Sawfish, Read in Tooth and Saw: rostral teeth as endogenous chemical records of movement and life-history in a critically endangered species

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Abstract

The ecology of endangered and rare species can be difficult to study due to their low abundances and legal limits on scientist's ability to catch, sample, and track them. This is particularly true of sawfish (family *Pristidae*) whose numbers have declined precipitously, placing all five species on the CITES list of critically endangered species worldwide. Best known for their distinctive, toothed rostrum the ecology, movement, and life-history of sawfish is poorly understood. Sawfish rostral teeth are modified placoid scales, which grow continuously throughout the life of the fish. This continuous growth, combined with their stable calcified makeup, makes sawfish teeth a potential source of temporal records of chemical and isotopic changes through the life of the fish. Rostral teeth can be removed non-lethally from living animals and are also often preserved in rostra housed in museums and as curios, potentially allowing both contemporaneous and historical sources of life-history data. Study of the potential for sawfish rostral teeth as endogenous chemical and structural records is extremely limited, however. Using archived samples of largetooth sawfish (*Pristis pristis*) we show that multiple chemical tracers can be recovered from sawfish teeth, and that these tracers can be used to understand movement across salinity gradients and between freshwater and the ocean. We further show that sawfish teeth contain repeated structures and indistinct banding which could potentially be used for aging or growth analysis of fish.

Introduction

Understanding the ecology of endangered and cryptic species can be challenging. Low abundance, and the ethical limitations on human interaction for highly endangered species, limit our ability to apply traditional techniques to understand the ecology of these species at appropriate temporal and spatial scales (Parris et al. n.d., Cuthill 1991, Mackenzie et al. 2005, Chadè et al. 2008). However, effective conservation of these species often requires detailed understanding of their life-history, migration patterns, and ontogeny. This leaves ecologists to find creative ways to uncover this important information.

The largetooth sawfish (*Pristis pristis*), a large ray with an iconic toothed rostrum, is one critically endangered species with extremely low abundances whose life-history is poorly understood (Burgess et al. 2009, Kyne, P.M., Carlson, J, Smith 2013, Fernandez-Carvalho et al. 2014). Along with habitat loss and black market trade in fins and rostra, a major threat to sawfish is entanglement in nets as bycatch for other species (Seitz and Poulakis 2006, Dulvy et al. 2016). All species of sawfish move across a range of salinities and are found in salt water as well as brackish and fresh water (Thorson 1982, Poulakis et al. 2011, Scharer et al. 2012, Faria et al. 2013). Historical populations in Lake Nicaragua and reports as far upstream as Manaus in the Amazon river indicate the extent of freshwater use of largetooth sawfish (Thorson 1982, Burgess et al. 2009). Yet the patterns of sawfish use of brackish and fresh water are not well understood, and many published accounts are limited to opportunistic sightings (Nunes et al. 2016, Feitosa et al. 2017, Schmid and Giarrizzo 2017). Uncovering these patterns of habitat use and migration could help target conservation and management actions to life-stages, locations, or seasons where the risk to sawfish is greatest. However, collecting the necessary data is currently difficult.

We propose that sawfish rostral teeth may provide a useful, non-lethal, record of age, growth and environmental chemistry which could hold lifetime information on life-history and movement individual fish. Rostral teeth are not true teeth but instead highly modified dermal denticles which grow through the life of the fish (Welten et al. 2015). Made of bio-apatite (Miller 2006), rostral teeth presumably trap similar trace-elements and isotopes in their chemical matrix as other calcium carbonate hard parts such as shells, true teeth, and otoliths (Campana and Thorrold 2001, Schöne et al. 2004, Begg et al. 2005, Thornton 2011). These characteristics indicate that sawfish rostral teeth may store useful ecological data as an endogenous record.

Further, sawfish rostra are often dried and kept as curios and many are kept in museums, academic and private collections worldwide (Seitz and Poulakis 2006, Melo Palmeira et al. 2013). These collections provide a reservoir of samples by which to uncover details of sawfish life-history without harming living individuals (Phillips et al. 2009, Whitty et al. 2014). Further, tooth removal is unlikely to harm a living individual, making rostral teeth a potential non-lethal source of detailed information on living fish (Field et al. 2009).

