

## Article

# Spatial Changes in Trace Elemental Water Chemistry in the Piedmont Ecoregion with Applications for Understanding Fish Movement

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**Abstract:** Trace elemental water chemistry in aquatic systems is primarily derived from the underlying geology of surrounding watersheds. Trace elements can be absorbed from the environment and become permanently incorporated into certain calcified structures of fish. Comparison of the trace elemental chemistry of these structures with the chemical signature of water from the watershed in which a fish was captured can help identify movement patterns. The goal of this study was to create a relatively fine scale baseline for the trace elemental water chemistry of the Chattahoochee, Ocmulgee, Oconee, and Savannah rivers within the Piedmont ecoregion of Georgia, U.S., and to distinguish any trace elements that may be useful in delineating different reaches. Principal component analysis was used to analyze ratios of Ba:Ca, Mg:Ca, Mn:Ca, Na:Ca, and Sr:Ca from samples in each river. Results suggest that Ba:Ca and Sr:Ca ratios may be useful in delimiting river reaches in each system. In addition, Na:Ca and Mn:Ca ratios may have some utility in further differentiating reaches in the Chattahoochee and Savannah rivers, respectively. The ability of these elements to differentiate reaches within these rivers lends support for their utility in fish microchemistry studies to help better understand and manage fish in these systems.

**Keywords:** trace element; strontium; barium; microchemistry; water chemistry; Georgia



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## 1. Introduction

Rivers provide an important ecosystem function by transporting organic compounds and nutrients that sustain aquatic organisms. These organic compounds and nutrients are derived from the surrounding watershed and give each river a specific chemical signature [1,2]. This signature, which can be described by the types and quantities of trace elements, becomes incorporated into the local ecosystem and the physiochemistry of organisms inhabiting those waters [3].

Trace elements in aquatic systems are found in minute concentrations, typically less than 1 mg/L, and are derived from natural and anthropogenic sources [2,4]. Natural sources of trace elements in aquatic systems typically arise from erosion and chemical weathering processes, leading to signatures that are representative of the local geology and can vary greatly based on the underlying lithology and catchment size [5–7]. For example, waters influenced by carbonate lithology (e.g., limestone) typically exhibit elevated levels of Ca, Mg, Sr, and Na, which vary depending on the specific type and quantity of carbonate rock in the catchment [8,9]. Anthropogenic factors such as mining, industry, agriculture, and urban runoff can also impact the trace elemental composition of an aquatic system.

These sources often lead to increased concentrations of trace heavy metals such as As, Ba, Cr, Pb, and Zn [4,10,11].

In fish, several dissolved trace elements, including Ba, Mg, Mn, Na, and Sr, have the potential to be absorbed from the water and subsequently integrated into calcified structures such as otoliths [12,13]. Otoliths are derived from calcium carbonate and grow continuously and incrementally throughout the life of a fish [14–16]. As the calcium carbonate matrix is being formed on the outer edge of the otolith, trace elements are permanently incorporated proportionally to their environmental availability and the physiology of the fish [13,16,17]. Through this incorporation, otoliths act as a permanent, sequential record of water chemistry [13]. Using a process known as hard-part microchemistry, the sequential deposition of these trace elements are analyzed, and the environmental history of a fish can be reconstructed. When coupled with trace element data from water samples, more specific life history characteristics of an individual fish may be elucidated, including natal origin, movement or migration patterns, and stock origin [12,18].

