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Assessing dorsal scute microchemistry for reconstruction of shortnose sturgeon life histories

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Abstract The imperiled status of sturgeons worldwide places priority on the identification and protection of critical habitats. We assessed the micro-structural and micro-chemical scope for a novel calcified structure, dorsal scutes, to be used for reconstruction of past habitat use and group separation in shortnose sturgeon (*Acipenser brevirostrum*). Dorsal scutes contained a dual-layered structure composed of a thin multi-layered translucent zone lying dorsally above a thicker multi-layered zone. Banding in the thick multi-layered zone correlated strongly with pectoral fin spine annuli supporting the presence of chronological structuring that could contain a chemical record of past environmental exposure. Trace element profiles (Sr:Ca), collected using both wavelength dispersive electron microprobe analysis and laser ablation inductively coupled mass spectrometry, suggest scutes record elemental information useful for tracing transitions between freshwater and marine environments. Moreover, mirror-image like Sr:Ca profiles were observed across the dual-zone structuring of the scute that may indicate duplication of the microchemical profile in a single structure. Additional element:calcium ratios measured in natal regions of dorsal scutes (Ba:Ca, Mg:Ca) suggest the potential for further refinement of techniques for identification of river systems of natal origin. In combination, our results provide proof of concept that dorsal scutes possess the necessary properties to be used as structures for reconstructions of past habitat use in sturgeons. Importantly, scutes may be collected non-lethally and with less injury than current structures, like otoliths and fin spines, affording an opportunity for broader application of microchemical techniques.

Keywords Acipenser · Sr:Ca · Trace elements · Habitat use · Natal origins

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Introduction

The depressed status of sturgeon species (Acipenseridae) throughout the world places great importance on understanding population structure, life history patterns and critical habitats (Birstein et al. 1997). These insights are often obtained by surveys in target habitats, active or passive telemetry and population genetic approaches. However, these approaches face important limitations for threatened and long-lived species, like sturgeon, that are often at low abundances, live in difficult to sample habitats, and range widely at some

life stages. The rarer or more dispersed a species, the more intensive field surveys must be to confidently ascertain presence or absence across the range of available habitats (Grogan and Boreman 1998; Pritt and Frimpong 2014). Telemetry can improve on this where individuals can be tracked as they move among habitats, but can be costly, present risks to individuals carrying tags (e.g., surgical injury or detection by predators), and be fundamentally constrained by limits of tag life and costs of deploying detection gear (Cooke et al. 2012; Heupel and Webber 2012). Population genetic approaches that might be effective in identifying large-scale and ancient patterns of genetic isolation and population structure often lack power to detect structure at finer spatial and temporal scales and do not provide direct insights into habitat associations (Campana and Thorrold 2001).

Microchemistry of calcified structures may provide complementary tools to address such limitations of standard approaches for species like sturgeon (Nelson et al. 2013). Microchemical patterns within calcified structures can often offer insight into past environmental exposures (Veinott et al. 1999; Elsdon and Gillanders 2003; Clarke et al. 2007). Changes in concentrations or ratios of elements in calcified structures have been used to identify the timing of important life history characteristics, such as first seawater entry in diadromous species (Limburg 1995; Allen et al. 2009). Difference in proportions of trace elements in natal growth regions of some calcified structures have been used to infer natal origins of fish species that range widely (Walther et al. 2008), but has only been applied to sturgeon in one study (Phelps et al. 2012). However, microchemistry of calcified structures has been historically constrained in fishes of high conservation concern due to reliance on structures that require lethal or injurious sampling. Hence, there is considerable need for identification and validation of alternative calcified structures that might permit broader use of microchemical approaches in such animals.

Saggital otoliths are the most commonly used microchemistry structure (Secor et al. 1991) for fish, but sample collection requires sacrificing the individual for dissection. Fin spines and scales can be collected non-lethally, and are gaining popularity for use in microchemical reconstructions in fishes (Wells et al. 2003; Clarke et al. 2007; Smith and Whitledge 2010; Woodcock and Walther 2014). However, concerns remain regarding the likelihood of fin spine sampling resulting in significant injury or secondary infections that compromise swimming performance or health in sturgeons and other fishes (Kahn and Mohead 2010; Baremore and Rosati 2014). As a consequence, sampling of otoliths and fin spines are often highly restricted when working with threatened or endangered fish species, including sturgeons. These sampling limitations are unfortunate because the limited microchemistry that has been conducted with sturgeon otoliths and fin spines have provided very useful insights into the life histories of these long-lived, extensively migratory and difficult to recapture fish. For example, using pectoral fin spine sections, Veinott et al. (1999) and Allen et al. (2009) characterized movements of white (*Acipenser transmontanus*) and green (*A. medirostris*) sturgeon respectively in association with transitions between fresh and marine environments. Saggital otoliths from sturgeon also appear to record past environmental exposure based on work conducted with Russian sturgeon (*A. guldenstadti*; Arai and Miyazaki 2001).

Scales have been used as a less injurious alternative to otoliths or fin spines for reconstruction of habitat use (Muhlfeld et al. 2005; Woodcock and Walther 2014). However, sturgeons, and a fair number of other fishes, lack typical bony scales (Helfman et al. 1997). Sturgeons do possess scutes, bony structures in the skin that share evolutionary and developmental homology with the scales of other fishes. Sturgeon scutes protrude through the integument and thus collecting material from scutes would not require sacrificing the fish, and it is possible, depending on project objectives and scute structure, that only a small portion of the scute might be required for microchemical reconstructions, avoiding significant injury to the integument. Hence, we hypothesized that these structures might provide an alternative to otoliths and fin spines for microchemical analysis. However, the use of scutes in this way this would depend upon a proof of concept that they possess the chronological and chemical properties required for such inference.

Sturgeons possess five rows of bony scutes that span the length of the body dorsally, laterally, and ventrally (Hilton et al. 2011). Scutes are formed in the dermis where osteoblasts deposit bone that is ossified beginning at the distal tips and ending proximally (Zhang et al. 2012). All scutes are present and ossified by 59 days post hatch in the dorsal row and 91 days post hatch in the ventral and lateral rows (Zhang et al. 2012). Previous descriptions of scute structure suggest they are composed of cellular, lamellar bone akin to dentine (Sire et al. 2009). Although sturgeon scutes have not been

validated as an ageing structure, the lamellar structure of sturgeon scutes has led others to propose that these layers may correspond to age (Brennan and Cailliet 1989; Jackson et al. 2007). Whether or not the lamellae directly correspond with annual age increments, the homology of sturgeon scutes with the scales of other fishes makes it likely these layers are deposited sequentially through time. However, this would not guarantee chronological preservation of water chemistry histories if scutes are for some reason more subject to tissue turnover than otoliths, fin spines, or scales.

