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Identification of Stocked Muskellunge and Potential for Distinguishing Hatchery-Origin and Wild Fish Using Pelvic Fin Ray Microchemistry

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Abstract The effectiveness of pelvic fin ray microchemistry of muskellunge *Esox masquinongy* Mitchill to identify stocked individuals along with the potential to identify naturally reproduced fish were evaluated. Fish and water samples were obtained from one hatchery and seven lakes with natural differences in water Sr:Ca to determine whether location-specific environmental signatures were recorded in sectioned muskellunge pelvic fin rays, including fish of known environmental history. Water and fin ray Sr:Ca were strongly correlated. Six lakes in Illinois possessed Sr:Ca signatures that were distinct from the hatchery where muskellunge were raised, resulting in pronounced shifts in Sr:Ca across sectioned fin rays of stocked fish. Hatchery and lake-specific Sr:Ca signatures were stable across years. Sixteen of 19 individual fish known to have been stocked based on PIT tags implanted at stocking were correctly identified as hatchery-origin fish using fin ray core Sr:Ca. Results also indicated that the hatchery Sr:Ca signal can be retained for at least seven years in fin rays of stocked fish. Fin ray microchemistry is a non-lethal approach for determining environmental history of muskellunge that could be used to assess movement patterns in lake and river systems and the degree to which muskellunge populations are supported by natural reproduction and stocking.

KEYWORDS: environmental history, *Esox masquinongy*, fin rays, LA-ICPMS, natural tag, strontium

Introduction

Stocking fish is an important management tool for fisheries professionals to enhance and maintain fish populations throughout North America (Halvorson 2008). Knowledge of stocking efficacy can help fisheries professionals further understand the cost-effectiveness of stocking individuals to enhance or supplement fisheries (Halvorson 2008). However, it is often difficult to distinguish between wild and hatchery-reared fish and thus difficult to determine the contribution of current stocking practices to fish populations. Typical methods available to differentiate wild from hatchery-reared fish include physical mutilation marks (e.g., fin clips and freeze branding; McNeil and Crossman 1979; Johnson & Margenau 1993; Conover & Sheehan 1999), external and internal tagging (e.g., T-bar anchor and passive integrated transponder; Younk *et al*. 2010; Rude *et al*. 2011), and fluorescent marks in hard-part structures (e.g., oxytetracycline; OTC, and calcein; Brooks *et al*. 1994; Conover & Sheehan 1999; Farrell & Werner 1999; Mohler 2003). Physical mutilation marks are relatively inexpensive and tend to work well in short-term studies (McNeil & Crossman 1979). However, clipped fins and freeze brands may regenerate, resulting in loss of the mark or a decreased confidence in identification (McNeil & Crossman 1979), and anglers tend to view missing or mutilated fins or burn marks as undesirable (Nielsen 1992). External and internal tags are a promising technique because they typically have high retention rates (>90% for PIT tags; Younk *et al*. 2010; Rude *et al*. 2011, and >70% in ≤ 2 years for T-bar anchor tags; Clugston 1996; Buzby & Deegan 1999; Rude *et al*. 2011) and offer the ability to distinguish specific cohorts along with individual fish (Guy *et al*. 1996). However, tagging fry and small fingerlings is challenging because tag size is often too large (Sutton & Benson 2003). Tagging advanced fingerlings is often a viable marking technique (PIT: Wagner *et al*. 2007; Younk *et al*. 2010; Rude *et al*. 2011), but it may not be cost-

effective to tag an entire batch of individuals due to a high cost per tag and post-stocking mortality (Johnson & Margenau 1993). Chemical batch marking using OTC and other fluorescent compounds is an effective and popular technique to mark large numbers of fry and fingerlings (Kayle 1992; Brooks *et al*. 1994; Mohler 1997) with low post-marking mortality (Brooks *et al*. 1994; Mohler 1997). However, the main disadvantage of chemical marking is that the tag cannot be detected externally and fish must be sacrificed for analysis of otoliths (and other bony-structures) in order to detect the fluorescent marks (Thorrold *et al*. 2002).

Otolith chemistry is an alternative technique for identifying fish origin that offers the potential to provide new insights into the efficacy of stocking. The premise of this technique is that the chemical composition of otoliths (at least for some elements) reflects that of the water in which a fish resides (Kennedy *et al*. 2002; Dufour *et al*. 2005; Whitledge *et al*. 2007; Zeigler & Whitledge 2010; Zeigler & Whitledge 2011). Associated changes in chemistry across the otolith enables retrospective reconstruction of fish environmental histories when an individual fish has resided in chemically distinct locations for a sufficient period of time to incorporate the unique chemical signatures of the water (Bickford & Hannigan 2005; Whitledge *et al*. 2007; Smith & Whitledge 2010; Zeigler & Whitledge 2011). Previous studies have used chemical differences between hatchery water and water where fish were stocked to distinguish between hatchery-reared and wild fish (Bickford & Hannigan 2005; Zitek *et al*. 2010), as well as identification of stocked fish from specific hatcheries (Bickford & Hannigan 2005; Gibson-Reinemer *et al*. 2009) using otolith chemistry. For example, Bickford & Hannigan (2005) were able to distinguish hatchery of origin for walleye *Sander vitreus* Mitchill residing in the Eleven Point River, AR with a high degree of accuracy (~90%) based on strontium:calcium (Sr:Ca), barium:calcium (Ba:Ca), and magnesium:calcium (Mg:Ca) concentrations from otolith cores (first year of life).

Despite its utility, the major drawback of otolith chemistry is that it requires sacrificing individuals, which can be problematic, especially when investigating imperiled species and long-lived, trophy fishes where sacrificing even relatively small numbers of fish is undesirable (DeVries and Frie 1996).

Fin ray chemistry is an alternative non-lethal technique to otolith chemistry that is effective for reconstructing fish environmental history and distinguishing among fish stocks (Veinott *et al*. 1999; Clarke *et al*. 2007; Allen *et al*. 2009; Smith & Whitledge 2010; Smith & Whitledge 2011; Phelps *et al*. 2012). However, fin ray chemistry has not been applied to distinguish hatchery-reared and wild fish. A non-lethal method to determine how hatchery-reared individuals enhance or supplement existing fisheries would be valuable to determine the relative value in supplemental stocking. Thus, the objective of this study was to determine if fin ray chemistry is a viable technique to identify hatchery-reared fish in populations of unknown origin fish using Sr:Ca signatures. The species used in this study was muskellunge, a species which sacrificing individuals is undesirable due to their relatively low population sizes and value as a trophy species in catch-and-release fisheries.

