Int. J. Aquat. Biol. (2018) 6(4): 242-247
ISSN: 2322-5270; P-ISSN: 2383-0956
Journal homepage: www.ij-aquaticbiology.com

Short Communication

Morphological comparison of western and eastern populations of Caspian kutum, *Rutilus kutum* (Kamensky, 1901) (Cyprinidae) in the southern Caspian Sea

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Abstract: This study aimed to investigate morphological differences of western and eastern populations of Caspian kutum *Rutilus kutum* in the southern Caspian Sea and its providing length-weight relationships and condition factor (CF). A 13-landmark based morphometric truss network system was used to investigate the hypothesis of population fragmentation of western and eastern populations of this species. The studied populations were differentmorphologically based on pre anal, body height, distance from pectoral fin to ventral fin, distance from pectoral fin to anal fin, caudal peduncle length, head length, pre orbital, pre ventral, and dorsal length. The results also revealed a negative allometry (b<3) of length-weight relationships for both sexes. Maximum condition factors was found in March. The results suggest distinct stocks in the western and eastern of Caspian Sea for fisheries management.

Article history:
Received 5 May 2018
Accepted 2 August 2018
Available online 25 August 2018

Keywords:
Morphological variation
Length-weight relationship
Condition factor
Stock

Introduction

Caspian Sea is unique ecosystem not only because of its size, but also due to its salinity which is brackish as well as those of freshwater fish species that adapted to live in such a salinity. Caspian kutum, *Rutilus kutum,* is a commercially important native fish species of the Caspian Sea basin representing over 50% of the total bony fish catch, and 60% of the fishermen's income in this basin (Abdolmaleki and Ghaninejad, 2007). The Iranian Fisheries Organization (IFO) has been releasing up to 200 million *R. kutum* fry (with average weight of 1 g) into its rivers annually since 1982, to rehabilitate their reduced populations in this basin (Abdolmaleki and Ghaninejad, 2007). Caspian kutum attains a maximum fork length and weight of 71 cm and 4 kg, respectively. Its spawning migration in the rivers and Anzali Wetland occurs from mid-March to mid-May, depending on water temperature, and ecological conditions (Valipour and Khanipour, 2006).

The relationship between body weight and length is a simple but essential parameter in fishery management (Chien-Chung, 1999). Length-weight relationships is used to convert growth-in-length equations to growth in weight in stock assessment models (Bobori et al., 2010). To estimate growth rates, determining age structure is necessary to obtain the condition of fish and comparative growth studies (Kolher, 1995). In addition, these relationships contribute to the comparison of life history and morphological aspects of populations between different regions. Hence, the present study aimed to study the morphological differences of western and eastern populations of *R. kutum* in the southern Caspian Sea in terms of morphometric and providing length-weight relationships and condition factor (CF) this species in the southern Caspian Sea.

Materials and Methods

The sampling was carried out on a monthly basis between December 2017 and March of 2018 in five regions, including Astara (38˚30'N, 48°88'E), Kiyashahr (37˚42'N, 49˚98'E), Anzali (37°46'E, 49°59'E) in the western part and Sari (36°48'N,

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DOI: https://doi.org/10.22034/ijab.v6i4.529

