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Life-history Traits of *Phoxinus strandjae* Drensky, 1926 (Actinopterygii: Leuciscidae) from Istranca Stream, Turkey

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Abstract: *Phoxinus strandjae* Drensky, 1926 is a cyprinid species distributed from the Veleka River (Bulgaria) to Gönen and Büyüksu Streams (Anatolia, Turkey). Because of the lack of biological knowledge on *P. strandjae* in its natural distribution area, life-history traits and feeding of this species were studied. During a 16-month sampling (from March 2012 to June 2013), specimens of *P. strandjae* were collected from the Istranca Stream in order to study age, growth, reproduction and diet composition of the species. The maximum age recorded was 6+. The length–weight relationships proved a positive allometric growth in females and an isometric growth in males, or lnW=-3.883+3.075lnSL and lnW=-3.857+3.044lnSL, respectively. The average length at first maturity of female was 2.99 cm, while in the male it was 2.89 cm. The mean absolute fecundity in the mature females was 839 eggs (ranged from 101 to 2392 eggs). Insects (93.16%) were the most prevalent food item in terms of the modified index of relative importance (MI%) and the consumed insect groups mostly composed of Diptera (IRI%=88.90).

Key words: Age, diet, growth, reproduction, *Phoxinus strandjae*.

Introduction

There are very limited data about the biological properties of small fish species that dominate the tributaries of running waters or lakes, except the commercial freshwater fishes (Kottelat & Freyhof 2007). In terms of ecosystem management, biological studies of small fish species are critical for the assessment of the ecological role of the species as well as the understanding of its position in the structure of the food web in ecosystems (Chrisafi et al. 2007). The members of the genus *Phoxinus* are small-bodied fishes. Recently, several new species of the genus were recognised in European freshwater systems (Kottelat 2007, Bianco & de Bonis 2015, Palandacic et al. 2017). *Phoxinus strandjae* was firstly described from the drainages of the Rezovska and Veleka Rivers, which are draining the waters from the Yıldız (Strandzha) Mountains to the Black Sea in Turkey and Bulgaria (Kottelat & Freyhof 2007). Nowadays, the species is in the "Endangered species" category of the IUCN due to its very limited distribution range (about 2000 km^2) and the expected population decline in the area (Freyhof & Kottelat 2008). However, a recent study that was carried out in Anatolia and Thrace (Turkey), the distribution area of the species was extended to the Gönen Stream in the south and the Büyüksu Stream in the east (Saç & Özuluğ 2015).

Until recently, the European populations of the genus *Phoxinus* were assigned to *Phoxinus phoxinus*; therefore, biological studies on currently recognised different species of *Phoxinus* had been published under the name of *P. phoxinus* (see Berg 1949, Gandolfi et al. 1991, Doadrio 2001). Zivkov & Trichkova (2006) studied the biology of *P. phoxinus* in

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high-mountain lakes in Bulgaria. However, the study by Dikov & Zivkov (2004) is the only publication on *P. strandjae* dealing with its abundance and biomass in the Veleka River, Bulgaria. Little is known on the biology of *P. strandjae* in Turkish inland waters. The aim of the present study was to explore key parameters such as age, growth, reproduction and feeding of *P. strandjae* in the Istranca Stream. The present study is the first attempt to identify the life-history traits and feeding strategy of this species.

Materials and Methods

Study area

The Istranca Stream flows through the forested area at a low altitude along the Istranca Mountains and drains to the Lake Durusu in the city of Istanbul. The watershed covers approximately 450 km^2 and the area is characterised by croplands and forestlands with a few small villages in the rural area. The sampling surveys were carried out at four different stations upstream and downstream the Istranca Stream (Fig. 1). Some of the other fish species inhabiting in the same habitat with *P. strandjae* are *Gobio bulgaricus*, *Alburnoides tzanevi*, *Barbus cyclolepis* and *Rhodeus amarus*.

Fish sampling and data analyses

Fish specimens were collected monthly between March 2012 and June 2013 using electrofishing (the grab net had a mesh size of 3 mm). Immediately after capture, fish specimens were killed with an overdose of clove oil and then transferred to the laboratory in cold conditions (portable freezer, -18°C). The water temperature was measured in situ with a portable thermometer. The specimens were measured fresh for standard length (SL) to the nearest 0.1 cm. The total body weight (W) was weighed using a digital balance with a 0.001 g accuracy. Otoliths (lapilli) were used for age readings. After otoliths were extracted from fish in the laboratory, they were rinsed in water to purify tissue and stored dry. For the analyses of age, otoliths were placed in glycerine on a black background with reflected light and observed under a stereo microscope. To validate the age, two independent operators performed concurringly the otolith readings. Mismatching readings were not included in the analyses. Fish was sexed through macroscopic or microscopic observations of the gonads. The observed sex ratio (females to males) was tested against theoretical ratio 1:1 using the chi-square test (Zar 1999).

Length–weight relationships were calculated using the equation: $W=aL^b$, where W was the total weight (g), L was the standard length (cm), a and b were regression parameters (Le Cren 1951). The relationship ($W=aL^b$) was converted into the natural logarithmic form ($ln W = ln a + b ln SL$) and parameters a (regression intercept) and b (slope) were calculated by the regression method of the least square (King 2007). 95% confidence limit (CL) of parameters a and b was estimated using the equation $95\%CL=x\pm(t_{0.05(n-}$

