Age validation of a tropical cyprinid, *Rastrineobola argentea* (Pellegrin 1904) by immersion in tetracycline hydrochloride and use of phi prime

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ABSTRACT

To verify that increments observed in the otoliths of *R. argentea* were deposited daily, fish were immersed in tetracycline hydrochloride (TC) of 100 - 600 mg L^{-1} between 2-24 hours. TC was found to be incorporated within 12 hours after immersion in 600 mg L^{-1}. Otoliths were marked with a band that fluoresced in ultraviolet (UV) light. Daily rings corresponded to the number of days the fish lived after marking. Strong dose of 600 mg L^{-1} TC did not produce any diffuse bands and weaker doses of 100-500 mg L^{-1} TC produced no rings. When daily increments were assumed to be deposited more than once per day, growth performance index (ø') did not lie within the calculated mean (± S.D). These results suggested that *R. argentea* likely deposits increments daily. Counts of daily increments provide more accurate estimates of *R. argentea* age than previously available. The most significant advantage of using otolith ageing technique is the ability to produce individual rather than population statistics which have been available for *R. argentea*.

Key words: age validation, daily increments, otoliths, *Rastrineobola argentea*, tetracyline hydrochloride, phi prime (ø')

INTRODUCTION

Marking individual fish is an effective means for obtaining various kinds of information on fish species and populations (Muth *et al.,* 1988; Takashima *et al.,* 2000). Many aspects of fish development are reflected in otolith structure. Short term and long-term changes in growth rate caused by either environmental fluctuations of life history (e.g. metamorphosis, spawning) and the events may also be incorporated into the otolith record (Takashima *et al.*, 2000).

Age estimation by daily increments of otolith has been recognized as a powerful method for investigating the ecology of fish larvae and juvenile (Itoh *et al.*, 2000). Escot and Granado-Lorencio, 2001). A common method of ageing larval fish is to count increments, or daily rings, on otoliths (Hettler, 1984). Validation of the rings as daily growth increments usually involves comparison of the actual age of larvae reared from eggs with the number of increments. When one works with larvae of unknown age, such as those collected in the field, validation of the ageing method is more difficult. A chemical time check, or mark, placed on the otolith can provide a reference point for subsequent time growth (Campana and Neilson, 1985; Geen, 1992; Hayashi *et al.*, 2001). Tetracycline is incorporation into calcium structure of fish during growth and this can be restricted to one day's increment on the otolith thus enabling an accurate identification of the treatment date (Alhossaini and Pitcher, 1988). Tetracycline marker is detectable in section of a bone by fluorescence microscopy.

Rastrineobola argentea (Pellegrin) an endemic cyprinid is the third commercially important fish in Lake Victoria after Nile perch, *Lates niloticus* (L.) and Nile tilapia, *Oreochromis niloticus* (L.) (Witte and Densen, 1995; Othina and Tweddle, 1999). There is no scientific information available on the use of daily increments in ageing the species. The objective of the present study was to validate if increments observed on *R. argentea* otoliths are deposited daily.

MATERIALS AND METHODS

Age validation using tetracycline hydrochloride

Fish caught from Nyanza Gulf of Lake Victoria (Fig. 1) by mosquito seines

(5mm) were first kept in large holding tanks (500 litres) with fresh lake water for 2 days to acclimatise. The holding tanks were positioned outside and exposed to normal photoperiod but sheltered from drastic temperature fluctuations, which might stress the fish. Fish were fed daily on aquarium food and wild zooplankton captured with 20 µm mesh size from Lake Victoria. The surviving fish were then divided for TC treatment. Individual fish were treated by placing them in 1-L glass beakers containing TC solutions of concentration ranging from 100-600 mg L^{-1} with exposure time of 2-24 hours.

