

## Growth and Reproduction Properties of the Doctor Fish, *Garra rufa* (Cyprinidae) Infested with *Paradiplozoon bingolensis* Civanova et al., 2013 (Monogenea: Diplozoidae) in the Göynük Stream (Bingöl, Turkey)

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Research Article

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### Abstract

This study was carried out to determine the growth and reproductive characteristics of *Garra rufa* Heckel, 1843 infested with *Paradiplozoon bingolensis* Civanova et al., 2013 in Göynük Stream, Bingöl. A total of 267 *G. rufa* specimens were caught by a casting net between March 2015 and February 2016. The total length of *G. rufa* ranged from 8.5-19.1 cm and the total weights ranged from 5.8-58.7 g. The most intensive catching was in *G. rufa* population of 12.0-12.9 cm (30.0%) cm and 15.0-19.9 (25.2%) g in the groups. The mean condition factor was determined as 1.126±0.151 (0.607-1.646). The length-weight relationships were calculated as  $W = 0.0205 \times TL^{2.758}$  ( $r^2 = 0.891$ ). The M: F ratio was 1:1.15. The sexual maturity length was recorded as 13 cm total length for males and 14 cm total length for females. Additionally, *Paradiplozoon bingolensis* was detected on only 218 *G. rufa* in this population. Of these positive samples, 173 *P. bingolensis* were isolated from male fish samples, while the females had 228 *P. bingolensis*. Therefore, female *G. rufa* specimens from this population were determined to be more predisposed to the infestation ( $p < 0.01$ ).

**Keywords:** Growth, reproduction, *Garra rufa*, length-weight relationships, *Paradiplozoon bingolensis*.

**Göynük Çayı (Bingöl, Türkiye)'nda Yaşayan ve *Paradiplozoon bingolensis* Civanova et al., 2013 (Monogenea: Diplozoidae) İnfestasyonuna Konak Olan Doktor Balık, *Garra rufa* (Cyprinidae)'nın Büyüme ve Üreme Özellikleri**

### Özet

Bu çalışmada Göynük Çayı'nda yaşayan *Garra rufa* Heckel, 1843 türünün büyüme ve üreme özellikleri ile *Paradiplozoon bingolensis* Civanova et al., 2013 infestasyonuna konak olma durumu araştırılmıştır. Mart 2015-Şubat 2016 tarihleri arasında toplam 267 adet *G. rufa* serpm ağ ile yakalanmıştır. Toplam boy 8,5-19,1 cm, ağırlık ise 5,8-58,7 g arasında belirlenmiştir. En yoğun avcılık 12,0-12,9 cm (30,0%) cm ve 15,0-19,9 (25,2%) g'lık gruplarda gerçekleşmiştir. Kondisyon faktörü 1,126±0,151 (0,607-1,646) olarak belirlenmiştir. Boy-ağırlık ilişkisi  $W = 0,0205 \times TL^{2,758}$  ( $r^2 = 0,891$ ) olarak hesaplanmıştır. Erkek: dişi oranı 1:1,15 olarak belirlenmiştir. Cinsel olgunluğun erkeklerde 13 cm, dişilerde ise 14 cm total boya ulaşıncaya gerçekleştiği tespit edilmiştir. Bununla birlikte, 218 *G. rufa* örneğinin *Paradiplozoon bingolensis* paraziti taşıdığı belirlenmiştir. Pozitif olan bu örneklerde 173 adet *P. bingolensis* erkek *G. rufa* örneklerinden izole edilirken, dişi *G. rufa* örneklerinin 228 adet *P. bingolensis* taşıdığı görülmüştür. Bu nedenle, dişi *G. rufa* örneklerinin enfeksiyona daha yatkın olduğu belirlenmiştir ( $p < 0,01$ ).

**Anahtar kelimeler:** Boy-ağırlık ilişkileri, büyüme, üreme, *Garra rufa*, *Paradiplozoon bingolensis*

### INTRODUCTION

It has been acknowledged that collecting data about the growth parameters of fish is a crucial step in the evaluation process of fish populations as it allows researchers to compare the condition and biomass of a specific population as well as the distribution of species in varied geographical areas (Martin-Smith, 1996).

The length-weight relationships (LWRs) provide a quick cost-effective method for assessing the weight of a particular fish species by estimating the weight from length observations obtained on the field (Kimmerer et al., 2005). Understanding the LWRs are of paramount importance in fishery resource management and useful in comparing life history and morphological aspects of populations

inhabiting in different regions. Besides, LWRs also provide information on the stock composition, life span, mortality, growth, and production of fish (Bolger and Connoly, 1989).

Fulton's Condition Factor (K) is used to know the variation between the observed and expected weight of fishes (Kund et al., 2011). K measures the deviation of an organism from the average weight in a given sample to assess the suitability of a specific water environment for the growth of fish (Yılmaz et al., 2012). An overall fitness for fish species is assumed when K values are equal or close to 1. Good growth condition of the fish is deduced when  $K \geq 1$ , while the organism is in poor growth condition compared to an average individual with the same length when  $K < 1$  (Jisr et al., 2018).

Gonadosomatic index (GSI) is one of the most essential parameters in studying reproductive investment, gonadal development and maturity of fish in relation to spawning. GSI is a parameter that is used to study the spawning biology of the fish. GSI is also helpful to assess the level of ripeness of the ovary. GSI serves as an indicator of the reproductive seasonality of fish species. Reproduction of fish stock is one of the important components of fishery biology as it has a direct bearing on fish production, stock-recruitment and stock management (Wootton, 1992; Çetinkaya et al., 2005; Shafi, 2012).

Belonging to the family of Cyprinidae, the members of the genus *Garra* were first found by Hamilton-Buchanan in 1822 from Southwest Asia and in the region extending from Africa to Southeast Asia (Krupp and Schneider, 1989); however, it was reported that *G. rufa* was first found in Aleppo. Originating from this region and reaching Southwest Asia, the distribution of *G. rufa* in Turkey is reported in varied regions such as Aras/Araxes River, Tigris-Euphrates River system, and Tigris River basins (Menon, 1964; Karaman, 1971; Coad, 1995; Geldiay and Balik, 2002). To the author's knowledge, there is no record of *G. rufa* living in Kızılırmak and Yeşilirmak rivers, both of which are located in Central Anatolia. Therefore, it is assumed that the distribution of *G. rufa* could not spread out of its original location (i.e., Mesopotamia, Eastern Turkey, and surrounding basins) (Koyun, 2011; Koyun et al., 2018).

