REPRODUCTION OF CRITICALLY ENDANGERED KILLIFISH *APHANIUS TRANSGREDIENS* UNDER LABORATORY CONDITIONS AND RELATED CONSERVATION STUDIES

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INTRODUCTION

Aphanius transgrediens (Figure 1) is a killifish which should most urgently be protected among other killifishes in Turkey because of being in danger of extinction. The spring system of Lake Acıgöl is the only natural habitat of *A. transgrediens* and the lake possesses Turkey's largest sodium sulphate reserves extensively used in the industry. In addition to industrial activities, a dense *Gambusia holbrooki* (Figure 2 and 3) population, which should be regarded as a serious threat to the population of *A. transgrediens* in this area due to its invasive characteristics and direct predation on fry, was recorded. To this respect, we carried out a conservation study including both in-situ and ex-situ methods to ensure viable stocks of *A. transgrediens* in order to be reintroduced or restocked into either their native habitat or suitable, alternative habitats. A *Gambusia*-free pond was used to create sheltered breeding unit for in-situ conservation. In this presentation we demonstrated some reproductive properties of *Aphanius transgrediens* maintained in aquaria and some other conservation actions.

Figure 1. Male (Left) and Female (Right) *A. transgrediens*

Figure 2. Male (L) and Female (R) *G. holbrooki*

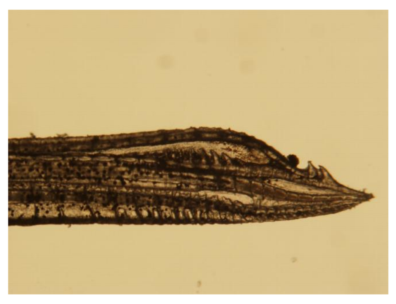


Figure 3. Male gonopodium of *G. holbrooki*

MATERIAL AND METHOD

Sampling procedure

The study has been being conducted since September 2012 in the spring system of the Lake Acıgöl (Figure 4 and 5). The first two surveys were for determining the distribution of *Gambusia* and *Aphanius* population in the area by CPUE (Catch per unit effort). Samplings were carried out by a hand seine net having 4 mm mesh size, 3 m length and 1 m height. The CPUE index is calculated as catch in fish number/area.

Figure 4. Acıgöl Lake

Figure 5. Acıgöl Lake

Acclimation and Breeding Procedure

A total of 20 individuals (10 males and 10 females) were transported to the FFBEL (Freshwater Fish Biology&Ecology Laboratory) in Hacettepe University (Figure 6, 7 and 8). Fish were kept in quarantine in 20 L plastic container for 2 days. After acclimation they all placed into a 120 L tank and fed by mosquito larvae and Tetrarubin flake food. Photoperiod was set as 14L-10D cycle and water temperature ranged between 22-24 °C. Two heaps of orlon mop were placed into the tank to imitate spawning substrate. Mops were controlled under a reading lamp day by day and eggs were collected into a petri dish containing commercial potable water having same temperature as the main tank. All eggs were photographed under Olympus SZX microscope day by day.

Figure 6. FFBEL

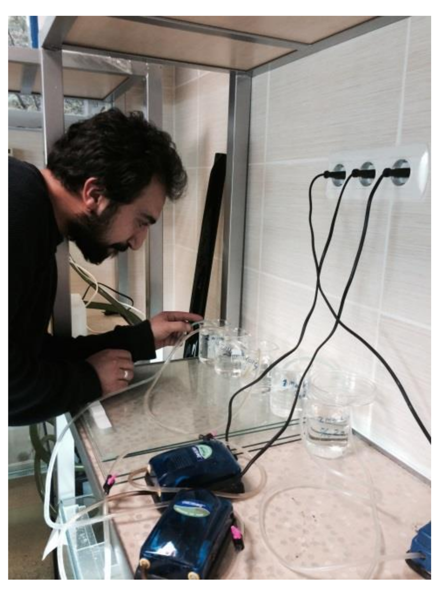


Figure 7. FFBEL

Figure 8. FFBEL

In-situ Conservation

In order to establish a continuous viable stock of *Aphainus transgrediens, a Gambusia*-free pond was used by the help of the local administrative authorities. The pond has a hemicycle shape with about 15 m diameter and about 1.5 m depth (Figure 9 and 10). Approximately 250 adult individual were transported into the pond in September 2013.

