Life history pattern and feeding habits of *Gobio bulgaricus* (Drensky, 1926) (Pisces: Gobionidae) in an endorheic stream (Istranca Stream, Turkey)

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Abstract
The Aegean gudgeon *Gobio bulgaricus* (Drensky, 1926) is a freshwater fish species restricted to the western parts (Thrace) of Turkey. The aim of the present study is investigating key parameters such as age, growth, reproduction and feeding habits of *G. bulgaricus* in the Istranca Stream to determine biological characteristics of the species. During monthly samplings from March 2012 to June 2013, *G. bulgaricus* specimens were collected from the endorheic Istranca Stream, which is draining to the Lake Durusu in İstanbul. The results showed that the lifespan of the species was 5 years. The length–weight relationships of *G. bulgaricus* indicated positive allometric growth and the relationship was $lnW = -3.976 + 3.080 lnSL$ for females, $lnW = -4.008 + 3.088 lnSL$ for males and $lnW = -4.049 + 3.118 lnSL$ for all individuals. The mean size at first sexual maturity ($L_{m}$) was 4.03 cm SL for females and 3.94 cm SL for males. Absolute fecundity ranged from 355 to 5556 eggs with mean of 1179±152 eggs. Prey analyses of digestive tract content indicated that *G. bulgaricus* was largely omnivorous (more precisely insectivorous) feeding mainly on aquatic insects and a small amount of plant and animal materials. Diptera larvae (IRI%=96.49) were the dominant prey of *G. bulgaricus* in terms of index of relative importance. It is considered that the present results will increase our knowledge of the life-history traits and feeding of *G. bulgaricus*.

Keywords: Age, Diet, Growth, Reproduction, Istranca Stream, Turkey

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Introduction
The members of the genus *Gobio* are small, benthic freshwater fishes and a total of 14 different species of the genus were recognised in Turkish freshwater systems (Naseka *et al.*, 2006; Turan *et al.*, 2012; Turan *et al.*, 2016; Turan *et al.*, 2017; Turan *et al.*, 2017). The Aegean gudgeon *Gobio bulgaricus* (Drensky, 1926) is known from the Aegean and Adriatic Sea basins in Europe, and it differs from the other gudgeon species with its breast scales between pectorals, the scales extend beyond the base of the pectoral fins (Kottelat and Freyhof, 2007). The distribution area of the species in Turkey is the Istranca Stream (D. Turan has confirmed that, this stream is not in the Lake Durusu basin, and it is draining to the Black Sea at Kırklareli) (Turan *et al.*, 2012). However, the species was identified as first record for the Lake Durusu basin with individuals captured during the present study period (Saç and Özuluğ, 2014).

As many as three hundred new species of fish are scientifically described and named every year, even in geographical areas where knowledge has been assembled over centuries (Kottelat and Freyhof, 2007). Most investigations on life history traits (age, growth, reproduction) and diet spectrum/feeding strategy generally refer to economically valuable fishes. However, there is a desperate lack of the rawest baseline biological data for most species: this is obvious for small-sizes species (Kottelat and Freyhof, 2007). The intention of the present study is to determine the age, growth, reproduction and feeding habits of *G. bulgaricus*, and to contribute to the understanding of the biology of this species. Thus, it is considered that, the result of the study will provide the first attempt knowledge of the considerably unknown biology of this species.

Materials and methods
Study area
The study was conducted in the endorheic Istranca Stream that located in the Lake Durusu basin in the city of Istanbul. The sampling surveys were carried out at six different stations along the stream: Station 1 (St. 1; 41.33098°, 28.24897°)-Taşlıgeçit creek; Station 2 (St. 2; 41.31415°, 28.24893°)-Danamandıra creek; Station 3 (St. 3; 41.41750°, 28.13845°)-Şeytan creek; Station 4 (St. 4; 41.39901°, 28.19366°)-Binkılıç creek; Station 5 (St. 5; 41.37920°, 28.29610°)-Karamandere creek; Station 6 (St. 6; 41.39946°, 28.38352°)-Karacaköy creek (Fig. 1).
Figure 1: Study area (Istranca Stream, Lake Durusu Basin, Turkey) and sampling stations.

