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The influence of environmental instability on the reproductive strategy of the critically endangered Acıgöl killifish (*Aphanius transgrediens*)

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Abstract

To investigate the variability in the reproductive tactics of Acıgöl killifish (*Aphanius transgrediens*), one of the most seriously threatened freshwater teleost species, this study compared its reproductive ecology in two contrasting habitats that differ substantially in terms of stability of environmental parameters, particularly salinity regime (stable *vs*. unstable). Fish were sampled monthly from October 2013 to September 2014 with the aim of testing whether the reproductive life-history response of fish to stable and unstable conditions differed. The reproductive effort (gonad weight) of both sexes did not differ significantly between the two habitats, but females in the unstable habitat had significantly lower fecundity and larger eggs. The relationship between fecundity and fish size was stronger in the stable habitat, whereas the relationship was quite variable and uncertain in the unstable habitat. Fish born in the unstable habitat reached their first maturity at a smaller size than those in the stable habitat. The gonado-somatic index and the duration of hydrated eggs showed that reproduction continued from February to May in both habitats; nonetheless, a second spawning event occurred during July and August in the unstable habitat, which included the reproductive contributions of YOY individuals and older generations. This study's results suggest that *A. transgrediens* employs varying reproductive strategies against environmental instability in its restricted unique range. This may have further implications for the ways in which habitat-specific conservation methods are used.

KEYWORDS
Acıgöl, conservation, fecundity, toothcarp, life history

1 | INTRODUCTION

Studies on variations in life-history strategies have long been of central importance for the conservation of endangered species, as life-history strategy has been suggested to be a strong predictor of how susceptible populations are to decline and extinction when disturbances are encountered (Angermeier, 1995; Chichorro *et al*., 2019; Olden *et al*., 2008). The constraints on life-history traits, particularly those associated with reproduction, are known to shape the strategies that species use to succeed in various kinds of environments and under disturbance (Winemiller and Rose, 1992; Wootton, 1992). Comparative approaches dealing with conspecific populations in this regard permit researchers to predict the adaptive abilities of species in response to several factors, such as habitat type or complex environmental stressors (Blanck and Lamouroux, 2007). For example, Panfili *et al.* (2006) compared the life-history traits of fish populations from two contrasting estuaries (with normal *vs*. inverse salinity gradients) and demonstrated that size at maturity was reduced with increasing salinity in populations living in high-salinity waters. Similarly, several populations of black-chinned tilapia (*Sarotherodon melanotheron*) were observed to have highly varying reproductive characteristics across coastal marine, estuarine and freshwater ecosystems (Guèye *et al*., 2012; Panfili *et al*., 2004). This plasticity in fish life-history traits against environmental variability has been independently described by several authors as an "opportunistic strategy" in which the individuals tend to be short lived and rapidly mature with frequent reproduction over an extended spawning season (Winemiller and Rose, 1992). As has been commonly suggested, this array of traits permits adapted recolonisation of habitats by species over relatively small spatial patches. One of the best examples of this opportunistic strategy is the killifishes of the genus *Aphanius* (Cyprinodontiformes: Aphaniidae), which are secondary freshwater fishes originating from a Tethyan ancestor (Hrbek and Meyer, 2003). The 45 valid members of the genus presently inhabit diverse ecosystems, from freshwater springs to brackish and saline waters in the eastern Mediterranean and southern Europe (Fricke *et al*., 2019; Hrbek and Meyer, 2003). Several species face an elevated risk of extinction because of threats, such as habitat loss and the introduction of invasive species (Darwall and Freyhof, 2016; Freyhof and Brooks, 2011). Globally, 10 species are listed as endangered or critically endangered (IUCN, 2020), and more than 30 species have not yet been assessed. In Anatolia, where the genus is remarkably diverse, there are 18 endemic species, 8 of which are represented by small isolated populations that are known from a single lake or spring and the remaining species are known from a single (or rarely two) watershed area (Freyhof *et al*., 2017; Yoğurtçuoğlu and Freyhof, 2018). Although only four of the Anatolian species are listed as threatened, it has been suggested that most of the species among those not yet assessed are endangered as a result of their highly localised occurrence and ongoing threats in their habitats (Yoğurtçuoğlu, 2018).

Aphanius transgrediens (Ermin, 1946), one of the two critically endangered killifish species (Freyhof, 2014), is a small-sized fish [reaching up to 60 mm total length (TL)] (Yoğurtçuoğlu and Ekmekçi, 2015) restricted to a few freshwater springs across Lake Acıgöl (south-western Turkey) and is considered among the world's 100 most threatened species based on its narrow range (<1 km^2) and low population size (Baillie and Butcher, 2012; Yoğurtçuoğlu and Ekmekçi, 2014). Since the early 1990s, the eastern mosquitofish (*Gambusia holbrooki*) has been introduced into the freshwater springs of Lake Acıgöl and is one of the main threats to killifish populations as it has outcompeted *A. transgrediens* in almost all of the small springs it inhabits (Wildekamp *et al*., 1999; Yoğurtçuoğlu and Ekmekçi, 2014). Other multiple ongoing threats that are believed to cause the species' decline in the area are pollution, desiccation and habitat modification (Freyhof, 2014; Yoğurtçuoğlu and Ekmekçi, 2014). Because of its critically endangered status, effective conservation measures are needed to ensure the survival of *A. transgrediens*. Practical conservation strategies, however, require knowledge of the species' life-history traits at the population level. The majority of the published literature is limited to studies that deal with a single population in a single habitat (Esmaeili and Shiva, 2006; Keivany and Soofiani, 2004; Yoğurtçuoğlu and Ekmekçi, 2013), whereas only a few studies have attempted to compare trait variability within *Aphanius* genus among contrasting environments (Kamal *et al*., 2009; Leonardos and Sinis, 1998). To the best of the authors' knowledge, almost no studies are currently available on the reproductive ecology of *A. transgrediens* in its only distribution range. Lake Acıgöl has been commercially exploited for brine production on its northern coasts. As a result, human-induced habitat modifications, particularly the opening of waterways from brine pits to freshwater springs, have caused instable environmental conditions such as rapid and severe changes in water salinity. Despite the occurrence of invasive *G. holbrooki* in these particular habitats (in small numbers relative to in stable habitats), *A. transgrediens* survives and thrives in quite high numbers. Reproductive trait plasticity in the species may be the underlying determinant of this success; however, this assumption remains hypothetical in the absence of quantitative data on the variability of its basic reproductive life history. Consequently, the main aim of the present study was to quantify the reproductive strategies of *A. transgrediens* in its only distribution range by comparing fine-scale differences in the basic reproductive parameters between two populations from stable and unstable environments. To this end, this study tested whether environmental instability could affect the life-history traits of the species. The potential benefits of using these results for the conservation management of *A. transgrediens* are also discussed here.