The objective of this project was to examine whether dorsal scutes from shortnose sturgeon (*A. brevirostrum*) possess the necessary structural and chemical properties to permit reconstruction of past environmental exposures of individuals. We were specifically interested in evaluating 1) whether shortnose sturgeon scutes incorporate a chronological structure comparable to that observed in pectoral fin spines, an age validated structure in other sturgeons; 2) if this is associated with chemical chronologies that are indicative of residence or transitions between water sources, including the major life history transition between early juvenile freshwater habitats and marine environments; 3) the degree to which these chronologies are repeatable with respect to analysis by alternate instrumentation, and 4) the potential for elemental signatures incorporated during the juvenile period to distinguish and classify sturgeon based on their freshwater rearing source.

Methods

Sample sources

The endangered status of shortnose sturgeon restricted our samples to dorsal scutes and pectoral fin spines obtained opportunistically from wild and hatchery-reared shortnose sturgeon. Samples from wild sturgeon were taken from recovered mortalities in two Gulf of Maine (GoM) rivers, the Penobscot River (n=4) and Kennebec River (n=3) between 2006 and 2009. Dorsal scutes and pectoral fin spines from hatchery-reared shortnose sturgeon were obtained from individuals reared and euthanized at the U. S. Geological Survey Conte Anadromous Fish Research Center (CAFRC; n=5) in Turners Falls, Massachusetts (B. Kynard, pers. comm.). Shortnose sturgeon from the CAFRC used in this study were raised from fertilized eggs of Savannah River, Georgia, USA origin (B. Kynard personal communication). Once hatched, the early-life-stage sturgeon were used in ontogenetic behavioral research at the CAFRC (Parker and Kynard 2014) and upon completion of the research, held in round flow-through tanks on Connecticut River water (M. Kieffer, pers. comm.). A subsection of the dorsal scute row consisting of at least four to five consecutive scutes was collected from GoM shortnose sturgeon mortalities while the entire dorsal scute row was collected from hatchery-reared individuals. Entire right pectoral fins were collected from all GoM and hatchery-reared shortnose sturgeon. All dorsal scutes and pectoral fin spines from shortnose sturgeon were stored frozen until processing.

Sample ageing

We examined correspondence in growth zone counts between dorsal scutes and pectoral fin spines from the same individuals using an approach similar to Brennan and Cailliet (1989) and Jackson et al. (2007). The use of pectoral fin spine sections as ageing structures has been validated for other sturgeon species including lake sturgeon (Rossiter et al. 1995) and Atlantic sturgeon (Stevenson and Secor 1999). In shortnose sturgeon, marginal increment analysis showed seasonal periodicity in the timing of annulus formation and suggests that annulus deposition occurs yearly (Woodland and Secor 2007). Preparation for chronological comparisons required both dorsal scutes and fin spines be separated from surrounding tissue, cleaned, and thin sectioned. Dorsal scutes were removed from the surrounding tissue with a scalpel and cleaned manually along the underside and periphery. The scutes were then air dried for 1–2 days after which any remaining tissue and residue was removed with a battery powered rotary tool and steel bristled brush. The entire leading pectoral fin spine was separated from the rest of the pectoral fin and allowed to air dry in the lab. No further cleaning of pectoral fin spines occurred prior to thin sectioning.

Whole dorsal scutes were placed in rectangular silicone molds (1×5×1.25”, Ted Pella Inc.) and covered in clear epoxy (Struers Epofix, Cleveland, Ohio). Multiple thin sections (0.5 mm) were taken medially along the transverse plane of the epoxy mounted dorsal scutes using an Isomet low speed saw (Buehler, Lake Bluff, Illinois, USA) with a diamond wafering blade (Fig. 1). Dried whole pectoral fin spines were also thin sectioned multiple times at 0.5 mm intervals within 1.0 cm of the

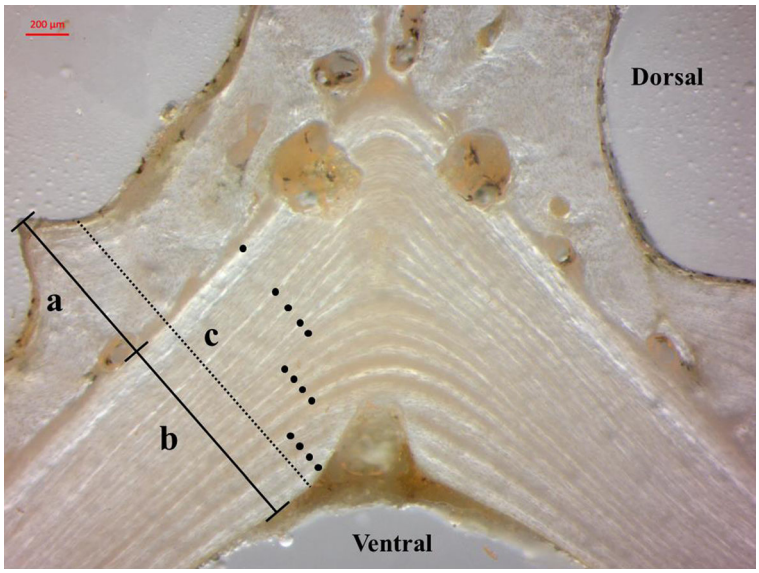

Fig. 1 Sectioned and polished dorsal scute from a wild shortnose sturgeon mortality. The structure of the scute appears dual-layered composed of a translucent thin zone (**a**) above thicker alternating translucent and opaque zones (**b**). Growth zones observed in zone b were enumerated (*single black circles*) and assumed annuli. The age estimated from this scute was 12+ years. The electron microprobe and laser ablation ICPMS profiles tracked perpendicularly through both zones (**c**)

articulating process. Sections of dorsal scutes and fin spines were lightly polished with dampened fine grit (1000 and 1500 grit) wet-dry sandpaper. One dorsal scute section and one pectoral fin spine section were chosen for further examination for each individual, based on initial observation of the apparent clarity of putative growth zones.

