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Flowing down the river: influence of hydrology on scale and accuracy of elemental composition classification in a large fluvial ecosystem

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Highlights

- Limitations of fish otolith chemistry analyses depend on regional water chemistry
- Hydrodynamics and geology alter the spatiotemporal composition of water
- Discharge–concentration relationships in the St. Lawrence River tributaries are weak
- Regional variations suggest that otolith chemistry analyses at the tributary scale are possible

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Abstract

Trace metals found in the calcified structures of fish (i.e. otolith, scales and vertebrae) serve as proxies for the ambient water composition at the time of mineralization, and these trace metals are increasingly used as a tool for assessing population structure and the migratory patterns of fish. However, the appropriate scale (e.g. resolution) for such applications can be uncertain because of a poor understanding of the spatiotemporal variations of metal-to-calcium ratios (Me:Ca) in the studied watersheds. This study aims to assess Me:Ca spatiotemporal variability within the St. Lawrence River and nine major tributaries and evaluate the ability of random-forest models to correctly identify rivers on the basis of their elemental composition. We tested the influence of daily discharge on four measured ratios (Sr:Ca, Ba:Ca, Mg:Ca and Mn:Ca) to document local and regional trace element sources and dynamics. The four element ratios displayed a low spatiotemporal variation, reflecting a marked stability over time. We observed that most element- and tributary-specific concentration–discharge relationships were either not significant or showed a weak influence, thereby confirming a stable point source dynamic. The classification performance based on a four-element model (Sr:Ca, Ba:Ca, Mg:Ca and Mn:Ca) produced a classification accuracy of 92.5%, which correspond to a small decrease of accuracy compared to the full model (25 elements, 96.6% of correct classification). A classification based on two elements (Sr:Ca and Ba:Ca) produced a lower classification accuracy (72.6%). Classification errors related mainly to tributaries in close proximity, a problem tempered by grouping these geochemically similar watersheds. Our results show that surveys of the elemental fingerprint of regional tributaries within a given region can provide critical information to determine the appropriate scale (tributary or watershed) for trace metal analysis of the hard-calcified parts of fish.

Keywords: Otolith chemistry, Concentration–discharge, Water chemistry, Random forests

1. Introduction

In freshwater ecosystems, bedrock chemistry, as a major source of weathering dissolution, can explain much of the water elemental composition (Humston and Harbor, 2006; Liu et al., 2000). Local and regional hydrodynamics can, however, have sufficient influence to preclude prediction based solely on geological information. Covariance among river discharge and weathering-diluted solutes have been widely observed (Moon et al., 2014), providing evidence of a relationship between hydrodynamics and water chemistry. In addition, other factors such as land use, landscape, industrial wastewater and riverine mixing patterns strongly influence the water chemistry of lakes, streams and rivers and could decouple covariance with discharge (Baronas et al., 2017). The monitoring of trace elements and their relationship to water flow—called concentration–discharge (C–Q) relationships (Baronas et al., 2017; O'Connor, 1976)—has successfully contributed to assess water sources and mixing patterns (Neal et al., 1996). Hence, analysis of C-Q relationship in a given freshwater ecosystem could inform on the processes contributing to observed water chemistry variations. This approach could inform the potential factors affecting accuracy of using water chemistry as an ecological tracer of fish origin and movements through the analysis of hard-parts (i.e. scales, otolith, vertebrae) elemental composition (Pracheil et al., 2014).

Otoliths (ear stones) are calcified structures of the inner ear of fish. These structures undergo continuous growth through the daily deposition of a new layer, proportional to the fish's somatic growth (Pannella, 1971). Otoliths are composed of calcium carbonate ($CaCO_3$, approximately 98%) and a smaller proportion (0.1%–2.3%) of organic matter (soluble and insoluble protein matrix), as well as approximately 45 elements in either minor (>100 ppm) or trace (<10 ppm) amounts (Campana, 1999; Sturrock et al., 2012). Some elements are incorporated into the otolith matrix at concentrations representative of the ambient water experienced by fish at that specific time (Doubleday et al., 2013; Izzo et al., 2018). Hence, the trace element composition of otoliths (or otolith chemistry) can provide information related to individual fish life histories (Campana, 1999). Otolith chemistry, assessed by bulk otolith analysis or precise point–based sampling, is increasingly used to clarify fish migratory history (Elsdon and Gillanders, 2003a; Morissette et al., 2016; Secor and Piccoli, 2007), define population structure (Lazartigues et al., 2018; Lazartigues et al., 2016; Wright et al., 2018) and assess mixed stocks composition (Tanner et al., 2016). The detection of biologically significant variations in otolith chemistry arises from the movement of virtually any teleost fish within a sufficient “elemental gradient” or from the fish using habitats characterized by divergent “elemental fingerprints”. Those elements gradients, however, are seldom described by measurements of trace elements in the water as a preliminary step of otolith chemistry studies (Pracheil et al., 2014), which could cause uncertainty on the appropriate scale to based inference on.

The most-studied chemical gradient experienced by fish is, without a doubt, the drastic freshwater-saltwater interface observed in estuarine habitats. The meeting of these two distinct water masses produces a steep gradient where elements such as strontium (Sr) and barium (Ba) vary by more than an order of magnitude across a short geographical distance (Taddese et al., 2019; Tanner et al., 2013). Elemental gradients in freshwater ecosystems are, however, generally weaker than estuarine gradients (Radigan et al., 2017; Zeigler and Whitledge, 2011) and are characterized by much less predictable, sporadic local variations (Chapman et al., 2013). For instance, large fluvial ecosystem such as the St. Lawrence River (Canada) exhibit significant spatial variations in elemental composition (Lum et al., 1991; Yeats and Loring, 1991). Understanding elemental composition dynamics is a prerequisite to develop applications for the management of various stressors on the fish community commonly observed in large fluvial ecosystem such as overexploitation, poaching, habitat fragmentation or invasion of exotic species.

Inferences related to fish migration that are derived from otolith chemistry are generally based on a few elements. Studies documenting the relationship between water and otolith trace metal concentrations, summarized as the partition coefficient $(Me: Ca)_{otolith}/(Me: Ca)_{water}$ (Campana, 1999), have demonstrated that Sr:Ca and Ba:Ca coefficients are positive for most species and environments (Brown and Severin, 2009). These conclusions leave little doubt that the concentrations of these elements in water influence the composition of the accreted otolith material to some extent. The published coefficients for manganese (Mn:Ca) are somewhat contradictory (Miller, 2009), although they suggest an environmental influence (Elsdon and Gillanders, 2003b); this is not the case for magnesium (Mg:Ca) because the partition coefficients of magnesium are generally low (<0.001) or nonsignificant (Elsdon and Gillanders, 2003a; Wells et al., 2003). The most parsimonious classification models should then be limited to the use of Sr:Ca and Ba:Ca. Nonetheless, adding Mg:Ca and Mn:Ca to the model can improve the accuracy of watershed classification, as observed in multiple studies, including ones in the St. Lawrence River

(Lazartigues et al., 2018; Morissette et al., 2016), and the possible contribution of these ratios to classification models should not be dismissed completely.

Otolith chemistry has been successfully used in the St. Lawrence River system to delineate the reproductive stock structure of yellow perch (*Perca flavescens*) fisheries (Lazartigues et al., 2018), identify the early reproductive and migratory behavior of the reintroduced striped bass (*Morone saxatilis*, Morissette et al., 2016; Vanalderweireldt et al., 2019) and determine the migratory behavior of the American eel (*Anguilla rostrata*, Benchetrit et al., 2015). These studies, however, have generally been conducted at a low resolution (i.e. salinity front). Finer resolution analyses can improve the delineation of the stock structure of numerous fish populations for which the relative importance of the St. Lawrence River and its tributaries during the various phases of their life cycle and consequent population dynamics remains to be quantified. Otolith chemistry also represents a promising tool for studies related to biological invasions (Crook et al., 2013; Morissette et al., submitted; Thibault et al., 2010) and wildlife forensic sciences (Bourret and Clancy, 2018), a field for which it remains underutilized.

