

**REPUBLIC OF TURKEY
FIRAT UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND
APPLIED SCIENCE**



**DETERMINATION OF MOST RELIABLE BONY
STRUCTURE FOR AGEING OF *ASPIUS VORAX*
INHABITING KARAKAYA DAM LAKE
(MALATYA-TURKEY)**

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**Master Thesis
Department: Fisheries Basic Sciences
Supervisor: Prof. Dr. Nuri BAŞUSTA**

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MASTER THESIS
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ABSTRACT

Determination of Most Reliable Bony Structure for Ageing of *Aspius Vorax* Inhabiting Karakaya Dam Lake (Malatya-Turkey)

The target of this study is to determination of the most reliable bony structure for ageing of *Aspius vorax* (Heckel, 1843) in Karakaya Dam Lake (Malatya, Turkey). The age determination method and its accuracy are the most important subjects for the evaluation of age, weight, sex and length relationships in fisheries biology. In this study, different kinds of “bony structures” are used such as scales, vertebrae, operculum otoliths and dorsal spine of *A. vorax* removed from each fish were examined by two readers three times each without reference to any data except the date of capture. A total of 73 specimens (27 female and 46 male) were examined. The highest percentage agreement (PA) and lowest index of avarage percentage error (APE) and coefficient of variance (CV) values were found between vertebrae and opeculum estimates and they were most suitable ageing structure in *A. vorax*. It was understood that operculums were the best bony structure for the age determination of *A.vorax* followed by vertebrae, dorsal spine and scale. In this work, we didn’t preferred the otolith for detect age because of it was impossible for ageing.

Key Words: Age determination, *Aspius vorax*, bony structure, Karakaya Dam Lake, Eastern Anatolia.

ÖZET

Karakaya Baraj Gölünde (Malatya -Türkiye) Yaşayan *Aspius vorax*'da Yaş Tespiti için En Güvenilir Kemiksi Yapının Belirlenmesi

Bu çalışmanın amacı, Karakaya Baraj Gölü'nde (Malatya, Türkiye) yaşayan *Aspius vorax* (Heckel, 1843)'de yaş tespiti için en güvenilir kemiksi yapıyı belirlemektir. Balıkçılık biyolojisinde; yaş, ağırlık, cinsiyet ve uzunluk ilişkilerinin değerlendirilmesinde yaş belirleme yöntemi ve doğruluğu en önemli konudur. Bu çalışmada, Sis balığının pul, omur, operculum, otolit ve dorsal diken gibi farklı kemiksi yapıları her balıktan çıkarıldı ve iki farklı okuyucu balığın yakalanma tarihi hariç herhangi bir verisi bilinmeden tarafından 3 kez incelendi. Toplam 73 balık örneği (27 dişi ve 46 erkek) incelendi. Enyüksek yüzde uyum (YU) ve endüşük ortalama yüzde hata indeksi (OYHI) ve varyasyon katsayısı değerleri omur ve operculum hesaplamaları arasında bulundu ve bu kemiksi yapılar yaş tayini için en uygun kemiksi yapılarıydı. Buradan *A. vorax* için en iyi kemiksi yapının operculum olduğu bunu sırasıyla omur, dorsal diken ve pul izlediği anlaşılmıştır. Bu çalışmada yaş tayininin imkansız olması nedeniyle otoliti yaş tayini için tercih etmedik.

Anahtar Kelimeler: Yaş tayini, *Aspius vorax*, kemiksi yapılar, Karakaya Baraj Gölü, Doğu Anadolu.

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ABBREVIATION

APE	: Average Percentage Error
CV	: Coefficient variation
D	: Deviation
IAPE	: Index Average Percentage Error
PA	: Percentage of Agreement
SD	: Standard Deviation



1. INTRODUCTION

Determination of fish age is definitely an important device in fisheries biology and it's also essential for the assessment of life history, growth rate, maturity and spawning times, growth at different age groups and mortality rates of a given population. Best way for estimating the age of fish are depend on certain structures, which possess patterns related to the annual growth [1].

The age of fish can be used in every studies concerning their biology. These studies including of the age-length keys, rate of survival, growth and mortality, age structure and reproduction rate of stock. Determination of age in fish assists us to learn the age in the beginning maturity, to study population dynamics, to estimate growth, and to optimize time that is harvesting. Age and growth studies are important for issues associated with management of fisheries. various ways are used in determining the age of fish. Probable The most accurate way of age determination under normal conditions could be the mark-release-recapture method; its application is limited in fisheries due to a number of constraints, such as time and money [2]. The other way is the analysis of the length-frequency data [3]. This method is benefit for that fish which is breed only once time in 12 months and it cannot be employed to determine the age of an individual fish [2].

The third method could be anatomical approach. It is suitable to determine the age of fish by assessment of the growth rings forming on bony structures such as scales, otoliths, the opercular bone, the vertebrae and the cross section of dorsal spine. Ages of fish are evaluated by variation of the readings from different bony structures and variant readers [4, 5, 6]. Age determination is not a procedure of calculate rings on bony structures. When bony structures are observed under a microscope, it is possible those various rings are noticed running concentrically around the of center. While some rings are express yearly growth, some rings also reflect stress or periods of spawning, starvation or migration. The annual rings are entirely indicators of age. It must be that the "true rings" be distinguished from other kinds of rings such as stock rings, false rings, larval rings and spawning rings through the repeated examination of samples to far away from any confusion and inaccuracy in age determination [2]. For getting proper age data, the ages of fish are evaluated by comparing readings from different bony structures and different readers [7]. As the most accurate ageing method may be vary among fish species, the precision of bony structures by readers should be studied for each species [8, 9].

Additionally, ageing errors should be considered before making decision on the most reliable bony structure for evaluate ageing of fish by establishing the rate of agreement and rate of ageing error, bony structures which have the highest agreements percentage and minimal ageing error must be determined. Thus, among various bony structures reliable ones could be distinguished from other bony structures [10, 11].

Many structures are used to estimate the age of fishes, consist of scales, otoliths, vertebrae, dorsal spines, opercular bones, cleithra, urohyal bone, and hyomandibular bone. One of the main dilemma in age and growth studies is the collection of the most suitable structure to age the fish. Ages of fish are estimated by the comparison of age estimates from various bony structures and by the different readers [12]. For this purpose, we must have accurate and updated basic biological information on the concerned fish species. But regrettably, such as information for several fish species is either incomplete or inaccurate. Age structure of population provides information that seems requirement for the understanding of several population parameters specially the dynamics of the target fish species, recruitment, population growth rates, and mortality. In the fishes without scales, age could be estimated via using a different of hard structures, they are consist of otoliths, dorsal spines, vertebrae and opercular bones. Some of the hard structures give more accurate and precise estimates of age than others. choosing the proper structure for ageing is important for supplying useful information to managers [17]. Comparison of age estimates among bone structures is an alternative technique to validation that will provideof benefit information on the precision and bias of age estimating structures [13].

