

Analysis of cod otolith microchemistry by continuous line transects using LA-ICP-MS

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Fish otoliths, also called ear stones or statoliths, are calcified structures functioning as movement and equilibrium indicators in the inner ear of fish (Fig. 1). From hatching to death these structures grow incrementally, with new material accreted daily (Pannella 1971) in successive layers of protein (1–8%, Degens *et al.* 1969) and calcium carbonate. The accretion rate of otoliths varies with fish growth, and in temperate species it is usually lowest during the winter season (Hüsey *et al.* 2010). This results in concentric growth resembling the ringed structure in trees (Fig. 1D), enabling the use of dendrochronological techniques to approximate the age and growth history of fish. During growth, certain elements are incorporated into the otolith structure, some associated with proteins and some with the calcium carbonate component (Thomas *et al.* 2017), supplying a valuable record of different aspects in fish life history and serving as a potential environmental record.

Previous studies show that trace element and isotopic compositions of otoliths can be used as a proxy for reconstructing water chemistry, temperature and salinity (Patterson *et al.* 1993; Thorrold & Shuttleworth 2000). Other studies demonstrate that elemental histories can be used to investigate fish spawning and migration patterns (e.g. Sturrock *et al.* 2012), and more recent studies use elements such as Zn, Cu and Mg as indicators of seasonality (Hüsey *et al.* 2016; Limburg *et al.* 2018). Combining this knowledge of elemental variation with the micro-beam capabilities of laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS) turns otolith microchemistry into a powerful tool for studying important parameters fundamental for establishing modern, sustainable fisheries management policies (e.g. stock identification, migration, pollution indicators, spawning habitats, duration of larval and juvenile stages, and magnitude and timing of spawning).

We present an analytical method developed by the Geological Survey of Denmark and Greenland (GEUS) in collaboration with the National Institute of Aquatic Resources, Technical University of Denmark (DTU Aqua), for element abundance analysis in otoliths. Analyses of otoliths from Baltic Cod (*Gadus morhua*; Fig. 1) are used as an example for its application.

Analytical approach

The microchemical analysis of otoliths focuses on Mg, P, Ca, Mn, Cu, Zn, Sr and Ba, as these elements are typically incorporated into otoliths, and are either subject to environmental control (e.g. Sr and Ba correlate with water salinity) or physiological control (e.g. Zn, Cu and Mg are useful to the interpretation of otolith growth history; Hüsey *et al.* 2016 and references therein; Limburg *et al.* 2018). The LA-ICPMS facility at GEUS employs a NWR213 laser system coupled to an ELEMENT 2 double-focusing, single-collector magnetic sector field ICPMS. Operating conditions, data acquisition and processing parameters are listed in Table 1. LA-ICPMS is often the preferred technique for rapid, *in-situ* analyses of trace elements and isotopes obtained from natural samples,

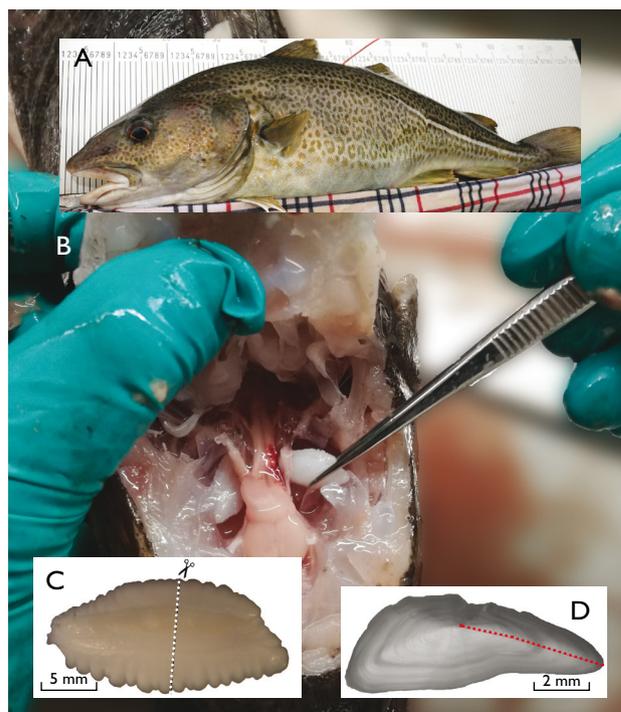


Fig. 1. A: Cod specimen caught in the Baltic Sea. B: Removal of otoliths, the cut is situated just above the eyes. C: Otolith, with dotted line showing the direction of a cross-section. D: Photograph (reflected light) of a polished cross-section of an otolith. The red line shows the position of the laser scan.

