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Growth and life history traits of Aegean chub, *Squalius fellowesii* (Günther, 1868) in streams in Muğla Province, Aegean coast, Turkey

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Summary

To aid in species' conservation, the aim of this study was to provide initial findings on age, growth and reproduction of an endemic species, Aegean chub *Squalius fellowesii* (Günther, 1868) populations from streams in the Aegean region of Muğla Province, Turkey. The species is relatively short-lived (maximum 6 years), attaining a size of about 200 mm total length with a rapid growth to first maturity (≈60 mm TL), and relatively little growth thereafter. The male:female ratio was 1.0 : 0.6, males significantly outnumbering females in the majority of the streams. General condition values of individual fish varied between 2.9 and 3.4. Sexual maturity was usually achieved later and at larger sizes in females than in males. Sexual maturation in most populations was at the age of 2 years in females and 1 year in males. The species spawns between early April and late May. Mean absolute and relative fecundity were about 4440 eggs and 57 eggs·g^{-1}, respectively. Mean egg diameter was 1.00 ± 0.03 mm, ranging from 0.70 to 1.20 mm. Suggestions for the conservation of Aegean chub are discussed.

Introduction

Within native fauna, endemic fish species are vulnerable (Crivelli, 1995). Although the biological diversity of freshwater fishes in Turkey is threatened mainly by the introduction of non-native organisms and overfishing (i.e. Tarkan et al., 2015), other causes such as habitat loss and degradation (pollution, eutrophication, channelization, damming, and other waterworks), drought, and excessive water extraction must also be considered (Fricke et al., 2007). These combined threats contribute as much as an estimated 32% to the endemic species under threat, of which at least 25% of the total freshwater fish fauna of Turkey are classified as Critically Endangered or Endangered (Hermoso and Clavero, 2011).

Aegean chub, *Squalius fellowesii* (Günther, 1868) is an endemic species in the Gediz, Bakır, Madra, Büyük Menderes, Eşen and Dalaman drainages in the Aegean region of Anatolia, Turkey (Özuluğ and Freyhof, 2011). Originally described as *Leuciscus fellowesii* from the nearby Eşen River, which is the westernmost Mediterranean drainage of Anatolia, the species was recently revised to be *Squalius fellowesii* by Özuluğ and Freyhof (2011). Based on molecular evidence, Durand et al. (2000) suggested that the group of 'short snouted chubs' from Western Anatolia, previously known as a subspecies of *Squalius cephalus,* should be considered as a separate species. Confirming this hypothesis, Özuluğ and Freyhof (2011) recently re-described six different valid species of the genus *Squalius* in Western and Central Anatolia, including *S. fellowesii.* Because these species are recent descriptions, very little information is available (e.g. Gianetto et al., 2012; Sülün et al., 2014); however, the IUCN Red List of Threatened Species (IUCN, 2013) has recently assessed *S. fellowesii* as being of 'Least Concern'.

Although one of the most common freshwater fish species in its area of distribution and consumed by the local people (Balık et al., 2004; Şaşı, 2004; Dirican and Barlas, 2007), only a few studies have been conducted in lakes and reservoirs regarding its basic biological features such as age, growth and sex ratio (Şaşı and Balık, 2003; Balık et al., 2004; Dirican and Barlas, 2007; Torcu-Koç et al., 2007; Gianetto et al., 2012). However, studies on the conservation of endemic species should require a detailed study of the biological and ecological characteristics. Information on growth and life history traits of an endemic species for management and conservation are important issues, but not readily available for Aegean chub in its native distribution range. In particular, except for a recent work on the distribution and ecology of freshwater fishes including the Aegean chub (Özdemir et al., 2015), virtually no information is available for Muğla Province, which falls inside the area of importance in the Mediterranean for fish biodiversity along with other Turkish regions bordering the Mediterranean and Aegean seas. To increase the knowledge on the Aegean chub as a means of aiding its conservation, the aim of the present study was to examine its environmental biology in various Muğla Province streams in Turkey. The specific goals were to evaluate the age-specific growth and body condition of Aegean chub and to assess the reproductive traits of the species (spawning duration, size at first maturity, fecundity and egg diameter).