*G. rufa* can live in a variety of habitats such as rivers, streams, ponds, and lakes, hiding under and among stones and vegetation. *G. rufa* generally feeds on algae and zooplankton. In addition, chrysophyta, chlorophyta, cyanobacteria, rotifera, and protozoa were detected in stomach contents of *G. rufa* populations (Krupp and Schneider, 1989; Jarvis, 2011).

*G. rufa* is named the 'doctor fish' due to its use as a therapeutic agent in human skin diseases/disorders (e.g., Psoriasis), but it is also known as nibble fish, Kangal fish, sucker, or licker fish (Koyun, 2011). Regarding morphological characteristics, the typical shape of *G. rufa* is a thin and long cylindrical structure covered with thin but large scales. There are tubercles present on its truncated nostrils. There are two pairs of short barbels on the crescent-shaped ventral mouth of this fish. A well-developed adhesive disc/organ located on the bottom/lower lip enables *G. rufa* to conveniently survive in fast-flowing water. During the fall and winter months, black spots emerge on their originally brown-colored bodies (Karaaslan, 2010; Jarvis, 2011).

Monogeneans, belonging to the family of Diplozoidae, are common parasites that have been found specifically in the gills of Cypriniformes. Their direct parasitic life cycle starts as free-swimming oncomiracidia, a larval stage (diporpa), and is completed with an adult stage. Two larvae (diporpa) permanently coexist by fusing their bodies to achieve sexual maturity. Vitellaria and most internal organs are distributed towards the cranial end in adult parasites, while reproductive organs of both sexes and distal point of the intestine are located towards the dorsal end. Additionally, adults have four pairs of hooks as attachment organs along with one small pair centered on the ventral side of the opisthaptor (Civanova et al., 2013).

This study was carried out for the determination of growth and reproduction properties of *Garra rufa* and the infestation status by *Paradiplozoon bingolensis* in the Göynük Stream.

## MATERIALS and METHODS

### Study area and sampling

The present study was carried out in Göynük Stream, which is a reach of Murat Stream (Figure 1). The river springs from Kargapazarı Village on the western slopes of the Bingol Mountains and merges with Mendo Creek and joins the Murat Stream. It has approximately 95 km in length and there are also 5 Hydro Electric Power Plants and 2 regulators on the river (Koyun et al., 2018). A total of 267 *G. rufa* specimens were monthly collected by a casting net in Göynük Stream from March 2015 to

February 2016. In Göynük Stream, water temperature and pH were measured for 12 months with AZ 8685 device in the sampling area, and dissolved oxygen (DO) values were taken with Portable Hanna Galvanic Dissolved Oxygen Meter - HI9147.

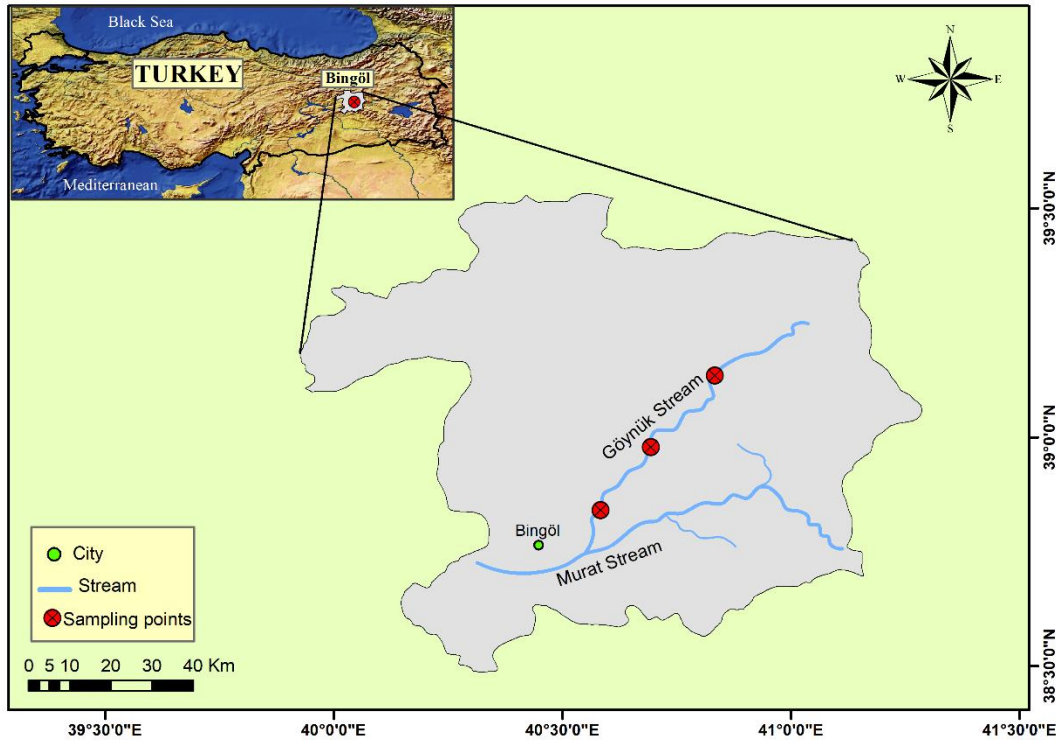


Figure 1. Sampling points at Göynük Stream

### Laboratory Procedures

Fish specimens were transported within a 20-liter plastic aquarium filled with stream water. After transport to the laboratory for every fish specimen, each gill arch was removed to examine monogeneans parasites within 24 h. At the same time samples were measured to the nearest 0.1 cm for total length (TL) and to the nearest 0.1 g for total weight (W). Total length-weight relationships (LWRs) were determined using the equation:  $W = a \times L^b$ , where W is the weight (W), L is length (L), a is the intercept, and b is the slope (Le Cren, 1951). Fulton's Condition Factor was calculated using  $K = (W / L^3) \times 100$ , where W = weight of fish (g), L = total length of fish (cm) (Ricker, 1975).

The gonads were removed and observed morphologically. Sex was recorded for each fish. The sex of the fish was categorized as male or female. The first reproduction length and weight of the fish were determined according to the maturity status of the gonads (Crim and Glebe, 1990). Gonadosomatic Index value was calculated using the formula  $GSI = (Gw / W) \times 100$ , where Gw = gonad weight of fish (g), W = total weight of fish (g) (Karataş et al., 2005).