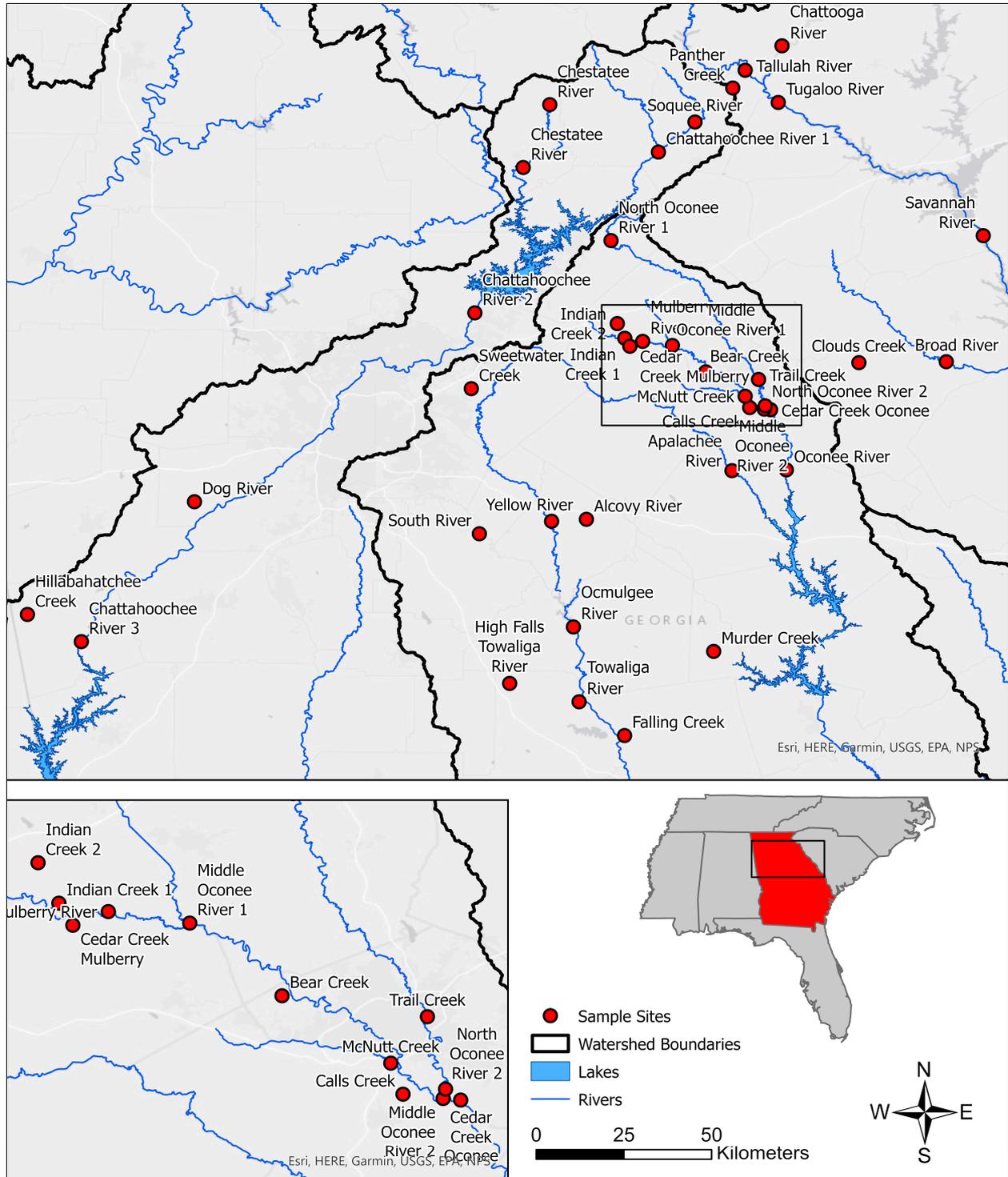
The Southeastern United States is a hotspot for freshwater biodiversity, containing 79% of the freshwater fish species found in the U.S. and Canada [19,20]. Due to this diversity and continued anthropogenic threats, the Southeastern United States also contains some of the greatest numbers of imperiled fishes [21–23]. Within the Southeast, the Piedmont ecoregion is defined as the area between the Appalachian Mountains and the Coastal Fall Line. In the state of Georgia, the Piedmont spans approximately 200 miles across the Blue Ridge Mountains in northern Georgia and extends between 150 and 200 km south and southeast towards the fall line. This section of the Piedmont is characterized by metamorphic or igneous lithology, landcover dominated by forests and agriculture, and a growing population with increasing urbanization [24–26]. The Piedmont region of Georgia contains 23 species listed as rare, threatened, or endangered by the state, 5 of which have threatened or endangered status under the federal government. As anthropogenic changes to these ecosystems exacerbate threats to these imperiled fish populations through habitat degradation and fragmentation, encroachment by invasive species, hybridization with non-native species, and overexploitation, a greater understanding of the habitat usage and movement of native and non-native fish is imperative to improve the management of imperiled species.