Materials and methods

Muğla Province (36°17′–37°33′N; 27°13′–29°46′E) is located in the southwestern part of Anatolia, surrounded by the Mediterranean Sea in the south and the Aegean Sea in the west. The province has some large river catchments and

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numerous small temperate streams, with two large lakes and several reservoirs covering circa 13 328 km^2 of surface area. The region features a typical Mediterranean climate with hot, dry summers and mild, wet winters. In Muğla Province there are several freshwater fish species endemic to the Aegean region: *S. fellowesii*, *Barbus pergamonensis*, *Capoeta bergamae*, *Ladigesocypris irideus*, *Petroleuciscus smyrnaeus*, and *Luciobarbus kottelati*, most of which are classified as threatened (IUCN, 2013). Additionally, non-native fish species (*Carassius gibelio*, *C. auratus*, *Lepomis gibbosus*, *Pseudorasbora parva*, *Gambusia holbrooki*, and *Coptodon zillii*) as part of the biotic degradation have been recently recorded (Önsoy et al., 2011; Tarkan et al., 2012; Karakuş et al., 2013; Özdemir et al., 2015).

Aegean chub specimens were collected monthly between March 2010 and July 2011 by electrofishing in 12 Mediterranean-type streams with very similar environmental and biological structures (Özdemir et al., 2015) (Table 1). After collection, fish were killed with an overdose of 2-phenoxyethanol, immersed in an ice water slurry and chilled. After transport to the laboratory, they were either examined fresh, or frozen and defrosted for later examination. The fish were measured individually for total length (TL) to the nearest 1 mm and for wet body weight (W_T) to the nearest 0.1 g. Gonads were examined for sex determination by visual examination for larger fish or with the aid of a lens (16x) for smaller fish, then weighed (W_G) to the nearest 0.01 g.

Age was determined from scales removed from the area between the dorsal fin and the left lateral line. Five or six scales per individual were placed on a 0.9 mm thick polycarbon plastic plate and flattened with a roller press. Prepared plates bearing the scale prints were read by a micro-projector (magnification: 48 × and 24 ×). Age determinations were cross-checked using independent readings from a second reader. The total scale radius (R) and radius of the annual rings were measured as the smallest distance between the scale center and distal edge. In order to determine which equations described the relationship between body length and scale radius, linear and non-linear models were fitted (Bagenal and Tesch, 1978). The body–scale relationships were best described by a linear equation, thus the TL at previous ages was back-calculated according to the Fraser-Lee equation (Francis, 1990): $L_t = c + (TL_c - c)(S_t/R)$, where L_t is TL when growth mark t was formed, TL_c is TL at the time of capture, S_t is the distance from scale center to the growth mark t, R is the scale radius, and c is the intercept on the length axis from linear regression between the total length and scale radius.

Growth trajectories were compared after Hickley and Dexter (1979) and calculated as: TL at mean ages (n) plotted against TL at age (n + 1) to obtain a straight line for the Walford (1946) method; TLs at age were then obtained from the formula $ln = L_\infty\ (1-k^n)$ where $L_\infty = l_t/(1-k)$; l_t = interception on the y axis; ln = TL at age n; k = slope of the Walford plot (Hickley and Dexter, 1979). The mean TLs at age for each year class were then expressed as a proportion (%) of the TLs from the Walford method. The proportions are then summed, and the resulting mean (i.e. growth index, GI) was used to determine relative growth for each population. The length-weight relationship is described by the equation: $W_T = a\mathrm{TL}^b$, where a and b are constants (Le Cren, 1951) and b from this equation is used as the estimator of 'generalized' (GCb) condition (*sensu* Pitcher and Hart, 1982).

The gonado-somatic index (GSI) was calculated from female fish just prior to spawning and for each population as: GSI = $(W_G/W_T) \times 100$ (Wootton, 1990). Female specimens with ovaries containing non-yolked or indistinguishable eggs were classified as immature; those with ovaries containing yolked eggs (even if spent) were classified as mature. Mean age at first maturity (AaM) was calculated from females collected just before reproduction using the formula from DeMaster (1978) as adapted by Fox (1994):

$$\alpha = \sum_{x=0}^{w}(x)[f(x) - f(x-1)]$$

where α is the mean age of first maturity, x is the age in years, f(x) is the proportion of fish mature at age x, and w is the maximum age in the sample. A modified version of this formula (10 mm TL intervals in place of age-classes) was used to calculate mean TL at first maturity (LaM) as per Fox and Crivelli (2001).

