Environ Biol Fish (2012) 95:191–194
DOI 10.1007/s10641-012-9979-3

Using energy dispersive x-ray fluorescence microchemistry to infer migratory life history of Atlantic sturgeon

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Received: 3 May 2011 / Accepted: 9 January 2012 / Published online: 26 January 2012

Abstract Atlantic sturgeon migrate between ocean and freshwater habitats to spawn, and juveniles spend several years in fresh/brackish water before returning to the ocean. Because strontium/calcium (Sr/Ca) ratios are diagnostic for freshwater and marine environments, we examined the utility of energy-dispersive x-ray fluorescence (EDXRF) to quantify Sr/Ca ratios of Atlantic sturgeon pectoral fin spines. Atlantic sturgeon spines from wild adults and experimental juveniles were analyzed along a linear transect from the primordium to the outermost point. To verify the technique hatchery juvenile Atlantic sturgeon were held in experimental tanks at <0.5, 13–15, or 33–35‰ and sampled after 5 months. Sr/Ca ratios of experimental hatchery fish increased with salinity, and Sr/Ca ratios in wild adults varied predictably along the measurement transect. However, the ratio decreased in the outermost region of the spine in mature fish collected during a return to freshwater for spawning. Therefore EDXRF is a useful tool to track individual movements of Atlantic sturgeons and other diadromous fish.

Keywords Atlantic sturgeon · Sturgeon spine · Sr/Ca ratio · Diadromy · EDXRF

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Introduction

Strontium/calcium (Sr/Ca) ratios of selected biogenic tissues (e.g. otoliths) are used to infer migration patterns between marine and freshwater environments for many fish species using laser ablation or wavelength dispersive microprobe analysis (Limburg 1995; Secor et al. 1995; Arai and Tsukamoto 1998; Allen et al. 2009). Elemental composition of fish tissues may be used to discriminate between marine and freshwater populations (or life history stages), determine links between natal rivers or nursery areas and adult stocks, and assess population structure in marine fishes (Sauer and Watabe 1989; Secor and Rooker 2000; Kraus and Secor 2004; Limburg et al. 2007). Energy dispersive x-ray fluorescence (EDXRF) is proposed as an alternative method that allows non-destructive and accurate determination of elemental composition of biogenic materials (Paiva et al. 1997; Lundblad et al. 2008). An EDXRF system works by detecting the

x-ray energy released due to electrons changing shell layers of an atom after it has been excited by an x-ray laser. A silicon detector is used to determine the amount of energy released which is known for most elements and therefore percent mass of elements in an area can be quantified.

Atlantic sturgeon (*Acipenser oxyrinchus oxyrinchus*) is an anadromous, long-lived (60+ y), iteroparous, historically fishery-targeted species, making it a good candidate for Sr/Ca analysis. Atlantic sturgeon populations are depleted in the United States (Atlantic Sturgeon Status Review Team 2007). A better understanding of migration patterns and life history may aid in management and recovery efforts.

We evaluated the use of EDXRF analysis of pectoral fin spines from Atlantic sturgeon to potentially provide migratory and life history information. The ratio of Sr to Ca is positively correlated with environmental salinity (Limburg 1995); we quantified this ratio as distance increases from the primordium in juveniles held under experimental salinity regimes and in wild adults during freshwater residence (Arai and Tsukamoto 1998; Secor and Rooker 2000).

Methods

We used 2 year old hatchery juvenile Atlantic sturgeon (295–340 mm fork length) to verify the instrument could detect variations of Sr/Ca ratios in the fish spines. The hatchery Atlantic sturgeon were acquired from the Maryland Department of Natural Resources Fisheries Division and held at the aquatics facility of Virginia Commonwealth University (VCU). The hatchery fish were maintained in freshwater prior to being moved to VCU and held at VCU for 3 months prior to salinity treatment. Richmond city water was filtered to remove chloramines, and salinity regimes were prepared with Instant Ocean© sea salt. Salinity was monitored using a refractometer (Bath et al. 2000). The ion ratio of Instant Ocean© sea salt mimics typical saltwater by 98.5% (US Aquatics Consumer Support). The three treatment tanks were maintained at identical temperature (16–18°C) and fed an identical diet (Zeigler Bros Inc. product # 306540-18-44) because both affect Sr/Ca uptake (Fowler et al. 1995; Secor et al. 1995; Gallahar and Kingsford 1996; Bath et al. 2000).

