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Growth and Feeding Ecology of Dnieper chub *Petroleuciscus* borysthenicus (Kessler, 1859) in Şahinkaya Reservoir, an Artificial Water Body of an Island Ecosystem (Gökçeada, Turkey)

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Article History		Abstract - Bio-ecological studies and life-history traits of small-bodied fishes are critical for the un-
Received:	14.10.2021	derstanding of their ecological role as well as the assessment of their position and continuity in the eco- systems. The current study goals to present initial data on growth and feeding habits of small-bodied
Accepted:	17.11.2021	Petroleuciscus borysthenicus inhabited in an island ecosystem (Gökçeada, Turkey). During the season-
Published:	10.03.2022	ally sampling surveys from May 2020 to January 2021, a total of 163 specimens were collected from
Research Article		Şahinkaya Reservoir using electrofishing. SL and W values of samples ranged from 2.6–10.0 cm and 0.32–25.68 g, respectively. LWR and K values were estimated for all specimens as W=0.017×SL ^{3.114} (r=0.988) and 2.04 (\pm 0.28), respectively. F:M sex ratio was calculated as 1:1.33 with no significant difference from the ratio of 1:1 (X ² =0.02; p>0.05). Diet of the species comprised of ten different food items. Plant (63.6%) was the most preferred food item from the point of frequency of occurrence (F%), pursued by Diptera (31.8%) and Odonata (12.7%). According to IRI (%) values of food items, Diptera was the dominant food item in all seasons. B and B _A values were estimated as 3.18 and 0.24, respectively. The results showed that <i>P. borysthenicus</i> was more selective on conveniently accessible food materials in the environment and its food preference was affected by seasonal food supply.

Keywords – Condition factor, feeding habit, growth, insular ecosystem, length-weight relationship

1. Introduction

A fish species' body size is the result of evolutionary processes such as speciation, genetic drift, and natural selection, and it is correlated with a remarkable array of morphological, physical, physiological, and behavioural characteristics that allow it to adapt to its environment (<u>Woodward & Warren, 2007; Albert & Johnson, 2012</u>). Small-bodied fish species predominate in freshwater systems, particularly in tributaries of running waters or lakes. These fish, unlike marine species, are generally not preferred as human food and are not subject to fishing pressure. Growth, which is linked to natural longevity, age at maturity, and reproductive efficiency, as well as feeding behaviour, which determines their ecological niche, are generally overlooked in these small-scale fish due to their lack of commercial value (<u>Kottelat & Freyhof, 2007</u>). Bio-ecological studies and biographical characteristics of small-scale freshwater fish species, on the other hand, are critical for understanding the ecological role of species, as well as evaluating their position and continuity in ecosystems, when it comes to ecosystem management and species conservation status assessment (<u>Chrisafi et al., 2007; Sac & Özuluğ, 2020a</u>).

Petroleuciscus borysthenicus (Kessler, 1859) is the only species of the genus *Petroleuciscus* in continental Europe with a wide range and is known from the west, north and east of Black Sea and Azov Seas, the Aegean Sea, and north/northwestern Turkey (Kottelat & Freyhof, 2007; Öztürk & Küçük, 2007; Saygun et al., 2017: Saç & Özuluğ, 2019; Sarı et al., 2019). This small-bodied fish prefers shallow areas with slow currents and a wide range of habitat tolerance, from montane streams to marshes and lakes (Kottelat

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<u>& Freyhof, 2007; Saç & Özuluğ, 2017</u>). This fish was assessed as Least Concern (LC) species according to the IUCN criteria due to it has been widespread and abundant (<u>Freyhof & Kottelat, 2008</u>). However, limited information is available on the life history and bio-ecological characteristics of *P. borysthenicus*. Some previous studies available on the species are mainly focusing on length-weight relationships (LWRs) (<u>Koutrakis & Tsikliras, 2003; Tarkan et al., 2006, 2009; İlhan et al., 2012</u>) whereas the only data on growth, age and reproductive traits of *P. borysthenicus* from Turkey are reported by <u>Serezli (2017)</u> for the population inhabiting the streams flowing into Sapanca Lake.

