Natural chemical markers identify source and date of introduction of an exotic species: lake trout (*Salvelinus namaycush*) in Yellowstone Lake

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Abstract: Exotic species invasions pose a pervasive threat to aquatic ecosystems worldwide, yet fundamental questions about the geographic origin and timing of invasions or introductions are frequently difficult to answer. We used natural chemical markers (Sr:Ca ratios) in otoliths to identify probable source and date of introduction of exotic lake trout (*Salvelinus namaycush*) into Yellowstone Lake, Wyoming, USA. Otolith Sr:Ca ratios were significantly different among lake trout known to have reared in three different Yellowstone National Park lakes (Yellowstone, Heart, and Lewis lakes) and showed little variation along the otolith axis, indicating that lake trout within each lake experienced a similar, and consistent, environmental history. In contrast, suspected transplants showed a large and rapid increase in otolith Sr:Ca ratios indicative of a marked shift to water of differing chemistry. Timing of the abrupt change in Sr:Ca ratios indicated that some lake trout were introduced into Yellowstone Lake during the late 1980s, but more recent transplants also occurred. A discriminant model identified Lewis Lake as the likely source lake for lake trout transplanted into Yellowstone Lake. Our results demonstrate that chemical signatures within otoliths can serve as an important forensic tool for identifying the probable source and date of exotic fish introductions.

Résumé : Bien que l'invasion d'espèces exotiques soit une menace omniprésente dans les écosystèmes aquatiques à l'échelle de la planète, les questions fondamentales concernant l'origine géographique et la date des invasions et des introductions restent souvent difficiles à résoudre. Nous avons utilisé des marqueurs chimiques naturels (rapports Sr:Ca) dans les otolithes pour identifier la source probable et la date d'introduction des touladis (*Salvelinus namaycush*) exotiques du lac Yellowstone, Wyoming, É.-U. Les rapports Sr:Ca des touladis élevés dans trois lacs différents du parc national de Yellowstone (Yellowstone, Heart et Lewis) sont significativement différents et ils affichent peu de variation le long de l'axe de l'otolithe, ce qui indique que les touladis de chacun des lacs y ont vécu une histoire environnementale semblable et uniforme. En revanche, les poissons soupçonnés d'avoir été transplantés montrent une augmentation rapide et importante des rapports Sr:Ca dans leurs otolithes, ce qui indique un passage subit dans une eau de caractéristiques chimiques différentes. La détermination de la date de ces changements abrupts des rapports Sr:Ca indique que certains touladis ont été introduits dans le lac Yellowstone à la fin des années 1980, mais qu'il y a eu aussi des introductions plus récentes. Un modèle discriminant identifie le lac Lewis comme la source probable des touladis introduits dans le lac Yellowstone. Nos résultats montrent que les signatures chimiques des otolithes peuvent être des outils de recherche intéressants pour identifier la source et la date probables d'introduction de poissons exotiques.

[Traduit par la Rédaction]

Introduction

Exotic species pose one of the most pervasive threats to fresh waters worldwide (Hall and Mills 2000; Rahel 2000; Kolar and Lodge 2002). Dramatic changes in species abundance and energy flow have been observed following the establishment of a single new species, even in large lakes (Zaret and Paine 1973; Vander Zanden et al. 1999). Although many exotic species have been intentionally introduced for commercial or recreational purposes, unauthorized transplants and invasions have also contributed substantially to exotic species expansion (McMahon and Bennett 1996; Fuller et al. 1999; Rahel 2000).

When a new exotic species is first discovered, questions about where it originated and when it was transplanted or invaded are frequently difficult to answer with confidence

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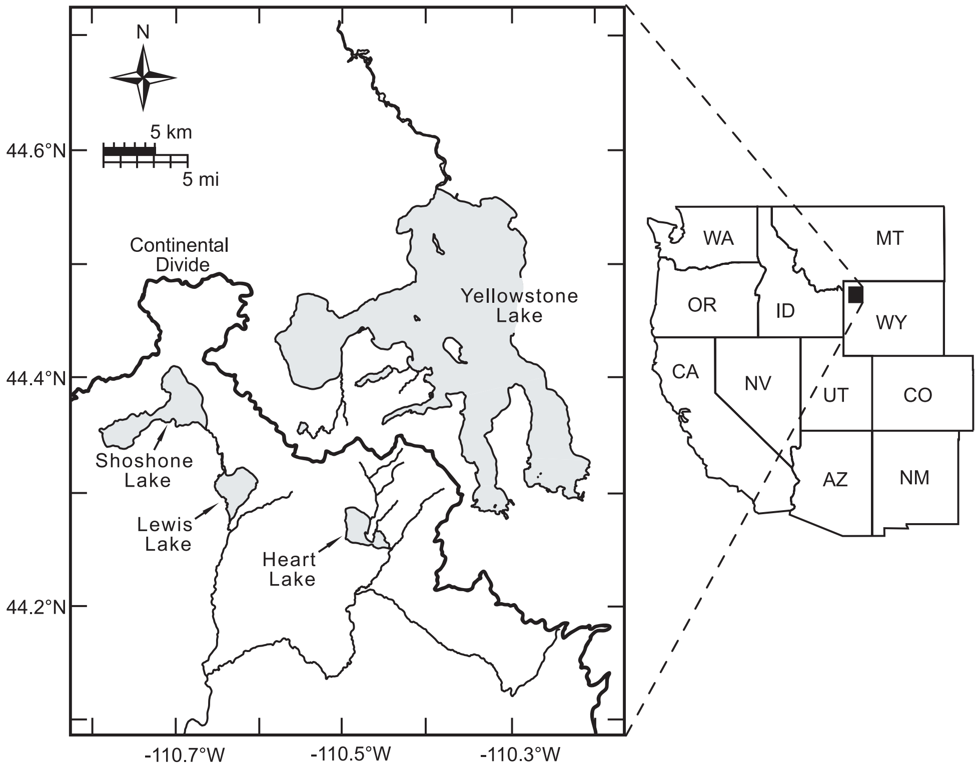
Fig. 1. Map of the major lakes in the study area, Yellowstone National Park.



