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Abstract White Sturgeon (*Acipenser transmontanus)* are a long-lived, slow-growing, and late-reproducing anadromous fish common in estuaries and coastal habitats along the North American West Coast. These life history characteristics make populations vulnerable to human impacts and a challenge to study and manage. Previous studies in the San Francisco Estuary, California have provided insights into rearing habitats and migratory patterns but are limited in spatial and temporal scope. Fin ray geochemical analysis can provide a non-lethal approach to reconstruct migratory patterns and environmental conditions experienced throughout an individual fish's lifespan. However, it is not known how soon post hatch age-0 White Sturgeon fin rays begin to calcify, reducing confidence in early life history temporal resolution using geochemical approaches. We used osteological (clear and stain) and geochemical techniques (laser-ablation-ICP-MS) to describe calcification initiation and completion, and element incorporation in the leading fin ray of known-age White Sturgeon reared at constant water temperature (18.6 °C) from 1 to 76 days post hatch (dph). We found that fin rays begin calcifying as early as ~20 dph (~27 mm total length) and are >95% calcified by ~72 dph (~70 mm total length). Consequently, the first ~20 dph are not likely to be recorded in the fin ray. Observed element (Li, Mg, Cu, Zn, Rb, Sr, Ba, Pb, U) incorporation patterns suggest that fin rays can provide a powerful tool to study White Sturgeon early movement and migratory patterns, habitat use, and environmental exposure.

Keywords *Acipenser transmontanus* · Fin ray · Microchemistry · Laser-ablation · Early development · Rearing

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Introduction

White Sturgeon (*Acipenser transmontanus*, Richardson) are the largest anadromous fish found in North America, inhabiting coastal waters and estuaries ranging from Ensenada, Mexico to north of the Gulf of Alaska (Kohlhorst and Cech 2001; Moyle 2002). Even with their wide distribution along the coastline of the North-East Pacific Ocean, spawning populations have only been documented in large rivers from San Francisco Bay and northward (Kohlhorst and Cech 2001; Moyle 2002). White Sturgeon have a long lifespan, slow

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growth, delayed sexual maturation, and extended spawning periodicity, making them exceptionally vulnerable to habitat loss, pollution, and over-exploitation (Boreman 1997; Helfman et al. 1997; Hildebrand et al. 2016). The San Francisco Estuary (SFE) White Sturgeon population was decimated by overfishing beginning in the late 1800's (Skinner 1962; Smith and Kato 1979; Kohlhorst and Cech 2001; Moyle 2002). Despite historical over-exploitation, the California population continues to support a recreational fishery under strict management; however, the population is still at risk. Understanding White Sturgeon spatial distribution, migratory behavior, habitat use, and environmental exposure over their lifetime is critical to enable fisheries managers to effectively protect and enhance critical habitat, develop informed fishing regulations, refine population models and assessments, and develop effective population recovery plans (Birstein et al. 2006).

White Sturgeon spawning and year-class recruitment are heavily reliant on early life stage survival which is influenced by environmental parameters such as legacy and emerging contaminants (Gundersen et al. 2017), river flow (Kohlhorst et al. 1991; Moyle 2002; Verhille et al. 2014; Jackson et al. 2016), temperature (Counihan and Chapman 2018; Rodgers et al. 2018) and sediment quality (McAdam 2011; Hatten et al. 2018;). Temperature and flow are highly variable in California's Mediterranean climate and predicted by climate models to become more extreme (Allan and Soden 2008; Dettinger 2011). California White Sturgeon are ~11 mm TL at hatch and immediately begin swimming in a vertical position, causing them to drift downstream, presumably into brackish waters of the SFE (Wang 1986; Moyle 2002). Although in-river spawning has been documented in the San Joaquin and Sacramento rivers and their tributaries, smaller age-0 sturgeon (10.9 to 65 mm TL) have occasionally been captured in the lower rivers and SFE (Jackson et al. 2016; Heublein et al. 2017). However, information is generally lacking regarding age-0 White Sturgeon distribution in the SFE. Higher densities of age-0 White Sturgeon in the SFE have been associated with high flow events, with initial hatch and early rearing assumed to occur in tributaries (Stevens and Miller 1970). Depending on how far upstream spawning occurs and how high river flows are, it is assumed that age-0 White Sturgeon drift downstream of natal spawning streams soon after hatching. However, age-0 White Sturgeon habitat use in the SFE and associated tributaries remains highly speculative and this information is essential for protecting and enhancing rearing habitat.

Previous California White Sturgeon studies have used electronic tag, video, and rotary screw trap monitoring, and disc tag recapture in recreational fishery creel surveys to infer migratory patterns and spatial distributions (Nelson et al. 2013; Faukner and Jackson 2014; Klimley et al. 2015; Jackson et al. 2016; Mytton et al. 2018; Miller et al. 2020). Although this research has provided key insights, many questions remain about this species' life history diversity, habitat use, migratory patterns, and potential exposure to environmental contaminants. There are currently no long-term monitoring efforts for early White Sturgeon life stages in the SFE and its tributaries, and the spatially and temporally limited monitoring studies that have been conducted to date have uncertain application for understanding early life stage habitat use in long-lived species (Stevens and Miller 1970; Nelson et al. 2013).

Analysis of elements and stable isotopes incorporated into calcified structures using laser-ablation inductively-coupled plasma mass spectrometry (LA-ICP-MS) is emerging as a valuable tool for reconstructing migratory patterns across an individual fish's entire lifespan (Campana 1999; Nelson et al. 2013; Willmes et al. 2016; Sellheim et al. 2017). For example, strontium isotope ratios ($^{87}Sr/^{86}Sr$) have been used to retrospectively determine natal origins and migratory patterns of several SFE fish species including Chinook Salmon (*Oncorhynchus tshawytscha,* Walbaum), Delta Smelt (*Hypomesus transpacificus*, McAllister), Longfin Smelt (*Spirinchus thaleichthys*, Ayres), and Sacramento Splittail (*Pogonichthys macrolepidotus*, Ayres) (Hobbs et al. 2005, 2010, 2019; Feyrer et al. 2007; Barnett-Johnson et al. 2008). Element/Ca ratios, such as Sr/Ca and Ba/Ca are commonly analyzed in otoliths ("ear stones") as a tool to reconstruct movement and natal origin of migratory fish species (Campana 1999; Sturrock et al. 2012; Kerr and Campana 2014; Walther and Thorrold 2006).

Otolith microchemistry is effective for bony fishes that have otoliths composed of aragonite calcium carbonate ($CaCO_3$) because otoliths form incremental layers and incorporate element signatures related to environmental and physiological conditions. Sturgeon otoliths, however, are relatively small compared to other fish and composed primarily of vaterite, a polymorph of $CaCO_3$ that results in indistinguishable growth rings and element incorporation that is difficult to relate to

environmental changes (Melancon et al. 2005; Pracheil et al. 2016; Sellheim et al. 2017). Furthermore, otolith collection is lethal, which is undesirable for species of management concern; particularly long-lived species such as sturgeon (Birstein 1993; Allen et al. 2009a).