Evaluation of accurate fish age is considered as an requirement step for age-depend on evaluation of fish population and successful resource administration . Comparison of age estimates from various ageing structures have already been reported to be undertaken in a number of fishes with a view to recognize the most reliable structure for a fish population [14-16]. selection between the bony structures for age determination varies from one species to another species. Generally, every bony structures in the fish body do not show the equal clarity and distinctness in their annual marks. for that reasen , it becomes essential to find out the most reliable ageing structures in each fish species. For getting proper age data, the ages of fish are evaluate by comparing readings among various bony structures and between different readers. As the most suitable ageing method could be vary among species, the accurate of bony structures by readers must be studied for each species [8]. Fish age determination essential for that growth marks or annuli be

identified and counted. For otoliths, vertebrae, opercular bones and fin rays observed under reflected light against a black background, the annulus recognized as the translucent zone and seems as a black band [1,19].

1.1. Biology of the *Aspius Vorax*

The *Aspius vorax* (Heckel, 1843) is a native and very commercial *cyprinid* species inhabiting Euphrates-Tigris basin in turkey, syria and iraq [24, 27]. On this species have already been conducted at different localities in iraq and syria [28, 29, 30 and 31]. Additionally, there are several studies in turkish freshwaters that were carried out to attain information on biological characteristics of *A. vorax* [27, 32] .

In spite of its commercial significant [33]. The studies on population structure of the fish species are insufficient in Karakaya Reservior. The key purposes regarding of the fisheries biology studies are to getting information belonging to population dynamics and advantage to get maximum point yield from natural fish stocks. Attaining these objectives is dependent on the evaluate of realistic population parameters [34]. Such as reproduction (sex ratio, fecundity, gonadosomatic index, spawning time and area), growth (growth characteristics, length, weight, quantity increase, length and weight relationship, condition factor and growth formulas), mortality (natural mortality, fishing mortality, total mortality and annual mortality) and stock evaluate [35]. Some 106 freshwater and euryhaline species fish have now been recorded in the non-marine waters of this basin, of which cyprinidae could be the major endemic freshwater family [36].

The species of *Aspius vorax* are relatively large *cyprinids*, spawning period for this species are come from april to may, whenever the water temperature among this degree 14-18°C [56].

Between native cyprinids, endemic asp, *Aspius vorax*, is the only representative of their genus with a geographical distribution limited to the Euphrates-Tigris Basin [24, 36, 37]. Because of their economic and ecological importance, growth and reproductive biology of the carnivorous asp has been the focus of few studies in the lower part throughout Iraq [38-40] and the upper part, moves through Turkey [27].

1.2. Aim of This Study

Age and growth studies are important for solving issues associated with the fisheries management. Determination age in fish assists us to learn the age in the beginning maturity, to study population dynamics, to estimate growth, and to optimize time that is harvesting.

The target of this study is to determination of the most reliable bony structure for ageing of *Aspius vorax*.



2. MATERIAL AND METHODS

2.1. Material

The study area, Karakaya Reservoir, is located southeast of anatolia in Turkey ($38^{\circ}8'-39^{\circ}13'$ E, $38^{\circ}47'-38^{\circ}08'$ N) (Figure 2. 1), that is the third largest dam lake in turkey having a 268 km^2 surface. It came into operation in 1987 as a hidro-electric dam lake [42]. It was reported and observed that the water temperature changed from $7, 67^{\circ} \text{C}$ to $24, 9^{\circ} \text{C}$ and average water temperature have been approximately 10°C [43]. Samples were caught with gillnets in dissimilar mesh sizes (40, 45, 50, 55, 60, 65, 75, 90, 110 and 120 mm), monthly between September 2016 and March 2017. During this study, an overall total of 73 samples had been collected and prepared individually to attain information on length, weight, sex determination, and ageing (Figure 2. 3, 2.4. and 2. 5). The total length (TL) and the total weight (W) were measured to the nearest 0.1mm and 1 g, respectively.

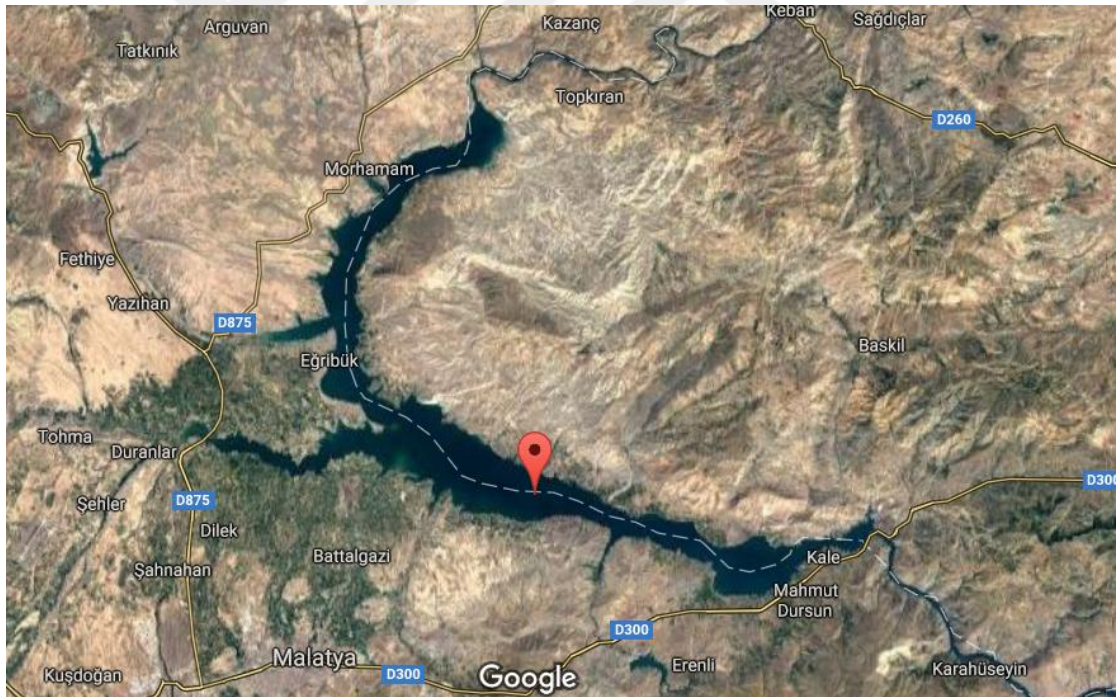


Figure 2.1. The sampling area, karakaya dam lake (Malatya-Turkey).

2.2. Methods

Gonads were macroscopically examined to determine of the sex gender [44]. Also total weight, total length, standard length and fork length of each sample were recorded

and otoliths, opercular bone (left and right side), scales, vertebrae and dorsal spine were received from each of fish and prepared for processing of age determination in *A. vorax* (Figure 0.3, 2.4, 2.5). as the following :

1. Scale: Preparation and reading techniques for scales in each fish we selected about twenty scales from the left side to right side were removed from above of the lateral line (Figure 2.9). At midpoint near the tip of the pectoral fin [57]. Scales were washed, cleaned and studied as dry mounts, after removing the extraneous matter and mucus by washing them in tap water and rubbing in between the finger tips to make scales more clear and soft (in case of large scales), immersed in 96% alcohol for approximately 5–10 min, then washed in tap water and dried in air. Small sized scales were mounted between two glass slides and studied with the help of compound microscope [85]. For scales, the annulus corresponds to the area of discordance the arrangement of circuli or a narrowing between them [23].