The obtained parasites were preserved in glycerine ammonium picrate and flattened in 70% ethanol for staining with iron acetocarmine. After dehydrated in an alcohol series and mounted in Canada balsam. Parasite specimens were observed under a light microscope and identified according to Pugachev et al. (2009) and Civanova et al. (2013). In addition, *P. bingolensis* was the first time described as a new species of genus in *Garra rufa* from Murat River (Civanova et al., 2013). So, the diagnosis of the mentioned parasite was done according to the descriptive features of Civanova et al. (2013) article, considering the host specificity. In another parasitic study conducted on 17 fish species in Iraq, it is reported that *P. bingolensis* was found only in the same host *Garra rufa* (Furhan et al., 2017). In addition, according to the distribution of the *P. bingolensis* in the host fish, sex, prevalence (P), abundances (A) and mean intensity (I) were investigated.

### Statistical Analysis

The growth types for the specimens were determined using the Student t-test. The t-test statistics values were calculated and compared with critical values from the T-Table to check if the growth type is isometric ( $b = 3$ ) or allometric ( $b \neq 3$ ) (Pajuelo and Lorenzo, 1998). The sex ratio was tested by the chi-square test ( $\chi^2$ ) to indicate whether there was a deviation from a 1:1 ratio (Zar, 1999). The prevalence, mean intensity and mean abundance levels of *P. bingolensis* were calculated according to Bush et al. (1997). Bivariate correlations between the mean intensity of parasites and the physicochemical parameters as well as the condition factor were tested to find out which parameters correlated significantly with the number of parasites, according to Pearson coefficients. The means, variance, standard deviation, regression, correlation values, and the comparisons of population parameters obtained from the study were performed with SPSS 21.0 and Microsoft Excel 2016.

## RESULTS

### Temperature, pH, oxygen

For 12 months at Göynük Stream, the mean values  $\pm$  standard deviation (SD) of water temperature, pH, and DO were measured as  $14.5 \pm 6.7$  °C,  $8.5 \pm 0.3$  and  $9.1 \pm 0.3$  mg/L, respectively (Table 1).

**Table 1.** Physico-chemical parameters of Göynük Stream.

| Months        | Temperature (°C) | pH            | DO (mg/L)     |
|---------------|------------------|---------------|---------------|
| Mar 2015      | 10.6             | 8.6           | 9.1           |
| Apr 2015      | 14.6             | 8.9           | 9.3           |
| May 2015      | 17.1             | 8.7           | 9.1           |
| June 2015     | 20.2             | 8.2           | 9.0           |
| July 2015     | 22.3             | 8.4           | 8.9           |
| Aug 2015      | 25.0             | 8.2           | 8.8           |
| Sep 2015      | 20.1             | 8.1           | 8.7           |
| Oct 2015      | 14.8             | 8.6           | 9.0           |
| Nov 2015      | 11.2             | 8.8           | 9.1           |
| Dec 2015      | 8.2              | 8.4           | 9.6           |
| Jan 2016      | 4.6              | 8.7           | 9.8           |
| Feb 2016      | 5.5              | 8.8           | 9.2           |
| Mean $\pm$ SD | $14.5 \pm 6.7$   | $8.5 \pm 0.3$ | $9.1 \pm 0.3$ |

### Growth in Length

The TL values of the *G. rufa* in this study ranged from 8.5 to 19.1 cm ( $n = 267$ ) for all population, 8.5 to 17.5 cm ( $n = 124$ ) for males, and 8.6 to 19.1 cm ( $n = 143$ ) for females. The samples were grouped in length (i.e., at 1 cm intervals) and the length-frequency distribution was examined. The length-frequency distribution of *G. rufa* indicated that the most frequent size class in the samples were 12.0-12.9 cm (30.0%) for all populations, 11.0-11.9 cm (21.3%) for males, and 12.0-12.9 cm (19.5%) for females (Figure 2).

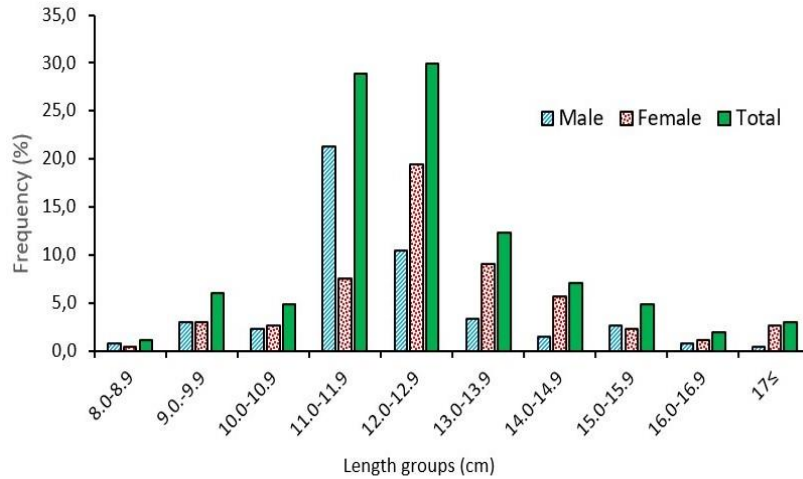


Figure 2. Total length frequency of *G. rufa* in the Göynük Stream

**Growth in Weight**

The ranges of total weight for *G. rufa* population were found between 5.8-58.7 g (n = 267), for all the samples, 7.6-42.6 g (n = 124) for males, and 5.8-58.7 g (n = 143) for females. All samples were investigated by grouping them at 5 g intervals. It was observed that the dominant weight groups were found as 15.0-19.9 g (25.5%) for all samples, 15.0-19.9 g (16.9%) for males, and 20.0-24.9 g (15.4%) for females (Figure 3).

