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Age Determination Technique According to the Calcium Accumulation in Bony Structures of *Capoeta umbla* (Heckel, 1843)

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Abstract: In this study, the ages of individuals belonging to *Capoeta umbla* (Heckel, 1843) populations obtained from Keban Dam lake and Hazar lake were determined from 5 different bony structures and the level of calcium mineral of each bony structure was measured by atomic absorption spectrophotometer. The results were compared between populations of Keban Dam lake and Hazar lake. In addition, the determined calcium element was statistically examined according to age, length and weight of fishes. The relationships between accumulation of calcium in some bony structures and fish size (both length and weight) and age were found insignificant ($p>0.05$). However, the best relationships between calcium accumulation and fish size and age were found in vertebra, otolith and dorsal fin ray. In conclusion, although there were some differences of calcium accumulation in the same bony structures of fishes obtained from Keban Dam lake and Hazar lake in general there were similarities. In addition, the accumulation of calcium on some bony structures was observed to increase with the increasing of age, length and weight of fishes but these increases were not statistically important.

Key words: *Capoeta umbla*, calcium, age determination, Keban Dam lake, Hazar lake, vertebra

INTRODUCTION

Mineral elements have a wide variety of uses within the animal body. Calcium is one of the most important among these elements. Calcium plays an important structural role in the development and maintenance of bone tissue. The most abundant mineral in living organisms, calcium has several important functions. There is strong evidence that low calcium intake can lead to fragile bones. More than 99% of total body calcium is stored in the bones and teeth where it functions to support their structure. The remaining 1% is found throughout the body in blood, muscles and the fluid between cells. Calcium is needed for muscle contraction, blood vessel contraction and expansion, the secretion of hormones and enzymes and sending messages through the nervous system. Fish take in such minerals from food and by gills from water (Lall, 1989; McCormick *et al*., 1992; Sari and Cakmak, 1996; Egemen and Sunlu, 1999; Calta and Canpolat, 2001; Ekingen, 2001; Aydin *et al*., 2008).

The ability to perform age determinations based on the examination of hard anatomical parts is of fundamental importance in fisheries research. The use of age information is an integral part of fisheries today. Thus, age determination has been done in many studies and the other results have been examined depending on age. Precise and accurate age determination is fundamental to the study of fish population structure and individual growth rates. The effect of inaccurate age determinations on population dynamics studies can lead to serious errors in stock assessment which results in overexploitation. Fish age and growth are also critical correlates in evaluating many other biological processes such as productivity, prey availability, habitat suitability and even feeding kinematics. Fish are cold blooded animals. Thus, their metabolic and physiologic activities depend on water temperature. Fish show various growth rates in a year depending on the seasons. This brings about the formation of annulus on some bony structures because of the different depositions of calcium in each season. Age determination in bony fishes can be carried out by counting seasonal growth annuli appearing on hard structures such as the otolith, scale, fin ray, vertebra and operculum. The readability of annulus changes depending on the amount of calcium deposition on the bony structure. In most of the cartilaginous fish, age determination is impossible using conventional structures because of less calcification of bony structures (Lagler, 1956; Chugunova, 1963; Tesch, 1968; Ekingen, 1983; Polat, 1986, 2000; Beamish and McFarlane, 1987;

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Sarihan, 1988; Celikkale,1991; Erkoyuncu, 1995; Polat and Gumus 1995; Geldiay and Balik, 1996; Avsar, 1998). Although many studies have been conducted on age determination from various bony structures in bony fish, only two studies have been conducted on the level of calcium deposition on the bone structures and the relationships of calcium deposition with annulus formation (Calta and Canpolat, 2001; Aydin *et al.*, 2008).

However, both of these studies have been carried out in the same species obtained from the same sampling sites. Therefore, the present study has been carried out in the same species obtained from the different locations as the calcium deposition on bony structures of fish may depend on the limnological characteristics of the water reservoir in which they live. The objectives of this study are to clarify the relationship between annuli formation and the higher concentration calcium deposition in bony structures of the *C. umbla* and to understand the readability of annulus changes depending on the amount of calcium deposition on the bony structures.