2 | MATERIALS AND METHODS

Fish collection was permitted by the Turkish Ministry of Food, Agriculture and Livestock, General Directorate of Fisheries and Aquaculture (codes for the protocols: B.12.0.KKG.0.17/106.01-11-01 and 76000869/150-4199); and laboratory procedures were approved by the Hacettepe University Animal Experimentations Local Ethics Board (Protocol number: 52338575-150).

2.1 | Study system and data collection

Lake Acıgöl is a hypersaline, Mediterranean-type shallow lake located in a closed basin in south-western Anatolia. The lake is situated in a tectonic depression between two fault lines striking northeast–southwest. Several freshwater springs (*ca*. 20) issue along these faults with various flow rates. Before ultimately reaching the saline lake, the springs flood the flat lowland and form wetland patches, which are the main habitat of *A. transgrediens*. The surface area of the lake, together with these wetlands, is *ca*. 80 km^2 with a maximum depth of *ca*. 2 m; nonetheless, the area of the main lake body varies considerably throughout the year. The lake is known for its sodium sulphate reserve and has been exploited for commercial brine production by operators since 1993.

Data were collected from two spatially close but ecologically contrasting habitats; one was stable (hereafter SH), and the other was unstable (hereafter UH) with regard to physical parameters, including water temperature, dissolved oxygen and salinity (Figure 1). The UH is located at the north coast of the lake (37° 50.642′ N, 29° 46.772′ E) and is connected to the discharge channel of one of the brine pits; therefore, it receives drained hypersaline water which gives rise to the unpredictable physical conditions, particularly salinity (Figure 1). The bottom of the UH is mostly covered by mud and clay without rooted aquatic plants and a small amount of riparian vegetation. The SH, however, is located on the southern shore (37° 49.088′ N, 29° 55.684 ′ E) and is one of the largest springs flowing directly into the lake. Almost all of the physical variables were stable throughout the study year (Figure 1), except in the delta area where the SH joins the hypersaline lake. The bottom structure consists of variable substrate from cobbles to sand. The dominant aquatic plant communities consist of epilithic and filamentous algae, and the riparian area comprises *Phragmites australis*, *Nasturtium officinalis*, *Typha* sp. and *Juncus* sp. *A. transgrediens* is the only native fish, together with rare *Cobitis phrygica* and *Petroleuciscus ninae*, in Lake Acıgöl; nonetheless, invasive eastern mosquitofish (*G. holbrooki*) were first observed in the early 1990s in one of the southern springs (Wildekamp *et al.*, 1999) and have spread to other springs over time (Yoğurtçuoğlu and Ekmekçi, 2017). Another non-native invasive species, *Carassius gibelio*, was recorded in some of the southern springs for the first time during the present study. Some of the springs become interconnected during the rainy season, and fish populations that are normally restricted to these small springs mix together on the southern side. Nonetheless, the lake itself constitutes a microgeographic barrier between the populations inhabiting the springs on the northern and the southern sides because of its hypersaline water (Kosswig, 1963). As the study

