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High resolution micromill sampling for analysis of fish otoliths by ICP-MS: Effects of sampling and specimen preparation on trace element fingerprints

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ABSTRACT

Otoliths are calcified structures in the head of fish that record environmental information about fish's life history. Gathering the elemental information from the core of an otolith corresponding to the juvenile period of fish's life is critical to discriminate the adult fish to their natal habitats reliably. A high resolution micromill has been used to isolate the otolith core from a whole otolith for elemental analysis. The effects of micromilling procedures (e.g., sectioning, embedding and drilling) on contamination to otolith trace element levels were examined using paired blackfin tuna (*Thunnus atlanticus*) otoliths. Otoliths were decontaminated by dilute hydrogen peroxide and nitric acid throughout to remove surface contamination. A preconcentration procedure was used to determine the trace elements from the small core material by ICP-MS. It was found that micromilling procedures introduce significant contamination to otoliths, especially for Al, Cu, Pb and Zn. The sectioning procedure caused significant contamination for Co and Cu, while the embedding procedure resulted in contamination for nearly all trace elements (Al, Cd, Co, Cu, Ga, Mn, Ni, Pb, V and Zn). The combined sectioning, embedding and drilling procedure also resulted in contamination for most trace elements. Despite the contamination across all procedural steps, the decontamination procedure effectively removed the surface contamination with the exception of Pb and Zn. Bias (e.g., residual contamination) on Pb was small in comparison to overall concentration of Pb expected to occur in fish otoliths, therefore, its effect may be minor in discriminating individuals. Bias on Zn was larger that could limit its application in discriminating individuals.

1. Introduction

Otolith chemical composition has been a particularly valuable tool for marine and fishery ecologists in understanding the spatial ecology of marine fish (Edmonds et al., 1991, 1992; Campana and Thorrold, 2001; Thorrold et al., 2001; Gillanders, 2002; Secor and Zdanowicz, 1998; Kraus and Secor, 2005). A principal application of otolith microchemistry is to assign widely distributed adults to their natal nursery habitats using otoliths as birth certificates (Secor, 2004) or natural tags (Campana et al., 2000). Classification accuracy using otolith microchemistry is moderate and often ranges between 60% and 80% (Forrester and Swearer, 2002; Secor et al., 2002; Rooker et al., 2003; Patterson et al., 2004; Arslan and Secor, 2005; Swan et al., 2006). Such classification rates show promise, but are often insufficient for intended applications. Curtailed classifications are in part due to analytical issues associated with the quadrupole inductively coupled plasma mass spectrometry (ICP-MS) which is the most commonly utilized technique for otolith microchemical analysis. ICP-MS is advantageous because of its high sensitivity, multi-element analysis capability, and high sample throughput (Campana, 1999; Thresher, 1999). For trace elements at picogram or nanogram per gram levels in otoliths, accurate determination is, however, hindered by insufficient detection limits, contamination, and spectral and non-spectral interferences from otolith matrix. Separation/preconcentration approaches have proved to be powerful means to overcome such issues in analysis of fish otolith by ICP-MS (Willie et al., 2001; Arslan and Paulson, 2002, 2003; Arslan and Secor, 2005).

Classifying adult fish to natal habitats requires isolating the otolith material associated with juvenile stage of the fish's life, so-called the "core" of otolith. Probe-based techniques, including laser ablation ICP-MS, electron probe microanalysis (EPMA) and micro-proton induced X-ray emission (micro-PIXE) are commonly used. Among these techniques, laser ablation ICP-MS is a powerful technique as it combines spot ablation feature of laser with high sensitivity of ICP-MS (Thresher, 1999; Thorrold et al., 2001; Arai and Hirata, 2006; Arai et al., 2007). However, laser ablation ICP-MS suffers from issues of calibration, ablation, fractionation and sample transport. Other techniques (e.g., EPMA and micro-PIXE) lack the sensitivity required for accurate detection of trace elements, such as Cd, Cu, Pb and Zn, which are usually present in otoliths at sub-ng/g levels (Thresher, 1999; Arai and Hirata, 2006). As an alternative, high resolution micromilling affords powerful tool to

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extract the temporal information from otoliths for quantitative analysis by conventional bulk solution ICP-MS (Wurster et al., 1999).

While isolation of the otolith core is now feasible, accurate detection of the small differences in trace element concentrations from tiny otolith core is limited by the detection capability of ICP-MS and contamination issues during the core isolation process. Micromill sampling has been successfully applied to the measurement of stable isotopes of $\delta^{13}C$ and $\delta^{18}O$ from otoliths of various fish (Høie et al., 2004; Rooker and Secor, 2004; Høie and Folkvord, 2006), but there is no information to date regarding the performance of the technique for trace elements. In this study, we utilized a high resolution micromill sampling technique and analyzed the otoliths of blackfin tuna (*Thunnus atlanticus*) for trace transition elements and heavy metals by ICP-MS with the aid of a solid phase preconcentration method. The objective of the study was (a) to investigate the contamination, accuracy and precision issues to otolith trace element measures from micromilling protocols, and consequently (b) to determine the utility of the elemental information gathered from micromilled (cored) otolith to discriminate among individuals.