Because of their unique traits and vulnerabilities, island ecosystems are of great significance for the longterm biological diversity conservation (<u>Balzan et al., 2018</u>). Thus, these unique ecosystems support a high level of biological diversity as well as privatized flora and fauna (<u>MacArthur & Wilson, 1967</u>). Because insular ecosystems are remote from the mainland, they can be used as natural experimental research areas to track changes in fauna and flora and understand the effects on habitat (<u>Velmurugan, 2018</u>).

Gökçeada, which is located near Çanakkale in the northwestern zone of the Aegean Sea, is the largest island of Turkey. The island covers an area of 290 km², with a 95 km coastline and the presence of *P. borysthenicus* was recently recorded from this island (Bakaç, 2018). The distribution of the species on the island is very limited; it inhabits only Çıkırım Stream and Şahinkaya Reservoir, which was built on this stream for irrigation purpose in the 1980s. Considering the current insufficient knowledge, there is a need to identify the life-history traits and bio-ecological characteristics of *P. borysthenicus*. The goal of the present study was to identify some growth patterns, feeding habits, and diet of *P. borysthenicus* living in Şahinkaya Reservoir.

2. Materials and Methods

Specimens of *P. borysthenicus* were collected seasonally from May 2020 to January 2021 from Şahinkaya Reservoir (40.1134000 N, 25.7721278 E; Gökçeada, Turkey) using electrofishing (SAMUS 1000 portable electroshocker; frequency 50-55 Hz). It is a shallow reservoir that was established for irrigation purposes on the Çıkırım Stream. Fish were sampled from approximately 100 m of shoreline with a max. depth of 1 meter in the reservoir's south region (Figure 1). Fish specimens were anaesthetized with clove oil and transferred to laboratory on ice. Total length (TL), fork length (FL) and standard length (SL) were all measured with a precision of 0.1 cm in the laboratory. On digital scales with a sensitivity of 0.01 g, total body weight (W) was weighed. Fish were sexed using a microscopic or macroscopic examination of the gonads. Observed sex ratios (female to male) were compared to the theoretical 1:1 ratio using the test of chi-square (Zar, 1999).



Figure 1. Map of sampling location in Şahinkaya Reservoir (Gökçeada Island, Çanakkale, Turkey)

The following equation was applied to estimate the LWR: $W=aL^b$, where W represents total weight, L represents standard length, and *a* and *b* represent parameters of regression (Le Cren, 1951; Froese, 2006). Equation($W=aL^b$) was converted to natural logarithmic form (lnW = lna + blnL), and the regression analysis calculated the parameters *a* (regression section) and *b* (slope). Equation was applied to estimate the confidence intervals (95%) for parameters *a* and *b* 95%CI=x±($t_{0.05}$ (n-2)×SE) (x: *a* and *b*; t: table value of t (t-test, 95% confidence); SE: standard error value of *a* and *b*; see King 2007). To be comparable to the results of several research utilizing different length measures, the LWR was determined using linear regression analyses. The calculation of condition was performed using the Fulton's Condition Factor K=(W/ L³)×100, where W represents total weight (g) and L represents standard length (cm) (<u>Ricker, 1975</u>).

Fish were dissected and their digestive tracts (DTs) were removed for the diet analyses. Before examinations, all DTs were fixed in formaldehyde solution (4%). The index of vacuity (VI) was calculated as a percentage of empty DTs to identify seasonal changes in feeding intensity; $VI\%=(N_e/N_t)\times100$, where N_e denotes point of empty DTs and N_t denotes total point of DTs (<u>Sarkhanizadeh et al., 2014</u>). Food objects were determined and classified to lowest probable taxonomic level by binocular microscope. After drying at 80°C for 2–4 hours, countable objects were counted and all food items were weighted (nearest 0.0001 g).

Each prey item's dietary value was determined by calculating its frequency of occurrence (F%) and relative importance index (IRI%, see <u>Hyslop 1980</u>) to identify the species' feeding habits: IRI%=[((N%+W%)×F%)/ Σ ((N%+W%)×F%)] × 100, where F% denotes frequency of occurrence percentage [(point of DTs involving a food object/total points of DTs with food)×100], N% represents the quantitative percentage of DTs including a specific food item in comparison to the total point of DTs, and W% is the gravimetric percentage of a specific food object in connection with the total weight of all consumed taxa. The significance of uncountable prey objects (e.g. plant materials) was assessed by taking their F (%) values into account. IRI (%) was used for only countable objects.

