Validation of the periodicity of increment formation in the otoliths of a cichlid fish from Lake Tanganyika, East Africa

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Tetracycline was used as a chemical tag in a mark-recapture study to examine the pattern of increment formation in the otoliths of Tropheus moorii, a rock-dwelling cichlid from Lake Tanganyika. A total of 256 fish were captured by divers and injected with tetracycline. Of these, nine were recaptured after either 1 or 2 years at liberty and eight retained tags within their otoliths. Comparison of the number of growth increments formed after the tag and the time at liberty demonstrated that increments were deposited on an annual basis in the otoliths of this species. Furthermore, there was a strong relationship between otolith mass and age suggesting that otoliths grew at a predictable rate throughout the life of the fish. Validation of an annual pattern of increment deposition allowed age and growth information to be derived from otoliths. This showed that T. moorii grew rapidly to attain adult size by 3 years of age. Males grew faster than females and also attained a larger size than females (8.74 v. 7.91 cm L50 respectively). The longevity of some of these small freshwater fish was surprising; the oldest individual had an age of 10 years, while the average age of adults was 4 years.

Key words: age; growth; Lake Tanganyika; otolith; Tropheus moorii.

INTRODUCTION

The Great East African Lakes, Victoria, Malawi and Tanganyika, are renowned as the most diverse freshwater ecosystems on earth, notably for their enormous diversity of cichlids. They are thus excellent model systems to study the process of speciation (Kosswig, 1947; Greenwood, 1991; Turner, 1994; Martens, 1997; Seehausen et al., 1997; Galis & Metz, 1998; Seehausen & van Alphen, 1998) and

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pathways of adaptive radiation (Fryer & Iles, 1972; Meyer, 1993; Rossiter, 1995; Kawanabe et al., 1997; Sturmbauer, 1998; Kornfield & Smith, 2000). While behaviour, ecology, taxonomy and evolutionary genetics of these fishes are topical areas of research, relatively little is known about the demographics of populations. This information is important since the demographic processes of recruitment, growth and mortality drive the dynamics of populations, and over long-time scales influence the rate and direction of evolution. For this reason, demographic studies may aid the understanding of the process of evolution in cichlids.

Age is the key variable in demography, as it forms the basis for calculations of growth and mortality rates and the productivity of populations. In fishes, the periodic increments deposited in calcified structures such as scales, vertebrae, fin rays, opercula and otoliths have been used to age individuals. Of these, otoliths have become the most popular and widely used source of age information (Fowler, 1995; Campana, 2001). The analysis of annual bands in otoliths has a long history in studies of fish populations of temperate marine and freshwater environments and has recently been used to examine the demography of tropical reef fishes (Fowler, 1995; Meekan et al., 1999, 2001). Few studies, however, have attempted to use otolith analysis to examine the demography of populations of tropical freshwater fishes (Bruton & Allanson, 1974; Hecht, 1980; Panfil & Tomás, 2001).

Prior to the analysis of otoliths, it is essential that the periodicity of formation of increments be known, in order to ascertain the accuracy of age estimates. While several methods exist to validate increment deposition patterns in fish otoliths (Campana, 2001), the best approach involves the release and recapture of chemically tagged fish in their natural environment. In this technique, chemical tags are applied either by immersion or injection that are incorporated into otoliths of fishes, providing a permanent mark at the time of tagging. Fish are then released and recaptured at a later date. The number of growth increments formed in the otolith after the application of the tag can then be compared to the time at liberty, allowing the periodicity of increment formation to be determined.

In the present study the periodicity of deposition of increments within the otoliths of a cichlid fish endemic to Lake Tanganyika, *Tropheus moorii* Boulenger was validated. The antibiotic tetracycline was used as a chemical tag in a mark-recapture study to show that the deposition pattern of increments in otoliths occurred at annual intervals. Age information was then used to derive estimates of growth, longevity, mortality rates and age structures of the population. This provides the first estimate of the demographic parameters of a population of an African cichlid species from the Great Rift Valley region.

