Journal of Fish Biology (2006) **69,** 1845–1848
doi:10.1111/j.1095-8649.2006.01214.x, available online at http://www.blackwell-synergy.com

BRIEF COMMUNICATIONS

Validation of daily increment deposition in otoliths of juvenile *Limnothrissa miodon* (Clupeidae)

J. Meisfjord*, F. Midtøy and A. Folkvord

Department of Biology, University of Bergen, PB 7800, N-5020 Bergen, Norway

(Received 14 November 2005, Accepted 9 June 2006)

Daily deposition of otolith increments was validated for juvenile *Limnothrissa miodon* in the Cahora Bassa reservoir, Mozambique, by the means of two successive chemical immersion markings.

Key words: alizarin red S; daily rings; kapenta; microstructure; sardine.

Limnothrissa miodon (Boulenger), a freshwater clupeid endemic to Lake Tanganyika, East Africa, has been introduced to lakes and reservoirs in the region as a commercial fisheries resource. Soon after its introduction to the Lake Kariba reservoir in the late 1960s, *L. miodon* (local name kapenta) passed the hydroelectric turbines at the Kariba Dam to establish itself on the lower River Zambezi and colonize the Lake Cahora Bassa reservoir that was formed as from 1975 (Gliwicz, 1984). The *L. miodon* stock in Lake Cahora Bassa currently constitutes, in terms of landings, the most important aquatic species in Mozambique, and is the focus of studies aimed at establishing key dynamic population parameters. Growth parameters may be derived from length frequency analysis (Marshall, 1987 based on data from Gliwicz, 1984), however, for the upstream Lake Kariba stock of *L. miodon* the method yielded only inconsistent estimates of growth parameters (Lake Kariba Fisheries Research Institute, unpubl. data), possibly due to continuous breeding, lack of distinct cohorts and size-dependent horizontal migration (Cochrane, 1984; Chifamba, 1992; Mtsambiwa, 1996). Otolith microstructure analysis has been suggested as a more reliable method for ageing and estimating growth parameters. This alternative approach requires, however, validation of the otolith micro-increment deposition rate (Campana, 2001). In a limited material of *L. miodon* from the Kariba stock, Mtsambiwa (1996) reached a preliminary conclusion that sagitta increments

*Author to whom correspondence should be addressed. Tel.: +47 9513 1085; fax: +47 5558 4450; email: jorgen.meisfjord@bio.uib.no

were laid down daily in larvae based on chemically marked fish in the laboratory. The objective of the present study was to validate increment deposition in juveniles under semi-natural conditions.

Limnothrissa miodon were captured, kept and marked in an *c*. 1000 l and 1 m deep plastic enclosure in a shallow bay in the Cahora Bassa reservoir, Mozambique (15°35′43″ S; 32°24′48″ E). A near optimum validation study environment (Geffen, 1992) was created by the natural cycles of photoperiod and temperature in the enclosure. Openings covered by mosquito netting extended from the brim of the enclosure to 30 cm above its bottom, allowing water exchange. The fish were captured on the night between 3 and 4 June 2001 by light attraction and by operating the enclosure as a lift net from *c*. 0·4 m depth.

Following capture, the fish were kept for 4 days to acclimate to experimental conditions. The acclimatization was followed by two alizarin red S (ARS) immersion markings (I_1 and I_2) that were separated by exactly 7 days. Three and a half days after I_2, the surviving fish were removed and fixed in 96% ethanol. The immersion marking procedure was identical on the two occasions: the enclosure was lifted until its openings were above the lake surface, reducing the water volume to *c*. 300 l. In this position, immersion marking in 100 mg l^{-1} ARS, dissolved in 1 M KOH (Blom *et al.*, 1994), took place in aerated water, starting at 1900 hours. After 12 h, the enclosure was lowered to its former position in which ARS was washed out over about 2 h by passive water exchange.

