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Age and growth of the Mesopotamian spiny eel, *Mastacembelus mastacembelus* (Banks & Solender, 1794), from southeastern Anatolia

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Abstract: Mesopotamian spiny eels, *Mastacembelus mastacembelus* (Banks & Solender, 1794), were collected from a large dam lake located on the Euphrates River in order to determine the most reliable ageing method among different hard structures and to estimate growth parameters. Vertebra, otolith, and opercle series were removed from 195 individuals. Vertebrae displayed the most interpretable annulus formations. Total length ranged from 14.4 cm to 76.9 cm and total weight ranged from 6.0 g to 950.0 g. The length and weight frequency distribution was significantly different between the 2 sexes (K-S test, $P < 0.001$). The length-weight relationship, determined as $W = 0.004TL^{2.84}$ ($r = 0.98$), revealed no sex-based significant difference (GLM, $P > 0.05$). Age classes ranged from 1 to 21 years in males and up to 9 years in females. Vertebra diameter was strongly correlated with total length and age, which were expressed by linear or cubic regression equations. The von Bertalanffy growth parameters were $L\infty = 81.7$ cm, $k = 0.13$ cm year^{-1} and $t_0 = -0.573$ years for the combined sexes, and the Ø' index was estimated as 6.766. Age interpretation revealed that a natural mark occurs on vertebrae in the third year of growth. This mark was composed of narrowly arranged numerous checks within the third annulus and it disappeared in subsequent annuli, which may be related to an environmental stress factor that occurred in that year.

Key words: Mesopotamian spiny eel, *Mastacembelus mastacembelus*, vertebra, age, growth

Güneydoğu Anadolu'dan Mezopotamya dikenli yılanbalığı (*Mastacembelus mastacembelus* (Banks ve Solender, 1794))'nda yaş ve büyüme

Özet: Mezopotamya dikenli yılanbalığı, *Mastacembelus mastacembelus* (Banks ve Solender, 1794) örnekleri, farklı sert yapılar arasından en güvenilir yaş tayini metodunu belirlemek ve büyüme parametrelerini tespit etmek amacıyla Fırat nehri üzerinde kurulu büyük bir baraj gölünden toplanmıştır. Toplam 195 örnekten omur, otolit ve operkül serisi çıkarılmıştır. Omur en kolay yorumlanabilir yaş halkalarını oluşturmuştur. Total boy dağılım aralığı 14,4-76,9 cm, ağırlık dağılım aralığı ise 6,0 g-950,0 g olarak tespit edilmiştir. Boy ve ağırlık frekans dağılımı eşeyler arasında önemli farklılık göstermiştir (K-S testi, $P < 0,001$). Total boy-ağırlık ilişkisi $W = 0,004TL^{2,84}$ ($r = 0,98$) şeklinde tespit edilmiş, erkek ve dişiler arasında anlamlı farklılık bulunmamıştır (GLM, $P > 0,05$). Yaş dağılımı 1-21 yıl arasında değişmiş ancak 9 yaşından büyük dişi bireylere rastlanmamıştır. Omur çapı ölçümleri ile total boy ve yaş arasındaki ilişkilere en iyi uyum sağlayan modeller doğrusal ve kübik regresyon denklemleriyle ifade edilmiştir. Von Bertalanffy büyüme denklemi parametreleri

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tüm veri grubu için L∞ = 81,7 cm, k = 0,13 cm $yıl^{-1}$ ve t_0 = -0,573 yıl, Ø' büyüme performans katsayısı ise 6,766 olarak hesaplanmıştır. Yaş yorumlaması esnasında omurda büyümenin üçüncü yılında 'doğal bir işaret' oluştuğu görülmüştür. Üçüncü yıl halkası içinde çok sayıda ince halkadan oluşan bu yapının daha sonraki annulus oluşumlarında kaybolduğu ve o yıl oluşan herhangi bir çevresel stress faktörüyle ilişkili olabileceği belirlenmiştir.

Anahtar sözcükler: Mezopotamya dikenli yılan balığı, *Mastacembelus mastacembelus,* omur, yaş, büyüme

Introduction

The Mesopotamian spiny eel, *Mastacembelus mastacembelus* (Banks & Solander, 1794), is a species of fish naturally distributed in the Euphrates–Tigris river basin (Coad, 1996; Froese and Pauly, 2009). This species is well known by local fishermen and is caught for selling, in some cases given as a gift for its delicious meat.