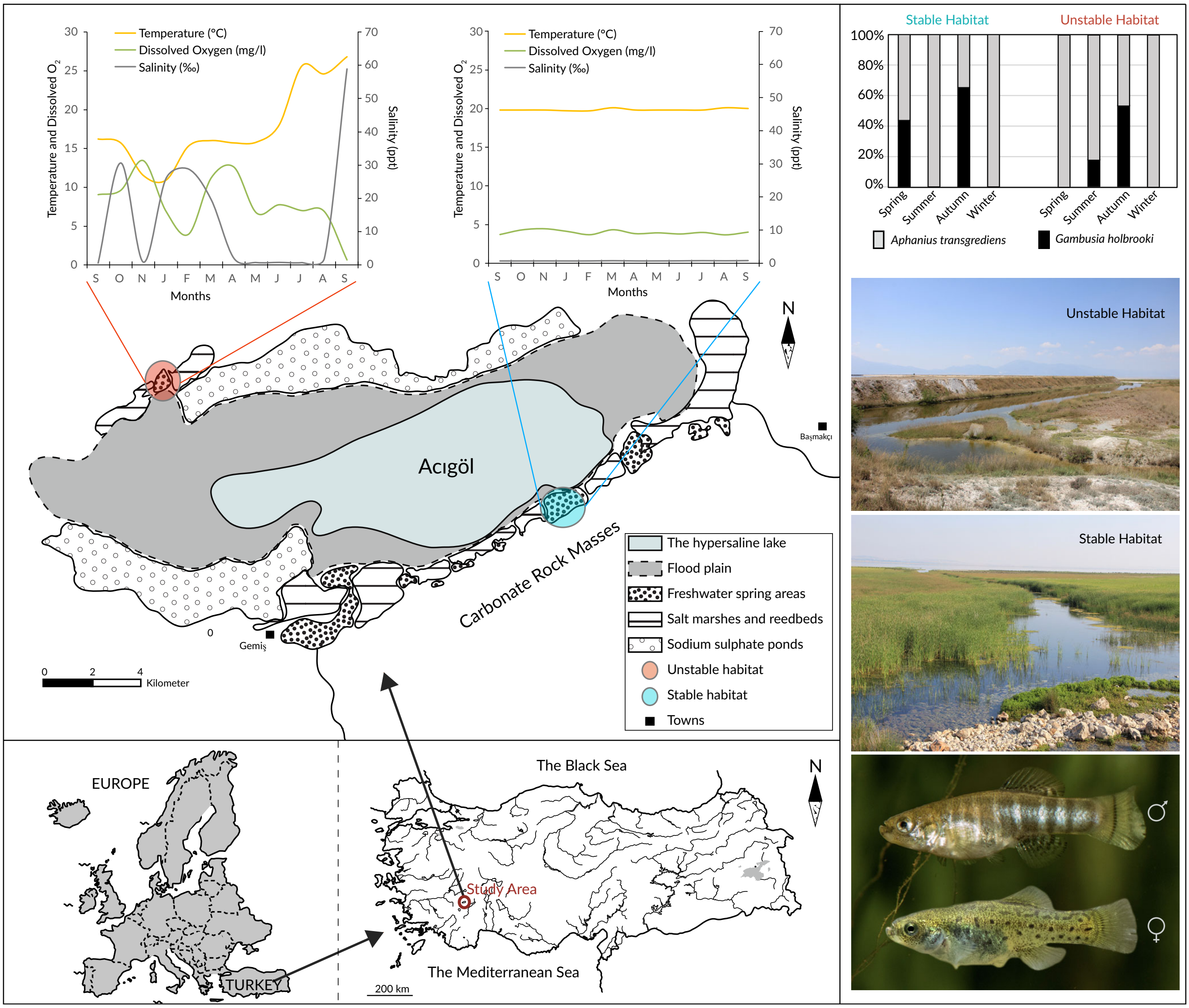

FIGURE 1 Location of the Lake Acıgöl in south-western Turkey and the sampling points with graphs demonstrating monthly change in water temperature, salinity and dissolved oxygen. The bar graph shows seasonal catch proportions of *Aphanius transgrediens* and *Gambusia holbrooki* in both the habitats. The photographs show sampling points and both sexes of *A. transgrediens*

species is critically endangered and in decline (Yoğurtçuoğlu and Ekmekçi, 2014), sample size was kept small (up to 30 individuals per month per habitat) to limit impacts on the population. Fish were captured using a beach seine (1.5 × 3 m) and a scoop net (both had a 4 mm knot-to-knot mesh size), immediately anaesthetised using clove oil and fixed in 10% formalin before being transported to the laboratory for further examination.

2.2 | Laboratory procedure

In the laboratory, fish were measured for total length (TL) to the nearest 0.01 mm and were subsequently weighed [body weight (BW)] to the nearest 0.01 g and sexed from external characteristics or microscopic examination of the gonads. Scales were used for age determination. Because there is scale reduction (in number and size) in the study species, scales were taken from the whole body between the base of the pectoral fin and the anterior end of the caudal fin. For preparations, scales were rinsed with water, dehydrated using 70% alcohol and mounted between two slides using a dissecting needle for examination under a stereomicroscope with transmitted light. The gonads were removed, and gonad weights (GW) were recorded to the nearest 0.001 g. Ovaries were placed on a counting chamber, and all of the oocytes in each ovary were spread under a stereoscope. Each chamber was photographed for subsequent counting and oocyte measurement using an image analysis software (ImageJ, National Institutes of Health, Bethesda, MD, USA), as described previously (Schindelin *et al*., 2012). Oocyte development was divided into five stages, as described in Table 1.

2.3 | Data analysis

Sex ratio, length at first maturity, fecundity, reproductive effort and spawning period of populations in the two habitats were analysed to characterise the reproduction of the species. A χ^2 test was used to determine whether the overall sex ratio differed from 1:1 in each habitat. The length at first maturation was estimated by calculating the proportion of mature fish in each 2 mm TL class and fitting the logistic function to the percentage data. The frequency of mature fish was used as the response variable with binary values (0, immature; 1, mature), and TL was used as the predictor variable. Female fish were classified as mature if the ovaries contained oocytes in stages IV and V (Table 1) during the reproductive season. The males were classified as mature if they exhibited nuptial colour and/or if advanced testes could be identified during the reproductive season. The logistic function used is given as follows:

$$P=\left(1+e^{-(a+b\mathrm{TL})}\right)^{-1}, \tag{1}$$

where *P* is the percentage of mature fish at given TL and *a* and *b* are the function parameters. Reproductive period was determined based on temporal variations in the gonado-somatic index (GSI) values and the developmental stages of the oocytes in the ovaries. GSI was calculated using the following equation:

$$\mathrm{GSI}=(\mathrm{GW}/\mathrm{BW})\times 100, \tag{2}$$

where GW is the gonad weight and BW is the body weight. Significant differences in monthly mean GSI values were tested using ANOVA following Games–Howell (GH) tests. GH tests are among the most powerful and robust *post hoc* multiple comparison methods for unequal variances (Day and Quinn, 1989). Fecundity was estimated based on the number of most advanced oocytes in the gonads collected from 43 and 49 females originating from SH and UH, respectively. Because the study species is a batch-spawner and carries oocytes that repeatedly mature throughout the spawning season, the oocyte size-frequency method described by Hunter *et al.* (1985) was used, in which the most advanced modal group of oocytes was inspected and counted for fecundity estimations after constructing the size-frequency distribution of oocytes. Bhattacharya's modal progression analysis was used to determine the most advanced modal groups (Yoğurtçuoğlu, 2019). The relationships between fecundity and age and between fecundity and fish size (TL and BW) were analysed using a linear regression model after log-transformation of the variables. The relationships were then expressed by the following power curve equation:

$$F=aX^b, \tag{3}$$

where *F* is the number of oocytes per female, *X* is either age or size (TL or BW) and *a* (constant) and *b* (slope) are the parameters (Bagenal & Braum, 1971).