Dorsal scute thin sections from hatchery-reared individuals were not used for age comparisons with fin spines because all of these individuals were the same age and erosion of growth zones was apparent. Growth zone enumeration was carried out visually on tissues from wild fish with an Olympus SZ60 microscope using transmitted or reflected light under 30X magnification. Glycerin was applied to increase the clarity of putative growth zones when needed. Growth zones in dorsal scutes and pectoral fin spines were defined as a pair of translucent and opaque zones as observed in previous studies (Brennan and Cailliet 1989; Jackson et al. 2007). Each structure was aged twice by one reader, with no reference to origin, date of collection, or the previous age estimate (Woodland 2005). Growth zone counts for both structures were then regressed against each other to examine the linearity of the relationship between the two metrics and any potential bias from a 1:1 expectation.

Electron microprobe analysis

Following chronological comparisons, twelve dorsal scute sections and ten corresponding pectoral fin spine sections (six of seven GoM individuals and four of five CAFRC reared individuals) were prepared for electron microprobe analysis. Fewer pectoral fin spine sections were analyzed due to the amount of time required for each fin spine analysis (approximately 7 hours) and resources available. Individual sections of each structure were placed at the bottom of 2.54 cm round molds and backfilled with Epofix clear epoxy. Hardened round epoxy blocks containing the sample sections were then hand polished using dampened 800, 1000, and 1500 grit wet-dry sandpapers and brought to a mirror-like finish with 0.3 μm alumina paste and a jewelers polishing cloth.

The surface of all mounted and polished dorsal scutes and fin spines was cleaned with commercial 409 cleaner (The Chlorox Company, Oakland California) to remove any surface residue and dried with compressed air. The 409 cleaner would not supplement any of the elements of interest (see later sections) based on its chemical composition. Moreover, the elements of interest substitute for calcium in the bony matrix of calcified structures promoting resilience to handling effects (Proctor and Thresher 1998; Rooker et al. 2001). A thin carbon coating was applied to each mounted sample using an Emitech K950X high vacuum evaporator. Carbon coated samples were then secured in an aluminum sample boat six at a time and electrically grounded to the boat using small pieces of carbon tape. A wavelength dispersive electron microprobe (Cameca SX-100, University of Maine School of Earth and Climate Sciences, Orono,

Maine) was used to measure concentrations of Ca and Sr. Acceleration voltage was 15 keV and the electron beam was focused at 5 μm to conduct spot analyses every 10 μm along a digitized profile. The digitized profile began at the extreme dorsal portion of a scute and traveled ventrally (presumably towards the most recently formed material; Zhang et al. 2012) traversing the entire width of the structure perpendicular to the orientation of presumed zone formation (Fig. 1c). Digitized profiles in fin spine sections began in the center of the spine and traveled along the longest axis of growth. Individual strontium and calcium concentrations at each spot analysis were reported as mass percentages (e.g., mass percent of 10 % = 10^5 ppm) and the final ratio was reported as Sr:Ca ppm (10^3).

Laser ablation ICPMS

Twelve additional dorsal scute thin sections (collected from the same individuals and same scutes as those used in the microprobe analysis) were used for analysis by laser ablation inductively coupled mass spectrometry (ICPMS). These sections were cleaned with ethanol, rinsed in distilled water and allowed to air dry prior to storage in plastic bags. Laser ablation ICMPS sampling was carried out using a New Wave UP-193 laser ablation system coupled with a Perkin Elmer SCIEX quadropole ICPMS (State University of New York's College of Environmental Science and Forestry, Syracuse, New York; SUNY ESF). Dorsal scute sections were affixed to petrographic microscope slides with double-sided tape and secured to a sample carrier boat. Digitized laser ablation profile paths were defined on each scute section in a similar position and orientation to those carried out during electron microprobe analysis. Laser power was set at 70 % and background element intensities were collected for 60 s prior to firing the laser. The laser traversed each dorsal scute at a rate of 3 μm per second and maintained a beam diameter of 100 μm. Dorsal scute sections were analyzed for multiple elements including Ca, Sr, Ba, Mg, Mn, and Hg.

Data reduction and determination of element concentrations was carried out according to the methods of Longerich et al. (1996). Mean background intensity was subtracted from gross mean analyte intensity to correct for gas blank background. Eighty-four replicates from two standard reference materials were analyzed in duplicate at least every six samples to examine instrument drift. These reference materials were a microanalytical calcium phosphate standard representative of true bone (MAPS-4, U.S. Geological Survey), and a pressed calcium carbonate otolith pellet (Karin Limburg, SUNY ESF). Estimates of precision (relative standard deviation; RSD) carried out using the MAPS-4 (n=14) and otolith pellet (n=12) reference materials were as follows (MAPS-4 / otolith pellet): Ca (6.4 % / 46.7 %), Sr (11.5 % / 16.6 %), Ba (12.8 % / 17.8 %), Mn (10.0 % / 43.6 %), Mg (10.9 % / 36.7 %) and Hg (26.2 % / NA). Instrumental drift was accounted for by adjusting the background corrected element intensities according to a linear relationship between time and element intensity using the MAPS-4 standard reference material (Longerich et al. 1996). Calcium was used as an internal standard and set to a concentration of 23 % (n=12; SD= 2.15) based on our electron microprobe derived mean calcium mass percent observed across dorsal scutes from all wild and hatchery-reared individuals used in this study. A similar approach has been used to determine calcium concentration in pectoral fin spines from Atlantic sturgeon (Stevenson and Secor 1999).

Limits of detection (LOD) were calculated for each analysis during background acquisition and based on the following equation in Longerich et al. (1996),

$$LOD = \frac{3 \cdot \sigma_{individual}}{S} \sqrt{\frac{1}{n_b} + \frac{1}{n_a}}$$

where $\sigma_{individual}$ = the standard deviation of the background intensity in the selected background interval, S = the sensitivity normalized to the amount of sample ablated (see below), n_b = number of determinations of the background accumulated, and n_a = the number of "slices" or sweeps in the ablation time interval that are integrated. With the exception of mercury, all dorsal scutes contained concentrations of the elements of interest above the LOD for 100 % of the readings. Mercury was subsequently excluded from further analyses.

