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Original Article

Ageing Nile tilapia (*Oreochromis niloticus*): A comparative study between scales and otoliths

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Abstract: This study is the first to compare age estimates based on scales and otoliths of the Nile tilapia, *Oreochromis niloticus,* from Lake Nasser, Egypt. Ageing precision between readers was estimated by calculating the percent agreement between three independent readers, and the coefficient of variation (CV) ages estimated from otoliths and scales. The relation between the total length of fish and the radius of its scale and otolith was determined and appeared to be linear. The estimated age composition of the *O. niloticus* included six age groups estimated using scales and five age groups estimated by otoliths. The relative precision (CV and SD) of ages estimated from otolith was higher than that from scales. Higher percentages of agreement of overall annuli identification and age assignment between readers were noted in otoliths comparing to that in scales. Scale showed some inaccurate estimation in the age of fish older than 4. The precision and bias information in this study will be beneficial to fisheries professionals in assessing the age of Nile tilapia and other cichlids in the future.

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Introduction

Growth and age studies investigate the essential demographic characteristics to examine and assess the structure of fish populations (Maceina and Sammons, 2006). Estimating the age of fish is a common technique used to model its population dynamics and productivity by understanding fish longevity, growth and mortality rates i.e. an accurate understanding of these metrics needs precise age information (Campana, 2001). If a population that is or will come under considerable fishing pressure is to be studied, it is imperative that ageing should be done accurately as it is the most important parameters upon which recommendations can be based for the rational exploitation of a fish species.

Many structures have been used for age estimation of fishes, including scales, various fin rays, fin spines, vertebrae, opercle, and otoliths. Ageing of fishes from subtropical and tropical regions have been reported via annual increments in such calcified structures, for example, scales (Ilieş et al., 2014), dorsal and pectoral spines (Metcalf and Swearer, 2005), vertebral centra (Branstetter and Stiles, 1987; Bahuguna, 2013), opercular bones (Gómez-Márquez et al., 2008), and otoliths (Pilling et al., 2003; Fowler, 2009). A comparison of these various calcified structures has been performed in many species to obtain valuable information on the accuracy and bias of such age estimating structures (Kruse et al., 1993; Abecasis et al., 2008; Lozano et al., 2014). Studying the variation in age estimations using different calcified structures provide collateral evidence for the validity of the ageing method (Campana, 2001). Significant variation in age estimates based on different calcified structures indicates inaccurate ageing methodologies, imprecise ageing structures, or problems associated with interpretation (Muir et al., 2008). Balancing the accuracy and precision of the ageing method with sample size limitation is the main criteria to select the proper method for determination of age and growth in fishes (Zymonas and McMahon, 2009).

Tilapias are the main species produced in Egypt

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Table 1. Coefficient of variation (CV), percentage of agreement, and standard deviation of age group estimation by different readers based on scales and otoliths reading.

Age group	Scales			Otoliths		
	CV	Agreement	SD	CV	Agreement	SD
I	34.2%	68.7%	0.56	16.2%	88.5%	0.38
II	30.2%	58.4%	0.68	11.1%	86.1%	0.39
III	17.2%	58.5%	0.59	12.2%	80.4%	0.50
IV	16.1%	61.0%	0.67	5.8%	82.7%	0.37
V	10.2%	70.1%	0.62	0.0%	100.0%	0.00
VI	10.4%	71.3%	0.62	-	-	-
Total	24.8%	64.7%	0.62	11.6%	87.5%	0.33

and contributed about 31% of the total wild fish catch in 2017 (GAFRD, 2017). Nile tilapia (*Oreochromis niloticus*) is widely distributed in all fresh and brackish water bodies in Egypt (El-Sawy, 2006). While various aspects of the biology of *O. niloticus* and other commercially important tilapias have been thoroughly studied in Egypt (El-Sawy, 2006; El-Bokhty et al., 2013; Hassan and El-Kasheif, 2013; El-Kasheif et al., 2015; Hussian et al., 2019; Shalloof et al., 2020), there were no comparative studies to validate used *O. niloticus* ageing methods based on calcified structures. The present work aims to compare scales and otoliths as reliable calcified materials for estimating the age of *O. niloticus* from Lake Nasser by obtaining information on the accuracy and bias of such age estimating structures which can provide collateral evidence for the validity of these ageing methods.

