Otolith and scale morphologies of doctor fish (*Garra rufa*) inhabiting Kangal Balıklı Çermik thermal spring (Sivas, Turkey)

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Abstract

Garra rufa is one of the most popular therapeutic and commercially important fish in ichthyotherapy. Otolith and scale morphology provide new and useful information for fish identification and classification. Left-right asteriscus and lapillus otoliths from juvenile and adult doctor fish, and the scales from 6 different regions of the juvenile and adult fish body have been examined in Kangal Balıklı Çermik thermal spring (Sivas, Turkey). The otolith and scale morphological characters such as type, size, shape, mesial surface, lateral surface, antirostrum and rostrum shapes, focus position, circuli appearance, radii type and posterior and anterior margin shapes were distinguishable features for the juvenile and adult *G. rufa* samples. Three different otolith shapes were observed such as squared and discoidal otolith shapes for asteriscus pairs and oval to elliptic otolith shapes for lapillus pairs in the juvenile and adult doctor fish. Seven different scale shapes were described from six different body regions of the *G. rufa* in the current study. The graphical illustration of wavelets was used for both asteriscus and lapillus pairs of the fish to discover otolith variabilities. This is one of the first otolith and scale morphology studies for the juvenile and adult doctor fish. All these otolith and scale characters and their morphologies could be used for best alternative tools to identification, classification, phylogenetic relationships among the different freshwater and marine fish species, genera, populations or stocks.

Keywords: *Garra rufa*, otolithology, Scale morphology, Shape indices

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Introduction

Otolith and scales are crucial and versatile research materials used in ichthyological studies due to their unique characters such as shape, contour, size and shape indices (Esmaeili *et al*., 2014; Bostancı *et al*., 2015). The otolith and scale morphologies have recently increased in importance with the development of image analysis systems and software programs using a Scanning Electron Microscope (SEM) (Razak, 2014; Bostancı *et al*., 2015). Previous studies have indicated fish scale and otoliths were used successfully for identification and discrimination of fish species and stocks (Jawad and Al-Jufaili, 2007; Esmaeili *et al*., 2014; Bostancı *et al*., 2015), and they widely used for age determination (Shimose *et al*., 2015; Elzey *et al*., 2015), growth (Romo-Curiel *et al*., 2015; Mayank *et al*., 2015), prey-predator (Esmaeili, 2001; Škeljo and Ferri, 2012) and genetic studies (Kumar *et al*., 2007; Annabi *et al*., 2013).

The genus *Garra* is represented 73 fish species in the family of Cyprinidae. *G. rufa* is one of them and is small tropical fish that live in the Syria, Iran, Iraq and Turkey (Esmaeili *et al*., 2009; Coad, 2014). *G. rufa* is also known as doctor fish because of the regular treatment specialties. The doctor fish is one of the therapeutic fish which is used in ichthyotherapy for therapeutic purposes (Özcelik *et al*., 2000), their regular treatment specialties can greatly improve skin treatment especially psoriasis and eczema (Fackler and Augustin, 2001; De Groot and Conemans, 2004; Grassberger and Hoch, 2006). Nowadays, *G. rufa* is becoming a much more popular fish species for therapeutic purposes and the doctor fish were listed as a locally and commercially vulnerable fish species for treatment and aquarium industry in Turkey.

Previous studies on *G. rufa* were limited and some of them are focused on length-weight, length-length relationship (Esmaeili and Ebrahimi, 2006; Gerami *et al*., 2013; Hamidan and Britton, 2013; Hashemzadeh *et al*., 2015), karyotypes (Gorshkova *et al*., 2012), microbiological risks for human (Heistinger *et al*., 2011), reproductive biology and age determination (Abedi *et al*., 2011), and mortality (Majtán *et al*., 2012). The uses of scales and otoliths morphological and morphometric characters have been shown as an important tool to determinate and discriminate marine and freshwater fish species (Esmaeili *et al*., 2014; Bostancı *et al*., 2015). There is little information related to the scale morphologies of *G. rufa* in Iran. For instance, Keivany *et al.,* (2015) examined 28 morphometric characters (standard length, body depth, body width, head length, etc.), 25 ratios (head length to standard length, body depth to standard length, pectoral fin length to standard length, etc.) and 10 meristic characters (lateral line scales, scale above the lateral line, scale below the lateral line, predorsal scales, keel scales, total vertebrae, etc.) of *G. rufa* in different basins of Iran. However, there are no studies about otolith morphology; therefore, the scales and otolith morphologies are largely unknown for *G. rufa* in many areas. The current study presents for the first time the comprehensive variation information on scales and left-right asteriscus and lapillus otoliths in the juvenile and adult *G. rufa* inhabiting Kangal Balıklı Çermik thermal spring (Sivas, Turkey).

Materials and methods

The doctor fish specimens were collected from Kangal Balıklı Çermik thermal spring in Sivas, Turkey (Fig. 1). Total length (TL), fork length (FL), and standard length (SL) of each doctor fish was measured to the nearest 0.1 cm, and their weight (W) was recorded to the nearest 0.1 g. Schematic shape of the *Garra rufa* and the six body regions for scale collection in the doctor fish were shown in Fig. 2. The fish were dissected and examined as a mature or immature and then they were considered as a juvenile or adult. The fish scales were gently removed from six different body regions of the fish. Asteriscus and lapillus otoliths pairs of each fish were removed using the otolith removal procedure (Secor *et al.,* 1991). The scales and otoliths were gently cleaned using a fine brush and then each otolith and scale were rinsed by distilled water (Chugunova, 1963; Campana, 2001). The cleaned scales of the fish were dehydrated in 30, 50, 70 and 90% ethanol for 30 minutes respectively and they were dried on filter paper to avoid curling (Lippitsch, 1990). Non-damaged otolith and scale samples were stored for further examination.

