LEPF 04-18

DETERMINING STREAM ORIGIN OF FOUR PURPORTED WALLEYE STOCKS IN LAKE ERIE USING OTOLITH ELEMENTAL ANALYSIS

FINAL REPORT

By

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ABSTRACT

Understanding stock structure and movement in aquatic organisms is necessary for successful population management. This is particularly important in exploited fishes, where stocks likely contribute differentially to the lake-wide population and the fishery. In Lake Erie, the walleye population is important, contributing both ecologically and economically to the lake and surrounding region. Although managed as a single unit, this population likely contains multiple stocks for which anecdotal evidence reveals that these stocks may exhibit different productivities, contributing differentially to the United States recreational and the Canadian commercial fishery.

In this study, we sought to quantify homing behavior of walleye stocks and evaluate their contribution to the recreational fishery. By collecting water and spawning adult walleye (N=332) during 2003-2005 from Maumee, Sandusky, and Grand rivers and Toussaint Reef located in the western and central basins of Lake Erie, we could identify otolith chemical characteristics and compare them to the water from these locations.

To assess their contribution, we collected three samples (N=300) from fish cleaning houses in Ottawa, Ashtabula, and Erie counties in Ohio during 2005. Again, otolith microchemistry identified element signatures, walleye were grouped, and the relative contribution of each purported stock was assessed.

Extent of homing differed among the four spawning stocks. The Sandusky River had the highest site fidelity (92%), followed by the Maumee (60%) and Grand rivers (40%), and finally Toussaint Reef (37%). Toussaint Reef contributed >50% of the fish sampled from the recreational fishery, whereas Maumee, Sandusky, and Grand rivers contributed only 46% combined.

By identifying stocks that exhibit high fidelity, we could protect them, thereby maintaining their genetic integrity. Armed with this stock-specific information, managers can protect or exploit populations in proportion to their contribution to the fishery.

ACKNOWLEDGMENTS

First and foremost I would like to thank my advisors, Drs. Roy Stein and Derek Aday, for their continual knowledge, guidance, and encouragement throughout this journey. These two have been a pleasure to work with, and I appreciate them both providing me with the opportunity to pursue this degree. In addition, I would also like to thank my committee members who always went above and beyond the call of duty. Dr. Elizabeth Marschall was always available for direction and support, specifically with revisions and her statistical expertise. Dr. Stuart Ludsin, the otolith microchemistry man, taught me a great deal about the finer details of work of this type, particularly important given it had not been done previously at the AEL.

I also would like to thank the numerous people at the Aquatic Ecology Lab for their technical help, advice, and support. I cannot express what a dynamic group of students, faculty, and staff are associated with this lab, making it a stimulating and entertaining place to work. Specifically, I would like to thank Jason Van Tassell (aka, my third advisor), Paris Collingsworth, Samantha Fedor, Ben Sylak, Jessica Best, Jamie Fagman, Mike Zimmerman, Ben Carr, and many others. I am also grateful to our administrative staff, Melissa Marburger and Barbara Fate, who guided me through the administrative details and formalities associated with the project.

In addition to intellectual support, I would like to thank my funding agencies who have made this project possible. I am grateful to the Division of Wildlife for not only the funds to complete this project, but for the countless hours they put into scoping meetings, field sampling, and preparation to make this project successful. Specifically, I would like to thank Roger Knight, Jeff Tyson, Chris Vandergoot, Tim Bader, Ann Marie Gorman, Larry Goedde, Carey Knight, and Travis Hartman. The Lake Erie Protection Fund (LEPF) provided additional funds that have contributed to project completion. Finally, I am grateful to Dr. John Olesik at the MARC facility on Ohio State University's campus for the amount of expertise and technical work he has put into this project, all the while ensuring samples were done in a timely manner.

Last, but certainly not least, I would like to thank my family and friends for their continual support. Specifically, I would like acknowledge my parents who have been there since day one and instilled in me to always strive for my goals. I would also like to thank Ryan Romero for his words of encouragement and understanding, and his continual support through all my endeavors.

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INTRODUCTION

Understanding movement, life history, and ecology of a population provide insight into its role and success within an ecosystem (Rubenstein and Hobson 2004, Koskinen et al. 2002, Campana and Thorrold 2001). This insight is particularly important to the management of exploited stocks, in which distribution, abundance, and movement all can be influenced by harvest. Simply knowing that populations are apportioned into distinct spawning stocks can dictate the need for additional information, such as stock-specific productivity, distribution, and vulnerability to exploitation. Clearly, if stocks are mixed at harvest, then understanding the relative contribution of each stock to this harvest is required for appropriate management. If stocks contribute differentially to the harvest (a likely scenario), managers can exploit productive stocks and protect others, but only if they know their distribution and abundance.

To provide insight into stock identification and distribution, considerable effort has been expended collecting and interpreting data on the movement patterns of organisms throughout their lives (Rubenstein and Hobson 2004). To this end, three major types of marker have been used to track organisms: extrinsic, biological, and biogeochemical markers. Although each has benefits and drawbacks, biogeochemical markers have real promise (Rubenstein and Hobson 2004). Use of biogeochemical markers is based on the premise that organisms will incorporate environmental elements into tissue and bone. As such, environmental variation in elemental concentration (due to geologic variation) is reflected in the organisms. For example, scientists now believe that the giant squid, *Architeuthis sp*., lives at depths of 125-500 m rather than 1,000 m as historically speculated (Landman et al. 2004). Similarly, Campana et al. (1995) successfully distinguished between two populations of cod, *Gadus morhua*, in a mixed stock fishery.

Another system in which use of biogeochemical markers could have practical benefits is Lake Erie, specifically for quantifying walleye (*Sander vitreus)* behavior and movement. The Lake Erie walleye population is extremely valuable, contributing both ecologically (Knight and Vondracek 1993, Knight et al. 1984) and economically (Hushak et al. 1988, Trautman 1981) to the lake and its surrounding communities. At present, the lake-wide population is managed as a single unit; however, accumulating evidence has begun to suggest that multiple stocks exist. This evidence, in the form of genetic, tagging, and microchemistry studies, is consistent with reduced gene flow and suggests that behavioral barriers, such as homing and philopatry, could be a source of genetic isolation (Stepien and Farber 1998; Todd and Haas 1993, Bartnik, 2005, Hedges 2002). Further, mitochondrial differences have been identified among some spawning stocks but not others, suggesting that fidelity may be stock-specific (Merker and Woodruff 1996). Aside from genetic research, microchemistry analyses have identified numerous stocks within the lake and concluded that these spawning stocks were contributing differentially to the lake-wide population (Hedges 2002, Bartnik 2005).