Muskellunge are large, long-lived esocids native to North America and are a highly sought after sport fish by anglers (Hall 1986). Enhancing and maintaining both native and introduced populations of muskellunge is considered a high management priority for fisheries professionals (Wingate 1986). Stocking juvenile muskellunge (fry to advanced fingerlings) is widely viewed as an effective method to enhance and maintain muskellunge populations (Larscheid *et al*. 1999; Margenau 1999; Wingate & Younk 2007); however, the value of stocking and the degree of natural reproduction remains poorly understood (Margenau 1999; Wahl 1999; Miller *et al*. 2009). Fin ray chemistry may provide an opportunity for fisheries professionals to

better understand muskellunge stocking practices and the extent of natural reproduction in water bodies where both natural reproduction and stocking may contribute to a fishery.

Methods

Adult muskellunge (300 – 1110 mm total length) were collected from six sites in Illinois, USA (Kinkaid Lake, Lake Mingo, North Spring Lake, Pierce Lake, Sam Dale Lake, and Shabbona Lake), and Elk Lake in northern Minnesota, USA during 2010 and 2011 using trap nets and direct current electrofishing (Table 1). Total length of each captured muskellunge was measured (nearest mm) and gender was determined for each fish based on the shape of the urogenital papilla (LeBeau & Pageau 1989). The leading pelvic fin ray was detached from the remainder of the fin and then cut off at the base (as close to the body as possible), after which fish were released. Leading pelvic fin rays were also collected from juvenile muskellunge obtained from Jake Wolf State Fish Hatchery (JWFH; this hatchery is the source of all muskellunge stocked into Illinois lakes) during late summer 2010 (Table 1). Hatchery-reared fish were fed a commercially available pelleted feed (from hatch until ~140 – 165 mm TL or ~110 d post-hatch) and then fed commercially available live fathead minnows *Pimephales promelas* Rafinesque from South Dakota until time of stocking (~280 mm). A combination of PIT tags, freeze brands, and fin clips applied at the time of stocking were used to identify and determine age of known-stocked fish (n = 19) from all sites. Age of each unmarked fish (hatchery-reared or wild unknown; n=29) was estimated from sectioned pelvic fin rays (Brenden et al. 2006). For fish from Kinkaid Lake that were not marked at the time of stocking (n = 17) but were tagged as adults as part of a separate mark-recapture study, age was also estimated with an age-length key developed using data for known-age fish from this population in combination

with knowledge of elapsed time since initial tagging. When the two age estimates did not agree, the latter method was used to assign an age to an individual fish, as fin rays may underestimate age, particularly in older fish (Johnson 1971).

A 20-ml water sample for analysis of Sr and Ca concentrations was collected from each of the seven lake sites at the time of fish collection, and a sample of water and juvenile muskellunge feed were obtained from JWFH during March 2010 and March 2011. Water samples were filtered using acid-cleaned polypropylene syringes and Whatman Puradisc 0.45-μm polypropylene syringe filters (Shiller 2003) and stored on ice or refrigerated until overnight shipment and analysis at the Center for Trace Analysis, University of Southern Mississippi. In the laboratory, water samples were acidified to pH 1.8 using ultrapure (Seastar Basline) HCl and allowed to sit acidified for at least one week before analysis. Samples were then diluted 11x in ultrapure (Seastar Baseline) 0.16 M HNO_3. The nitric acid contained 2 ppb scandium, indium, and thorium as internal standards. External certified reference standards were also prepared using the same HNO_3 used for sample dilutions. Samples were analyzed for ^{44}Ca and ^{88}Sr in medium resolution using a Thermo-Finnigan Element 2 inductively coupled plasma mass spectrometer (ICPMS). Precision of analyses based on repeated measurements of standards was better than ±2% (2 SD). Feed samples were ground into a fine powder using a mortar and pestle and stored refrigerated prior to analysis. Ground feed samples were dissolved in a small quantity of ultrapure concentrated HNO_3, diluted to the same acid strength as for the water samples, and analyzed for ^{44}Ca and ^{88}Sr using methods described above for water samples. Measurements of the spiked indium internal standard were consistent among standards, dissolved fish feed samples, and water samples, suggesting minimal matrix effect on measurement of Sr and Ca in

feed samples. Elemental concentration data for water and feed samples were converted to Sr:Ca ratios (mmol/mol).

The leading pelvic fin ray from each fish was embedded in epoxy and sectioned at the articulating process (the widest portion at the base of the fin ray) using a Buehler ISOMET^{TM} low-speed saw (Buehler Inc., Lake Bluff, IL, USA). Pelvic fin ray sections were prepared for analysis of Sr:Ca under a class 100 laminar flow hood and handled with non-metallic acid-washed forceps. Fin ray sections were mounted on acid-washed glass slides using double-sided tape, ultrasonically cleaned for 5 min in ultrapure water, and dried for 24 h under the laminar flow hood. Mounted and cleaned pelvic fin ray sections were stored in acid washed polypropylene Petri dishes in a sealed container until analyses. Pelvic fin ray sections were analyzed for ^{88}Sr and ^{43}Ca using a Perkin-Elmer DRC II ICPMS coupled with a CETAC Technologies LSX-500 laser ablation system. A transect was laser ablated along the long axis of the pelvic fin ray from the core to the edge of the fin ray (beam diameter = 25 μm, scan rate = 5 μm/s, laser pulse rate = 10 Hz, laser energy level = 9 mJ, wavelength = 266 nm, 7 data points per second). A standard developed by the U. S. Geological Survey (MACS-1, $CaCO_3$ matrix) was analyzed by laser ablation every 12-15 samples to adjust for possible instrument drift; several previous studies investigating fin ray microchemistry have used glass or $CaCO_3$ standards (Clarke *et al*. 2007; Allen *et al.* 2009; Smith & Whitledge 2011; Phelps *et al.* 2012; Woodcock *et al.* 2013), although these are not perfectly matrix-matched with fin rays. Each sample was preceded by a 60 second gas blank measurement. Isotopic counts were converted to elemental concentrations (μg/g) after correction for gas blank and drift effects. Strontium concentrations were normalized to Ca concentration based on the consideration of Ca as a pseudo-internal standard (Clarke *et al*. 2007; Allen *et al.* 2009; Phelps *et al.* 2012). Calcium concentration (μg/g)

was set at 27% based on previous research investigating fin ray microchemistry (Veinott *et al.* 1999; Allen *et al.* 2009; Phelps *et al.* 2012). Mean limit of detection for Sr was 0.06 μg/g; concentrations of Sr in all pelvic fin rays were well above the detection limit. Strontium and Ca concentrations were used to calculate molar Sr:Ca ratios (μmol/mol). Elemental concentrations for the pelvic fin ray core (reflecting a fish's early life history) and edge (reflecting a fish's most recent environmental history) were calculated for each adult fish from integrations over the first 10 μm and last 35 μm of laser transects, respectively. Elemental concentrations were integrated over the entire laser transect for juvenile fish from JWFH.

