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Habitat Fingerprints for Lake Superior Coastal Wetlands Derived from Elemental Analysis of Yellow Perch Otoliths

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Abstract.—Assessing the ecological importance of coastal habitats to Great Lakes ecosystems requires an understanding of the ecological linkages between coastal and offshore waters. Elemental analysis of fish otoliths has emerged as a powerful technique that can provide a natural tag for determining nursery area affiliation, population structure, and movement of individual fish. Since the elemental composition of fish otoliths reflects some of the environmental conditions under which a fish was reared, otolith chemistry can record differences in ambient water conditions specific to habitats used during a fish's life history. Although few studies have been conducted in freshwaters, trace element analysis of marine fish otoliths has proven useful in identifying the chemical signatures unique to particular spawning and nursery habitats. To examine the utility of this method in freshwater, sagittae were removed from 275 young-of-the-year yellow perch *Perca flavescens* captured from eight wetlands in western Lake Superior during August 2001. They were analyzed for Ca and 13 minor and trace elements using inductively coupled plasma mass spectrometry (ICPMS) and inductively coupled atomic emission mass spectrometry (ICPAES). Otolith concentrations of Ba, K, Mg, Mn, Na, and Sr differed significantly among wetlands (ANOVA, $P < 0.001$). Interwetland differences were also pronounced when analyzed as a multivariate fingerprint (MANOVA, $P < 0.001$). Discriminant function analysis revealed relatively distinct chemical fingerprints associated with each wetland. Wetland classification accuracy based on a five-element model (Sr, Mn, K, Ba, and Mg) ranged from 62% to 100% and averaged 76%. Differences in fingerprints between wetland types (river-influenced versus lagoon) were also distinct (MANOVA, $P < 0.001$). Classification accuracy for wetland type was 81% based on a five-element model that included Ba, Mg, Mn, Na, and Sr. Our results suggest that otolith elemental fingerprints may be useful for quantifying the relative contributions of different wetland nursery areas to recruitment in adjacent lake populations.

To accurately assess the influence of coastal wetlands on adjacent large lake ecosystems, ecological linkages between coastal and offshore waters need to be quantified. Because coastal wetlands occupy only a fraction (<1%) of the total surface area of the Great Lakes, it has generally been assumed that ecological linkages between wetlands and the adjacent lake would be unimportant. However, recent investigations of linkages among wetlands and adjacent offshore waters in the Great Lakes suggest coastal wetlands may support adjacent lake fisheries and mediate trophic interactions in open-lake food webs through the export of organic matter, nutrients, and organisms (Keough et al. 1996; Brazner et al. 2000). A number of studies suggest the movement of fishes, such as yellow perch *Perca flavescens*, between Great Lakes coastal wetlands and adjacent offshore waters is common and potentially important in an ecosystem context (for review, see Brazner et al. 2001). However, there is little hard evidence because of difficulties in quantifying fish movements in such large open systems.

An important step toward quantifying fish movements in the Great Lakes is the development of a readily sampled and accurate natural tag, one

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of the primary goals of this study. Elemental analysis of fish otoliths has emerged as a potentially powerful technique for providing a natural tag for fishes and their habitats. Otoliths are paired, calcified structures used for balance and hearing in all teleost fishes. Elemental fingerprinting based on a chemical analysis of these structures is possible, in part because fish otoliths are acellular and metabolically inert. Their continuous growth and formation of daily and annual growth rings provide a record of age and growth, but it is the lack of resorption or alteration of material deposited within the otolith that allows them to serve as natural tags (Campana and Neilson 1985).

Thirty-one elements have been detected in otoliths (Campana 1999). Ions from these major, minor, and trace elements from the endolymphatic fluids surrounding the otolith bind to the otolith's protein and calcium carbonate matrix in a variety of ways (Campana 1999), and reflect the physical and chemical conditions of the water mass a fish has occupied throughout its life. Otolith concentrations do not necessarily track ambient conditions in a straightforward manner since the physiological regulation of some elements can be strong (e.g., Kalish 1989; Fowler et al. 1995; and see Campana 1999 for review). However, differences in water chemistry among sites, whether due to anthropogenic effects or natural factors, are reflected in the elemental composition of the otolith, and these differences can be used to identify fish that have lived in different habitats for at least part of their lives.

Virtually all of the published studies to develop and test this technique have been conducted with fish that are part- or full-time residents in marine environments (e.g., Edmonds et al. 1989; Thresher at al. 1994, Campana et al. 1995, 2000). An exception is a study by Bronte et al. (1996) that attempted to discriminate among different spawning populations of lake herring *Coregonus artedi* in Lake Superior. The microchemical analysis of otoliths to develop elemental fingerprints has not always provided unequivocal results (e.g., Proctor et al. 1995; Gillanders et al. 2001). However, elemental fingerprinting of fish otoliths has been used to discriminate among different fish stocks or nursery areas (e.g., Campana et al. 1995, 2000; Gillanders and Kingsford 1996, 2000; Kennedy et al. 1997; Thorrold et al. 1998a, 1998b; Rooker et al. 2001) and to identify migration patterns (e.g., Pender and Griffin 1996; Secor et al. 2001; Kennedy et al. 2002), including movement between nursery areas and offshore waters (Thorrold et al. 1997; Gillanders 2002a).

Although the ultimate goal of our research is to evaluate the utility of elemental fingerprinting for quantifying fish movement between coastal wetland and offshore waters, there were two primary objectives for our present study: (1) to quantify variation in trace element concentrations in the otoliths of young-of-the-year (age-0) fish from different coastal wetland nursery areas in western Lake Superior; and (2) to determine if this variation was distinct enough to allow fish to be accurately classified to nursery areas based on otolith elemental fingerprints.