Only one unpublished study has investigated the use of sawfish rostral teeth as an endogenous record of growth and provenance (Field et al. 2009). This work indicated that the trace element chemistry of whole sawfish teeth could be used to distinguish sawfish populations around the coast of Australia. The authors also demonstrated that growth banding could be observed on the outside of whole teeth which could be used to estimate age. Despite this, no work has been published investigating whether rostral teeth record higher-resolution chemical or structural data throughout the life of the fish. Sawfish vertebra, much like those of other sharks and rays, have been shown to contain yearly growth bands as well as a chemical record of salinity (Scharer et al. 2012).

In this study we present data from the analysis of rostral teeth taken from two rostra housed in a university collection in the United States. Our first objective was to demonstrate methods of sectioning, treating and imaging sawfish teeth to quantify age and growth information they contain. Our second objective was to document the potential of sawfish rostral teeth as a chemical record of location and movement using laser ablation-inductively coupled plasma mass spectrometry (LA-ICPMS) of $^{87}Sr/^{86}Sr$ isotope ratio, as well as trace element ratios of Sr/Ca, Ba/Ca, Mn/Ca, Mg/Ca. Finally, we analyzed these chemical and growth data to investigate their utility in understanding lifetime sawfish movement across salinity and the existence of other environmental or ontogenetic information recorded during the life of the fish.

Methods

Sample Information

All data in this study were taken from two sawfish rostra included in the ichthyology teaching collection at University of Idaho in Moscow, ID USA (Figure 1). The first was the rostrum of a largetooth sawfish embryo. Information associated stated that the rostrum was taken in Belize in 1969, the total length (TL) of the embryo was approximately 45 cm, and the mother was approximately 244 cm in length. This rostrum has a total rostral length (TRL) of 14.2cm and a standard rostral length (SRL, length from tip of rostrum to the most distal tooth) of 13.6cm (Figure 1A)

The second sample is an adult largetooth sawfish rostrum with a TRL of 57cm and a standard rostral length of 55cm (Figure 1B). No information was available for the provenance of this rostrum. Using the mean SRL to total length (SRL/TL) relationship from Whitty et al. (2014) results in a TL of 7 feet 10 inches for the fish from which the rostrum was taken. Given that the adult and embryo rostra were archived together it was assumed that the adult was the rostrum of the mother mentioned in the information attached to the embryo.

Structural Analysis

The most distal tooth on the left-hand side each rostrum was removed using a utility knife and a Dremel tool. The embryo tooth was in pristine condition. The adult tooth was highly worn on the anterior edge of the tooth extending beyond the rostrum and significantly pitted and eroded on the posterior edge. Teeth were cleaned of tissue by soaking in water followed by scraping with a scalpel blade. Prior to sectioning, each tooth was stained using silver nitrate to highlight growth ridges as described by Field et al. (2009).

Teeth were then sectioned using an Isomet low speed saw and a diamond wafering blade (Beuhler, beuhler.com). The adult tooth was curved both distally and along the dorsal-ventral axis. To allow sectioning through the center of the entire tooth it was cut in half near the point at which it protruded from the rostrum. The embryo tooth and the two pieces of the adult tooth were embedded in epoxy and an approximately 1mm section in the dorsal-ventral plane was taken that included the center core of each tooth (Figure 2). A further two sections were taken from the adult tooth and a second section was taken from the embryo tooth to facilitate staining. Each section was polished on 3600 grit and 6000 grit Micro Mesh wet-dry sandpaper (Micro Mesh, micromesh.com)

The adult tooth sections were stained using both silver nitrate and Mutvei's solution (Dunca et al. 2005) to enhance any growth banding present in the teeth. Sections were submerged in silver nitrate for approximately 5 minutes prior to light exposure. The second section was submerged in Mutvei's solution (dilute acetic acid, gluteraldahyde, and alcian blue) for 6 hours. The remaining embedded sample was burned using a handheld butane micro-torch (BernzOmatic, bernzomatic.com) until the sample surface was golden brown. The sample was then polished using the same sequence of wet-dry sandpaper to bring out rings. The embryo tooth was not stained or analyzed for growth.