**MATERIALS AND METHODS**

**STUDY SPECIES**

The evolutionary history of fishes in the genus *Tropheus* has been studied extensively (Sturmbauer & Meyer, 1992; Sturmbauer et al., 1997; Baric et al., 2003). A total of six nominal species have been described (Poll, 1986), all of which are endemic to Lake Tanganyika, although >70 distinctly coloured ‘races’ are recognized in the genus (Brichard, 1989; Konings, 1992). All of these fishes are confined to rock habitats for foraging and mating and have a limited capacity for dispersal either as adults or larvae.
across open water (Yanagisawa & Nishida, 1991; Sturmbauer & Dallinger, 1995). Adult *Tropheus* are found at varying depths feeding on epilithic algae and their maximum size seems to depend on environmental variables such as the amount of food available and competition (Axelrod, 1993). *Tropheus moorii* is a maternal mouth-brooder, and females of this species 10 cm standard length (*L*<sub>S</sub>) lay from five to 20 eggs (Fryer & Iles, 1972). These eggs are c. 5–6 mm long and hatch after c. 4 weeks of incubation. At hatching, young fish have an average *L*<sub>S</sub> of 6 mm (B. Taborsky, pers. comm.). In contrast to the average female *L*<sub>S</sub> of 10 cm argued by Fryer & Iles (1972), female fish in this study attained an average size of 7.91 cm.

**STUDY SITE AND FIELD TECHNIQUES**

The study site at Mpulungu, Zambia, consisted of a steep cobble shore and a pier that had been naturally colonized by a number of species of rock cichlids in the years following its construction. The habitat was isolated from neighbouring cobble shores by a stretch of muddy shore of c. 50 m on each side. In July 1998 the population of *T. moorii* at the pier underwent secondary admixis, which was induced by the accidental introduction of at least 200 individuals caught for the aquarium trade at several localities in the south of the lake. The otolith study was part of a systematic survey of the genetic and phenotypic consequences of this human-induced secondary admixis.

Divers used small gillnets to collect 93 *T. moorii* in February 1999 and 163 individuals in February 2000. On both occasions, each fish that was collected was photographed, weighed, measured (*L*<sub>S</sub> and total length, *L*<sub>T</sub>) and injected with a 10 mM solution of tetracycline (0.15 mg 10 g<sup>−1</sup> fish mass). Tetracycline was dissolved in boiled lake water that also contained 179 mosmol NaCl. After injection, a fin clip was taken from the upper edge of the caudal peduncle and stored in 99% ethanol for genetic analysis. Fish were placed in 300 l tanks to allow recovery for 1 h prior to their release at the same location from which they were originally collected. Recaptures commenced in February 2001, when a total of 41 individuals were collected. All individuals were again weighed, measured and fin clips were taken and preserved in 99% ethanol. Fish were then killed and stored in 99% ethanol. As catches in February 2001 were low due to turbid water, more fish were collected during October 2001, when an additional 51 individuals were captured and treated as described above, with the exception of fin clipping.

**LABORATORY TECHNIQUES**

Both sagittal otoliths were removed from each fish, cleaned of adhering tissue and stored dry. Otoliths were weighed to 0.01 mg and each was mounted on a glass slide using thermoplastic cement so that the distal end protruded over the edge of the slide. The end of the otolith was then ground using wet sandpaper (800 grade) and polished with 12 µm lapping film. The glue was then reheated and the polished face of the otolith was mounted face down on the slide so that the rostral end of the otolith protruded from the slide. This was ground down to produce a thin transverse section incorporating the nucleus. Otoliths were viewed under transmitted white light and under reflected light against a dark background using a dissecting microscope at ×60 magnification. Sections were also examined under reflected ultraviolet light at ×100 magnification using a compound microscope to detect tetracycline marks. This microscope was linked to a video camera and image analysis system, which allowed the images of the otoliths to be recorded and the positions of the tetracycline marks to be measured. Three counts of all increments within each of the otolith pairs were made by the same reader. Between the short periods of observation, sections were stored in darkness.