Methods

Species selection and sample collection.—We used yellow perch for this study for several reasons. It is one of the most common fishes that utilize coastal wetlands for spawning and nursery areas in the Great Lakes (Jude and Pappas 1992), and provides a significant sport and commercial fishery (e.g., Milliman et al. 1992; Baldwin et al. 2002). In addition, yellow perch are quite abundant and trophically important in the nearshore waters of most of the lakes, and are known to move between wetland and lake habitats (Brazner et al. 2001).

Otoliths (sagittae) were removed from age-0 yellow perch (31–93 mm in length) that were captured using a combination of fyke nets, electrofishing, and shoreline seining from eight wetland sites along the south shore of Lake Superior in Wisconsin during August 2001(Figure 1). Honest John Slough, Bark Bay Lagoon, East Flag Lagoon, and Bibon Slough are all lagoonal wetlands, and Bad River, Kakagon River, Flag River, and Allouez Bay are river-influenced sites. These sites are typical of other barrier-beach lagoons and river-influenced coastal wetlands throughout Lake Superior and comprise the majority of coastal wetland habitat on the western end of the lake (Maynard and Wilcox 1997). We attempted to capture at least 30 fish from each site but did not succeed at Honest John and Kakagon Sloughs (Table 1). Fish were iced during daily transport to the laboratory and held frozen for 6 months, until otoliths were removed.

Elemental analysis.—The preparation and cleaning of otoliths for elemental analysis were done following procedures described by Campana et al. (2000). Briefly, this included removal of the otolith and cleaning of any debris or connective tissue using a dissecting microscope. Otolith samples

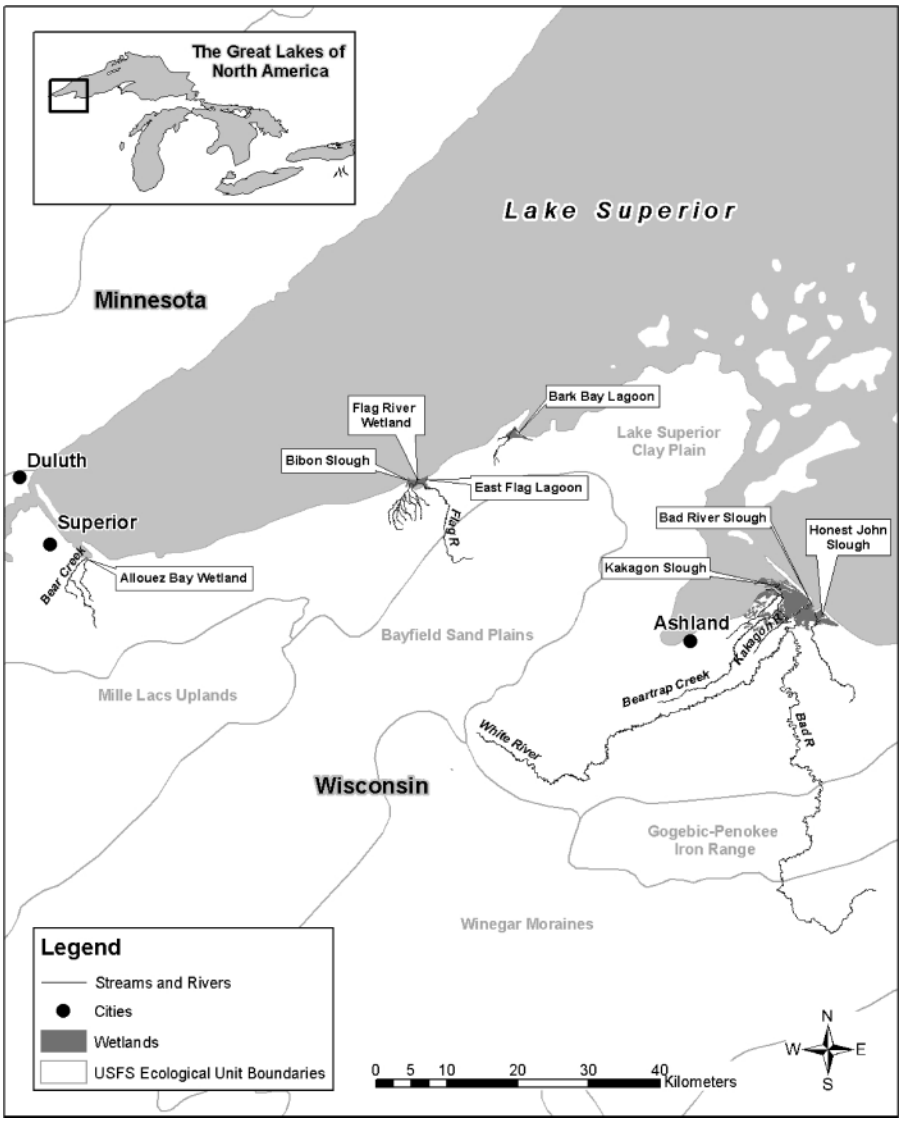

FIGURE 1.—Map of western Lake Superior coastal wetland sampling locations.

TABLE 1.—Yellow perch mean length and otolith mean weight summary statistics by wetland. Parenthetical values are ±SE.