(Radtke 1995; McMahon and Bennett 1996; Hebert and Cristescu 2002). This uncertainty hinders possible management actions for reducing future occurrences and, in some instances, raises questions as to whether a presumed invader is in fact native or else has resided in the system longer than suspected but at low abundance (Kaeding et al. 1996; Waters et al. 2002). Recent investigations of fresh water zooplankton illustrate the utility of genetic markers as a forensic tool for studying invasion biology (Cristescu et al. 2001; Hebert and Cristescu 2002). In this paper, we demonstrate the use of natural chemical markers in fish otoliths to identify the probable source and date of introduction of an exotic fish species.

Exotic lake trout (*Salvelinus namaycush*) were first discovered in Yellowstone Lake, Yellowstone National Park, Wyoming, USA, in 1994 (Kaeding et al. 1996). This 250 000-ha high-elevation lake near the headwaters of the Yellowstone River drainage is one of the largest relatively intact lake ecosystems in the United States and is the primary remaining habitat for Yellowstone cutthroat trout (*Oncorhynchus clarki bouvieri*) (Gresswell and Varley 1988). The clear, deep, cold waters and abundant prey base of Yellowstone Lake provide prime habitat for piscivorous lake trout, and by 1996, their population was estimated at several thousand, including individuals as large as 91 cm (Ruzycki et al. 2003). Development of an abundant lake trout population was anticipated if left unchecked, with the resultant high predation pressure causing a significant decline in the cutthroat trout population (Varley and Schullery 1995; Ruzycki et al. 2003). Cutthroat trout generally evolved in the absence of competing top predators (Behnke 1992), and similar declines have been documented for several western North American lakes following lake trout introduction (Cordone and Frantz 1966; Marnell 1988; Donald and Alger 1993). Consequently, an aggressive lake trout removal program was initiated in Yellowstone Lake in 1995 to protect a valuable recreational fishery and the integrity of the lake's terrestrial and aquatic foodwebs, which are heavily dependent on cutthroat trout (Varley and Schullery 1995; Koel et al. 2003).

The origin of the lake trout in Yellowstone Lake is unknown. Although lake trout from the Great Lakes were introduced into Yellowstone National Park's Shoshone and Lewis lakes in the late 1800s and later spread to Heart Lake, these lakes are in the Snake River (Pacific) drainage and lack connection to the Yellowstone River (Atlantic) drainage (Fig. 1) (Varley and Schullery 1983). Prior to 1994, no lake trout had been reported in Yellowstone Lake despite extensive population sampling and angler survey records dating back more than 50 years (Gresswell and Varley 1988; Kaeding et al. 1996). Based on the age and size of lake trout when they were first discovered in 1994 (≤5 years and 43 cm), it was estimated that lake trout had reproduced in Yellowstone Lake since at least 1989, but when the original transplant occurred was unknown (Kaeding et al. 1996).

We used chemical analysis of otoliths to estimate where the lake trout originated and when they were transplanted into Yellowstone Lake. Because trace elements of ambient waters are incorporated into otoliths as a fish grows, analysis of natural chemical markers in otoliths can be used to reconstruct environmental history, including timing of movements and stock origins (Campana 1999; Limburg et al. 2001; Thorrold et al. 2001). However, to our knowledge, there have been no previous reports of using the elemental compo-

Table 1. Summary of length, age, and year-class of the known-origin lake trout and suspected transplants collected from three lakes in Yellowstone National Park.

			Total length (mm)		Age (years)		
Group	Lake	*n*	Median	Range	Median	Range	Year-class range
Known-origin	Heart	10	490	327–691	15	8–22	1977–1991
	Lewis	10	503	416–949	14	9–26	1973–1990
	Yellowstone	10	720[a]	700–767	9	8–11	1988–1991
Suspected transplants	Yellowstone	20	805	765–890	18	10–32	1965–1987

[a]Length data were not available for five of the known-origin Yellowstone Lake fish.

sition of otoliths to assess introductions of exotic species. The Sr:Ca ratio has been the most widely used marker in otolith composition studies because (*i*) of the strong correlation between otolith and ambient water ratios, (*ii*) substitution of Sr for Ca in the otolith's crystalline structure, and (*iii*) Sr is more apt to reflect environmental concentrations than other elements owing to a lack of physiological regulation (Campana 1999).

