

# New Insights into Fish Ecology via Nuclear Microscopy of Otoliths

K.E. Limburg\*, M. Elfman<sup>†</sup>, P. Kristiansson<sup>†</sup>, K. Malmkvist<sup>†</sup>, and J. Pallon<sup>†</sup>

\*State University of New York, College of Environmental Science & Forestry, Syracuse, NY 13210 USA

<sup>†</sup>Department of Nuclear Physics (Lund Institute of Technology), Lund University, Lund, Sweden

**Abstract.** Otoliths, or earstones, are small, biogenic concretions of aragonitic calcium carbonate precipitated on a protein matrix. Otoliths form part of the hearing and balance system in teleost fishes, and grow as the fish grow, providing a continuous biochronology of growth. Various elements are entrained in minor and trace quantities. In particular, strontium is a useful scalar of habitat use when variable environmental gradients exist. By mapping elemental concentrations and ratios with the Lund nuclear microprobe, we have used strontium in many cases as a proxy for salinity, because Sr:Ca values are roughly an order of magnitude higher in marine vs most fresh waters. In addition, zinc shows strong seasonal variations in salmoniform fishes (salmons, charrs, and whitefishes have been tested to date). We present case studies of several species, and discuss exciting future directions in this research that is revolutionizing fisheries ecology.

## INTRODUCTION

Otoliths (earstones), found in the inner ear of teleost fish, are biogenic structures composed of calcium carbonate minerals [1]. Otoliths are part of the hearing/balance system in fishes. There are three pairs, which reside in semicircular canals beneath the brain. The shape and growth features of otoliths are species-specific, but a characteristic they all share is a temporal periodicity in accretion. Increments are daily when growth is fast, primarily at early life stages; annual increments are created by variations in environment produced by seasonal changes in temperature, precipitation, or both. Because of these chronometric properties, otoliths have become a valuable tool for understanding age, growth, and life history characteristics of fish populations [2].

Microanalysis of otolith chemistry is one of the most important fields in otolith research [3,4]. Otolith elemental and stable isotopic composition yields information about the habitat, thermal regimes, and salinities experienced by fish [4]. Trace and minor elements, combined with stable isotopic ratios of C and O, in otoliths have proven to be reliable as “chemical fingerprints,” identifying the past environments of many fishes [5,6], and even past environmental conditions [7].

Over 40 elements have been identified in otoliths, many of them at trace levels [4]. Whereas bulk analysis with methods such as ICP mass spectrometry yield results that integrate over the life of the fish (or, if an area is cored out and analyzed, that portion of the otolith: [cf.

8,9]), other techniques, such as microbeam analyses, are superior for fine-scale spatial resolution of the incremental growth zones. One difficulty with the latter approach is detecting the small amounts of trace elements in the spot of the microbeam. Conventional electron microprobe analyses can, for example, analyze the strontium in otoliths, but the concentrations are near the instrument limits of detection.

Our work over the past 5 years has focused on the use of the Lund Nuclear Microprobe (LNMP) to investigate past life histories of a number of fish species. Sven Johansson and colleagues developed Particle Induced X-ray Emission (PIXE) analysis at the Department of Nuclear Physics at Lund University [10]. PIXE, an accelerator-based technique, combines the analytical breadth of energy dispersive spectroscopy (EDS) with the quantitative resolution of wavelength dispersive spectroscopy (WDS), but has far greater resolving power due to much lower background noise. Whereas electron microprobes (WDS) can resolve elemental concentrations into the parts per thousand range, PIXE can approach the parts per million (ppm) range, while at the same time scanning the full spectrum of elements heavier than Al, and with modifications, even lighter elements.

Our investigations have focused mostly on the application of the use of strontium to calcium ratios as an indicator of habitat use. Sr:Ca ratios, measured from the otolith core through the outer edge, often provides a reliable record of the salinity history of diadromous fish species (fishes that use both marine and freshwater

habitats as part of their life cycle). Compared to marine water (with a fairly constant Sr:Ca of 8-10), many inland waters are depleted in Sr:Ca [11, 12], although exceptions occur due to variations in local geological conditions. Here, we present some examples, and discuss future directions.