As suggested above, genetic divergence within the Lake Erie walleye population could be stimulated by reproductive site fidelity (Stepien and Farber 1998, Hendry et al. 2000). Although walleye migration patterns during non-spawning periods have been documented (Wolfert 1963, Todd and Haas 1993, Smith et al. 1952, Kershner et al. 1999), little is known about their spring spawning movements. Further, research on walleye homing behavior in other systems has proven ambiguous; some studies provide evidence for strong homing behavior (Crowe 1962, Jennings et al. 1996), and others suggest this behavior is weak or inconsistent (Smith et al. 1952, Olson and Scidmore 1962). Despite a consensus, these studies suggest that site fidelity exists, but only in some populations. Further, given the genetic evidence, we believe that divergence may be taking place, emphasizing the need for identifying potential stocks.

A relatively new biogeochemical technique in fisheries that has been used to distinguish stocks is otolith microchemistry. Fish otoliths, paired calcium carbonate structures in the head, provide a record of environmental history. Because fishes 1) absorb environmental elements into their otoliths and 2) do not reabsorb elements due to inertial properties of the otolith (Campana and Thorrold 2001), they provide a detailed record of their origin plus their subsequent, chronological habitat use. Because of its promising results, this technique has been widely used to classify stocks within populations (Bronte et al. 1996, Campana et al. 1995), quantify migration patterns (Wells et al. 2003, Kennedy et al. 2002, Thresher et al. 1994), identify homing behavior (Thorrold et al. 2001), and determine relative spawning-site contributions to adult populations (Gillanders and Kingsford 1996, Brazner et al. 2004). Given this success, we use otolith microchemistry to examine walleye movement patterns and stock structure. Specifically, we apply otolith microchemistry to identify spawning stocks of Lake Erie walleye, explore their reproductive isolation, and quantify their relative contribution to the recreational fishery.

METHODS

Sample Collection

Spawning adult walleye (N=500) were collected from the western (Maumee and Sandusky rivers; Toussaint Reef) and central (Grand River) basins of Lake Erie during March-May (2003-2005). Fish were collected at night via electrofishing in rivers, whereas reef fish were caught in overnight gillnet sets.

Simultaneously, water samples (N=66) were collected during March-June (2004-2005) from the four spawning locations with acid-washed syringes (25-mL) and were immediately filtered using Whatman® 0.45-µm syringe filters into acid-washed containers. Finally, 2% nitric acid was added as a standard, following the Environmental Protection Agency (EPA) water-sampling protocol (Method #1669; 1996). Water samples were stored at -40°C until analysis.

Sample Preparation

Sagittal otoliths were extracted from walleye, cleaned, dried, and embedded in Spurr's low viscosity resin. Cut into 130-µm transverse sections with a diamond saw (Struers Minitom), otoliths were polished on both sides using aluminum oxide lapping paper of descending roughness (i.e., 30µm, 10µm, 3µm, and 1µm) to expose the core and annual rings. Oriented with the clearest side up, polished sections were mounted on a microscope slide (~28 per slide), held firmly by permanent doubled-sided tape. Finally, each slide was placed in an acid-washed Petri dish filled with ultrapure water (Ohio State University Reagent Laboratory), sonicated three times for 5 min, and dried in positive air flow produced in a Class-100 hood. During these processes (and all remaining), slides were handled with non-metal, acid-washed utensils, and stored in acid-washed polypropylene vials (two per vial) until processed.

Sample Processing

Laser Ablation-Inductively Coupled Plasma Mass Spectrometry (LA-ICPMS) was used to quantify otolith elements at the Trace Element Research Laboratory, located on The Ohio State University campus (OSU). A ThermoFinnigan Element 2 Inductively Coupled Plasma Mass Spectrometer (ICP-MS), using helium as a carrier gas, was operated with low mass spectral resolution (ΔR=300) to maximize sensitivity. This instrument was coupled to a New Wave Research UP-193HE 193 nm Excimer laser with two 9x9 arrays of lenses and provided a fully homogenized laser beam for sample ablation (Güther et al 1997, Kuhn and Güther 2004). Further, a 30-µm laser spot size

with an energy pulse of 0.11 mJ and repetition rate of 10 Hz provided sufficient sensitivity over the ablated area. The laser scanned across the sample at a rate of 5 µm/s. We measured four points on the middle 10% of the flat-topped mass spectral peak for each isotope with a dwell time of 9 ms per point (36 ms per isotope). Time between measurements for each set of isotopes was 0.93 s. Sectioned otoliths were viewed and measured under ~100x to 500x magnification on a computer monitor to ensure proper sample selection and to define laser sampling line scans. Before analysis, elemental mapping was performed on a few samples to gain experience in finding otolith primordia. Based on these observations and the experience of others (Ludsin et al. 2006, Bartnik 2005, Brophy et al. 2004), we could clearly pinpoint the exact origin by a strong Mn spike. Thus, samples were ablated across areas thought to be the natal origin in search of a Mn spike; when spikes were not found, additional lines were scanned until these spikes were found. Data were acquired for the following isotopes: ^{7}Li, ^{25}Mg (to avoid overlap due to $^{48}Ca^{2+}$ at m/z 24), ^{43}Ca, ^{44}Ca, ^{55}Mn, ^{66}Zn, ^{85}Rb, ^{88}Sr, ^{118}Sn, ^{120}Sn, ^{137}Ba, and ^{208}Pb; all isotope signals were normalized to the ^{43}Ca.

A NIST 612 glass reference material was used to calibrate the instrument and check for drift. We used Glitter software (version 4.0) to analyze data. After the Mn peak was identified, data within ~50 µm of each side of the peak were selected and integrated. Sample elements were calculated from the sample and standard signals by the Glitter software using NIST 612 standard concentration values compiled by Pearce et al. (1997). Finally, the limit of detection (LOD) was calculated by the Glitter software for isotopes measured in each sample using an algorithm developed by Longerich et al. (1996); any sample concentration falling below this value was excluded.

Finally, water concentrations were measured by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES). The elements Mg, Mn, Cu, Zn, Rb, Sr, Ba, and Pb, were measured by with a PerkinElmer Sciex ELAN 6000 instrument using the following isotopes: ^{25}Mg, ^{55}Mn, ^{65}Cu, ^{66}Zn, ^{85}Rb, ^{88}Sr, ^{137}Ba, and ^{208}Pb. Instruments were calibrated using a set of standard solutions made by appropriate dilution and mixing of single element standards. Three replicates were measured for 10 s, each consisting of nine sweeps with a dwell time of 100 ms.