Fish sampling and data analyses
Electrofishing (the grab net has a mesh size of 3 mm) surveys were monthly conducted at each station to collect fish specimens from March 2012 to June 2013. The water temperature was measured in situ with a portable thermometer. Immediately after capture, fish specimens were killed with an overdose of clove oil and then transferred to the laboratory in cold condition (portable freezer, -18°C). In the laboratory, for each individual, standard length (SL) was measured to the nearest 0.1 cm, and total body weight (W) was weighed on a digital balance with 0.001 g accuracy. The lapilli otoliths were used for age readings because the sagittal otoliths of the species are not available for reading. After otoliths were extracted from fish, they were rinsed in water to purify tissue, and stored dry. For age readings, otoliths were placed in glycerine on a black background with reflected light and observed under a stereo zoom microscope. To validate the ages, two independent operators performed concurrently the oolith readings. Gonads were sexed by macroscopic or microscopic examination. The chi-square test was used to test the observed sex ratio (females to males) against theoretical ratio 1:1 (Zar, 1999).

Length–weight relationship was determined by the equation: \( W=aL^b \), where \( W \) is the total weight (g), \( L \) is the standard length (cm), \( a \) and \( b \) are regression parameters (Le Cren, 1951; Froese, 2006). The relationship \( (W=aL^b) \) was converted into the logarithmic form \( (\ln W=a+b\ln L) \) and parameters \( a \) (regression intercept) and \( b \) (slope) were calculated by the regression method of least square. 95% confidence limit (CI) of parameters \( a \) and \( b \) was estimated by the equation: 95%CI=\( x \pm (t_{0.05(n-2)}SE) \) (\( x \): \( a \) and \( b \); \( t \): table value of \( t \) (t-test at 95% confidence level)).
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confidence); SD: standard error value of \( a \) and \( b \) (King, 2007). The null hypothesis of the isometric growth was tested by t-test using the equation: \( t_s = \frac{(b - 3)}{SE_b} \) (\( SE_b \) is the standard error of the slope) (Zar, 1999). Fish condition was calculated by Fulton’s Condition Factor (\( K = \frac{W}{L^3} \times 100 \)) (Ricker, 1975). Growth in length was expressed by the von Bertalanffy function; \( L_t = L_\infty \left[1 - e^{-k(t-t_0)}\right] \), where \( L_t \) is length as a function of time \( t \), \( L_\infty \) is the theoretical asymptotic length, \( t \) is the age, \( t_0 \) is the theoretical time at zero length and \( k \) is the rate constant (Cailliet et al., 1986). Relative growth in length (RGL\%) and relative growth in weight (RGW\%) were computed according to \( \text{RGL} = \frac{[L_{t+1} - L_t]}{L_t} \times 100 \) and \( \text{RGW} = \frac{[W_{t+1} - W_t]}{W_t} \times 100 \), where \( L_{t+1} \) is the standard length at age \( t+1 \), \( L_t \) is the standard length at age \( t \), \( W_{t+1} \) is the total weight at age \( t+1 \) and \( W_t \) is the total weight at age \( t \) (Chugunova, 1963).

The gonads were detached from the fish and weighed to the nearest 0.0001 g. Gonadosomatic index (GSI) was estimated to determine the spawning period of females and males using the formula \( \text{GSI} = \frac{G}{W} \times 100 \), where \( G \) is gonad weight and \( W \) is total body weight (Ricker, 1975). For the estimation of mean length at 50% maturity (\( L_m \)), a logistic function was fitted to the proportion of the mature individuals by size class using non-linear regression. The function used for calculating length at first maturity was \( P = \frac{1}{\left[1 + \exp\{-r(L-L_m)\}\right]} \), where \( P \) is the proportion of mature individuals in each size class, \( r \) (-\( b \) slope) is a parameter controlling the slope of the curve and \( L_m \) is the size at 50% maturity (King, 2007).