Inferred life history transition

A sequential regime shift algorithm was applied as a less-subjective means to identify scute regions with different chemical properties indicative of the known freshwater to estuarine/marine transition of GoM sturgeon (Rodionov 2004). This algorithm uses a sequential t-test to identify significant changes in the mean of a given variable, in this case the Sr:Ca ratio data obtained from electron microprobe and laser ablation ICPMS

analysis. The potential beginning of a new regime occurs when a significant difference in Sr:Ca is observed between the current regime mean and that of a consecutive point. However, a shift is only declared if the difference in means is maintained upon inclusion of subsequent data points (Rodionov 2004). Serial correlation in the dataset was taken into account by removing red noise through a "prewhitening" procedure (Rodionov 2006). The initial input parameters of this procedure were established as follows: cut-off length = 10, significance level = 0.05, and Huber's weight parameter = 1. As in Barausse et al. (2011), the influence of the cut-off length and Huber's weight parameter on the results was determined using a sensitivity analysis where model parameters were changed one by one: cut-off length varied from seven to thirteen in increments of one, and Huber's weight parameter varied from 1 to 3 in increments of one. Significance level was maintained at 0.05 across all analyses.

Regime shift timing was assessed using only the Sr:Ca data associated with the portion of the scute used for ageing. All measurements of Sr:Ca across the dorsal scute profile were scaled to percentages (i.e., regime shift positions in the profiles are reported as percentages across the whole). This facilitated regime shift position comparisons where the number of profile points differed between individuals and instrument runs. The position of the first regime shift was noted for each individual and referenced to a corresponding growth zone in the associated scute. The mean (± SE) of Sr:Ca values prior to the first regime shift for each GoM individual is considered representative of the assumed freshwater residency period. A paired *t*-test was used to examine overall differences in mean Sr:Ca before and after the first regime shift in wild individuals for both instruments. A *t*-test was also used to examine differences in mean freshwater baseline Sr:Ca values between wild and hatchery-reared individuals by instrument.

Group separation

Multivariate analysis of element-to-calcium ratios was used to determine if groups of sturgeon might ultimately be distinguished based on distinct signatures arising from their water sources during early rearing. Sample sizes for this comparison were limited to the subset of individuals processed with laser ablation ICPMS. We used a comparison of wild GoM sturgeon (n=7) and hatchery-reared CAFRC fish (n=5). For wild GoM fish we used data collected within the earliest observable growth zone to target the most-probable region of natal freshwater residency (i.e., young of year). Hatchery-reared fish showed evidence of erosion of superficial scute material due to rearing in tanks, with associated loss or disruption of very early growth zones. However, outside of the short period of time when the hatchery-reared CAFRC sturgeon were being used in ontogenetic behavioral research (and held in city water for approximately 4 months), all CAFRC sturgeon were reared in ambient Connecticut River water for their entire lives (Parker and Kynard 2014), so we simply used data from their earliest remaining growth zones for this test case.

Element-to-calcium ratios were examined for univariate normality and equal variance using the Shapiro Wilk W and Levene's test respectively. Deviations from either assumption were addressed using a Box-Cox power transformation (Box and Cox 1964; Schaffler et al. 2015). Multivariate normality was then assessed using Mardia's test (Schaffler et al. 2015). A multivariate analysis of variance (MANOVA) was used to identify element-to-calcium ratios exhibiting significant differences between wild and hatchery-reared individuals. We then applied linear discriminant function analysis to assess group separation (Program R v. 3.1.0). A leave-one-out cross validation procedure was used to characterize assignment capacity.

Results

Sample ageing

Thin sectioning and polishing of dorsal scutes revealed a dual-zoned structure: a thin multi-layered translucent zone composed of many thin and only subtly distinct layers (Fig. 1a) lying above a thicker multi-layered zone composed of heavier alternating translucent and opaque bands that we treat here as presumptive growth zones (Fig. 1b). The number of presumptive growth zones observed in this region of the dorsal scutes and in pectoral fin spines from wild shortnose sturgeon ranged from 3 to 12 and 3 to 15 respectively. Regression analysis indicates there is a significant and strong correlation between the number of growth zones found in dorsal scutes and the number of growth zones in pectoral fin spines from the same individual (r^2=0.85; p<0.05; Fig. 2). The slope of this relationship was not different from unity (p=0.202), suggesting scutes do not provide

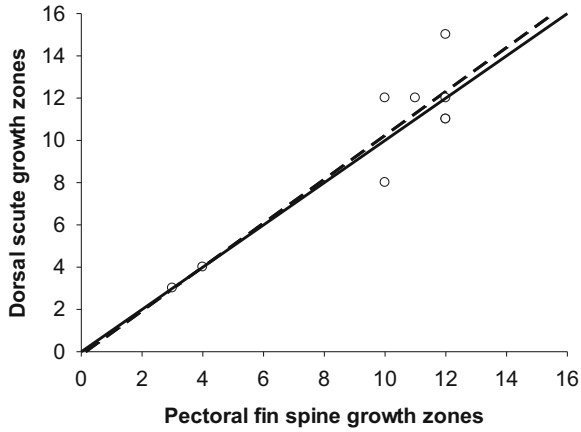

Fig. 2 Regression of pectoral fin spine and dorsal scute growth zone counts for wild GoM shortnose sturgeon (n=8; r^2=0.85, p<0.05). The *dashed line* represents the regression and the *solid line* has a slope of one. The regression slope did not differ significantly from one (p=0.202)

systematically biased chronology with respect to fin spines.

Electron microprobe

Electron microprobe profiles traversed the entirety of the dual-zoned scute section and revealed what appeared to be mirror-like images of Sr:Ca profiles with the reflection point occurring at the division of the two distinct composition layers (Fig. 3a). Differences between these distinct zones in their relative thickness resulted in different spatial resolution and number of scan points. However, separating the two profiles at the point of structural division, inverting the profile order of Sr:Ca reads from the upper translucent zone, and relativizing the two zones onto a proportional scale (0 to 1), revealed that these profiles are indeed similar in magnitude and pattern (Fig. 3b). This general pattern was observed in all dorsal scutes from wild GoM shortnose sturgeon (not assessed in CAFRC fish due constant rearing environment and erosion of translucent zone).

