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Reconstructing Habitat Use and Wetland Nursery Origin of Yellow Perch from Lake Superior using Otolith Elemental Analysis

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ABSTRACT. *The use of otolith elemental composition as a natural tag has emerged as a powerful tool for managing and understanding the ecology of marine fish populations. The approach remains relatively untested in fresh waters, so we examined its utility for reconstructing habitat use and wetland nursery origin in Lake Superior. We analyzed the otolith margin of adult yellow perch,* Perca flavescens*, as an indicator of recently occupied habitat, and the juvenile region of the otolith core as an indicator of nursery area. To characterize elemental fingerprints, all otolith samples were analyzed for Ca and 13 minor and trace elements using mass spectrometry. We found differences in the otolith concentrations of several elements between yellow perch inhabiting coastal wetlands and those inhabiting the adjacent nearshore waters of Chequamegon Bay. The most striking difference was the high concentration of Sr in the sagittal margins of wetland-caught fish relative to those captured in the bay. Based on differences in otolith Sr concentrations alone, fish from bay and wetland habitats could be distinguished with 100% accuracy. We also found that elemental fingerprints derived from otolith cores of adult yellow perch were similar among fish captured from wetlands adjacent to Chequamegon Bay but quite distinct for one site outside of the bay, suggesting these fish came from a separate population from those in Chequamegon Bay. Overall, these results encourage us that elemental fingerprinting techniques will be useful for estimating the relative importance of different coastal wetland habitats to wetland-dependent species in the Great Lakes.*

INDEX WORDS: *Elemental fingerprints, yellow perch, Perca flavescens, otolith, sagittae, lapilli, Great Lakes coastal wetlands, Lake Superior, nursery areas, habitat use.*

INTRODUCTION

Otolith elemental composition has proven quite useful for managing and understanding the ecology of a number of marine and estuarine fish populations in recent years. Elemental "fingerprinting" based on the analysis of trace elements in fish otoliths has been used to identify migration and life history patterns (Northcote *et al.* 1992, Halden *et al.* 1996, Tsukamoto *et al.* 1998, Zimmerman and Reeves 2002, Goto and Arai 2003), movement within estuaries (Secor and Piccoli 1996, Secor et al 2001) and rivers (Howland *et al.* 2001, Kennedy *et al.* 2002, Weber *et al.* 2002), seasonal changes in habitat use (Pender and Griffin 1996), and nursery origins of adult fishes captured offshore or other locations

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away from the nurseries (Thorrold *et al.* 2001, Gillanders 2002a). Typically, the relative abundance of trace elements, such as Sr, Ba, and Mg, in the otoliths of individual fish vary depending on capture location or age of the fish, providing a permanent marker or "fingerprint" which is then used as a natural tag (for reviews, see Campana 1999, Gillanders *et al.* 2001).

Elemental fingerprinting studies have been possible because fish incorporate minor and trace elements from the water into their otoliths, although not necessarily in direct proportion to environmental concentrations due to physiological regulation of certain elements (Kalish 1989, Campana 1999). Differences in otolith element concentrations among fishes can reflect either natural or anthropogenically-induced differences in the fishes' environment. Since the protein and calcium carbonate structure of fish otoliths is acellular and metabolically inert, once elements are incorporated from the endolymphatic fluid their concentrations remain fixed over the life of the fish. By tracking changes in elemental concentrations over time in an individual fish or among fish captured from different locations, it is often possible to deduce much about their environmental history, such as previous habitat use or nursery location. Although these sorts of studies have become relatively common in marine environments, few have been conducted with freshwater fishes. The work of Bronte *et al.* (1996) on discriminating among lake herring spawning populations in Lake Superior, and Patterson *et al.* (1993) on establishing the oxygen isotope temperature fractionation relationship for Great Lakes fishes are notable exceptions.

Although the vast majority of fishes in the Great Lakes are thought to be dependent on coastal wetland habitats for some portion of their life history (Whillans 1992) and coastal wetlands are considered to be critical nursery areas in the Great Lakes (Jude and Pappas 1992), very little is actually known about the *relative* importance of different wetlands for spawning, or the frequency of movement of fish among coastal wetlands and other habitats in the adjacent lake (Brazner *et al.* 2001). Despite the belief that coastal wetlands are "centers of organisation" in the Great Lakes and have a much more important role in regulating trophic interactions than their size alone would suggest (Steedman and Regier 1987), there is little supporting evidence (e.g., Keough *et al.* 1996, Brazner *et al.* 2000). This is primarily because of the difficulty in conducting mark-recapture or other studies that track fish movements in such large open systems. Attempts to use genetic markers as natural tags have had poor success (e.g., Wirgin *et al.* 1995, Epifanio *et al.* 1995). However, the emergence of elemental analysis of fish otoliths holds great potential for developing habitat and nursery fingerprints for Great Lakes fishes. In fact, Brazner *et al.* (2004) recently developed elemental fingerprints for several western Lake Superior coastal wetlands using yellow perch otoliths. Ultimately, these fingerprints should be useful for assessing the relative importance of coastal wetlands for different species.

There were three main objectives for this study: 1) To determine if there were differences in the elemental fingerprints between adult fish living in coastal wetlands and those captured in adjacent nearshore waters. Establishing that there is a difference in elemental fingerprints among wetland and other nearshore habitats is the first step in confirming that elemental fingerprints can be used to reconstruct habitat history; 2) To examine elemental fingerprints in the cores of adult yellow perch caught in different wetlands in western Lake Superior to assess the likelihood that these fish came from the same nursery area. Otolith cores are comprised of material deposited in the first summer in the life of each fish, so distinctive elemental fingerprints from the cores of adults would be indicative of the degree of mixing among adult populations after YOY leave different nursery areas and mature. In general, elemental fingerprints from cores of fishes captured at a particular wetland should be more similar to each other than to those from cores of fishes captured at other sites if the adults tend to live near their nursery grounds; 3) Finally, because conducting these analyses required the use of untested techniques that could have influenced results, the effect of micromilling and sample mounting methods on elemental fingerprints were examined.

Yellow perch was selected as a test species for these initial studies for a number of reasons. Yellow perch commonly utilize Great Lakes coastal wetlands as spawning and nursery areas (Jude and Pappas 1992), and they are fished commercially and for sport in the Great Lakes. In addition, yellow perch are known to move between wetland and lake habitats (Brazner *et al.* 2001), and their abundance in the nearshore waters of most of the lakes suggests a potentially important trophic role (Brazner *et al.* 2000). Although our focus was on yellow perch, these results should be helpful in guiding studies to determine the relative importance of coastal wetlands to other Great Lakes fishes.