MATERIALS AND METHODS

A total of 100 *Capoeta umbla* (Heckel, 1843) samples were caught in Keban Dam lake and Hazar lake for the purposes of this study. In order to obtain fish samples of various sizes, gill nets, trammel nets with 18, 28, 36 and 44 mm mesh sizes were used in the catches. In this study, 100 individuals belonging to *C. umbla* populations obtained from two different habitats (Keban Dam lake and Hazar lake) were examined in terms of the levels of calcium mineral of each bony structure. The calcium accumulation in five different bony structures taken from each fish was measured by atomic absorption spectrophotometer.

Captured fish were placed in freezer plastic bags with ice and immediately transported to the laboratory. The total length and weight of each fish were determined and then the scale, vertebra, otolith, dorsal fin ray and operculum bones were removed. They were prepared for age determination using the techniques in Lagler (1956) and Chugunova (1963). Firstly, they were cleaned from soft integuments and dried. The scale, vertebra, otolith and operculum bones were used as a whole and a small part of the dorsal fin ray was crosscut for microscopic examination. In order to determine age, all bony structures were read several times by three experts independently. The readings which repeated most frequently for each of bony structures were accepted as the age of the fish. The ages of these bones were determined using a stereomicroscope (Kyowa Optical, Model SD-2PL, Nagano, Japan) and then weighed by means of a balance (Shimadzu, Model UW620H, Kyoto, Japan) with 0.001 g sensitivity. Finally, they were transferred individually to 4 mL glass vials washed previously with 0.1 N nitric acid, dried and weighed and then they were dried in an oven at 105°C for 24 h and digested on a hot plate by adding 2 mL of nitric acid (65%, Merck, Whitehouse Station, NJ, USA). Digested samples were kept at room temperature for 24 h and then diluted to 50 mL with double-distilled water. Standard solutions for calibration graphs were prepared. Blanks were also prepared using the procedure as above. The diluted samples and blank solutions were analysed using an atomic absorption spectrophotometer (Perkin Elmer Model 370, Perkin Elmer, Wellesley, MA, USA) for determining the calcium level.

The concentrations of calcium were determined from the calibration graphs and calculated on a milligram per gram bone weight basis (mg g^{-1}). All statistical analyses were done using SPSS 12.0 software (SPSS, Chicago, IL, USA). The amount of calcium in different bony structures was tested using one-way analysis of variance (ANOVA, Duncan's multiple range test) to determine differences at 5% ($p<0.05$). Regression analysis was used to predict the significance level of the relationship between calcium concentration and age groups. The significance difference between male and female accumulation of calcium was checked using a bivariate correlation test.

RESULTS AND DISCUSSION

Some characteristics of experimental animals: In this study, the ages of individuals belonging to *C. umbla* (Heckel, 1843) populations obtained from Keban Dam lake and Hazar lake were determined from 5 different bony structures. Ages of the fish obtained from both water reservoirs ranged between 3 and 8.

The mean length and weight of the fish from Keban Dam lake and Hazar lake were found as 300.32 and 297.28 cm; 247.07 and 241.04 g, respectively. In both water reservoirs, the most abundant age group was observed to be the groups IV, V VI and the less abundant age group was VIII. Both Keban Dam lake and Hazar lake fishes in terms of the age groups distribution have been observed alike.

Calcium amounts in bony structures: In general, the highest calcium levels in the Keban Dam lake fishes were found on dorsal fin ray followed by the otolith, vertebra, operculum and scale and the highest calcium levels in the Hazar lake fishes were found on vertebra followed by otolith, dorsal fin ray, operculum and scale (Fig. 1). There was an increase in the accumulation of calcium in all bony structures with an increase in the weight, length and age

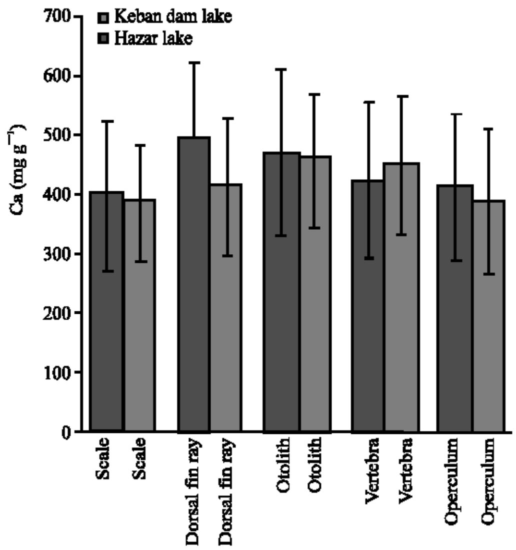

Fig. 1: Calcium amounts (mean value, n = 50 each of area) in various bony structures of the *Capoeta umbla* (Heckel, 1843) populations obtained from Keban Dam lake and Hazar lake. Bars represent±SD. Difference letters above the bars indicat significant differences (p>0.05; ANOVA, Duncan's multiply range test)

