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Growth and reproduction of a marine fish, *Atherina boyeri* Risso 1810, in a freshwater ecosystem

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Abstract: *Atherina boyeri* (Risso, 1810) has established abundant and successful populations in inland waters in Anatolia, although it is known as a marine species. In this study, the growth and reproduction properties of *A. boyeri* in the Hirfanlı Reservoir were studied. Age determinations based on scale readings showed that the population had a 4-year life cycle. Sampled individuals ranged between 5.76 and 115.65 mm in total length and 0.01 and 10.48 g in total weight. The von Bertalanffy growth parameters for females were $L_\infty = 156.78$, $k = 0.197$, and $t_0 = -0.197$; for males, $L_\infty = 151.02$, $k = 0.148$, and $t_0 = -0.148$. Mean condition factor values, estimated from eviscerated weight, varied between 0.49 and 0.60. The length–weight relationship of *A. boyeri* indicated allometric growth, whereas the *b* value for females, males, and juveniles was 3.29, 3.23, and 3.50, respectively. The sex ratio was 1:1.14 in favor of females. The reproductive season, evaluated by the gonadosomatic index, extended from May to July. Mature egg diameter ranged from 0.64 to 1.73 mm. The mean total fecundity increased with age. In the Hirfanlı Reservoir, certain life-history features of *A. boyeri*, such as short life cycle and increasing fecundity, indicated that this species has the potential to become dominant in freshwater systems.

Key words: Translocated fish, Central Anatolia, life-span, fecundity, length–weight relationship, Hirfanlı Reservoir

1. Introduction

The sand smelt, *Atherina boyeri*, is an atherinid fish that principally inhabits coastal and estuarine waters, including coastal lagoons, over a wide range of salinities from freshwater to hypersaline conditions (Henderson and Bamber, 1987). This species inhabits coasts of the northern Atlantic Ocean and basins of the Mediterranean Sea, Black Sea, and Caspian Sea (Kottelat and Freyhof, 2007). In Turkey, *A. boyeri* is naturally distributed along all the surrounding coasts of Anatolia, lagoons, and sea-connected lakes (Altun, 1991; Balık et al., 2005; Küçük et al., 2006). However, in recent studies it has been reported that *A. boyeri* has extended its distribution to inland waters, including natural lakes (Küçük et al., 2006) and some reservoirs that are isolated from the sea (Özuluğ et al., 2005; Ekmekçi et al., 2006; Kırankaya and Ekmekçi, 2006; Küçük et al., 2006; Becer Özvarol et al., 2009).

In Turkey, the distribution of this species in inland waters has been expanding for the last decade due to accidental or intentional introductions, and the species has become more popular among fishermen due to increasing demand in the international market. Although sand smelt is known as a marine species, in 2002 a large amount of *A. boyeri* was caught from fresh waters (6677 t), exceeding that caught in marine environments (993 t). In the year 2002, the amount of *A. boyeri* caught only in fresh waters was 1733 t, whereas in 2005 it increased dramatically to 6677 t. Almost half of the *A. boyeri* species harvested in Turkey came from Hirfanlı, Kapulukaya, and Gelingüllü, Reservoirs, located in the Kızılırmak basin (TurkStat, 2006). The abundance of the newly introduced *A. boyeri* into inland waters in Turkey suggests a potential threat of becoming an invader, and hence it is necessary to study its biological characteristics in a Central Anatolian reservoir. Primarily, the life-history characteristics of *A. boyeri*, established in huge populations in freshwater habitats, should be determined in order to assess the invasive potential of this species in inland water systems. The aim of this study is to describe the growth and reproduction properties of *A. boyeri* in the Hirfanlı Reservoir, a freshwater environment.

1.1. Study area

The study was carried out in the Hirfanlı Reservoir, established in 1959 on the Kızılırmak River (39°16′22.2″N, 33°31′07.16″E; Figure 1) with an average surface area of 218.81 km^2 and an average depth of 20.15 m. The reservoir is surrounded by agricultural land, principally for cereal production (DSI, 2005).