As awareness of the utility of fish hard-part microchemistry becomes more widespread and the process becomes increasingly affordable, the frequency of microchemical studies will likely increase. However, for these studies to be effective it is critical for water chemistry to vary at either spatial or temporal scales, and it is imperative that researchers verify these differences prior to undertaking a study [12]. While countless studies quantify trace elements or describe trends in water chemistry, few stand-alone water chemistry studies are conducted that either have the spatiotemporal resolution necessary or analyze trace elements that are applicable to fish microchemical analysis. This results in researchers collecting water chemistry data that are narrow in scope and typically only applicable to the species of interest. Moving forward, fine scale, regional baselines of trace element values can become an invaluable tool for future studies on the ecology and movement of local fish populations. Thus, the aim of this study was to characterize the trace elemental composition of the major rivers and tributaries of the Piedmont ecoregion within the state of Georgia, with a focus on its utility for future microchemical analysis.

## 2. Methods

Water samples were collected across major river systems within the Chattahoochee, Ocmulgee, Oconee, and Savannah river basins in the Piedmont region of Georgia (Figure 1; specific coordinates can be found in the Supplementary Material [Table S1]). Because

bedrock composition, soil, and land use can influence water chemistry, replicate samples from the selected sites were collected seasonally. This approach was employed to provide a reliable method for characterizing stable patterns in water chemistry and to identify areas that may fluctuate due to seasonal differences or anthropogenic inputs (e.g., wastewater treatment effluent).



**Figure 1.** Map of study sites from the Chattahoochee, Ocmulgee, Oconee, and Savannah River watersheds. Bottom left map shows a subset of sites from the Oconee. Each circle represents a distinct sampling site ( $n = 40$ ).

Water sampling was conducted in the spring, summer, and fall of 2024 in approximate three month intervals to assess spatiotemporal variations in trace element concentrations [27]. Due to the humid, subtropical nature of the region, which produces mild winters and little variation in seasonal precipitation, trace elemental variation between fall and winter were likely to be negligible, thus the winter sampling period was omitted due to time constraints. From the mainstem river within each river basin, one-liter water samples were collected longitudinally from the mouth of the river upstream at approximately 50 km intervals or when the interface between water bodies (e.g., tributary mouths) or potential anthropogenic inputs or features were encountered. The number of sample site locations for medium and small tributary rivers varied based on stream length, river connectivity, and perceived importance. However, the target number of sample sites was 40. Water samples were collected using a 250 mL polyethylene bottle, and a syringe filter was used to filter approximately 15 mL of sample water. All water sampling supplies were acid washed prior to use, and sampling procedures followed Shiller [28]. Samples were analyzed for Ba, Mg, Mn, Na, and Sr using inductively coupled plasma mass spectrometry at the University of Southern Mississippi's Center for Trace Analysis. A standard curve was created from standards purchased through High Purity Standards, specifically standards T209, T211, T241, and T251. Standards were then checked against USGS reference waters. Data were reported as the molar concentration for each trace element and converted to element:Ca (mmol/mol) ratios (i.e., Sr:Ca and Ba:Ca).

To assess how elemental concentrations between and within reaches of a river differ, a principal component analysis (PCA) was applied to each system using the `prcomp()` function in base R [29]. PCA works by reducing the dimensionality of the data set, creating principal components (PCs) that are linear combinations of variables that maximize explained variance in data [30,31]. This requires the assumption that there is some linearity between variables, which was assessed both visually and with a correlation plot using the `ggpairs()` function in the `GGally` package [32]. Despite showing the least amount of linearity between itself and other variables, the ratio of Mn:Ca was included in the analysis as it shows potential utility in microchemical studies and its potential relation to other variables, as well as its importance in describing the overall variation in these data, was deemed necessary [12,16,33]. Each PCA used all available elemental ratios (Ba:Ca, Mg:Ca, Mn:Ca, Na:Ca, and Sr:Ca), centered and scaled, reducing them to linear principal components (PCs). The number of principal components selected to describe each system was determined to be the fewest number of PCs that accounted for 80% or greater of the variance for a river [34]. The loadings on individual PCs were considered strong when loading values were above 0.75; moderate loadings were between 0.74 and 0.5, and weak loadings were 0.49 and below [35].