An alternative approach utilizes calcified sturgeon pectoral fin rays (Veinott and Evans 1999; Allen et al. 2009a, b; Phelps et al. 2012; Sellheim et al. 2017). Unlike otoliths, fin rays can be collected nonlethally, with minimal effects on growth, survival, or swimming performance (Collins and Smith 1996; Nguyen et al. 2016). Allen et al. (2018) showed that juvenile Atlantic Sturgeon (*A. oxyrinchus,* Mitchill) fin rays are able to heal and fully regenerate within 6 weeks (partial fin ray removal) or 1 year (entire fin ray removal), further indicating that fin rays can be collected nonlethally. Fin rays have been used for sturgeon aging and life history reconstructions in a variety of different watersheds (Brennan and Cailliet 1989; Rien and Beamesderfer 1994; Veinott et al. 1999; Allen et al. 2009a; Phelps et al. 2012). For example, Sr/Ca ratios in Fraser River White Sturgeon fin rays suggested that most remained in fresh water, with limited migration to marine or estuarine environments (Veinott et al. 1999). Sr/Ca and Ba/Ca ratios in Klamath River Green Sturgeon (*A. medirostris*, Ayres*)* fin rays indicated that most migrate to marine environments at 2.5–3.5 years of age (Allen et al. 2009a). Furthermore, laboratory experiments have demonstrated that changes in fin ray $^{87}Sr/^{86}Sr$ isotope ratios, reflecting individual movements between waters with different $^{87}Sr/^{86}Sr$ isotope ratios, can be detected in White Sturgeon fin rays within 11–36 days (Sellheim et al. 2017). A laboratory study by Phelps et al. (2012) found that Pallid Sturgeon (*Scaphirynchus albus*, Forbes and Richardson) fin ray Sr/Ca ratios tracked water Sr/Ca changes at 1 day post hatch (dph), suggesting that short-term habitat changes can be recorded. This implies that fin ray microchemistry analysis can be applied to young fish and allows reconstruction of rapid environmental changes. However, most fin ray microchemistry studies were performed on older fish (> age-1), and thus the applicability of these methods to age-0 fish remain largely unknown.

While study results using older fish suggest fin ray microchemistry holds great promise for improving our current understanding of White Sturgeon life history characteristics, several uncertainties must be addressed to establish the technique's validity and determine its potential limitations. Element incorporation into calcified fish tissues such as otoliths and fin rays is a physiologically regulated, multi-stage process and follows complex biochemical pathways that can lead to element/Ca ratios differences for different calcified structures within a fish, even in a homogeneous environment (Campana 1999; Gillanders 2001; Tzadik et al. 2017). Pectoral fin rays consist of calcium-phosphate (hydroxyapatite) and elements can occur within the hydroxy-apatite as substitutes for calcium, in interstitial spaces, and/or in association with the more organic-rich layers (Stavri and Zarnescu 2013; Tzadik et al. 2017). Furthermore, the fin ray chemical composition is controlled by several factors including the chemical composition of the water, ambient water salinity and temperature, diet, and fish physiology, including metabolic rate, growth rate, and reproductive state (Kalish 1991; Campana 1999; Clarke et al. 2007; Sturrock et al. 2012; Kerr and Campana 2014). Consequently, tissue and element-specific distribution coefficients are needed to link chemical analyses in calcified parts of fish to the water chemistry of a specific watershed. In addition, the presence of accessory lobes (additional rays absorbed within the leading pectoral fin ray) on some fin rays could affect aging and microchemistry profiles if laser-ablation transects intersect them (Brennan and Cailliet 1989; Sellheim et al. 2017). Resorption of the fin ray may occur after initial deposition due to vascularization but the extent to which this affects White Sturgeon fin rays has not been resolved (Veinott et al. 1999; Clarke et al. 2007; Nelson et al. 2013). Furthermore, it is unclear when age-0 White Sturgeon fin rays begin to calcify and provide sufficient ossification to reliably measure their microchemistry (Sellheim et al. 2017). Dillman and Hilton (2015) found that the earliest evidence of ossification in White Sturgeon fin rays can occur as early as 25.3 to 29.9 mm total length (TL); however, it is uncertain whether the $CaCO_3$ concentration in newly-ossified fin ray tissues is sufficient to reliably measure element ratios and infer natal origins of these early life stages.

To determine how early fin rays begin to calcify and the feasibility of microchemistry use in juvenile White Sturgeon, an experimental series was conducted to examine physical development and element incorporations of leading pectoral fin rays in age-0 White Sturgeon. The objectives of this validation study were to (*i*) observe the initiation of fin ray calcification as a function of age and length, (*ii*) observe the extent of fin ray

calcification as a function of age and length, (*iii*) quantify the calcium concentration incorporated into newly developing fin rays, and (*iv*) determine the relationship between water element concentrations and element uptake in calcified fin rays. Understanding fin ray calcification and how elements are incorporated into bony structures during the earliest portion of White Sturgeon life history will inform the interpretation of microchemical patterns; specifically, this information will determine how soon after hatching it is possible to infer White Sturgeon habitat use, movement, and environmental exposure.

Materials and methods

Experimental

White Sturgeon rearing conditions used in this study are described in detail in Zarri et al. (2019) and are briefly summarized below. White Sturgeon were reared at the Center for Aquatic Biology & Aquaculture (CABA) located on the University of California Davis campus in a 1.8 m diameter tank capable of holding 455 L. Water was supplied from a well at the CABA facility and aerated prior to being pumped into the tank. Water temperature was held constant at 18.6 ± 0.03 °C (mean ± SD) throughout the rearing period. White Sturgeon were acquired as fertilized eggs from Sterling Caviar LLC (Wilton, CA) and hatched at the CABA facility. Roughly 7 days post hatch (dph), White sturgeon were fed a sturgeon starter feed (55% protein, 17% fat, <2% fiber, <10% ash; Rangen, Buhl, Idaho), ad libitum via 24-h belt feeder (Pentair, #BFS24A). White Sturgeon were then transitioned onto semi-moist feed (45% protein, 19% fat, <2% fiber, <9% ash, Rangen) at an optimal feed rate. Average fish mass in the tank was measured weekly to calculate optimal feed rates as fish grew (Deng et al. 2003; Lee et al. 2015). All fish used in this experiment were held in the same tank under constant environmental conditions and diet throughout the study duration. Over a nine-week period, 15–20 fish were randomly collected twice weekly from 12 to 76 dph for a total of 346 individuals during 18 sampling events (Fig. 1). 50-mL tank water samples were taken at each sampling event, except on the last sampling event (76 dph), for a total of 17 samples for element analysis. Each water sample was filtered through a 0.45 μm Millex® syringe-driven filter unit into a sterile vial with 0.5% diluted trace metal grade nitric acid (Barnett and Mallory 1971). Water samples were then stored in a refrigerator at ~4 °C.

Sample preparation

Fish were euthanized with Tricaine Methanesulfonate (MS-222). The MS-222 solution was prepared following UC Davis Institutional Animal Care and Use Protocol #18767 (0.5 g/L, MS-222 buffered with 0.42 g/L sodium bicarbonate, and 6 g/L NaCl. Total length (TL) was measured to the nearest mm, wet weight was measured to the nearest 0.1 mg, and individuals were assigned a unique ID number immediately following euthanization. Left pectoral fins (for clearing and staining) and right pectoral fins (for laser-ablation microchemistry) were surgically removed with a scalpel at the proximal edge of the pectoral girdle. Detached fins were set flat on a strip of Rite in the Rain® paper which was then folded over to secure the fin. Fins were then placed in Whirl-Pak® bags labeled with sample ID and date collected and stored in a freezer at −18 °C.

