Fisheries Research 143 (2013) 67–80

Contents lists available at SciVerse ScienceDirect

Fisheries Research

journal homepage: www.elsevier.com/locate/fishres

Chemical analysis of otoliths: Cross validation between techniques and laboratories

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ARTICLE INFO

Article history:
Received 31 October 2012
Received in revised form 21 January 2013
Accepted 22 January 2013

Keywords:
Argyrosomus regius
ICPMS
Intercalibration
Laser-ablation
Microchemistry
Performance

ABSTRACT

Otolith microchemistry is a powerful tool for fisheries biology, but many applications stretch the limits of analytical techniques. Sources of measurement error were evaluated with a methodological comparison of solution and laser ablation inductively coupled plasma mass spectrometry at two laboratories, using otoliths and certified reference materials NIES-22 and FEBS-1. The intercalibration exercise was effective for Na, Ca, Zn, Cu, Sr, Ba, and Pb. Measurements of Sr were most robust, i.e. sensitivity, accuracy, and precision were least affected by laboratory, technique or sample type. Calcium and Ba measurements varied more between laboratories than between techniques. Lead and Cu measurements were more dependable across laboratory and technique while Zn measurements were more sensitive to these effects. Because the measurement of otolith elements may vary with methodology, it is imperative to consider the sensitivity, accuracy and precision for each application as the suite of elements of interest may differ. The development of reference materials has been the first step in standardization of the procedures, but there is a need for wider ranging calibration and comparison studies.

1. Introduction

Otolith microchemical analysis, the chemical analysis of otolith elemental and isotopic composition, is an important source of information about the physiology and life history (e.g. habitats, feeding, etc.) of individual fish, mixing and movement between populations and areas, and certain aspects of the environment such as temperature and salinity (Campana, 1999, 2005; Secor, 2010). The number of otolith microchemistry studies has increased exponentially since the 1980s (Campana, 2005), with continued refinements in terms of methodology and applications (Elsdon et al., 2008). It is an activity where methodology is critical, because of the heterogeneity of the samples (Payan et al., 1999) and the low concentrations of many of its constituents (Geffen et al., 2002). In addition, the supporting matrix of calcium carbonate, which makes up the majority of the mineralized portion of the otolith, causes interference and presents particular problems for analytical determination (Sturgeon et al., 2005). As microchemistry studies are often applied to management issues, it is increasingly important that methods are standardized so that results from different laboratories can be compared across distances and years. The standardization should cover procedures and processes from postmortem contamination of otoliths (i.e. handling and storage methods (Swan et al., 2006), cleaning procedures (Davies et al., 2011)); otolith preparation (Barnett and Patterson, 2010); to detection limits and accuracy and precision of the analytical equipment (Campana et al., 1997).

One important methodological achievement has been the production of certified/standard reference materials (CRM, SRM) for fish otoliths, available from Atlantic (FEBS-1 (Sturgeon et al., 2005) and Pacific (NIES No.22 (Yoshinaga et al., 2000)) fish. These powdered otolith standards allow direct comparisons between analysis sessions, between laboratories and methodology (Yoshinaga et al., 1999). It is also important to make such comparisons of performance using the species of interest, because of the wide variation in otolith composition observed between species and life stages (Morales-Nin et al., 2005). There are many methodological approaches for microchemistry studies, and the best approach depends on the particular application and its requirements for resolution in space and time (Chang et al., 2012), as well as the need for detecting particular elements (Secor et al., 2002). With continued

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http://dx.doi.org/10.1016/j.fishres.2013.01.005

method development, it is important to evaluate performance of the different methodologies with updated intercalibration exercises.

There are surprisingly few technique comparisons or intercalibration studies in the otolith microchemisty literature. In the first published large scale inter-laboratory comparison of otolith microchemistry techniques, Campana et al. (1997) distributed artificial otolith beads made from homogenized otoliths and whole otoliths of reared juvenile fish to participants who analyzed the samples using their own protocols by one or more of four different surface methods (i.e. wavelength-dispersive electron microprobe (WD-EM), energy-dispersive electron microprobe (ED-EM), proton-induced X-ray emission (PIXE), or laser ablation (LA) inductively coupled plasma mass spectrometry (ICPMS)). Accuracy, precision, limits of detection, linearity, and spot/crater size were compared between methods and laboratories. The results were valuable as the first step in establishing microchemistry as a standard technique, rather than a novel exploratory approach. However, at the time of that study, the lack of reference material increased the variation between laboratories. In an exploratory study of the identification of natal origin of bluefin tuna juveniles using ICPMS and isotope dilution (ID) ICPMS left and right otoliths were used as replicates to compare both techniques at two laboratories (Secor et al., 2002). Although ID-ICPMS was the more accurate of the two ICPMS techniques, inter-laboratory precision was moderately high (3–18%) for individual elemental concentrations (Li, Na, Mg, K, Ca, Mn, Sr, and Ba), and multi-variate elemental fingerprints were similarly ordinated between laboratories ($r = 0.75$). Ludsin et al. (2006) compared solution-based to LA-ICPMS in pairs of otoliths within one laboratory, to determine the feasibility of the method to determine the nursery areas of larval yellow perch (*Perca flavescens*), concluding that both methods were appropriate but recommending LA-ICPMS due to more precise estimates, even with the limitation of the high limits of detection for some elements.

