2011

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Recommended Citation
Smith, Kurt T. and Whitledge, Gregory. "Evaluation of a Stable Isotope Labeling Technique for Mass-Marking Fin Rays of Age-0 Lake Sturgeon." *Fisheries Management and Ecology* 18 (Jan 2011): 168-175. doi:10.1111/j.1365-2400.2010.00771.x.

Evaluation of a stable isotope labeling technique for mass-marking fin rays of age-0 lake sturgeon

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Abstract The effectiveness of marking age-0 lake sturgeon, *Acipenser fulvescens* Rafinesque, pectoral fin rays with a stable strontium isotope was evaluated. Age-0 lake sturgeon were reared in water spiked with 0 (control), 25, 50, or 100 μg L^{-1} $^{86}SrC0_3$ for 10 and 24 d; fish from each treatment group were retained for up to 120 d post-labeling to assess mark retention. Enriched isotope marks imparted to fin rays were distinct from fin ray $^{88}Sr/^{86}Sr$ ratios of control fish immediately following marking, with the 100 μg L^{-1} $^{86}SrCO_3$ treatments consistently yielding the highest rate of marking success (83-92%). Lower marking success (25-69%) was observed with the 25 and 50 μg L^{-1} $^{86}SrCO_3$ treatments. Isotopic marks in fin rays were retained for 120 d post-labeling. Immersion marking of juvenile fish pectoral fin rays with distinct strontium isotope ratios is possible and does not require sacrificing fish to check for marks.

KEYWORDS: *Acipenser fulvescens*, chemical marking, enriched isotopes, LA-ICPMS, mark retention, strontium

Introduction

Lake sturgeon, *Acipenser fulvescens* Rafinesque are listed as endangered, threatened, or as a species of conservation concern by multiple Great Lakes, USA fisheries management agencies (U.S. Fish and Wildlife Service 2003). Although improvement and protection of critical habitat are of primary importance to achieving goals for lake sturgeon restoration, stocking is a significant component of rehabilitation plans (e.g., Hay-Chmielewski & Whelan 1997; Wisconsin Department of Natural Resources 2000; Auer 2003). Stocking (including streamside rearing) has been conducted with various life stages, including larvae, fry and fingerlings (Hay-Chmielewski & Whelan 1997; Wisconsin Department of Natural Resources 2000; Auer 2003; U.S. Fish and Wildlife Service 2005). Recent workshops identified the principle research needs to advance lake sturgeon rehabilitation that included assessments of habitat requirements and movement and dispersal patterns for age-0 fish; investigations of the most suitable life stages for stocking based on imprinting, straying, survival and return rates; and methods for differentiating stocked from wild fish for all life stages (U.S. Fish and Wildlife Service 2003, 2005)

Efforts to describe fish dispersal and movement patterns and evaluate stocking success require appropriate tagging or marking methods for the life stage of interest. Tags or marks that have been applied to lake sturgeon include radio transmitters (Auer 1999; Benson *et al.* 2005), coded wire tags (U.S. Fish and Wildlife Service 2005), passive integrated transponder (PIT) tags (U.S. Fish and Wildlife Service 2005), visible implant elastomer (VIE) tags (Caroffino *et al.* 2010) and naturally-occurring genetic markers (McQuown *et al.* 2003; DeHaan *et al.* 2006). Each of these tags or marks has

limitations. Radio transmitters, coded wire tags and PIT tags are generally restricted to fingerling-size and larger fish; external radio transmitters should not exceed 1.25% of body weight (Sutton & Benson 2003). The inability of conventional internal and external tags to mark early life stage lake sturgeon prohibits their use for addressing key questions regarding fish movement and dispersal and success of stocking early life stages. Tag loss, non-detection of tags and alteration of behaviour or growth of tagged fish also represent potential problems when using conventional tags. The suitability of visible implant elastomer (VIE) tags may be reduced in small fish due to a limited amount of adipose tissue for implantation (Hale & Gray 1998) and reduction in tag visibility over time (Fitzgerald *et al.* 2004). With a few exceptions, microsatellite DNA markers can be used to identify the population of origin for lake sturgeon in the Great Lakes (DeHaan *et al.* 2006), but individual fish within populations cannot be distinguished. Larval and juvenile fishes can also be marked with fluorescent compounds such as oxytetracycline or calcein (Brooks *et al.*1994), but marking with fluorescent compounds may require pH adjustment and buffering (Butcher *et al.* 2003; van der Walt & Faragher 2003) that may not be practical or desirable in some cases.

Stable (non-radioactive) isotopes offer the potential to efficiently produce multiple differentiating marks that can be detected from pectoral fin rays removed non-lethally from fishes (including fry and fingerlings) and are not subject to the shortcomings of current tagging and marking methods for early life stage individuals. Recent research has demonstrated that exposure of fishes to enriched stable isotopes of an element (e.g. strontium or barium) can impart isotopic ratios to otoliths that are distinct from naturally occurring stable isotope ratios (Thorrold *et al.* 2006; Munro *et al.* 2008).

