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Water Sampling in the Mississippi River Basin to Inform Calcified Structure Chemistry Studies on Fishes

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Final Report to the Mississippi River Basin Panel on Aquatic Nuisance Species

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Introduction

Analyses of otolith stable isotope and elemental compositions can provide insights regarding environmental history of individual fish in a variety of environments, and are particularly useful for identifying environments used by fishes during early life stages (Pracheil et al. 2014). Otoliths are calcareous concretions in the inner ear of fishes that contain a permanent record of age and growth and are metabolically inert (Campana and Thorrold 2001). Concentrations or stable isotope ratios of some chemical elements in otoliths are strongly correlated with those in water where a fish lives. If locations that a fish may have occupied during its lifetime are chemically distinct and the fish spends sufficient time in each location to acquire location-specific chemical “signatures” in its otolith, it is possible to infer which locations a fish occupied by analyzing samples taken from specific locations within the fish’s otolith.

Hard-part (otolith or bone) chemistry has been used to identify natal environments and infer movement patterns for several native and invasive fish species in parts of the Mississippi River basin, primarily in the Upper and Middle Mississippi, Illinois, and lower Ohio rivers (Norman and Whitledge 2015; Laughlin et al. 2016; Phelps et al. 2017; Spurgeon et al. 2017; Pracheil et al. 2018; Schiller 2018; Rude and Whitledge 2019; Whitledge et al. 2019; Snyder et al. 2022; Whitledge et al., in review). Water sampling to characterize chemical ‘signatures’ of main-stem rivers and their tributaries was conducted as part of each of these studies. Thus, water chemistry of the Upper Mississippi River, Middle Mississippi River, Illinois River, the lower Ohio River, and tributaries of these rivers or river segments has been well-characterized (in many instances, water sampling has been conducted since 2006). Some water chemistry data relevant to otolith microchemistry studies have also been collected from the lower Missouri River and a few of its major tributaries and from the Lower Mississippi River and tributaries (Whitledge, unpublished; J. Spurgeon, unpublished). However, water chemistry data are much more limited in these areas, and additional samples would be useful to assess temporal variability in relevant water chemistry parameters. There are also several tributaries in parts of the Mississippi River basin (most notably in the Missouri, upper Ohio, and Tennessee-Cumberland drainages) where water chemistry data relevant to otolith microchemistry studies have not yet been obtained. There is strong potential for invasive fishes (e.g., bigheaded carps) to expand their ranges into these areas of the Mississippi River basin where water chemistry data are not currently available. Thus, whether otolith microchemistry might be informative for inferring natal environments of invasive fishes in these parts of the Mississippi River basin is unknown. Water samples from main-stem rivers and tributaries where relevant water chemistry data are currently limited or lacking (particularly in the Lower Mississippi, Missouri, upper Ohio, and Tennessee-Cumberland drainages) is needed to assess potential applicability of otolith microchemistry techniques to invasive (and native) fishes in these parts of the Mississippi River basin.

Objective

The objective of this study was to assess water chemistry (strontium, barium, and calcium concentrations and stable oxygen isotope ratio) of main-stem rivers and tributaries in the Mississippi River basin during 2021, focusing on rivers where limited or no water chemistry data relevant to fish hard-part chemistry studies were available.

Methods

Water samples were collected during June through early September 2021 from 54 locations across the Mississippi River basin. Water sampling locations and collection dates are listed in Table 1. Water sampling was conducted during summer because age-0 fishes are present at this time of year and otolith microchemistry is typically focused on inferring environments used by fish during early life. Two water samples were collected from near the river surface at each location on each of two sampling dates. One sample was used for stable oxygen isotope analysis and the second sample was used for analysis of elemental concentrations (different instrumentation is required for these analyses, thus requiring two sets of samples to be obtained). Water samples were collected using a syringe filtration technique described in Shiller (2003). Water sampling 'kits' (acid-cleaned bottles and syringes with filter discs) were purchased from Dr. Alan Shiller's lab at the University of Southern Mississippi and distributed to state and federal agency personnel who volunteered to collect water samples; instructions for sample collection, storage, and shipping accompanied each kit. Vials containing filtered water samples for stable oxygen isotope analysis were filled to the rim and sealed tightly to curtail evaporative loss and associated isotopic fractionation (Kendall and Caldwell 1998).

Water Samples were analyzed for stable oxygen isotopic composition using a high-temperature conversion elemental analyzer interfaced with a Thermo Finnigan Delta V isotope ratio mass spectrometer at the Southern Illinois University Mass Spectrometry Facility. Stable oxygen isotope ratios were expressed in standard delta notation, defined as the parts per thousand deviation between the isotope ratio of a sample and standard material (Vienna Standard Mean Ocean Water for water $\delta^{18}O$):

$$\delta^{18}O\ (‰) = [(Rsample – Rstandard) – 1\ ] \times 1000;$$

where R represents $^{18}O/^{16}O$. Analytical precision estimated from analysis of laboratory standards was 0.07‰.

Analysis of water samples for calcium (Ca), strontium (Sr) and barium (Ba) concentrations was conducted at the Center for Trace Analysis, University of Southern Mississippi. These elements were chosen for analysis because Sr:Ca and Ba:Ca are the most commonly used natural chemical markers in otolith chemistry studies (Pracheil et al 2014), are strongly correlated with corresponding elemental ratios in water, and have been informative in published hard-part chemistry studies in the Mississippi River basin (Zeigler and Whitledge 2010, 2011; Norman and Whitledge 2015; Laughlin et al. 2016; Phelps et al. 2017; Spurgeon et al. 2017; Pracheil et al. 2018; Schiller 2018; Rude and Whitledge 2019; Whitledge et al. 2019; Snyder et al. 2022; Whitledge et al., in review). Some elements (e.g., Mg, Mn) are also strongly physiologically regulated by fishes; concentrations of these elements in water are uncorrelated or only weakly correlated with their concentrations in fish calcified structures (Pracheil et al. 2014; Tuner and Limburg 2015). In the laboratory, water samples were acidified to pH 1.8 using ultrapure HCl and allowed to sit acidified for at least 1 week before analysis. Samples were then diluted 11x in ultrapure 0.16 M HNO_3. The nitric acid contained 2 ppb scandium, indium, and thorium as internal standards. External certified reference standards were also prepared using the same HNO_3 used for sample dilutions. Samples were analyzed for ^{44}Ca, ^{88}Sr, and ^{137}Ba using a Thermo-Finnigan Element 2 inductively coupled plasma mass spectrometer (ICPMS). Precision of analyses based on repeated measurements of standards was better than ± 2% (2 SD). Water strontium, barium, and calcium concentration data were converted to molar Sr:Ca and Ba:Ca ratios (mmol/mol) for comparison with data from prior studies.

Results and Discussion

Water chemistry data from samples collected for this study are shown in Table 1. Data are also available at: https://opensiuc.lib.siu.edu/fiaq_data/13. Water Sr:Ca and Ba:Ca across all samples collected for this study ranged from 0.57 to 9.51 mmol/mol and 0.13 to 3.53 mmol/mol, respectively. Water δ^{18}O ranged from -2.69‰ to -12.83‰. For rivers in which prior water Sr:Ca, Ba:Ca, or δ^{18}O data were available, values observed in this study were within previously measured ranges (Coplen and Kendall 2000; Phelps et al. 2012; Whitledge, unpublished data). Broad ranges of water Sr:Ca and Ba:Ca are indicative of differences in geology across the study area (Wells et al. 2003), whereas the broad range of river water δ^{18}O among sampling locations reflects geographic differences in precipitation δ^{18}O contributing to watersheds; δ^{18}O of precipitation and surface waters decreases with increasing latitude, altitude, and distance from the ocean (Kendall and Coplen 2001). A few rivers in the study area had water Sr:Ca, Ba:Ca or δ^{18}O values that were unique among the locations sampled. For example, the Scioto River was the only location where water Sr:Ca was > 7 mmol/mol, the Obion River was the only location where water Ba:Ca exceeded 2 mmol/mol, and water δ^{18}O values < -10‰ were only observed in the Missouri River. However, rivers with unique Sr:Ca, Ba:Ca, or δ^{18}O values, or combinations thereof, are rare at the geographic scale of the Mississippi River basin. Nonetheless, data from this study and published studies indicate that differences in water Sr:Ca, Ba:Ca, and δ^{18}O are present among many rivers across the Mississippi River basin, especially between main-stem rivers and tributaries, that will enable some inferences to be made regarding fish environmental history using calcified structure chemistry data.