**DATA ANALYSIS**

The relationship between otolith mass and age was examined using regression analysis (SigmaPlot software). Mortality rates were calculated using In-linear regression analyses. The natural logarithm of the number of fish in each age class was plotted against their
corresponding age. As the youngest age classes of fish (0, 1 and 2 years) were inadequately targeted by sampling, the year classes to the left of the age-frequency mode were excluded from the analysis (Pauly, 1984; Meekan et al., 2001). The ‘von Bertalanffy growth function’ (VBGF; von Bertalanffy, 1957) was fitted to length and age data using non-linear least squares estimation procedures. The VBGF is defined by the equation: 

\[ L_t = L_\infty \left[1 - \exp \left(-K( t - t_0 )\right)\right], \]

where \( L_t \) is the length at age \( t \), \( L_\infty \) the asymptotic length, \( K \) the ‘Brody growth coefficient’ (Brody, 1945) that defines the growth rate towards \( L_\infty \), \( t \) the age of the fish and \( t_0 \) the hypothetical age at which fish have zero length. As few juvenile fish were included in samples, the intercept of the VBGF model was constrained to the size at hatching (6 mm) in these analyses. VBGFs were plotted separately for males and females and then compared using plots of 95\% CI around least squares estimates of \( K \) and \( L_\infty \) following Kimura (1980).

RESULTS

RECOVERIES OF MARKED FISH

Of the 256 tetracycline-marked individuals, eight recaptured fish carried a tetracycline mark, yielding a recovery rate of 3.1%. Genetic analyses of five microsatellite loci [TmoM11 and TmoM25 (Zardoya et al., 1996), Pzeb3 (van Oppen et al., 1997), UME002 (Parker & Kornfield, 1996) and US-780/783 (Schliewen et al., 2001)] confirmed that these fish had been tagged (unpubl. data) and the date on which tagging occurred could be identified. Five tagged fish were recaptured in February 2001. Genetic analysis revealed that one of these did not retain the tetracycline tag in its otoliths. Of the remaining fish, one was at liberty for 12 months and the other three fish at liberty for 24 months. Four marked fish were recaptured in October 2001. Times at liberty for these fish were 12 + 6 months for three fish and 24 + 6 months for one individual.

OTOLITH GROWTH AND INTERPRETATION OF TETRACYCLINE MARKS

The otolith sections showed a consistent pattern of a densely opaque nucleus in the centre followed by alternating opaque and translucent zones [Fig. 1(a)]. The opaque zones were slightly narrower than the translucent zones. Tetracycline marks were visible under UV light [Fig. 1(b)]. While it was possible to observe the succession of opaque and translucent zones under UV light, they were less obvious in photographs [Fig. 1(b)]. Fig. 1(c) shows the same otolith depicted in Fig. 1(b) under visible light. The presence of two opaque zones distal to the tetracycline mark can be seen by comparing these two photographs.

In each of the eight recaptured specimens, the number of increments distal to the tetracycline mark corresponded to the known times at liberty [Fig. 1(c)]. Fish that were at liberty for 1 year had one complete increment (an opaque and a translucent zone) following the tetracycline mark, while those at liberty for 2 years had two complete increments.

The opaque zones and tetracycline marks of all eight tagged specimens are shown in Fig. 2. The extensions of translucent and opaque zones varied within and among individuals. The two younger individuals (3 years; T55, A01) laid down relatively wide increments in their otoliths in comparison to older fish. Additionally, the onset of the formation of the opaque zone relative to the tetracycline mark varied among
individuals. Opaque zones in four fish (A38, A22, A50 and T45) were formed immediately after the date of injection (end of February), whereas in the remaining individuals (T28, T42, T55, A01) the opaque zone began to form somewhat later,

![Diagram showing measurements of opaque zones and tetracycline marks](image)

Fig. 2. Measurements of the position of opaque zones and the tetracycline marks made on the sulcal axis of the otolith. Dates of injection are shown, dates of the fish killed are February 2001 for samples A 01, A 38, A 22, A 50 and October 2001 for T 28, T 55, T 45 and T 42. Otoliths marked on the same date (February 1999, February 2000) are arranged with tetracycline marks aligned vertically.
possibly indicating a slight variation of the growth cycle among individuals. Consequently, the opaque zone probably begins to form during the period March to April in the otoliths of *T. moorii*.

Despite the difference in time at liberty of fish recaptured in February and October 2001 (12 and 24 v. 18 and 30 months, respectively), there were no differences in distances from the tetracycline marks to the edge of the otolith of these fish.

