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RESEARCH ARTICLE

Morphometric Variations Among Three Populations of *Alburnus zagrosensis* (Coad, 2009) in the Zagros Mountain Basin, Iran

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Abstract In the present study, the morphometric differentiation among three populations of *Alburnus zagrosensis* (Coad, 1) in Zagros mountains basin is analyzed. Fish specimens were sampled from three habitats namely Cheshme Ali, Hamze Ali and Choghakhour wetland in Chaharmahal Va Bakhtiari Province, Iran. ANOSIM (Analysis of Similarity), SIMPER (Similarity percentage analysis) and Canonical discriminant analysis were employed in order to define the morphological features responsible for population discrimination. Ten morphometric variables were measured. An Analysis of the sampling sites suggested a partial separation among three populations. The SIMPER analysis revealed that the total length and the head length were the main features that differed among populations. In conclusion, geographical isolation is suggested to be the main explanation for the morphological differences among *A. zagrosensis* specimens from different stations.

Keywords Morphometric variables · *Alburnus zagrosensis* · Stock identification · Chaharmahal and Bakhtiari

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Introduction

Alburnus zagrosensis, Coad [1], is a member of the cyprinid fish genus *Alburnus* which is endemic in several rivers of the Zagros mountains. In the Zagros mountains of Iran, a series of rivers is draining westward and southward towards the river Tigris, or heading to the Persian gulf [1]. *A. zagrosensis* is found in the upper reaches of the river basin Karun. River Karun rises in the Bak̲tiāri Zagros mountains, west of Isfahan Province, flows out of the central Zagros range, traverses the Khuzestan plain, and joins the Arvandrud [2]. The average temperature in this basin is 10 °C (minimum and maximum average temperature 3.1 and 18.7 °C, respectively) with 120 frost days per year, 300 mm annual rainfall, 3361.6 total monthly sunshine hours and 2153 m average height above sea level [3]. As a result of these extreme conditions, the habitat of *A. zagrosensis* experiences seasonal fluctuations of the surface water temperatures. Due to topography conditions in the basin, the habitat of this species is restricted.

Genetics and morphometrics are tools for the discrimination and identification of fish stocks [4]. The effects of genetic and environmental parameters on different growth and developmental processes cause a variation of the body shape among stocks [5]. Morphometric analysis is applicable for studying short-term environmentally induced disparities, species differences, stock identification, practical morphology, ontogeny and improving fisheries management [6]. Morphometric studies and variation among *Alburnus*s species have been considered in many studies including *Alburnus chalcoides* and *Alburnus alburnus* of the Caspian sea basin [5], *Alburnus mossulensis* in river Beshar [7] and *Alburnus filippii* in Anzali lagoon [8]. *A. zagrosensis* was only recently described, and no data about morphometric variation of this species in its habitat have been published. It is well known that the phenotypic plasticity of fishes is often correlated with an ontogenetic response to environmental changes resulting in physiological, behavioural and morphological modifications [9–15]. The present study aims to investigate the morphometric variation within the three populations of A. *zagrosensis*, and to examine for differences among geographic populations.

Material and Methods

One hundred and thirty specimens of *A. zagrosensis* were collected from three locations from Chaharmahal Va Bakhtiari Province, Iran (Table 1), including Choghakour wetland, Hamzeh Ali and Cheshmeh Ali. These three sites are located in the upper reaches of Tigris basin, and *A. zagrosensis* is considered as endemic to these habitats. The authors distinguished this species from other Iranian and Tigris-Euphrates basin *Alburnus* by its 67–83 lateral line scales, 9–10 branched anal fin rays, the total gill raker count of 12–14, and the absence of a prominent mid-flank stripe [1, 2].

In terms of water flow, Choghakour wetland was different from Hamzeh Ali and Cheshmeh Ali. In addition, Hamzeh Ali and Cheshmeh are geographically separated and do not have any confluence or actual water exchange (Fig. 1). Choghakour wetland is lentic and Hamzeh Ali and Cheshmeh Ali are both lotic. Choghakour wetland has lake characteristics but Hamzeh Ali and Cheshmeh Ali both have river conditions.

The fish specimens were collected using different fishing gear (hand net, seine net, electrofishing). They were initially preserved in 5% formalin and transferred to the laboratory for further examination. Ten morphometric variables were measured using a digital caliper to the nearest 0.001 mm and a digital weight scale with an accuracy of 0.001 g. In the present study, the variables namely head length (HD), body depth (BD), postorbital length (POL), snout length (SL), eye diameter (ED), total length (TL), dorsal fin base length (LAD), anal fin base length (LAA), standard length (STL) and total weight (TW) were measured (Fig. 2).