A section from the left leading fin spine was taken from each fish for pre-treatment analysis. The following day we measured fish for fork length and placed them in experimental tanks. The fish were separated into three treatment groups with three fish per treatment, freshwater (<0.5‰), brackish (13–15‰) or saltwater (33–35‰) tanks for 5 months. Fish were measured, and the right leading pectoral spine was removed (VCU IACUC AT20127) at the termination of the experiment.

Pectoral fin spines were removed from carcasses of 13 recently killed wild adult male Atlantic sturgeon found in September, 2008 and 2009 during a putative spawning period in the freshwater portion of the James River, Virginia. The carcasses were found by researchers examining the shoreline for Atlantic sturgeon mortalities. These fish were confirmed as adult males due to fork length measurement and having fully developed gonads.

A 2 mm thick section of the leading fin spine was cut within 1 cm of the articulation point with an isomet saw. A section from the left spine was used when available; however, the right spine was used if the left spine was not present. Extreme care was taken to insure the sample section was cut orthogonally. Soft tissue on the spine was removed with a fine brush. The spine was then rinsed with deionized water and air-dried.

Samples were analyzed for elemental analysis on a Horiba X-Ray Guide Tube XGT-7000V EDXRF microscope with 50 kV of energy at 1 mA with a 100 μm probe held under vacuum. Each sample point was ~100 μm in diameter. The machine was calibrated using protocols and samples provided by the microscope's manufacturer. To support the sample with minimal background, plastic wrap (Fisher Scientific) was placed on a flat stage with a 5 cm×5 cm hole in the middle of the stage. Two millimeter thick spine samples were attached to the plastic wrap using double sided tape. Analysis with a copper plate backing indicated 1.9 mm thick samples were sufficient to block the copper signature, i.e. laser excitations are restricted to the spine sample. Sampling points were measured equidistant along a linear transect across the spine section for 30 s per point, and each point measurement was repeated to verify precision (Fig. 1). For all samples extreme care was taken to ensure the most peripheral portion of the spine section was sampled. The Sr/Ca ratio from both transects was averaged for each point.

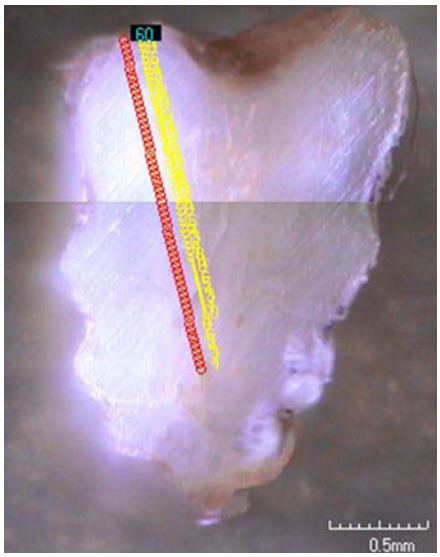

Fig. 1 Photograph of Sr/Ca analysis points on an Atlantic sturgeon pectoral spine. The red circles show loci where Sr/Ca was analyzed, and the yellow number (0–60 in this case) is the point number along the transect. Point one is at the primordium and point 60 is at the spine edge. Each point was measured twice and the average was used for analysis

Results

The salt and brackish water tanks had one fatality each leaving an $n=2$ for these treatments. After 5 months in the aquatic center, the average fork length of the hatchery fish increased 31 mm (23–50 mm). An ANOVA ($F=1.62$, $p=0.13$) indicated no significant difference in the Sr/Ca ratio among the pre-treatment samples (Fig. 2a). The ratios of freshwater-control fish stayed flat between 0.2×10^{-3} and 0.3×10^{-3} (Fig. 2b). In the salt and brackish treatments Sr/Ca values increased toward the edges of the spine and leveled off. The brackish water treatment maximum ratio was 0.9×10^{-3} (greater than 3× freshwater values), and the saltwater treatment maximum was 1.7×10^{-3} (a further doubling compared to brackish water). The lack of overlap in Sr/Ca ratios between treatments is a strong result and additional statistics are not necessary (Yoccuz 1991). Variation in the replicate point runs averaged 3.7% and ranged between 0 and 6% indicating reasonable precision.