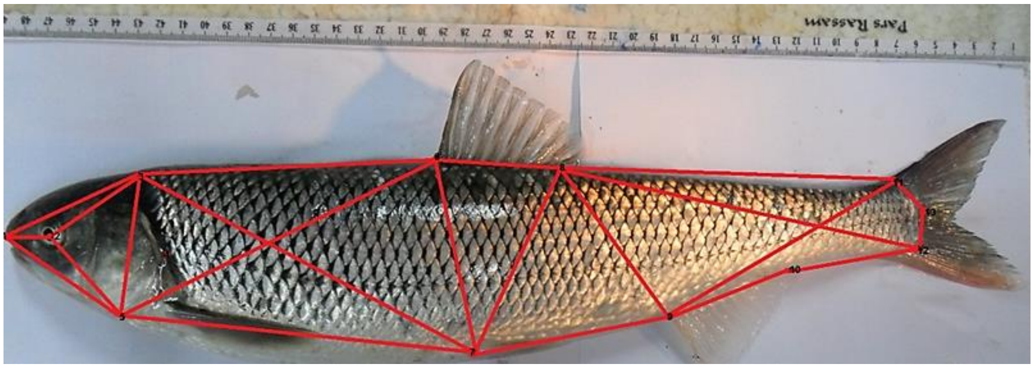

Figure 1. Locations of the 13 landmark points for constructing the truss network on *Rutilus kutum*. 1. Tip of the snout; 2. Center of the eye; 3. End of the frontal bone; 4. Ventral end of the gill slit; 5. Origin of the pectoral fin; 6. Insertion of the dorsal fin; 7. Origin of the pelvic fin; 8. Insertion of the dorsal fin; 9. Origin of the anal fin; 10. Insertion of anal fin; 11. Dorsal end side of caudal peduncle, at the nadir; 12. Ventral end of the caudal peduncle, at the nadir; 13. End of body lateral line.

53°6'E) and Bandar-e Turkmen (36°33'N, 55°50'E) in the eastern part of the Caspian Sea. For morphological comparison, those of western and eastern were merged and considered as two populations. A total of 240 fish were collected and fixed into 10% buffered formalin and transferred to the laboratory of Ichthyology, University of Guilan, Rasht, Iran. Total length (TL) and whole body wet weight (g) were recorded for each specimen. The length-weight relationship was estimated using following equation:

$$W = a L^b$$

Where W is the whole body weight (g), L is the total length (mm), a is the intercept of the regression and b is the regression coefficient (slope) (Ricker, 1975). The parameters a and b of the length-weight relationship were estimated by the least-squares method based on logarithms (Zar, 1999):

$$\text{Log}(W) = \log(a) + b \log(L)$$

When b = 3, increase in weight is isometric and when the value of b is other than 3, weight increase is allometric (positive if b>3, negative if b<3). Also a t-test was used to compare b value in the linear regression of males and females (Zar, 1999).

$$t = \frac{b_1 - b_2}{S_{b_1 - b_2}}$$

These parameters (a, b) are important in stock assessment studies (Froese, 1998). Data analysis was performed by Excel and SPSS version 19 software. The condition factor (CF) was computed by the following equation:

$$k = \frac{100w}{l^3}$$

Where W = the observed total weight for each fish, L = the observed standard length for each fish and K= the condition factor (CF).

For morphological study, measurements of specimens were extracted by collecting X–Y coordinate data for relevant morphological features, followed by the three-step process as described below (Turan, 1999). The fish were placed on a white board with dorsal and anal fins held erect by pins. The right body profile of each fish was photographed using a 300-dpi, 32-bit color digital camera (Cybershot DSC-F505; Sony, Japan). Images were saved in jpg format and analyzed by TPS dig2 (Ver. 2.04) to digitize 13 landmark points (Fig. 1). A box truss of 26 lines connecting landmark points was generated for each fish to extract the basic body shape (Cardin and Friedland, 1999). All data were transferred to a spreadsheet file (Excel 2010), and the X–Y coordinate data transformed into linear distances by computer for subsequent analysis (Turan, 1999). Size-dependent variation was corrected by adopting an allometric method based on Elliott et al. (1995):

$$M_{adj} = M (L_s / L_0)^b$$

Where M is the original measurement, Madj = size adjusted measurement, L_0 the standard length of fish, Ls = the overall mean of the standard length for all fish from all samples in each analysis, and b = estimated for each character from the observed data as the slope of the regression of log M on log L_0 using all fish in

Table 1. Length and Weight parameters of the studied *Rutilus kutum*.