Temperature, dissolved oxygen and pH were measured using portable multi-meters (HACH 51154, USA) with three replications at each site. Phosphate was analyzed using a modified ascorbic acid reduction method [16], and the concentration of chlorophyll a was assessed by spectrophotometric method [17].

In order to eliminate any size effect in the data set, an allometric formula was used [18, 19] as following.

$$M_{adj} = M\left(\frac{L_S}{L_O}\right)^b$$

where, M is original measurement, M_{adj} is size adjusted measurement, L_o is standard length of the specimen, L_s is overall mean standard length for all specimens in all samples in each analysis, and *b* is the allometric growth rate (regression coefficient among logM and log L_o). The variation coefficient was calculated with the following formula [20].

$$C.V_p = 100\sqrt{\frac{\sum S^2}{\sum X^2}}$$

where S^2 is variance of morphometric features, X^2 is mean square of the same morphometric features. A condition factor for individual species was used to calculate Fulton's Condition Factor (CF) Index [19] as given below.

$$CF = \frac{W}{L^3} \times 100$$

where L is the length in centimeters (cm) and W is the weight in gram (g). Both a univariate and multivariate variance analyses were carried out to test the significance of morphometric differences among populations. A One-Sample Kolmogorov–Smirnov Test was used to test the data normality, and non-parametric k-independent sample tests were used to analyze significant differences of the CF at the three locations at a 5% level.

Potential differences among the communities of the three sites were then evaluated with a non-parametric variance analysis ANOSIM (Analysis of Similarity) and SIMPER (Similarity percentage analysis) with Bray-Curtis method [21]) of similarities of morphometric features. Before analyzing, the data were square-root transformed. In addition, the authors plotted the data in two dimensions

Table 1 Location and physico-chemical properties of three studied area in autumn 2014

Site	Coordinates	N	T	O_2	pH	Phos	Chl.a
Cheshmeh Ali	51° 11′ E 31° 37′ N	30	13.8 ± 8.3	8.8 ± 1.1	7.9 ± 0.5	25 ± 0.015	2.72 ± 1.38
ChoghakhourWetland	50° 54′ E 31° 55′ N	50	14.4 ± 7.5	9.8 ± 0.9	8.30 ± 0.5	24 ± 0.015	2.94 ± 1.28
Hamzeh Ali	51° 02′ E 31° 56′ N	50	14 ± 8.2	9.3 ± 0.7	8.1 ± 0.5	23 ± 0.015	2.53 ± 1.42

N number of samples, *T* temperature °C, O_2 oxygen concentration mg.l^{-1}, *Phos* total phosphate µg.l^{-1}, *Chl.a* chlorophyll a µg.l^{-1}, the mean values are indicated with ±SD (standard deviation)