We compared the Sr:Ca ratios in otoliths from suspected transplants with those in (*i*) otoliths of lake trout from more recent year-classes, thought to have been spawned and reared in Yellowstone Lake, and (*ii*) otoliths of lake trout from the two most likely source lakes from within Yellowstone National Park (Lewis and Heart lakes). We hypothesized that lake trout reared in a single lake would have similar otolith Sr:Ca ratios throughout their lives, from the early-growth zone near the nucleus to the outer edge. In contrast, we predicted that lake trout transplanted into Yellowstone Lake would have a significantly different chemical composition between the two zones, reflective of a change in environmental history, and that the Sr:Ca ratio of the early-growth zone could be used to identify the probable source lake of the transplant. We further surmised that among suspected transplants, the timing of the change in Sr:Ca ratio in relation to the age of the fish could provide an estimated date of when transplantation had occurred.

Materials and methods

Otolith collection and preparation

Two groups of otoliths were analyzed for this study. The first group consisted of archived otoliths from lake trout that had been collected from Yellowstone Lake during early stages of the lake trout removal program in 1996 and 1997. It was surmised that the largest fish in these samples were likely some of the original fish transplanted to Yellowstone Lake and smaller sizes were offspring of these suspected transplants. Twenty otoliths, 10 from each year, were randomly selected from among the 164 largest lake trout that constituted the suspected transplant group. These fish were >70 cm total length and comprised the upper 10%–20% of length range of lake trout collected during 1996 and 1997 gillnet sampling (Table 1). The second group consisted of otoliths from lake trout of known origin: suspected offspring of the original founding population in Yellowstone Lake and lake trout of various ages from Heart and Lewis lakes (Table 1). Otoliths from this group were randomly selected from fish gillnetted in 1999 from all three lakes ($n = 10$ for each lake).

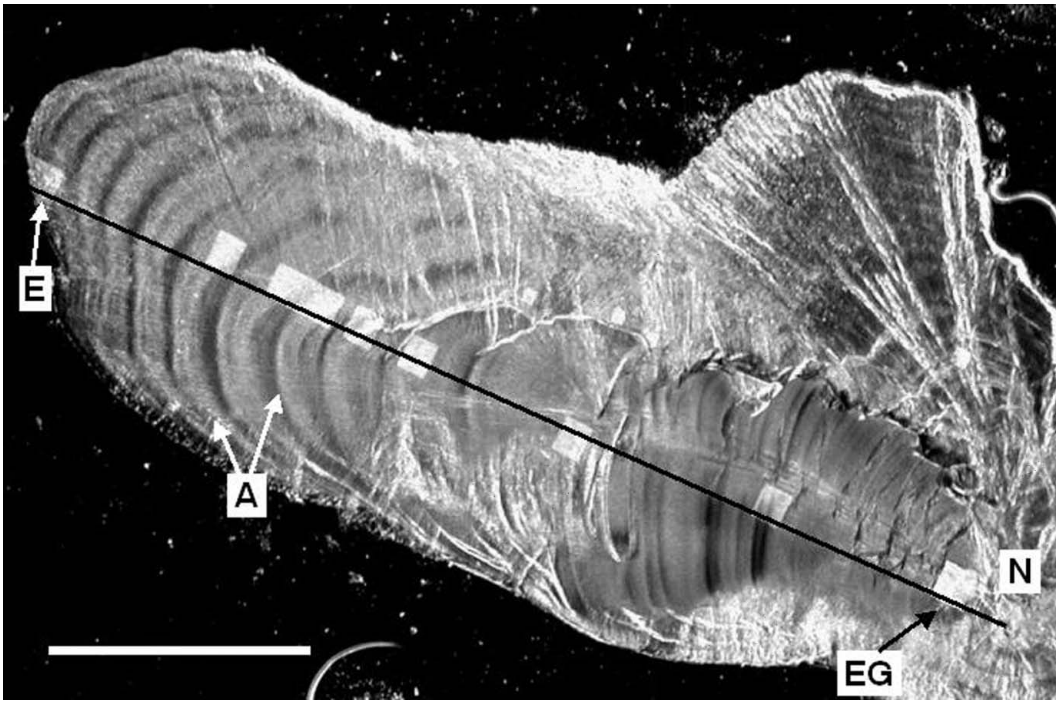
Otoliths were extracted, cleaned, and stored in polyethylene vials soon after collection. One otolith from each fish was sectioned, ground, and polished to expose the nucleus following the techniques of Secor et al. (1992). Prior to chemical analysis, otolith sections were ultrasonically cleaned in a series of baths of Milli-Q water, analytical-grade hexane, and analytical-grade methanol (<1 min each) to remove surface contaminants. Otoliths were then sputter-coated with 15 nm of Au–Pd alloy to reduce the buildup of an electrical charge on the sample during analysis.