Each section was imaged in sections at 10X magnification using a Leica DFC450 digital camera attached to a Zeiss AxioImager A.1 microscope. These images were subsequently stitched into a larger mosaic using the Photomerge tool in Photoshop (Adobe, adobe.com) with the "collage" layout option and "blend images together" option checked. These color images were then transformed into grayscale as well as inverting the color palette to determine an imaging method which would best highlight any banding or sequential growth structures that were present. Each image was subsequently optimized for contrast using a levels adjustment.

The number of bands and/or sequential structures inside the adult tooth were counted by two readers using ImageJ analysis software (https://imagej.nih.gov). Points and distances between them were measured along a line adjacent to the proximal edge of the tooth, on the outside of the dentin layer and inside the enamel layer where it was still present. Distances were standardized to the length of the laser scan for comparison with the chemical data.

Trace-element and Isotopic Analysis

The unstained sections of the tip and base of the adult rostral tooth, as well as the embryo, were analyzed for strontium ratio ($^{87}Sr/^{86}Sr$) as well as concentration of a suite of trace elements (^{86}Sr, ^{138}Ba, ^{55}Mn, ^{25}Mg). Calcium was used as the internal standard and all trace element data is expressed as a ratio to calcium (Sr/Ca, Ba/Ca, Mn/Ca, Mg/Ca).

Analysis was conducted at the Radiogenic Isotope and Geochronology Laboratory (RIGL) at Washington State University. Strontium ratio and trace element data were collected simultaneously using a laser ablation split stream (LASS) methodology (Hegg, Fisher, and Vervoort, in prep). Laser ablation was conducted using a Teledyne Analyte Excite 193nm ArF laser using a 35μm spot diameter, 10μm/sec rastering speed, operating at 20Hz, and with laser power of ~4J/cm^2. Ablated material was then split between a NeptunePlus multicollector inductively coupled mass spectrometer (MC-ICP-MS) for $^{87}Sr/^{86}Sr$ measurement and Element2 ICP-MS for trace element measurement.

Samples were corrected using a single analysis of a NIST610 standard as well as a marine shell standard. Strontium ratio analysis corrected for interferences from Kr and Rb as described in (LASS paper citation). Trace element analysis was calibrated using the NIST 610 standard as described in (LASS paper citation).

All data reduction was completed using a custom-built version of Iolite software as described in Fisher et al. (Fisher et al. 2017). All plotting and analysis was completed in R statistical software (https://www.r-project.org).

Results

Structural Analysis

Whole teeth, both unstained and after silver nitrate staining, did not exhibit the external growth banding described by Field et al. (2009). The interior of both teeth contained an inner, clear layer and an outer opaque layer in areas which had not been subjected to wear (Figure 2A). The teeth contain a mesh-like network of interconnected tubules as described by Miller (2006). These tubules followed a general pattern of intertwining tubes near the central core, with downward “legs” curving distally toward the edge of the tooth (Figure 2A&D). These descending legs appear to be spaced somewhat regularly, suggesting that they could mark growth or age.

Burning of the sample showed some evidence of layering within the tooth but it was too indistinct to be easily quantified. Silver nitrate staining of the tooth sections did not reveal additional layering or banding structure. Mutvei’s solution was moderately successful in bringing out faint alternating banding in the middle portion of the adult tooth (Figure 2B, D&E). The alcian blue stain in Mutvei’s solution infiltrated the tubules which broke the surface of each tooth section, making clear which tubules were on the surface and which were below.

Both readers agreed that the inverted image of the section stained with Mutvei’s solution provided the best image for measurement (Figure 2C). This image rendered the surface tubules in a distinct orange which was easier to discern than the blue of the non-inverted image. Quantifying of the apparent banding created by the tubule legs and indistinct light-dark bands was done by both readers. Both readers picked 26 growth bands. The first 15 of these bands, starting from the youngest growth at the tooth tip, matched between the readers (Figure 2D). In the basal section of the tooth, where the tubules were most indistinct, 6 of the remaining 11 points matched between the readers (Figure 2E). A consensus measurement was then created which contained 29 bands. When plotted the distance between measurements displayed a linear relationship.