To adjust size variation in trait data, the authors used regression models with ANCOVA. They could fix the influence of covariate (*e.g*., fish length) (Zar, 2010) to test inter-population differences in the reproductive strategies of the species between the two habitats.

TABLE 1 Description of the stages of oocyte development in *Aphanius transgrediens* according to size and macroscopic appearance

Classification	Maturity state	Size and macroscopic appearance
Stage I	Immature/ resting	Roundish and translucent/white granulated oocytes; often <0.45 mm in diameter
Stage II	Early developing	Round and opaque/brown oocytes 0.45–0.85 mm in diameter
Stage III	Developing	Round and translucent or opaque/ brown to yellow oocytes 0.85–1.15 mm in diameter
Stage IV	Late developing	Round and translucent/yellow oocytes 1.15–1.45 mm in diameter
Stage V	Mature/ripe	Hydrated round and transparent or pale-yellow oocytes >1.45 mm in diameter

TABLE 2 TL ranges of *Aphanius transgrediens* in stable and unstable habitats with respect to the age groups

	Stable habitat				Unstable habitat			
Age	***n***	**♀ TL range**	***n***	**♂ TL range**	***n***	**♀ TL range**	***n***	**♂ TL range**
0^+	169	15.4–37.1 (27.3 ± 4.4)	41	18.7–30.7 (24.4 ± 3.1)	99	17.3–40.5 (27.5 ± 5.5)	95	17.7–37.0 (26.8 ± 3.5)
1^+	53	23.3–42.4 (33.7 ± 4.1)	15	25.3–36.0 (30.3 ± 3.1)	76	26.4–51.0 (37.1 ± 5.1)	67	24.6–33.3 (32.9 ± 4.3)
2^+	4	38.1–45.1 (42.2 ± 3.0)	–	–	14	38.3–54.3 (44.1 ± 4.1)	2	32.2–34.1 (33.1 ± 1.3)

Note. Values are given accompanied by mean ± s.d. in brackets. TL, total length.