**LONGEVITY, AGE STRUCTURE, GROWTH AND MORTALITY**

For calculations of longevity, age structure, growth and mortality, a total of 33 males and 37 females was used that were aged on the basis of the validation described above. There was a strong linear relationship (regression analysis; \( r^2 = 0.867, P < 0.001 \)) between otolith mass and age, suggesting that otolith mass may provide a reliable estimate of age in this species and that otoliths grew at a predictable rate over the life span of the fish (Fig. 3). Increment counts suggest that the fish sampled in the study attained a maximum age of 10 years. Fish >6 years of age, however, were relatively rare and comprised only 9% of the sample (Fig. 4). The age distribution of *T. moorii* showed that the age group of 3 year-old fish was the most abundant and accounted for 29% of the sample (Fig. 4). The observation that younger individuals were less abundant than 3 year-old fish is probably an artefact of the sampling technique, which did not capture small fish with the same efficiency as adults. Unlike the larger adults that swam above the bottom, small fish tended to flee into crevices among boulders when approached, making their capture by divers far more difficult.

The overall growth curve of *T. moorii* is shown in Fig. 5(a) and sex-specific curves are shown in Fig. 5(b). In general, fish grew rapidly during the first 2 years after hatching and attained an average \( L_S \) of 7.1 cm by the age of 3 years. As a consequence of the rapid growth rate, numerous age classes accumulated in a relatively narrow adult size range. Plotting of CI around least squares estimates of \( K \) and average adult \( L_S \), [Fig. 5(c)] showed that males grew significantly faster than females and attained a larger average size. Mortality rate

![Otolith mass at age for *T. moorii*. The curve was fitted by \( y = 940x \) \( r^2 = 0.867, P < 0.001 \).](image)
of *T. moorii* was calculated by excluding the age classes of 1 and 2 year-old fish as these were ‘under-sampled’ by the collection technique (Fig. 6). This gave an estimate of average longevity of 4·1 years.

**DISCUSSION**

It has been suggested that tropical fishes may not have interpretable increments due to dampened cycles of temperature and productivity in low latitudes (Longhurst & Pauly, 1987). Recent studies, however, show this not to be the case. Many tropical reef fishes show increment patterns that can be reliably interpreted (Meekan et al., 1999, 2001; Cappo et al., 2000, Marriott & Cappo, 2000). The present results show for the first time, that otoliths can be used to derive the age structure of a population of tropical cichlids in Lake Tanganyika. All individuals of *T. moorii* that were analysed showed a clear pattern of otolith increments in the form of an opaque zone followed by a slightly wider translucent zone. The mark-recapture study showed that these increments were deposited on an annual basis and the strong relationship between otolith mass and age suggested that otoliths grew at a predictable rate throughout the life of the fish. Thus, the otoliths of *T. moorii* conformed to the criteria necessary for estimating age-based demographic variables (Fowler & Doherty, 1992).

Studies on a variety of species from different climatic and geographical regions have shown that the timing and pattern of annuli formation within otoliths may differ with respect to species, climate and locality (Hill & Radtke, 1988; Fowler, 1990; Ferreira & Russ, 1992; Lou, 1992; G. McPherson, L. Squire & J. O’Brien, pers. comm.). Many tropical reef fishes, however, lay down opaque zones in spring when water temperatures rise, while translucent zones are formed during the rest of the year (Fowler, 1995). Thus, the timing of the onset of the deposition of the opaque zone in *T. moorii* during spring (most probably March to April) is in agreement with the findings on tropical reef fishes. Johnson (1983) and Fowler (1990) suggested that deposition of opaque zones is related to the onset of reproductive activity or to accelerated somatic...
growth that occurs in spring. Other authors question this hypothesis or suggest the opposite (Loubens, 1978; Fowler & Doherty, 1992).