Parameter	**Sex**	
	M	F
Length range (cm)	29.5-47	32-51
Mean length (cm)±SE	38.71.2	43.7±0.8
Weight range (g)	260-1010	330-1324
Mean weight (g)±SE	628.1±55.9	892.1±46.9

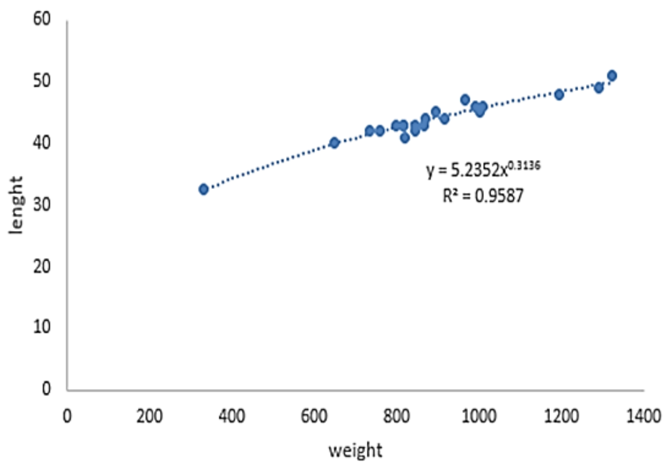

Figure 2. Length-weight relationship of the studied *Rutilus kutum* (male).

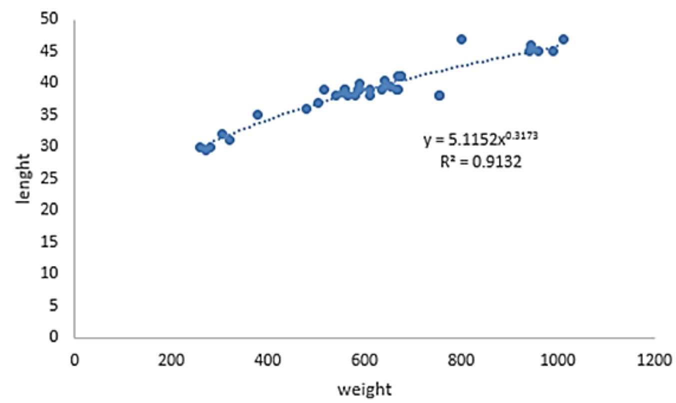

Figure 3. Length-weight relationship of the studied *Rutilus kutum* (female).

any group. The derived data from the allometric method by testing significance of the correlation between transformed variables and standard length were confirmed (Turan, 1999).

Univariate ANOVA was performed for each morphometric character to evaluate the significant differences between the populations (Zar, 1984). The morphometric characters that showed significant difference (P<0.05) were used for further analysis. In addition, the recommended ratio of the number of organisms (N) measured to the parameters (P) were included in the analysis to be at least 3-3.5 (Kocovsky et al., 2009) for obtaining the stable outcome from the multivariate analysis. Linear discriminant function analysis (DFA) was used to calculate the percentage of correctly classified (PCC) fish. A cross-validation using PCC was carried out to estimate the expected actual error rates of the classification functions. To examine the suitability of the data for PCA, Bartlett's test of sphericity and the Kaiser–Meyer–Olkin (KMO) measures were performed. The Bartlett's test of sphericity can be used to determine if the value of the correlation matrix is equal to zero, while the KMO measures sampling adequacy tests whether the partial correlation among variables is sufficiently high (Nimalathasan, 2009). The KMO statistics varies between 0 and 1. Kaiser (1974) suggested that values greater than 0.5 are acceptable. Statistical analyses for morphometric data were performed using the SPSS software (version 16).

Results and Discussion

Length and weight parameters of the studied *R. kutum* are shown in Table 1. The minimum and maximum length of females were 32 and 51 (cm), while those of weight were 330 and 1324 (g), respectively. The minimum and maximum length of males were 29.5 and 47 (cm), while those of weight were 260 and 1010 (g), respectively (Table 1).