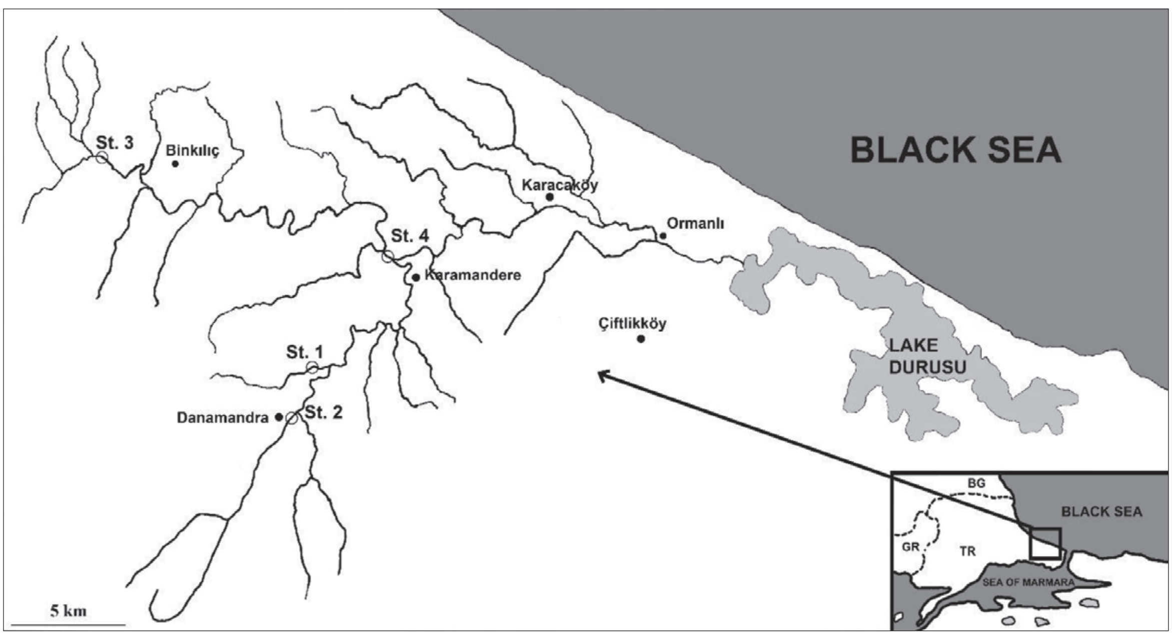

Fig. 1. The study area (Istranca Stream, Lake Durusu Basin, Turkey) and sampling stations: Station 1 (St. 1; N 41.33098°, E 28.24897°) – *Taşlıgeçit Creek;* Station 2 (St. 2; N 41.31415º, E 28.24893º) – *Danamandıra Creek;* Station 3 (St. 3; N 41.41750º, E 28.13845º) – *Şeytan Creek;* Station 4 (St. 4; N 41.37920º, E 28.29610º) – *Karamandere Creek*.

$_{2)}$×SE) (*x*: *a* and *b*; t:table value of t (t-test at 95% confidence); SE: standard error value of *a* and *b*) (King 2007). The null hypothesis of the isometric growth was tested with t-test using the equation $t_s=(b-3)/SE_b$ (SE_b is the standard error of the slope) (Zar 1999). Fish condition was assessed with the Fulton's Condition Factor ($K=(W/L^3)\times100$) (Ricker 1975). Growth in length was expressed with the von Bertalanffy function $L_t=L_\infty[1-e^{-k(t-to)}]$, where L_t was length as a function of time t, L_∞ was the theoretical asymptotic length, t was the age, t_o was the theoretical time at zero length and k was the rate constant (Cailliet et al. 1986). Relative growth in length (RGL%) and relative growth in weight (RGW%) were calculated according to $RGL=[(L_{t+1}-L_t)/L_t]\times100$ and $RGW=[(W_{t+1}-W_t)/W_t]\times100$, where L_{t+1} was the standard length at age t+1, L_t was the standard length at age t, W_{t+1} was the total weight at age t+1 and W_t was the total weight at age t (Chugunova 1963).

To determine the spawning period, the gonadosomatic index (GSI) was estimated for females and males using the formula $GSI=(G/W)\times100$, where G was gonad weight and W was total body weight (Ricker 1975). For the estimation of mean length at 50% maturity, a logistic function was fitted to the proportion of the mature individuals by size class using a non-linear regression. The function used for calculating length at first maturity was $P=1/\{1+\exp[-r(L-L_m)]\}$, where P was the proportion of mature individuals in each size class, r (-*b* slope) was a parameter controlling the slope of the curve and L_m was the size at 50% maturity (King 2007).

The absolute fecundity (F) was determined in 50 females of the species captured from March to May, as the number of all eggs (ripe eggs and small oocytes) in each ovary. Relative fecundity was calculated using the equations RF=(F/W) and RF=(F/L), where F was the absolute fecundity, W – total weight and L – standard length. The relationships between fecundity and fish size (standard length/weight) were calculated using regression analysis: $F=aX^b$, where F was fecundity, X was the standard length (cm) or weight of fish (g), *a* was the regression constant and *b* was the regression coefficient.

To determine the feeding strategy and diet composition of the species, fish specimens were dissected in the laboratory. The digestive tracts were removed and fixed in 4% formaldehyde solution until analyses. To determine the seasonal differences in feeding activity, the vacuity index (VI%) was estimated as a percentage of empty digestive tracts: $VI\%=(N_e/N_t)\times100$, N_e was the number of the empty digestive tracts, N_t was 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. Each taxonomic group was counted individually, oven-dried at 80°C and dried items weighed to the nearest 0.0001 g. The modified index of relative importance (MI%) of each uncountable prey items (major groups such as plant and detritus) and the index of relative importance (IRI%) of each countable prey items (insects groups) were estimated as follows: $MI\%=[(F\%\times W\%)/\Sigma(F\%\times W\%)]100$ and $IRI\%=[((N\%+W\%)F\%)/\Sigma((N\%+W\%)F\%)]100$, where F% was the percentage of frequency of occurrence [(number of digestive tracts containing a food item/total number of digestive tracts with food)×100], N% - the numerical percentage and W% – 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 method of Costello (1990) 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 $P_i=(\sum S_i/\sum S_{ti})\times100$, where P_i was the prey-specific abundance of prey i, S_i – the digestive tract content (volume, weight or number) comprised of prey *i* and S_{ti} – 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' standardised (Hurlbert 1978) indices: $B=1/\sum(P_j)^2$ and $B_A=(B-1)/(n-1)$, where *B* was Levins' measure of niche breadth, P_j – the proportion of individuals found using resource *j*, B_A – the Levins' standardised niche breadth and *n* – the number of possible resource states. Levins' *B* and B_A are minimal when all the individuals occur in only one resource state (minimum niche breadth, maximum specialisation). 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