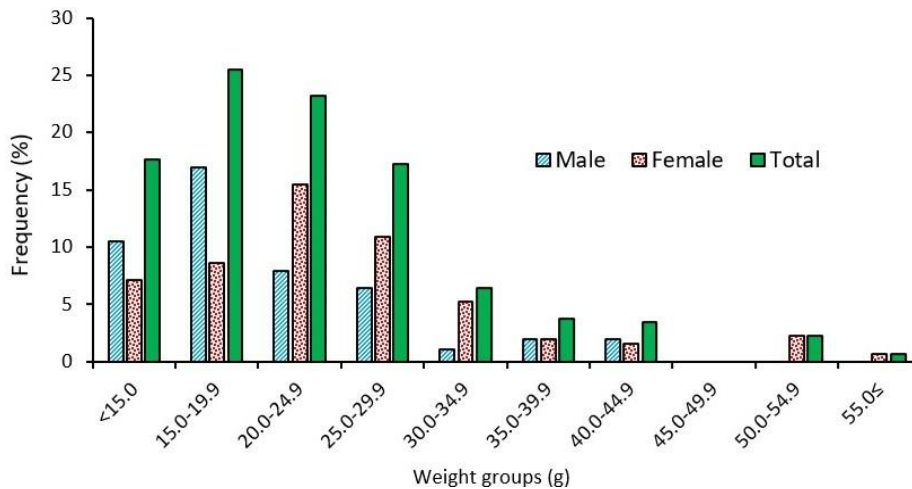


Figure 3. Total weight frequency of *G. rufa* in the Göynük Stream

**Length-weight Relationship**

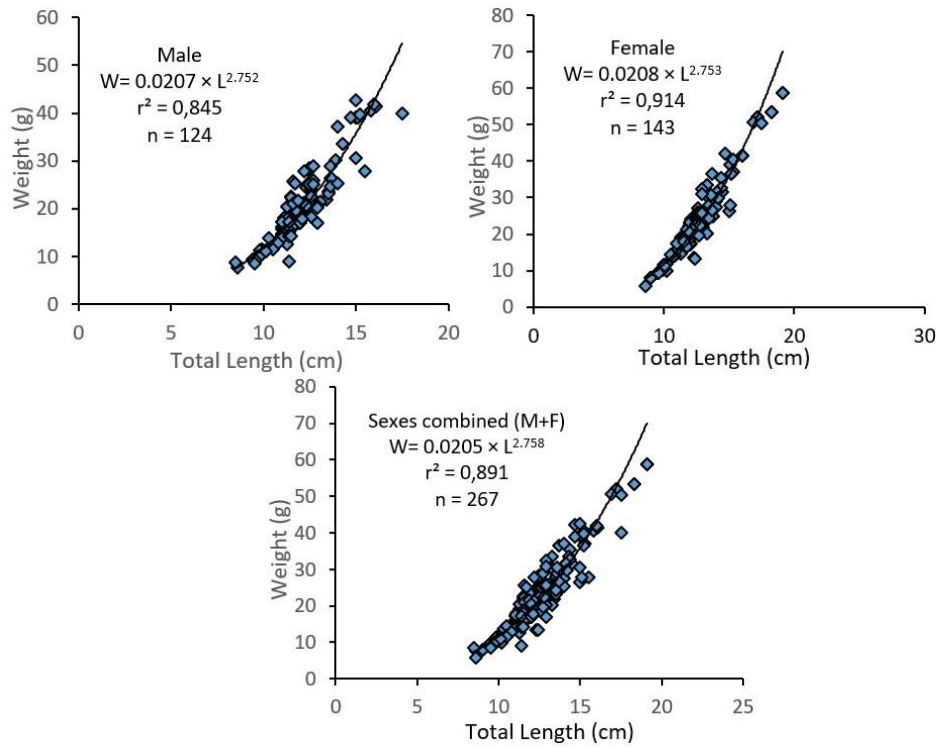
The LWRs for *G. rufa* are shown in Table 2. The LWRs evaluated by using the lengths and weights were found to be significant with the high regression coefficient for each considered group ( $r^2 = 0.851$  to  $0.922$ ).

The LWRs of *G. rufa* population from Göynük Stream was calculated as  $W = 0.0205 \times TL^{2.758}$  ( $r^2 = 0.891$ ) for all samples,  $W = 0.0207 \times TL^{2.752}$  ( $r^2 = 0.845$ ) for males, and  $W = 0.0208 \times L^{2.753}$  ( $r^2 = 0.914$ ) for females (Figure 4). The b values imply that the body shape of the sexes and of the population displays isometric form, because the b values were not significantly different from 3 ( $p > 0.05$ ) (Table 2).

**Table 2.** The descriptive statistics and estimated parameters of length-weight relationships of *G. rufa* populations in the Göynük Stream.

| Sex    | n   | Regression parameters |       |                 |                | Student's t-test | p     | Growth type |
|--------|-----|-----------------------|-------|-----------------|----------------|------------------|-------|-------------|
|        |     | a                     | b     | SE <sub>b</sub> | r <sup>2</sup> |                  |       |             |
| Male   | 124 | 0.0207                | 2.752 | 0.138           | 0.845          | -1.341           | 0.207 | I           |
| Female | 143 | 0.0208                | 2.753 | 0.107           | 0.914          | -0.994           | 0.341 | I           |
| Total  | 267 | 0.0205                | 2.758 | 0.102           | 0.891          | -1.379           | 0.195 | I           |

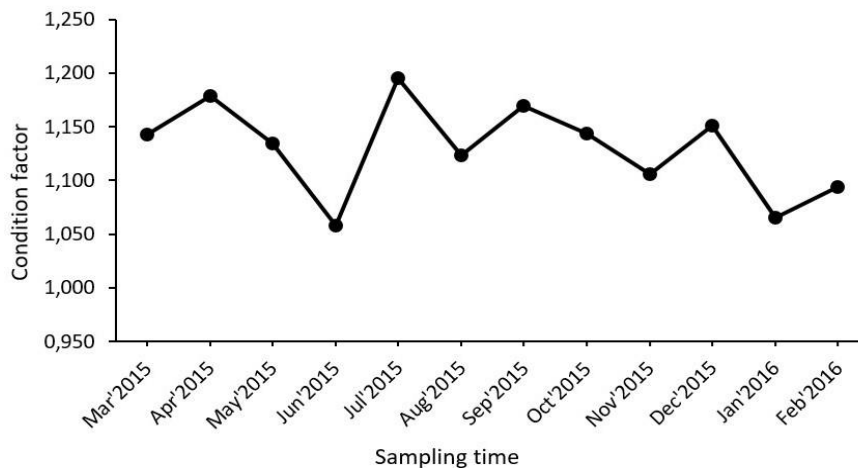
n = number of individuals, a = proportionality constant, b = slope of the relationship; SE<sub>b</sub>, standard error of b; r<sup>2</sup> = coefficient of determination; I = isometric growth; \* Statistically different (p<0.05).



**Figure 4.** Length-weight relationships for *G. rufa* in the Göynük Stream

**Fulton’s Condition Factor**

Fulton’s Condition Factor of *G. rufa* was calculated as 1.126±0.151 (0.607-1.646) for all samples, 1.134±0.168 (0.607-1.646) for males, and 1.119±0.135 (0.698-1.509) for females. The mean lowest condition value was determined in June (1.058±0.124), whereas the highest was in July (1.195±0.105) (Figure 5).



**Figure 5.** Fulton’s Condition Factor changes for *G. rufa* in the Göynük Stream

### Reproduction and Sexual Maturation

In the population of *G. rufa*, 124 (46.4%) of the specimens collected were male and 143 (53.6%) were female. The M: F ratio was calculated as 1:1.15. The sex ratio was only found to be significantly different from equality 1:1 in June ( $\chi^2$  test,  $p < 0.05$ ) (Table 3).