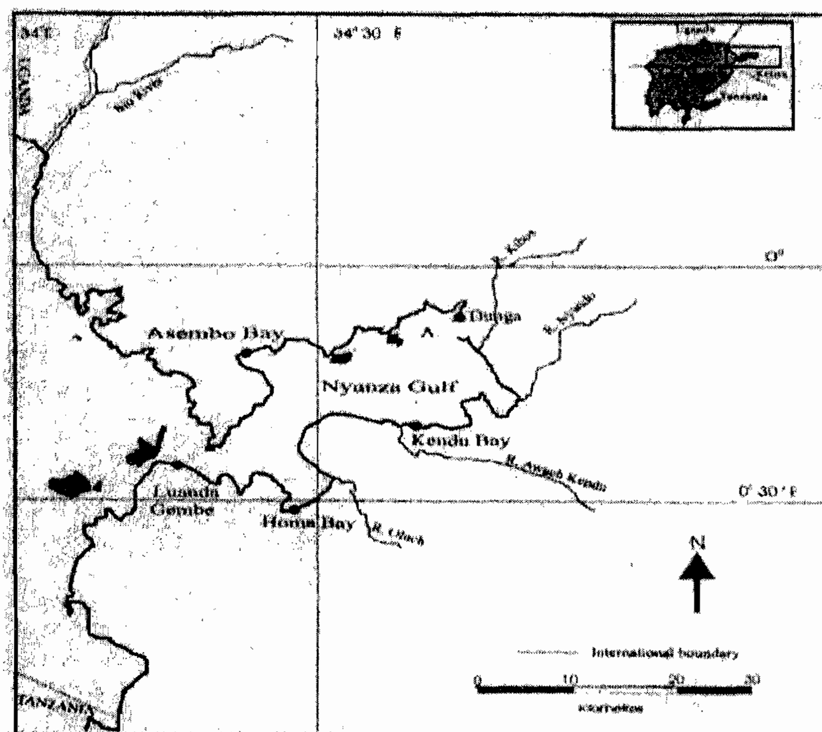


Figure 1. Map showing the Nyanza Gulf of Lake Victoria, Kenya and the sampling site A

The test solution was prepared using distilled water because TC binds to calcium ions in hard water, hindering the uptake of TC by otolith (Muth *et al.*, 1988). The test solution was adjusted to pH 7.8-8 with tris-buffer to correspond to the lake water. During treatment the water was aerated but the fish were not fed. After each prescribed exposure the fish were returned to 500 litres holding tanks to resume normal growth. Three tanks each with 2 untreated fish distributed among tanks with treated fish acted as control. Water in the tanks was replaced with fresh lake water three times a week. Surviving fish from each experimental group length was measured (TL) and otoliths removed. Otoliths were mounted on a microscope slide with thermoplastic glue and kept in lightproof slide holders to prevent degradation of tetracycline marks (Hall, 1992). Otoliths preparation for viewing under microscope followed procedure outline by Hall (1992). Otoliths were viewed under fluorescent ultraviolet light (UV360 nm) and bright light illumination with a compound microscope. Under UV light an ocular marker was aligned with the fluorescent band on the otolith. Incremental rings were counted under bright light illumination from the marker to the edge of the otoliths to verify if they do correspond to the number of days since the fish was tagged and the time it was killed.

Age validation using growth performance index

Second approach to validation (to establish increments were deposited daily) was to compare the values of the growth parameters (L∞(cm), K(yr^{-1})) using the growth performance index (Ø') (Pauly and Munro, 1984), defined;

$$Ø' = \log_{10} K + 2 \log_{10} L\infty,$$

where K the growth constant and L∞ the asymptotic length are from von Bertalanffy growth model. Growth parameters were estimated using commercial catch data collected from Nyanza Gulf in January to May 1994. The data analysis was based on Electron Length Frequency Analysis (ELEFAN) computer programs incorporated in FAO-ICLRAM Stock Assessment Tool (FISAT) (Sparre and Venema, 1998).

For the same L∞, values of K were fitted to the size of the fish under three oneassumptions about ring deposition rate: one ring per day, two rings per day and ring per 3 days. For each assumption, Ø' was calculated. The three Ø'estimates were then compared with the mean Ø ' calculated from the literature on this species.