Table 1. Instrument operating conditions, data acquisition and processing parameters

<i>Instrumentation:</i>	
Thermo-Fisher Scientific Element 2 double focusing SF-ICP-MS	
Forward power:	1470 W
Cones:	Ni
Plasma gas:	16 l min ⁻¹
Auxiliary gas (He):	0.85 l min ⁻¹
Nebuliser gas (Ar):	0.95 l min ⁻¹
New Wave Research NWR 213 solid state Nd:YAG laser ablation system	
Laser wavelength:	213 nm
Laser fluence:	~ 9.5 J cm ⁻²
Spot size:	40 µm
Repetition rate:	10 Hz
Scan speed:	5 µm s ⁻¹
<i>Data acquisition and processing</i>	
Analyte isotopes: ²⁵ Mg, ³¹ P, ⁴³ Ca, ⁴⁴ Ca, ⁵⁵ Mn, ⁶⁵ Cu, ⁶⁶ Zn, ⁸⁸ Sr, ¹³⁷ Ba	
Sampling time, ms:	10 10 10 10 10 10 10 10 10
Samples per peak:	10 10 10 10 10 10 10 10 10
Acquisition: Time resolved (continuous analysis) along transects	
Mass resolution: 300 (low)	
Oxide production rate tuned to ≤0.3% UO ₂ (²⁵⁴ UO ₂ / ²³⁸ U)	
Single analysis duration and setup: 30 s blank, 2–20 min ablation (sample dependent), 30 s washout	
<i>Software for data reduction:</i>	
Iolite version 2.5 (Paton <i>et al.</i> 2011; Hellstrom <i>et al.</i> 2008)	
<i>Standards:</i>	
Internal standard isotope: ⁴³ Ca	
External standardisation: NIST-612 glass	
Secondary standards: NIST-614 glass, FEBS-1 otolith powder and MACS-3 carbonate powder pressed as tablets	

as it requires little sample preparation and can produce high sample throughput, extracting elemental and isotopic information at a micrometre scale. Most conventional LA-ICPMS analysis is performed by spot analyses, following a bracketing analysis protocol using well-characterised standard materials. This is a powerful method when studying specific areas in solid materials. However, for compositional variations along millimetre- to centimetre-scale transects, the spot approach becomes very time-consuming. For example, a 5 mm long transect requires about one hundred spots 40 µm in diameter, taking 3–4 hours to complete. Spot analysis also introduces difficulties like downhole-element fractionation when the laser drills into the material. Instead, research of fish otolith microchemistry favours faster sampling approaches such as line scans across the sample to acquire continuous time-resolved compositional profiles (a 5 mm long line takes *c.* 17 min, using a fixed scan speed of 5 µm s⁻¹; e.g. Søndergaard *et al.* 2015; Hüseyin *et al.* 2016). This approach is rapid, suppresses depth heterogeneity and avoids downhole elemental fractionation, as it ablates only to a depth of a few microns. The potential interfering effects of varying scan speeds, washout times and debris blanketing from the ablation are not yet

studied, but this is intended in the near future. For ongoing otolith studies, we used a line-scan LA-ICPMS approach to measure 325 cod otoliths (15–30 otoliths per day).

The otoliths were embedded in epoxy resin and cut along the dorsoventral profile to expose the core and annuli. For streamlined, high-throughput analyses, custom-made epoxy mounts 7 × 0.8 × 0.8 cm large were cast, each holding 10 otoliths. A custom-made 10 cm² sample holder with four mounts allows for analysis of 40 otoliths per day. For each otolith, a reflected light optical photo depicting a line from core to edge (Fig. 1D) was imported as an overlay image into the laser ablation software. The image was fitted to the otolith position in the live image, using reference points to facilitate the exact positioning of the line scans.