Direct uptake from the water (rather than through the diet) is the principal route by which strontium and barium are incorporated into hard structures in fishes (Walther & Thorrold 2006). Stable isotope labels are non-toxic at the low concentrations required to impart a distinct chemical signature to otoliths (Thorrold *et al.* 2006; Munro *et al.* 2008). Unlike otoliths, fin rays can be sampled non-lethally (Clarke *et al*. 2007; Allen *et al.* 2009).

Natural variation in strontium concentration across sectioned pectoral fin rays has been successfully used as an indicator of white sturgeon, *Acipenser transmontanus* Richardson, and green sturgeon, *A. medirostris* Ayres, migrations (Veinott *et al.* 1999; Allen *et al.* 2009) and has also been found to be an effective means of determining Arctic grayling, *Thymallus arcticus* Pallas, origins and migratory history in British Columbia streams (Clarke *et al*. 2007). Stable isotope labels will persist in pectoral fin rays provided that tissue containing the label is not totally resorbed after deposition. Fin ray tissue is metabolically active and therefore subject to some resorption in the event of a protracted period of nutritional stress (Veinott & Evans 1999); the degree of fin ray resorption in age-0 lake sturgeon is unknown, but the probability that all of the labeled fin ray material would be resorbed is low.

The objectives of this study were to determine the concentration of strontium carbonate ($SrCO_3$) enriched with the stable isotope ^{86}Sr ($^{86}SrCO_3$) and immersion time in water enriched with $^{86}SrCO_3$ required to incorporate distinct enriched-isotope marks (measured as $^{88}Sr/^{86}Sr$ ratios) in pectoral fin rays of age-0 lake sturgeon and to evaluate retention rate for enriched isotope marks applied to fin rays up to 120 d post-labeling.

Methods

Age-0 lake sturgeon (mean total length 162.5 mm ± 0.89 mm SE, range 110-210 mm) were obtained from Genoa National Fish Hatchery, Genoa, WI, USA and transported to the Fisheries and Illinois Aquaculture Center at Southern Illinois University-Carbondale in a tank aerated with pressurised oxygen. In the laboratory, fish were initially held in 1,000-L tanks equipped with water recirculation, aeration, temperature control, and biofiltration systems. Fish were fed to satiation twice daily with frozen bloodworms (chironomid larvae) during an initial acclimation period to the laboratory (approximately 2 weeks) and throughout the study. Water temperature was maintained at 20 ± 1°C, pH was 7.5-7.8, photoperiod was held at 12 h light:12 h dark and dissolved oxygen concentrations were maintained at ≥ 7 mg L^{-1} throughout laboratory experiments. Immediately prior to experiments, 10 fish were euthanized with an overdose of MS-222, and one pectoral fin ray was obtained from each of these fish to characterise pre-experimental (natural) $^{88}Sr/^{86}Sr$ ratios and to measure pre-experimental fin ray radius (see fin ray preparation and analytical methods below).

Lake sturgeon (*n*=8/tank) were randomly assigned to each of 26 randomly distributed, 5-L treatment tanks for stable isotope labeling experiments. Treatments consisted of all combinations of 25, 50, and 100 μg L^{-1} $SrCO_3$ enriched in the stable isotope ^{86}Sr and 10- and 24-d immersion times. Four replicate tanks were used for each treatment to control for possible tank effects. A control group was represented by two tanks with fish reared in ambient water. Treatment-group tanks were spiked with solutions of known concentration of $SrCO_3$ enriched in ^{86}Sr (~97% ^{86}Sr and 2.5% ^{88}Sr) was obtained from Oak Ridge National Laboratory, Oak Ridge, TN. The $^{88}Sr/^{86}Sr$ ratio

of the ^{86}Sr-enriched $SrCO_3$ (hereafter referred to as $^{86}SrCO_3$) was distinct from strontium isotope ratios of natural substances, including fresh waters; natural abundances of ^{86}Sr and ^{88}Sr are 9.86% and 82.58%, respectively (Moore *et al*. 1982). $^{86}SrCO_3$ solutions were prepared by dissolving $^{86}SrCO_3$ in a small amount of ultrapure water and concentrated HCl, then diluting when all carbonate was dissolved. The appropriate amount of solution was then pipetted into each treatment tank to give the desired final concentration. Fifty percent of the water in each tank was changed daily, and solid waste was siphoned from the bottom of each tank to maintain good water quality (nitrite and nitrate concentrations were < 0.20 and 20 mg L^{-1}, respectively, in all tanks). Treatments were spiked with appropriate amounts of $^{86}SrCO_3$ solution following water changes to maintain desired $^{86}SrCO_3$ concentrations in treatment tanks. After the specified immersion time for each treatment, eight fish were randomly selected from each treatment group (2 fish/tank, including controls) and anesthetised using a lethal dose of MS-222 (~200-250 mg L^{-1}). The right leading pectoral fin ray was removed at the point of articulation from each of these eight fish from control and three treatment groups.

