Validation of age estimation and back-calculation of fish length based on otolith microstructures in tilapias (Pisces, Cichlidae)

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Abstract—Two tilapia species, *Sarotherodon melanotheron* (brackishwater fish) and *Oreochromis niloticus* (freshwater fish), were marked with tetracycline and reared in Côte d'Ivoire (West Africa) in intensive (fish fed) and extensive (fish unfed) conditions. Juvenile and adult otoliths of the two species were examined. They were cut into transverse thin sections (10 to 40 µm), and otolith microincrements were counted on the sulcus along the ventral axis. Results for both species showed that microincrements are laid down daily. The number of days of growth reflects the number of microincrements (regression with slope not different from 1 and intercept not different from 0; $P>0.05$). This technique has a tendency to underestimate age ($P<0.05$): for *S. melanotheron*, the mean bias error is 4.4 d for juveniles (48 to 169 d of growth) and 8.1 d for adults (34 to 185 d of growth); for *O. niloticus*, the mean bias error is 0.9 d for juveniles (31 to 62 d of growth) and 5.1 d for adults (36 to 65 d of growth). Back-calculation of individual length at marking is very sensitive to an uncoupling between otolith and fish-specific growth rates. With the present data, back-calculated lengths overestimated actual size. When otolith and fish growth were coupled, length was back-calculated accurately.

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"Tilapia" is the common name for three genera of endemic cichlids from Africa, *Oreochromis*, *Sarotherodon*, and *Tilapia*. Species belonging to these three genera are widely distributed in tropical areas and have colonized all kinds of continental waters as natural or introduced species. Their adaptative capabilities have also been used in developing an aquaculture for these species worldwide (Lowe-McConnell, 1982; Wootton, 1984; Pullin et al., 1988). Both the reproduction and growth strategies in tilapias, however, differ among populations depending on environmental conditions (Kolding, 1993). Therefore, correct estimates of age is of great importance in assessing different growth strategies, as well as in characterizing different populations, by means of parameters such as size at first maturity. Despite the need for accurate age estimates, age at first maturity has not been the subject of many studies in tilapias and the question remains whether the observed differences in size at maturity are due to growth or to age differences (Eyeson, 1983; Legendre and Ecoutin, 1989; Duponchelle and Panfili, 1998). Moreover, tilapias can be sexually active a few months after hatching as shown in studies on tilapia reared in aquaculture (Eyeson, 1983).

Since Pannella's (1971) work, otolith microincrements have been widely used to estimate the age of fish in days, useful for studies on larvae and juvenile fish (Jones, 1992). Several authors have studied microincrements in tilapia otoliths, but their results have not been applied to field research. One possible reason is that there is not a standard and simple method available for choosing and preparing the otolith. Previous works have used different otoliths and preparation techniques: sagitta cut transversally and acid etched for *Tilapia guineensis* (Fagade, 1980), sagitta cut transversally and observed with scanning electron microscope for *Oreochromis niloticus* (Tanaka et al., 1981), lapillus observed whole in photonic microscopy for *Tilapia mariae* (Rosa and Ré, 1985), sagitta cut sagittally and observed in scanning electron microscopy for *Oreochromis aureus* (Karakiri and Hammer, 1989), and sagitta cut transversally, etched and stained for *Oreochromis niloticus* (Zhang and Runham, 1992). Each of these authors, except Fagade (1980), has validated the daily deposition of microincrements. Nevertheless, all fish used for validation were juveniles and were reared under controlled conditions in aquaria, far from their natural environment. To date, no attempt has been made to estimate growth for tilapia in natural waters nor age at maturity by using microincrements.

The first aim of our study was to assess whether examination of microincrements in otoliths yields valuable estimates of age (in days) for both juveniles and adults of *Sarotherodon melanotheron* (brackishwater species) and *Oreochromis niloticus* (freshwater species). To obtain results that could be applied to field studies, fish were reared

in an environment as similar as possible to natural conditions. The second aim was to develop a standard method for otolith preparation and examination to be used for both species. Both objectives are particularly useful in the case of *S. melanotheron* for which no validation has previously been published. We chose to carry out experiments in two aquaculture stations in Côte d'Ivoire (Africa), where the fish and the otoliths were simultaneously marked with external tags and tetracycline, respectively, to follow fish and otolith growth. Tetracycline labels have been used widely to mark calcified structures since the first assays (Weber and Rigway, 1967; Meunier, 1974), and we improved this universal marker for marking tilapia otoliths. During the validation process, special attention was paid to the accuracy of the age estimations (Campana and Jones, 1992). Geffen (1992) reported in her review on validation that there is a lack of analysis of the variation in increment number at a given age and we focused on that particular point. Our data on individual fish and otolith tagging were useful to test a growth back-calculation model commonly used for fishes (Francis, 1990, for review; Campana and Jones, 1992; Smedstad and Holm, 1996) and particularly to test the problem of uncoupling between somatic and otolith growth reported in previous studies (Mosegaard et al., 1988; Reznick et al., 1989; Secor and Dean, 1989). The final aim of our study was to obtain an accurate, precise, and simple tool for age estimation for future life history studies on tilapias.

Materials and methods

Rearing experiments

Fish were reared in two aquaculture stations in Côte d'Ivoire (West Africa), a country that experiences a transitional equatorial climate with two dry and two rainy seasons (Durand and Skubich, 1982; Durand and Guiral, 1994). Juveniles and adults of *Sarotherodon melanotheron* and *Oreochromis niloticus* were used in the experiments. Juvenile fish were obtained from synchronous layings, whereas adults, males and females, were caught in the natural environment. *Sarotherodon melanotheron* was reared at the Layo station (Centre de Recherches Océanologiques) located on the Ebrié lagoon. One-hundred and ninety-eight adults of *S. melanotheron* ranging between 90 and 130 mm FL (fork length) were marked and randomly assigned to three 4-m^3 cages (C1, C2, C3) immersed in the lagoon. Another forty four adults between 170 and 210 mm FL were marked and kept in a 25-m^3 cage (C4). After hatching, juveniles were transferred to a 3-m^3 concrete tank supplied with a constant flow of water from the lagoon. Fish were fed daily with formulated pellets. *Oreochromis niloticus* was reared at the Bouaké station (Institut des Savanes). One-hundred and fifty-two adults of *O. niloticus* were marked and released in two 400-m^2 ponds (A1 and A2) that had been previously enriched with organic matter (density of 0.2 fish/m^2). Juveniles were kept in two 50-m^2 ponds (J1 and J2) after hatching. No food was supplied; fish were sustained by natural resources.

