GREAT LAKES FISHERY COMMISSION

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Exploration of the existence of natural reproduction in Lake Erie lake trout using otolith microchemistry

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EXECUTIVE SUMMARY

Lake Erie fishery management agencies have undertaken a cooperative program to reestablish lake trout (*Salvelinus namaycush*) as a self-sustaining predator in the eastern basin of Lake Erie. This restoration program has included efforts to reduce parasitic sea lamprey (*Petromyzon marinus*), supplement lake trout through a hatchery-stocking program, and conduct annual assessment surveys. Overall, this program has demonstrated some successes in rehabilitating the Lake Erie lake trout population. For example, hatchery supplementation has led to the recolonization of the eastern basin by juveniles and mature adults, and the successful natural production of eggs has been documented. To date, however, an understanding of whether these naturally produced eggs successfully recruit to older life stages remains unknown. Herein, we sought to help Lake Erie agencies better understand the success of their lake trout restoration program by using otolith microchemistry as a tool to determine whether 28 individuals of unknown origin (i.e., they lacked both a coded-wire tag and fin clip that is typical of a hatchery fish) were produced in the hatchery (and released as fry) or in the wild. Our specific objectives, as outlined in our proposal to the Great Lakes Fishery Commission, were to:

1) Use hatchery-reared lake trout that were recaptured in Lake Erie to quantify differences in otolith elemental "signatures" between periods of open-lake vs. hatchery residence.
2) Quantify temporal stability of hatchery-reared lake trout otolith elemental signatures.
3) Use these characteristic elemental signatures to identify whether lake trout of unknown origin, which were collected in Lake Erie, were hatchery-reared or naturally produced (wild).

To ensure that physiological processes were not causing the Lake Erie elemental signature (developed from edges of hatchery recaptures) to differ from the hatchery elemental signature (developed from cores of hatchery recaptures), we analyzed the micro-elemental composition of otoliths cores and edges from individuals that spent their entire existence in Allegheny National Fish Hatchery (the source of stocked Lake Erie lake trout), as well as in Keuka Lake (NY). We also used an independent cross-validation dataset consisting of known-origin individuals (cores and edges of resident hatchery broodstock) to verify our ability to correctly identify the origin of lake trout.

Large differences in elemental composition existed between otolith cores (hatchery phase) and edges (Lake Erie phase) of recaptured individuals. Using the additional lake trout that spent their entire life in hatchery and in Keuka Lake (NY), we found that two elements (Zn, Mn) likely differed between core and edge only because of physiology; hence, these elements were not included in our final classification model. Remaining elements (Li, Mg, Rb, Sr, Sn, Ba, and Pb), however, varied more likely as a function of the environment (i.e., water chemistry), allowing us to discriminate between environments with 95% accuracy (based on our independent, cross-validation set consisting of known-origin fish) (i.e., **Objective 1 was accomplished**). Barium was the most important discriminator between the hatchery and Lake Erie environments, followed by Sr, Mg, and Li. Rubidium, Sn, and Pb were unimportant for differentiating between environments. Our success in discriminating between the hatchery and Lake Erie environments was in large part due to individual elements not varying much in either environment during 1984-2003. Given the stability of elemental signatures during this 19-yr period, we are confident that the discriminant functions generated herein could be used to identify the origin of any additional unknown lake trout caught in the future (i.e., **Objective 2 was accomplished**). Ultimately, using a four-element model consisting of Ba, Mg, Sr, and Li, we classified 24 of the 28 unknown individuals as hatchery-reared, whereas the remaining four fish were classified as non-hatchery reared (i.e., wild). Because the likelihood is small that these four "wild" fish migrated into Lake Erie from another system, our results strongly suggest that the resident Lake Erie lake trout population does not consist solely of hatchery-reared lake trout, and that successful recruitment of naturally produced fish has occurred (i.e., **Objective 3 was accomplished**). Thus, although the current level of natural reproduction is still low, agencies are progressing ever closer towards achieving a self-sustaining lake trout population in Lake Erie.

ABSTRACT

Lake Erie fishery management agencies have been attempting to reestablish lake trout (*Salvelinus namaycush*) as a self-sustaining predator in eastern Lake Erie, primarily through stocking. Although this program has led to the recolonization of eastern Lake Erie by mature adults, and natural egg production has been documented, an understanding of whether these naturally produced eggs successfully recruit to older life stages is lacking. To assess the likelihood of successful recruitment of wild fish, we used laser-ablation inductively coupled plasma-mass spectrometry to develop characteristic otolith elemental "signatures" for the hatchery (from cores) and Lake Erie (from edges) using hatchery-reared lake trout that were recaptured in Lake Erie during 1984-2003. In turn, discriminant functions were developed to determine whether 28 lake trout of unknown origin were produced in the hatchery or wild.

Large differences in elemental composition existed between otolith cores (hatchery phase) and edges (Lake Erie phase) of recaptured individuals. Using additional lake trout that spent their entire life in the hatchery or in Keuka Lake (NY), we found that two elements (Zn, Mn) likely differed between core and edge only because of physiology; hence, these elements were not included in our final classification model. The remaining elements (Li, Mg, Rb, Sr, Sn, Ba, and Pb), however, varied more likely as a function of water chemistry, and allowed us to discriminate between environments with 95% accuracy (based on an independent, cross-validation set consisting of known-origin fish). Barium was the most important discriminator, followed by Sr, Mg, and Li. Ultimately, using these four elements, we classified 24 of 28 unknown individuals as hatchery-reared, whereas the other four fish were classified as wild. Our results strongly suggest that the resident Lake Erie lake trout population no longer consists solely of hatchery-reared Lake trout, and that successful recruitment of naturally produced fish has occurred.

INTRODUCTION

Lake trout (*Salvelinus namaycush*) were once an abundant piscivore in the Laurentian Great Lakes. During the mid-1900s, however, all Great Lakes populations began to collapse, owing primarily to the invasion of sea lamprey (*Petromyzon marinus)* and overexploitation. Since that time, lake trout rehabilitation efforts, which have included sea lamprey control, reduced exploitation, stocking programs, and annual monitoring surveys have commenced in all of the Great Lakes (Cornelius et al. 1995, Elrod et al. 1995, Eshenroder et al. 1995, Hansen et al. 1995, Holey et al. 1995). The success of these efforts has varied considerably among lakes (Cornelius et al. 1995, Elrod et al. 1995, Eshenroder et al. 1995, Hansen et al. 1995, Holey et al. 1995). In Lake Superior and Georgian Bay (Lake Huron), self-sustaining populations once again exist, although these populations still remain below historical levels. In main Lake Huron, Lake Michigan, and Lake Ontario, efforts to rehabilitate lake trout have been less successful in that self-sustaining populations still do not exist. There is, however, evidence that natural reproduction is occurring. By comparison, Lake Erie's restoration program has been the least effective, given that no natural reproduction of lake trout has yet been documented. Importantly, however, evidence of natural egg deposition at levels equal to that of natural spawning areas of lakes Michigan, Huron, and Ontario has been documented (Fitzsimons and Williston 2000).

Despite the seeming failure of Lake Erie's rehabilitation efforts relative to the other Great Lakes, they continue unabated. For example, the U.S. Fish and Wildlife Service (USFWS), New York State Department of Environmental Conservation (NYSDEC), and the Pennsylvania Fish and Boat Commission (PFBC) have remained involved in a cooperative stocking effort since 1982. Since that time, ~200,000 yearling lake trout have been stocked annually into Lake Erie, although levels have declined to ~120,000 yearling/year since 1994 because of concerns of a reduced forage base (Ryan et al. 2002). The fact that the lake trout historically was the dominant large-bodied predator in deep, profundal waters explains why its rehabilitation remains a Fish Community Objective in Lake Erie (Lake Erie Committee 2002), as well as in the other Great Lakes (Great Lakes Fishery Commission 2001).

In addition to this stocking effort, a lake trout monitoring program exists on Lake Erie. Standardized interagency coldwater assessment surveys (Ryan et al. 2002) have been conducted by the NYSDEC (since 1986), the PFBC (since 1991), the USFWS (since 1992), and the Ontario

Ministry of Natural Resources (OMNR; since 1992). These efforts have been facilitated by the fact that all individuals are tagged before being released into Lake Erie. Specifically, hatchery-reared yearling (~18 months of age) lake trout receive a coded-wire tag (CWT) and adipose fin-clip, whereas otoliths of hatchery-reared fry, which are stocked on occasion, are temperature marked (Paré 1993). These tagging and monitoring programs were viewed as critical to evaluating the success of the rehabilitation effort.

Unfortunately, even with tagging and monitoring programs, Lake Erie resource management agencies remain somewhat uncertain as to how successful the rehabilitation effort has been. Admittedly, through monitoring, they know that the one goal of the "Strategic Plan for the Rehabilitation of Lake Trout in Eastern Lake Erie"—to build adult stocks to 75,000 individuals that can produce 10,000 yearlings annually (Lake Trout Task Group 1985)—has not been met. They are, however, less certain as to whether natural reproduction has been occurring given that lake trout of unknown origin have been collected in Lake Erie during assessment surveys conducted in recent years. The inability to determine whether these individuals of unknown origin were hatchery-reared or naturally produced appears due to a lack of sufficient tools and technologies available to Lake Erie agencies (J. Markham, pers. comm.). For example, although individuals that were released as fry were temperature-marked, Lake Erie agencies do not have a means to look for the temperature marks. Likewise, Lake Erie agencies lack an ability to identify the origin of individuals released as yearlings that never received or lost their tags.

The analysis of the elemental composition of lake trout otoliths provides a cost-effective, alternative means to potentially determine the origin of these unknown lake trout without suffering from the same deficiencies of traditional, artificial tagging techniques (e.g., non-existent or unreadable tags, tag loss). Using otoliths as "natural" environmental tags (Campana 1999, Thresher 1999) is made possible by the fact that otoliths grow incrementally throughout life, are metabolically inert, and incorporate trace elements from the surrounding water into their calcium-carbonate matrix as they grow (Campana and Neilson 1985; Campana 1999; Thresher 1999; Campana and Thorrold 2001). In this way, the chemistry in and near the otolith core should characterize the water where an individual hatched and spent its first days (Rieman et al. 1994; Mugiya and Tanaka 1995; Farrell and Campana 1996; Thorrold et al. 1998; Bath et al. 2000; Gillanders and Kingsford 2000; Milton and Chenery 2001b). In turn, it is reasonable to expect that differences in otolith core chemistry should exist between lake trout produced in the

wild versus those reared at the Allegheny National Fish Hatchery (Warren, PA), where the majority of lake trout stocked in Lake Erie have been reared since 1976 (Paré 1993). Indeed, analysis of otolith microchemistry has allowed for determination o f migration histories (Rieman et al. 1994; Limburg 1995; Thorrold et al. 1997; Kafemann et al. 2000; Secor et al. 2001; Kennedy et al. 2002; Milton and Chenery 2003), discrimination among local spawning populations (Campana et al. 1995; Gillanders and Kingsford 1996; Begg et al. 1998; Thorrold et al. 1998; Edmonds et al. 1999; Pontual et al. 1999; Gillanders and Kingsford 2000; Kennedy et al. 2000; Milton and Chenery 2001a), and identification of production areas (sources) of recruits that survive to the fishery (Gillanders and Kingsford 1996; Thorrold et al. 1998; Campana et al. 1999; Thorrold et al. 2001).

