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Comparison of Otolith, Scale and Vertebrae for Age Estimation in Freshwater Exotic Fish *Oreochromis mossambicus*

KEYWORDS: Comparison, age, longevity, exotic fish, *Oreochromis mossambicus*

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ABSTRACT *Otolith, scale and vertebrae sections of freshwater exotic fish O. mossambicus (N = 133) were compared to ascertain the best aging material. Different body sized fishes revealed the presence of 1 - 7 growth marks. Agreement between otolith and scale ages was 96.96% and otolith and vertebrae ages were 88.36% in 33 fishes used for comparative studies. Vertebrae ring counts (VRC) were under estimated the age in (13.6%) older individuals compared to otolith and scale ring counts (SRC). The results revealed that otoliths and scales are reliable calcified materials for estimating age compared to vertebrae in O. mossambicus. There was a highly positive correlation between body length (BL) and body weight (BW). Further, otoliths ring count (ORC), otoliths weight (OW) and otoliths diameter (OD) showed positive correlation with BL and BW. This fish may live for a maximum of 7 years in the natural population.*

INTRODUCTION

Freshwater exotic fish *O. mossambicus* was noticed in the fish catch, probably on account of accidental entry with Indian major carp seed in 1990s. This fish population has been continuous increasing in the total number of caught in Indian major Rivers (Jain and Gupta, 1994) which cause of worry for the fishery biologists (Anon, 1995; Biju Kumar, 2000). Moreover, as per 2006 Global Invasive Species Database *O. mossambicus* is listed being as in the top 100 invasive alien species on the planet (Canonico *et al*., 2005). Invasive populations are now causing environmental and ecological problems in many countries including India (Canonico *et al*., 2005). *O. mossambicus* is widely distributed and now forms a part of fish fauna in the Godavari, Krishna, Cauvery, Yamuna and Ganga Rivers (Lakra *et al*., 2008).

Growth and age studies provide important demographic parameters to analyze and asses fish populations (Maceina & Sammons, 2006). Ageing of fishes from tropical regions have been reported through annual increments in calcified structures such as, scales (Werder and Soares, 1985; Mayekiso & Hecht, 1988; Sudarshan & Kulkarni, 2013), dorsal and pectoral spines (Bio & Ikusemiju, 1981; Pantulu, 1961), vertebral centra (Brown & Gruber, 1988; Bahuguna, 2013), and otoliths (Fowler & Doherty, 1992; David & Pancharatna, 2003). Most of the earlier age estimation studies of Indian fishes have been concentrated on scales (Singh & Sharma, 1998; Dua & Kumar, 2006; Kanwal & Pathani, 2011; Ujjania *et al*., 2013) due to the easiest to collect, process and avoids sacrificing the specimens. Recent reviews have suspected on scale aging due to difficulties in reading annuli, low precision (Lowerre-Barbieri *et al*., 1994), and that scale ages may become inaccurate when growth becomes asymptotic (Beamish & McFarlane, 1987; Shepherd, 1988). Scales have generally been found to underestimate ages relative to other structures, especially for older individuals and in slow-growing populations (Campbell & Babaluk, 1979; Mills & Beamish, 1980; Erickson, 1983; Kocovsky and Carline, 2000). Since scale growth is assumed to be proportional to body growth (Whitney & Carlander, 1956; Hile, 1970; Bagenal, 1974; and Erickson, 1983), annuli become crowded on the scale edges in slow-growing populations and in older fish, making scale interpretation difficult. To overcome from these difficulties, comparison of various calcified materials have been performed in many species, including yellow perch *Perca flavescens* (Niewinski & Ferreri, 1999), river carp suckers *Carpiodes carpio* (Braaten *et al*., 1999), and white suckers *Catostomus commersonii* (Scidmore & Glass, 1953; Ovchynnyk, 1969; Quinn & Ross, 1982; Sylvester, 2006). Although, some studies are also available on age and growth of *O. mossambicus* by using scales (Ujjania *et al*., 2013) and otoliths (Booth & Merron, 1996; Panfili & Tomas, 2001). There were no comparative studies for selection of reliable calcified material for aging in this species. Present investigation aims to select the most reliable calcified material for estimating the age of exotic freshwater fish *O. mossambicus* inhabiting Krishna River, Southern India.