Table 1
Number, latitude, longitude, altitude, temperature (T), dissolved oxygen (DO) and pH of study sites, Muğla Province (Aegean Region, Turkey)

No	Name	Latitude	Longitude	Altitude	T	DO	pH
1	Balıklı Stream	37°00′26″N	28°32′55″E	83	16.8 (±1.2)	5.6 (±2.1)	7.9 (±0.7)
2	Dalaman Stream	36°50′24″N	28°47′45″E	18	15.6 (±4.2)	8.4 (±0.3)	7.9 (±1.5)
3	Yuvarlakçay Stream	36°54′24″N	28°44′32″E	12	15.0 (±1.2)	8.1 (±1.7)	8.1 (±0.6)
4	Kargı Stream	36°42′24″N	29°02′52″E	19	12.2 (±0.1)	11.2 (±0.7)	9.0 (±0.3)
5	Tahliye Stream	37°00′48″N	28°30′34″E	115	16.9 (±0.1)	6.7 (±0.3)	8.3 (±0.1)
6	Gelibolu Stream	36°56′59″N	28°17′ 25″E	25	17.0 (±2.9)	8.4 (±1.8)	8.0 (±1.2)
7	Namnam Stream	37°00′41″N	28°31′02″E	117	18.4 (±0.8)	4.6 (±1.4)	8.2 (±1.3)
8	Sarıöz Stream	37°00′27″N	28°30′43″E	119	15.1 (±1.7)	6.4 (±1.6)	7.9 (±0.2)
9	Tersakan Stream	36°47′30″N	28°49′45″E	16	17.7 (±4.3)	7.5 (±2.6)	8.5 (±0.2)
10	Kınık Stream	36°49′36″N	28°19′28″E	20	20.2 (±6.5)	7.1 (±0.1)	7.6 (±1.3)
11	Eşen Stream	37°44′52″N	29°23′16″E	45	15.2 (±2.3)	7.1 (±1.2)	8.3 (±0.1)
12	Sarıçay Stream	37°20′38″N	27°43′45″E	20	20.6 (±4.6)	8.4 (±1.9)	7.6 (±0.7)

For fecundity analyses, ovarian samples were taken from anterior, middle, and posterior pieces to test whether the ovaries were homogenous with respect to follicle diameter and density (number of follicles per gram of ovary). The fecundity of females was estimated gravimetrically (Bagenal, 1978): F = GW × D where:

F is the number of mature oocytes spawned by a female in a single spawning, GW is the weight of the ovary and D is the density of mature oocytes (number of oocytes per g of ovarian tissue). Relative fecundity (RF) was calculated as $RF = AF/W_T$ (Bagenal, 1978). The diameters of circa 50 randomly sampled oocytes from each female were measured under a binocular microscope and the pooled data used to determine the size frequency for consecutive months over the year.

Fork length (FL) values derived from the literature were converted to TL (mm) for proper comparisons using the formula for Aegean chub given in Gianetto et al. (2012). A chi-square (χ^2) test was used to examine the male-to-female ratio. Comparisons of growth index, absolute and relative fecundity, egg diameter and size at first maturity among populations were undertaken with one-way analysis of variance (ANOVA) using population means for each variable. When significant differences among populations were detected (a P value <0.05 was accepted as the level of significance), the Tukey HSD test was used to determine which populations were different. Analysis of covariance (ANCOVA) was used to determine the significant differences between generalized conditions among the populations. Data were checked for normality and homogeneity of variances. If neither criterion was met, then the variables were log-transformed prior to analysis. To test the significant deviation of the slope (*b*) value of length-weight relationships from 3, the Students' *t*-test was employed (Zar, 1999).

Results

A total of 1217 individuals was collected. The male:female ratio was 1.0 : 0.6, which deviated statistically from unity ($\chi^2 = 60.42$, $P < 0.001$); in most cases males significantly outnumbered females. Only in the Tersakan Stream ($\chi^2 = 0.07$, $P > 0.05$) and Kargı Stream ($\chi^2 = 0.40$, $P > 0.05$) the sex ratio did not deviate from unity, with the Aegean chub females being dominant in the latter (Table 2).

In all populations the TL ranged from 11 mm to 237 mm (Table 2). As ANOVA did not show any significant differences in TL between sexes (P > 0.05), data on female and male TLs were pooled. The Kargı Stream had by far the highest growth index value (Tukey HDS, P < 0.01). No significant differences were observed in the growth index in other examined Aegean chub populations (ANOVA, P > 0.05) (Table 2). The minimum growth indexes were 116 and 117 in the Tahliye and Namnam streams, respectively (Table 2). Maximum age across study sites was 6 years in the Tersakan and Balıklı streams, whereas most other populations had a maximum 4-year lifespan (Table 2). Growth increments of individuals in all Aegean chub populations were similar, with progressively declining growth increments with increase in age (Table 2). Generalized condition values of individual fish varied

