Age determination of *Scardinius erythrophthalmus* (Cyprinidae) inhabiting Bafra Fish Lakes (Samsun, Turkey) based on otolith readings and marginal increment analysis

by

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ABSTRACT. - Age and growth of *Scardinius erythrophthalmus* (Linnaeus, 1758) collected from Bafra Fish Lakes between January 2000 and December 2000 were determined by examination of five hard structures. Scales, vertebrae, opercular bones, lagenar and utricular otoliths were aged by three readers independently. The precision in age estimations, which is derived by percent reader agreement and coefficient of variation indicated that otoliths were the most reliable structures for age determination. The estimated ages ranged from two to five years. The radius length of each annulus was measured in lagenar and utricular otoliths and the mean values per age were calculated. Otolith dimensions were found to be highly correlative with the fork length. The weight-length relationship of *S. erythrophthalmus* was described as $W = 0.0105FL^{3.23}$ (r = 0.98) for combined sexes. Monthly changes in the frequency of appearance of an opaque margin on the outer edge of the otolith and the annual variation of marginal increment length indicated that only one growth zone formed from March to October per year.

RÉSUMÉ. - Détermination de l'âge des *Scardinius erythrophthalmus* (Cyprinidae) vivant dans les lacs de Bafra (Samsun,Turquie) par lecture de stries annuelles sur les otolithes et validation par les incréments marginaux.

L'âge et la croissance de *Scardinius erythrophthalmus* (Linné, 1758) dans les lacs de Bafra (Turquie) entre janvier et décembre 2000 ont été déterminés par l'examen de cinq types de structures dures. Les écailles, les vertèbres, les opercules, l'otolithe lagenaire (sagitta) et l'otolithe utriculaire (lapillus) ont été observés par trois personnes de façon indépendante. La précision des estimations d'âge, estimée par la proportion de lectures concordantes et le coefficient de variation, indiquent que les otolithes constituent la méthode la plus fiable. L'âge estimé varie entre deux et cinq ans. Le rayon de chaque annulus a été mesuré sur les deux types d'otolithes et une valeur moyenne a été calculée. Les dimensions des otolithes étaient fortement corrélées à la longueur à la fourche. La relation longueur (FL)-poids (W) de *S. erythrophthalmus* est $W = 0{,}0105FL^{3,23}$ (r = 0,98) pour les deux sexes confondus. Les variations mensuelles de l'apparition d'une marge opaque sur le bord extérieur de l'otolithe et la variation annuelle de la largeur de cet accroissement marginal indiquent qu'une seule strie opaque se forme chaque année, entre mars et octobre.

Key words. - Cyprinidae - *Scardinius erythrophthalmus* - Turkey - Bafra Lake - Age - Otolith - Marginal increment.

The rudd, *Scardinius erythrophthalmus* (Linnaeus, 1758) is a common freshwater fish species, which is widely distributed throughout streams and lakes in Europe, North Caucasia and in the Black Sea Basin. In Turkey, it is abundant especially in Middle and Northern Anatolia and Thrace where it inhabits both streams and lake systems (Slastenenko, 1956; Geldiay and Balik, 1999). It is known in Turkey by the local name 'kizilkanat' meaning 'having red fins'. The catch of *S. erythrophthalmus* is not so heavy as of other commercially valuable species inhabiting the similar lakes, but it is always marketable and is also important as food for carnivorous fish species living in the same habitat.