Clear and stain analysis

A representative subsample ($n = 44$) was selected for clearing and staining from all samples ($n = 346$) maximizing representation across the full range of age classes (Table 1). Three to seven fish were selected for each week and size-at-age of the subsample was contrasted with the full dataset to confirm that the subsample was representative of the full dataset (Fig. 2). Left pectoral fins from the 44 subsampled fish were removed from the Rite in the Rain® paper and placed in 1.5 mL centrifuge tubes and stored in 70% ethanol (ETOH). In order to observe calcification onset in the leading pectoral fin ray, the 44 left fins were cleared and single stained for calcium detection using methods adapted from multiple studies (Wassersug 1976; Dingerkus and Uhler 1977; Taylor and Van Dyke 1985; Ellis and Miller 2016; Eshaghzadeh et al. 2018). Due to their small size, fins were not stained for cartilage detection using acidic alcian blue to eliminate the risk of bone demineralization (Walker and Kimmel 2007). Prior to analysis, fins were removed from 70% ETOH and fixed in 10% buffered formalin for 48 h. Following fixation, fins were transferred to distilled water for an additional 48 h to remove traces of formalin. After the distilled water rinse,

Fig. 1 Images of young White Sturgeon with associated total lengths (TL) and ages (days post hatch, dph) at different developmental stages throughout the time frame of this experiment

Table 1 Sample sizes for clear and stain and laser-ablation analysis by sample week and days post hatch (dph)

Sample week	Age (dph)	Clear and stain	Laser-Ablation
1	12	4	1
	16	3	1
2	20	3	1
	23	1	1
3	26	2	2
	30	3	2
4	33	1	0
	36	3	2
5	44	3	1
6	47	2	2
	51	1	1
7	54	4	2
8	61	3	0
	65	4	1
9	68	5	0
	76	2	1
	Total	44	18

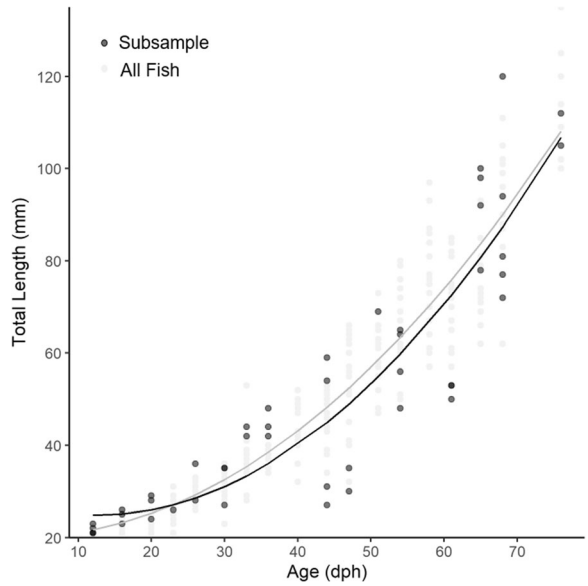

Fig. 2 The size-at-age quadratic growth function for laboratory-reared White Sturgeon used in this study. Functions for all fish (n = 346, light grey) and the subsample (n = 44, black) were examined to ensure the subsample was representative

larger fins (from fish greater than 50 mm TL) were incubated in a trypsin solution (2.5 g 1:100 trypsin powder in 30% saturated sodium borate solution in 175 mL distilled water) for 1–5 days depending on fin size and clearing status. If necessary, 1–2 drops of 3% hydrogen peroxide (H_2O_2) was added to aid in tissue decolorization. Smaller fins (i.e., fins that did not require additional tissue clearing) and larger fins following decolorization were moved into a staining solution containing 0.5% potassium hydroxide (KOH) with distilled water and saturated alizarin red S dye with 70% ETOH. Fins were left in solution until bone was sufficiently stained red (2–24 h depending on size; average 4–6 h; Fig. 3). Following staining procedures, fins were rinsed with distilled water and transferred to a graded series of 0.5% KOH-glycerin for 24 h at each step (i.e. 3:1, 1:1, 1:3) to continue clearing, and finally into 100% glycerin to preserve and permanently store specimens.

Left fin rays were imaged before and after clear and stain procedures with Image Pro-Premier® using a Motic BA310 compound microscope with a Motic Cam 5+ camera attached to the trinocular port. Each fin was calibrated, photographed, and measured under 40X total magnification with proximal end of fin facing left, distal end of fin facing right, and leading pectoral fin ray along the top of each image (Fig. 3). Additional images at magnifications of 100X were taken as needed for visualization only and not used for measurements. Calcification was measured from proximal to distal end of the stained lead pectoral fin ray. If the leading pectoral fin ray had no indication of calcification following clear and stain procedures, measurements were recorded as 0 μm (Fig. 3). Proportion of fin ray calcified was calculated by dividing the calcification length by the total extent of possible calcification length for a fin at a given size. Extent of possible calcification length was measured at the top edge of the fin between the beginning of the calcified structure and the point where the fin begins to angle downward (Fig. 3). If fin ray calcification length was equal to the extent of possible calcification length, proportion of fin ray calcified was equal to one.

Fin ray microchemistry

For microchemistry, right pectoral fin rays from 18 individuals were subsampled from the 44 individual sample set used in clear and stain analysis to validate clear and stain estimates using independent chemical analyses. One to four samples from each sampling week

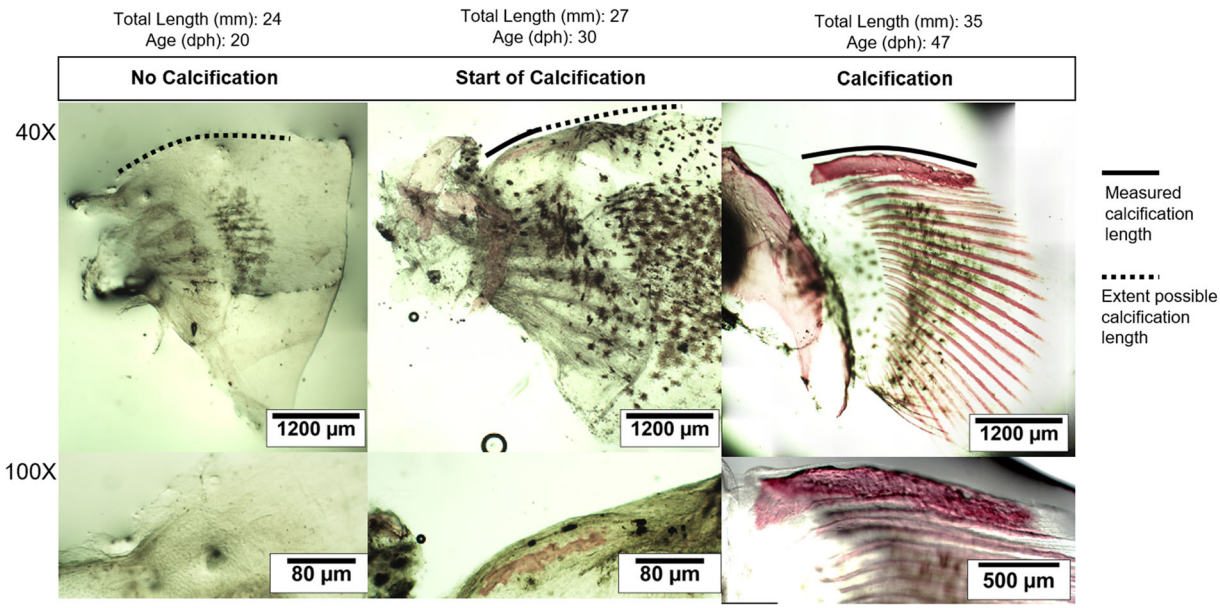

Fig. 3 Images of clear and stained White Sturgeon fin rays showing increasing calcification in fish from 20 to 47 days post hatch (dph). Images of whole fins (top, 40x total magnification) and the leading fin ray (bottom, 100x total magnification) are shown (solid black line = calcified portion; dashed line = uncalcified portion). No calcification (no pink stain) is present in the leading fin ray at 20 dph, whereas partial calcification (slight pink stain) and full calcification (solid pink stain) are shown at 30 dph and 47 dph, respectively