## METHODS

At the LNMP facility, a 2 to 3 MeV ion beam composed of protons, deuterons, or alpha particles is focused down to a size of 1 to 10 microns to produce a micro-beam. This is scanned across a specimen and elemental maps are generated by correlating PIXE readings with spatial coordinates on the specimen. For our studies, otoliths are mapped on a 128 x 128 grid, with inter-point distances ranging from 6 to 64 microns depending on the size of the otolith to be mapped. Otoliths are first prepared by removal from the fish head, cleaning, sectioning, and fine polishing to expose all of the growth layers down to the core.

Typically, X-rays are detected with a KeveX Si(Li) detector with a 50 mm<sup>2</sup> active area and a measured energy resolution of approximately 155 eV at the 5.9 keV Mn K<sub>α</sub> peak. A thick absorber (mylar + aluminum) is used during the analysis to suppress the Ca X-ray peaks; this allows us to increase the current to enhance the signals of Sr and other trace elements, optimizing the Ca/Sr count rate very close to 1.

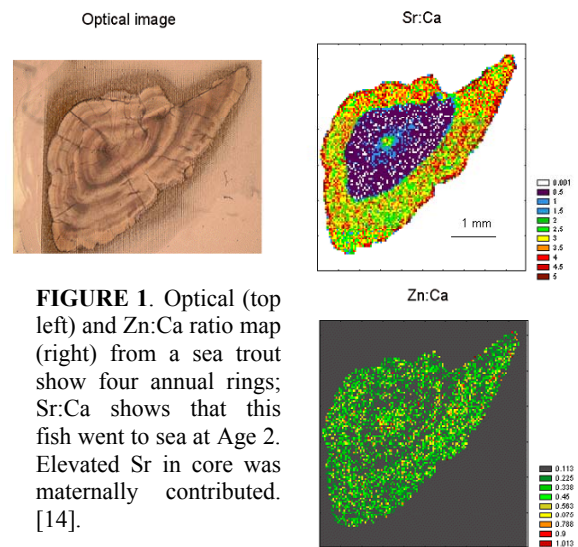
## CASE STUDIES

### 1. Flexible Modes of Anadromy Discovered in Baltic Sea Trout

Seagoing brown trout or sea trout (*Salmo trutta*) have been considered to be obligate anadromous, that is, requiring a period of freshwater residency before emigrating to the sea. Recently, we discovered that this species possesses far more variation in habitat use than previously thought [13]. In studies of fish from the Island of Gotland in the central Baltic Sea, we found that sea trout show a wide variety of habitat use, ranging from 100% freshwater residency (a known trait) to 100% marine (previously unknown). Fish that used smaller, ephemeral tributaries often were born in a coastal zone, probably near the confluence of the stream and the sea, while individuals from larger streams had longer freshwater residencies.

In addition to Sr:Ca being an excellent tracer of salinity (due to the low Sr:Ca in Gotland

waters and the stable salinity gradient in the Baltic), we found Zn:Ca ratios to track with seasons, as noted by Halden et al. [14] (Fig. 1). In fact, we have now analyzed several species in the Salmon family, including Atlantic salmon (*S. salar*) and whitefish (*Coregonus albula*) and all show Zn:Ca oscillations corresponding to seasons. This appears thus to be a family trait, and one we have not observed in other fish families.



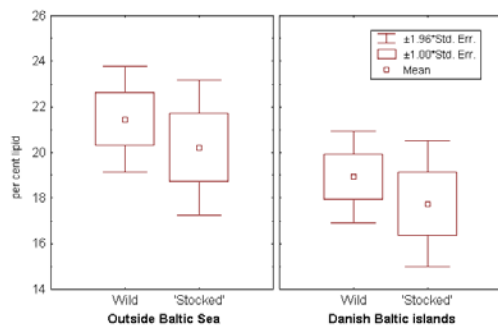
**FIGURE 1.** Optical (top left) and Zn:Ca ratio map (right) from a sea trout show four annual rings; Sr:Ca shows that this fish went to sea at Age 2. Elevated Sr in core was maternally contributed. [14].