Table 2
Site number (N°; see Table 1), number (n) of Aegean chub sampled, minimum (Min) and maximum (Max) total length (TL, in mm) of fish captured, mean back-calculated TLs at ages (in mm), growth index (GI), generalized condition (GC*b*), sex ratio (M:F), mean age at first maturity (AaM, in years), mean TL at first maturity (TLaM, in mm), mean absolute (AF) and relative fecundity (RF) and mean egg diameter (ED, in mm) with standard deviations (±) from various water bodies, Muğla Province, Aegean Region, Turkey

	Fish at capture		Back-calculated TL at age										Males		Females				
N°	n	Min–Max	A1	A2	A3	A4	A5	A6	A7	GI	GC*b*	M:F	AaM	TLaM	AaM	TLaM	AF	RF	ED
1	89	53–198	66	89	107	142	157	177		126	3.1	1 : 0.5	1.0	60	1.8	80	4514 (±1502)	54 (±12)	0.87 (±0.6)
2	72	53–154	57	83	106	154				125	3.3	1 : 0.4	1.1	58	1.2	60	4679 (±1667)	65 (±14)	0.90 (±0.5)
3	70	53–179	55	83	110	155				124	3.1	1 : 0.3	1.1	56	1.7	80	4770 (±1234)	67 (±9)	1.11 (±0.7)
4	171	55–210	89	134	137					190	3.1	1 : 1.1	1.2	93	1.6	121	2334 (±567)	42 (±8)	1.01 (±0.6)
5	38	33–144	49	79	115	132				116	3.0	1 : 0.2	1.0	50	1.7	75	5643 (±1345)	55 (±10)	0.95 (±0.4)
6	76	54–163	54	85	115	140				123	3.4	1 : 0.5	1.4	67	2.2	90	2081 (±635)	41 (±11	1.20 (±1.1)
7	12	76–127	50	80	100					117	2.9	1 : 0.7	2.0*	86*	2.0*	81*	1391 (±499)	58 (±13)	1.02 (±0.9)
8	78	43–175	56	87	115					130	3.3	1 : 0.6	0.8	48	2.5	72	6012 (±2045)	73 (±15	1.08 (±1.0)
9	90	11–237	60	92	122	162	185	224		138	3.1	1 : 0.6	1.2	65	2.1	96	9676 (±1986)	71 (±14)	1.10 (±1.2)
10	29	72–191	68	97	149	171				151	3.1	1 : 0.3	2.0*	98*	2.0*	102*	4958 (±1267)	50 (±12)	0.70 (±0.8)
11	101	59–223	62	99	134	167				136	3.2	1 : 0.3	1.3	70	2.1	106	4344 (±1178)	45 (±8)	1.08 (±1.0)
12	391	51–205	67	88	117	141				145	2.9	1 : 0.9	0.6	46	0.7	49	2902 (±889)	59 (±12)	0.92 (±0.9)
13	528	140–244	157	180	197	214	228				3.0	1 : 0.7							
14	414	122–241	135	152	163	182	216	254			2.9	1 : 1.4							
15	332	147–261	110	156	175	208	239	258	276			1 : 2.7	2.0	154	2.0	153	9142		1.30

*No immature fish below the indicated length or age were captured, thus this is assumed to indicate their length and age at first maturity values. 13: Lake Işıklı (Balık et al., 2004), 14: İkizcetepeler (Torcu-Koç et al., 2007); 15: Topçam Reservoir (Şaşı, 2004).

between 2.9 and 3.4, with Aegean chub in Gelibolu and Sarıöz streams being significantly larger than those in other water bodies (ANCOVA, $P < 0.001$) and showing positive allometric growth (*t*-test, $P < 0.05$), while other populations showed isometric growth (*t*-test, $P > 0.05$).

Assessment of the main spawning period of Aegean chub for all studied sites was based on the GSI and direct observation of the gonads. In all populations examined, the highest GSI value occurred in April and May, with a decrease in late May and June when spawning took place. After spawning, there was a 6-month quiescent period. From January to March there was a rapid growth of the gonads. Spawning is in early April to late May for Aegean chub in the water bodies of Muğla Province, a time period that did not largely change among the populations (Fig. 1).