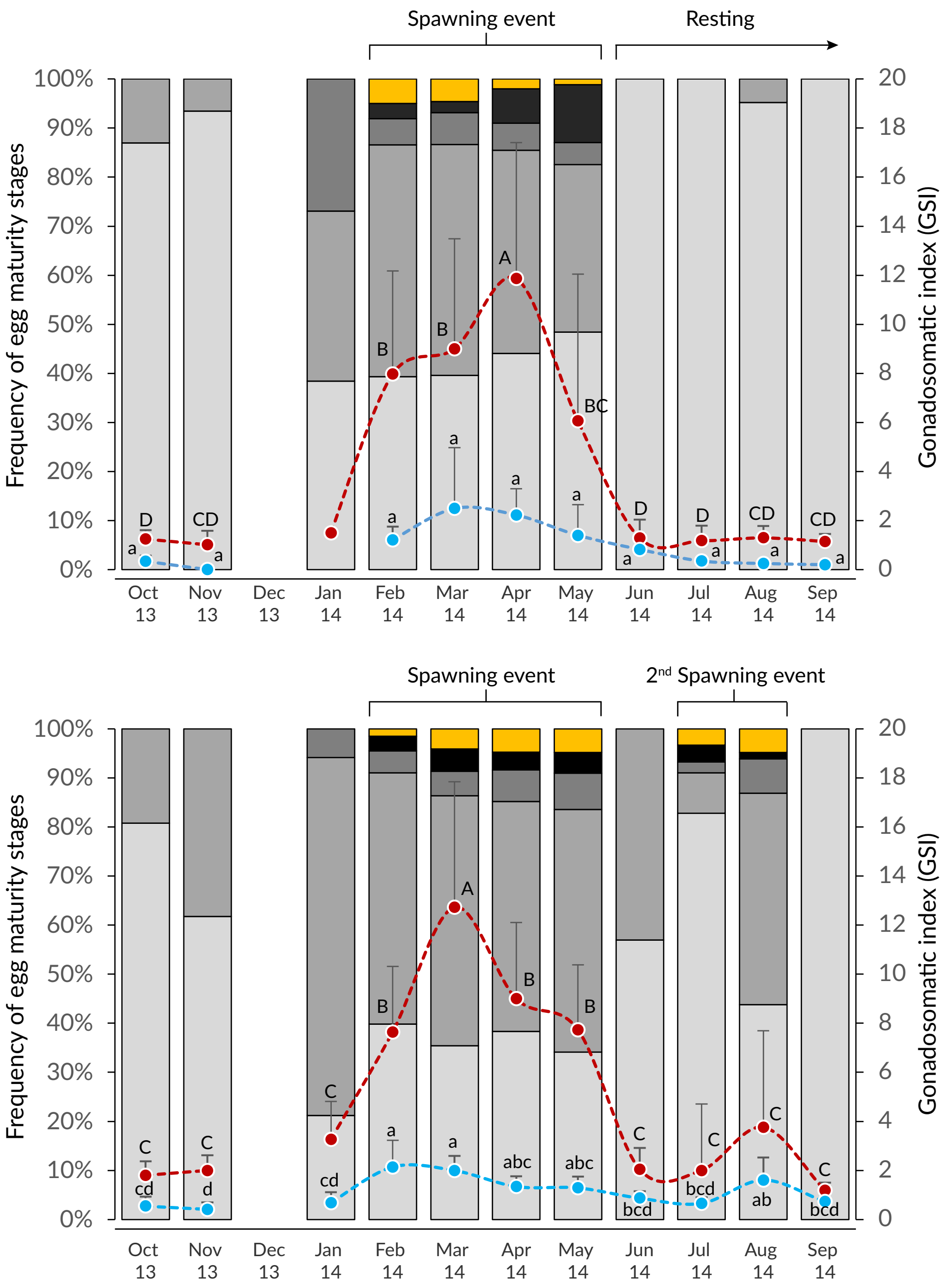

FIGURE 2 Monthly variations in the gonado-somatic index (GSI) and the percentage of eggs corresponding to each developmental stage in *Aphanius transgrediens* (above for SH and below for UH). GSI is given as mean ± s.d. Mean values that do not share a letter (A–D for females; a–d for males) differ significantly (*P* < 0.05, *GH* test). □ Stage I (Immature); ▨ Stage II (Early developing); ▩ Stage III (Developing); ■ Stage IV (Late developing); 🟨 Stage V (Mature); --●-- Female GSI; --●-- Male GSI

Here, the models were used by selecting habitat and sampling month (during spawning period) as the explanatory variables; the reproductive effort (as GW of both sexes), fecundity and eviscerated BW of females as the response variables; and TL as the covariate (all dependent variables and the covariate were log-transformed) (Zar, 2010).

Where appropriate, data are presented as mean ± s.d.

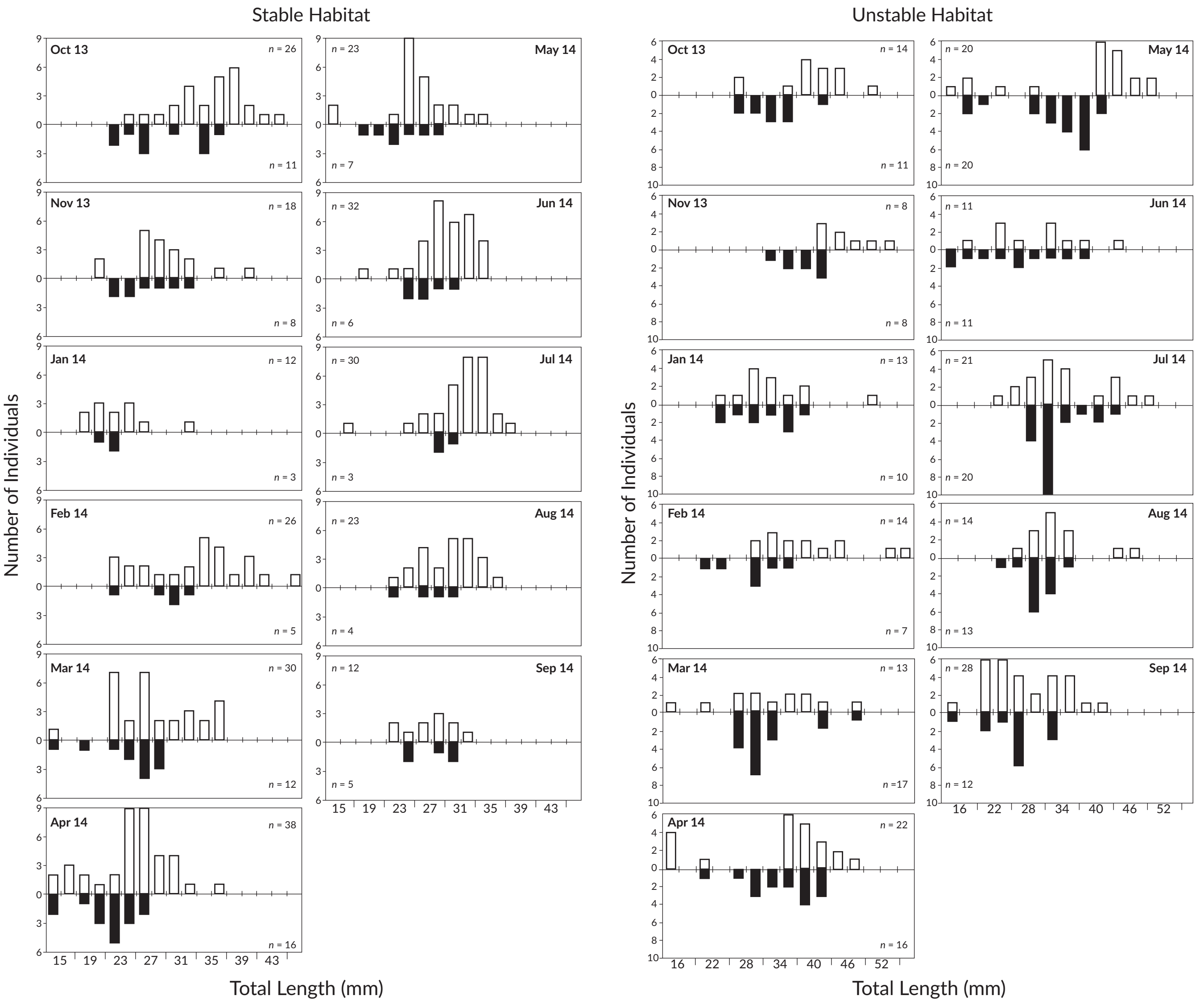

FIGURE 3 Monthly variation in the length–frequency distribution of *Aphanius transgrediens*. Open bars indicate females; black bars indicate males

3 | RESULTS

3.1 | Study habitats

The seasonal changes in the catch proportions of the two species in the SH and UH are given in Figure 1. Some environmental parameters remained nearly constant throughout the sampling period in SH; the water temperature ranged between 20 and 21°C, dissolved oxygen between 4 and 5 mg l^{-1} and salinity between 0.6 and 0.7 ppt. In the UH, however, the water parameters exhibited great fluctuation year-round. In the UH, the lowest temperature was recorded in January (11°C) and the highest in September (27°C). Salinity varied dramatically with the current direction of water flow (from brine pits to the spring or *vice versa*). After salinity fluctuated from 0.6 to 30.6 ppt between September 2013 and March 2014, the UH showed fresh- to brackish water characteristics from April to August 2014 (salinity ranged from 0.7 to 1.9 ppt) before ultimately reaching maximum salinity in September 2014 (58.9 ppt). Dissolved oxygen in the UH also showed similar monthly fluctuations but in an inverse pattern to the salinity pattern (ranging between 1 and 13 mg l^{-1}) (Figure 1). Day length was <12 h from October to February, exceeded 12 h from late February and peaked in June (14.8 h).