A two-sample t-test was used to assess whether mean pelvic fin ray Sr:Ca for juvenile muskellunge from JWFH was significantly different from the mean pelvic fin ray core Sr:Ca value of known-stocked, adult muskellunge collected from Illinois lakes. Kruskal-Wallis one-way analysis of variance was used to test for a significant difference in mean pelvic fin ray core Sr:Ca of known-stocked, adult muskellunge from Illinois lakes among years in which fish were stocked (2005, 2006, 2007, and 2008). The relationship between lake water Sr:Ca and pelvic fin ray edge Sr:Ca for adult muskellunge captured from Illinois lakes and Elk Lake, MN was characterized using least-squares linear regression. To determine the accuracy with which individual, known-stocked adult muskellunge could be identified as hatchery-origin fish using fin ray core Sr:Ca, mean (± 3 standard deviations) pelvic fin ray Sr:Ca was calculated for fish obtained from JWFH and compared with fin ray core Sr:Ca for each known-stocked fish collected from Illinois lakes. Mean fin ray Sr:Ca ± 3 SD for fish obtained from JWFH was chosen to approximate the upper and lower limits of expected fin ray Sr:Ca for individual, hatchery-origin fish. Known-stocked adult muskellunge with pelvic fin ray core Sr:Ca values within ± 3 SD of the mean fin ray Sr:Ca of hatchery fish (hereafter referred to as the "hatchery

signature") were considered to be correctly identified as hatchery-origin and the percentage of correctly-classified individuals was determined. For unmarked adult muskellunge collected from Illinois lakes (which may be stocked or wild fish), pelvic fin ray core Sr:Ca was used to classify individual fish as being of hatchery reared (fin ray core Sr:Ca consistent with hatchery signature) or potentially naturally reproduced (fin ray core Sr:Ca outside of the defined hatchery signature limits). All statistical analyses were performed using SAS 9.2 (SAS Institute, Inc., Cary, North Carolina). *P*-values ≤ 0.05 were considered significant for all statistical tests.

Results

A broad range of water Sr:Ca was observed among individual sites, with JWFH, North Spring Lake, Lake Mingo, Shabbona Lake, Pierce Lake, and Elk Lake, MN having water Sr:Ca values ranging from 0.45-1.05 mmol/mol, while Kinkaid Lake and Sam Dale Lake had water Sr:Ca > 1.50 mmol/mol (Table 1). The feed sample from JWFH had a Sr:Ca value of 2.23 mmol/mol. A total of 19 known-stocked adult muskellunge were collected from four lakes in Illinois, including Kinkaid Lake (n = 3), Lake Mingo (n = 5), Pierce Lake (n = 5) and Sam Dale Lake (n = 6). There was no significant difference between mean pelvic fin ray Sr:Ca for juvenile fish from JWFH (301.8 μmol/mol ± 5.7 μmol/mol SE; n=10) and mean fin ray core Sr:Ca for known-stocked adult muskellunge (287.2 μmol/mol ± 7.9 μmol/mol SE) captured from Illinois lakes ($t = 1.26$; d.f. = 27; $P = 0.22$). No significant difference in mean pelvic fin ray core Sr:Ca of known-stocked muskellunge among the four stocking years (2005-2008) was detected ($\chi^2 = 7.80$; d.f. = 3; $P = 0.0503$; Fig. 1). Mean fin ray core Sr:Ca values of hatchery-origin adult muskellunge stocked during 2005, 2006, 2007, and 2008 all fell within 3 SD of mean pelvic fin ray Sr:Ca of juvenile muskellunge obtained from JWFH (Fig. 1). Pelvic fin ray edge Sr:Ca

values of adult muskellunge captured from Illinois lakes and Elk Lake, MN were strongly correlated with water Sr:Ca values ($r^2 = 0.80$; $P = 0.0067$; Fig. 2). However, mean fin ray Sr:Ca for juvenile muskellunge from JWFH plotted well above the regression line relating water and fin ray edge Sr:Ca developed using data from lake-caught adult muskellunge (Fig. 2).

Pelvic fin ray core Sr:Ca values of known-stocked, adult muskellunge collected from Illinois lakes ranged from 229-347 μmol/mol. Mean SD of Sr:Ca measurements within fin ray cores (first 10 μm of laser transect) of individual fish was 17.75 μmol/mol. Sixteen of the 19 known-stocked fish (84%) had fin ray core Sr:Ca values that fell within the hatchery signature (247-356 μmol/mol) defined by the mean ± 3 SD of fin ray Sr:Ca of fish from JWFH (Figs. 3 and 4), and three known-stocked fish (one each from Kinkaid Lake, Pierce Lake, and Sam Dale Lake) had pelvic fin ray core Sr:Ca values that fell outside of the JWFH Sr:Ca signature (242.8, 228.5, and 229.5 respectively). Seventeen unknown-origin adult muskellunge were collected from Kinkaid Lake, of which three fish (18%) had pelvic fin ray core Sr:Ca values lower than the JWFH Sr:Ca signature (205.4, 194.6, and 191.3, respectively; Fig. 5a). Nine unknown-origin muskellunge were collected from North Spring Lake, of which three fish (33%) had pelvic fin ray core Sr:Ca values lower than the JWFH Sr:Ca signature (142.9, 118.2, and 103.5 respectively; Fig. 5b). All unknown-origin fish from Pierce Lake (n = 2) and Shabbona Lake (n = 1) had pelvic fin ray core Sr:Ca values that were within 3 SD of mean fin ray Sr:Ca for juvenile muskellunge from JWFH (Fig. 5c).