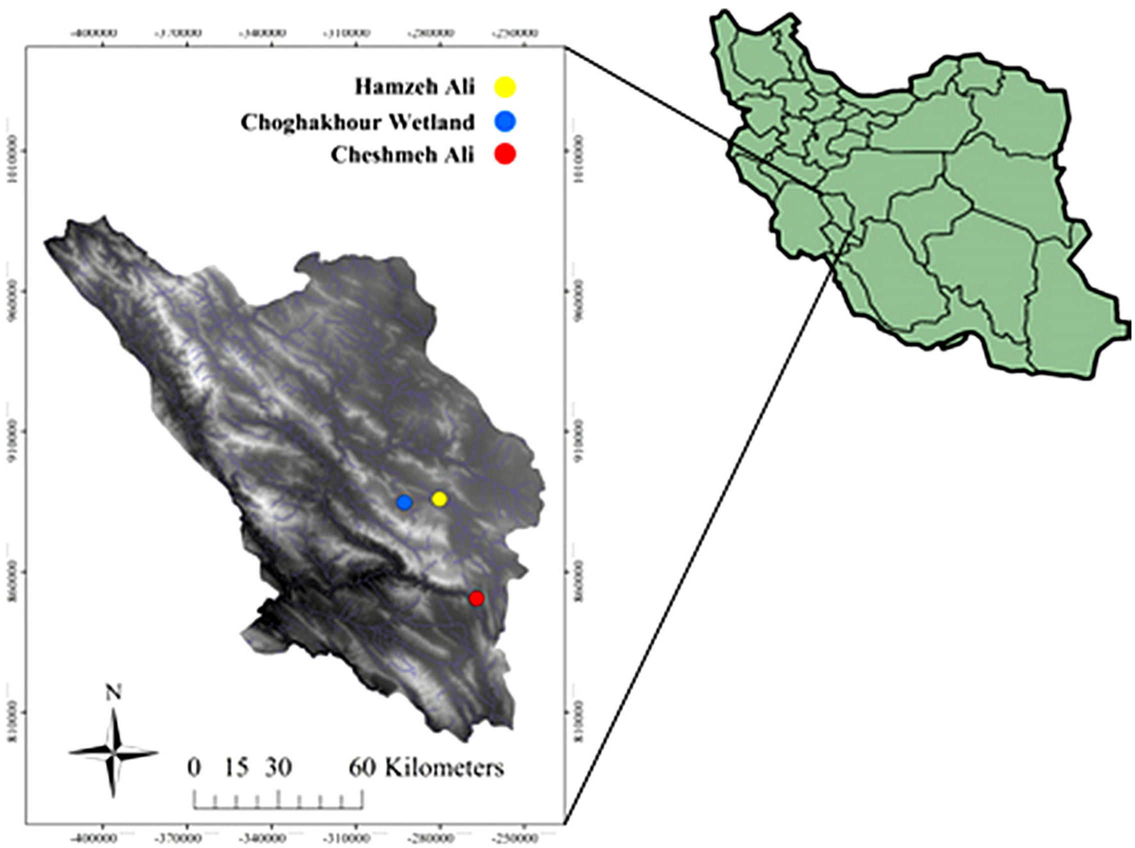

Fig. 1 The map of the sampling of *A. zagrosenisi* in Chaharmahal and Bakhtiari province, Iran

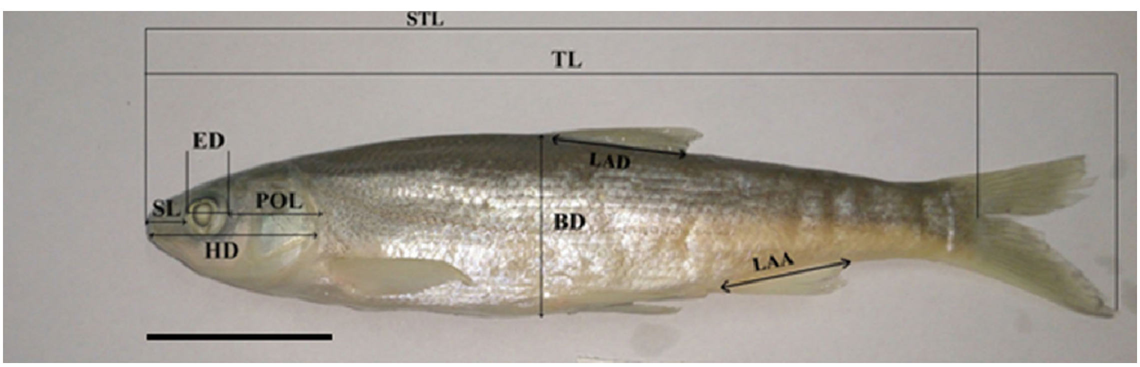

Fig. 2 Morphometric characters of *A. zagrosensis* used in the present study. *Scale bar* is 10 mm

using a canonical discriminant analysis. Statistical analyses were performed using R statistical package (version 3.1.3).

Results and Discussion

The morphometric characters of the specimens of *A. zagrosensis* at three sites have been shown in Table 2. The variance analysis showed that the populations differed significantly in their HD, ED, SL and LAD.

The condition factors have been presented in Table 3. The maximum condition factor was found at Cheshmeh Ali. The K-independent sample test showed that the populations at the three sites differed significantly in their CF ($p < 0.05$).