**Table 3.** M: F ratio of the *Garra rufa* populations in the Göynük Stream.

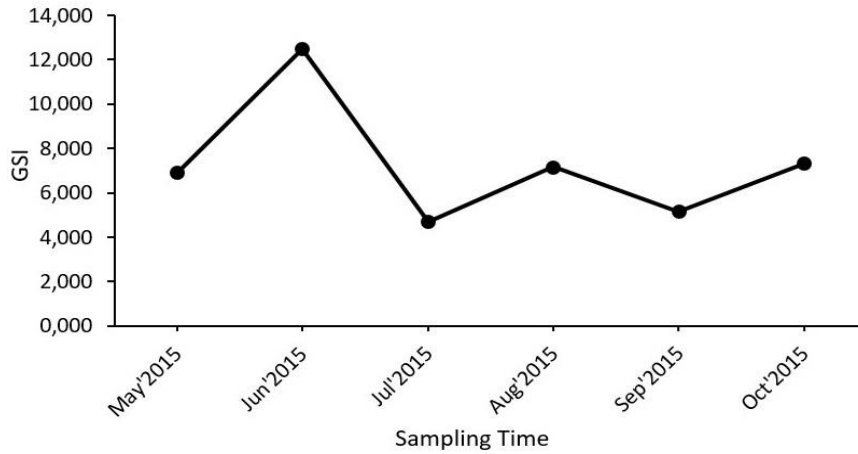
| Months    | Male |      | Female |      | M: F   | $\chi^2$ | Result     |
|-----------|------|------|--------|------|--------|----------|------------|
|           | n    | %n   | n      | %n   |        |          |            |
| Mar 2015  | 5    | 50.0 | 5      | 50.0 | 1:1    | 0.00     | $P > 0.05$ |
| Apr 2015  | 16   | 53.3 | 14     | 46.7 | 1:0.88 | 0.13     | $P > 0.05$ |
| May 2015  | 6    | 33.3 | 12     | 66.7 | 1:2    | 2.00     | $P > 0.05$ |
| June 2015 | 8    | 27.6 | 21     | 0.0  | 1:2.63 | 5.83     | $P < 0.05$ |
| July 2015 | 4    | 40.0 | 6      | 60.0 | 1:1.50 | 0.40     | $P > 0.05$ |
| Aug 2015  | 12   | 52.2 | 11     | 47.8 | 1:0.92 | 0.04     | $P > 0.05$ |
| Sep 2015  | 13   | 54.2 | 11     | 45.8 | 1:0.85 | 0.17     | $P > 0.05$ |
| Oct 2015  | 13   | 43.3 | 17     | 56.7 | 1:1.31 | 0.53     | $P > 0.05$ |
| Nov 2015  | 13   | 48.1 | 14     | 51.9 | 1:1.08 | 0.04     | $P > 0.05$ |
| Dec 2015  | 10   | 41.7 | 14     | 58.3 | 1:1.40 | 0.67     | $P > 0.05$ |
| Jan 2016  | 17   | 58.6 | 12     | 41.4 | 1:0.71 | 0.86     | $P > 0.05$ |
| Feb 2016  | 7    | 53.9 | 6      | 46.1 | 1:0.86 | 0.08     | $P > 0.05$ |
| Total     | 124  | 46.4 | 143    | 53.6 | 1:1.15 | 1.35     | $P > 0.05$ |

The sexual maturity for *G. rufa* was determined on 62 samples. When the sexual maturation was examined according to 1 cm total length groups, it was determined that maturation occurred at 13 cm (55.6%) TL for males and 14 cm (53.3%) TL for females (Table 4).

**Table 4.** Sexual maturity groups of *G. rufa* in the Göynük Stream.

| Total Length groups (cm) | Male          |                 | Female        |                 |
|--------------------------|---------------|-----------------|---------------|-----------------|
|                          | Mature n (%n) | Immature n (%n) | Mature n (%n) | Immature n (%n) |
| ≤9                       | 0             | 10 (100.0)      | 1 (11.1)      | 8 (88.9)        |
| 10                       | 0             | 6 (100.0)       | 3 (42.9)      | 4 (57.1)        |
| 11                       | 3 (5.3)       | 54 (94.7)       | 6 (30.0)      | 14 (70.0)       |
| 12                       | 2 (7.1)       | 26 (92.9)       | 13 (25.0)     | 39 (75.0)       |
| 13                       | 5 (55.6)      | 4 (44.4)        | 11 (45.8)     | 13 (54.2)       |
| 14                       | 1 (25.0)      | 3 (75.0)        | 8 (53.3)      | 7 (46.7)        |
| 15                       | 1 (14.3)      | 6 (85.7)        | 0             | 6 (100.0)       |
| 16≤                      | 0             | 3 (100.0)       | 8 (80.0)      | 2 (20.0)        |
| Total                    | 12            | 112             | 50            | 93              |

The total GSI for *G. rufa* was calculated between 0.000 and 27.460 with an average of  $7.280 \pm 0.766$ . This value was determined as  $6.327 \pm 1.824$  (0.000-22.439) for males and  $8.196 \pm 1.145$  (0.000-27.459) for females. The mean GSI for all samples peaked in June ( $12.480 \pm 1.423$ ), whereas the lowest in July ( $4.699 \pm 2.201$ ) (Figure 6).

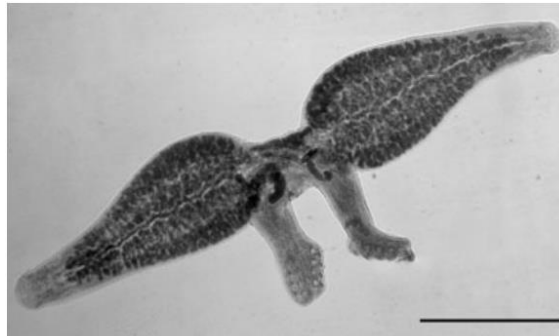


**Figure 6.** GSI changes for *G. rufa* in the Göynük Stream

**Ectoparasite, *Paradiplozoon bingolensis*** (Civanova et al., 2013)

218 (81.6%) fish specimens were infested by *P. bingolensis* in the *G. rufa* population (Table 5). Females were found to be more commonly infested with the parasite *P. bingolensis* ( $p < 0.01$ ). The highest prevalence value in all populations of *G. rufa* was found as 95.7% in August, while the lowest as 40.0% in March (Table 5). The highest mean abundance and mean intensity values in all populations of *G. rufa* were determined in June (1.8) and in June (2.2) and January (2.2), respectively (Table 5).