Previous regional studies concerning *S. erythrophthalmus* were only dealing with its distribution and some biological characteristics (Slastenenko, 1956; Geldiay and Balik, 1999) but did not considered its age characteristics. The aim of the present study is to describe a reliable age determination method for *S. erythrophthalmus* inhabiting this shallow brackish water ecosystem and to validate the ageing method by means of marginal increment analysis. There is no doubt that accurate age determination is one of the most crucial topics required for rational fishery management. Beamish and McFarlane (1983) had pointed out the great negligence in age validation and mentioned the limited number of studies on this topic. In recent years however, the number of studies of validation in ageing process has increased. Some authors have applied direct validation methods such as known-age fish and mark-recapture studies (Jones and Brothers, 1987; Leaman and Nagtegaal, 1987; Hammers and Miranda, 1991; Oxenford *et al.*, 1994; Rien and Beamesderfer, 1994; Masuda *et al.*, 2000) and some others obtained indirect validation by marginal increment analysis (Campana, 1984; Ferreira and Russ, 1994; Gillanders *et al.*, 1999; Massuti *et al.*, 2000; Swan and Gordon, 2001) by length-fre-

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quency analysis and progression of dominant year classes (Eltink and Kuiter, 1989; Horn, 1993; Hanchet and Uozumi, 1996) and by comparison of different bony structures (Barber and McFarlane, 1987; Morales-Nin *et al.*, 1999; Sinis *et al.*, 1999, Panfili *et al.*, 2002).

This paper represents a comparison of the age interpretability on five hard structures of *S. erythrophthalmus*, the relationships between otolith dimensions and fish size and the validation of annulus formation per year by means of marginal increment analysis.

MATERIALS AND METHODS

Study area

The study area is a part of the Kizilirmak Delta (Samsun, Turkey), which is located in the north of Bafra County (41°36'N-36°04'E) and the highway connecting Samsun and Sinop city. Kizilirmak Delta has a total area of 50,000 ha and consists of a number of different sized lakes known as Bafra Fish Lakes. The samples were collected from two of these lakes named Sweet and Gici. The lakes are generally at sea level but may fall lower during dry seasons. They are connected with Fish Lake and Long Lake to the east by narrow marshy channels and with the sea by Kumcagiz Strait. The salinity level of the lakes is extremely low and its western side is covered by dense reed vegetation. These lakes are rich in plankton and have a maximum depth of 3 m at high water levels and an average depth of 1.5 m (Environment Foundation of Turkey, 1993).

Sampling and preparation of ageing material

A total of 160 specimens of *Scardinius erythrophthalmus* were caught in the study area using gillnets with a mesh size ranging from 10 mm to 26 mm at monthly intervals between January 2000 and December 2000. Each fish was measured for fork length (FL) to the nearest mm, weighed to the nearest g, and sex was recorded. Five hard structures: scales, vertebrae, opercular bones and lagenar (lapillus) and utricular (sagitta) otoliths were removed from individuals and cleaned by appropriate procedures. Scales were treated in 4% NaOH for 24 h, rinsed with water, left in 96% alcohol for one hour, rinsed with water again, dried and fixed between two slides, respectively. Excess tissue was removed from vertebrae boiling in water for 2-3 min. and cleaned before heating by 100ºC for 30 min. Opercular bones were cleaned in the same way as vertebrae but dried at room temperature for 2-3 days. Lagenar and utricular otoliths were relatively clean; the membranous material surrounding some of the specimens was removed with a fine forceps and scalpel in water and preserved dry in labelled envelopes (Chugunova, 1963).

Examination and Measurement of Hard Structures

Scales were viewed under a stereo binocular microscope with transmitted light and a magnification strength of 10X and vertebrae and opercular bones with 20X and 10X respectively, using reflected light.

Each pair of lagenar and utricular otoliths were weighed to the nearest 0.0001 g as right and left ones separately, to detect any significant difference. Total lengths and heights of lagenar and utricular otoliths were measured by a micrometric scale ocular placed in stereo microscope with a magnification strength of 15X and 20X, respectively in a black concave dish filled with alcohol. Lengths of lagenar and utricular otoliths were measured along the linear axis between anterior and posterior tips and the heights along the horizontal axis through the central core. Age counts on both lagenar and utricular otoliths were made along the axis between primordium and the anterior tip, because annuli were more visible on this area when compared to the posterior area, which is thicker and less translucent. Radius of each presumed annular mark on lagenar and utricular otoliths was measured and the length of marginal increment was also recorded. Any significant difference between the radius measurements within age groups was controlled by means of analysis of variance (ANOVA, $\alpha = 0.05$). The marginal increment is the growth zone at the outer part of the last completed annulus. The variation in marginal increment length gives information about the time of annulus formation throughout the year and also proves that each individual is laying down only one annulus per year.