MATERIALS AND METHODS

Different body sized freshwater fishes (*O. mossambicus,* N = 133) were collected from the Krishna River, Sangli District, Maharashtra, India with the help of local fisherman and purchased from local market in 2012 - 2013. Total body length (BL) of each fish was measured from the tip of snout to tip of the caudal fin (in cm). Body weight (BW) was recorded by using single pan balance nearest to 0.01gm, simultaneously lateral line scales, otoliths and vertebrae of each specimen was collected and preserved in different vials for further studies.

Scale study:

Lateral line scales of each specimen was collected with the help of forceps, cleaned in water by rubbing gently with fingers, fixed in 10% formalin solution for 24 hours and washed in water for 2 hours. Cleaned scale was kept in between two clean slides and slides were tied with rubber band on either side and observed under binocular microscope (Magnus MSZ-BI) for enumerating the ring counts present on the scales and photographed by digital camera (ABBOT DEC-2000).

Otolith Studies:

Otoliths were collected by taking an incision on the dorsal side of the head, to expose the brain on either side of which the otic capsules are located. The sagittal otoliths were removed from the otic capsules by opening the otic bulla. Both sagittae were retrieved intact from each specimen, washed in water and cleaned from all extraneous tissue. Then, each otolith was weighed to the nearest 0.001 mg the diameter was measured to the nearest 0.01 mm using a standard micro screw gage (Newman *et al*., 2000). Otoliths were then immersed in 50% glycerol and observed under binocular microscope (Magnus MLXB). Growth rings were clearly visible as alternate opaque and translucent zones that were enumerated.

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Vertebrae Section:
Different body sized 33 (BL 6.4 – 35.5 mm) fishes were used for vertebrae sections. Central 5th and 10th vertebrae of each fish were excised, cleaned and fixed in 10% formaldehyde solution for 24 hours, then washed in water for 1 hour and decalcified with 10% nitric acid (duration of decalcification depends on size of vertebrae). Decalcified vertebrae were washed under running tap water for 24 hours to remove the traces of formaldehyde and nitric acid and preserved in 70% alcohol. These vertebrae were embedded in paraffin wax and sectioned (10μ thickness) by using a rotary microtome (Model GE-70). Mid-diaphyseal sections were stained with Harris haematoxyline and observed under compound microscope for enumeration of growth marks (Buckmeier *et al.*, 2002).

Comparison of otoliths, scales and vertebrae sections of 33 fishes were undertaken for the selection of reliable calcified material for accurate assessment of age and longevity. For each fish, growth marks were counted on the aging structures independently by two readers without prior information of body length and body weight. Then percent agreement was calculated between otolith ring counts with scales and vertebrae ring counts.

Statistical analysis:
The relationship if any, between BL and BW, BL and OW, BL and OD, BW and OW, BW and OD was assessed by plotting scatter diagrams and using regression analysis (Zar, 1984). The relationship between ORC and BL, ORC and BW, ORC and OW and the ORC and OD was determined by calculating the correlation coefficient '*r*' by Karl Pearson's method (Zar, 1984).

RESULTS
Monthly variation in ambient temperature and rainfall in the year 2012 - 2013 is shown in figure 1. Although the mean monthly temperature varied between 21 – 30°C and that of rainfall from 0 to 121 mm (Fig. 1)

The otoliths, scales and vertebrae of *O. mossambicus* showed annual rings, each ring composed of a faint broader growth zone and a dark opaque line (Lines of arrested growth) the LAG (Plate 1A-F). One to seven growth marks were observed in the otoliths, scales, and vertebrae of different body sized fishes (N = 133). Out of 133 fishes, 21 (15.79%) fishes with mean body length (13.9 cm) have not exhibited any LAG (Table 1, Plate 1A, C, E). Thirty fishes (22.56%) with mean body length (16.28 cm) showed 1 LAG (Table 1), 38 fishes (28.57%) with mean body length (16.71 cm) exhibited 2 LAGs, 29 fishes (21.80 %) with mean body length (15.57 cm) showed 3 LAGs, 8 fishes (6.02%) with mean body length (17.21cm) exhibited 4 LAGs, 4 fishes (3.00 %) with mean body length (18.95 cm) showed 5 LAGs and 3 fishes (2.26%) with mean body length (23.66 cm) exhibited 6 LAGs (Table 1 & Plate 1B, D, F). It was generally observed that in older fish growth marks appeared sharp at the periphery of the otoliths, scales, and vertebrae sections (Plate 1B, D, F).

Comparison of otolith ring count with scale and vertebrae ring count in 33 large body sized fishes were done, the number of rings remained identical in otoliths, scales and vertebrae sections of 26 (78.78%) fishes. In one (3.03%) fish, the scales showed short on one growth ring compared to otolith in 3rd year individual. In 4 (12.12%) fishes, the vertebrae showed one growth ring lesser compared to otolith rings in 3rd year age groups and 2 (6.06%) fishes were showed short on two growth rings in 4th and 7th year age group individuals.