were used in the microchemistry sample set (Table 1). Right pectoral fins from the 18 subsampled fish were removed from the Rite in the Rain® paper and a bony fin ray was surgically extracted from each fin prior to analysis. If a distinct, bony fin ray was not visible for extraction (i.e. <30 mm TL), the entire right fin was used in analysis. Fin rays (or entire fin) were mounted on 27 × 46 mm petrographic slides using double-sided Scotch™ tape. Each fin ray was mounted proximal end of fin facing left, distal end of fin facing right. If the whole fin was used, the leading pectoral fin ray was positioned at the top. Right fin rays were imaged before and after microchemistry analysis with Image Pro-Premier® using a Motic BA310 compound microscope with a Motic Cam 5+ camera attached to the trinocular port. Each fin was calibrated, photographed, and measured under 40X total magnification with proximal end of fin facing left, distal end of fin facing right, and leading pectoral fin ray along the top of each image.

Element concentrations of the right pectoral fin rays were analyzed by LA-ICP-MS using a Photon Machines 193 nm ArF Excimer laser with a HelEx dual-volume LA cell coupled to an Agilent 7700x Quadrupole ICP-MS at the department of Earth and Planetary Sciences, UC Davis. Laser repetition rate was set at 10 Hz and fluence was ~3 J/cm^2. A line with a spot size of 40 μm was ablated at 5 μm/s along the proximal to distal edge of the fin ray or along the entire leading edge if the whole fin was mounted. Before data collection, a cleaning run (pre-ablation) was performed across the same trajectory with a larger (150 μm) spot size and fast scan speed (100 μm/s). The following isotopic masses for elements were measured: ^{7}Li, ^{24}Mg, ^{43}Ca, ^{44}Ca, ^{63}Cu, ^{66}Zn, ^{85}Rb, ^{88}Sr, ^{114}Cd, ^{138}Ba, ^{208}Pb, and ^{238}U. Analysis cycle time was set to 1 s so that all elements were measured every second and concentrations thus reported for each second of analysis and time was converted to distance using the known laser run speed.

Data were reduced using Iolite (Paton et al. 2011) and normalized to ^{43}Ca using the *Trace Element* data reduction schema following standard best practices (Longerich et al. 1996; Jochum et al. 2011). Fin ray calcium concentration was assumed to contain 27 wt% Ca based on prior sturgeon studies (Veinott et al. 1999; Stevenson and Secor 2000; Allen et al. 2009a; Phelps et al. 2012). NIST 610 and 612 glasses were measured prior to and between each sample and used as external reference materials to correct for instrument drift. Element-specific limits of detection (Table 2) were calculated as 3 times the standard deviation of the element-specific background value. Measured concentrations of an element below its respective detection limit were set to zero, indicating negligible abundance. Elements (X) were then ratioed to calcium X/Ca and expressed in μmol/mol. To account for the potential influence of fish development on element incorporation we averaged the first 200 μm of each calcified fin ray when comparing them to the water samples from the experimental tank.

Water microchemistry

Water samples (N = 17) were analyzed for element concentrations at the Interdisciplinary Center for Plasma Mass Spectrometry at the University of California, Davis. Sample aliquots, external reference materials, and blanks were mixed at a ~ 16:1 ratio with an internal reference solution and then introduced into an Agilent 8900 QQQ-ICP-MS (Agilent Technologies Inc. ©, Palo Alto, CA) via a peristaltic pump using a 0.4 mL/min MicroMist nebulizer. The QQQ-ICP-MS instrument was tuned and calibrated prior to analysis and operated in MSMS mode using a 3-point peak pattern with three replicates per injection and 50 sweeps per replicate. H2, He, and no-gas modes were used in the collision/reaction cell during the measurement. NIST 1640a and a blank were analyzed for calibration and blank verification. SPEX CertiPrep *2A* at 50 ppb and SPEX CertiPrep *Calibration Standard 3* at 1000 ppb were analyzed every 10th sample as quality controls along with a blank to monitor instrument performance and provide continuing calibration and blank verification. All reference materials were prepared in 3% HNO3 (v:v, conc. Trace Metal grade nitric acid (Fisher Scientific™):18.2 MΩ/cm water). The raw data was processed using MassHunter ICP-MS software (G7201C, Version C.01.03, Agilent Technologies Inc. ©). Detection limits for elements in water samples are shown in Table 2.

Statistical analysis

To quantify the allometric relationships between age-0 White Sturgeon age and length, a quadratic regression model was calculated to predict TL (mm) based on age (dph). Quadratic regression analyses were used to model the initiation of fin ray calcification as a function of age and length (i), and to assess the proportion of fin ray

Table 2 Average limits of detection of the isotopic masses for elements present in water from rearing tank, fin rays, and external reference materials

Element	Water (μg/ml)	Fin Ray (μg/g)	NIST610 (μg/g)	NIST612 (μg/g)
^{7}Li	3.57E-07	3.22E-01	6.16E-02	8.17E-02
^{24}Mg	1.56E-04	4.37E-01	3.01E-01	8.51E-01
^{44}Ca	9.58E-05	35.44	9.98	13.73
^{63}Cu	6.73E-06	1.90E-01	1.66E-01	2.87E-01
^{66}Zn	1.94E-05	2.98E-01	8.89E-02	2.71E-02
^{85}Rb	1.46E-06	2.97E-02	1.16E-01	1.68E-01
^{88}Sr	4.48E-06	3.44E-02	1.05E-02	1.48E-02
^{114}Cd	1.47E-06	2.48E-01	4.57E-02	7.15E-02
^{138}Ba	1.33E-05	1.27E-02	6.80E-03	9.80E-03
^{208}Pb	9.24E-07	4.20E-02	6.80E-03	9.80E-03
^{238}U	2.57E-07	1.57E-02	6.80E-03	9.80E-03

calcified as a function of age and length (*ii*). In addition, a logistic regression analysis was used to predict the probability of a fin ray exhibiting any calcification as a function of length and age (*ii*). Age (dph) was treated as a continuous variable for each regression analysis. To determine the relationship between water element concentration and element uptake in calcified fin rays (*iv*), distribution coefficients were calculated using the following equation (Campana 1999):

$$D_{Element} = \frac{\left[\frac{Element}{Ca}\right]_{fin\ ray}}{\left[\frac{Element}{Ca}\right]_{water}}$$

A distribution coefficient of 0 indicates that the element was available in ambient water but not incorporated into the fin ray, while a distribution coefficient = 1 indicates the element was available in ambient water was incorporated into the fin ray without discrimination (Campana 1999; Zimmerman 2005). All statistical analyses were conducted in R version 3.5.2 (R Core Team 2019).