While the scales were viewed under a stereo binocular microscope with transmitted light and magnification strength of 10×, the asteriscus and lapillus otolith pairs were photographed using Leica S8APO brand microscope with Leica Application Suit (Ver. 3.7.0) software. The otoliths and scales morphometric parameters such as length, width, perimeter, and area were measured using Digimizer image analysis software (Ver. 4.5.1) program. The left-right asteriscus and lapillus otolith shape indices such as form factor (FF), roundness (RD), aspect ratio (AR), circularity (C), rectangularity (R), and ellipticity (E) were calculated to describe the otolith shape for the juvenile and adult fish (Tuset *et al*., 2003; Ponton, 2006). Mesial and distal surfaces of the left-right otoliths were photographed for each sample. Eleven otolith characters such as width, depth, shape, rostrum-antirostrum size and shape, anterior-posterior regions, mesial-lateral surfaces were determined for each *G. rufa.* SHAPE software (Ver. 1.3) program was used to extract the contours of the *G. rufa* otolith outline (Iwata and Ukai, 2002) and AFORO database system (Lombarte *et al.,* 2006) was used to determine contour wavelets of the otolith for the juvenile and adult fish.

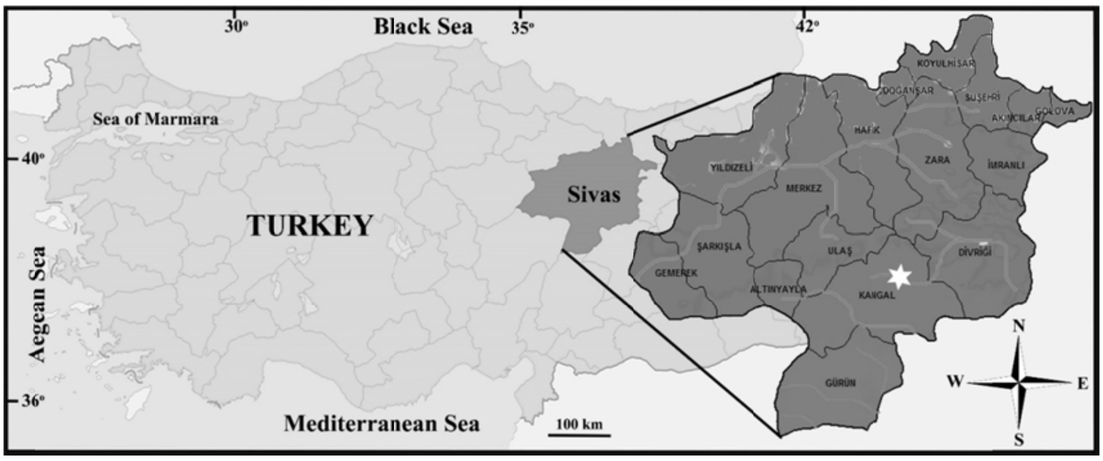

Figure 1: Sampling location of the *Garra rufa* in Kangal Balıklı Çermik thermal spring (Sivas, Turkey).

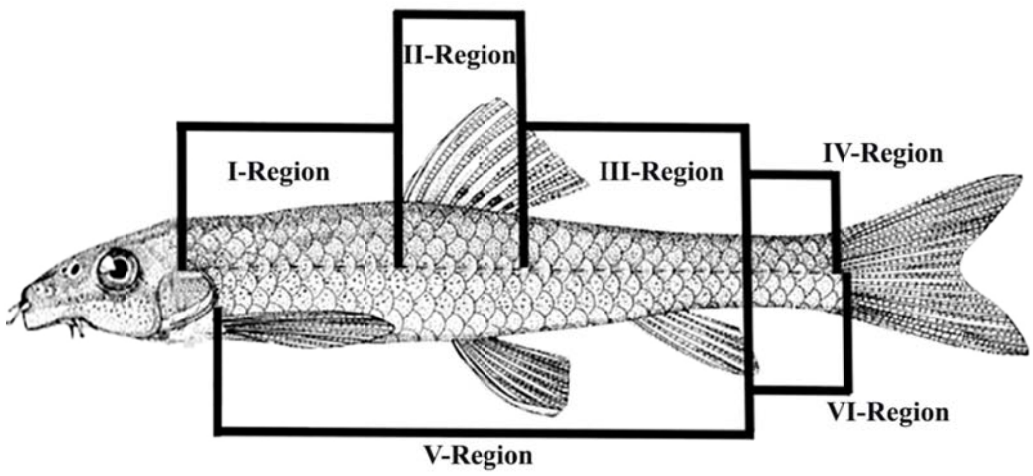

Figure 2: Schematic shape of the *Garra rufa;* showing the six body regions for scale collection (modified from Coad, 2014).

Due to the determination of graphical illustration of wavelets in the left-right asteriscus and lapillus otolith contours, a total of 512 equidistant cartesian coordinates of the left and right otolith were extracted and they were analyzed using the wavelet transformed.

General otolith (A) and scale (B) features of the doctor fish and the terminology used in the current study were presented in Fig. 3. The asteriscus and lapillus otolith pairs morphological characters such as overall otolith shape, otolith with, otolith depth, rostrum size and shape, antirostrum size and shape, mesial and lateral surfaces, anterior and posterior regions were examined for each *G. rufa*. Due to the small body of the fish, six body regions were selected for scale morphology of *G. rufa*.

