

First Attempt at Conservation of a Critically Endangered Cyprinodontid in Turkey*

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Received: 23.12.2014; Accepted: 23.12.2014; Published Online: 06.01.2015

ABSTRACT

This study has been conducted for conservation of an endemic killifish, *Aphanius transgrediens* (CR), in Lake Acıgöl since spring 2013. The only distribution area of *Aphanius transgrediens* is the ground water dependent wetland which consists of about 20 small freshwater springs. Habitat degradation and invasive mosquitofish (*Gambusia holbrooki*) are the most threatening factors in the area. The main purpose of initial attempts was based on the assessment of the general situation of the fish populations and habitat. The number and location of spring outlets occupied by *G. holbrooki* and *A. transgrediens* were determined by fish samplings and census. According to the investigations, only six of the 20 springs were found to host *Aphanius transgrediens*, and almost all springs were occupied by *Gambusia holbrooki*. Another two important outcomes of the study were environmental education for local elementary schools and determining some reproductive properties of *Aphanius transgrediens* under laboratory conditions. As a conclusion of this ongoing study, it was assumed that constructing integrated suitable shelters (e.g. *Gambusia*-free ponds) which serve as viable stock for *Aphanius transgrediens* are needed.

Key Words: *Aphanius transgrediens*, *Gambusia holbrooki*, Acıgöl, Invasive species, Conservation

Kritik Düzeyde Tehlike Altında Olan Bir Dişlisazancık Türünün Türkiye'deki İlk Koruma Girişimi

ÖZET

Bu çalışma Acıgöl'deki endemik bir dişlisazancık türü olan *Aphanius transgrediens* (CR)'in korunması amacıyla 2013 baharından beri yürütülmektedir. *Aphanius transgrediens*'in tek dağılım alanı yaklaşık 20 küçük kaynaktan oluşan Acıgöl akifer sistemidir. Habitat bozulması ve istilacı sivrisinek balığı (*Gambusia holbrooki*) alandaki en önemli tehdit faktörleridir. Yürütülen bu ilk koruma girişiminin başlıca amacı balık popülasyonları ve habitat yapısının genel değerlendirmesi üzerine dayanmaktadır. *Gambusia* ve *Aphanius* bireyleri tarafından kullanılan kaynakların sayısı ve kaynakların konumları balık örnekleme ve sayımı ile gerçekleştirilmiştir. Yapılan çalışmanın sonucuna göre, *Aphanius transgrediens*'in 20 kaynağın sadece altısında bulunduğu ve *Gambusia holbrooki*'nin tüm kaynakları işgal ettiği ortaya konulmuştur. Çalışmanın diğer önemli iki çıktısı da yerel ilköğretim okullarında çevresel eğitim seminerleri verilmesi ve *Aphanius transgrediens*'in bazı üreme özelliklerinin laboratuvar koşullarında belirlenmesidir. Devam eden bu çalışmanın sonucu olarak *Aphanius transgrediens* türünün popülasyon stoklarının devamlılığı için bölgede doğal yapıyla bütünleşik uygun sığınakların yapılması (Örneğin *Gambusia* içermeyen havuzlar) gerektiği düşünülmektedir.

Anahtar Kelimeler: *Aphanius transgrediens*, *Gambusia holbrooki*, Acıgöl, İstilacı tür, Koruma

INTRODUCTION

Turkey is assumed to be the center of diversification of *Aphanius* genus by owing 12 endemic and 2 widely-distributed species (Pfleiderer *et al.* 2014, Yoğurtçuoğlu and Ekmekçi 2013a). As hypothesized by several authors the genus has evolved from a common ancestor distributed along the Tethys Sea (Kosswig 1967, Hrbek and Meyer 2003). Many of the species have been threatened by several factors such as habitat destruction, water abstraction, pollution and invasive species, especially by *Gambusia holbrooki* (Carmona-Catot 2013, Alcaraz 2008, Rincon 2002). The endemic *Aphanius transgrediens* should most urgently be protected among other killifish species since its population has dramatically declined in recent years.

The distribution of *Aphanius transgrediens* is known only from springs around the Lake Acıgöl in southern central Turkey. The fish schools generally do not inhabit the lake itself due to high salinity, but they exhibit a regular movement between the lake shore and the spring outlets. Occurrence of *Gambusia holbrooki* in

* This study was presented at "6th National Limnology Symposium" between 25 and 28 August 2014 in Bursa, TURKEY

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this area has been recorded in 1992 (Wildekamp *et al.* 1999) and its population number has gradually been increasing (Yoğurtçuoğlu and Ekmekçi 2014). As shown in many case studies *Gambusia* is probably one of the main detrimental factors influencing the survival of native *Aphanius* species due to its competitive pressure and direct predation on fries (Alcaraz 2008, Caiola and Sostoa 2005, Rincon 2002).

In this study we aimed to determine the actual distribution and proportions of *Aphanius transgrediens* and *Gambusia holbrooki* in the area to find out the invasion impact and potential station(s) to be selected for in-situ stocking program. In addition, a preliminary survey about spawning of *Aphanius transgrediens* was conducted under laboratory conditions.