Discussion

Results indicated that known-stocked muskellunge could be identified as JWFH-reared individuals with a high degree of accuracy (84%) based on pelvic fin ray core (first 10 μm of

laser ablation transect) Sr:Ca values. Three known-stocked individuals were not classified as being of JWFH origin, although the Sr:Ca values of the first 5 μm of the laser ablation transect near the fin ray core for each of these individuals were within the range of the JWFH Sr:Ca signature. This finding suggests that only the outer portion of the fin ray core bearing the hatchery signature may have been sampled during the ablation process for these particular fish due to imprecise placement of the starting point for the laser ablation transect. More thorough sampling of the fin ray core with a pattern of laser-ablated spots or a raster would likely increase the probability of detecting the hatchery Sr:Ca signature in the fin ray core. Alternatively, it is possible that some reabsorption of fin ray material may have occurred, which can happen during periods of stress (Veinott & Evans 1999). Inclusion of additional intrinsic chemical markers (e.g., Ba:Ca, $\delta^{34}S$, or $^{87}Sr/^{86}Sr$) may also potentially improve detection rates of the hatchery signature in fin ray cores (Kennedy *et al.* 2002; Bickford & Hannigan 2005; Coghlan *et al.* 2007; Gibson-Reinemer *et al.* 2009; Johnson *et al.* 2012). Nevertheless, the results of this study are consistent with previous investigations which demonstrated that otolith Sr:Ca (and other naturally-occurring markers) can be used to identify hatchery-reared individuals with a high degree of accuracy in populations consisting of hatchery-origin and wild fish (Bickford & Hannigan 2005; Zitek *et al*. 2010). Results of this study indicate that fin rays are a suitable non-lethal alternative to otoliths for identifying stocked fish pending consistent differences in Sr:Ca signatures imparted to the fin ray during the period of hatchery residency and by the environment into which the fish is stocked.

Fin ray edge Sr:Ca values of lake-resident muskellunge were strongly correlated with corresponding water values and reflected differences in water Sr:Ca among all lakes sampled. Strong correlations between environmental water Sr:Ca and fish hard-part structure Sr:Ca are

consistent with previous research investigating fin rays (Veinott *et al*. 1999; Clarke *et al*. 2007; Smith & Whitledge 2011; Phelps *et al*. 2012) and otoliths (Wells *et al*. 2003; Whitledge *et al*. 2007; Zeigler & Whitledge 2010; Smith & Whitledge 2011; Zeigler & Whitledge 2011). However, fin ray edge Sr:Ca values for juvenile muskellunge obtained from JWFH were much higher than would be expected based on hatchery water Sr:Ca and deviated strongly from the regression line relating water Sr:Ca and fin ray Sr:Ca for lake-resident muskellunge. We postulate that muskellunge incorporate Sr into their fin rays from a combination of dietary sources and environmental water. Juvenile fish from JWFH were fed a commercial pellet feed consisting primarily of marine-derived fish meal and oils, which likely accounts for the high Sr:Ca value (2.23 mmol/mol) of the feed (Gibson-Reinemer *et al*. 2009). Thus, a substantial contribution of dietary Sr to fin rays is likely responsible for the relatively high fin ray Sr:Ca values observed in fish obtained from JWFH. In contrast, fin ray edge Sr:Ca values from adult, lake-resident fish were strongly correlated with lake water Sr:Ca because their diet (prey fishes) was also likely reflecting Sr:Ca of the lake environment. Published studies have indicated that >80% of Sr and Ba in fish otoliths is derived from environmental water, with the remainder derived from diet (Walther & Thorrold 2006; Gibson-Reinemer *et al*. 2009). Elevated dietary Sr concentrations lead to elevated Sr concentrations in otoliths (Limburg 1995; Buckel *et al.* 2004). For example, Limburg (1995) found that otolith Sr:Ca increased significantly in American shad *Alosa sapidissima* Wilson after switching from a freshwater zooplankton diet to a high Sr:Ca marine fishmeal diet. Although dietary influence on fin ray Sr has not been investigated, a recent study by Woodcock *et al*. (2013) indicated that dietary Ba was incorporated into fin rays of juvenile red drum *Sciaenops ocellatus* Linnaeus in sufficient amounts to detect differences in $^{138}Ba/^{137}Ba$ between fish fed with diets enriched with 0.25 μg ^{137}Ba/g diet and fish fed a control

diet. Previous research by Bath *et al*. (2000) and Wells *et al*. (2000) indicates that sources and factors influencing deposition of Ba and Sr in fish hard-part structures are similar, suggesting that incorporation of dietary Sr is likely responsible for the elevated fin ray core Sr:Ca values in muskellunge from JWFH. Additional research should be conducted to quantify the relative contributions of environmental water and diet to fin ray Sr and other commonly-applied elemental markers of fish environmental history using isotopically distinct water and feed, as has been done for otoliths (Walther and Thorrold 2006).

Regardless of the mechanisms responsible for elevated fin ray core Sr:Ca values in JWFH-reared individuals, a unique signature was present that identified hatchery-origin fish and could potentially be used to detect naturally-reproduced muskellunge in Illinois lakes. Data from this study suggest that a limited number of individuals collected from Kinkaid Lake and North Spring Lake may have been wild fish based on fin ray core Sr:Ca values well below the JWFH signature, although natural reproduction of muskellunge has not been documented in these systems (Smith 1979; S. Hirst and W. Herndon, personal communication). Natural reproduction is theoretically possible in both lakes, as individuals produce sufficient gametes for reproduction (e.g., North Spring Lake serves as a brood-stock collection lake for JWFH), and fish are often observed exhibiting behaviors and habitat use suggestive of spawning activity (S. Hirst, personal communication). Although fin ray Sr:Ca data suggest the presence of naturally-reproduced muskellunge in Kinkaid and North Spring lakes, potential misidentification of naturally-reproduced fish may have occurred due to several factors. Individuals classified as naturally reproduced may have been due to loss of the JWFH Sr:Ca signature by reabsorption of fin ray material (Veinott *et al*. 1999); however, data from known-stocked individuals indicate that the JWFH Sr:Ca signature can persist for at least seven years. Fish identified as potentially naturally