Herein, we explored whether natural lake trout reproduction has been occurring in Lake Erie using a common micro-chemical technique, laser ablation-inductively coupled plasma-mass spectrometry (LA-ICPMS). Specifically, we used LA-ICPMS to analyze the cores of hatchery-produced lake trout, which were recaptured in Lake Erie as juveniles or adults during 1984-2003, to develop a characteristic otolith elemental "signature" for the hatchery. Likewise, we used LA-ICPMS to analyze the edges (i.e., non-core areas) of these same individuals to characterize the otolith micro-elemental signature associated with residence in Lake Erie. Using these two characteristic signatures, we developed discriminant functions that could be used to classify unknown lake trout as hatchery-reared or naturally produced (wild). Additional lake trout that spent their entire life in either the Allegheny National Fish Hatchery (i.e., the hatchery production site of Lake Erie lake trout) or Keuka Lake (a pure wild, naturally-reproducing population) were analyzed to explore the assumption that otolith micro-chemical differences between the core (hatchery residence) and edge (Lake Erie residence) of recaptured Lake Erie lake trout were more likely due to water chemistry differences than physiological ones (e.g., age, growth rates, maturation).

METHODS

Lake trout collections. Juvenile and adult lake trout used in this study were collected via annual interagency assessment surveys conducted by member agencies of the Great Lakes Fishery Commission's Coldwater Task Group (i.e., NYSDEC, PFBC, USGS, OMNR) during

1984-2003. A stratified random sampling design for lake trout has been in place since 1986. The design divides the eastern basin of Lake Erie into eight equal areas, each containing 13 equidistant north-south transects (Markham et al. 2004). Within each area, transects are randomly sampled for lake trout during late August or early September. Sampling within each transect consists of fishing 10 net panels, each 15.2 m long by 1.8 m high, which have been tied together into 10-panel gangs. Each diamond-shaped panel is of uniform mesh (ranging from 38 mm to 152 mm on a side, in 12.7 mm increments), and is arranged randomly within a gang. Gangs are set perpendicular to a randomly selected north-south transect, where they fish overnight on the bottom. All juvenile and adult lake trout are examined for length (nearest mm TL), weight (nearest g), sex, and artificial tags. Fin clips and coded-wire tags were used to verify hatchery origin, while the coded-wire tag also allowed for assessment of age (i.e., year-class). Sagittal otoliths from a subsample of lake trout were stored dry in envelopes during most years since 1984.

Although coded-wire tags provided ages of hatchery recaptures, age information was lacking for the lake trout of unknown origin (n = 28). Thus, any model developed to classify these unknown individuals as hatchery- versus wild-produced would need to be robust enough to account for inter-annual variability in hatchery otolith elemental "signatures" (developed from otolith cores of recaptured fish) that might be due to natural variation in water chemistry at the Allegheny National Fish Hatchery (Warren, PA). To develop as robust a model as possible, we analyzed the otolith micro-elemental composition lake trout from a subset of year-classes, spanning 1984-2003 (Table 1). In addition, because sexes of these unknown individuals were not always recorded, we selected an equal number of males and females from each year class, when possible, to avoid potential confounding effects of sex on otolith chemical signatures (Table 1).

To determine whether individuals of unknown origin were produced in the hatchery or the wild, we needed to build a predictive model that could accurately classify them to a natal site. A hatchery signature could easily be produced from analysis of recaptured lake trout otolith cores. However, we obviously did not have a sample of otolith cores from wild lake trout from which a Lake Erie signature could be developed. As such, we used the non-core (edge) regions of recaptured Lake Erie lake trout to develop a wild-type otolith elemental signature. A potential drawback to this approach is that we must assume that core and edge chemistries in these

recaptured fish differ because of water chemistry, and not because of inherent physiological mechanisms (e.g., maturation, ontogenetic shifts in diet) that could artificially cause elemental signatures to vary between the core (i.e., hatchery residence) and edge (i.e., Lake Erie residence). To alleviate these concerns, we made use of two additional collections of lake trout. First, otoliths from individuals (n = 25) that were reared in Allegheny National Fish Hatchery as part of the 1992 year-class, and which ultimately were used as hatchery broodstock through 2003, were analyzed to assess whether core chemistry differs from edge chemistry. Because these individuals resided in the hatchery from birth to senescence, we expected little difference between the core and edge otolith microchemistries. Any detected differences would suggest non-water chemistry (i.e., physiological) regulation of otolith chemistry (assuming water chemistry had remained constant during this 11-yr period). Second, otoliths from pure-wild Keuka Lake (NY) lake trout (ages ranged 2 to 9; n = 26) were analyzed to provide a second assessment of physiological effects on otolith core versus edge chemistry. Again, because these lake trout spent their entire life in a single system, core and edge chemistries were expected to not differ greatly.

Allegheny National Fish Hatchery provided an additional sample of hatchery-reared lake trout fry, from the 2000, 2001, and 2002 year-classes, at sizes they normally would be released into Lake Erie (total lengths ranged 23 to 31 mm). By measuring the dimensions (length and width) of these otoliths, we could determine the maximum LA-ICPMS transect length that should be used to serve as the "core" in our analyses. From analysis of 15 random fry (n = 5 per year class), we found that the smallest otolith was 211 μm wide and 333 μm long. Thus, in our analysis of otolith cores in both recaptured and unknown individuals, we only integrated elemental concentrations in the core over a distance $\leq$ 200 μm (100 μm on each side of the core).

Otolith preparation and LA-ICPMS analysis. After embedding otoliths in epoxy (West Coast Marine), transverse sections (~350 μm wide) were cut using a Buehler ISOMET™ saw such that each section contained the full growth chronology (including the core). Subsequent to mounting sections to a piece of an overhead transparency sheet with Krazy Glue®, we polished the upper surface using a combination of 20-, 12-, 1-, and 0.3-μm aluminum-oxide 3M lapping film to improve optical quality and to ensure the otolith core was at the exposed surface of the section. Afterwards, sections were randomly mounted (with a small piece of the transparency) to acid-

washed glass slides with Krazy Glue® (n = 12-14 otoliths per slide) and left to dry for 24 h. Slides were then sonicated for 10 min in ultra-pure Milli-Q water, rinsed three times in Milli-Q water, dried for 24 h in under a Class 100 laminar flow hood, and then stored in a covered Petrie dish until LA-ICPMS analysis. All post-polishing processing (including storage) occurred in a Class 100 clean room, using only non-metallic instruments.

Elemental concentrations were quantified using a customized Continuum Surelite I solid state Nd:YAG laser (wavelength: 266 nm; maximum power: 20mJ; pulse rate: 20 Hz; pulse width: 4-6 ns; laser spot diameter: 15 μm) coupled to a Thermo-Elemental X7 ICPMS (peak-jumping mode, 10 ms dwell time per isotope). Micro-elemental analysis was conducted both inside and outside of the otolith core in lake trout from Lake Erie (hatchery recaptures), Keuka Lake, and the Allegheny National Fish Hatchery (hatchery broodstock). Because we were only interested in determining the natal origin of unknown lake trout, elemental concentrations were quantified only in the otolith cores of these fish. Core signatures were determined by averaging concentrations along a single transect (average length = 200 μm, per above) that was equidistant on each side of the core. We also ablated two different sections of the otolith edge (non-core area) to account for potential non-homogeneity in elemental composition, which might be due to the formation of different calcium-carbonate structures (aragonite and vaterite) in the same otolith (see below). Ablation distances in the edge ranged 47 μm to 1,980 μm, and varied with otolith size (fish age). To eliminate potential confounding effects associated with integrating across edges consisting of both vaterite and aragonite, only sections consisting of stable (i.e., no obvious directional changes) elemental concentrations were used to characterize edge signatures. Thus, actual distances used to calculate mean edge concentrations ranged from only 28 μm to 334 μm. In cases where both transects were either aragonite or vaterite, a single average elemental concentration was calculated for that otolith. In this way, all known-origin lake trout would have a core signature plus a signature for aragonite, vaterite, or perhaps both.

A glass reference standard (NIST 612) with known concentrations of elements was analyzed before and after every 16 samples (n = 2 replicates before and after), which allowed for quantification and correction of instrumental drift. This same standard also was used to determine precision in estimating elemental concentrations. The Ar carrier gas (i.e., background) was

analyzed for 60 sec before every sample, allowing limits of detection (LODs) to be calculated for individual samples, using the following formula,

1) $$LOD = \frac{3 * \sigma_{bgd}}{S * Y} * \sqrt{\left(\frac{1}{N_{bgd}} + \frac{1}{N_{pk}}\right)}$$

where σ_{bgd} = SD of the pre-ablation determination of the background; N_{bgd} and N_{pk} = replicate determinations used in the integration of the background and ablation signal, respectively; S = mean sensitivity (counts per second per unit concentration) for the NIST reference standard; and Y = ablation yield relative to the NIST reference standard, determined from the measured count rates and known concentrations of the internal standard (Jackson 2001).

Not all elements were suitable for post-processing analysis, owing to elevated detection limits and imprecise quantification. For an element to be included in our analyses, it had to be precisely measured; i.e., the average CV (i.e., SD/mean*100%) for at least one measured isotope, as determined from our NIST samples, had to be < 10.5% (Gillanders and Kingsford 1996). In addition, its concentration had to be above the LOD for 90% of the samples. Based on these criteria, ^{7}Li, ^{25}Mg, ^{55}Mn, ^{85}Rb, ^{86}Sr, ^{88}Sr, ^{120}Sn, ^{137}Ba, ^{138}Ba, and ^{208}Pb were suitable for use in our analyses, whereas ^{65}Cu, ^{111}Cd, ^{140}Ce, and ^{238}U were not (Table 2). Because both Sr isotopes were highly correlated (r = 0.96, df = 368), as were both Ba isotopes (r = 1.0, df = 368), we only used ^{86}Sr and ^{138}Ba to estimate Sr and Ba concentrations, respectively.

Aragonite versus vaterite formation. Previous work has demonstrated that otoliths of salmonines, including lake trout, can be simultaneously comprised of two different calcium-carbonate crystalline structures, aragonite and vaterite (Gauldie 1986; Casselman and Gunn 1992; Bowen II et al. 1999; Brown and Severin 1999), which can differ dramatically in their elemental composition (Brown and Severin 1999). Although areas of aragonite and vaterite can be differentiated visually under both reflected (see Figure 1 in Casselman et al. 1992) and transmitted (Figure 1) light, we used Raman spectroscopy (Truchet et al. 1995; Gauldie et al. 1997), which is a non-destructive technique that measures the wavelength and intensity of scattered light in molecules, to verify our ability to differentiate these two calcium-carbonate polymorphs. Raman spectra were recorded in otolith edges from broodstock lake trout using a Renishaw inVia Reflex Raman with spectrometer. The exciting source was a He:Ne laser operating at 633 nm with a power of about 27 mW and a focus of 3.5 mW on the otolith. The

system was equipped with a Charged-Coupled Device (CCD) detector. Afterwards, we used LA-ICPMS, as described above, to quantify elemental concentrations in edges consisting of aragonite and vaterite (in the same annulus) to generalize differences in composition. In this way, we sought a means to definitively identify areas consisting of vaterite versus aragonite in the remainder of otoliths, without having to conduct Raman spectroscopy.