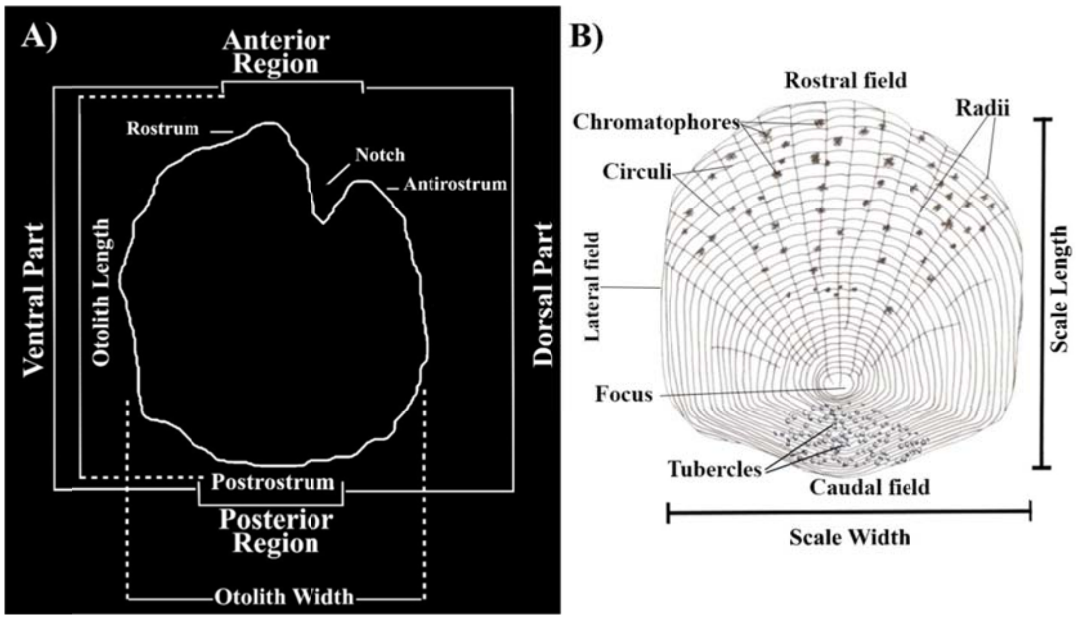

Figure 3: Otolith (A) and scale (B) features of the *Garra rufa,* and their terminology used in the current study.

The scales morphological characters such as scale type, overall scale shape, scale size, focus position, circuli appearance, radii type, rostral and caudal margins were determined in six different body regions of the doctor fish.

Results

In this study, *G. rufa* samples weight (W) and total length (TL) and the mean values of their left-right asteriscus and lapillus otoliths measurements are shown for the juvenile and adult fish (Table 1). The mean values of otolith shape indices such as FF, RD, AR, C, R, and E were calculated and the results are shown for not only asteriscus and lapillus otolith pairs but also the juvenile and adult fish (Table 1).

The mean values of the scale measurements in six different body regions of the juvenile and adult doctor fish are shown in Table 2 and also fork length (FL), standard length (SL), W and TL are presented in Table 2. The left-right asteriscus and lapillus otoliths morphological characters of the juvenile and adult fish were separately determinated. In this study, 11 otolith morphological characters such as overall otolith shape, otolith with, otolith depth, rostrum size and shape, antirostrum size and shape, mesial and lateral surfaces, anterior and posterior regions were used, and the results are presented in Table 3. The overall otolith surface views are shown for left and right asteriscus (a;b) and lapillus (c;d) otoliths in the doctor fish (Fig. 4).

Table 1: The mean values of the left - right asteriscus and lapillus measurements and the otoliths shape indices of the juvenile and adult *Garra rufa*.

			Otolith Measurements				Otolith Shape Indices					
Species	**Otolith Type**	**Side**	**Otolith Area**	**Otolith Perimeter**	**Otolith Length**	**Otolith Width**	**Form factor**	**Roundness**	**Aspect ratio**	**Ellipticity**	**Circularity**	**Rectangularity**
Garra rufa **Juvenile**	Asteriscus	Left	0.157472	1.676127	0.506155	0.467697	0.704367588	0.782612	1.082228	0.039491	17.84064	0.665205
		Right	0.165401	1.656890	0.515218	0.465100	0.757112892	0.793353	1.107757	0.051124	16.59775	0.690241
	Lapillus	Left	0.167274	1.620323	0.545638	0.420177	0.800635998	0.715368	1.298591	0.129902	15.69549	0.729611
		Right	0.164867	1.617943	0.540573	0.406844	0.791438485	0.718348	1.328698	0.141151	15.87789	0.749638
Adult	Asteriscus	Left	0.782242	3.846612	1.079345	0.989114	0.664345856	0.854930	1.091224	0.043622	18.91540	0.732714
		Right	0.785028	3.767128	1.110066	1.041649	0.695143166	0.811143	1.065681	0.031796	18.07738	0.678914
	Lapillus	Left	0.659788	3.183537	1.019115	0.846896	0.818077547	0.808850	1.203353	0.092293	15.36086	0.764454
		Right	0.643000	3.165159	1.009782	0.892159	0.806547174	0.802908	1.131841	0.061844	15.58045	0.713742

Table 2: The mean values of the scale measurements in six different body regions of the juvenile and adult *Garra rufa* with fish weight (W), total length (TL), fork length (FL), and standard length (SL).

Species		**W (g)**	**TL (cm)**	**FL (cm)**	**SL (cm)**	**Scale Measurements**	**I-Region**	**II-Region**	**III-Region**	**IV-Region**	**V-Region**	**VI-Region**
Garra rufa	Juvenile	0.7	4.6	4.2	3.7	Area	2.419	5.709	4.582	4.442	5.691	4.593
						Perimeter	6.615	9.531	8.780	8.648	10.014	8.814
						Length	1.898	3.009	3.052	2.935	2.973	2.976
						Width	1.797	2.678	1.942	2.004	2.799	1.981
	Adult	4.9	8.0	7.2	6.5	Area	4.021	7.380	7.090	5.460	8.231	5.534
						Perimeter	8.063	11.141	12.120	10.341	12.156	9.986
						Length	2.444	3.493	3.637	3.229	3.664	3.358
						Width	2.235	3.129	2.834	2.229	3.339	2.182

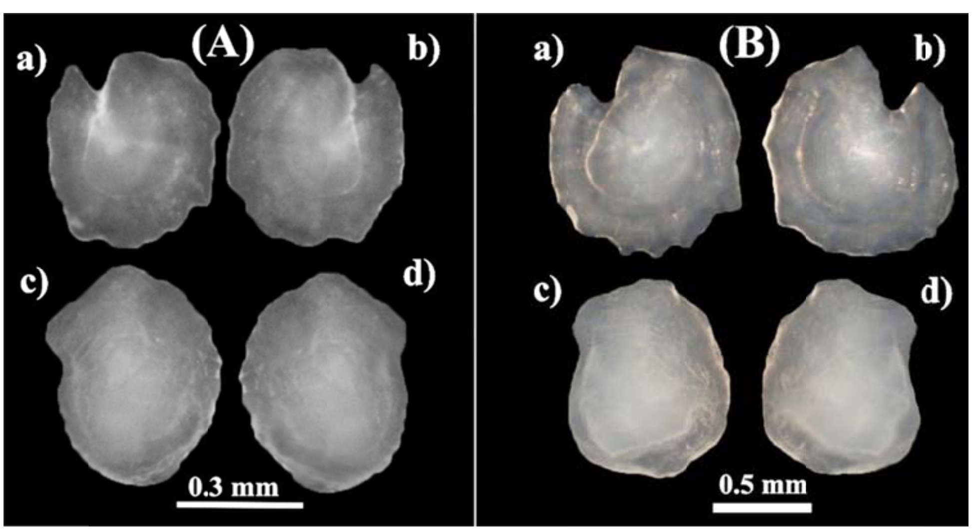

Figure 4: Surface views of the asteriscus (a;b) and lapillus (c;d) otolith pairs in the *Garra rufa* ((A)-TL: 4.6 cm; (B)-TL: 8.0 cm).

