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Age Structure and Length-Weight Relationship for Four Species of *Aphanius* Nardo, 1827 (Actinopterygii: Aphaniidae) Endemic to the Lake District, Central Anatolia, Turkey

Deniz Innal[1*], Salim Serkan Güçlü[2], Mehmet Can Ünal[1], Buğrahan Doğangil[1] & Daniela Giannetto[3]

[1]Department of Biology, Mehmet Akif Ersoy University, Burdur, Turkey
[2]Eğirdir Fisheries Faculty, Isparta University of Applied Sciences, Isparta, Turkey
[3]Department of Biology, Faculty of Science, Muğla Sıtkı Koçman University, Muğla, Turkey

Abstract: Age structure and length-weight relationship are examined for four species of the genus *Aphanius* Nardo, 1827 endemic to the Lake District, Central Anatolia, Turkey: *A. iconii* Akşiray, 1948, *A. saldae* (Aksiray, 1955), *A. sureyanus* (Neu, 1937) and *A. transgrediens* (Ermin, 1946). Specimens were sampled by a shore seine net from Lake Eğirdir (*A. iconii*), Lake Salda (*A. saldae*), Lake Burdur (*A. sureyanus*) and Lake Acıgöl (*A. transgrediens*) during 2014-2015. A total of 1246 specimens are examined (*A. iconii:* n=206; *A. saldae*: n=525; *A. sureyanus*: n= 350; *A. transgrediens*: n=165). The maximum age for *A. iconii*, *A. saldae* and *A. sureyanus* was 4 years and for *A. transgrediens* was 5 years. The values of the b parameter of the length-weight equations for *A.transgrediens*, *A. saldae*, *A. sureyanus* and *A. iconii* populations are 3.027, 2.587, 3.221 and 2.713, respectively. A new maximum total length of 6.1 cm for *A. transgrediens* is reported.

Key words: Anatolia; endemic species; killifish; Aphaniidae; Cyprinodontidae; length-weight relationship; age.

Introduction

The genus *Aphanius* Nardo, 1827 is native to North Africa, South-Western Asia and Europe. It formerly belonged to the family Cyprinodontidae but recently Freyhof et al. (2017) suggested its retention in the distinct monotypic family of Aphaniidae as previously proposed by Parenti (1981). The taxonomy of the genus has been widely discussed with the description of new species and the debate on their validity (Akşiray 1948, Wildekamp 1993, Wildekamp et al. 1999, Hrbek et al. 2002, Geiger et al. 2014, Pfleiderer et al. 2014, Freyhof et al. 2017). Geiger et al. (2014) reported 14 species of *Aphanius* from Turkey, most of them endemic only to a single or a few basins. Due to this richness, Anatolia has been recognised as one of the diversity hotspots for the genus *Aphanius* (Wildekamp et al. 1999).

Aphanius iconii Akşiray, 1948, *A. saldae* (Aksiray, 1955), *A. sureyanus* (Neu, 1937) and *A. transgrediens* (Ermin, 1946) are endemic to the Lake District, a wide area in South-Western Turkey characterised by several saline and freshwater lakes (Atalay 1987, Kazanci et al. 2004). *Aphanius iconii* (also reported as Konya killifish by Çiçek et al. 2015) is endemic to the Lake Eğirdir Basin where it is widely distributed (Freyhof et al. 2017). Although *A. iconii* is now the name definitively accepted for the species known from Lake Eğirdir (Freyhof et al. 2017), the taxon is still reported as *A. anatoliae* in FishBase and not yet evaluated by the IUCN. *Aphanius saldae* (also reported as Salda killifish by Çiçek et al. 2015) is endemic to Lake Salda, one of the largest and deepest enclosed saline lakes characterised by a high alkalinity and magnesium content