### 3. Results

Trace elemental water chemistry varied within each river basin of the Piedmont section of Georgia. For all river systems, incorporation of the top three PCs met the 80% threshold for described variance. While a large portion of the variance was described by the first two PCs of each PCA (67.6 to 74.4%), incorporation of the third PC increased the percentage of variance described to between 85.5 to 92.7% (Table 1). Trends in the loadings of individual PCs varied among systems; however, either Sr:Ca or Ba:Ca was always moderately loaded on the first PC (Table 2).

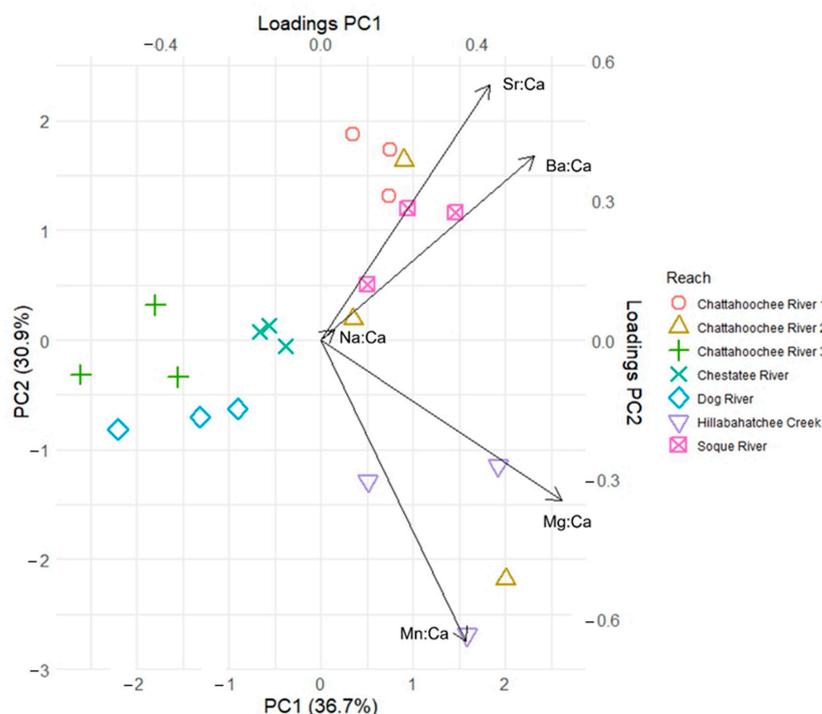
**Table 1.** Proportion of variance accounted for by the top three principal components for each watershed.

Watershed	Chattahoochee	Ocmulgee	Oconee	Savannah
PC 1	0.367	0.533	0.438	0.484
PC 2	0.309	0.204	0.274	0.260
PC 3	0.210	0.144	0.142	0.184
Total	0.886	0.881	0.855	0.927