METHODS

Habitat Differences Study

To determine if wetland and bay habitats produced distinct elemental fingerprints, sagittal otoliths were removed from adult yellow perch captured from lower Chequamegon Bay, near Ashland, Wisconsin, and from the three primary coastal wetland nursery areas (Maynard and Wilcox 1996) adjacent to the lower and middle portions of Chequamegon Bay (East and West Fish Creek Wetlands, and Sioux River Slough, Fig. 1). Each of these wetlands are river-influenced to some degree, although the small creek that feeds East Fish runs only intermittently. Trebitz *et al.* (2002) provides an excellent description of the morphologic and hydrologic character of these wetlands. Perch from the bay (mean length = 15.7 cm, Table 1) were captured with gill-nets set at 3-4 m depth (n = 34) on 11 June 2001. Wetland fish (mean length = 17.9 cm, Table 1) were captured by electrofishing throughout the wetlands on 28 August 2001 (n = 10 per site). Fish were iced during transport to the lab and held frozen for 6 months, until otoliths were removed.

After removal, sagittae were cleaned of debris and tissue under a dissecting microscope, and decontaminated with the sonification-Super Q water (distilled, millipore-filtered, reverse osmosis water) rinse protocol described in Campana *et al.* (2000), although we were not able to scrub the otoliths due to their small size. 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 milling. Before milling, sagittae were attached to acid-washed glass slides with thermoplastic glue, so that their outer margin

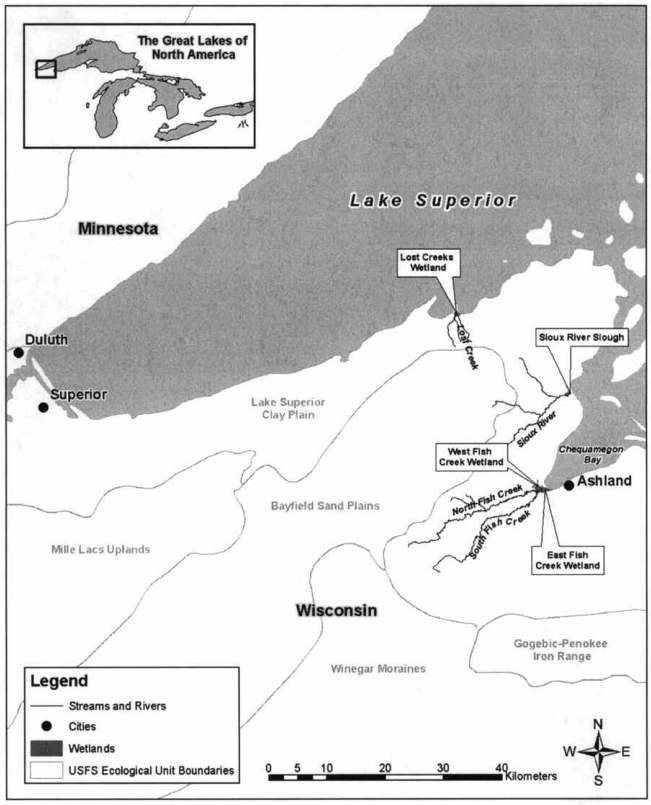

FIG. 1. Map of western Lake Superior coastal wetland sampling locations.

TABLE 1. Yellow perch length and otolith weight summary statistics for the Habitat and Core Differences Studies by sampling location (for each study, differences in means with no superscripts in common were significantly different, ANOVA, $p < 0.01$; otolith weight is the calculated weight used for elemental analysis; note that fish from East and West Fish Creek Wetlands and Sioux River Slough are the same for both studies).

Sampling Location	n	Fish Length (cm) mean (se)	Otolith Weight (mg) mean (se)
Habitat Differences Study			
Chequamegon Bay	34	15.7 (0.3)[a]	0.37 (0.02)
East Fish Creek Wetland	10	17.8 (0.6)[b]	0.35 (0.05)
West Fish Creek Wetland	10	17.8 (0.6)[b]	0.28 (0.04)
Sioux River Slough	10	18.1 (0.5)[b]	0.33 (0.04)
Core Differences Study			
East Fish Creek Wetland	10	17.8 (0.6)	2.25 (0.13)
West Fish Creek Wetland	10	17.8 (0.6)	2.21 (0.13)
Sioux River Slough	10	18.1 (0.5)	2.58 (0.10)
Lost Creeks Wetland	10	16.6 (1.8)	2.44 (0.19)

TABLE 2. Detection limits (LOD based on reagent blanks) and precision (CV based on homogenized otolith reference powder) for all elements by analytical method.

	ICPMS								ICPAES					
	Al	Ba	B	Cu	Li	Pb	Sr	Zn	Ca	Fe	K	Na	Mg	Mn
LOD (ppm)	3.06	0.10	1.24	0.61	0.03	0.08	0.15	0.70	37.44	1.38	29.47	32.71	1.45	0.11
CV (%)	47.8	8.8	65.5	44.5	118.3	17.5	3.5	11.4	0.6	13.2	8.7	3.9	5.8	15.4

could be removed for elemental analysis. We assumed that the outer margin of the sagittae would represent growth and deposition of material from the capture site and would therefore have recorded the fish's recent history of habitat use.

Milling was completed with a programmable microsampling device with submicron stage resolution (New Wave Research Merchantek MicroMill) integrated with a high-resolution stereomicroscope and linked to a video camera and display that allowed precise on screen selection and programming of drilling location, direction, depth, and speed. Multiple passes along the outermost 100–150 microns of the posterior sagittal margin at a drill speed of 80% of maximum and at a depth of 40 microns were completed until the entire margin of the otolith could be removed. Small pieces of sagitta edge and powder that were on the slide after drilling were retrieved with a fine-bristle brush and placed in acid-rinsed vials. The outer margins milled from three fish were polished and examined under an image analysis system to determine how many days of growth the milled material represented. Our estimates ranged between 30 and 45 days, based on counts of daily growth increments.

Elemental Analysis

Milled otoliths were digested with sub-boiling double-distilled nitric acid in the original acid-washed sample containers and diluted to a final volume of 2.0 mL. Based on preliminary studies with YOY 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). Ba, B, Cu, Mg, Mn, Sr, and Zn were simultaneously analyzed by both inductively coupled plasma mass spectrometry (ICPMS) and inductively coupled atomic emission spectrometry (ICPAES). K, Na, Ca, and Fe were analyzed only by ICPAES, and Al, Li, and Pb only by ICPMS for logistical reasons. 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. For elements analyzed by both methods we selected results from the method that provided the best combination of low detection limit and high precision (Table 2). Microconcentric nebulizers with natural aspiration rates of approximately 0.4 mL per minute 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 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 of the extremely small sample sizes and weighing difficulties associated with milled (e.g., powder) samples, all reported element concentrations are based upon a sample weight referenced to calcium concentration. This approach produces accurate estimates of sample weight for all samples, including those below the limits of the balance, since the otolith is composed of nearly pure calcium carbonate (40% calcium by weight) (Campana 1999). Based on the thirteen analyzed reagent blanks, detection limits (LOD = 3 SD in µg/g of otolith weight; U.S.EPA 2003) were well below blank-corrected mean concentrations at each site except for B (Fig. 2). Estimates of precision (coefficient of variation) based on nineteen samples of finely ground cod, *Gadus morhua*, otolith reference powder (see Campana *et al.* 2000 for details on this material) were well below 20% for most elements, but ranged as high as 118% for Li (Table 2). Although there are no