Data from this study and prior studies indicate that water Sr:Ca, Ba:Ca, and δ^{18}O values in the unimpounded section of the Missouri River (downstream from Gavins Point Dam) differ from those of several of the major tributaries that flow into this section of the Missouri River. Changes in water Sr:Ca, Ba:Ca, and δ^{18}O along the length of the unimpounded section of the Missouri River also offer some potential utility for calcified structure chemistry studies on fishes. Several tributaries have lower Sr:Ca than the Missouri River (Figure 1); exceptions include the Kansas River and Platte River (Nebraska) whose ranges of water Sr:Ca overlap that of the Missouri River. The Big Nemaha River had higher Sr:Ca than the section of the Missouri River into which it flows. The Vermillion and James rivers had lower Ba:Ca than the Missouri River (Figure 2); tributaries with higher Ba:Ca than the Missouri River included the Moreau, Lamine, Platte (Missouri-Iowa), Nishnabotna, and Platte (Nebraska) rivers. With the exception of the Platte River (Nebraska), available data indicate that Missouri River tributaries have higher (less negative) water $\delta^{18}O$ than the section of the Missouri River into which they flow (Figure 3). Based on water chemistry data from this study and prior studies, distinguishing fish use of the Missouri River versus its tributaries would likely be feasible using calcified structure Sr:Ca, Ba:Ca, and $\delta^{18}O$. Whether one, two, or all three of these chemical markers would be needed for a particular study would depend on the set of locations relevant to study objectives and the species and life stages of interest. Inferring fish use of a few particular tributaries based on unique combinations of Sr:Ca, Ba:Ca, and $\delta^{18}O$ also appears possible. The Missouri River exhibits increasing Sr:Ca and decreasing Ba:Ca and $\delta^{18}O$ with increasing distance upstream from its mouth (Figures 1-3), which may provide some potential to infer fish use of different sections of the river.

The range of water Sr:Ca for the Lower Mississippi River (downstream of the Ohio River confluence) overlaps that of many tributaries of the Lower Mississippi River (Figure 4). However, the ranges of water Sr:Ca for the Arkansas, Big Black, and Red rivers include values that are higher than those measured in the Lower Mississippi River. The White River has lower water Sr:Ca than the Lower Mississippi River and is unique among Lower Mississippi River tributaries in having water Sr:Ca values < 1 mmol/mol. Five tributaries of the Lower Mississippi River (Obion, Hatchie, St. Francis, Yazoo, and Big Black) have higher water Ba:Ca than the Lower Mississippi River (Figure 5). Like the Missouri River, the Lower Mississippi River also differs in water $\delta^{18}O$ from nearly all of its major tributaries; only the White River has a partially overlapping range of $\delta^{18}O$ with the Lower Mississippi River (Figure 6). Thus, $\delta^{18}O$ and Ba:Ca appear to be the natural chemical tracers with the best potential to distinguish fish use of the Lower Mississippi River and its tributaries in calcified structure chemistry studies, although Sr:Ca would also likely be useful for inferring fish use of the limited set of tributaries whose range of water Sr:Ca is partially or completely non-overlapping with that of the Lower Mississippi River.

The observed range of water Sr:Ca for the Ohio River overlaps that of many of its tributaries, although there are also several tributaries with higher or lower Sr:Ca than the Ohio River (Figure 7). Most of the tributaries with lower water Sr:Ca than the Ohio River flow into the J.T. Myers, Smithland, or Olmsted pools (Tennessee, Cumberland, Saline, Tradewater, Wabash, and Green rivers). Other tributaries with lower water Sr:Ca than the Ohio River include the Salt, Kentucky, Little Miami, and Licking rivers. The Kanawha, Big Sandy, and Scioto Rivers had higher Sr:Ca than the Ohio River; one sample from the Great Miami River also had Sr:Ca > 4 mmol/mol. The relatively high Sr:Ca in the Scioto River (and perhaps the sample from the Great Miami River mentioned above) is likely due to the presence of $SrCO_3$ deposits in northwestern Ohio, which are responsible for high water Sr:Ca in the Sandusky River and other tributaries to western Lake Erie (Whitledge et al. 2021). The range of water Ba:Ca for the Ohio River partially or fully overlaps that of many of its major tributaries (Figure 8). However, samples collected from the Salt, Kentucky, and Licking rivers had lower Ba:Ca than the Ohio River and the Allegheny, Kanawha, and Little Kanawha rivers had higher Ba:Ca than the Ohio River. Water samples from this study and prior studies indicate that the range of Ohio River water $\delta^{18}O$ partially or completely overlaps that of many of its tributaries, although water $\delta^{18}O$ values > -5‰ only occurred in tributaries (Tennessee, Cumberland, Saline, Tradewater, Wabash, Salt, Licking, and Little Kanawha rivers) and values < -8‰ were only measured in the upstream section of the Ohio River (Belleville Pool) and its two source rivers (Allegheny and Monongahela rivers). Calcified structure chemistry appears to have some potential utility in the Ohio River basin, as several major tributaries would likely be distinguishable from the Ohio River using Sr:Ca, Ba:Ca, or $\delta^{18}O$. As in the Missouri River basin, whether one, two, or all three of these chemical markers would be needed for a particular study would depend on the set of locations relevant to study objectives and the species and life stages of interest. However, some tributaries (e.g., the Blue River) appear to be indistinguishable from the Ohio River using these three natural chemical markers.

Rivers sampled for this study also included a few tributaries to the Upper Mississippi (Minnesota River), Illinois (Vermilion River), Wabash (Tippecanoe River), Cumberland (Red River), and Tennessee (Duck and Buffalo rivers) rivers. Water Sr:Ca in the Minnesota River is higher than that of the Upper Mississippi River (upstream of the mouth of the Missouri River) and all other tributaries that enter pools 2-17 of the Upper Mississippi River (Whitledge et al. 2019; Whitledge, unpublished data). Thus, Sr:Ca will likely be a useful marker of fish use of the Minnesota River in the Upper Mississippi River system. The Duck River's lower water Sr:Ca and Ba:Ca compared to the Tennessee River may also prove useful for calcified structure microchemistry studies. The Vermilion River (Illinois River tributary), Tippecanoe River (Wabash River tributary), Red River (Cumberland River tributary), and Buffalo River (Tennessee River tributary) have overlapping ranges of water Sr:Ca, Ba:Ca, and $\delta^{18}O$ with their respective main-stem rivers and thus will likely be indistinguishable from the rivers into which they flow. Prior sampling has found that some tributaries of the Illinois and Wabash rivers are distinguishable from each of these rivers using Sr:Ca, whereas other tributaries are not (Zeigler and Whitledge 2010; Whitledge, unpublished data).

The applicability of calcified structure chemistry studies to infer environmental history of fishes depends on the presence of persistent differences in water chemistry among relevant locations. The set of relevant locations will differ among studies depending on objectives (e.g., identification of natal environments or inter-river movement patterns, locations that may be of particular focus for management actions) and potential for use by the species and life stages of interest. The likelihood of being able to distinguish among relevant locations using one or more natural chemical markers generally declines as the number of locations increases due to the limited set of natural markers that meet the fundamental criteria for use in calcified structure chemistry studies (persistent spatial differences and strong correlations between water and calcified structure values). Individual fish also need to remain in a location long enough to accrue enough calcified structure growth to acquire the chemical 'signature' of that location. Water chemistry in individual rivers is subject to temporal variability. Thus, although the data presented in this report provide some insight regarding which natural chemical markers may be most effective for distinguishing among a particular set of locations (or whether calcified structure chemistry will likely be applicable at all), additional water sampling should be conducted as part of any calcified structure chemistry study, particularly in rivers where limited data are available. Future studies should also consider assessing the potential utility of strontium isotope ratios ($^{87}Sr/^{86}Sr$), which have proven useful in several otolith chemistry studies (Pracheil et al. 2014), although no published studies have used strontium isotope ratios in the study area encompassed by this project.

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Table 1. Water sampling locations and dates and water Sr:Ca, Ba:Ca, and stable oxygen isotope data for each sample.