reproduced were all ≤ age-7 in Kinkaid Lake (with the exception of one ~age-11 female) and ≤ age-4 in North Spring Lake, so it is unlikely that all of these relatively young fish reabsorbed all of their fin ray cores. Additional evaluation of fin ray microchemistry using known-age, known-stocked muskellunge > age-7 would be valuable to assess persistence of the hatchery signature in older fish. It is doubtful that Sr:Ca signatures of JWFH juveniles changed over the course of the study and resulted in incorrectly identifying naturally-reproduced fish, as data indicated that the JWFH Sr:Ca signature was similar among fish known to have been reared at JWFH during different years (2005-2008) across all six Illinois lakes sampled. Furthermore, the diet and rearing practices for JWFH muskellunge remained consistent over the course of this study (S. Kreuger, personal communication). It is also unlikely that inter-annual variation in environmental water Sr:Ca signatures contributed to erroneous identification of naturally-reproduced fish, as water Sr:Ca data for North Spring Lake and Kinkaid Lake were consistent with prior water Sr:Ca data (Zeigler & Whitledge 2010). In addition, fin ray Sr:Ca values in the portion of laser-ablation transects distal to the pronounced drop in Sr:Ca from the hatchery signature in samples from known-stocked fish were relatively stable across multiple annuli and consistently much lower than the JWFH signature, indicative of sufficient inter-annual stability in lake-specific Sr:Ca signatures that would enable distinction of naturally-reproduced and stocked fish.

The high degree of accuracy with which JWFH-reared muskellunge could be identified in lakes throughout Illinois using non-lethal sampling of pelvic fin ray Sr:Ca signatures demonstrates the potential applicability of this technique for identifying wild or hatchery-reared muskellunge in systems where differences in water or food Sr:Ca result in distinct fin ray Sr:Ca between fish that reside in the hatchery and at stocking location(s). Fin ray and fin spine

chemistry has been successfully applied to reconstruct environmental history of individuals for a variety of fish species (Veinott *et al*. 1999; Clarke *et al*. 2007; Allen *et al*. 2009; Smith & Whitledge 2010, 2011; Phelps *et al*. 2012), but has not been previously used to distinguish between hatchery-reared and wild fish. Application of this technique may help fisheries professionals non-lethally document the presence of natural reproduction and estimate the contributions of supplemental stocking to populations of wild fishes. Knowledge of the extent to which fish populations are supported by natural reproduction is important because unnecessary supplemental stocking may have negative effects on the genetic legacy of native, naturally-reproducing muskellunge strains (Miller *et al*. 2009; Jennings *et al*. 2010; Miller *et al*. 2012), may potentially artificially inflate predator densities (Bozek *et al*. 1999), and may be not be cost effective, as stocking on top of naturally reproducing populations is often ineffective at increasing recruitment and adult population density (Laarman 1978; Jennings *et al*. 2005). In addition to assessing the degree to which muskellunge populations are supported by natural reproduction versus stocking, fin ray microchemistry may also prove valuable for assessing environmental history of muskellunge in lake and river systems where spatial differences in water Sr:Ca exist, provided that fish reside in chemically-distinct environments for a sufficient period of time to incorporate detectable shifts in fin ray Sr:Ca..

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Table 1. Location (latitude and longitude) of sites where muskellunge and water samples were collected and water Sr:Ca (mmol/mol) at the time of fish collection at each lake site. Water Sr:Ca for Jake Wolf Fish Hatchery is the mean (SE in parentheses) of samples (n=2) taken during March 2010 and March 2011.

Site	Location	Water Sr:Ca
Jake Wolf Fish Hatchery, IL	40° 25' 47.94" N; 89° 53' 33.49" W	0.46 (0.02)
Kinkaid Lake, IL	37° 47' 54.10" N; 89° 24' 59.81" W	1.75
Lake Mingo, IL	40° 12' 34.59" N; 87° 43' 41.16" W	0.64
North Spring Lake, IL	40° 28' 17.70" N; 89° 51' 49.72" W	0.45
Pierce Lake, IL	42° 20' 42.42" N; 88° 59' 00.14" W	0.84
Sam Dale Lake, IL	38° 32' 29.25" N; 88° 35' 01.31" W	1.55
Shabbona Lake, IL	41° 44' 48.27" N; 88° 51' 37.48" W	0.95
Elk Lake, MN	47° 11' 34.15" N; 95° 13' 15.90" W	1.03

Figure Captions

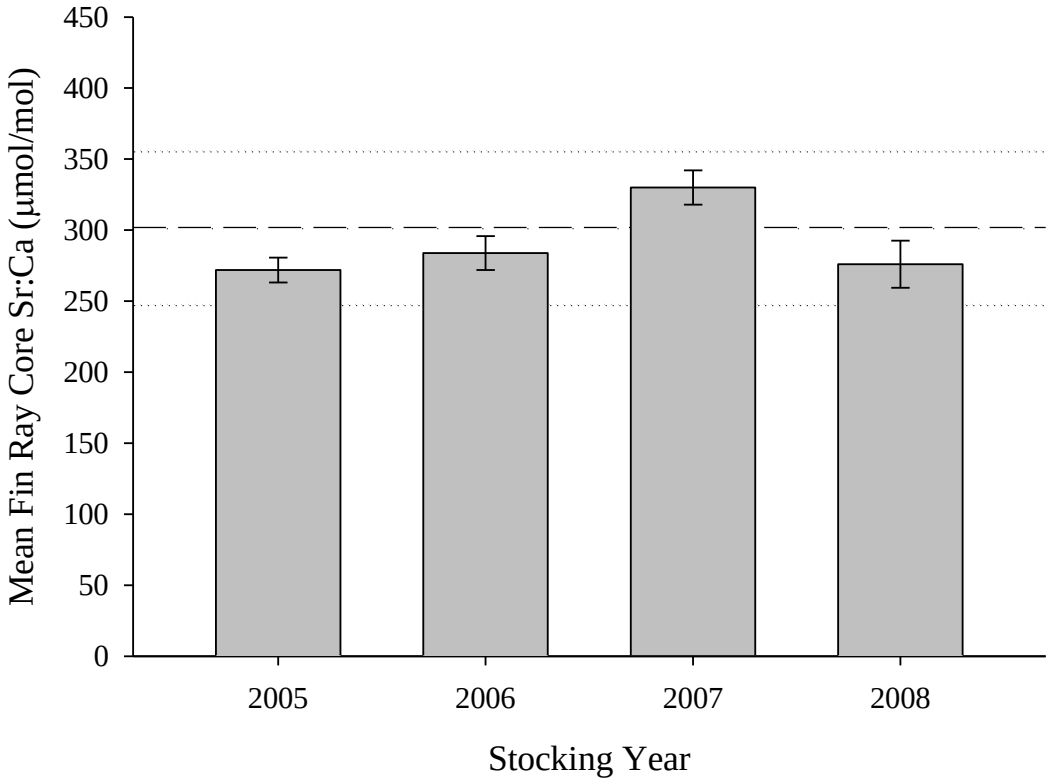
Figure 1. Mean pelvic fin ray core Sr:Ca (± SE) by year of stocking for adult muskellunge raised at Jake Wolf Fish Hatchery (JWFH) and stocked into Illinois lakes during 2005 (n = 5), 2006 (n = 6), 2007 (n = 4), and 2008 (n = 4). All fish were known to have originated from JWFH based on tags or marks applied at the time of stocking. Dashed and dotted horizontal lines indicate mean ± 3 SD of pelvic fin ray Sr:Ca for juvenile muskellunge obtained from JWFH in 2010.