Wetland	*n*	Fish length (cm)	Otolith weight (mg)	Wetland type
Allouez Bay Wetland	33	6.91 (0.08)	1.30 (0.03)	Riverine
Bad River Slough	37	6.50 (0.12)	1.29 (0.04)	Riverine
Bark Bay Lagoon	37	4.47 (0.17)	0.77 (0.06)	Lagoon
Bibon Slough	40	6.13 (0.12)	1.24 (0.05)	Lagoon
East Flag Lagoon	43	6.14 (0.16)	1.17 (0.05)	Lagoon
Flag River Wetland	38	3.73 (0.07)	0.31 (0.01)	Riverine
Honest John Slough	26	6.62 (0.14)	1.34 (0.05)	Lagoon
Kakagon Slough	21	7.92 (0.13)	1.75 (0.08)	Riverine

were placed in 50-mL, acid-washed vials and immersed in Super Q water (distilled, millipore filtered, reverse osmosis processed), sonicated (Cole-Parmer 8892), and rinsed. Otoliths were handled with acid-washed equipment made of polyethylene or polypropylene in a Class 100 laminar flow, positive-pressure fume hood. Decontaminated otoliths were stored in acid-washed plastic vials until assayed.

Decontaminated otoliths were digested with subboiling, double-distilled nitric acid in the original sample containers and diluted to a final volume of 2.0 mL. Based on preliminary studies with age-0 yellow perch sagittae collected from western Lake Superior wetlands, we analyzed Ca and 13 trace elements (Al, Ba, B, Cu, Fe, K, Li, Mg, Mn, Na, Pb, Sr, and Zn). We used inductively coupled plasma mass spectrometry (ICPMS) on a VG PlasmaQuad II STE for all elements except Fe, K, Na, and Ca, which were assayed using inductively coupled atomic emission mass spectrometry (ICPAES, Varian Vista Pro) to avoid some of the isobaric interferences and other logistical problems of assaying these elements on ICPMS. We used conventional analytical methods as opposed to isotope dilution (ID) techniques because the small sample sizes (and associated weighing error) and proximity to detection limits for many elements would limit the opportunity for data improvement that isotope dilution can provide (Campana et al. 1995). ID-ICPMS also requires the addition of stable isotopes as spikes, which would have made simultaneous quantification by ICPAES impossible. Microconcentric nebulizers with natural aspiration rates of approximately 0.4 mL/min were used for sample introduction to both the ICPAES and ICPMS instruments. Sample acquisition times were 30 s for ICPAES and 40 s for ICPMS. Simple dilute acid standard solutions were used for the initial instrument calibrations, but high calcium matrix-matched standards were used to monitor and correct for ionization interferences and instrument drift. ^{103}Rhodium was added as an internal standard during initial sample preparation to provide an additional opportunity to compensate for instrument drift. The assay sequence was systematically randomized across sample sites to eliminate bias associated with any instrument drift that was not accounted for by monitoring standards.

Because errors associated with weighing otoliths increased as sample size decreased (Figure 2), all reported element concentrations (μg/g) are based upon a sample weight, calculated from blank-corrected solution concentrations of calcium. This approach should produce accurate estimates of sample weight for all samples (including those below the limits of the balance), assuming otolith matrix material was nearly pure calcium carbonate and approximated 40% calcium by weight (Campana 1999). Based on the 10 analyzed reagent blanks, limits of detection (LOD = 3 SD in μg/g of otolith weight; USEPA 2003) for Ba, Ca, K, Mg, Mn, Na, Sr, and Zn were well-below mean blank-corrected concentrations in the otolith (Table 2; Figure 3). LODs for Al, Cu, Fe, Li, and Pb were near mean concentrations, and the LOD for B was above mean concentrations in the otolith at all sites (Figure 3). Estimates of precision (coefficient of variation [CV = 100 × SD/mean]) based on 24 samples of homogenous reference powder derived from Atlantic cod *Gadus morhua* otoliths used in previous studies (see Campana et al. 2000) were as low as 0.8% for Ca, less than 30% for six other elements, and ranged as high as 285% for B and 196% for Fe (Table 2). Although there are no strict criteria for retaining elements in these types of studies, we used the following guidelines: the mean value for at least one wetland had to exceed the LOD, and the precision (CV) of replicate assays of the homogeneous reference powder had to be less than 30%. Based on these criteria Al, B, Cu, Fe, Li, Pb, and Zn were eliminated, while Ba, K, Mg, Mn, Na, and Sr were

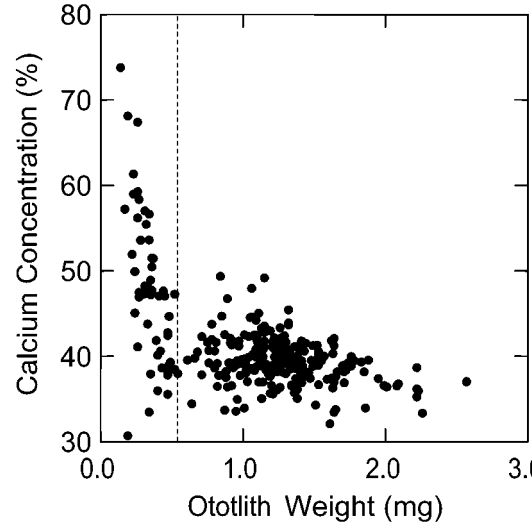

FIGURE 2.—Relationship between measured otolith weight and calcium concentration (dotted line depicts measurement error threshold where calcium concentrations become inaccurate due to inaccurate otolith weights).