Marking and sampling

Juveniles were not marked, therefore the "date of marking" actually refers to the date of birth. Adults caught in the field were marked by injecting tetracycline into the peritoneal cavity (50 mg/kg of live weight) and tagged with plastic T-bar anchor tags. Fish were measured (standard length at marking (SL_m, millimeters), weighed (grams), and sex was determined at the date of marking (Table 1). All *O. niloticus* adults and only *S. melanotheron* adults kept in cage C4 were tagged. After marking, both species were sampled in cages and ponds at monthly intervals. After capture, all individuals were measured (standard length at capture (SL_c, millimeters), weighed (grams), sexed and their otoliths (sagittae) removed. Randomly selected otoliths were prepared for analysis. Table 1 shows the dates of marking and sampling, otolith subsamples, and the number of days between marking and recapture for the two species.

Otolith preparation

Only the right otolith was prepared according to the technique described by Secor et al. (1992). After testing all possible planes of the section (i.e. sagittal, transverse, frontal), we chose the transverse section plane. Each otolith was then embedded in polyester resin before being sectioned transversally (with an Isomet saw) to avoid extra polishing and taking care to leave material on both sides of the core's plane. The resulting section was attached to a glass slide with thermoplastic glue (CrystalBond), ground with wet sand paper (grit ranging from 400 to 1200 per paper), and polished (polishing cloth with alumina paste ranging from 3 to 1/3 µm) on one side until reaching the primordium. The block was then turned over, affixed again to a slide with the polished face down, and ground and polished to remove extra material until the core area was reached. The thickness of the resulting sections ranged between 10 and 40 µm. Microincrement readability was improved by polishing the surfaces with 1/3 µm alumina paste. Otoliths with over-ground surfaces or with damage in the reading axis were discarded.

Otolith interpretation and variables measured

Terminology used in our study refers to that of Kalish et al. (1995). Microincrements were interpreted and counted as number of D-zones (reading) along the sulcus, chosen as the standard axis for reading (Fig. 1). Otoliths of adults were read under an epifluorescent microscope (Leica, 50W HBO lamp, 355–420 nm D filter) because the tetracycline deposit emits a yellow-green fluorescence under UVB at 390 nm. Microincrements were counted between the tetracycline mark and the otolith edge on a monitor coupled with a video with 1250× magnification. In juvenile otoliths, microincrements were counted from the primordium to the outer edge of the otolith under 400× and 1000× magnifications on the monitor coupled with video.

Each otolith was read twice by the same reader, first from the primordium to the edge and then back from the

Table 1

Number of otoliths for each sampling date and number of days between marking and sampling (in brackets) for *Sarotherodon melanotheron* and *Oreochromis niloticus* marked in Layo and Bouaké. [1] = adults tagged with anchor tags.

Species	Date of marking or birthdate	Date of sampling					
Sarotherodon melanotheron							
Adults		22 Nov 93	22 Dec 93	20 Jan 94	25 Feb 94	31 Mar 94	22 Apr 94
Cage C1	19 Nov 93	1 (34 d)	1 (64 d)	1 (93 d)	1 (129 d)	1 (163 d)	1 (185 d)
Cage C2	19 Nov 93	1 (34 d)	1 (64 d)	1 (93 d)	1 (129 d)	1 (163 d)	1 (185 d)
Cage C3	19 Nov 93	1 (34 d)	1 (64 d)	1 (93 d)	1 (129 d)	1 (163 d)	1 (185 d)
Cage C4[1]	19 Nov 93	4 (34 d)	4 (64 d)	4 (93 d)	4 (129 d)	4 (163 d)	4 (185 d)
Juveniles			22 Dec 93	20 Jan /94	26 Feb 94	31 Mar 94	22 Apr 94
Tank	4 Nov 93		5 (48 d)	5 (77 d)	5 (114 d)	5 (147 d)	5 (169 d)
Oreochromis niloticus							
Adults						3 Mar 94	1 Apr 94
Pond A1[1]	26 Jan 94					15 (36 d)	15 (65 d)
Pond A2[1]	26 Jan 94					15 (36 d)	15 (65 d)
Juveniles							
Pond J1	2 Feb 94					7 Mar 94	5 Apr 94
						15 (33 d)	16 (62 d)
Pond J2	14 Feb 94					17 Mar 94	15 Apr 94
						15 (31 d)	16 (60 d)

edge to the primordium following the same growth axis (Campana, 1992). If no significant difference was found between these two readings for the whole sample after a paired *t*-test, the mean was used to estimate the age (Campana and Jones, 1992). To maintain unbiased readings, physical data (size, date of capture) were not given to the reader.

Three variables were measured on each otolith: 1) the otolith diameter at capture (OD_c) which corresponds to the maximum length on the anteroposterior axis of the otolith before sectioning; 2) the otolith radius at capture (R_c) which corresponds to the distance between the primordium and the edge along the sulcus axis; and 3) the otolith radius at marking (R_m) which corresponds to the distance between the primordium and the tetracycline mark along the sulcus axis for the adults marked (Fig. 1). Variables were measured with TNPC image processing software (Visilog software platform, Noesis, France).