Pectoral fin rays were embedded in epoxy resin and sectioned at the articulating process (the widest portion at the base of the spine) using a Buehler ISOMET™ low-speed saw (Lake Bluff, IL). Sectioned fin ray samples were ground with silicon carbide paper until the core was exposed and polished. Fin ray sections were then sonicated for 5 min in ultrapure water to remove surface contamination and dried in a class 100 laminar flow hood for 24 h. Once dry, fin ray sections were mounted on glass slides using 3M™ (St. Paul, MN) double-sided tape. Fin ray samples were analyzed for ^{88}Sr and ^{86}Sr using laser ablation-inductively coupled plasma mass spectrometry (LA-ICPMS). The laser

ablated a line transect along the longest axis of the fin ray cross section from the fin ray core to its edge (beam diameter = 22 μm, scan rate = 5 μm/s). A standard (NIST 610, National Institute of Standards and Technology, Gaithersburg, MD) was analyzed every 10-15 samples to adjust for possible mass bias and instrument drift. Each analysis was preceded by a gas blank measurement. Mean $^{88}Sr/^{86}Sr$ ratio for the standard was 8.355 ± 0.007 SE (min = 8.09, max = 8.58, n = 112), very similar to the natural ratio of $^{88}Sr/^{86}Sr$ (8.375; Moore *et al.* 1982 Munro *et al.* 2008). Blank-corrected abundances of ^{88}Sr and ^{86}Sr were used to calculate isotopic ratios for samples. Line transect $^{88}Sr/^{86}Sr$ ratio data for fin ray samples were smoothed using a 10-point running average (Munro *et al.* 2008; Allen *et al.* 2009). Mean fin ray $^{88}Sr/^{86}Sr$ ratios of the last 50 μm of laser transects (nearest the fin ray edge) were then calculated for each fish in control and treatment groups. This section of the fin ray was used for comparisons of $^{88}Sr/^{86}Sr$ ratios among control and treatment groups immediately following the labeling experiment because it reflected growth during the experiment (mean fin ray radius of fish prior to the experiment was 245.8 μm ± 30.9 μm SE; mean fin ray radius at the conclusion of labeling experiments was 406.0 μm ± 40.9 μm SE).

The effects of $^{86}SrCO_3$ concentration and immersion time on mean $^{88}Sr/^{86}Sr$ ratios in pectoral fin rays of age-0 lake sturgeon were assessed using three-way ANOVA with concentration and immersion time treated as fixed factors and tank (nested within the concentration × immersion interaction term) as a random factor. Tukey's HSD test was applied to assess differences among treatment group means when the overall ANOVA indicated significance. Mark success was determined by setting a successful mark criterion of 7.30, the mean $^{88}Sr/^{86}Sr$ ratio of control fish minus two standard deviations.

All treatment group individuals with $^{88}Sr/^{86}Sr$ ratios < 7.30 were considered successfully marked.

At the end of the experimental labeling period, remaining fish from treatment groups were returned to ambient water and maintained in their replicate tanks to assess retention time and retention rate of altered $^{88}Sr/^{86}Sr$ ratios. Remaining control fish were also retained in ambient water. One pectoral fin ray was obtained from a sub-sample of 7-8 fish from control and treatment groups at 30, 60, and 120 d post-labeling; fish were anesthetized with MS-222 prior to fin ray removal. Sample preparation and analysis of fin ray samples for $^{88}Sr/^{86}Sr$ ratios were as described above. Differences in the mean fin ray $^{88}Sr/^{86}Sr$ ratios among control (unmarked) fish sacrificed immediately prior to the labeling experiment and on days 30, 60, and 120 following the experiment were assessed using one-way ANOVA. The minimum value of the smoothed $^{88}Sr/^{86}Sr$ ratio profile (smoothed using a 10-point running average as previously described) across each sectioned fin ray was also determined and was used to assess mark retention for treatment-group fish (Munro *et al.* 2008); individuals with minimum $^{88}Sr/^{86}Sr$ ratios < 7.30 were considered successfully marked. Correlations between proportion of fish retaining enriched isotope marks and number of days post-labeling were assessed for treatment groups using Spearman rank correlations. Differences in mean minimum ratios of $^{88}Sr/^{86}Sr$ (hereafter referred to as minimum $^{88}Sr/^{86}Sr$ ratios) in fin ray profiles among fish from control and treatment groups and over time (30, 60, and 120 d post-labeling) were assessed using repeated measures ANOVA followed by least squares means to assess differences among treatment groups and dates when the overall ANOVA indicated significance. To further test the ability to distinguish between fish in marked (all

treatments) and unmarked (control) groups, linear discriminant function analysis (LDFA) with a leave-one-out jackknife procedure was used to determine the accuracy with which individual fish could be classified as a member of the marked or unmarked groups based on minimum ratios of $^{88}Sr/^{86}Sr$ in fin ray profiles. A second LDFA and cross-validation was performed using data from control fish and treatment groups that were exposed to the highest concentration of $^{86}SrCO_3$ (100 μg L^{-1}). Statistical analyses were conducted using SAS version 9.1 (SAS 2004), and statistical significance was declared at $P<0.05$ for all tests.