TABLE 2.—Limits of detection (LOD from reagent blanks) and precision (CV = 100 × SD/mean; based on homogenized otolith reference powder) for all elements analyzed by inductively coupled plasma mass spectrometry (ICPMS) and inductively coupled atomic emission mass spectrometry (ICPAES). [BC mean] represents the blank-corrected mean concentration for each element across all sites.

	ICPMS						
Statistic	Al	Ba	B	Cu	Li	Mg	Mn
LOD (mg/L)	1.44	0.12	0.69	0.11	0.09	0.54	0.04
CV (%)	68	12	285	160	54	18	26
[BC mean] (mg/L)	1.65	14.1	0.02	0.32	0.07	28.31	3.99
[BC mean] > LOD (number of wetlands)	3	8	0	7	3	8	8

retained for statistical analyses. While the development of broadly applicable selection criteria is a worthy goal, it is important to note that there is a trade-off between strict requirements for low LODs–high precision and the elimination of elements that might improve the clarity of elemental fingerprints. The range of variability among site means and the high number of site means above LODs for Pb and Zn suggested these elements might improve the clarity of elemental fingerprints in this study; however, they were eliminated because precision was low.

Statistical analysis.—Concentration of Mg was reciprocal transformed, and Mn and Sr concentrations were natural log-transformed to normalize their distributions prior to statistical analysis (Wilkinson et al. 1996). Ba, K, and Na concentrations were not transformed because their distributions were normal. Based on these distributions, the data were checked for outliers (points separated from the main distribution by >5 SD; Tukey 1977), but none were found.

Because there were obvious differences in the mean lengths of fish among sites (Table 1), we tested for relationships between elemental concentration and fish length within sites to determine if these differences might confound any wetland-specific differences in elemental compositon. Plots of Na, K, and log-transformed Sr concentrations versus fish length indicated a significant positive correlation ($P < 0.05$) across all sites and for many within-site plots, so we detrended the concentrations of these elements by methods described in detail by Campana et al. (2000). Briefly, we subtracted the product of fish length and the common within-group linear slopes obtained with analysis of covariance (ANCOVA; Na: $b_1 = 185.74$; K: $b_1 = 19.69$; $\log_e$ (Sr): $b_1 = 0.137$) from each sample point to detrend the element concentrations. There was no residual relationship between element concentrations and fish length following this detrending (e.g., see Figure 4). All statistical analyses reported here are based on detrended concentrations, although additional analyses revealed that we would have obtained virtually the same conclusions without detrending. Similar conclusions were also obtained if analyses were restricted to fish of sizes that overlapped across all sites (between 5 and 8 cm).

Both univariate and multivariate approaches were used to analyze and describe elemental fingerprints. Analysis of variance (ANOVA) with Tukey's multiple comparison procedure was used to test for differences in element concentrations among individual sites. Multivariate ANOVA (MANOVA) and linear discriminant function analysis (LDFA) were used to quantify and illustrate the distinctness of the multivariate fingerprint. MANOVA with Pillai's trace statistic quantified the significance of overall differences in the fingerprints in multivariate space. Plots of canonical variates from the LDFA provide a graphical representation of site differences for visualizing the multivariate fingerprints and, along with 95% Gaussian confidence ellipsoids around the centroids of each site distribution (Wilkinson et al. 1996), provide an a posteriori means of visualizing the significance of intersite differences in multivariate space. LDFA was also used to determine how accurately the elemental fingerprints can be used to classify individual age-0 yellow perch to their coastal wetland nursery areas. A classification algorithm (Wilkinson 1999) which uses a jackknife procedure was used to estimate classification accuracy. The procedure removes each sample sequentially from the dataset, reestimates the discriminant function from remaining samples, and uses the resulting function to classify the data point that was removed. We completed these analyses using all variables that were significant in the univariate tests. We also sequentially removed the least explanatory elements to examine the possibility that fewer elements could be used in future studies without sacrificing discriminatory power.

TABLE 2.—Extended.

Statistic	ICPMS			ICPAES			
	Pb	Sr	Zn	Ca	Fe	K	Na
LOD (mg/L)	0.01	0.09	0.26	204	1.13	1.15	2.63
CV (%)	119	6	83	0.8	196	4	2
[BC mean] (mg/L)	0.043	255.81	1.65	410,000	3.40	498.4	2,470.0
[BC mean] > LOD (number of wetlands)	7	8	8	8	3	8	8

Results

All six elements retained for statistical analysis (Ba, Mg, Mn, Na, K, and Sr) differed significantly among sites (ANOVA, $P < 0.001$). Elements with the greatest number of significant intersite differences (Tukey's HSD, $P < 0.05$) were Sr (24 site pairs) and Mn (19 site pairs). Among the most notable intersite differences were the Sr peak at Honest John Slough; Mn at Allouez Bay Wetland; Ba spikes at Flag River Wetland, Honest John Slough, and E. Flag Lagoon; high K concentrations at Kakagon Slough; and low Na concentrations at Bark Bay and Flag River (Figure 3).

Intersite differences were highly significant when analyzed as a multivariate fingerprint (MANOVA, Pillai's trace statistic, $P < 0.001$). All six elements that differed among sites in ANOVAs were also identified as significant contributors to the separation among elemental fingerprints in multivariate discriminant space (LDFA, Wilk's lambda, $P < 0.0001$). However, Na did not improve classification success, so it was removed from the LDFA, leaving a five-element model that included Ba, Mg, Mn, K, and Sr. The first three discriminant functions from this model accounted for 90% of the total dispersion in the dataset (CV1 = 49.4%; CV2 = 25.0%; and CV3 = 15.3%). The highest correlations between individual trace elements and the canonical functions were for Sr and CV1 (0.94), Mn and Mg and CV2 (0.75 and 0.60), and K and CV3 (0.89). The most statistically influential elements in the LDFA model (based on high *F*-statistics) were Sr (87.3), Mn (21.5), K (23.4), and Ba (17.8), indicating these elements were the most important in defining elemental fingerprints in multivariate discriminant space.