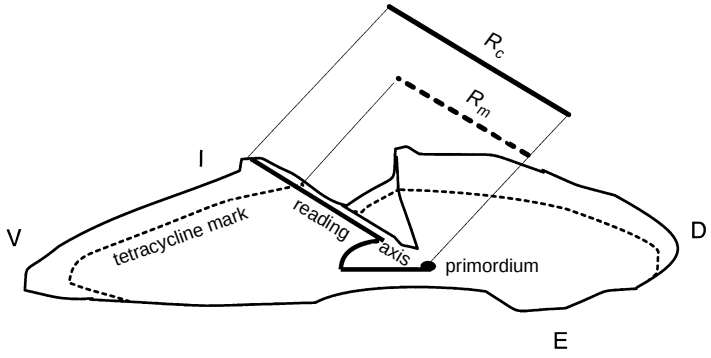

Figure 1

Diagram of the variables measured and of the reading axis used in reading thin otolith sections of tilapias. R_c = otolith radius at capture, R_m = otolith radius at marking (tetracycline mark), D = dorsal face, E = external face, I = internal face, V = ventral face.

Validation of the time of deposition of microincrements

The term validation refers here to the accuracy of the age estimation in tilapias by counting the number of microincrements in the otoliths (N_{inc}). The number of days involved in the validation experiment (D in days) refers to the microincrements deposited between hatching and the date of capture for juveniles and between the date of tetracycline labeling and the date of capture for adults. Pannella's hypothesis (1971) of a daily deposition rate for microincrements was tested for the two development stages of both species.

The accuracy of the method was tested by establishing the relation between the number of microincrements counted and the number of days involved in the experiment. A Student test on the linear regression was used to establish if the microincrements were deposited daily (slope equal to 1) and if the deposition started on the

first day (intercept equal to 0). The difference between the number of microincrements and the number of days of growth was plotted against time.

Validation of back-calculation for *Oreochromis niloticus*

Using tagged individuals, we assessed the validity of back-calculating fish length at marking. Several back-calculation models are described in the literature and each one assumes a different relationship between fish growth and otolith growth (Francis, 1990). Francis (1990) recommends Whitney and Carlander's model where a constant proportionality between fish growth and otolith growth throughout the life of the fish is assumed. Moreover, Francis (1990) recommends the regression of fish length against otolith length. Two approaches were considered in this study.

If the relationship between fish length and otolith length was linear,

$$SL_c = a + b \times R_c \quad (1)$$

with a body proportional hypothesis (BPH), then the back-calculation formula would be

$$SL_{mb} = \left(\frac{a + b \times R_m}{a + b \times R_c} \right) \times SL_c. \quad (2)$$

If the relationship between fish length and otolith length was nonlinear and for example multiplicative,

$$Log(SL_c) = c + d \times Log(R_c) \quad (3)$$

with a body proportional hypothesis (BPH), then the back-calculation formula would be

$$Log(SL_{mb}) = d \times Log\left(\frac{R_m}{R_c} \right) + Log(SL_c), \quad (4)$$

where SL_c = standard length at capture;
SL_{mb} = back-calculated standard length at marking;
R_c = otolith radius at capture;
R_m = otolith radius at marking (tetracycline mark); and
a, b, c, d = constants.

The validation of the back-calculation model was carried out on otoliths of adult *O. niloticus* labeled with tetracycline. For each individual, fish length measured at marking (SL_m, millimeters) was compared with the individual fish length back-calculated at marking from the otolith (SL_{mb}, millimeters). The mean of the differences between measured and back-calculated fish lengths was compared by means of a Student test with a theoretical mean equal to 0.

The specific growth rates of the fish length (G_{SL} in %/d) or of the otolith radius length (G_{OL} in %/d) were calculated following Ricker´s formula (1975):

$$G_{SL}(\%/d) = \frac{\log(SL_c) - \log(SL_m)}{D} \times 100 \quad (5)$$

$$G_{OL}(\%/d) = \frac{\log(R_c) - \log(R_m)}{D} \times 100 \quad (6)$$

where SL_c = standard length at capture;
SL_m = standard length observed at marking;
R_c = otolith radius at capture;
R_m = otolith radius at marking; and
D = number of days of growth.

The relationships between these specific growth rates and the differences between observed and back-calculated lengths at marking were established.

Results

Microincrement identification and tetracycline labeling

Preparing tilapia otoliths for examination is time-consuming; it takes sixty to ninety minutes to prepare one otolith. A final polishing with 1/3 µm alumina powder is an important improvement for microstructure reading when dealing with thin sections ranging between 10 and 40 µm of thickness. The central core of the otolith of both species seems to correspond to the fusion of several primordia (up to six) even though it remains a small structure easily recognizable during the grinding process. Two accessory growth centers are visible on any transverse section of juvenile otoliths between the 13th and the 28th microincrements. They are located on both sides of the core on the dorsal and ventral halves of the otolith, where they appear to control the growth of the otolith on the dorsoventral axis. Microstructures are more clearly identified along the sulcus or along the dorsoventral axis as typically alternated L-zones and D-zones. Cross-checking and narrower microstructures are more common on the dorsoventral axis than in the sulcus region. As a result, we chose to interpret the microincrements along the sulcus axis (Fig. 1). Microincrements were counted from the hatching check which was clearly identifiable in the core area. For at least the first 30 microincrements the otolith grows predominantly along the dorsoventral axis and towards the external face. To avoid underestimating the number of microstructures during this first growth stage, the first 15 to 20 microincrements were counted along the core-ventral axis. Other microstructures along the ventral axis, interpreted as subdaily increments, rapidly increased in number and made the reading difficult. The shift to the sulcus axis was completed by following any conspicuous microincrement along the core-ventral axis to the sulcus region where the reading was finished (Fig. 1), provided accessory growth centers had not been encountered in this area.

The tetracycline mark was present in all otoliths examined, except one otolith of *S. melanotheron* sampled in April 1994. The mark was more intense on *O. niloticus*

Table 2

Results of microincrement readings for adults and juveniles *Sarotherodon melanotheron*. D = number of days between marking (adults) or birth (juveniles) and capture; SD = standard deviation; CI (95%) = confidence interval for mean at 95%; CV = coefficient of variation.