Figures 2 and 3 show length–weight relationships of male and female Caspian kutum, respectively. Figures 4 and 5 present length–length relationships in males and female Caspian kutum, respectively. All the three relationships were positively correlated at 0.05% level (P<0.05%). A negative allometry (b<3) in length-weight relationship were found for *R. kutum* in the Caspian Sea. According to Weatherley and Gill (1987), the annual length-weight relationships could differ between seasons and years and also many

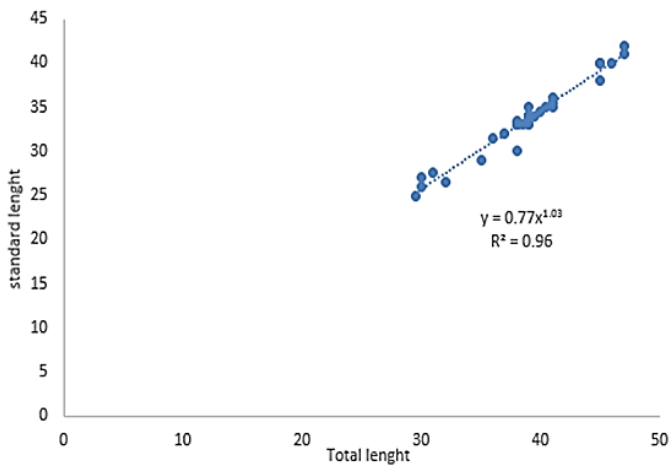

Figure 4. Length- length relationship the studied *Rutilus kutum* (male).

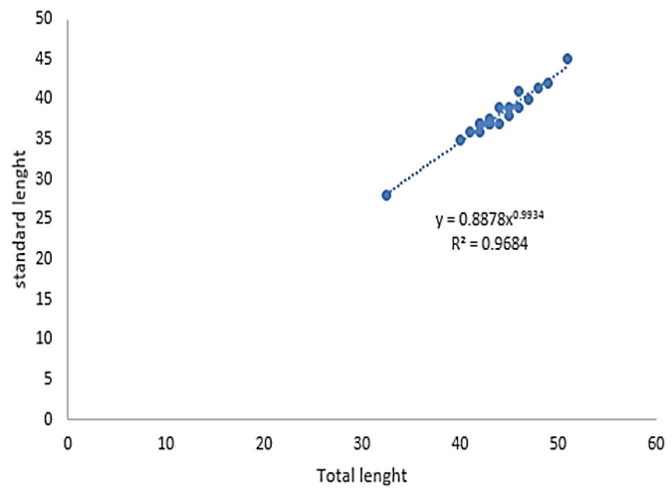

Figure 5. Length- length relationship the studied *Rutilus kutum* (female).

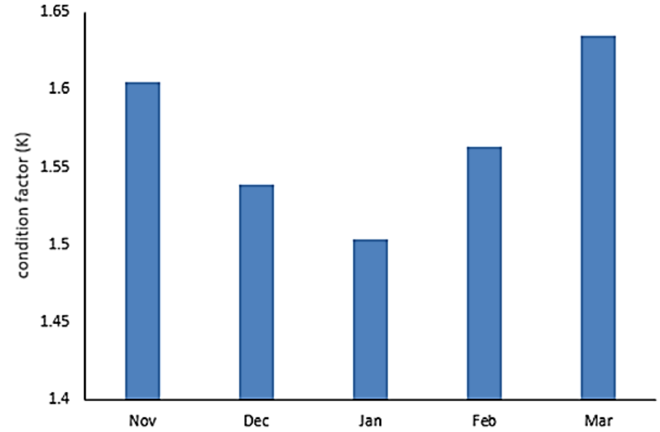

Figure 6. Monthly changes of the condition factor in the studied *Rutilus kutum* in the Caspian Sea.

factors could contribute to these differences such as maturity, temperature, salinity, food availability and size. Length-weight relationship may vary seasonally according to the degree of sexual maturity, sex, diet, stomach fullness, sample preservation techniques (Wootton, 1992), number of specimens examined, area/season effects and sampling duration (Moutopoulos and Stergiou, 2002).

Figure 6 shows the mean monthly CF of the Caspian kutum from November 2017 till March 2018. The monthly CFs were higher in March reflecting better conditions of fish in this month. The highest value (1.63) was observed in March, and the lowest value (1.5) in January.

Significant differences were found between eastern and western populations of *R. kutum* in 42 morphometric characters out of 78 standardized traits ($P \leq 0.05$).