Sexual maturity was usually achieved later and at larger sizes in females than in males (Table 2). In most populations sexual maturity was achieved in 2-year-old females and 1-year-old males. The oldest sexual maturity was for males and females from Gelibolu, at the age of 1.4 and 2.2, respectively (Table 2). The youngest size at maturity was observed in Sarıçay Stream, at the age of 0.6 in males and 0.7 in females (ANOVA, $P < 0.05$) (Table 2). Length and age at first maturity values were similar for the remaining studied populations, although no immature fish below some certain sizes were captured in Namnam and Kınık streams, thus the minimum lengths and ages that could be obtained were assumed to be their length and age at first maturity (Table 2).

Mean fecundity in mature female Aegean chub was 4442 eggs (S.E. = 627), ranging from 1391 (an age-2 female from Namnam Stream) to 9676 eggs (an age-6 female from Tersakan Stream), and tended to increase with age classes in all stream populations (ANOVA, $P < 0.001$). Mean relative fecundity was 57 eggs$\cdot$g^{-1} (S.E. = 3.03), and varied from 41 (Gelibolu Stream) to 73 (Sarıöz Stream), while mean egg diameter was 1.00 ± 0.03 mm (ranging from 0.70 to 1.20), with no differences in mean egg diameter observed between age classes (ANOVA, $P > 0.05$). The egg diameter was not significantly correlated (Spearman's Rank correlation test, $P > 0.05$) to TL or W for any Aegean chub populations, whereas the fecundity and body size (both length and weight) correlated significantly in all Aegean chub populations ($P < 0.01$).

Discussion

Despite their high presence in Aegean region water bodies (Şaşı and Balık, 2003; Balık et al., 2004; Şaşı, 2004; Dirican and Barlas, 2007; Torcu-Koç et al., 2007; Gianetto et al., 2012; Özdemir et al., 2015), there is paucity of regional information on the environmental biology of the Aegean chub (Table 2). Previous studies on the biology and ecology of the species *Leuciscus cephalus* were conducted only in lakes and reservoirs within different basins of the Aegean region rather than in Muğla Province (Table 2).

Among populations, variations in growth and reproduction are common in fishes (e.g. Mann, 1991), which is apparent in the available data. Growth and reproduction characteristics are widely variable within our stream populations as well as in other types of environments in the Aegean region (Table 2). This can be seen not only in the geographically nearby streams of Muğla Province, but also in the habitat-dependent variations in age and growth features of Aegean chub also recognizable through literature comparisons and suggesting that growth is better for the lentic than the lotic populations (Table 2). This is also the case for other *Squalius* species distributed in various regions of Anatolia (Türkmen et al., 1999; Kalkan et al., 2005). These observed variations may simply reflect environmental differences such as temperature, competition, food availability or quality in the absence of exploitation, as is the case in the present study. Influence from these factors was noted for Aegean chub in areas of its distribution, where growth patterns have been shown to vary in relation to these abiotic and biotic

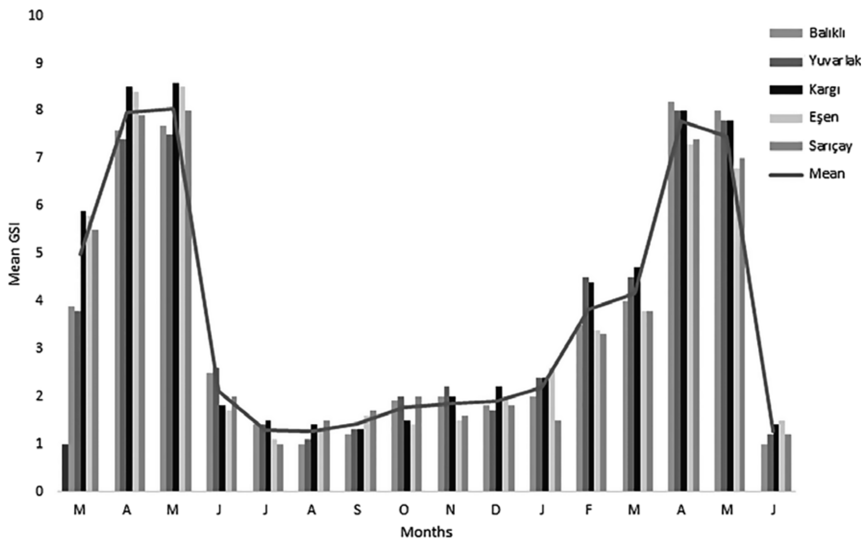

Fig. 1. Mean female gonadosomatic index (GSI) variations in several Aegean chub *Squalius fellowesii* populations from Muğla Province, Aegean Region, Turkey, March 2010–June 2011