The asteriscus and lapillus otolith shapes are characterized as squared and oval to elliptic for asteriscus and lapillus otolith in juvenile doctor fish, and discoidal and oval to elliptic for adult doctor fish asteriscus and lapillus otolith shape, respectively (Table 3). The rostrum and antirostrum are present for asteriscus otolith for the *G. rufa*, but they are absent for lapillus otoliths of the juvenile and adult fish (Fig. 4). Moreover the lapillus width is determined as thick, asteriscus width is thin in the *G. rufa*, while anterior margin of the otoliths is characterized by double-peaked for asteriscus and rounded for lapillus in the juvenile and adult fish (Fig. 4). Whereas the posterior margin of the otoliths is determined as an oblique for lapillus, it is rounded for asteriscus (Table 3; Fig. 4). Nine wavelet functions were determined for asteriscus and lapillus otoliths using the AFORO in the juvenile and adult fish. The wavelet 5-6 gave high variations of the otolith contour and the wavelet 6 was selected as an intermediate function in the current study. The graphical illustration of wavelet number 6 showed specific variations associated with prominent features of the left-right lapillus and asteriscus otolith contours (Fig. 5).

Six different body regions were selected based on the removed scales areas in the *G. rufa* (Fig. 2). General scale shapes from six different body regions were presented for the juvenile and adult fish, separately (Fig. 6). Eight scales morphological characters such as scale type, overall scale shape, scale size, focus position, circuli appearance, radii type, rostral and caudal margins were used during the study and the determined scales morphological characters are shown in Table 4. Different types of cycloid scale shape were found in the six different body regions of the doctor fish and generally juvenile and adult fish morphological scales characters are varied in six body regions especially overall shape, rostral and caudal margins (Table 4; Fig. 6). While the scale focus is distinct for II-Region on both the juvenile and adult fish bodies, the focus is indistinct for III-Region on juvenile fish body (Table 4; Fig. 6). Moreover, the scale size is varied in six different body regions of the fish (Table 2; Fig. 6). The circuli appearance on the scales are district for the six different body regions of the fish. In addition, the radii are determined as primary and secondary for each region scale from the juvenile and adult doctor fish (Fig. 6).

Table 3: The morphological characters of the asteriscus and lapillus otolith pairs for the juvenile and adult *Garra rufa.*

Otolith Characters	Juvenile				Adult			
	Asteriscus		Lapillus		Asteriscus		Lapillus	
	Left	Right	Left	Right	Left	Right	Left	Right
Otolith Shape	Squared	Squared	Oval to elliptic	Oval to elliptic	Discoidal	Discoidal	Oval to elliptic	Oval to elliptic
Otolith Width	Thin	Thin	Moderately thick	Moderately thick	Moderately thin	Moderately thin	Thick	Thick
Otolith Depth	Shallow	Shallow	Shallow	Shallow	Shallow	Shallow	Shallow	Shallow
Rostrum Size	Short	Short	Absent	Absent	Short	Short	Absent	Absent
Rostrum Shape	Round	Round	Absent	Absent	Moderately Pointed	Moderately Pointed	Absent	Absent
Antirostrum Size	Well development	Well development	Absent	Absent	Well development	Well development	Absent	Absent
Antirostrum Shape	Moderately Pointed	Pointed	Absent	Absent	Pointed	Pointed	Absent	Absent
Mesial Surface	Concave	Concave	Concave	Concave	Concave	Concave	Concave	Concave
Lateral Surface	Convex	Convex	Concave	Concave	Convex	Convex	Concave	Concave
Anterior Region	Double Peaked	Double Peaked	Rounded	Rounded	Double Peaked	Double Peaked	Rounded	Rounded
Posterior Region	Rounded	Rounded	Oblique	Oblique	Rounded	Rounded	Oblique	Oblique

Table 4: The scale morphological characters in six different body regions of the juvenile and adult *Garra rufa.*

Scale Characters		I-Region	II-Region	III-Region	IV-Region	V-Region	VI-Region
Scale Type	Juvenile	Cycloid	Cycloid	Cycloid	Cycloid	Cycloid	Cycloid
	Adult	Cycloid	Cycloid	Cycloid	Cycloid	Cycloid	Cycloid
Scale Size	Juvenile	Small	Moderately Large	Medium	Moderately Medium	Moderately Large	Moderately Medium
	Adult	Medium	Large	Large	Medium	Large	Medium
Scale Shape	Juvenile	Rectangular	Square	Elongate Pentagonal	Oblong	Square	Oblong
	Adult	Rounded	Pentagonal	Pentagonal	Pentagonal	Hexagonal	Elongate Pentagonal
Focus Position	Juvenile	Centric	Non-centric	Indistinct	Indistinct	Non-centric	Non-centric
	Adult	Non-centric	Non-centric	Non-centric	Non-centric	Non-centric	Non-centric
Circuli Appearance	Juvenile	Distinct	Distinct	Distinct	Distinct	Distinct	Distinct
	Adult	Distinct	Distinct	Distinct	Distinct	Distinct	Distinct
Radii Type	Juvenile	Primary Secondary	Primary Secondary	Primary Secondary	Primary Secondary	Primary Secondary	Primary Secondary
	Adult	Primary Secondary	Primary Secondary	Primary Secondary	Primary Secondary	Primary Secondary	Primary Secondary
Rostral Margin	Juvenile	Relatively Smooth	Undulate	Undulate	Relatively Wavy	Lobed	Relatively Smooth
	Adult	Lobed	Relatively Wavy	Wavy	Wavy	Relatively Wavy	Undulate
Caudal Margin	Juvenile	Roundly	Roundly	Triangular	Roundly	Roundly	Roundly
	Adult	Roundly	Roundly	Triangular	Triangular	Roundly	Triangular