MATERIALS AND METHODS

Sampling Procedure

A total of 20 spring outlets (Figure 1) were sampled monthly from July 2013 to September 2013 in order to determine fish distribution in the area and to decide further stations to be investigated for the next 1 year. Samplings were carried out by a beach seine net having 3 m length, 1 m height, 2 depth and 4 mm mesh size. Fish were counted per unit area and classified by means of their species. Some physico-chemical characteristics of water such as temperature, salinity, pH and dissolved oxygen were measured in-situ for each station.

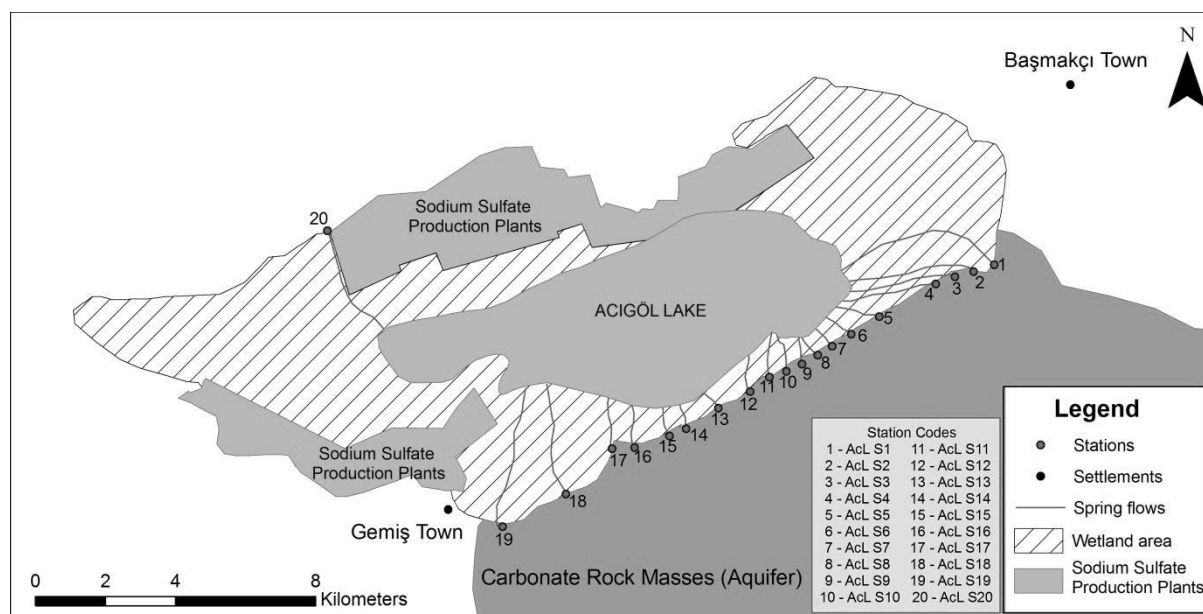


Figure 1. Study area and Sampling points.

Laboratory Study

Some observations on the spawning traits such as egg diameter, batch number and size hatching success of *Aphanius transgrediens* were carried out in the laboratory. Ten pairs of fish were acclimated to aquarium conditions in 40x40x80 cm aquaria. After one-month acclimation period orlon mops were placed into the aquaria as spawning substrata. The water temperature and photoperiod was adjusted to 23±2 °C and 14L:10D, respectively. Mops were scanned and eggs were collected and photographed daily. The eggs were maintained in 200 ml glass containers by pumping air for gas exchange and water in the containers was changed daily.

In situ Conservation and Public Awareness

A *Gambusia*-free pond which has been constructed for recreational purpose was used to stock development trial. The surface of the pond was covered by filamentous algae and it was cleaned up by local authority before fish

introduction. Our first environmental education study was organized for elementary schools of Başmakçı which is the closest village to our study area.

RESULTS

According to the fish samplings it was found that all the spring outlets were invaded by *Gambusia holbrooki*, and 6 spring outlets hosted *Aphanius transgrediens* at different proportions (Table 1). The average temperature and salinity values of the stations those of which include *Aphanius transgrediens* were given in the Table 2. Hatching success of 66 eggs collected from mops was 63.6 %. Hatching time was ranged between 6 and 10 days and the batch size ranged between 5 to 11 eggs (Table 3). In September 2013, 250 fish were collected from the AcL S20 station and introduced to the *Gambusia*-free pond. This procedure was accomplished by the participation of the local authority.

Two consecutive environmental education seminars were given for about 500 elementary school students. During presentations basic information about the importance of biodiversity, life cycle of *A. transgrediens* and impact of invasive species were emphasized. T-shirts demonstrating *Aphanius transgrediens* comics were distributed after presentation.

Table 1. Sampling locations and fish ratios per station.