The fish were fed live natural zooplankton and dry pellets (produced for marine fry), which were added morning and late afternoon in accordance with what has been suggested to be their natural feeding rhythm (de Iongh *et al.*, 1983; Mandima, 1999). Through passive water exchange, dissolved oxygen and temperature in the upper part of the enclosure were at ambient surface levels (6–7 mg l^{-1} and 23–26° C, respectively) while dissolved oxygen was *c*. 0·7 mg l^{-1} lower near the enclosure bottom and during marking. Water transparency was very low due to high clay load. One hundred and forty-four juvenile *L. miodon* (*c*. 50%) survived until the end of the 2 week experiment. One hundred and forty dead individuals were recovered during the same period, most (134) in association with capture and I_1. Mortality was estimated at 40 and 13% for those events, respectively.

Sagittae were extracted and mounted in clear Crystalbond™ 509 on microscope slides. Radial distance and number of increments between the outer edges of ARS marks were recorded with a Zeiss Axioskop fluorescence microscope equipped with a ×40 objective lens and fitted with an Olympus DP11 high resolution digital camera. Increment counting was carried out by two readers, one of whom was not aware of the duration of the experiment (reader 2; Table I).

In a random sample of 38 individuals, 95% were successfully marked, displaying two red ARS zones both under natural and fluorescent light (Fig. 1). About 90% of these individuals displayed an otolith microstructure clear enough to be interpreted with some degree of confidence without prior grinding. In the resulting material ($n = 32$, Table I), total fish length and sagittae length at the end of the experiment ranged from 16 mm and 270 μm in the smallest individual to 23 mm and 530 μm in the largest. Similarly sized fish collected at the same time of the year were up to 3 months of age. The mean

TABLE I. Distribution of otoliths among categories of microstructure clarity and categories of increment counts (values in parenthesis reflect inconclusive increment counts). For a given otolith, mean daily otolith growth between alizarin red S (ARS) marks was computed as the distance between the inner edges of the ARS marks, divided by the number of days separating markings (seven)

Microstructure clarity	Number of otoliths	Outcomes of increment counts (reader 1/reader 2)	Number of occurrences	Range of mean daily otolith growth between ARS marks (μm day^{-1})
Good	12	7/7	12	1·6–3·4
Fair	18	7/7	10	1·7–2·7
		7/(7–8)	3	1·9–2·6
		7/6	1	2·0
		6/(6–7)	1	2·3
		6/(6–8)	1	1·6
		6/6	2	1·7–1·9
Poor	2	7/(6–8)	2	2·0–2·6
Total	32		32	1·6–3·4

individual otolith growth in the period between ARS marks ranged from 1·6 to 3·4 μm day^{-1}. Otoliths were characterized as displaying 'good', fair' or 'poor' microstructure clarity (Table I).

In 70% of the individuals, readers unanimously reached the expected count of seven between inner edges of the ARS marks and in only one instance did the two readers differ categorically in their counts. No count departed from the expected value with more than one increment. Loss of precision seemed to have two principal explanations. First, in otoliths displaying indistinct ARS marks, it is possible that one of the increments adjacent to the ARS marks was indiscernible. Second, and not surprisingly, loss of precision was linked to poor

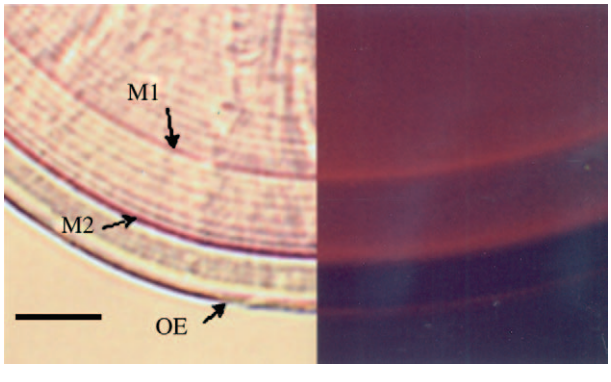

FIG. 1. Sagitta microstructure of juvenile *Limnothrissa miodon* (total length = 19 mm) subject to two alizarin red S markings 7 days apart: marks appeared as red rings in natural light (left) and corresponded to the fluorescent marks (right). Scale bar = 15 μm. M1 and M2, marks produced from alizarin red S immersions I_1 and I_2, respectively; OE, otolith edge. The red 'mark' at the otolith edge (right) is an optical artefact. The otolith was neither ground nor polished.

