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Dear NASPS Governing Board,

First and foremost, I would like to thank the society for providing me with the funding to travel to Oak Ridge National Laboratory to conduct my research quantifying otolith calcium carbonate ($CaCO_3$) polymorph composition of Lake Sturgeon and White Sturgeon in collaboration with Dr.'s Brenda Pracheil and Bryan Chakoumakos. I was able to further hone my skills to operate X-ray and neutron sources and learn the programs used to integrate and interpret the resulting data. To date, the data from this collaborative research has been published in two peer reviewed journals (Loeppky AR, Chakoumakos BC, Pracheil BM, and Anderson WG. 2019. **Otoliths of sub-adult Lake Sturgeon *Acipenser fulvescens* contain aragonite and vaterite calcium carbonate polymorphs**. *J Fish Biol*. 1-5. and Chakoumakos BC, Pracheil BM, Wood SR, Loeppky AR, Anderson WG, Koenigs R, and Bruch R. 2019. **Texture analysis of polycrystalline vaterite spherulites from Lake Sturgeon otoliths**. *Scientific Reports*. 9:7151.) as well as presented at multiple international and national conferences. In addition, there are several manuscripts currently being drafted and are anticipated to be submitted to high impact journals in the coming months. The work that had initiated this collaboration has enabled future plans to further pursue the environmental factors influencing otolith precipitation and how this effects the resulting behaviour of individuals. The following report outlines the details of this research.

I look forward to presenting and discussing the data at the 2019 NASPS meeting in Hecla, Manitoba.

Regards,

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Otoliths are calcified structures that form by the accumulation of biominerals deposited on a proteinaceous matrix in the inner ear of fish and function as hearing and balance structures (Secor et al., 1995; Campana, 1999). Calcite, aragonite and vaterite are crystallized biomineral polymorphs of calcium carbonate ($CaCO_3$) and make up the primary composition of otoliths (Pracheil et al., 2017). Otoliths of primitive fishes, including sturgeons, were previously described as being composed entirely of vaterite (Carlström, 1963; Gauldie, 1993). Recently however, it was demonstrated that the otoliths of adult Lake Sturgeon, *Acipenser fulvescens*, also contained significant proportions of calcite (18-30%; Pracheil et al., 2017). These findings were surprising given that the ability to form calcite in otoliths was not thought to have evolved until the separation of teleosts from ray-finned fishes (Gauldie, 1990; Pracheil et al., 2017). It was unclear, however, if this shift in $CaCO_3$ polymorph phase was due to ontogenetic development or a physiological response to changes in environmental conditions. My research focused on investigating these potential influencing factors by conducting two controlled experiments.

First, variations in percent otolith polymorph composition were measured throughout the ontogenetic development of larval Lake Sturgeon and White Sturgeon, *A. transmontanus*. Both species were sampled at regular intervals throughout development from immediately post hatch to two months post fertilization (Figure 1). Sagittal and lapilli otoliths were then dissected from each individual and transported to Oak Ridge National Laboratory for polymorph quantification via X-Ray microdiffraction (μXRD). This technique uses the scattering of X-rays off the atoms of a polycrystalline structure (i.e., otolith). The scattered X-rays register on a detector producing a Debye–Scherrer diffraction pattern that can be used to gain information on the structure of the crystals or the identity of a crystalline substance (i.e., polymorph composition). The diffraction patterns are then radially integrated generating intensity plots. A technique