The absolute fecundity (\( F \)) was determined in 50 females (in spawning stage) captured from March to May, as the number of the all eggs (ripe eggs and small oocytes) in each ovary. Relative fecundity was calculated using the equations; \( \text{RF} = \frac{F}{W} \) and \( \text{RF} = \frac{F}{L} \), where \( F \) is absolute fecundity, \( W \) is total weight and \( L \) is standard length. The relationships between fecundity and fish size (standard length/weight) was calculated by regression analysis; \( F = a X^b \), where \( F \) is fecundity, \( X \) is standard length (cm) or weight of fish (g), \( a \) is the regression constant and \( b \) is the regression coefficient.

To determine the feeding strategy and diet composition of the species, the digestive tracts were removed and fixed in 4% formaldehyde solution until analyses. To determine the seasonal differences in feeding activity, vacuity index (VI\%) was estimated as a percentage of empty digestive tracts: \( \text{VI} = \frac{N_e}{N_t} \times 100 \), \( N_e \) is the number of the empty digestive tracts, \( N_t \) is the total number of examined digestive tracts. The prey items were identified to the lowest possible taxonomic level using a binocular microscope and then grouped. The each taxonomic group was counted individually, oven dried at 80°C and weighed to the nearest 0.0001 g. The modified index of relative importance (MI\%) of each uncountable prey items (major groups such as plant, detritus etc.) and the index of relative importance (IRI\%) of each countable prey items (insects groups) were
estimated as follows:

\[ \text{MI}\% = \left[ \frac{F\% \times W\%}{\Sigma (F\% \times W\%)} \right] \times 100 \]

and

\[ \text{IRI}\% = \left[ \frac{(N\% + W\%)F\%}{\Sigma ((N\% + W\%)F\%)} \right] \times 100, \]

where \( F\% \) is the percentage of frequency of occurrence [(number of digestive tracts containing a food item/total number of digestive tracts with food)\( \times 100 \)], \( N\% \) is numerical percentage and \( W\% \) is the percentage of gravimetric composition (Hyslop, 1980; Hayse, 1990).

To interpret the prey importance in digestive tract contents data and to assess the feeding strategy of the species studied, the modified Costello's (1990) method was used (Amundsen et al., 1996). In this method, the prey-specific abundance \( (P_i) \) was plotted against the frequency of occurrence \( (F_i\%) \). The calculation of the prey-specific abundance was carried out by the formula \( P_i = \left( \frac{\Sigma S_i}{\Sigma S_{ij}} \right) \times 100 \); where \( P_i \) is the prey-specific abundance of prey \( i \), \( S_i \) is the digestive tract content (volume, weight or number) comprised of prey \( i \), and \( S_{ij} \) is the total digestive tract content in only those predators with prey \( i \) in their digestive tract.

Niche breadth of the species was estimated using Levins (1968) and Levins' standardized (Hurlbert, 1978) indices: \( B = 1/\Sigma (P_j)^2 \) and \( B_A = (B-1)/(n-1) \), where \( B \) is Levins' measure of niche breadth, \( P_j \) is proportion of individuals found using resource \( j \), \( B_A \) is Levins' standardized niche breadth and \( n \) is the number of possible resource states. Levins' \( B \) and standardized \( B_A \) are minimal when all the individuals occur in only one resource state (minimum niche breadth, maximum specialization). The range of \( B \) is from 1 to \( n \), where \( n \) is the total number of resource states and \( B_A \) varies between 0 (species consume a single food item) and 1.0 (species exploits available items in equal proportion) (Krebs, 1998). Values of \( B_A \) are considered high when higher than 0.6, intermediate, when between 0.4 and 0.6 and low when below 0.4 (Novakowski et al., 2008).

**Results**

*The age and growth of G. bulgaricus*

A total of 875 *G. bulgaricus* specimens were captured from all stations (St.1: 59 specimens, St.2: 583 specimens, St.3: 150 specimens, St.4: 70 specimens, St.5: 4 specimens, St.6: 9 specimens) in the Istranca Stream during the study period.