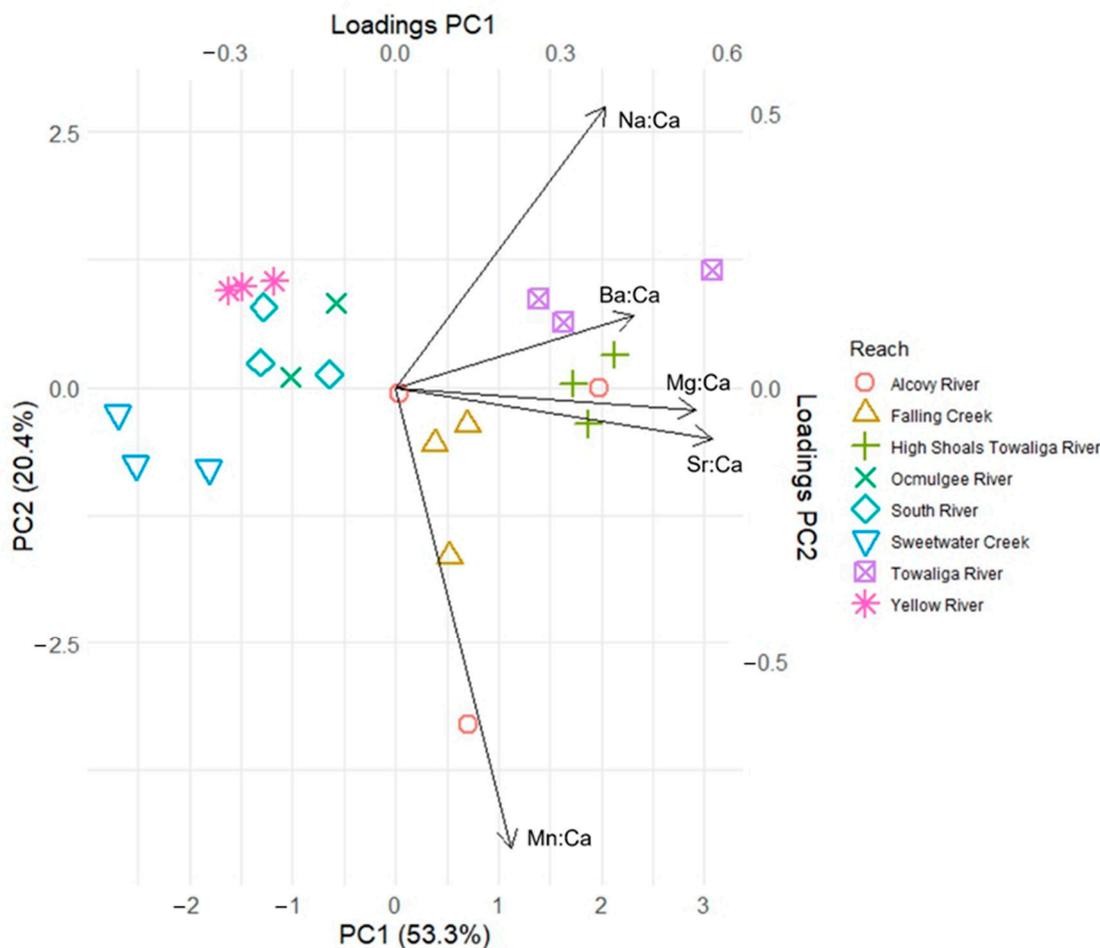
**Table 2.** Individual loadings of each elemental ratio for individual principal components (PCs) of each river system.

	Chattahoochee			Ocmulgee			Oconee			Savannah		
	PC 1	PC 2	PC 3	PC 1	PC 2	PC 3	PC 1	PC 2	PC 3	PC 1	PC 2	PC 3
Na:Ca	0.034	0.022	-0.966	0.380	0.513	-0.335	0.006	-0.675	-0.716	-0.526	0.407	-0.171
Mg:Ca	0.616	-0.346	0.161	0.544	0.040	0.517	-0.098	-0.667	0.669	-0.378	0.657	0.156
Mn:Ca	0.371	-0.649	-0.108	0.210	-0.842	-0.361	0.503	-0.271	0.175	0.238	0.209	-0.934
Sr:Ca	0.431	0.550	0.107	0.574	-0.092	0.328	0.593	0.157	0.053	-0.466	-0.508	-0.217
Ba:Ca	0.545	0.396	-0.131	0.431	0.132	-0.618	0.621	-0.029	-0.080	-0.554	-0.317	-0.163

For the Chattahoochee River basin, the first three PCs accounted for 88.6% of the variance in the watershed's data. Principal components 1, 2, and 3 described 36.7, 30.9, and 21.0% of the variance, respectively. PC 1 showed a moderate positive loading for Mg:Ca and Ba:Ca ratios, all other ratios were weakly positively loaded (Figure 2). PC 2 showed a moderate positive loading for Sr:Ca and a moderate negative loading for the Mn:Ca ratio. Mg:Ca was weakly negatively loaded and the remaining ratios were weakly positively loaded. PC 3 showed a strong negative loading for Na:Ca, while Mg:Ca and Sr:Ca were weakly positively loaded, and Mn:Ca and Ba:Ca were weakly negatively loaded.