Otolith chemistry

Otolith chemical composition was measured with a Phi-Evans (Chanhassen, Minnesota) time-of-flight secondary ion mass spectrometer (ToF-SIMS) (Schueler 1992) at the Image and Chemical Analysis Laboratory, Montana State University, Bozeman, Montana. Positive ion spectra were collected in high-mass resolution mode using a pulsed 15-keV primary Ga^+ ion beam focused to 9 μm × 9 μm. Ion counts for ^{88}Sr and ^{44}Ca were measured from the spectra and reported as Sr:Ca ratios. A laboratory standard consisting of ground and pressed otoliths from hatchery-reared rainbow trout (*Oncorhynchus mykiss*) was analyzed at the start of each session to assess instrument precision, and the ToF-SIMS was recalibrated if the Sr:Ca ratio was >2 SD of the baseline mean of the standard. Within-site sampling variation was estimated by repeat sampling of the same site on 10 randomly selected otoliths. Prior to sampling, a 60 μm × 60 μm area was sputtered for 4 min to remove the alloy coating. Ion counts were measured at the center of the sputtered area.

For each otolith, ion counts were measured at two sites, the early-growth zone near the nucleus and a zone near the outer edge (Fig. 2). For a subset of otoliths from each group (suspected transplants into Yellowstone Lake, $n = 5$; offspring of transplants, $n = 1$; Heart Lake, $n = 2$; Lewis Lake, $n = 1$), additional ion counts were measured at three equidistant points between the early-growth and edge sample sites. If large changes were detected between adjacent sample sites, further sites were sampled to pinpoint the location of temporal changes in Sr:Ca ratio along the otolith axis (Fig. 2). Abrupt changes in Sr:Ca ratio have been correlated with rapid changes in water chemistry (e.g., Limburg et al. 2001). The date of a large change in Sr:Ca ratio, indicative of a movement to new waters, was estimated by comparing the location of the change on the otolith axis with the age of the fish at that time. Annuli were identified using ageing criteria developed for lake trout otoliths by Sharp and Bernard (1988).

Fig. 2. Transverse section of a lake trout (85 cm, 18 years) otolith from a suspected transplant into Yellowstone Lake. Analysis sites for ^{88}Sr and ^{44}Ca ion counts were located in the early-growth zone (EG), near the nucleus (N), and the edge zone (E) in each otolith. Sr:Ca ratio data were obtained from a 9 μm × 9 μm area within the areas cleared of the Au–Pd coating (open squares). Analyses along a transect (solid line) were used to pinpoint the location of any temporal changes in Sr:Ca ratios. A, annuli. Scale bar = 0.5 mm.



Water chemistry

Sr:Ca ratios of water from each of the three study lakes were measured to assess if geochemical differences existed among the lakes and the degree to which these differences were imparted to lake trout otoliths. Surface water samples were collected from Heart and Lewis lakes in 2000 (n = 4 per lake) following standard protocols (American Public Health Association 1998). Water was collected in 1-L polyethylene acid-washed bottles and 100 mL of each sample was immediately filtered through a 0.45-μm-pore membrane filter into an opaque acid-washed polyethylene bottle. Water samples were then preserved with 1 mL of analytical-grade concentrated nitric acid and refrigerated until analyzed. Yellowstone Lake water samples were collected at different depths in the water column in four areas of the lake (Southeast Arm, West Thumb, Mary Bay, and Stevenson Island) in 1997 (n = 30) and 1998 (n = 41) using a hydrobottle clean of trace metals (see Balistrieri et al. 2004). The water samples were filtered and preserved using the same methods described above. Total dissolved Sr and Ca concentrations (milligrams per litre) were measured with a Perkin-Elmer (Wellesley, Massachusetts) Sciex Elan 6000 inductively coupled plasma mass spectrometer (Lamothe et al. 1999) and converted to molar concentrations for calculation of the Sr:Ca ratio.

Statistical analyses

A type III mixed model analysis of variance (ANOVA) and Tukey's multiple comparison tests (SAS Institute Inc. 2000) were used to compare Sr:Ca ratios among known-origin lake trout from Heart, Lewis, and Yellowstone lakes. Lake and otolith zone (early growth and edge) were included as fixed factors and fish, zone × fish interaction, and zone replication as random factors in the ANOVA. Compound symmetry covariance structure (constant variance and covariance) was used to account for possible correlation among multiple measurements from the same otolith (Littell et al. 1998). The Satterthwaite approximation was used to calculate the degrees of freedom for the statistical tests (Littell et al. 1996). For all significance tests, α = 0.05.

Nearest-neighbor discriminant analysis, a nonparametric discrimination procedure (Johnson 1998; SAS Institute Inc. 2000), was used to determine the probable source of lake trout in Yellowstone Lake. Mean otolith Sr:Ca ratios for known-origin fish, weighted by number of sites sampled in each otolith zone, were used to construct the discriminant model (PROC DISCRIM, METHOD = NPAR, k = 9; SAS Institute Inc. 2000). This discrimination procedure assigns each new observation into the group to which the majority of its k nearest neighbors belong (those with the smallest Mahalanobis distances) (Johnson 1998). Model accuracy was evaluated by cross-validation. The Sr:Ca ratios from lake trout otoliths of suspected transplants were then classified using the discriminant model developed for the known-origin data set.