River	Basin	Location	Lat	Lon	Date	Sr:Ca (mmol/mol)	Ba:Ca (mmol/mol)	δ18O (‰)
Minnesota River	Upper Mississippi	Chaska, MN	44.7773	-93.5945	7/2/2021	2.02	0.26	-7.35
Minnesota River	Upper Mississippi	Chaska, MN	44.7773	-93.5945	8/19/2021	2.56	0.37	-5.95
Minnesota River	Upper Mississippi	Kinney Access near Granite Falls, MN	44.7743	-95.5304	7/16/2021	2.31	0.25	-5.46
Minnesota River	Upper Mississippi	Kinney Access near Granite Falls, MN	44.7743	-95.5304	8/17/2021	2.40	0.27	-5.06
Loutre River	Missouri	Katy Trail bridge near McKittrick, MO	38.73314	-91.44922	7/6/2021	1.03	0.65	-5.63
Loutre River	Missouri	Katy Trail bridge near McKittrick, MO	38.73314	-91.44922	8/20/2021	1.20	0.65	-4.43
Gasconade River	Missouri	Gasconade, MO	38.66755	-91.55551	7/6/2021	0.70	0.59	-5.69
Gasconade River	Missouri	Gasconade, MO	38.66755	-91.55551	8/20/2021	0.66	0.53	-5.64
Osage River	Missouri	Mari-Osa Access	38.4921	-92.00998	8/27/2021	2.11	0.49	-4.24
Osage River	Missouri	Mari-Osa Access	38.4921	-92.00998	9/9/2021	2.12	0.47	-4.46
Moreau River	Missouri	Moreau 50 Access	38.5414	-92.10673	7/6/2021	1.44	1.18	-4.99
Moreau River	Missouri	Moreau 50 Access	38.5414	-92.10673	8/20/2021	1.53	1.08	-3.99
Lamine River	Missouri	De Bourgmont Access	38.94112	-92.87166	7/6/2021	2.29	0.87	-4.88
Lamine River	Missouri	De Bourgmont Access	38.94112	-92.87166	8/20/2021	2.80	0.79	-4.08
Grand River	Missouri	Brunswick, MO	39.42175	-93.13269	8/27/2021	1.92	0.61	-3.90
Grand River	Missouri	Brunswick, MO	39.42175	-93.13269	9/9/2021	2.03	0.58	-4.27
Missouri River	Missouri	Noren Access (Jefferson City)	38.58919	-92.17926	7/6/2021	2.95	0.71	-7.11
Missouri River	Missouri	Noren Access (Jefferson City)	38.58919	-92.17926	8/20/2021	4.00	0.51	-9.61
Chariton River	Missouri	Rathbun Lake tailwater	40.82167	-92.89168	6/8/2021	2.02	0.64	-6.37
Chariton River	Missouri	Rathbun Lake tailwater	40.82176	-92.89201	8/6/2021	2.06	0.62	-4.89
Kansas River	Missouri	DeSoto, KS	38.9851	-94.9741	6/9/2021	4.04	0.44	-5.19
Kansas River	Missouri	DeSoto, KS	38.9851	-94.9741	7/14/2021	3.91	0.40	-4.64
Platte River	Missouri	Iowa Highway 2	40.71187	-94.40414	6/29/2021	2.47	0.94	-5.31
Platte River	Missouri	Iowa Highway 2	40.71187	-94.40414	8/19/2021	2.45	0.83	-5.26
Missouri River	Missouri	Atchison, KS	39.5656	-95.1123	6/9/2021	3.96	0.37	-10.61
Missouri River	Missouri	Atchison, KS	39.5656	-95.1123	7/14/2021	4.06	0.43	-10.31
Nodaway River	Missouri	Iowa Highway 2	40.72762	-94.98024	6/29/2021	2.11	0.72	-5.12
Nodaway River	Missouri	Iowa Highway 2	40.72749	-94.98024	8/19/2021	2.13	0.73	-3.70
Nishnabotna River	Missouri	Hamburg, IA	40.6019	-95.64508	6/29/2021	1.93	0.78	-5.69
Nishnabotna River	Missouri	Hamburg, IA	40.60144	-95.6456	8/19/2021	2.66	0.99	-5.63
Little Sioux River	Missouri	near Smithland, IA	42.25394	-95.90452	6/29/2021	2.55	0.48	-6.61
Little Sioux River	Missouri	near Smithland, IA	42.25394	-95.90452	8/16/2021	2.78	0.49	-5.20
Missouri River	Missouri	Sioux City-Omaha reach	42.21016	-96.35841	6/29/2021	4.48	0.22	-12.83
Missouri River	Missouri	Sioux City-Omaha reach	42.21021	-96.35834	8/16/2021	4.59	0.22	-12.78
Big Nemaha River	Missouri	near Preston, NE	40.04378	-95.52017	7/6/2021	4.72	0.57	-5.10
Big Nemaha River	Missouri	near Preston, NE	40.04378	-95.52017	8/25/2021	5.45	0.56	-4.40
Big Sioux River	Missouri	Akron, IA	42.83939	-96.56102	6/4/2021	2.79	0.29	-7.39
Big Sioux River	Missouri	Akron, IA	42.82974	-96.56132	8/10/2021	2.28	0.24	-6.68
Vermillion River	Missouri	Highway 19	42.9901	-96.96385	6/4/2021	2.83	0.14	-5.48
Vermillion River	Missouri	Highway 19	42.9901	-96.96385	8/10/2021	2.99	0.17	-4.24
James River	Missouri	Highway 81 Access	43.05687	-97.40017	6/14/2021	3.62	0.16	-5.54
James River	Missouri	Highway 81 Access	43.05687	-97.40017	8/10/2021	4.15	0.13	-3.98
Obion River	Lower Mississippi	Highway 89 access near Kenton, TN	36.24679	-88.9733	6/24/2021	2.88	1.03	-5.11
Obion River	Lower Mississippi	Highway 89 access near Kenton, TN	36.24679	-88.9733	8/19/2021	3.28	3.53	-5.10
Hatchie River	Lower Mississippi	Highway 76 Access	35.52283	-89.25379	6/24/2021	3.30	1.07	-3.90
Hatchie River	Lower Mississippi	Highway 76 Access	35.52283	-89.25379	8/19/2021	3.36	0.81	-4.41
St. Francis River	Lower Mississippi	Madison Access	35.01384	-90.71942	6/16/2021	1.86	1.18	-4.48
St. Francis River	Lower Mississippi	Madison Access	35.01384	-90.71943	8/3/2021	1.87	1.16	-4.14
White River	Lower Mississippi	DeValls Bluff, AR	34.79065	-91.44441	6/16/2021	0.65	0.36	-5.08
White River	Lower Mississippi	DeValls Bluff, AR	34.79065	-91.44441	8/3/2021	0.57	0.28	-5.53
Arkansas River	Lower Mississippi	Sheppard Island Access	34.24436	-91.89947	6/15/2021	3.49	0.56	-4.19
Arkansas River	Lower Mississippi	Sheppard Island Access	34.24436	-91.89947	8/3/2021	3.33	0.53	-3.79
Yazoo River	Lower Mississippi	Belzoni, MS	33.17028	-90.49028	7/16/2021	2.87	0.98	-2.86
Yazoo River	Lower Mississippi	Belzoni, MS	33.17028	-90.49028	8/26/2021	2.85	0.97	-2.70
Big Black River	Lower Mississippi	Highway 16 Access	32.70711	-90.09409	8/9/2021	5.04	1.82	-2.85
Big Black River	Lower Mississippi	Highway 49 Access	32.60453	-90.36533	8/10/2021	4.66	1.47	-2.69
Atchafalaya River	Red-Atchafalaya	Butte La Rose	30.28032	-91.68589	6/30/2021	3.12	0.59	-4.36
Atchafalaya River	Red-Atchafalaya	Butte La Rose	30.28032	-91.68589	8/31/2021	2.81	0.50	-4.87
Red River	Red-Atchafalaya	Coushatta Access	32.0145	-93.35231	6/21/2021	4.05	0.70	-3.25
Red River	Red-Atchafalaya	Coushatta Access	32.0145	-93.35231	7/19/2021	4.64	0.62	-3.08
Vermilion River	Illinois	near Manville, IL	41.04799	-88.79979	6/17/2021	1.22	0.19	-7.18
Vermilion River	Illinois	Pontiac, IL	40.87776	-88.63189	8/17/2021	1.14	0.18	-6.18

Table 1 (continued)