The relationships between dimensions of lagenar and utricular otoliths and the fork length were investigated by regression analysis to reveal the relation between otolith growth and fish growth. In addition, the weight-length relationship was estimated by the commonly used equation (Ricker, 1975): $W = aFL^b$, where W is total weight (g), FL is fork length and a and b are constants.

Precision of age estimates

All ageing structures were examined by three readers. Age readings were undertaken without any reference to fish length, weight, etc. Only the date of capture was known by readers and January 1 was accepted as the birthday while assigning age classes. One opaque and one translucent zone were considered as one annulus in age readings (Panfili *et al.*, 2002). Readings were performed by at least a two weeks interval to minimize subjectivity. Age counts were evaluated to determine the level of precision, which reveal whether adequate ageing criteria was assessed by all readers. Precision is well defined by many authors (Kimura and Lyons, 1991; Campana *et al.*, 1995) as the reproducibility of age estimations between readers for a certain species. Here, we are going to use the coefficient of variation (CV) to assess consistency in repeated age determinations. Chang's CV which can be written as;

$$CV_j = 100x \frac{\sqrt{\sum_{i=1}^{R} \frac{\left(X_{ij} - X_j\right)^2}{R-1}}}{X_J}$$

where CV_j is the CV of the age estimate for a single fish (jth fish). This coefficient determines the relative ease of age interpretation from different ageing structures (Beamish and Fournier, 1981). Certainly, the ageing structure having the lowest CV is preferred as a more reliable ageing material and used in validation procedure. Therefore a mean CV for each bony structure was estimated from age readings of three readers and compared by ANOVA for any significant difference.

RESULTS

Annulus interpretation and precision of age estimates

In scale readings, two handicaps appeared affecting the age interpretation. The first was the high frequency of checks those can be described as discontinuity in circulus and the second was scattered absorptions of calcified tissue (Fig. 1). Inevitably, the quality of age readings reduced in scales revealing a full agreement of 33.75% between readers.

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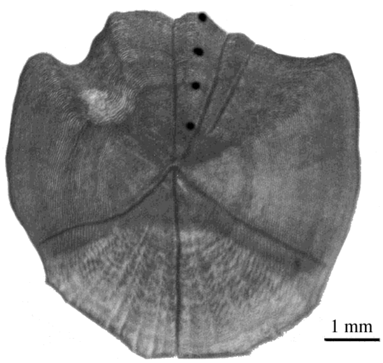

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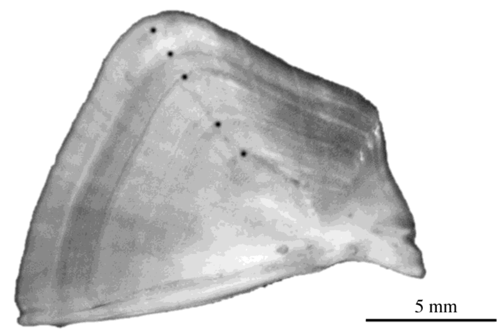

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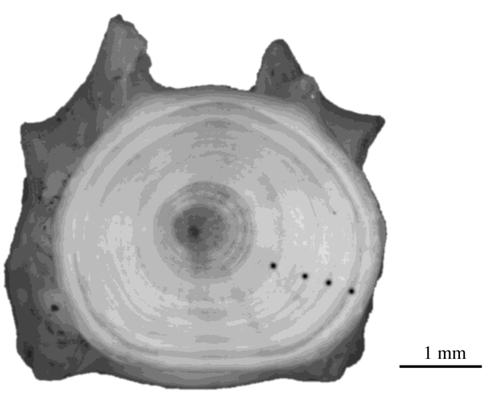

