Otolith features and utility of lapillus for daily increment analysis in Malawian Characidae *Hemigrammopetersius barnardi* (Herre, 1939) (Pisces: Characidae) collected from Lake Malawi

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Abstract

The suitable otolith for increment analysis in Malawian characin *Hemigrammopetersius barnardi* was investigated, and increment formation pattern of otolith was validated using otolith marking technique using Alizarin Complexone (ALC). The sagitta was fragile and increments in rostra were not well readable. The asteriscus had an ambiguous core structure and the first increment was impossible to be identified. These observations indicated that the sagitta and asteriscus are not suitable for increments analysis. The lapillus had the visible increments from the core to marginal portion and is considered the suitable otolith for increments analysis. The lapilli in fish treated by ALC showed the daily periodicity in increments formation outside ALC mark. Consequently, the lapillus is resulted the most appropriate otolith for daily increments analysis in *H. barnardi*.

Key words: *Hemigrammopetersius barnardi*, otoliths, lapillus, increments validation

Introduction

*Hemigrammopetersius barnardi* is one of two characin species distributing in Lake Malawi and its tributaries. This species grows to 7 cm in total length and inhabits in shallow weedy areas (Eccles 1992). *H. barnardi* has not been commercially important in Malawi due to its size and small amount of catch despite being locally consumed as dried fish in the lakeshore communities. However, the fish catch in the lake has tended to decline since 1970’s (Bulirani et al. 1999; Zidana 2000), and it is expected that the commercially invaluable fish up to the present, such as *H. barnardi*, would have more commercial value. In this background, the importance of the biological information of this species in terms of resource management aspects is currently pointed out.

In order to obtain the biological information of fish, analyses on otolith daily increments have been broadly applied since Pannella (1971), because the breeding period and growth pattern can be analyzed using the age information in days obtained from otoliths. The sagitta has been generally applied for daily increments analyses in most species reported so far (Campana & Neison 1985; Nishimura 1993a etc.). However, for using the otolith increment counts as an indicator of age in days, the suitable otolith is needed to be detected.
among the sagitta, lapillus and asteriscus, since the sagitta are occasionally not appropriate due to structural complexity in several species (Hoff et al. 1997; Morioka & Machinadiarena 2001; Morioka & Kaunda 2001). In addition, the validation on the increment formation pattern whether increments are formed on daily basis or not is needed. This study aimed at detecting suitable otolith for the increments reading and to validate the increment formation pattern in otoliths of *H. barnardi*.

**Materials and Methods**

A total of seventy-three specimens of *H. barnardi* juveniles were collected from Chia beach on the southwestern shoreline of Lake Malawi (Fig. 1). A seine net (1 mm mesh, 1 m height, 8 m width) was used for fish collection, which was operated along the shoreline (0.8 – 1.5 m depth). Fish collection was made on 28 February, 5 April, 3 May and 5 June 2001. Total length (TL) of fish ranged between 20.15 and 52.20 mm.

Fish were preserved in 70% ethanol immediately after capture, and the otoliths, i.e. sagitta, lapillus and asteriscus, were extracted. Extracted otoliths were embedded on the glass slides with epoxy resin. When otoliths were opaque, they were ground with sand paper (1500) and lapping films (6, 9 and 12 µm mesh) for being thin proximal sections, following the method described by Nishimura (1993b). Microstructures of the otoliths were observed under the optic microscope with transmitted light (x 200 – 400).

Seven specimens were kept alive and used for otolith marking treatment by Alizarin Complexone (ALC) for the validation of increments formation pattern. ALC treatment was taken up for 22 hrs from 11:00 of 1 October to 09:00 of 2 October 2001on fish immersed in 100 ppm ALC solution in 10 l plastic tank. After ALC treatment, fish were reared in 30 l aquaria and given diet (TETRA CICHLID FOOD) twice a day. Fish were sacrificed on 09:00 of 12 October, 10 days after ALC treatment. Otoliths of fish were treated as mentioned above.

**Results**

**Otolith features**

The sagitta were arrow-head shaped with an obvious core (Fig. 2). It had the anterior- and posterior-rostra that elongated as fish grew (Fig. 2). Maximum radii of the sagittae ranged from 340 to 740 µm (n = 40), and the relationship between radii and TL was expressed by $R = 46.08L^{0.70}$ (r=0.94, n = 40), where $R$ and $L$ are radii (µm) and TL (mm), respectively (Fig. 3). Although the increments were observable in the sagittae from the core up to ca. 20 - 30th increment deposition at the bases of both anterior- and posterior-rostra, they were invisible in rostra (Fig. 2). Rostra, in addition, were fragile and often destroyed by otolith extracting/grinding procedure. These features demonstrate that the sagitta is not a suitable otolith for increment analysis.