River	Basin	Location	Lat	Lon	Date	Sr:Ca (mmol/mol)	Ba:Ca (mmol/mol)	δ18O (‰)
Blue River	Ohio	near Leavenworth, IN	38.18301	-86.32834	9/9/2021	2.82	0.33	-6.03
Blue River	Ohio	Indiana highway 462 bridge	38.23011	-86.2531	10/25/2021	2.15	0.20	-5.99
Ohio River	Ohio	McAlpine Pool	38.68393	-85.1869	9/7/2021	2.78	0.37	-6.28
Ohio River	Ohio	McAlpine Pool	38.68371	-85.18682	10/25/2021	3.12	0.31	-6.74
Ohio River	Ohio	Markland Pool	39.05461	-84.89783	9/7/2021	3.06	0.36	-6.26
Ohio River	Ohio	Markland Pool	39.05462	-84.89787	10/25/2021	3.16	0.35	-6.56
Great Miami River	Ohio	Heritage Park	39.2923	-84.6642	6/29/2021	4.24	0.33	-5.46
Great Miami River	Ohio	near Lawrenceburg, IN	39.1161	-84.8284	7/13/2021	2.94	0.28	-6.62
Little Miami River	Ohio	Rogers Park	39.3675	-84.2156	6/29/2021	2.13	0.29	-6.53
Little Miami River	Ohio	Magrish Recreation Center	39.0859	-84.4192	7/13/2021	1.93	0.22	-6.24
Ohio River	Ohio	Meldahl Pool	38.79026	-84.13327	6/8/2021	3.41	0.35	-7.76
Ohio River	Ohio	Meldahl Pool	38.6859	-83.59612	8/27/2021	3.54	0.42	-6.93
Kentucky River	Ohio	Pool 4	38.20226	-84.88183	6/28/2021	1.87	0.19	-5.46
Kentucky River	Ohio	Pool 4	38.20226	-84.88183	8/30/2021	2.77	0.25	-5.87
Licking River	Ohio	Frederick's Landing	39.04954	-84.49287	6/29/2021	1.37	0.14	-5.97
Licking River	Ohio	Frederick's Landing	39.04954	-84.49287	8/25/2021	1.59	0.19	-6.02
Scioto River	Ohio	Chillicothe, OH	39.34111	-82.98139	6/7/2021	9.51	0.26	-7.30
Scioto River	Ohio	Chillicothe, OH	39.34111	-82.98139	8/18/2021	9.47	0.28	-4.98
Muskingum River	Ohio	Marietta Aquatics Center	39.43255	-81.47147	6/21/2021	2.19	0.24	-7.31
Muskingum River	Ohio	Marietta Aquatics Center	39.43255	-81.47147	7/27/2021	2.07	0.25	-6.85
Kanawha River	Ohio	Leon, WV	38.75022	-81.96141	6/21/2021	4.04	0.64	-7.68
Kanawha River	Ohio	Leon, WV	38.75022	-81.96141	7/28/2021	4.80	0.60	-6.57
Little Kanawha River	Ohio	Parkersburg, WV	39.22727	-81.51747	6/21/2021	2.75	0.97	-7.19
Little Kanawha River	Ohio	Parkersburg, WV	39.22727	-81.51747	7/27/2021	3.02	0.88	-6.13
Ohio River	Ohio	Greenup Pool - Green Bottom WMA	38.58925	-82.22277	6/21/2021	2.73	0.34	-7.17
Ohio River	Ohio	Greenup Pool - Green Bottom WMA	38.58925	-82.22277	7/28/2021	2.61	0.36	-7.41
Ohio River	Ohio	Belleville Pool - Williamstown ramp	39.40718	-81.45009	6/21/2021	2.72	0.44	-9.05
Ohio River	Ohio	Belleville Pool - Williamstown ramp	39.40718	-81.45009	7/27/2021	2.40	0.43	-8.09
Big Sandy River	Ohio	Virginia Point Park	38.35693	-82.59827	6/29/2021	6.78	0.43	-6.83
Big Sandy River	Ohio	Virginia Point Park	38.35693	-82.59827	8/23/2021	5.60	0.44	-6.34
Allegheny River	Ohio	River mile 3.5	40.47282	-79.96786	6/16/2021	2.53	0.53	-9.46
Allegheny River	Ohio	River mile 3.5	40.47282	-79.96788	8/13/2021	2.37	0.46	-8.67
Monongahela River	Ohio	River mile 4.7	40.4172	-79.95421	6/16/2021	3.01	0.37	-8.06
Monongahela River	Ohio	River mile 4.7	40.4172	-79.95421	8/13/2021	3.33	0.33	-7.09
Tennessee River	Tennessee	Pickwick State Park boat ramp	36.05702	-88.2325	6/22/2021	1.66	0.30	-4.54
Tennessee River	Tennessee	Pickwick State Park boat ramp	36.05702	-88.2325	8/26/2021	1.80	0.32	-4.21
Duck River	Tennessee	Dyer Road boat ramp	35.93206	-87.7476	6/21/2021	1.28	0.13	-4.74
Duck River	Tennessee	Dyer Road boat ramp	35.93206	-87.7476	8/25/2021	1.18	0.15	-4.78
Buffalo River	Tennessee	Gladden Road boat ramp near Linden, TN	35.61394	-87.83041	6/21/2021	1.78	0.36	-5.31
Buffalo River	Tennessee	Gladden Road boat ramp near Linden, TN	35.61394	-87.83041	8/25/2021	1.85	0.42	-5.10
Cumberland River	Cumberland	Cheatham Reservoir	36.31691	-87.20563	6/17/2021	1.87	0.17	-5.32
Cumberland River	Cumberland	Cheatham Reservoir	36.31691	-87.20563	8/25/2021	2.10	0.20	-5.17
Red River	Cumberland	Clarksville, TN	36.54329	-87.36316	6/17/2021	2.12	0.26	-5.16
Red River	Cumberland	Clarksville, TN	36.54329	-87.36316	8/25/2021	2.56	0.22	-4.77
Tippecanoe River	Wabash	Oakdale Access	40.65317	-86.75622	6/9/2021	1.41	0.29	-6.92
Tippecanoe River	Wabash	Oakdale Access	40.65317	-86.75622	8/17/2021	1.66	0.33	-5.93

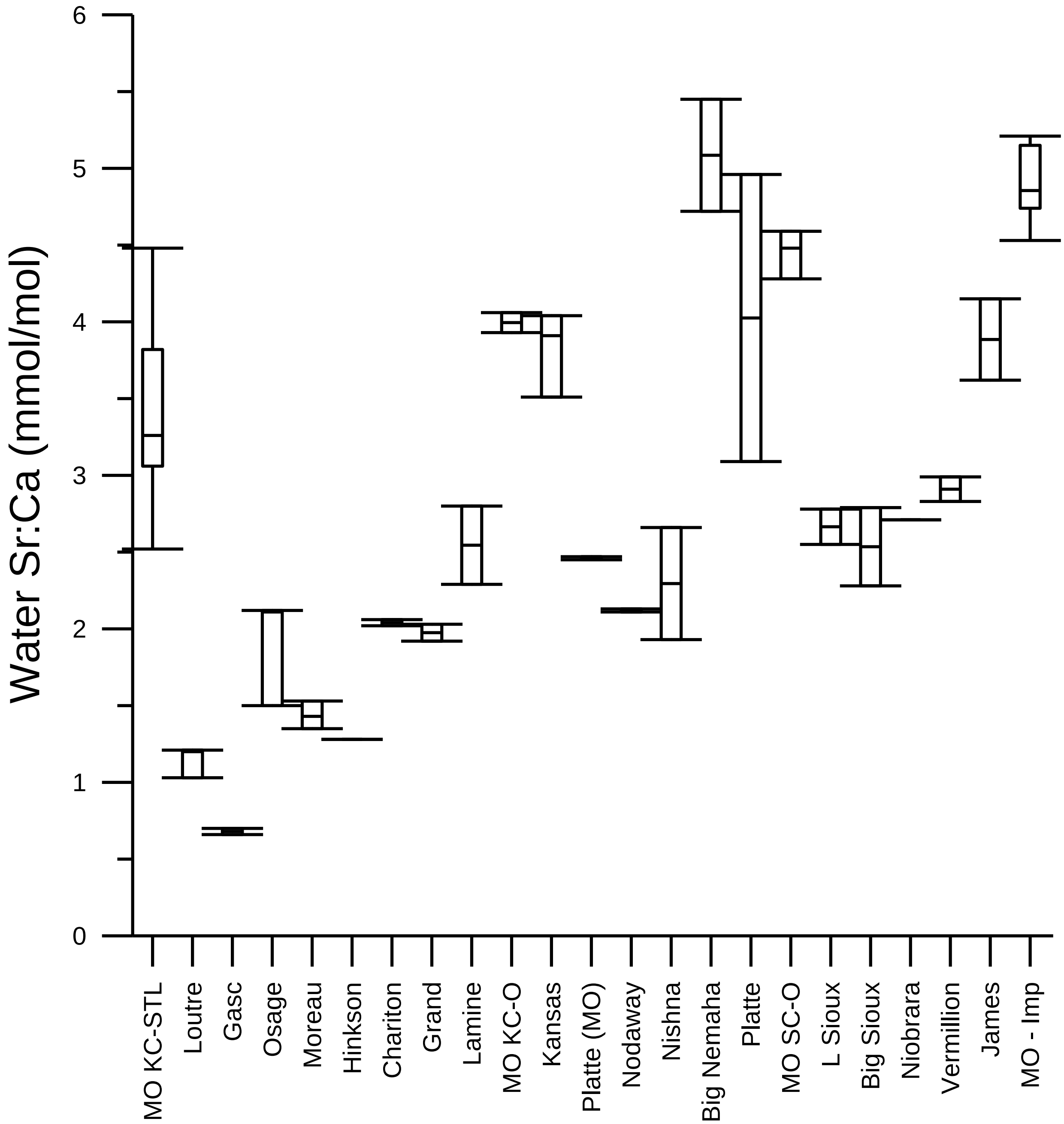

Figure 1. Boxplot showing ranges of water Sr:Ca for the Missouri River and major tributaries; interquartile ranges and medians also shown for sites with sufficient sample sizes. Data are from samples collected for this study and prior collections by the Whitledge lab. Site names on the x-axis are (from left to right): Missouri River between Kansas City and St. Louis, Loutre River, Gasconade River, Osage River, Moreau River, Hinkson Creek, Chariton River, Grand River, Lamine River, Missouri River between Kansas City and Omaha, Kansas River, Platte River (Missouri-Iowa), Nodaway River, Nishnabotna River, Big Nemaha River, Platte River (Nebraska), Missouri River between Sioux City and Omaha, Little Sioux River, Big Sioux River, Niobrara River, Vermillion River, James River, and the impounded section of the Missouri River (upstream of Gavins Point Dam).