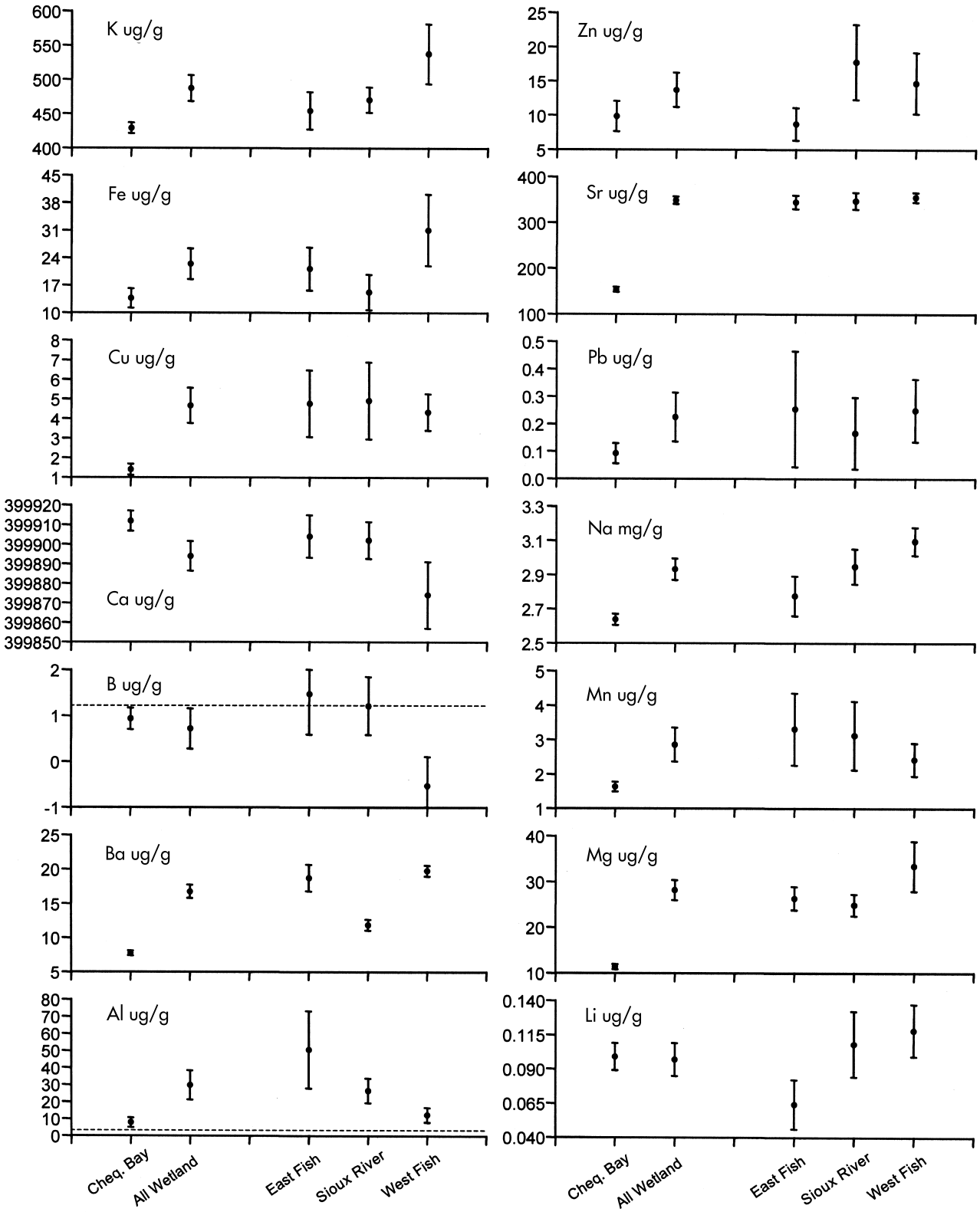

FIG. 2. Mean element concentrations (± 1 s.e.) by habitat and site for the **Habitat Differences Study** *(East Fish, Sioux River, and West Fish were the three wetlands averaged to produce the "All Wetland" values; dotted horizontal lines depict LODs for elements where site means were close to the LOD).*

strict criteria for retaining elements in these types of studies, the following guidelines were used by Brazner *et al.* (2004); 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%. We adopted these criteria for this study and suggest that they should be broadly applicable to other studies of elemental fingerprints. Based on these criteria Al, B, Cu, and Li were eliminated from inclusion in elemental fingerprinting models, while Ba, Fe, K, Mg, Mn, Na, Pb, Sr, and Zn were retained for further statistical analyses. Calcium concentrations were used only as a reference to derive other element concentrations (as described above). They were not included as part of the statistical analysis of elemental fingerprints.

Statistical Analysis

Pb concentrations were reciprocal transformed and Fe, Mn, Na, and Zn concentrations were natural log transformed to normalize skewness in their distributions prior to statistical analysis (Wilkinson *et al.* 1996). Ba, K, Mg, and Sr concentrations were not transformed because their distributions were normal. Outliers were removed from the data when they were separated from these distributions by more than five standard deviations (Tukey 1977). Only five points were removed as outliers—one each from the Fe, Na, and Sr data sets, and two from the Pb data set.

Both univariate and multivariate approaches were used to analyze and describe elemental fingerprints. Analysis of variance (ANOVA) was used to test for differences in element concentrations between habitats and as a screening tool to help guide which elements to include in additional multivariate analyses. Multivariate analysis of variance (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 using only those elemnts that were significant in the ANOVAs. Plots of the first two canonical variates provide a two-factor graphical representation of habitat or site differences for visualizing the multivariate fingerprints and, along with 95% Gaussian confidence ellipsoids around the centroids of each distribution (Wilkinson *et al.* 1996), provide an *a posteriori* means of estimating the significance of inter-habitat or inter-site differences in multivariate space. Stepwise LDFA based on the same elements included in the MANOVAs was used to determine how accurately the elemental fingerprints can be used to classify individual yellow perch to the habitats or sites from which they were sampled. A classification algorithm (Wilkinson 1999) which uses a jack-knife procedure was used to estimate classification accuracy. The procedure removes each sample sequentially from the data set, re-estimates 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 ($p < 0.05$) and also by sequentially removing the least explanatory elements (based on F-statistics) to provide reduced variable models that were evaluated for statistical significance using Pillai's trace statistic. This is the most robust of the multivariate test statistics to assumptions of multivariate normality and homogeneity (Wilkinson *et al.* 1996). We determined the best stepwise LDFA model by conducting both forward and backward variable entry approaches, retaining only those variables with significant F-statistics ($p \leq 0.05$), and then comparing the classification accuracy of reduced models to see which one had the highest classification success. The model with the fewest variables and highest classification success was selected.