		Number of microincrements				
	D (d)	*n*	Mean	SD	CV (%)	CI (95%)
Adults	34	6	28.3	2.8	10.4	25.4–31.3
	64	6	56.1	8.9	16.6	46.7–65.5
	93	3	88.5	10.0	12.2	63.7–113.3
	129	7	119.4	7.7	6.7	112.3–126.2
	163	4	150.7	23.5	16.5	113.4–188.1
	185	3	176.7	11.0	6.7	149.4–203.9
Juveniles	48	4	48.6	2.3	5.0	45.0–52.3
	77	4	76.9	2.2	3.0	73.3–80.4
	114	5	109.9	2.1	2.0	107.3–112.5
	147	4	132.9	17.1	13.7	105.6–160.1
	169	5	164.5	6.1	3.9	156.9–172.1

Table 3

Linear regressions between the number of microincrements (N_{inc}) and the number of days between marking or birth and capture (D), $D = a + b \times N_{inc}$, and Student tests for slope (b=1) and intercept (a=0) for *Sarotherodon melanotheron*. F = result of model ANOVA; ns = no significant difference (P>0.05); r^2 = coefficient of determination.

				Intercept			Slope		
Group	*n*	*F*	r^2 (%)	*a*	*t*	a = 0	*b*	*t*	*b* = 1
Juveniles	22	524.7	96.3	4.348	0.88	ns	0.923	–1.91	ns
Adults	29	658.4	96.1	–4.844	–1.11	ns	0.968	–0.85	ns
Juveniles and adults	51	1176.3	96.0	–1.899	–0.57	ns	0.957	–1.54	ns

otoliths than *S. melanotheron* even though the quantities injected in both species were identical (50 mg per kg of live weight). In all cases the tetracycline deposit coincided with a check in the otolith structure, confirming that the marking is synonymous with stress to the fish. Other checks present along the sulcal axis interrupted the microstructure deposition pattern without any regularity. The growth of the otoliths in the region between the tetracycline deposit and the edge (i.e. between marking and capture) was always smaller for *S. melanotheron* than for *O. niloticus* for comparable sizes regardless of the date of capture.

Validation of microincrement deposition in otoliths of *Sarotherodon melanotheron*

Microincrement interpretation on adult *S. melanotheron* otoliths is difficult and requires a minimum magnification of 1000× for the microincrements between the tetracycline mark and the outer edge. Results of otolith reading for adults and juveniles are summarized in Table 2. The mean of the two readings was used as the value of the microincrement count of each otolith because the difference between the two readings was not significant in the whole sample (paired *t*-test, t=0.02, P>0.05). Results showed a tendency to underestimate true age, even though this underestimation was less pronounced in juvenile fish than in adults (Table 2). The mean underestimation was 8.1 d for adults and 4.4 d for juveniles; both means were significantly different from 0 (respectively t=4.19 and t=2.36, P<0.05). The number of increments were plotted against the days of growth. An ANOVA conducted with the resulting linear models (Table 3) with adults or juveniles, or both, showed a coefficient of determination (r^2) significantly different from 0 (P<0.001). The slopes were not different from 1 and the intercepts were not different from 0 (Table 3). Thus the microincrements counted on the transverse section of *S. melanotheron* otoliths can be considered structures that are deposited daily. The technique appears to accurately estimate the age of *S. melanotheron* in days.

The dispersal of residuals in Figure 2 was more important in large individuals. Underestimation slightly in-

Table 4

Results of microincrement readings for adults and juveniles *Oreochromis niloticus*. D = number of days between marking (adults) or birth (juveniles) and capture; SD = standard deviation; CI (95%) = confidence interval for mean at 95%; CV = coefficient of variation.

		Number of microincrements				
	D (d)	*n*	Mean	SD	CV (%)	CI (95%)
Adults	36	25	31.1	2.5	8.2	30.0–32.1
	65	27	59.7	2.6	4.4	58.7–60.8
Juveniles	31	12	31.9	2.5	8.2	30.2–32.1
	33	9	30.5	1.3	4.5	29.5–31.5
	60	9	59.4	3.7	6.4	56.6–62.3
	62	13	60.4	2.3	3.9	59.0–61.8

creased with the duration of the experiment although the coefficient of variation did not increase with time (Table 2). Variability in precision was nevertheless higher for adults than for juveniles. Figure 2 also shows that the difference between the number of increments and the number of growth days was equal to zero for only a few individuals.

Validation of microincrement deposition in otoliths of *Oreochromis niloticus*

The distinction of microincrements was much greater in *O. niloticus* than in *S. melanotheron* otoliths resulting in an easier interpretation of microincrement deposition in *O. niloticus*. Results of microincrement counts for adults and juveniles of *O. niloticus* are summarized in Table 4. As for *S. melanotheron*, the two readings of each otolith were not significantly different across the whole sample; thus the mean of the two counts was used as the otolith microincrement value (paired *t*-test, t=0.85, P>0.05). Microincrement values in otoliths of adults after 36 and 65 d underestimated the true age of the fish by 4.9 and 5.2 d, respectively. Both values significantly differed from 0 (respectively t=9.78 and t=10.44, P<0.05) but were not significantly different from each other (P>0.05). Therefore the deviation between the true age and the estimated age was similar between 36 and 65 d and approximately equal to five days.

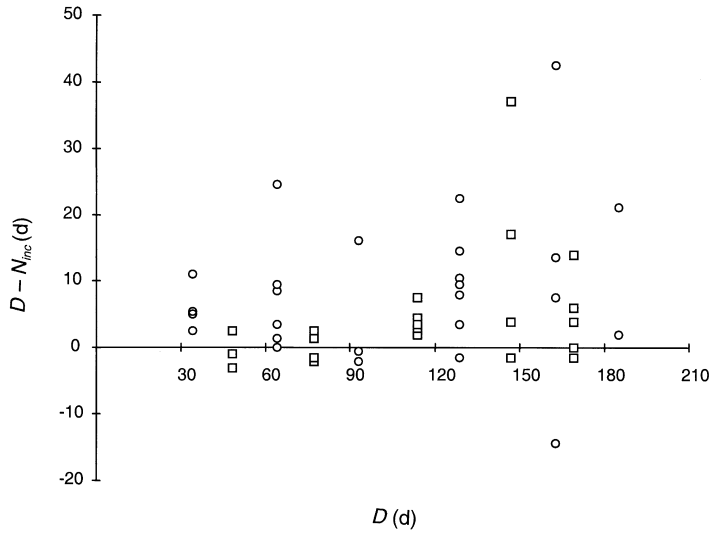

Figure 2

Individual differences between the number of days of growth (D) and the number of microincrements counted on transverse otolith section (N_{inc}) for adults (○) and juveniles (□) *Sarotherodon melanotheron*.