Studies about the biology and ecology of this species are scarce, and only a few papers about its age and growth (Pazira et al., 2005), morphological and molecular characteristics (Çakmak, 2008), parasites (Jalali et al., 2008), heavy metal bioaccumulation (Karadede et al., 1997), and embryonic development and milt quality (Şahinöz et al., 2006a, 2006b) have been published. Kılıç (2002) investigated some of the species' biological characteristics and Eroğlu (2004) examined its reproductive features; both of them used the synonym '*Mastacembelus simack*' in referring to this species. However, there is a lack of information about the biology and ecology of the northern populations of the Mesopotamian spiny eel inhabiting the Euphrates-Tigris river basin in the southeastern Anatolian region of Turkey.

Age data in fish populations is of great importance as the majority of fisheries' biological estimations are based on this parameter (Campana, 2001). Determination of the most reliable ageing method depends on the species itself, the availability of its skeletal structures for estimating age, the sample size and sampling strategy, and the experience of the researcher. The application of a validation method is required for accurate age estimations (Beamish and McFarlane, 1983), but the method of counting annuli in hard structures and measuring the level of precision between structures and between researchers is the best way to corroborate age estimations if a method of validation cannot be established (Kimura et al., 2006).

Different ageing methods have already been accepted as routine, such as marginal increment analysis (Peres and Haimovici, 2004), growth curve comparisons (Kerstan, 2000), length frequency analysis (Bellido et al., 2000), and the interpretation of annuli on hard structures (Morales-Nin et al., 1999; Maceina and Sammons, 2006; Gümüş et al., 2007). Of these methods, the last was used in this study.

The objectives of this study were to determine a reliable ageing method for the Mesopotamian spiny eel by comparative analysis of hard structures; to estimate the precision of the method; to determine the relations between structure size, fish size, and age; and, finally, to assess growth parameters.

Materials and methods

All specimens were collected in June 2006 to avoid variation in appearance of the opaque and hyaline edges on hard structures throughout the year. Age classes were assessed independently of the timing of the annulus formation. A total of 195 individuals (77 male and 118 female) were captured from Atatürk Dam Lake (37°23´N, 38°34´E) from a depth range of 0-5 m. Fish were collected using gillnets with 22, 28, 34, 42, and 55 mm mesh sizes, fishing baskets with conical openings (35 cm diameter, 20 cm height, and 17 mm mesh size), and fish traps formed of 5 loops (where the largest loop had a diameter of 45 cm and a height of 35 cm, and the others had a 27 cm diameter; all had a 17 mm mesh size). The average water temperature was 23.5 °C during the sampling period.

The eels were sexed and their total length (TL, mm) and total body weight (W, 0.1 g) were recorded. The paired sagittal otoliths, approximately the first 10 vertebrae beginning from the base of the skull, and the pairs of opercles, subopercles, and preopercles were extracted. Otoliths were washed in fresh water and preserved dry without any other treatment.

Vertebrae and opercular bones were soaked in boiling water for 2-5 min depending on the size of the hard structure. The excess tissue was removed with a piece of soft cloth and the hard structures were rinsed with alcohol. Larger vertebrae samples were treated with bleach for 60 s and rinsed again with water. Vertebrae and opercular bones were dried at 105 °C for 15 min to enhance the visibility of annular patterns. All hard structures were examined in alcohol against a dark background with a stereo-microscope using reflected light and magnification of 10× and 15×. This was a preliminary examination to observe the annulus characteristics of the structures and to project their usefulness for age assessment. Age counts were conducted using a stereo-microscope (Leica MZ 125) attached to a video camera monitor system and an image analysis system (Leica IM50).

Annular patterns on hard structures were interpreted within each sample by evaluation of certain criteria such as clarity, continuity, and identification of the first annulus. Ages were counted by considering one opaque (period of rapid growth) and one hyaline (period of slow growth) zone as a single annulus. Age counts were repeated 3 times at intervals of at least 10 days by the first author of the present study without reference to length data. The precision of the age estimates was calculated by using the Index of Average Percent Error (IAPE) (Beamish and Fournier, 1981) and the coefficient of variation (CV) (Chang, 1982).