Core Differences Study

To assess the likelihood that fish from different wetlands originated from the same nursery area, sagittae were extracted from ten adult yellow perch (12.7 - 28.3 cm in length, mean length = 17.6 cm, Table 1) sampled from each of four western Lake Superior coastal wetlands (Lost Creeks, West Fish Creek, East Fish Creek, and Sioux River) by electrofishing throughout the entire wetland during the week of 29 August 2001. These are the same wetlands utilized for the *Habitat Differences Study* with the addition of Lost Creeks Wetland, which is another river-influenced site that provides a contrast outside of Chequamegon Bay (Fig. 1). Fish were handled in the field and otoliths processed and decontaminated in the lab in the same manner as described for the *Habitat Differences Study*. However, for this study we needed to identify and remove the core of the otolith prior to decontamination and elemental analysis.

To identify the proper size of the core to extract, we measured the sizes of twenty YOY yellow perch sagittae from a different elemental fingerprinting study in western Lake Superior coastal wetlands

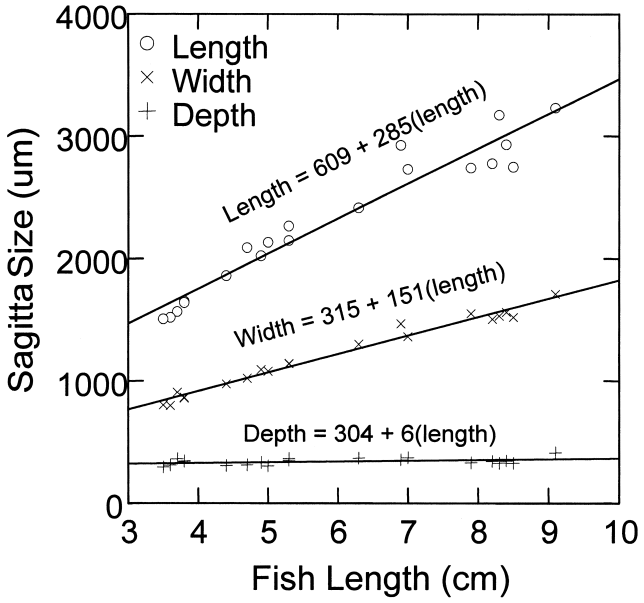

***FIG. 3. YOY fish length vs. otolith size regression relationships for sagitta length, width, and depth used in the* Core Differences Study.**

(Brazner *et al.* 2004) and developed regressions between YOY fish length and sagittae length, width, and depth (Fig. 3). From this, we calculated that the otolith core size of a mid-summer YOY yellow perch (5 cm total length) was approximately 1 mm by 2 mm and 350 μm thick. Differences in elemental fingerprints from cores of this size should reflect differences in nursery habitats, assuming YOY spend their entire lives in a single nursery area. A diamond wafering saw (Isomet 11-1180 low speed saw, Buechler Ltd., Evanston, IL) was used to cut the 1 × 2 mm cores out of the adult sagittae, while metallurgical lapping film was used to sand the cores to a thickness that approximated YOY sagitta depth (350 μm).

After cores were extracted and decontaminated, elemental analysis was completed in the same manner as described for the *Habitat Differences Study*. The same LODs and precision estimates (Table 2) apply to the core data since blanks and reference samples were part of the same analytical run. Statistical analyses to test for differences in elemental fingerprints among sites and to develop classification models based on the fingerprints were also nearly identical to those used in the *Habitat Differences Study*. Since all element concentrations were well above LODs (Fig. 4), the same set of elements were analyzed in the *Core Differences Study*. Because the skewness of individual element concentrations was slightly different than for the *Habitat Differences Study* we applied different transformations for certain elements. Fe, Mg, and Zn concentrations were natural log transformed; Pb concentrations were reciprocal transformed; and Ba, K, Mn, Na, and Sr concentrations did not require transformation to normalize their distributions. Only one point was removed as an outlier. This was from the Mg data set. Since there were no significant differences in fish length or otolith weight among sites ($p > 0.25$, Table 1), all element concentration data were analyzed without detrending for length or weight.

Micromilling Effects Study

To examine the influence of micromilling and sample mounting on elemental fingerprints, 50 pairs of lapilli were extracted from adult yellow perch captured in several Lake Superior and Lake Michigan coastal wetlands in August of 1995 and 2001. Lapilli are one of the three types of otoliths in teleost fishes, intermediate in size between the larger sagittae and the asteriscii. Thirty-five of the pairs were used to test for effects of micromilling, and fifteen pairs were used to test for the effects of glueing otoliths to slides for micromilling. All inter-fish variability was eliminated through the use of a matched-pair design, which tests for differences in elemental concentration within pairs of otoliths from the same fish using one otolith of each pair as the test and the remaining otolith as a control. As a result, details about collection sites and differences among these sites become irrelevant and will be omitted for brevity. We selected lapilli for this study partly because some were available from fish whose sagittae we had already extracted, but also because we felt their smaller size would maximize the likelihood of detecting a milling effect, if there was one, when analyzing the whole otolith. All of the lapilli were run through the same decontamination procedures as were used in the other two studies.

To examine micromilling effects, the thirty-five lapilli pairs were split into two groups. Both groups were glued to glass slides with the heat-sensitive thermoplastic glue (Crystal bond) used for the *Habitat Differences Study*. The test group was milled with our high-resolution micromilling device, while the control group was not. Milled lapilli were milled with a nine-hole square grid pattern that covered approximately one-third of the surface area of each lapillus, and drilled to a depth of approximately 40% of maximum depth. Both drilled and undrilled lapilli