RESULTS

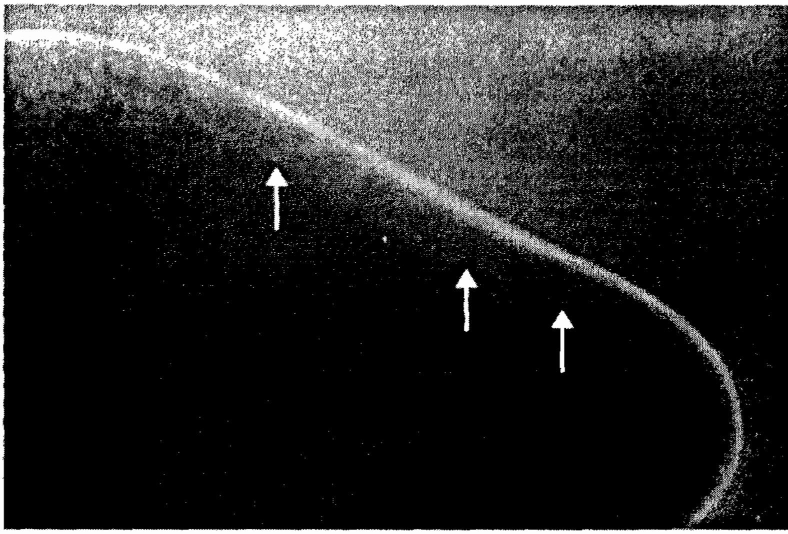
Age Validation using tetracycline

Table I shows the concentration of tetracycline hydrochloride (TC) used *R. argentea*, the number of fish used and the hours of exposure. Only fish exposed to 600 mg L^{-1} TC for 12 hours and 21 hours were marked (Figure 2a). The appearance of a second ring at the edge of the lapillus was due to a second immersion in 600 mg L^{-1} TC for 12 hours after fish had survived for 30 days, After the second immersion the fish lived for three days. Under high magnification (1000x) 30 increments on the lapillus of fish immersion in 600 mg L^{-1} TC for 21 hours were counted (Figure 2b). A clear checks on the lapillus on the lapillus of fish exposures to 600 mg L^{-1} TC for 21 hours is evident. There were no stress marks (check) in fish reared in control tanks and after immersion in TC, suggesting TC did not affect fish normal growth. Fish immersed in 600 mg L^{-1} TC for 12 hours had no visible rings under LM (Figure 3).

Table I. Concentration of tetracycline hydrochloride (TC) administered to *Rastrineobola argentea*, exposure time and survival time in holding tanks.

TC (mg L^{-1})	**Fish no**	**exposure time (hrs)**	**survival time (days)**	**comments**
100	1	4	15	died*
200	3	2	17	died*
200	1	6	1	died*
200	1	24	26	killed+
300	2	18	25	killed+
400	2	24	30	killed+
500	1	24	6	died*
500	1	24	27	killed+
500	1	6	26	killed+
500	1	6	8	killed+
600	3	21	30	killed+
600	2	12	30	killed+
600	1	12	7	died
600	1	6	7	died*

*Dead fish covered by mould +Fish killed for otolith removal a



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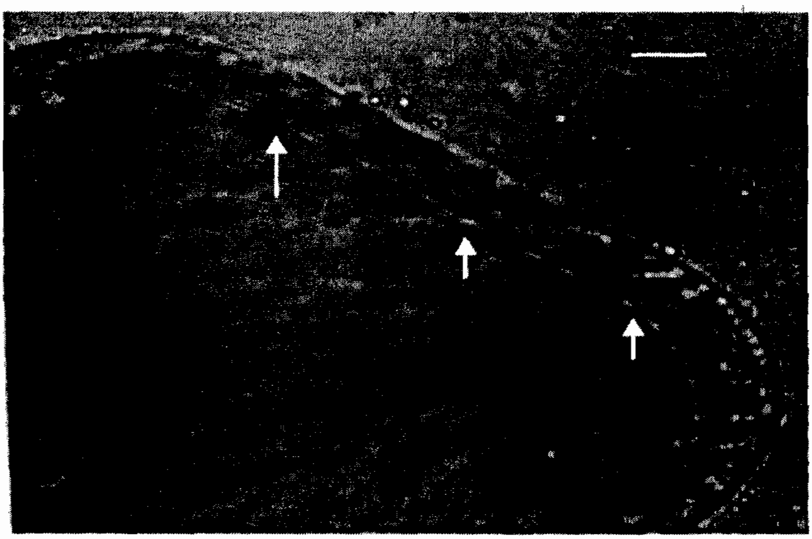

Figure 2. Lapillus of 24.1 mm *Rastrineobola argentea* immersed in 600 mg L^{-1} TC for 21 hours. a) Under ultraviolet light showing tetracycline fluorescent bands, b) Same as photography (a) under bright illumination. Arrows indicates a check corresponding to the tetracycline fluorescent band. Scale bar 10 µm.