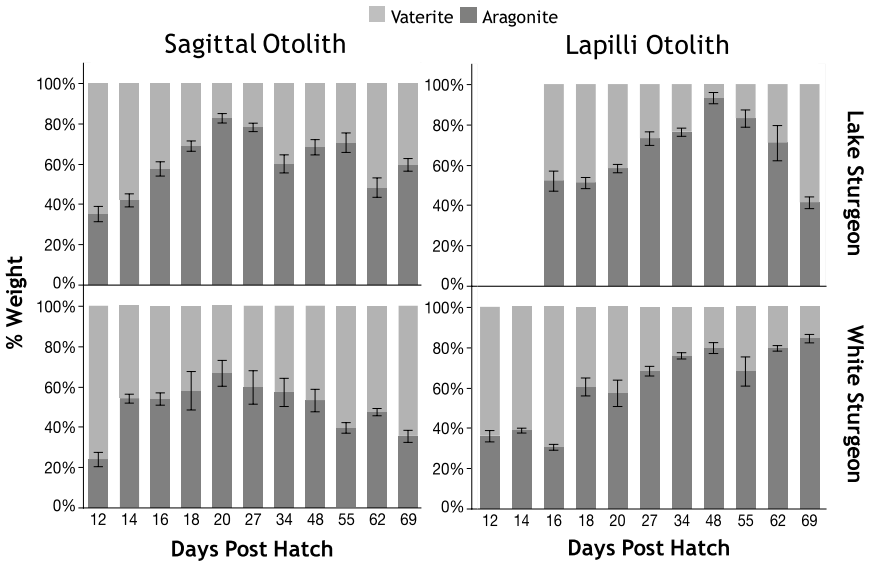

Figure 1 Average (±SE) percent polymorph composition of sagittal and lapilli otoliths from Lake Sturgeon and White Sturgeon sampled at regular intervals throughout ontogenetic development.

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called Rietveld analyses was then used to quantify the percent composition of each polymorph using reference structural data for known patterns of calcite, aragonite and vaterite. Results identified both species showed similarly changing patterns of otolith polymorph composition with increasing proportions of aragonite forming as larvae developed. This, however, did not account for the calcite crystallization observed in the adult otoliths.

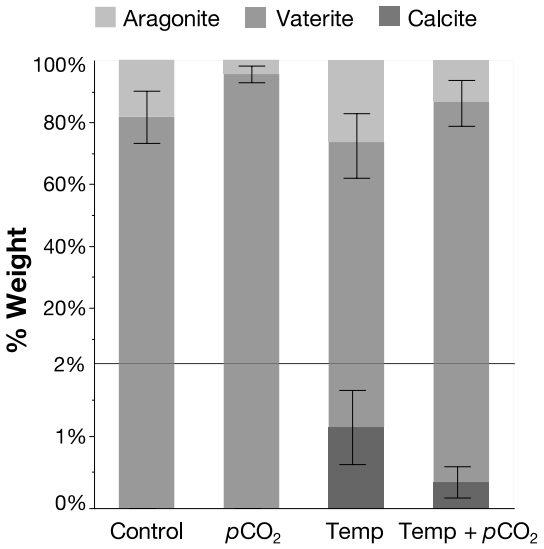

Figure 2 Average (±SE) percent polymorph composition of sagittal otoliths from Lake Sturgeon reared for six months in varying temperature and pCO_2 concentrations.

To identify whether the environment influences polymorph precipitation, Lake Sturgeon larvae were reared in varying temperature and pH conditions with either elevated temperature (22°C), elevated pCO_2 (2500 µatm) or a combination of both (Figure 2). After six months, otoliths were removed and analyzed for percent polymorph composition using µXRD as described above. Interestingly, pH had no effect on polymorph composition, however, fish raised in elevated temperatures had significantly different aragonite and vaterite proportions as well as inclusions of calcite crystals. These results suggest both ontogenetic development and environmental conditions play a significant role in the precipitation of otolith $CaCO_3$ polymorphs. In addition, this data indicates that sturgeon have the capacity to precipitate all three forms of $CaCO_3$ polymorphs countering long standing classifications of otolith composition among fish species. The ability to quantify polymorph composition in the otoliths of larval and juvenile sturgeons using µXRD provides a unique opportunity to investigate these mechanisms in Acipenserids and could potentially be applied to any fish species. The capacity for this technique to be used on small sized otolith samples (< 500 µm) in a non-destructive manner allows for an understanding of the role development and the environment may play on otolith composition throughout early life history. Furthermore, identifying the molecular mechanisms involved in protein expression linked to polymorph precipitation in primitive fishes would provide insight into the evolution of otoliths and their role in hearing and balance among fish species and higher vertebrates.

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