In otoliths of juveniles, the mean of the differences between the number of days and microincrements counted did not differ significantly from 0 in ponds J1 (0.9 d) and J2 (0.6 d) (t=1.18 and t=0.45 [P>0.05], respectively). For juveniles reared in pond J1, age was underestimated after 33 and 62 d of growth (Table 4), whereas ages of juveniles in pond J2 (31 and 60 d of growth) were accurately estimated (Table 4). A one-level nested ANOVA was carried out to check whether the pond or the age, or both, had had an effect in the difference between the true age and the estimated age. Results showed a significant effect of the pond ($F_{(1,39)}$=7.57, P<0.05) but not of age ($F_{(2,39)}$=1.13, P>0.05). Furthermore, a multiple rank test showed that no significant differences existed between the means at 31, 60, and 62 d and between the means at 33, 60, and 62 d (P>0.05), but that a significant difference existed between 31 and 33 d (P<0.05). Thus an effect of the pond on otolith deposition could not be confirmed.

The relation between the number of microincrements and the number of days before capture was established to test the accuracy of the age estimation (Table 5). The resulting r^2 was significantly different from 0 for adults or juveniles, or for both (P<0.001). The slopes of the model were not different from 1 (Table 5, P>0.05), which shows that the deposition rate of microincrements is daily. The num-

Table 5

Linear regressions between the number of microincrements (N_{inc}) and the number of days between marking or birth and capture (D), $D = a + b \times N_{inc}$ and Student tests for slope (b=1) and intercept (a=0) for *Oreochromis niloticus*. F = result of model ANOVA; ns = no significant difference (P>0.05); r^2 = coefficient of determination; s = significant difference (P<0.05).

				Intercept			Slope		
Group	n	F	r^2 (%)	a	t	a = 0	b	t	b = 1
Juveniles	43	1122.6	96.5	0.293	0.21	ns	0.975	–0.85	ns
Adults	52	1264.9	97.0	–4.522	–3.47	s	0.989	–0.45	ns
Juveniles and adults	95	1696.5	94.8	–1.347	–1.12	ns	0.963	–1.59	ns

ber of days of growth explains the number of microincrements counted. Nevertheless, the intercept differed from 0 for adults (P<0.05). It therefore seems that the deposition of new increments did not start on the first day after marking and that this difference (5 d) remained constant for one to two months of growth (Tables 4 and 5), suggesting that the increment technique is accurate for estimating the age of *O. niloticus* in days.

Residual dispersal was similar for adults and juveniles and seems constant through time (coefficient of variation in Table 4 and Fig. 3). Age was especially overestimated in juvenile fish (Fig. 3). As for *S. melanotheron*, a difference between the number of increments and the number of growth days equal to zero was very rare with *O. niloticus* otoliths. The trend in the deviation of the age estimation was the same over time for a given pond (Fig. 3).

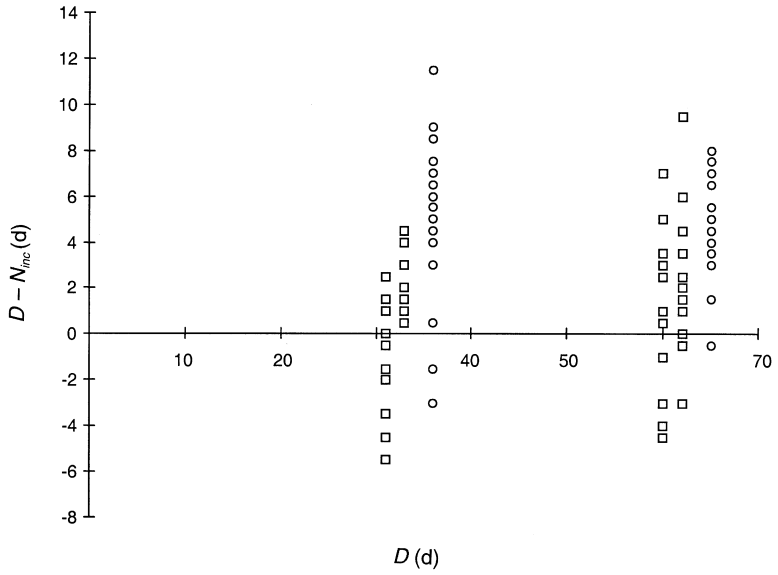

Figure 3

Individual differences between the number of days of growth (D) and the number of microincrements counted on transverse otolith section (N_{inc}) for adults (○) and juveniles (□) *Oreochromis niloticus*.

Validation of back-calculation of length in *Oreochromis niloticus*

The relation between fish length and otolith length was determined by establishing the regression of fish standard length at capture on the otolith radius at capture (Table 6). Both the linear and the multiplicative models were tested by an ANOVA (F calculated, Table 6) and had highly significant relationships (P<0.001). A comparison of the variances suggested that the coefficient of determination in the multiplicative model was significantly higher than that in the linear model (P<0.05). As a result, the regression used for the back-calculation of fish length was the multiplicative form. The observed dispersion of residuals reinforced this choice.

The observed low value of r^2 for the linear regression was due to the importance of the dispersion of points around the model for adults (Fig. 4). Nevertheless, this dispersion was also observed in the relation between fish length and otolith diameter at capture (Fig. 4), prior to any otolith preparation. In this case the linear regression had a higher coefficient of determination (Table 6). The dispersion can be explained by the natural growth variation which appears with age, and which is strong for this species.