Age and growth of *Phoxinus strandjae*

A total of 608 specimens of *P. strandjae* was captured from four stations (St.1: 247 specimens, St.2: 374 specimens, St.3: 5 specimens, St.4: 2 specimens) in the Istranca Stream. The standard length and total body weight of the specimens varied between 1.2-7.2 cm and 0.039-10.596 g, respectively. The sex ratio of female to male was found to be 1:1.15 with no significant difference from the ratio of 1:1 (x^2=1.47; $p>0.05$). A total of 489 specimens were used for aging and the age composition of the population ranged from age class 0 to VI. It was observed that age class III (33%) and II (29%) were dominant (Fig. 2).

Length- and weight-range distributions, relative growth in length and relative growth in weight of different ages and sexes of *P. strandjae* are given in Table 1. The maximum relative growth in length and also weight was observed for age class II, while the relative growth rates decreased with age.

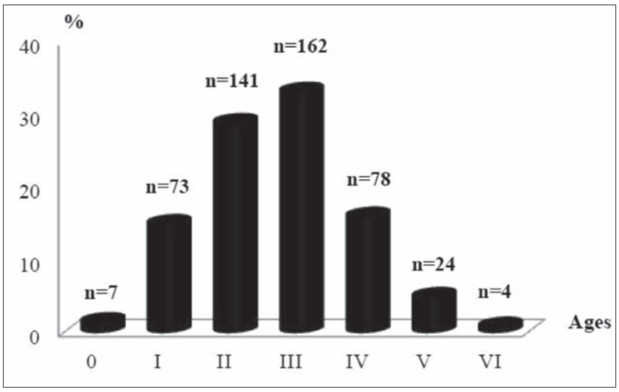

Fig. 2. The frequency (%) distribution of age classes by numbers of individuals (n) of *Phoxinus strandjae* in the Istranca Stream.

Length-weight relationship of *P. strandjae* was calculated for females and males as lnW=-3.883+3.075lnSL and lnW=-3.857+3.044lnSL, respectively. In terms of growth types according to *b* values, female individuals had positive allometric growth and male individuals had isometric growth (Table 2).

The Von Bertalanffy growth parameters were calculated as $L_t=10.89(1–e^{-0.121(t+1.986)})$ for females, $L_t=15.32(1–e^{-0.069(t+2.537)})$ for males and $L_t=11.78(1–e^{-0.106(t+2.103)})$ for all individuals. The mean condition factor (±SD) was calculated as 2.29 (±0.25) for females, 2.26 (±0.23) for males and 2.25 (±0.25) for all individuals. The analyses of the average condition factor showed that the condition increased with age and the highest value was for age class III (K=3.07) for all individuals (Table 1). Monthly variation of condition factor was also calculated for females, males and all individuals (Fig. 3). Especially in females, the condition rose up to 3.16 in March (2013) due to an increase in gonad weight.

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

Age classes	Sexes	n	SL (cm) (min.-max.)	RLR (%)	W (g) (min.-max.)	RWR (%)	K (min.-max.)
0	Juvenile	7	1.2–2.5	-	0.039-0.332	-	1.77–2.40
I	Juvenile	19	1.9–2.9	35.3	0.130-0.428	119.9	1.43–2.44
	Female	22	2.0–2.9		0.162–0.623		1.98–2.63
	Male	32	1.9–2.8		0.135–0.506		1.62–2.73
	All ind.	73	1.9–2.9		0.130–0.623		1.43–2.73
II	Juvenile	5	2.8–3.1	43.5	0.360–0.690	188.9	1.64–2.38
	Female	51	2.7–3.9		0.370–1.445		1.50–3.00
	Male	85	2.7–3.9		0.398–1.369		1.63–2.75
	All ind.	141	2.7–3.9		0.360–1.445		1.50–3.00
III	Female	75	3.5–4.6	21.2	0.955–2.796	94.5	1.81–3.07
	Male	87	3.5–4.6		0.884–2.660		1.83–2.86
	All ind.	162	3.5–4.6		0.884–2.796		1.81–3.07
IV	Female	49	4.3–5.5	22.5	1.567–4.179	72.9	1.74–3.16
	Male	29	4.5–5.6		1.988–3.467		1.76–2.71
	All ind.	78	4.3–5.6		1.567–4.179		1.74–3.16
V	Female	14	5.3–6.2	14.3	3.266–5.150	44.4	1.90–2.42
	Male	10	5.2–5.9		3.276–4.286		1.74–2.86
	All ind.	24	5.2–6.2		3.266–5.150		1.74–2.86
VI	Female	4	5.8–6.6	10.7	3.970–6.569	41.5	1.84–2.62

Table 2. The descriptive statistics and estimated parameters of length-weight relationships of *Phoxinus strandjae* 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).