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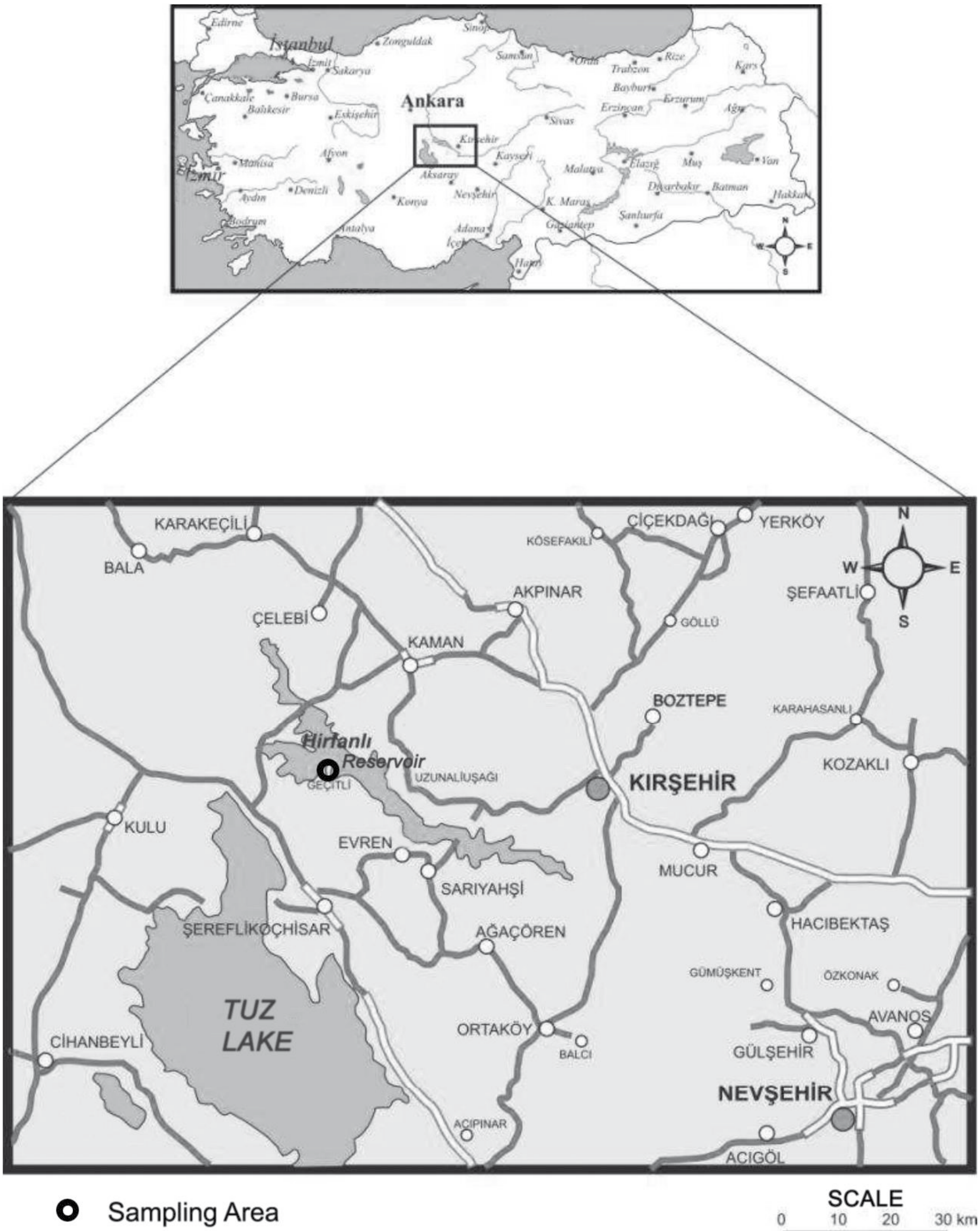

Figure 1. Hirfanlı Reservoir (modified from Ekmekçi et al., 2010).