Results

Allometric relationships

Across all 346 samples, sturgeon sizes ranged from 20 to 135 mm TL (mean = 50.5 mm, SD = 24.9) and ages ranged from 12 to 76 dph (mean = 42 dph). Sturgeon sizes from the 44 subsampled group ranged from 21 to 120 (mean = 52.3 mm, SD = 28.2) and ages ranged from 12 to 76 dph (mean = 42) (Fig.2; raw data available in Online Resource 1). Fish length increased allometrically with age, with an ontogenetic acceleration of growth following approximately 55 dph across the entire data set (n = 346) and subsampled group (n = 44). Allometric relationship between TL and age for both data sets are shown in Fig. 2 and model coefficients are available in Online Resource 2.

Calcification of fin rays – Initiation

Fin ray staining demonstrated that leading pectoral fin ray calcification occurred as early as 16 dph and 25 mm TL (Fig. 4). On average, calcification began by 20 dph and 27 mm TL. By 26 dph and 29 mm TL, all fins showed signs of calcification in the leading pectoral fin ray. Leading fin rays showed a 50% and ≥ 95% probability exhibiting *any* sign of calcification at 21.2 dph and ≥ 34 dph (McFadden's R^2 = 0.61) or of 26.7 mm and ≥ 32 mm, respectively (McFadden's R^2 = 0.71) (Fig. 4, model coefficients and predicted probabilities and shown in Online Resource 3 and Online Resource 4, respectively).

Calcification of fin rays – Length and extent

Quadratic regression models revealed non-linear relationships between length of the calcified portion of a fin ray (calcification length) and TL or age. Calcification length increased as a near-linear asymptotic function of length but accelerated as a function of age (Fig. 5, model

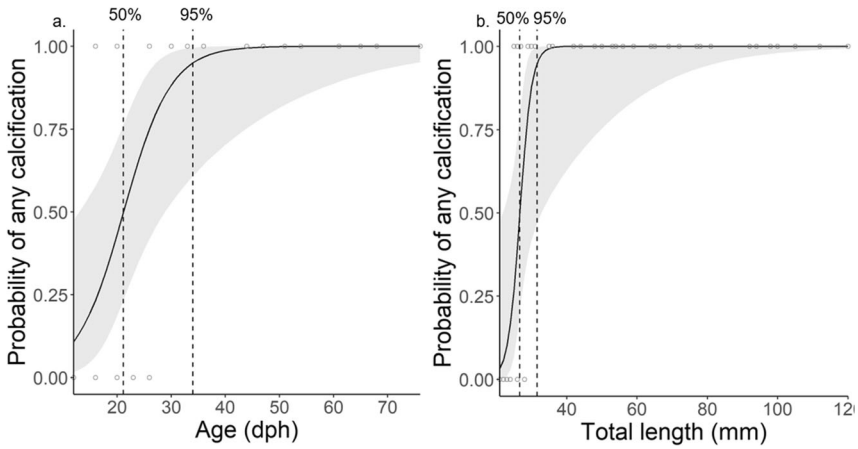

Fig. 4 Logistic regression plots showing the predicted probabilities of observing any fin ray calcification as a function of (**a**) age or (**b**) total length. Shading indicates 95% confidence intervals. Vertical lines indicate ages and total lengths when fin rays exhibit 50% and 95% probability of observing any calcification

coefficients shown in Online Resource 5). Leading fin rays became 50% and 95% fully calcified at 32 and 72 dph, or 38.5 and 70 mm TL, respectively. Thus, calcification of the leading fin ray progressed isometrically with length, attaining full calcification within two months (Fig. 6, model coefficients and proportions shown in Online Resource 6 and Online Resource 7, respectively).

Fin ray calcium detection

The laser-ablation analysis path followed the same trajectory as the visual calcification measurement during clear and stain analysis. We accounted for uncalcified pieces of the fin ray breaking off during analyses by using the original measurement of the fin ray (marked as a grey dot in Fig. 7). Laser-ablation analysis revealed low Ca concentration ($^{43}Ca < 300{,}000$ counts per second, cps) in the entire leading fin ray of young fish <26 dph (<30 mm TL). This threshold value was based on analyzing non-calcified tissues that exhibited low ^{43}Ca counts consistently below 200,000 cps. In contrast, Ca was abundant at the proximal end of the fin ray in fish ≥26 dph (≥30 mm TL) (Fig. 7). The proportions of the fin ray that were calcified were compared between clear and stain and laser-ablation methods. Overall, clear and stain and laser-ablation methods identified similar proportions of the fin ray as calcified with the mean difference (Δ) between calcified proportion in clear and stained fins and laser-ablation being $\Delta_{mean} = 0.004$ ($\Delta_{minimum} = -0.07$, $\Delta_{maximum} = 0.09$) (Fig. 8; Online Resource 8). The extent of calcification observed by clear and stain

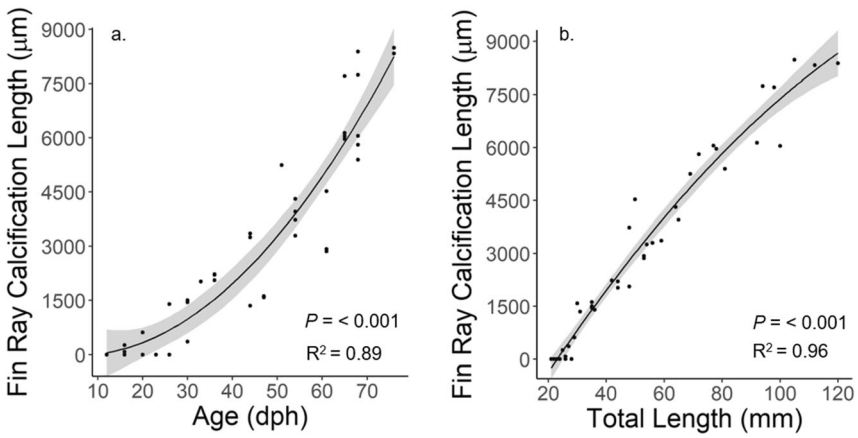

Fig. 5 Fin ray calcification length as a quadratic function of age (**a**) and total length (**b**). Shading indicates 95% confidence intervals

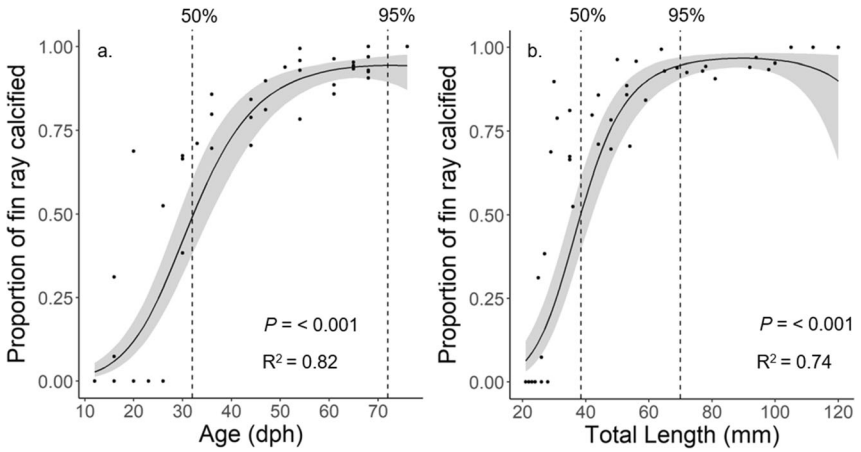

Fig. 6 Proportion of fin ray calcified as a quadratic function of age (**a**) and total length (**b**). Shading indicates 95% confidence intervals. Vertical lines indicate ages and total lengths when 50% and 95% of the entire fin ray was calcified

explained 99% of the variation in calcification estimated by laser-ablation element analyses ($p < 0.001$), demonstrating good agreement between the two methods (Fig. 8; model coefficients shown in Online Resource 9).