Sexes	n	Standard length (SL, cm)		Body weight (W, g)		Regression parameters		Confidence limits		r^2
		Min.	Max.	Min.	Max.	*a*	*b*	95% CL of *a*	95% CL of *b*	
Female	253	2.0	7.2	0.162	10.596	-3.883	3.075 (A+)	0.068	0.049	0.984
Male	291	1.9	5.9	0.135	4.286	-3.857	3.044 (I)	0.059	0.045	0.984
All ind.	608	1.2	7.2	0.039	10.596	-3.926	3.099 (A+)	0.038	0.029	0.986

"A+" means positive allometric growth, "I" means isometric growth.

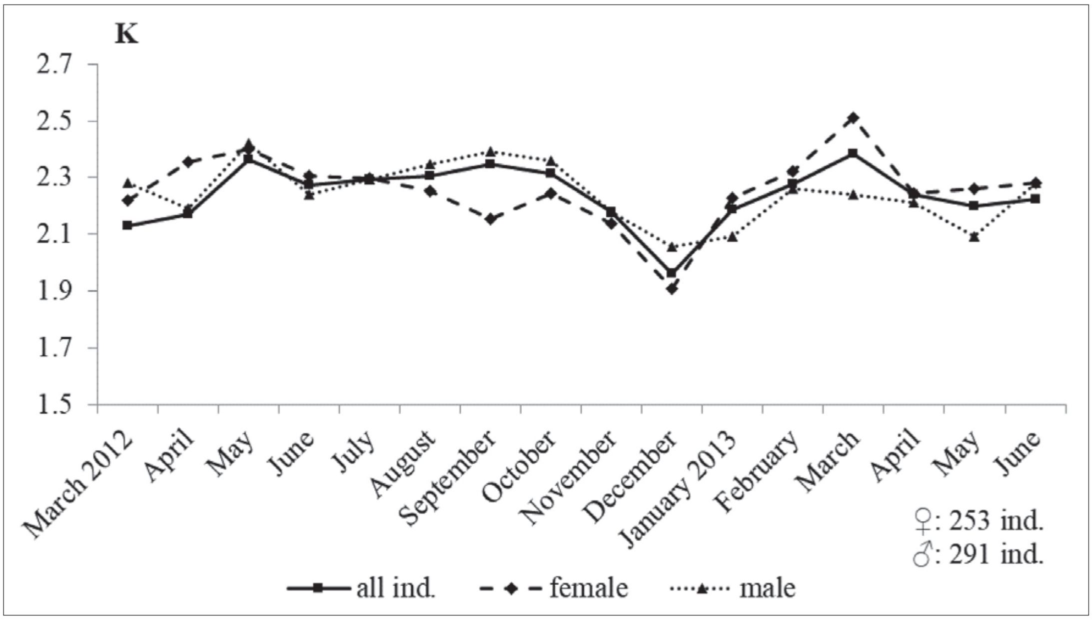

Fig. 3. The mean monthly condition factors (K) of *Phoxinus strandjae* in the Istranca Stream.

Reproduction of *Phoxinus strandjae*

The gonadosomatic index of *P. strandjae* ranged from 0.10 (August 2012) to 28.55 (March 2013) for females, and from 0.13 (August 2012) to 7.74 (March 2013) for males. The species spawned from April to July, the main spawning activity was in late April and June. The highest GSI values for both sexes were observed in April (2012) and March (2013) during the two spawning seasons (Fig. 4). In 2012, the spawning activity finished in July and no individual carrying eggs ready for spawning was captured.

Observation on gonads showed that there were always small oocytes beside the ripe eggs during the spawning period. While the number of 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, *P. strandjae* released eggs in batches and exhibited multiple spawning within a season. The mean absolute fecundity in mature females was 839 eggs (SD=529), ranging from 101 eggs (SL of 3.4 cm) to 2392 eggs (SL of 6.6 cm). The absolute fecundity increased with body size (both length and weight) and the relationship of absolute fecundity versus body size (both length and weight) was estimated as $F=4.079L^{3.257}$ (r^2=0.710) and $F=211.1W^{1.193}$ (r^2=0.801), respectively. Mean relative fecundity was calculated as 162 eggs.cm^{-1} (SD=82) (ranged from 30 to 365 eggs.cm^{-1}) and 268 eggs.g^{-1} (SD=78) (ranged from 92 to 422 eggs.g^{-1}). The size at first maturity, where 50% of the individuals had attained gonad development, was 2.99 cm SL for females and 2.89 cm SL for males. The mean age at maturity of both males and females was determined as age class II.

Diet spectrum and feeding strategy of *Phoxinus strandjae*

A total of 583 fish specimens were dissected to determine the diet spectrum and feeding strategy of *P. strandjae*. As some of the digestive tracts of *P. strandjae* assessed in the study were empty, a total