The analysis of morphometric characters showed significant differences among the three populations (ANOSIM: $p = 0.001$, Fig. 3). The dissimilarity average among three sites is shown in Table 4. The results indicate that TL and HD were the most important contributors in

Table 2 Morphometric characters of *A. zagrosensis*at three sites in this study (2014)

Parameter	Mean ± STD(min–max)			C.V %		
	Cheshmeh Ali	Hamzeh Ali	Choghakhour wetland	Cheshmeh ALi	Hamzeh Ali	Choghakhour wetland
TL (mm)	55.80 ± 18.58[a] (40–115)	53.08 ± 13.85[a] (35.54–85.84)	55.80 ± 17.44[a] (31.12–97.3)	33.29	26.09	31.25
STL (mm)	47.57 ± 16.46[a] (31.94–99.2)	43.01 ± 12.01[a] (27.76–70.24)	45.97 ± 15.35[a] (22.72–82.06)	34.60	27.92	33.38
HD (mm)	9.17 ± 2.71[a] (5.98–15.76)	8.35 ± 2.19[ab] (5.6–13.48)	6.21 ± 2.66[b] (2.98–12.1)	29.57	26.30	42.95
BD (mm)	9.91 ± 3.80[a] (5.56–20.58)	10.68 ± 4.02[a] (6.2–19)	8.83 ± 5.58[a] (3.24–23.72)	38.38	37.7	63.27
LAD (mm)	7.81 ± 3.24[a] (3.78–17.26)	7.17 ± 1.17[a] (5.24–8.84)	2.92 ± 2.01[b] (0.84–7.38)	41.51	16.33	68.91
LAA (mm)	5.40 ± 2.16[a] (3.24–12.28)	3.05 ± 1.1[a] (2–6.06)	5.23 ± 2.19[a] (2.54–9.72)	40.16	36.13	41.86
SL (mm)	1.58 ± 0.89[a] (0.26–3.32)	2.97 ± 0.68[b] (2.38–4.8)	1.55 ± 0.69[a] (0.28–2.7)	56.27	23.04	44.83
ED (mm)	1.74 ± 0.99[a] (0.68–4.46)	4.34 ± 1.2[b] (3.05–7.12)	1.24 ± 0.75[a] (0.24–3.42)	57.13	27.67	61.07
POL (mm)	4.05 ± 2.05[a] (1.96–9.7)	6.95 ± 3.06[a] (2.5–14.12)	2.48 ± 2.02[b] (0.64–6.86)	50.66	44.08	81.48
TW (gr)	2.16 ± 2.99[a] (0.533–12.55)	1.71 ± 1.87[a] (0.26–5.94)	1.81 ± 2.32[a] (0.224–9.075)	138.29	109.61	128.45

Different superscript letters indicate significant ($p < 0.05$) difference among the groups

Table 3 Condition factor of *A. zagrosensis* in three habitats

Location	Cheshmeh Ali	Choghakhour wetland	Hamzeh Ali
CF	0.933064*	0.740501*	0.824393*

* Shows significant difference by sample t test ($p < 0.05$)

dissimilarity among Cheshmeh Ali and other two sites. Meanwhile, more than 5 morphometric features (TW, LAA, STL, POL and HD) were contributed as dissimilarity factors among Choghakour wetland and Hamzeh Ali (Table 4).

Fulton's condition factor had higher values in the Cheshmeh Ali population which indicates good growth performance conditions in this region. The K-independent sample test shows a significant difference in the CF at the three sites ($p < 0.05$) which suggests different conditions in three regions. This fluctuation for same species may be caused by environmental conditions such as temperature and salinity [3], population dynamics, predators, competitors [22] and trophic relationships [23]. Such findings were also observed in *Gadus morhua* [24] and *Gymnocephalus cernuus* [25]. The results of the CF analysis (Table 3) showed that the populations of Cheshmeh Ali and Hamzeh Ali had a better condition factor than that of Choghakour wetland. This was due to food availability in the region. Fathi et al. [26] reported that water quality of the wetlands are in two pollution categories, moderate and severe. Therefore, the fish species in this habitat cannot grow well due to habitat degradation.

Size-related traits play a dominant role in the morphometric analysis [27]. The ANOSIM analysis revealed that there was a significant difference among the populations of *A. zagrosensis* at the three sites ($p < 0.05$). However, the differentiation was only moderate ($R = 0.696$, Fig. 3). The dissimilarity analysis showed that a morphometric differentiation among the samples was mainly located in the head of *A. zagrosensis*. The head length had a strong contribution in the three compared sites. Although the total length was the most important dissimilarity factor among Cheshmeh Ali and other sites, it was not significant for dissimilarity among Choghakour wetland and Hamzeh Ali (Table 4).