The asteriscus had an ambiguous core (Fig. 2). It was star-shaped with notches on the margin (Fig. 2). Maximum radii of the asterisci ranged from 250 to 600 µm (n = 73), and the relationship between radii and TL was $R = 9.94L + 76.80$ (r=0.92), where $R$ and were radii (µm) and TL (mm), respectively (Fig. 3). Ambiguous cores of the asterisci led to the difficulty in discerning the first increment identification.

The lapillus was round a-shaped with an obvious core (Fig. 2). Maximum radii of the lapilli ranged from 220 to 550 µm (n = 73), and the relationship between radii and TL was expressed by $R = 9.04L + 62.50$ (r=0.95) where $R$ and L were radii (µm) and TL (mm), respectively (Fig. 3). Increments in the lapilli were well visible from the core to marginal portion throughout its development (Fig. 2), indicating the lapillus being applicable for otolith increments analysis.

**ALC treatment**

All fish (seven specimens) survived after ALC treatment, and the checks formed by ALC treatment were observed in all lapilli under normal transmitted light (Fig. 4). These checks were not observed under fluorescent light. Increment counts in the lapilli outside ALC check were $10.14 ± 0.38$ (n = 7, mean ± SD), not significantly differing from number of days after ALC treatment, 10 days (t-test, P>0.05). This indicated the increments in the lapillus being formed on a daily basis.
Discussion

The sagittae of ostariophysans are generally elongate (Rosen and Greenwood 1970), and unsuitable features for increment reading, i.e. fragile rostral structures and invisible increments in rostra, are commonly reported in the sagitta of cyprinid (Hoff et al. 1997; Bestgen & Bundy 1998; Morioka et al. 2002). In Hemigrammopetersius barnardi, one of characins as in ostariophysans, inutility of the sagitta observed in this study agreed with results obtained in cyprinids cited above.

The asteriscus is also considered inappropriate because of its ambiguous core (Fig. 2B). The asteriscus, in addition, is reported to be absent at hatching and formed later than the sagitta and lapillus in several fish species (Campana et al. 1987; Oxenford et al. 1994; Hoff et al. 1997). This also suggests the asteriscus being unsuitable for otolith increment reading. As a result, with considering a structural suitability and increment visibility (Fig. 2C) of the lapillus, the lapillus is only appropriate for otolith increments analysis in this species as well as cyprinids in earlier studies (Hoff et al. 1997; Bestgen & Bundy 1998; Morioka & Kaunda 2001; Morioka et al. 2007).
Increments in the lapillus were elucidated being deposited daily on the basis of ALC treatment (Fig. 4), indicating the utility of the lapillus for determining age in days of this species. The first daily increment in otolith is occasionally observed to be deposited a few days after hatching in several species, such as reported in Sardinops melanostictus (Hayashi et al. 1989), Clupea harengus (Moksness & Wespestad 1989). Therefore, the timing of the first increment deposition in this species should be identified. Although ALC checks were observable under normal transmitted light but not under fluorescent light in this study, Mugiya & Muramatsu (1982) reported the check formation in otoliths by various stresses, e.g. injection of chemicals, cold shock. Checks observed in this study were thus considered being deposited by stress induced by ALC treatment. In Chanos chanos larvae and Genypterus blacodes juveniles, checks formed by ALC treatment were observed under transmitted normal light (S. Moriioka, unpubl. data).

Although the sagitta is the most applied otolith for aging teleost fish apart from ostariophysans, better utility of the lapillus than the sagitta for daily increment reading was also reported in several other species, e. g. Hirundichthys affinis of Beloniformes (Oxenford et al. 1994), Genypterus blacodes of Ophidiiformes (Morioka & Machinandiarena 2001). In addition, it is well known that subdaily increments often occur in the sagitta (Marshall & Parker 1982; Morales-Nin 1987; Prince et al. 1991; Moriioka & Machinandiarena 2001). Moriioka & Machinandiarena (2001) observed that numerous subdaily increments occurred in the sagitta but did not in the lapillus of Genypterus blacodes. These observations suggest that the lapillus may be more appropriate structures than the sagitta for determining age in not only the ostariophysans but in most teleost species.

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References