Differences in lake water Sr:Ca ratio among the three study lakes were evaluated using a one-factor ANOVA, and Tukey's multiple comparison test was used to test for pairwise differences. Simple linear regression was used to assess the relationship between otolith and lake water Sr:Ca ratio. Only Sr:Ca data from the otolith edge zones were used to best match otolith composition with lake water composi-

Fig. 3. Relation between mean (±2 SE) lake water Sr:Ca ratios and mean (±2 SE) otolith edge Sr:Ca ratios of lake trout from Heart (▲), Lewis (●), and Yellowstone (■) lakes, Yellowstone National Park. The solid line denotes the fitted linear regression line: $Sr:Ca_{otolith} = 0.0047 + 0.0088[Sr:Ca]_{water}$ ($r = 0.997$, $p = 0.037$).

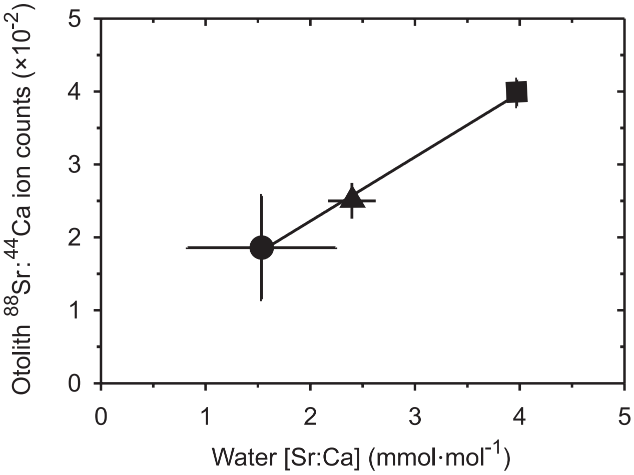


Table 2. Lake water Sr:Ca ratios (mmol·mol^{-1}) from Heart, Lewis, and Yellowstone lakes, Yellowstone National Park.

Lake	Year	*n*	Mean (SD)
Heart	1999	4	2.40 (0.23)
Lewis	1999	4	1.53 (0.71)
Yellowstone	1997	30	3.92 (0.10)
	1998	41	4.01 (0.14)

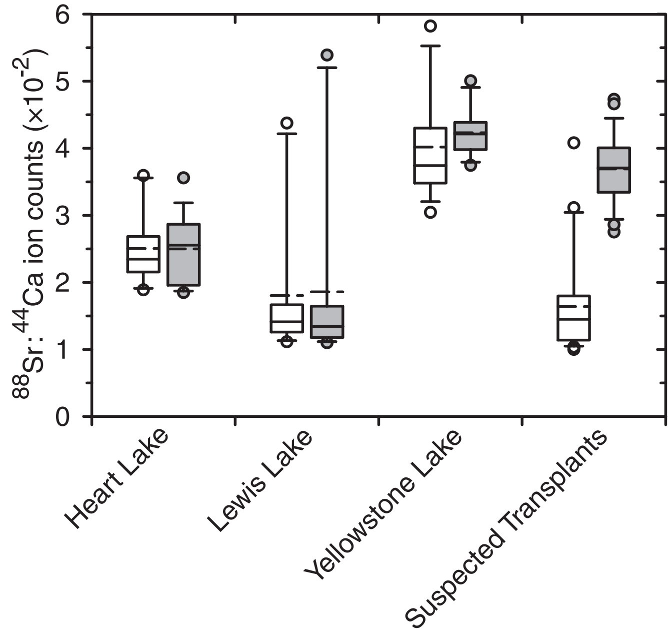
tion at the times of sampling (within 1 year for Heart and Lewis lakes and 2 years for Yellowstone Lake).

Results

Lake water Sr:Ca ratios were significantly different among Heart, Lewis, and Yellowstone lakes (ANOVA, $F = 402.21$, df = 2, 76, $p < 0.001$) with differences in mean values among the lakes ranging from 160% to 270% (Fig. 3; Table 2). Tukey's multiple comparison tests indicated that there were significant differences ($p < 0.05$) for all pairwise comparisons among the lakes. There was a significant linear relation between the otolith Sr:Ca ratios of known origin lake trout and the lake water Sr:Ca ratios ($r = 0.997$, $p = 0.037$) (Fig. 3). Although possible year effects could not be ruled out because water samples from Lewis and Heart lakes were collected in a different year than Yellowstone Lake samples, small differences in lake water Sr:Ca between the 1997 and 1998 water samples in Yellowstone Lake suggest that annual variation in water chemistry was minor compared with the among-lake differences (Table 2).

Otolith Sr:Ca ratios of known-origin lake trout were significantly different among the three lakes ($F = 28.84$, df = 2, 27.9, $p < 0.001$) (Fig. 4). Mean otolith Sr:Ca ratios of Yellowstone Lake lake trout were significantly different from lake trout otolith Sr:Ca ratios from Heart Lake (Tukey's test, $t = -5.10$, df = 28, $p < 0.001$) and Lewis Lake ($t = -7.43$, df = 28, $p < 0.001$). Differences in otolith Sr:Ca ratios between lake trout from Lewis and Heart lakes were not significant at the $\alpha = 0.05$ level ($t = 2.33$, df = 27.8, $p = 0.068$). However, the Sr:Ca ratio for Lewis Lake fish was strongly influenced by one fish with a Sr:Ca ratio much greater (3.7 SD) than that observed in the other nine fish sampled (Fig. 4). When this outlier was removed, the pairwise difference in otolith Sr:Ca ratios between Lewis Lake and Heart Lake lake trout was highly significant ($p < 0.001$).