Raman spectroscopy identified marked differences between aragonite and vaterite, many of which were similar to those documented for coho salmon *Oncorhynchus kisutch* (Gauldie et al. 1997). In addition to differential shifts in lattice modes, vaterite had triplet bands of symmetric stretching ($\square_{1)}$, whereas aragonite consisted of a single peak (see Figure 2; Melancon et al. in prep). In addition, $\square_4$, in-plane bending, is characterized by a narrow doublet in aragonite instead of a broader doublet for the Raman spectrum of vaterite (Figure 2). (The bands $\square_2$ and $\square_3$ were not visible, as their intensities are too weak and fluorescence overshadows the peaks. However, they were not needed to differentiate between these two calcium-carbonate polymorphs.)

Large, consistent differences in elemental concentrations also existed between aragonite and vaterite of broodstock. Most importantly, Sr concentrations in vaterite were 17-fold lower than in aragonite, Mg levels in vaterite were 37-fold higher than in vaterite, and Ba levels were 19-fold higher in aragonite than vaterite (Table 3). When the suite of significant elements (Mg, Mn, Rb, Sr, and Ba) were evaluated together using principal components analysis (based on a correlation matrix), the differences between aragonite and vaterite become even more obvious, as does the realization that the core is comprised of aragonite (Figure 3). These differences in elemental concentration can largely be explained by the ionic sizes of elements in relation to the bond length in the structure of the two crystalline polymorphs (Melancon et al. in prep). Because the core chemistry of all lake trout, regardless of location, more closely matched that of aragonite than vaterite (Table 3, Figure 3), vaterite samples were not included in our effort to identify natal origins of unknown lake trout.

Data analysis. To identify differences between (aragonite) cores (representing the hatchery) and edges (representing Lake Erie) of otoliths from recaptured lake trout, we conducted two-sample t-tests (2-sided) for all usable elements. Two-sample t-tests were used, as opposed to paired t-tests, because not every otolith had an aragonite edge, owing to vaterite formation. When

variances differed between samples (Levene's test of heterogeneity, $\alpha = 0.05$), separate variance estimates were used in the analysis. Because nine separate t-tests were conducted, a Bonferroni adjustment to the α-level was made (i.e., $\alpha = 0.05/9 = 0.0056$).

To explore consistency in core and aragonite edge signatures in both the hatchery and Lake Erie through time, we conducted univariate one-way ANOVAs, using year-class as the main factor. Differences among years were quantified using a Tukey's honestly significant difference test (hsd) for unequal sample sizes.

Paired t-tests were conducted to determine whether elemental composition varied between cores and (aragonite) edges in both the hatchery broodstock and Keuka Lake fish. If a significant difference did not exist for an element in either sample ($\alpha = 0.05/7 = 0.007$), we assumed that physiological regulation of that element was unimportant and that this element could potentially be used in our discriminant functions used to classify unknowns. To determine whether elements that demonstrated similar differences between cores and edges in all three datasets (hatchery broodstock, Keuka Lake, hatchery recaptures) should be included in predictive classification models, we compared the relative magnitude of the differences in the Keuka Lake and broodstock lake trout to those observed between the core (i.e., hatchery) and edge (i.e., Lake Erie) of the recaptures. We also used our hatchery recaptures (hatchery cores versus Lake Erie edges) to build several linear discriminant function models (Statistica v. 6.0, StatSoft, Inc.) to assess the relative importance of individual elements in classifying an independent, cross-validation set of known-origin lake trout. Specifically, with each LDFA, we tested our ability to classify both the cores and edges (independent of one another) of the hatchery broodstock (i.e., known-origin fish). The model that best classified both the cores and edges of these broodstock as "hatchery" fish would be the one that would be used to predict the origin of our sample of unknowns. Initially, we included all elements in a LDFA, and subsequently removed elements (one by one) that may have differed between core and edge simply because of physiology (i.e., those elements that varied similarly in all three datasets). For an element to be included in any of the final models, its p-value had to be < 0.05.

Once we settled upon a final model that could best classify both the cores and edges of broodstock as "hatchery" fish, we used that LDFA to predict the origin (hatchery versus wild) of

our 28 lake trout. To help assess our confidence in these predictions, we evaluated the posterior probability associated with the classification for each unknown individual.

All data were tested for normality *a priori* and appropriately transformed to achieve normality for each test (Kolmogorov-Smirnov normality test, all $p \geq 0.20$): no transformation was necessary for Rb; Li, Zn, Sn, Pb were $\log_{10}$-transformed; Sr and Mg were reciprocal-transformed; Ba was square-root transformed; and Mn was 1/square-root transformed. In addition, because outliers can unduly influence the results of multivariate analyses (Tabachnick and Fidell 2001), we removed highly significant multivariate outliers (> 3.0 SD) based on a relative Euclidian distance measure (McCune and Grace 2002). Finally, substituting element means for missing values, which occurred when a sample was below the LOD, reduced our ability to accurately classify the broodstock in our cross-validation set. Therefore, only samples that contained a concentration for all usable elements (Li, Mg, Mn, Zn, Rb, Sr, Sn, Ba, and Pb) were included in our LDFAs, which can explain discrepancies in sample sizes among analyses.

RESULTS

Hatchery versus Lake Erie signatures. Univariate two-sample t-tests revealed much variation in otolith elemental concentrations between the hatchery (i.e., cores of recaptured fish) and Lake Erie (i.e., edges of recaptured fish). Magnesium, Mn, Zn, and Ba were higher in cores (hatchery) than in edges (Lake Erie) of recaptured lake trout (Figure 4). Although the ratio of the difference between the average otolith core and edge concentrations was small for both Mn (core:edge = 2.6) and Zn (core:edge = 3.5), it was >2-fold higher for Ba (core:edge = 6.9). By contrast, Li and Sr demonstrated the opposite pattern, with edge (Lake Erie) concentrations being higher than core (hatchery) concentrations (Figure 4). Only a weak statistical difference was found for Rb ($p = 0.04$), whereas no differences between these two environments were observed for Sn and Pb (both $p > 0.07$). Hence, we would expect Sn and Pb to be poor discriminators between the hatchery and Lake Erie.

These differences in chemistry were quite consistent across years as well (Figure 5). In fact, only trivial differences were found among years: 1) Li concentrations were higher in the hatchery (cores) during 1992 than in 1984; 2) the Sn signal in 1990 was greater in the hatchery (core) than during 1984 and 1996; 3) Pb concentrations were greater in the hatchery (core) during 1990 than

during 1984 (one-way ANOVAs, Tukey's hsd test for unequal sample sizes). No element demonstrated any difference among years in Lake Erie (edges) (one-way ANOVAs, Tukey's hsd test for unequal sample sizes). Ultimately, these results indicate that both the hatchery and Lake Erie elemental environments have remained relatively stable during 1984-2003.

Confounding effects of physiology. Given that otolith edges were used to develop a Lake Erie signature, whereas cores were used to develop a hatchery signature, we were concerned that differences detected between the hatchery and Lake Erie (i.e., Li, Mg, Mn, Zn, Rb, Sr, and Ba) may not have been due to water chemistry, but instead to physiological effects on otolith elemental deposition. A comparison of mean elemental concentrations in cores versus edges of both hatchery broodstock and Keuka Lake lake trout revealed the potential for some physiological regulation in both Zn and Mn. For both elements, concentrations were greater in the core than the edge in both systems (Figure 4), which is consistent with differences found between the cores (hatchery) and edge (Lake Erie) of recaptured fish (Figure 4). In addition, the relative magnitude of these differences was similar for all three comparisons for both Zn (core:edge = 3.5, 3.0, and 2.5 for recaptures, broodstock, and Keuka Lake fish, respectively) and Mn (core:edge = 2.7, 2.6, and 1.8 for recaptures, broodstock, and Keuka Lake fish, respectively). Barium also demonstrated similar patterns in all three comparisons (i.e., core higher than edge); however, the differences in both the broodstock (core:edge = 1.9) and Keuka Lake lake trout (core:edge = 1.8) were small when compared to the magnitude of the difference between the hatchery and Lake Erie (core:edge = 6.9). Thus, although Ba may in part differ between the core and edge because of physiological effects on otolith elemental deposition, environmental (i.e., water chemistry) differences appear to have an overwhelming effect on otolith elemental composition.

As for the remaining elements (Li, Mg, Rb, and Sr), we feel confident that physiological processes only have a minimal effect on the relationship between core and edge chemistry. A significant difference in Sr was not found in the broodstock lake trout (Figure 4), while the Sr differences in the Keuka Lake lake trout were minor (~2-fold smaller) relative to the Sr differences between the hatchery and Lake Erie. No differences were detected for both Li (in Keuka Lake) and Rb (in broodstock), and inconsistent patterns between the core and edge existed for both elements (e.g., Li was higher in the core than edge in broodstock, but lower in the core (hatchery) than edge (Lake Erie) in recaptures).

As a further check on whether to retain Ba, but eliminate Zn and Mn, when classifying unknown fish, we conducted a series of stepwise linear discriminant function analyses, using different combinations of elements, to see which combination(s) allowed us to best classify an independent cross-validation dataset consisting of broodstock cores and edges. If an element was included that was indeed governed more by physiology than water chemistry, we would expect our model to do poorly in classifying both cores and edges of broodstock. For example, if Zn is always lower in the edge than core because of physiology alone, a disproportionate number of broodstock edges would be classified as Lake Erie fish (again, the Lake Erie signature was derived from otolith edges of recaptured fish) instead of hatchery fish. From these analyses, we feel confident in our decision to remove Zn from our final linear discrimination model; when Zn is retained in the model, our ability to classify broodstock edges diminished relative to most other models without Zn (Table 4). In fact, whereas our best models allowed 86% correct classification of broodstock edges (2 misidentifications; Figure 6), the model containing Zn only allowed 50% correct classification. The fact that half of the broodstock edges were misclassified as "Lake Erie" individuals, instead of "hatchery" individuals, indicates strong regulation of Zn levels in otoliths by non-water chemistry effects. By contrast, models involving Ba and Mn were not subject to the same deficiencies (Table 4). Barium was especially valuable for distinguishing between the hatchery and Lake Erie, as indicated by its large F-values in all models (Table 4). Further, when Ba was not allowed into the final model, our ability to classify both cores and edges of broodstock declined to no better than a coin toss (Table 4). Although our results demonstrate two models could be used to classify the origins of unknown lake trout, one with Ba, Sr, Mg, and Li and a second one with only Ba and Mn, we were conservative and used the model without Mn (Table 4). This decision was in part based on research involving other Lake Erie fishes (i.e., yellow perch *Perca flavescens* and walleye *Sander vitreum*), wherein we have documented large Mn peaks in the center of the core that can be an order of magnitude greater than in the edges (see Ludsin et al. submitted). Similar high Mn peaks also have been documented in the cores of Atlantic cod *Clupea harengus* (Brophy et al. 2004).

Classification of unknown lake trout. Using the linear discriminant functions developed in our model that included Ba, Sr, Mg, and Li, we classified 4 of 28 unknown-origin lake trout as wild, whereas the others were classified as hatchery-reared (Figure 6). To evaluate our confidence in these classifications, we examined posterior probabilities associated with each

classification. With regard to the four wild fish, two had posterior probabilities $\geq$ 0.92; thus, we feel confident that these fish were indeed not produced in the hatchery ($\leq$ 8% chance they were misclassified). Our confidence in the classification of the other two lake trout is lower, given that posterior probabilities were 0.51 and 0.68. By contrast, the posterior probabilities for the remaining 24 lake trout classified as hatchery-reared were all $\geq$ 0.73 (with most above 0.90) indicating that no other unknowns were remotely close to being designated as wild.