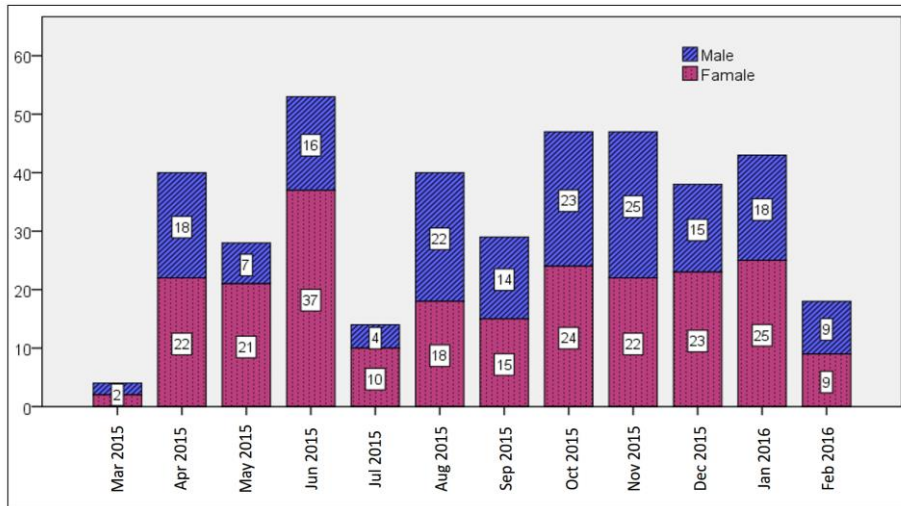
Additionally, a total of 401 *P. bingolensis* (Figure 7) were detected on *G. rufa* samples in this study. While 173 (43.1%) of them were male, 228 (56.9%) of them were female, indicating that *P. bingolensis* were more inclined ( $p < 0.01$ ) to prefer female *G. rufa* as hosts in this population from Göynük Stream (Table 5).



**Figure 7.** *P. bingolensis*; total view (scale bar 1 mm) (from Civanova et al., 2013)

The highest amount of *P. bingolensis* in this study was recorded in June, while the lowest in March (Figure 8).





**Figure 8.** The amount of *P. bingolensis* isolated on *G. rufa* by months and sex

Although the results obtained from the correlation analysis are not statistically significant ( $p > 0.05$ ), it shows that increasing water temperature, as well as the oxygen level, has a positive correlation with the number of parasites, while the pH level causes a negative correlation. On the other hand, a positive correlation was detected between the present *P. bingolensis* in the environment and the fish population ( $p < 0.05$ ).

A negative correlation was found between the mean intensity levels and the condition factor of all populations in *G. rufa*, but it was not statistically significant ( $p > 0.05$ ) (Table 6).

**Table 5.** Prevalence, mean intensity and mean abundance of *P. bingolensis* infection of *G. rufa*.

| Months   | Number of examined fish samples-<br>Number of infected fish samples |         |         | P (%) |        |       | The number of <i>P. bingolensis</i> determined on<br>male, female, and all population of <i>G. rufa</i> |            |             | A-I         |               |              |
|----------|---|---------|---------|-------|--------|-------|---|------------|-------------|-------------|---------------|--------------|
|          | Male  | Female  | Total   | Male  | Female | Total | Male (%)  | Female (%) | Total (%)   | Male<br>A-I | Female<br>A-I | Total<br>A-I |
| Mar 15   | 5-2   | 5-2     | 10-4    | 40.0  | 40.0   | 40.0  | 2 (0.5)   | 2 (0.5)    | 4 (1.0)     | 0.4-1.0     | 0.4-1.0       | 0.4-1.0      |
| Apr 2015 | 16-11   | 14-12   | 30-23   | 68.8  | 85.7   | 76.7  | 18 (4.5)  | 22 (5.5)   | 40 (10.0)   | 1.1-1.6     | 1.6-1.8       | 1.3-1.7      |
| May 15   | 6-5   | 12-10   | 18-15   | 83.3  | 83.3   | 83.3  | 7 (1.8)   | 21 (5.2)   | 28 (7.0)    | 1.2-1.4     | 1.8-2.1       | 1.6-1.9      |
| Jun 2015 | 8-7   | 21-17   | 29-24   | 87.5  | 81.0   | 82.8  | 16 (4.0)  | 37 (9.2)   | 53 (13.2)   | 2.0-2.3     | 1.8-2.2       | 1.8-2.2      |
| Jul 2015 | 4-3   | 6-4     | 10-7    | 75.0  | 66.7   | 70.0  | 4 (1.0)   | 10 (2.5)   | 14 (3.5)    | 1.0-1.3     | 1.7-2.5       | 1.4-2.0      |
| Aug 2015 | 12-12   | 11-10   | 23-22   | 100.0 | 90.9   | 95.7  | 22 (5.5)  | 18 (4.5)   | 40 (10.0)   | 1.8-1.8     | 1.6-1.8       | 1.7-1.8      |
| Sep 2015 | 13-8  | 11-10   | 24-18   | 61.5  | 90.9   | 75.0  | 14 (3.5)  | 15 (3.7)   | 29 (7.2)    | 1.1-1.8     | 1.4-1.5       | 1.2-1.6      |
| Oct 2015 | 13-13   | 17-15   | 30-28   | 100.0 | 88.2   | 93.3  | 23 (5.7)  | 24 (6.0)   | 47 (11.7)   | 1.8-1.8     | 1.4-1.6       | 1.6-1.7      |
| Nov 2015 | 13-12   | 14-12   | 27-24   | 92.3  | 85.7   | 88.9  | 25 (6.2)  | 22 (5.5)   | 47 (11.7)   | 1.9-2.1     | 1.6-1.8       | 1.7-2.0      |
| Dec 2015 | 10-9  | 14-12   | 24-21   | 90.0  | 85.7   | 87.5  | 15 (3.7)  | 23 (5.7)   | 38 (9.4)    | 1.5-1.7     | 1.6-1.9       | 1.6-1.8      |
| Jan 2016 | 17-9  | 12-11   | 29-20   | 52.9  | 91.7   | 69.0  | 18 (4.5)  | 25 (6.2)   | 43 (10.7)   | 1.1-2.0     | 2.1-2.3       | 1.5-2.2      |
| Feb 2016 | 7-6   | 6-6     | 13-12   | 85.7  | 100.0  | 92.3  | 9 (2.2)   | 9 (2.2)    | 18 (4.5)    | 1.3-1.5     | 1.5-1.5       | 1.4-1.5      |
| Total    | 124-97  | 143-121 | 267-218 | 78.2  | 84.6   | 81.6  | 173 (43.1)  | 228 (56.9) | 401 (100.0) | 1.4-1.8     | 1.6-1.9       | 1.5-1.8      |

Total: male+female individuals (all population) of *G. rufa*; P: prevalence; A: mean abundance, I: mean intensity.