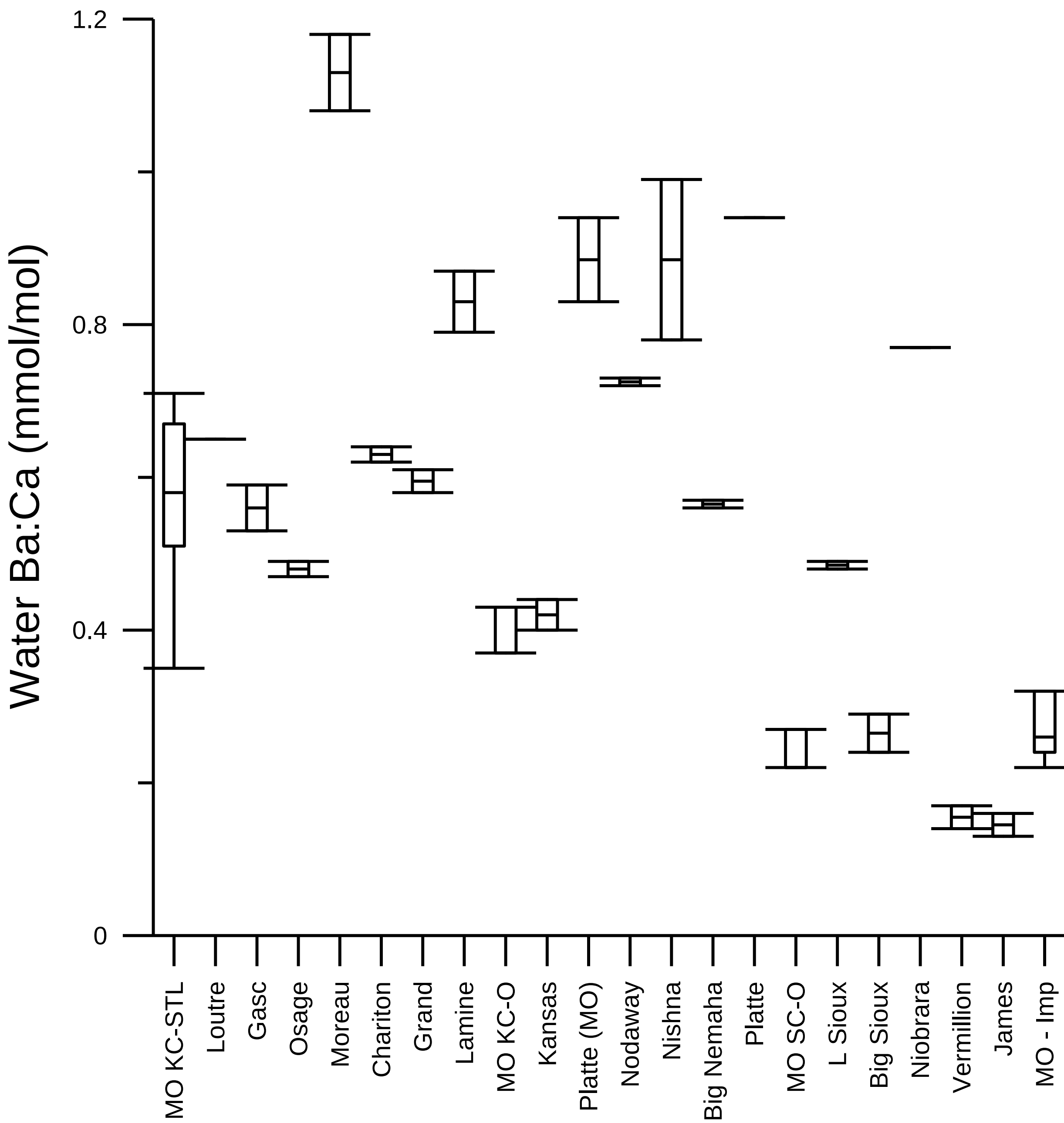

Figure 2. Boxplot showing ranges of water Ba:Ca for the Missouri River and major tributaries; interquartile ranges and medians also shown for sites with sufficient sample sizes. Data are from samples collected for this study and prior collections by the Whitledge lab. Site names on the x-axis are (from left to right): Missouri River between Kansas City and St. Louis, Loutre River, Gasconade River, Osage River, Moreau River, Chariton River, Grand River, Lamine River, Missouri River between Kansas City and Omaha, Kansas River, Platte River (Missouri-Iowa), Nodaway River, Nishnabotna River, Big Nemaha River, Platte River (Nebraska), Missouri River between Sioux City and Omaha, Little Sioux River, Big Sioux River, Niobrara River, Vermillion River, James River, and the impounded section of the Missouri River (upstream of Gavins Point Dam).