Trace-element and Isotopic Analysis

Laser ablation and ICP-MS analysis was successful, with high Sr and Ca voltages for both analyses (Figure 3). The marine shell standard measured 0.70915 (±0.00038 2SE, Global Marine Signature 0.70918). Measurements exceeded the limits of detection (LOD) by at least three orders of magnitude in all cases (Average LOD across standards and samples: Sr=0.24 ppm, Ba=0.07 ppm, Mn=0.36, ppm, Mg=0.54 ppm). All analyses exhibited repeated, low level, spikes in signal. While tubules at the base of the fish created voids which unavoidably resulted in changes to the signal, the smaller, repeated spikes appeared not to correlate with voids in the surface of the tooth or with changes in the signal and were therefore assumed to be environmental.

Strontium ratio (Figure 3A) exhibited a convex pattern in the adult tooth, starting below 0.708 in the earliest growth periods, before increasing in the middle of the fish’s life toward the Global Marine Signature (0.70918), then gradually moving down to near 0.707 at the base of the tooth. In the embryo $^{87}Sr/^{86}Sr$ ratio was very steady and near the Global Marine Signature of .70918 (Figure 3A).

The Sr/Ca ratio was relatively steady in the adult fish, with slightly higher values in the earliest period of growth and at mid-life (Figure 3B). In comparison the Sr/Ca ratio was significantly elevated in the embryo, exhibiting lower values at the start of growth, increasing to their highest values between 5 and 10 mm from the tip, and decreasing slightly at the tooth base (Figure 3B).

In the adult tooth, Ba/Ca exhibited the highest values at the earliest growth stages, decreasing at mid-life, and increasing again toward the end of life at the tooth base (Figure 3C. The embryo tooth contained very low levels of barium throughout (Figure 3C).

Magnesium to calcium ratios showed pronounced, repeated peaks throughout the life of both fish, with large decreases near the tooth base (Figure 3D). Manganese to calcium ratios showed similar repeated spikes throughout the life of the fish (Figure 3E). The highest Mn/Ca values were recorded at the tip and base of each tooth.

Discussion

The highly endangered status of sawfish presents challenges to researchers and conservationists (Simpfendorfer 2000, Kyne, P.M., Carlson, J, Smith 2013, Dulvy et al. 2016). Modern, long-term, studies of living sawfish in the wild are rare and are often skewed toward smaller, juvenile fish because they are most likely to be captured (Thorson 1982, Peverell 2008, Poulakis et al. 2011). Further, conservation status and abundance preclude most lethal sampling of sawfish, negating the promise of vertebra as an aging and microchemical tool for understanding sawfish life history. Developing non-lethal methods which can address life-history questions with larger sample sizes are required to understand sawfish ecology and inform conservation efforts of sawfish.

Chemical analysis of the two sawfish teeth in this study indicate that they store highly useful chemical records of movement, environment, and life-history. A combination of $^{87}Sr/^{86}Sr$, Sr/Ca, and Ba/Ca (determined simultaneously, Hegg, Fisher, and Vervoort, in prep) show movement across fresh, brackish, and ocean water in for these fish. Strontium ratio is well mixed in ocean water, such that all ocean water exhibits a signature of 0.70918 (Faure and Mensing 2004). Deviations from this indicate movement into fresh or brackish water. Further, barium contentration quickly decrease as salinity increases in estuaries, signaling the transition from fresh to brackish water (Kalish 1990, Walther and Limburg 2012). Strontium concentrations exhibits the opposite dynamic, increasing quickly as brackish water transitions to the higher salinity of the ocean (Zimmerman 2005). Taken together, this indicates that the adult in our study spent significant periods in low salinity water.

High Ba/Ca, low Sr/Ca, and $^{87}Sr/^{86}Sr$ far below 0.70918 indicate the earliest recorded period of the adult fish was spent in low salinity, likely fresh water (Figure 3A, B&C). During the middle part of its life (15-35mm) Ba/Ca decreases and $^{87}Sr/^{86}Sr$ increases toward the global marine signature. This indicates a movement toward more saline water. The lack of significant increase in Sr/Ca and the fact that $^{87}Sr/^{86}Sr$ stays slightly below the global marine value indicates the fish was in saline water, but not in the open ocean. By the end of its life the fish appears to have moved back into fresh or mildly saline water. The smaller spikes and troughs in these signals may indicate smaller-scale forays into different salinities, either through movement or through seasonal changes in salinity as reported by Sharer et al. (Scharer et al. 2012). High Sr/Ca, extremely low Ba/Ca, and a stable $^{87}Sr/^{86}Sr$ signature very near the global marine average indicates that the embryo spent the entirety of the gestation in the open ocean.