Results

Immersion of age-0 lake sturgeon in water spiked with $^{86}SrCO_3$ produced enriched-isotope marks (identified by shifts in $^{88}Sr/^{86}Sr$ ratios) in pectoral fin rays that were distinct from fin ray $^{88}Sr/^{86}Sr$ ratios of control fish reared in ambient laboratory water. Neither immersion time in water spiked with $^{86}SrCO_3$ ($F_{1,34} = 2.61$, $P = 0.12$) nor tank ($F_{6,34} = 0.32$, $P = 0.92$) had a significant effect on $^{88}Sr/^{86}Sr$ ratios immediately after the $^{86}SrCO_3$ labeling period; the $^{86}SrCO_3$ immersion time-concentration interaction term was also non-significant ($F_{2,34} = 0.99$, $P = 0.38$). Therefore, data from fish in all tanks with 10- or 24-d immersion times within each $^{86}SrCO_3$ treatment were combined in subsequent statistical analyses.

The $^{86}SrCO_3$ concentration to which fish were exposed had a significant effect on $^{88}Sr/^{86}Sr$ ratios ($F_{3,34} = 10.72$, $P < 0.001$). Treatment fish exposed to water containing 25, 50, or 100 μg L^{-1} of $^{86}SrCO_3$ exhibited fin ray $^{88}Sr/^{86}Sr$ ratios that were significantly

lower than that of control fish immediately after the $^{86}SrCO_3$ labeling period (Fig. 1). Fish immersed in 100 μg L^{-1} of $^{86}SrCO_3$ had a significantly lower fin ray $^{88}Sr/^{86}Sr$ ratios than fish exposed to either 25 or 50 μg L^{-1} of $^{86}SrCO_3$, but $^{88}Sr/^{86}Sr$ ratios did not differ between 25 or 50 μg L^{-1} of $^{86}SrCO_3$. Fin ray $^{88}Sr/^{86}Sr$ ratios for control fish reared in ambient laboratory water (8.05) was slightly lower than the average natural ratio of $^{88}Sr/^{86}Sr$ (8.375), and treatment-group fish immersed in water spiked with $^{86}SrCO_3$ for 10 or 24 d exhibited fin ray $^{88}Sr/^{86}Sr$ ratios that were substantially lower than the average natural $^{88}Sr/^{86}Sr$ ratio.

Marking success differed among treatment groups exposed to different concentrations of $^{86}SrCO_3$, with the 100 μg L^{-1} $^{86}SrCO_3$ treatment groups exhibiting consistently higher percentages of successfully marked individuals following the $^{86}SrCO_3$ labeling period than the 25 and 50 μg L^{-1} $^{86}SrCO_3$ treatment groups (Table 1). Percentages of fish within treatment groups that retained enriched isotope marks in fin rays were not significantly correlated with the number of days post-labeling (Spearman rank correlations, $P > 0.05$ for each group). Number of days post-labeling did not significantly affect $^{88}Sr/^{86}Sr$ ratios within fin ray profiles ($F_{3,163} = 1.07$, $P = 0.36$), nor did the interaction between days post-labeling and $^{86}SrCO_3$ concentration to which fish were previously exposed ($F_{9,163} = 0.80$, $P = 0.62$). However, $^{86}SrCO_3$ concentration had a significant effect on minimum $^{88}Sr/^{86}Sr$ ratios ($F_{3,163} = 32.54$, $P < 0.001$), indicating that differences in fin ray $^{88}Sr/^{86}Sr$ ratios of enriched isotope marks among treatment groups were preserved throughout the 120-d post-labeling period. Fin ray $^{88}Sr/^{86}Sr$ ratios of control fish did not change significantly over time from the start of the $^{86}SrCO_3$ labeling experiment through 120 d after the conclusion of the labeling period ($F_{4,32} = 1.10$, $P =$

0.37). Minimum $^{88}Sr/^{86}Sr$ ratios within fin ray profiles were significantly different between controls and fish in the 100 μg L^{-1} $^{86}SrCO_3$ treatment groups at 30, 60, and 120 d post-labeling (Fig. 2), demonstrating that fish immersed in 100 μg L^{-1} $^{86}SrCO_3$ for 10 or 24 d retained distinct enriched isotope marks in pectoral fin rays for up to 120 d following the marking period. Minimum $^{88}Sr/^{86}Sr$ ratios in laser-ablated transects across sectioned fin rays were significantly different between controls and fish in the 50 μg L^{-1} $^{86}SrCO_3$ treatment group at 30 and 120 d post-labeling but not on day 60 post-labeling. Minimum $^{88}Sr/^{86}Sr$ ratios for the 25 μg L^{-1} $^{86}SrCO_3$ treatment group were significantly different from control values only on day 120 post-labeling . The lack of consistent differences between minimum $^{88}Sr/^{86}Sr$ ratios for fish in the 25 and 50 μg L^{-1} $^{86}SrCO_3$ treatment groups and controls reflected the lower percentages of successfully marked fish in these treatment groups compared to the 100 μg L^{-1} $^{86}SrCO_3$ treatment group.