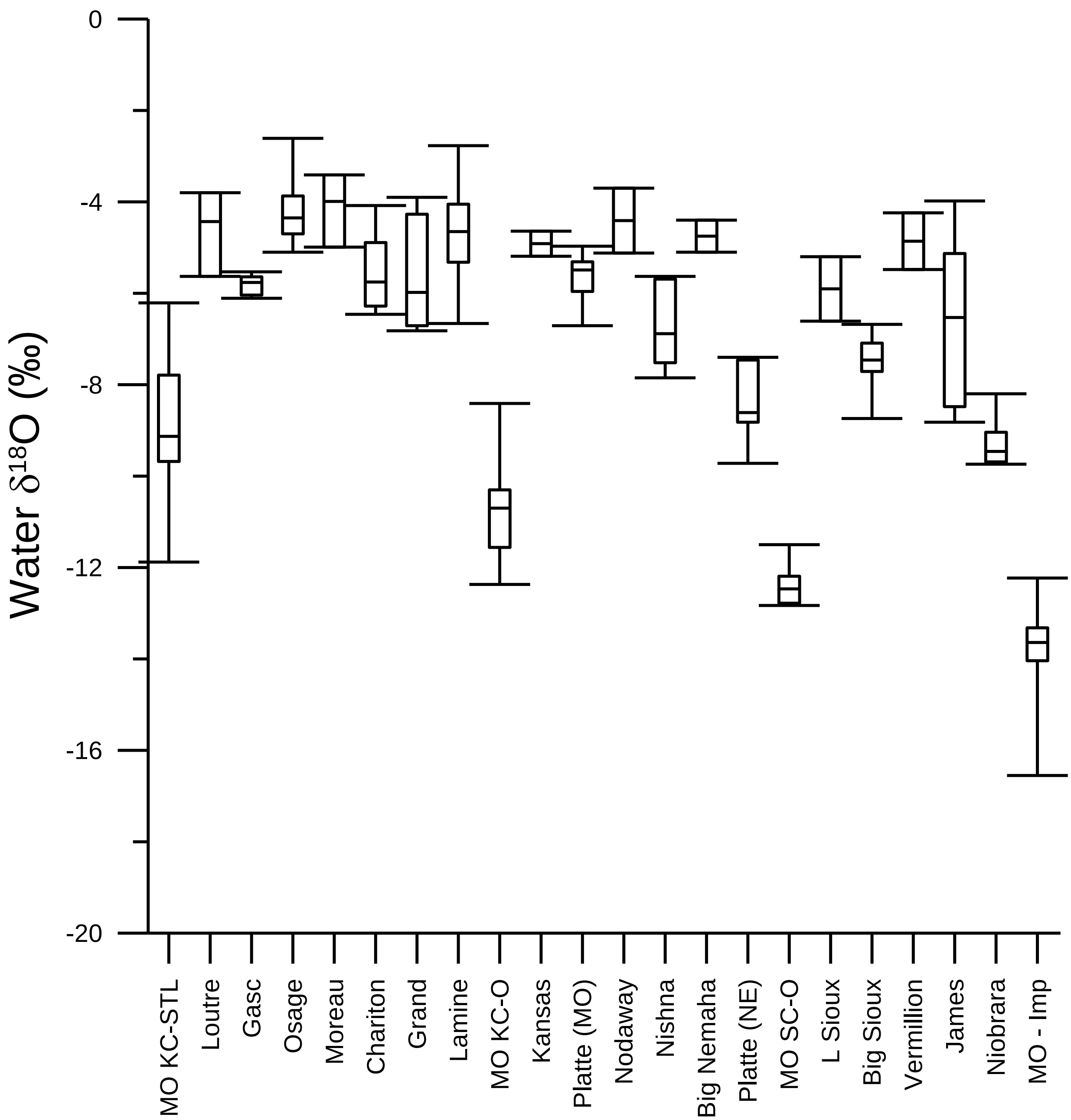

Figure 3. Boxplot showing ranges, interquartile ranges, and median water $\delta^{18}O$ for the Missouri River and major tributaries. Data are from samples collected for this study, prior collections by the Whitledge lab, and Coplen and Kendall (2000) (excluding winter collections). Site names on the x-axis are (from left to right): Missouri River between Kansas City and St. Louis, Loutre River, Gasconade River, Osage River, Moreau River, Chariton River, Grand River, Lamine River, Missouri River between Kansas City and Omaha, Kansas River, Platte River (Missouri-Iowa), Nodaway River, Nishnabotna River, Big Nemaha River, Platte River (Nebraska), Missouri River between Sioux City and Omaha, Little Sioux River, Big Sioux River, Vermillion River, James River, Niobrara River, and the impounded section of the Missouri River (upstream of Gavins Point Dam).

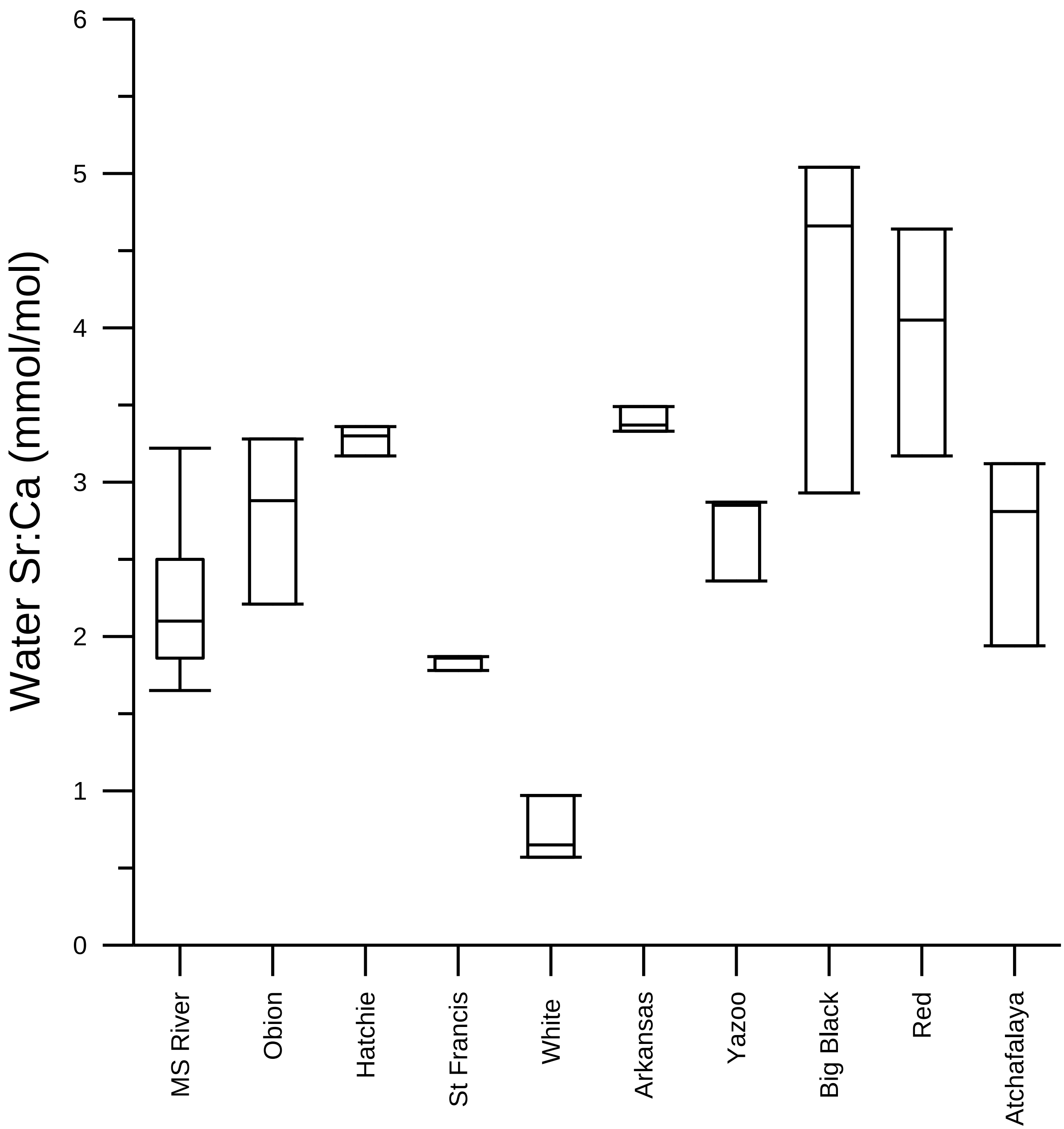

Figure 4. Boxplot showing ranges of water Sr:Ca for the Lower Mississippi River and major tributaries and distributaries; interquartile ranges and medians also shown for sites with sufficient sample sizes. Data are from samples collected for this study and prior collections by the Whitledge lab.

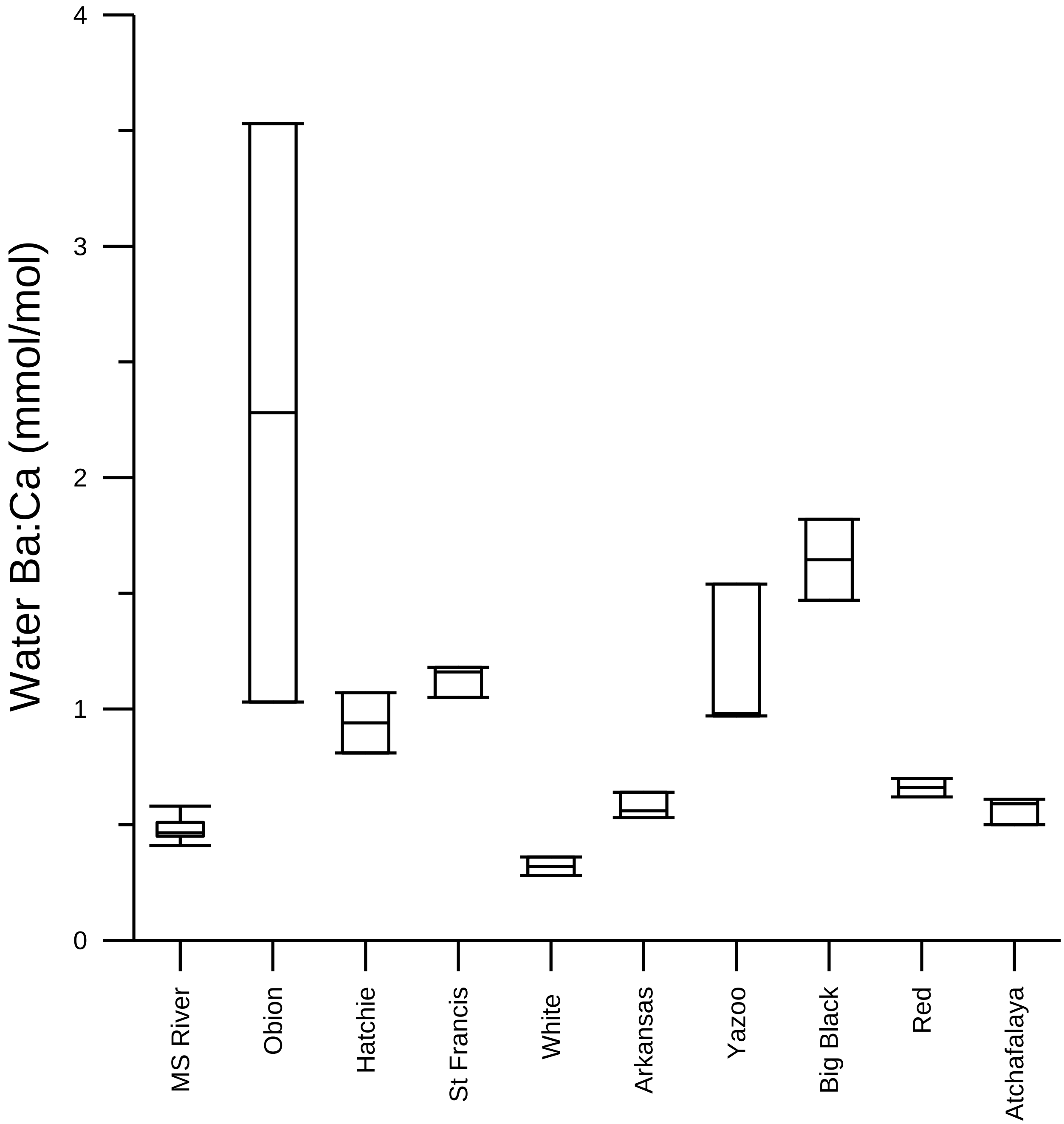

Figure 5. Boxplot showing ranges of water Ba:Ca for the Lower Mississippi River and major tributaries and distributaries; interquartile ranges and medians also shown for sites with sufficient sample sizes. Data are from samples collected for this study and prior collections by the Whitledge lab.