This study has the objective of summarizing the elemental composition of the fluvial section of the St. Lawrence River and its major tributaries in order to explore regional spatiotemporal variations in elemental composition and the consequent effects on the accuracy of identifying and classifying rivers based on their water chemistry. We analyzed element concentrations in the various rivers to quantify the intra- and interannual variability of elemental compositions in the tributaries and isolate the influencing factors. Additionally, we assessed the C–Q relationships for Sr:Ca, Ba:Ca, Mg:Ca and Mn:Ca in the studied tributaries in relation to estimates of daily discharge to define elemental source dynamics and the origin of temporal variations. Finally, we tested the accuracy of multiple classification models to explore the potential of using fish hard-parts chemistry for identifying rivers at the tributary scale. This study aimed to provide a detailed depiction of the trace metal fingerprints of a large fluvial ecosystem and its tributaries and improve the understanding of trace metal dynamics to promote diverse applications in fisheries management, and conservation for large river ecosystems.

2. Methods

We obtained the trace metal concentration data from the *Banque de données sur la qualité du milieu aquatique* (BQMA) database compiled by the province of Québec's *Ministère de* *l'environnement et de la lutte contre les changements climatiques* (MELCC, 2020). This database includes concentrations (µg/L) of 25 trace elements (Ag, Al, As, Ba, B, Be, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, Pb, Sb, Se, Sr, U, V, Zn) and the physicochemical parameters for each sample station. Concentrations of trace metal have been quantified by inductively coupled plasma mass spectrometry (ICP-MS). Raw data and detailed analytical protocols are available via open access through an interactive interface (MELCC, 2020). To quantify the spatiotemporal variation of trace metal concentrations, we restricted our analysis to freshwater tributaries for which we had more than four years of monitoring. Sites of water sampling were chose to be easily accessible and exempt of any exogenous source of water (i.e. small tributaries or storm water channel). Our data set, therefore, covers nine tributaries of the St. Lawrence River, all located between Montréal and Québec City (Fig. 1). Between May 2009 and October 2017, water samples were retrieved on a monthly basis from a single station near the mouth of each sample tributary (Fig. 1). In addition to these sites, we created a single site by combining two St. Lawrence River sampling regions (St. Thérèse and Tracy), each including three sites (North Shore, Center and South Shore). We chose to pool these data into a single site, as high intersite similarities in St. Lawrence River produced high confusions in subsequent classifications models (data not shown).

We normalized trace metal concentrations (µg/L) according to the calcium concentration measured at the same sampling site (mg/L). Metal-to-calcium ratios (Me:Ca) were expressed as molar concentrations ($mmol \cdot mol^{-1}$). Since the uptake of trace elements by fish gills decreases as the concentration of dissolved calcium increases (Mayer et al., 1994), the absolute concentration of trace metals is generally a poor predictor of their environmental availability and their eventual accretion in otoliths (Campana, 1999). Ratios of trace element concentrations as a function of calcium offer a better representation of the elements available for integration into the carbonate matrix of the otolith, and these ratios should be privileged when available. We therefore conducted subsequent analyses using the Me:Ca values for every studied element.

We tested the differences of Sr:Ca, Ba:Ca, Mn:Ca and Mg:Ca between sites (sites, $N = 10$) using one-way analysis of variance (ANOVA). Intersite differences were identified by post hoc Tukey HSD tests. We tested for intrasite variability using repeated-measure analyses of variance (RM-ANOVA), where the response variables (Me:Ca) were a function of sampling years (fixed factor) and date of sampling nested in years (fixed factor). We treated tributaries (sites) as the repeated factor to account for the non-independence of the monitoring data. We ran both ANOVA models using the function *aov* in the R software (R Development Core Team, 2008) base package.

Linear mixed-effects models assessed the intrasite variations of trace metal C–Q relationships as a function of the adjusted discharge at the site of water sampling. This modelling approach have been chose as its offers possibility of considering intrasite variability and producing variable slopes and intercept within a single model. We retrospectively estimated the adjusted discharge using observed discharge at the available gauge stations and their relative position to water sampling stations, following the method of Lachance-Cloutier et al. (2017). No discharge data were available for Des Prairies River and the St. Lawrence River site. The influence of a large dam complex on the St. Maurice River prevented an accurate estimate of daily discharge at the sampling station. We therefore excluded those three sites from the subsequent analysis of C–Q relationships. We modeled Me:Ca ratios (dependent variable) as a function of adjusted daily discharge (dependent variable, natural-logarithm transformation) nested in sites, with adjusted discharge nested in sites treated as a random factor to account for study design and allow varying intercepts and slopes for every site. We considered slopes of site-specific regressions to be significantly different from 0 when $p < 0.05$ (t-test). Linear mixed-effects models were run using the 'nlme' R package (Pinheiro et al., 2018).

To estimate the identification accuracy of the elemental composition of the tributaries, we built three different classification models using the random forest (RF) algorithm. Classification by RF was shown to be outperforming parametric models (ex. linear discriminant analysis) for otolith chemistry data when raw data are not respecting multi-normality assumptions (Jones et al., 2016; Mercier et al., 2011), which is the case for the present study. Three classification models were built to evaluate the accuracy of identification on the basis of (1) the full elemental fingerprints (25 elements) of the sites, which represent the higher classification accuracy reachable with this dataset; (2) the most frequently used trace elements in otolith chemistry, as noted in the published literature (Sr:Ca, Ba:Ca, Mn:Ca and Mg:Ca); and (3) elements exhibiting the most robust partition coefficients between water and otolith concentrations (Sr:Ca and Ba:Ca). To assess the performance of each model, we subdivided the data set at random to create training (75% of the data) and test (25% of the data) data sets. The RF model was adjusted a priori, using the sampling sites as a classification factor, and the adjustment was carried out by 1000 classification trees. We reclassified the test data set and estimated the correct reclassification to test the performance of the

RF model. Random-forest modeling was run using the 'randomForest' R package (Breiman and Cutler, 2018).

3. Results

Our water chemistry data set consisted of 580 samples distributed over ten sites. Each site was sampled for at least four years (5–6 monthly samples per year) and for a maximum of eight years. ANOVA showed significant between-site differences for the concentrations of the four elements (Fig. 2). Moving upstream to downstream, the Sr:Ca differences between sites (ANOVA, $F_{9,569}$ = 229, p < 0.001) increased, with the St. Charles River having the highest value in the data set ($Sr:Ca_{avg} = 4.85 \pm 0.56$ mmol·mol^{-1}). Conversely, Ba:Ca (ANOVA, $F_{9,570}$ = 859, p < 0.001) values generally decreased from upstream to downstream, although the St. Maurice River showed the highest values in the studied tributaries ($Ba:Ca_{avg} = 1.13 \pm 0.17$ mmol·mol^{-1}). The main steam of the St. Lawrence River and all the tributaries of the floodplain displayed high Mg:Ca values (ANOVA, $F_{9,580}$ = 265, p < 0.001), except for the St. François, Chaudière and St. Charles rivers. Finally, Mn:Ca (ANOVA, $F_{9,569}$ = 271, p < 0.001) tended to increase heading downstream, with the exception of sites located within the St. Lawrence River, which had low and variable values ($Mn:Ca_{avg} = 0.07 \pm 0.06$ mmol·mol^{-1}). The analysis of intrasite variability showed that there were no significant interannual or intra-annual differences for Sr:Ca ($RM\text{-}ANOVA_{inter}$, $F_{4,2}$ = 4.31, p = 0.20; $RM\text{-}ANOVA_{intra}$, $F_{3,2}$ = 0.51, p = 0.72) and Mg:Ca ($RM\text{-}ANOVA_{inter}$, $F_{4,2}$ = 2.95, p = 0.27; $RM\text{-}ANOVA_{intra}$, $F_{3,2}$ = 0.24, p = 0.86). However, Ba:Ca exhibited a significant intra-annual variability ($RM\text{-}ANOVA_{intra}$, $F_{3,2}$ = 238.89, p = 0.004), and Mn:Ca was variable both inter- and intra-annually ($RM\text{-}ANOVA_{inter}$, $F_{4,2}$ = 24.55, p = 0.04; $RM\text{-}ANOVA_{intra}$, $F_{3,2}$ = 88.83, p = 0.01). These patterns are attributable mainly to the large variations observed in the St. Maurice and St. Charles rivers for this element (Fig. 3).