2. Opercular: Bone preparation and reading techniques the opercular bone were detached with the help of scalpel and immersed in boiling water for few minutes to get rid of extraneous tissue (Figure 2.8,2.6). A bristled brush was used to remove tissue that boiling water did not loosen. Cleaned opercular bones were dried at room temperature and examined under transmitted fluorescent light with naked eye [15, 45].

3. Otolith: Preparation and reading techniques Sagittal otoliths were eliminate from otic capsules by opening the otic bulla (Figure 2.11). In *A. vorax* .otoliths were washed, cleaned and read whole by immersion in alcohol while in *A. vorax*, otoliths were studied by immersion in 50 % glycerol (Figure 2.10). And analyzed under microscope using reflected light. Otoliths with unclear annual rings were ground with sand paper to make the annuli more distinct for age reading [85,48].

4. Vertebrae: Preparation and reading techniques for vertebrae by taken out the (4th to 10th) pieces of vertebrae and placed in boiling water for 30 minutes and then a bristled brush was used to remove extraneous matter (Figure 0.7,2.6). To clear the attached muscles. They were dried at room temperature for 5-6 days to count annual rings after that

they were putted in glycerine and examined under the microscope over a black background using reflected light to illuminate annuli under dissecting microscope [15,49 and 46].

5. Dorsal spine: Preparation and reading techniques for dorsal fin in general the third dorsal one were removed and placed in boiling water for a few minute to remove the attached muscles (Figure 0.1,2,4). and then immersed in 96% alcohol and dried in air. parts of 0.35-0.60 mm were taken by a jewellery saw and dried at 103°C in the oven for 15 min and then Sections were placed on a microscopic slide and viewed under dissecting microscope [15,47].

All bones structure were immersed in alcohol, and some of the structure when we worked they had so fatty surrounding of them and then we dropped in to 3- 4 % solution of Sodium Hydroxid. To remove the extra attached fatty.

In spite of all structure after process of washing, cleanin and dried we were preserved in a referenced packet then recorded some note on this packets about date , number and name of the structure till reading under microscopic .

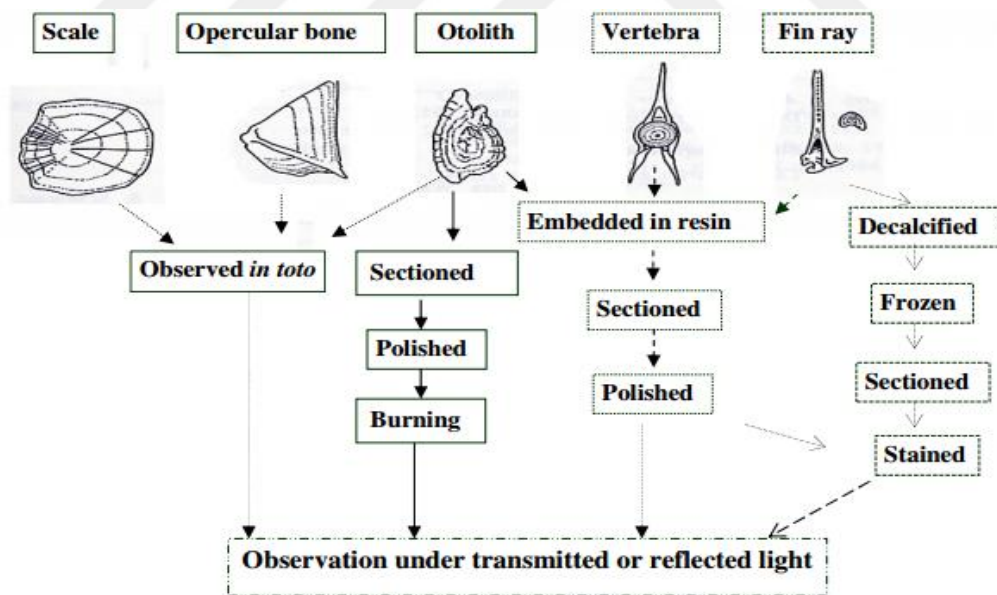


Figure 2.2. Detection and calcified of the fish structure.

Fish age determination requires that growth marks or annuli be identified and counted. For otoliths, vertebrae, opercular bones and dorsal spine observed under reflected light against a black background [19, 20, and 21].

For stained preparations, the annuli correspond to the chromophilic rings representing the arrested-growth lines [22].

For every preparation mode, at least two observations are made by one or more readers. The age determination adopted in the Sclerochronology Laboratory is dependent on the following three steps:

- Selection of the most reliable and suitable calcified structure and its matching preparation mode.
- Validation of this timing of annulus formation.
- Age determination, establishment of the age-length key, and study associated with the growth [18].



Figure 2.3. Length measurement of *A. vorax*.



Figure 2.4. Recieving of the dorsal spine in *A.vorax* .



Figure 2.5. Weight measurement in *A.vorax* .



Figure 2.6. Boiling of dorsal spine, vertebrae and operculum in *A. vorax*.



Figure 2.7. Washing of vertebrae in *A. vorax*.



Figure 2.8. Cutting the operculum from *A.vorax*.



Figure 2.9. Remove scale from *A.vorax*.



Figure 2.10. Otolith placed into glycerol in *A. vorax*.



Figure 2.11. Otolith receiving into left side of *A. vorax*.

The bony structures were photographed using Leica S8APO brand microscope with Leica Application Suit (Ver.4.8.0) software. For every fish, annuli were counted on all these structures separately by two readers without prior knowledge of fish length and age evaluate from other structures [41].

Calculations and statistical analyses: Age estimates were compared by calculating the coefficient of variation, percentage agreement (PA) and index average percentage error (IAPE) between the readers and between the pairs of ageing structures. IAPE was derived using the formula presented by [51]. Described IAPE to be sensitive not only to age disagreement but also to the magnitude in the difference in age assignment between or among readers.

Precision was measured by calculating the percent agreement (PA), coefficient of variation (CV) [50]. And index of average percent error (IAPE). Between the readers and between the pairs of ageing structures for the selected fish species. IAPE was derived using the formula presented by [51].

$$IAPE = \frac{1}{R} \sum_{i=1}^R \frac{|x_{ij} - x_j|}{x_j} \times 100 \quad (2.1)$$

Where x_{ij} is the i^{th} age determination of the i^{th} fish, x_i the average age calculated for the i^{th} fish and R the number of times each fish is aged. Where x the coefficient of variation [52]. Is calculated as the ratio of standard deviation over the mean, and can be written as

$$CV_j = 100\% \times \frac{\sqrt{\frac{\sum_{i=1}^R (x_{ij} - x_j)^2}{R-1}}}{x_j} \quad (2.2)$$

Where CV_j is the age precision estimate for the i^{th} fish. Mean age readings (consensus data) attain from various bony parts were subjected to one-way analysis of coefficient of variation [53]. So that you can explain the readings from various bony structure of the fish showed significant differences between themselves. Although mean age evaluate is not an indicator for the reliability of ageing structure, it may provide useful information regarding over or under estimation of age by a structure irrespective of fish size-class [54]. This is may prove beneficial in choosing the structures which may give statistically indifferent readings when size-class is not taken into account.