Plots of the first three canonical variates, including 95% Gaussian confidence ellipsoids around wetland centroids, revealed significant differences among the mean values for most sites ($P < 0.05$; Figure 5), although there was some overlap between the centroids for Bad River and Flag River along axes 1 and 2, as well as for Flag River and Bibon Slough along axes 1 and 3. LDFA classification success averaged 76% for the five-element model, with the highest accuracy for Kakagon Slough (100%) and Allouez Bay Wetland (94%), and the poorest accuracy for Bad River (62%) and Bibon Slough (65%), which tended to be misclassified as their neighboring wetlands (Table 3). Sequentially removing the least explanatory elements (based on associated *F*-statistics) from the full model maintained classification success at a relatively high level (≥70%) even when only three elements were included (Table 4). A two-element model that included only Sr and Mn had 56% accuracy overall, but was still an excellent predictor for some wetlands (e.g., Allouez Bay and Kakagon Slough). The most significant loss in separation among wetlands in the two-element model was between Bark Bay and Honest John Slough, where approximately half the fish were classified as the opposite wetland, and between Bad River Slough and Flag River Wetland, where 25% of the fish from Flag River were classified as Bad River fish. Loss of power to discriminate between these sites is not surprising since both Bark Bay and Honest John Slough are lagoonal wetlands with nearly identical hydrogeomorphology, and Bad River and Flag River wetlands are both at the mouths of relatively large rivers with high suspended sediment loads. A moderate correlation ($r = 0.47$, $P = 0.013$) between interwetland distance and classification success suggests that differences in elemental fingerprints among sites were at least in part related to geographic separation among sites. However, some of the separation also seems to be related to wetland type in that the four river-influenced wetlands (Allouez Bay, Flag River, Bad River, and Kakagon) and the four lagoonal wetlands (Bark Bay, Honest John, Bibon, and East Flag) clustered in different portions of discriminant space (Figure 6). An LDFA model testing for differences in the elemental fingerprints among