Modeling the effect of the estimated daily discharge on Me:Ca values showed an overall marginal influence. Hence, most site/element combinations (57.1%, 16/28 tests) displayed weak or nonsignificant C–Q relationships (Appendix A). We observed significant relationships for the Assomption, Yamaska, St. François, Chaudière and St. Charles rivers (Fig. 4). Specifically, Sr:Ca values correlated negatively with discharge in the Yamaska, Chaudière and St. Charles rivers. Ba:Ca values showed a positive correlation with discharge in the Chaudière and Des Prairies rivers and a negative correlation with discharge in the St. François and St. Charles rivers. Mn:Ca values correlated positively with discharge in the Yamaska, Chaudière and St. François rivers, but this pair of variables were negatively correlated in the St. Charles River. Finally, Mg:Ca and discharge had a positive correlation for the Assomption River. Me:Ca values in the Des Prairies and Richelieu rivers were weakly influenced by discharge through the observed range of discharge levels. Calcium concentrations were negatively correlated with discharge for all rivers except the Des Prairies and Richelieu Rivers, where concentrations were mostly constant for all estimated discharges. The absence of an influence of daily discharge on Me:Ca in most tributaries showed that calcium and trace metal concentrations remain highly coupled under fluctuating hydrologic conditions, thereby explaining the stability of the ratios.

The accuracy of the RF model classification varied depending on the number of elements considered in the model. The full model (25 elements) produced a classification accuracy of 96.6%. Most classification errors in the model were attributed to the Des Prairies River (91.8% correct) and Richelieu River (93.8% correct). Ba:Ca and Mn:Ca were, respectively, the first and second most important elements for classification within the full model. The four-element (Sr:Ca, Ba:Ca, Mn:Ca and Mg:Ca) model produced a classification accuracy of 92.5%. We observed most classification errors for the Des Milles-Iles (78.1% correct) and Des Prairies rivers (89% correct), two rivers originating upstream of the Ottawa River. Eight of ten sites showed classification accuracy greater than or equal to 90%. The RF classification model based on the concentration of the two major trace elements (Sr:Ca and Ba:Ca) were 72.6% accurate. The classification accuracy of the Richelieu River was the lowest (61.9% correct), most often confused with the two adjacent tributaries (St. François and Yamaska rivers, Table 1). These three rivers are all located along the south shore of the St. Lawrence River and are located relatively close to each other. Six sites had classification accuracy greater than 85%. The St. Charles and St. Maurice rivers were perfectly classified (100% correct), a result potentially attributed to their high Sr:Ca and Ba:Ca values, respectively (Fig. 5). Pooling the data of the Des Prairies and Des Milles-Iles rivers (Ottawa River water mass) and the Richelieu, St. François and Yamaska rivers (south shore tributaries) increased the classification accuracy of the two-element model to 84.1%.

4. Discussion

Assessment of the geographical and seasonal variability of trace elements represents an opportunity for a more objective evaluation of the appropriate resolution for any analyses of otolith chemistry or other fish hard-parts (Walther, 2019). Knowledge of the local, regional or continental elemental gradients can also establish the possibilities and limits of achievable inferences (Pracheil et al., 2014; Whitledge, 2009). Such portraits also represent an important step for identifying redundancy and anomalies within the trace element landscape; for example, the identification of the Sr-rich waters of the Sandusky River (western basin of Lake Erie) was vital for using otolith chemistry to assess the recruitment and migration of invasive grass carp in the Great Lakes (Chapman et al., 2013). In our study, the spatiotemporal variability of dissolved Me:Ca values, assessed over a major portion of the ice-free season, was relatively low. Most intrasite variations, if present, were lower than the observed intersite variations over the study period (>5 years). This result highlights the relative stability of the observed Me:Ca values of the major St. Lawrence River tributaries. Our results showed that the limited intrasite variability and the consistent intersite differentiation will most likely allows for accurate identification of rivers elemental fingerprint and the resulting fish hard-parts chemistry.

4.1 Elemental landscape of the St. Lawrence River system

We observed the presence of positive (Sr:Ca and Mn:Ca) and negative (Mg:Ca) upstream–downstream gradients among the study sites. Such gradients are concordant with previous observations from the St. Lawrence main stem and tributaries (Benchetrit et al., 2015; Lum et al., 1991; Yeats and Loring, 1991). Most studies of trace element concentrations in the system, however, targeted water quality assessments and did not apply *per se* to fish hard-parts chemistry. This specificity caused key information to be either missing (i.e. temporal variability, ratios to calcium content and missing elements) or inappropriate. Our study is, to the best of our knowledge, one of the first to evaluate the potential of tributary- or watershed-scale classifications through the analyses of otolith chemistry in the St. Lawrence system. Admittedly, such conclusions have to be confirmed using actual otolith data in a similar study design. The observed large-scale variations reflect the potential influence of regional geology of the St. Lawrence River valley, characterized by a pronounced difference between North shore and South shore bedrock composition and a gradual upstream/downstream succession. Our result suggest the relatively high likelihood of predicting water composition based on geological data (Liu et al., 2000). In general, tributaries in close proximity, originating and flowing over similar rock formations (e.g. same shore), will share a similar chemical composition, which could highly differs from a close tributary located on the opposite shore.

In our data set, the St. Maurice River did not follow the upstream–downstream trend, showing notably higher Sr:Ca and Ba:Ca values relative to the other studied tributaries. Such a marked difference in water chemistry for this river is likely due to the particular geography and geological characteristics of this watershed. Located on the north shore of the St. Lawrence River, the St. Maurice River flows through the black spruce–dominated forest lying upon the Grenville Province of the Canadian Shield (Saint-Jacques and Richard, 2002). The river therefore contains a high concentration of humic acid and a low concentration of calcium; this contrasts with the calcium-rich waters of the St. Lawrence River and its south shore tributaries situated on top of the limestones of the St. Lawrence Lowland Platform. The latter originate from within the Appalachian Province, characterized mainly by sedimentary rocks (i.e. limestone, shale, mudstone), with the river flowing over surface deposits of clay and silt. The low calcium content of the St. Maurice River produces higher Me:Ca values, hence trace element availability. Classification of the St. Maurice River was therefore efficacious, attributed mainly to its comparatively unique bedrock as it was the sole boreal shield river in the data set. Other tributaries flowing over the Canadian Shield, albeit smaller streams originating from southern parts of the Shield, likely share similar trace metal compositions; therefore, the probable accuracy of our model would be reduced with the addition of these other streams. Incidentally, the St. Charles River could have also been considered as a northern shore boreal shield stream, although its watershed is more restricted and mostly urbanized, reducing boreal forest contribution to water chemistry, hence acidity, and increasing Ca concentrations, which is consistent with its less extreme composition. Difference in morphology and landform feature between St. Maurice and St. Charles Rivers are likely to exacerbate the discrepancy in water trace elements compositions. These results also emphasize the importance of using metal-to-calcium ratios when considering ambient water chemistry values and its relationship with otolith chemistry (i.e. elemental availability for deposition), especially in systems where water chemistry varies over short distances owing to differences in geology and land use (e.g. forest vs urban vs agriculture).