*Corresponding author: innald@gmail.com

(Kazanci et al. 2004). *Aphanius saldae* is not yet evaluated by the IUCN and only scarce information is reported about this species (Yoğurtçuoğlu & Ekmekçi 2015). The Burdur toothcarp *A. sureyanus* is endemic to the saline Lake Burdur. It can be found in schools near the lake shore and springs, in water that varies from relatively fresh to strongly brackish and sometimes even sulphurous (Güçlü et al. 2007). The slow drought of the lake, due to massive water abstraction and presence of dams, was identified as the major threat to this species (Freyhof 2014a). *Aphanius sureyanus* is currently assessed as Endangered according to the IUCN Red List of Threatened species (Freyhof 2014a). The Acıgöl toothcarp *A. transgrediens* is known only from a spring field in Lake Acıgöl (Freyhof 2014b). The lake is important for its sodium sulphate reserves that are massively used in the industry (Güçlü & Küçük 2012). During heavy rainfalls, when the salinity concentration is low, the species can also be found in the lake near the shores (Wildekamp 1993, Güçlü & Küçük 2012). Reduction in rainfall, water abstraction and the abundant presence of the alien *Gambusia holbrooki* has impacted the species across its former range (Yoğurtçuoğlu & Ekmekçi 2014). It has been observed in our field studies that the alien species *Carassius gibelio* has formed dense populations. Currently, *A. transgrediens* is assessed as Critically Endangered according to the IUCN criteria (Freyhof 2014b).

The aim of this study was to provide information on the population and age structures and length-weight relationships for *A. iconii* (Lake Eğirdir), *A. saldae* (Lake Salda), *A. sureyanus* (Lake Burdur) and *A. transgrediens* (Lake Acıgöl).

Materials and Methods

Samples of the four species (*A. iconii:* n= 206; *A. saldae*: n= 525; *A. sureyanus*: n= 350 and *A. transgrediens*: n= 165, for a total of 1246 specimens) were collected from four lakes in the Lake District

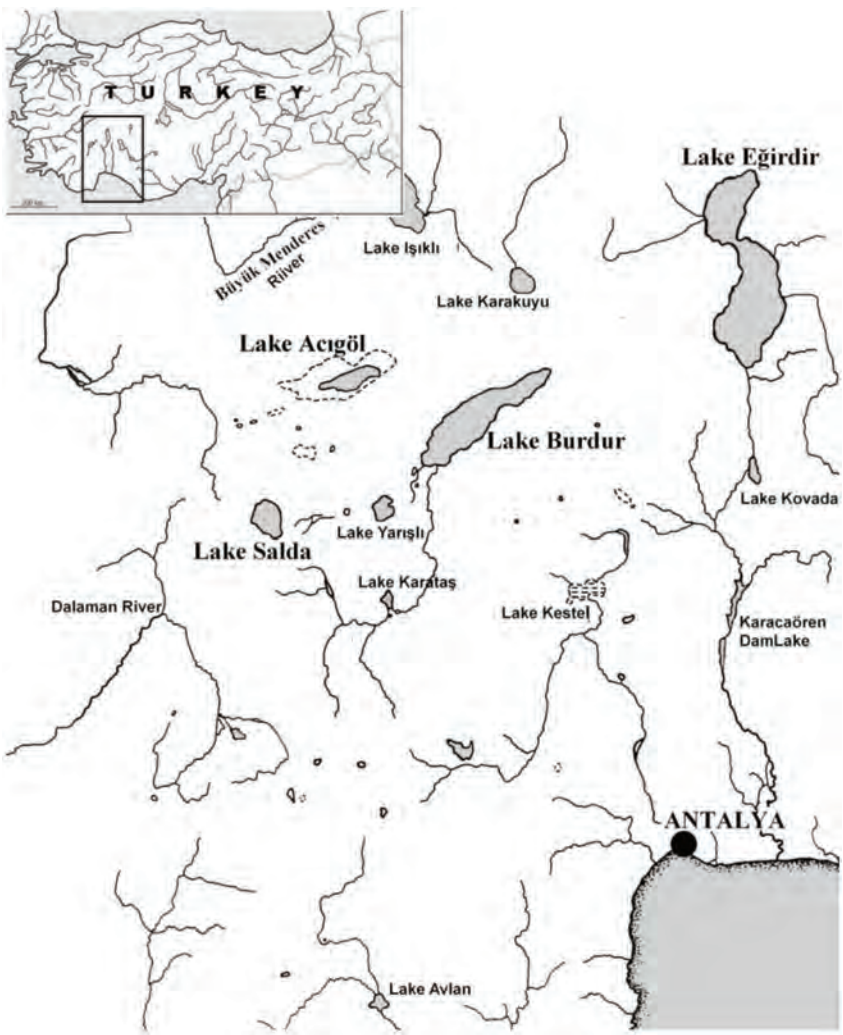

Fig. 1. Map of the Lake District showing localities of the four endemic species of the genus *Aphanius*.

Fig. 2. *Aphanius iconii* from Lake Eğirdir.