The standard length and body weight of the specimens varied between 2.0-9.8 cm and 0.137-22.429 g, respectively. The sex ratio of female to male was found to be 1:1.18 with significant difference from the expected ratio of 1:1 (\( \chi^2 = 4.88; p<0.05 \)). The age composition of the population ranged from age class 0 to V. It was observed that age class III (36%) was dominant (Fig. 2).

![Figure 2: The age classes frequency (%) distribution by numbers of individuals (n) for Gobio bulgaricus in the Istranca Stream.](image-url)
Length and weight range distributions, relative growth in length and relative growth in weight of different ages and sexes of *G. bulgaricus* are given in Table 1. The maximum relative growth in length and also weight was observed in age class II, and the relative growth rates decreased with age.

**Table 1:** The range of standard length (SL, cm), body weight (W, g) and condition factor (K) values with relative growth in length (RGL %) and relative growth in weight (RGW%) of *Gobio bulgaricus* in the Istranca Stream.

<table>
<thead>
<tr>
<th>Age classes</th>
<th>Sexes</th>
<th>n</th>
<th>SL (cm) (min. - max.)</th>
<th>RLR (%)</th>
<th>W (g) (min. - max.)</th>
<th>RWR (%)</th>
<th>K (min. - max.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Juvenile</td>
<td>10</td>
<td>2.4 - 3.3</td>
<td>-</td>
<td>0.254 - 0.710</td>
<td>-</td>
<td>1.63 - 2.07</td>
</tr>
<tr>
<td>I</td>
<td>Juvenile</td>
<td>29</td>
<td>2.4 - 3.7</td>
<td>16.3</td>
<td>0.343 - 1.263</td>
<td>88.4</td>
<td>1.80 - 2.60</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>25</td>
<td>2.9 - 5.3</td>
<td></td>
<td>0.450 - 3.121</td>
<td></td>
<td>1.64 - 3.13</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>22</td>
<td>2.8 - 4.9</td>
<td></td>
<td>0.374 - 2.577</td>
<td></td>
<td>1.71 - 2.47</td>
</tr>
<tr>
<td></td>
<td>All ind.</td>
<td>76</td>
<td>2.4 - 5.3</td>
<td></td>
<td>0.343 - 3.121</td>
<td></td>
<td>1.64 - 3.13</td>
</tr>
<tr>
<td>II</td>
<td>Female</td>
<td>37</td>
<td>4.4 - 6.0</td>
<td></td>
<td>2.010 - 5.256</td>
<td></td>
<td>1.71 - 2.84</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>41</td>
<td>4.1 - 6.5</td>
<td>51.8</td>
<td>1.094 - 6.332</td>
<td>234.5</td>
<td>1.59 - 2.71</td>
</tr>
<tr>
<td></td>
<td>All ind.</td>
<td>78</td>
<td>4.1 - 6.5</td>
<td></td>
<td>1.094 - 6.332</td>
<td></td>
<td>1.59 - 2.84</td>
</tr>
<tr>
<td>III</td>
<td>Female</td>
<td>62</td>
<td>5.3 - 7.1</td>
<td></td>
<td>2.966 - 8.537</td>
<td></td>
<td>1.78 - 2.96</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>70</td>
<td>5.2 - 7.2</td>
<td>23.5</td>
<td>2.704 - 8.506</td>
<td>91.5</td>
<td>1.72 - 2.81</td>
</tr>
<tr>
<td></td>
<td>All ind.</td>
<td>132</td>
<td>5.2 - 7.2</td>
<td></td>
<td>2.704 - 8.537</td>
<td></td>
<td>1.72 - 2.96</td>
</tr>
<tr>
<td>IV</td>
<td>Female</td>
<td>17</td>
<td>7.0 - 8.6</td>
<td></td>
<td>7.213 - 14.059</td>
<td></td>
<td>1.90 - 2.72</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>40</td>
<td>6.8 - 8.1</td>
<td>17.3</td>
<td>6.765 - 12.666</td>
<td>62.6</td>
<td>1.88 - 2.54</td>
</tr>
<tr>
<td></td>
<td>All ind.</td>
<td>57</td>
<td>6.8 - 8.6</td>
<td></td>
<td>6.765 - 14.059</td>
<td></td>
<td>1.88 - 2.72</td>
</tr>
<tr>
<td>V</td>
<td>Female</td>
<td>6</td>
<td>7.7 - 8.4</td>
<td></td>
<td>9.849 - 13.900</td>
<td></td>
<td>2.16 - 2.52</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>11</td>
<td>7.5 - 9.5</td>
<td>10.6</td>
<td>9.886 - 20.846</td>
<td>37.6</td>
<td>2.12 - 2.43</td>
</tr>
<tr>
<td></td>
<td>All ind.</td>
<td>17</td>
<td>7.5 - 9.5</td>
<td></td>
<td>9.849 - 20.846</td>
<td></td>
<td>2.12 - 2.52</td>
</tr>
</tbody>
</table>