of fish. However, these increases in the accumulation of calcium in all bony structures were not found to be statistically significant (p>0.05).

Relationships between calcium amount in bony structures and fish size: There were some differences between the accumulation of calcium in some bony structures with the fish size (both length and weight) in the Keban Dam lake and Hazar lake fishes. Although, the best relationship between the accumulation of calcium in some bony structures with the fish size was found in the vertebra, otolith and dorsal fin ray, this relationship in the scale and operculum was observed weak (Fig. 2 and 3). Among all bony structures, the best correlations between the accumulation of calcium and the fish weight in the Keban Dam lake fishes were found in the otolith (r = 0.69), followed by the dorsal fin ray (r = 0.61), vertebra (r = 0.53), scale (r = 0.49) and operculum (r = 0.41) while it was in the vertebra (r = 0.74) followed by otolith (r = 0.66), dorsal fin ray (r = 0.55), operculum (0.43) and scale (r = 0.37) for the Hazar lake fishes (Fig. 2).

Among all bony structures, the best correlations between the accumulation of calcium and the total length in the Keban Dam lake fishes were found in the otolith (r = 0.62) followed by the dorsal fin ray(r = 0.51), vertebra (r = 0.46), scale (r = 0.45) and operculum (r = 0.35), as for in the Hazar lake fishes was found in the otolith (r = 0.57), followed by the vertebra (r = 0.54), dorsal fin ray (r = 0.47), scale (r = 0.29) and operculum (0.27) (Fig. 3). There was an increase in the accumulation of calcium in all bony structures with an increase in the fish size in the both water reservoirs. However, the relationships between the accumulation of calcium in all bony structures were not found to be statistically significant (p>0.05; both Keban Dam lake and Hazar lake).

Relationships between calcium amounts in bony structures and age of fish: The best linear correlation between the accumulation of calcium and the age of fish in Keban Dam lake was found in the otolith (r = 0.74), followed by the dorsal fin ray (r = 0.68), scale (r = 0.44), operculum (r = 0.43) and vertebra (r = 0.34) as for in the Hazar lake fishes was found vertebra (r = 0.71) followed by dorsal fin ray (r = 0.55), otolith (r = 0.48), scale (r = 0.38) and operculum (0.37) (Fig. 4). Although the best correlation between the accumulations of calcium and the fish age was found in the vertebra of the Hazar lake fishes, this correlation was found the least level in the vertebra of Keban Dam lake fishes. This difference may be due to the fact that the fish live in different habitats. In general, all the rest bony structures in the both areas fishes were showed resemblances in point of the Ca accumulation in the bony structures. In addition, the correlations between calcium amounts in five different bony structures and sex of *C. umbla* in both areas were not found to be different between males and females (p>0.05).

Calcium is one of the most essential mineral for fish as it is in all vertebrates. Calcium plays an important structural role in the development and maintenance of bone tissue. It assures the strength and firmness of the bone and makes teeth harder. Low calcium levels have been correlated with abnormal bone crystal formation and osteoporosis in the vertebrates. Trace elements in calcified tissues have been suggested as one of the most powerful means for the age determination of fishes.

In this study, the ages of individuals belonging to *C. umbla* populations obtained from Keban Dam lake and Hazar lake were determined by examining hard structures such as the otolith, scale, dorsal fin ray, vertebra and operculum and then the levels of calcium accumulation in each bony structure were measured with atomic absorption spectrometer.