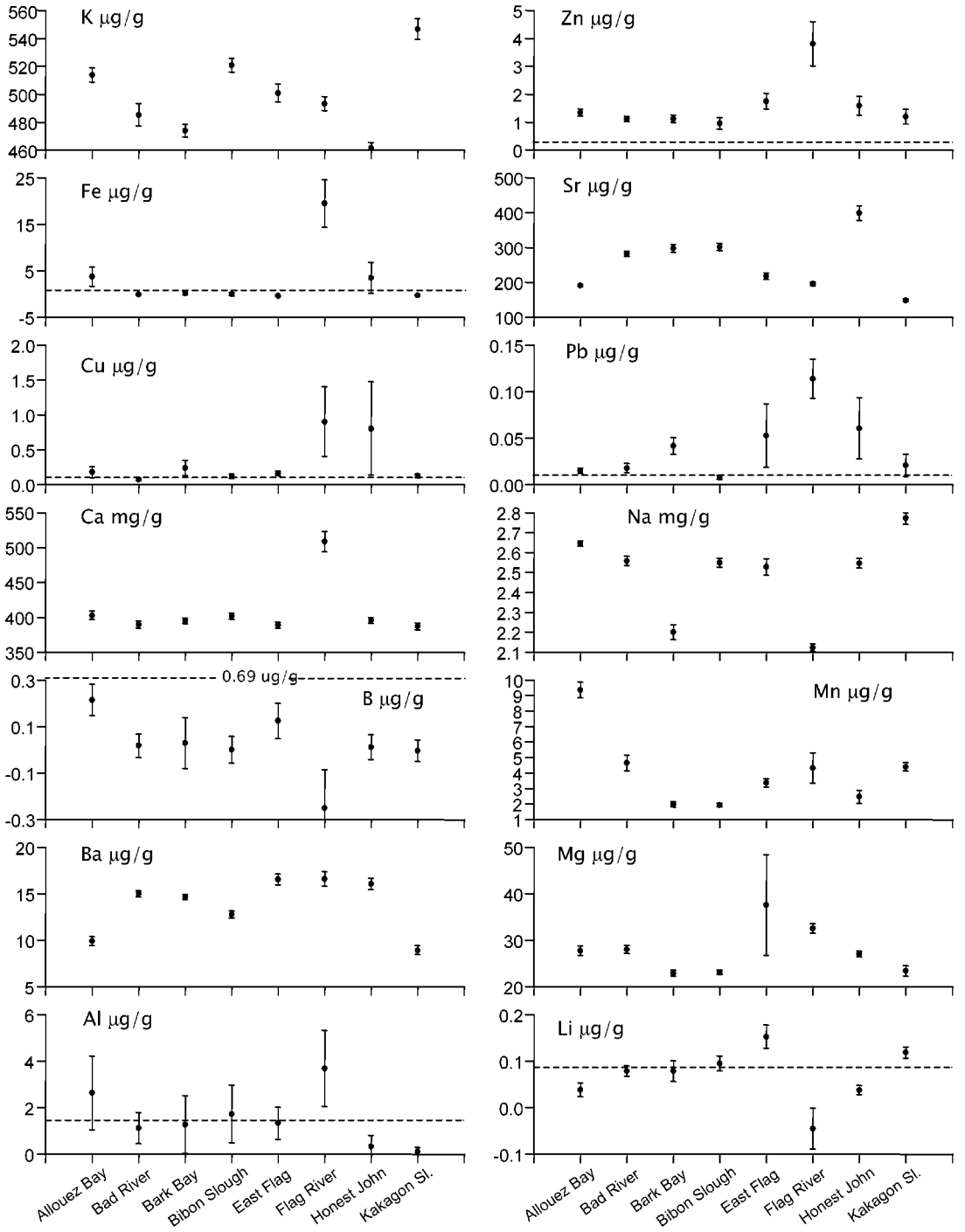

FIGURE 3.—Mean element concentrations (±SE) at each coastal wetland (dotted horizontal lines depict limits of detection [LODs] for elements where site means were close to the LOD; note that the LOD line for B of 0.69 µg/g is not to scale).

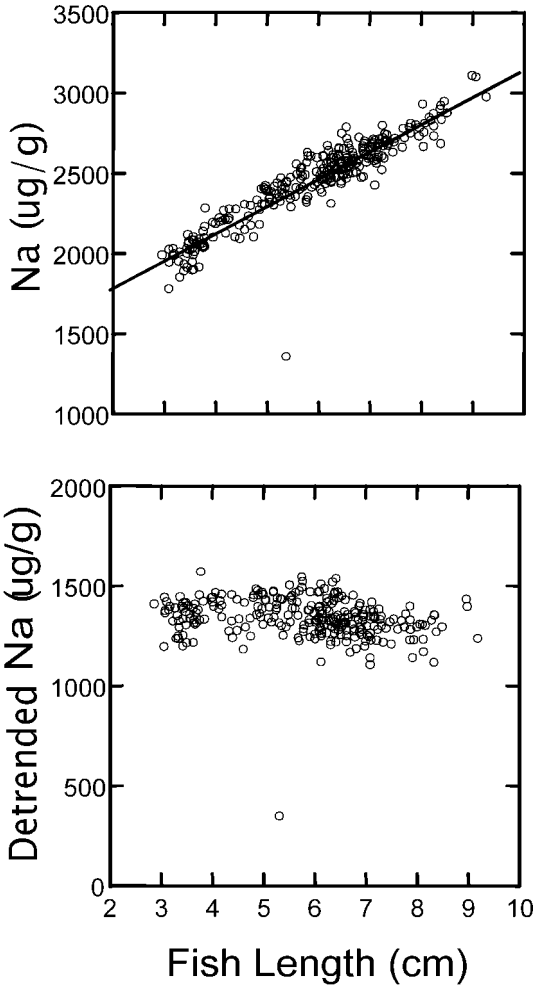

FIGURE 4.—Detrended and undetrended Na concentrations as an example of the effects of detrending element concentration data with the use of the within-group slope coefficients from analysis of covariance.

riverine and lagoonal wetlands was able to correctly classify 79% of yellow perch from lagoonal wetlands and 84% from river-influenced sites, with a highly significant five-element fingerprint that included Mg, Mn, Sr, Na, and Ba (Wilk's lambda, $P < 0.0001$). MANOVA to test for differences among wetland types was also highly significant ($P < 0.001$). This test revealed that four elements (Ba, Mg, Mn, and Sr) contributed significantly to separation among wetland types in multivariate space.

Discussion

Although the mechanism(s) which led to the differences in fingerprints is (are) uncertain, our re-

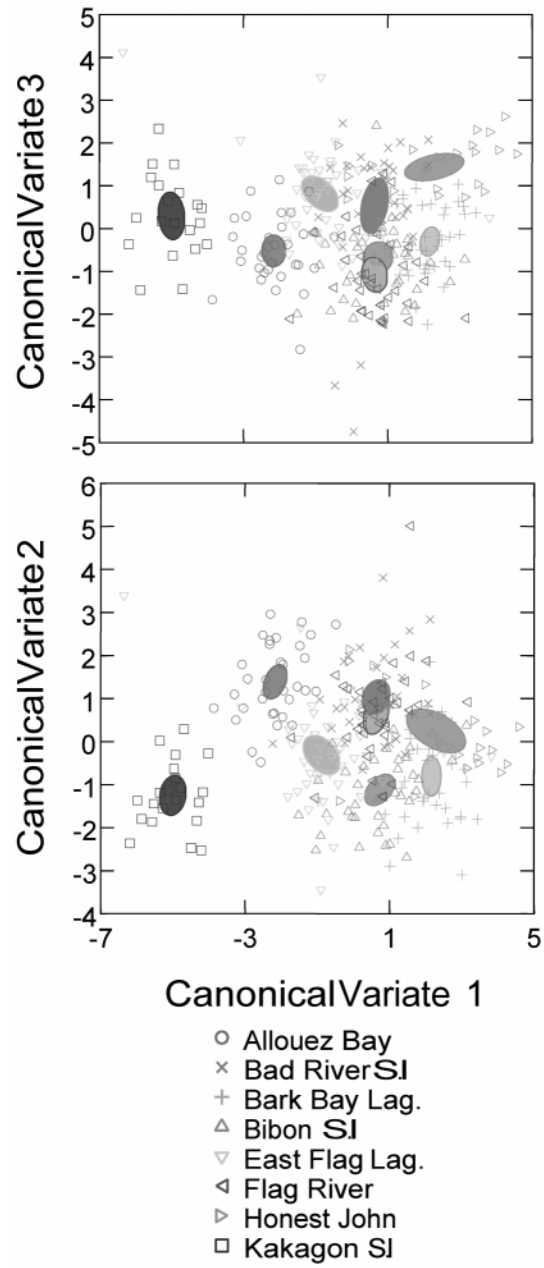

FIGURE 5.—Elemental fingerprints for age-0 yellow perch otoliths from western Lake Superior coastal wetlands based on the first three canonical variates obtained through linear discriminant function analysis including the five most explanatory elements (Sr, Mn, K, Ba, and Mg [ordered from most to least explanatory based on associated *F*-statistics in the model]). Observations for each wetland depicted with 95% confidence ellipsoids around the distribution centroids.