The reason for differential otolith growth may be due to seasonal changes of the lake environment as a result of rainfall or upwelling, both of which may generate cycles of temperature and productivity. The rainy season extends from October to the end of February in the south of Lake Tanganyika, resulting in increased inflow of sediments and nutrients (Plisnier et al., 1999). This input of minerals and detritus is likely to have a positive influence on the growth rate of

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epilithic algae, the major food source of *T. moorii* (Sturmbauer & Dallinger, 1995) and may thus explain a higher growth rate of fish from October to March. Upwelling, which is important in other large lake systems (*e.g.* Lake Baikal; Kipfer *et al.*, 2000), has been recorded in the south of Lake Tanganyika during the dry, windy season from May to September (Coulter, 1991; Plisnier *et al.*, 1999). In May to June, warm surface water is driven towards the north of the lake, resulting in a tilting of the epilimnion and an upwelling of deep water along the southern coast. At Mbulungu, upwelling was inferred from the presence of colder, nutrient rich water that averaged 24·4°C at the surface during the dry season, compared to the warmer waters (27·7°C) that occur during the wet season (Plisnier *et al.*, 1999). This seasonal change in the average surface temperature of 3·3°C might lead to a decelerated growth rate during the dry season. The present results favour the hypothesis of opaque zones being correlated to decelerated growth of *T. moorii*, since both water temperature and sediment input are higher during the wet season when the translucent increments are deposited. Although the seasonal variation of 3·3°C is less than the 4–5°C argued by Longhurst & Pauly (1987) to be necessary for the deposition of growth increments in hard structures of tropical marine fishes, it appears sufficient to produce interpretable increments in the otoliths of *T. moorii*. Moreover, the combination of more than one seasonal environmental factor correlating with water temperature such as nutrient input may provide enough variability to promote the development of regular cycles in deposition of increments. Clearly, further studies at different localities and on other littoral cichlid species are necessary to provide an answer to the question of the precise growth conditions that are correlated with opaque zone formation and the extent to which this is influenced by water temperature, nutrients and sediment input.

Recent reviews conclude that chemical tagging is one of the best methods available to validate the periodicity of otolith increment deposition (Fowler, 1995; Campana, 2001). As tagged fish must be recaptured within a few years of release to ensure reasonable rates of tag return, the number of increments

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Fig. 6. Log-linear regression of abundance at age. Year classes 1 and 2 were excluded from the analyses as they were under-sampled by the collection technique. The curve was fitted by: $y = 4.24 - 0.44x$. 

between the tetracycline mark and the edge of the otolith is often small. This can lead to misinterpretation of the last increments at the edge of the otolith. Despite the low recapture rate in the study (3.1%) this was not found to be a problem. In the future, recapture rates might be increased through better external tags. Most of the ‘visible implant tags’ were lost within 1 year.

Unfortunately, not a single individual tagged at a young age (<1 year) was recaptured, so that the periodicity of the deposition of the first increment could not be validated. This first growth increment differed from subsequent increments in both shape and size and was often difficult to interpret.

*Tropheus moorii* was shown to be fast-growing and attain adult sizes at c. 3 years of age. This rapid rate of growth to asymptotic size is typical of other small, herbivorous fishes on coral reefs such as pomacentrids (Meekan *et al.*, 2001). Differential growth of males and females was also demonstrated [Fig. 5(b)]. The maximum size of the female fishes is clearly smaller than that of the males. Sexual dimorphism is a consequence of the reproductive implications of being male or female (Magurran & Macías García, 2000). In African cichlids, sexual size dimorphism increases with age in species in which males are the larger sex, but decreases with age whenever females are larger (Erlandsson & Ribbink, 1997). Another interesting finding was the longevity of some members of the population. Although relatively small, the oldest individual collected in the samples had an age of 10 years, while the average age of adults was 4 years. This provides the first estimate of the generation time of a rock-dwelling cichlid.

*Tropheus moorii* is an important target of aquarium trade collectors, a factor that may determine maximum ages and growth rates as these demographic variables can be strongly affected by fishing pressure (Hutchings, 2000). The ornamental fish trade comprises an important part of commercial fisheries in Lake Tanganyika, although at present little is known about either the intensity of fishing pressure or the extent to which stocks are exploited in Lake Tanganyika (Coenen *et al.*, 1998). The demographic information presented here not only gives insights into the ecology and evolution of *T. moorii*, but also provides important data for sustainable management of cichlid populations in Lake Tanganyika.

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