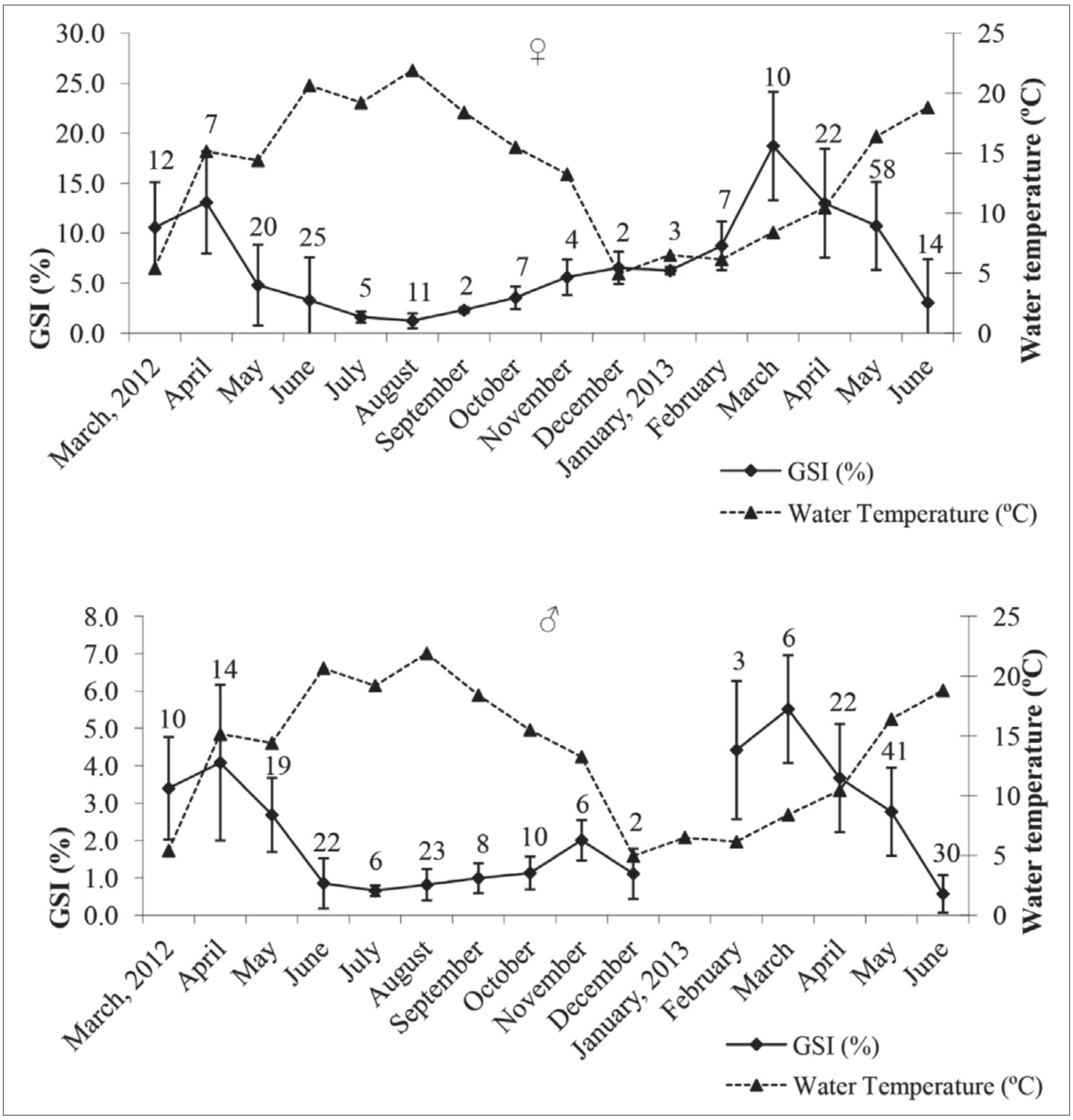

Fig. 4. The monthly changes in the gonadosomatic index (GSI) of both female and male *Phoxinus strandjae* and mean water temperature (°C) in the Istranca Stream.

of 277 specimens were examined for the diet analyses. The vacuity index (VI%) of *P. strandjae* specimens changed seasonally and accounted for 26% in spring (2012), 29% in summer, 25% in autumn, 62% in winter and 68% in spring (2013). In the second summer (2013) period, the data were excluded from the examination as fish specimens were sampled only in June.

According to the analysis, the diet spectrum of *P. strandjae* contained a total of seven major groups: insects (Diptera, Ephemeroptera, Plecoptera, Odonata, Trichoptera, Coleoptera and Hymenoptera), crustaceans (Gammaridae), molluscs (Gastropoda), plants (terrestrial plants, algae), arachnids (Acaridae), worms (Annelida) and detritus (organic detritus). Insects (93.16%) were the most prevalent food item in terms of modified index of relative importance (MI%), while the consumed insect groups mostly composed of Diptera (IRI%=88.90; Table 3).

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 the axis (P_i and F_i%). In addition to the indices of relative importance (IRI% and MI%), this diagram also proved that *P. strandjae* was rarely fed on these food items. However, Diptera had been eaten by more than half the individuals (F_i%=64%) making Diptera the dominant prey in the diet of *P. strandjae*. The Levins' niche breadth (B) and the standardised niche breadth (B_A) values were estimated as 1.97 and 0.16, respectively. The results showed that *P. strandjae* fed on a limited range of prey.

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

Prey items		**F(%)**	**W(%)**	**N(%)**	**IRI (%)**	**MI (%)**
Insecta		79.42	72.20			93.16
	Diptera	64.26	34.24	82.28	**88.90**	
	Ephemeroptera	23.47	18.68	10.77	**8.20**	
	Plecoptera	5.05	1.50	0.39	**0.11**	
	Trichoptera	16.97	4.77	2.22	**1.41**	
	Odonata	1.81	1.62	0.13	**0.04**	
	Coleoptera	10.11	6.99	4.11	**1.33**	
	Hymenoptera	1.08	0.27	0.08	**<0.00**	
Plant material		22.02	16.62			5.95
Crustacea		5.78	5.21			0.49
Annelida		1.44	2.07			0.05
Arachnida		0.72	0.07			<0.00
Gastropoda		0.36	0.05			<0.00
Detritus		5.78	3.77			0.35