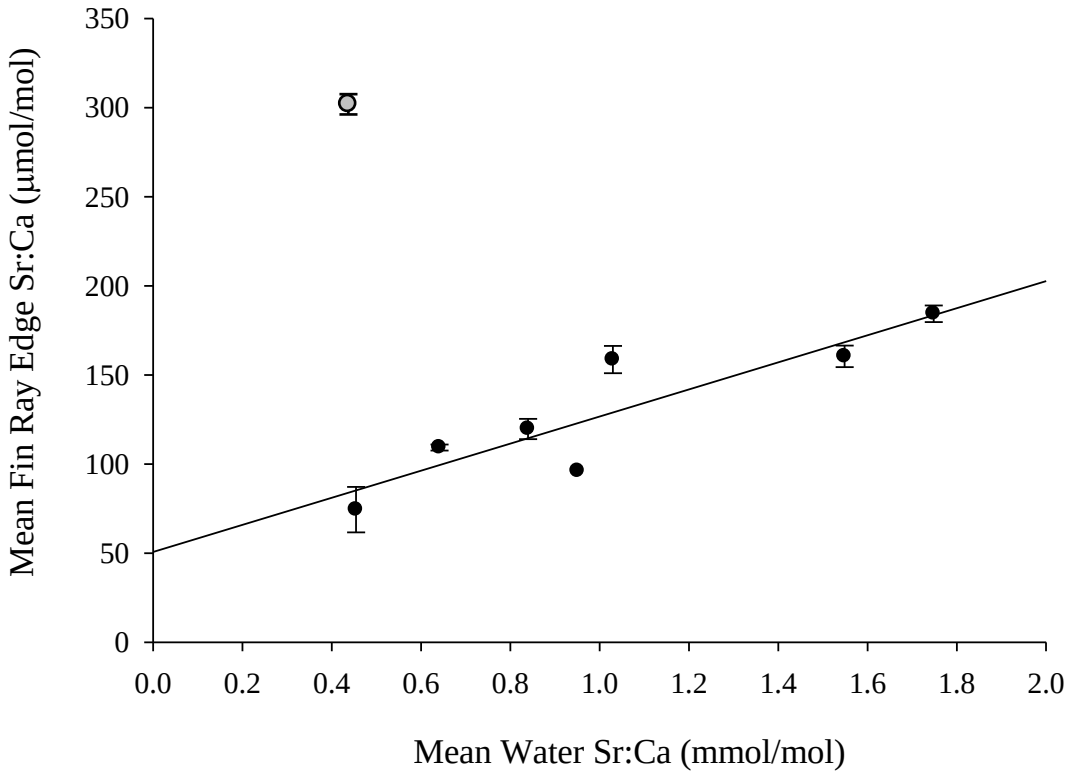
Figure 2. Relationship between water Sr:Ca and mean pelvic fin ray edge Sr:Ca (± SE) for adult muskellunge (both known-stocked and unknown origin) collected from Illinois lakes and Elk Lake, MN. Solid line indicates least-squares linear regression fit to data ($y = 75.99 x + 50.64$). Gray shaded point indicates mean fin ray Sr:Ca (± SE) of juvenile muskellunge obtained from JWFH for comparison (not included in regression).

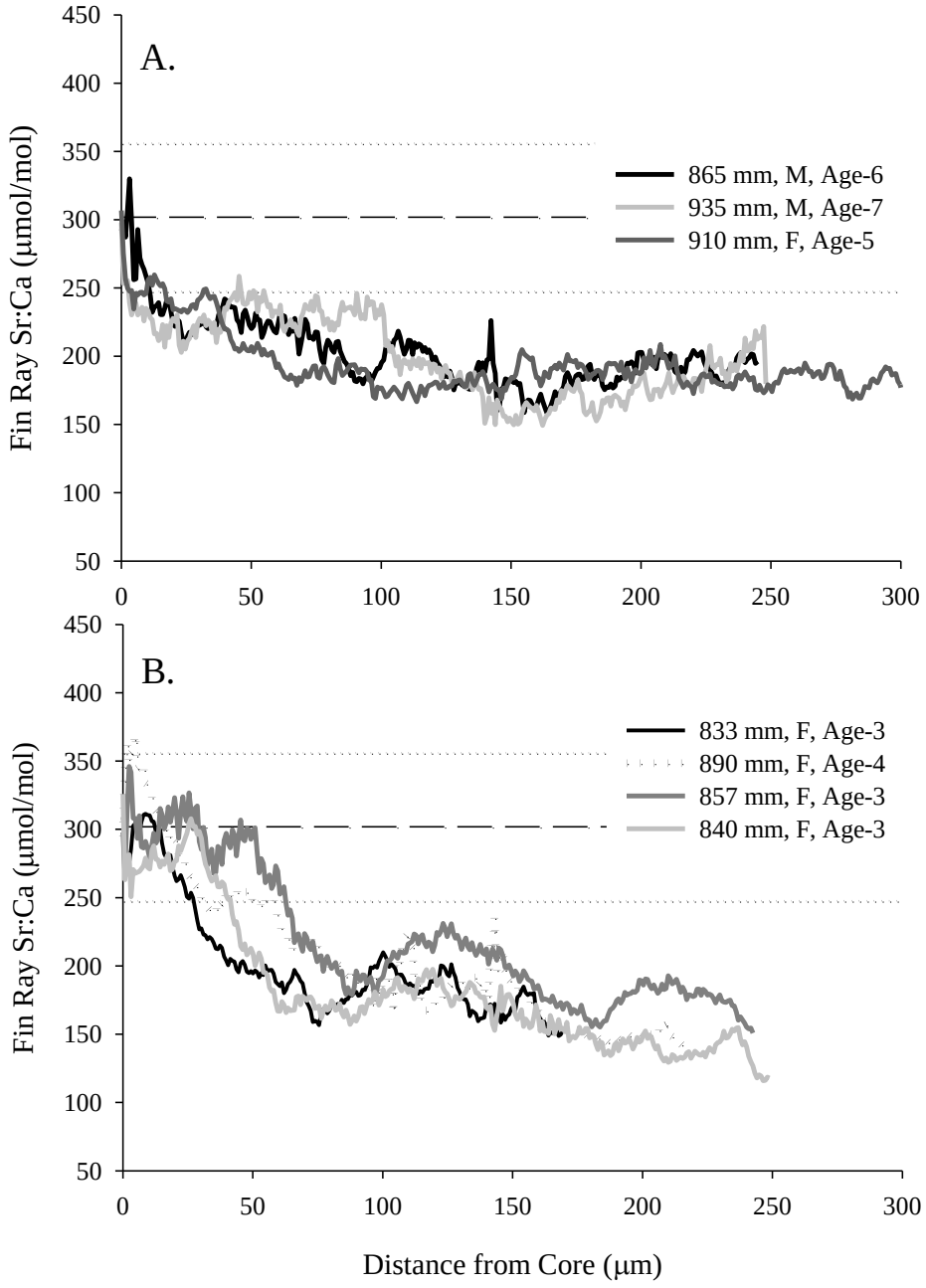
Figure 3. Patterns of Sr:Ca along laser-ablated transects from core to edge of sectioned adult muskellunge pelvic fin rays for representative known-stocked, known-age fish collected from Kinkaid Lake (panel A) and Sam Dale Lake (panel B). Total length at capture, sex, and age are shown for each fish. Dashed and dotted horizontal lines indicate mean ± 3 SD of pelvic fin ray Sr:Ca for juvenile muskellunge from JWFH.