**Figure 2.** Principal component analysis of the Chattahoochee River. The primary set of axes describes observation scores for each principal component. The secondary set of axes represents scaled loadings of individual variables.

The top three PCs for the Ocmulgee River basin accounted for 53.3, 20.4, and 14.4% of the variance, totaling 88.1%. PC 1 showed positive loadings for all elemental ratios; all ratios were weakly positively loaded except for Mg:Ca and Sr:Ca, which were moderately loaded (Figure 3). PC 2 showed a strong negative loading for Mn:Ca and moderate positive loading for Na:Ca. Sr:Ca and Mg:Ca were weakly negatively loaded and Ba:Ca was weakly positively loaded on PC 2. PC 3 showed moderate positive loadings for Mg:Ca, moderate negative loadings for Ba:Ca, weak negative loadings for Na:Ca and Mn:Ca, and weak positive loadings for the Sr:Ca ratio.

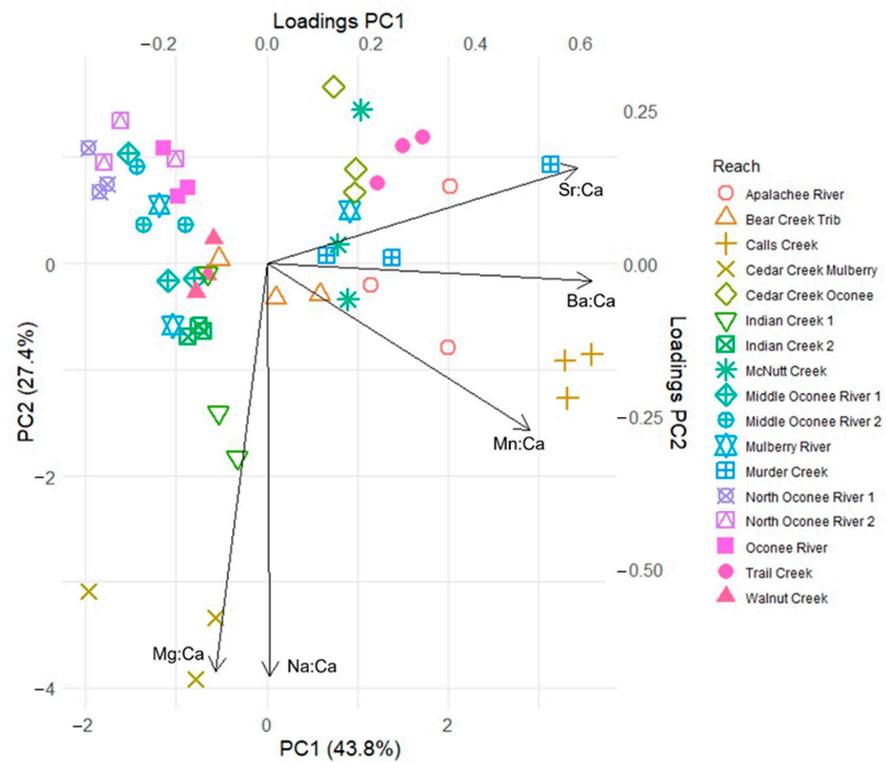


**Figure 3.** Principal component analysis of the Ocmulgee River. The primary set of axes describes observation scores for each principal component. The secondary set of axes represents scaled loadings of individual variables.