DISCUSSION

Lake trout populations in the Laurentian Great Lakes experienced a variety of anthropogenic perturbations during the past century. Most prominent were the effects of overexploitation and sea lamprey predation, which caused substantial declines in stocks throughout the basin, including complete extirpation in both Lake Ontario and Lake Erie (Cornelius et al. 1995, Elrod et al. 1995). In an effort to rehabilitate native lake trout populations, restoration efforts were initiated in all systems that consisted of reduced exploitation, hatchery supplementation, sea lamprey control, and annual assessment surveys (Cornelius et al. 1995, Elrod et al. 1995, Eshenroder et al. 1995, Hansen et al. 1995, Holey et al. 1995). Overall, each program has demonstrated some successes, although great disparities exist in the magnitude each program's achievements. For example, in Lake Superior and Georgian Bay (Lake Huron), self-sustaining populations of lake trout now thrive (Eshenroder et al. 1995, Hansen et al. 1995), whereas this is not the case in Lake Michigan, Ontario, or Erie (Cornelius et al. 1995, Elrod et al. 1995, Holey et al. 1995). In fact, Lake Erie could be viewed as the least successful program, given that it remained the only Great Lake in which natural reproduction has not been documented.

Production of wild lake trout. Results from this investigation, however, indicate that the lake trout restoration program in Lake Erie actually may be doing better than once thought. Using otolith microchemistry as a tool to identify the natal origin of recently captured fish of unknown origin, we provide the first compelling evidence that the naturally produced (wild) lake trout have recruited to mature (fishable) life stages in Lake Erie, which has not occurred since the 1950s. Specifically, 4 of 28 (14%) lake trout of unknown origin were classified as naturally produced instead of hatchery-reared. It also is important to recognize that this percentage may

be somewhat conservative, given that our discriminant functions tended to err on the side of misclassifying Lake Erie fish (otolith edges) as hatchery fish (otolith cores), and not vice versa. Thus, if a misclassification occurred it is more probable that a wild-type fish would be accidentally classified as hatchery-reared, than the reverse.

Because we used an indirect method to determine the natal origins of unknown fish, there is no way to "prove" that these four fish were indeed produced in the wild. Other considerations, however, lend support to our conclusion. First, using length information, which was available for only two of the four unknown lake trout classified as naturally produced, we estimated that these two fish were produced naturally as part of the 1993 (age 9 at capture) and 1995 (age 5 at capture) year-classes (length-age relationships were developed from Lake Erie recaptures sampled during 1984-2003). Interestingly, this period coincides with the first reports of lake trout egg sightings in the wild. Cornelius et al. (1995) reported incidental lake trout egg collections on trap nets in Barcelona Harbor, NY during both 1992 (680,000 eggs) and 1993 (450,000 eggs), whereas egg densities at levels equivalent with lakes Michigan, Huron, and Ontario were recorded at several natural spawning reefs in eastern Lake Erie during 1994 and 1995 (Fitzsimons and Williston 2000). Unfortunately, no information exists on egg deposition rates for other years. Second, 1988-1995 is the only period in Lake Erie's recent (post-1980) history in which sea lamprey wounding rates on lake trout remained at consistently low levels. During this time, annual time wounding rates averaged less than 6 wounds per 100 lake trout, which is considerably lower than during both 1980-1987 (15 to >60 wounds per 100 lake trout) and 1996-2001 (8 to 25 wounds per 100 lake trout) (Sullivan et al. 1993; Cornelius et al. 1995; NYSDEC 2004). This ebb in wounding rates can most certainly be attributed to the application of lampricide to Lake Erie tributaries during 1986, which caused major reductions in sea lamprey nests, larval abundance, and spawning-phase adults relative to the years before and after (Sullivan et al. 2003; Markham et al. 2004; NYDEC 2004). Finally, 1988-1995 was a period during which large numbers of mature ($\geq$ age 5) lake trout existed in the lake, owing to development of a coordinated hatchery supplementation program 1982 (Cornelius et al. 1995; Markham et al. 2004). In addition, adult lake trout survival (S) was higher during 1988-1992 (S = 0.32) than in the preceding period (S = 0.26) of high sea lamprey abundance (Sullivan et al. 2003). Ultimately then, the years during which at least two of the four wild lake trout were produced coincided with a period when conditions were favorable for lake trout growth and

survival (due to a reduction in sea lamprey), and fertilized eggs of hatchery-reared lake trout were being found on natural Lake Erie spawning reefs.

Fry stocking success. In addition to providing insight into the existence of lake trout natural reproduction in Lake Erie, our research can provide a coarse evaluation of the success of the fry- (20-30 mm TL) stocking program. Since a coordinated lake trout restoration program was begun on Lake Erie in 1982, yearlings (or yearling equivalents) have been stocked into Lake Erie annually at rates of ~200,000 fish per year (1982-1995) or ~120,000 fish per year (1996-2003) (NYSDEC 2004). Each fish receives both a fin clip and coded-wire tag, which allows agencies to identify which stocking event produced survivors during annual summer assessment surveys. Supplemental stocking of fingerlings (1982-1983, 1989, 1991, 1993, 1995, and 1999-2000) and fry (1990, 1993-1994, 1997-1998, and 2000-2003) has occurred during many of these years as well at levels of 7,000-120,000 fingerlings per year and 81,000-301,000 fry per year (NYSDEC 2004). Whereas the fingerlings also are externally tagged, thus allowing agencies to identify their origin and age, the stocked fry are not. Instead, fry are temperature-marked (Paré 1993) in the hatchery before release into Lake Erie.

Although fry are temperature-marked before release, Lake Erie agencies lack the technologies and expertise to read these tags (J. Markham, pers. comm.). Based on rates of return of tagged to untagged (i.e., unknown-origin) lake trout in annual assessment surveys, agencies had already grasped the notion that survival rates of stocked fry are low. Again, only ~30 untagged individuals had been recovered out of 1000s of lake trout sampled since fry stocking began in 1990. However, what agencies could not know until our investigation is whether any fry-stocked individuals actually survived to juvenile or adult life stages. If we ignore the low probability that these unknown fish were actually stocked into Lake Erie without a fin clip or coded-wire tag (J. Markham, unpub. data), one can only assume that the remaining 86% of the unknowns were survivors of fry stockings. We have ruled out the possibility that these 24 individuals were produced in a different system, given that 1) not one lake trout tagged in another system has ever been recovered in annual Lake Erie assessment surveys (J. Markham, pers. comm.), and 2) the 24 unknown individuals classified as hatchery-reared had otolith elemental signatures that closely matched the other fish produced in Allegheny National Fish Hatchery (and had posterior classification probabilities that generally exceeded 0.90). Given that our LA-ICPMS analyses only utilized one of the otolith per fish, it still may be possible to make

a true determination of whether these 24 individuals were stocked as fry or yearlings (assuming a means to read temperature tags is found). However, even if it was confirmed that these 24 unknown individuals, out of hundreds sampled annually (Markham et al. 2004), were stocked as fry, the ultimate fact would remain—fry stocking contributes only a small fraction of recruits to fishable stages.

It is beyond the scope of this investigation to evaluate the reasons why rates of natural reproduction and fry stocking success have been low; however, one interesting observation is worth mentioning. Our analysis of otolith edges of lake trout from Keuka Lake, the hatchery broodstock, and Lake Erie (recaptures) varied greatly in the percentages of fish that had vaterite in their otoliths. Specifically, only 4% (1 of 26) lake trout from Keuka Lake had otoliths with vaterite. By contrast, 68% (17 of 25) lake trout that spent their entire life in the hatchery (broodstock) had vaterite in at least one of the edge transects, whereas 59% (60 of 101) recaptured lake trout had otoliths with vaterite in them. Although the exact mechanisms that cause vaterite formation in otoliths are unknown (Bowen II et al. 1999), as are the consequences, it has been suggested that the formation of vaterite arises because of external stress on the individual (Casselman 1986, 1990; Bowen II et al. 1999). Similar to our findings, Bowen II et al. (1999) found higher incidences of vaterite in hatchery-reared lake trout in Lake Superior (66%), Lake Huron (75%), and Lake Ontario (86%) than in wild-caught lake trout captured in Lake Superior (37%), Lake Huron (22%), or northern Canada (49%). Casselman (1986) also documented high rates (>70%) of vaterite formation ("crystallization") in stocked Lake Ontario lake trout, but no vaterite in otoliths from 26 wild lake trout. Given these differences between hatchery-reared and wild lake trout, it has been suggested that stocking stress is the root cause of vaterite formation in hatchery-reared individuals (Casselman 1986, 1990; Bowen II et al. 1999). Although a mechanistic test of this hypothesis is clearly needed, our findings support this hypothesis, given that Keuka Lake fish, which were produced naturally, had a very low incidence of vaterite, whereas the broodstock that spent their entire existence in an unnatural setting had the highest incidence of vaterite in their otoliths.

Clearly, if vaterite formation is an indicator of stress, then recaptured individuals are sending a strong signal that something is wrong either during late stages of hatchery development, or in the lake proper. Unfortunately, because we only sampled the innermost 200 μm of the core

(which corresponds with fry development), we cannot determine whether vaterite formation typically begins in the hatchery (during fingerling or yearling stages) or afterwards. Interestingly, 3 of the 101 lake trout recaptured in Lake Erie and 1 of 26 wild Keuka Lake fish did have inner cores consisting of vaterite (these fish were removed as multivariate outliers from our analyses), indicating that vaterite can form early in life (at sizes < 30 mm TL). Similarly, Bowen II et al. (1999) documented vaterite formation in lake trout fingerlings, both wild and hatchery-reared, that were only 3 to 4 months of age. Thus, if vaterite formation begins immediately at the onset of stress, then the hatchery environment itself (at least through the fry stage) may not be all that stressful, given that 98 of 101 hatchery-reared otoliths did not have vaterite in their core. If true, this would mean that stress is induced during later stages of hatchery development, during the process releasing individuals in the lake environment, or perhaps during the process of adapting to and living in a new, natural environment. Certainly, a better understanding of the linkage between stress and vaterite formation, including the timing of the onset of vaterite formation relative to the onset of stress, would be valuable in helping evaluate these alternative hypotheses.

Value of otolith microchemistry. Supplementing wild populations with hatchery-reared individuals is a common practice in both freshwater and marine systems. In turn, fishery management agencies have sought ways in which to identify the relative contributions of hatchery-produced versus naturally produced individuals to the general fishable population. Until recently, agencies relied largely on artificial tags to demarcate populations (e.g., fin clips, coded-wire tags, visual implant tags, PIT tags, temperature, alizarin, or tetracycline marking otoliths) (Pawson and Jennings 1996), choosing a technique that balanced information needs with both the ease of use and costs (Hammer and Blankenship 2001). Although conceptually simple, and in most cases, relatively inexpensive, investigations that implement artificial tagging programs typically suffer from one or more of the following vagaries: 1) it is impossible to tag all individuals in a population, and hence, the origins of some individuals will never be known; 2) artificial tags can be lost; 3) fish can accidentally not be marked; and 4) recovering tagged individuals is difficult, especially in large populations.