Station Code	Latitude	Longitude	Surface Area (m ²)	<i>Gambusia holbrooki</i> (%)	<i>Aphanius transgrediens</i> (%)
AcL S1	37° 50.906'K	29° 59.435'D	15	100	None
AcL S2	37° 50.869'K	29° 58.959'D	9	100	None
AcL S3	37° 50.830'K	29° 58.829'D	45	100	None
AcL S4	37° 50.809'K	29° 58.685'D	1	100	None
AcL S5	37° 50.110'K	29° 57.440'D	87	100	None
AcL S6	37° 49.802'K	29° 56.940'D	77	100	None
*AcL S7	37° 49.476'K	29° 56.411'D	16	95	5
AcL S8	37° 49.434'K	29° 56.332'D	5	100	None
AcL S9	37° 49.209'K	29° 55.857'D	7	100	None
*AcL S10	37° 49.139'K	29° 55.760'D	132	90	10
*AcL S11	37° 49.080'K	29° 55.688'D	9000	30	70
*AcL S12	37° 49.031'K	29° 55.601'D	10800	60	40
AcL S13	37° 48.478'K	29° 54.523'D	5	100	None
AcL S14	37° 48.044'K	29° 53.755'D	28	100	None
AcL S15	37° 47.984'K	29° 53.635'D	23	100	None
*AcL S16	37° 47.749'K	29° 52.984'D	16	99	1
AcL S17	37° 47.702'K	29° 52.552'D	17	100	None
AcL S18	37° 46.941'K	29° 51.751'D	14	100	None
AcL S19	37° 46.346'K	29° 50.593'D	544	100	None
*AcL S20	37° 50.643'K	29° 46.769'D	3744	10	90

Table 2. The average water temperature and salinity values of the stations which include *Aphanius transgrediens*.

	Temperature (C°)	Salinity (ppt)
	Av. ± Sd(Min-Max)	Av. ± Sd(Min-Max)
Acl S7	21.0 ± 0.2 (20.7-21.5)	0.90 ± 0.05 (0.77-0.93)
Acl S10	20.5 ± 0.1 (20.1-20.6)	0.76 ± 0.05 (0.66-0.8)
Acl S11	19.7 ± 0.1 (19.4-19.8)	0.67 ± 0.04 (0.6-0.71)
Acl S12	21.7 ± 0.3 (21.1-22)	0.66 ± 0.05 (0.58-0.74)
Acl S15	19.1 ± 1.9 (15.8-21)	0.88 ± 0.06 (0.76-0.94)
Acl S20	17.2 ± 5.5 (11-25.9)	12.6 ± 13.8 (0.54-30.62)

Table 3. Some hatching parameters and egg diameter of *Aphanius transgrediens*.

N	Hatching Success (%)	Av. Hatching Time (Min-Max) - Day	Av. Daily Batch Number per Female (Min-Max)	Min and Max Egg Diameter (mm)
66	63.6	7.66 (6-10)	7.75 (5-11)	1.2 – 1.5

DISCUSSION

We presented the first conservation attempt for an endemic fish in its native range. The most important aim of the study was to ensure long-term survival of *Aphanius transgrediens* populations. However, it is an ongoing project which has been planned to continue until self-insurance of the species is provided although the options are limited. The findings of the first stage have only pictured the general view yet by making it clear that *Gambusia holbrooki* has dominated the habitat. Many studies claimed that mosquitofish have the potential to displace *Aphanius* through interference competition especially in small isolated microhabitats where spatial overlap is high (Riberio and Leunda 2012, Ruiz-Navarro 2013). As it is seen in the Table 1, *Aphanius* was dominant only in Acl S20 station that has exceptionally very uncertain salinity profile which may not be tolerated by *Gambusia holbrooki*. Other springs are almost identical in terms of many characteristics such as salinity, temperature or pH, but some have remarkably larger surface area, i.e. less confrontation chance which eventually means less competition. Thus, *Aphanius* was found to displace in small springs, but found in high proportions in relatively large ones.

The first spawning data for *Aphanius transgrediens* was also obtained by this preliminary study. Similar to all other members of the genus males become aggressive to each other and get brighter and darker colors in breeding condition. The eggs of *Aphanius transgrediens* are slightly smaller and less transparent than that of *A. mento* (Yoğurtçuoğlu *et al.* 2008) and *A. sureyanus* (Personal observation of the first author), and also smaller than non-spawned eggs of *A. marassentensis* (1.5 to 1.9 mm) (Yoğurtçuoğlu and Ekmekçi 2013b) and *A. villwocki* (Av. 1.80 mm) (Erbay 2014). 36.4 % of the eggs were unable to hatch. This reduced success may result from small incubation containers which provided small gas exchange surface. However, this parameter should be repeated with larger sample and in larger hatching chambers.

The next stages of the study which include assessing feeding competition and constructing more effective breeding ponds have already been commenced. Besides, an effective conservation effort needs to involve establishment of a conceptual strategy based upon a system approach considering the spatio-temporal dynamics of surface and groundwater systems, and these are also started. It is also important to strengthen communication with local community and authority to maintain in-situ conservation through long-time, for this purpose in order to increase public awareness further seminars at different schools are planned.

ACKNOWLEDGEMENTS

We would like to thank the Rufford Small Grants for their financial support through 12983-1 numbered project. We would also like to thank Dr. Mehmet Ekmekçi of the International Research Center for Karst Water

Resources for his support in hydrogeological assessment of the area and to Mr. Umut Uyan for his valuable help in field study.

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