The fish fauna of the Hirfanlı Reservoir has changed significantly over the years due to anthropogenic effects. By the end of the 1960s the native fish fauna of the reservoir consisted of *Cyprinus carpio*, *Squalius sp.*, *Capoeta capoeta*, *Silurus glanis*, *Barbus tauricus*, *Alburnus escherichii*, and *Chondrostoma nasus*. Within the scope of the stocking program of the State Hydraulic Works (Turkish abbreviation: DSI) for fishery management, carp (*C. carpio*), sander (*Sander lucioperca*), tench (*Tinca tinca*), and crayfish (*Astacus leptodactylus*) were introduced into the reservoir after the 1970s (DSI, 2005). Even though the origin of the introduction is not known, *A. boyeri* appeared to be the dominant species (97.74%) in the reservoir according to the 2005 DSI stock report. Several of the native species, such as *C. carpio* and *S. glanis*, are still present in the reservoir after the establishment of the dam; however, several are absent or scarce (DSI, 2005). Additionally, the nonnative fish species topmouth gudgeon (*Pseudorasbora parva*) and native killifish (*Aphanius danfordii*) are also abundant in the reservoir and share the same habitat as *A. boyeri* (Ekmekçi et al., 2010). Recently, the genus *Aphanius* has been revised and *Aphanius* in the Kızılırmak was named as *A. marassentensis* by Pfleiderer et al. (2014). In previous studies from the Hirfanlı Reservoir, *A. marassentensis* was not recorded, as it could not be caught due to gear selectivity. However, the nets used for *A. boyeri* have the ability to catch this species (Yoğurtçuoğlu and Ekmekçi, 2013).

2. Materials and methods

Between April 2008 and April 2009, 674 sand smelt specimens were collected from the Geçitli village shore (39°09′32.4″N, 33°36′42.5″E; Figure 1) on a monthly basis, using a trawl net with a mesh size of 5 mm. *A. boyeri* specimens were preserved in 4% formaldehyde solution. Water temperature (°C), dissolved oxygen (mg/L), conductivity (µS/cm), salinity, and pH measurements were taken monthly in the field from the surface (Gençoğlu, 2010; Ekmekçi et al., 2010).

In the laboratory, total length (TL) was measured in mm. Total body weight (W) and gonad weight (GW) were recorded to the nearest 0.01 g. In the majority of fish, age is determined from the scales. Scales are simply collected and enable the age to be determined fairly certainly (Nikolsky, 1978). Thus, age determination was performed using scales (Lagler, 1966; Bagliniere and Le Louarn, 1987; Steinmetz and Müller, 1991) and validated using the Bhattacharya method, available in FISAT software (Gayanilo et al., 2005). For age determination, the scales were removed, placed on two slides after cleansing with water, and read twice by independent researchers under a binocular microscope (Lagler, 1966). The relationship between TL and W was determined by the equation $W = aL^b$, where W is the weight in g, L is the total length in mm, and *a* and *b* are the parameters to be estimated from the regression graphic. In order to test the difference of the length–weight relationship between the sexes, analysis of variance (ANOVA) was used. Length-at-age was modeled using three key parameters of the von Bertalanffy growth model described as $L_t = L_\infty [1 - e^{-k(t - t_0)}]$, where L_t is length at age t, L_∞ is the maximum theoretical length, *k* is the body growth coefficient determining the rate at which L_∞ is attained, and t_0 is the age of zero-length fish (Ricker, 1980). Condition factor (K) was estimated by $K = (W / TL^3) \times 10^5$, where W is the eviscerated weight. ANOVA was carried out using Microsoft Excel (v. 2007) to determine significance in length, weight, and condition factor values between sexes, whereas Student's t-test was used for condition factor values between sexes in age groups (Zar, 1996).