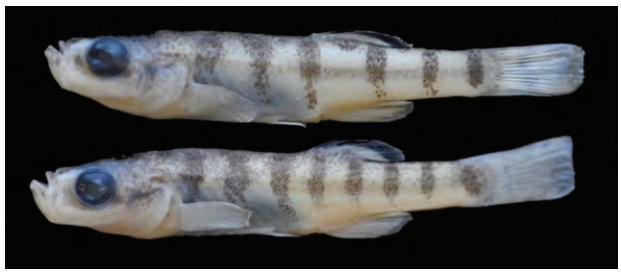

Fig. 3. *Aphanius saldae* from Lake Salda.

Fig. 4. *Aphanius sureyanus* from Lake Burdur.

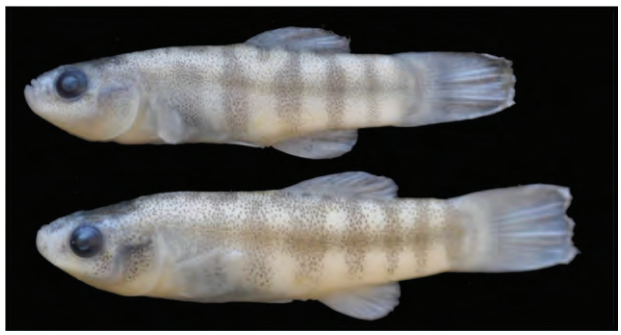

Fig. 5. *Aphanius transgrediens* from Lake Acıgöl.

(SW Turkey): the saline lakes Acıgöl, Burdur and Salda and the freshwater Eğirdir (Fig. 1). Sampling was carried out seasonally, between March 2014 and February 2015, by shore seine net (10 m long and 2 m high; 1.2 x 2 mm mesh size). After capture, the fish were preserved in 4% formaldehyde and transported to the Fish Biology Laboratory of the Biology Department of the Mehmet Akif Ersoy University, Burdur, Turkey. The total length of each fish was measured with 0.01 mm sensitive callipers and weight was recorded by an electronic balance at the nearest 0.01 g. The sex was determined by external observation since the species of *Aphanius* exhibit sexual dimorphism. The age was determined from scales taken from the left side of the body, between the end of the pectoral fin and the beginning of the dorsal fin. Age was estimated by two different operators (Bagenal 1978). For each species the overall ratio of males to females was evaluated using χ^2 test ($P > 0.05$). A preliminary step in the computation of the length- weight relationship was the validation of the data and potential outliers were excluded as they were likely derived by wrong measurements or aberrant data. The length-weight relationships were estimated for the total sample and separated by sex by means of the exponential regression equation, $W = a\ TL^b$, where W is the weight in g, TL – the total length in cm, a – the intercept of the regression, and b – the slope or regression coefficient (Ricker 1975).

Results

During the study, totally 1246 specimens were examined: *A. iconii* – n=206 (Fig. 2), *A. saldae* – n=525 (Fig. 3), *A. sureyanus* – n=350 (Fig. 4) and *A. transgrediens* – n=165 (Fig. 5). The age of the fish ranged from 0-IV years for *A. sureyanus*, I-V years for *A. transgrediens*, I-IV years for both *A. saldae* and *A. iconii* (Table 1). The overall ratios of females : males were 0.76 for *A.iconii*, 0.17 for *A. saldae*, 2.39 for *A. sureyanus* and 1.84 for *A. transgrediens*. The sex ratio was statistically different for all species except for *A. iconii* (Table 1). The sex composition differed between the age classes with a higher percentage of females in the older age classes for all the species (Table 1). The total length class (5 mm interval) composition for age (estimated years) was analysed (Table 2). The estimated b values of the total length-weight relationships varied from 2.58 for the total sample of *A. saldae* to 3.41 for the male sample of *A. sureyanus* (Table 3).

Discussion

Currently, freshwater fish may be considered the most threatened group of vertebrates based on the IUCN assessments (McGregor Reid et al. 2013). Endemic freshwater fish species are the most sensitive because they are often distributed over a small area and they are the most exposed to human impacts (Crivelli

Table 1. Age and sex distribution of females (F), males (M) and all *Aphanius iconii, A. transgrediens, A. sureyanus* and *A. saldae* (N: Number of samples; N%: Percentage of samples).