Fig. 4. Comparison of Sr:Ca ratios of known-origin lake trout otoliths and otoliths of suspected transplants. Two zones were analyzed for each otolith: early-growth (open boxes) and edge zones (shaded boxes) for both known-origin lake trout from Heart, Lewis, and Yellowstone lakes ($n = 10$ per lake) and suspected transplants gillnetted from Yellowstone Lake ($n = 20$). Boxes show the mean Sr:Ca ratios (broken line), median (central solid line), first and third quartiles (box edges), and individual outliers (circles) outside the 10th and 90th percentiles (whiskers).



Otolith Sr:Ca ratios between the early-growth and edge zones among known-origin lake trout from each lake varied little (ANOVA, $F = 1.17$, df = 1, 28.5, $p = 0.289$) (Fig. 4), the average percent difference ranging from 0.1% to 5.3%, despite a wide range in the age of fish sampled (8–26 years) (Table 1). Variation of Sr:Ca ratios obtained from multiple sampling within a sample site on the otolith was also low, averaging 3.92% ($n = 25$). Accordingly, there was no significant interaction between lake and otolith zone as factors in the ANOVA ($F = 0.15$, df = 2, 28.5, $p = 0.857$). Additional samples taken between the early-growth and edge zones also revealed consistent Sr:Ca ratios across the otolith growth axis among lake trout sampled from different lakes (Fig. 5). Nearest-neighbor discriminant analysis correctly classified 90%–100% of lake trout into their home lake (Table 3).

In sharp contrast with lake trout of known origin, 18 of 20 suspected transplants, ranging in age from 13 to 32 years of age at the time of their collection in 1996 and 1997, exhib-

Fig. 5. Patterns of Sr:Ca ratios along the otolith axes of four known-origin lake trout from (*a*) Lewis Lake (26 years, 949 mm), (*b*) Heart Lake (10 years, 481 mm), (*c*) Heart Lake (15 years, 490 mm), and (*d*) Yellowstone Lake (10 years, 767 mm). Analysis sites were classified by discriminant analysis: ●, Lewis Lake; ▲, Heart Lake; ■, Yellowstone Lake. The dotted lines show the location of annuli and the broken line the otolith edge.

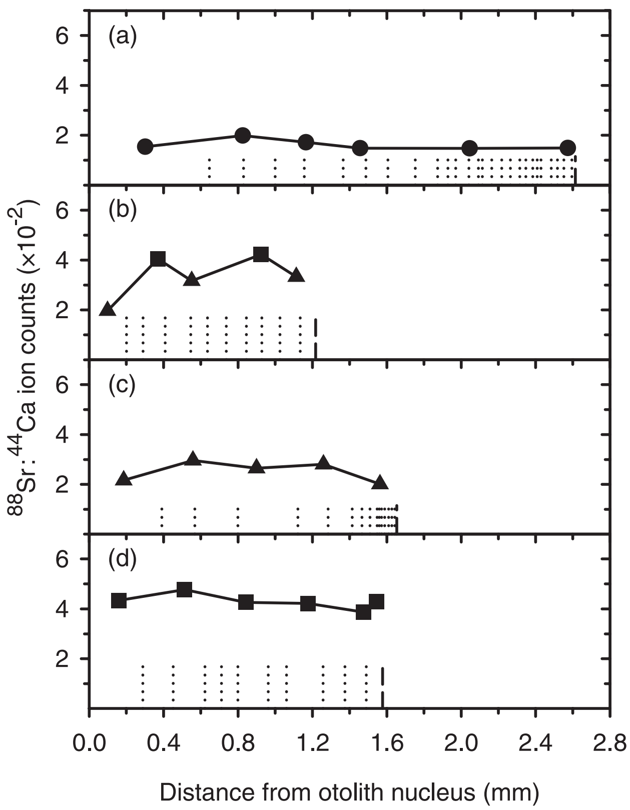
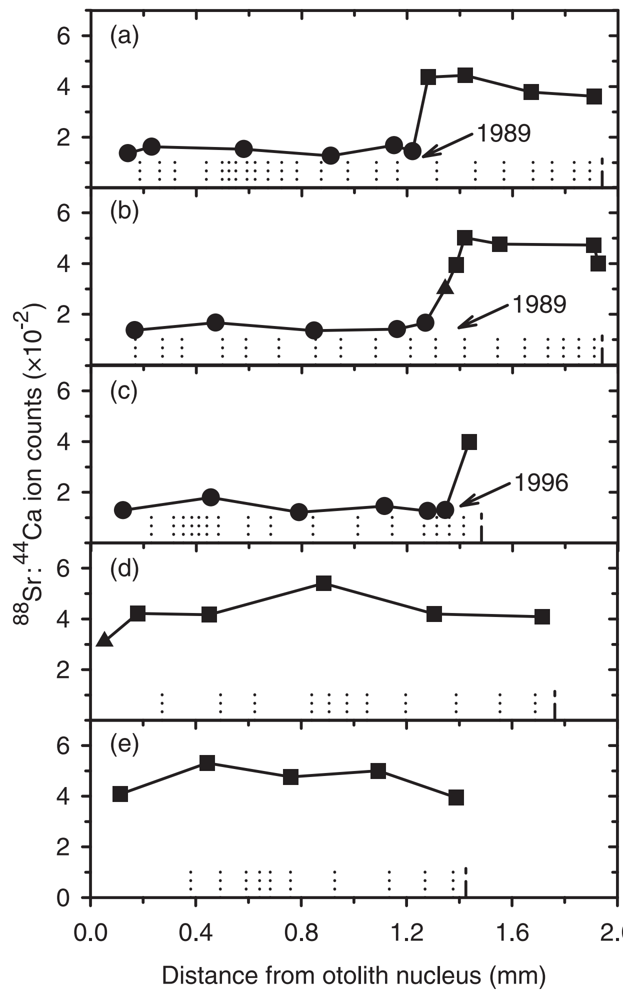


Table 3. Classification of lake trout into probable source lakes.