LDFA correctly classified 74% of treatment group fish as individuals that were immersed in water spiked with $^{86}SrCO_3$ and 81% of control fish as unmarked based on fin ray $^{88}Sr/^{86}Sr$ ratios (Table 2). Classification accuracy for identifying individual fish as members of treatment or control groups was higher when the LDFA was restricted to data from fish in the 100 μg L^{-1} $^{86}SrCO_3$ treatment and controls, the two groups that consistently exhibited the most distinct fin ray $^{88}Sr/^{86}Sr$ ratios. Eighty-three percent of fish from the 100 μg L^{-1} $^{86}SrCO_3$ treatment were correctly identified as members of this treatment group, while 97% of control fish were correctly classified as unmarked based on fin ray $^{88}Sr/^{86}Sr$ ratios (Table 3).

Discussion

Results of this study demonstrated that stable strontium isotope ratios ($^{88}Sr/^{86}Sr$) in age-0 lake sturgeon pectoral fin rays can be altered by immersion of fish in water spiked with $^{86}SrCO_3$. This is consistent with recent studies that have established that fish otoliths can be marked with distinct $^{138}Ba/^{137}Ba$ or $^{88}Sr/^{86}Sr$ ratios when the fish are reared in water containing enriched isotopes (Walther & Thorrold 2006; Munro *et al.* 2008). Marks that were distinguishable from $^{88}Sr/^{86}Sr$ ratios in fin rays of fish reared in ambient laboratory water were produced in as few as 10 d by immersion of fish in water spiked with 100 μg L^{-1} of $^{86}SrCO_3$. Munro *et al.* (2008) were able to successfully mark juvenile golden perch, *Macquaria ambigua* Richardson, otoliths with as little as 4 d exposure to 15 μg L^{-1} of ^{137}Ba or 24 d exposure to 25 μg L^{-1} of ^{86}Sr. Unlike Munro *et al.* (2008), concentrations of enriched isotopes in this study were expressed as concentrations of $^{86}SrCO_3$ rather than concentrations of just the isotopically enriched element (^{86}Sr). Thus, the low concentrations of enriched ^{86}Sr used in this study (25 μg L^{-1} $^{86}SrCO_3$) were lower than those used by Munro *et al.* (2008). Whereas Munro *et al.* (2008) found that otolith $^{138}Ba/^{137}Ba$ and $^{88}Sr/^{86}Sr$ ratios and marking success were affected by both the concentration of enriched isotopes and the immersion time, results of this study indicated that $^{86}SrCO_3$ concentration influenced fin ray $^{88}Sr/^{86}Sr$ ratio and marking success, but no measurable differences were observed between the 10- and 24-d immersion times of fish exposed to water spiked with $^{86}SrCO_3$. The absence of significant effects of immersion time on enriched isotope marks in fin rays or marking success suggests that age-0 sturgeon fin ray $^{88}Sr/^{86}Sr$ ratios may have reached equilibrium with water $^{88}Sr/^{86}Sr$ ratios

prior to day 10 of the labeling period and, therefore, that immersion times < 10 d may also have produced distinct enriched strontium isotope marks in fin rays of age-0 sturgeon.

As in the otolith marking study by Munro *et al.* (2008), water $^{86}SrCO_3$ concentration had a significant effect on marking success and fin ray $^{88}Sr/^{86}Sr$ ratios. Immersion of fish in 100 μg L^{-1} of $^{86}SrCO_3$ produced relatively high marking success (83-92%). Exposure to 25 or 50 μg L^{-1} of $^{86}SrCO_3$ yielded significantly lower mean $^{88}Sr/^{86}Sr$ ratios in fin rays compared to control fish, but marking success was generally poor for these treatment groups (< 70% of fish were successfully marked). Results of this study indicated that 10-d immersion in 100 μg L^{-1} of $^{86}SrCO_3$ produced discernible marks on at least 83 % of age-0 lake sturgeon. Additional studies should assess minimum concentrations of $^{86}SrCO_3$ and immersion times and evaluate isotopic marking of different sizes of age-0 lake sturgeon.

Stable strontium isotopic marks applied to pectoral fin rays of age-0 lake sturgeon were retained for 120 d post-labeling. While it is unknown how long strontium isotope-enriched marks will persist in fin rays, strong potential for long-term retention of stable-isotope marks applied to fin rays is suggested by previous studies that have documented retention of naturally occurring trace element signatures (e.g., Sr:Ca, Ba:Ca, Sr:Ba) indicative of natal environments and migration history in fin rays of adult green and white sturgeons and Arctic grayling for periods of several years (Veinott *et al*. 1999; Clarke, *et al*. 2007; Allen *et al.* 2009). Unlike otoliths, fin rays are metabolically active and subject to elemental turnover in addition to the possibility of resorption in the event of a protracted period of nutritional stress (Veinott & Evans 1999; Clarke *et al.* 2007).

Further evaluation of the ability of fish fin rays to retain artificially induced stable-isotope marks as well as natural trace elemental and stable-isotope signatures throughout the life span of fishes is needed to assess the potential of these artificial and natural marks to provide information on location of origin and movement and dispersal patterns of wild or stocked fish throughout their lifetimes.