Fin ray element analysis

Of the original 18 fin rays analyzed for calcium concentration, 13 (26–76 dph and 30–112 mm TL) had sufficient calcification to determine their element compositions (Fig. 9). To account for the different ages and different developmental stages of the fish we averaged the first 200 μm of fin ray material for each fish, comparing only the early life independent of the total age. All analyzed elements were present within the fin rays above the detection limit, with the exception of cadmium (Cd), which was below detection limit in most of the fin ray and water samples and removed from further analyses (Fig. 9, detection limits in Table 1). On average, Ca, Mg and Sr had the highest concentrations across all calcified fin rays, while Li, Pb, and U had the lowest elemental concentrations (Online Resource 10). Element/Ca ratios revealed two different patterns with Li/Ca, Mg/Ca, Sr/Ca, Ba/Ca, and U/Ca showing lower ratios than the water, while Cu/Ca, Zn/Ca, Rb/Ca, Pb/Ca and had similar ratios between fin ray and water (Fig. 9).

Distribution (D) coefficients were calculated for each element using the average element/Ca ratios of all fin rays and the average element/Ca of all water samples from the experimental tank which was at a constant temperature of 18.6 ± 0.03 °C (mean ± SD, $n = 17$). D values ranged from 0.002 to 1.434 across all elements (Online Resource 11). U/Ca (0.002) and Li/Ca (0.004) showed the lowest D value, followed by Mg/Ca (0.027), Ba/Ca (0.03), and Sr/Ca (0.16). Zn/Ca showed the highest D value across all element/Ca ratios (1.434) followed by Rb/Ca (1.182), Cu/Ca (0.964), and Pb/Ca (0.952) which were all close to a D value equivalent of one.

Discussion

Fin ray microchemistry has the potential to provide crucial information regarding early rearing habitat use, migratory patterns, and environmental exposure of juvenile White Sturgeon throughout their entire range of distribution (Hildebrand et al. 2016). However, to accurately interpret early fin ray microchemistry data, it is important to first understand how soon post hatch and at what fish size age-0 White Sturgeon fin rays can be used as geochemical archives.

Fin ray calcification

Dillman and Hilton (2015) explored the fin ray development and ossification of four different sturgeon species; Lake Sturgeon (*A. fulvescens*, Rafinesque), Green Sturgeon, Pallid Sturgeon, and White Sturgeon using clear and stain methods. Results from that study indicate White Sturgeon fin rays calcified at 25.3 to 29.9 mm TL, which was earlier than some other sturgeon species but similar to Green Sturgeon. Similarly, our study revealed that age-0 White

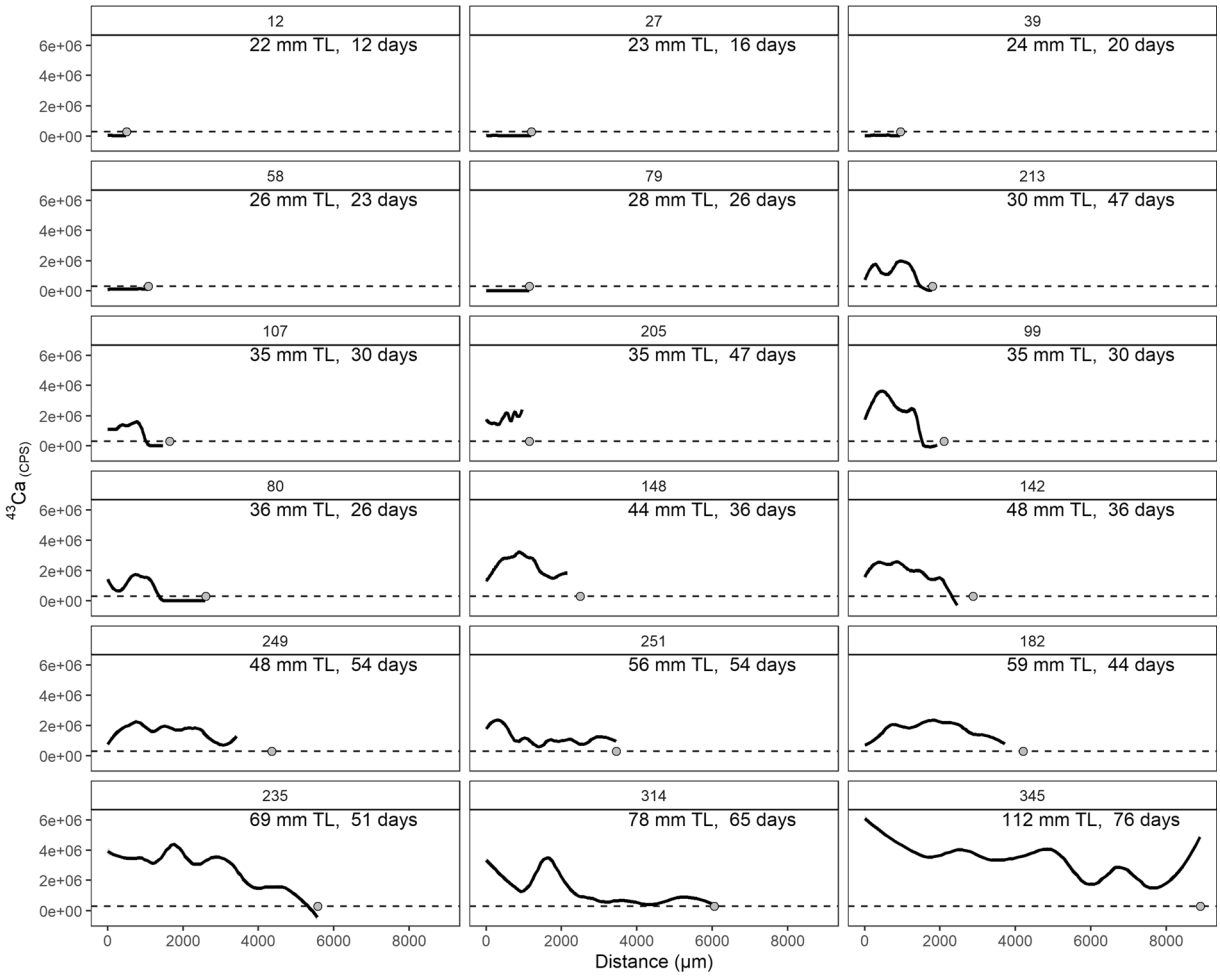

Fig. 7 Calcium (^{43}Ca in counts per second) profiles of entire leading fin rays (base to tip) of 18 juvenile White Sturgeon ages 12–76 days post hatch (22–112 mm total length). Sample IDs shown at the top of the graph. The grey dot indicates the end of the fin ray; in some samples the non-calcified portion of the ray broke off during the laser-ablation analysis. Dashed line indicates a threshold to differentiate calcified vs. non-calcified parts (^{43}Ca < 300,000 cps)

Sturgeon begin fin ray calcification as early as 25 mm TL. In addition, our study provides information about the minimum age (16 dph) that calcification begins. Further, we determined that White Sturgeon exhibit ≥95% probability of fin rays showing *any* calcification at ≥34 dph or ≥ 32 mm TL.