Materials and Methods

Sampling and data collection: A total of 266 Nile tilapia were collected from Lake Nasser during 2018 from local fishermen. They were caught using trammel and gill nets which are the main commercial fishing gears used to catch this species in the lake. For each fish specimen, total length (TL) was measured to the nearest 0.1 cm and total body weight (W_t)) to the nearest 0.1 g. Fish samples were transported to the laboratory and stored at -20ºC.

In the laboratory, several scales (8–10) were removed from each fish sample from the area below the pectoral fin (Abecasis et al., 2008) and stored in individually labelled envelopes. Scales were then cleaned in dilute aqueous ammonia solution, rinsed, and dried. All scales showing signs of regeneration were eliminated. Cleaned scales were kept between two clean slides. Digital images of at least three scales for each fish sample were obtained using a camera (ABBOT DEC2000) mounted on a binocular stereomicroscope (Zeitess 530). The radius of the scale was measured on the digital image.

Both sagittas were recovered intact from each fish sample by exposing the otoliths capsules by applying an incision on the dorsal side of the head. Otoliths then washed in water and cleaned from all extraneous tissue, and dried, labeled, then stored in plastic vials. Digital images were obtained using a camera (ABBOT DEC2000) mounted on a binocular stereomicroscope (Zeitess 530) for each pair of otoliths, submerged in 50% glycerol and illuminated with oblique reflected light. The clearest image was chosen for interpretation. The radius of the otolith was measured on the digital image.

Age determination: Each scale and otolith were read twice by three different readers separately (no previous knowledge of count, or length of the sample). Each reader assigned each fish to an annuli class based on the number of opaque zones of the scale or the otolith (Murie and Parkyn, 2005). Aging precision between readers was estimated by calculating: (1) the percent agreement between three independent readers and (2) The coefficient of variation (CV) (Kimura and Lyons, 1991).

Results

Out of 266 examined fish, 136 were females (51.1%), and 130 males (48.9%). The total length (TL) of the samples ranged 13.5-48.0 cm with an average of 20.75±8.61 cm. The total weight (TW) varied 46.6-

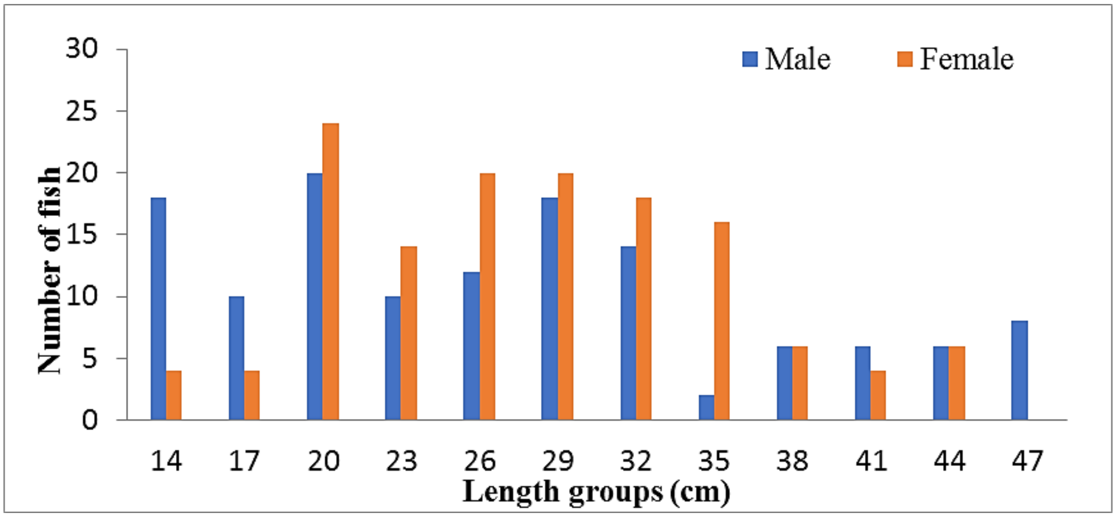

Figure 1. Length frequency of *Oreochromis niloticus* from Lake Nasser during 2018.