Schoener's Index (α ; see Schoener 1970) was used to calculate the overlap in diet between seasons; α =1-0.5($\Sigma(P_{xi}-P_{yi})$), where P_{xi} and P_{yi} denote points proportions of food category *i*, in diets group *x* and group *y*. F (%) and IRI (%) values of food objects were used as point proportions for the index, respectively. Index values from 0 to 1, with overlap values of α =0.6 or larger considered biologically relevant from the point of prey objects ingested by groups of *x* and *y* (Macpherson et al., 2010).

Costello's (1990) modified procedure was applied as a visual test to understand the significance of prey in the diet data and to evaluate the species' feeding strategy (<u>Amundsen et al., 1996</u>). Prey-specific abundance (P_i°) was figured against the F_i° in this procedure. The estimation of the prey-specific abundance; $Pi=(\Sigma S_i/\Sigma S_{ii})\times 100$; where P_i denotes prey-specific abundance of prey *i*, S_i represents the DT content (numerical) composed of prey *i*, and S_{ii} denotes total DT content in only those predators with prey *i* in their DT.

Levins' measure of niche breadth (Levins, 1968) and Levins' standardized (Hurlbert, 1978) index were used to calculate the species' dietary niche breadth; $B=1/\Sigma(P_j)^2$ and $B_A=(B-1)/(n-1)$, where B denotes Levins' measure of niche breadth, P_j represents the numerical proportion of individuals discovered using source *j*, B_A denotes Levins' standardized and *n* is the point of probable source states. When all specimens consist of only one source state, B and B_A are minimal (maximum specialization and minimum niche breadth). B values range from 1 to n, where n is total point of source states and B_A values range from 0 to 1.0 (Krebs, 1998). B_A values are specified higher when they are higher than 0.6, medium when they ranged from 0.4 and 0.6, and lower when they are less than 0.4 (Novakowski et al., 2008).

3. Results and Discussion

A total of 163 *P. borysthenicus* specimens were caught from Şahinkaya Reservoir. Specimens' SL and W ranged from 2.6 to 10.0 cm and 0.32 to 25.68 g, respectively (<u>Table 1</u>). The sex ratio was estimated to be 1:1.33, with no significant difference from the ratio of 1:1 ($\chi 2 = 0.02$; p > 0.05). The LWR of *P. borysthenicus* was calculated as W=0.017×SL^{3.114} (r=0.988). For all individuals, the slopes of relationships (*b* values) pointed out a positive allometric growth (b>3). <u>Table 1</u> shows the SL and W distributions, as well as the calculated parameters of LWRs for both sexes. The LWR results are SL=0.801×TL+0.076(r=0.996), SL=0.870×FL+0.099(r=0.996) and FL=0.919×TL-0.018(r=0.998), respectively.

Table 1

Distribution of SL and W and calculated values of LWRs of *P. borysthenicus* inhabited in Şahinkaya Reservoir

Sex	Sexes	n	SL, cm	W, g Regression Parameters		95% CL of a	95% CL of <i>b</i>	r	
			MinMax.	MinMax.	а	b	_		
	Female	54	3.8 - 10.0	1.01 - 25.68	0.012	3.304	0.015 - 0.019	3.160 - 3.449	0.988
	Male	72	3.9 - 9.0	0.97 - 16.12	0.017	3.088	0.013 - 0.024	2.917 - 3.259	0.974
	All ind.	163	2.6 - 10.0	0.32 - 25.68	0.017	3.114	0.014 - 0.019	3.038 - 3.190	0.988

Average K (\pm SD) values for female, male and all individuals were 2.09 (\pm 0.25), 2.04 (\pm 0.30) and 2.04 (\pm 0.28), respectively. For both sexes and all specimens, seasonal variation in K value was calculated (<u>Table 2</u>). The K value increased to maximum values for both sexes in the summer.