Sex was determined by examining gonads, either visually in the larger specimens or by using a binocular stereomicroscope in specimens smaller than 20 mm after dissection. The significance of difference among the sex ratios was assessed using the chi-square test (Zar, 1996). Monthly gonadosomatic index (GSI) was calculated with the equation $GSI = (GW / W) \times 100$. Eggs were examined under a stereomicroscope and PC-based image analysis with commercially available software BAB-Bs200Pro was used for the measurement of egg diameter. Total fecundity was determined following the gravimetrical method given by Ricker (1980).

3. Results

During this study, temperature ranged from 5.82 to 23.09 °C, dissolved oxygen from 3.65 to 12.98 mg/L, conductivity from 1688 to 1832 µS/cm, salinity from 0.85‰ to 0.92‰, and pH from 6.62 to 8.18 (Table 1).

Five age groups (0–4) were identified in both sexes. Females were predominant in the 2 and older age groups, whereas males were abundant in the 0 (7.14%) and 1 (17.86%) age groups (Figure 2). Juveniles were merged into the group including all the specimens, which was named as 'total'.

The TL of *A. boyeri*, including juveniles, ranged from 5.76 to 115.65 mm, and W ranged from 0.01 to 10.48 g. For females, TL and W varied between 34.29 and 115.65 mm and 0.23 to 10.48 g, respectively, whereas for males they ranged from 29.20 to 88.92 mm and 0.12 to 5.42 g, respectively. Females were significantly longer (ANOVA, $F = 3.86$, df = 1, $P < 0.001$) and heavier (ANOVA, $F = 3.86$, df = 1, $P < 0.001$) than males. The TL and W of the juvenile specimens varied between 5.76 and 35.53 mm and 0.01 and 0.42 g, respectively. Females were dominant in length groups of 70 mm and longer (Figure 3).

The von Bertalanffy equations for females and males were $L_t = 156.78 [1 - e^{-0.1965(t + 1.0976)}]$ and $L_t = 151.02 [1 - e^{-0.1478(t + 1.8004)}]$, respectively.

The length–weight relationship was calculated separately for juveniles, males, and females. The *b* values of the relationships imply that the body shape of both sexes and juveniles displayed a positive allometric form (Student's t-test, t_{female}: 11.6, t_{male}: 176.92, $t_{juvenile}$: 793.7) (Table 2). There were significant differences in coefficient *b* between mature females and males (ANOVA; $F(1, 548) = 177.49$, $P < 0.01$).

Mean condition factor values for both females and males ranged between 0.49 (Age 1) and 0.60 (Age 4), with a tendency to increase with age. This difference in condition factors was not observed in the same age group between sexes (Student's t-test, $t_0 = 0.053$, $t_1 = 0.234$, $t_2 = 0.083$, $t_3 = 0.111$, $t_4 = 0.908$, $P > 0.05$). Mean monthly condition factor values, which started to increase in spring and decreased

Table 1. Variations of some physicochemical parameters of the Hirfanlı Reservoir during the sampling period (summarized from Ekmekçi et al., 2010; Gençoğlu, 2010).

	T (°C)	EC (25 °C, µS/cm)	DO (mg/L)	pH
April 2008	6.99	1803	12.98	7.51
May	12	1802	8.49	7.6
June	20.01	1832	5.22	8.18
July	23.09	1688	8.61	8.01
August	23.09	1688	8.61	8.01
September	21.62	1749	3.65	8.07
October	17.72	1740	3.81	7.98
November	15.54	1722	3.55	7.85
December	11.25	1716	4.88	7.28
January	5.82	1700	12.24	6.62
February	6.01	1698	11.88	7.1
March	6.68	1697	12.64	7.34
April 2009	10.36	1686	11.54	7.44

by the end of the summer, were estimated using eviscerated weight. It was noted that these values fluctuated in a narrow range. Mean condition factor ranged from 0.46 (in March) to 0.62 (in May) in females and from 0.43 (in January) to 0.61 (in April) in males. Throughout the year, females had higher mean condition values than males (ANOVA, F = 3.86, df = 1, P < 0.001).