Figure 9. *Gambusia*-free pond

Figure 10. *Gambusia*-free pond

RESULTS

The proportions given in Table 1 were obtained by CPUE results, i.e. fish counts per unit area for each station. According to this table, all of the springs were occupied by *Gambusia holbrooki* and 6 of the 20 spring possesses *Aphanius transgrediens*. Two of these 6 springs (AcL S11 and AcL S20) were important in terms of being main reproduction areas for *Aphanius transgrediens*.

Table 1. Proportions of *Aphanius transgrediens* and *Gambusia holbrooki* in the springs

Station Code	*Aphanius tansgrediens* (%)	*Gambusia holbrooki* (%)
AcL S1	None	100
AcL S2	None	100
AcL S3	None	100
AcL S4	None	100
AcL S5	None	100
AcL S6	None	100
AcL S7	**5**	**95**
AcL S8	None	100
AcL S9	None	100
AcL S10	**10**	**90**
AcL S11	**70**	**30**
AcL S12	**40**	**60**
AcL S13	None	100
AcL S14	None	100
AcL S15	**1**	**99**
AcL S16	None	100
AcL S17	None	100
AcL S18	None	100
AcL S19	None	100
AcL S20	**90**	**10**

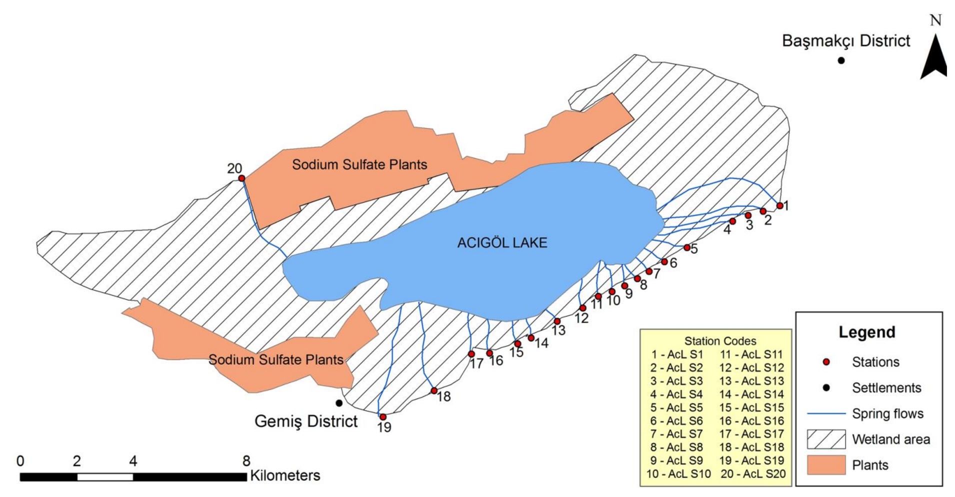


Figure 3. Study area and the stations

After 2 weeks of acclimation and maintenance, some male individuals started to exhibit mating behavior and their color turned to darker and brighter pattern. Spawning occurred within two days from this observation. The findings about hatching success, average hatching time and batch size per female is depicted in Table 2. In addition, egg development is demonstrated in Figure 10.