Table 2

Seasonal variations of SL and W distribution and K values (±SD) for female, male and all ind. of *P. borys-thenicus* inhabited in Şahinkaya Reservoir

			Spring			Summer				
Sex	n	SL, cm	W, g	K±SD	n	SL, cm	W, g	K±SD		
		MinMax.	MinMax.			MinMax.	MinMax.			
Female	15	4.4 - 12.31	1.49 - 12.31	2.08 ± 0.15	12	5.6 - 10.0	4.65 - 25.68	2.41 ± 0.13		
Male	22	4.0 - 7.2	1.46 - 8.00	2.11 ± 0.21	13	5.1 - 8.0	3.47 - 12.00	2.38 ± 0.22		
All ind.	44	3.5 - 8.3	0.91 - 12.31	2.08 ± 0.20	26	3.6 - 10.0	1.20 - 25.68	2.40 ± 0.18		
			Autumn				Winter			
Sex	n	SL, cm	W, g	K±SD		SL, cm	W, g	K±SD		
		MinMax.	MinMax.	K±5D	n	MinMax.	MinMax.	K±5D		
Female	16	6.0 - 8.6	4.78 - 12.24	2.08 ± 0.13	11	3.8 - 6.3	1.01 - 4.61	1.76 ± 0.13		
Male	21	6.3 - 9.0	6.00 - 16.12	2.05 ± 0.19	16	3.9 - 6.6	0.97 - 4.97	1.67 ± 0.16		
All ind.	57	2.6 - 9.0	0.32 - 16.12	2.07 ± 0.17	36	3.4 - 6.6	0.72 - 4.97	1.71 ± 0.14		

Among 163 investigated individuals, only 110 fish had stomach contents. Vacuity index (VI, %) has differed by seasons: it was calculated as the highest in autumn (42.1%) and the lowest in summer (3.8%; see Table 3). The B and the B_A values were estimated as 3.18 and 0.24, respectively. The results showed that *P. borysthenicus* mainly fed on a limited range of prey. B and B_A in the diet of the species are indicated in Table 2. B values ranged from 1.4 (in winter) to 5.2 (in summer), while the B_A values varied between 0.1 (in winter) and 0.6 (in summer).

Table 3

Seasonal values of VI (%), B and B_A indices for P. borysthenicus inhabited in Şahinkaya Reservoir

	Spring (n=44)	Summer (n=26)	Autumn (n=57)	Winter (n=36)
Vacuity index (VI, %)	38.6	3.8	42.1	30.6
Levins' measure of niche breadth (B)	5.2	2.2	2.8	1.4
Levins' standardised niche breadth (B_A)	0.6	0.4	0.4	0.1

The species' diet consisted of 10 different food items; Diptera, Ephemeroptera, Odonata, Trichoptera, Hemiptera, Coleoptera, Cladocera, Bivalvia, Hirudinea and Plant (macrophytes and algae). The IRI% could not be calculated in the plant because it is an uncountable food type. However, Plant (63.6%) was the most preferred food item from the point of frequency of occurrence (F%), pursued by Diptera (31.8%) and Odonata (12.7%). According to IRI (%) values of food items, Diptera predominated in all seasons (<u>Table 4</u>) and it was followed by Odonata in spring (37.6%), Hemiptera in summer (19.6%) and autumn (1.3%), and Hirudinea in winter (17.0%, see <u>Table 4</u>).

Table 4

IRI (%) values of food items in diet of *P. borysthenicus* inhabited in Şahinkaya Reservoir (Bold numerals show the highest importance values of food items according to F% and IRI% values)

	Spring				Summer			Autumn				Winter				
	F	W	N	IRI	F W N IRI		F (%) W	N	N IRI	F (%)	W	N	IRI			
	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	F (70)	(%)	(%)	(%)	F (70)	(%)	(%)	(%)
Diptera	44.4	25.7	38.2	45.6	28.0	0.7	57.9	72.9	42.4	7.6	89.2	97.7	8.0	0.6	50.0	66.5
Ephemer- optera	7.4	2.2	3.6	0.7	-	-	-	-	-	-	-	-	-	-	-	-
Odonata	37.0	45.0	18.2	37.6	8.0	5.3	15.8	7.5	6.1	1.5	2.7	0.6	-	-	-	-
Trichop- tera	7.4	0.4	3.6	0.5	-	-	-	-	3.0	1.1	1.4	0.2	-	-	-	-
Hemiptera	22.2	14.2	23.6	13.5	16.0	1.3	26.3	19.6	9.1	0.4	5.4	1.3	-	-	-	-
Coleop- tera	3.7	1.8	1.8	0.2	-	-	-	-	-	-	-	-	-	-	-	-
Cladocera	11.1	0.3	10.9	2.0	-	-	-	-	-	-	-	-	4.0	< 0.1	25.0	16.5
Plant	18.5	10.6	-	-	88.0	92.7	-	-	60.6	87.4	-	-	92.0	98.5	-	-
Bivalvia	-	-	-	-	-	-	-	-	3.0	2.1	1.4	0.2	-	-	-	-
Hirudinea	-	-	-	-	-	-	-	-	-	-	-	-	4.0	0.9	25.0	17.0