microstructure clarity which again may have been linked to alignment errors during mounting or the lack of grinding: Whereas unanimous counts of the expected number of increments were obtained in all otoliths characterized as displaying 'good' microstructure clarity, inconsistency occurred for the categories 'fair' and 'poor'. There was no apparent link between precision and otolith growth (Table I).

It is concluded that periodicity of increment deposition is daily, at least in juveniles. The tentative conclusion that this is also the case in larvae from a stock in nearby Lake Kariba (Mtsambiwa, 1996) indicates that microstructure analysis can be used to determine the age after first ring deposition in *L. miodon*, at least up to the size class studied here. Increment counts did not involve increments near the edge of the otolith thus potential errors due to the 'edge effect' (Campana & Neilson, 1985) were avoided.

This work was supported by the Norwegian Research Council, Project No. 124079/730. We wish to express our gratitude to the staff at the Oceanographic Museum, Monaco, for invaluable advice on collection of live sardines and to G. 'Bad' who provided the fishing vessel used in the study.

References

Blom, G., Nordeide, J. T., Svåsand, T. & Borge, A. (1994). Application of two fluorescent chemicals, alizarin complexone and alizarin red S, to mark otoliths of Atlantic cod, *Gadus morhua* L. *Aquaculture and Fisheries Management* **25** (Suppl. 1), 229–243.

Campana, S. E. (2001). Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. *Journal of Fish Biology* **59,** 197–242. doi: 10.1006/jfbi.2001.1668

Campana, S. E. & Neilson, J. D. (1985). Microstructure of fish otoliths. *Canadian Journal of Fisheries and Aquatic Sciences* **42,** 1014–1032.

Chifamba, P. C. (1992). Daily rings on the otoliths as a method of ageing the sardine *Limnothrissa miodon*. *Transactions of the Zimbabwe Scientific Association* **66,** 15–17.

Cochrane, K. L. (1984). Seasonal fluctuations in the catches of *Limnothrissa miodon* (Boulenger) in Lake Kariba. Master of Philosophy Thesis, University of Zimbabwe.

Geffen, A. J. (1992). Validation of otolith increment deposition rate. In *Otolith Microstructure Examination and Analysis* (Stevenson, D.K. & Campana S.E., eds). *Canadian Special Publication of Fisheries and Aquatic Sciences* **117,** 101–113.

Gliwicz, Z. M. (1984). Limnological study of Cahora Bassa reservoir with special regard to sardine fishery expansion. *FAO/GCP/MOZ/006/SWE, Field Document 8*. Rome: FAO.

de Iongh, H. H., Spliethoff, P. C. & Frank, V. G. (1983). Feeding habits of the clupeid *Limnothrissa miodon* (Boulenger), in Lake Kivu. *Hydrobiologia* **102,** 113–122.

Mandima, J. J. (1999). The food and feeding behaviour of *Limnothrissa miodon* (Boulenger, 1906) in Lake Kariba, Zimbabwe. *Hydrobiologia* **407,** 175–182.

Marshall, B. E. (1987). Growth and mortality of the introduced Lake Tanganyika clupeid, *Limnothrissa miodon*, in Lake Kariba. *Journal of Fish Biology* **31,** 603–615.

Mtsambiwa, M. Z. (1996). Some management aspects of the pre-recruitment ecology of the freshwater sardine *Limnothrissa miodon* in Lake Kariba. Doctor of Philosophy Thesis, University of British Columbia, Canada.