All the bone structures (otoliths, scales, vertebrae, opercular bone and dorsal spine sections) were aged stand alose by two readers without the information of fish length. In case of disagreement between readers the ageing structures were re examined together by the two readers until a consensus was reached.

3. RESULTS

In this study we worked with 73 fish of *A. vorax* individuals which were (27 female and 46 male). The highest body length was 69.5 cm and the lowest was 38.7 cm in female. And in the other side the highest length was 58.8 cm and lowest length was 41.1 cm in male. And about weight measurement the highest weight was 3.176 g and the lowest weight was 454 g. in female. In spite of the highest weight was 1580 g with the lowest 534 g in male. To determine age composition we used the five bone structure scale, otolith, vertebrae, operculum and dorsal spine also from each of them we received the right and left side of otolith, vertebrae and operculum. In spite of total weight, total length, standard length and fork length for each sample were recorded.

Nevertheless after prepared and finished from washing, cleaning and dried we were protecting all the structure in a special of nylon then recorded some note on them about date, number and name of the structure until reading time under the microscopic. Between all the structures were analyzed in the current study, operculum structure was the most reliable method for ageing *A.vorax*. Annual growth rings were the most clear and sharper leading to lesser errors in the age estimation (Figure 3.12). After the ageing structures we observed, operculum were the most reliable and suitable structure for ageing *A.vorax* depend on the PA, Index of APE and CV values.

PA of ages between the three readers was the highest for operculum (28.77 %) followed by dorsal spin (24.66 %), scale (16.44 %) and vertebrae (13.70 %) as (Table 3.2). Also operculum had the lowest in IAPC (3.79 %). And CV (7.8 %) and SD (0.5 %). Values followed by scale, vertebrae, dorsal spin (Table 3.3).

When age estimation were compared between operculum and vertebrae it was observed that the highest PA was 71.23%. And the lowest APE between them was (2.70 %). Relationships between all of the methods are summarized in the (Table 3.1). Age composition of all the examined specimens throughout the this study ranged between 4 and 8 years of age.

In this study, when various bony structures were compared, the least age difference was found between operculum with vertebrae and followed by operculum with scale and vertebrae with scale and dorsal spine with vertebrae and dorsal spine with operculum and scale as in the (Table 3.1).

According to this work the otoliths were not possible for age determination because of it was covered with dark opaque and we could not to see clearly under the microscopic (Figure 3.10). The reliability was decreased followed by scales ,dorsal spine, in (Table3.2).

Table 3.1. Age differences between structure.

Bony structures compared	Age differences								Total N
	0		1		2		3		
	N	%N	N	%N	N	%N	N	%N	
Dorsal spine-Vertebrae	9	12.33	45	61.64	18	24.66	1	1.37	73
Dorsal spine-Operculum	6	8.22	35	47.90	30	41.09	2	2.70	73
Dorsal spine-Scale	6	8.22	30	41.09	28	38.35	9	12.33	73
Vertebrae-Operculum	52	71.23	19	26.02	2	2.70	-	-	73
Vertebrae-Scale	32	43.83	35	47.90	6	8.22	-	-	73
Operculum-Scale	42	57.53	30	41.09	1	1.37	-	-	73

Table 3.2. Compliance groups.

Bony structures	Compliance groups						Total N
	3/3		2/3		1/3		
	N	N%	N	N%	N	N%	
Dorsal spine	18	24.66	51	69.86	4	5.48	73
Vertebrae	10	13.70	60	82.19	3	4.11	73
Operculum	21	28.77	46	63.01	6	8.21	73
Scale	12	16.44	50	68.49	11	15.07	73

Table 3.3. Age comparative.

	Dorsal spine	Vertebrae	Operculum	Scale
Min	3	3	4	4
Max	8	8	8	9
Mean	5.1	5.9	6.4	6.4
SD	0.5	0.5	0.5	0.6
CV	10.5	9.5	7.8	9.0
D	6.1	5.5	4.5	5.2
IAPE%	7.95	7.01	3.79	2.84

IAPE: Index Average Percentage Error , **PA:** Percentage of Agreement , **CV:** coefficient of variation, **APE:** Average Percentage Error , **SD:** Standard Deviation and **D:** Deviation).

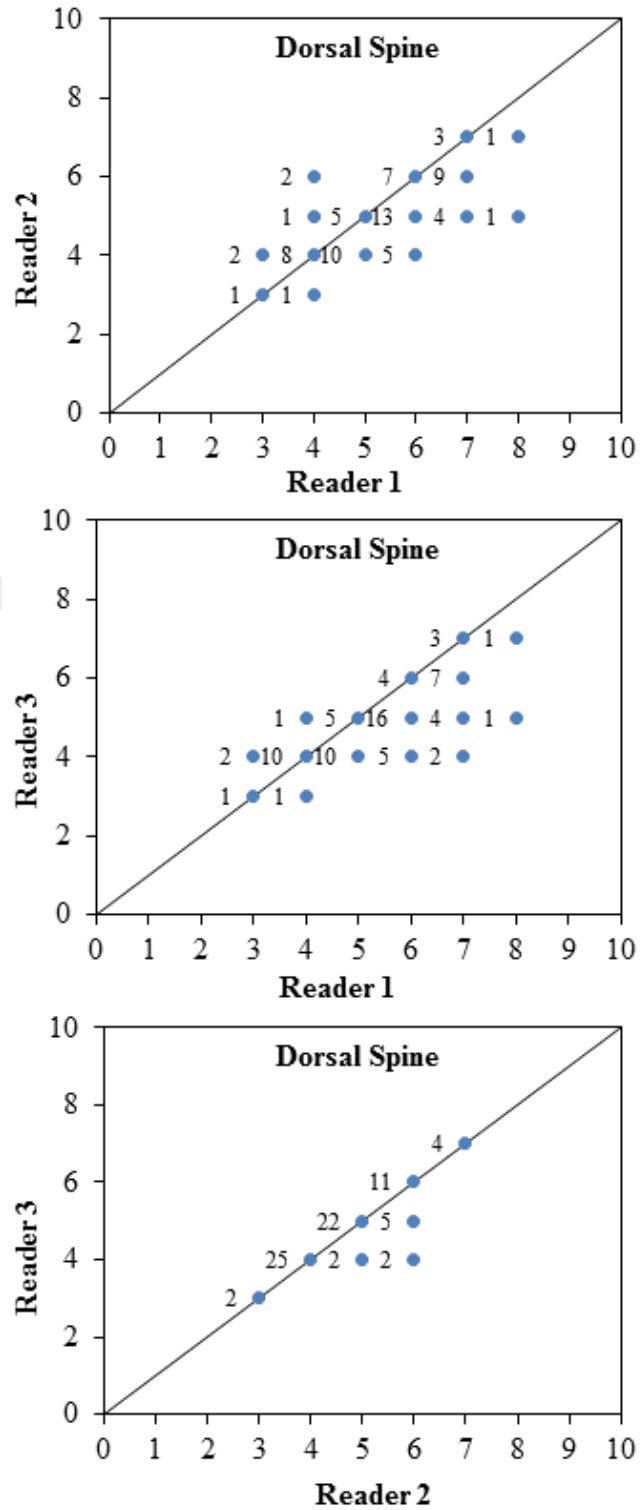


Figure 3.1. The comparison of dorsal spine age between 3 readers on *A. vorax* from Karakaya Dam Lake