In recent years, there has been a growing reliance on "natural" tags to identify origins of individuals (Pawson and Jennings 1996). Most common have been the use of genetic techniques. However, owing to a slow rate of neutral marker differentiation, the use of genetics

in stock discrimination studies can be limited, especially in situations where the groups to be differentiated are genetically young (e.g., wild progeny of hatchery-reared lake trout; Smith and Tibbles 1980). This technique also is restricted by the fact that even minor exchanges of individuals among local breeding populations can lead to genetic homogeneity (Allendorf and Phelps 1981; Slatkin 1987; Hartl and Clark 1989; Rymer et al. 1995). For these reasons, genetic approaches have generally not provided information that could be readily used by management agencies for stock discrimination purposes (Pawson and Jennings 1996).

In this study, we relied on an alternative "natural" tagging approach, otolith microchemistry, to identify the origins of our unknown lake trout (see Campana 1999 and Thresher 1999 for reviews). Our approach had a clear advantage over a genetics approach because all Lake Erie lake trout, both of hatchery and wild origin, emanated from the same hatchery-stocked lineage only a short time ago; hence, we would not expect much (if any) difference in genetic structure between the two groups (not to mention that wild fish did not exist from which to sample DNA). Further, although otolith microchemistry has not been used in the same manner as we have done here (i.e., using both cores and edges to develop unique signatures for two environments), it has been used successfully in other systems to determine origins of naturally producing locals spawning populations (e.g., Gillanders and Kingsford 1996; Thorrold et al. 1998; Campana et al. 1999; Thorrold et al. 2001).

There were only two foreseeable limitations to using an otolith micro-chemical approach to identify the origins of these unknown lake trout. First, because water samples from the hatchery and Lake Erie had not been collected, we had to assume that sufficient water chemistry variation existed between the hatchery environment and Lake Erie to allow discrimination. Second, owing to a lack of naturally produced individuals from which to develop a Lake Erie signature using otolith cores, we were forced to use otolith edges from lake trout recaptured in Lake Erie to develop a Lake Erie signature, and the core of those same otoliths to characterize the hatchery signature. Thus, we had to assume that differences between core (hatchery) and edge (Lake Erie) chemistry were due more to the environment (i.e., water chemistry) than to physiological processes that might differentially alter how elements are incorporated into the edge versus core.

Clearly, based upon our ability to discriminate between the hatchery and Lake Erie environments, sufficient variation in elemental composition exists between environments. As to

the cause(s) of this variation, we are uncertain. Most likely, variation in elemental signatures between the hatchery and Lake Erie is due to the influence of local geology, given that the rivers that feed into eastern Lake Erie drain watersheds with glacial deposits (recent to late Illonoian, Wisconsinan), whereas the Allegheny River, which feeds the Allegheny National Fish Hatchery, does not (http://www.dcnr.state.pa.us/topogeo/map13/map13.aspx). In addition, erosion along the eastern shoreline of Lake Erie, at least in Pennsylvania, brings unconsolidated materials (e.g., sand, gravel) into Lake Erie, whereas sandstones and shales that line the valleys of the Allegheny River erode into that system (http://www.dcnr.state.pa.us/topogeo/map13/map13.aspx).

Despite lacking a full understanding of the causes of variation in micro-elemental signatures between environments, it is clear from our analysis of both hatchery and Lake Erie signatures during 1984-2003 that both environments are temporally stable. The fact that we could pool data from both environments across nearly 20 yr and still discriminate between them is testament to this notion. Based on a recent review conducted by Gillanders (2002), we are fortunate to have such stability in otolith elemental chemistry. In fact, with few exceptions, high inter-annual variability in otolith microchemistry is the strict rule, at both short (2-3 yr) or long (up to 13 yr) time scales (see Gillanders 2002 for a review). This high variability is easily understood when considering that the elemental composition of water can be highly influenced by a variety of factors, including watershed inputs, temperature, salinity, and atmospheric deposition. We suggest that stability in our systems is due to the fact that eastern Lake Erie is a large, deep basin with a long residence time that also does not receive much direct runoff from the watershed, while the hatchery environment is highly controlled relative to natural water bodies. Regardless of the cause, a major implication of stability in otolith elemental signatures is that any future collections of unknown-origin lake trout can be classified using extant discriminant functions, without having to re-characterize both the hatchery and Lake Erie signature (although we would recommend doing so periodically to account for any unforeseen shifts in either environment). In any case, by only having to process unknown individuals, much time and money could be saved. Additionally, the fact that both the hatchery and Lake Erie signatures remained stable, at least enough to differentiate between environments, bodes well for the use of otolith microchemistry as a stock discriminating tool for other species in the Great Lakes.

One surprising aspect of our otolith chemical work involved the potential influence of physiology on core and edge concentrations. We had no doubt entering this investigation that

physiology plays a role in regulating elemental deposition in otoliths. In fact, physiological regulation of elements is expected to occur at several interfaces (e.g., water-gill, blood-endolymph, endolymph-otolith) as they move from the water into the otolith (Campana 1999; Thresher 1999). For this reason, most otolith micro-chemical studies have tended to focus more upon cations that are unimportant to physiological processes (e.g., Li, Mg, Mn, Zn, Sr, Ba) than those that are (e.g., Na, K, Cl) (Campana 1999; Thresher 1999). Our comparison of both Zn and Mn in the cores and aragonite edges of our broodstock and Keuka Lake fish, which seemingly were exposed to the same water chemistry during their entire life, raises some doubt, however, about the use of these elements in otolith micro-chemical investigations. Indeed, our own research with both Lake Erie yellow perch and walleye has demonstrated extremely high Mn spikes in the exact core of otoliths, which can sometimes be more than 10-fold higher than in non-core areas (Ludsin et al. submitted). Manganese spikes also have been documented in Atlantic cod *Clupea harengus* otoliths captured in the Irish and Celtic seas (Brophy et al. 2004). Given the consistency of Mn spikes in the core of Atlantic cod otoliths, and their non-environmental origin (via experimentation), Brophy et al. (2004) has suggested that differences between core and non-core areas of their larval otoliths might arise from the otolith core consisting not of aragonite, but calcite, which has a higher affinity for Mn than aragonite (Raiswell and Brimblecombe 1977).

The explanation for the difference in Zn between the core and edge also is tenuous. We do know that Zn can track water chemistry (Saquet et al. 2002), and therefore, is not always under strict physiological regulation. However, it is possible that changes in diet that occur with ontogeny might be partly responsible, given that feeding is a primary uptake rout of Zn in marine fishes (Renfro et al. 1975; Milner 1982; Willis and Sunda 1984). Alternatively, the metabolic requirement for Zn, which changes with ontogeny, has been proposed as mechanism or non-homogeneity of Zn in otoliths of Arctic char *Salvelinus alpinus* (Halden et al. 2000). Given that several processes, including feeding, metabolic changes, and ambient concentrations in the environment, can influence Zn concentrations in otoliths (Halden et al. 2000), caution should be exhibited when attempting to use Zn in otolith core-to-edge transects to reconstruct migration histories of adults from birth. However, because stock discrimination studies have relied primarily on cores or edges of similar-aged individuals, which was not possible in our investigation, the continued use of Zn in those types of investigations is warranted.

CONCLUSIONS

In summary, our investigation has successfully demonstrated that otolith microchemistry can be used as a tool to identify natal origins, wild versus hatchery-reared, of Lake Erie lake trout, even in the absence of a known sample of wild-produced fish from which to develop a Lake Erie "signature". More importantly, however, we have provided compelling evidence that natural reproduction of lake trout has been occurring in Lake Erie, which had not yet been documented since their extirpation. Further, the period during which at least two of the four naturally produced lake trout were produced (1992 and 1994) happens to correspond with a time when conditions were favorable for lake trout—sea lamprey were at low levels in Lake Erie, while an abundance of reproductively active lake trout were in the system. Thus, although current levels of natural reproduction is still low in Lake Erie, we are optimistic that continuation of the current restoration program (except maybe fry stocking) will continue the progression toward a rehabilitated lake trout population in Lake Erie, barring potential setbacks due to global warming (Magnuson et al. 1997) or invasive species (e.g., round goby *Neogobius melanostomus* predation on lake trout eggs; Chotkowski and Marsden 1999). Our optimism seems well justified when considering that the integrated Lake Erie lake trout restoration, including sea lamprey control, efforts only began recently (1982 and 1986, respectively) relative to other systems.

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Table 1. Sample sizes of recaptured lake trout used to determine otolith elemental signatures for the hatchery (from otolith cores) and Lake Erie (from otolith edges). For each year class, information on sex and age range (at capture) of lake trout analyzed is provided.

Year Class	Males	Females	Youngest Age	Oldest Age
1984	6	6	11	16
1986	8	8	9	14
1990	8	7	5	9
1992	8	7	3	7
1996	8	8	3	5
1998	7	7	2	5
2000	5	5	2	2
2003	1	2	1	1
Totals	51	50		

Table 2. Limits of detection (LOD), coefficients of variation (CV), and maximum percentage of otoliths above LODs (% > LOD) in the cores of all lake trout analyzed. Otolith elemental concentrations were determined using laser-ablation ICPMS. Bolded values indicate that this isotopic attribute met our selection criteria for potential inclusion in analyses.

Isotope	LOD	CV	% > LOD
^{7}Li	0.0332	**4.9**	**98.3**
^{25}Mg	0.4146	**2.4**	**100.0**
^{55}Mn	0.1473	**3.2**	**99.3**
^{65}Cu	0.2261	**4.8**	80.6
^{66}Zn	0.1008	**4.1**	**100.0**
^{85}Rb	0.0187	**3.8**	**98.7**
^{86}Sr	0.2412	**1.7**	**100.0**
^{88}Sr	0.0146	**2.5**	**100.0**
^{111}Cd	0.0413	**4.6**	19.6
^{120}Sn	0.0188	**3.8**	**98.0**
^{137}Ba	0.0478	**2.4**	**100.0**
^{138}Ba	0.0068	**2.1**	**100.0**
^{140}Ce	0.0032	**2.2**	59.5
^{208}Pb	0.0025	**4.4**	**99.5**
^{238}U	0.0260	**4.6**	18.1

Table 3. Mean (± 1 SE) elemental concentrations (parts per million) of vaterite and aragonite in Allegheny National Fish Hatchery lake trout (broodstock). Elemental concentrations were determined by laser-ablation ICPMS, whereas Raman spectroscopy was used to quantify areas as vaterite versus aragonite, prior to ICPMS analysis. Core elemental concentrations also have been provided for the same otoliths. Means with similar letters for an element (i.e., within a row) do not differ (one-way ANOVA, Tukey's hsd test). Those elements in which vaterite differed from both aragonite and the core are denoted in bold. Results from principal components analysis using only bolded elements also are provided (see Figure 3). Included are each element's correlation with PCA axis 1 and the relative contribution (%) of each element to determining PCA axis 1 scores.