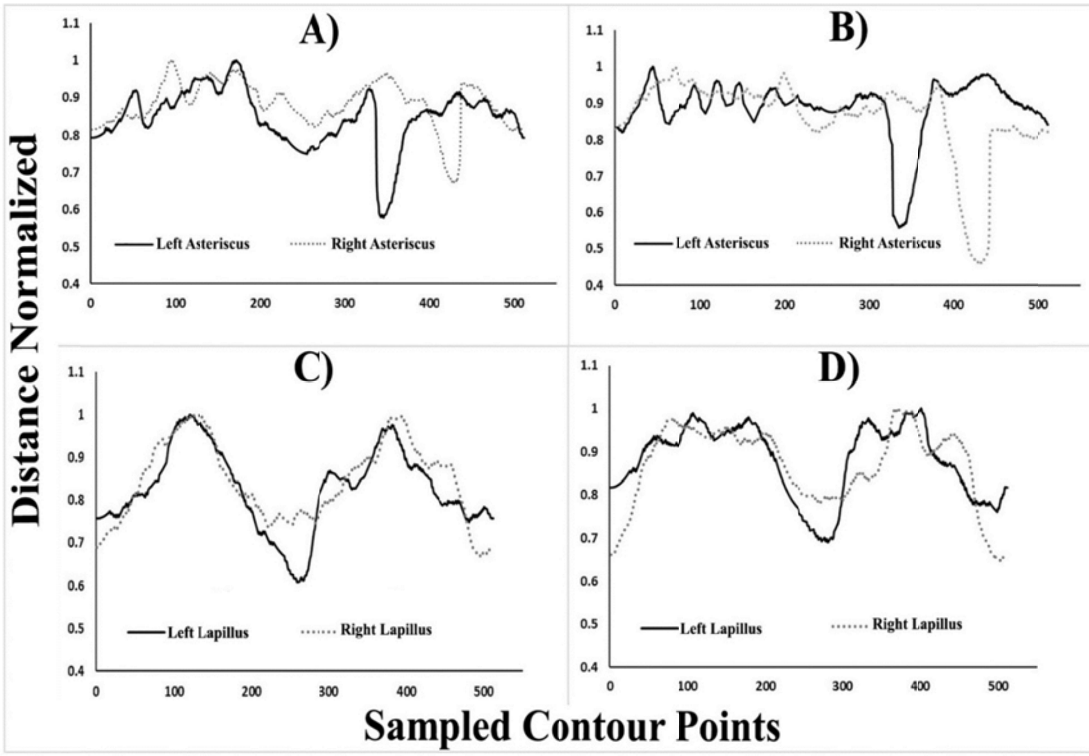

Figure 5: Signals of wavelet 6 from the asteriscus and lapillus otolith pairs in the juvenile (A, C) and adult (B, D) *Garra rufa*.

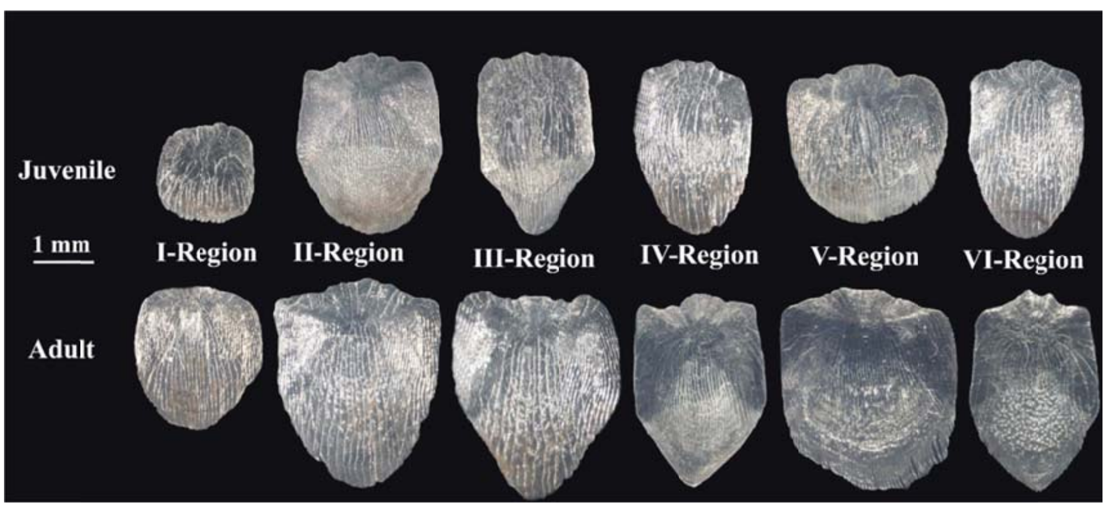

Figure 6: Scales from six different body regions of the juvenile and adult *Garra rufa*. (Juvenile- TL: 4.6 cm; Adult-TL: 8.0 cm).

Discussion

The current study describes the otolith and scale morphologies of juvenile and adult doctor fish, *G. rufa* from Turkey. In addition, this is the first time that 10 morphometric and 11 morphological features for left and right asteriscus and lapillus otoliths were examined in juvenile and adult fish. Additionally, 4 morphometric and 8 morphological features were determined for scales of the juvenile and adult *G. rufa*. The general architectural pattern of a cycloid scale of doctor fish having focus, circuli and radii. Esmaeili *et al.,* (2012) reported that focus of the scale in *Garra rossica* is distinct and located in the anterior field and the focus is the first part of the scale to be formed during ontogenesis. Similarly, the focus of the scale in *G. rufa* is also distinct for body regions I, III, and IV. Scale shape in different fish species may vary such as circular, triangular, rectangular and oval (Helfman *et al.,* 2009). In the current study, six different scale shapes were determined such as rectangular, rounded, square, pentagonal, hexagonal, elongate and oblong in juvenile and adult fish.