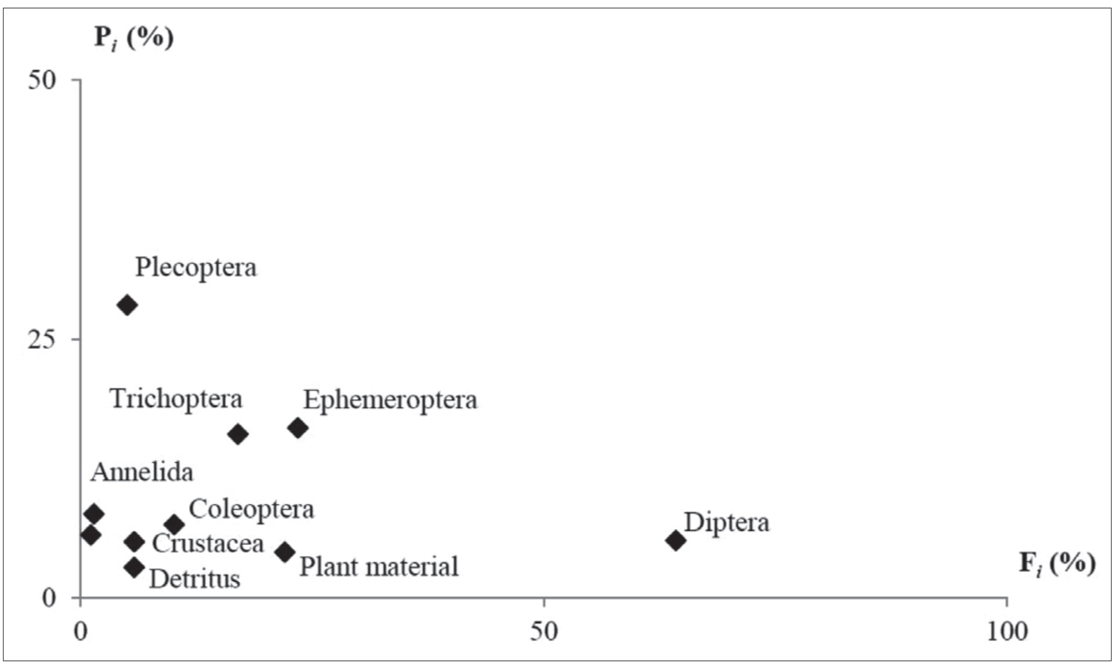

Fig. 5. The modified Costello feeding strategy diagram for *Phoxinus strandjae* in the Istranca Stream. Prey-specific abundance (P_i) plotted against frequency of occurrence (F_i%) of food items in the diet of the species.

Discussion

The members of the genus *Phoxinus* are short-lived fish species. According to Kottelat & Freyhof (2007), *P. phoxinus* lives 4-5 years in general but can live up to 11 years and *P. lumaireul* lives up to 6 years. The lifespan of *P. strandjae*, estimated as 6 years, is similar to the literature presented for the members of the genus.

The age distribution of the species showed that age class III was predominant, while the younger age classes (I, II) were represented by a large number of specimens (Fig. 2). Juveniles of the species were poorly represented in this study because the mesh size of the electrofishing grab was too large to provide a quantitative estimation of age class 0. The observations made in the field also showed that the individuals of the species moved in small groups that consisted of similarly-sized classes. Taşlıgeçit (St.1) and Danamandıra (St.2) creeks, where *P. strandjae* has been captured in large numbers, are very small and shallow creeks, including riffle and pool habitats. The average water depth was approximately 20 cm and reached up to 45 cm in the pool habitats. The individuals of the older age classes (V, VI) mostly preferred these deeper pool habitats and lived solitary to hide from their predators. They were also represented by a small number, because of the difficulties of catching the fish and natural or predation-caused deaths.

The members of the genus *Phoxinus* are small cyprinids and limited studies have estimated the growth parameters of this genus. The asymptotic length for *P. phoxinus* has been measured as 10.8 cm fork length (Froese & Pauly 2014), although the maximum recorded length is 14.0 cm SL (Muus & Dahlström 1968). In the present study, the asymptotic length for females of *P. strandjae* could be considered realistic for this population as the largest specimen caught had a standard length of 7.2 cm. However, the estimated value for males of *P. strandjae* appeared to be quite higher than the observed value. These results are likely caused by the lack of 6-year-old males participated in the calculations.

In the present study, the exponents of the length-weight relationships of the species (all individuals; female, male and juveniles) indicated a positive allometric growth (Table 2). The value of *b* varies according to the species, as well as some factors such as food availability, feeding rate, gonad development and spawning period; and it is mostly used to compare the growth of the same fish species living in different habitats (Bagenal & Tesch 1978). Tarkan et al. (2009) studied the length-weight relationship of *P. strandjae* (the species was recorded as *P. phoxinus*) from the small streams flowing into Lake Sapanca and determined that the population had an allometric growth (b=3.23), similar to the results of the present study.

According to Kottelat & Freyhof (2007), *P. colchicus, P. lumaireul* and *P. phoxinus* start to reproduce in April and the spawning activity continues until July. Similarly, the spawning period of *P. strandjae* living in the Istranca Stream occurred from April to July, the main spawning activity being in May. However, we recorded temporal difference between the two spawning seasons (2012 and 2013) of the species and this could be explained with differences in water temperature, as it was warmer early in 2013 (Fig. 3). Besides, it was observed in the surveys that *P. strandjae* used the riffle (fast-flowing stream habitats on gravel bottom) areas on the streams as a spawning site during these months, while it preferred shallow areas with aquatic vegetation in the other months of the year.

In the spawning period, males developed some visible breeding ornamentations, such as dark lateral coloration, bright red abdomen, red lips, metallic green flanks and also breeding tubercles on the head. As reported earlier (Kottelat & Freyhof 2007, Lai et al. 2013), male ornamentation was developed for intrasexual competition, either for breeding opportunities or to attract females. Moreover, we surprisingly observed that many of the females also had bright red abdomens and we have not found any data about this pattern in the available literature.