Element	Edge-Vaterite	Edge-Aragonite	Core-Aragonite	Correlation with PCA axis 1	% Contribution To PCA axis 1
Li	0.233 ± 0.123 [a]	0.088 ± 0.032 [a]	0.166 ± 0.032 [a]		
Mg	**596.273 ± 29.345 [a]**	**16.078 ± 2.088 [b]**	**17.075 ± 1.802 [b]**	**0.94**	**24.7**
Mn	**9.358 ± 0.729 [a]**	**1.010 ± 0.216 [b]**	**1.853 ± 0.270 [c]**	**0.81**	**18.3**
Zn	7.661 ± 1.958 [a]	10.693 ± 1.973 [a]	32.571 ± 2.338 [b]		
Rb	**0.075 ± 0.010 [a]**	**0.120 ± 0.011 [b]**	**0.112 ± 0.014 [b]**	**-0.57**	**8.9**
Sr	**44.984 ± 1.774 [a]**	**780.431 ± 26.554 [b]**	**720.791 ± 42.567 [b]**	**-0.98**	**26.2**
Sn	0.084 ± 0.007 [a]	0.103 ± 0.016 [a]	0.076 ± 0.005 [a]		
Ba	**0.393 ± 0.040 [a]**	**7.309 ± 0.409 [b]**	**14.244 ± 0.629 [c]**	**-0.89**	**21.9**
Pb	0.061 ± 0.024 [a]	0.045 ± 0.008 [a]	0.042 ± 0.091 [a]		

Table 4. Results from different linear discriminant function analyses used to classify an independent cross-validation set of known-origin lake trout (hatchery broodstock). Discriminant functions were developed from the cores (hatchery residence) and edges (Lake Erie residence) of hatchery-reared lake trout that were recaptured in Lake Erie during 1984-2003. By testing our ability to accurately classify both cores (N = 24) and aragonite edges (N = 14) of these broodstock, we could determine which elements might differ between the cores and edges of recaptured individuals simply because of physiology (not water-chemistry). Otolith micro-elemental concentrations were quantified using laser-ablation ICPMS. Only elements with a p-value ≤ 0.05 were included in the final model.

Potential model elements[†]	**Final model elements**[††]	**% of hatchery cores classified accurately**	**% of hatchery edges classified accurately**
Mn, Zn, Ba	Ba (132.4) Mn (25.1) Zn (18.2)	100%	50%
Mn, Ba	Ba (303.5) Mn (23.8)	100%	86%
Ba	Ba (534.7) Sr (8.6) Mg (7.9) Li (4.2)	100%	86%
None	Sr (34.7) Mg (18.8) Li (12.2)	46%	50%

[†] Other elements available for potential inclusion in models were Li, Mg, Rb, Sr, Sn, Pb.

[††] Elements are listed in order from most important to least important as indicated by each element's F-values in parentheses.

FIGURE LEGENDS

Figure 1. Photograph of an 11-yr old hatchery lake trout (broodstock) otolith under transmitted light. Aragonite is the calcium-carbonate structure above the transition zone, whereas vaterite is the calcium-carbonate structure below the transition zone. Excessive "waviness" in the vaterite growth zone relative to the aragonite growth zone could be used to help visually differentiate these two calcium-carbonate polymorphs.

Figure 2. Wave numbers from the Raman spectroscopy analysis of aragonite and vaterite portions of a hatchery-reared lake trout that spent its entire existence in the Allegheny National Fish Hatchery as a broodstock individual. The patterns presented here were typical of 12 other broodstock analyzed with Raman spectroscopy.

Figure 3. Principal components analysis ordination diagram of otolith cores (black circles), aragonite edges (gray squares), and vaterite edges (white triangles) from age-11 lake trout that had resided in Allegheny National Fish Hatchery since birth. The high degree of overlap between the cores and aragonite edges indicates that the cores also are aragonite. Generalizations of elemental gradients along PCA axis 1, which explained the majority of variation in the date, are provided.

Figure 4. Mean ($\pm$ 1 SE) elemental concentrations of a) Li, b) Mg, c) Mn, d) Zn, e) Rb, f) Sr, and g) Ba in the cores versus edges of lake trout recaptured in Lake Erie during 1984-2003 (cores = hatchery residence, n = 95; edges = Lake Erie residence, n = 69), hatchery-reared broodstock (i.e., n = 15), and wild Keuka Lake lake trout (n = 25). Laser-ablation ICPMS was used to determine elemental concentrations in the (aragonite) cores and edges. Results of paired t-tests conducted on hatchery recaptures, broodstock lake trout, and Keuka Lake trout, are provided. NS denotes a non-significant difference between the core and edge in a comparison, whereas ** denotes a highly significant result ($\alpha = 0.05/7 = 0.007$).

Figure 5. Mean ($\pm$ 1 SE) elemental concentrations of a) Li, b) Mg, c) Mn, d) Zn, e) Rb, f) Sr, g) Sn, h) Ba, and i) Pb in the cores versus edges of hatchery-reared lake trout that were recaptured in Lake Erie during 1984-2003. The x-axis indicates the year-classes of the individuals, not the year collected. Laser-ablation ICPMS was used to determine elemental concentrations in the (aragonite) cores (hatchery residence, gray symbols) and edges (Lake

Erie residence, white symbols) of these lake trout. Sample sizes each year-class are provided in panel a).

Figure 6. Results from our linear discriminant function analysis wherein Ba, but not Zn and Mn, were included in the model. Positions along LDFA root 1 for the cores (hatchery residence) and edges of (Lake Erie residence) of recaptured lake trout provided. Recaptured lake trout were used to develop the classification functions to identify the natal origins of unknown-origin lake trout. Accuracy of classification functions were tested from *post hoc* predictions of known-origin hatchery broodstock. Generalizations of elemental gradients along LDFA root 1 are provided.