The calcium accumulations in the same bony structures of the individuals obtained from different habitats (Keban Dam lake and Hazar lake) were compared. In addition, the determined elements were statistically examined according to the age, length and

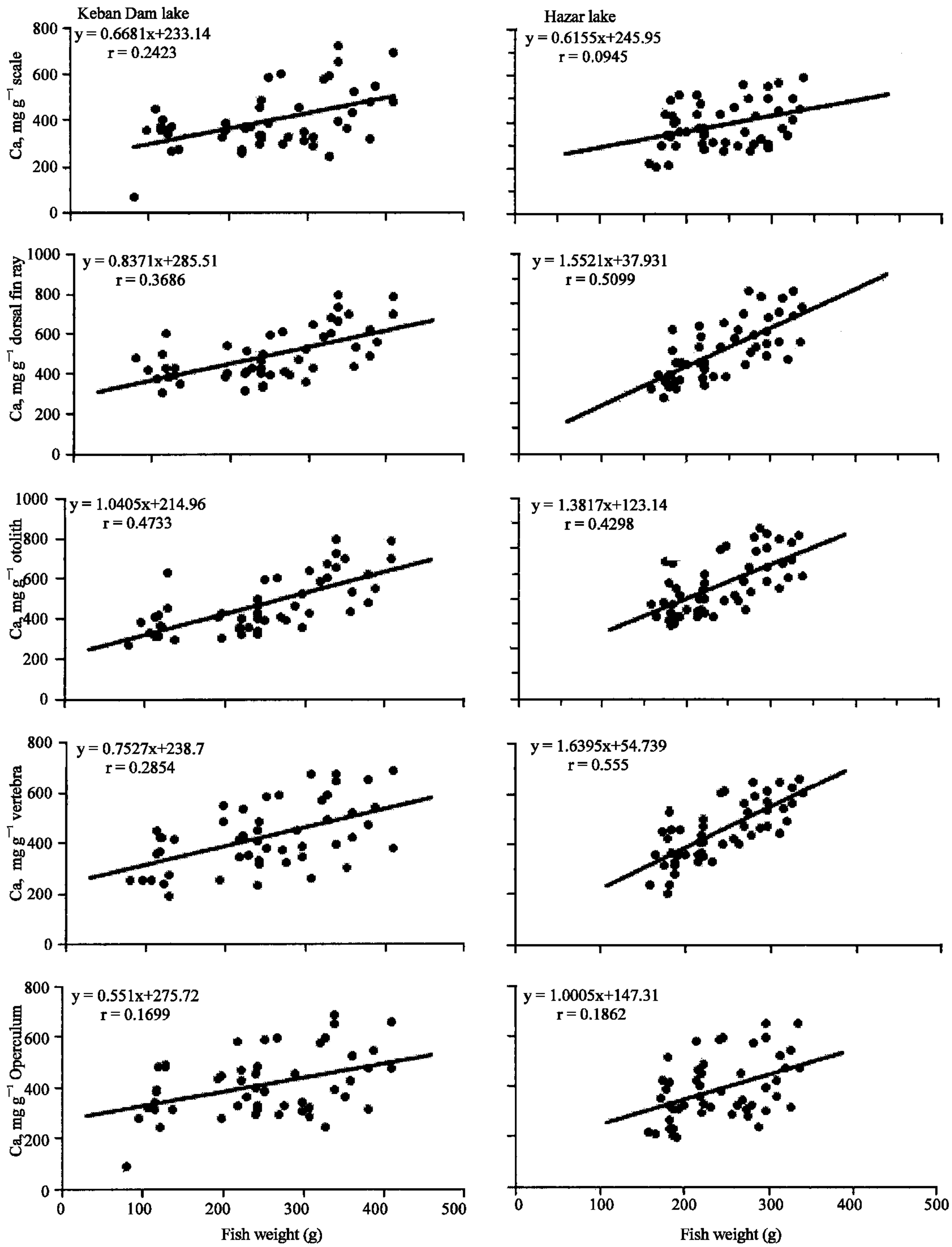

Fig. 2: Relationships between calcium amounts in various bony structures and body weights of *Capoeta capoeta umbla* obtained from Keban Dam lake and Hazar lake

weight of fishes. These results show that the fish from both sites have less calcium on their scales than they have in their other bony structures. In general, the highest calcium levels in the Keban Dam lake fishes were found in the dorsal fin ray followed by the otolith, vertebra, operculum and scale, the highest calcium levels in the Hazar lake fishes were found in their vertebra followed by otolith, dorsal fin ray, operculum and scale. There was an