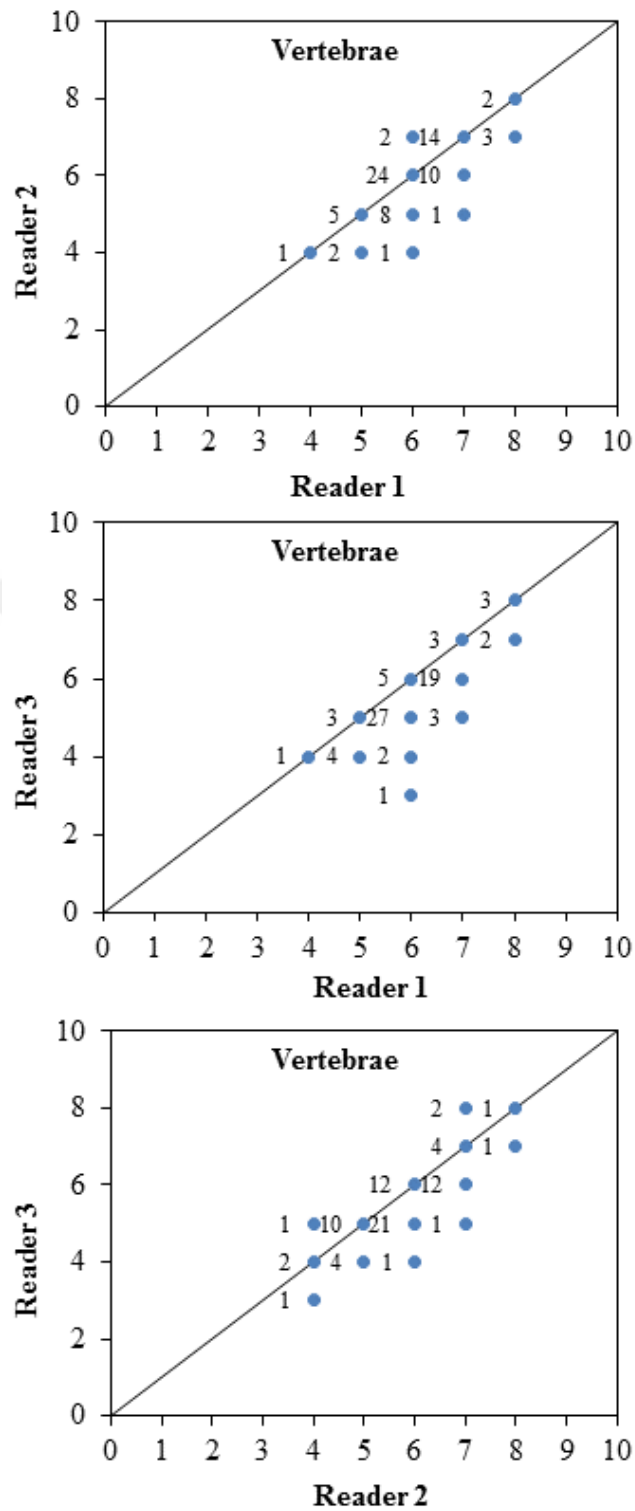


Figure 3.2. The comparison of vertebrae age between 3 readers on *A. vorax* from Karakaya Dam Lake

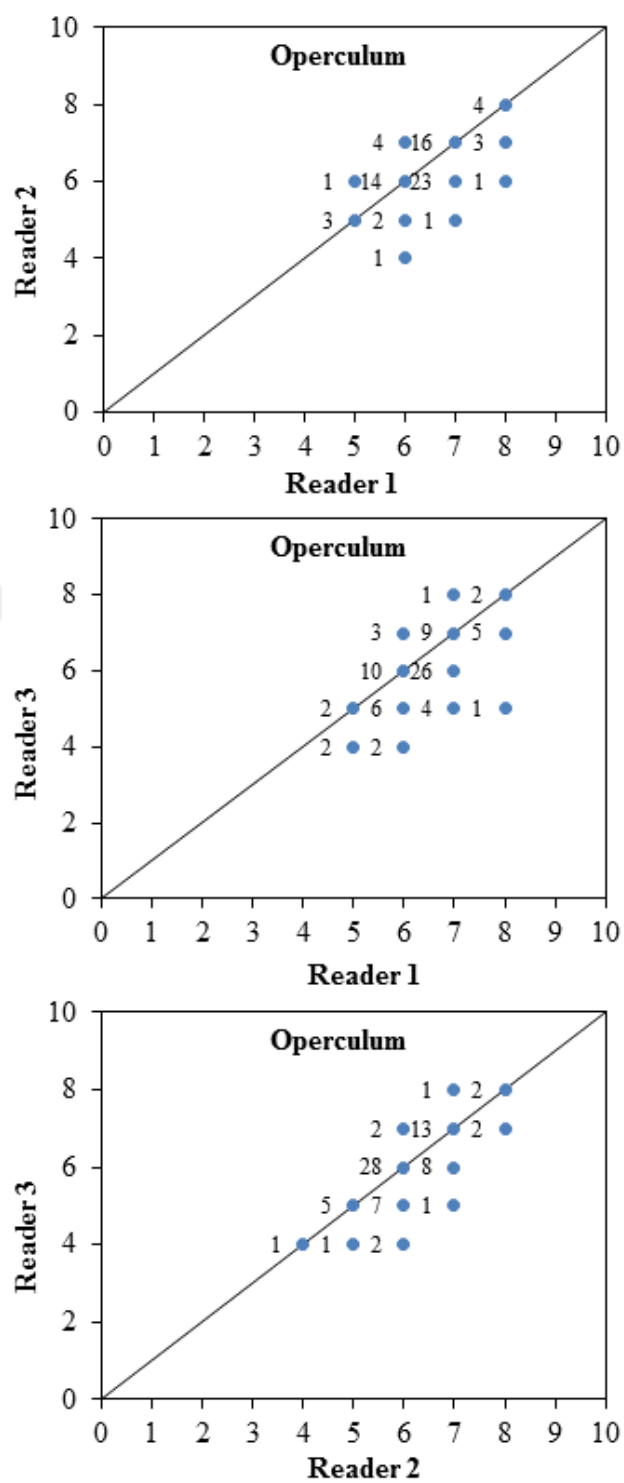


Figure 3.3. The comparison of operculum age between 3 readers on *A. vorax* from Karakaya Dam Lake.