The overall sex ratio was 1:1.14 (M:F; 322 females, 282 males). The sex ratio of the Hirfanlı population was not significantly different from the ideal Mendel ratio [χ^2 (1, N = 491) = 7.95, P > 0.05], yet sex ratio changes were found to be significant within age classes. Males were significantly dominant in the 0 and 1 age groups, whereas females were significantly dominant in 2 and older age groups [χ^2_{age0} (1, N = 76) = 5.26, P > 0.05; χ^2_{age1} (1, N = 200) = 8.00, P > 0.05; χ^2_{age2} (1, N = 181) = 4.64, P > 0.05; χ^2_{age3} (1, N = 97) = 12.62, P > 0.05; χ^2_{age4} (1, N = 48) = 27.00, P > 0.05]. All the specimens were found to be sexually mature in the first spring after hatching.

Mean GSI ranged from 0.02 to 12.07 in females and 0.20 to 9.99 in males (Figure 4). According to these values, March and April were the ripening phase of gonads, and spawning occurred between the beginning of May and the end of July, when the mean GSI of females was 8.08, 12.07, and 7.19, respectively. The period between August and February was accepted as the quiescence period.

During the spawning period, immature, maturing, and mature eggs were present all together in the ovaries. However, mature eggs could only be observed in May, June, and July; other than in those months, mature eggs were not found in the ovaries. Mean mature egg diameter increased during the spawning period, although it did not

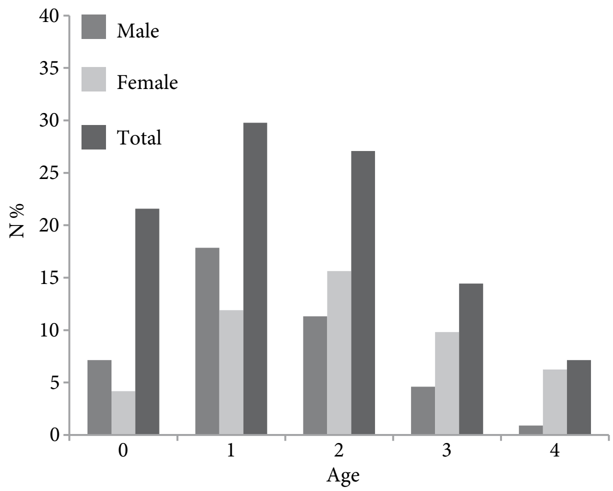

Figure 2. Age frequency of *Atherina boyeri* in the Hirfanlı Reservoir.

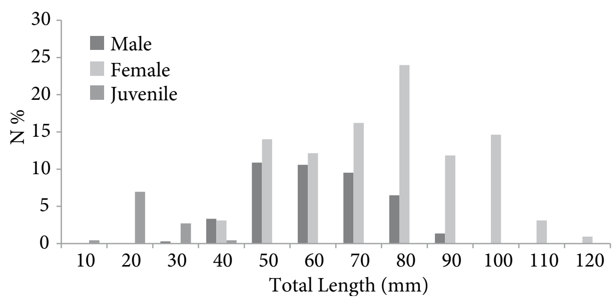

Figure 3. Length frequency of *Atherina boyeri* in the Hirfanlı Reservoir.

Table 2. Regression of body weight on total length for *Atherina boyeri* from the Hirfanlı Reservoir.

	a	SE	b	SE	r^2	N	P
Females	2×10^{-6}	0.184	3.2929	0.025	0.9763	288	<0.001
Males	2×10^{-6}	0.072	3.2313	0.013	0.9783	264	<0.001
Juveniles	2×10^{-6}	0.012	3.5001	<0.001	0.9726	69	<0.001

differ significantly among months (ANOVA, $F = 67.59$, df = 2, P < 0.001).