The magnesium calcium ratio has been called the 'chemical clock' in some fish species due to its association with seasonal growth rates (Limburg et al. 2018). Without additional information about sawfish in particular it is impossible to determine, but the repeated sharp peaks in this tracer are suggestive of periods of higher and lower growth (figure 3D), a relationship which merits further study. The marked decrease in Mg/Ca near the base of the tooth (30-45mm) coincides with the onset of cloudiness in the tooth matrix. Since this pattern occurs, on a smaller scale, in the embryo tooth as well it and may be related to some unknown process of tooth formation occurring near the root.

Spikes in Mn/Ca have been shown to be an indicator of exposure to hypoxia in multiple fish species (Altenritter et al. 2018, Limburg and Casini 2018). This is due to a switch in redox state which releases Mg into the water column from the substrate under hypoxic conditions (Thorrold and Shuttleworth 2000). Sawfish are known to inhabit shallow, nearshore habitats and mangrove swamps which could potentially experience hypoxic conditions. The peaks in Mn/Ca exhibited in both the adult and embryo rostral teeth (Figure 3D) rise above the threshold set by Limburg and Casini (2018). This suggests the possibility that sawfish may be experiencing some level of hypoxic stress. Altenritter et al. (2018) showed a tradeoff between exposure to hypoxia and feeding opportunity in Atlantic croaker. A similar behavior could be indicated by the peaks in Mn/Ca apparent in the rostral teeth in this study.

Analysis of banding structures in the adult tooth showed that the distance between the putative growth bands formed a linear relationship rather than characteristic decrease in distance with age expected from a von Bertalanffy growth function. There are two possible reasons for this. The first is that these structures form at regular intervals along the tooth and are not influenced by growth. The second is related to tooth wear. Sawfish rostral teeth are often heavily worn, as the adult sample in our study was. The amount of tooth that has worn away is difficult to know, but it is possible that the only remaining growth record in our sample is the relatively linear, adult portion of the growth curve. The furthest distal teeth in our rostrum were also some of the most worn, indicating that future studies should target teeth with less wear and curvature, elsewhere on the rostrum.

While the length-at-age relationships of sawfish are poorly understood for larger adults, comparisons of our data to existing growth curves suggest that the putative growth bands are sub-annual. Based on the SRL/TL relationship reported by Whitty et al. (2014), the adult sample was taken from a 238 cm fish. Simpfendorfer (2000) predicts the adult would have been ~5 years old, or ~12 years old based on an alternative growth curve assuming a halved growth rate. Tanaka et al. (1991) predicts the fish would have been 11-12 years old. More recent growth curves from Peverell (2008) all predict ages less than 6 years old, however this data comes from other species of sawfish and does not include *Pristis pristis*. Thus, if the structures observed are related to age or growth, the relationship would need to be established through a relationship to other age information such as counts of annual vertebral bands.

Taken together this study presents compelling evidence that sawfish rostral teeth are a recorder of chemical information throughout the life of individual fish. Further, this chemical data records long-term, high-resolution, information about sawfish life-history and movement that is otherwise difficult to obtain from a live specimen. Our data further suggest that the structures in sectioned rostral teeth are periodic in nature and may be useful in determining growth or age, though they do not appear to be annual in nature.

The results reported here are necessarily preliminary given the limitations of our study. Larger samples sizes, including fish of known location and origin, are necessary to confirm our findings. Further, more must be known about the rate of wear on sawfish teeth to better constrain the amount of information missing due to tooth wear. Despite these limitations, our data support further investigation of sawfish rostral teeth as a non-lethal method of reconstructing movement, life-history, age and growth. The large numbers of rostra housed at museums, academic institutions, and in private collections worldwide could potentially be employed to study both current and historic sawfish ecology. Further, if tooth removal is indeed not detrimental to sawfish condition and survival (Field et al. 2009), sawfish teeth from captured specimens could provide increased understanding of sawfish life-history and movement in conjunction with tagging and tracking studies.