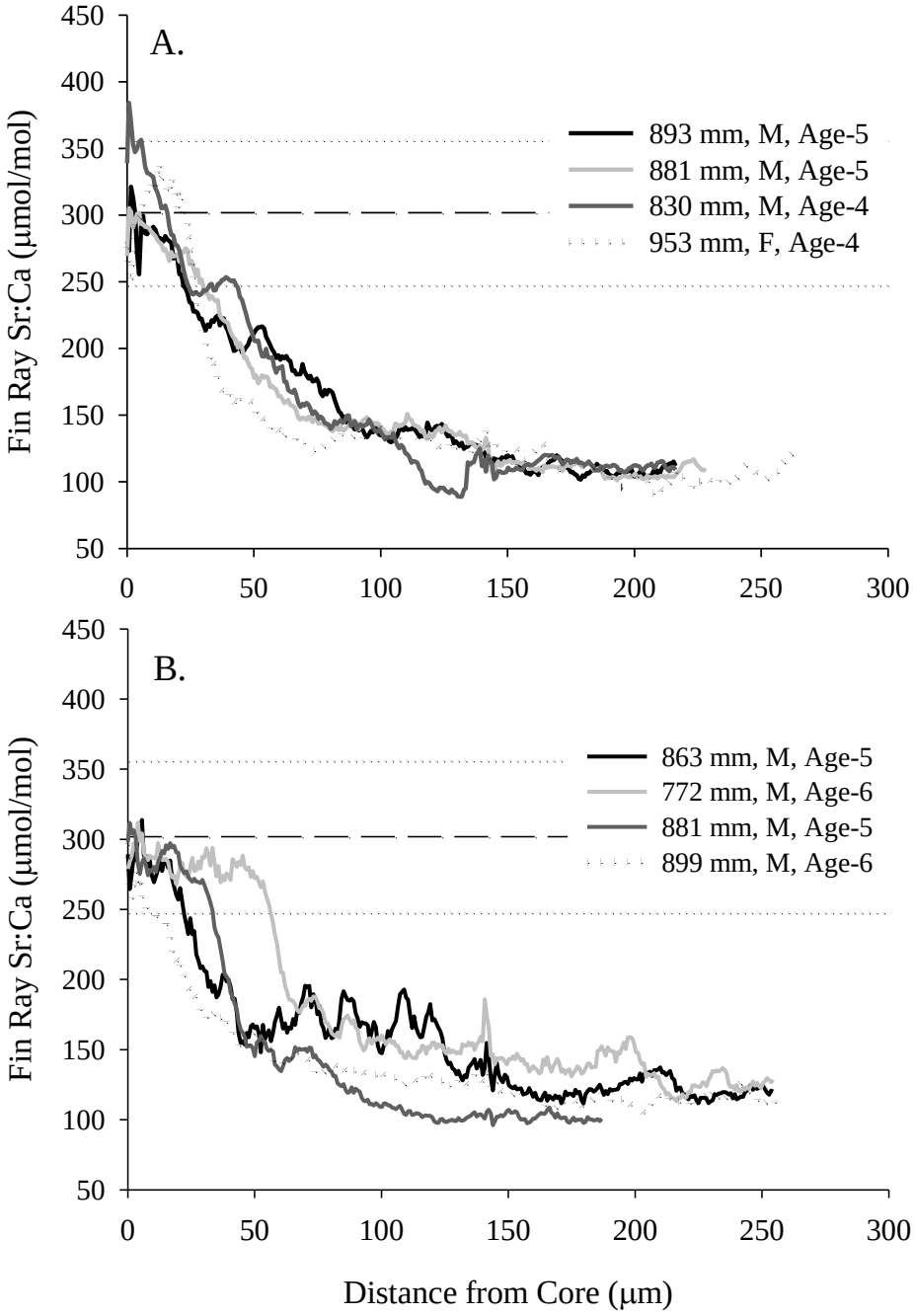
Figure 4. Patterns of Sr:Ca along laser-ablated transects from core to edge of sectioned adult muskellunge pelvic fin rays for representative known-stocked, known-age fish collected from Lake Mingo (panel A) and Pierce Lake (panel B). Total length at capture, sex, and age are shown for each fish. Dashed and dotted horizontal lines indicate mean ± 3 SD of pelvic fin ray Sr:Ca for juvenile muskellunge from JWFH.