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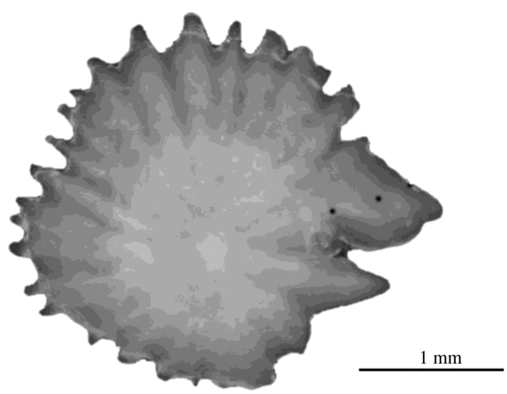

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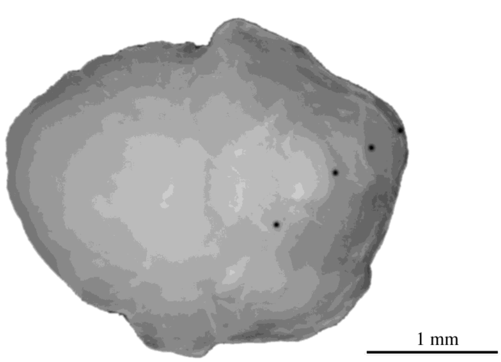

Figure 1. - Scale with absorbtion and indistinct annuli from a female of 13.2 cm fork length. *[Ecaille avec résorption et annuli indistincts sur une femelle de 13,2 cm de longueur à la fourche.]*

Figure 2. - Opercular bones from a female of 16.8 cm fork length. *[Opercule d'une femelle de 16,8 cm de longueur à la fourche.]*

Figure 3. - Vertebra displaying double rings from a female of 21.7 cm fork length. *[Vertèbre montrant des anneaux doubles sur une femelle de 21,7 cm de longueur à la fourche.]*

Figure 4. - Lagenar otolith from a female of 16.5 cm fork length. *[Lapillus d'une femelle de 16,5 cm de longueur à la fourche.]*

Figure 5. - Utricular otolith from a female of 19.2 cm fork length. *[Sagitta d'une femelle de 19,2 cm de longueur à la fourche.]*

Table I. - Precision level of age estimations obtained from readings of three readers (SE, the standard error of the mean). *[Niveau de précision des âges obtenus par trois observateurs (SE, Erreur Standard).]*

Structure	n	% of full agreement	CV (%)	SE
Opercular bone	107	14.66	21.47	0.26
Vertebra	107	30.13	15.52	0.24
Scale	107	33.75	13.09	0.26
Lagenar otolith	107	49.29	13.36	0.32
Utricular otolith	107	54.76	10.90	0.24

Opercular bones of young individuals were highly translucent and this makes it difficult to distinguish true annuli from checks (Fig. 2). Larger opercules were interpreted more easily due to enhancing contrast between translucent and opaque ones as a result of denser calcification.

Alternating broad opaque and narrow translucent zones around the centre of the vertebrae were observed. Some of the samples laid down double rings, which can be defined as two rings spaced too closely being different from usual annulus deposition (Fig. 3). These types of rings were accepted as one annulus.

Lagenar otoliths were larger and flatter than utricular ones and typically show broad opaque zones and narrow translucent zones concentric around the central core (Fig. 4). The first translucent zone surrounding the opaque central nucleus was always distinct and was interpreted as first annulus. The clarity of annuli was poorer at the dorsoventral axis of lagenar otolith than at their anteroposterior axis. Therefore age readings were done at the horizontal axis between the core and the rostral tip.

The anterior half of the utricular otolith is flat to slightly concave, but the posterior half is thick, even forming an upward projection (Fig. 5). It was impossible to observe annuli on this posterior part. Therefore all counts and radius measurements were carried out on the anterior part where opaque and translucent zones had a sharp contrast and easily interpretable.