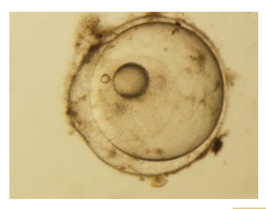
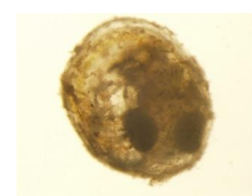


Table 2. Some spawning parameters of *Aphanius transgrediens*

N	Hatching success (%)	Av. Hatching Day (Min-Max) - Day	Batch size per female (Min-Max)
66	63,6	7,66 (6-10)	7,75 (11-5)



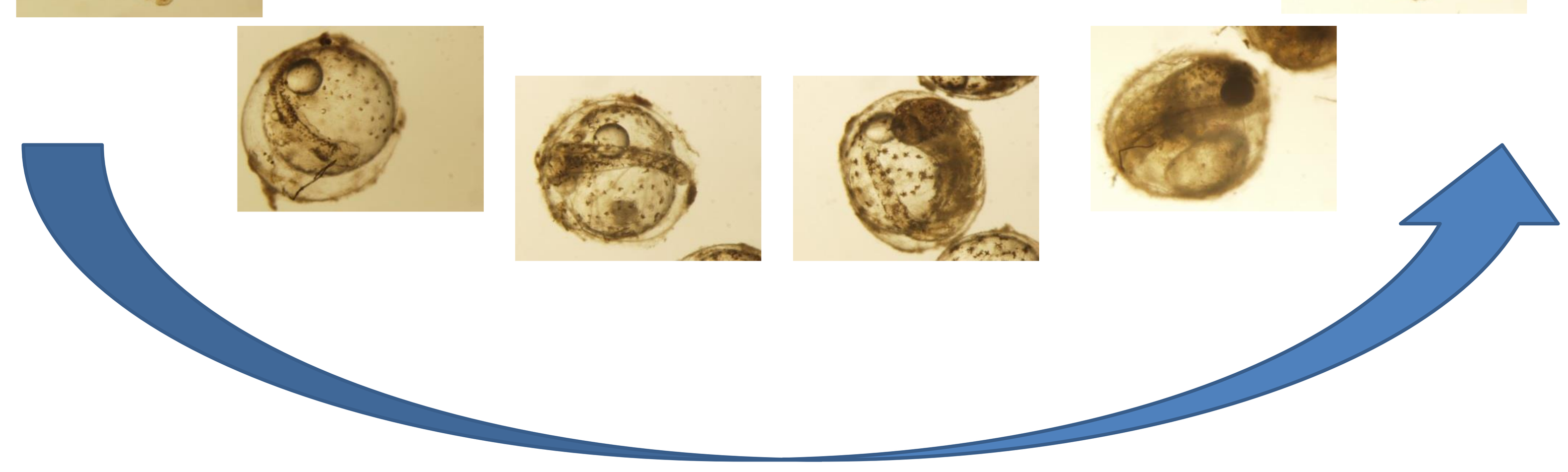


Figure 11. Egg development of *Aphanius transgrediens*

In-situ conservation efforts were not fully accomplished since the pond had not constructed for fish maintenance. Following months after fish introduction, water surface has rapidly covered with macro-algae and we could not able to get macro-algae under control. So we came to an agreement with the local administrative authorities on constructing new pond which will particularly function as stock development medium.

DISCUSSION

Fish samplings were carried out in almost all the springs around the lake. Since both species are small-sized fishes it was not easy to estimate exact proportions, even to prove occurrence of species. Thus, some relatively large springs are planned to be scanned by using environmental DNA.

Captive breeding is not a reliable tool for conservation as it may often results in genetic restrictions. However, if conservation genetic studies are applied properly, breeding viable stocks can be established as insurance for imperiled species. Laboratory studies applied in this study did not intended to breed *Aphanius transgrediens* for further reintroductions, instead it was for understanding mating behavior, hatching success and egg and larval development of the species.

In-situ conservation, especially creating new *Gambusia*-free habitat patches is much more effective than ex-situ captive breeding. Therefore, our future plans will be focused mainly on constructing new ponds which are able to support viable stocks of *Aphanius transgrediens* in its native habitat.

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