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Short Note

# Validation of growth zone formation in *Oreochromis mossambicus* otoliths collected from an irrigation pond in the Sundays River Valley, Eastern Cape, South Africa

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**In fisheries science, life-history trait information is widely used to estimate fish population recruitment, growth, and mortality. The aging and growth of *Oreochromis mossambicus* from the Sundays River Valley irrigation pond in the Eastern Cape province of South Africa were determined using otoliths. Mark recapture of chemically-tagged wild fish was used to confirm the periodicity of growth zone creation. A total of 150 specimens of *O. mossambicus* were obtained using a combination of seine and fyke nets. Female total lengths ($L_t$) ranged from 20 to 340 mm, while male $L_t$ ranged from 82 to 374 mm. Growth zone deposition rates of wild *O. mossambicus* otoliths fluorochrome-marked with oxytetracycline hydrochloride (OTC) and recaptured after one year was consistent with the deposition of one growth increment annually. According to the three-parameter von Bertalanffy model, growth was described using different coefficients for females and males, respectively. The maximum age estimate for both females and males was 12 years. *Oreochromis mossambicus* in this warm temperate pond had relatively long life spans compared to subtropical populations, which suggest likely long-term population persistence within cooler, novel environments.**

**Keywords:** impoundment, oxytetracycline, chemical tagging, ring formation, von Bertalanffy model

Knowledge of fish biology, such as age and growth, is critical for gaining a better understanding of fish population dynamics and effective fishery management (Carlson et al. 2006; Weyl and Booth 2008; Gebremedhin et al. 2021). The counting of growth increments in otoliths, scales or spines are frequently employed to determine the age of fish (Winker et al. 2010a; Sabah and Khan 2014). Estimating age is critical for determining how fish species respond to environmental variations over time, as well as determining age at sexual maturity, growth and mortality (Campana 2001; Winker et al. 2010a; Taylor and Weyl 2012).

The consistency of age interpretation and the proximity of repeated measurements of the same quantity are dependent on the verification and precision of structural readings (Cailliet et al. 2006). Panfili et al. (2002) defined age validation as the proximity of a quantity estimate to its true value. As a result, Beamish and McFarlane (1983) emphasised the necessity of age validation, claiming that it proves that the individual fish has been appropriately aged. Chemical marking and edge analysis are the only two procedures that are commonly used to validate increment periodicity (Campana 2001; Campana 2005). It is important to validate otolith increment formation, mainly because of the potential variation in the temporal pattern of increment formation (Hernaman et al. 2000). In addition, validation is considered a fundamental requirement mainly through the interplay of environmental (e.g. temperature, water flow), behavioural (e.g. feeding, spawning) and biological response (e.g. metabolism) factors that influence the deposition of growth zones (Campana 1999). While, a variety of methods are available for validating growth increment formation, there are only two methods that are often applied in validating increment periodicity, which are chemical marking and edge analysis (Campana 2001; Campana 2005).

Chemical marking of wild fish involves injecting a fluorescing, calcium-binding chemical such as oxytetracycline hydrochloride (OTC) into calcified structures to form a permanent fluorescing band (Campana 1999). Edge analysis is based on the assumption that a growth increment is formed on a yearly cycle, and that the outermost increment state frequency (opaque zone present or absent) should form a yearly sinusoidal cycle when plotted against time (Campana 2001). The Mozambique tilapia, *Oreochromis mossambicus* (Peters, 1852) belongs to the Cichlidae family, and its natural range extends from Mozambique's lower Zambezi River system to South Africa's Eastern Cape province's Bushmans River (Skelton 2001). This is the first study in an austral warm-temperate zone to determine and validate the age and growth of *O. mossambicus*.

The Eastern Cape's Department of Economic Development and Environmental Affairs (DEDEA permit no. CRO 35/17CR and CRO 36/17CR) approved the fish collection. The National Research Foundation–South African Institute for Aquatic Biodiversity (NRF–SAIAB reference number 25/4/1/7/5/2017-03) and the Rhodes University Ethical Standards Committee (Reference no. 25/4/5) both gave their approval for the current work.