Data Analysis

Univariate and multivariate techniques were used to examine temporal and spatial differences in water and otolith elemental concentrations. Before statistical analyses, the coefficient of variation (CV) was calculated for each element or isotope. Because elements of interest occurred in minute quantities in the otolith (i.e., < 3% of total otolith composition), we only included elements with CVs ≤ 10%(Campana 1999). In turn, at least 90% of each measured element exceeded the limit of detection (LOD; Gillanders and Kingsford 1996). After identifying the elements that fit these criteria, all element:calcium ratios were tested for normality *a priori* and, if necessary, data were transformed using Box-Cox transformation (Kolmogorov-Smirnov Normality Test; p ≥

0.05). We used two-way analysis of variance (ANOVA) to test for effects of site (i.e., Maumee, Sandusky, and Grand rivers, Toussaint Reef) and year (i.e., 2004-2005) on element concentration. When this test was significant, Tukey's Pairwise Comparisons identified where differences occurred. In water samples, multi-element signatures were analyzed with multiple analysis of variance (MANOVA) and, when significant ($p \leq 0.05$), was followed by discriminant function analysis via a step-wise model (JMP; Version 6.0.0). Contrary to water signatures, otolith elemental signatures were analyzed with a clustering model in which data with similar element values were grouped. Specifically, hierarchical clustering where the model begins with each point as an individual cluster, and at each step a distance is calculated between clusters with most similar points combined or clustered. This process is repeated until the assigned number of clusters (N=4 locations in our case) has been reached. This method of grouping points (i.e., versus discriminant function analysis, See Appendix C) was chosen because the data reflect a group of fish with known spawning location, but unknown natal origin.

Mixed Stock Analysis

We collected adults during June, August and October (N= 3 x 100) in 2006 from recreational fish houses around Lake Erie. Based on creel data from the Ohio Department of Natural Resources, Division of Wildlife (Sandusky and Fairport Lake Erie Biological ResearchStations) and movement assessment from previous researchers (Kershner 1999), we could follow walleye movement through fishing effort from the western to the central basin through summer. Three haphazard samples (N=100) were collected during June, August and October from recreational fish houses in Ottawa (western basin), Ashtabula (eastern central basin), and Erie (central basin) counties in Ohio, respectively. Sagittal otoliths were extracted and gonads provided sex information. Because carcass lengths had an unknown bias, we measured heads and extrapolated these measures to calculate total lengths (i.e., TL [mm] = 18.5 + 4.1 x HL; Isermann and Vandergoot 2005). Finally, otoliths were prepared for LA-ICPMS following methods set forth in *Sample Preparation* (i.e., p. 5-6) and *Sample Processing (*i.e., p.6-8) and data were analyzed following methods set forth in *Data Analysis* i.e., p. 8-9).

RESULTS

Water Samples

Elements from water samples differed across sites (two-way ANOVA, $p < 0.001$, Appendix B, Figure 1), with the exception of Zinc (Zn 66). The Grand River was classified as follows: high concentrations of manganese (Mn 55), barium (Ba 137), and lead (Pb 208), coupled with low concentrations of magnesium (Mg 25) and strontium (Sr 88). Toussaint Reef water was classified by low Mn, Sr, and Ba concentrations and high rubidium (Rb 85). The Maumee River contained low copper (Cu 65) and Rb coupled with high Sr. Finally, the Sandusky River was classified as high Sr concentrations and low Cu and Pb. Although numerous elements differed, strontium (Sr 88) provided the most variability, leading to clear distinction between sites (Figure 1.6). In addition, Sr exhibited relatively strong temporal stability, despite the fact that concentrations varied across years (Figure 1.6, Appendix B).

Classifying water correctly is critical because these site-specific elemental concentrations reflect those in the otolith. Thus, our ability to correctly classify water samples allows us to identify the environment in which otoliths were formed. To classify water, we used Linear Discriminant Function Analysis (LDFA) to predict the location of each sample based on its chemical characteristics, and comparing the "predicted" location to its "actual" location. With this information, a sample was labeled "correctly classified" when its predicted location was the same as its actual location and "misclassified" when it was not. Further, we developed numerous models in an attempt to identify those element combinations with the highest classification accuracy. In the ideal model, two significant canonicals were used for classification; Sr and Mg corresponded to the x- and y-axes, respectively (Figure 2). Using these two elements, water samples were classified with 79 - 100% accuracy (Table 1). The Maumee River was classified with the highest percent accuracy (i.e., 100%), followed by the Sandusky River (i.e., 88%; two misclassifications as the Maumee River), the Grand River (i.e., 85%; two misclassifications as Toussaint Reef), and finally Toussaint Reef (i.e., 79%; two misclassifications as the Maumee River and one as the Grand River).

Adult Walleye: Homing

Before elements could be included in the analyses, both the coefficient of variation (CV) and the limit of detection (LOD) had to be calculated to determine if each had a CV <10% and 90% of the calculated concentration exceeded the LOD. Elements that fulfilled these requirements were Mg, Mn, Rb, Sr, Sn, Sn, and Ba (Table 2); these then were used in various combinations in the cluster analysis. From this suite of candidate elements, we chose to focus on Sr. First, Sr served as an excellent discriminator for water samples (Figure 1.6). Second, walleye otolith microchemistry in Lake Erie reveals that Sr most strongly distinguishes among spawning locations (Hedges 2002, Bartnik 2005). Finally, Sr alone separated our data into discrete clusters (Figure 3). Further, because Sr concentrations in these clusters were directly proportional to Sr

concentrations in the water (Figure 1.6), we could predict natal origin of walleye as corresponding to one of our four sites (Table 3). Collection site was identified as the spawning location, given that fish had migrated to these locations to spawn. With this information, the predicted natal origins were compared to the adult spawning location. Site fidelity was determined by identifying the percentage of homing behavior at each location.

The Sandusky River exhibited the highest site fidelity (92% returning to their natal origin to spawn), followed by the Maumee River (60%), the Grand River (40%), and finally Toussaint Reef (37%; Table 3). Despite the low walleye numbers collected from Toussaint Reef, (N=82) ~50% of all fish sampled from the spawning grounds (N=332) were linked to this location.

Adult Walleye: Mixed Stock Analysis

Although we do not know exactly how many stocks exist in Lake Erie, we sought to determine which walleye from the recreational fishery could be associated with one of our four 'stocks' (Merker and Woodruff 1996, Bartnik 2005). To accomplish these comparisons, we first determined core Sr concentration ranges from our four clusters in the homing analysis, then used these ranges to group our mixed-stock data. Based on these ranges, each walleye was identified as to natal origin: Sandusky River, Maumee River, Toussaint Reef, Grand River, or unknown (Table 4). Strontium concentration of unknown walleye did not overlap the ranges of any of our four identified 'stocks.' Based on these ranges, the Sandusky River contributed 1% (N=4), the Maumee River contributed 42% (N=126), the Toussaint Reef contributed 54% (N=161), and the Grand River contributed 1% (N=3; Table 4). Six fish were unknown, contributing 2%. Toussaint Reef was the major contributor of samples collected in Curtice, Ottawa County (55%) and Geneva, Ashtabula County (67%); the Maumee River contributed most of the collection from Vermillion, Erie County (58%).