Marking pectoral fin rays of fish with distinct stable-isotope ratios offers several advantages over other tagging or marking techniques. Unlike stable isotope or other chemical marks applied to otoliths (e.g., Brooks *et al.* 1994; Brown & Harris 1995; Munro *et al.* 2008), stable-isotope marks imparted to fin rays can be assessed without sacrificing the fish. Pectoral fin rays are routinely removed from lake sturgeon and other sturgeon species for non-lethal determination of fish age (LeBreton & Beamish 2004). In contrast to physical tags (e.g. radio transmitters, coded wire tags or PIT tags), stable isotope and other chemical marking techniques are applicable to very small juvenile or even larval fishes (Brothers 1990; Munro *et al.* 2008). Chemical marking methods such as stable isotope labeling require minimal handling of fish, and large batches of fish can be tagged simultaneously (Brothers 1990). Unlike marking fish hard structures with fluorescent compounds, stable isotope marking does not require pH adjustment and buffering of water in fish holding tanks (Butcher *et al.* 2003; van der Walt & Faragher 2003). Stable isotope immersion marking also requires lower concentrations of Sr to produce distinctly artificial marks than elemental (Sr:Ca) marking (Brown & Harris 1995). Strontium concentrations required to produce detectable stable-isotope marks in otoliths or fin rays are non-toxic, and there are no drinking water guidelines for strontium. Although isotopically marking fish fin rays conveyd several advantages, stable

isotope marking of pectoral fin rays would be best suited for marking small fishes that are too small to carry physical tags in situations in which non-lethal mark recovery is necessary or highly desirable. This technique requires LA-ICPMS, which is relatively costly (~$15 US per fish for analysis) compared to mark detection using other tagging or marking methods and is available at relatively few laboratories, to detect marks. While enriched $^{86}SrCO_3$ is relatively expensive (US $11 mg^{-1}), the cost of producing stable strontium isotope marks in fish is comparable to other chemical marking methods for small fishes due to the small amount of $^{86}SrCO_3$ required to mark otoliths (Munro *et al.* 2008) or fin rays. In this study, the cost associated with marking 100 lake sturgeon fin rays with100 μg L^{-1} $^{86}SrCO_3$ over a 10-d immersion period was ~US $350. This study was conducted with relatively large age-0 fish (mean total length 162.5 mm ± 0.89 mm SE); smaller individuals could be maintained at higher densities, thus potentially reducing the cost of isotopic marking per individual if fish were marked at smaller sizes.

Isotopic batch marks in fin rays of age-0 lake sturgeon offer a non-lethal means of distinguishing stocked fish from naturally reproduced individuals, particularly for fish that are too small to carry conventional tags. Releases of marked fish could be used to assess factors influencing early life stage dispersal rates and patterns, habitat use and mortality rates (Secor & Houde 1995), which are frequently critical processes affecting recruitment to older life stages. Isotopic marking of fin rays could also potentially be used to differentiate stocked from wild fish and evaluate relative success of stocking fish at different life stages.

Additional research is needed to assess retention of stable-isotope marks imparted to fin rays of age-0 fish beyond the 120-d duration of this study. If stable-isotope marks

in fin rays are retained for several years, isotopic marks could potentially be used to investigate fidelity of older juvenile and adult fish to their natal or stocking site. Given that stable-isotope marks can be induced in pectoral fin rays, we suggest that production of multiple differentiating marks may be possible by immersion of fish in water enriched with both barium and strontium stable isotopes, as has been demonstrated for fish otoliths (Munro *et al.* 2008). Multiple differentiating marks would enable identification of different batches of fish stocked from a single source, facilitating assessment of factors influencing stocking success for individuals stocked at early life stages. Additional studies should evaluate whether production of multiple differentiating isotopic marks in fin rays is possible. Stable isotopic marking of fin rays will also likely be applicable to fish species other than lake sturgeon. Additional evaluation of this technique for other species is recommended.

Acknowledgments

The Great Lakes Fishery Commission (GLFC) provided funding for this project. Findings or conclusions presented in this article are not necessarily endorsed by the GLFC. Doug Aloisi, manager of Genoa National Fish Hatchery (U.S Fish and Wildlife Service) provided the lake sturgeon used in this study. Analysis of fin rays by LA-ICPMS was performed by the Great Lakes Institute for Environmental Research, University of Windsor.

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Table 1. Percentage of age-0 lake sturgeon exposed to three different concentrations of $^{86}SrCO_3$ for 10 or 24 days that were considered successfully marked (minimum $^{88}Sr/^{86}Sr$ ratios < 7.30 in laser ablation transects across sectioned fin rays) at four time intervals following the $^{86}SrCO_3$ labeling period. Data from 10- and 24-d exposure times within each level of $^{86}SrCO_3$ concentration were pooled.

$^{86}SrCO_3$ concentration ($\mu g\ L^{-1}$)	*n*	Days post-labeling	Fish successfully marked (%)
25	13	0	38
25	12	30	25
25	12	60	58
25	14	120	57
50	15	0	33
50	12	30	64
50	12	60	42
50	13	120	69
100	12	0	83
100	12	30	92
100	12	60	92
100	13	120	85

Table 2. Results of linear discriminant function analysis showing classification accuracy as marked or unmarked for age-0 lake sturgeon marked with 25, 50 or 100 μg/L $^{86}SrCO_3$ for 10 or 24 days or unmarked (control) based on fin ray $^{88}Sr/^{86}Sr$ ratio. The 'marked' group includes all fish exposed to enriched ^{86}Sr.