The back-calculated formula used to compare the observed length at marking and the back-calculated length with otolith transverse sections was therefore

$$Log(SL_{mb}) = 0.899278 \times Log\left(\frac{R_m}{R_c}\right) + Log(SL_c). \qquad (7)$$

Back-calculated lengths at marking were overestimated in the whole sample and this tendency did not depend on fish size at marking (Fig. 5). The mean of the differences

(mean=8.5 mm, standard deviation=8.7 mm) was significantly different from 0 (t=6.94, P<0.05). As a result, back-calculation of fish length overestimated the length of fish that had grown for one or two months.

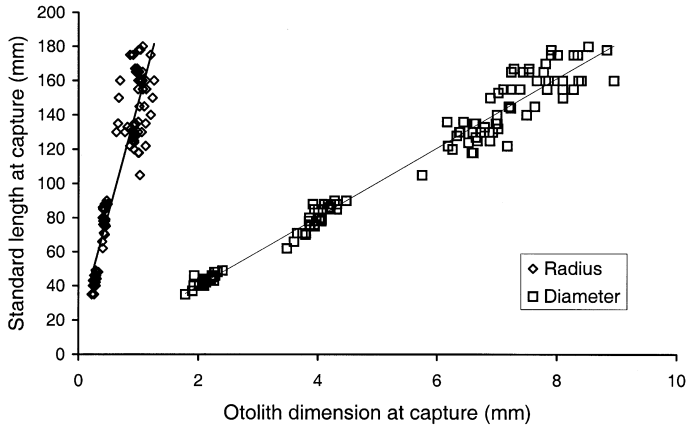

Figure 4

Relationship between standard length and otolith dimension (radius or diameter) observed at capture for *Oreochromis niloticus*. The lines show the linear regression models.

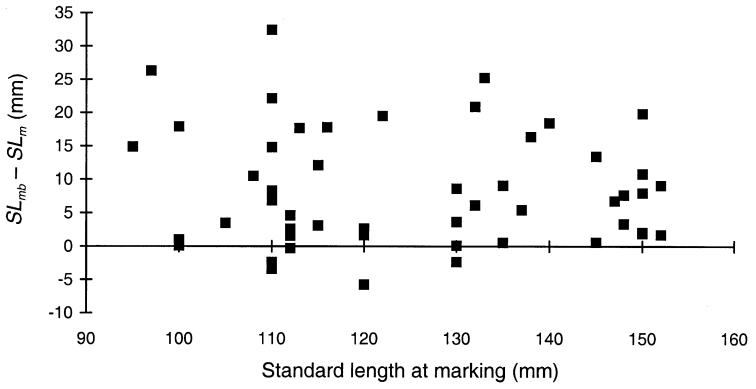

Figure 5

Differences between standard length back-calculated at marking (SL_{mb}) and standard length observed at marking (SL_m) for each *Oreochromis niloticus* (36 and 65 days after marking).

The relation between the specific growth rate of the fish (G_{SL}) and the difference between back-calculated and observed length at marking was positive because overestimation increases with the specific growth rate, regardless of the duration of the experiment (Fig. 6). The actual under- or overestimation of back-calculated length seems to depend on the coupling between the specific growth rates of the otolith and the fish (Fig. 7). An uncoupling between somatic and otolith growth rates would explain the deviation of the back-calculated length from the measured value (Fig. 7). That is to say, if the specific growth rates of the fish and the otolith are identical, the back-calculation gives a very good approximation of the length at marking (i.e. the difference is almost equal to zero), whereas if the otolith growth rate is higher than the fish growth rate, then the back-calculation underestimates the length at marking. Inversely if the somatic growth rate is higher than the otolith growth rate, the back-calculation will overestimate the length at marking. Moreover, when the uncoupling between otolith and somatic growth rates rises, the overestimation of the back-calculation increases (Fig. 7).

Discussion

Otolith preparation and interpretation of microincrements in tilapias

Transversal sections (in contrast to sagittal or frontal sections) of otoliths are the clearest and most reliable way to interpret microincrements in tilapia otoliths. Although some authors have worked on sagittal sections (Fagade, 1980; Karakiri and Hammer, 1989) the concavoconvex shape of the otoliths in adults makes it very difficult to obtain a plane that would include both the core area and the otolith edge. Zhang and Runham (1992), who prepared transverse sections of adult *O. niloticus* otoliths with their histological technique (Zhang et al., 1991), obtained sections with clear microincrements. Rosa and Ré (1985) reported that sagittae in

Table 6

Relations between SL_c (standard length at capture) and R_c (otolith radius at capture) or OD_c (otolith diameter at capture) for *Oreochromis niloticus*. F is calculated from the ANOVA for testing the model. r^2 = coefficient of determination.

Model	n	Regression	F	r^2 (%)
Linear	108	$SL_c = 17.13 + 130.31 \times R_c$	612.5	85.1
Multiplicative	108	$Log(SL_c) = 5.02 + 0.899 \times Log(R_c)$	1117.3	91.3
Linear	119	$SL_c = -0.609 + 20.33 \times OD_c$	4163.8	97.2

juvenile *Tilapia mariae* were too thick to allow observation of the microstructures without previous preparation and decided to work on lapilli. Despite the long and time-consuming process (about 1 h for preparing each transverse section of a sagitta), it appears to be the best way to observe otolith microincrements for both juvenile and adult tilapia. To avoid parallax when observing microstructures on thick preparations (Campana, 1992; Neilson, 1992), a thickness of 10 to 40 µm and a fine polishing of the sections are necessary.

Zhang and Runham (1992) observed microincrements of juvenile *O. niloticus* otoliths under a maximum magnification 400× with light microscopy, whereas Rosa and Ré (1985) used magnifications ranging from 600× to 1250× while working on *Tilapia mariae* lapilli. Our results show that microincrement observation and interpretation require a minimum magnification of 1000× for adult tilapias. These high magnifications with compound microscopes are required to observe microincrements in the sulcus area where they are found to have such a compressed arrangement that no space is left for subdaily increments (Zhang and Runham, 1992). Therefore, to interpret microincrements in tilapia otoliths accurately, we strongly recommend preparing thin transverse sections (10–40 µm), polishing them finely ($^1/_3$ mm), and observing them under high magnifications (minimum of 1000×).