DISCUSSION

The walleye population contributes greatly to sport and commercial fisheries in Lake Erie and, therefore, understanding its life history is essential to making sound management decisions (Hushak et al. 1988, Trautman 1981). Effective management requires an understanding of how the population is structured into spawning stocks and the contribution of these stocks to the adult population (Moritz et al. 1995). Until recently, these data have been difficult to obtain. However, the use of otolith microchemistry has provided a reliable method for assessing these important population parameters. Using this tool, our data reveal that stock-specific site fidelity may exist within the Lake Erie walleye population. Further, we believe that these spawning stocks contribute differentially to the recreational fishery, information vital to how the fishery could be managed.

Despite the importance of understanding walleye movement patterns, equivocal results have hampered management (Smith et al. 1952, Crowe 1962, Olson and Scidmore 1962, Jennings et al. 1996). Using otolith microchemistry, our study illustrates that homing behavior may be stock-specific. For example, we identified high site fidelity in the Sandusky River, moderate fidelity in the Maumee River, and low site fidelity in both the Grand River and Toussaint Reef. In addition to these findings, our results provide insight into previous studies documenting genetic divergence in Lake Erie walleye (Stepien and Farber 1998; Merker and Woodruff 1996). For example, high fidelity in the Sandusky River stock may be the mechanism for apparent genetic divergence between the Maumee and Sandusky rivers (Merker and Woodruff 1996). Further, few genetic differences between these locations and the Grand River could have derived from low site fidelity in the Grand River stock (Merker and Woodruff 1996).

Equally important is the possibility that these identifiable spawning stocks contribute differentially to the adult population, and thus the recreational fishery. This could be the result of geography (Smith et al. 1952), physical barriers (Zheng 2005), genetics (Jennings et al. 1996), or local adaptation (Hendry et al. 2000). From our data, the Toussaint Reef stock appears substantially larger than any other stock, e.g., >50% of all the fish in our mixed stock analysis were identified as Toussaint Reef fish. Further, two of three samples, collected in separate basins (i.e., Curtice, OH, western basin and Geneva, OH, central basin), were almost entirely comprised of Toussaint Reef fish. Surprisingly, even our homing study identified most walleye as Toussaint Reef fish, despite having a small sample size (N=82) in comparison to the other sample locations.

Knowledge of stock fidelity and productivity is essential if the recreational fishery is to be managed effectively. Given that the largest identifiable spawning stock in Lake Erie, Toussaint Reef, appears to also be the major contributor to the recreational fishery is vital information for managers. In this case, managers may find it important to exploit these intermixing stocks, i.e., Toussaint Reef and Maumee River, by making the spawning grounds available to anglers during spawning season. Simultaneously, information about the Sandusky River, a smaller spawning stock with high fidelity, suggests to managers that perhaps this spawning ground should be closed to anglers

during spawning season so that these walleye can reproduce, thus protecting the genetic integrity of the Sandusky stock.

In addition to otoliths, we used water to quantify chemical signatures among our sample sites, setting the foundation for our otolith analyses. Although we identified numerous appropriate elements that allowed for characterization, we ultimately chose Sr as the only discriminator, given that it clearly distinguished water collected from our sample sites. Likewise, other researchers have indicated that Sr is the most powerful discriminator in walleye residing in these locations. For example, one microchemistry study identified Sr as the only significant discriminator among larval walleye from the Maumee and Sandusky rivers and the Toussaint Reef (Hedges 2002). Further, another microchemistry study in Lake Erie chose to use this element in a mixed stock analysis (Bartnik 2005). As a result of our water analyses and previous microchemistry findings, we chose to only use this element in both the homing study and mixed stock analysis.

Despite the advantages of using Sr as our only classification variable, we had to make some assumptions about this element, as well as the technique in general. For example, we assumed Sr was temporally stable, in spite of the variation in our two years of water samples. In addition, fish and water were concurrently collected during only a few years. To justify these assumptions, we examined our data and water quality data from United States Geological Survey (USGS). Data were available from the Maumee, Sandusky and Grand rivers during 1962-1999. In both data sets variation was present; however, concentrations in our sample sites remained relatively proportional to one another over time (i.e., Sandusky River always exhibited highest Sr concentration, followed by Maumee River, Toussaint Reef, and Grand River, respectively). Despite the temporal stability described, we recommend implementing a long-term water sampling program that would evaluate chemical stability as well as allow for collection of concurrent water and otolith data. Collecting water samples from more locations around the entire lake will help us identify all site-specific signatures and associated overlap.

In addition, we made a specific assumption about the number of spawning stocks. Despite research (Hedges 2003, Bartnik 2005, Merker and Woodruff 1996, Stepien and Farber 1998), the exact number that exists lake-wide is unknown. For example, Bartnik (2005) suggests that four stocks contribute to the western basin. Others believe that the Ontario reefs, in Canada, are major spawning sites, contributing substantially to the adult population (Bartnik 2005, Hedges 2003). Despite this ambiguity, we chose to assume four stocks exist based on our water samples. Because we clearly distinguished four sites from our water samples, we assumed walleye collected from these sites could be distinguished. In addition, genetic evidence (Todd and Haas 1993), site-specific parental attributes (Van Tassell 2006), and microchemistry research indicates that at least these four spawning stocks do, in fact, exist. Long-term annual water sampling, as suggested above, will contribute to this issue.

Finally, as is always the case in otolith microchemistry, assumptions concerning the general technique are necessary. The main assumption is that chemical concentrations

are deposited in the otolith in proportion to those exhibited in the environment, thus reflecting the fish's residency (Campana 1999). In the past, scientists have discovered factors that may affect the rate at which these elements are substituted for calcium into the otolith. The list includes exogenous factors such as temperature, salinity and diet; and endogenous factors including growth rate, gonadal development, and stress. Although the exact influence of these factors on otolith absorption is unknown, they appear to have some effect on elemental uptake and are discussed in the subsequent paragraphs.

Numerous papers have examined the effects of exogenous factors, particularly temperature regime. Some experiments have illustrated a positive correlation between temperature and otolith absorption (Bath et al. 2000, Fowler et al. 1995), whereas others have found no relationship (Sadovy and Severin 1994). Further, researchers have found inconclusive results when examining the interactions of multiple exogenous factors (Gallaher and Kingsford 1996, Martin et al. 2004, Secor et al. 1995). Certain endogenous factors may also play a role in otolith uptake of elements. For example, some researchers have found that gonadal development is important (Gallahar and Kingsford 1996, Kalish 1991). On the contrary, Sadovy and Stevenson (1994) concluded that gonadal development and reproductive activity was not a source of differentiating Sr uptake in otoliths. Physiological factors such as stress also have been examined. Gallahar and Kingsford (1996) concluded that although they found a positive relationship with diet and no relationship with temperature in their experiment, stress could have had a major impact on their results. In addition, they explained the difficulty in conducting experiments to control environmental factors and, inadvertently inducing stress on organisms.