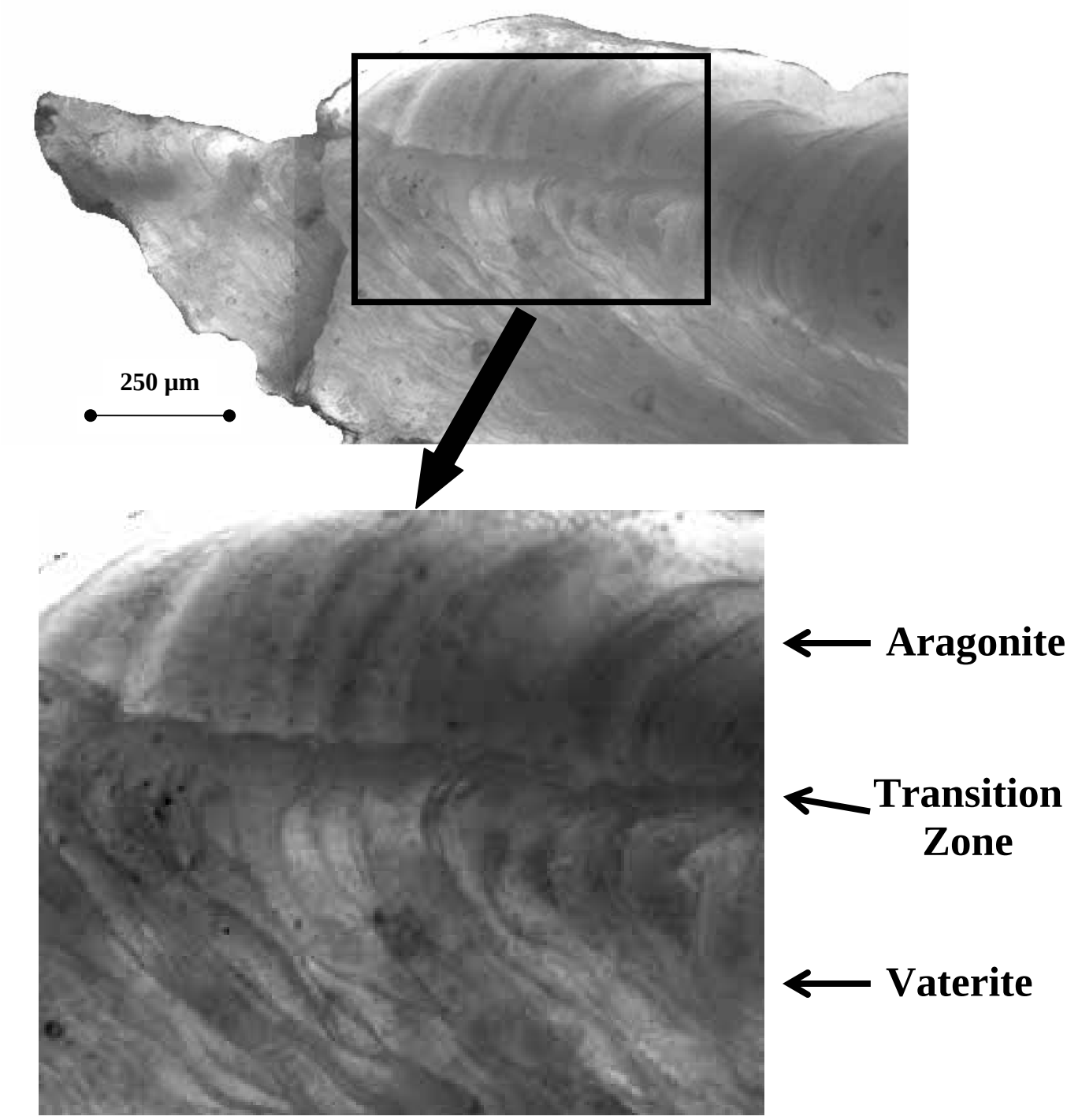

Figure 1

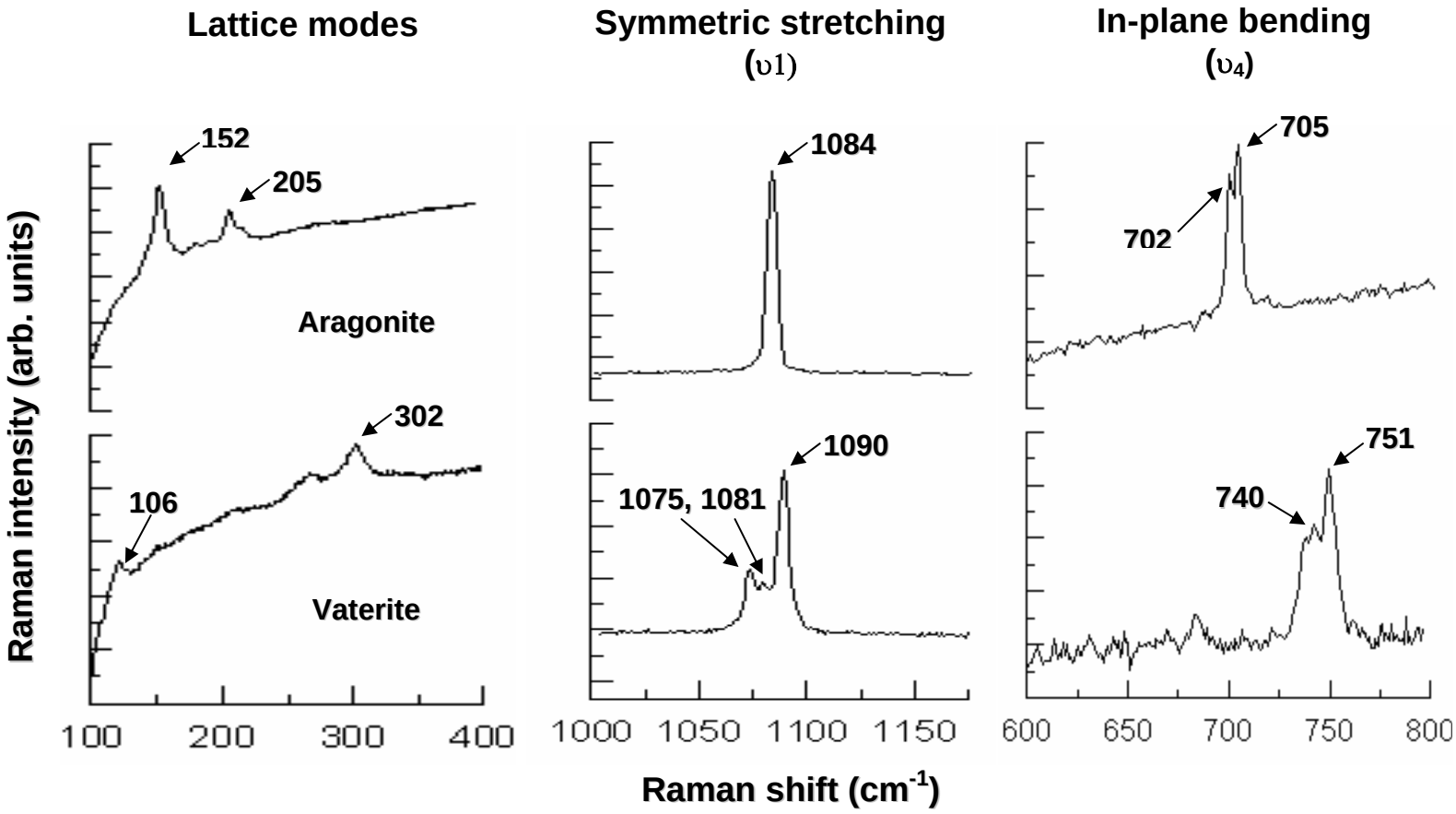
Lattice modes
Symmetric stretching
(υ1)
In-plane bending
(υ4)
Raman intensity (arb. units)
152
205
Aragonite
302
106
Vaterite
1084
1090
1075, 1081
705
702
751
740
100
200
300
400
1000
1050
1100
1150
600
650
700
750
800
Raman shift (cm-1)

Figure 2

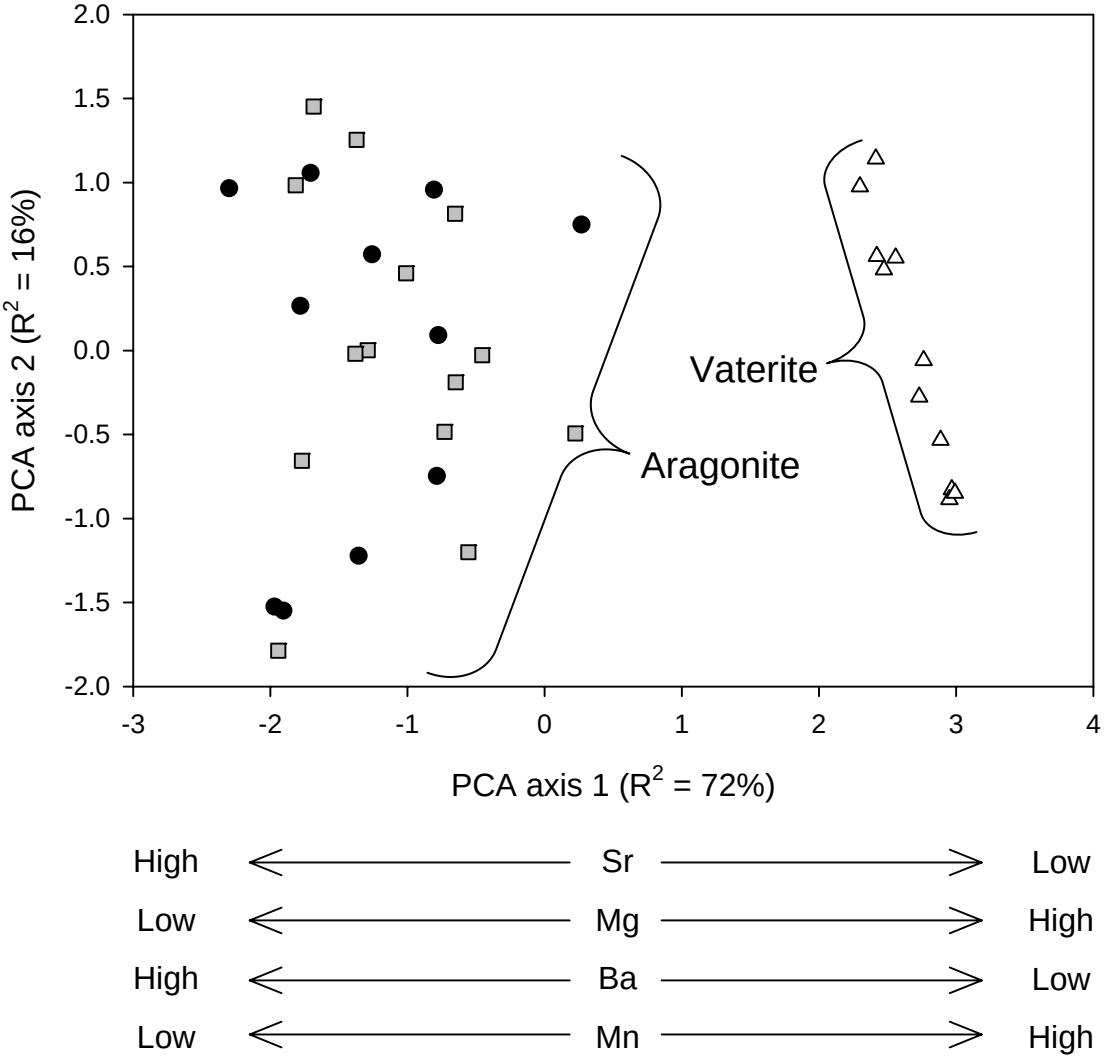
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
PCA axis 2 (R2 = 16%)
-3
-2
-1
0
1
2
3
4
PCA axis 1 (R2 = 72%)
Vaterite
Aragonite
High
Sr
Low
Low
Mg
High
High
Ba
Low
Low
Mn
High

Figure 3

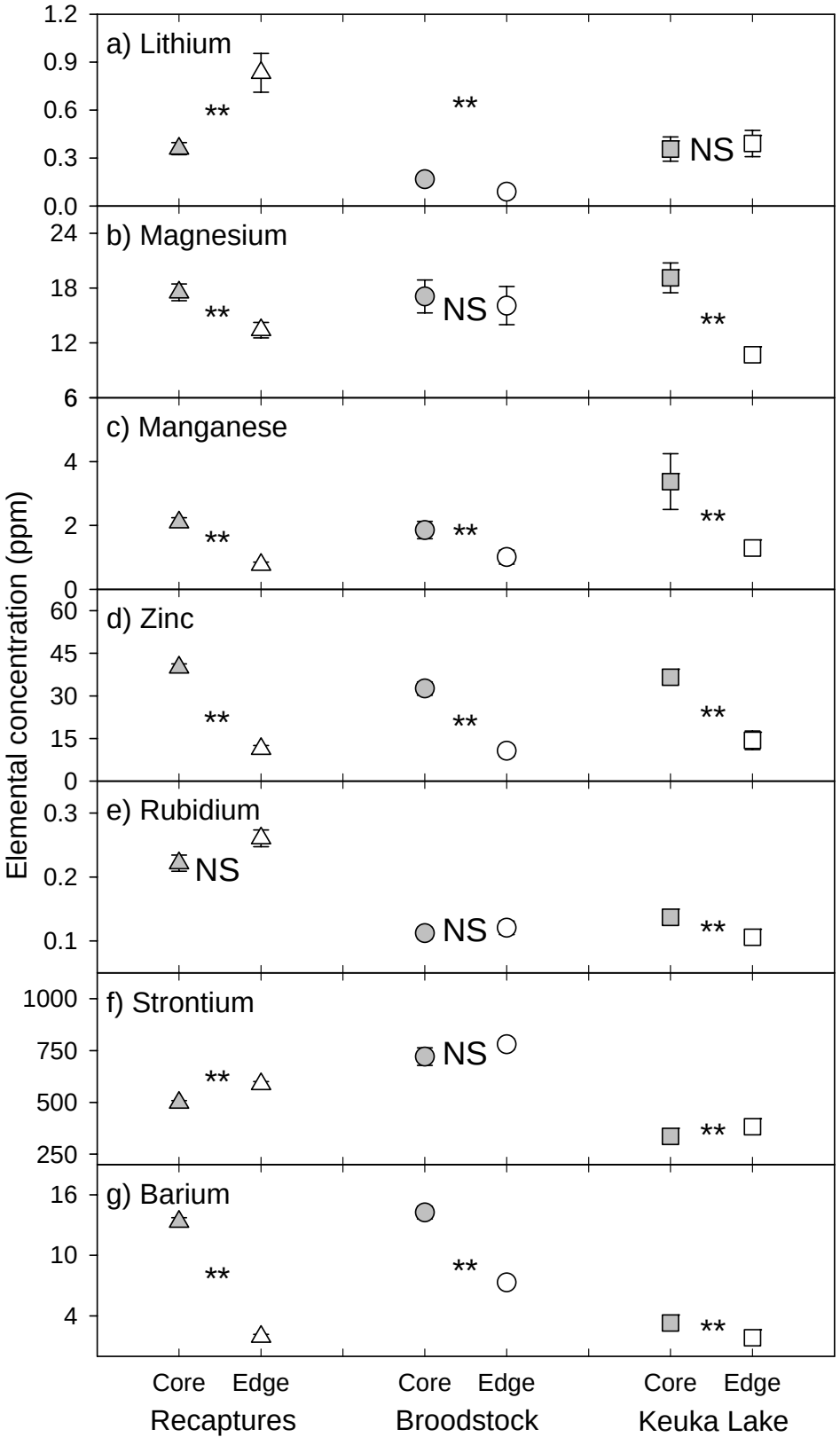
a) Lithium
b) Magnesium
c) Manganese
d) Zinc
e) Rubidium
f) Strontium
g) Barium
Elemental concentration (ppm)
1.2
0.9
0.6
0.3
0.0
24
18
12
6
4
2
0
60
45
30
15
0
0.3
0.2
0.1
1000
750
500
250
16
10
4
**
NS
Core
Edge
Recaptures
Broodstock
Keuka Lake