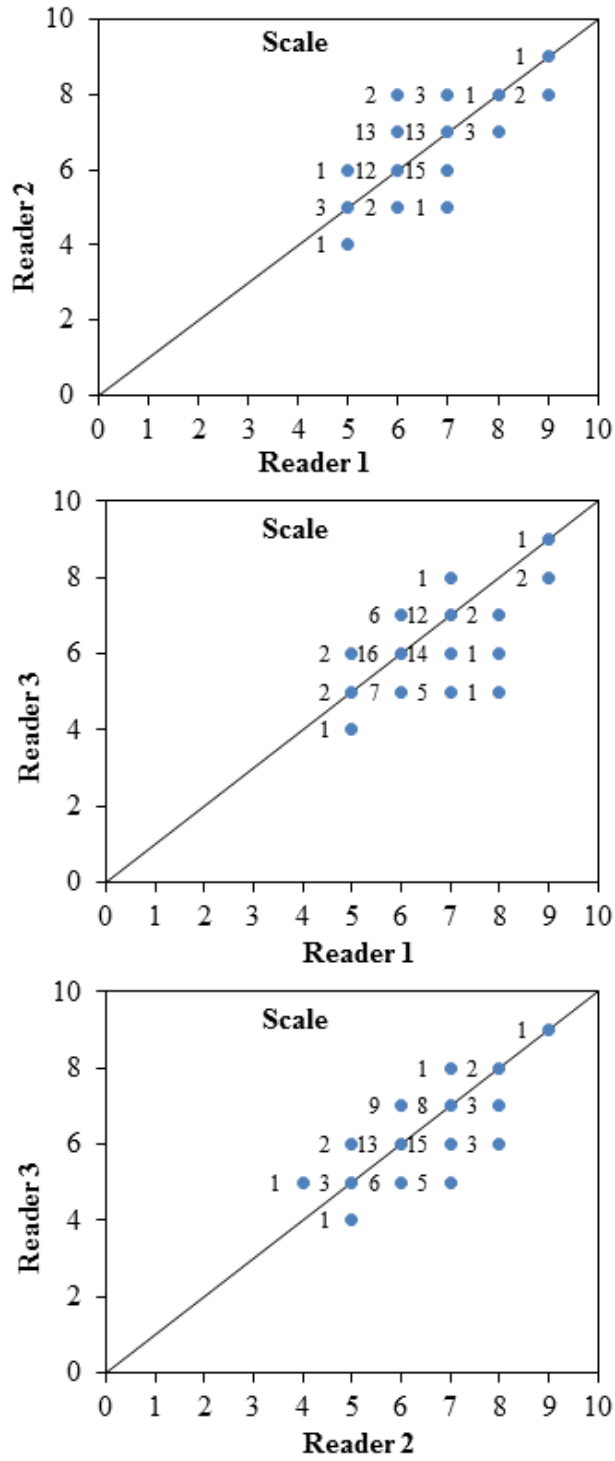


Figure 3.4. The comparison of scale age between 3 readers on *A. vorax* from Karakaya Dam

Lake

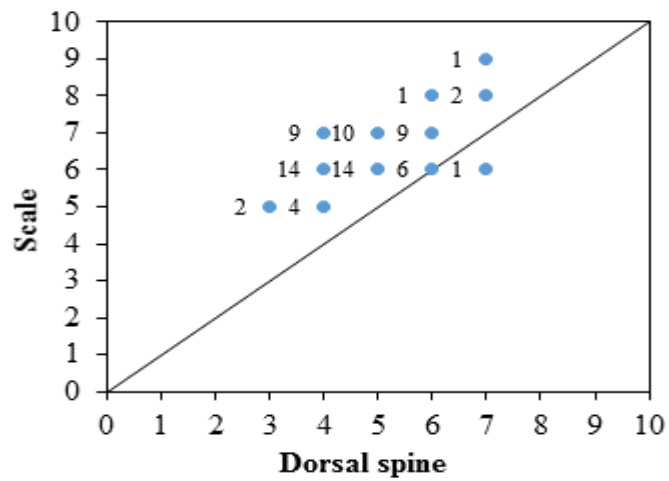
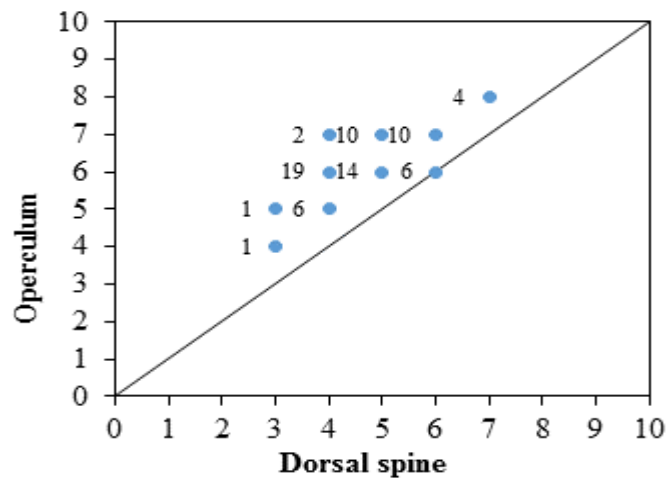
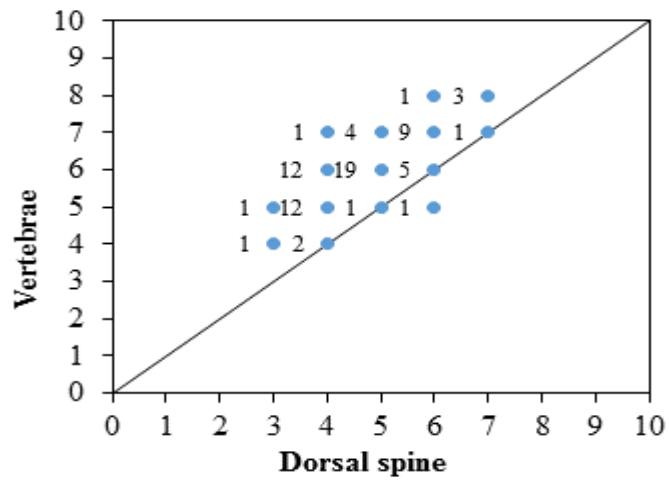


Figure 3.5. Differeens between dorsalfin with sacle ,operculum and vertebrae.