The present study was based on 150 specimens of *O. mossambicus* collected from an irrigation pond in the Sundays River Valley (33°28′46″ S, 25°35′34″ E). The pond is 3 870 $m^2$ in size and is located within a semi-arid region, with average air temperatures of between 5 °C and 18 °C in winter and 15 °C and 45 °C in summer and rainfall is about 350–600 mm (Lombard et al. 2001). The water level fluctuates as a function of the inflow from the Sundays River, which peaks in September and is low in January. The mean surface water temperature and pH were 21 °C (range 20.9–21.1 °C) and 8.29 (range 8.1–8.6), respectively. All fish were caught using a seine net with 12 mm mesh wings and an 8 mm mesh size cod-end, as well as double-ended fyke nets (8 m guiding net, first ring diameter of 55 cm, 10 mm mesh size at the cod end) in March 2017. To reduce post-capture stress, fish were transported to an oxygenated holding tank containing a dosage of 0.2 mg $l^{-1}$ of anaesthetic clove oil. Fish were then injected with 60 mg $kg^{-1}$ mass oxytetracycline hydrochloride (OTC) (Hitet 120; Bayer, Leverkusen, Germany), then tagged with a T-bar anchor tag (model TBA-2) and released to validate the periodicity of otolith growth (Winker et al. 2010a, 2010b). Fish were re-captured after 12 months in April 2018, measured to the nearest millimetre. Fish were then dissected and the sex was determined through visual assessment of the gonads. Sagittal otoliths were removed and dried for later age determination.

In the laboratory, cleaned otoliths were set in clear polyester casting resin, sectioned transversely through the nucleus (0.3–0.6 mm with a double-bladed diamond-edged saw), mounted on a microscope slide with DPX microscopy mountant (Merck, Darmstadt, Germany) and viewed under a dissecting microscope using transmitted light (variable magnification: ×10–40). Growth zones were found as alternating opaque and translucent bands. Under transmitted light, the number of opaque rings from the nucleus to the margin of the otolith were counted to determine the age. A von Bertalanffy growth function (VBGF) was used to estimate growth to the sex-specific length-at-age data. The VBGF is expressed as:

$$L_t = L_\infty(1 - e^{-k(t - t_0)})$$

where, $t_0$ is the age-at-'zero'-length, $L_t$ is the length at age $t$, $L_\infty$ is the predicted asymptotic length and $K$ is the Brody growth co-efficient (Ricker 1975).

Sex ratio was assessed using Chi-square analysis on the sex of all specimens examined. Analyses were performed in R (R Development Core Team 2020). A likelihood ratio test was used to test the null hypothesis that there were no differences in growth parameters between sexes.

The overall sex ratio of females to males was 1:1.19, which was not significantly different from unity ($\chi^2$ = 1.04, df = 1, $p$ = 0.31). Sex-specific growth parameters were not statistically different ($\chi^2$ = 2.85, df = 1, $p$ = 0.09). The size ($L_t$) of females ranged from 20 to 340 mm and males ranged from 82 to 374 mm. The age of *O. mossambicus* from sagittal otoliths ranged from one to 12 years for both males and females (Figure 1). The observed length-at-age data obtained from otolith readings, the von Bertalanffy growth parameters, standard errors, and 95% confidence intervals are summarised in Table 1. Growth was described by the von Bertalanffy model illustrated in Figure 1 and the visible fluorescent mark was confirmed (Figure 2). Males attain a greater length-at-age than females after one year (Table 1). Of the 97 *O. mossambicus* that were injected with OTC, a total of 40 fish were recaptured from the pond.

This study validates that the growth zone deposition rate in *O. mossambicus* is annual. *Oreochromis mossambicus* in the Sundays River Valley irrigation pond grow slowly and reach maximum lengths of 374 mm total length. The annual growth zone deposition rate in sagittal otoliths of *O. mossambicus* was consistent with research conducted in a subtropical Lake Chicamba in Mozambique using otoliths (Weyl and Hecht 1998). However, a study by Weyl and Hecht (1998) showed that a single opaque band was