3.2 | Population structure

The samples were significantly dominated by females in both habitats (SH: n = 282, 1M:4.0F, χ^2 = 102.5, $P < 0.05$; UH: n = 353, 1M:1.2F, χ^2 = 20.6, $P > 0.05$). The TL of the samples ranged from 15.3 to 45.1 mm in the SH and from 17.3 to 54.3 mm in the UH. BW ranged from 0.04 to 1.56 g in the SH and from 0.05 to 2.38 g in the UH. The majority of individuals belonged to the 0^+ and 1^+ age groups, which made up 98.3% of the population in the SH and 92.4% in the UH. A

TABLE 3 Analysis of covariance results on the effects of habitat, month and their interaction on the fecundity, female and male gonad weight and eviscerated female body weight of *Aphanius transgrediens*

Source of variation	*df*	*F*	*P*-value
Fecundity (number of oocytes in stages IV and V)			
Covariate	1	14.25	<0.001
Habitat	1	10.13	0.002
Month	3	2.17	0.098
Habitat × month	3	2.62	0.056
Error	79		
Female gonad weight (GW)			
Covariate	1	231.12	<0.001
Habitat	1	1.24	0.268
Month	3	10.13	0.000
Habitat × month	3	3.82	0.012
Error	128		
Male GW			
Covariate	1	58.95	<0.001
Habitat	1	1.20	0.277
Month	3	0.36	0.781
Habitat × month	3	2.18	0.100
Error	59		
Eviscerated female body weight			
Covariate	1	3202.16	<0.001
Habitat	1	7.34	0.008
Month	3	4.19	0.007
Habitat × month	3	3.82	0.293
Error	128		

Note. Fish length was used as covariate. *F*-value; *df*, degrees of freedom; significant *P*-values are highlighted in bold.

TABLE 4 Relationship between fish size (length and eviscerated body weight) and fecundity in *Aphanius transgrediens*

Habitat	Equation	*N*	95% c.i. of b	R^2	*F*	*P*
Relationship between fecundity (F) and total length (TL) (cm)						
Stable	$F = 0.71\text{TL}^{2.90}$	43	2.00–3.80	50.8	42.4	<0.001
Unstable	$F = 2.07\text{TL}^{1.49}$	49	−0.03–3.00	7.6	3.9	0.055
Relationship between fecundity (F) and body weight (BW) (g)						
Stable	$F = 57.86\text{BW}^{0.87}$	43	0.62–1.12	54.7	22.2	<0.001
Unstable	$F = 19.91\text{BW}^{0.40}$	49	−0.07–0.86	5.8	2.9	0.094
Relationship between fecundity (F) and age (t) (years)						
Stable	$F = 13.08t^{0.94}$	43	0.46–1.43	27.4	15.5	<0.001
Unstable	$F = 10.09t^{0.53}$	49	−0.32–1.38	3.2	1.55	0.219

Note. 95% c.i. is the lower and upper 95% confidence levels for the slope of the equation.

second annulus was observed in 4 females from the SH and in 14 females and 2 males from the UH. The TL range and fish numbers found for each age group and sex are listed in Table 2.

3.3 | Reproduction

The monthly variation in average GSI and the occurrence of hydrated oocytes showed that *A. transgrediens* reproduces between February and May in both habitats, with a second spawning event in the UH in July and August (Figure 2). In the second spawning event in the UH, 2 out of the 10 females (20%) carrying mature oocytes were the YOYs. The juveniles first appeared in March in both habitats and continued to appear until July in the SH and until September in the UH because of recruitment from the second spawning event (Figure 3). The YOYs which hatched at the beginning of the reproductive period (February) reached sexual maturity towards the end and participated in reproduction in April. At both sites, female GSI varied over the