Age and growth in fin rays are recorded as distinct annuli due to the incremental and additive ossification, as opposed to growth through elongation like other exoskeletal bones (Tzadik et al. 2017). However, during the earliest stages of development, fin ray calcification occurs outward from the base. The degree to which the ray is completely calcified may play a role in whether it can be used to reconstruct migratory history using microchemistry. We used total calcification length in leading fin rays and total length of the leading edge of the pectoral fin to calculate the proportion of fin ray calcified in relation to White Sturgeon age or length. Calcification length appeared to increase as a near-linear function of TL; in contrast, calcification length accelerated with age. High calcium (Ca^{43} cps) concentrations were detected through laser-ablation in the proximal part of fin rays as early as 26 dph or 30 mm TL in White Sturgeon, a result that is in agreement with the clear and stain method (26 dph, 29 mm TL).

Sturgeon in this study were reared under controlled laboratory conditions and wild sturgeon are likely to be more developmentally variable due to spatially and temporally variable environmental conditions. Age-0 White Sturgeon growth rate relies

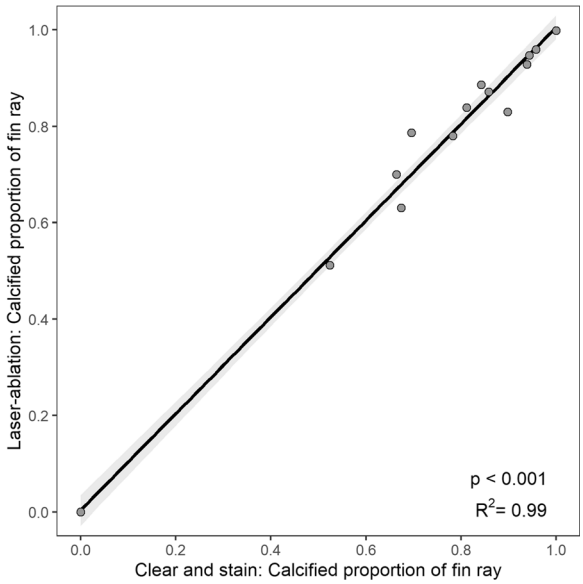

Fig. 8 Comparison of clear and stain versus laser-ablation techniques to quantify the proportion of the fin ray that is calcified. Note that there are five data points at location 0,0 (see Online Resources 8 and 9)

heavily on environmental conditions including temperature, dissolved oxygen, salinity, and diet (Cech et al. 1984; Lee et al. 2015; Zheng et al. 2015). Young White Sturgeon generally exhibit a rapid growth rate in early life stages that gradually decelerates with age (Israel et al. 2009). The calcification length data suggest that age-0 White Sturgeon pectoral fin rays are likely ≥95% fully calcified by ≥70 mm TL or ≥ 72 dph, at least under the optimal growth conditions of this study. Temperature in natural rearing environments can be highly variable and can directly influence these age-size relationships. Therefore, similar studies should be performed to understand fin ray calcification rates in colder, less optimal, growth temperatures.

Geochemical tracers in fin rays

Compared to otoliths, which are highly calcified (~98%) and consist of calcium-carbonate, fin rays are much less calcified (23–29%) and consist of calcium-phosphate (hydroxyapatite) and contain a large organic component (Veinott et al. 1999; Stevenson and Secor 2000; Allen et al. 2009a; Phelps et al. 2012; Tzadik et al. 2017). Consequently, elements can occur within the hydroxyapatite as substitutes for calcium, in interstitial spaces, and/or in association with the organic-rich layers (Stavri and Zarnescu 2013; Tzadik et al. 2017). In addition to the chemical composition of the water, other factors such as ambient water salinity and temperature, diet, and fish physiology, including metabolic rate, growth rate, and reproductive state, can influence element/Ca ratios (Kalish 1991; Campana 1999; Sturrock et al. 2012; Kerr and Campana 2014; Hüssy et al. 2020). Sr/Ca and Ba/Ca are well established geochemical tracers in otoliths and fin rays to reconstruct movement in freshwater systems, across estuarine salinity gradients, and into the ocean (Veinott et al. 1999; Allen et al. 2009a; Arai et al. 2002; Rude et al. 2014; Allen et al. 2018). Mg/Ca ratios in otoliths and fin rays have also been used to track movement, but physiological processes and ambient water temperature may also be important (Clarke et al. 2007; Sturrock et al. 2015; Allen et al. 2018). Diet may also influence these element/Ca ratios but in many natural systems the diet has similar element/Ca ratios as the surrounding water making it difficult to quantify dietary influence (Walther and Thorrold 2006; Rude et al. 2014).

The distribution coefficients for Mg/Ca, Sr/Ca, and Ba/Ca determined in this study are similar to values reported from other sturgeon fin rays and otoliths of anadromous species (Wells et al. 2003; Zimmerman 2005; Allen et al. 2009a). For example, for Green Sturgeon fin rays in freshwater systems, Allen et al. (2009a) found D values for Sr/Ca of 0.11, and Ba/Ca of 0.02. Phelps et al. (2017) also tested Sr/Ca ratios in water samples and Lake Sturgeon and Shovelnose Sturgeon (*S. platorynchus*, Rafinesque) pectoral fin-rays across a large range of concentrations and found comparable distribution coefficients of approximately 0.2 over a range of varying concentrations (Phelps et al. 2017). These finding suggests that while these elements are following different uptake pathways between otoliths and fin rays and are under physiological control, their tendency to substitute for Ca during mineral deposition into calcified structures (Blumenthal 1990; Walther and Thorrold 2006; Allen et al. 2009a), makes them promising environmental tracers.