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Figures

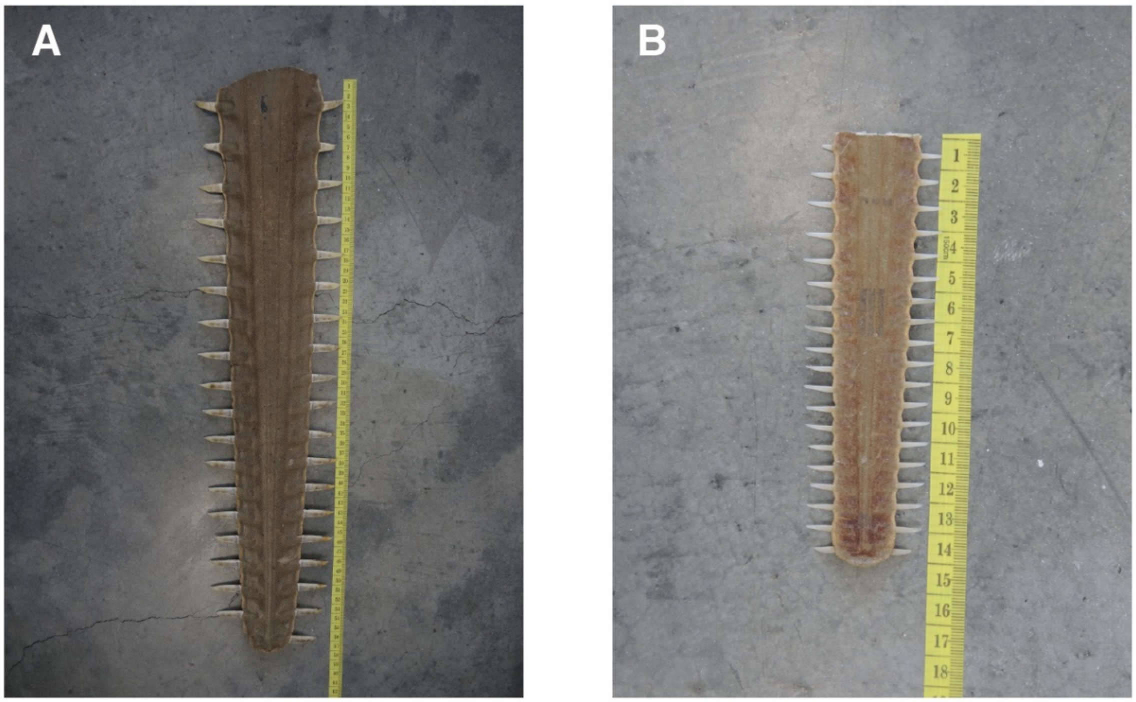

Figure 1 – Rostra from two largetooth sawfish were used in this study. An adult rostrum (A) of unknown origin had a TRL of 57cm and a SRL of 55cm. The embryo rostrum collected in Belize in 1969 had a TRL of 14.2cm and a SRL of 13.6cm. The most distal tooth on the left-hand side was taken for analysis from each rostrum.