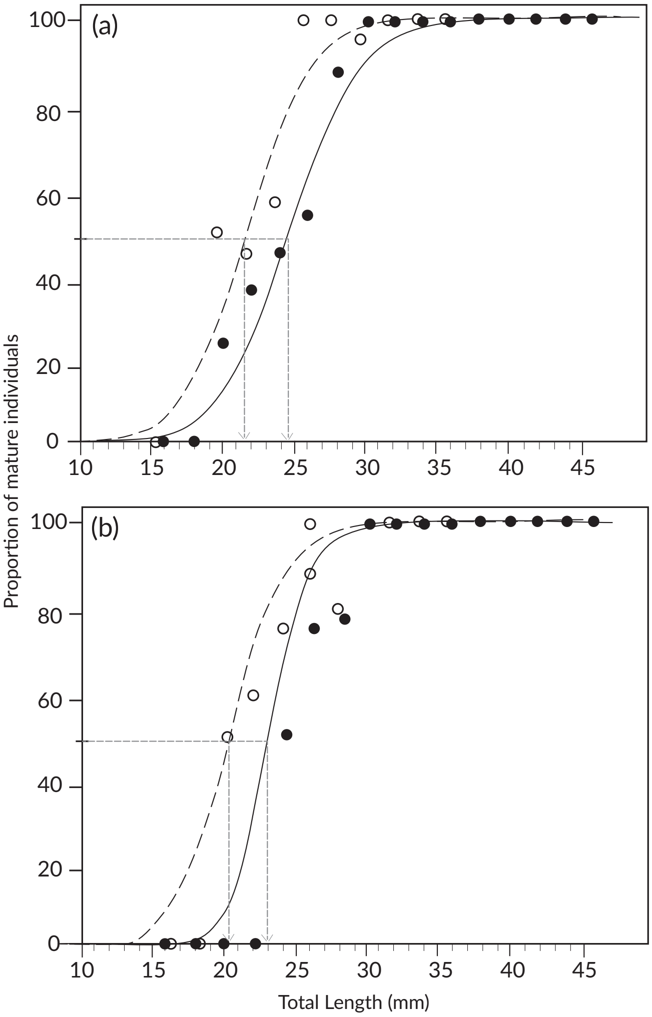

FIGURE 4 Percentage of mature individuals of *Aphanius transgrediens* fitted to logistic function (a, stable habitat; b, unstable habitat). Arrows represent size at 50% maturity. ○ Male; ● Female; —— Female curve; ------ Male curve

sampling period and was significantly higher during the reproductive season (ANOVA, $F = 28.97$ and $P < 0.001$ for SH; $F = 29.51$ and $P < 0.001$ for UH; see Figure 2 for GH test), peaking in April in the SH (11.9%) and in March in the UH (12.7%). The mean male GSI followed a similar pattern to that of females in both sites, with peaks occurring in March in the SH (2.8%) and in February in the UH (2.1%). The mean male GSI differed significantly across the months of the sampling period in the UH (ANOVA, $F = 8.33$ and $P < 0.001$) but did not differ significantly in the SH (ANOVA, $F = 1.26$ and $P = 0.331$; see Figure 2 for GH test). The models testing whether habitat had an effect on the GW (reproductive effort) revealed no significant effect for either sex (Table 3). Nonetheless, the effects of sampling month and the interaction between sampling month and habitat on reproductive effort were significant for females (Table 3; higher in SH than in UH in April). In the coinciding reproductive months, female-eviscerated BW was significantly higher in the UH than in the SH (Table 3; ANCOVA-adjusted mean-eviscerated BWs, 0.33 *vs.* 0.36 g, respectively). TL at 50% maturity for females was 24.5 and 23.0 mm and for males 21.6 and 20.1 mm (Figure 4) in the SH and UH, respectively. The relationships between fecundity and fish size (length and weight) and between fecundity and age were significant in the SH but were insignificant in the UH (Table 4), with a stronger relationship between fecundity and fish size in the SH than in the UH (Figure 5). Absolute fecundity ranged from 3 to 86 oocytes in the SH and from 2 to 66 oocytes in the UH. Relative fecundity ranged from 15.7 to 128.9 oocytes per gram BW in the SH subsample (mean 52.2 ± 23.2) and from 1.6 to 71.7 oocytes per gram BW (mean 29.0 ± 17.9) in the UH subsample. The model analysing the effects of habitat on fecundity was significant ($P = 0.002$), with the mean number of mature oocytes significantly higher in the SH than in the UH (mean oocyte numbers adjusted for covariate 22.0 *vs.* 12.1 oocytes, respectively). The effect of the covariate on fecundity was also significant, whereas no significant differences were found across the months of the spawning season (Table 3). The mean diameter of mature oocytes (stages IV and V) was significantly different across the months during the reproductive period in both of the studied habitats (ANOVA, $F = 45.43$ and $P = < 0.001$ for SH and $F = 9.89$ and $P < 0.001$ for UH). The maximum egg diameter was 1.76 mm in the SH and 1.90 mm in the UH. The mean diameter of mature oocytes was estimated to be 1.40 ± 0.16 mm in the SH and 1.43 ± 0.14 mm in the UH. The result of the two-way ANOVA testing the effects of habitat, month and their interaction on the mean diameter of mature oocytes was significant for each of the factors (ANOVA, $F = 5.61$ and $P = 0.018$ for habitat; $F = 13.69$ and $P < 0.001$ for month; $F = 48.96$ and $P < 0.001$ for habitat × month).

4 | DISCUSSION

The reproductive life history of *A. transgrediens* conforms to an opportunistic strategy that is often characterised by early maturation, short life span and high reproductive effort. *Aphanius transgrediens* individuals matured in their first year of life in Acıgöl, and YOYs participated in reproduction only 2 months after hatching in both of the studied habitats. The difference in length at maturity between the two habitats (individuals were slightly smaller in the UH; Figure 5), along with the extended reproduction enabled by a second spawning event in the UH, may reflect the adaptation of the species to high adult mortality caused by highly fluctuating salinity. The second spawning episode in the UH occurred at the end of a freshwater phase (from April to August; Figure 1), which likely allowed some of the individuals to recover from the previous spawning event that took place under high salinity and for some of the YOYs to become capable of spawning. As suggested by Lewontin (1965), small-bodied opportunistic fishes are well equipped with rapid maturation and prolonged batch spawning to recolonise habitats following disturbances that may cause high adult mortality. According to Bye (1984), in environmentally fluctuating habitats, later stages of oocyte development may be triggered or adjusted by environmental factors, such as tides, floods or salinity changes, which could be the underlying abiotic mechanism behind the second spawning event in the UH. Yet the second spawning event