The morphological otolith characters can be used to distinguish fish species in different habitats, the same measurements may also be used to predict unknown fish species (Zischke *et al.,* 2016). Bostancı *et al.,* (2015) demonstrated that otolith characters were varied among four valid species of the genus *Alburnus* in different habitats of Turkey. In addition, the present study revealed that the otolith characters are also varied in juvenile and adult doctor fish. Morphometric and meristic characters of hard parts such as otolith and scale can be used as a tool for identification of fish species in marine and freshwater ecosystems. As the otolith and scale being important parts of fish species, the study reveals that these bony structures can be used successfully to determine fish species at juvenile and adult stages. The results of present study suggest that differences in asteriscus - lapillus otoliths and scales measurements and their morphologies are detectable in *G. rufa*. Environmental and ecological factors might also influence fish otolith and scale morphologies defining fish species and populations for inhabiting natural waters (Volpedo and Echeverria, 2003; Vignon *et al*., 2008; Bendoy *et al*., 2011). However, the doctor fish is inhabiting thermal spring; therefore, the environmental and ecological factors are more stable with 7.8 pH, dissolved oxygen 2.9 ppm, and 35°C (Timur *et al*., 1983) and they are not directly influence the fish otoliths and scales. In addition, their scale properties are varied in that habitat and they may be a useful tool to identify the doctor fish in different habitat. Comparing the scales from six different body regions in the same *G. rufa*, the best body region is II-Region for the fish scale with weak deformation and distinct scale characters such as focus, circuli, and radii. Otolith morphology is the most crucial criteria in ichthyological studies, whereby a number of identification guides and atlases are published on scale and otolith morphologies (Patterson *et al*., 2002; Tuset *et al*., 2008; Lin and Chang, 2012). Nowadays, otolith morphology (Jemaa *et al*., 2015a,b; Tuset *et al*., 2015) and scale morphology (Ganzon *et al*., 2012; Chapman *et al*., 2015; Keszka *et al*., 2015) are much more popular and several studies have been published for a variety of fish species.

The graphical illustration of wavelets was used for asteriscus and lapillus pairs in the doctor fish to discover variabilities that could indicate different type of otolith shape. The current study demonstrates that wavelet analysis is a convenient method for determination and discrimination of the juvenile and adult doctor fish. Although, the method is reliable approach for species discriminations in ichthyological studies, it was rarely used in the previous studies (Parisi-Baradad *et al*., 2010, Sadighzadeh *et al*., 2012). For instance, the analysis was successfully used to discriminate for ten species of snappers *Lutjanus* spp. (Sadighzadeh *et al*., 2014). In the present study, wavelet analysis was detected as an alternative method to identify juvenile and adult *G. ruffa*.

The previous ichthyological studies indicated that molecular and genetic methods are also important for determination and discrimination of fish species and populations (Perrier *et al*., 2011; Shafee *et al*., 2013; Dorafshan *et al*., 2014). However, using the morphometry and morphology of versatile research materials are cheaper and more time-efficient methods than genetic and molecular methods (Tracey *et al*., 2006).

In conclusion, this is the first time that the left-right asteriscus-lapillus otoliths and scales of the juvenile and adult doctor fish are examined. In addition, the otolith, scale shapes, and their morphometric and morphological features were used to determination of one of the therapeutic fish in the thermal spring (Turkey). The features might be considered importantly for ichthyologist, biologists, and scientists and they might be used for identification or discrimination of other freshwater and marine fish species in further studies.

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References

Abedi, M., Shiva, A.H, Mohammadi, H. and Malekpour, R., 2011. Reproductive biology and age determination of *Garra rufa* Heckel, 1843 (Actinopterygii: Cyprinidae) in central Iran. *Turkish Journal of Zoology,* 35(**3**), 317-323.

Annabi, A., Said, K. and Reichenbacher, B., 2013. Inter-population differences in otolith morphology are genetically encoded in the killifish *Aphanius fasciatus* (Cyprinodontiformes). *Scientia Marina*, 77(**2**), 269-279.

Bendoy, C.P., Torres, M.A.J. and Demayo, C.G., 2011. Image analysis of fish intraspecific scale variations. The First International Conference on Interdisciplinary Research and Development Bangkok, Thailand.

Bostancı, D., Polat, N., Kurucu, G., Yedier, S., Kontas, S. and Darcin,

M., 2015. Using otolith shape and morphometry to identify four *Alburnus* species (*A. chalcoides, A. escherichii*, *A. mossulensis* and *A. tarichi*) in Turkish inland waters. *Journal of Applied Ichthyology,* 31(**6**), 1013-1022.

Campana, S.E, 2001. Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. *Journal of Fish Biology,* 59(**2**), 197-242.

Chapman, B.B., Hulthén, K., Brönmark, C., Nilsson, P. A., Skov, C., Hansson, L.A. and Brodersen, J., 2015. Shape up or ship out: migratory behaviour predicts morphology across spatial scale in a freshwater fish. *Journal of Animal Ecology,* 84(**4**), 1187-1193.

Chugunova, N.I., 1963. Age and growth studies in fish. Washington: National Science Foundation. 132P.

Coad, B.W., 2014. Freshwater fishes of Iran. June 2014; [cited 2015 Nov 16] Available from http://www.briancoad.com/species%20accounts/Cyprinidae%20Garra%20to%20Vimba.htm

De Groot, A. and Conemans, J., 2004. Doctor fish for psoriasis? *Ned Tijdschr Dermatol Venereol,* 14, 406-408.

Dorafshan, S., Shafee, Z. and Keivany, Y., 2014. A study on genetic differentiation in two species of Iranian bleaks, *Alburnus mossulensis* and *Alburnus caeruleus* (Teleostei, Cyprinidae) using simple sequence repeats. *Caspian Journal of Environmental Sciences,* 12(**2**), 197-204.

Elzey, S.P., Rogers, K.A. and Trull, K.J., 2015. Comparison of 4 aging structures in the American shad (*Alosa sapidissima*). *Fishery Bulletin,* 113(**1**), 47-54.