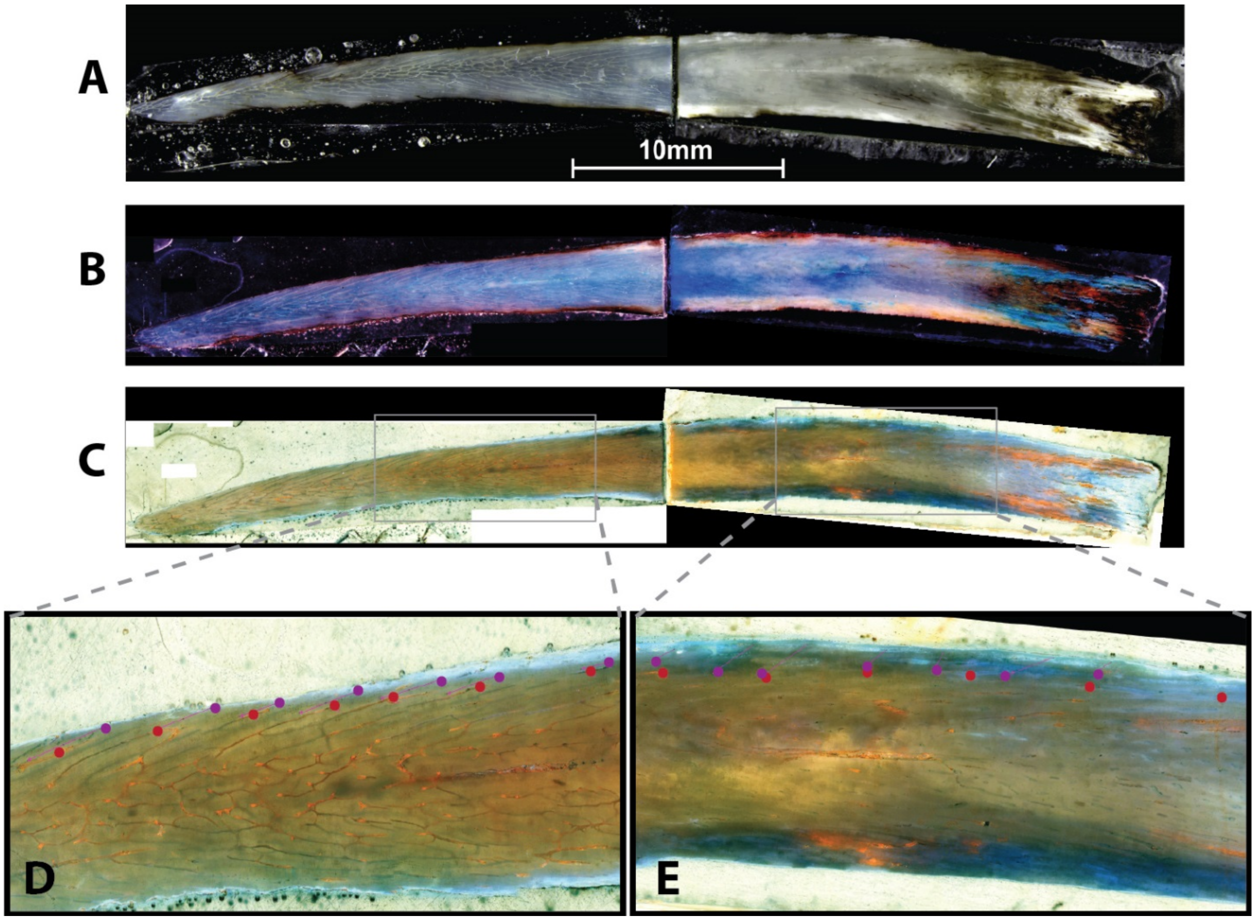

Figure 2 – Adult largetooth sawfish rostral tooth sectioned on the dorsal-ventral plane (A) shows internal tubule structures and faint banding. Staining with Mutvei's solution (acetic acid, glutaraldehyde, and alcian blue) highlighted the tubules which broke the surface in darker blue (B). Inverting the color spectrum of the stained section (C) further improved visualization of the surface tubules. Tubules overlapped in curved bands (D), with "legs" descending distally from the core of the tooth to the edge. These tubules were less distinct near the base of the tooth, however faint banding was also visible. Putative growth was quantified by two readers (reader 1 – red dots, reader 2 – purple dots). Measurements agreed despite being offset where descending tubule "legs" were prominent (D). Agreement was lower in sections with less prominent tubules (E).