**Table 6.** Correlation coefficients between the mean intensity (I) and the condition factor of *G. rufa* in the Göynük Stream.

|                         | Parameter           | Male condition factor |
|-------------------------|---------------------|-----------------------|
| Male I                  | Pearson Correlation | -0.149                |
|                         | Sig. (2-tailed)     | 0.643                 |
|                         | N                   | 12                    |
| Female condition factor |                     |                       |
| Female I                | Pearson Correlation | 0.080                 |
|                         | Sig. (2-tailed)     | 0.804                 |
|                         | N                   | 12                    |
| Total condition factor  |                     |                       |
| Total I                 | Pearson Correlation | -0.169                |
|                         | Sig. (2-tailed)     | 0.600                 |
|                         | N                   | 12                    |

\*. Correlation is significant at the 0.05 level (2-tailed).

\*\*Correlation is significant at the 0.01 level (2-tailed) and the other parameters were non-significant, I: mean intensity.

## DISCUSSION

The maximum total length was reported as 29.9 cm by Birecikligil and Çiçek (2011). However, the maximum total length was found as 19.1 cm in this study and this result is higher than the previous studies except for Birecikligil and Çiçek (2011) (Table 7). The variation in the maximum size of a fish population could be explained based on ecological conditions. Population characteristics of *G. rufa* presented different studies are given in Table 7.

The LWRs are used to ascertain the condition of fish and to determine whether the growth pattern is isometric or allometric and the b value used in the calculation of the length-weight relationship indicates nutritional, thereby optimum growth conditions of fish (Sakar et al., 2013). The ideal growth pattern represents an isometric growth due to good environmental conditions or good condition of the fish (Ricker and Carter, 1958). Allometric growth can be either positive or negative. When the growth pattern shows negative allometric ( $b < 3$ ), fish become slimmer with increasing length and growth. When the growth pattern shows positive allometric ( $b > 3$ ), fish become heavier reflecting optimum conditions for growth (Jisr et al., 2018). In this study, the LWR of *G. rufa* population was calculated using the equation of  $W = 0.02979 \times L^{2.68}$  ( $r^2 = 0.898$ ). Calculated b values were not significantly different from 3 and the growth types of *G. rufa* for all the samples, males and females were isometric growth ( $p > 0.05$ ). However, it was also reported as negative and positive allometric growths in some habitats and the calculated coefficient b varied among the localities from a minimum of 2.74 to a maximum of 3.196 in the previous studies (Table 7). The ideal b value for fish is determined 3 or close to 3 (Wootton, 1992). On the other hand, Froese (2006) proposed that the ideal b value should range from 2.5 to 3.5. The discrepancy between recorded b values in varied studies can be attributed to ecological factors such as temperature, food supply, spawning conditions, and habitat characteristics. In our study, the value of b remained within the expected range.

Fulton's Condition Factor, based on the analysis of length-weight data, is effectively used to compare the feeding and reproduction activity of populations thriving under similar or different conditions of nutrient density and climate (Bolger and Connolly, 1989). In this study, the mean Fulton's Condition Factor was calculated as  $1.126 \pm 0.151$  (0.607-1.646) for all samples. The mean condition factor for all samples peaked in July ( $1.195 \pm 0.105$ ), whereas the lowest was in June ( $1.058 \pm 0.124$ ). The condition factors of *G. rufa* populations were reported as 2.03 (0.87-3.14) in Armand River (Abedi et al., 2011) and  $1.218 \pm 0.18$  (0.651-2.198) in Cholvar River (Gerami et al., 2013). These results were higher than our study. Being one of the key criteria in fish growth and development, the ideal condition factor value has been accepted as 1 (Martinez and Vazquez, 2001), and it is proposed that it may vary due to the species, sex, age, and maturation of fish, as well as fat and muscle deposition in the host (Barnham and Baxter, 1998).

Sex determination, which is used to calculate the sex ratio, can be described as the differentiation in sexes. The sex ratio of a water source can vary due to fish species and populations inhabiting the environment (Nikolsky, 1963). In this study, the M: F ratio was calculated at 1:1.15 and the chi-square analysis showed that the sex ratio was not significantly different from the expected 1:1 ratio ( $\chi^2 = 1.35$ ,

$p>0.05$ ). Only females were dominant in June ( $\chi^2= 5.83$ ,  $p<0.05$ ). The M: F ratio was found as 1:0.91 in Armand Stream, 1:1.09 in Dalaki River and 1:1.46 in Cholvar River (Table 7).

Evaluation of ideal length and weight values as indicators of sexual maturity is a crucial process to sustain and manage current fish stocks. When sexually mature fish (with a certain length and weight) reproduces, the values are defined as the length or weight at first sexual maturity (Avşar, 2005). The first sexual maturity size for *G. rufa* population in Göynük Stream was observed at 13 cm TL for males and 14 cm TL for females.

Gonadosomatic index (GSI) is utilized to determine or detect the reproduction season of fish (Karataş et al., 2005). In this study, the mean GSI for all samples peaked in June ( $12.480\pm 1.423$ ), whereas the lowest in July ( $4.699\pm 2.201$ ). Abedi et al. (2011) reported that GSI values of *G. rufa* samples from Armand River peaked in April for males and May for females and the GSI values of both genders kept declining until November. Patimar et al. (2010) reported the GSI indicated that the reproduction of *G. rufa* in the Kangir River occurred around April-May. The variation in these studies due to different peak times may be attributed to the water temperature. Monitoring gonad development ratio and determining the reproductive season of fish present in natural habitats are important both economically and scientifically (Martinez and Vazquez, 2001).

218 (81.6%) *G. rufa* samples were *P. bingolensis* positive in the current population (121 females and 97 males). Additionally, 401 *P. bingolensis* were detected on the current *G. rufa* population (173 (43.1%) male and 228 (56.9%) female). The highest mean intensity of *P. bingolensis* in the *G. rufa* population was recorded in June (2.2) and in January (2.2), while the lowest in March (1.0). A negative correlation was found between the mean intensity of *P. bingolensis* parasites and the condition factor of the fish population, indicating when the number of parasites increased in the host, the condition factor of the *G. rufa* population declined ( $p>0.05$ ). *P. bingolensis* parasites were more inclined to prefer female *G. rufa* fish as hosts. As is seen in this study, diplozoid parasites more commonly prefer the members of the family Cyprinidae as hosts. Aside from the afore-mentioned parasite, there are other examples of diplozoids found in different fish species inhabiting varied water sources. In the other studies, *Paradiplozoon ichthyoxanthon*, *Paradiplozoon hemiculteri*, *Paradiplozoon yunnanensis*, and *Paradiplozoon chazarikum* were isolated from *Labeobarbus aeneus* (Avenant-Oldewage et al., 2014), *Hemiculter leucisculus* (Jirsova et al., 2018), *Sikukia gudgeri* (Fan et al., 2018), and *Rutilus frisii kutum* (Mohamamad and Mahsa, 2019), respectively.