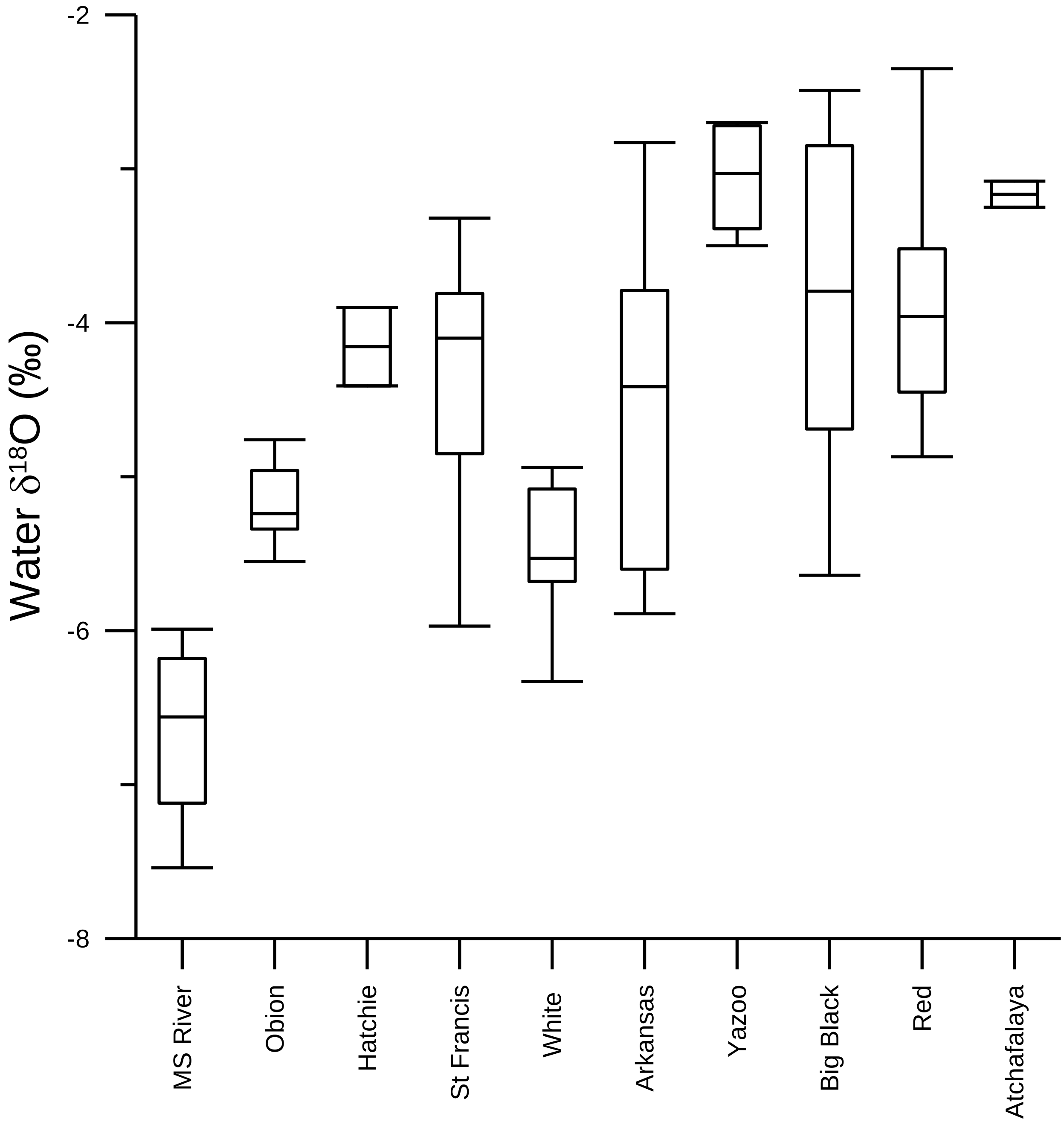

Figure 6. Boxplot showing ranges, interquartile ranges, and median water $\delta^{18}O$ for the Lower Mississippi River and major tributaries and distributaries. Data are from samples collected for this study, prior collections by the Whitledge lab, and Coplen and Kendall (2000) (excluding winter collections).

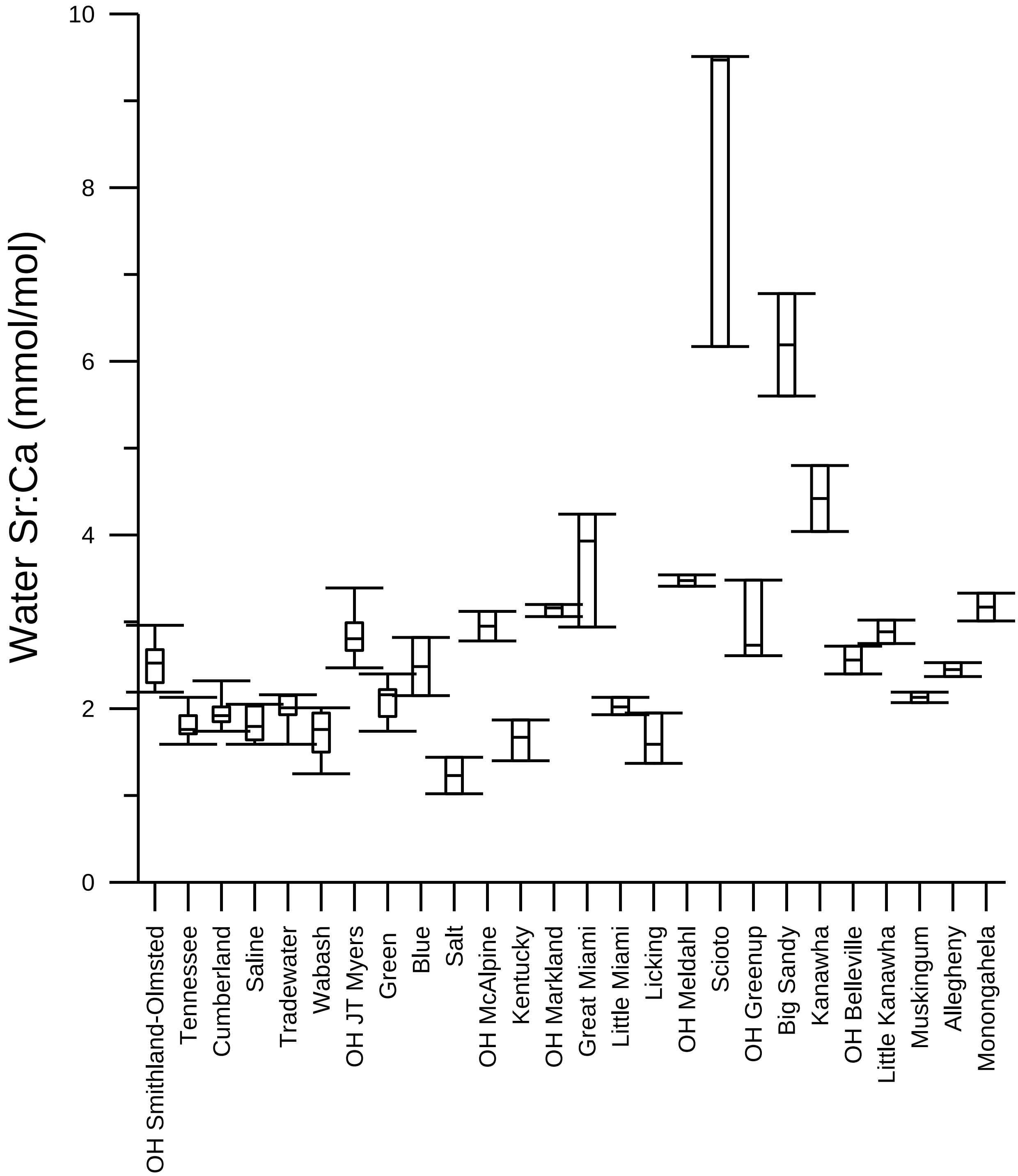

Figure 7. Boxplot showing ranges of water Sr:Ca for the Ohio River and major tributaries; interquartile ranges and medians also shown for sites with sufficient sample sizes. Data are from samples collected for this study and prior collections by the Whitledge lab. Ohio River (OH) sampling sites are listed by navigation pool where samples were collected.