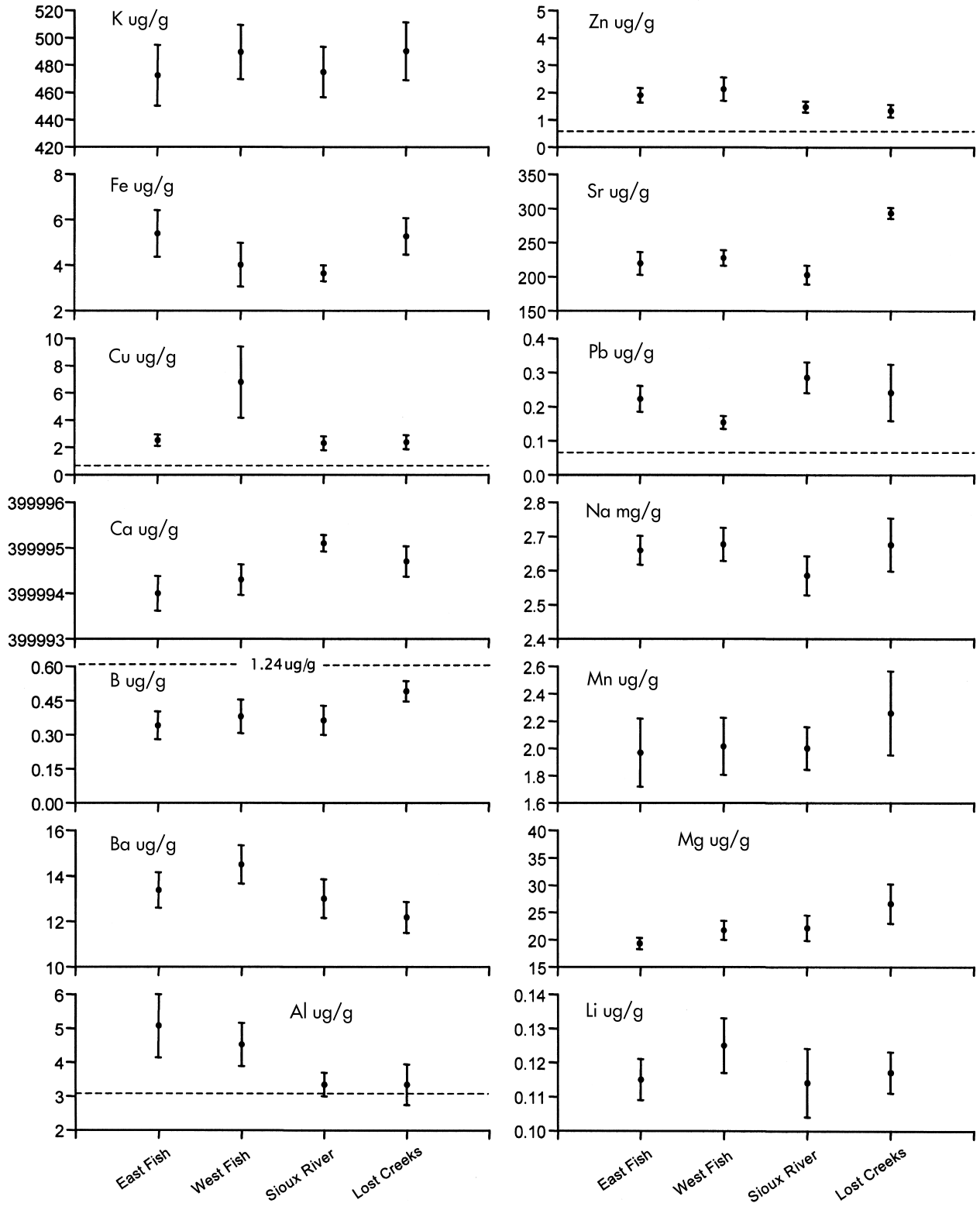

FIG. 4. Mean element concentrations (± 1 s.e.) by wetland for the **Core Differences Study** *(dotted horizontal lines depict LODs for elements where wetland means were close to the LOD; note LOD for B not to scale).*

were subsequently removed from the glue by heating to 135°C. and placed back into acid-washed plastic vials, including all otolith pieces or powder left over from drilling.

To determine the effect of glueing on otolith chemistry, another fifteen pairs of lapilli were split into two groups. The test group was glued to a slide and removed, while the control group was not glued or altered in any way. Both glued and unglued lapilli were placed back into separate acid-washed plastic vials before elemental analysis. Although only fifteen pairs could be included for elemental analysis, twenty-nine pairs of glued lapilli were weighed to the nearest 0.01 mg, before and after glueing, to get an estimate of the weight of residual glue.

The same elements analyzed for the *Habitat and Core Differences Studies* were analyzed in the lapilli to determine if milling or glueing effects were apparent. Because preliminary results revealed unusual patterns for Al, we included it in these analyses as well. Significance of treatments on element concentrations were based on whether the mean within-pair differences in milled vs. unmilled, or glued vs not-glued pairs of lapilli were significantly greater than zero ($p \leq 0.05$). Since none of the distributions of differences in element concentrations between otolith pairs was skewed, we analyzed these data without transformations.

RESULTS

Micromilling Effects Study

Overall, neither micromilling or glueing had much effect on the elemental concentrations we measured. However, micromilling did elevate Zn concentrations substantially, with mean drilled concentrations 73% higher in one otolith (18.2 vs. 10.55 µg/g) than undrilled concentrations in the matching otolith (mean difference = 7.65 µg/g, 95% CI = 0.1 <[Zn diff] < 15.2). In addition, micromilled Ba concentrations were slightly but significantly higher (11%) than unmilled Ba concentrations in matching lapilli (17.9 vs. 16.1 µg/g, mean difference = 1.8 µg/g, 95% CI = 0.4 <[Ba diff] < 3.2). All other elements appeared to be unaffected by micromilling.

None of the final suite of elements selected based on detection limits and precision were significantly affected by glueing, despite the fact that the mass of residual glue on glued lapilli accounted for an average of 12% (s.e. 0.03%) of the unglued lapilli weight (mean preglue weight = 0.58 mg). However, it seems worth noting that Al concentrations were below LOD in samples without glue, but much higher (mean = 34.6 µg/g) in glued samples (mean [Al diff] = 36.1 µg/g, 95% CI = 1.5 < [Al diff] < 70.7), suggesting that bias due to glueing and overall variability would have been too great to allow reliable determination of Al concentrations in micromilled samples if the element had not already been eliminated due to mediocre precision (CV = 48%).

Habitat Differences Study

There were six elements (Ba, K, Mg, Mn, Na, and Sr) that differed significantly among habitats (ANOVA, wetland vs. bay, $p < 0.02$) and all of these except Mn differed significantly among the four sites (ANOVA, 3 wetlands and 1 bay, $p < 0.01$). Differences between wetland and Chequamegon Bay concentrations were most striking for Sr, Ba, Mg, and Cu (Fig. 2). Although Cu was eliminated from fingerprint analysis due to inadequate precision (CV = 45%), the habitat differences we observed suggests it may hold potential for habitat discrimination in future studies. Otolith weight did not differ among habitats or sites. Even though the range of fish lengths was between 15 and 22 cm at all sites (Table 1), fish from the bay were slightly but significantly smaller than wetland fish as a group ($p < 0.01$, Fig. 5). However, fish length was not correlated with any element concentration in either habitat ($p > 0.10$), suggesting it did not have an important influence on the results of this study.