The mean total fecundity of mature females during the spawning season increased with age; however, the mean relative fecundity decreased. While the mean total fecundity was 549 ± 352.94 in females at age 1, it reached 1168 ± 517.70 at age 4. The mean relative fecundity was 343.85 ± 212.37 in females at age 1 and it decreased to 148.71 ± 49.21 at age 4. The relationship of fecundity with TL and W of the *A. boyeri* population in the Hirfanlı Reservoir was evaluated and no significant relationship was found.

4. Discussion

In the Hirfanlı population, the life-span of *A. boyeri* was found to be between 2 and 4 years, which indicates a short life-span. Similar findings have been reported in other studies, given in Table 3. In the Hirfanlı population, females have a longer life-span than males, which is in accordance with the findings of Leonardos and Sinis (2000), Andreu-Soler et al. (2003), and Koutrakis et al. (2004) in other regions of the Mediterranean. The rapid early growth rate was typical of most atherinids (Henderson and Bamber, 1987; Fernández-Delgado et al., 1988; Creech, 1992). In our study, the growth rate indicated by the von Bertalanffy growth parameters ($k_{female} = 0.1965$, $k_{male} = 0.1478$) in the Hirfanlı Reservoir was lower and L_∞ of both sexes was higher than that reported for other populations in the studies given in Table 3.

The growth pattern of a fish species is measured using the length–weight equation. If the value of *b* is significantly higher than 3 for individuals, this indicates an allometric increase in growth (Ricker, 1980). Even though the *b* values differed among studies, the growth was reported to be allometric in all the previous records, as well as in our study (Table 3).

The mean monthly condition factor values, calculated from the eviscerated weight, had a tendency to decrease towards the end of the reproduction period (July). Thereafter, *A. boyeri* gradually recovered during fall and winter, reaching its highest values during spring, when food was abundant. Generally, in freshwater bodies, the diet composition of *A. boyeri* is dominated by zooplankton (Chrisafi et al., 2007; Doulka et al., 2013), and in the Hirfanlı Reservoir, zooplankton density increases in spring (Baykal et al., 2006). Nikolsky (1980) stated that rapid feeding enables many fish to grow rapidly, which is also the case for *A. boyeri* in the Hirfanlı Reservoir. In the Hirfanlı Reservoir, the mean monthly condition factor values, calculated using eviscerated weight, varied in a narrow range, exhibiting a peak in spring. However, some authors reported more than one peak (Fernández-Delgado et al., 1988; Andreu-Soler et al., 2003; Koutrakis et al., 2004), which could be attributed to different feeding and environmental conditions.

According to the overall sex ratio of the population, neither sex was dominant. This result reveals that the sex ratio of the *A. boyeri* population in the Hirfanlı Reservoir is close to the ideal Mendelian ratio. However, the sex ratio significantly differed within age classes, with females being dominant in age classes 3 and 4. This indicates that in the

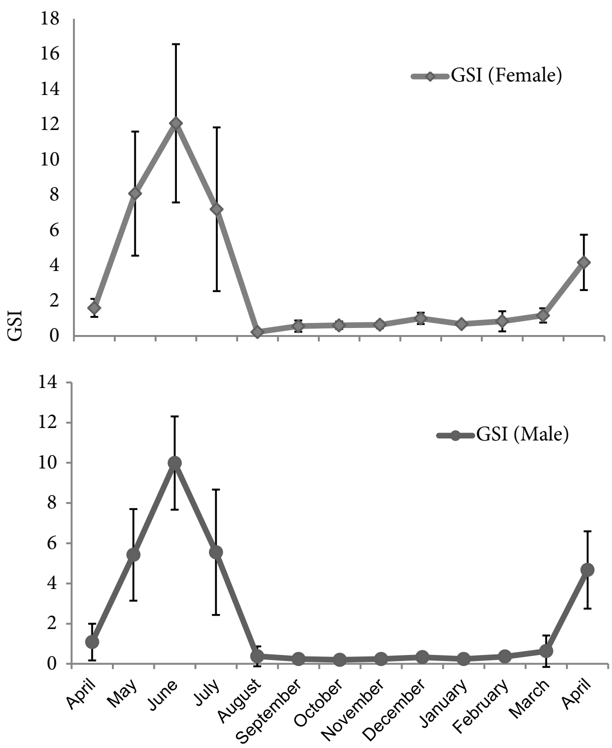

Figure 4. Variation of GSI for females and males of *Atherina boyeri* in the Hirfanlı Reservoir.