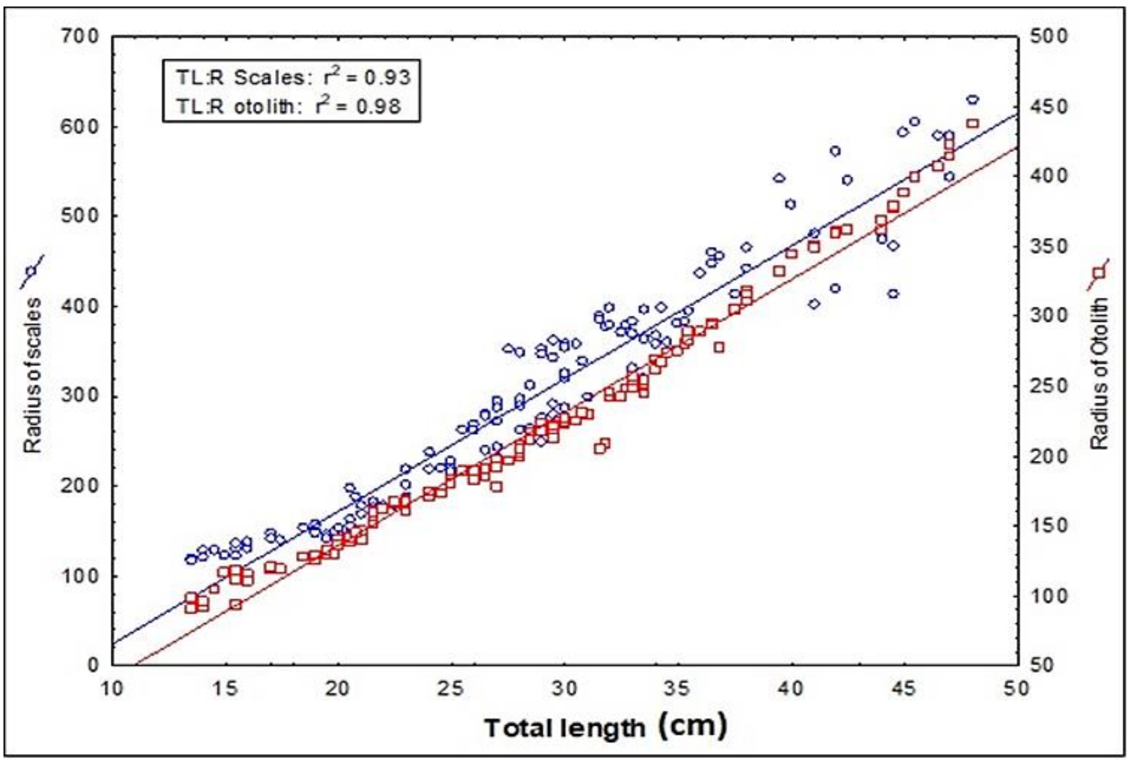

Figure 2. The relation between scales and otoliths radius and the total body length of *Oreochromis niloticus* from Lake Nasser during 2018.

2301.7 g with an average of 652.96±537.2 g. The total length of males ranged between 13.5 and 48.0 cm and their total weight between 46.6 and 2301.7 g while females TL ranged between 14.0 and 45.5 cm TW between 56.3 and 2055.3 g. The TL for females and males did not differ significantly (T-test t=0.66, $P<0.05$) (Fig. 1).

The relationship between total length and the radius of its scales and otolith was determined and appeared to be linear. The correlation coefficient of the linear relationship was lower in scales (r^2=0.93) than that of otolith (r^2= 0.98) (Fig. 2).

The otoliths and scales of *O. niloticus* showed annual rings, each ring composed of a faint broader growth zone and a dark opaque line (Fig. 3). The estimated age composition of the *O. niloticus* represented by the samples, including six age groups estimated using scales and five age groups estimated by otoliths (Table 1). The relative precision (CV and SD) of ages estimated from otolith was higher than

Table 2. Mean and standard deviation of total body weight, total body length, hard structure radii, and number and frequency (%) of fishes of *Oreochromis niloticus* assigned to each age group based on scales and otoliths reading.