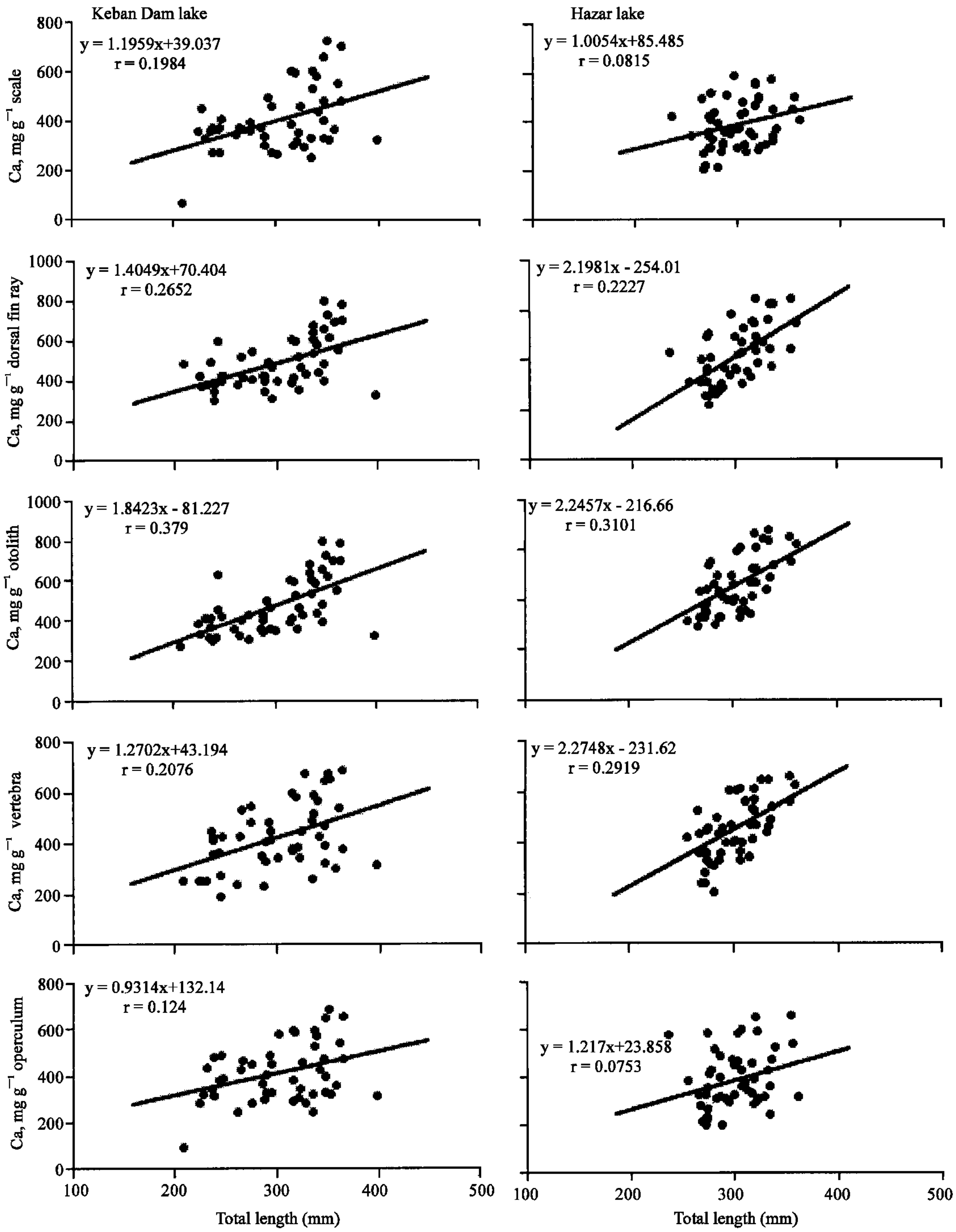

Fig. 3: Relationships between calcium amounts in various bony structures and total lengths of *Capoeta capoeta umbla* obtained from Keban Dam lake and Hazar lake

increase in the accumulation of calcium in all bony structures with an increase in the weight, length and age of fish. The relationships between the accumulation of calcium in all bony structures with an increase in the weight, total length and age of fishes in the both areas were not found to be statistically significant ($p>0.05$). There was some evidence that the high calcium level was linked with readability of bone structures. In the light of