In interpreting microstructures in tilapia otoliths, four types of problems were encountered: 1) difficulty in interpreting microstructures in the otolith region that correspond to first-growth stages; 2) difficulty in having to switch the reading axis (starting in the dorsoventral area and finishing along the sulcus area); 3) difficulty in reading some zones; and 4) difficulty in identifying microstructures near the outer edge of the otolith. Microincrements around the hatching check are very faint and narrow and require high magnifications to be identified. Narrow increments were also reported on *Tilapia mariae* lapilli by Rosa and Ré (1985). The presence of accessory growth centers on both sides of the core area in the dorsoventral plane of the otolith were also observed by Karakiri and Hammer (1989) and Zhang and Runham (1992) on *O. niloticus* otoliths. These authors estimated the date of formation of these accessory growth centers to be between 21 and 30 days and between 16 and 28 days after hatching, respectively. Secondary growth centers in Ivorian tilapia otoliths were located between the 13th and the 28th microincrements; therefore our results agree with observations made by these authors. It is likely that the presence of accessory growth centers represents a shift in the growth of the otolith, meaning that growth along the dorsoventral axis is favored at this stage.

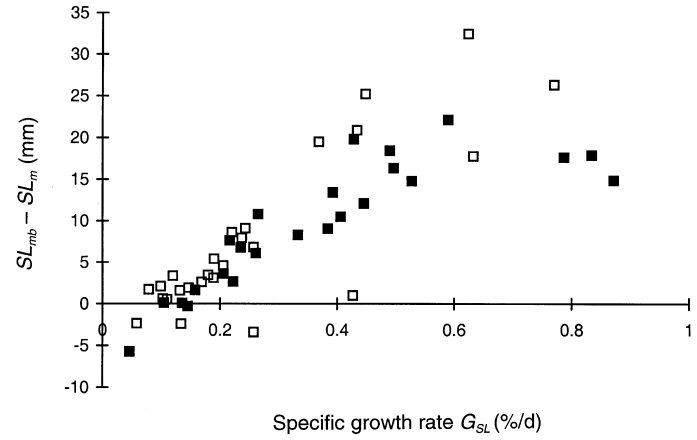

Figure 6

Relation between the difference of standard length back-calculated at marking (SL_{mb}) and standard length observed at marking (SL_m) versus the specific growth rate of standard length (G_{SL}) for *Oreochromis niloticus*, 36 days (■) and 65 days (□) after marking.

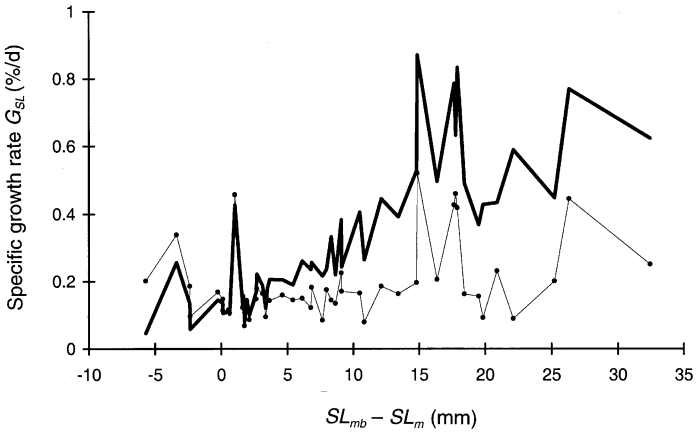

Figure 7

Relations between the specific growth rate (otolith length [thin curve] or standard length [bold curve]) and the individual difference of the standard length back-calculated at marking (SL_{mb}) and the standard length observed at marking (SL_m) for *Oreochromis niloticus*.

The use of the dorsoventral axis to read microincrements may induce reader error. Ambiguities arise because of the numerous subdaily structures deposited during the fast growing period (Zhang and Runham, 1992); thus reading along the sulcus region is recommended. However, as the growth of the otolith along the sulcus is relatively indistinct during the first 15 to 20 microincrements, proper information can only be gathered along the core-ventral axis. Certain regions in otoliths, especially near the edge, have been difficult to read for numerous other species (Campana, 1992). Tilapia otoliths also exhibit unreadable

parts as reported and observed by Zhang and Runham (1992). Otoliths of both species are difficult to interpret and attention should be paid to the need for trained readers capable of properly interpreting microincrements in tilapia otoliths.

Validation of microincrement deposition in tilapias

Tetracycline remains a universal marker for otoliths (Beamish and McFarlane, 1987; Brothers, 1990; Geffen, 1992). In our study only one *S. melanotheron* did not reveal any tetracycline deposit on its otolith. Because the fish is necessarily handled, injecting tetracycline induces a strong calciotraumatic effect on the otolith (Meunier and Boivin, 1978; Pannella, 1980; Campana and Neilson, 1985; Panfili and Ximénès, 1992). As a result, the mark is easily recognizable in the otolith and corresponds to a check in the otolith structure. This check probably reflects a cessation in the growth of the otolith that could last several days. In our study, underestimation of age was constant (equal to 5 days) for adult *O. niloticus* which indicates that the growth of the otolith was stopped with the tetracycline injection and resumed five days later. When using microincrement counts, the effect of marking should be considered, but it is difficult to estimate the time elapsed between injection and resumed growth of the otolith.

We estimated the age of juvenile and adult *O. niloticus* and *S. melanotheron* accurately using the same microincrement examination and interpretation on thin otolith transverse preparations. Tanaka et al. (1981) and Zhang and Runham (1992) had similar results on transverse sections of juvenile *O. niloticus* otoliths. The former observed otoliths with scanning electron microscopy and the latter observed stained otoliths. Karakiri and Hammer (1989) also reported daily increment deposition on sagittal sections of *Oreochromis aureus* otoliths observed with scanning electron microscopy. Our technique of preparation is the only one that has been validated for two different species and for several developmental stages.