Length-weight relationship of *G. bulgaricus* was calculated for females, males and all individuals (female, male and juvenile) as

\[
\ln W = 3.976 + 3.080 \ln SL
\]

and

\[
\ln W = 4.008 + 3.088 \ln SL
\]

\[
\ln W = 4.049 + 3.118 \ln SL
\]

respectively. In terms of growth types according to \( b \) values, the population had positive allometric growth (Table 2).

**Table 2:** Descriptive statistics and estimated parameters of length-weight relationships of *Gobio bulgaricus* in the Istranca Stream. (n, number of individuals; SL, standard length; W, body weight; Min, minimum; Max, maximum; \( a \), intercept; \( b \), slope; CL, confidence limits; \( r^2 \), coefficient correlation).

<table>
<thead>
<tr>
<th>Sexes</th>
<th>n</th>
<th>Standard length (SL, cm)</th>
<th>Body weight (W, g)</th>
<th>Regression parameters</th>
<th>Confidence limits</th>
<th>r²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Min.</td>
<td>Max.</td>
<td>Min.</td>
<td>Max.</td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>Female</td>
<td>316</td>
<td>2.7</td>
<td>9.6</td>
<td>0.408</td>
<td>20.548</td>
<td>-3.976</td>
</tr>
<tr>
<td>Male</td>
<td>374</td>
<td>2.8</td>
<td>9.8</td>
<td>0.374</td>
<td>20.846</td>
<td>-4.008</td>
</tr>
<tr>
<td>All ind.</td>
<td>875</td>
<td>2.0</td>
<td>9.8</td>
<td>0.137</td>
<td>22.429</td>
<td>-4.049</td>
</tr>
</tbody>
</table>

“+” means positive allometric growth.
Von Bertalanffy growth function was 
\[ L_t = 10.31(1 - e^{-0.271(t+1.515)}) \] for females, 
\[ L_t = 13.25(1 - e^{-0.163(t+1.984)}) \] for males and 
\[ L_t = 10.80(1 - e^{-0.259(t+0.203)}) \] for all individuals of *G. bulgaricus*. The mean condition factor (±SD) was calculated as 2.17 (±0.25) for females, 2.15 (±0.21) for males and 2.13 (±0.24) for all individuals. Analyses of the average condition factor showed that the condition increased with age and the highest value of the average condition factor was in the age class IV (K=2.33) for females and age class V for males (K=2.28) (Table 1). Monthly variation of condition factor was also calculated for females, males and all individuals (Fig. 3). Especially in females, the condition increased up to 3.13 in April (2013) due to increase in gonad weight.