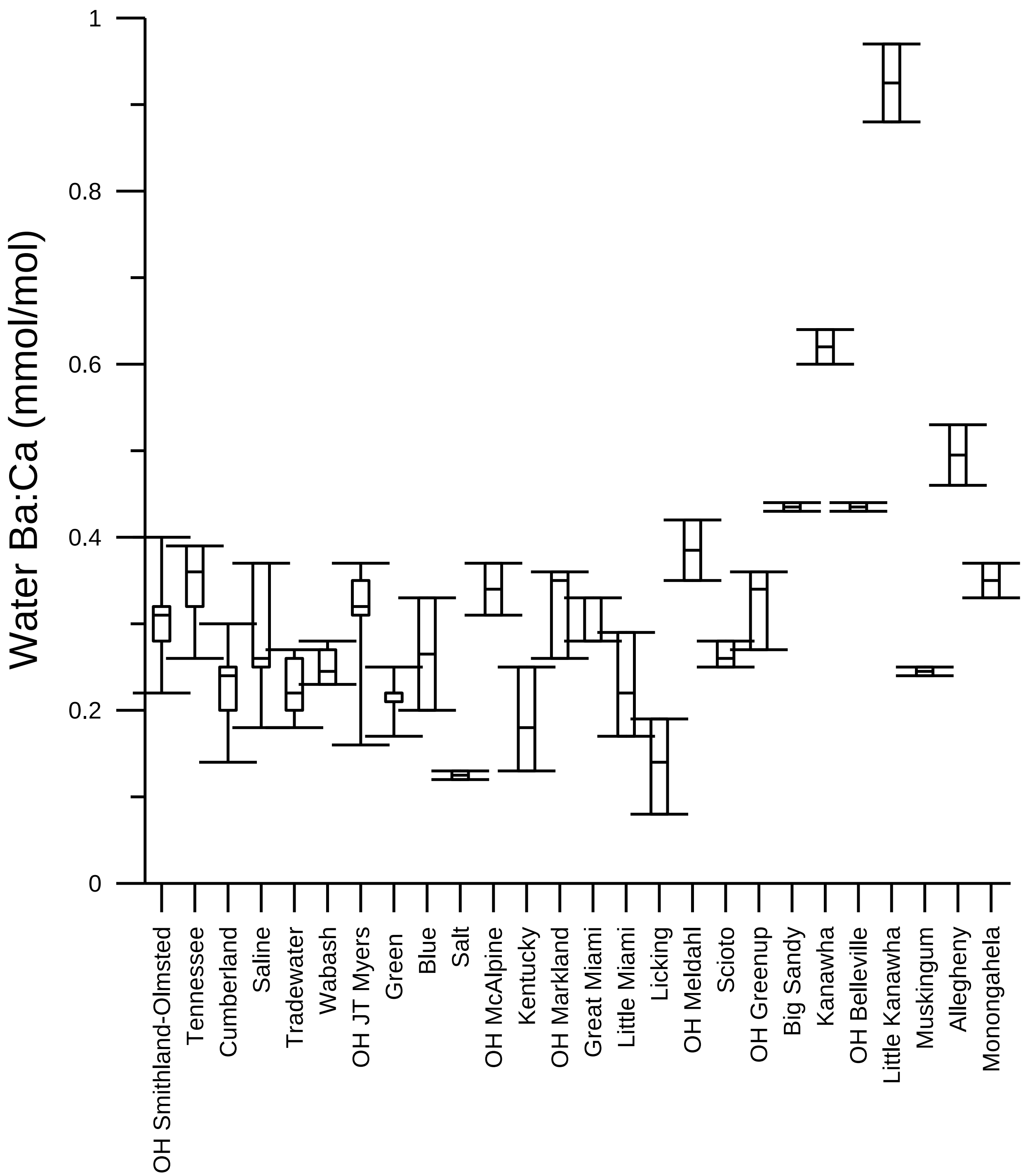

Figure 8. Boxplot showing ranges of water Ba:Ca for the Ohio River and major tributaries; interquartile ranges and medians also shown for sites with sufficient sample sizes. Data are from samples collected for this study and prior collections by the Whitledge lab. Ohio River (OH) sampling sites are listed by navigation pool where samples were collected.

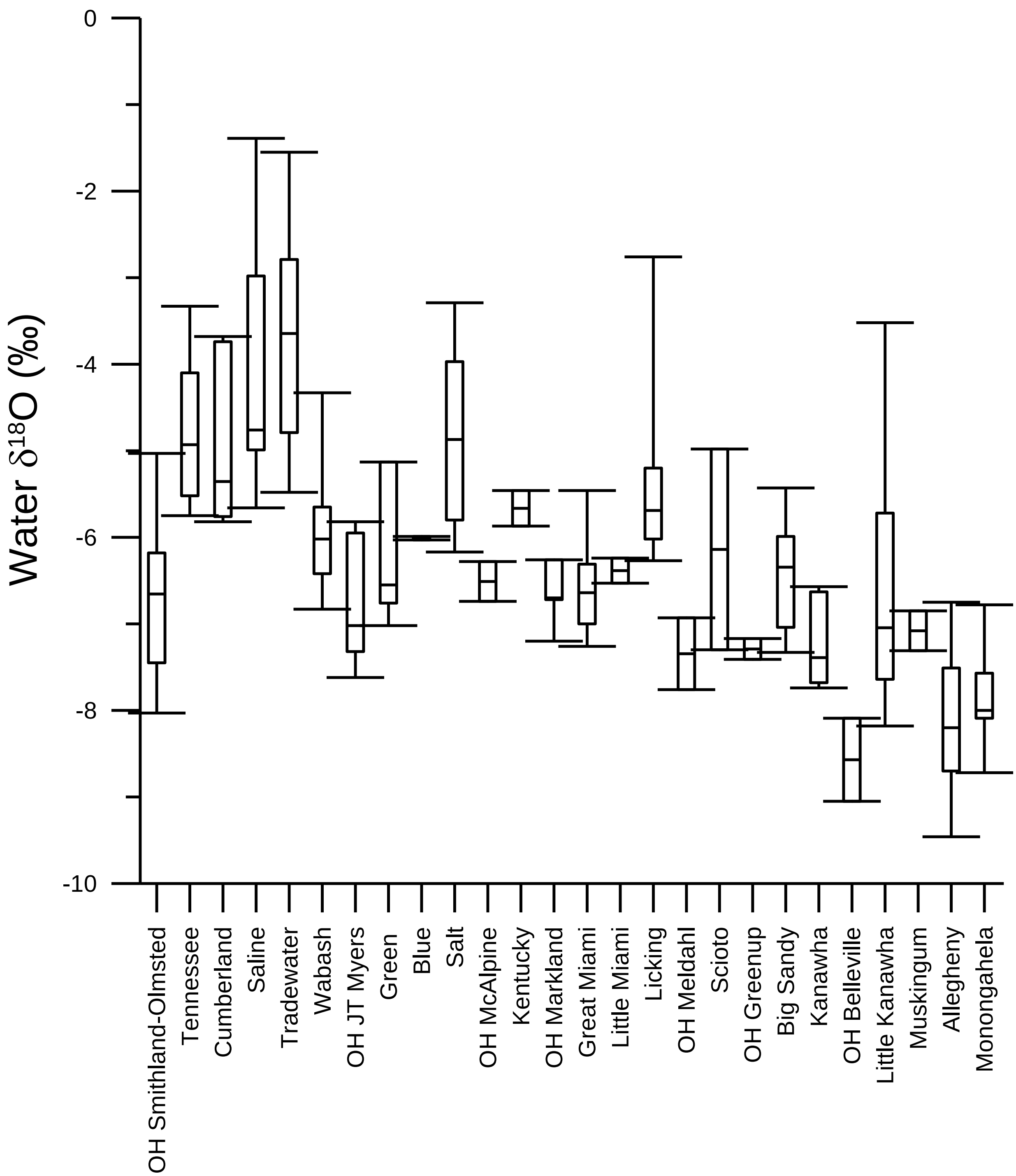

Figure 9. Boxplot showing ranges, interquartile ranges, and median water $\delta^{18}O$ for the Ohio River and major tributaries. Data are from samples collected for this study, prior collections by the Whitledge lab, and Coplen and Kendall (2000) (excluding winter collections). Ohio River (OH) sampling sites are listed by navigation pool where samples were collected.