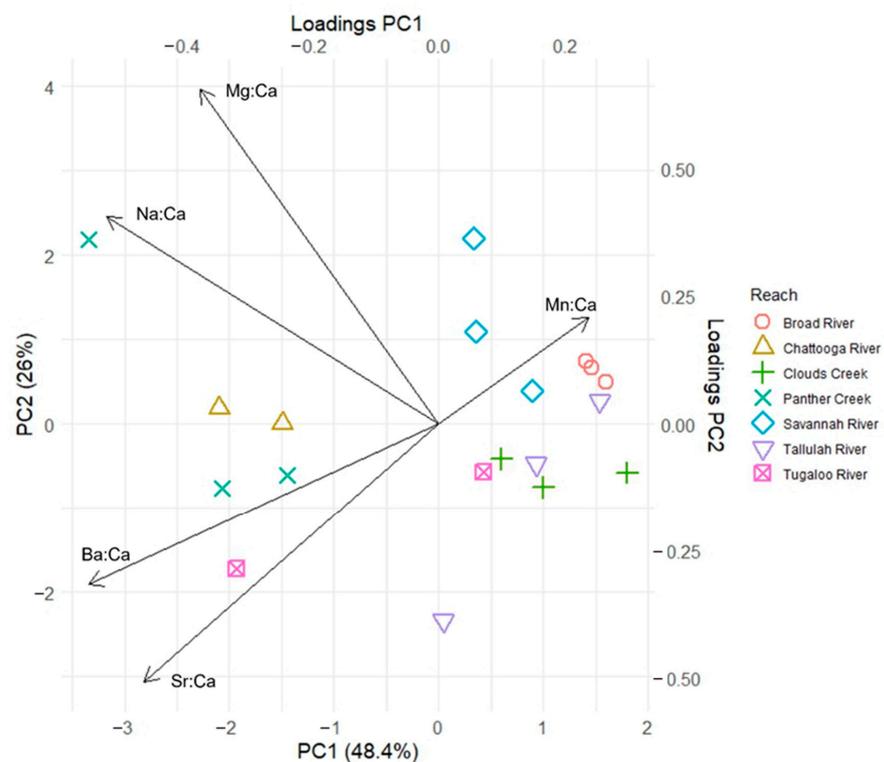
For the Oconee River basin, the top three PCs accounted for 43.8, 27.4, and 14.2% of the variance. Mn:Ca, Sr:Ca, and Ba:Ca ratios all contributed moderate, positive loadings to PC 1; Na:Ca and Mg:Ca showed weak positive and negative loadings, respectively (Figure 4). PC 2 had moderate negative loadings for Na:Ca and Mg:Ca, weak negative loadings for Mn:Ca and Ba:Ca, and weak positive loadings for Sr:Ca. PC 3 showed moderate positive loading for Mg:Ca, while Na:Ca had a moderate negative loading. Sr:Ca and Mn:Ca contributed weak positive loadings, and Ba:Ca contributed weak negative loadings to PC 3.

Within the Savannah River basin, PCs 1, 2, and 3 accounted for 48.4, 26.0, and 18.4% of the variance, respectively. PC 1 had moderate negative loadings for Na:Ca and Ba:Ca, weak negative loadings for Mg:Ca and Sr:Ca, and a weak positive loading for Mn:Ca (Figure 5). PC 2 had moderate positive loading for Mg:Ca and a moderate negative loading for Sr:Ca. Mn:Ca and Na:Ca contributed weak positive loadings, while Ba:Ca contributed a weak

negative loading on PC 2. PC 3 had a strong negative loading for Mn:Ca, weak negative loadings for Na:Ca, Sr:Ca, and Ba:Ca, and a weak positive loading for Mg:Ca.



**Figure 4.** Principal component analysis of the Oconee River. The primary set of axes describes observation scores for each principal component. The secondary set of axes represents scaled loadings of individual variables.



**Figure 5.** Principal component analysis of the Savannah River. The primary set of axes describes observation scores for each principal component. The secondary set of axes represents scaled loadings of individual variables.