The ICPMS analysis was optimised for dry plasma conditions through continuous linear ablation of the NIST 612 standard. The signal-to-noise ratios were maximised for the isotopic mass range from Mg to Ba, while opting for low element-oxide production levels by minimising the ²⁵⁴UO₂/²³⁸U ratio. Instrumental drift was minimised by following a standard-sample-standard analysis protocol, bracketing every sample analysis by line analyses of the NIST-612 and NIST-614 glass standards (Jochum *et al.* 2011), while the FEBS-1 otolith (Sturgeon *et al.* 2005) and the MACS-3 carbonate powder tablets (Jochum *et al.* 2012) provided quality control of the NIST-612 standard measurements. The averaged 2σ accuracy and uncertainty of the standards were typically <5% for element abundances >1–3 ppm. Data processing was done with software Iolite v. 2.5 (Hellstrom *et al.* 2008; Paton *et al.* 2011) using the Trace Elements IS data reduction routine. Calculation of abundances were based on ⁴³Ca isotope as the internal standard, assuming 38.3 wt% Ca in all otoliths, comparable to the certified Ca concentration reported for the FEBS-1 otolith standard (Sturgeon *et al.* 2005).

Application of the analytical approach on Baltic cod

The main purpose of the analyses is to answer research questions important to the fisheries management in Denmark and Greenland. The specific approach presented here and modified versions of this method are used in several projects addressing a range of management questions relating to stock structure, migrations and age determination. Here, we highlight some preliminary results of the most advanced project.

The project *Tagging Baltic Cod* (TABACOD) is a joint Baltic collaboration aiming to develop a new age-estimation method based on seasonal variations in element concentrations along a gradient from the core (birth) to the edge (death) of otoliths. Fish age is one of the key variables in stock

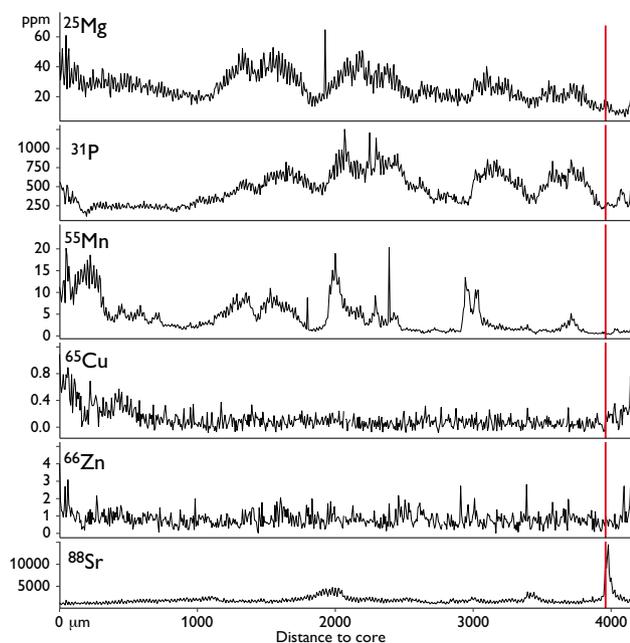


Fig. 2. Trace-element concentration profiles in ppm of the cod otolith shown in Fig. 1D. The x-axis indicates the concentration along the profile from 0 μm , when the fish was born, to 4300 μm , when it died. The red line marks the position of the Sr peak induced by SrCl_2 injection. Data from Nielsen *et al.* (2018). As this cod was tagged in April, the Sr timestamp corresponds to the coldest water temperatures experienced by the cod over a year, where Mg and P concentrations are at a minimum.

assessment and has traditionally been obtained by visual examination of otolith cross-sections, where seasonal fluctuations in growth are visible as optically contrasting growth zones much like the rings in cross-sections of trees. In recent years, this traditional method has failed to provide reliable age information, thus posing severe management problems for the Eastern Baltic cod stock. Initial results from the TABACOD project on the seasonality in the otolith chemical composition are presented as an example of the application of the LA-ICPMS approach. All analysed otoliths were acquired from cod that were subjected to a mark-recapture experiment. A total of *c.* 500 cod specimens were captured, externally marked, injected with SrCl_2 and released again. When the cod were recaptured they were sent to DTU Aqua for analysis. The SrCl_2 is incorporated into the otolith as it grows and acts as an internal timestamp. The chemical signals from timestamp to edge corresponds to the time the fish spent at sea between capture and recapture. Combining information on how many days the fish had been at sea, what time of year it was released/recaptured and how much it had grown since tagging allows us to validate our hypotheses on seasonally varying element concentrations. Concentrations of elements like Mg and Zn vary with season (Hüssy *et al.*