It is obvious that more research is critical to clearly understand the influence of these factors on otolith composition. In the meantime, we do know that some factors (e.g., salinity and diet) do not appear to be as influential in freshwater systems. For example, most freshwater species are not passing through different salinity gradients (i.e., ocean to tributaries to spawn) and so salinity does not appear to be a problem. Further, unlike marine species, freshwater fish receive trace elements through their gills, and so diet is less likely to be important as well (Campana 1999). Endogenous factors (e.g., growth rate, gonadal development, and stress) appear to have some effect on otolith elemental composition in freshwater systems, however, their impact is so minor that, given geological differences among systems are high, physiological effects do not appear to confound the data (Kalish 1991, Sadovy and Stevenson 1994). Currently, temperature appears to be the most important factor in freshwater systems and may have an effect on how elements are absorbed and incorporated into the otolith. However, more research is necessary to quantify its impact so that these data may be interpreted more effectively. In the meantime, we may not be able to quantify the impact of these factors on our otolith samples; however, we do know that researchers have been able to actually distinguish larval walleye from our sample sites, suggesting that element differences among sites are larger than the influence of the described variables (Hedges 2002, Bartnik 2005).

In this study, we compared spawning stocks by quantifying site fidelity and measuring the contribution of each to the mixed stock fishery. By quantifying elements in both water and otoliths, we used water to characterize site elemental signatures which, in turn, set the foundation for our otolith analyses. Thus, using Sr as our only discriminator, we identified four spawning stocks in the homing study and determined their stock-specific fidelity. In addition, our homing study allowed us to quantify the range of Sr concentration in identified fish and apply these ranges to our mixed stock analysis. Rather than use water or larvae as priors, we used adult walleyes and applied their Sr differences to the mixed stock analysis. These data will give rise to new research opportunities in Lake Erie, such as comparing the contribution of these spawning stocks to the commercial fishery in Canada. Equally important, managers will be able to make well-informed decisions on how to manage this fishery effectively so that it will continue to thrive.

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Collection Site (Rows)	**Predicted from Model** (Columns)				
	Grand	**Maumee**	**Reefs**	**Sandusky**	**Percent Correct**
Grand	11	0	2	0	85%
Maumee	0	16	0	0	100%
Reefs	1	2	11	0	79%
Sandusky	0	2	0	15	88%

Table 1. Discriminant function analysis of water (n=60) from Grand, Maumee, and Sandusky rivers, all Ohio tributaries to Lake Erie, and Toussaint Reef (west basin, Lake Erie) in 2004 and 2005. Samples are classified by elements 25 Mg and 88 Sr. 'Predicted from Model' indicates how well the data were classified by assigning each data point to a group that best describes its characteristics

Element	CV	LOD	% > LOD
Sr 88	1.91	13.10	**100**
Ba 138	8.42	0.16	**100**
Mg 25	7.67	3.87	**98**
Mn 55	5.05	0.10	**98**
Rb 85	9.06	0.03	**98**
Sn 120	8.85	0.07	**97**
Sn 118	8.95	0.10	**96**
Zn 66	7.38	0.86	88
Pb 208	9.05	0.06	83
Li 7	3.39	0.09	69

Table 2. Coefficient of variation (CV), limit of detection (LOD), and percent above LOD for individual chemical elements from water and otoliths. Only elements with a CV <10 and with 90% of the calculated concentration >LOD were included in our analyses. Elements fulfilling both requirements appear in bold.

		Adult Spawning Location					
Cluster #	Predicted Natal Origin	Sandusky	Maumee	Reef	Grand	Total	% Homing
1	Sandusky	47	1	3	0	51	92%
4	Maumee	19	58	15	5	97	60%
2	Reef	14	72	63	20	169	37%
3	Grand	4	4	1	6	15	40%
	Sample Size	84	135	82	31	332	

Table 3. Results of the cluster analysis from the homing experiment using the element Sr 88 quantified in the otoliths as the only discriminator. Predicted natal origin was determined by examining box plots of the four clusters in Figure 3. To interpret the table, read across the columns. For example, "Cluster 1 was identified as fish of Sandusky River natal origin. Of those fish, 47 spawned in the Sandusky River, 1 spawned in the Maumee River, 3 on Toussaint Reef, and 0 spawned in the Grand River, illustrating a homing rate of 92%."

Core Sr Concentration	Predicted Natal Origin	Sample			Total Contribution
		Curtice	Geneva	Vermillion	
1043.68 - 3342.7	Sandusky	1% (1)	2% (2)	1% (1)	1% (4)
440.6 - 959.8	Maumee	39% (39)	29% (29)	58% (58)	42% (126)
213.8 - 437.4	Reef	55% (55)	67% (67)	39% (39)	54% (161)
63.1 - 196.7	Grand	3% (3)	0% (0)	0% (0)	1% (3)
	Unknown	2% (2)	2% (2)	2% (2)	2% (6)

Table 4. Distribution of walleye (N=300) in the mixed stock analysis separated by sample 1 (Curtice, Ohio; Ottawa County, western basin) collected in June, 2 (Geneva, Ohio; Ashtabula County, central basin) collected in August, and 3 (Vermillion, Ohio; Erie County, central basin) collected in October, 2006. Predicted natal origin was determined by identifying the ranges of core otolith Sr concentrations of the four identified clusters in the homing cluster analysis (See Figure 3). Unknown fish (N=6), which did not fall into any of the core Sr concentration ranges listed above, were excluded from the analyses.

