ENVIRONMENTAL LIFE HISTORIES OF FRESHWATER FISH USING OTOLITH MICROCHEMISTRY

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ABSTRACT

Using otolith microchemistry (fish ear stones) we explored whether elemental difference in freshwater fish otoliths could isolate game fish populations. Specifically we assessed whether otolith microchemical signatures in trout (Brown, Rainbow, and Cutthroat) otoliths to isolate stocked from resident populations. In all cases otolith microchemistry was sufficiently variable to allow classification of the fish to the stream or hatchery natal habitat. Our study firmly demonstrates the utility of otolith microchemistry in freshwater systems showing that this technique can provide valuable insights into the environmental life histories of freshwater resident fish.

INTRODUCTION

Otolith, or fish ear stone, microchemistry has become an important tool for tracking fish movement in aquatic systems (Thorrold et al., 2001; Dorval et al., 2002; Kennedy et al., 2002). Although this tool has been validated independently through controlled experimentation and field collections (Kalish 1991; Thorrold et al. 1998; Bath et al. 2000; Wells et al. 2003), some of the mechanics of otolith chemistry and growth are unknown. Otoliths are composed of $CaCO_3$ in the form of aragonite. Divalent cations of similar ionic radii to calcium (e.g. Mg^{2+}, Sr^{2+}, and Ba^{2+}) can substitute for calcium in the otolith matrix (e.g., Kalish, 1989). Other trace metals work their way into the spaces within the aragonite crystal lattice or are incorporated into the protein in the otolith matrix (Milton and Chenery, 1998; Campana, 1999; Sanchez-Jerez et al., 2002). The mechanism of substitution and incorporation of trace metals into the otolith matrices are a function of abiotic and biotic conditions such as salinity and fish growth rate (Thresher, 1999).

The otolith forms through concentric additions of mineralized tissue around a central nucleus with daily additions during the larval and early juvenile stages of life (Pannella, 1980; Campana, 1999). These daily increments become spatially resolvable only as seasonal or yearly bands over time (Fig. 1). Because the otolith is acellular, it is physiologically static; in other words, once deposited, otolith material is not resorbed or metabolically reworked to any significant degree. Therefore, otoliths remain relatively unaffected by short-term changes in fish condition. Consequently, otolith banding provides an accurate means of determining age and growth. Age and growth determinations are possible because precipitation rates for otoliths of some species may exceed 1 mm/yr and daily growth banding is evident; therefore, it is possible to time events in the life history of fish much the same way that tree rings are used to age trees.

Otoliths have been used recently to identify natal habitat for salmon, weakfish, and spotted sea trout (Thorrold et al., 2001; Dorval et al., 2002; Kennedy et al., 2002), study of fish life history, and stock delineation (Milton and Chenery, 2001, Bath et al. 2000, Thorrold et al., 1998 and 1997). Although these applications have been successful several unanswered questions about otolith microchemistry

remain. While not tested directly researchers assume that the rate at which a fish grows does not alter the elemental ratios in the otolith. The question that this study addressed is how might growth affect otolith microchemistry?

Figure 1. Sagittal otolith of a Brown Trout. A. Otolith under plane light showing visible banding in the lower left quadrant. B. After thin-sectioning to expose the core of the otolith under polarized light, yearly bands are visible.

Zimmerman and Reeves (2002) used otolith microchemistry of anadromous and resident rainbow trout to determine the maternal origin of fish. The Sr/Ca of the cores of the resident and anadromous rainbow trout otoliths allowed for the classification of fish to specific rearing habitats. Numerous studies have estimated that juveniles and adults move less than 30 km during their natal residence (Annett, 1998; Whittier et al. 1999) and this close association results in the potential of otolith microchemistry signatures that yield geographic specificity. In this study we were limited by the variations otolith microchemistry and not by the spatial extent of roaming. Researchers understand how to use otolith microchemistry to identify natal habitat, but the question remains: Are there changes in growth that impart a signature in the chemistry of the otolith? In this study, we compared otolith microchemistry in juvenile trout over a six-month period in three congener trout (Rainbow trout – *Salmo gairdneri*, Brown trout – *Salmo trutta*, and Cutthroat trout – *Salmo clarki*). We identified temporal changes in otolith chemistry, over time, within species as well as differences in otolith microchemistry between species.

MATERIALS AND METHODS

Fish- Ninety trout of each species (Rainbow, Brown, and Cutthroat) were used in this study. The two-week-old trout were placed into two raceways with 45 of each species in each raceway. The raceways were located in an inside facility at the Spring River State Hatchery. The raceways were side by side and had the same water source. The trout were fed a constant diet. Ten to twenty of each trout species were taken from each raceway at one, three, and six months. Over the sampling period some predation (trout on trout) was observed.