Food overlap values between the seasons are given in <u>Table 5</u>. The food overlap, calculated using the F (%), was significant for summer vs. autumn and summer vs. winter. A significant overlap in food composition, calculated using the IRI (%), was also observed between all the seasons except spring vs. autumn and spring vs. winter.

Table 5

Dietary overlap values (Schoener's Index) between the seasons. Significant values are those that are equal to or higher than 0.6 (* implying that overlap is significant)

		F (%)			IRI (%)	
	Summer	Autumn	Winter	Summer	Autumn	Winter
Spring	0.25	0.41	0.01	0.67*	0.48	0.48
Summer	-	0.72*	0.72*	-	0.75*	0.67*
Autumn	-	-	0.52	-	-	0.67*

<u>Figure 2</u> indicates a schematic of the modified Costello graphical approach, and *P. borysthenicus* appears to have a generalist feeding strategy, with all prey points on the lower side of the diagram. From the point of prey importance, Bivalvia, Hirudinea, Coleoptera, Odonata, Cladocera, Tricoptera and Ephemeroptera had low axis values (P_i % and F_i %), indicating a rare feeding behavior. Plant status on the diagram (<u>Figure 2</u>) and to be consumed by more than half of the specimens (F_i %=63.6%) contributes to the specialist feeding strategy of *P. borysthenicus* on it.

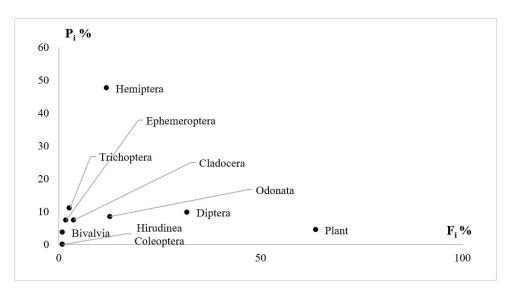


Figure 2. Feeding strategy diagram of modified Costello for the specimens of *P. borysthenicus* inhabited in Şahinkaya Reservoir.

The expected range for the value of the *b* value in the LWR equations is 2.5 - 3.5 (Froese, 2006) and for this research, *b* values for *P. borystenicus* population inhabited in Şahinkaya Reservoir are within this range. The growth type of *P. borystenicus* which calculated as positively allometric (*b*>3) in this research is similar to the results of other works performed in Sapanca Lake, Ömerli Reservoir, Küçükçekmece Lagoon and the streams of Biga Peninsula (Tarkan et al., 2006, 2009; İlhan et al., 2012). In contrast, Koutrakis & Tsikliras (2003) reported a negative growth type for *P. borysthenicus* population inhabited in the Strymon Estuary, while Tarkan et al., 2006 and Serezli (2017) have reported an isometric growth for the populations in Terkos Lake and in the streams flowing into Sapanca Lake. Within-species variance in variables of LWR of fish are influenced by a number of conditions involving size range and growth phase, sex, level of stomach fullness, gonad matureness, general fish health, season and annual differences in environmental conditions (Tesch, 1971; Froese, 2006). However, in Şahinkaya population, different growth types were observed between females and males; while females had positive allometry, males showed isometric growth (Table 1). It is thought that sex and gonad maturity caused different growth patterns for females and males within the same population.

The correlation coefficients (r) for all the LLR equations calculated for *P. borystenicus* population were higher than 0.95 and significantly linear. LLRs calculated for the species are thought to be comparable to the results of different studies, which used or will use different length measures.