Of the 133 fishes sampled, the body length (BL) ranged from 6.0 – 35.5 cm with an average of 20.75 cm and body weight (BW) varied between 5 and 760 g with a mean of 382.5 g (Table 1). There was a high degree of positive correlation (r = 0.736) between BL and BW (Fig. 2). The scatter diagrams indicated that BL correlated highly positively with OD (r = 0.841; Fig. 3) and OW (r = 0.647; Fig. 4). Further, BW also showed positive correlation with OD (r = 0.628; Fig. 5) and OW (r = 0.759; Fig. 6). While, the ORC showed a lesser correlation with BW (r = 0.447), BL (r = 0.220), OW (r = 0.273) and OD (r = 0.144).

DISCUSSION
Age and growth studies of fish provide information of rate of survival, growth, mortality, age composition, and reproduction rate (Polat *et al.*, 2001; Campana, 2001). In the present study, one to seven broader light growth zones and one to six opaque sharp growth-arrested zones were found in fishes of varying body size (Table 1 and Plate 1). The comparative studies of otolith, scale, and vertebrae of freshwater exotic fish *O. mossambicus* revealed the presence of growth marks similar to that reported for other tropical species namely, *Sillago japonica* (Sulistiono *et al.*, 1999); Indian whiting, *S. indica* (David & Pancharatna, 2003); *Catla catla* (Ujjania, 2012); and *Notopterus notopterus* (Sudarshan & Kulkarni, 2013). The meteorological data indicate that, the annual temperature variation of Sangli is within 9°C, and the rainfall exhibited considerable annual variation (Fig. 1), further, during monsoon season there were increased temperature and maximum rainfall, consequently, cyclic pattern of growth in hard calcified material is common in fishes which is generally attributed to enforced seasonal feeding activity owing to marked variations in surrounding climatic factors, e.g. the extreme seasonal shifts in temperature of temperate areas, and the typical wet and dry conditions of the humidity cycle in the tropics (Johal & Tandon, 1992; Seshappa, 1999; Campana, 2001). Most of the freshwater fishes inhabiting Southern India exhibits a clear seasonality in body growth, breeding activities and gametogenesis (Saidapur *et al.*, 1989). Even though, *O. mossambicus* is spawning in July – August (rainy season) when spawning of most of the Indian major carps (*C. catla*, *L. rohita*, and *C. mrigala*) occurred (Ganie, 2013). Similar observations on the other fishes have reported (Seshappa, 1958; 1972; Alam & Pathak, 2010). Moreover, experimental studies have been confirmed that the formation of growth marks are annual in many species including, *Cirrhinus mrigala* (Hanumantha Rao, 1974), *O. niloticus* (Abdel-Hadi *et al.*, 2000); *Clarias gariepinus* (Wartenberg *et al.*, 2011), *Puntius conchonius* (Bahuguna, 2013); and *Labeo rohita* (our personal observation). Therefore, number of growth marks present in scale, otolith and vertebrae section is directly depict the age of individual fish.

Among 133 fish samples, 21 fishes showed no LAG in its calcified materials indicating that the fishes may be in their first year of growth. One to third year age group individuals are dominant with varied body length (6.0 – 35.2 cm). Further, the positive correlation between body length and growth mark of these fishes indicate that larger individuals have experienced a greater number of growth marks and therefore may be older. Larger fishes exhibited increased number of growth marks, thus there was a positive correlation between OW, OD with BL and BW, whether the body length and weight influences age needs further elucidation.

Comparison of the three ageing structures within the current study revealed that otolith is the most precise material for age estimation in *O. mossambicus* due to clear and distinctness in growth marks. The suitability of otolith for age estimation is also supported by several workers (Seshappa, 1999; Campana, 2001) the fact that otolith do not show reabsorption and their growth is acellular rather than by calcification (Secor *et al.*, 1995) and also because otoliths are reported to be metabolically inert and thus do not reflect physiological changes that may occur throughout the life of fish (Phelps *et al.*, 2007). Otoliths continue to grow and form annuli even as body growth slows and asymptotic length is reached, and annuli reasbsorption does not appear to occur during periods of food limitation or stress (Devries & Frie, 1996). Several workers were reported that otoliths to be the most reliable ageing structure in a number of temperate as well as tropical species such as, *Chelidonichthys kumu* (Staples, 1971),