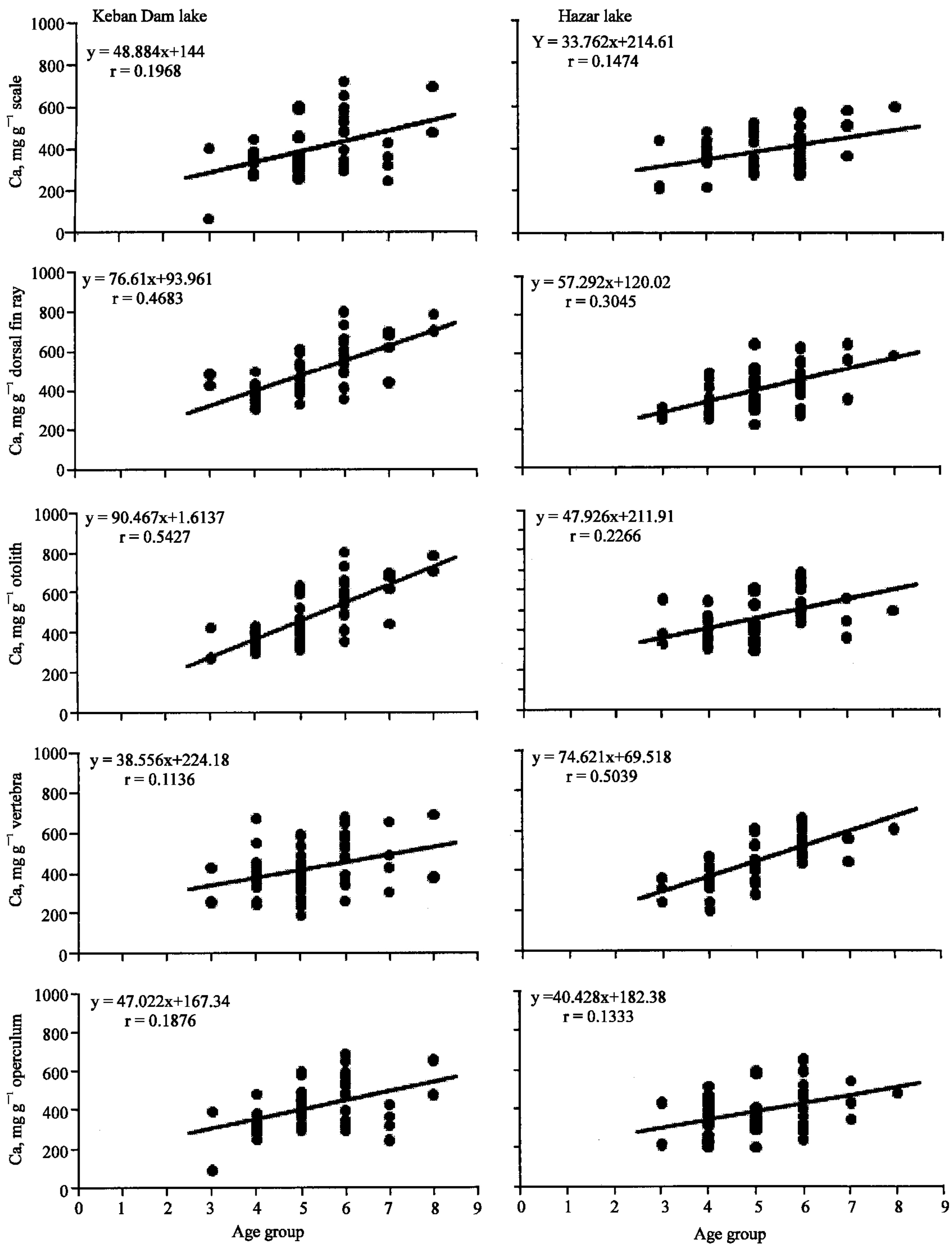

Fig. 4: Relationships between calcium amounts in various bony structures and age groups of *Capoeta capoeta umbla* obtained from Keban Dam lake and Hazar lake

this, vertebras collected from Hazar lake were significantly higher in terms of calcium accumulation than those of collected from Keban Dam lake. Thus, the Hazar lake vertebras were more easily determined than those obtained from Keban Dam lake in terms of readability. In general, the ages of the bony structures with high calcium levels were more easily estimated than those of the bony structures with less calcium level.