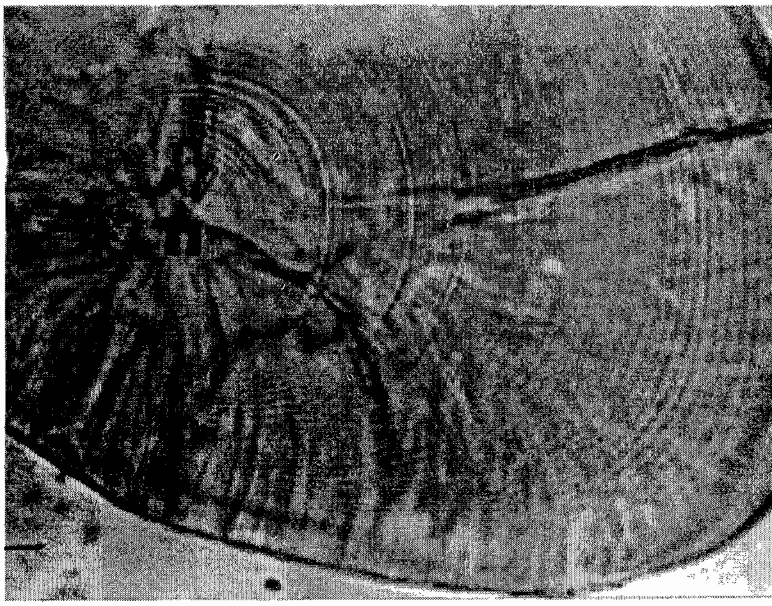

Figure 3. Light microscope micrographs of *Rastrineobola argentea* exposured to 600 mg L^{-1} TC.of tretracycline hydrochoride for 12 hours. No checks were evident. At the edge increments were less visible, refocusing solved the problem. Scale bar = 10 µm, n= nucleus.

Age validation using growth performance index

Phi prime from this study and from other authors has a mean and standard deviation of 1.62±0.05 cm (Table II). When increments are assumed to be formed daily, phi prime (Ø '= 1.7cm) lies within this estimated mean from published data. (Table 3). When otolith formation is assumed to be two per day (Ø '=2.0cm) or one per every three days (Ø '=1.2cm), the estimates do not lie within the estimated mean phi prime derived from growth parameters estimated by length-frequency method (Table III). These results suggest that the otolith rings seen in *R. argentea* were mostly likely deposited daily.

Table II. Growth parameters ($L\infty$, K) and phi prime of *Rastrineobola argentea* from this study compared with other published data. SL= Standard length.

Source	$L\infty$ (SL cm)	K (yr^{-1})	Phi prime
Wanink 1989	5.2	1.1	1.5
Wandera 1992	6.5	0.9	1.6
Wandera and Wanink 1995	6.5	0.9	1.6
Nyanza Gulf	5.0	1.8	1.7
Mean±SD			1.62±0.05

Table III. Growth parameters ($L\infty$ K) and phi prime of *Rastrineobola argentea* when increments are assumed to be deposited twice a day and once per three days in Nyanza Gulf.

Otolith ring assumed formation	$L\infty$ (cm)	K (yr^{-1})	Phi prime
Daily	5.0	1.8	1.7
Twice daily	5.0	3.6	2.0
1 per 3 days	5.0	0.6	1.2

DISCUSSION

In this study tetracycline hydrochloride (TC) was incorporated into *R. argentea* otoliths within 12 hours after immersion. Incorporation was only in 600 mg L^{-1} TC. Incorporation of TC only in strong dose of 600 mg L^{-1} TC was attributed to immersion instead of injection. Geffen (1992) recommends juvenile and adult fish be marked by injection. In their studies Secor et al. (1991) found that incorporation rates of oxytetracycline hydrochloride (OTC) by immersion was low and time for exposure had to be increased up to 40 hours. Injection with TC was not possible because *R. argentea* were very fragile and a few minutes exposures lead to their death.