In conclusion, the information provided here on the studied population as representative of *G. rufa* from the Göynük Stream shows that the specific characteristics include a isometric growth resulting in ideal shape ( $b=3$ ) and a shortened duration of the spawning season. The mean condition factor value of  $1.126\pm 0.151$  which indicates that fish were in good condition during the study period. It was established that *G. rufa* attained sexual maturity when they reached to 13 cm TL for males and 14 cm TL for females. It has been observed that *G. rufa* population reproduced at similar periods from various regions. When the mean intensity value increased in the population, the condition factor of the *G. rufa* population declined.

**Table 7.** Population characteristics of *Garra rufa* presented different studies.

| Locality                        | N   | M   | F   | M:F    | Total Length (cm)<br>(min-max) | Total Weight (g)<br>(min-max) | a        | b     | r <sup>2</sup> | References                          |
|---------------------------------|-----|-----|-----|--------|--------------------------------|-------------------------------|----------|-------|----------------|-------------------------------------|
| Göynük Stream, Turkey           | 247 | 124 | 143 | 1:1.15 | 8.5-19.1                       | 5.8-58.7                      | 0.0205   | 2.758 | 0.891          | The present study                   |
| Iran                            | 291 | -   | -   | -      | 2.90-13.00                     | -                             | 0.0119   | 3.139 | 0.992          | Esmaeili and Ebrahimi (2006)        |
| Kangir River, Iran              | 433 |     |     | 1:1.21 | 4.8-10.2                       | 1.53-14.45                    | -        | -     | -              | Patimar et al. (2010)               |
| Armand Stream, Iran             | 364 | 191 | 173 | 1:0.91 | 2.91-15.13                     | -                             | 0.0063   | 3.112 | 0.984          | Abedi et al. (2011)                 |
| Euphrates River, Turkey         | 161 | -   | -   | -      | 4.75–29.90                     | 7.6–13.9                      | 0.0075   | 3.149 | 0.996          | Birecikligil and Çiçek (2011)       |
| Dalaki and Shapour rivers, Iran | 324 | 156 | 168 | 1:1.09 | 3.0-17.1                       | 2-35                          | 0.0223   | 2.91  | 0.93           | Pazira et al. (2013)                |
| Cholvar River, Iran             | 535 | 217 | 318 | 1:1.46 | -                              | -                             | 0.000005 | 3.196 | -              | Gerami et al. (2013)                |
| Tange River, Iran               | 27  | -   | -   | -      | 5.07-9.60                      | 2.45-13.32                    | 0.00005  | 2.74  | 0.95           |                                     |
| Beshar River, Iran              | 21  | -   | -   | -      | 5.48-10.16                     | 1.97-13.98                    | 0.00002  | 2.86  | 0.93           |                                     |
| Mazoo River, Iran               | 19  | -   | -   | -      | 5.89-12.34                     | 2.66-28.31                    | 0.00001  | 2.99  | 0.99           |                                     |
| Palangan River, Iran            | 51  | -   | -   | -      | 6.3-17.8                       | 11.5-239                      | 0.05     | 2.95  | 0.98           |                                     |
| Sirvan River, Iran              | 27  | -   | -   | -      | 6.0-14.8                       | 3.6-57.8                      | 0.01     | 3.00  | 0.97           |                                     |
| Kheirabad River, Iran           | 70  | -   | -   | -      | 5.07-12.48                     | 1.96-28.1                     | 0.00002  | 2.96  | 0.98           |                                     |
| Gamasiab River, Iran            | 10  | -   | -   | -      | 5.25-10.57                     | 2.10-18.06                    | 0.00001  | 3.19  | 0.98           | Segherloo et al. (2015)             |
| Ghalate River, Iran             | 31  | -   | -   | -      | 6.94-15.39                     | 4.46-55.85                    | 0.00001  | 3.16  | 0.99           |                                     |
| Cheshme gerdab River, Iran      | 12  | -   | -   | -      | 7.60-16.96                     | 5.87-73.71                    | 0.00001  | 3.08  | 0.99           |                                     |
| Maroon River, Iran              | 12  | -   | -   | -      | 5.30-10.02                     | 1.84-13.45                    | 0.00001  | 3.14  | 0.97           |                                     |
| Dashte chenir River, Iran       | 17  | -   | -   | -      | 4.82-12.06                     | 1.53-23.74                    | 0.00001  | 3.02  | 0.99           |                                     |
| Kheirak-shekarak River, Iran    | 20  | -   | -   | -      | 5.48-11.11                     | 1.91-15.78                    | 0.00002  | 2.82  | 0.98           |                                     |
| Tange faryab River, Iran        | 47  | -   | -   | -      | 4.94-10.51                     | 1.64-14.15                    | 0.00002  | 2.86  | 0.98           |                                     |
| Jarrahi River, Iran             | 108 | -   | -   | -      | 2.72-13.54                     | 0.18-23.0                     | 0.0044   | 3.06  | 0.98           | Keivany and Zamani-Faradonbe (2017) |
| Tigris Basin, Iran              | 147 | -   | -   | -      | 3.22-12.28                     | -                             | 0.0093   | 3.10  | 0.97           |                                     |
| Karkheh Basin, Iran             | 121 | -   | -   | -      | 1.98-17.28                     | -                             | 0.0115   | 3.02  | 0.99           |                                     |
| Karun Basin, Iran               | 62  | -   | -   | -      | 3.69-13.09                     | -                             | 0.0099   | 3.06  | 0.98           | Keivany et al. (2015)               |
| Persis Basin, Iran              | 103 | -   | -   | -      | 1.98-17.28                     | -                             | 0.0095   | 3.06  | 0.98           |                                     |
| Hormuz Basin, Iran              | 19  | -   | -   | -      | 3.35-7.57                      | -                             | 0.0059   | 3.34  | 0.99           |                                     |
| Merzimen Stream, Turkey         | 365 | -   | -   | -      | 2.9-16.8                       | 0.21-69.27                    | 0.0124   | 2.989 | 0.96           | Çiçek et al. (2021)                 |

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