Table 3. Comparison of growth parameters of *Atherina boyeri* found in various studies.

Authors	Study area	Maximum age (years)	N	a	*b*	$L_{\infty a}$(mm)	k	t_0
Altun, 1986 **	Küçükçekmece Lake, Turkey	3	900	1.61×10^{-2}	2.97	115.3	-	-
Henderson and Bamber, 1987 **	English Channel populations	4				138	0.7	
Creech, 1992 **	Aberthaw Lagoon, Wales	2	329	3.5×10^{-6}	3.27	92	-	-
Rosecchi and Crivelli, 1992 ***	Camargue Wetland, France	4	350	1.84×10^{-12}	2.98	-	-	-
Leonardos and Sinis, 2000 *	Mesolongi and Etolikon Lagoon, Greece	3	4269	4.168×10^{-3}	3.15	115.79	0.24	−1.27
Leonardos, 2001 *	Trichonis Lake, Greece	4				112.40	0.42	−0.40
Andreu-Soler et al., 2003 ***	Mar Menor Lagoon, Iberian Peninsula	3	1936	7.29×10^{-6}	3.07			
Koutrakis et al., 2004 *	Vistonis Estuarine System, Greece	4	1056	2×10^{-6}	3.22	116.97	0.35	−0.99
Bartulović et al., 2004 *	Mala Neretva River, Croatia	4	1200	3.43×10^{-3}	3.24	135.03	0.37	0.97
Sezen, 2005 *	Homa Lagoon, Turkey	3	1640	5.2×10^{-3}	3.08	134.5	0.23	−1.89
Pombo et al., 2005 *	Aveiro River, Portugal	3	350	-	3.4	116	0.099	−3.797
Gaygusuz, 2006 *	İznik Lake, Turkey	4	1138	3.6×10^{-3}	3.3	128.83	0.31	−0.89
Patimar et al., 2009 *	Gomishan Wetland, Iran	4	2256	0.5×10^{-2}	3.06	-	-	-
Özeren, 2009 *	İznik Lake, Turkey	4	922	0.4×10^{-2}	3.21	141.11	0.27	−0.49
Present study *	Hirfanlı Reservoir, Turkey	4	674	3×10^{-6}	3.16	156.78 (f) 151.02 (m)	0.197 (f) 0.148 (m)	−0.197 (f) −0.148 (m)

*: Total length.
**: Standard length.
***: Fork length.

Hirfanlı Reservoir, male *A. boyeri* have a shorter life-span compared to females. In many fish species, females live longer than males. This fact was found to be true for *A. boyeri*, not only in sea and brackish waters, but in inland waters as well (Creech, 1992; Sezen, 2005; Gaygusuz, 2006; Tarkan et al., 2007; Özeren, 2009; Patimar et al., 2009).

The GSI values showed that the duration of the reproductive period was about 3 months. In the Hirfanlı Reservoir, this period was shorter than the period reported by other studies in different locations (Table 4). Water temperature has a significant influence on the duration of the spawning period of fish (Nikolsky, 1978). *A. boyeri* in the Hirfanlı Reservoir could spawn within a year in the spring following hatching, and by the end of the first summer it was possible to identify the sex of the young-of-the-year by macroscopic examination of the gonads. An extended reproductive period is an important strategy for the life history of *A. boyeri*, achieving invasiveness in inland waters (Bogutskaya and Naseka, 2002).

Batch spawning was observed in *A. boyeri* populations (Rosecchi and Crivelli, 1992). During the spawning period, immature, maturing, and mature eggs were

Table 4. Reproductive parameters of *Atherina boyeri* found in various studies.