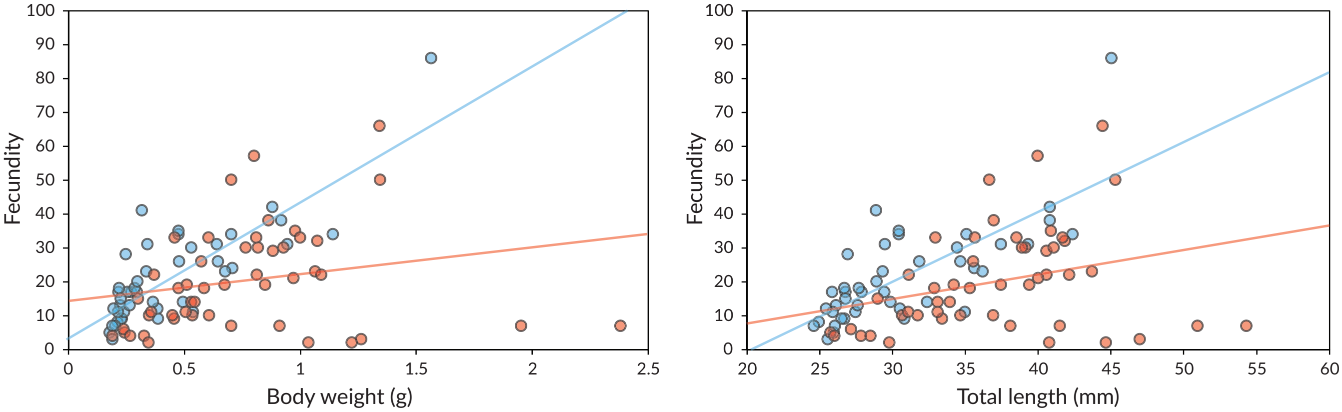

FIGURE 5 Relationship between absolute fecundity and body weight (left) and between fecundity and total length (right) in *Aphanius transgrediens* from stable and unstable habitats. ● UH (R^2 = 5.8); ● SH (R^2 = 54.7); ● UH (R^2 = 7.6); ● SH (R^2 = 50.8)

(or protracted spawning) in the UH needs to be further investigated using additional data taken over much longer periods to determine whether it is a general or occasional pattern. The water temperature was nearly constant throughout the year in the SH (*ca*. 20°C) and differed from that in the UH (*ca*. 15°C) at the beginning of reproductive activity (February), whereas the day length was identical (*ca*. 12 h per day) in February in both habitats, which suggests that photoperiod, rather than the water temperature, stimulated the commencement of spawning activity. Similar results were also demonstrated in two simultaneously reproducing *Aphanius* species (*Aphanius fontinalis* and *Aphanius saldae*) in the neighbouring Lake Salda (Yoğurtçuoğlu, 2019).

In the course of the study period, females in both habitats were proportionally better represented than males, especially in the SH, where males in the 2^+ age group were not observed. This might be a result of higher male mortality in older-age classes as they exhibit nuptial colours, making them more visible to predators. The more complex habitat structure in the SH, comprising dense vegetation including a mixed stand of reed and sedge beds, supports various terrestrial and aquatic predators, such as waterbirds and *Natrix* spp. Biased sex ratios in favour of females have been reported in several lakes, rivers (Esmaeli and Shiva, 2006; Kamal *et al*., 2009; Yoğurtçuoğlu and Ekmekçi, 2013) and estuaries (Leonardos and Sinis, 1998; Vargas and De Sostoa, 1997) inhabited by the *Aphanius* population. Although the significantly smaller females in the SH (Table 3) could be associated with a life-history strategy of *A. transgrediens*, where more energy is invested in reproduction than in growth, it could also simply be linked to the higher level of predation causing selection for larger individuals.

Fecundity estimations were achieved by considering only the number of mature oocytes, by deciding delimitation of maturity using Bhattacharya's frequency method, which in turn resulted in relatively lower estimates of fecundity than in other studies of *Aphanius* spp. (Esmaeli and Shiva, 2006; Keivany and Soofiani, 2004; Yoğurtçuoğlu and Ekmekçi, 2013). The significantly lower fecundity observed in the UH (Table 3) could be attributed to high and fluctuating salinity, causing a trade-off between the number of oocytes produced and the amount of energy needed for osmoregulation. The rapid and severe change in salinity and dissolved oxygen, likely in combination with resource-poor conditions, may also inhibit oocyte maturation in the UH. Leonardos and Sinis (1998) also estimated lower fecundity in *Aphanius fasciatus* in their study associated with salt works, which caused highly fluctuating and unpredictable conditions similar to the present study (*vs*. higher fecundity in environmentally stabile stations). As previously hypothesised and tested by a number of empirical studies, salinity restricts the aggressive behaviour of *Gambusia* spp. towards native cyprinodonts and reduces foraging efficiency under coexistence (Alcaraz and García-Berthou, 2007; Nordlie, 1996). In contrast, native cyprinodonts do not alter their aggressive behaviour and feed more efficiently with increased salinity (Alcaraz *et al*., 2008) under coexistence with invasive mosquitofish. In addition, Brigolin *et al*. (2016) reported that *A. fasciatus* had a higher number of spawning events and lower per-spawning fecundity in habitats associated with lower levels of predation risk. This empirical evidence may explain why females are significantly larger bodied, carry fewer oocytes and are able to extend spawning to a second event in the UH. Several large females (>40 mm TL) in the UH sample, therefore, carried only a few number of mature oocytes, which led to a substantially lower correlation between fecundity and fish size than in the SH (Figure 5). Decreased oocyte number per unit body size in the UH, however, seemed to be compensated by significantly larger eggs and prolonged spawning, which could be associated with ensuring a higher probability of survival of offspring (Bagenal, 1969; Wooton, 1990).

In conclusion, *A. transgrediens* exhibits plastic reproductive traits in its restricted and unique geographic range, with a longer reproductive period, larger eggs and faster maturation in a UH. The unpredictable conditions caused by artificial waterways and associated extreme salinity fluctuations in the UH are not always treated as threatening factors. Rather, they could serve as a competitive refuge against the antagonistic impacts of invasive mosquitofish and could be acknowledged as a model for alternative conservation management options in the area.

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AUTHOR CONTRIBUTIONS

B.Y. contributed to ideas, data collection, data analysis and manuscript preparation; U.U. contributed to data collection; and F.G.E. contributed to ideas, data collection and manuscript review.

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