Esmaeili, H.R., 2001. Biology of an exotic fish, silver carp, *Hypophthalmichthys molitrix* (Val., 1844) from Gobind sagar Reservoir, Himachal Pradesh, India. [PhD Thesis]. Panjab University. 287P.

Esmaeili, H.R. and Ebrahimi, M., 2006. Length-weight relationships of some freshwater fishes of Iran. *Journal of Applied Ichthyology*, 22(**4**), 328-329.

Esmaeili, H.R., Ebrahimi, M., Ansari, T.H., Teimory, A. and Gholamhosseini, G., 2009. Karyotype analysis of Persian stone lapper, *Garra persica* Berg, 1913 (Actinopterygii: Cyprinidae) from Iran. *Current Science,* 96(7), 959-962.

Esmaeili, H.R., Gholamifard, A., Zarei, **N. and Arshadi**, **A., 2012.** *Iranian Journal of Science and Technology,* A4 (**4**), 487-492

Esmaeili, H.R., Khaefi, R., Sayyadzadeh, G., Tahami, M.S., Parsi, B. and Gholamifard, A., 2014. Scale surface microstructure and scale size in three Mugilid fishes (Teleostei, Mugilidae) of Iran from Three Different Habitats. *IUFS Journal of Biology,* 73(**1**), 31-42.

Fackler, R. and Augustin, M., 2001.

Natural therapy of psoriasis. *Biology and Medicine,* 30, 121-125.

Ganzon, M.A.M., Torres, M.A.J., Gorospe, J.J. and Demayo, C.G., 2012. Variations in scale morphology between sexes of the spotted barb, *Puntius binotatus* (Valenciennes, 1842) (Actinopterygii: Cyprinidae). 2nd International Conference on Environment and Bio Science. IPCBEE Vol. 44. Singapore: IACSIT. pp. 80-84.

Gerami, M.H., Abdollahi, D. and Patimar, R., 2013. Length-weight, length-length relationship and condition factor of *Garra rufa* in Cholvar River of Iran. *World Journal of Fish and Marine Sciences,* 5(**4**), 358-361.

Gorshkova, G., Gorshkov, S., Abu-Ras, A. and Golani, D., 2012. Karyotypes of *Garra rufa* and *G. ghorensis* (Pisces, Cyprinidae) inhabiting the inland water systems of the Jordan basin. *Italian Journal of Zoology,* 79(**1**), 9-12.

Grassberger, M. and Hoch, W., 2006. Ichthyotherapy as alternative treatment for patients with psoriasis: a pilot study. *Evidence-Based Complementary and Alternative Medicine,* 3(4), 483-488.

Hamidan, N. and Britton, J.R., 2013. Length-weight relationships for three fish species (*Capoeta damascina*, *Garra rufa*, and *Nemacheilus insignis*) native to the Mujib Basin, Jordan. *Journal of Applied Ichthyology,* 29(**2**), 480-481.

Hashemzadeh, I., Tabatabaei, S., Mansouri, A., Abdoli, A., Ghalenoei, M. and Golzarianpour, K., 2015. Length-weight relationships of *Garra rufa*, in the Tigris and Persian Gulf basins of Iran. International *Journal of Aquatic Biology*, 3(**1**), 25-27.

Heistinger, K., Heistinger, H., Lussy, H. and Nowotny, N., 2011. Analysis of potential microbiological risks in ichthyotherapy using kangal fish (*Garra rufa*). *Egyptian Journal of Aquatic Biology and Fisheries,* 15(**3**), 93-98.

Helfman, G.S., Collette, B.B., Facey, D.E. and Bowen, B.W., 2009. The diversity of fishes: Biology, Evolution and Ecology. John Wiley & Sons Publication. 720 p.

Iwata, H. and Ukai, Y., 2002. SHAPE: a computer program package for quantitative evaluation of biological shapes based on elliptic Fourier descriptors. *Journal of Heredity,* 93(**5**), 384-385.

Jawad, L.A. and Al-Jufaili, S.M., 2007. Scale morphology of greater lizardfish *Saurida tumbil* (Bloch, 1795) (Pisces: Synodontidae). *Journal of Fish Biology,* 70(**4**), 1185-1212.

Jemaa, S., Bacha, M., Khalaf, G. and Amara, R., 2015a. Evidence for population complexity of the European anchovy (*Engraulis encrasicolus*) along its distributional range. *Fisheries Research*, 168, 109-116.

Jemaa, S., Bacha, M., Khalaf, G.,

Dessailly, D., Rabhi, K. and Amara, R., 2015b. What can otolith shape analysis tell us about population structure of the European sardine, *Sardina pilchardus,* from Atlantic and Mediterranean waters? *Journal of Sea Research,* 96, 11-17.

Keivany, Y., Nezamoleslami, A. and Dorafshan, S., 2015. Morphological diversity of *Garra rufa* (Heckel, 1843) populations in Iran. *Iranian Journal of Ichthyology*, 2(**3**), 148-154

Keszka, S., Czerniejewski, P., Raftowicz, M. and Ficek, M., 2015. Intraspecific scale variation among different populations of vendace (*Coregonus albula* L.) in some Polish Lakes. *Turkish Journal of Fisheries and Aquatic Sciences (TrJFAS),* 15(**2**), 193-200.

Kumar, R., Singh, P.J., Nagpure, N.S., Kushwaha, B., Srivastava, S.K. and Lakra, W.S., 2007. A non-invasive technique for rapid extraction of DNA from fish scales. *Indian Journal of Experimental Biology,* 45(**11**), 992-997.

Lin, C.H. and Chang, C.W., 2012. Otolith atlas of Taiwan fishes. Taiwan: National museum of marine biology and aquarium. 415P.

Lippitsch, E., 1990. Scale morphology and squamation patterns in cichlids (Teleostei, Perciformes): A comparative study. *Journal of Fish Biology,* 37(**2**), 265-291.

Lombarte, A., Chic, Ò., Parisi-Baradad, V., Olivella, R., Piera, J. and García-Ladona, E., 2006. A web-based environment from shape analysis of fish otoliths. The AFORO database. *Scientia Marina,* 70(**1**), 147-152.