### 2. The Fate of Eels Stocked into the Baltic Sea

European eels (*Anguilla anguilla*) all spawn in the Sargasso Sea and then undertake a spectacular, long-distance migration as larvae to coastlines along Africa, the Mediterranean, and Europe including Scandinavia. Fishing pressure and habitat removal due to damming of rivers has caused the Baltic Sea eel populations to decline dramatically over the past 30 yr. In response, the Swedish Fisheries Board initiated a stocking program in the late 1970s [15] but was unable to determine its success at contributing fish to the spawning stock.

We collected eels in the silver stage (mature) in an area presumed to be a migration route out of the Baltic; we also obtained silver eels from a site west of Denmark, where it was hypothesized that stocked eels might accumulate if they could not find their way out of the Baltic with previously learned olfactory cues [16]. We calibrated the Sr:Ca found in eel otoliths to salinity by sampling eels in the stable salinity gradient along the Swedish coast (Limburg et al.

2002 in press). A substantial fraction (26.7%) of eels showed Sr:Ca patterns consistent with having been stocked in stocking programs,



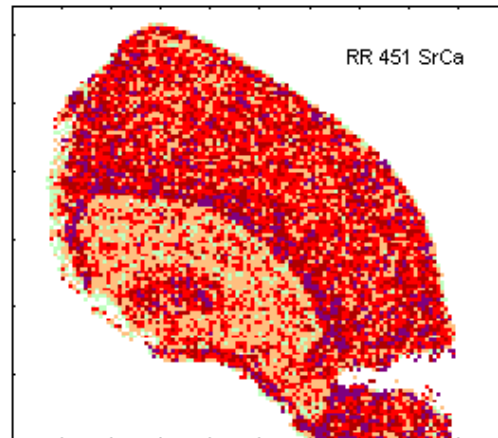
**FIGURE 2.** Lipid contents of wild and presumed stocked silver eels caught outside of the Baltic, compared to fish collected within the Baltic. [17]

which included placement in both freshwater and Baltic Sea habitats. Naturally catadromous (eels that enter fresh water to feed and grow) individuals were also found, bringing up the number of eels that used inland waters to 31.4%. By combining eel habitat use from otolith microchemistry with indexes of maturity, especially lipid content, we found that eels collected outside the Baltic were fatter, but that wild fish were in better condition (indexed by lipid content) than were those presumed to be stocked (Fig. 2). By calculating a “migration potential” based on lipid content and energy required in swimming and reproduction, we found that most of the eels stocked into fresh waters would not endure the trip back to the Sargasso, but that some of the sea-stocked fish, or those that had left fresh water and lived in the Baltic for several years, had sufficient energy resources.

### 3. Movements of an Endangered Species in the Southeastern U.S.

The robust redhorse (*Moxostoma robustum*) is a large, rare sucker fish (Catastomidae) that was presumed to be extinct until its rediscovery in 1990. Its current distribution is a small portion of its former range, consisting of a few rivers in the Carolinas and Georgia. Although tagging studies have turned up this fish in several rivers below the fall line, it has not been caught at lengths from 14 – 400 mm (late larval to juvenile phase). Given its rarity, robust redhorse biology and ecology are to a large degree unknown. Noting their absence above the first impassable dam (at the fall line), and the

abundance of related species above this, the question arose whether the “missing” form was dependent on brackish water habitats in the



**FIGURE 3.** Strontium:calcium map of a lapillar otolith from a robust redhorse. Darker zones have higher Sr:Ca. A light zone is visible in the period when this fish is “missing” and cannot be captured.

estuarine portions of southeastern river systems.

Collaborating with D. Coughlan of Duke Energy, we have scanned approximately a half dozen otoliths from robust redhorse and confirmed that they undertake movements into different habitats during their lifetimes. However, patterns such as the one in Fig. 3 have caused us to re-evaluate our assumptions about the constancy of Sr relative to Ca in southeastern river systems. Recently, a longitudinal water chemistry transect of the Altamaha River in Georgia showed Ca depletion in two major tributaries (J. Andrus, pers. comm.), which causes the ratios of Sr and many other elements to Ca to be elevated. Oxygen stable isotopic data from a parallel study also does not indicate movement into marine waters by this fish. Other tracers may eventually aid us in pinpointing which portions of this large and complex river system are being used by juvenile redhorses.