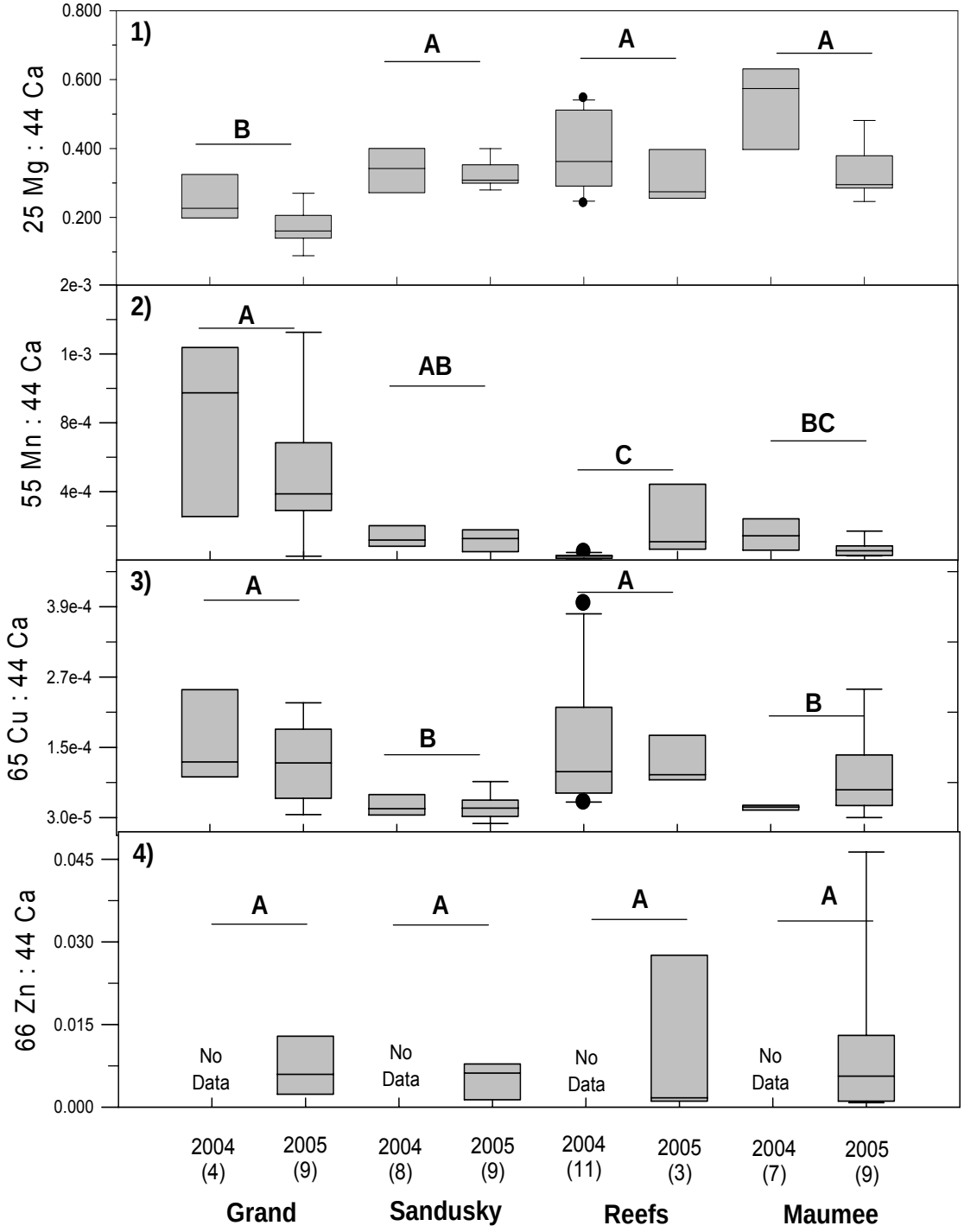

Figure 1. Element concentrations 1) 25 Mg, 2) 55 Mn, 3) 65 Cu, and 4) 66 Zn in water samples (sample size in parentheses along x-axis) separated by location (Grand River, Sandusky River, Maumee Rver and Toussaint Reef) and year (2004 and 2005) in Lake Erie, Ohio. Boxes denote the 25th and 75th percentiles with a center horizontal line corresponding to the median. Whiskers represent the 10th and 90th percentiles; outliers are shown as points outside the box plots. Letters above plots denote element differences by site identified by Tukey's HSD test. No data were collected for 66 Zn in 2004 for all locations.