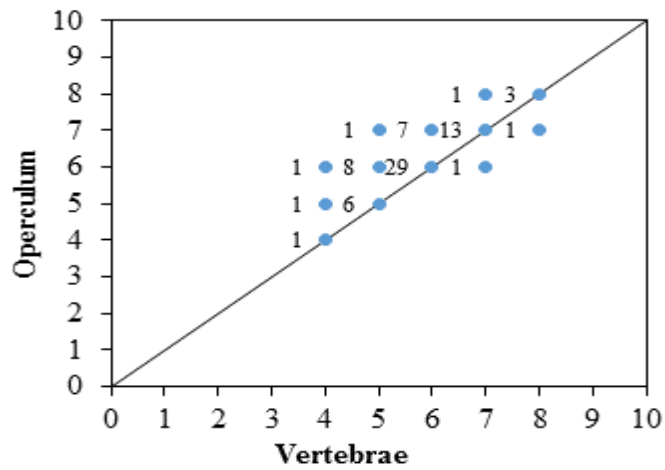


Figure 3.6. Comparative between operculum and vertebrae.

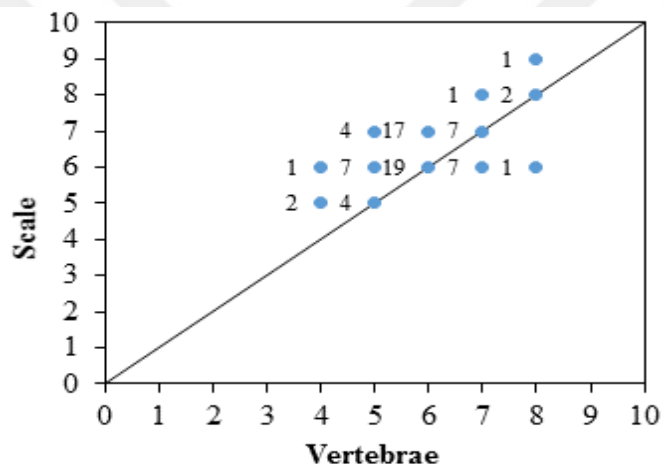


Figure 3.7. Comparative between operculum and scale.

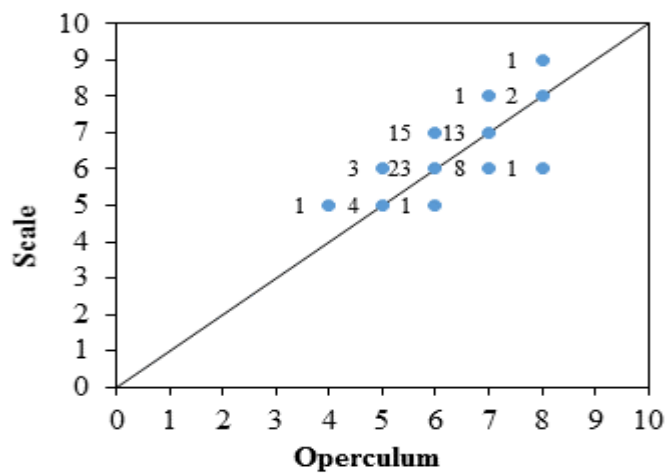


Figure 3.8. Comparative between vertebrae and scale.



Figure 3.9. Dorsal fin 6 years old of *A. vorax*

Dorsal spine had been found to be suitable than the otoliths for ageing there is parts of 0.35-0.60 mm were received by a jewellery saw and then Sections were placed on a microscopic slide and observed under dissecting microscope to evaluated aging (Figure 0.1.)

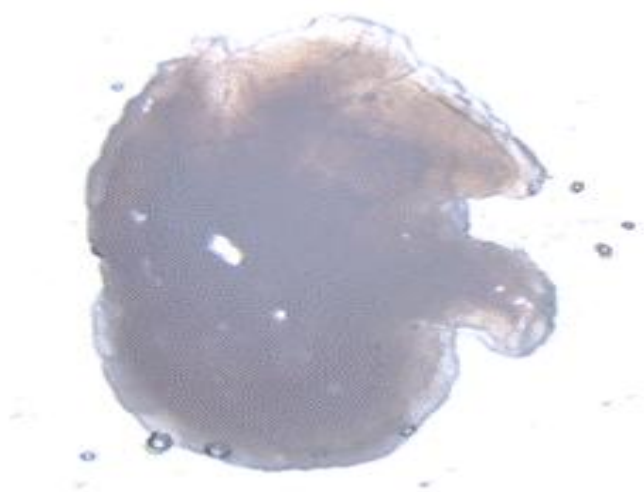


Figure 3.10. Otolith 4 years old of *A. vorax*.

Otoliths were not possible for age determination because of it was covered with dark layer of opaque and then we could not to observed annuli rings clearly under the

microscopic like(Figure 0.2) for that reason we excluding it for using in this study current.



Figure 3.11. Scale 5 years old *A. vorax*

Scales were putted between two glass slides and then studied uneder microscope to estimate ageing which was easy for preparing and suitable for age determination compared with otolith .Observed of annuli rings of scales in *A. vorax* under microscop (Figure 3.3)



Figure 3.12. Operculum 8 years old of *A.vorax* .

Anuli rings in opercular bone it was very clear and facilitate observed for age reading and it was more reliable than other structure for ageing of *A. vorax* it was most precision and accurate bone (Figure 0.4.).

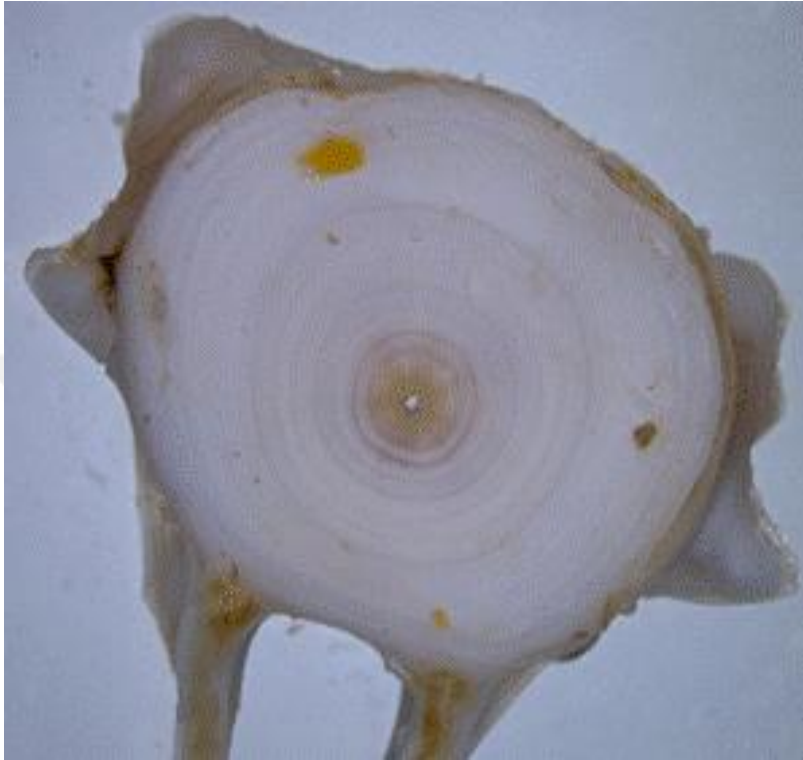


Figure 3.13. Vertebrae 8 years old of *A. vorax* .