4.2 Temporal variation and hydrological influence

Analyses of temporal variations provided evidences that annual variations were mostly negligible for Sr:Ca and Mg:Ca. Annual and seasonal Ba:Ca and Mn:Ca variations were important only in tributaries (i.e. St. Charles and Chaudière) exhibiting significant concentration-discharge relationships (C-Q). The modeled C–Q relationships showed that most Me:Ca concentrations were either not influenced by discharge or presented evidence of stable point-source dynamics. Decreases of Me:Ca in relation to discharge suggest that trace metal inputs are likely from deep (i.e. bedrock) or single point sources (i.e. industrial or municipal wastewater or city effluent) contributing at a relatively constant rate (Neal et al., 1996). Increased flow, associated with waters intermittently flooding lowlands and/or increased sediment mobilization and leaching, appears to have only a marginal influence on the observed Me:Ca values in our studied tributaries and the St. Lawrence River. The positive C–Q relationship observed for the Chaudière River, however, provides possible evidence of intermittently accessible external sources of Ba and Mn that are linked to the flooding regime. The Chaudière River is characterized by a high flooding frequency due to its shallow channel and large (approximately 1 km wide) alluvial plain over a 60 km stretch, which ends only 35 km from the river mouth (Hamelin, 1958). This hydrological regime releases important quantities of dissolved trace metals, which could have a high residency time given the large dam and reservoir complex located approximately 3 km upstream of the river mouth. Even if Me:Ca varied with discharge, we did not observe much overlap with other tributaries in periods of either high or low flow. In this model, intermittent variations of water chemistry related to discharge of the Chaudière River were not sufficient to affect the classification performance at a tributary scale.

4.3 Classification and potential use in otolith chemistry

The St. Lawrence River is a large unhindered freshwater system composed of two highly structured parallel-flowing water masses (Great Lakes and Ottawa River water mass). Previous studies have suggested a limited longitudinal mixing of those water masses from their initial confluence at the eastern tip of the island of Montréal to Lake St. Pierre. From this point, mixing increases until arriving at the St. Lawrence estuary, slightly downstream of Québec (Lum et al., 1991). We observed that, at least for the studied fluvial section, limited variability in chemical composition, in agreement with previous studies (Benchetrit et al., 2015; Yang et al., 1996; Yeats and Loring, 1991). Contributions of most tributaries were marginal to the trace element composition of the St. Lawrence River, except for stations very near the outflow of the tributaries. In light of these results, a tributary-scale reconstruction of fish displacement or analyses of stock structure is possible; nonetheless, fish movement within the main stem could be missed owing to a lack of a sufficient element gradient, at least in the studied freshwater reach. Our results also demonstrate that tributary-based classification is difficult or unlikely on the basis of a single element classification.

The Chaudière, St. Maurice and St. François rivers are, for example, nearly indistinguishable (Fig. 3) when only considering Sr:Ca, despite these rivers being located within separate watersheds characterized by different geology. However, adding only one other element (Ba:Ca) overcomes this problem and provides a 100% success in identifying the rivers (Table 1).

Admittedly, identification of substantial trace element gradients in the studied system is only a part of the overall assumption supporting otolith chemistry (Pracheil et al., 2014). This assumption states that ambient variability in the water environment will be reflected in the accreted increments of the otoliths (Campana, 1999) to provide a useful proxy of habitat use. As most trace element concentrations in otolith are not representative of surrounding water (partition coefficient null or non-significant), the full model (25 elements) is presented here as the maximum attainable accuracy, but is not a plausible option for use in otolith chemistry analyses. The elemental composition of water is the major influence on Sr and Ba concentrations in otoliths, an aspect that is particularly relevant in freshwater where salinity, the second most influential factor (Izzo et al., 2018), is absent. Manganese has been related to environmental concentrations (Dorval et al., 2007; Elsdon and Gillanders, 2003b), although the accretion mechanism needs to be better defined (Sturrock et al., 2015). Studies assessing the Mg partition coefficients show that $Mg:Ca_{otolith}$ is not influenced by Mg concentrations in water (Miller, 2011; Wells et al., 2003), although other extrinsic (e.g. water temperature) and intrinsic (i.e. ontogeny and growth) factors appear responsible for the accretion rate (cited in Hüssy et al., 2020). The influence of these intrinsic and extrinsic factors (such as hypoxia), which affect the rates of elemental uptake and otolith deposition in its inorganic and organic components (Limburg et al., 2015), also need to be considered for improved comprehension and predictive power and to avoid too simplistic views of such complex phenomena (Hüssy et al., 2020). Such developments are certainly not trivial but could lead to much more effective applications. Luckily, the decrease of classification accuracy of a three-element RF model (Sr:Ca, Ba:Ca, Mn:Ca; 85.6% correct) was minimal compared to our four-element RF model (92.5% correct) and likely provides sufficient definition to envision a tributary-scale resolution.

5. Conclusion

Based on water regional chemistry data, we suggest that otolith chemistry analyses in the St. Lawrence River system could be possible at a tributary-scale resolution. Our analysis of the interannual variations of water samples collected from the St. Lawrence River and its tributaries highlighted the relative stability of the chemical composition of water flowing in the tributaries. The simple experimental design applied in this study could be replicated in other aquatic ecosystems. Trace elements concentrations and discharge gauging stations are generally part of routine analyses from federal or states environmental agencies and could provide, even if spatio-temporally limited, valuable a priori information before initiating otolith chemistry studies. We acknowledge that our analysis of elemental composition was limited to the main tributaries of the St. Lawrence Lowlands, located between Montréal and Québec City. These analyses should be extended to the more than 40 tributaries that enter into the freshwater portion of the St. Lawrence River. Moreover, it have been assumed that surface water chemistry of St. Lawrence main stem and tributaries is representative of the whole water column, owing to the high water velocity, however, local vertical variations of water chemistry in main stem deep channels of the St. Lawrence River may exist and will need to be characterized. It is likely that the inclusion of a greater number of tributaries and extreme (deep) habitats into the models will require creating groups of proximal streams/rivers of similar elemental composition, which will decrease the study resolution (e.g. the creation of classification groups combining streams of similar elemental composition). Future studies should aim to refine and improve the spatiotemporal (inter- and intra-tributary) elemental data from large fluvial ecosystems to better understand partition coefficients for the most common elemental species of the system and test classification models at the tributary scale on actual otolith chemistry data. Such developments would increase the value and usefulness of otolith chemistry beyond its actual state in the St. Lawrence River and in several other fluvial ecosystems around the world.

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5. References

Baronas JJ, Torres MA, Clark KE, West AJ. Mixing as a driver of temporal variations in river hydrochemistry: 2. Major and trace element concentration dynamics in the Andes-Amazon transition. Water Resources Research 2017; 53: 3120-3145.

Benchetrit J, Béguer-Pon M, Sirois P, Castonguay M, Fitzsimons J, Dodson JJ. Using otolith microchemistry to reconstruct habitat use of American eels *Anguilla rostrata* in the St. Lawrence River–Lake Ontario system. Ecology of Freshwater Fish 2015; 26: 19-33.

Bourret SL, Clancy NG. Using forensic geochemistry via fish otoliths to investigate an illegal fish introduction. Canadian Journal of Fisheries and Aquatic Sciences 2018; 75: 1778-1783.

Breiman L, Cutler A. Package 'randomForest' : Breiman and Cutler's Random Forests for classification and regression, 2018.

Brown RJ, Severin KP. Otolith chemistry analyses indicate that water Sr:Ca is the primary factor influencing otolith Sr:Ca for freshwater and diadromous fish but not for marine fish. Canadian Journal of Fisheries and Aquatic Sciences 2009; 66: 1790-1808.