Age	Scales					Otolith				
	Total weight (g)	**Total length (cm)**	**Radius (mm)**	**No**	**%**	**Total weight**	**Total length**	**Radius (mm)**	No	**%**
	(Min-Max) Mean±SD	(Min-Max) Mean±SD	(Min-Max) Mean±SD			(Min-Max) Mean±SD	(Min-Max) Mean±SD	(Min-Max) Mean±SD		
I	(46.6-336.0) 116.8±35.3	(13.5-20.0) 17.5±2.4	(0.116-0.157) 0.138±0.012	56	21.1	(46.6-336.0) 116.8±35.3	(13.5-20.0) 17.5±2.5	(0.091-0.139) 0.120±0.016	56	21.1
II	(157.4- 551.7) 332.5±118.3	(20.5-29.5) 25.2±3.1	(0.156-0.372) 0.242±0.059	100	37.6	(157.4- 639.0) 366.1±139.2*	(20.5-30.8) 25.9±3.3	(0.139-0.230) 0.188±0.027	116	43.6
III	(480.7-891.2) 680.9±59.4	(30.0-35.0) 32.1±1.6	(0.286-0.397) 0.357±0.038	54	20.3	(555.8-947.5) 749.5±95.4*	(31.0-36.0) 33.4±5.5*	(0.185-0.289) 0.253±0.037	46	17.3
IV	(803.0-1236.0) 995.8±135.7	(35.3-39.5) 36.9±3.2	(0.366-0.540) 0.436±0.045	24	9.02	(888.0-1904.0) 1240±406.2**	(36.5-42.5) 39.2±7.5*	(0.258-0.361) 0.326±0.031	28	10.5
V	(1082.2-2059.4) 1649.4±304.5	(40.0-45.0) 42.8±4.1	(0.401-0.592) 0.486±0.062	22	8.27	(1582.0-2301.7) 1985.7±223.7*	(44.0-48.0) 45.6±10.8*	(0.361- 0.437) 0.395±0.0.24	20	7.5
VI	(1939.5-2301.7) 2118.4±170.7	(45.5-48.0) 46.8±6.8	(0.543-0.630) 0.591±0.036	10	3.76	-	-	-	-	-

Significant compared with scales * $P<0.05$, ** $P<0.01$

that of scales (Fig. 4). A higher percentage of agreement of overall rings identification and age assignment between readers was noted in otoliths (87.5%) comparing to that of scales (64.7%) (Table 1). Calculating the agreement percentage between readers in the identification of each ring and assignment of each age group in both otolith and scales showed that higher percentages were recorded in otoliths reading reaching to 100% in the determination of age group V while the same group in scale had 71.1% agreement and the lowest percentage of agreement was recorded in the age group III with 84.4% in otoliths and 58.5% in scales (Table 1).

The relationship between the assigned age groups and the total body length was determined and appeared to be linear. The correlation coefficient of this linear relationship for of scales (r^2=0.91) was lower than that of otolith (r^2= 0.93) (Fig. 5).

The proportion of fish in each age group increased from the group I to group II, then it decreased gradually with increasing age groups where the second year of the age (age II) had the highest frequency percentage of 37.6 and 43.6% for age estimation by scales and otoliths, respectively (Fig. 6). Fifty-six samples were assigned to age group I using both scales and otoliths and they had mean TL of 17.5 cm while group II had some differences where the number of assigned samples were less when scales were used instead of otoliths (100 and 110, respectively) and the mean TL were 25.2 and 25.9, respectively, but these differences were not significant. Furthermore, significant differences ($P<0.05$) was noted in samples assigned to age group

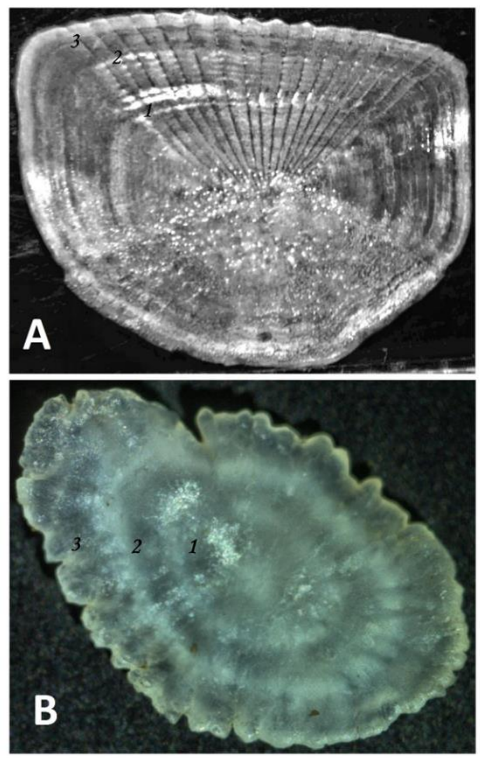

Figure 3. A scale (A) and an otolith (B) of *Oreochromis niloticus* with annual rings, each ring composed of a faint broader growth zone and a dark opaque line.