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Capoeta capoeta umbla (Ekingen & Polat, 1987), *Trachurus trachurus* (Polat & Kukul, 1990), *Pylodictis olivaris* (Nash & Irwin, 1999), *Indian whiting* (David & Pancharatna, 2003) and *Ictalurus punctatus* (Buckmeier *et al*., 2002; Colombo *et al*., 2010). In the comparative studies, out of 33 fishes 32 fishes the number of growth marks was identical in scales and otoliths indicating that scale aging is reliable in > 97% of fishes. In other hand among 33 fishes 4 fishes one number of growth marks were short and another 2 fishes two growth marks were lesser in vertebrae sections compared to otolith ring counts in older individuals. Result indicating that vertebrae ring count is not that much reliable in this fish, this might be due to overcrowding of growth marks at the periphery of vertebrae sections, which was difficult to differentiate and count.

Our study found variation in the otolith, scale, and vertebrae ages of *O. mossambicus*. In this fish, vertebrae section underestimated the ages when compared with the otolith and scale ages. However, otolith and scale ring counts are more distinct and easy to enumerate, even in the older fish, than the vertebrae ring count. Therefore, we assume that the otolith and scale provide a reliable source for aging in *O. mossambicus* inhabiting Southern India.

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Table 1 Showing number of fishes, percentage, mean body weight, length, otolith weight, diameter and number of lines of arrested growths (LAGs) and age frequencies of freshwater exotic fish *O. mossambicus* (N = 133).

Number of Fishes	Percentage (%)	Body Weight (gm)	Body Length (cm)	Otolith Weight (mg)	Otolith Diameter (mm)	No. of LAGs	Age Frequency
21	15.79	83.57	13.9	21.11	0.276	0	1st Year
30	22.56	108.9	16.28	27.53	0.350	1	2nd year
38	28.57	151.94	16.71	39.35	0.366	2	3rd year
29	21.80	179.20	15.57	39.87	0.344	3	4th year
08	6.02	314.62	17.21	64.00	0.323	4	5th year
04	3.00	249.25	18.95	70.67	0.417	5	6th year
03	2.26	394.66	23.66	39.8	0.421	6	7th year

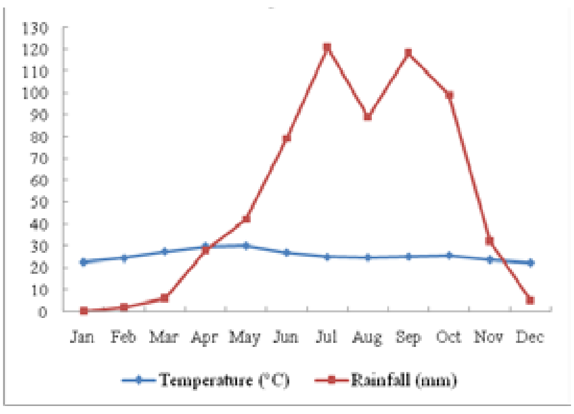

Figure 1: Figure shows monthly variation in mean temperature and rainfall of Sangli District from January 2012 to December 2013.

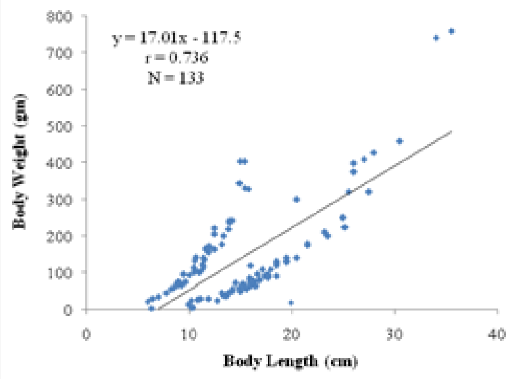

Figure 2: Relationship between Body Length (BL) and Body Weight (BW) in *O. mossambicus.*

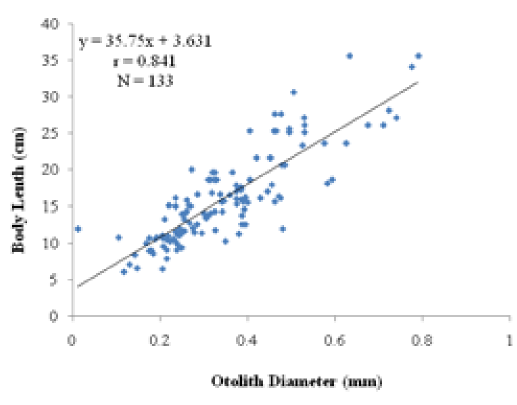

Figure 3: Relationship between Body Length (BL) and Otolith diameter (OD) in *O. mossambicus.*