Campana SE. Chemistry and composition of fish otoliths: pathways, mechanisms and applications. Marine Ecology Progress Series 1999; 188: 263-297.

Chapman DC, Davis JJ, Jenkins JA, Kocovsky PM, Miner JG, Farver J, et al. First evidence of grass carp recruitment in the Great Lakes Basin. Journal of Great Lakes Research 2013; 39: 547-554.

Crook DA, Macdonald JI, McNeil DG, Gilligan DM, Asmus M, Maas R, et al. Recruitment sources and dispersal of an invasive fish in a large river system as revealed by otolith chemistry analysis. Canadian Journal of Fisheries and Aquatic Sciences 2013; 70: 953-963.

Dorval E, Jones CM, Hannigan R, Montfrans JV. Relating otolith chemistry to surface water chemistry in a coastal plain estuary. Canadian Journal of Fisheries and Aquatic Sciences 2007; 64: 411-424.

Doubleday ZA, Izzo C, Woodcock SH, Gillanders BM. Relative contribution of water and diet to otolith chemistry in freshwater fish. Aquatic Biology 2013; 18: 271-280.

Elsdon TS, Gillanders BM. Reconstructing migratory patterns of fish based on environmental influences on otolith chemistry. Reviews in Fish Biology and Fisheries 2003a; 13: 217-235.

Elsdon TS, Gillanders BM. Relationship between water and otolith elemental concentrations in juvenile black bream *Acanthopagrus butcheri*. Marine Ecology Progress Series 2003b; 260: 263-272.

Hamelin L-E. Observations concernant les crues de la Chaudière (Canada). Cahiers de géographie du Québec 1958; 2: 217-231.

Humston R, Harbor D. Geologic analyses for evaluating watershed heterogeneity: Implications for otolith chemistry studies. Proceedings of the Southeastern Association of Fish and Wildlife Agencies 2006: 132-139.

Hüssy K, Limburg KE, de Pontual H, Thomas ORB, Cook PK, Heimbrand Y, et al. Trace element patterns in otoliths: The role of biomineralization. Reviews in Fisheries Science & Aquaculture 2020: 1-33.

Izzo C, Reis-Santos P, Gillanders BM. Otolith chemistry does not just reflect environmental conditions: A meta-analytic evaluation. Fish and Fisheries 2018; 19: 441-454.

Jones CM, Palmer M, Schaffler JJ. Beyond Zar: the use and abuse of classification statistics for otolith chemistry. Journal of Fish Biology 2016; 90: 492-504.

Lachance-Cloutier S, Turcotte R, Cyr J-F. Combining streamflow observations and hydrologic simulations for the retrospective estimation of daily streamflow for ungauged rivers in southern Quebec (Canada). Journal of Hydrology 2017; 550: 294-306.

Lazartigues A, Girard C, Brodeur P, Lecomte F, Mingelbier M, Sirois P. Otolith microchemistry to identify sources of larval yellow perch in a fluvial lake: an approach towards freshwater fish management. Canadian Journal of Fisheries and Aquatic Sciences 2018; 75: 474-487.

Lazartigues AV, Plourde S, Dodson JJ, Morissette O, Ouellet P, Sirois P. Determining natal sources of capelin in a boreal marine park using otolith microchemistry. ICES Journal of Marine Science 2016; 73: 2644-2652.

Limburg KE, Walther BD, Lu Z, Jackman G, Mohan J, Walther Y, et al. In search of the dead zone: Use of otoliths for tracking fish exposure to hypoxia. Journal of Marine Systems 2015; 141: 167-178.

Liu Z-J, Weller DE, Correll DL, Jordan TE. Effects of land cover and geology on stream chemistry in watersheds of Chesapeake Bay. Journal of the American Water Resources Association 2000; 36: 1349-1365.

Lum KR, Kaiser KLE, Jaskot C. Distribution and fluxes of metals in the St. Lawrence River from the outflow of Lake Ontario to Québec City. Aquatic Sciences 1991; 53: 1-19.

Mayer FLJ, Marking LL, Bills TD, Howe GE. Physicochemical factors affecting toxicity in freshwater: hardness, pH and temperature. In: Hamelink JL, Landrum PF, Bergman HL, Benson WH, editors. Bioavailability: physical, chemical and biological interactions. Lewis Publishers, London, 1994, pp. 256.

MELCC. Banques de données sur la qualité du milieu aquatique (BQMA). In: Direction générale du suivi de l'état de l'environnement, editor, Québec, 2020.

Mercier L, Darnaude AM, Bruguier O, Vasconcelos RP, Cabral HN, Costa MJ, et al. Selecting statistical models and variable combinations for optimal classification using otolith microchemistry. Ecological Applications 2011; 21: 1352-1364.

Miller JA. The effects of temperature and water concentration on the otolith incorporation of barium and manganese in black rockfish *Sebastes melanops*. Journal of Fish Biology 2009; 75: 39-60.

Miller JA. Effects of water temperature and barium concentration on otolith composition along a salinity gradient: Implications for migratory reconstructions. Journal of Experimental Marine Biology and Ecology 2011; 405: 42-52.

Moon S, Chamberlain CP, Hilley GE. New estimates of silicate weathering rates and their uncertainties in global rivers. Geochimica et Cosmochimica Acta 2014; 134: 257-274.

Morissette O, Lecomte F, Vachon N, Drouin A, Sirois P. Migratory capacity and secondary dispersal of the Eurasian tench (*Tinca tinca*), a St. Lawrence River invader, using otolith chemistry. Canadian Journal of Fisheries and Aquatic Sciences submitted; na: na.

Morissette O, Lecomte F, Verreault G, Legault M, Sirois P. Fully equipped to succeed: Migratory contingents seen as an intrinsic potential for striped bass to exploit a heterogeneous environment early in life. Estuaries and Coasts 2016; 39: 571-582.

Neal C, Smith CJ, Jeffery HA, Jarvie HP, Robson AJ. Trace element concentrations in the major rivers entering the Humber estuary, NE England. Journal of Hydrology 1996; 182: 37-64.

O'Connor DJ. The concentration of dissolved solids and river flow. Water Resources Research 1976; 12: 279-294.

Pannella G. Fish otoliths: daily growth layers and periodical patterns. Science 1971; 173: 1124-1127.

Pinheiro J, Bates D, DebRoy S, Sarkar D, R development Core Team. *nlme* : Linear and Nonlinear Mixed Effects Models, 2018.

Pracheil BM, Hogan JD, Lyons J, McIntyre PB. Using hard-part microchemistry to advance conservation and management of North American freshwater fishes. Fisheries 2014; 39: 451-465.

R Development Core Team. R: A language and environement for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, 2008.

Radigan WJ, Carlson AK, Graeb BDS, Fincel MJ. Assessing the utility of otolith chemistry for management of six freshwater fishes from a river–reservoir system. North American Journal of Fisheries Management 2017; 38: 316-326.

Saint-Jacques N, Richard Y. Le bassin de la rivière Saint-Maurice : les communautés ichtyologiques et l'intégrité biotique du milieu. Ministère de l'environnement, Québec, 2002, pp. 75.

Secor DH, Piccoli PM. Oceanic migration rates of Upper Chesapeake Bay striped bass (*Morone saxatilis*), determined by otolith microchemical analysis. Fishery Bulletin 2007; 105: 62-73.

Sturrock AM, Hunter E, Milton JA, Eimf, Johnson RC, Waring CP, et al. Quantifying physiological influences on otolith microchemistry. Methods in Ecology and Evolution 2015; 6: 806-816.

Sturrock AM, Trueman CN, Darnaude AM, Hunter E. Can otolith elemental chemistry retrospectively track migrations in fully marine fishes? Journal of Fish Biology 2012; 81: 766-795.

