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Marine and Freshwater Research

Supplementary Material

Physicochemical mechanisms of FT-NIRS age prediction in fish otoliths

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Supplementary material

Confirmation of crystal structure: Wide Angle X-ray Scattering (WAXS)

Thin sections of three otoliths aged 2, 10, and 17 years were analyzed using a SAXS Lab Ganesha instrument at the South Carolina SAXS Collaborative to evaluate potential age-related differences in otolith crystal structure. Whole, clean otoliths were embedded in epoxy and cross-sectioned in the transverse plane through the otolith core according to the methods described in VanderKooy (2009). The resulting thin sections (~ 0.5 mm thick) were individually irradiated at a standardized position on the convex surface near the sulcus for each section (Figure S1.A) using a Xenocs GeniX3D microfocus source with a Cu target, generating a monochromic beam with a 0.154 nm wavelength. The instrument was calibrated using National Institute of Standards and Technology (NIST) reference material 640c silicon powder with the peak position at $2\theta=28.44^{\circ}$ where 2θ is the total scattering angle. A Pilatus 300 K detector (Dectris) was used to collect the two-dimensional (2D) scattering patterns. All WAXS data were acquired with the sample positioned 112.1 mm from the source with an X-ray flux of ~38.5 M photons/s incident upon the sample. Each WAXS measurement was acquired with a 20 min exposure. Each sample was measured with a transmission geometry having the beam normal to the sample plane. The resulting 2D images were azimuthally integrated with SAXSGUI to yield the scattering vector and intensity.

WAXS measurements were also acquired using ground otolith powder to eliminate potential effects of crystal orientation upon peak intensities. Following evaluation of intact thin sections, the approximate area of focus for each otolith was excised from the sections and ground gently with a mortar and pestle to a uniform particle size. A subsample of each powdered otolith was then evaluated again with WAXS using the settings outlined above. The resulting intensity measurements were normalized across samples before plotting for comparison relative to 2θ. Peaks were compared to published standards for aragonite (AMCSD# 0000233), and related polymorphs such as vaterite

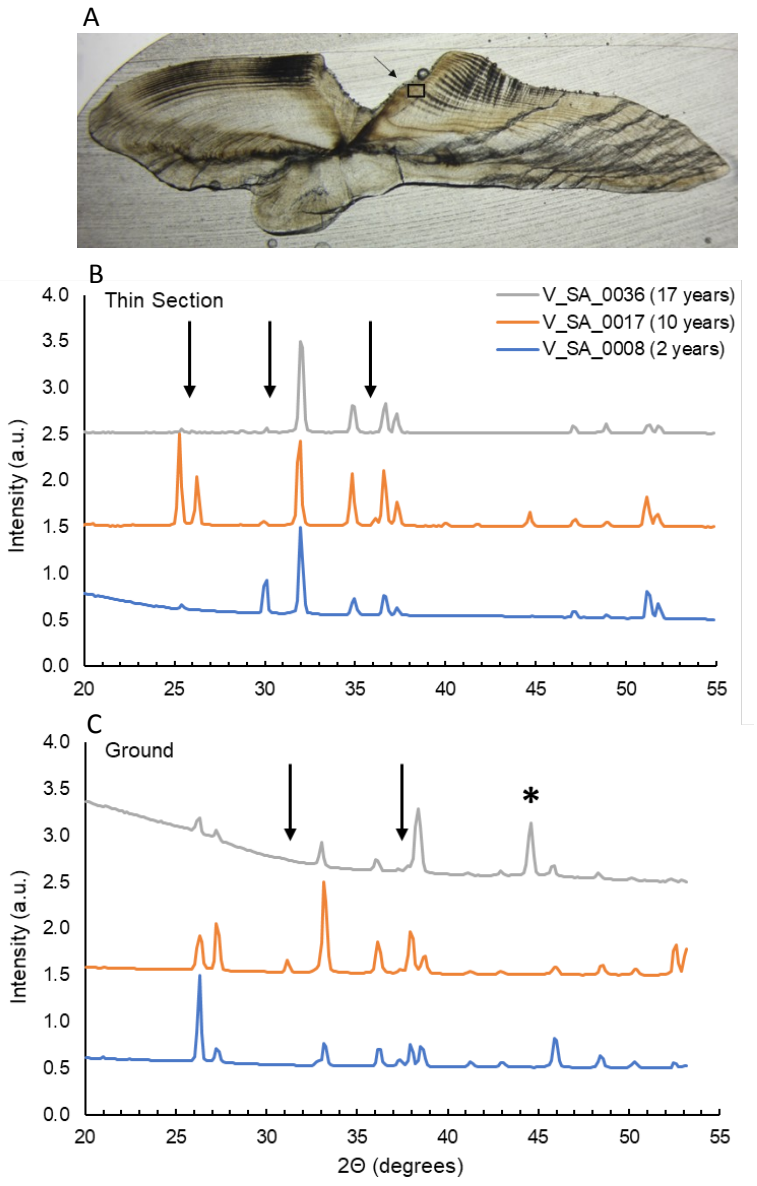
(AMCSD# 0004854) and calcite (AMCSD# 0000098), as well as hydrated forms ikaite (AMCSD# 0010853) and monohydratecalcite (AMCSD# 0004611).

Results

WAXS

The observed diffraction patterns obtained for whole otolith sections and corresponding ground powders were consistent with an expected polycrystalline aragonite structure, with no evidence of vaterite, calcite, or hydrated crystal structures (Figure S1.B-C). Some variations in the relative peak intensities were observed for all the otolith samples analyzed regardless of preparation method. As these variations persistent after grinding the otolith sections, the differences are not attributable to crystal orientation but perhaps are associated with e.g. fractional atomic substitutions. For example, atomic substitutions such as strontium replacing calcium are well-documented in otoliths (e.g., Doubleday *et al.*, 2014) and are not necessarily age-related. One peak in the ground sample from V_SA_0036 was not consistent with any reference crystal structures evaluated (Figure S.1C, starred), and was attributed to diamond residue from the blades used in the cutting process (AMCSD 0011242). Overall, the WAXS results do not indicate any predictable, age-related changes to the crystal lattice, and confirm that any water molecules present in the otoliths did not alter the crystal structure.

Figure S1. A) Red snapper otolith section from V_SA_0032, a 17 year old fish evaluated using WAXS. Black box (indicated by arrow) shows the approximate location of WAXS sampling, which was standardized among the three sections analyzed. B) Results of WAXS measurements from thin sectioned and C) ground red snapper otoliths aged 2, 10, and 17 years. Arrows in B and C denote peaks where differences were evident between samples, and * denotes a peak attributed to diamond residue from the cutting process (AMCSD 0011242).



Literature Cited

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