TABLE 3.—Classification accuracy (determined by a jackknife procedure) of age-0 yellow perch samples based on otolith fingerprints used in linear discriminant function analyses that identified Sr, Mn, K, Ba, and Mg as the significant explanatory elements (in order of their significance to the model based on associated *F*-statistics).

Actual wetland	Predicted wetland membership								Percent correct
	AB	BR	BB	BS	EF	FR	HJ	KS	
Allouez Bay Wetland (AB)	31	1	0	0	0	1	0	0	94
Bad River Slough (BR)	2	23	1	0	2	3	6	0	62
Bark Bay Lagoon (BB)	0	1	31	3	0	0	2	0	84
Bibon Slough (BS)	0	3	7	26	3	1	0	0	65
E. Flag Lagoon (EF)	1	2	1	4	32	2	0	1	74
Flag River Wetland (FR)	0	4	1	4	2	27	0	0	71
Honest John Slough (HJ)	0	7	0	0	1	0	17	0	68
Kakagon Slough (KS)	0	0	0	0	0	0	0	21	100
Total	35	41	34	39	42	34	27	22	76

sults suggest that elemental fingerprints can distinguish between juvenile yellow perch that were reared in different coastal wetland nursery habitats with considerable accuracy. Classification accuracy was comparable to other successful habitat fingerprinting studies to date (e.g., Campana et al. 1995; Thorrold et al. 1998b, Gillanders and Kingsford 2000), and considerably higher than the only other otolith fingerprinting study in the Great Lakes (Bronte et al. 1996). Since elemental fingerprints of whole otoliths represent the lifelong integration of an individual fish's exposure to the environment (Campana et al. 1995), we believe differences in otolith elemental fingerprints among wetlands were most likely due to a combination of environmental factors, including: differences in the physical-chemical character of their watersheds, the degree of connectivity of wetlands to their watersheds and the lake, and differences in the physical-chemical character of the wetlands. However, supporting environmental data are quite limited, and other factors that we did not account for may have been important (e.g., diet; see Fowler et al. 1995).

Watersheds along the western end of Lake Superior span several ecological unit boundaries (Figure 1; Albert 1995), so they have diverse geology, topography, vegetation, and soils (Detenbeck et al. 2000), enhancing the probability that differences in otolith chemistry might be derived ultimately from differences in watershed character that translated into differences in ambient water quality in the wetlands. In addition, only half of our sites are connected to their watersheds by rivers that flowed through or immediately adjacent to them (Allouez Bay, Bad River, Flag River, and Kakagon Slough). The other half are lake connected but had no direct river influence (Bark Bay, Bibon Slough, East Flag, and Honest John). Although there are almost no published data on trace element water chemistry for our study area, lake water chemistry is clearly different from tributary chemistry in most locations (Trebitz et al. 2002; Anne Cotter, U.S. Environmental Protection Agen-

TABLE 4.—The effect of input variable reduction on overall classification accuracy of age-0 yellow perch samples based on otolith fingerprints used in linear discriminant function analyses (LDFA). LDFA models (including 2–5 of the most explanatory elements as input variables) were examined. ''Most explanatory'' based on *F*-statistics associated with each element in the full model utilized for Table 3; elements ordered from most to least explanatory. Site abbreviations are defined in Table 2.

Wetland	Five-element model (Sr, Mn, K, Ba, Mg)	Four-element model (Sr, Mn, K, Ba)	Three-element model (Sr, Mn, K)	Two-element model (Sr, Mn)
AB	94	94	91	94
BR	62	59	62	54
BB	84	70	65	38
BS	65	68	50	53
EF	74	77	72	67
FR	71	50	71	39
HJ	68	64	62	8
KS	100	100	100	100
Total	76	71	70	56

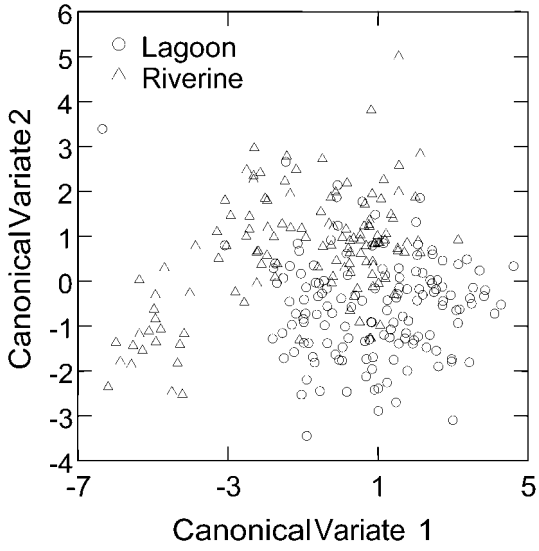

FIGURE 6.—Elemental fingerprint as presented in Figure 5 (canonical variates 1 and 2 only), but with sites coded by wetland type.