Inter-habitat and inter-site differences were also quite pronounced when analyzed as a multivariate fingerprint using MANOVA and discriminant analy-

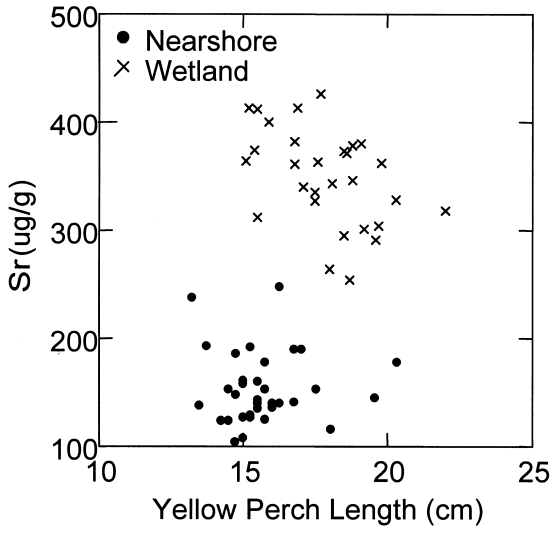

***FIG. 5. Otolith Sr concentration by fish length vs. habitat in the* Habitat Differences Study.**

sis. MANOVA revealed a more significant difference than some of the individual ANOVAs ($p < 0.001$), but the same elements that differed among habitats and sites in univariate ANOVAs were also identified as significant contributors to the separation among elemental fingerprints by MANOVA. Discrimination in multivariate space was even more distinct with LDFA ($p < 0.0001$), especially in identifying differences among habitats. Discrimination among habitats was quite distinct using only one element (Sr, Fig. 5). Four elements (Ba, Mn, Na, and Sr) were retained as significant discriminators among sites by stepwise LDFA, but a two variable site model including Sr and Ba was just as powerful statistically as the four variable model ($p < 0.0001$). Sr and Ba were the most important variables in LDFA (F-statistics = 73.6 for Sr and 45.6 for Ba) and the most highly correlated with canonical variables (Sr with CV1 =87.1%, Ba with CV2 = 74.0%). Plotting Sr vs. Ba demonstrates how Sr clearly separates fish from the three wetland sites from those captured in Chequamegon Bay and that differences in Ba provide some separation among wetlands, although the fingerprints from West Fish and East Fish Creek Wetlands overlap considerably (Fig. 6).

LDFA classification success for differences between habitats (wetland vs. bay) based on Sr as the lone explanatory variable was 100%, and classification success among sites was 86% overall for the two element (Sr-Ba) model, with the highest accuracy for Chequamegon Bay (97%) and the poorest for East and West Fish Creek Wetlands (67 and 70%), which tended to be misclassified as each other (Table 3). The four variable site model had slightly better overall classification success (89%), but East Fish and West Fish Creek Wetlands still tended to be misclassified as each other, suggesting movement of juveniles or adults between these sites may not be uncommon.

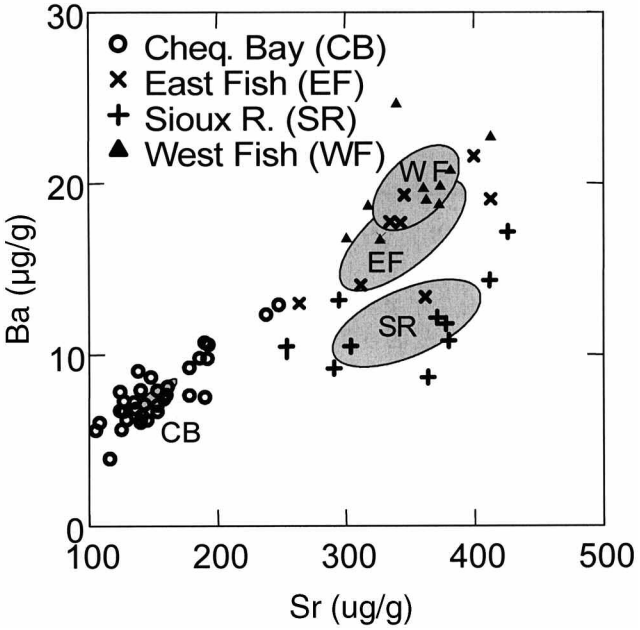

FIG. 6. Otolith Ba vs Sr concentrations by site in the* Habitat Differences Study *(95% Gaussian confidence ellipsoids around site centroids).

Core Differences Study

Sr was the only element that was strikingly different among sites (ANOVA, $p < 0.001$), with fish from Lost Creeks having the highest values (Fig. 4). However, differences in otolith concentrations of Pb among the four sites approached significance (ANOVA, $p = 0.09$). Although the overall elemental concentration differences among sites were more subtle than we found in the *Habitat Differences Study*, the multivariate fingerprint based on Sr and Pb concentrations was still significant (MANOVA, $p < 0.001$). Discrimination among sites using LDFA

TABLE 3. Jack-knifed classification accuracy of number of yellow perch samples identified to their actual capture locations (individual wetlands or Chequamegon Bay proper) based on linear discriminant function analyses of elemental fingerprints from adult otolith margin samples using Sr and Ba as explanatory elements. Overall percentage correct included for each site.

	LDFA Predicted Site				
Actual Site of Capture	CB	EF	WF	SR	% Correct
Chequamegon Bay (CB)	33	1	0	0	97
East Fish Creek Wetland (EF)	0	6	2	1	67
West Fish Creek Wetland (WF)	0	3	7	0	70
Sioux River Slough (SR)	1	1	0	8	80
Total	34	11	9	9	86

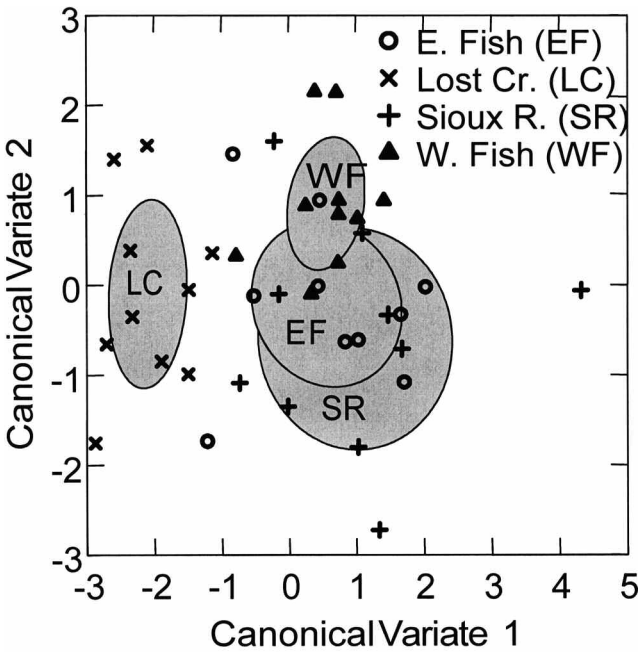

FIG. 7. Canonical variate plot based on LDFA results for a three element stepwise model using Sr, Ba, and Pb for the* Core Differences Study *(95% Gaussian confidence ellipsoids around wetland centroids).

revealed that the elemental fingerprint from fish captured at Lost Creeks Wetland was quite distinct from the other wetlands ($p < 0.0001$), but the plot of the first two canonical variates demonstrates that distinctions are less clear among the wetlands located adjacent to Chequamegon Bay (Sioux R., East Fish and West Fish) (Fig. 7). The best stepwise LDFA model included Sr, Pb and Ba. Although Ba concentrations were not significantly different in the ANOVA or MANOVA results, we included it (as well as Fe and Mg) in the stepwise LDFAs because we wanted to identify a greater number of fingerprinting elements, if possible, and differences in mean concentrations among sites appeared large enough to potentially provide discriminatory power. As it turned out, the F-statistic associated with Ba in the final LDFA model was significant ($p < 0.02$). The strong correlation between Sr and the first canonical variate (–0.70) indicates Sr differences were primarily responsible for separation along the first discriminant axis, while differences in both Pb and Ba concentrations were most influential along the second discriminant axis based on their positive correlations (≥ 0.44) with the second canonical variate. Jack-knife classification accuracy from LDFA using the three variable model was only 54% for the four wetlands overall, but fish from Lost Creeks Wetland were classified correctly 100% of the time. Accuracy for fish from the wetlands adjacent to Chequamegon Bay was much poorer, ranging from 11 to 60% (Table 4).