The vertebrae of the Mesopotamian spiny eel had the most interesting hard structure in this study. The anterior part of each vertebra was conical, but the posterior was almost flat (Figure 1a). The annuli on each vertebra were observed as continuous concentric bands and the first annuli were always detectable. Annuli were widely spaced on the anterior surface, whereas they were very closely spaced on the posterior (Figure 1b). As a result, the latter were considered inappropriate for age estimation and all measurements and age counts were carried out on the anterior surface. The only problem with the vertebrae was the sharpness of the image in microscopic examination, especially in larger vertebrae. Small vertebrae were clearly viewed from focus to edge with a single focusing. Large vertebrae required examination in 2 steps because of the conical shape. The first step focused on the center of the vertebra and the second focused on an imaginary line where the slope of the vertebral surface changed. The change in slope occurred at the point where the edge of the vertebra continues to grow in an outward direction. The width of the vertebra, rather than its height, increased, and this made the structure less conical along this imaginary line (Figure 1c).

The diameter of the first 10 vertebrae from each fish was measured and a mean vertebra diameter (VD, ±0.05 mm) was estimated for each individual. The relation between VD and TL was determined by regression analysis, using linear and non-linear

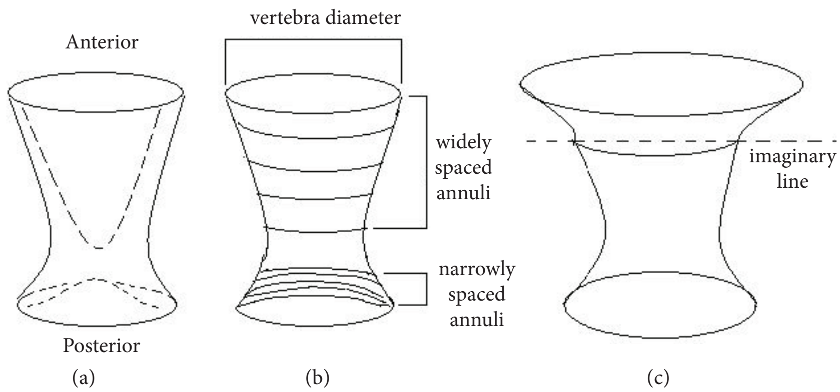

Figure 1. Diagram of vertebrae in *M. mastacembelus*: (a) deeply conical anterior and slightly conical posterior surface, (b) appearance of annuli on both sides, (c) change of slope in vertebral cone at the imaginary line observed in old specimens.

models for a better comprehension of age and growth characteristics and the use of vertebrae in age estimates.

The relation between length and weight was described by a power relationship (Ricker, 1975):

$W = aL^b$

where W is the total weight (g) and L is the total length (mm). The exponent b was controlled for any significant difference from the value 3 by means of a one-sample t-test. A regression model for total length versus total weight was compared by means of analysis of the covariance using the General Linear Model (GLM) procedure in SPSS to determine any significant difference in the length-weight relationship between the sexes. Transformations of length and weight data to a natural logarithm function ($\log_e X$) were performed to satisfy assumptions of normality and homogeneity prior to the application of parametric tests.

The length-at-age values obtained from vertebra interpretation were analyzed for any significant sex-based difference. A 2-sample independent t-test was performed within each age group.

The von Bertalanffy growth function (VBGF) was fitted to the observed length and age data derived from vertebra readings using nonlinear least squares estimation procedures. The VBGF is defined by the following equation:

$L_t = L_\infty \{1\text{-exp} [-K (t\text{-}t_0)]\}$

where L_t is the total length at age t; L_∞ (cm) is the asymptotic length, i.e. the length a fish would reach if it lived indefinitely; K (year^{-1}) is the rate at which the asymptotic length is approached; t (year) is the age of the fish; and t_0 (year) is the hypothetical age at zero length. Overall growth performance was estimated by the index Ø’ (phi prime test) (Munro and Pauly, 1983; Pauly and Munro, 1984):

$Ø' = \ln K + 2 \ln L_\infty$.

All statistical significance was based on α = 0.05.

Results

Comparison of hard structures and age interpretation

The otoliths were extremely small and were surrounded by very hard otic capsules. It was almost impossible to open these capsules without damaging the otoliths. The annuli on the otoliths could not be viewed clearly. The spiny eel otoliths displayed a similar appearance in both young and old individuals, and no thickening was evident. All otoliths were almost transparent, even those belonging to the largest individuals. Thus, we did not evaluate the otoliths further.