Taddese F, Reid MR, Closs GP. Direct relationship between water and otolith chemistry in juvenile estuarine triplefin *Forsterygion nigripenne*. Fisheries Research 2019; 211: 32-39.

Tanner SE, Reis-Santos P, Cabral HN. Otolith chemistry in stock delineation: A brief overview, current challenges and future prospects. Fisheries Research 2016; 173: 206-213.

Tanner SE, Reis-Santos P, Vasconcelos RP, Fonseca VF, França S, Cabral HN, et al. Does otolith geochemistry record ambient environmental conditions in a temperate tidal estuary? Journal of Experimental Marine Biology and Ecology 2013; 441: 7-15.

Thibault I, Hedger RD, Dodson JJ, Shiao JC, Iizuka Y, Tzeng WN. Anadromy and the dispersal of an invasive fish species (*Oncorhynchus mykiss*) in Eastern Quebec, as revealed by otolith microchemistry. Ecology of Freshwater Fish 2010; 19: 348-360.

Vanalderweireldt L, Winkler G, Mingelbier M, Sirois P. Early growth, mortality, and partial migration of striped bass (*Morone saxatilis*) larvae and juveniles in the St. Lawrence estuary, Canada. ICES Journal of Marine Science 2019; 76: 2235-2246.

Walther BD. The art of otolith chemistry: interpreting patterns by integrating perspectives. Marine and Freshwater Research 2019; 70: 1643-1658.

Wells BK, Rieman BE, Clayton JL, Horan DL, Jones CM. Relationships between water, otolith, and scale chemistries of Westslope Cutthroat Trout from the Coeur d'Alene River, Idaho: The potential application of hard-part chemistry to describe movements in freshwater. Transactions of the American Fisheries Society 2003; 132: 409-424.

Whitledge GW. Otolith microchemistry and isotopic composition as potential indicators of fish movement between the Illinois River drainage and Lake Michigan. Journal of Great Lakes Research 2009; 35: 101-106.

Wright PJ, Régnier T, Gibb FM, Augley J, Devalla S. Assessing the role of ontogenetic movement in maintaining population structure in fish using otolith microchemistry. Ecology and Evolution 2018; 8: 7907-7920.

Yang C, Telmer K, Veizer J. Chemical dynamics of the “St. Lawrence” riverine system: δDH2O, δ18OH2O, δ13CDIC, δ34Ssulfate, and dissolved 87Sr/86Sr. Geochimica et Cosmochimica Acta 1996; 60: 851-866.

Yeats PA, Loring DH. Dissolved and particulate metal distributions in the St. Lawrence estuary. Canadian Journal of Earth Sciences 1991; 28: 729-742.

Zeigler JM, Whitledge GW. Otolith trace element and stable isotopic compositions differentiate fishes from the Middle Mississippi River, its tributaries, and floodplain lakes. Hydrobiologia 2011; 661: 289-302.

Tables

Table 1. Classification accuracy of the training data of the sample trace metal composition from the different stations using the two-element RF model (Sr:Ca, Ba:Ca).

	DPR	DMI	SLR	LAS	RIC	YAM	STF	STM	CHA	STC	Error
Des Prairies (DPR)	**18**	0	3	0	0	0	0	0	1	0	0.18
Des Milles-Iles (DMI)	2	**11**	4	0	0	0	0	0	7	0	0.54
St. Lawrence (SLR)	4	3	**74**	10	0	1	0	0	0	1	0.20
L'Assomption (LAS)	0	1	15	**8**	0	1	0	0	1	0	0.69
Richelieu (RIC)	0	0	0	1	**16**	2	7	0	0	0	0.38
Yamaska (YAM)	0	0	0	2	3	**22**	0	0	2	0	0.24
St. François (STF)	0	0	0	0	8	0	**12**	0	0	0	0.40
St. Maurice (STM)	0	0	0	0	0	0	0	**25**	0	0	0.00
Chaudière (CHA)	1	8	0	1	0	3	0	0	**12**	0	0.52
St. Charles (STC)	0	1	0	0	0	0	0	0	1	**22**	0.08

* The numbers represent the water samples from a site (lines) assigned by the model to the established elemental fingerprint by model (columns). The numbers in bold are the correct classifications.

Figures

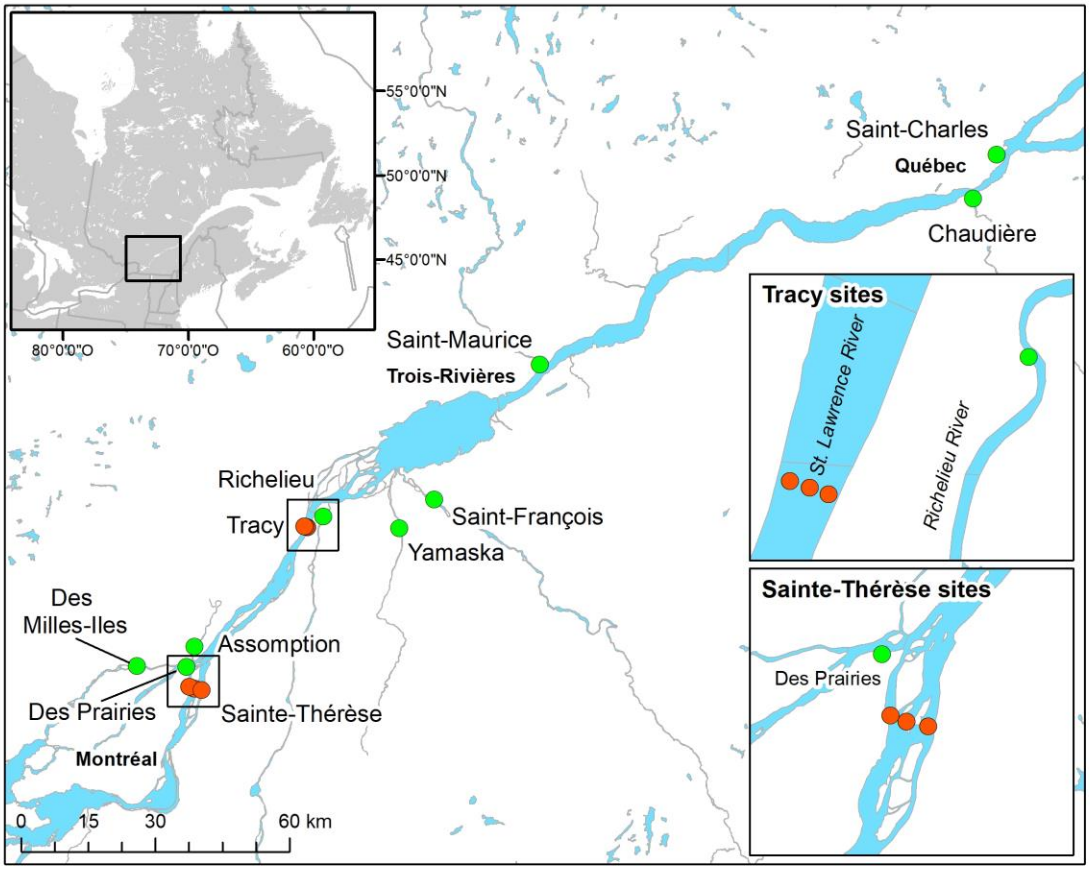

Fig. 1. Sampling sites located in tributaries (green circles) and the St. Lawrence River main stem (orange circles). Inset maps show the locations of the Tracy and St. Thérèse sites (orange circles), which were pooled as St. Lawrence River sites (see text).