Otoliths- Otolith analyses of juvenile fish: Both sagittal otoliths were extracted from juvenile Rainbow, Brown, and Cutthroat trout using our standard technique that ensures that the otoliths are maintained under Class 100 Clean laboratory with all otolith extraction and preparation done in a purpose-built clean lab at Arkansas State University. All tools used for extraction were nonmetallic and acid washed prior to use to minimize contamination. The otoliths were triple rinsed with Millipore Milli-Q® water (18.2 Mohm), then rinsed for five minutes with ultrapure hydrogen peroxide (36%) to remove organics, triple rinsed again with Millipore Milli-Q water, sonicated for five minutes in Millipore Milli-Q water, triple rinsed with Millipore Milli-Q water, and dried under a laminar flow hood for 24 hours. One sagittal otolith was digested for analysis on the ELAN 9000 ICP-MS (Perkin Elmer) located in the Water-Rock-Life Laboratory at Arkansas State University. The remaining sagittal otolith was cleaned and prepared for aging. (Shuttleworth, 2000). Otoliths were digested in Teflon using 0.2 mL ultra-pure HNO_3. The digested otolith samples were diluted to 2 mL and spiked with 40 ppb internal standard (^{75}Ge, ^{115}In). Samples were introduced to the ICP-MS using a microconcentric nebulizer (MCN 100) coupled with a PFA Scott – type spray chamber. A five-point calibration curve was established using standards ranging on concentration form 10 ppb to 1000 ppb. We used NIST 1640 (Trace Elements in Natural Waters) as an external standard to monitor precision. In addition, the process was monitored by a calibration blank (1% of ultrapure- HNO_3) and re-calibration every 6 samples. Concentrations of all elements were calculated from the calibration curve after proper correction for control blank, matrix and drift effects. Based on measurements of NIST 1640 our reported values are better than 3% for all elements of interest. The following isotopes were monitored with isobaric

correction equations built-into the analytical method as specified by EPA 200.8. ^{24,25,26}Mg, ^{44}Ca, ^{55}Mn, ^{63,65}Cu, ^{86,87,88}Sr, ^{135,137,138}Ba, ^{206,207,208}Pb, and ^{235,238}U. Whole element concentrations were calculated based on calibrations and relative abundance of isotopes. In the case of multi-isotope elements the reported concentration represents and average of the measured concentrations calculated independently for each isotope. All multi-isotope concentrations were within 1% of each other. T-Tests were performed on ratios comparison and comparisons between ratios and growth rate. T-Tests were used to compare ratioed data sets to one and other.

RESULTS

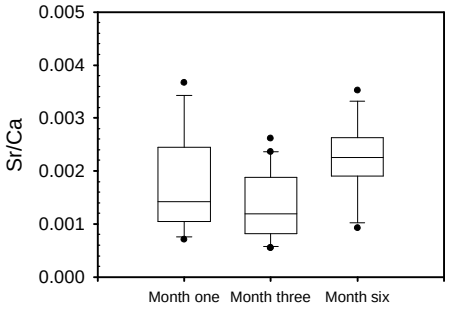

Figure 2. Sr/Ca ratios in Rainbow Trout are marginally statistically different from month one to month six (p = 0.056) and statistically different from month three to month six (p= 0.002).

Comparison of Ca ratioed data allows assessment of variations in relative uptake of metals by the otolith over time. In Figure 2 Sr/Ca data of whole Rainbow Trout otoliths, collected over 6 months from raceways, shows that, over time, Sr/Ca varies as the fish ages. No other differences in Ca ratioed elements were noted in the Rainbow Trout raised in the raceways. These relationships were consistent within all species tested.

DISCUSSION

There were no statistically significant differences between ratios in otoliths for the different fish in the raceways. The ratios become much more variable when we look at the differences over time. Rainbow Trout had one ratio that changed over time and Brown Trout and Cutthroat Trout had four and three ratios that changed over time respectively. This pattern does not necessarily fit into contemporary theory on otolith microchemistry, where the ratios in the otoliths will stay the same if the input of metal stays the same. Water was not collected during this experiment, so the water chemistry could have changed in a six-month period. Or there could be some developmental changes within the fish that could lead to a change in metal exchange and accumulation. When the ages are plotted against weight and length and age vs. growth rate, an interesting pattern emerges (Figure 3). Rainbow Trout grew slower and the changes from start to finish are very slight, but Rainbow Trout were larger at the beginning of the experiment. Both Brown and Cutthroat Trout were smaller in size at the beginning of the experiment than Rainbow Trout but grew quicker. Cutthroat Trout actually increased in size quicker than Brown Trout and achieved an overall size larger than the other trout species. Because Brown and Cutthroat Trout grew quicker than Rainbow Trout there could be some developmental mechanism that affects the otolith ratios. Microchemistry ratios for the trout otolith were linearly correlated to otolith weight (Rainbow Trout R^2 = 0.77, Brown Trout R^2