All opercular bones (opercle, subopercle, and preopercle) displayed annuli formations on their surfaces. Opaque zones were more distinct in opercular bones than in otoliths. In particular, the bases of the preopercles displayed reasonably clear annuli. Even though we were able to count annuli in a number of specimens, we noted that the annuli disappeared in the central core as the opacity increased in older specimens. This made it difficult to develop objective age-reading criteria for all sizes of fish, and the opercular bones were eliminated from the age estimation process.

Annuli on the vertebrae were mostly discernible. However, an unusual ring formation appeared in the third year of growth (Figure 2), involving thin checks at various frequencies. We assessed this regular formation as a natural mark. No natural mark was detected on samples smaller than 23.0 cm in TL (27% of females). The frequency of observation of this natural mark was 53.4% among females larger than 23.0 cm in TL. However, only 3 males (3.9%) displayed this irregular pattern on their vertebrae. The formation of the next annulus, which corresponded to the fourth year of growth, was of the usual pattern. Many other false annuli were investigated in the subsequent annuli, but none was as regularly arranged as the natural mark.

The vertebrae of older individuals were examined in more detail. The clear and distinct annulus formation in the first years of life became more complicated after 5 years (Figures 3a and 3b). The shape of the vertebral cone began to alter through the axis indicated by the imaginary line and the annuli grew closer to each other. This primarily occurred at age 6 or 7. Vertebrae removed from one of the oldest individuals displayed highly crowded but still discernible annuli on the outer edge (Figure 3c).

The IAPE and CV describing the precision of age estimates obtained from repeated age readings were

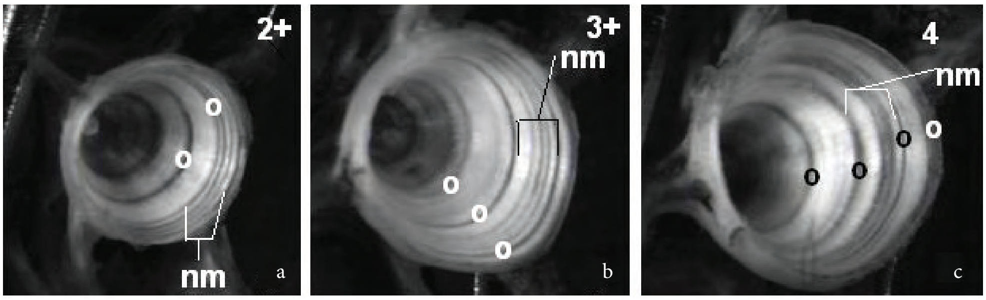

Figure 2. The appearance of a natural mark (nm) within the third year of growth on vertebrae of (a) 2+-year-old, (b) 3-year-old, and (c) 4-year-old specimens of *M. mastecembelus*.