The condition factor (K) value calculated for the female, male and all individuals of *P. borysthenicus* inhabited in Şahinkaya Reservoir is close to each other and over the value of 2.0. Similarly, Serezli (2017) has found very close values (2.01 for females, 2.07 for males and 2.01 for all specimens; calculated values from data) for the K values of the species in the streams flowing into Sapanca Lake. Fulton's K value is expressed as the ratio of body mass and the cube of length (Froese, 2006) and since this morphometric index is a calculation involving the length and weight of a fish, it could be influenced by the same factors effected on LWR (Hamid et al., 2015). The K value reflects the well-being of fish and varies according to the season, sex, size range, degree of gonad development, environmental conditions, food supply and parasitism (Froese, 2006). When seasonal variation of K value in this study is examined, it is seen that in both sexes the K values, which are low in winter, rise in the spring and reach their peak in the summer (Table 2). It is an expected result that the K value increases in the spring depending on the development of the gonads. It is thought that the higher values of the K in the summer may be related to the fish regaining the energy lost during reproductive activity in spring and increasing the degree of feeding activity. According to De Giosa et al. (2014), in water temperature of summer is in suitable values for growing and effective feeding action begins for many species. In addition, the relatively higher length and weight values of the fish examined in the summer may also be effective in the increase in the K value (Table 2).

Although there is no specific study on the feeding characteristics and diet of *P. borysthenicus*, it is thought that the species shows an omnivorous feeding characteristic like many other cyprinids and primarily feeds on insects and their larvae, as well as benthic invertebrates, algae and plankton (Kottelat & Freyhof, 2007). This study supported that P. borysthenicus has a wide food spectrum from zooplankton to Bivalvia, but it prefers mainly aquatic plants and insect larvae. There were no temporary changes in feeding intensity of P. borysthenicus and vacuity index values were very close to each other in four seasons (Table 3), but the food spectrum and preference of *P. borysthenicus* were changed seasonally. In the spring, a wide variety in the food spectrum was observed and especially Diptera and Odonata represented with high values of food importance index in the diet. However, over the summer, there was a noticeable decrease in food variety in the diet and the frequency occurrence values of aquatic plants in the stomach increased considerably. This has been associated with increasing the preference of the fish on easily accessible plants in the environment as a food resource to rapidly regain the lost energy after reproductive activity. In autumn, the aquatic plants were the main food item that had the highest value of F (%) in the diet of the species. Although there was an increase in the diversity of insect groups in the food spectrum, Diptera had the highest value as the index of relative importance. However, in winter, only Diptera was consumed among insect groups, and the fish still mainly fed on the plant (Table 4). Dipterans are the most abundant insects groups in aquatic systems and form large populations (Keiper et al., 2002) and their larvae or nymphs became important as easy prey due to their abundance and nutritional value, as can be seen in the diets of many freshwater fish species (Blanco-Garrido et al., 2003; Piria et al., 2005; Pompei et al., 2014; Saç et al., 2017; Saç & Özuluğ, 2020a, 2020b). Similarly, it has been determined that Diptera is an essential food item for *P. borysthenicus* in all four seasons.

Although *P. borysthenicus* can consume different food groups, it has shown maximum specialization on aquatic plants and Diptera. The positions of Plant and Diptera in the diagram support the view that fish having a general feeding strategy is selective over these two food items. The low values of Levins' indices (B and B_A) also contribute to this inference. According to <u>Krebs (1998)</u>, the broadest possible niche is possible when the fish consume all food types equally without discrimination and, the niche is narrow when all individuals only consume more of one food item against the other foods. The seasonal variation on the food spectrum and preference of *P. borysthenicus* conclude different levels of niche breadth. In spring, the variety of food consumed and their high values resulted in a wide niche size, while in winter the feeding with only a few food items and selectivity on the plant caused a narrow niche size (Table 3, Table 4).

4. Conclusion

The current study has exhibited base line data on LWR and condition parameters of *P. borysthenicus*, where there have been few studies on the growth characteristics. It has been determined that this population living in a reservoir shows positive allometric growth and has a good condition. Furthermore, for the first time, comprehensive dietary research on this small-bodied fish has been reported in this study. The results showed that *P. borysthenicus* was more selective on easily reachable food items in the environment and the seasonal change in its food preference was affected by seasonal variations in the food supply. Moreover, the occurrence of this species on Gökçeada inland waters has also yielded important findings for the freshwater biodiversity of Turkey, and also the island.

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Author Contributions

Sevan Ağdamar: Collected data, conceived the study, designed the manuscript and wrote the introduction and conclusion.

Gülşah Saç: Performed data analysis and wrote the results and discussion.

Conflicts of Interest

The authors indicated that they have no conflicts of interest.

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