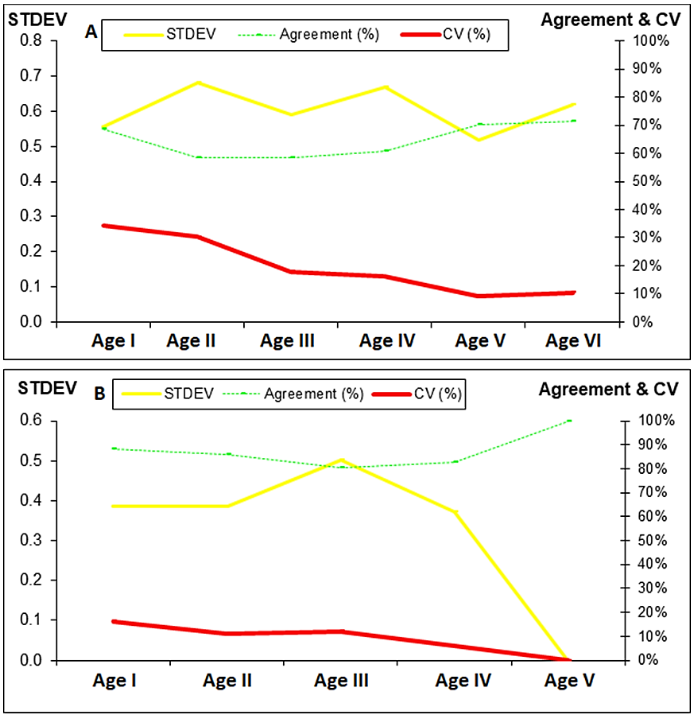

Figure 4. The coefficient of variation (CV%), percent agreement, and the standard deviation (STDEV) in age estimation by different readers based on scales (A) and otoliths (B).

III and higher (Table 2) where the frequency of samples and mean TL of each age group varied significantly between scale and otolith-based age estimation (Table 2). This variation is noted in the last assigned age group which was the group V in scales-based estimation while it was group VI in otolith-based estimation.

Discussions

In the present study, the otoliths and scales of *O. niloticus* showed rings, each ring composed of a faint broader growth zone and a dark opaque line. Many factors were reported to be correlated with ring formation in fish's hard structures, including changes in temperature, total dissolved solids, food quantity, and changes, associated with the cycle of wet-dry seasons (Karakiri and von Westernhagen, 1989; Lecomte et al., 1989; Gauldie et al., 1990; Yosef and Casselman, 1995; Admassu and Casselman, 2000). Indeed, seasonal variations are less intensive in the subtropics and tropics than in the temperate regions. However, regular climatic fluctuation occurs in many tropical freshwaters (Oppenheimer, 1989). This

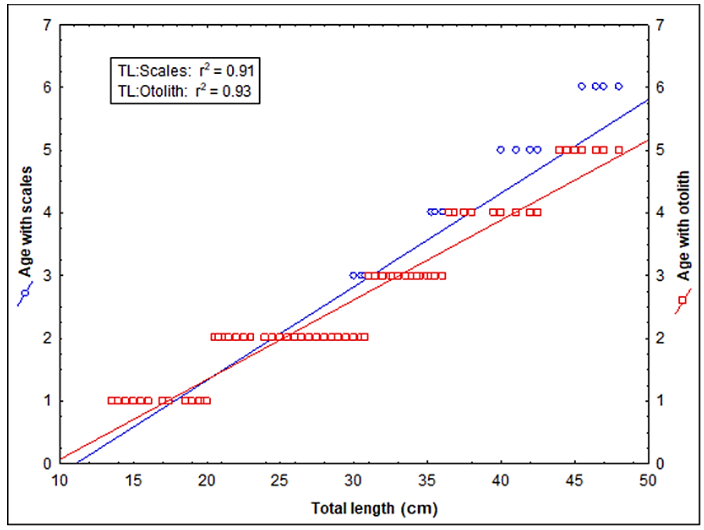

Figure 5. The relation between total body length and the assigned age group using scales and otoliths of *Oreochromis niloticus* from Lake Nasser during 2018.