#### 4. Discussion

Within each of the river basins, most replicate water samples from individual sites grouped together well, lending support to these sites having temporally stable trace elemental signatures. Of the sites where samples did not group, many were from smaller rivers and creeks where only a single sample of each set of replicates appeared to be an outlier. In these instances, differences in trace elemental concentrations were thought to be caused by seasonal variation in discharge, anomalous precipitation events, or anthropogenic influence exacerbated by the reaches smaller size [36–38]. For example, Murder Creek in the Oconee River watershed showed an elevated Sr:Ca ratio for a single sample, which may be attributed to the seasonal variation in discharge. During the summer months, discharge at this site is dominated by groundwater that flows through gneiss lithology (specifically biotite), which has been shown to produce elevated levels of strontium [39].

Within each river basin, many sites showed spatial differences in trace elemental composition. For example, Calls Creek and McNutt Creek both feed into the Middle Oconee River less than three river kilometers apart, but contain different trace elemental signatures, not only from each other, but from the river they feed into as well. In addition, trace element composition varied within the mainstem Chattahoochee River, where sites 1 and 2 were similar, but could be differentiated from site 3, which is downstream of a major metropolitan U.S. city (Atlanta, Georgia).

Previous research has indicated that Sr and Ba have the best applicability for pairing water concentrations to fish hard-part structures for the assessment of environmental life history [12,16,17]. In our study, Sr:Ca or Ba:Ca was associated with moderate loadings on the first or second PC of each PCA, suggesting that these elements were influential in differentiating water chemistry among rivers within each basin. In addition, Mn, which has been shown to have some utility in differentiating environmental life histories in fish [16,33], was found to contribute a strong loading to PC 3 of the Savannah system, while all other elemental ratios showed very weak loadings, suggesting that it may be useful in further differentiating this system. Similarly, Na was strongly loaded to PC 3 of the Chattahoochee system; however, Na is less frequently used in microchemistry studies and may have limited utility [12,40]. Despite this, it is likely that Ba, Mn, and Sr can be analyzed in fish hard parts to address ecological and management questions in the Piedmont regarding fish dispersal and movement, spawning behaviors, and natal origin. This is encouraging, as the Piedmont ecoregion hosts a variety of mobile native fishes, many of which have declined in abundance due to various anthropogenic activities that have led to alteration of flow regimes, degradation of habitat, and competition with non-native species [41–43].

This study has shown evidence of spatiotemporal trends in trace elements within the Piedmont ecoregion of Georgia that lend support for their utility in fish hard-part microchemical studies. In addition, these data will serve as an important baseline for the river systems of this region, where the expansion of population centers and accelerated climate change may result in widespread changes in water chemistry. Because little is known about the trace elemental water chemistry in most of the rivers we assessed, continued monitoring of these sites can better inform the understanding of the baseline water chemistry values. Long-term monitoring can also provide more nuanced insights into the magnitude and variability seen in seasonal trace elemental compositions and how these trends may be impacted by climate change. We recommend using trends described in this paper to formulate hypotheses and encourage researchers exploring specific questions to collect additional trace element samples related to their study, as baseline values may shift due to climate change, land use, or other anthropogenic factors. Additionally, finer spatiotemporal scales may be necessary depending on the life history characteristics of the species of interest. We also recommend the expansion of water chemistry sampling

across the Piedmont ecoregion, which covers a vast area in the United States, ranging as far north as Maryland and south to Alabama. Based on the heterogeneity of the underlying lithology, as well as spatiotemporal trends seen in this study, it is likely that similar patterns exist across the region, lending its potential utility to future microchemistry studies. Lastly, establishment of a range-wide reference map of trace elemental water chemistry among river basins in the Piedmont would be beneficial for researchers working in this important ecosystem to conserve and enhance aquatic systems.

**Supplementary Materials:** The following supporting materials can be downloaded at: <https://www.mdpi.com/article/10.3390/hydrobiology4020009/s1>, Table S1: List of each stream and river collection site along with its watershed and specific latitude and longitude.

**Author Contributions:** A.T.B. contributed to the statistical analysis, creation of graphics, writing, and editing of the manuscript. W.L.G. contributed to project design, graphics creation, sample collection, and editing of the manuscript. S.F.M. contributed to project design, sample collection and preparation, and editing of the manuscript. M.J.H. secured funding for and designed this project and contributed to the writing and editing of the manuscript. All authors have read and agreed to the published version of the manuscript.

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