There was higher mortality in fish exposed to low and high concentration (100-200 mg L^{-1} TC and 600 mg L^{-1} TC) compared to medium concentration (300-400 mg L^{-1} TC), which could not be accounted for. There were no stress marks (checks) evident for fish immersed in TC, suggesting TC did not affecting fish growth after tagging. Of the two marked fish, the fish immersed in 600 mg L^{-1} TC for 21 hours and lived for 30 days had 30 countable increments, while the one immersed for 12 hours had no increment beyond the fluorescence band. This suggested that *R. argentea* likely deposits increments daily. If the assumption of a daily periodicity of increments were correct, one would expect a reasonable agreement between the values of the growth parameters derived from the present work and those given in the literature. This is in fact is the case, values of phi prime from this study was 1.7 and are in agreement with 1.5, 1.6 and 1.6 from Wanink (1989), Wandera (1992), Wandera and Wanink (1995) respectively. When daily increment are assumed to be deposited more than once per day, phi prime does not lie within the calculated mean (± SD) (1.62 ± 0.05).

Tetracycline although most commonly used in marking otoliths (Geffen, 1992), is toxic, cause disorders in the digestive system, and inhibits protein synthesis affecting growth in animals (Winstein, 1975). It is possible that *R. argentea* immersed in 600 mg L^{-1} TC for 12 hours growth was affected and deposition of daily increments did occur, but they were beyond the resolution of light microscope. Yoklovich and Boehlert (1987) failed to observe daily rings in most otoliths of *Sebastes melanops* after injection with TC. Hetler (1984) failed to determine increment beyond the fluorescence band after immersion of fish in oxytetracycline hydrochloride (OTC).

The lapillus of fish immersed in TC showed low contrast between the continuous and discontinuous zones under a light microscope. The poor deposition of daily rings in *R. argentea* could be related to TC immersion. Such faint increments are a common phenomenon among several species in the laboratory. Fish reared in constant temperature in the laboratory were found to produce faint increments, whereas fish subjected to diel temperature cycle are characterised by more easily observed growth increments (Alhossaini and Pitcher, 1988; Hayashi, *et al.*, 2001).

However, there are drawbacks to the use of otoliths increment data for estimating age and mortality. Most apparent is the extensive effort required extracting them, preparing and counting growth increments. Increments are inherently difficult to locate and this problem increases with size of the fish, where increments disappear or are difficult to see on the edge and near the nucleus of otolith because of refraction of the transmitted light (Campana, 1992). Over grinding of lapillus lead to loss of microstructure and inaccurate age determination may be caused by non-dairy deposition of rings, or failure to detect all rings within an otolith due to the resolution problem of light microscopy (Campana and Jones, 1992). Increment width in this study ranged from 1.3 µm near the nucleus to 4.5 µm towards the edge of the lapillus indicating that the light microscope resolution was adequate for counting the increments. The theoretical resolution of a light microscope is 0.2 µm although for practical applications it is really close to 1 µm (Neilson, 1992).

Implication of the study

Counts of daily increments can provide more accurate estimates of *R. argentea* age and growth than has previously been available. Size, which has previously been used, is a poor indicator of cohort membership and age estimated from daily increments is a better age for *R. argentea*. This information allows computation of age-dependent mortality rates resulting in more accurate estimates of larval mortality in the lake. The most significant advantage of using otolith ageing technique is the ability to produce individual rather than population statistics which have not been available for *R. argentea*.

The knowledge of more accurate age, growth and mortality of juvenile *R. argentea* is fundamental to sustainability of the present *Rastrineobola* fishery. Information on age structure can be used to clarify the effects of changes in the environment, growth and survival of the juveniles, resulting in improved understanding of factors affecting recruitment success. In adults, the information can be used to determine the effects of fishing on the stocks, to understand life history events, and to maximise yield while ensuring future stocks of *dagaa* are maintained. If *dagaa* is to be cultured, knowledge of growth rates of cultured versus wild fish can be useful in determining the feasibility, potential, and profitability of rearing the fish in captivity.

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