Similarly, U/Ca and Li/Ca both had very low distribution coefficients, indicating that there is strong selectivity against incorporating these elements from the water into the calcified fin ray. These elements are not commonly measured in fish otoliths and their occurrence at measurable quantities in age-0 fin rays suggests they might be good additional elements to include in fin

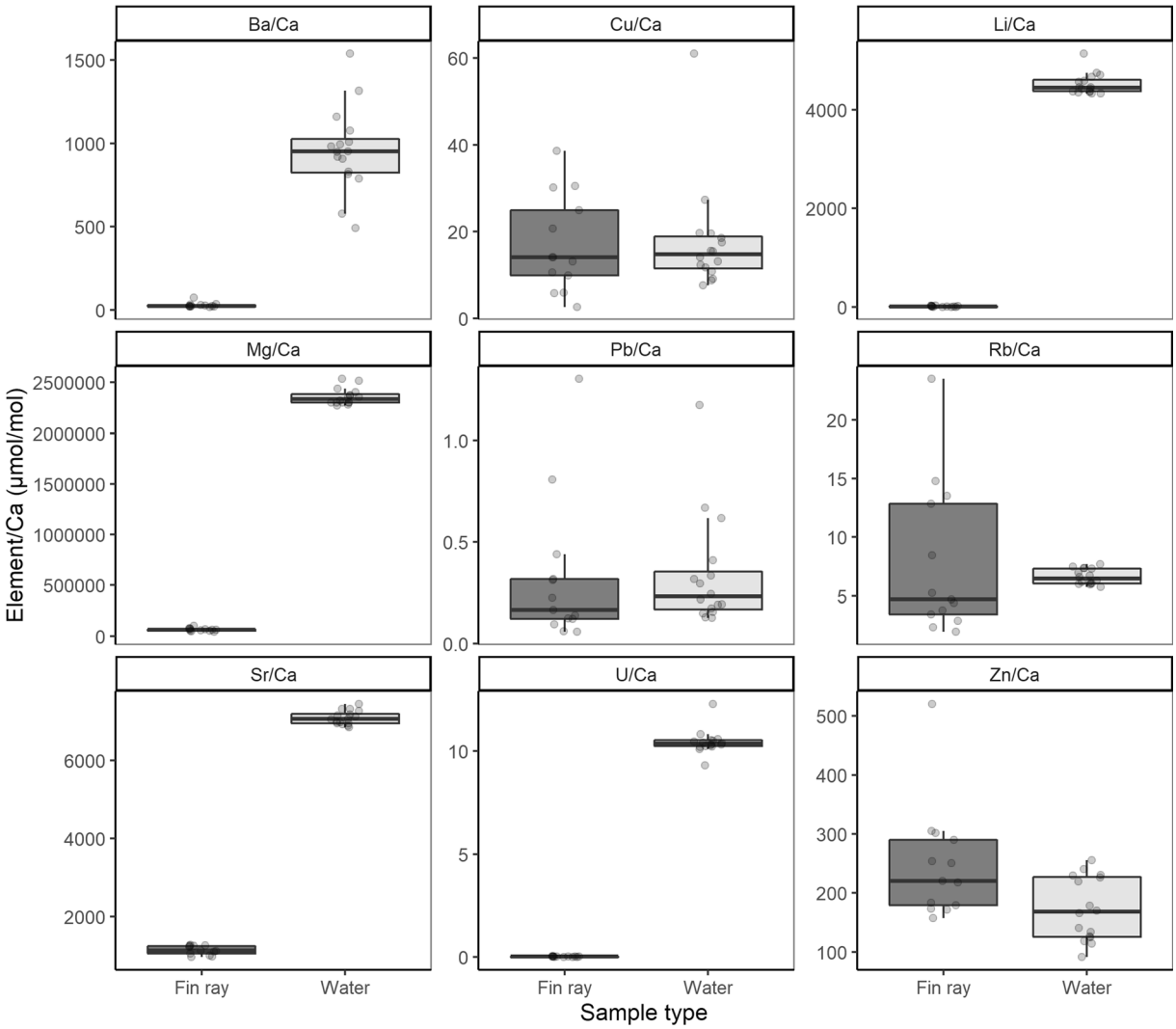

Fig. 9 Element/Ca ratios for fin rays and water samples. Grey points are individual fin ray or water samples, boxplots show the median and interquartile range (dark grey for fin ray, light grey for water)

ray studies when trying to differentiate watersheds and to reconstruct salinity gradients.

In contrast, Cu, Zn, Rb, and Pb showed D coefficients close to one, indicating that these elements are either incorporated in interstitial spaces in the hydroxyapatite or in the organic-rich layers. Cu and Pb are both potentially linked to environmental exposure and have been analyzed in fish soft tissues (liver, gill, muscle) to provide information about the heavy metal exposure a fish may have experienced due to prolonged residence time and/or ineffective excretion mechanisms (Williams 1971; Ay et al. 1999; Kalay et al. 1999). The occurrence of Cu and Pb at low but measurable concentrations (~7.1 and ~0.45 μg/g, respectively) in fish held in uncontaminated water provides a baseline for their use as tracers for heavy metal exposure. Cu/Ca and Pb/Ca in fin rays could provide a useful tracer because fin rays may preserve a permanent record of heavy metal exposure, which may disproportionately affect health and/or reproductive success of long-lived species such as sturgeon (Ugarte et al. 2012; Gundersen et al. 2017; Tzadik et al. 2017).

Another potential geochemical tracer in fin rays is Zn/Ca which is potentially related to diet but is also thought to be highly dependent on environmental variability and water temperature to which fish are exposed

(Ward et al. 2010; Sturrock et al. 2012; Avigliano et al. 2015a, b; Allen et al. 2018). The Rb/Ca ratio is important for strontium isotope studies because Rb is a direct analytical interference during the laser-ablation process, thus samples with high Rb/Ca ratios can be very difficult to analyze for their strontium isotope ratio (Woodhead et al. 2005; Vroon et al. 2008; Müller and Anczkiewicz 2016; Willmes et al. 2016).

A potential fin ray microchemistry limitation is the uncertainty surrounding resorption and vascularization processes in early sturgeon fin ray development. Vascularization occurs at the center of the fin ray, which leaves the earliest fin ray development zones susceptible to resorption during times of stress (Beamish and Chilton 1977; Beamish 1981; Tzadik et al. 2017). However, studies documenting developmental processes of fin rays have not been conclusive, and resorption was not the primary objective of these studies (Veinott and Evans 1999; Tzadik et al. 2017; Sellheim et al. 2017). Veinott and Evans (1999) suggest that areas within the fin rays that are actively involved in resorption are likely to contain bone cells rich in Potassium (K). Therefore, enriched K in fin rays may provide an indicator of stress/negative growth and allow researchers to filter out fin rays where migratory history information might be compromised by resorption. Additional long-term laboratory experiments are necessary to document development and potential resorption within the fin ray as fish are exposed to a variety of physiological stressors (e.g. temperature, starvation, disease, and contaminant exposure).

Conclusions

The results of this study provide data to address key assumptions about early life history fin ray development when reconstructing natal origin, early habitat use, migratory history, and environmental exposure in age-0 White Sturgeon. We found that age-0 White Sturgeon fin rays begin to calcify and incorporate elements commonly used to trace movement patterns at a relatively early age and small size. However, age-0 sturgeon dispersal is potentially rapid and variable, depending on the river flow conditions (Stevens and Miller 1970; Moyle 2002; Jackson et al. 2016). Consequently, early (1–20 dph) dispersal before fin ray calcification may remain undetected. Despite this limitation, fin ray microchemistry is a valuable tool to understand early migratory behavior of long-lived and difficult to monitor species, especially because fin rays can be collected non-lethally. Furthermore, fin rays can provide insight into an individual's habitat use and movement pattern throughout almost its entire life history, and address questions about exposure and response to stressors and water contaminants. In addition, elements and stable isotope ratios that are related to food sources (i.e. C, N, and S) could be analyzed to gain additional insights into sturgeon diet, and would leverage the greater fraction of organic material present in fin rays compared to otoliths. This validation study provides an indication of approximately how early in development we can expect to detect migratory history in White Sturgeon, which will inform the interpretation and application of fin rays as geochemical archives.

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