For subsequent analyses we considered only the Sr:Ca profile from the thicker underlying zone because of the ability to more directly establish its apparent chronological properties. Sr:Ca profiles for hatchery-reared individuals were consistent with exposure to a controlled water supply. Specifically, Sr:Ca in dorsal scutes and pectoral fin spines from hatchery-reared individuals remained relatively stable across their entire life history profiles (n=5; Fig. 4a). Though stable

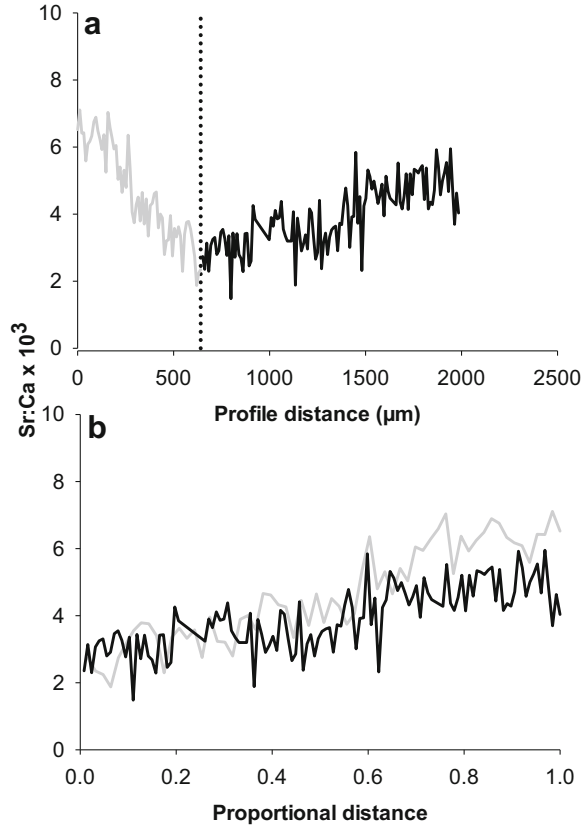

Fig. 3 Example dorsal scute Sr:Ca ppm (10^3) profile illustrating potential mirroring of profile across thin, multi-layered translucent zone and thicker zone containing heavier alternating translucent and opaque bands. The data was collected using laser ablation ICPMS. The entire Sr:Ca profile is illustrated in panel (**a**) where the gray line denotes profile position in the thin translucent zone, the *black line* denotes profile position in the heavily structured zone, and the *dotted line* indicates the delineation between structural layers. Reversing the Sr:Ca profile from the thin translucent zone (*gray line*) and scaling profiles from both thin translucent and thick zone (*black line*) to the same scale suggests Sr:Ca correspondence in both magnitude and pattern (**b**)

within individuals, Sr:Ca values did vary somewhat among individuals (Table 1). Patterns in Sr:Ca ratios along microprobe profiles in dorsal scutes and pectoral fin spines varied within and among wild GoM shortnose sturgeon (Fig. 4). Three general patterns were apparent in Sr:Ca profiles; a stable period of Sr:Ca followed by an increase (Fig. 4b; n=1), a gradual increase in Sr:Ca across the scute (Fig. 4c; n=3), and an increase in Sr:Ca followed by a decrease (Fig. 4d; n=3). Visual comparisons of Sr:Ca profiles between dorsal scutes and pectoral fin spines within individuals showed these patterns were broadly consistent across both structures (Fig. 4a–d).