DISCUSSION

Micromilling Effects Study

Our results suggest the effects of milling and glueing on elemental fingerprints are minor. The process of glueing otoliths to slides prior to milling renders Al concentrations suspect, while the milling process itself altered only Zn concentrations in any important way. Since neither of these elements were integral to any of the multivariate fingerprint discrimination or classification models developed as part of the *Habitat Differences Study* and no milling was done for the *Core Differences Study*, it seems unnecessary to dwell on these results much further. Depending on the methodology employed and the trace elements that are of interest in future studies, concern over the effects of glue on elemental finger-

TABLE 4. Jack-knifed classification accuracy of number of yellow perch samples identified to their actual capture locations (individual wetlands) based on linear discriminant function analyses of elemental fingerprints from adult otolith core samples using Sr, Ba, and Pb as explanatory elements. Overall percentage correct included for each wetland.

	LDFA Predicted Wetland				
Actual Wetland of Capture	LC	EF	WF	SR	% Correct
Lost Creeks Wetland (LC)	10	0	0	0	100
East Fish Creek Wetland (EF)	1	1	2	5	11
West Fish Creek Wetland (WF)	1	3	6	0	60
Sioux River Slough (SR)	1	3	2	4	40
Total	13	7	10	9	54

prints may or may not be important and will need to be assessed on a case by case basis. Although micromilling of fish otoliths for elemental analysis has been used in other studies recently (Wurster *et al.* 1999, Kennedy *et al.* 2002), effects on elemental fingerprints, other than those we observed here, remain virtually unexplored. There is one relevant study we are aware of that examined potential contamination of elemental fingerprints from cutting otoliths with a diamond saw (Dove *et al.* 1996). They found no detectable effect of cutting. More work of this nature is needed to verify that milling and cutting effects are indeed minimal.

Habitat Differences Study

Our results suggest there are important differences in the concentrations of several elements from the recent growth portions of otoliths of adult yellow perch that inhabited coastal wetlands compared to those that inhabited the adjacent nearshore waters of Chequamegon Bay. The most striking difference was the high concentration of Sr in wetland-caught fish relative to those captured in the bay, but Ba, Mg, and Cu were also consistently higher in otoliths from wetland fish. Although this study will have to be expanded to verify the generality of these element concentration differences elsewhere in Lake Superior and with different age classes of fish, our results suggest otolith chemistry differences between wetland and nearshore dwelling adult yellow perch will be helpful in identifying patterns of habitat use for yellow perch and possibly other wetland-dependent species in the Great Lakes.

Trace element water chemistry data is extremely sparse for coastal habitats in Lake Superior, so we cannot be certain that the differences in otolith chemistry between fish from wetland and bay habitats reflected ambient water chemistry differences. However, watersheds associated with the sites we sampled span two ecological unit boundaries (Fig. 1, Albert 1995), 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. There is limited evidence that supports the idea that trace elements are generally more concentrated in coastal wetlands than in their adjacent nearshore waters. Previously unpublished ICPMS water chemistry data collected for a different study in western Lake Superior in 1996 indicates that Ba is more concentrated in four coastal wetlands than in the adjacent lake waters (Anne Cotter, U.S.EPA-Duluth, personal communication), and Trebitz *et al.* (2002) present evidence that coastal wetland Mg concentrations are often double what they are in adjacent lake water. Otolith margin concentrations reflected these ambient water chemistry differences, in that Ba and Mg were also more concentrated in yellow perch captured from wetlands. Future efforts to elucidate differences in water chemistry between wetland and nearshore waters of the Great Lakes will be necessary to determine the generality of this pattern and potential relevance to our results.

It is also possible that growth differences among fishes occupying different habitats was, at least in part, responsible for the differences in otolith Sr concentrations we observed. It has been shown that if all other factors are equal, slower growing fish will tend to accumulate more Sr in their otoliths than faster growing fish (e.g., Otake *et al.* 1994, Tzeng 1996). The reasons for this relationship are complex and not fully understood, but probably tied to rates of protein synthesis and its linkage to otolith crystallization rates. Campana (1999) provides a detailed review of mechanisms responsible for Sr uptake, including the effect of reduced growth rate on enhanced Sr uptake. Although we found slight differences in the size of yellow perch captured from wetland and bay habitats, it was wetland fish that were larger that had higher otolith Sr concentrations. We cannot say the larger size of wetland fish was due to faster growth because we did not make direct growth measurements for this study. However, coastal wetlands are typically much warmer and more productive than their adjacent nearshore waters in the Great Lakes (Brazner and Beals 1997, Brazner *et al.* 2000), and therefore, more conducive to fast growth. So, it seems unlikely the otolith Sr concentration differences we observed were related to growth differences and more likely were related to differences in ambient water chemistry between habitats. Bath *et al.* (2000) found that otolith Sr was not only related to ambient water concentration, but also positively correlated with temperature. Therefore, warmer temperatures in wetlands may have also contributed to the higher Sr concentrations in the ototliths of fish from these habitats relative to fish from Chequamegon Bay. Temperature-driven physiological differences may also explain the differences in Mg concentrations between wetland and nearshore habitats, but mechanisms that influence Mg incorporation into otoliths are not well understood. However, differences in otolith Mg concentrations between habitats seem less likely to reflect differ-

ences in ambient concentrations since they are under strict physiological control and must be maintained within narrower limits to assure the fishes survival (Campana 1999).

Water chemistry differences also provide a reasonable explanation for the pattern we observed for Ba, since its relative concentration in otoliths appears to reflect ambient concentrations in the environment (Bath *et al.* 2000, Dove 1997). In addition, it probably shares a common mode of inclusion into the otolith with Sr (Campana 1999) without the temperature dependence (Bath *et al.* 2000), suggesting deposition patterns would be similar.