Species	Age (years)	Females		Males		All		F:M
		N	N%	N	N%	N	N%	
A. iconii	I	5	2.43	21	10.19	26	12.62	0.23:1.00 (P<0.05)
	II	33	16.02	61	29.61	94	45.63	0.54:1.00 (P<0.05)
	III	45	21.84	33	16.02	78	37.86	1.36:1.00 (P>0.05)
	IV	6	2.91	2	0.97	8	3.88	3.00:1.00 (P<0.05)
	Total	89	43.20	117	56.80	206	100	0.76:1.00 (P>0.05)
A. saldae	I	0	0	4	0.76	4	0.76	0.00:4.00 (P<0.05)
	II	12	2.29	190	36.19	202	38.48	0.06:1.00 (P<0.05)
	III	58	11.05	248	47.24	306	58.29	0.23:1.00 (P<0.05)
	IV	10	1.90	3	0.57	13	2.48	3.33:1.00 (P<0.05)
	Total	80	15.24	445	84.76	525	100	0.17:1.00 (P<0.05)
A. sureyanus	0	20	5.71	2	0.57	22	6.29	10.0:1.00 (P<0.05)
	I	51	14.57	21	6	72	20.57	2.42:1.00 (P<0.05)
	II	131	37.43	76	21.71	207	59.14	1.72:1.00 (P>0.05)
	III	37	10.57	4	1.14	41	11.71	9.25:1.00 (P<0.05)
	IV	8	2.29	0	0	8	2.29	8.00:1.00 (P<0.05)
	Total	247	70.57	103	29.43	350	100	2.39:1.00 (P<0.05)
A. transgrediens	I	27	16.36	23	13.94	50	30.30	1.17:1.00 (P>0.05)
	II	61	36.97	30	18.18	91	55.15	2.03:1.00 (P>0.05)
	III	13	7.88	5	3.03	18	10.91	2.06:1.00 (P>0.05)
	IV	5	3.03	0	0	5	3.03	5.00:1.00 (P<0.05)
	V	1	0.61	0	0	1	0.61	1.00:0.00 (P>0.05)
	Total	107	64.85	58	35.15	165	100	1.84:1.00 (P>0.05)

1995). Furthermore, little attention is usually given to the local populations of the endemic species and this lack of interest can represent a higher risk for their conservation and survival. Although the genus *Aphanius* has received considerable attention, the most of the studies focus on the taxonomy of the species (Akşıray 1948, Wildekamp 1993, Wildekamp et al. 1999, Hrbek et al. 2002, Geiger et al. 2014, Pfleiderer et al. 2014, Freyhof et al. 2017) and, to date, the available knowledge on the biology and ecology of some species of the genus is scarce and limited to a few studies (Güçlü et al. 2007, Güçlü 2012, Freyhof 2014a,b, Yoğurtçuoğlu & Ekmekçi 2015, Sarı et al. 2017).

In the present study, the maximum total length for *A. iconii* is 4.2 cm. For *A. iconii*, the maximum total length has been reported as 5.03 cm (reported as *A. anatoliae*, see Güçlü 2012) (Table 4). For *A. saldae* and *A. sureyanus*, the maximum total lengths observed (5.2 and 4.5 cm, respectively) are in line with those reported by Yoğurtçuoğlu & Ekmekçi (2015) (6.01 and 4.67 cm, respectively; Table 4). For *A. transgrediens*, a new maximum total length of 6.1 cm is recorded in this study. The value is slightly bigger than 5.41 cm reported by Yoğurtçuoğlu & Ekmekçi (2015) (Table 4). Examining the estimated length-weight equations, the R^2 of the equation are >0.90 for all the species as suggested by Froese (2006) and the b values are all within the expected range of 2.5-3.5 recommended by Carlander (1969). The estimated b values for *A. sureyanus* are higher than 3, in line with the results reported by Güçlü et al. (2007) and Yoğurtçuoğlu & Ekmekçi (2015) (Table 4). For *A. saldae*, the estimated b values are lower than 3 as reported by Yoğurtçuoğlu & Ekmekçi (2015) (Table 4). In contrast, the estimated b value for *A. transgrediens* is close to 3 and smaller than those reported by Yoğurtçuoğlu & Ekmekçi (2015). These dissimilarities can be due to the different length-range and sample size (Froese et al. 2011). Usually a reduced length range and the abundance of smaller fish can result in a higher b value because small and juvenile fish usually have a more "pumpkin''-shaped body and become more fusiform with age (Froese 2006). Moreover, Froese (2006) has reported that only when length-weight estimates cover reasonable geographic and inter-annual variation, it is possible to discuss isometric versus allometric growth of the species as a whole by using the value of *b*. Froese et al. (2011) have reported that the ideal sampling period for studies on length-weight of fish species should extend over a full year cycle and cover all seasons. Thus, it is avoided the inclusion of data that could derive from an "abrupt change of shape'' (Le Cren 1951). These aberrant data can be due to the huge mass of gonads