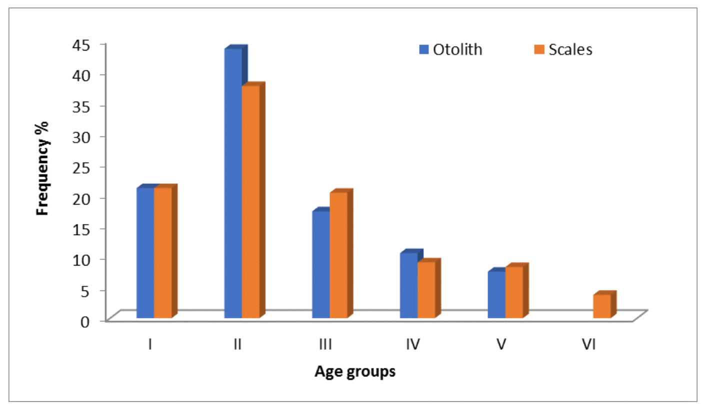

Figure 6. Age composition of *Oreochromis niloticus* from Lake Nasser based on scales and otolith reading.

fluctuation may follow either a uniannual or multiannual cycle, lead to the formation of one or more macrozones or checks in the calcified structures of the fish (Admassu and Casselman, 2000). In addition, fluctuation in body condition associated with spawning activity has also been considered an important factor especially in tropical and subtropical areas, where ring formation has often been attributed to the reproduction, particularly in cichlid fishes (Garrod, 1959; Pannella, 1974; Hecht, 1980).

The results of both scales and otoliths-based age estimation in the present study showed that the growth of *O. niloticus* in Lake Nasser followed the pattern shown by cichlids in other systems (El-Sawy, 2006; El-Bokhty et al., 2013; Hassan and El-Kasheif, 2013;

El-Kasheif et al., 2015). Growth during the first year is extremely rapid, reaching almost one third (17.5 cm) of its maximum length. The advantage of this growth pattern is the ability of juveniles to avoid the intense predation by rapidly attaining a size large enough (Hecht, 1980). After sexual maturation, the growth rate decreases with an asymptotic length being approached early on in life (Booth et al., 1995).

This study is the first to use two different tools simultaneously to assess the quality of ageing information of *O. niloticus*. All age estimation of this species in different water bodies in Egypt used scale method except Anonymous (1997) and Khalifa (2000) who used otoliths for ageing *O. niloticus* from Qarun and Wadi El-Raiyan Lakes. Generally, most of the earlier studies of fishes age and growth used scales for age estimation due to ease of collection, process, and avoids sacrificing the specimens. Emphasis on proper animal care in the fisheries profession would justify use of the scale in areas where other tools provide similar results, or even in areas where scales are slightly less precise (Kruse et al., 1993).

In this study, the comparison between age estimates using scales and otoliths showed that otoliths were more accurate for age determination of *O. niloticus*. It was found that otolith-based age estimates would be more precise than scale-based estimates as the average precision estimates for scales (CV=24.8%) is twice more than that of otoliths (11.6%). Our results are in accordance with the growing evidences that the scale as a tool of age estimation may be unreliable under certain growth conditions.

Many studies have suspected on scale ageing due to difficulties in reading annuli, low precision (Lowerre-Barbieri et al., 1994), and that scale ages may become inaccurate when growth becomes asymptotic. As scale growth is proportional to body growth, in older fish annuli become crowded on the scale edges making scale interpretation difficult. As a result, true age can be misestimated, especially in species that growth is concentrated in early life history like *O. niloticus* (Beamish and McFarlane, 1987; Shepherd, 1988). Moreover, several studies have reported that scales can provide unreliable estimates of fish age because of the lack of a distinct cold season, as annuli are formed during cold months when growth is slow, likely results in scale annuli that are indistinguishable for some fish populations in subtropical and tropical areas (Huish, 1954; Schramm and Doerzbacher, 1985). Several studies reported that otoliths are the most reliable ageing structure in several temperate as well as tropical fish species (Beamish, 1979; Brothers, 1979; Kalish, 1989; Cailliet et al., 2001; Phelps et al., 2007; Gunn et al., 2008; Fowler, 2009).