2. Materials and methods

2.1. Otolith samples

Sagittal otolith pairs were extracted from blackfin tuna (69–84 curved fork length) collected by angling from Texas Gulf of Mexico waters during February–March, 2002. No effort was made to use clean procedures in extraction. Adhering tissue was removed in the field and otoliths were stored dry until further processing.

2.2. Core isolation procedure

Isolation of the core from otolith was performed using a Mercantek Micromill which consists of a microscope and imaging system, an automated stage controlled precisely by computer software, and a tungsten milling drill embedded in the nose piece of the microscope. Prior to milling, sagittal otoliths were first embedded in epoxy resin and then sectioned using a low speed Isomet saw to obtain a 2 mm transverse section through the core. This section was glued to a thin plastic block with thermoplastic glue and then attached to the microscope stage of the micromill system. The intervening plastic block beneath the sectioned otolith allowed the drill to completely transverse the otolith without striking the stage. Following attachment to the microscope stage, the portion of the otolith corresponding to the first year of life was identified (via measurements from sectioned otoliths of yearling bluefin tuna), and the drill path was programmed into the micromill system. Approximately 20 passes were made at 30 microns depth per path to completely isolate the core of the otolith (Fig. 1). The cored material (a prism of intact material) was then removed carefully from the section using forceps and stored in a plastic vial for analysis.

2.3. Otolith decontamination procedure

According to trial specifications (Fig. 2), sagittal otoliths were rigorously decontaminated to remove surface contamination. This was performed by sequential treatment of pairs separately with H_2O_2 and HNO_3. First, otoliths were soaked in deionized water to hydrate the surface of the sample. Next the otoliths were soaked in 3% H_2O_2 for 5 min to dissolve any biological residue. They were then immersed for 5 min in 1% v/v HNO_3 acid to remove surface contamination, and then flooded with deionized water for 5 min to remove the acid. Finally, they were dried under a Class 100 laminar flow hood, weighed to the nearest 0.01 mg, and stored in plastic vials.

2.4. Tests of contamination from micromilling procedures

The micromilling procedure to isolate the otolith's core involves multiple steps (e.g., sectioning, embedding and milling) that increase the risk of contamination on trace elements. A series of carefully designed experiments (Fig. 2) were carried out on paired otoliths to test the magnitude of contamination from individual treatments and to examine the ability of the otolith decontamination procedure to remove surface contamination prior to instrumental analysis.

2.4.1. Test of otolith decontamination procedure

In this experiment, one otolith of each pair was deliberately contaminated by immersing in a multi-element solution that contained 1000 ng/mL (ppb) of Cu, Mn and Zn, and 10 ng/mL (ppb) of Al, Bi, Cd, Co, Ga, Mn, Ni, Pb and V. These levels were about two to

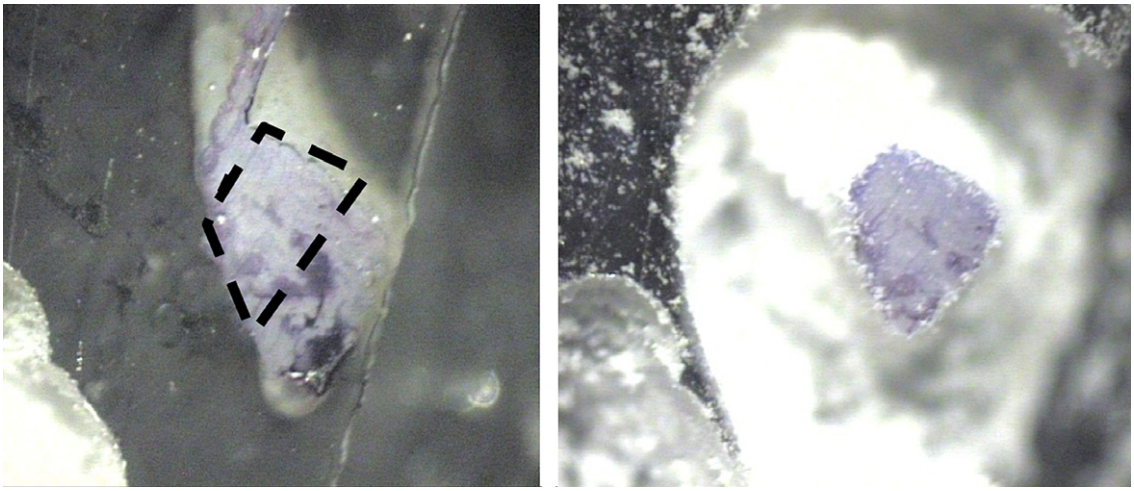

Fig. 1. Isolation of core material in blackfin tuna otolith. Shown in left panel is medial wing in sectioned sagittal otolith. The dashed line indicates target region to be isolated. Right panel shows isolated core region following micromilling procedure. Note that "moat" surrounding core section is ~2 mm deep. The otolith has been stained to better show micromilling result.