J. D. Wehr. 2017. How important are terrestrial organic carbon inputs for secondary production in freshwater ecosystems? Freshwater Biology 62:833–853.

Cabana G., and J. B. Rasmussen. 1996. Comparison of aquatic food chains using nitrogen isotopes. Proceedings of the National Academy of Sciences of the United States of America 93:10844–10847.

Christian, A. D., B. N. Smith, D. J. Berg, J. C. Smoot, and R. H. Findlay. 2004. Trophic position and potential food sources of 2 species of unionid bivalves (Mollusca:Unionidae) in 2 small Ohio streams. Journal of the North American Benthological Society 23:101–113.

Delong, M. D. 2010. Food webs and the Upper Mississippi River: contributions to our understanding of ecosystem function in large rivers. Hydrobiologia 640:89–101.

Finlay, J. C. 2001. Stable-carbon-isotope ratios of river biota: implications for energy flow in lotic food webs. Ecology 82:1052–1064.

Fritts, A. K., J. T. Peterson, P. D. Hazelton, and R. B. Bringolf. 2015. Evaluation of methods for assessing physiological biomarkers of stress in freshwater mussels. Canadian Journal of Fisheries and Aquatic Sciences 72:1450–1459.

Gustafson, L. L., W. Showers, T. Kwak, J. Levine, and M. Stoskopf. 2007. Temporal and spatial variability in stable isotope compositions of a freshwater mussel: implication for biomonitoring and ecological studies. Oecologia 152:140–150.

Gustafson, L. L., M. K. Stoskopf, A. E. Bogan, W. Showers, T. J. Kwak, S. Hanlon, and J. F. Levine. 2005. Evaluation of a nonlethal technique for hemolymph collection in *Elliptio complanata*, a freshwater bivalve (Mollusca: Unionidae). Diseases of Aquatic Organisms 65:159–165.

Herwig, B. R., D. H. Wahl, J. M. Dettmers, and D. A. Soluk. 2007. Spatial and temporal patterns in the food web structure of a large floodplain river assessed using stable isotopes. Canadian Journal of Fisheries and Aquatic Sciences 64:495–508.

Hornbach, D. J., D. C. Allen, M. C. Hove, and K. R. MacGregor. 2018. Long-term decline of native freshwater mussel assemblages in a federally protected river. Freshwater Biology 63:243–263.

Layman, C. A., D. A. Arrington, C. G. Montana, and D. M. Post. 2007. Can stable isotope ratios provide for community-wide measures of trophic structure? Ecology 88:42–48.

Lefebvre, S., C. Harma, and J. -L. Blin. 2009. Trophic typology of coastal ecosystems based on σ13C and σ15N ratios in an opportunistic suspension feeder. Marine Ecology Progress Series 390:27–37.

McKinney, R. A., J. L. Lake, M. Allen, and S. Ryba. 1999. Spatial variability in mussels used to assess base level nitrogen isotope ratio in freshwater ecosystems. Hydrobiologia 412:17–24.

Naimo, T. J., E. D. Damschen, R. G. Rada, and E. M. Monroe. 1998. Non-lethal evaluation of the physiological health of unionid mussels: methods for biopsy and glycogen analysis. Journal of the North American Benthological Society 17:121–128.

Nichols, S. J., and D. Garling. 2000. Food-web dynamics and trophic-level interactions in a multispecies community of freshwater unionids. Canadian Journal of Zoology 78:871–882.

Novais, A., E. Dias, and R. Sousa. 2016. Inter- and intraspecific variation of carbon and nitrogen stable isotope ratios in freshwater bivalves. Hydrobiologia 765:149–158.

Oulhote, Y., B. Le Bot, S. Deguen, and P. Glorennec. 2011. Using and interpreting isotope data for source identification. Trends in Analytical Chemistry 30:302–312.

Post, D. M. 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology 83:703–718.

Raikow, D. F., and S. K. Hamilton. 2001. Bivalve diets in a midwestern U.S. stream: a stable isotope enrichment study. Limnology and Oceanography 46:514–522.

Rounick, J. S., M. J. Winterbourn, and G. L. Lyon. 1982. Differential utilization of allochthonous and autochthonous inputs by aquatic invertebrates in some New Zealand streams: a stable carbon isotope study. Oikos 39:191–198.

Satterthwaite, F. E. 1946. An approximate distribution of estimates of variance components. Biometrics Bulletin 2:110–114.

Thorp, J. H., M. D. Delong, K. S. Greenwold, and A. F. Casper. 1998. Isotopic analysis of three food web theories in constricted and floodplain regions of a large river. Oecologia 117:551–563.

Vander Zanden, M. J., J. M. Casselman, and J. B. Rasmussen. 1999. Stable isotope evidence for the food web consequences of species invasions in lakes. Nature 401:464–467.

Weber, A. M., J. E. Bauer, and G. T. Watters. 2017. Assessment of nutritional subsidies to freshwater mussels using a multiple natural abundance isotope approach. Freshwater Biology 62:615–629.