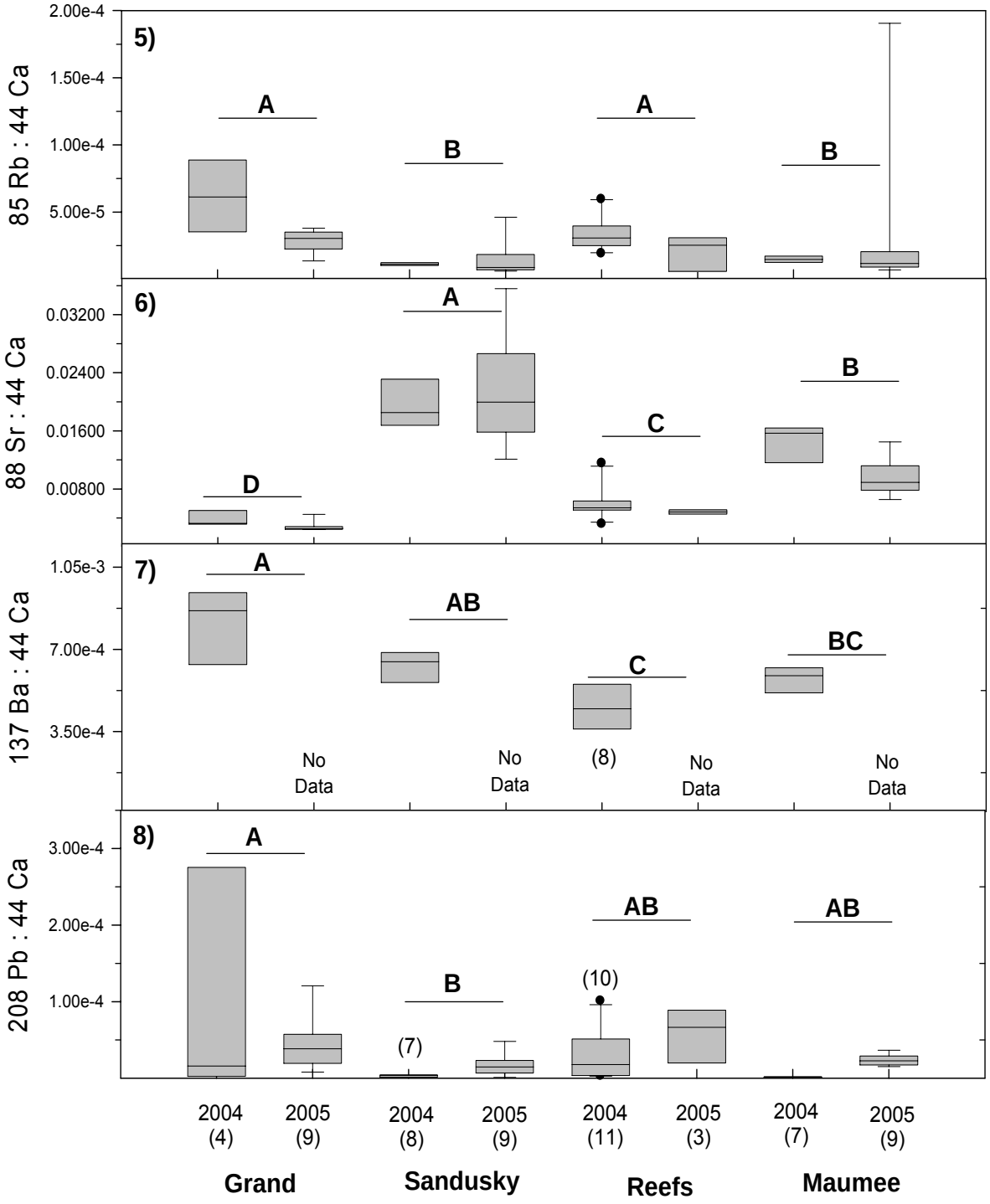

Figure 1 (cont.). Element concentrations 5) 85 Rb, 6) 88 Sr, 7) 137 Ba, and 8) 208 Pb, in water samples (sample size in parentheses) separated by location (Grand River, Sandusky River, Maumee River, and Toussaint Reef) and year (2004 and 2005) in Lake Erie, Ohio. Boxes denote the 25th and 75th percentiles with a center horizontal line corresponding to the median. Letters above Whiskers represent the 10th and 90th percentiles; outliers are shown as points outside the box plots. plots denote element differences by site identified by Tukey's HSD test.. No data are reported for 137 Ba in 2005 for all locations.

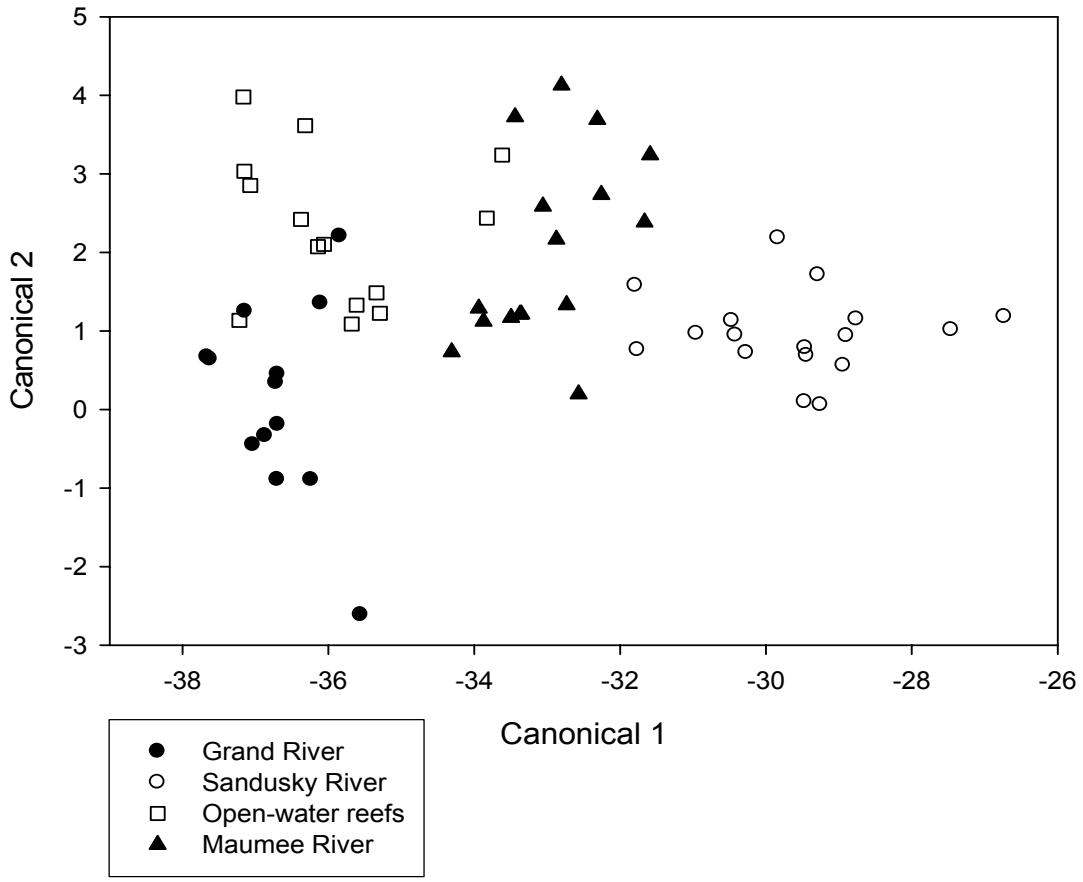

Figure 2. Discriminant function analysis of water data (n=60), collected from Grand, (central basin), Maumee, and Sandusky rivers (western basin), all Ohio tributaries to Lake Erie, and Toussaint Reef (western basin) in 2004 and 2005. Samples are classified by elements 25 Mg and 88 Sr.