In many cases, otoliths are too small to achieve repeat sampling for successive validation measurements, even though careful planning of sampling strategies and improved instrumentation can reduce this source of variation (Ludsin et al., 2006). In contrast, the otoliths of meagre *Argyrosomus regius* (Asso, 1801) are large, with clear annual growth increments ranging from 0.4 to 1 mm in width (Morales-Nin et al., 2012). The size of these otolith increments makes it feasible to take multiple samples for separate analyses. For example, material can be milled from the core or annual increments in sufficient quantity for solution-based techniques, allowing direct comparison with surface techniques.

This study comprises a methodological comparison of solution and laser ablation inductively coupled plasma mass spectroscopy (SO-ICPMS and LA-ICPMS) to evaluate the precision and accuracy of the analytical methods and measurements on meagre otoliths and on certified reference materials. The most influential factors determining the precision of element measurements were evaluated with respect to differences between laboratories, between techniques, and between sample types. The accuracy of the measurements was evaluated by comparison of the recovery rates for certified values of the reference materials. The results highlighted the level of measurement error that can be expected, to help quantify the resolution possible with standardized methodology, for observed variations in wild populations.

2. Materials and methods

The validation experiment used the two commercially available otolith certified reference materials (CRMs) (NIES-22 and FEBS-1) and sagittal otoliths of meagre (*A. regius*), a valuable commercial species, fished primarily in local artisanal fisheries (González-Quirós et al., 2011). The otoliths were provided by Centro del Toruño (Cádiz, Spain) from fish captured in the Gulf of Cadiz (SW Spain) between April and July 2007 near the Guadalquivir estuary, and sampled at the fish markets of Chipiona and Rota. The fish were weighed (W; g), measured (TL; cm) and the otoliths removed and stored dry in plastic vials. The right-hand otoliths were weighed (OW; g) before being embedded in epoxy resin and sectioned through the core in the transverse plane with a diamond wafering saw. Three or four 1 mm thick sections were mounted on a petrographic glass slide using an epoxy resin (Fig. 1), ground with 1200, 2400 and 4000 grit paper using ultrapure water type I (MilliQ) and polished using progressively finer diamond pastes (3 and 1 μm) with an automated grinding plate. Two sections from each otolith were prepared as replicate slides. To remove any contamination during collection and preparation, prior to sampling of otolith material, each slide was rinsed with MilliQ water, cleaned in an ultrasonic bath with MilliQ water for 1 min, immersed in 2% HNO_3 Suprapur (Merck) for 10 sec and triple rinsed in MilliQ water. After cleaning, the slides were dried overnight at room temperature in a laminar flow hood in a clean room.

The experimental design called for comparison of measurements of elemental concentrations to evaluate the variability within and between CRMs and otoliths, caused by factors such as the method of preparation, laboratory, analytical technique, and in the case of the otoliths, the location of the samples i.e. core vs. edge (Fig. 2). Ontogenetic effects were expected to be small for most elements, since the otoliths were obtained from young fish (age 1+), although otolith cores can have a markedly different signature representing the larval or pelagic stage (Macdonald et al., 2008; Ruttenberg et al., 2005). Otolith material was extracted from otoliths in the core and edge areas by micromilling (New Wave Research), and then analyzed in solution by ICPMS (Thermo Scientific Element2 and Thermo Scientific ElementXR). The core and edge areas of the same otoliths were analyzed directly by LA-ICPMS (LA system Nd:YAG UP-213 (New Wave Research) coupled to either a Thermo Scientific Element2 or ElementXR ICPMS). The two CRMs, FEBS-1 (NRC-CNRC) and NIES-22 (NIES, Japan) were analyzed directly in solution (SO-ICPMS) and pressed into pellets at 750 MPa (10T) with a laboratory press, for the surface analysis technique (LA-ICPMS). In addition, for direct comparison with the milling procedure used for the otoliths, the pellets were milled and the resulting material was prepared and analyzed by SO-ICPMS (Fig. 2).

The results of the analyses were compared between LA-ICPMS and SO-ICPMS at two laboratories (Lab A and Lab B). Although the protocols were standardized as much as possible, there were some differences in the sample preparation and analytical protocols between the different techniques and laboratories (see below).

2.1. Sample preparation for SO-ICPMS

The otolith sections were photographed for measurement and mapping of the core and edge areas for milling. A micromill (New Wave Research) fitted with a diamond bit (Horico H539F007 at Lab A, and Diatech H845-008 at Lab B) was used to extract powder sample for analysis (Fig. 2). The drill bits used in the micromill were 3 mm in length, and tapered from tip to top. With the milling depth set at 150 μm, the bits tapered from 194 μm diameter (Lab A) and 347 μm diameter (Lab B) at the tip out to 217 μm diameter (Lab A) and 442 μm diameter (Lab B) at the point level with the sample surface. With the milling method used in both laboratories (Høie et al., 2004), only the edge of the drill bit is used to shave the pellet or otolith material, so that the sampling is not affected by the diameter of the drill bit. This method maximizes the amount of material extracted and