Table 2. Size and age composition for the total sample of *Aphanius iconii* from Lake Eğirdir, *A. saldae* from Lake Salda, *A. sureyanus* from Lake Burdur and *A. transgrediens* from Lake Acıgöl.

Species	TL class (cm)	0	I	II	III	IV	V	Total
A. iconii	2.0-2.49		12					12
	2.5-2.99		14	37	2			53
	3.0-3.49			57	51			108
	3.5-3.99				25	6		31
	4.0-4.49					2		2
	Σ		26	94	78	8		206
	Range TL		2.1-2.7	2.5-3.4	2.9-3.7	3.6-4.2		2.1-4.2
	Mean TL±SD		2.45±0.16	2.96±0.15	3.37±0.18	3.89±0.17		3.09 ±0.37
A. saldae	TL class (cm)		I	II	III	IV		**Total**
	2.5-2.99		3					3
	3.0-3.49		1	1				2
	3.5-3.99			99	3			102
	4.0-4.49			102	212	2		316
	4.5-4.99				91	4		95
	5.0-5.49					7		7
	Σ		4	202	306	13		525
	Range TL		2.80-3.30	3.20-4.20	3.80-4.90	4.30-5.20		2.80-5.20
	Mean TL±SD		2.95±0.24	3.92±0.13	4.34±0.21	4.89±0.26		4.18 ±0.31
A. sureyanus	TL class (cm)	0	I	II	III	IV		Total
	1.0-1.49	3						3
	1.5-1.99	19	24					43
	2.0-2.49		48	43				91
	2.5-2.99			113				113
	3.0-3.49			51	17			68
	3.5-3.99				21			21
	4.0-4.49				3	7		10
	4.5-4.99					1		1
	Σ	22	72	207	41	8		350
	Range TL	1.2-1.8	1.7-2.28	2.2-3.4	3.1-4.1	4.0-4.5		1.2-4.5
	Mean TL±SD	1.62±0.16	2.02±0.14	2.74±0.31	3.50±0.24	4.25±0.19		2.64 ±0.60
A. transgrediens	TL class (cm)		I	II	III	IV	V	Total
	2.0-2.49		33					33
	2.5-2.99		17	46				63
	3.0-3.49			44	8			52
	3.5-3.99			1	9			10
	4.0-4.49				1	2		3
	4.5-4.99					2		2
	5.0-5.49					1		1
	5.5-5.99							0
	6.0-6.49						1	1
	Σ		50	91	18	5	1	165
	Range TL		2.0-2.6	2.5-3.6	3.0-4.0	4.0-5.1	6.1-6.1	2.0-6.1
	Mean TL±SD		2.36±0.17	2.96±0.23	3.48±0.266	4.54±0.48	6.1±0	2.90 ±0.57

(especially in the species of bigger size) but they can also derive from wrong measurements during the sampling activity (Froese 2006). The sampling in the present study has been carried out seasonally for one year in order to cover the entire annual cycle and to provide accurate and comprehensive parameters of the length-weight equations for the four species. Overall, the age composition is characterised by a higher number of females in the older age classes for all the species. The same unequal sex ratio for mature fish has been observed by Leonardos & Sinis (1999) for *A. fasciatus* from the Mesolongi and Etolikon Lagoons (Greece) and the authors have speculated that this imbalance could reflect different survival rates for males and females. As possible reasons are identified the higher exposition of males to predation due to their courtship coloration (Leonardos & Sinis 1999) and the generally higher survival rate of females, especially in extreme environmental conditions (Leonardos 1996). Further detailed studies are encouraged in order to understand if the imbalanced sex ratio of mature fish observed for the four

Table 3. Estimated parameters of the total length (TL)-weight (W) regressions for the total sample (All) and separately for females (F) and males (M) for *Aphanius sureyanus*, *A. transgrediens*, *A. iconii* and *A. saldae* (N: number of individuals).