Known group	*n*	Assigned group		% Correct
		Marked	Unmarked	
Marked	150	111	39	74
Unmarked	37	7	30	81

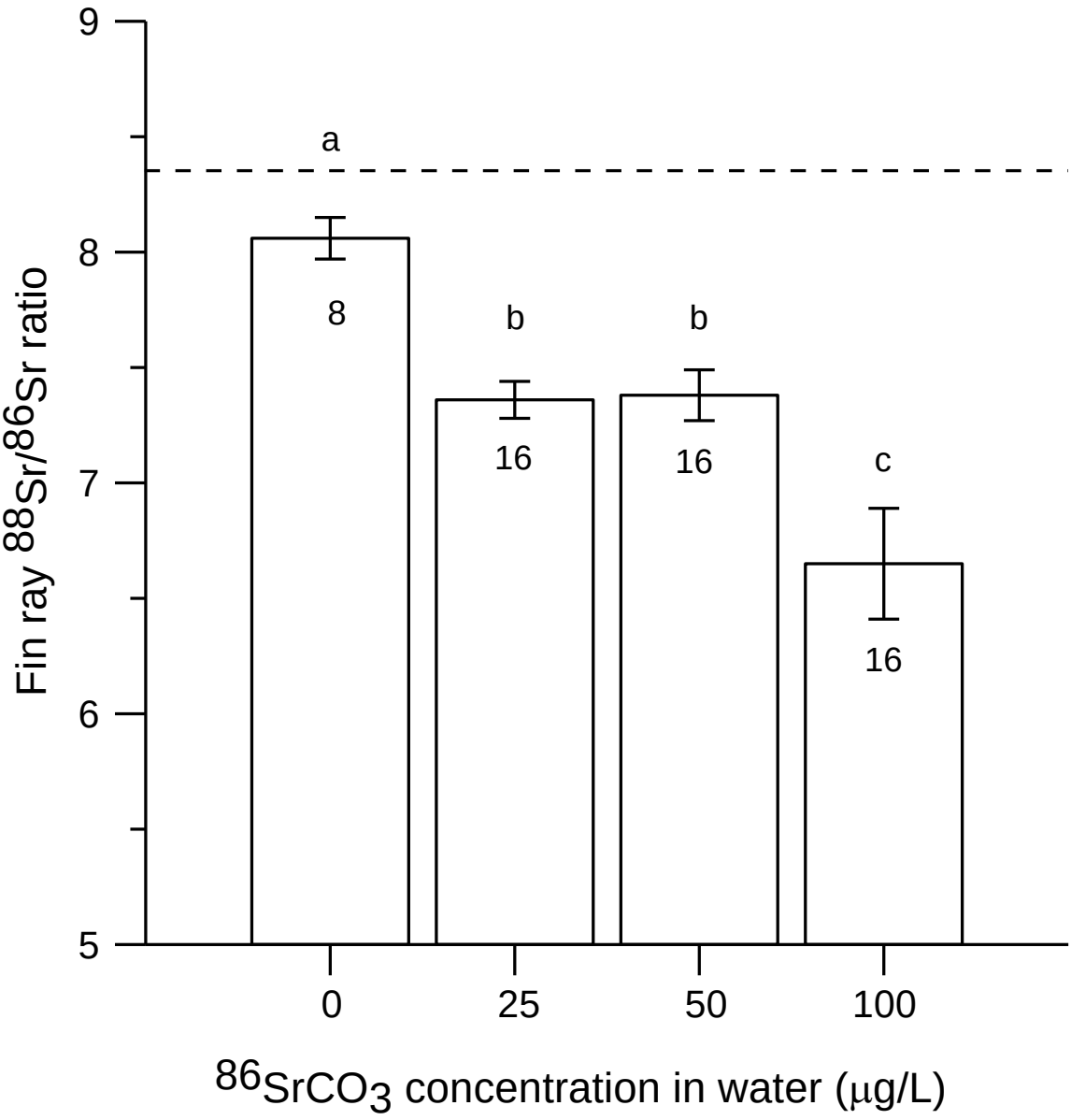
Table 3. Results of linear discriminant function analysis showing classification accuracy as marked or unmarked for age-0 lake sturgeon marked with100 μg/L $^{86}SrCO_3$ for 10 or 24 days or unmarked (control) based on fin ray $^{88}Sr/^{86}Sr$ ratio.