The specimens (n = 160) ranged in age from two to five years. The most dominant age group was two (49.3%) followed by age three (33.9%), age four (16.9%) and age five (9.8%). The maximum length recorded was 22.3 cm and the minimum was 12.1 cm.

Age readings from three readers were evaluated and a mean CV and percent agreement for each ageing structure was calculated (Tab. I). The CV values were significantly different (ANOVA, F = 9.50, $p < 0.001$) among five structures. A oneway multiple comparison test was performed accepting utricular otoliths as the control group which presents the lowest CV (Dunnett's test, $t_d = 2.44$, D = 0.045). Mean CV of opercle and vertebra were both significantly different ($p < 0.05$) from mean CV of utricular otolith. Difference was not significant for scale and lagenar otolith ($p > 0.05$). At this stage, we concluded that opercular bones and vertebra were not reliable enough for ageing of this species. Furthermore, we also neglected scales for the low percent of agreement and the high frequency of checks when compared to lagenar and utricular otoliths. So, we concentrated on lagenar and utricular otoliths for validation procedure and marginal increment analysis.

Relationships between fork length and otolith dimensions

Mean weights of right and left lagenar otoliths were the

Table II. - Mean radius lengths at each annulus in lagenar and utricular otoliths of *Scardinius erythrophthalmus*. The standard deviation is represented in parenthesis. *[Rayon moyen des annuli sur les otolithes sagitta et lapillus. L'écart type est donné entre parenthèses.]*

Age (years)	n	Mean radius length (mm)				
		r_1	r_2	r_3	r_4	r_5
		Lagenar otolith				
2	22	1.1713 (0.0656)	1.7852 (0.0666)	_	_	_
3	11	1.1589 (0.1231)	1.7781 (0.2783)	2.2298 (0.1611)	_	_
4	12	1.1133 (0.0550)	1.6912 (0.0752)	2.1353 (0.0632)	2.5550 (0.01245)	_
5	3	1.1437 (0.0421)	1.6547 (0.0843)	2.0927 (0.0421)	2.6523 (0.0421)	3.1390 (0.0)
$\bar{x}$		1.1522 (0.0807)	1.7519 (0.1494)	2.1703 (0.1231)	2.5740 (0.1186)	3.1390 (0.0)
ANOVA		F = 1.42, $p > 0.05$	F = 1.62, $p > 0.05$	F = 2.69, $p > 0.05$	F = 1.70, $p > 0.05$	_
		Utricular otolith				
2	22	0.7443 (0.0437)	1.0895 (0.0511)	_	_	_
3	11	0.7696 (0.0424)	1.1035 (0.0524)	1.3585 (0.0766)	_	_
4	12	0.7369 (0.0474)	1.0582 (0.0442)	1.3047 (0.0341)	1.5087 (0.0341)	_
5	3	0.7480 (0.0294)	1.0540 (0.0294)	1.3090 (0.0589)	1.5470 (0.0779)	1.7510 (0.0779)
$\bar{x}$		0.7485 (0.0441)	1.0827 (0.0507)	1.3280 (0.0620)	1.5164 (0.0451)	1.7510 (0.0779)
ANOVA		F = 1.20, $p > 0.05$	F = 2.14, $p > 0.05$	F = 2.61, $p > 0.05$	F = 1.52, $p > 0.05$	_

Table III. - Parameters of the regression equations between otolith size and fork length of *Scardinius erythrophthalmus*. *[Paramètres des équations de régression entre la taille de l'otolithe et la longueur à la fourche.]*