Phoxinus strandjae lays its eggs in batches and exhibits multiple spawning within a season. Batch spawning is related to body size or age (Fitzhugh et al. 2012) and this trait of *P. strandjae* is thought to result from being small-bodied and short-lived fish species. Especially in 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 & Munday 2005). Early maturation is common for the members of the genus *Phoxinus*: *P. lumaireul* and *P. phoxinus* start to spawn at 2 years of age (Mills & Eloranta 1985, Gandolfi et al. 1991, Kottelat & Freyhof 2007). Similarly, *P. strandjae*, living in the Istranca Stream, was matured in age class II. Small-bodied species generally exhibit an early maturation trait for increasing the reproductive capacity and surviving/settling in variable and temporary habitats (Hernaman & Munday 2005, Mims et al. 2010, Uusi-Heikkilä et al. 2015).

The vacuity index (VI%) was the highest during the winter (2012) and spring (2013) months. The high values of VI% indicated that the feeding intensity was low throughout these seasons. In the winter months, the feeding intensity decreased due to the falling water temperature. On the other hand, the remarkable difference in the springs of 2012 and 2013 was an unexpected finding. Because in both springs no structural changes or habitat losses were found at the sampling stations and fish specimens were collected at the same time of the day. This difference may have resulted from: 1) The reduced food resources in the environment, 2) A possible increase in food competition due to the increasing number of individuals in the study area (the number of specimens caught from the sampling stations in the first year was 122, while during the second year it was 213).

The digestive tract survey verified that *P. strandjae* was largely omnivorous (more precisely insectivores) feeding mainly on aquatic insects and a small amount of plant and animal material. The mouth of *P. strandjae* is a terminal mouth that enables it to take in food at the water surface. Nevertheless, the diet spectrum of the species contains insect groups as Diptera larvae.

The recent identifications of the European populations of *Phoxinus* based on morphological and genetic characteristics (Kottelat 2007, Kottelat & Freyhof 2007, Geiger et al. 2014), prevent us from comparing our results with previous studies. A recent study (Gaygusuz et al. 2011) has determined the diet spectrum of *P. strandjae* (the species was recorded as *P. phoxinus*) in the Hepçe Stream (Darlık Reservoir, İstanbul, Turkey): the Hepçe population fed mainly on Diptera but rarely on other food items such as detritus, crustacean, worm, etc.

In accordance with the explication of trophic strategies based on Costello's modified method (1990) and niche breadth analyses (Levins 1968, Hurlbert 1978), the species shows a specialist feeding strategy. However, the position of Diptera in the diagrams contributes to the narrow niche width of this species. Having a specialist feeding strategy could be stressful under condition of food scarcity, or competition for food with other species (feeding mainly on the same food items. i.e. Diptera).

In conclusion, the present study provides basic information about the biological traits of *P. strandjae*. No previous data exist about the life-history traits and the feeding of the species from the distribution area in Turkey. Therefore, our results will contribute to understanding the function of this species in nature.

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References

Amundsen P.A., Gabler H.M. & Staldvik F.J. 1996. A new approach to graphical analysis of feeding strategy from stomach contents data – modifycation of the Costello (1990) method. Journal of Fish Biology 48: 607–614. doi: 10.1111/j.1095-8649.1996.tb01455.x

Bagenal T.B. & Tesch F.W. 1978. Methods for assessment of Fish Production in Fresh Waters, IBP Handbook No. 3 (3rd ed.). Bagenal T.B. (Ed.). Blackwell Scientific Publications, Oxford, London, Chapter 5. 101-136 pp.

Berg L.S. 1949. Freshwater Fishes of the U.S.S.R. and adjacent countries. Moskva and Leningrad: Izdatel'stvo Akademii Nauk SSSR, vol. 2 [translation: Israel Program for Scientific Translations, Jerusalem, 1965].

Bianco P.G. & De Bonis S. 2015. A taxonomic study of the genus *Phoxinus* (Acthinopterygii, Cyprinidae) from Italy and western Balkans with description of four new species: *P. ketmaieri, P. karsticus, P. apollonicus* and *P. likai*. Researches on Wildlife Conservation 4: 1–17.

Cailliet G.M., Love M.S. & Ebeling A.W. 1986. Fishes. A Field and Laboratory Manual on Their Structure, Identification, and Natural History. Wadsworth Publishing Company, 194 p.

Chrisafi E., Kaspiris P. & Katselis G. 2007. Feeding habits of sand smelt (*Atherina boyeri* Risso, 1810) in Trichonis Lake (Western Greece). Journal of Applied Ichthyology 23: 209–214. doi: 10.1111/j.1439-0426.2006.00824.x

Chugunova N. I. 1963. Age and growth studies in fish. Israel Prog. Sci. Transl. No: 610, Natl. Sci. Found Washington, D.C. 132 p.

Costello M. J. 1990. Predator feeding strategy and prey importance: a new graphical analysis. Journal of Fish Biology 36: 261–263. doi: 10.1111/j.1095-8649.1990.tb05601.x

Dikov T. & Zivkov M. 2004. Abundance and biomass of fishes in Veleka River, Bulgaria. Folia Zoologica 53(1): 81–86.

Doadrio I. 2001. Atlas y libro rojo de los peces continentales de Espaňa. Direccion General de Conservación de la Natureleza and Museo Nacional de Ciencias Naturales, Madrid.

Fitzhugh G.R., Shertzer K.W., Kellison G.T. & Wyanski D.M. 2012. Review of size- and age-dependence in batch spawning: implications for stock assessment of fish species exhibiting indeterminate fecundity. Fishery Bulletin 110(4): 413–425.

Freyhof J. & Kottelat M. 2008. *Phoxinus strandjae*. In: IUCN 2013. IUCN Red List of Threatened Species. Version 2013.2. <www.iucnredlist.org>. Downloaded on 16 January 2014.

Froese R. & Pauly D. 2014. FishBase. World Wide Web electronic publication. www.fishbase.org, 20 November, 2014.

Gandolfi G., Zerunian S., Torricelli P. & Marconato A. 1991. I pesci delle acque interne Italiane. Istituto Poligrafico e Zecca dello Stato, Roma.