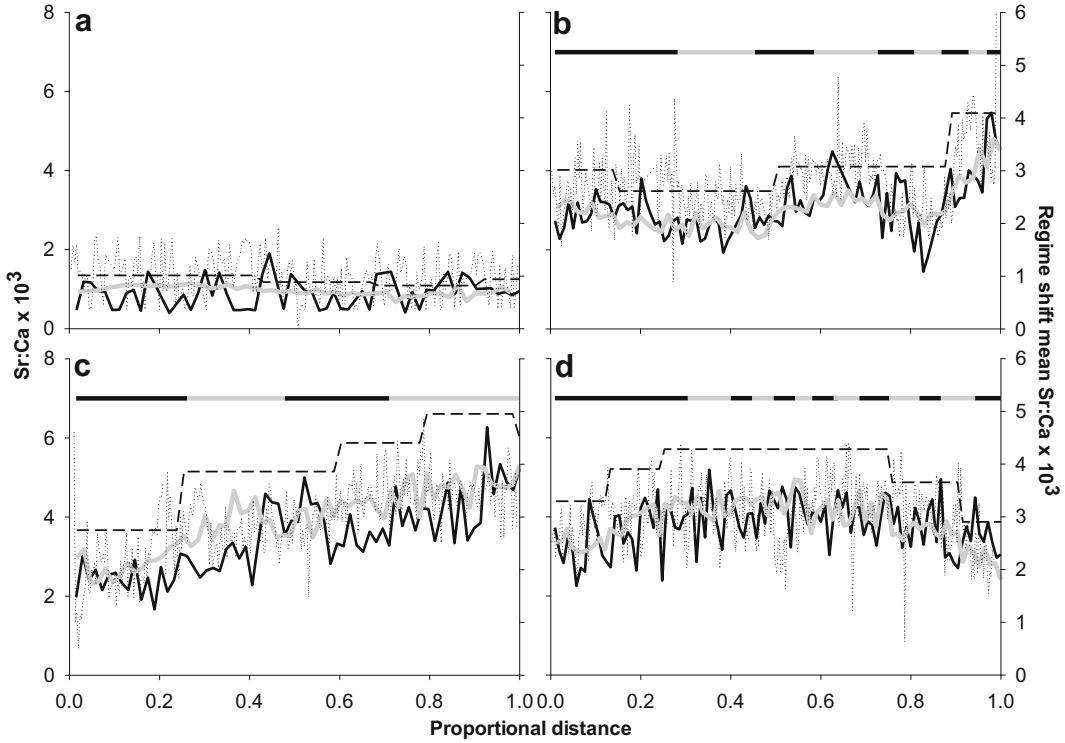

Fig. 4 Examples of the four general Sr:Ca ppm (10^3) profiles observed in dorsal scutes and fin spines from hatchery-reared individuals (**a**) and wild GoM individuals (**b**–**d**). Proportional distance is Sr:Ca profile distance scaled from 0 to 1 to account differences in profile distance between instruments. The alternating *black* and *gray line* at the top of figures **b**–**d** identify growth zones in the scutes. *Solid black lines* represent dorsal scute Sr:Ca measured using the electron microprobe, *solid gray lines* represent dorsal scute Sr:Ca measured using laser ablation ICPMS, *dotted lines* represent pectoral fin spine Sr:Ca measured using the electron microprobe, and *dashed lines* represent the mean Sr:Ca ratio observed in a given regime as defined using the Rodionov (2004, 2006) approach. General patterns of Sr:Ca include stability across the entire structure (**a**), a period of stability followed by an increase (**b**), a gradual increase over time (**c**), and a gradual increase and then decrease (**d**). Greater variability in Sr:Ca profiles was observed using the electron microprobe, but correspondence in signal magnitudes and patterns were observed across instruments and structures

Laser ablation ICPMS

Mirror-image like Sr:Ca profile profiles across the two major scute structural layers (as observed using the electron microprobe) were also observed in data from the laser ablation ICPMS. Laser ablation ICPMS Sr:Ca profiles from within the thicker, banded zone appeared similar in profile pattern to those returned using the electron microprobe for individuals from both the GoM and CARFC (Fig. 4a–d). However, differences in the overall magnitudes of the Sr:Ca signals were observed across instruments in scutes from three of seven GoM individuals and three of five hatchery-reared individuals, likely corresponding to some drift in instrument baselines. The patterns of Sr:Ca profiles between instruments were similar despite the differences in magnitude (Fig. 5).

Inferred life history transition

In dorsal scutes from all seven wild individuals, the position of the first regime shift (suggesting initial saltwater exposure) in Sr:Ca profiles fell within growth zones one through three corresponding to young-of-year through year two. The mean and range of Sr:Ca values observed prior to and after the first regime shift in wild GoM individuals were used to estimate baseline concentrations indicative of freshwater and saltwater (e.g. estuarine or marine exposure) occupancy respectively. Only mean Sr:Ca values greater than those of the freshwater baseline were associated with presumed saltwater exposure. Given evidence of possible instrumental differences in Sr:Ca profile magnitude, separate freshwater and saltwater baselines for all individuals are reported. Mean Sr:Ca values were lower during the

Table 1 Biological and Sr:Ca ppm (10^3) micro-chemical data for all shortnose sturgeon dorsal scutes examined using electron probe microanalysis (EPMA) or laser ablation ICPMS

Source	TL (mm)	Age	First break point age	EPMA (FW)	Laser ablation ICPMS (FW)	EPMA (SW)	Laser ablation ICPMS (SW)
Wild	835	12	1	2.93	2.95	4.14	4.45
Wild	966	12	1	4.81	2.39	5.79	3.38
Wild	935	11	1	4.23	2.68	5.26	3.75
Wild	994	12	1	3.29	3.37	3.68	3.95
Wild	655	3	1	2.58	2.82	3.97	4.43
Wild	747	10	3	2.72	3.02	4.03	3.59
Wild	895	12	3	4.28	3.14	5.54	4.01
Mean (SE)				3.55 (0.33)	2.91 (0.12)	4.63 (0.33)	3.94 (0.15)
Hatchery	723	5	.	0.56	0.95	.	.
Hatchery	635	5	.	3.21	0.83	.	.
Hatchery	520	5	.	2.07	0.88	.	.
Hatchery	574	5	.	0.90	0.93	.	.
Hatchery	704	5	.	1.17	0.90	.	.
Mean (SE)				1.58 (0.48)	0.90 (0.02)		

Data collected prior to the first break point age was assumed indicative of freshwater (FW) residency whereas data compiled after this point was assumed indicative of possible marine or saltwater (SW) exposure

presumed freshwater period than during the presumed saltwater period within both instruments (paired *t*-test; $p<0.001$; Table 1). The variation around the means was significantly greater for the microprobe than the laser ablation ICPMS, also leading to wider range of estimates of regime-shift transitions (Table 1). Freshwater baseline values of Sr:Ca for hatchery reared individuals were lower than those observed in wild individuals (*t*-test; $p<0.01$), but again the electron microprobe tended to return greater variation in signal magnitude than the laser ablation ICPMS, (Table 1).

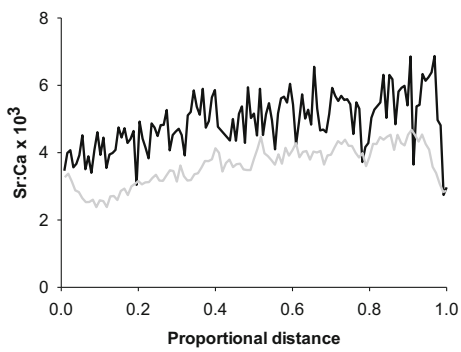

Fig. 5 Sr:Ca ppm (10^3) profile from scute section of wild individual showing relative correspondence in profile pattern but not in overall profile magnitude across instruments. The *black line* represents Sr:Ca measured using the electron microprobe while the *gray line* represents Sr:Ca measured using laser ablation ICPMS. Proportional location across the full profile was used for comparison given differences in profile length/resolution between the electron microprobe and laser ablation ICPMS runs. In some cases, instruments did show similar Sr:Ca magnitude

Group separation

The ratio of Mg:Ca did not initially meet normality or equal variance assumptions and thus was transformed according to the Box-Cox power transformation ($\lambda = 3.787$). After transformation, Mg:Ca met both assumptions. Both Sr:Ca and Ba:Ca ratios met univariate normality and equal variance assumptions without transformation. Mardia's test suggested the subsequent data did not deviate from multivariate normality ($P=0.155$). Comparisons of early-life dorsal scute chemistry between wild GoM sturgeon and hatchery-reared CAFRC sturgeon suggest that the scutes of these groups do differ in their early life multivariate elemental signatures (MANOVA: Pillai's trace $F_{3,8}=0.964$; $P<0.001$). Specifically, mean Sr:Ca, Mg:Ca and Ba:Ca ratios were found to differ significantly between wild GoM, and CAFRC sturgeon with canonical axis one loading positively on Ba:Ca (24.66) and Sr:Ca (3.06) and negatively on Mg:Ca (−0.00011). This elemental difference is noteworthy given the small sample sizes available to us

(GoM n=7, CAFRC n=5). Indeed, percentage of correct classifications based on a leave-one-out cross-validation procedure indicated 100 % correct classification for both GoM and CAFRC scutes.