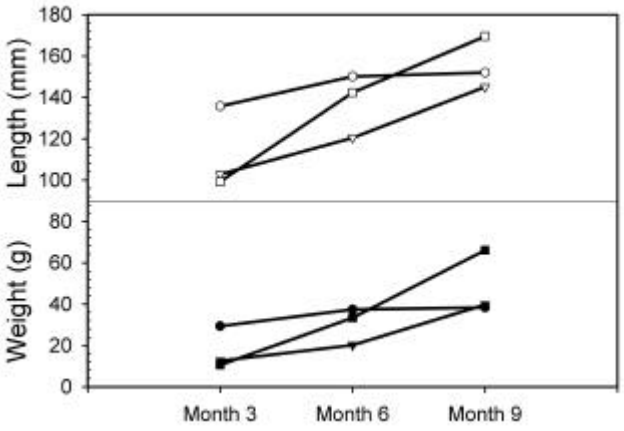

Figure 3. Weight (g) and Length (mm) vs. age in all trout species. Circle – Rainbow, Square – Brown, Triangle – Cutthroat.

= 0.65, and Cutthroat Trout R^2 = 0.70). As the fish and otolith grow the ratio also changes in a linear pattern to relative to growth. One interesting pattern that emerges is the slopes of the linear line (otolith weight vs. Sr/Ca) for Rainbow Trout with a positive slope while the slope is negative for Brown and Cutthroat. The sign of the slope indicates that as the Rainbow Trout otolith grows the Sr/Ca ratio increases. The opposite is true for Brown and Cutthroat Trout. These relations lead us to explore the linear correlation of otolith weight vs. Ca and vs. Sr. When we plot these linear correlations we see that the for otolith weight vs. Ca in Rainbow Trout the slope is negative and positive for Brown and Cutthroat Trout. When otolith weight vs. Sr is plotted the Rainbow Trout's slope is positive and Negative in Brown and Cutthroat Trout.

If we compare the number of ratios that were affected in each species we see that there are two for Rainbow Trout, Five for Brown Trout, and Six for Cutthroat Trout. Thereafter we can compare the growth to number of ratios affected temporally and we see that Rainbow Trout grow more slowly, and also have the least number of ratios affected. Brown and Cutthroat Trout have triple the number of affected ratios and they grew much quicker than Rainbow Trout. The reason for the differences are unknown but we think it may have to do with growth and the elements that easiest and quickest to get from the environment.

This leads the discussion to variation between trout congeners. We would expect to see very little difference between species so similar, but many of the ratios were affected when the individual species were grouped together and analyses vs. each other. But again this could be a matter of the growth changes affecting microchemistry. Therefore we then only compared trout in the same size class and found a much different picture. Before the size class comparison there were nine ratios that were different when conducting species comparisons. By taking a closer look we find that most of the differences are between Rainbow Trout and the other Trout in this study, this is also indicating a size class change in chemistry because of the growth difference in the trout species. When we compared ratios of trout in the same size class we have two ratios that are different from species to species.

Conclusion

Growth in juvenile trout appears to be playing a very important role in otolith microchemistry. Consequently any comparisons by otolith microchemistry need to take into account the size classes before analysis. We also found the ratios Sr/Ca and Mg/Mn may be more sensitive to species effects. Sr/Ca is very sensitive to any changes, whether those are species or size class. Of the ratios used in the study Mg/Ca, Ba/Ca, Sr/Ca, Mg/Sr, Mg:Mn, Mn/Ba, and Mn/Sr are sensitive size class fluctuations. Whereas Pb/Ca, Mn/Ca, Mg/Ba, and Ba/Sr are more resilient to size class fluctuation, and are also resilient to species fluctuations.

In any study the question that is being asked is the control for what tools you need. Using otolith microchemistry to delve into fish movement is a powerful tool and there are many ways to use this tool. The researchers using otolith microchemistry have to understand and use the correct procedures and ratios to fully utilize this powerful technique.

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