Dependent-independent variable	n	Type of regression equation	Intercept (a)	Slope (b)	Correlation coefficient (r)
Lagenar otolith weight (mg)-FL (cm)	142	curvilinear	0.007	2.33	0.86
Lagenar otolith length (mm)-FL (cm)	141	linear	3.225	2.65	0.87
Lagenar otolith height (mm)-FL (cm)	141	linear	10.433	1.82	0.87
Utricular otolith weight (mg)-FL (cm)	125	curvilinear	0.005	2.49	0.84
Utricular otolith length (mm)-FL (cm)	124	linear	12.801	2.13	0.82
Utricular otolith heigth (mm)-FL (cm)	124	linear	7.644	1.83	0.76

same ($\bar{x}$ = 0.0056 g, SD = 0.0017, n = 81). Mean weights of right and left utricular otoliths were also found to be equal ($\bar{x}$ = 0.0062 g, SD = 0.0019, n = 89). As there is no significant difference in weight between the pairs of otoliths, we used any of the right or the left one in measurements and age readings when the other is unpresent or deformed.

The length measurements of each radius in lagenar and utricular otoliths are shown in table II. The mean distance from core to each annulus were compared for any significant difference within age groups by means of ANOVA. No significant difference ($p > 0.05$) was obtained between radius measurements within each age group. Furthermore, there was no overlapping in radius lengths of successive age groups supporting the consistency in annulus interpretation. Another case that can be observed from table II is the gradual decrease in length of each distance at annulus as the fish age increases. This can be accepted as a natural result of slowing down in the growth rate of fish by increasing age. Only the absolute increase in radius length at age five seems to be biased with this exception probably due to insufficient sampling (only three) at this age group.

The relationships between fork length and otolith size were investigated (Tab. III). The weight of lagenar and utricular otoliths display curvilinear relationships with the fork length but the length and height of both structures were found to be linearly related to the fork length by high correlation coefficients.

The lagenar and utricular otolith weight was significantly related to age. Both weights are found to be linearly increased with fish age by following equations and correlation coefficients.

Weight of lagenar otolith = 0.0013 + 0.0015 age
(r = 0.86; n = 72; $p < 0.001$)

Weight of utricular otolith = 0.0015 + 0.0017 age
(r = 0.84; n = 72; $p < 0.001$)

Furthermore, the weight-fork length relationship (Fig. 6) in *Scardinius erythrophthalmus* was developed with the equation:

$W = 0.0105\ FL^{3.23}$ (r = 0.98; n = 142; $p < 0.001$)

Annulus formation and marginal increment analysis

The lagenar otoliths of 140 and utricular otoliths of 126

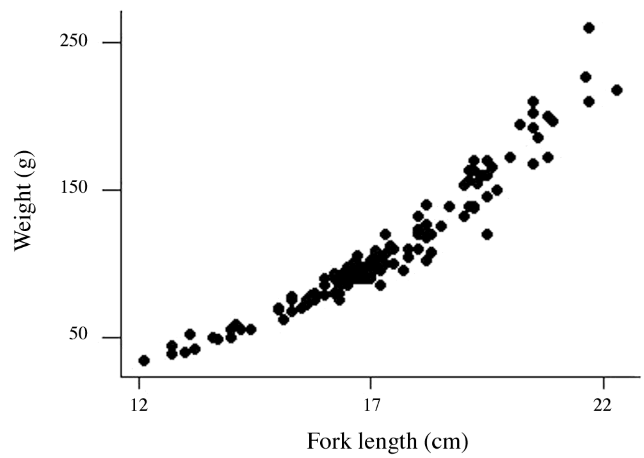

Figure 6. - Length-weight relationship of *Scardinius erythrophthalmus* (n = 142). *[Relation longueur - poids.]*