![Figure 3: Mean monthly condition factors (K) of *Gobio bulgaricus* in the Istranca Stream.](image)

**Reproduction of G. bulgaricus**
The gonadosomatic index of *G. bulgaricus* ranged from 0.06 (August, 2012) to 21.00 (April, 2013) for females, and from 0.04 (August, 2012) to 3.24 (April, 2013) for males. The highest GSI values for both sexes were observed in May (2012) and April (2013) during two spawning seasons, and the species spawned from May to July, the main spawning activity being in late May and June (Fig. 4). In 2012, the spawning activity finished in July, and no individual carrying eggs ready for spawning was captured.
Figure 4: Monthly changes in the gonado-somatic index (GSI) of both female and male *G. bulgaricus* and mean water temperature (°C) in the Istranca Stream.

Observations on gonads also showed that there were always small oocytes beside the ripe eggs during the spawning period. While the number of the small oocytes was higher than the large ripe eggs’ number at the beginning of the spawning period, that number decreased considerably at the end of the period. According to this finding, *G. bulgaricus* releases eggs in batches and exhibits multiple spawning within a season. Mean absolute fecundity in mature female was 1179 eggs (SD=1152), ranging from 355 eggs (SL of 4.3 cm) to 5556 eggs (SL of 8.2 cm). Absolute fecundity and body size (both length and weight) relationships were positively correlated; absolute fecundity-length relationship was $F=7.937L^{2.915}$ ($r^2=0.807$) and absolute
fecundity and weight relationship was \( F = 271.9W^{1.018} \) (\( r^2 = 0.864 \)). Mean relative fecundity was calculated as 272 eggs cm\(^{-1}\) (SD=126) (ranged from 83 to 678 eggs cm\(^{-1}\) and 287 eggs g\(^{-1}\) (SD=62) (ranged from 161 to 436 eggs g\(^{-1}\)). The size at first maturity (\( L_m \)), where 50\% of the individuals had attained gonad development, was 4.03 cm SL for females and 3.94 cm SL for males. Mean age at maturity of both male and female was determined as age classes I and II.

**Diet spectrum and feeding strategy of G. bulgaricus**

A total of 740 fish specimens were dissected to determine the diet spectrum and feeding strategy of *G. bulgaricus*. As some of the digestive tracts of the species were empty, a total of 374 specimens were examined for the analyses. The vacuity index (VI\%) of *G. bulgaricus* specimens changed seasonally and accounted for 31\% in spring (2012), 49\% in summer, 39\% in autumn, 41\% in winter and 54\% in spring (2013). In the second summer (2013) period, the data were excluded from the examination because fish specimens were sampled only in June.

The analyses showed that, the diet spectrum of *G. bulgaricus* contained a total of seven major groups as feed item: Insect (Diptera, Ephemeroptera, Plecoptera, Odonata, Trichoptera, Coleoptera, Hymenoptera, Lepidoptera), crustacean (Gammaridae, Ostracoda, Cladocera), mollusc (Gastropoda), plant (terrestrial plants, algae), arachnid (Acaridae) and detritus (organic detritus). Insect (91.77\%) was the most prevalent feed item in terms of modified index of relative importance (MI\%), and the consumed insect groups mostly composed of Diptera (IRI\%=96.49) (Table 3).

**Table 3:** The diet composition of *Gobio bulgaricus* in the Istranca Stream (F (%): The percentage of frequency of occurrence, W (%): The percentage of gravimetric composition, N (%): Numerical percentage, IRI (%): The index of relative importance for countable prey items, MI (%): The modified index of relative importance for uncountable prey items).