Discussion

The findings of this study suggest that dorsal scutes from shortnose sturgeon possess chronological and microchemical properties that may permit reconstruction of past environmental exposures. Dynamic patterns in Sr:Ca across dorsal scutes from wild fish and relatively stable patterns of Sr:Ca across dorsal scutes from hatchery-reared individuals indicate a link to ambient water conditions and individual life history experience. Moreover, evidence suggests that elemental signatures preserved in freshwater regions of scutes are different enough to have potential for classifying fish to their natal water source, where water sources differ in elemental profiles.

Structure chronology

Strong evidence exists that annuli observed in shortnose sturgeon fin spines are deposited on a yearly basis (Woodland and Secor 2007) and fin spines are currently in use as an ageing surrogate in shortnose sturgeon field studies. We found a strong correlation between the number of growth zones observed in dorsal scutes and the presumptive growth zones and ages inferred from pectoral fin spines. This implies that dorsal scutes provide the chronological microstructure necessary to infer age and stage-specific microchemical histories. A comparative age examination of multiple calcified structures in white sturgeon also found a positive linear relationship between age estimated using dorsal scutes and pectoral fin spines (r^2=0.83; Brennan and Cailliet 1989), supporting that scutes may have general utility as chronological structures in other sturgeon species. Demonstrating the absolute accuracy of dorsal scutes as ageing structures in shortnose sturgeon was not an objective of the current study, but our results provide encouragement for doing so. However, this may require an indirect approach. Hatchery-reared fish of known age are often used in direct age validation studies for other structures (Koch et al. 2011), but such direct validation may prove difficult with scutes given that all hatchery-reared shortnose sturgeon showed signs of severe scute erosion that was not encountered in wild fish. Hence, validation of scute age for shortnose sturgeon might only be possible through correlational comparisons to fin spine ages (akin to analysis in the present study), following initial independent validation of fin spine ages in captivity.

Dorsal scute structure and composition

Examinations of sectioned dorsal scutes from shortnose sturgeon in this study suggest the presence of two distinct structural zones (Fig. 1a and b) with a relatively thin, multi-layered translucent zone lying above a thicker structural zone composed of sequential opaque and translucent bands. Previous descriptions of scute development in sturgeon suggest that scutes are of mesodermal origin and grow through layering of bone by osteoblasts on both the dorsal and ventral surfaces of the scute (Sewertzoff 1926; Zhang et al. 2012). Development begins medially and proceeds laterally with each layer of bone (Sewertzoff 1926). An alternative description of scute structure and composition suggests scutes are composed exclusively of parallel fibered lamellar bone (Sire et al. 2009). However, profiles of Sr:Ca from both structural zones (Fig. 1a and b) of each scute appear to be mirror images during at least early life phases, with some compression of the profile recorded in the upper translucent zone (Fig. 3). Mirror-image comparisons of these elemental profiles support the growth model of Sewertzoff (1926) of two opposing developmental fronts for bone deposition.

This finding is interesting, as it suggests scutes may offer the added benefit of providing replicate environmental histories from developmentally distinct portions of a single structure, and in so doing increase certainty with respect to life history reconstruction or natal elemental signatures. Indeed, unlike repeat transects through an otolith, fin spine, or scale, which merely replicate the same developmental tissue in a slightly different orientation, the developmental and structural distinctness of these layers makes their comparison closer (in terms of independence) to what one might expect from comparison of two different structures of the same fish (e.g., fin spine and otolith). Mirror-image microchemical profiles in dorsal scutes also suggests the possibility of superficial sampling that removes only a small portion of the scute, but this remains to be tested. All that said, the fine layering in the translucent zone did not permit a simple comparison with banding in fin

spines and so more work needs to be conducted to better confirm and assess potential chronology in this layer. We suggest that controlled manipulations of rearing conditions and elemental exposures by age would be particularly helpful in this regard.

The chemical composition of dorsal scutes is not known in detail. However, the concentration of calcium in dorsal scutes (23 % by mass) is quite similar to that measured in hydroxyapatite-based fin spines of Atlantic sturgeon (29 % by mass; Stevenson 1997) and white sturgeon (26 % by mass; Veinott et al. 1999). This is to be expected given that actinopterygian fin rays, scales and scutes are all forms of dermal bone that likely share some evolutionary and developmental homology (Zhang et al. 2012). We found clear indication of corresponding elemental profiles in scutes and fin spines. In conjunction with previous work illustrating the efficacy of sturgeon fin spines to record environmental exposure histories and maintain these signals over time (Allen et al. 2009), this strongly suggests that scutes hold promise as a homologous structure useful for reconstructions of past habitat use.

Inferred life history transition

The concentration of strontium relative to calcium in marine waters is often higher than that in freshwater environments (Ingram and Sloan 1992), with very few exceptions as noted in Kraus and Secor (2004). Given that strontium often substitutes for calcium in the hydroxyapatite matrices comprising bony structures (Rokita et al. 1993) and that correspondence between environmental exposure and Sr:Ca ratios has been demonstrated repeatedly for various bony structures of fishes (Veinott et al. 1999; Arai et al. 2002; Allen et al. 2009; Scharer et al. 2012), it is highly likely that the ontogenetic Sr:Ca patterns observed in the bony dorsal scutes of shortnose sturgeon reflect environmental exposures to the estuarine mixing gradient these fish transition into during early life. The position of the first regime shift in dorsal scute Sr:Ca profiles from GoM shortnose sturgeon suggests these individuals begin to experience some level of increased saltwater exposure in the first 1.5 to 3.5 years of life. This is consistent with the observation of Dadswell et al. (1984) that shortnose sturgeon remain inland of full saltwater between the ages of 2 and 8 years.