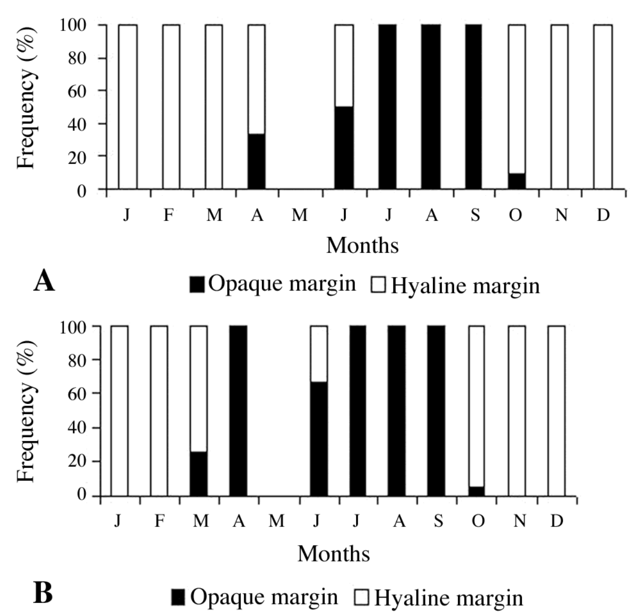

Figure 7. - Monthly frequency of opaque and translucent margins in lagenar (**A**) and in utricular (**B**) otoliths of *Scardinius erythrophthalmus*. *[Fréquence mensuelle des marges opaques et transparentes sur les lapilli (**A**) et les sagittae (**B**).]*

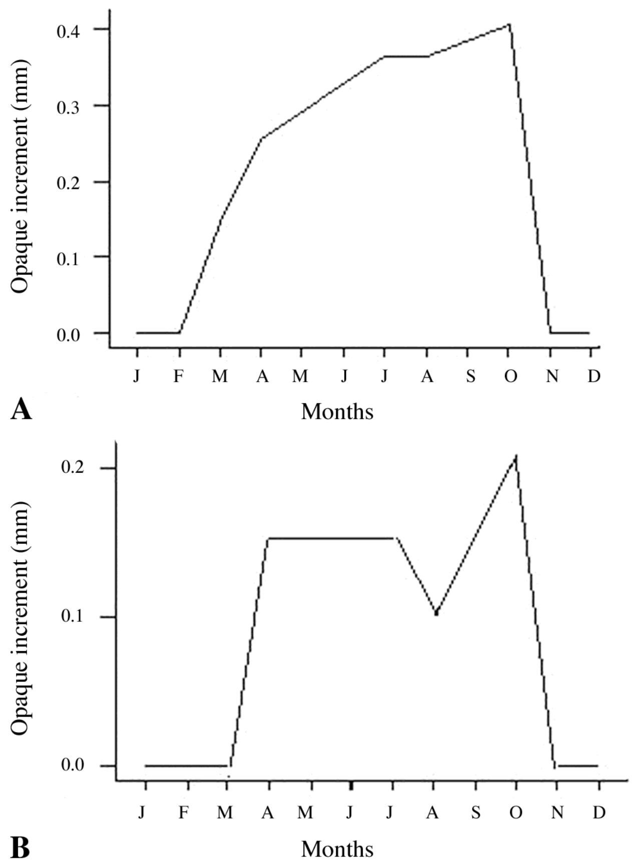

Figure 8. - Monthly change in mean length of opaque increment in lagenar (**A**) and utricular (**B**) otoliths of *Scardinius erythrophthalmus*. *[Variations mensuelles de la longueur moyenne des incréments opaques sur les lapilli (**A**) et sagittae (**B**).]*

individuals were investigated in order to define the yearly variation of percentage of opaque and translucent edges. The first opaque formation at the edge of lagenar otolith occurred in April and of utricular otolith in March. Opaque margins reached the maximum in July and remained at full percent in the following two months The frequency of translucent edges increased in October. All of the samples revealed hyaline edges at their outer margins from November to March for lagenar otolith and to February for utricular otolith (Fig. 7A, B). These data reveal that this species has only one rapid growth period corresponding to summer months throughout a year.

The yearly variation in length of the opaque increment both in lagenar and utricular otoliths was represented in figure 8A, B. The length of opaque increment in both structures increased steadily during growing period of fish reaching its maximum in October. The reason for the sudden decrease in values for utricular otolith in August may be the limited number of samples (only two) investigated at this month. It may be more explanatory to describe the yearly variation of opaque increment length for each age group separately but specimens representing each month within age groups were not sufficient.