factors (e.g. Balık et al., 2004; Torcu-Koç et al., 2007). However, the most influential factor seems to be habitat differentiation, since lake and reservoir populations had remarkably higher growth rates and total lengths compared to stream populations examined in the present study (Table 2). Even the fastest growing population in our sampling had dramatically shorter length-age data and length increments than lentic populations. This might suggest that stagnant water conditions provide more food availability and/or less inter- and intra-specific interactions, and consequently would appear to be more favorable environments for Aegean chub. Indeed, all of these lentic systems were reported as highly productive and which are eutrophic in character (Şaşı and Balık, 2003; Balık et al., 2004; Torcu-Koç et al., 2007). Another reason for the observed growth differences could be that the examined streams are unpredictable environments, partially drying up in the summer months. Hence, fish species are forced to migrate upstream and compete with other fishes for the limited space and food. Water temperature variations between lakes and streams would also be high enough to produce remarkable growth differences. Within stream populations, the Aegean chub usually demonstrated similar growth patterns, except for the Kargı, Kınık and Sarıçay streams, probably due to the small size range studied in the former and nutrient rich waters in the latter. A rapid growth pattern was noted in the pumpkinseed fish species, *Lepomis gibbosus,* due to high nutrient levels as a result of several fishbait plants on the Sarıçay Stream (Top, 2012).

The sex ratio was male-biased in the Aegean chub stream populations in Muğla Province, and also showed considerable differences in other Aegean chub populations; Balık et al. (2004) reported a similar pattern. However, Şaşı and Balık (2003) recorded a female-biased sex ratio, and Torcu-Koç et al. (2007) found a balanced sex ratio. Given that a sex ratio is environmentally linked and that water bodies have different environmental characters, such variations are likely. Sex ratios can also be influenced by many other factors such as selective sampling, behavior, migration, season and different growth rates (Paxton et al., 1999), all not considered in the present study.

Information on the reproduction of Aegean chub is crucial for any species management and the protection thereof is restricted to the lone work of Şaşı (2004). Our analyses on stream populations of Aegean chub revealed a spawning period between early April to late May, which is not consistent with Şaşı (2004), as between March and April for Topcam Reservoir. This can be explained simply by the well-known effect of water temperature at the onset of spawning (e.g. Lappalainen and Tarkan, 2007), as the necessary water temperatures for Aegean chub spawning in Topçam Reservoir (around 13°C) were reached at an earlier time (Şaşı, 2004) than for the streams in Muğla Province (Özdemir et al., 2015).

Sexual maturity in stream populations of Aegean chub suggest that males attained maturity within 1 year and females within 2 years, with the exception of some populations where growth is relatively faster. This is in contrast to the finding of Şaşı (2004) from Topcam Reservoir, suggesting that males and females reach sexual maturity in their second year of life. This difference is more prominent when comparing length at first maturity values between stream and reservoir populations, since the reservoir population matured at around 150 mm TL whereas this was around 50 mm TL (in males) and 80 mm TL (in females) for the stream populations (Table 2). Life-history theory (Atkinson, 1994) predicts that ectotherm organisms will experience faster juvenile growth and precocious maturity in response to elevated water temperatures. Therefore, the slow-growing stream populations in Muğla Province should demonstrate delayed maturity, yet the contrary was observed although true when compared with only the stream populations (Table 2). This can be attributed to the adaptive response to variable and unpredictable environmental factors confronting Aegean chub stream populations.

As observed in growth patterns, fecundity, as one of the most essential life history traits of a fish species, is much higher in a reservoir population than in stream populations, with the exception of Tersakan Stream (Table 2). Whilst variations in absolute fecundity among stream populations are obvious, comparisons with reservoir populations may result in biased outcomes, as this is largely size-dependent (i.e. larger females tend to produce more eggs).

In conclusion, endemic Aegean chub populations in Muğla Province demonstrated slow growth, early maturity, low fecundity, and a delayed spawning period. The species is widely distributed in Muğla Province, where the most abundant fish species are usually found (Özdemir et al., 2015). However, comparisons with other Aegean chub populations and its congeners suggest that stream populations are more vulnerable, with reduced growth and reproduction features. In predicted future climate scenarios elevated temperatures and habitat degradation may constrain habitat availability for the species and escalate a potential density-dependent impact on other fishes associated with non-native fish introductions that are being increasingly reported in Muğla Province (e.g. Top, 2012; Tarkan et al., 2012; Karakuş et al., 2013). Hence, conservation effort should focus on the streams where growth rate and offspring potential are low and where these vulnerable habitats should be strictly protected.

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