Figure 4

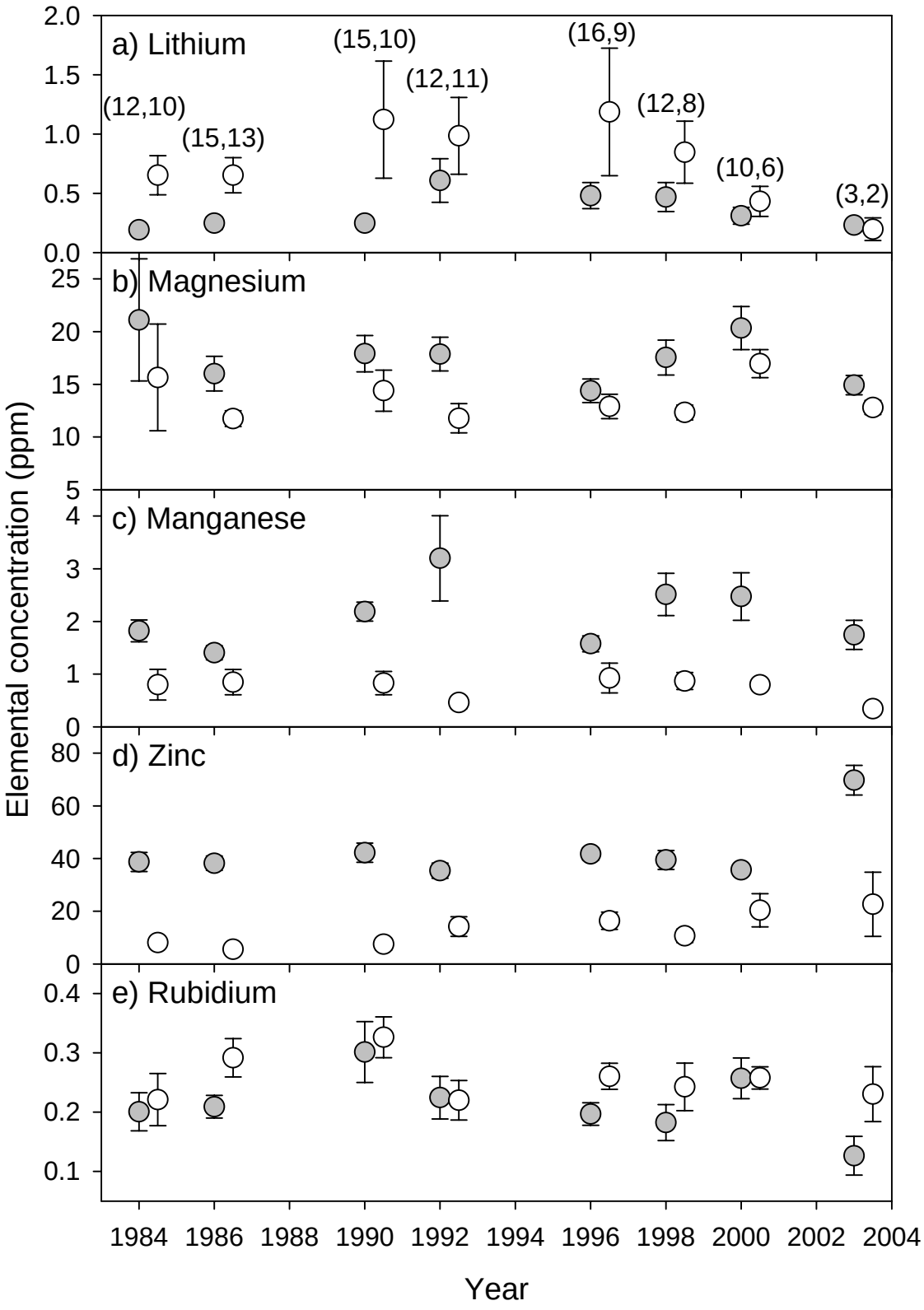
a) Lithium
(12,10)
(15,13)
(15,10)
(12,11)
(16,9)
(12,8)
(10,6)
(3,2)
b) Magnesium
c) Manganese
d) Zinc
e) Rubidium
Elemental concentration (ppm)
Year
1984
1986
1988
1990
1992
1994
1996
1998
2000
2002
2004

Figure 5

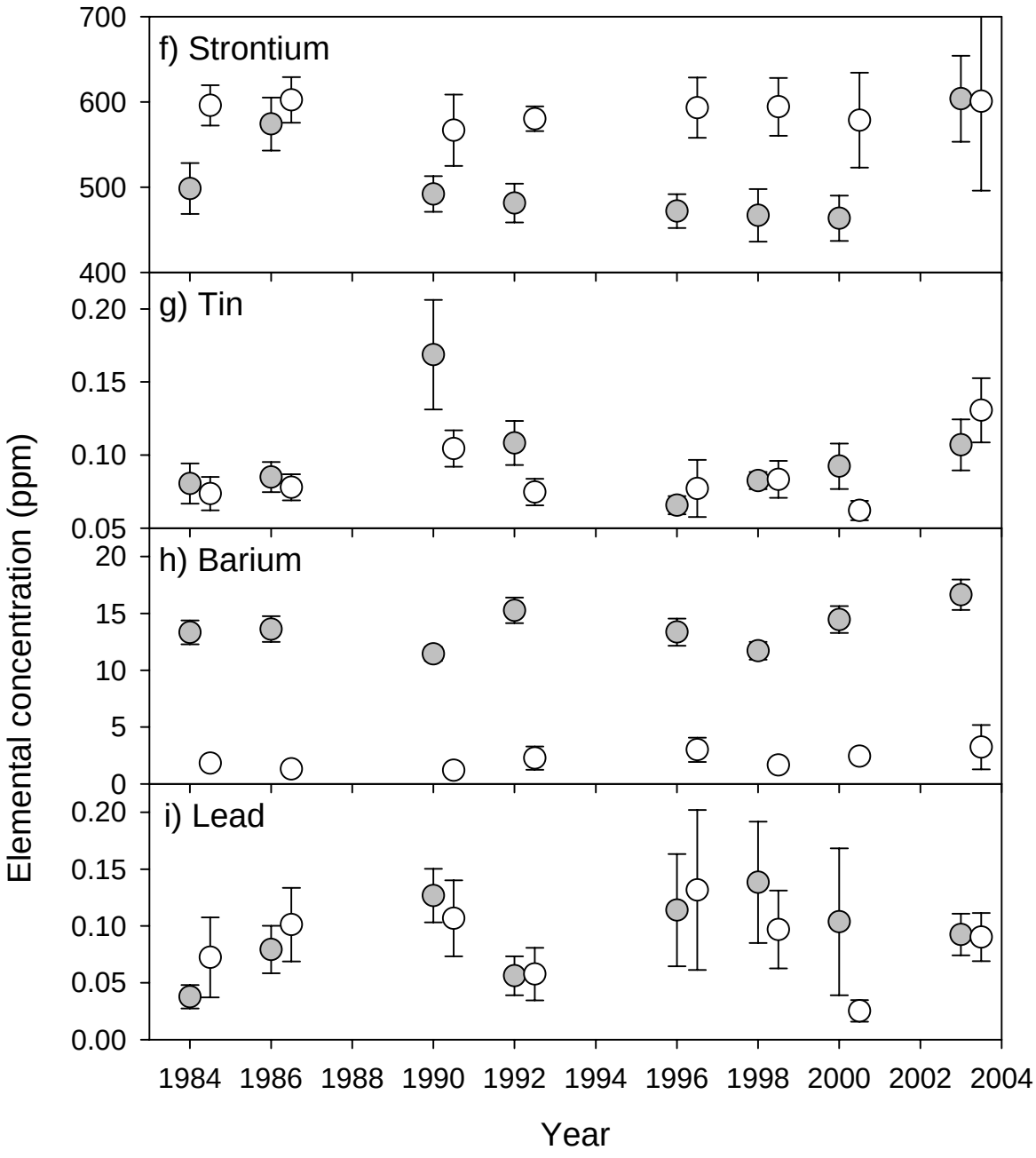

Figure 5 (cont)

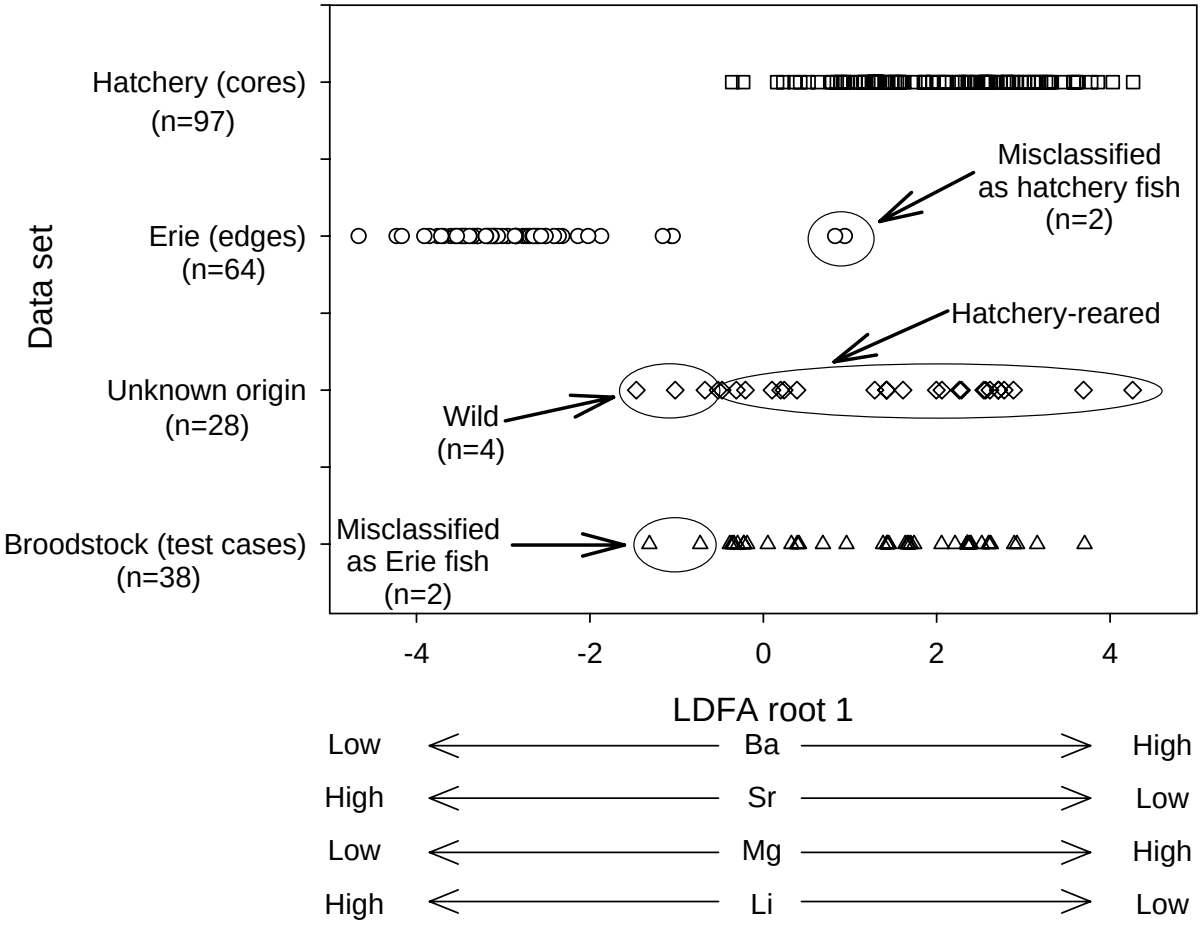
Hatchery (cores)
(n=97)
Misclassified
as hatchery fish
(n=2)
Erie (edges)
(n=64)
Data set
Hatchery-reared
Unknown origin
(n=28)
Wild
(n=4)
Misclassified
as Erie fish
(n=2)
Broodstock (test cases)
(n=38)
-4
-2
0
2
4
LDFA root 1
Low
Ba
High
High
Sr
Low
Low
Mg
High
High
Li
Low

Figure 6