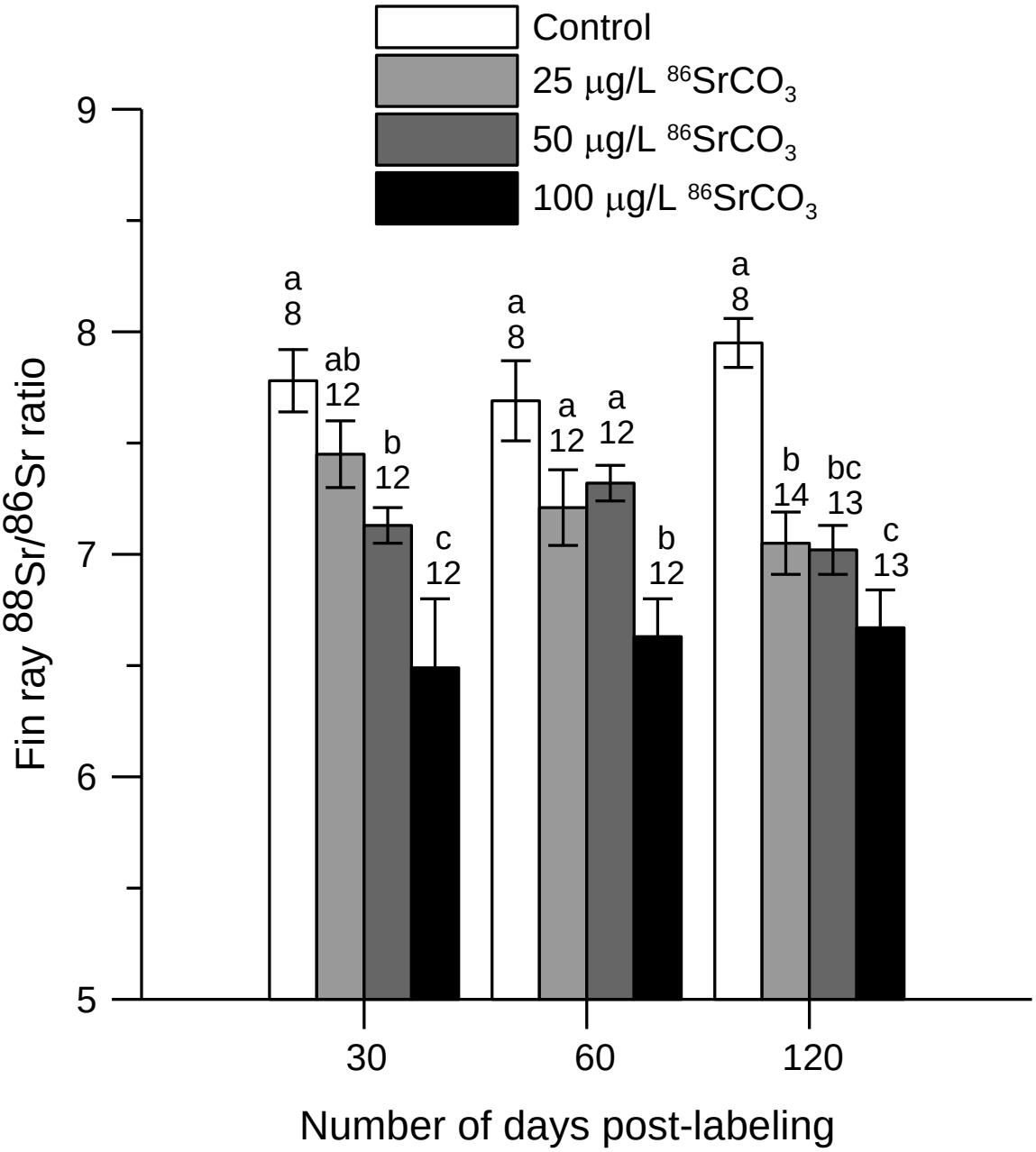
Known group	*n*	Assigned group		% Correct
		Marked	Unmarked	
Marked	48	40	8	83
Unmarked	37	1	36	97

Figure captions

Figure 1. Mean (± SE) pectoral fin ray $^{88}Sr/^{86}Sr$ ratios for age-0 lake sturgeon reared in ambient water (controls) and ambient water spiked with three concentrations of $^{86}SrCO_3$ for 10 or 24 days. Ten- and 24-d treatments for a given $^{86}SrCO_3$ concentration were pooled because immersion time in water enriched with ^{86}Sr did not significantly affect mean fin ray $^{88}Sr/^{86}Sr$ ratios. Means bearing the same letter are not significantly different (ANOVA followed by Tukey's HSD test, $P > 0.05$). Numbers within bars are sample sizes. The horizontal dashed line indicates the natural water $^{88}Sr/^{86}Sr$ ratio.

Figure 2. Mean (± SE) pectoral fin ray $^{88}Sr/^{86}Sr$ ratios 30, 60, and 120 days after the labeling experiment for age-0 lake sturgeon reared in ambient water (control) and for fish exposed to 25, 50, or 100 μg L^{-1} $^{86}SrCO_3$ for 10 or 24 days. Means bearing the same letter within dates are not significantly different (ANOVA followed by least squares means test, $P > 0.05$). Numbers above bars are sample sizes.


9
8
7
6
5
Fin ray 88Sr/86Sr ratio
a
b
b
c
8
16
16
16
0
25
50
100
86SrCO3 concentration in water (μg/L)


Control
25 μg/L 86SrCO3
50 μg/L 86SrCO3
100 μg/L 86SrCO3
Fin ray 88Sr/86Sr ratio
9
8
7
6
5
a
8
ab
12
b
12
c
12
a
8
a
12
a
12
b
12
a
8
b
14
bc
13
c
13
30
60
120
Number of days post-labeling