Despite an incomplete understanding of the mechanisms that underlie the differences in elemental fingerprints we observed between habitats, the significance of our results is not diminished; the utility of elemental fingerprints as natural habitat tags is not at all dependent on a mechanistic understanding of the differences observed, only that the differences can be accurately quantified analytically and statistically. We expect to use elemental fingerprints from fish otoliths as habitat-specific markers in future studies. If we are able to further validate the results from this study across a broader geographic range, we plan to use age-specific information on the otolith to obtain elemental fingerprints that we can use to estimate the relative importance of coastal wetland habitats to yellow perch and other wetland-dependent species in the Great Lakes. This will require intensive microsampling of otoliths (e.g., laser-ablation ICPMS or micromilling) across transects that span the life history of individual fish, so that the proportion of time spent in a particular habitat type (wetland vs. lake) over the life of a fish can be estimated. The ability to track macrohabitat use by barramundi, *Lates calcarifer* (Pender and Griffin 1996), striped bass, *Morone saxatilis* (Secor and Piccoli 1996, Secor *et al.* 2001, Zlokovitz *et al.* 2003), and Atlantic croaker *Micropognias undulatus* (Thorrold *et al.* 1997) with microsampling-based elemental fingerprinting methodologies provides encouragment that this will be possible. Stable isotope analysis, especially with ^{18}O and ^{87}Sr, also holds promise as a tool for determining macrohabitat use in the Great Lakes. The temperature-dependence of ^{18}O deposition on otoliths and the large disparity in temperatures between coastal and offshore Great Lakes waters has already demonstrated the utility of ^{18}O for these purposes (Patterson *et al.* 1993). In addition, ^{18}O (along with ^{13}C) markedly improved discrimination among weakfish (*Cynoscion regalis*) nursery habitat fingerprints (Thorrold *et al.* 1998). The ability to reconstruct inter-habitat movements during the freshwater phase of Atlantic salmon (*Salmo salar*) by measuring $^{87}Sr/^{86}Sr$ isotope ratios suggests the utility of Sr isotopes should be investigated as a habitat discrimination tool in the Great Lakes as well (Kennedy *et al.* 2002).

Core Differences Study

We found that elemental fingerprints derived from juvenile otolith cores of adult yellow perch were similar among fish captured from wetlands adjacent to Chequamegon Bay, but quite distinct for the one site (Lost Creeks Wetland) outside of the bay. This overlap for Chequamegon Bay wetlands suggests substantial mixing among juveniles after they leave their natal wetlands and mature as adults, or a common source nursery that supplies at least some of the adults to all wetlands. The distinctness of the core elemental fingerprint from adults captured at Lost Creeks suggests these fish come from a separate population from those in Chequamegon Bay. Accurate interpretation of these results is dependent upon the assumption that the fish used in this study were from the same age class, otherwise differences in fingerprints may be attributable to inter-annual differences. Since we did not do a complete age analysis of these fish we cannot be sure they were in the same age class. However, the mean size of fish from the four sites was quite similar (insignificant ANOVA differences), the close proximity and similar geomorphology of the four wetlands provide very similar growth conditions, and the few fish ($n = 5$) we aged using scale annulus counts were all age IV individuals. Future efforts should include more precise age estimates and larger sample sizes from each wetland to improve the accuracy and precision of fingerprint differences. Further core analysis of adult fish captured from other wetlands near Lost Creek (e.g. Bark Bay Slough or Flag River Wetland) combined with additional elemental fingerprinting of juveniles will be necessary to determine if the Lost Creeks fishes come from a population associated with the bay immediately adjacent to that wetland or from a wider geographic area. As discussed for the *Habitat Differences Study*, it is likely that differences in Sr and Ba concentrations among elemental fingerprints reflect differences in ambient water chemistry among the three Chequamegon Bay wetlands and Lost Creeks Wetland. Differences in otolith Pb concentrations among sites may reflect differences in anthropogenic Pb contamination among wetlands (Geffen *et al.* 1998, Spencer *et al.*

2000), but we have no water or sediment chemistry data to corroborate this possibility. It will be necessary to develop fingerprints from fish captured at different times during the year to determine the stability of site-specific signatures and identify the most appropriate sampling frequency. Laser-ablation transects across a single otolith could also be used to assess fingerprint stability through time and quantify the temporal scales which will provide valid fingerprint comparisons.

Since we already know from previous work (Brazner *et al.* 2004), that YOY yellow perch from different coastal wetland nursery areas in western Lake Superior have distinct elemental fingerprints (Fig. 8), a goal for the future is to match fingerprints from adult cores to the YOY fingerprints we already identified, to estimate recruitment differences among wetlands and sources of adults captured in the lake proper. This has been done successfully in a number of marine applications (e.g., Gillanders and Kingsford 1996, Thorrold *et al.* 2001, Gillanders 2002a), so we think it will also be possible in the Great Lakes. Establishing that there were differences in fingerprints among adult cores from different wetlands was a first step in this direction. Since there can be considerable temporal variability associated with elemental fingerprints, it is likely that a library of fingerprints will have to be catalogued over time for each wetland, so that natal nurseries for adult fish of a variety of ages can be accurately determined (Gillanders 2002b). If we succeed in using elemental fingerprinting to quantify which wetlands are responsible for exporting fish to the adjacent lake, our results will increase understanding of coastal wetland habitat function in the Great Lakes and could be used to help set priorities for wetland protection and coastal wetland restoration efforts.

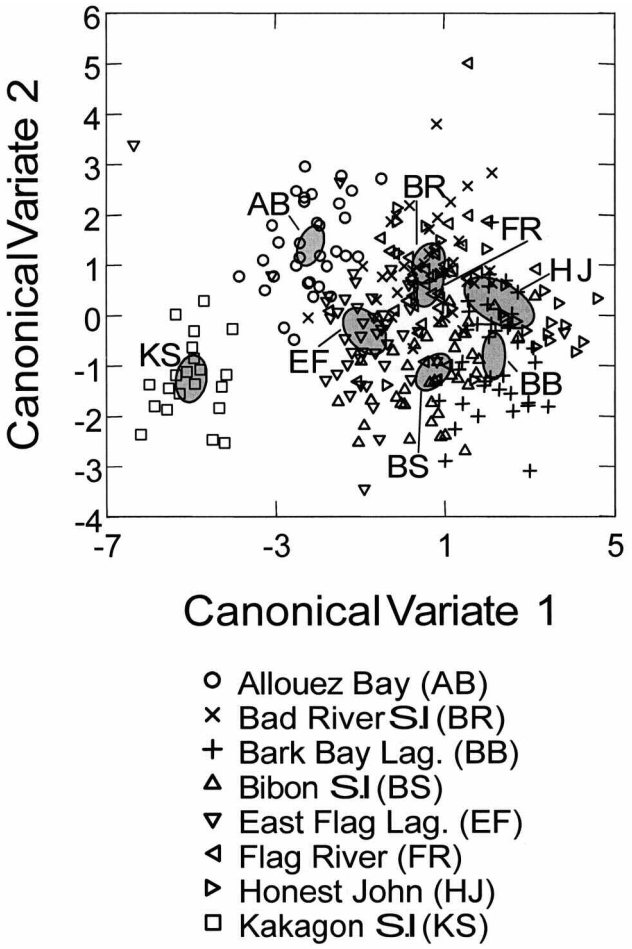

FIG. 8. Elemental fingerprints for YOY yellow perch otoliths from western Lake Superior coastal wetlands (taken from Brazner et al. 2004) based on canonical variates 1 and 2 obtained through linear discriminant function analysis that included Sr, Mn, K, Ba, and Mg as explanatory variables. Observations for each wetland depicted with 95% confidence ellipsoids around the distribution centroids.

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