The relatively slow increase in Sr:Ca profiles from wild GoM shortnose sturgeon might suggest a muted salinity signal in scutes. However, elemental profiles were similar in scutes and fin spines and so we suggest this pattern is more consistent with the relatively gradual transition from freshwater to estuarine and marine environments in the complex mixing environment of the estuaries these fish inhabit. We did note some abrupt ratio changes in scute profiles suggesting that scutes have the potential to document short-term changes in environmental conditions. Also, the transition pattern we observed is consistent with the gradual development of salinity tolerance found in captive studies (Jenkins et al. 1993) and the suggested negative effects abrupt salinity exposure may have on growth and survival of early juveniles (Jarvis et al. 2001). This pattern of gradual change has also been observed in other species and bony structures (Allen et al. 2009). Future studies, with larger sample sizes, will be useful in more precisely defining Sr:Ca values indicative of particular salinity habitats and the parameters for instrumentational comparisons.

Group separation

Microchemical analysis of calcified structures has been applied broadly across many fish species to provide group separation and assignment (e.g., natal origins; Walther and Thorrold 2008; Pangle et al. 2010). To the best of our knowledge, such group assignment has only been attempted in one other study of sturgeon, when Phelps et al. (2012) used Sr:Ca ratios in fin spines as a marker to delineate Missouri and Mississippi River sources of *Scaphirhynchus* sturgeons (*S. albus* and *S. platorynchus*). Shortnose sturgeon inhabiting coastal rivers throughout the Gulf of Maine are known to undertake marine migrations and enter other river systems (Fernandes et al. 2010; Zydlewski et al. 2011; Dionne et al. 2013). This stands in contrast to the previously held perception that shortnose sturgeon do not leave their river of origin and highlights the need for a tool that can assign individuals back to a natal river (Peterson and Farrae 2011; Dionne et al. 2013). Likewise, larger river systems can support multiple shortnose sturgeon spawning and rearing areas (Kynard et al. 2000), and such sites may differ in their elemental profiles. In both cases, populations may be insufficiently isolated and divergent to provide genetic resolution of natal origin. A method to delineate natal origins would thus be of considerable value for shortnose sturgeon research and management.

Our results suggest that elemental profiles in scutes may provide a practical alternative to assign individuals to their natal habitats. However, our test of this must be regarded as largely heuristic, given that it only sought to distinguish wild and hatchery-reared fish. This was a constraint of the limited number of scutes available from salvaged fish. However, these findings support that natal growth in sturgeon scutes occurs superficially in the region of the spinous process, which might be sampled with little or no injury to soft tissues through methods like clipping or coring. Even if whole lateral scute removal is conducted, removal of a dermal structure is likely less injurious and detrimental to performance than surgical removal of the leading pectoral fin spines. Indeed, Rien et al. (1994) used whole scute removal as an external marking technique for wild-caught white sturgeon and successfully recaptured marked individuals that had been at large for over two years. Scute removal is also procedurally outlined for Atlantic sturgeon (Damon-Randall et al. 2010). Nonetheless, we believe our work provides the necessary impetus to conduct research on alternative scute sampling methodologies that could substitute for taking whole scutes.

Alternate instrumentation

Some differences in magnitudes and variances in Sr:Ca values are not unexpected across different analytical instruments (Campana et al. 1997), particularly when examining a modest number of samples in a new structural material. In studies carried out by Arai and Hirata (2006) and Arai et al. (2007), significant correlations were observed between Sr:Ca measured in otoliths of Japanese eels (*Anguilla japonica*) and chum salmon (*Oncorhynchus keta*) respectively, using both laser ablation ICPMS and wavelength dispersive electron microscopy, but both studies also showed variability in Sr:Ca values across instruments. A direct comparison of the Sr:Ca profiles between the electron microprobe and laser ablation ICPMS in this study illustrates correspondence in Sr:Ca profiles, but greater variation in signal magnitude when using the electron microprobe. We feel this is likely attributable to the spatial scale at which each instrument sampled material from the scute, given that both instruments have high sensitivity and precision. The electron microprobe sampled scute material at very small points (5 μm diameter) that were spaced closely (every 10 μm) but not overlapping. This resulted in a relatively fine scale examination of the microchemistry throughout the scute. The laser ablation ICMPS was maintained at a beam diameter of 100 μm, traversed the sample at 3 μm per second, and element concentrations at each replicate were the average values of five sweeps per reading or replicate. This approach does reduce noise in the data and facilitates differentiation between analyte and background signals, but also may conceal signals associated with fine scale temporal changes (Longerich et al. 1996). In this respect, signals from the electron microprobe are more discrete whereas signals from the laser ablation ICPMS are best viewed as an integration across space. For the purposes of the present study, the most important outcome is that we did indeed observe general correspondence in Sr:Ca profiles across instruments, suggesting either tool may be effective in future studies.

Given the likelihood of a relatively gradual transition from freshwater to marine environments by shortnose sturgeon, the integration of signal intensity across sample space using the laser ablation ICPMS likely still provides the resolution necessary to identify the approximate period in time (e.g., year) when this transition occurs. Laser ablation ICMPS can also be adapted for higher spatial resolution than we used here, and has been used previously to characterize microchemical properties within relatively small saggital otoliths (Woodhead et al. 2005). The biggest advantage of ICPMS, however, may be for its utility in obtain data most useful for assigning fish to particular water sources, given that some trace elements (e.g., Ba, and Mg) detectable by ICPMS in this and other studies are often found at concentrations below detection limits of the electron microprobe (Campana et al. 1997). As noted by others (Campana et al. 1997) for different structures, the choice of which instrument to employ for scute analysis should likely be made based upon resources and the specific nature of the questions at hand (e.g., life history chronology versus water source assignment).

Conclusions

This study provided proof of concept that dorsal scutes possess the chronological and chemical features required for microchemical reconstructions of past habitat associations of sturgeon. We feel this is important as it provides impetus for further work to develop scutes as a potentially minimally invasive structure for characterizing age, life history patterns, natal origins or other habitat associations of this imperiled group. This would have broad importance for defining critical habitat for these animals that live in environments that are difficult to survey, range widely and often show complex population structure. Importantly, such a tool could complement and strengthen inferences from other technologies used to study these fish. For example, the outlined microchemical approach for assignment of natal water sources could in theory be coupled with existing approaches to corroborate genetic baselines and assignments used in mixed-stock analyses, or to strategically deploy telemetry tags and receivers to better study possible philopatry or mixed migratory strategies.

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Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted. This article does not contain any studies with human participants performed by any of the authors.

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