cy–Duluth, personal communication), so it seems likely that these differences could have contributed to differences in elemental fingerprints that we observed. Connectivity differences almost certainly were reflected in the differences in fingerprints among lagoon and riverine wetlands that we detected, but probably also contributed to differences among individual sites even within wetland classes, since connectivity to the lake (e.g., size and depth of inlet/outlet) and tributary influence (e.g., discharge) vary considerably for these wetlands (Trebitz et al. 2002). Although we did not quantify physical-chemical differences among wetlands for this study, previous estimates suggest there are differences in temperature, suspended and bottom sediments, and macrophyte cover (Trebitz et al. 2002; J. C. B. and D. K. T., unpublished data) that may also have contributed to differences in otolith chemistry (e.g., Fowler et al. 1995). Identifying the mechanisms that led to differences in fingerprints that we observed will be a goal of future studies.

Discriminant models using different numbers of elements as explanatory variables revealed that overall site classification success was nearly as good with three elements as it was with five, although accuracy changed slightly for particular wetlands depending on which elements were left in the model. This suggests that it may be possible to focus on a smaller number of elements in future studies to increase analytical efficiency. However, the suite of elements within acceptable analytical limits for this study was fairly low, so it was advantageous to have started with a larger suite and be able to select those with the greatest accuracy and precision. Until more is known about which elements are typically present in detectable quantities with acceptable analytical precision in otolith studies of freshwater fishes, analyzing a wider variety of elements may be the best choice.

The number of elements analyzed is related to the choice of analytical instrument. Eliminating the need to run analyses on both ICPMS and ICPAES instruments would obviously improve analytical efficiency, but given the need to calculate sample weights based on Ca concentrations rather than from actual weights, it was prudent for us to use ICPAES. Calcium analysis is much more precise and requires considerably less sample with ICPAES when calcium concentrations are as high as they are in otoliths (R. Kean, Royal Productivity Council, personal communication). Using ICPAES also simultaneously provided data on K and Na, which improved overall classification success. However, new ICPMS technologies (e.g., flow injection) with greater sensitivity and fewer problems with interferences may eliminate the advantage currently provided by ICPAES.

Elements that were the most important explanatory variables in our study have also been important in other elemental fingerprinting studies. Most commonly, Sr, Ba, Mg, and Mn, along with K, and Na in various combinations, have all helped discriminate among estuarine nursery areas (Gillanders and Kingsford 1996, 2000), riverine nurseries adjacent to estuaries (Thorrold et al. 1998a, 1998b), Atlantic cod populations in a mixed-stock fishery in the North Atlantic (Campana et al. 1995, 2000), and in a variety of other studies in marine environments (for review see Gillanders et al. 2001). It is interesting that the suite of elements we found to be important for discriminating among sites in Lake Superior were very different from those analyzed by Bronte et al. (1996) in their attempt to discriminate among lake herring populations in Lake Superior. The two studies shared no elements that were significant discriminators. It seems likely that differences in lake and wetland chemistry would lead to differences in the importance of various elements in the fingerprints from the two habitats. It may also be that the reduced ability to discriminate among spawning sites in the Bronte et al. (1996) study was due to a greater mixing of open Lake Superior waters where lake herring spawn compared with the wetland areas

that we studied which are relatively discrete habitats. Although there are no other elemental fingerprinting studies for comparison in the Great Lakes or other freshwater lakes, it seems likely that element choice will be critical to obtaining the most discriminating fingerprint. Further work needs to be done in freshwater ecosystems to better define the best suite of elements and the mechanisms that lead to differences among them. However, it is important to note that lack of mechanistic understanding does not detract from the utility of using elemental fingerprints as natural tags. Trace element differences that can be detected analytically and statistically in the otoliths of fish from different sites are all that is necessary.

Regardless of the mechanisms that led to differences in elemental fingerprints, the key implication of our study is that we should be able to use elemental fingerprints from fish otoliths as wetland-specific markers in future studies. We hope to use this tool to determine the magnitude and timing of the movement of yellow perch and other wetland-dependent fish populations between coastal wetlands and the adjacent lake, as well as to identify which wetlands are responsible for recruitment to these populations in the lake. This use of elemental fingerprinting has been successful in a number of marine applications (Forrester and Swearer 2002; Gillanders 2002a), and we expect it will also be successful for fishes using Great Lakes coastal wetlands. It will require sampling age-0 fish from all likely nursery areas in an area of interest in the Great Lakes (e.g., western Lake Superior) to establish a complete library of nursery fingerprints that can be matched against fingerprints from the otolith cores of adult fish captured offshore. Since nursery fingerprints have been shown to vary between years (Gillanders 2002b), it will be critical to age-match the adult and age-0 fingerprints (e.g., if adults are sampled 2 years after the age-0 fingerprint is established, only fish in the 2+ age-class should be examined). Ultimately, we hope to establish age-specific elemental fingerprints to determine the amount of time yellow perch and other wetland-dependent species spend in coastal wetlands relative to other habitats. This will help us gain a better understanding of coastal wetland habitat function and the ecological linkages between wetlands and adjacent Great Lakes waters. In addition, based on the results of studies in estuarine rivers (e.g., Thorrold et al. 1997), the techniques we are developing with wetland fish may be useful for establishing the fingerprints and origins of river run fish stocks in the Great Lakes.

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