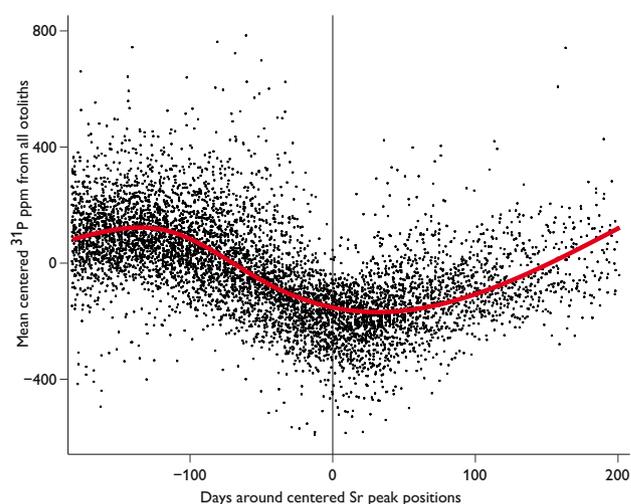


Fig. 3. Variations of phosphorous concentration in all measured otoliths *c.* 200 days before and after the SrCl_2 tagging and release experiment. On the x-axis, measurements are centred on the injection-induced Sr peak. On the y-axis, measurements have been centred on the mean P concentration of each otolith transect. The red line is a Generalised Additive Model smoothed curve.

2016). Figure 2 depicts core-to-edge compositional profiles of Mg, P, Mn, Cu, Zn and Sr from the otolith shown in Fig. 1D, corresponding to the entire life of the fish. The red line in Fig. 2 marks where the Sr concentration dramatically increases, representing the SrCl_2 -tagging event. The Mg, P and Mn concentrations show clear and similar variations throughout the otolith structure on a scale of tens to hundreds of ppm. Zn and Cu concentrations vary around our analytical resolution threshold of *c.* 1–3 ppm and do not show significant systematic variations.

The LA-ICPMS data are currently undergoing statistical analysis to quantitatively identify seasonal variations. However, some analysed elements indicate clear patterns resembling seasonality. If a seasonality in element concentration occurs, superimposing individual transects (like the ones shown in Fig. 2) of all cod in one plot will result in a generic signal. If element signals are random in relation to time, no such signal will be evident. Figure 3 shows P concentrations from all analysed otoliths, standardised by dividing each measured value by the mean profile P concentration to remove the effect of differences in average P levels between individuals. All profiles were centred at the Sr peak, since all cod were tagged during the same season (April to May of the same year). The time scale on the x-axis is estimated, assuming linear growth within years, similar growth in individuals across the year, and that all specimens were tagged and released on the same date of a given year. Although there are individual differences between fish, Fig. 3 indicates a general seasonal variation in

P concentrations. The total number of minima occurring in the profiles from birth to death thus corresponds to the number of winters the fish has experienced and hence its age.

Other ongoing projects

Migration patterns of the Kattegat cod: This project seeks to map migration patterns of cod captured in the Kattegat, which were genetically identified to belong to the North Sea or western Baltic stock. Elemental profiles of 400 cod captured along a geographic gradient covering the entire Kattegat will be compared with baseline samples from adjacent areas. Comparing the results to information about the otoliths' annual growth zones can reveal at what time in its life the cod has migrated to and from the Kattegat.

Stock structure in capelin: With partners from Greenland's fishing industry this project investigates stock structure, migration and natal homing (the return to a birthplace to reproduce) of capelin (*Mallotus villosus*) from 18 areas along the coasts of South and West Greenland. The aim of this project is to provide counsel on sustainable management of a species that plays a vital role in the marine food chain.

Final remarks

A LA-ICPMS approach for quantitative, high-throughput transect measurements of otoliths was successfully set up at GEUS. Data from 325 otoliths are being thoroughly examined, and only an 'appetizer' of the data is presented here. The LA-ICPMS approach is adaptable for most solid carbonate (e.g. bivalves) and phosphate (e.g. teeth, horn) materials showing cross-surface compositional variations. Analyte isotopes include most major, minor and trace elements and acquisition parameters are easily optimised for the specific sample type, thus providing a rapid and extremely versatile *in-situ* analytical approach for comparable natural materials.

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