Gaygusuz Ö., Temel M. & Gürsoy Gaygusuz Ç. 2011. Hepçe Deresi'nde (Darlık-İstanbul) Yaşayan *Phoxinus phoxinus* Linnaeus, 1758) (Pisces: Cyprinidae) Türünün Beslenme Ekolojisi, X. Ulusal Ekoloji ve Çevre Kongresi, 04–07 Ekim 2011, Çanakkale (in Turkish).

Geiger M.F., Herder F., Monaghan M.T., Almada V., Barbieri R., Bariche M., Berrebi P., Bohlen J., Casal-Lopez M., Delmastro G.B., Denys G.P.J., Dettai A., Doadrio I., Kalogianni E., Kärst H., Kottelat M., Kovačič M., Laporte M., Lorenzoni M., Marčič Z., Özuluğ M., Perdices A., Perea S., Persat H., Porcelotti S., Puzzi C., Robalo J., Šanda R., Schneider M., Šlechtová V., Stoumboudi M., Walter S. & Freyhof J. 2014. Spatial heterogeneity in the Mediterranean Biodiversity Hotspot affects barcoding accuracy of its freshwater fishes. Molecular Ecology Resources, doi: 10.1111/1755-0998.12257.

Hayse J.W. 1990. Feeding habits, age, growth, and reproduction of Atlantic spadefish *Chaetodipterus faber* (Pisces: Ephippidae) in South Carolina. Fishery Bulletin 88(1): 67–83.

Hernaman V. & Munday P.L. 2005. Life-history characteristics of coral reef gobies. II. Mortality rate, mating system and timing of Maturation. Marine Ecology Progress Series 290: 223–237.

Hurlbert S.H. 1978. The measurements of niche overlap and some relatives. Ecology 59: 67–77.

Hyslop E.J. 1980. Stomach contents analysis-A review of methods and their application. Journal of Fish Biology 17: 411–429.

King M. 2007. Fisheries Biology, Assessment and Management (2nd ed.). Oxford: Blackwell Scientific Publications, 381 p.

Kottelat M. 2007. Three new species of *Phoxinus* from Greece and Southern France (Teleostei: Cyprinidae). Ichthyological Exploration of Freshwaters 18(2): 145–162.

Kottelat M. & Freyhof J. 2007. Handbook of European freshwater fishes. Berlin, 640 p.

Krebs C.J. 1998. Ecological methodology. Addison-Wesley Educational Publishers, Menlo Park, CA.

Lai Y., Kekäläinen J. & Kortet R. 2013. Male ornamentation in the European minnow (*Phoxinus phoxinus*) signals swimming performance. Ethology 119: 1–9. doi: 10.1111/eth.12167

Le Cren E.D. 1951. The length-weight relationship and seasonal cycle in gonad weight and condition in the perch (*Perca fluviatilis*). Journal of Animal Ecology 20: 201–218.

Levins R. 1968. Evolution in changing environments. Princeton University Press, Princeton.

Mills C.A. & Eloranta A. 1985. The biology of *Phoxinus phoxinus* (L.) and other littoral zone fishes in Lake Konnevesi. central Finland. Annales Zoologici Fennici 22: 1–12.

Mims M.C., Olden J.D., Shattuck Z.R. & Poff N.L. 2010. Life history trait diversity of native freshwater fishes in North America. Ecology of Freshwater Fish 19: 390–400.

Muus B.J. & Dahlström P. 1968. Süβwasserfische. BLW Verlagsgesellschaft, München. 224 p.

Novakowski G.G., Hahn N.S. & Fugi R. 2008. Diet seasonality and food overlap of the fish assemblage in a pantanal pond. Neotropical Ichthyology 6(4): 567–576.

Palandacic A., Naseka A.M., Ramler D. & Ahnelt H. 2017. Contrasting morphology with molecular data: an approach to revision of species complexes based on the example of European (Cyprinidae). Biodiversity Data Journal 5: e21772. doi: 10.3897/BDJ.5.e21772

Ricker W.E. 1975. Computations and interpretation of biological statistics of fish populations. Bulletin of the Fisheries Research Board of Canada 191: 1–382.

Saç G. & Özuluğ M. 2015. New data on the distribution and conservation status of *Phoxinus strandjae* (Teleostei: Cyprinidae). Ichthyological Exploration of Freshwater 25(4): 381–383.

Sangun L., Akamca E. & Akar M. 2007. Weight-length relationships for 39 fish species from the north-eastern Mediterranean coast of Turkey. Turkish Journal of Fisheries and Aquatic Sciences 7: 37–40.

Tarkan A.S., Özuluğ M., Gaygusuz Ö., Gaygusuz Ç.G. & Saç G. 2009. Length–weight relationships of six freshwater fishes from small streams flowing into Lake Sapanca, NW Turkey. Journal of Applied Ichthyology 25: 230–231. doi:10.1111/j.1439-0426.2008.01201.x

Uusi-Heikkilä S., Whiteley R.A., Kuparinen A., Matsumura S., Venturelli P.A., Wolter C., Slate J., Primmer C.R., Meinelt T., Killen S.S., Bierbach D., Polverino G., Ludwig A. & Arlinghaus R. 2015. The evolutionary legacy of size-selective harvesting extends from genes to populations. Evolutionary Applications 8: 597–620. doi:10.1111/eva.12268

Zar J.H. 1999. Biostatistical Analysis. Englewoods Cliffs. N. J., Prentice Hall, New Jersy, 663 p.

Zivkov M.T. & Trichkova T.A. 2006. Biology of European minnow (*Phoxinus phoxinus*, Pisces: Cyprinidae) in high mountain lakes, the Rila Mountains, Bulgaria. Acta Zoologica Bulgarica 58 (3): 371–386.

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