The relative position of the eyes has been related to vertical habitat preference [28]. A smaller HD, SL and POL were found in the Choghakour wetland population, while a maximum ED was observed in Hamze Ali mineral spring. Gatz [29] stated that a short head length was correlated with a small prey size in stream fishes. Wetlands have been considered as the biologically most diverse ecosystems, serving as home to a wide range of plant and animal life. It is suggested that changes in head

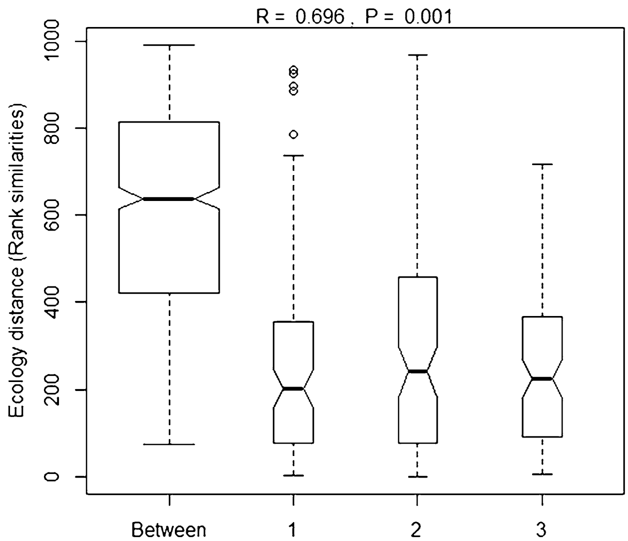

Fig. 3 ANOSIM graph with R and *P* values for the three study sites (*1* Cheshmeh Ali, *2* Choghakour Wetland, *3* Hamzeh Ali)

Table 4 Dissimilarity percentages and factor contributions in the three study sites

Factors	1 versus 2 Contribution %	1 versus 3	2 versus 3
TL	38.33	40.38	6.91
STL	6.98	6.82	12.11
BD	4.91	3.50	7.31
HD	22.52	22.68	11.79
LAD	4.36	4.40	9.80
LAA	4.92	–	13.04
POL	5.55	–	11.86
TW	–	9.19	14.28
SL	6.34	3.66	6.72
ED	–	–	–
Average	27.72	24.69	14.37

The canonical discriminant analysis clearly showed a differentiation among the populations (Fig. 4). In the discriminant space, all populations were clearly distinct from each other

1 stands for Cheshmeh Ali, *2* for Choghakour Wetland, and *3* for Hamzeh Ali

morphometric variations were due to the morphological plasticity. In addition, the LAA was the main component separating the populations from Choghakour wetlands and Hamzeh Ali. In addition, this factor was minimum and maximum in Hamzeh Ali and Cheshmeh Ali, respectively. Buj et al. [30] stated that a character often used for the discrimination of *Alburnus* species is the position and length of the anal fin. They reported much variation among the anal fin positions of *Alburnus* sp in Adriatic basin populations, which represented three different species. The present results suggest that this character is highly overlapping among the Zagros mountain basin populations of *A. zagrosensis*.

The causes of morphological differences among populations are often quite difficult to explain [31]. The present morphometric analysis of the *A. zagrosensis* in the Zagros mountain basin showed a considerable distinctiveness of the populations in the 3 studied sites. While a variance analysis did not result in significant differences among the morphometric features of the three populations, a canonical discriminant analysis clearly separated the populations based on the morphological features examined. Similar differences among populations of *Alburnus* sp were reported from the southern Caspian sea basin [5, 32] and the Adriatic basin [33]. Cheshmeh Ali and Hamzeh Ali have similar water sources (spring mineral water), but they are geographically separated (Fig. 1). The populations of *A. zagrosensis* at these two sites live in habitats with strong current and sporadic aquatic vegetation, while the Choghakhour wetland population lives in a gentle current and dense aquatic vegetation. Turan [33] stated that morphological variation among fish populations is influenced by a mixture of environmental factors including temperature, salinity, radiation, dissolved oxygen, water depth and current flow but not limited to these factor only. Such morphological differences among different populations of a species may be related to differences in habitat factors such as food availability, turbidity, water depth, temperature and flow [34]. It is well known that morphometric characters may express a high degree of plasticity in response to habitat conditions [34]. In addition, the morphological characteristics of fishes are determined by genetics, environment, and their interaction [35]. Fish species which lives in different environmental and habitat conditions may express diverse phenotypes within a population. The habitat structure is an important factor for the early life stages of fishes. In fact, the environmental factor prevailing during the early development stages when the individual's phenotype is more amenable to environmental influence is of particular importance [36]. Morphometric variations due to habitat conditions develop more quickly than genetic variations because latter are formed by several genes [37]. Variation in fish morphology has repeatedly been shown to be correlated with or be caused by changes in environmental and ecological factors such as feeding regimes and salinity [38], temperature [39, 40], current velocity [41], turbidity [32] and predation risk [39]. However, all sites in the present study were very similar according to physico-chemical examinations (Table 1). Therefore, the main factor causing themorphometric differences seems to be the lack of dispersal by geographic isolation. The intraspecific effects of population separation and isolation on morphometric variation have been extensively documented in recent years. Yamamoto et al. [42]