### FUTURE DIRECTIONS

The LNMP has proved itself a capable workhorse for rapid analyses of otoliths for certain elements, in particular, Sr, Ca, Zn, and in some cases, Fe. Typically our scans are completed in under 1 hr compared to data collection times of 12+ hr with other systems. A new nano beam line is planned and a new HPGe detector is under construction. This new detector will be mounted on the nano beam line and will

reduce the analysis time (not including setup) to around 15 minutes with increased statistics. The detector consists of eight independent segments, which requires modifications of data handling. Each sample will have eight spectra rather than one, and will need to be added together, preferably on-line.

Many other elements are present in otoliths, but typically at trace levels [4]. The elements Ba, K, Mg, Mn, and Zn have proven to be useful ancillary tracers in addition to Sr:Ca [5,6,8,9]. Recently we determined that elevated levels of sodium are present in most otoliths, creating gamma radiation that prevents detection of many low concentration peaks. We will need to solve this problem if we wish to analyze the full range of elements present.

## ACKNOWLEDGMENTS

Funding for this project was from the Swedish Council for Forest and Agricultural Research. We acknowledge our collaborators, fisheries biologists D. Coughlan, H. Wickström, L. Westin, and H. Svedäng.

## REFERENCES

1. Popper, A. N., and Lu, Z., *Fisheries Research* **46**, 15-25 (2000)
2. Campana, S. E., and Neilson, J. D., *Can. J. Fish. Aquat. Sci.* **42**, 1014-1032 (1985).
3. Secor, D. H., Dean, J. M., and Campana, S. E., *Recent Developments in Fish Otolith Research*. Columbia, South Carolina: University of South Carolina Press, 1995.
4. Campana, S. E., *Mar. Ecol. Progr. Ser.* **188**, 263-297 (1999).
5. Thorrold, S. R., Jones, C. M., Swart, P. K., and Targett, T. E., *Mar. Ecol. Progr. Ser.* **173**, 253-265 (1998).
6. Gillanders, B. M., and Kingsford, M. J., *Mar. Ecol. Progr. Ser.* **201**, 273-286 (2000).
7. Patterson, W. P., *Palaeogeogr., Palaeoclimatol., Palaeoecol.* **138**, 271-303 (1998).
8. Secor, D. H., Rooker, J. R., Zlokovitz, E., and Zdanowicz, V. S., *Mar. Ecol. Progr. Ser.* **211**, 245-253 (2001).
9. Thorrold, S. R., Latkoczy, C., Swart, P. K., and Jones, C. M., *Science* **291**, 297-299 (2001).
10. Johansson, S. A. E., and J. L. Campbell, *PIXE – A Novel Technique for Elemental Analysis*, London: Wiley, 1988.
11. Andersson, P. S., Wasserberg, G. J., and Ingri, J., *Earth Planet. Sci. Letters* **113**, 459-472 (1992).
12. Ingram, B. L., and Sloan, D., *Science* **255**, 68-72 (1992).
13. Limburg, K. E., Landergren, P., Westin, L., Elfman, M., and Kristiansson, P., *J. Fish Biol.* **59**, 682-695 (2001).
14. Halden, N. M., Mejia, S. R., Babaluk, J. A., Reist, J. D., Kristofferson, A. H., Campbell, J. L., and Teesdale, W. J., *Fisheries Research* **46**, 289-298 (2000).
15. Svedäng, H., *Polish-Swedish Symposium on Baltic Coastal Fisheries Resources and Management*, 255-267 (1996).
16. Westin, L., *Fisheries Res.* **38**, 257-270 (1998).
17. Limburg, K. E., Wickström, H., Svedäng, H., Elfman, M., and Kristiansson, P., *Biology, Management and Protection of Catadromous Eels* (in press).