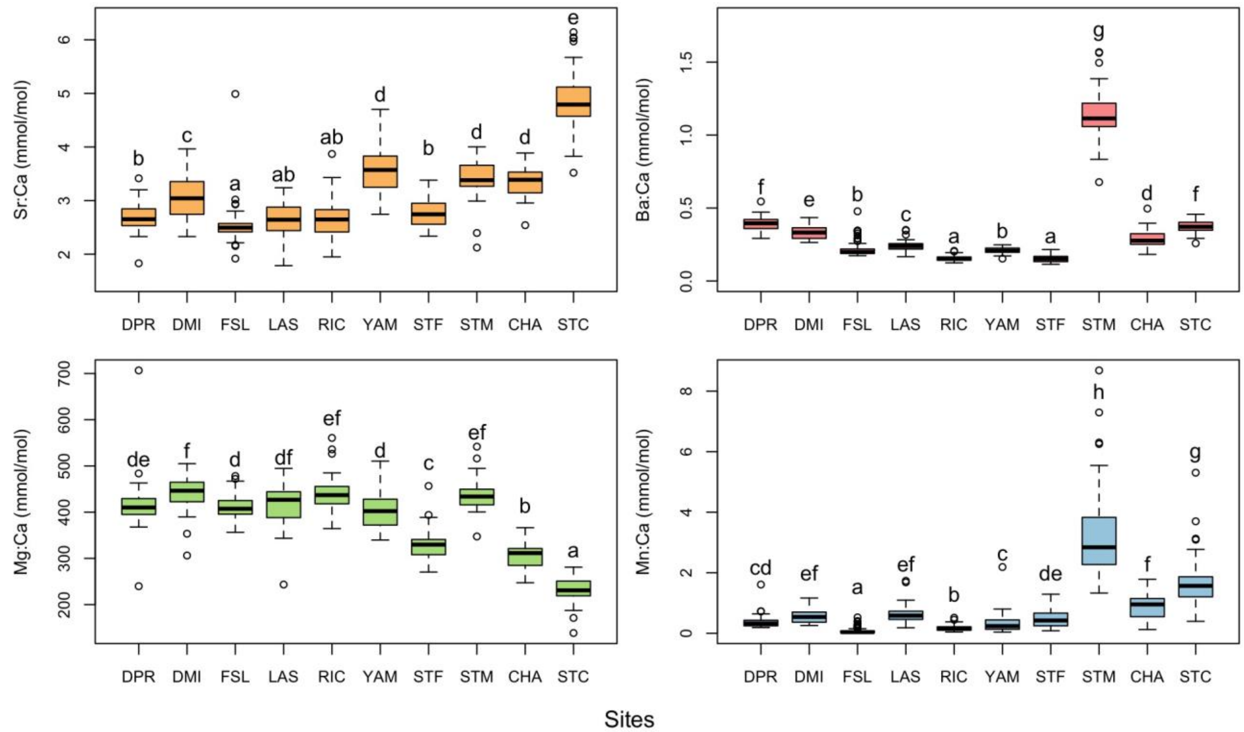

Fig. 2. Average concentrations of Sr:Ca, Ba:Ca, Mn:Ca and Mg:Ca ($mmol \cdot mol^{-1}$) from the ten study sites. In the figure, sites are organized upstream to downstream (from left to right) along the St. Lawrence River. The Fleuve St. Laurent (FSL) station corresponds to the combined North, South and Center stations of the St. Thérèse and Tracy Island sites. Letters show significant post hoc differences between sites.

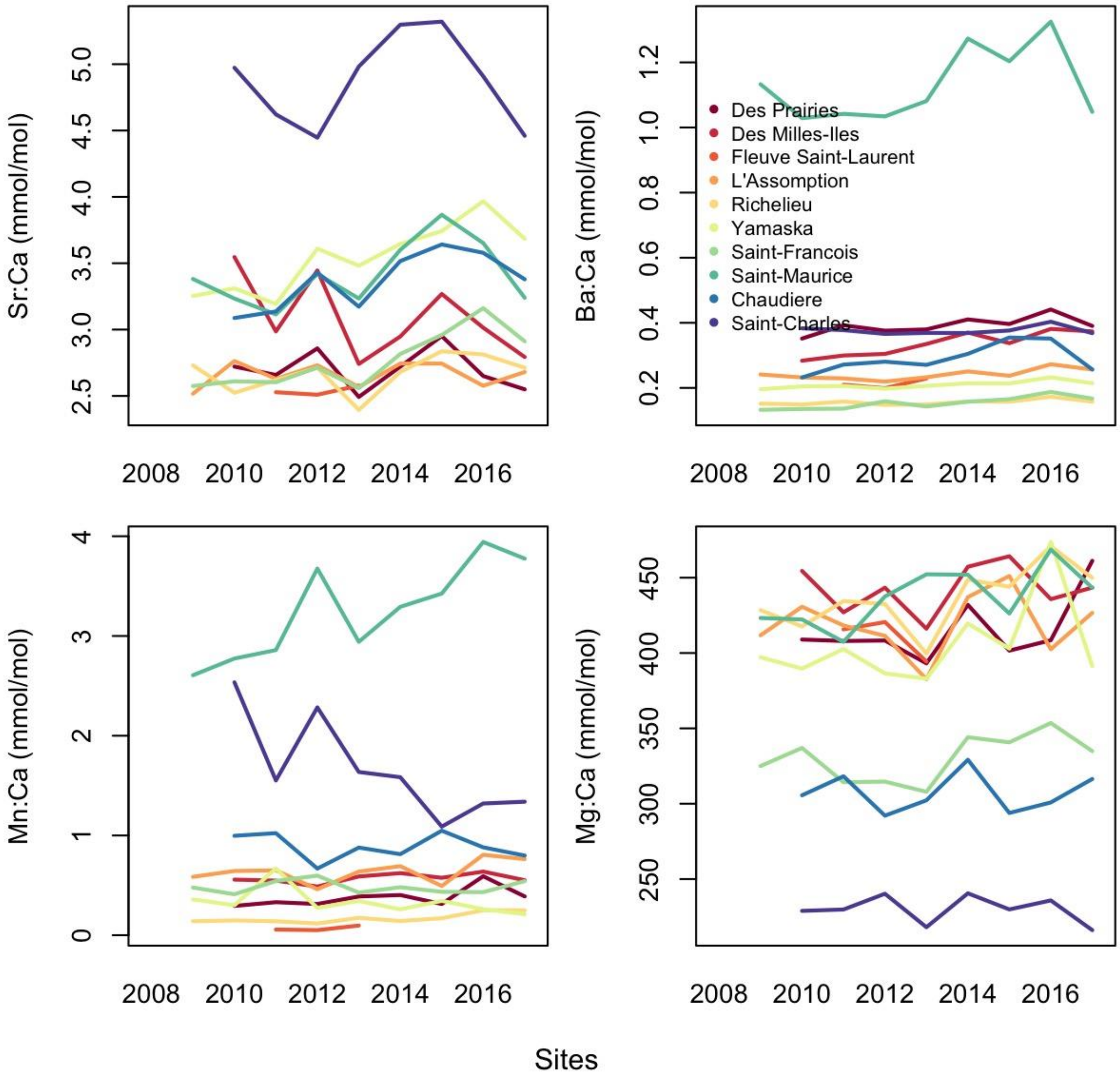

Fig. 3. Annual variations in mean concentrations of Sr:Ca, Ba:Ca, Mn:Ca and Mg:Ca ($mmol \cdot mol^{-1}$) for the ten study sites. Each color represents a tributary along the downstream (warmer colors) to upstream (cooler colors) gradient.