	% classification into lake		
From lake	Heart	Lewis	Yellowstone
Known-origin			
Heart	100.0	0.0	0.0
Lewis	0.0	90.0	10.0
Yellowstone	0.0	0.0	100.0
Suspected transplants			
Early growth	5.0	90.0	5.0
Edge	20.0	0.0	80.0

Note: Cross-validation results for the known-origin lake trout calibration data set (n = 10 fish per lake) were used to assess classification accuracy. Early-growth and edge zones of the otoliths from the group of suspected transplants captured in Yellowstone Lake (n = 20) were classified into one of the three lakes. The probable origin of lake trout in Yellowstone Lake was based on the early-growth zone Sr:Ca ratios of suspected transplants.

Fig. 6. Patterns of Sr:Ca ratios measured along the otolith axes of five lake trout from the group of suspected transplants collected from Yellowstone Lake: (*a*) 23 years, 832mm, (*b*) 18 years, 850 mm, (*c*) 16 years, 768 mm, (*d*) 11 years, 782 mm, and (*e*) 10 years, 765 mm. Three of the fish show a rapid increase in Sr:Ca ratios corresponding to transplant dates of 1989 (Figs. 6*a* and 6*b*) and 1996 (Fig. 6*c*), whereas the two youngest fish (Figs. 6*d* and 6*e*) show little variation in Sr:Ca ratios, suggesting that they had lived in Yellowstone Lake throughout their lives. Analysis sites were classified by discriminant analysis. ●, Lewis Lake; ▲, Heart Lake; ■, Yellowstone Lake. The dotted lines show the location of annuli and the broken line the otolith edge. Arrows mark the estimated year that the increase in Sr:Ca ratios occurred.



ited substantial increases (mean = 256%) between the early-growth and edge zones (Fig. 4). Eighty percent of the edge zones of suspected transplants were classified by the discriminant model as Yellowstone Lake, whereas 90% of the Sr:Ca ratios measured in the early-growth zone were classified as Lewis Lake (Table 3). This percentage increased to 100% if the two fish that had similar early-growth and edge zone Sr:Ca ratios were excluded from the classification analysis. Sampling along the otolith axis of a random subset (n = 3) of lake trout exhibiting the abrupt shift in Sr:Ca ratio revealed that Sr:Ca ratio increases occurred within a short time period (Fig. 6). Timing of the increase in Sr:Ca ratios was estimated to occur in 1989 for two fish (Figs. 6*a* and 6*b*) and in 1996 for one fish (Fig. 6*c*) of the subset sampled. The other two fish analyzed, representing the lake trout with similar early-growth and edge zone Sr:Ca ratios, showed little variation in Sr:Ca ratios along the otolith axis (Figs. 6*d* and 6*e*). These fish were the youngest fish in the group of suspected transplants (age 10 and 11).

Discussion

Our work demonstrates that otolith chemical composition can be used to identify a probable source and date of exotic species introductions. In the three large lakes that we studied, water chemistry differed significantly among lakes and these differences were directly imparted to lake trout otoliths. The low variation in Sr:Ca ratios of known-origin lake trout along the otolith axis from the early-growth to the edge zone, despite a wide range of ages, established that lake trout from each lake lived in a similar water chemistry throughout their lives. This temporal and spatial stability in otolith chemical signatures was reflected by the high discriminatory power to classify lake trout by their home lake based on unique otolith Sr:Ca ratios. These findings corroborate previous work demonstrating (*i*) a strong association between chemical composition of water and otolith chemistry (Bath et al. 2000; Wells et al. 2003) and (*ii*) that source waters, even in fresh water environments, can be identified with a moderate to high degree of precision based on otolith chemical composition (Thorrold et al. 1998; Wells et al. 2003). Both attributes of otoliths are important in determining stock origins, and our findings indicate that this is particularly relevant for exotic species where stock origin is frequently unknown.