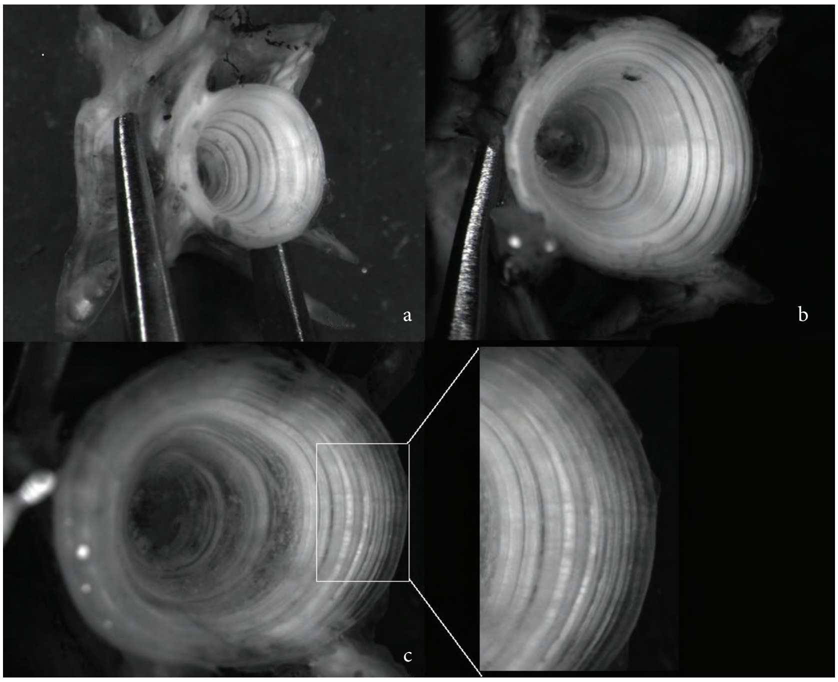

Figure 3. Vertebrae belonging to (a) 5-, (b) 10-, and (c) 16-year-old specimens of *M. mastacembelus*.

computed as 2.9% and 6.5%, respectively. As these 2 values are reasonably low, the precision could be considered to be high.

Age counts revealed a wide range of age classes, from 1 to 21, with very few representatives after 9-10 years and a significantly different distribution for males and females (K-S test, $z = -0.037$, $P < 0.001$). No female was older than 9 years old and the most dominant group was age 2 (51.7%), followed by age 3 (37.3%). Males attained older ages than females, with the most dominant age group being 2 years old (26.0%). The oldest individual was a 21-year-old male, 76.9 cm in TL and 950.0 g in W.

Fish size, VD, and age relations

Of a total sample comprising 195 individuals, 77 were male and 118 were female. Descriptive statistics of total length, weight, and vertebra diameter are presented in Table 1 for both sexes and for combined data. The length-weight relationships were calculated as follows:

$W = 0.0022\ L^{2.996}$ n = 77 R = 0.98 for males,

Table 1. Descriptive statistics of TL (cm), W (g), and VD (mm) of *M. mastacembelus* for each sex and combined sexes.

	Male				Female				Combined data			
	Min.	Max.	Mean	$S.e._m$	Min.	Max.	Mean	$S.e._m$	Min.	Max.	Mean	$S.e._m$
TL	14.4	76.9	43.3	2.17	14.9	57.3	26.3	0.58	14.4	76.9	33.0	1.09
W	6.0	950.0	285.2	30.9	8.0	367.0	58.9	5.25	6.0	950.0	147.5	14.8
VD	1.11	7.68	3.72	0.22	1.18	4.94	2.14	0.05	1.11	7.68	2.75	0.11

$W = 0.0054\ L^{2.792}$ n = 118 R = 0.96

for females, and

$W = 0.0044\ L^{2.835}$ n = 195 R = 0.98

for both sexes combined.

The value of b for females (t = 13.12, $s.e_b = 0.080$, $P < 0.001$) and for the combined data (t = 8.46, $s.e_b = 0.043$, $P < 0.001$) was significantly different from 3.0. This was not the case for males (t = 0.557, $s.e_b = 0.065$, $P > 0.05$). ANCOVA of weight-at-length was not significantly different between the sexes ($F_{1,194} = 3.327$; $P > 0.05$).

The relationship between VD and TL is presented in Figure 4. There was a strongly correlated linear relationship between VD and TL described by the following equation:

$VD = -0.037 + 0.095\ TL$ r = 0.990

The diameter of vertebrae increased positively with specimens' TL by a constant ratio for the whole lifespan. Another strong correlation existed between VD and age. The best fitting regression model for the data of VD versus age was a cubic equation calculated as follows:

$VD = 0.387 + 0.749\ Age - 0.029\ Age^2 + 0.0005\ Age^3$ r = 0.987

The rate of increase in VD was high in the earlier period of life but apparently slowed down as the fish aged.

An age-length key is given in Table 2 for males and females separately because of the highly different age ranges within sexes.

The relationship between TL and age is presented in Figure 5. The parameters of the von Bertalanffy growth equation, which was fitted to TL-age data, were determined as follows:

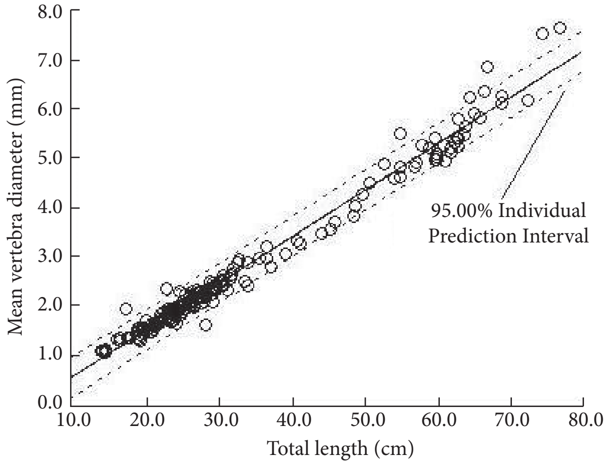

Figure 4. The scatter plot of TL versus VD and associated regression line and 95.00% confidence interval for *M. mastacembelus.*