In the present study, the value of KMO for overall matrix was 0.6 and the Bartlett's test of sphericity is significant ($P<0.05$). Principal component analysis of 42 morphometric measurements extracted 12 factors with eigenvalues >1, explaining 96% of the variance. The first principal component (PC1) accounted for 26.84% of the variation, while the second one (PC2) for 16.33%. The most significant loadings on PC1 were 1-3, 1-4, 1-5, 1-8, 1-11, 1-12, 1-13, 2-3, 2-4, 2-7, 3-5, 3-6, 3-7, 3-9, 3-10, 4-6, 4-8, 4-9, 4-11, 4-12, 5-6, 5-8, 5-9, 5-11, 5-12, 6-12, 7-9, 7-10, 8-9, 8-10, 9-13, 10-11, 10-13, while on PC2 were 1-7, 1-10, 1-12, 2-6, 2-9, 2-10, 2-11, 3-8, 3-9, 3-10, 3-11, 4-7, 4-10, 4-12, 5-7, 5-8, 5-10, 6-10, 6-11, 7-8, 7-9, 7-11, 7-12, 8-11, 9-10, 9-11, 9-12, 9-13. In this analysis, the characteristics with an eigenvalues exceeding 1 were included and others discarded. Also, in female visual examination of plots of PC1 and PC2 scores, specimens were grouped into two areas with moderate overlap between two stations (Fig. 7). Wilks's lambda distribution of discriminant analysis indicated significant differences in 13 morphometric characters of the two populations. In this test, one function was highly significant ($P<0.01$). The linear discriminant analysis gave an average PCC (Percentage of specimens classified) of 86.5% for morphometric characters indicating a high rate of correct classification of individuals into their original populations.

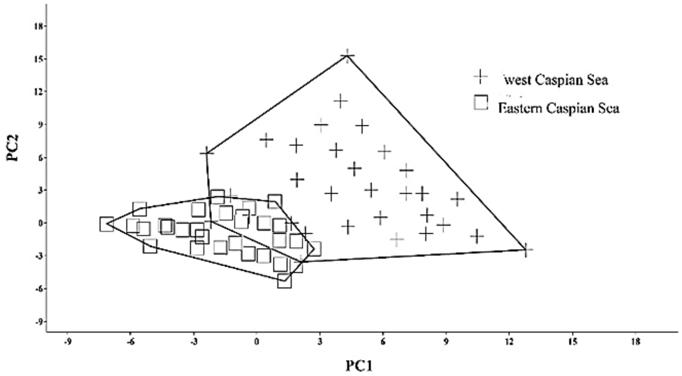

Figure 7. Plot of the factor scores for PC1 and PC2 of all morphometric measurements for *Rutilus kutum*.

The studied populations were distinguished from each other by morphologic differences especially in pre anal, body height, distance from pectoral fin to ventral fin, distance from pectoral fin to anal fin, caudal peduncle length, head length, pre orbital, preventral and predorsal length. Geographical isolation can also affect growth pattern and reproductive strategy of fish species. The importance of such factors on producing morphological differentiation in fish species is well-known (Yamamoto et al., 2006; Pollar et al., 2007). The causes of morphological differences among populations are often quite difficult to explain (Bookstein 1991). It was suggested that morphological characteristics of fishes are determined by genetic, environment and the interaction between them (Kohestan-Eskandari et al., 2014). The environmental factors prevailing during the early development stages, when the individual's phenotype is more amenable to environmental influence is of particular importance (Eschmeyer and Fong, 2011; Burgos et al., 2016). The phenotypic variability may not necessarily reflect population differentiation at the molecular level (Bookstein 1991). Apparently, different environmental conditions can lead to an enhancement of pre-existing genetic differences, providing a high inter-population structuring (Eschmeyer and Fong, 2011; Burgos at al., 2018).

These morphologically different populations can be considered as distinct stocks in the Caspian Sea for fisheries management. Nevertheless, future studies on determination of population structure will be elucidated using biochemical and molecular genetic methods or not.

Acknowledgments

This study was financially supported by The Caspian Basin Research Center, University of Guilan, Rasht, Iran, file number 21195170

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