Species	Sex	N	TL range	W range	a	b	95% conf b	R^2
A. iconii	All	206	2.1-4.2	0.102-0.800	0.0152	2.7132	2.6993-2.7324	0.92
	F	89	2.2-4.2	0.110-0.800	0.0122	2.8836	2.8630-2.9100	0.93
	M	117	2.1-3.8	0.102-0.632	0.0162	2.6708	2.6494-2.6950	0.92
A. saldae	All	525	2.8-5.2	0.180-1.030	0.0133	2.5869	2.5791-2.5897	0.91
	F	80	3.7-5.2	0.410-1.030	0.0089	2.8512	2.8403-2.8694	0.91
	M	445	2.8-5.0	0.180-0.960	0.0143	2.5324	2.5274-2.5388	0.90
A. sureyanus	All	350	1.2-4.5	0.029-1.025	0.0077	3.2207	3.1870-2.3807	0.96
	F	247	1.2-4.5	0.032-1.025	0.0081	3.1834	3.1399-3.2623	0.97
	M	103	1.4-3.6	0.029-0.557	0.0062	3.4097	3.3724-3.4709	0.92
A. transgrediens	All	165	2.0-6.1	0.088-2.330	0.0118	3.0274	3.0060-3.0453	0.97
	F	107	2.0-6.1	0.088-2.330	0.0118	3.0236	3.0007-3.0492	0.97
	M	58	2.0-4.0	0.091-0.670	0.0116	3.0459	3.0103-3.0789	0.96

Table 4. Estimated parameters of total length-weight regressions for other Turkish populations of *Aphanius* spp. reported in other studies.

Species	Sex	N	TL range	a	b	R	Location	References
A. anatoliae	All	522	1.67-5.31	0.0136	3.19	0.82	Lake Eğirdir	**Güçlü (2012)**
A. saldae	M	43	4.02-5.47	0.0116	2.90	0.97	Lake Salda	**Yoğurtçuoğlu & Ekmekçi (2015)**
	F	33	3.87-6.01	0.0105	2.94	0.98		
	All	76	3.87-6.01	0.0121	2.86	0.97		
A. sureyanus	M	77		0.0078	3.49	0.96	Lake Burdur	**Güçlü et al. (2007)**
	F	119		0.0076	3.47	0.97		
A. sureyanus	M	30	2.37-4.49	0.0071	3.32	0.97	Lake Burdur	**Yoğurtçuoğlu & Ekmekçi (2015)**
	F	23	2.41-4.67	0.0096	3.02	0.98		
	All	53	2.37-4.67	0.0090	3.11	0.97		
A. transgrediens	M	50	2.16-4.44	0.0098	3.26	0.98	Lake Acıgöl	**Yoğurtçuoğlu & Ekmekçi (2015)**
	F	63	1.78-5.41	0.0098	3.29	0.99		
	All	113	1.78-5.41	0.0095	3.30	0.99		
A. transgrediens	M	16	2.3-3.4	0.0145	3.056		Lake Acıgöl	**Sarı et al. (2017)**
	F	144	2.0-4.7	0.0262	2.661			
	All	160	2.0-4.7	0.0237	2.732			

examined species of *Aphanius* could be related to the peculiar environmental conditions and high disturbances (such as water abstraction, presence of translocated or non-indigenous species) characterising the four Turkish lakes studied.

Currently, Lake Acıgöl is impacted by habitat loss and modification and by the introduction of non-indigenous species (i.e. *Gambusia holbrooki* and *Carassius gibelio*). Lake Eğirdir has been impacted by water abstraction for agricultural use, water pollution, especially due to domestic waste, overfishing and introduction of alien species (*Atherina boyeri, Knipowitschia caucasica*, *Carassius gibelio*, *Gambusia holbrooki* and *Sander lucioperca*). Lake Burdur and Lake Salda are currently impacted by spring water reduction for use in agriculture and drying-up.

The Anatolian populations of *Aphanius* are currently in decline due to degradation of habitats, pollution of inland and coastal waters and the presence of introduced exotic fish (Freyhof 2014 a,b, Freyhof et al. 2017). Often these factors work in synergy and this represents the real "threat'' brought about by human activities (McGregor Reid et al. 2013). For these reasons, further studies on life history traits of these endemic species are needed to increase the basic knowledge on their population biology and ecology, assess the population-level responses to ecosystem disturbance and monitor the population trend and the status of these endemic species.

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