$L_\infty = 80.0$, $k = 0.14$, $t_0 = -0.452$ for males,

$L_\infty = 83.6$, $k = 0.12$, $t_0 = -0.615$ for females, and

$L_\infty = 81.7$, $k = 0.13$, $t_0 = -0.573$ for combined data.

The growth performance index (Ø') was calculated as 6.798 for males, 6.732 for females, and 6.766 for the combined sexes.

Discussion

We were not able to interpret the otoliths even though this method is common and highly reliable. Both the otoliths of the old and the young individuals were unavailable for reading because of high transparency due to insufficient calcification, which resulted in indistinct annuli. The loss of most of the primary annuli on opercular bones that represent the early period of life history compelled us to reject these hard structures too, as it could lead us to underestimate the ages.

Table 2. Age-length key for males and females (in parentheses) of *M. mastecembelus* (ages 17, 18, and 20 had no representatives).

	Age (year)																				
TL(cm)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
10.0-15.0	4(1)																				
15.0-20.0	- (1)	5 (12)																			
20.0-25.0		15(36)	- (1)																		
25.0-30.0		- (13)	3(35)																		
30.0-35.0			4 (8)	-(4)																	
35.0-40.0				3(1)	1(-)																
40.0-45.0					3(-)																
45.0-50.0						2(2)	1(-)														
50.0-55.0							2(1)	2(-)	1(-)												
55.0-60.0								4(-)	2(1)	2(-)											
60.0-65.0									2(-)	6(-)	2(-)	1(-)									
65.0-70.0												2(-)	1(-)	1(-)	2(-)						
70.0-75.0																1(-)			1(-)		
75.0-80.0																					1(-)
Mean	15.1	22.6	28.8	34.9	41.6	47.6	52.0	57.4	59.6	61.8	63.8	65.1	66.6	68.9	68.0	72.5	-	-	74.5	-	76.9
N	6	81	51	8	4	4	4	6	6	8	2	3	1	1	2	1	-	-	1	-	1
$S.e._m$	0.37	0.28	0.30	0.82	1.05	1.07	1.29	0.99	1.21	0.58	0.75	0.58	*	*	1.0	*	-	-	*	-	*

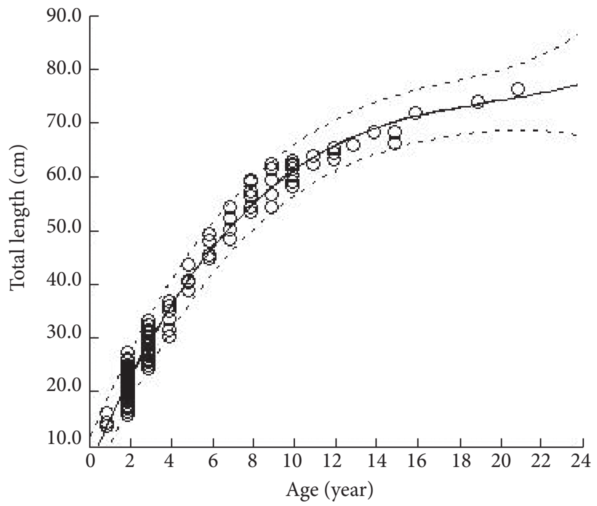

Figure 5. The scatter plot of age versus TL for males and females of *M. mastacembelus.*

The precision in age estimates obtained from repeated vertebra readings in the present study could be considered high, as a value below 5% has been suggested by Campana et al. (1995) as acceptable for ageing accuracy. Vertebrae and opercles were suggested by Pazira et al. (2005) as appropriate structures for age determination rather than otoliths, but no information was presented about the level of precision.