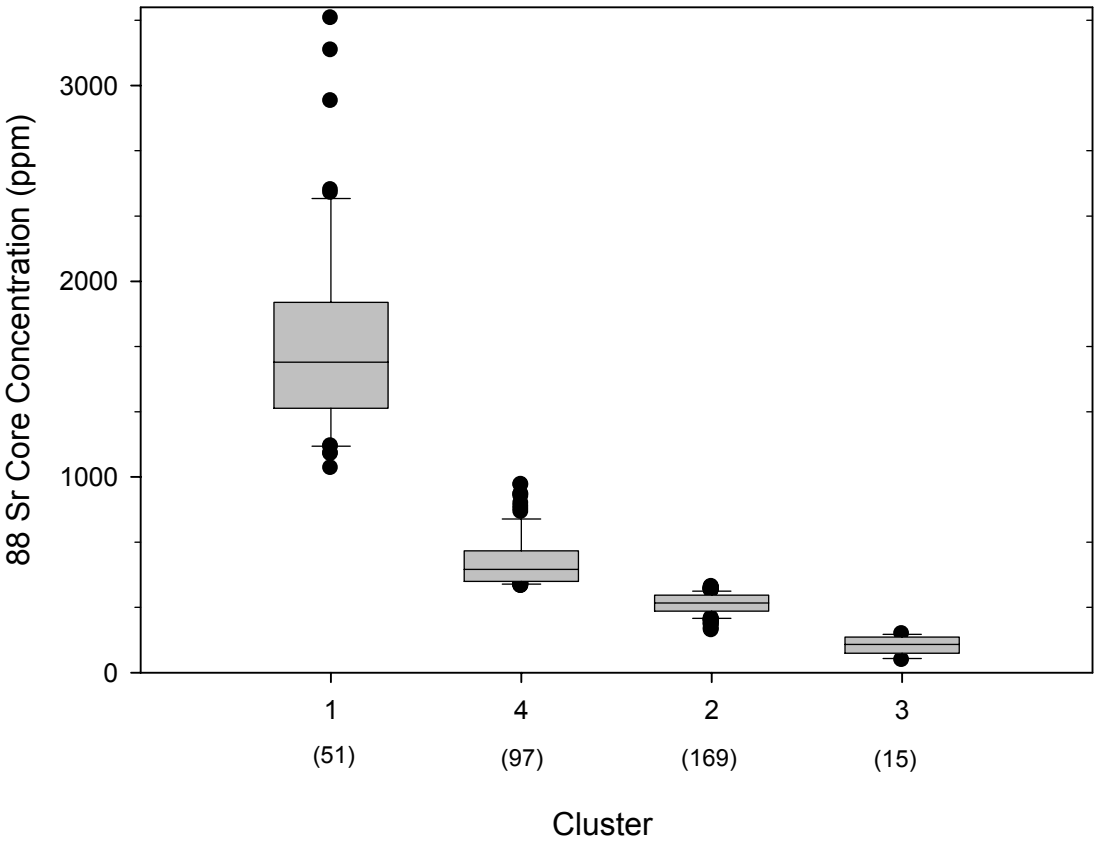

Figure 3. Comparison of core Sr concentration in the four clusters identified in Table 3 by cluster analysis (with sample sizes in parentheses along the x-axis. Samples (N=332) were collected during 2003-2005 from the Grand (central basin), Maumee and Sandusky rivers (west basin), all tributaries to Lake Erie, and Toussaint Reef (West basin, Lake Erie). Boxes denote the 25th and 75th percentiles with a center-line corresponding to the median. Whiskers represent the 10th and 90th percentiles;outliers are shown as points outside the box plots. Clusters are ordered by decreasing core Sr concentration.

APPENDIX A

Advances in Otolith Microchemistry

Advances in Otolith Microchemistry

Otolith microchemistry has the proven potential to reshape our understanding of aquatic population dynamics. For example, scientists now believe that the giant squid, *Architeuthis sp*., lives at depths of 125-500 meters rather than 1,000 meters as historically speculated (Landman et al. 2004). Campana et al. (1995) successfully distinguished between two populations of cod, *Gadus morhua*, in a mixed stock fishery. Movement patterns of blue groper, *Achoerodus viridis*, from estuarine seagrass to rocky reefs were identified with high accuracy (Gillanders and Kingsford 1996). Kennedy et al. (2000) used stable isotopes of Atlantic salmon, *Salmo salar*, to find 11 unique Sr signatures of fish from 18 regions of the Connecticut River, concluding that isotopic analysis was effective at distinguishing between regions with different geologic variation. Although the majority of otolith microchemistry research has been conducted in marine systems, numerous ecologists have begun to pave the way for freshwater systems. For example, Wells et al. (2003) successfully used otolith microchemistry to describe movements in cutthroat trout (*Oncorhyncus clarki lewisi*) with 100% accuracy. Thorrold et al. (1998) were able to correctly classify juvenile American shad (*Alosa sapidissima*) to their natal origins of the Connecticut, Hudson, and Delaware rivers with 90% accuracy. Ludsin et al. (in press) identified the presence of naturally produced lake trout (*Salvelinus namaycush*) in Lake Erie, signifying success of an ongoing rehabilitation program. In Lake Superior, freshwater ecologists were able to develop site-specific signatures of spawning grounds for yellow perch (*Perca flavescens;* Brazner et al. 2004) and lake herring (*Coregonus artedi*; Bronte et al. 1996). Brothers and Thresher (2004) correctly identified stream origins of the invasive sea lamprey (*Petromyzon marinus*) in Lake Huron. Clearly, this technique has allowed for numerous advances in both marine and freshwater systems and, because of its utility, has grown exponentially, resulting in 157 peer-reviewed papers published during1980-1998 (Campana 1999).

APPENDIX B

Additional Tables

1) 25 Mg : Ca	df	SS	F value	Prob > F	Figure
Site	3	0.596	15.507	< 0.0001	Figure 1.1
Year	1	0.167	13.062	0.001	
Site*Year	3	0.065	1.694	0.180	
Error	52	0.666			
2) 55 Mn : Ca					
Site	3	5.243	7.380	0.0003	Figure 1.2
Year	1	0.349	1.472	0.231	
Site*Year	3	2.026	2.852	0.046	
Error	52	12.314			
3) 65 Cu : Ca					
Site	3	1.740	10.623	< 0.0001	Figure 1.3
Year	1	3.7E-04	0.007	0.935	
Site*Year	3	0.301	1.837	0.152	
Error	52	2.840			
4) 66 Zn : Ca					
Site	3	9.8E-05	0.228	0.876	Figure 1.4
Year	-	-	-	-	
Site*Year	-	-	-	-	
Error	26	0.00373			
5) 85 Rb : Ca					
Site	3	2.062	11.419	< 0.0001	Figure 1.5
Year	1	0.262	4.347	0.042	
Site*Year	3	0.342	1.892	0.142	
Error	52	3.130			
6) 88 Sr : Ca					
Site	3	1.9E-03	110.412	< 0.0001	Figure 1.6
Year	1	3.2E-05	5.642	0.021	
Site*Year	3	4.4E-05	2.546	0.066	
Error	52	3.0E-04			
7) 137 Ba : Ca					
Site	3	3.6E-07	10.427	0.0002	Figure 1.7
Year	-	-	-	-	
Site*Year	-	-	-	-	
Error	26	2.6E-07			
8) 208 Pb : Ca					
Site	3	5.471	7.561	0.0003	Figure 1.8
Year	1	5.595	23.198	< 0.0001	
Site*Year	3	1.328	1.836	0.153	
Error	49	11.818			

Table 1. Two-way ANOVA testing for effects of site (Sandusky, Maumee and Grand rivers, and open-water reefs located in Lake Erie, Ohio) and year (2004 and 2005) on elements 1) 25 Mg, 2) 55 Mn, 3) 65 Cu, 4) 66 Zn, 5) 85 Rb, 6) 88 Sr, 7) 137 Ba, and 8) 208 Pb in water samples (n=60). No data were reported for 66 Zn in 2004 and 137 Ba in 2005; therefore, effects of year on these two elements were unknown.

APPENDIX C

Discriminant Function Analysis

	Adult Spawning Location					
Most Comparable Group	Grand	Maumee	Reef	Sandusky	Total	% Homing
Grand	17	17	17	6	57	30%
Maumee	8	71	28	12	119	59%
Reef	5	35	29	3	72	40%
Sandusky	1	12	4	63	80	78%
Sample Size	31	135	78	84	328	

Table 1. Discriminant Function Analysis (DFA) of adult spawning walleye collected in the Sandusky, Maumee, and Grand rivers, tributaries of Lake Erie, and Toussaint Reef during 2003-2005. Sr 88, Mg 25, and Rb 85 were used as discriminators. DFA compares the chemical characteristics (i.e., Sr, Mg, and Rb) of each sample to the characteristics of the four groups (location) and identifies the group it is most similar to. If the group a sample is placed into is the same as its adult spawning location, the fish is considered "homing." If the group a sample is placed into is different than its adult spawning location, it is considered "straying." To interpret the table, read across columns