For age estimation vertebrae centra was second structure which is coming for ageing of *Aspius vorax* next of operculum it was possible and easy for observed annuli rings under microscope and suitable for age determination in *A. vorax* (Figure 0.5)

4. DISCUSSION

Shafi and Jasim [58] were reported that scales were used for the determination of age with back-calculation of length for *A. vorax*. Duman and Gul [84] used vertebrae for age determination on *Aspius vorax* in Kara kaya Reservoir (in Euphrates River), Turkey and Oymak et al. [27] used ten to fifteen scales were removed from the right side of each fish body just between the lateral line and dorsal fin base on *Aspius vorax* in Atatürk Dam Lake (Euphrates River), Turkey. Szypula et al. [40] was reported total length (TL) for *Aspius vorax* from the Middle Reaches of Euphrates River ranged between 19 and 70 cm corresponding with 46 to 2824.5 g weight, respectively. The mean weight of *A. vorax* was reported higher than those stated for each age group. Oymak et al. [27] was reported, the relationship between total length and total weight of *Aspius vorax* in Atatürk Dam Lake (Euphrates River), Turkey.

Opercular: Opercular bones had been reported to be better than scales for age estimation of *C. carpio* [65]. Used opercular bones of *C. catla* for age reading. The determination of age and growth of fish from opercular bone is well established in fishes of temperate waters and has been discovered to be more satisfactory than other ways such as scales, vertebrae, spines or other hard sections in common pike, *Esox lucius* [66,67]. And European perch, *Perca fluviatilis* [68]. Rings on opercular bone of younger age group fishes were clearer and much more effortlessly recognizable than in the older age groups. Similar observations have also been reported by other researchers [66,69]. Mentioned that the extraction, preparation and reading of opercular bones in comparison with scales were relatively easier and cited that the rings on scales and opercular bones were due to periods of quick or slow growth imposed by reproductive energetic demands in blue tilapia, *Oreochromis aureus* [70].

Vertebrae: Vertebrae had been reported to be the most reliable structure for age determination in Nile perch, *Lates niloticus* [71,72]. Compared different bony parts of north Atlantic flounder, *Pleuronectes flesus luscus* for age determination were reported vertebrae has the most reliable age structure having minimal ageing error. [73]. Compared vertebrae, otoliths and scales for ageing fall chum salmon, *Oncorhynchus keta*. The researcher observed that time required to process and read vertebrae (20 times as long as scales) made them less practical to use but the precision and accuracy associated with vertebrae made them best of the three structures researched. Vertebrae have rarely been

used to study age estimation in fishes which is displayed clear growth marks in other ageing structures that lead to lesser or no damage to the fish. In the present study, the band of rings on vertebral centra were not very clear and displayed numerous minute marks unrelated to cyclic events which corroborates with the observations made by [74].

Dorsal spine: Dorsal spine were reported to be most suitable for ageing *Salmo trutta* than otoliths [47]. Dorsal spine had been found to be more ideal than the otoliths for ageing *brown trout, Salmo trutta* [47]. Also for other structures to determination of ageing *Barbus rajanorum mystaceas* and *C. trutta* [64]. In *thin-lipped grey mullet, Liza ramada* population from Mersin bay, dorsal spine were discovered to be the most reliable bony structure for age determination followed by scales, vertebrae, otoliths and operculum [75]. Dorsal spine were reported to be more suitable for ageing *Barbus rajanorum mystaceus* and *Capoeta trutta* [64]. The researcher also explained that a lot of ages in *C. carpio* were overestimated by scales, vertebrae and opercles through age 6 but underestimated beyond age 10. Use dorsal spine does not need sacrificing the fish, and dorsal spine can be eliminated without any obvious harm to the fish [76]. Dorsal spine annuli are reported to keep prominent for older fish when scale annuli are not recognizable [77]. Dorsal spine were reported most suitable for ageing *shabut, Barbus grypus* [86].

Scale: There are a lot of studies reported that scales have been most suitable method for age determination [59,60]. because of they are more practical and easier to prepare. However, in that work, the annual rings on scales of *L. ramada* could not be easily distinguished as also reported for *Grey Mulletts* [61]. In most of the *cyprinids*, age has been estimated from scales [78]. while scales and vertebrae were better as compared with otoliths in *Leuciscus cephalus* [63]. In common carp, *Cyprinus carpio* comparison with the various bone structures (scales, opercular bone, dorsal spines and sagittal otoliths) indicated scales to be the best structure for evaluate age but in some case of fish with unreadable scales using of the fin parts was recommended [79]. Amongst all age structures, scales were found to be the most suitable ageing structure in *L. rohita* and *C. marulius* in the present study. In addition to having clear and sharp annuli, scales also provide the advantages such as easy collection, preparation and being non-destructive to the fish. This is in corroboration with the findings of other studies. In *shir mahi, Capoeta trutta* [80]. Found rather clear annuli on scales though best annuli were reported in dorsal spine.

The annuli on scales of *C. trutta* were reported better than those on the otoliths and operculum [63]. In some scientific reports, the use of scales was criticized mainly because of the frequent underestimation of the ages of older fish [81].

Otolith: Otolith sometimes, the interpretation of the otoliths is complicated due to existence of false rings [82]. Which are often deposited corresponding to the crucial moments of the fish life cycle such as sexual maturity. There are reports that the use of whole otoliths, as used in the present study, can lead to underestimation of the ages when compared with sliced otoliths [83]. Otoliths were reported to be the most reliable ageing structure in transcaucasian barb, *Capoeta capoeta umbla* (Heckel) [55]. It is comparably more difficult to recognize annual rings on otoliths as they are opaque [62]. for that reason, otoliths are generally used together with scales in age determination in fish.

Between all structures when we used throughout the work of age determination of *A. vorax*, operculum had been discovered to be the most reliable bony structure and then vertebrae have the high precision and used to study age evaluation in fishes which was showed clear growth marks in ageing structures (Figure 3.13). Followed by ,dorsal spine,scale and otolith in the current study. Annual rings on the operculum were better defined and easier to observe as compared to those on the other structures. As this ageing method resulted in less ageing error, and highest average percentage of agreement, it is suggested to be the most reliable method in the age determination of *A. vorax* . The reliability was decreased in the order of vertebrae, dorsal spine,scale and otoliths bones.

In this circumstances , it is obviously appear that operculum and vertebra have the highest percentage of agreement and the lowest average percentage of ageing error (Table 3.1). for that reason, the operculum and vertebra are the most reliable bony structure for the age determination of *Aspius vorax*.

5. CONCLUSION

Vertebral centra and operculum were estimate to be most reliable bony structure for determine ageing of *A. vorax* from 4 to 8 of years old and they were very close relationship between vertebral centra and opercular bones in this study and also because of several advantages discussed elsewhere vertebral centra may be recommended as ageing structure, if non-destructive method is to be used.



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