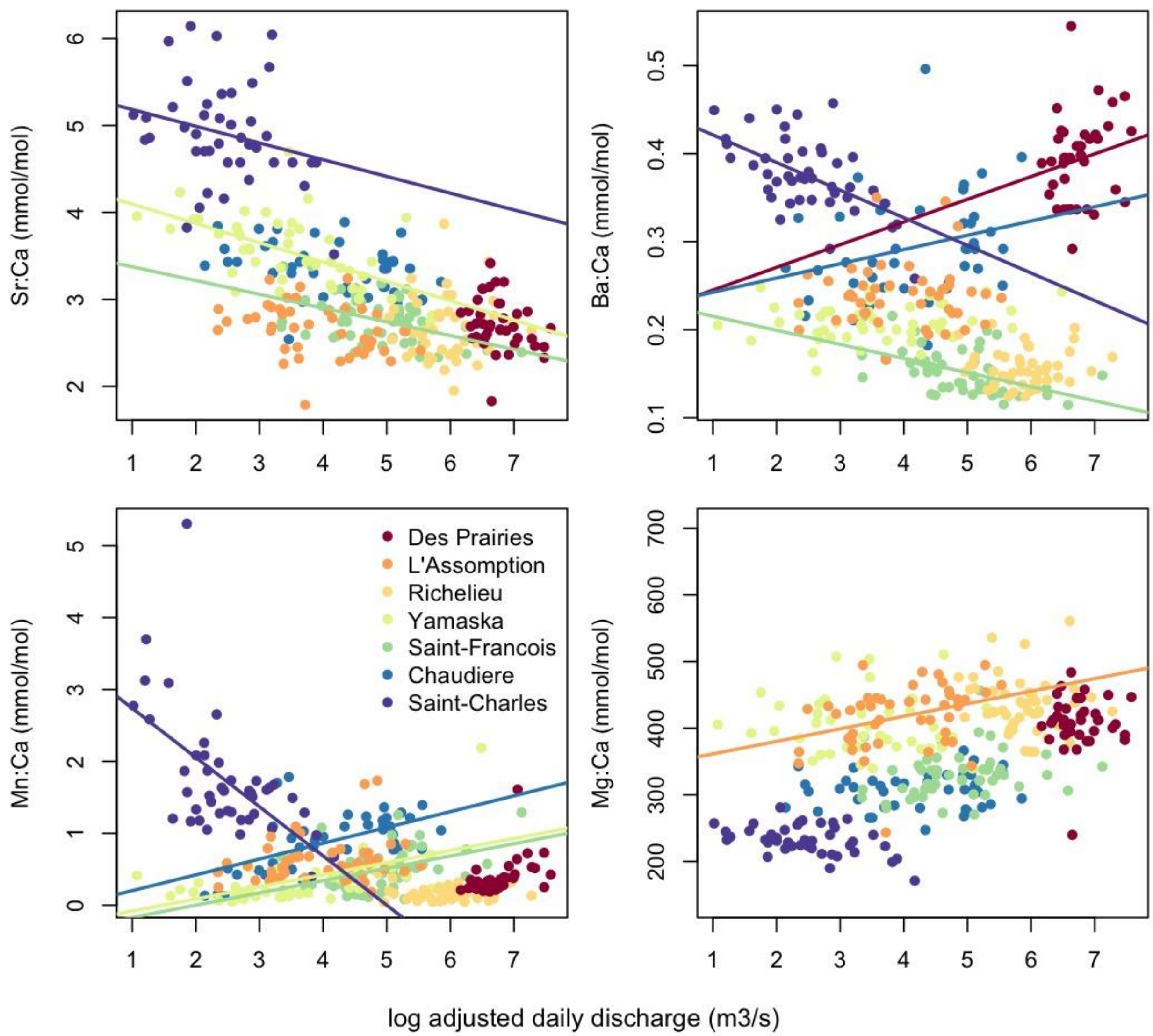

Fig. 4. Regressions of measured concentrations of Sr:Ca, Ba:Ca, Mn:Ca and Mg:Ca in relation to the adjusted discharge (natural logarithm). Regression lines are displayed for significant relationships only, according to linear mixed-effect modeling.

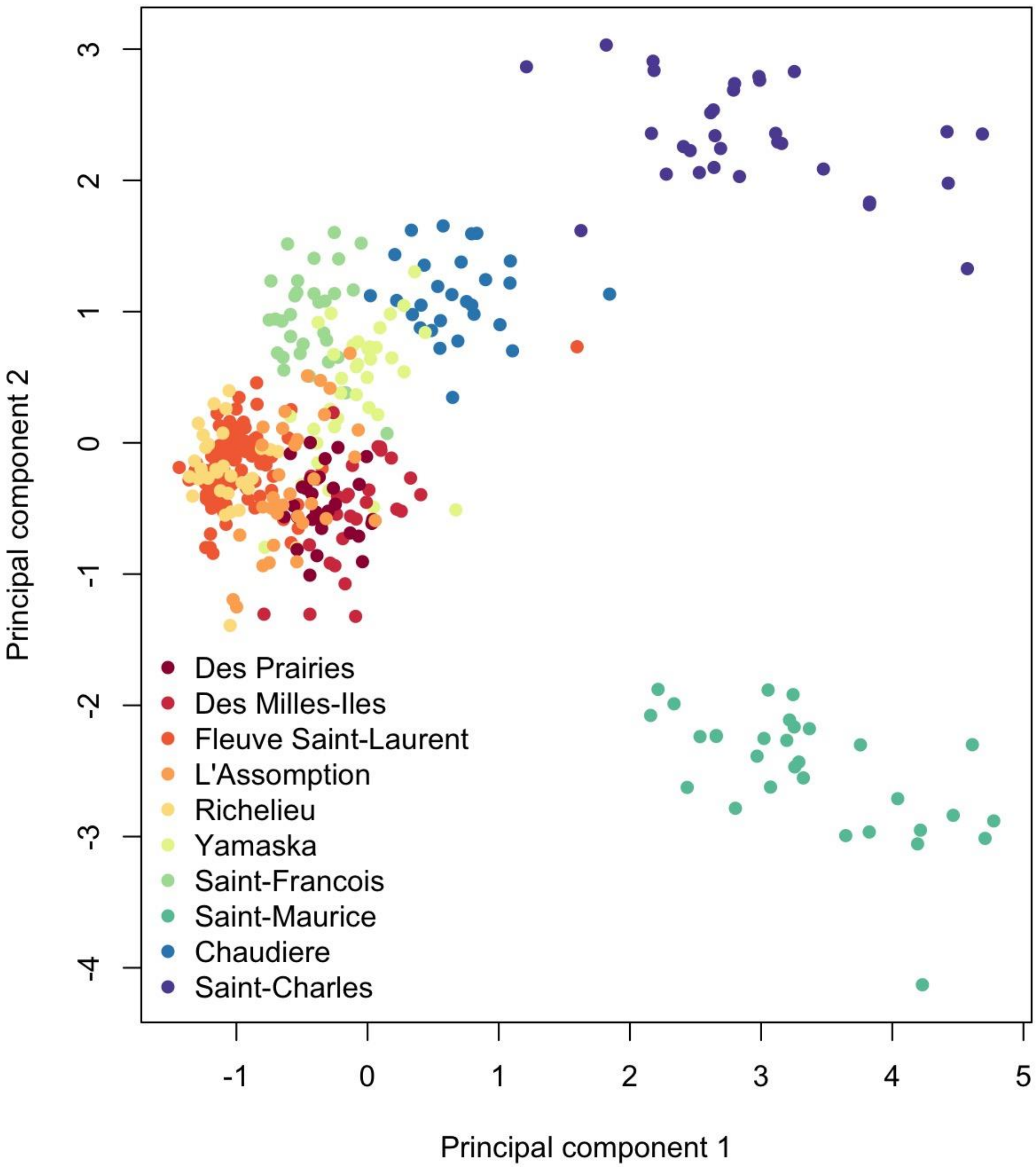

Fig. 5. Principal component analysis ordination of the ten sample sites, according to the elemental fingerprint of Sr:Ca, Ba:Ca, Mn:Ca and Mg:Ca.