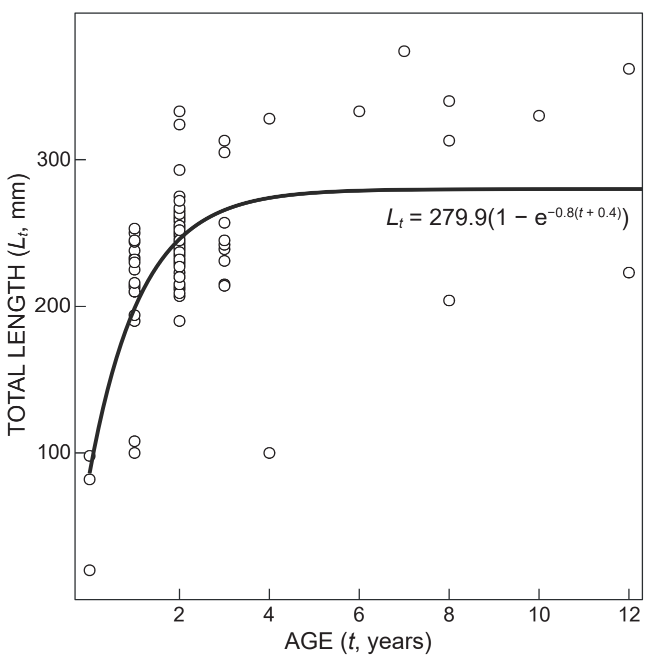


**Figure 1:** Observed total length-at-age (years) of *Oreochromis mossambicus* sampled from the Sundays River Valley irrigation pond in the Eastern Cape province of South Africa

**Table 1:** Von Bertalanffy growth model parameter estimates, standard error (SE) and 95% confidence intervals (95% CI), of *Oreochromis mossambicus* from the Sundays River Valley irrigation pond, Eastern Cape province of South Africa

| | Parameter | Estimate | SE | 95% LCI | 95% UCI |
|---|---|---|---|---|---|
| Entire population | $L_\infty$ | 279.91 | 11.15 | 257.78 | 302.04 |
| | $K$ | 0.87 | 0.16 | 0.54 | 1.19 |
| | $t_0$ | –0.42 | 0.17 | –0.76 | –0.07 |
| Male | $L_\infty$ | 294.98 | 25.33 | 243.99 | 345.97 |
| | $K$ | 0.69 | 0.25 | 0.18 | 1.21 |
| | $t_0$ | –0.64 | 0.36 | –1.38 | 0.09 |
| Female | $L_\infty$ | 267.20 | 10.33 | 246.48 | 287.92 |
| | $K$ | 1.11 | 0.22 | 0.67 | 1.56 |
| | $t_0$ | –0.18 | 0.15 | –0.48 | 0.11 |