DISCUSSION

Among five hard structures of *Scardinius erythrophthalmus*, we rejected the use of opercular bones, scale and vertebra in age determination. Mann and Steinmetz (1985) had discussed the accuracy of scale method for *S. erythrophthalmus* and reported a reader agreement less than 50%. Authors mentioned that 12 of 80 incorrect ages were the result of misinterpretation of false annuli and the other 68 arose from the missing of first annulus that was close to the centre of scales. Same authors also reported a mean length of 6.65 cm for age 1 but we have no available information for any comparison due to lack of this age group.

The absence of younger and older age classes limited our understanding of growth in *S. erythrophthalmus*. We tried to fit the length-at-age data to von Bertalanffy growth curve but the resulting parameters were not realistic with a L_{∞} of 63.2 cm and a K value of 0.040. So we could not achieve a curve describing accurately the growth of *S. erythrophthalmus*. The mean length-at-ages calculated from observed length measurements and validated age counts were as 14.5 cm, 17.2 cm, 18.05 cm and 20.5 cm for ages 2-5, respectively. Slastanenko (1956) reported the mean lengths at ages 2-5 from the river Dinieper as 9.5 cm, 12.7 cm, 17.2 cm and 21.4 cm, respectively.

The results of morphometric relationships showed that growth of lagenar and utricular otoliths was strongly correlated with fish growth. The length and height of otoliths in relation to fish growth indicated positive allometry while the weight of both structures revealing a negative allometry. It is known that prediction of age is possible by relating the size of calcified structures to age (Worthington, 1995). For example, Cardinale *et al.* (2000), Labropoulou and Papaconstantinou (2000), Newman *et al*. (2000), and Araya *et al*. (2001) reported that otolith weight is a good predictor of age. In this study we suggest that otolith weight may be a better dimension for age prediction of *S. erythrophthalmus* accounted for 74% of the variability of age in lagenar otoliths and 71% in utricular otoliths and may be preferred for its more reliable and quick measurement when compared to length and height.

The period of annulus formation for *S. erythrophthalmus* was similar to many other fish species of temperate zone. Opaque deposition is first evident in spring and broadens during summer till the first occurence of translucent edge in October. The annulus formation may be considered as complete only with the appearance of a new opaque increment representing the next growth period. The yearly variation of marginal structure of both lagenar and utricular otoliths indicated one peak of translucent and one peak of opaque margin percent corresponding only one annulus per year. Nevertheless, validation of an ageing method requires proving the periodicity of rings formed in all age classes. As the growth rate generally reduces as fish grows older, it may be difficult to validate ring periodicity by means of marginal increment analysis. Despite the above limitation, this method is an effective tool at least to determine the timing of annulus formation.

The factor, which governs the timing of annulus formation for *S. erythrophthalmus*, needs to be investigated. It is well known that increasing water temperature is the most important environmental factor that induces fish growth and therefore opaque zone and translucent zone formation (Barber and Walker, 1988; Das, 1994; Tzeng, 1994). The case here for *S. erythrophthalmus* does not contradict with this knowledge. Furthermore, food availability and reproductive activity were widely recorded as the other factors affecting annulus formation (Bilton and Robins, 1971; Quinn and Ross, 1982; Iizuka *et al*., 1985). The spawning time of *S. erythrophthalmus* reported as April-June (Geldiay and Balik, 1999) seems to be synchronous with the period of opaque zonation and to have no hindering effect on marginal growth. Certainly we need more information to prove these assumptions and to make out the physiological and environmental factors causing annulus formation in *S. erythrophthalmus*.

In conclusion we suggest that lagenar and utricular otoliths may be used as reliable ageing structures for age determination of the concerned species inhabiting these lakes. A direct age validation method such as labelling that will probably improve the information about the age and growth characteristics of *S. erythrophthalmus* needs to be conducted involving younger and older age groups.

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