<table>
<thead>
<tr>
<th>Prey items</th>
<th>F(%)</th>
<th>W(%)</th>
<th>N(%)</th>
<th>IRI (%)</th>
<th>MI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insecta</td>
<td>83.96</td>
<td>67.79</td>
<td></td>
<td>91.77</td>
<td></td>
</tr>
<tr>
<td>Diptera</td>
<td>76.20</td>
<td>43.37</td>
<td>97.09</td>
<td>69.49</td>
<td></td>
</tr>
<tr>
<td>Ephemeroptera</td>
<td>18.98</td>
<td>8.29</td>
<td>1.69</td>
<td>1.71</td>
<td></td>
</tr>
<tr>
<td>Plecoptera</td>
<td>5.88</td>
<td>0.75</td>
<td>0.27</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>Trichoptera</td>
<td>14.17</td>
<td>12.50</td>
<td>0.81</td>
<td>1.70</td>
<td></td>
</tr>
<tr>
<td>Odonata</td>
<td>2.94</td>
<td>1.51</td>
<td>0.09</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>Coleoptera</td>
<td>1.07</td>
<td>0.13</td>
<td>0.03</td>
<td>&lt;0.00</td>
<td></td>
</tr>
<tr>
<td>Hymenoptera</td>
<td>0.53</td>
<td>0.06</td>
<td>0.01</td>
<td>&lt;0.00</td>
<td></td>
</tr>
<tr>
<td>Lepidoptera</td>
<td>0.27</td>
<td>0.04</td>
<td>0.01</td>
<td>&lt;0.00</td>
<td></td>
</tr>
<tr>
<td>Plant materials</td>
<td>23.80</td>
<td>10.51</td>
<td></td>
<td>4.03</td>
<td></td>
</tr>
<tr>
<td>Crustacea</td>
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<td>8.20</td>
<td></td>
<td>2.23</td>
<td></td>
</tr>
<tr>
<td>Annelida</td>
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<td>5.17</td>
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<tr>
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<td>0.01</td>
<td></td>
<td>&lt;0.00</td>
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</tr>
<tr>
<td>Bivalves</td>
<td>0.80</td>
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<tr>
<td>Detritus</td>
<td>13.90</td>
<td>7.88</td>
<td></td>
<td>1.77</td>
<td></td>
</tr>
</tbody>
</table>

The diagram of the modified Costello graphical method is shown in Fig. 5. In terms of prey importance, all the prey items except Diptera presented low values in axis (\( P_i \) and \( F_i \)). In addition to the indices of relative importance
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(IRI% and MI%), this diagram also proves that *G. bulgaricus* is rarely feeds on these feed items. However, Diptera have been eaten by more than half the individuals (F_i%=76%) and this result indicates that Diptera has a dominant prey importance in the diet of *G. bulgaricus*. Levins' niche breadth (B) and standardized niche breadth (B_A) values were 2.50 and 0.25, respectively. The results showed that *G. bulgaricus* feeds with a limited range of prey.

Figure 5: Modified Costello feeding strategy diagram for *Gobio bulgaricus* in the Istranca Stream. Prey-specific abundance (P_i) plotted against frequency of occurrence (F_i%) of food items in the diet of the species.

Discussion

The members of the genus *Gobio* are short-lived fish species and lives up to 5 years (Miñano *et al.*, 2003; Kottelat and Freyhof, 2007; Özdemir and Erk’akan, 2012). But, Özdemir (2012) reported that the maximal age of the endemic species *G. gymnostethus*, that lives in Melendiz Creek (Anatolia, Turkey) was 7 years. However, the lifespan of *G. bulgaricus*, estimated as 5 years, is similar to the literature presented for the other members of the genus.

The age distribution of the species showed that age class III was predominant, and the younger age classes (I, II) in the species are represented by a large number of specimens (Fig. 2). Juveniles of the species are poorly represented in this study because the mesh size of the electroshocker’s grab was too large to retain the age class 0 quantitatively. In the Istranca Stream, hydrological characteristics of streams (e.g. stream width, water depth, flow rate, substrate) are affected on the distribution of *G. bulgaricus* from upstream to downstream, directly. The observations made in the field showed that the young individuals of the species inhabit riffle habitats (fast-flowing, shallow waters with gravely bottoms: especially the upper and middle parts of the Istranca...
Stream) and moved in small groups consisted from close size classes. However, individuals of the older age classes (IV, V) had mostly preferred pool habitats (stagnant or slow-flowing, deeper waters with sandy bottoms) and lived solitary to hide from their predators. Individuals of older age classes of the species are also represented by a small number because of the difficulties of obtaining the fish and, natural or predation-caused deaths.