Author	Study area	Sex ratio male:female	Spawning period	GSI (Max. average)		Max. ripe oocyte diameter (mm)
				Female	Male	
Rosecchi and Crivelli, 1992	Camargue Wetland, France	-	March–July	13.3	9.16	1.81
Creech, 1992	Aberthaw Lagoon, Wales	1:1.01	March–July	-	-	2.08
Tomasini et al., 1996	Maugio, Perols and Mejean Lagoons, France	-	February–August	17.2	-	1.94
Tomasini and Laugier, 2002	Maugio, Perols and Mejean Lagoons, France	-	February–August	-	10.60	-
Özeren, 2009	İznik Lake, Turkey	1:1.6	March–June	16.91	16.39	1.1
Sezen, 2005	Homa Lagoon, Turkey	1:1.76	March–July	-	-	-
Andreu-Soler et al., 2006	Mar Menor Lagoon, Iberian Peninsula	-	March–August	-	-	-
Bartulović et al., 2006	Mala Neretva River, Croatia		March–August	5.4	4.7	
Gaygusuz, 2006	İznik Lake, Turkey	1:3.01	April–August	8.96	6.15	1.21
Tarkan et al., 2007	İzmir Bay, Turkey	1:1.96	April–July	-	-	-
	Homa Lagoon, Turkey	1:1.28	April–July	-	-	-
	Ömerli Reservoir, Turkey	1:12.5	April–September	-	-	-
Patimar et al., 2009	Gomishan Wetland, Iran	1:1.30	March–July	7.12	3.16	0.20
Present study	Hirfanlı Reservoir, Turkey	1:1.14	May–July	12.07	9.99	1.73

observed all together in single ovaries of the females in the Hirfanlı Reservoir, which suggests the possibility of batch spawning. As can be seen in Figure 4, there is great variation in GSI among females during the spawning period, which indicates that some individuals have already laid their eggs while others are still maturing. Apart from the spawning period, the variation of GSI was found to be very low, and only immature eggs were present in the ovaries. This suggests the batch spawning of *A. boyeri* in the Hirfanlı Reservoir. Mature egg diameters in the Hirfanlı Reservoir were similar to those reported by various authors (Creech, 1992; Rosecchi and Crivelli, 1992; Tomasini et al., 1996).

Fecundity of *A. boyeri* increased linearly with age. No clear trend towards latitude or environmental conditions emerged from a comparison with the published data, because the criteria of the fecundity evaluation were different.

A. boyeri, a nonnative species for the inland waters in Turkey, has established abundant populations. During the period of 1964–1970, the dominant fish in the Hirfanlı Reservoir was *Alburnus escherichii* according to the reports of the DSI (2005). *A. boyeri* has been fished intensely in the Hirfanlı Reservoir. On the contrary, during a study period of 3 years and despite all efforts, *A. escherichii*, an endemic fish species that is pelagic, similar to *A. boyeri*, could not be found (Ekmekçi et al., 2010). These observations suggest that the *A. escherichii* population has decreased dramatically in the last 40 years, and the introduction of *A. boyeri* may have had an adverse effect on *A. escherichii*, as they share a common niche. *Alburnus* species are pelagic fishes and, similar to *A. boyeri*, mainly feed on zooplankton, along with insect nymphs and adults (Politou et al, 1993; Geldiay and Balık, 2007).

Several features of *A. boyeri* in the Hirfanlı Reservoir, such as their short life-span, rapid growth, extended reproductive period, early sexual maturation, and batch spawning, are typical invasive characteristics ensuring establishment success. However, Bamber and Henderson (1988) stated that many atherinids show a high degree of intraspecific morphological variability, which can be linked to estuarine habitats. Such environments are physically highly variable and this has acted to select for generalist genotypes able to adjust their morphology, physiology, and behavior to a wide range of conditions. Additionally, such plasticity preadapts atherinids to invade and rapidly populate fresh waters containing vacant niches. Our results, obtained from the sand smelt population of the Hirfanlı Reservoir, support the hypothesis of Bamber and Henderson (1988).

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