Age estimation of *O. niloticus* from tropical and subtropical lakes using calcified structures are more challenging when compared to temperate species, where, in many cases, the between-reader agreement is above 90% (Bwanika et al., 2007). Nevertheless, the ageing precision (CV=11.6 %, the agreement between readers=87.5%, and SD=0.33) for *O. niloticus* using otoliths in this study indicated comparable to that of the majority of studies ageing fish using otoliths (Campana, 2001). This precision estimate is reflected by 100% agreement obtained between readers for age 5 years. The ageing precision results for scales and otoliths in the present study were similar to those of different species (Hecht, 1980; Boxrucker, 1986; Welch et al., 1993; Booth et al., 1995; Abecasis et al., 2008) which reported that the percent of accurate agreement for scale readers is always less than that of otolith readers.

The age composition of the catch is used in age-structured stock assessments, which can be used to estimate exploitable biomass. The results of this study showed that the proportion of fish in each age group increased from the group I to group II, then it decreased gradually with increasing age groups. This pattern is following the classical dome shaped catch curve which is an indicator of two oppositely directed drives: the process of recruitment and the influence of mortality. The process of recruitment results in the formation of the left (rising from group I to group II) region on the catch curve. At a sufficiently mature age, the age group to which recruits belong almost completely transits into the harvested stock. Approximately at this age, the catch curve goes past

Table 3. Length at age (cm) of *Oreochromis niloticus* reported by different authors in Lake Nasser.

I	II	III	IV	V	VI	VII	Tool	Size parameter	Author
18.9	31.1	41.4	47.8	52.3	54.7	56.7	Scales	Total length	Talaat (1979)
19.4	27.0	32.6	37.2	39.0			Scales	Standard length- 1984/1985	Adam (2004)
6.9	20.3	29.8	36.5	41.3	44.7	47.2	Scales	Standard length	Latif and Khallaf (1987)
17.3	25.4	30.9	34.7	37.3	39.0	40.2	Scales	Total length -Males	Yamaguchi et al. (1990)
16.8	25.2	31.1	32.9	34.6	35.5	36.1	Scales	Total length -Females	Yamaguchi et al. (1990)
21.8	26.3	30.6	39.0				Scales	Total length	Agaypi (1992)
14.3	19.4	23.7	29.4	33.5	37.8		Scales	Total length	Mekkawy et al. (1994)
17.7	24.2	31.3	36.7				Scales	Standard length-1994/1995	Adam (2004)
17.3	25.8	32.2	37.7				Scales	Total length	Shenouda et al. (1995)
15.6	23.2	27.6	31.8				Scales	Standard length- 1999/2000	Adam (2004)
17.5	25.2	32.1	36.9	42.8	46.8		Scales	Total length	Present study
17.5	25.9	33.4	39.2	45.6			Otoliths	Total length	Present study

the maximum and starts to decrease monotonously (Sukhanov, 2016).

Age-length keys for *O. niloticus* developed in this study showed high variability between tools in assigning ages to fish >30 cm TL. Great variation in the length-at-age key of *O. niloticus* from Lake Nasser is noted in the previous longevity and age estimation studies as they all were solely based on scale (Table 3). This variation may have resulted from the inaccuracy of the estimation method and /or a combination of both extrinsic factors such as the variable abiotic conditions experienced by the fishes in different areas of the lake and intrinsic factors such as genetic variation between the individuals themselves. Much of the extrinsic intra-year variation in the growth of *Oreochromis* spp. was linked to flood intensity and duration (Booth et al., 1995).

In the present study, age frequency distributions of *O. niloticus* based on scales were different from those based on otoliths, nevertheless, scale ages can fulfil a manager's need for information about population age composition if the oldest age-groups (>4) are combined. However, the scale is not sufficiently precise to assess the ages of individuals in a population, and does not, for the most part, accurately recognize older *O. niloticus* in Lake Nasser.

Conclusion

This study is the first to compare age estimates based on scales and otoliths of the Nile tilapia. Otoliths are recommended for the best age estimates due to its higher precision of age estimates compared to scales. If nonlethal techniques for estimating age are required, scales can provide close age estimates for fish in comparison to otolith age estimates, but they may still inaccurately estimate the age of fish older than age 4. The precision and bias information in this study will be beneficial to fisheries professionals in assessing age of Nile tilapia and other cichlids in the future.

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