The spines of all 13 wild fish had similar patterns, and transects from three representative individuals are shown in Fig. 2c. Mean Sr/Ca

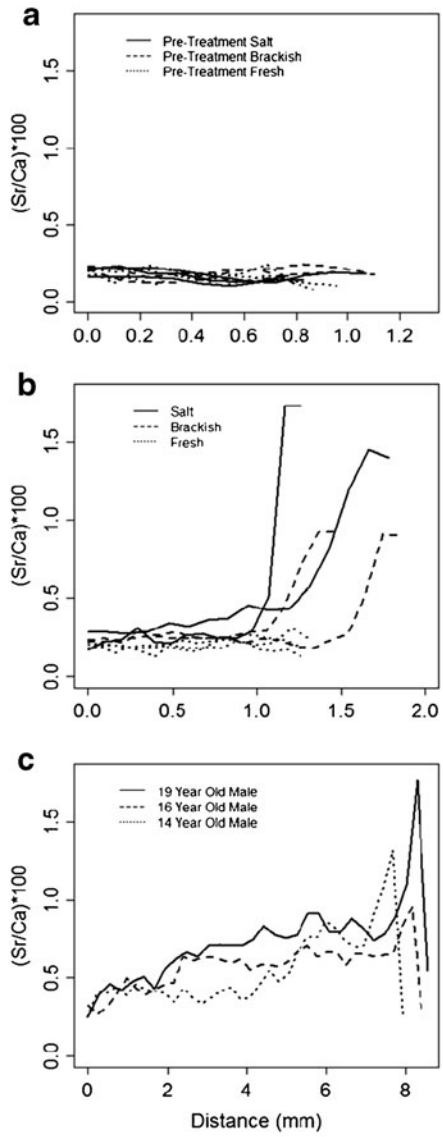

Fig. 2 The percent mass of Sr/Ca ratios at different distances from the primordium of Atlantic sturgeon spines. **a**. The percent mass of Sr/Ca ratios of experimental juveniles prior to salinity level treatment. **b**. The percent mass of Sr/Ca ratios of experimental juveniles maintained at different salinity levels. **c**. The percent mass of Sr/Ca ratios of three representative wild adult male Atlantic sturgeon captured in the James River

ratios in wild Atlantic sturgeon increased from 0.3×10^{-3} at the spine primordium to 1.5×10^{-3} at the periphery (paired $t_{12}=-16.0949$, $p<0.0005$). Ratios decreased at the outermost portion of the spine consistent with a return to a freshwater environment (Fig. 2c).

Discussion

This study successfully demonstrates the ability of EDXRF to indicate an ontogenetic change in Sr/Ca ratios of Atlantic sturgeon consistent with migration across an environmental salinity gradient. It has an advantage over other methods because it is non-destructive to the sample. However, similar results have been found in green sturgeon (*A. medirostris*) using laser ablation on spines and Russian sturgeon (*A. guldenstadi*) using wavelength dispersive x-ray electron microprobe analysis on otoliths (Arai and Miyazaki 2001; Allen et al. 2009). Ratios in experimental juveniles in this study increased with salinity indicating that 5 months and 23 mm of growth are sufficient for a salinity signature to imprint on an Atlantic sturgeon spine. By comparing annuli (Balazik et al. 2010) of the 13 wild fish with the position of increased Sr/Ca ratios our data suggest Atlantic sturgeon out migrate from natal rivers between 1 and 4 years of age. The ratio increased by age 4 for the 14 years old, by age 3 for the 16 years old, and by age 2 for the 19 years old (Fig. 2c). These findings agree with previous catch data on Atlantic sturgeon outmigration (Bain 1997; Kynard and Horgan 2001). Sr/Ca ratio decreased at the spine periphery in the three adult male samples collected in freshwater, indicating that water chemistry can be imprinted on the spine during inward migration. With increased sampling point density this new technique could be used to determine natal immigration to brackish environments, further movement from brackish to ocean environments and perhaps even spawning events.

Acknowledgements We thank the Virginia Atlantic sturgeon restoration team and the US Army Engineer Research and Development Center (ERDC) Geospatial Research and Engineering Division – Photonics Imaging and Spectroscopy Laboratory located at George Mason University. We thank Brianna Langford for laboratory assistance. This is VCU Rice Center contribution number 20.

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