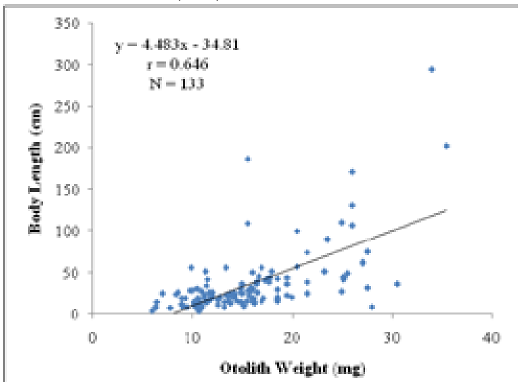

Figure 4: Relationship between Body Length (BL) and Otolith weight (OW) in *O. mossambicus.*

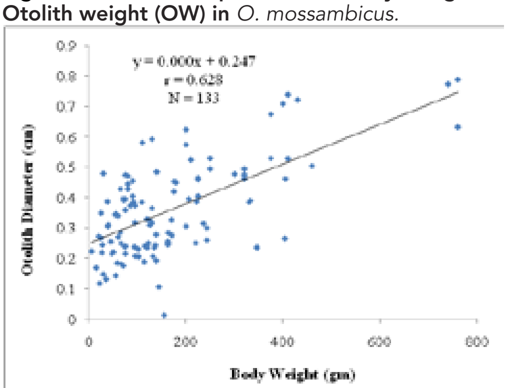

Figure 5: Relationship between Body Weight (BW) and Otolith diameter (OD) in *O. mossambicus.*

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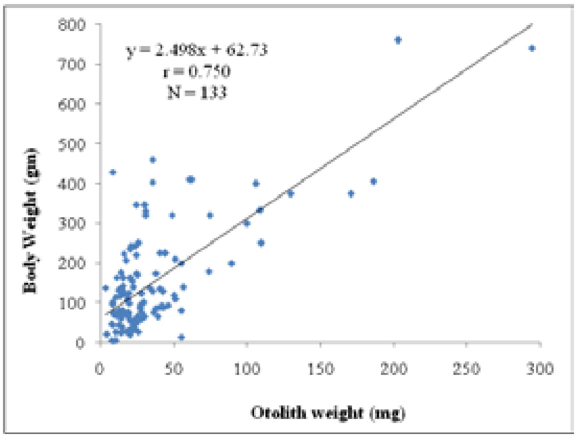
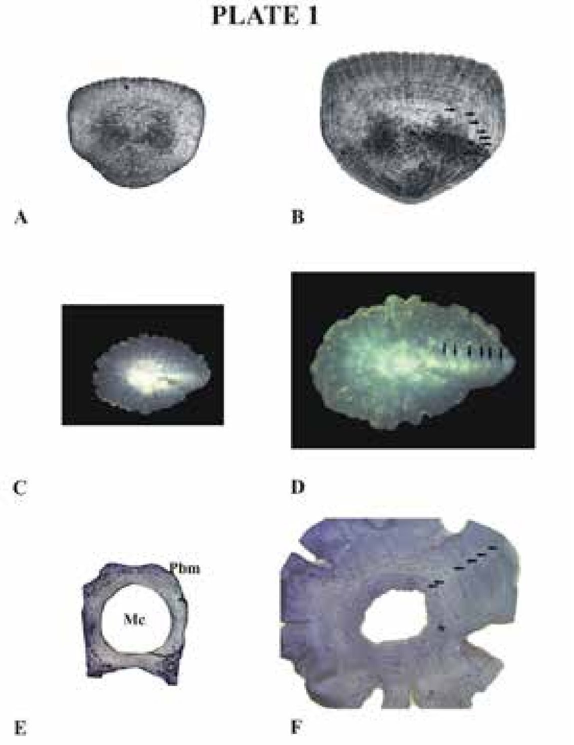


Figure 6: Relationship between Body Weight (BW) and Otolith weight (OW) in *O. mossambicus.*



A - Scale with no LAG, B - Scale with 6 LAGs
C - Otolith with no LAG, D - Otolith with 6 LAGs
E - Vertebra section with no LAG, F - Vertebra Section with 6 LAGs in *O. Mossambicus*
Mc - Marrow Cavity, Pbm - Periosteal Bone Margin
Arrow - LAG

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