Unlike known-origin lake trout, the large and rapid change in Sr:Ca ratio along the otolith axis of suspected transplants demonstrates that these fish experienced a rapid change in water chemistry. The magnitude of the change in otolith Sr:Ca ratio among suspected transplants (256% increase) mirrors that shown by anadromous fish migrating from fresh water to seawater. For example, Limburg (1995) found that otolith Sr:Ca ratios of age-0 American shad (*Alosa sapidissima*) increased by 250%–620% during movement from fresh water to seawater. Such a large and rapid change in otolith chemistry among lake trout from older year-classes supports the hypothesis that lake trout had been transplanted to Yellowstone Lake. All Yellowstone Lake lake trout from younger age-classes, ≤11 years (1986 estimated year-class and later) at the time of collection in 1996–1999, had similar early-growth and edge zone Sr:Ca ratios, indicating a constant environmental history. In contrast, all lake trout from older year-classes had a marked increase in Sr:Ca ratios between the early-growth and edge zones, indicating that these fish had reared in waters of distinctly different water chemistry during their life-span. These results therefore support the assertion that initial transplanting and natural reproduction of lake trout in Yellowstone Lake likely occurred during the mid- to late 1980s (Kaeding et al. 1996). Although our sample size was not large enough to pinpoint the exact number and timing of transplants, Ruzycki et al.'s (2003) estimate of 298 lake trout >10 years old in 1996 (year-class 1986 and earlier) suggests that a rather large number of individuals were transplanted. Moreover, the dating of the abrupt shifts in otolith chemistry as occurring in 1989 and 1996 suggests that multiple transfers may have occurred.

The classification of 90% of the early-growth zone Sr:Ca ratios of the suspected transplants into Lewis Lake by discriminant analysis suggests that, of the two lakes considered to be the most probable source lakes within Yellowstone National Park, Lewis Lake is the likely source of transplanted lake trout. Lewis Lake, unlike Heart Lake, is accessible by road, which may have facilitated the unauthorized transfer of lake trout into Yellowstone Lake.

Change in Sr:Ca ratios with age or maturation (ontogenetic or physiologic effects) are possible alternatives to the transplant hypothesis. An age- or maturation-induced Sr:Ca ratio increase was unlikely given that lower Sr:Ca ratios would be expected in the early-growth zone among all lake trout. However, the pattern of increased Sr:Ca ratio was only observed in suspected transplants and not in the early-growth zone or among younger age groups of other lake trout sampled from Yellowstone Lake or from any of the lake trout sampled from Heart and Lewis lakes, which varied greatly in age.

Another possible explanation for the increase in Sr:Ca ratios in the otoliths of the suspected transplant group of lake trout is temporal or spatial variation in lake water Sr:Ca ratios of Yellowstone Lake. The large increase in otolith Sr:Ca ratios in 1989 observed in some lake trout from the suspected transplant group coincided with the intense wildfire in Yellowstone National Park in 1988 that altered dissolved ion concentrations of some streams in Yellowstone National Park (Minshall et al. 1997). Although Sr was not measured, other dissolved ions in the lake showed only minor changes in concentration, and Lathrop (1994) and Theriot et al. (1997) found no evidence for significant changes in water chemistry resulting from the 1988 wildfire; therefore, temporal changes in water chemistry seem unlikely to account for the 256% increase in otolith Sr:Ca ratios for Yellowstone Lake lake trout. Yellowstone Lake has many hydrothermal vents that may be a source of local enrichment of Sr and Ca (Balistrieri et al. 2004). However, we found that lake water Sr:Ca ratios varied little (<3.5%) with depth or among lake subbasins; therefore, it is unlikely that the increase in otolith Sr:Ca ratios was a result of fish inhabiting different areas within Yellowstone Lake with different Sr:Ca ratios.

There are two important caveats when assessing the implications of this study. First, the long life-span of lake trout facilitated a long-term retrospective analysis of their environmental history. Detection of unique chemical marks would have been more difficult in species with higher turnover rates or with extensive migrations between waters of differing chemical signatures. Second, although Lewis Lake was identified as the source lake for transplanted lake trout with a high degree of probability, not all ambient waters have unique Sr:Ca signatures (Gillanders et al. 2001; Wells et al. 2003; Munro 2004). Therefore, we cannot eliminate the possibility that lake trout were transplanted from some other (unknown) lake with Sr:Ca ratios similar to those of Lewis Lake. In future studies, use of isotopes or other elements in addition to Sr could enhance the accuracy of identifying source waters (Kennedy et al. 2002; Wells et al. 2003).

There is growing appreciation for just how extensive introductions of exotic species have been and the formidable problem they present for aquatic ecosystem management (Hall and Mills 2000; Rahel 2000; Kolar and Lodge 2002).

For instance, in Montana alone, 375 cases of unauthorized introductions of fishes have been documented in 224 different waters comprising 45 different species (Vashro 1995). Discovery of a new species often poses questions about when the invasion occurred and the geographic origin of the exotic, but thus far, few tools have been available to answer such key questions (McMahon and Bennett 1996; Hebert and Cristescu 2002; Waters et al. 2002). Better knowledge of where exotic species originated and the relative risks they pose is essential for the design of educational and regulatory programs aimed at stemming the tide of future unauthorized introductions (McMahon and Bennett 1996; Kolar and Lodge 2002). Genetic markers have recently been shown to be a useful forensic tool for studying invasion biology (Cristescu et al. 2001; Hebert and Cristescu 2002; Waters et al. 2002). Our study demonstrates how chemical analysis of otoliths can provide a novel forensic tool to estimate geographic origin and timing of exotic fish introductions. Because chemical and genetic analysis techniques each have distinct advantages and limitations (Cristescu et al. 2001; Thorrold et al. 2001; this study), a combination of both tools could provide important new insights into the study of invasion biology.

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