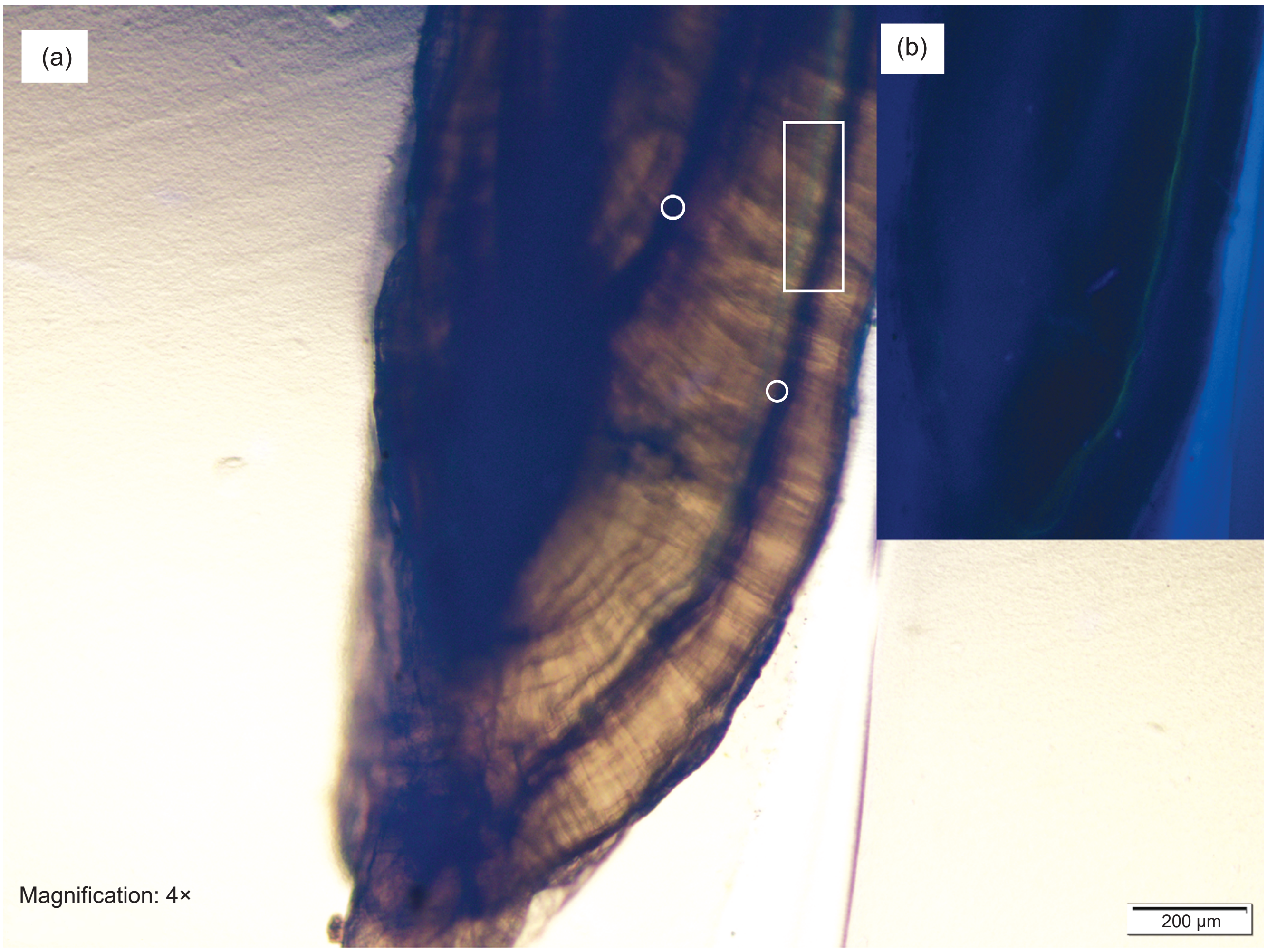


**Figure 2:** Composite photomicrographs of a sectioned *Oreochromis mossambicus* sagittal otolith injected with oxytetracycline. (a) The base image is of the otolith viewed using transmitted light with position of opaque zones indicated with circles and rectangle shows yellow wavelength. (b) optically filtered image highlighting yellow wavelength

formed in late winter and that *O. mossambicus* from Lake Chicamba had rapid growth with asymptotic length being reached within three years. These results differ from the current study where it was found that *O. mossambicus* had a slightly slower growth rate and reached an asymptotic length in four years.

Booth and Khumalo (2010) also showed that *O. mossambicus* from a reservoir in eSwatini reached asymptotic length within three years. This formation of a single annulus in a year has been shown in other cichlids (Peel et al. 2016). The present study showed that *O. mossambicus* in warm-temperate regions of southern

Africa live long and reach a maximum age of 12 years. This is longer than the maximum age of *O. mossambicus* populations observed in warmer subtropical regions by Weyl and Hecht (1998) (maximum age = 10 years) and by Booth and Khumalo (2010) (maximum age = six years).

The differences in growth rates and maximum age have been attributed to various factors such as reservoir size, food availability, food condition, and/or population density have been shown for the disparities in growth rates and maximum age (De Silva 1986). Furthermore, research has revealed that tilapias can shift their energy expenditure toward reproduction at the expense of growth, resulting in variances in growth rates between water bodies depending on environmental factors (Lowe-McConnell 1982). Moreover, there are fewer predators and negligible fishing pressure in this pond and therefore, this would result in *O. mossambicus* inhabiting these waterbodies living longer than those in other studied systems (i.e. Weyl and Hecht 1998; Booth and Khumalo 2010). This work provides significant evidence that growth rates vary among populations of the same fish species in different climate zones, emphasising the need for age validation as a prerequisite for understanding *O. mossambicus* life history in diverse geographical locations.

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*Data availability* — Data is readily available upon request from the authors

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