Aydin *et al.* (2008) investigated on the accumulation of calcium in some bony structures such as the otolith, scale, dorsal fin ray, vertebra, cleithrum and operculum with the fish size (both length and weight) and age of *Cyprinion macrostomus*. In their study, very strong correlations were found between calcium deposition and fish age in some bony structures. This correlation was seen especially between the vertebra and otolith and fish age in their study.

However, they conducted their study on the fish of a certain species obtained from the same sampling site. In the present study, the same species fish are obtained but obtained from the different habitats. Aydin *et al.* (2008)'s findings match up with those of the present study in terms of accumulation of calcium in the same bony structures such as vertebra, otolith and dorsal fin ray. In addition, they too found the lowest correlations in the scale and operculum.

The qualitative calcification profile of bony structures of rainbow trout during the early development stage has been determined by Calta and Aydin (1998). They found that only a small part of the skull was calcified in the just hatched larvae. It was followed by the vertebrae on day 3, dorsal fin rays on day 8, anal fin rays on day 12 and operculum on day 30. During this time, scales had not yet formed. These qualitative profiles of calcification have similarities to the quantitative profiles of calcification in the present study.

The scales are the bony structure mostly commonly used for age determination in fish, as it is more practical and can be cleaned easily. However, the scale does not form together with the embryo. It forms when the fish reaches a certain size, depending on the fish species (Chugunova, 1963; Tesch, 1968; Ekingen, 1983; Avsar, 1998). In addition, it was reported that the calcium was reabsorbed from the scale in some fish species (Polat, 2000). All these may be the reasons for the lowest level of calcium in the scale and operculum compared with the other bony structures examined.

A previous study (Cailliet, 1990) showed that conventional structures used for age determinations of teleost fishes (e.g., fin rays, otoliths, scales) cannot be used for elasmobranches which have cartilaginous structures. However, vertebral centre with systematic deposits of calcium phosphate have been used for age estimation in a number of elasmobranches species such as the tiger shark (*Galeocerdo cuvier*), scalloped hammerhead (*Sphyrna lewini*) and several smoothhound sharks (*Mustelus* sp.). Therefore, this new technique may provide an alternative age determination method for species in which the vertebral centre or dorsal fin rays are poorly calcified.

CONCLUSION

In conclusion, although there were some differences of calcium accumulation in the same bony structures of fishes obtained from Keban Dam lake and Hazar lake in general there were similarities. Very strong correlations were found between calcium deposition and the same bony structures (especially the vertebra, otolith and dorsal fin ray).

The correlation between calcium deposition and fish age may be helpful in determining the ages of fish with an unclear annulus in the same population because some individuals in a fish population do not have a clear annulus in any bony structures in their bodies because of the occurrence of fake annulus.

This technique may be an alternative age determination method for these fish. In addition, this technique may be applied on cartilaginous fishes. However, the application of this technique on the same fish species obtained from various locations may be disadvantageous as the calcium deposition on bony structures of fish may depend on the water quality of the water reservoir in which they live because it should be kept in mind that water reservoirs have different limnological characteristics.

Especially, water temperature and food availability in a reservoir have an important effect both on the growth of fish and surely on the calcium deposition in bony structures (Aydin *et al.*, 2008). Therefore, the present study has been carried out on the same species obtained from the different habitats. There were some differences between the accumulation of calcium in some bony structures and the fish size in the Keban Dam lake and Hazar lake fishes.

However, both in this study and the previous one, the best relationship between the accumulation of calcium in some bony structures and the fish size were found in the vertebra, otolith and dorsal fin ray.

Therefore, the correlations between calcium deposition with fish size (both length and weight) and age may be helpful in determining the ages of fish. Since there have been no other studies on this, any comparison couldn't be done. Therefore, it believe that the present study may prove to be a useful model for similar future studies. We are of the opinion that this technique may be more useful and practical in determining the ages of cartilaginous fish.

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