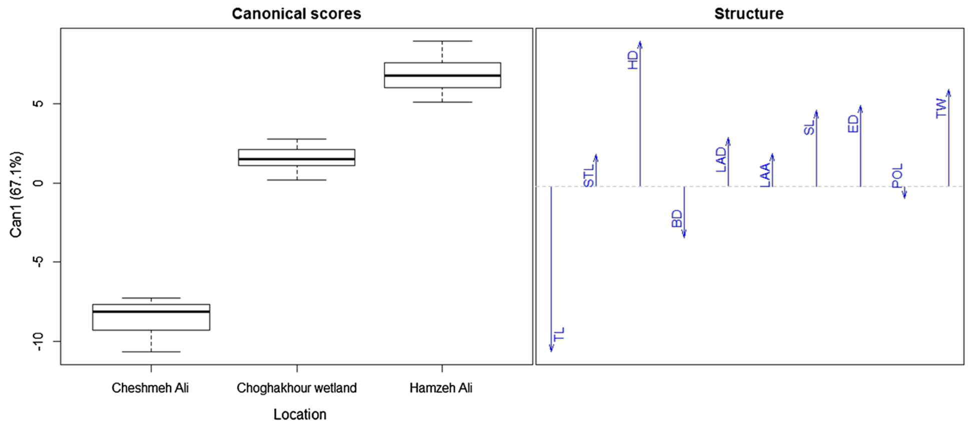

Fig. 4 Canonical discriminant analysis plot for *A. zagrosensis* population in Cheshmeh Ali, Choghakour wetland and Hamzeh Ali sites

showed that habitat fragmentation resulted in different body shape in populations of *Salvelinus leucomaenis*. Khan et al. [43] stated that different population of *Channa punctata* from three Indian rivers had different morphometric features which resulted in different stocks. Cadrin and Friedland [44] found that population of *Limanda ferruginea* in southern New England has a shorter snout length, head length and pectoral fin as compared to the Scotian shelf population. In addition, Heidari et al. [35] stated that the long-term isolation of populations and interbreeding lead to morphometric variation among populations, and provide a basis for population differentiation. Population isolation among same species causes reproductive isolation. Salles et al. [45] proposed that the morphometric discrimination among the two stocks of *Lutjanus purpureus* was probably a result of the feeding/reproductive strategies adopted in each of their respective subareas. When a population is isolated and has no regular gene exchange with the next population, the balance among gene flow and the forces responsible for population differentiation, such as genetic drift or differential selection, result in clines, while genetic differentiation increases with geographic distance [46, 47]. Furthermore, interactive effects of the environment, selection, and genetics on individual ontogenies produce morphometric differences within a species in isolated populations [48].

Conclusion

The genus *Alburnus* is an excellent example for high diversity and endemism in western Palaearctic freshwater fishes and its karyotype varies significantly on a spatial scale [2, 49]. Published data on the confluence or water exchange among three sites in the present study in flooding or even glacial periods is scarce. Therefore, the time scale of former genetic exchange among these populations is unknown. However, according to local observations, a mixture and possible genetic exchange among the populations in massive flooding events is expected. According to the results of the present study, geographic isolation and habitat differences are considered to be the main factors for the different growth rate and morphological differences among the disjunct populations of *A. zagrosensis*. However, to further corroborate the findings, a genetic examination is needed to determine the genetic isolation of the fish stocks from different stations and populations. The present study provides a baseline for biological information for *A. zagrosensis* and suggests that different populations in selected habitats exist, which should be considered separately for species management and conservation.

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Compliance with ethical standards

Conflict of interest There is no other conflict of interest to declare.

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