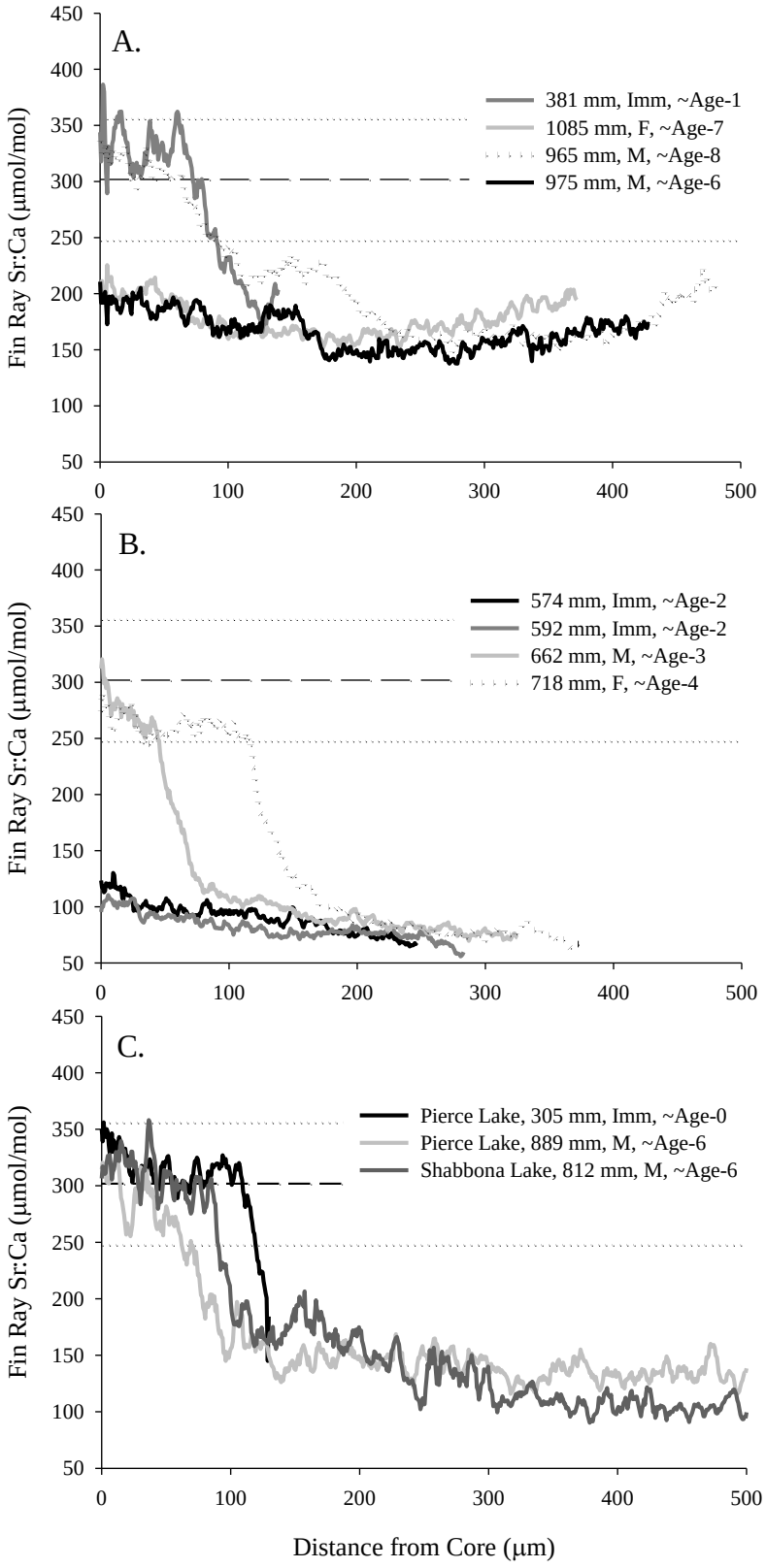
Figure 5. Patterns of Sr:Ca along laser-ablated transects from core to edge of sectioned adult muskellunge pelvic fin rays for representative unknown-origin fish collected from Kinkaid Lake (panel A), North Spring Lake (panel B), and Pierce and Shabbona lakes (panel C). Total length at capture, sex (M=male, F=female, IMM=immature), and estimated age are shown for each fish. Dashed and dotted horizontal lines indicate mean ± 3 SD of pelvic fin ray Sr:Ca for juvenile muskellunge from JWFH.


Mean Fin Ray Core Sr:Ca (µmol/mol)
450
400
350
300
250
200
150
100
50
0
2005
2006
2007
2008
Stocking Year


Mean Fin Ray Edge Sr:Ca (µmol/mol)
350
300
250
200
150
100
50
0
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
Mean Water Sr:Ca (mmol/mol)


A.
B.
865 mm, M, Age-6
935 mm, M, Age-7
910 mm, F, Age-5
833 mm, F, Age-3
890 mm, F, Age-4
857 mm, F, Age-3
840 mm, F, Age-3
Fin Ray Sr:Ca (µmol/mol)
Distance from Core (µm)
450
400
350
300
250
200
150
100
50
0
50
100
150
200
250
300


A.
893 mm, M, Age-5
881 mm, M, Age-5
830 mm, M, Age-4
953 mm, F, Age-4
Fin Ray Sr:Ca (µmol/mol)
450
400
350
300
250
200
150
100
50
0
50
100
150
200
250
300
B.
863 mm, M, Age-5
772 mm, M, Age-6
881 mm, M, Age-5
899 mm, M, Age-6
Fin Ray Sr:Ca (µmol/mol)
Distance from Core (µm)


A.
381 mm, Imm, ~Age-1
1085 mm, F, ~Age-7
965 mm, M, ~Age-8
975 mm, M, ~Age-6
B.
574 mm, Imm, ~Age-2
592 mm, Imm, ~Age-2
662 mm, M, ~Age-3
718 mm, F, ~Age-4
C.
Pierce Lake, 305 mm, Imm, ~Age-0
Pierce Lake, 889 mm, M, ~Age-6
Shabbona Lake, 812 mm, M, ~Age-6
Fin Ray Sr:Ca (µmol/mol)
Distance from Core (µm)
50
100
150
200
250
300
350
400
450
0
100
200
300
400
500