Journal of Fish Biology (1999) **54,** 223–225
Article No. jfbi.1998.0855, available online at http://www.idealibrary.com on IDEAL

BRIEF COMMUNICATION

A new method for three-dimensional otolith analysis

S. F. HAMRIN*††, E. ARNERI†, P. DOERING-ARJES*, H. MOSEGAARD‡, A. PATWARDHAN§, A. SASOV‖, M. SCHATZ¶, D. VAN DYCK**, H. WICKSTRÖM* AND M. VAN HEEL§

**Institute of Freshwater Research, SE-178 93 Drottningholm, Sweden; †Istituto di Ricerche sulla Pesca Marittima, CNR, Largo Fiera della Pesca, I-60100 Ancona, Italy; ‡Danish Institute for Fisheries Research, Charlottenlund Castle, DK-2920 Charlottenlund, Denmark; §Imperial College of Science, Technology & Medicine, Wolfson Laboratories, Biochemistry Department, London SW7 2AY, U.K.; ‖Sky Scan b.v.b.a., Antwerpsesteenweg 28, B-2630 Aartselaar, Belgium; ¶Image Science, Heilbronner Str. 10, D-10711 Berlin, Germany and **Department of Physics, University of Antwerp (RUCA), Groenenborgerlaan 171, B-2020 Antwerpen, Belgium*

(*Received 2 June 1998, Accepted 31 August 1998*)

The three-dimensional (3-D) shape and density distribution of a cod otolith was determined using a newly developed micro X-ray tomograph. The technique requires no special treatment of the otolith and could yield the entire 3-D growth history in a single analysis.

Key words: fish ageing; otolith shape; 3-D analysis; X-ray tomography.

Fish ageing is a basic tool for cohort analysis in fish stock assessment. The quality of fish ageing procedures directly influences the reliability of scientific advice in fisheries management. Presently, no fast, objective, and generally applicable method exists to determine the age of fish. The most commonly used ageing technique is that of counting annual structures in the hard parts of fishes. This technique is elaborate and to a great extent subjective.

Significant correlations have been found between otolith shape and genotype, sex, size, growth rate, and stock membership (Campana & Casselman, 1993; Colura & King, 1995). The age of fish of some species can be predicted, to a certain extent, from the two-dimensional (2-D) otolith shape alone (Aps *et al.*, 1990; Castonguay *et al.*, 1991; Campana & Casselman, 1993; Doering & Ludwig, 1990).

For a better understanding of otolith growth, an approach is needed which includes all aspects of the form of an otolith, i.e. its full three-dimensional (3-D) shape and density distribution. Three-dimensional analysis may yield insights into how age, environmental factors, and genotype influence growth and shape of otoliths. More reliable and faster ageing techniques may be developed when inner structures can be included in the reconstruction. Our basic working hypothesis is that fish age information is coded in the 3-D shape and density distribution of otoliths primarily hidden behind environmentally induced and physiologically mediated variations in the local bio-mineralization rate on the otolith surface. From the study of the 3-D information we expect to be able to validate simpler 2-D criteria for reliable and fast age determination of fish.

††Author to whom correspondence should be addressed. Tel.: +46 8 620 04 01; fax: +46 8 759 03 38; email: stellan.hamrin@fiskeriverket.se

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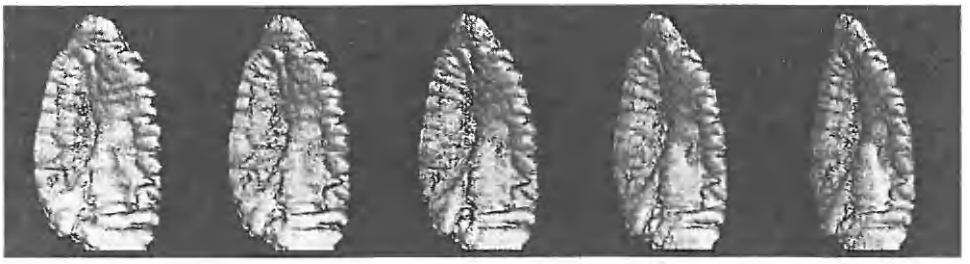

FIG. 1. A cod *Gadus morhua* L. otolith was scanned with a micro X-ray tomograph (Sasov & Van Dyck, 1998) and processed with the IMAGIC-5 image analysis system (Van Heel *et al.*, 1996) to evaluate the outer surface and the 3-D growth pattern. The images, representing the otolith densities approximately at the outer surface, are shown as a continuous sequence of stereo pictures, which allow the observer to merge any two neighbouring pictures for 3-D visualization.

Until recently it was possible to capture and reconstruct the 3-D surface of an otolith only from serial sections (Bailey *et al.*, 1995). This technique loses part of the otolith due to the cutting. Sky Scan b.v.b.a. in collaboration with the University of Antwerp has recently developed a micro X-ray tomography instrument, which produces a very detailed 3-D density distribution. With this tomograph an object such as an otolith can be analysed non-destructively to reveal its 3-D structure (Fig. 1). There are no alignment problems as with the common technique since no physical partitioning into slices is necessary. The system consists of a microfocus X-ray tube, an object rotation table, a sensitivity improved CCD-camera and on-line computer control. The currently attainable resolution of $\sim 8\ \mu m$ is sufficient to reveal the outer shape of the otolith to high precision. In order to improve the visibility of the internal details of the 3-D reconstruction (say, the annuli), we plan to make use of X-ray phase-contrast microtomography (Van Dyck *et al.*, 1998), using an submicron spatial resolution system currently under development.

The digital 3-D density information of the otoliths may be stored in databases which can be accessed through the internet. Such databases can thus play an important role in an educational context. Moreover, the databases may be used as calibration standards among fisheries institutes.

The authors thank J. Modin for supplying the cod otoliths. Our project is part of the contract *Fish Ageing by Otolith Shape Analysis* (FAIR PL97-3402) with the European Commission, Directorate-General XIV (Fisheries).

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