Majtán, J., Černy, J., Ofúkana, A., Takáč, P. and Kozánek, M., 2012. Mortality of therapeutic fish *Garra rufa* caused by *Aeromonas sobria. Asian Pacific Journal of Tropical Biomedicine*, 2(**2**), 85-87.

Mayank, P., Tyagi, R.K. and Dwivedi, A.C., 2015. Studies on age, growth and age composition of commercially important fish species, *Cirrhinus mrigala* (Hamilton, 1822) from the tributary of the Ganga river, India. *European Journal of Experimental Biology,* 5(**2**), 16-21.

Özcelik, S., Polat, H.H., Akyol, M. and Yalcin, A.N., 2000. Kangal hot spring with fish and psoriasis treatment. *Journal of Dermatology,* 27(**6**), 386-389.

Parisi-Baradad, V., Manjabacas, A., Lombarte, A., Olivella, R., Chic, Ò., Piera, J. and García-Ladona, E., 2010. Automated taxon identification of Teleost fishes using an otolith online database. *Fisheries Research*, 105(**1**), 13-20.

Patterson, R.T., Wright, C., Chang, A.S., Taylor, L.A., Lyons, P.D., Dallimore, A. and Kumar, A., 2002. Atlas of common squamatological (fish scale) material in coastal British Columbia, and an assessment of the utility of various scale types in paleofisheries reconstruction. *Palaeontologia Electronica,* 4(**1**), 1-88.

Perrier, C., Daverat, F., Evanno, G., Pecheyran, C., Bagliniere, J.L. and Roussel, J.M., 2011. Coupling genetic and otolith trace element analyses to identify river-born fish with hatchery pedigrees in stocked Atlantic salmon (*Salmo salar*) populations. *Canadian Journal of Fisheries and Aquatic Sciences*, 68(**6**), 977-987.

Ponton, D., 2006. Is geometric morphometrics efficient for comparing otolith shape of different fish species? *Journal of Morphology,* 267(**6**), 750–757.

Razak, A., 2014. Scale morphology of cuning fish (*Caesio cuning* Bloch, 1791) (Caesionidae) using dekstop Scanning Electron Microscope. Proceeding of International Conference on Research, Implementation and Education of Mathematics and Sciences; Yogyakarta, Indonesia.

Romo-Curiel, A.E., Herzka, S.Z., Sosa-Nishizaki, O., Sepulveda, C.A. and Aalbers, S.A., 2015. Otolith-based growth estimates and insights into population structure of white seabass, *Atractoscion nobilis*, off the Pacific coast of North America. *Fisheries Research*, 161, 374-383.

Sadighzadeh, Z., Tuset, V.M., Valinassab, T., Dadpour, M.R. and Lombarte, A., 2012. Comparison of different otolith shape descriptors and morphometrics in the identification of closely related species of *Lutjanus* spp. from the Persian Gulf. *Marine Biology Research,* 8(9), 802-814.

Sadighzadeh, Z., Otero-Ferrer, J.L., Lombarte, A., Fatemi, M.R. and Tuset, V.M., 2014. An approach to unraveling the coexistence of snappers (Lutjanidae) using otolith morphology. *Scientia Marina,* 78(**3**), 353-362.

Secor, D.B., Dean, J.M. and Laban, E.B., 1991. Manual for otolith removal and preparation for microstructural examination. Belle W. Baruch Institute, Univ. South Carolina Press, Columbia, SC, 85P.

Shafee, Z., Dorafshan, S., Keivany, Y. and Qasemi, S.A., 2013. Genetic structure of Mosul bleak (*Alburnus mossulensis* Heckel, 1843) using microsatellite marker in Tigris basin. *Taxonomy and Biosystematics,* 5(**4**), 9-22.

Shimose, T., Yokawa, K. and Tachihara, K., 2015. Age determination and growth estimation from otolith micro-increments and fin spine sections of blue marlin (*Makaira nigricans*) in the western North Pacific. *Marine and Freshwater Research,* 66(**12**), 1116-1127.

Škeljo, F. and Ferri, J., 2012. The use of otolith shape and morphometry for identification and size-estimation of five wrasse species in predator-prey studies. *Journal of Applied Ichthyology,* 28(**4**), 524-530.

Timur, M., Çolak, A. and Marufi, M., 1983. A study on the systematic identification of the Balıklı thermal spring (Sivas) fish and the curative effects of the fish on dermal diseases. *Veterinary Journal of Ankara University,* 30(**2**), 276-282.

Tracey, S.R., Lyle, J.M. and Duhamel, G., 2006. Application of elliptical fourier analysis of otolith form as a tool for stock identification. *Fisheries Research*, 77(**2**), 138-147.

Tuset, V.M., Lozano, I.J., González, J.A., Pertusa, J.F. and García-Díaz, M.M., 2003. Shape indices to identify regional differences in otolith morphology of scomber, *Serranus cabrilla* (L., 1758). *Journal of Applied Ichthyology,* 19(**2**), 88-93.

Tuset, V.M., Lombarte, A. and Assis, C.A., 2008. Otolith atlas for the western Mediterranean north and central eastern Atlantic. *Scientia Marina,* 72(S1), 7-198.

Tuset, V.M., Imondi, R., Aguado, G., Otero-Ferrer, J.L., Santchi, L., Lombarte, A. and Love, M., 2015. Otolith patterns of rockfishes from the northeastern Pacific. *Journal of Morphology,* 276(**4**), 458-469.

Vignon, M., Morat, F., Galzin, R. and Sasal, P., 2008. Evidence for spatial limitation of the bluestripe snapper *Lutjanus kasmira* in French Polynesia from parasite and otolith shape analysis. *Journal of Fish Biology,* 73(**10**), 2305-2320.

Volpedo, A.V. and Echeverria, D.D., 2003. Ecomorphological patterns of the sagitta in fish on the continental shelf off Argentine. *Fisheries Research*, 60(**2-3**), 551-560.

Zischke, M.T., Litherland, L., Tilyard, B.R., Stratford, N.J., Jones, E.L. and Wang, Y-G., 2016. Otolith morphology of four mackerel species (*Scomberomorus* spp.) in Australia: Species differentiation and prediction for fisheries monitoring and assessment. *Fisheries Research,* 176, 39-47.