Precision in age estimation with otolith microincrements was calculated for both species and showed that the error ranges between 4.4 d in juveniles and 8.1 d in adults for *S. melanotheron*, and between 0.9 d in juveniles and 5.1 d in adults for *O. niloticus*.

Validation of back-calculation and influence of individual growth rates

We validated the back-calculation model with a body proportional hypothesis (BPH) developed by Whitney and Carlander (1956, in Francis, 1990) and commonly recommended and used in the literature (Francis, 1990; Smedstad and Holm, 1996; Horppila and Nyberg, 1999). Back-calculation models rely on the assumption that otolith size and fish size are related and that a relation between them can be established. It is assumed that 1) the frequency of formation of each structure is constant and 2) the width of each increment is proportional to the growth of the fish (Campana and Jones, 1992). Caution should be taken when calculating the relationship because, as stated by Francis (1990) and Campana and Jones (1992), if the aim is to backcalculate a mean fish length from any otolith dimension, the resulting regression must have fish length as a dependent variable and otolith dimensions as independent variables. It is therefore very important to set up the most suitable relationship relating fish length and otolith length. Some works have shown that many factors influence this relationship. Wright et al. (1990) reported that the relation of fish length to otolith length was linear for smolts belonging to the high mode (fast growth) and curvilinear for smolts belonging to the low mode (slow growth). The relationship is also affected by food supply (Rice et al., 1985) or seasonal changes (Thomas, 1983). Furthermore, Reznick et al. (1989) showed that slow growing guppies (*Poecilia reticulata*) have larger otoliths than fast growing guppies of similar lengths, even though both groups of fish shared the same genetic background, had the same feeding schedule, and were reared under the same conditions. In this context, the use of the model of Whitney and Carlander is particularly suitable because it assumes that if a fish is 10% smaller than the mean length of the population for a given otolith size, this deviation will be constant throughout the life of the fish. We chose the curvilinear model for the relationship between fish length and otolith length because it fitted the existing data better. Bradford and Geen (1987) also found no significant difference between the curvilinear model and the linear model and therefore used the former because it adjusted total data better. Smedstad and Holm (1996) compared several back-calculation formulae for cod otoliths and concluded that the nonlinear one was better. These relationships seem to depend on the axis of the otolith chosen for back-calculation. Back-calculation results could have been less variable if the diameter of the whole otolith had been used in the relationship with fish length instead of the radius on the transverse otolith section (Fig. 4; Table 6). Unfortunately, because it was impossible to interpret the microincrements along the anteroposterior axis (diameter), that axis was discarded for back-calculation. A prerequisite of back-calculation is the assumption that the frequency of formation of microincrements is constant along the axis of analysis (Campana and Jones, 1992) and that assumption could not be made in the anteroposterior axis otoliths of our study.

Our study shows that back-calculated fish lengths are greater than measured fish lengths at marking among fish that have grown between one and two months. These results agree with those obtained by Rijnsdorp and Visser[1] on plaice (*Pleuronectes platessa*) grown for 19 months, although the back-calculation model used by these authors was the Dahl-Lea model (1920, in Francis, 1990). Two main points can be related to the observed overestimation. First, it is related to fish growth: the larger the fish growth, the larger the overestimation, although previous authors found an inverse relationship. This overestima-

[1] Rijnsdorp, A. D., and T. A. M. Visser. 1987. Tetracycline labelling of otoliths in plaice. ICES, C.M. 1987/G:33, 12 p.

tion may be result of back-calculation model being different or the marking-recapture interval being longer. Second, rather than fish growth rates alone, it appears that the coupling between fish growth and otolith growth plays a major role in explaining the observed overestimation of fish length. As shown in our study, underestimation of back-calculated fish length corresponds more to otolith growth rates compared with fish growth rates, and vice versa. These aspects must be discussed in light of the relationship between fish size and otolith size, the influence of fish growth on the overestimation of fish length, and the evidence of uncoupling between fish and otolith growth rates (Mosegaard, et al., 1988; Reznick, et al., 1989; Secor and Dean, 1989). Figure 6 shows that the higher the fish growth rates, the higher the deviation of the back-calculated fish length, implying that the faster a fish grows the further the fish is from the model. Secor and Dean (1989) considered that the ratio of otolith size to fish size increases in starved fish, as well as in fish with slow growth rates. Thomas (1983) considered this relationship to correct the underestimation resulting from using the Lee back-calculation model. Because fish growth rates have an influence on the observed estimations, they raise the question of what was the influence of fish growth rates on otolith growth rates along the sulcus axis in our study? Geffen (1992) proposed establishing the relationship between fish and otolith growth rates prior to back-calculation. Unfortunately this method is difficult to achieve in the field because it would mean marking fish from any studied populations, which unfortunately rarely happens.

Finally, Bradford and Geen (1987) advised caution when back-calculating fish length because otolith growth seems to be more conservative than fish growth. In our study, this assumption takes force because tilapias used in the experiments were starved before the beginning of the rearing experiments and experienced high growth rates after placement in ponds. Otolith growth rates followed fish growth rates within a certain range. When fish growth decreased below a certain limit, the otolith continued to grow. When fish growth increased, otolith growth also increased to a certain extent. This finding confirmed that the rate of growth in otoliths is conservative compared with the rate of somatic growth. Furthermore, the otolith represents an essential part of the equilibrium and sensory system of fish and thus cannot follow only fish growth rates. As a result, high growth rates in fish will imply a bigger dispersion of the data, or heterocedasticity around the relation of fish length to otolith length, which is observed here for larger individuals. The uncoupling of fish and otolith growth thus explains the difference between back-calculated and measured fish lengths at marking. Therefore caution should be taken when establishing the relationship of fish length to otolith length by using a representative sample of the individuals in their natural environment. In conclusion, the model developed by Whitney and Carlander represents a valid model for studies in the field because it considers individual variability in the relationship of fish length to otolith length but further work is needed to validate the use of other back-calculation models.

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