The von Bertalanffy growth model is widely used to determine fish growth and life-history traits, and the model is typically focused on long-living species since short-lived species are often overlooked (Guo et al., 2016). The members of the genus *Gobio* are small fish and limited studies have estimated the growth parameters of this genus. According to Froese and Binohlan (2003), the asymptotic length of *G. gobio* was calculated as 21.1 cm TL, although the maximum length is recorded as 13.0 cm SL (Kottelat and Freyhof, 2007). In the present study, the asymptotic length for *G. bulgaricus* was estimated as 10.8 cm and this can be considered realistic for this population as the largest specimen caught had a standard length of 9.5 cm.

In the present study, the exponents of length-weight relationships of the species showed that the population had positive allometric growth ($b > 3.0$). Tarkan et al. (2006) studied the length-weight relationship of *G. bulgaricus* (the species was recorded as *G. gobio*) from Büyükçekmece Lake (İstanbul) and determined that the $b$ value of the population was 3.01. The value of $b$ varies according to the species as well as some factors such as feed availability, feeding rate, gonad development and spawning period, and it is mostly used to compare the growth of same fish species living in different habitats (Bagenal and Tesch, 1978). However, the main factor in the differences for these two populations is related to trophic levels of the two different habitats (stream and lake) where the populations live in.

The members of genus *Gobio* in European inland waters reproduce from April to August (Kottelat and Freyhof, 2007). The spawning seasons of *G. hettitorum* and *G. gymnostethus* living in Anatolian part of Turkey were determined as March-June and April-July (Özdemir, 2012; Özdemir and Erk’akan, 2012). Similarly, the spawning season of *G. bulgaricus* occurred from April to July with the main spawning activity being in May and June. The spawning periods of fish may vary from species to species, or different populations of same species which live in different ecological conditions (water temperature, current, etc.). The impact of temperature on the beginning of the breeding season was also monitored in *G. bulgaricus*. The spawning of *G. bulgaricus* took place one month earlier in 2013 depending on the water temperature and the species had used the fast-flowing habitats with gravel bottom as spawning site.

*G. bulgaricus* lays its eggs in batches and exhibits multiple spawning within a season. According to Fitzhugh et al. (2012), batch spawning is related to body size or age, and this trait of *G.
*Gobio bulgaricus* is thought to result from being short-lived and also small-bodied fish species. Especially in these small-bodied fishes, the number of eggs produced at each spawning is limited by the volume of the body cavity available to carry the ripe ovaries and thus, the size of the body cavity can impose a substantial limitation on batch fecundity of those fishes (Hernaman and Munday, 2005).

The members of the genus *Gobio* spawn for the first time at 1-3 years; most individuals spawn 1-2 years. Sexual maturity age of endemic *G. hettitorum* population (Yeşildere Stream, Turkey) was determined as 1 and 2 years (Özdemir and Erk'akan, 2012). According to Özdemir (2012), the other endemic species *G. gymnostethus* (Melendiz Creek, Turkey) matured at 1 years. In *G. bulgaricus*, the age at first maturity is similar to the other species of the genus *Gobio* with one and two years of age.

The digestive tract survey verified that *G. bulgaricus* was largely omnivorous (more precisely insectivores) feeding mainly on aquatic insects and a small amount of plant and animal materials. The mouth of *G. bulgaricus* is ventral and also barbelled so that the species can also survey deeper areas of the water bodies. The diet spectrum of the species contains mostly insect groups (especially Diptera larvae). However, the higher consumption of Diptera larvae, Annelida and detritus by *G. bulgaricus* is probably related to the fact that this species prefers a benthic position in the water bodies. However, relative abundance of prey types, narrow niche width and the positions of prey types in the Costello's modified method indicate that the fish has a specialist feeding strategy.

In conclusion, the present study has provided basic information about the biological traits of *G. bulgaricus* because no any other studies about the age, growth, reproduction and feeding of the species from the distribution area in Turkey exist, and it is hoped that the results will contribute to understand the function of this species in the nature.

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