The annulus character observed on the vertebrae was mostly unproblematic, but age-reading required exceptional care in the Mesopotamian spiny eel vertebrae. The numerous checks within the opaque zone of the third-year growth, named the "natural mark" in this study, could be related to an environmental stress factor occurring that year and needs to be investigated with a larger number of specimens on a sex- and age-specific base.

The measurement of VD and its relation to TL indicates that the increase in VD was directly proportional to the increase in TL throughout the life history of the Mesopotamian spiny eel.

The length-weight regression from our study, which revealed a negative allometric b value of 2.835 for the combined sexes, was in accordance with previously published data. Kılıç (2002) estimated the value of b as 1.923 for the combined sexes and Pazira et al. (2005) reported values of 2.524 for males, 2.124

for females, and 2.275 for the combined sexes, where all values implied negative allometric growth. A significant difference was detected between b values of the 2 sexes in this study. It is known that differences in b-values can be attributed to many factors, such as the number of specimens examined, area or seasonal effects, differences in the observed length ranges of the specimen, gonad maturity, diet, stomach fullness, health, and even preservation techniques (Moutopoulos and Stergiou, 2002; Oscoz et al., 2005; Leunda et al., 2006). The different size ranges in males and females and especially the dominant narrow size range in females could explain the difference in this study.

The maximum age determined in this study suggests that the spiny eel has a longer lifespan than previously thought. Kılıç (2002) reported an age range of 1-5 years for females (25.0-62.0 cm) and 1-7 years for males (21.0-70.0 cm) from a total of 66 specimens in a river system connected to a large dam lake, and also emphasized that the larger individuals were caught in the dam lake while the smaller ones were caught in the rivers. Eroğlu (2004) measured the age range as 1-8 years for females (26.6-68.5 cm) and 1-9 years for males (23.7-80.6 cm) where all samples (n = 187) were collected from a lentic system. Pazira et al. (2005) reported the lifespan of the spiny eel to be 0-6 years for both females (4.2-42.5 cm) and males (4.2-43.2 cm) collected from 2 rivers in southern Iran (n = 120). The differences in age ranges could not be from a size-dependent capture as the fishing methods were similar (gill nets, fish traps, and fishing baskets) in all mentioned studies. We think that the difference could be an outcome of lotic and lentic habitat differences. Fishing pressure could also be another factor affecting the age range, but we had no available data for any comparison in this case. Kılıç (2002) and Eroğlu (2004) also reported a slight difference between the age ranges of the sexes, which was highly remarkable in this study, where the oldest female was 9 years old while the oldest male was 21 years old. It is certainly possible that such high age estimates may involve some error in ageing and need to be validated.

The length-at-age values obtained from vertebra interpretation in this study are similar only to those suggested by Pazira et al. (2005). A TL of 43.2 cm was recorded by Pazira et al. (2005) at age 6. The mean TL for the same age group was 47.58 cm in this study. The difference in length between this study and that by Pazira et al. (2005) may be explained by the trophic levels of the 2 different aquatic systems, where the former is a lotic but the latter is a lentic system. The mean values of TL that were estimated for age groups younger than 6 years in this study were also consistent with those reported by Pazira et al. (2005).

Spiny eel growth was rapid up to age 9, but the rate of somatic growth slowed with increasing age from 10 years onward. The asymptotic lengths were reported to be 98.6 cm for the combined sexes by Kılıç (2002) and 92.3 cm for males and 87.3 cm for females by Pazira et al. (2005). All reported values are higher than the asymptotic length estimated in this study.

Instead of comparing k and t_0 values independently, Ø' was derived from growth equations that were given in other studies. The highest Ø' was obtained by Kılıç (2002), at 7.355 with no sex differentiation. The value of Ø' estimated for males and females by Pazira et al. (2005) was 6.537 and 6.439, respectively. In this study, Ø' was estimated as 6.798 for males and 6.732 for females. The values of Ø' obtained by Pazira et al. (2005) and this study indicate that the Mesopotamian spiny eel has a similar growth performance in these 2 habitats and among the sexes.

In conclusion, the Mesopotamian spiny eel can be considered a slow-growing and long-lived species. All kinds of fishery management, commercial or recreational, should be based on this fact to maintain the current status of this endemic and mysterious river fish. Furthermore, we recommend vertebrae as a reliable structure for ageing of the Mesopotamian spiny eel in studies regarding its biology or management of the population. Further studies are required for validation of the ageing method and the longevity of the spiny eel.

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