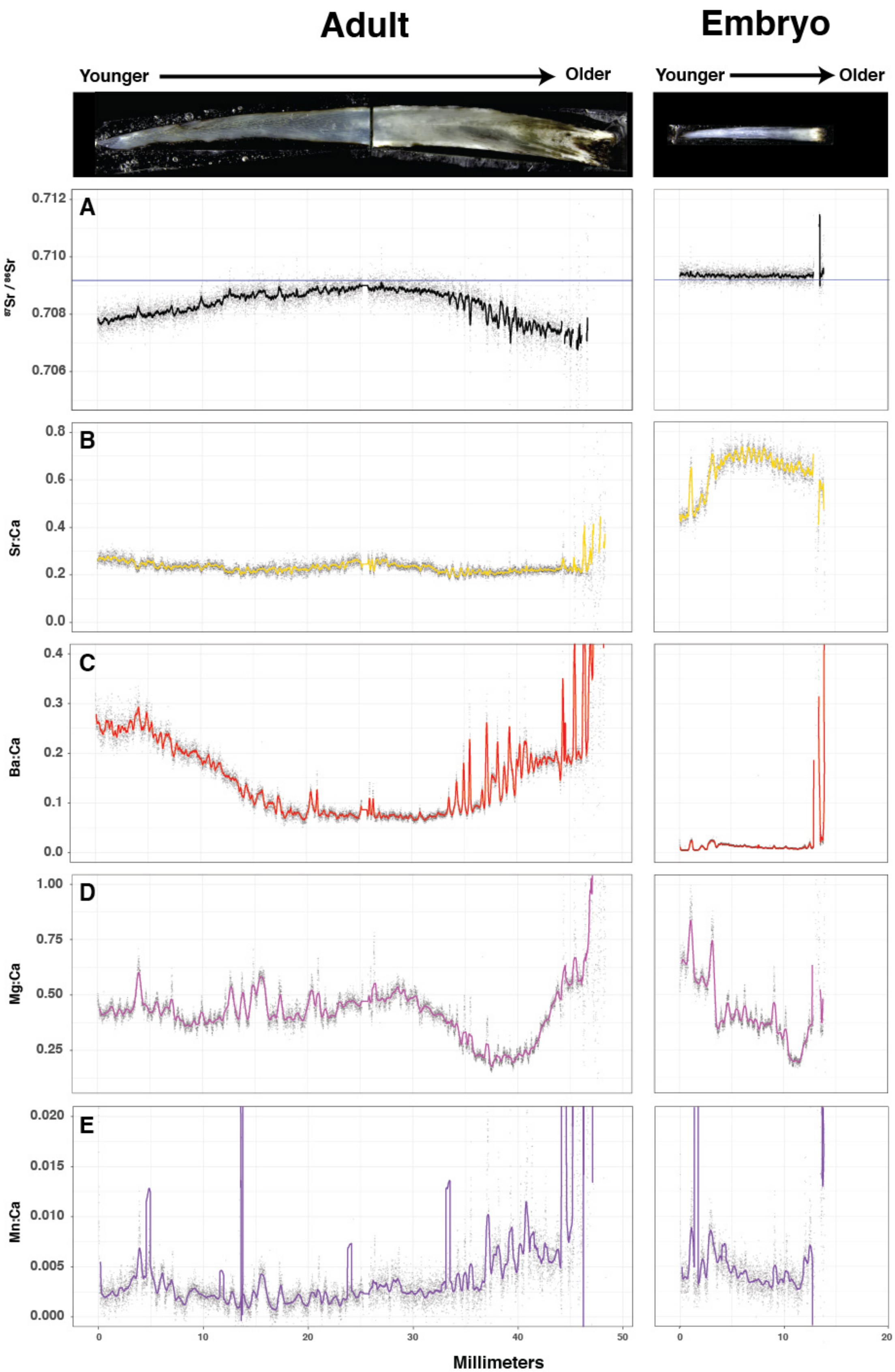
Adult
Embryo
Younger
Older
Younger
Older
A
B
C
D
E
⁸⁷Sr / ⁸⁶Sr
Sr:Ca
Ba:Ca
Mg:Ca
Mn:Ca
Millimeters

Figure 3 – Results of split stream LA-ICP-MS analysis of sectioned rostral teeth from to largetooth sawfish specimens, and adult (left-hand column) and an embryo (right-hand column) showed significant variation across the tooth. Strontium ratio ($^{87}Sr/^{86}Sr$, A) indicated movement within fresh or brackish water, with movement toward the global marine average (blue horizontal line) in mid-life indicating movement into higher salinity water. Meanwhile the embryo $^{87}Sr/^{86}Sr$ appears to match the global marine signature throughout life. Strontium to calcium ratio (Sr/Ca, B) did not rise significantly during mid-life in the adult, confirming residence in brackish rather than fully saline water. The embryo Sr/Ca, in contrast, is elevated, confirming a likely ocean residence. Barium to calcium ratio (Ba/Ca, C) rise in fresh water and decrease in salt water, confirming adult and embryo movements inferred from the prior tracers. Magnesium to calcium ratios (Mg/Ca, D) are linked to growth rate in other species. Peaks in Mg/Ca could indicate growth rate changes as well as movement into new locations. Manganese to calcium ratios (Mn/Ca, E) are linked to anoxic conditions as Mn is released into the water column due to a redox reaction in low oxygen environments. Peaks in Mn/Ca rise above values signaling hypoxia in teleost fish species, which could indicate hypoxia tolerance in sawfish, however the dynamics of Mn incorporation into sawfish teeth requires further study.