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Research Article

Red-bellied Piranha *Pygocentrus nattereri* Kner, 1858 (Characiformes: Serrasalmidae) in open waters in R. Macedonia

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Abstract
There are so many reports of introduction of non-native fish species in Europe but reports including introduction of species from Serrasalmidae, especially from piranhas fish are scarce. This paper documents the first record of the occurrence of the exotic red bellied piranha, in Dojran Lake, the southern part of Republic of Macedonia. The morphological taxonomy revealed that the specimen is *Pygocentrus nattereri* Kner, 1860.

Key words: piranha, morphology, taxonomic identification.

Introduction

Alien fishes have been introduced with a variety of motives in Europe: ornament, sport, improvement of wild stocks, aquaculture, biological control and accident. In many cases fish introductions have been carried out for more than one motive. The trade industry of ornamental fishes have been appointed as a great source of introduced species worldwide (Padilla & Williams 2004). Recently, in Europe it seems attractive to have Amazonian ornamental fishes (piranha and pacu) in aquariums, even though due to irresponsible handling they end up in the open waters (Hanel 2011). Ellis (2006) has confirmed red piranha (genus *Pygocentrus*) for water bodies in UK. Gherardi et al. (2009) reported introduction of *Pygocentrus nattereri* to Europe without providing any detailed information. The presence of pacu fish (genus *Piaractus*) in open waters in Europe has been registered in Slovakia (Hensel 2004), United Kingdom (Zieba et al. 2010) and Croatia (Caleta 2011).

It is well known that both pacu and red piranha as ornamental fish can be reared in aquarium (Czeczuga 2010). Often this two species are confused with one another due to the similar coloration pattern of the body (Reenamole & D'cruz 2015). Knowing the facts that during juvenile stages *Piaractus brachypomus* has almost the same colour as *P. naterreri*, both species are easily mistaken for one another (Fink 1993). However, certain firmly established characteristics such as dentations in jaws, geometric shape of jaw teeth, size and coloration of adult fish, shape of the gas blader indisputably discriminate these two species into two different genera (Machado-Alison & Fink, 1996; Mirande 2010). Moreover, phylogenetic relationships between pacu and piranha groups within the Serrasalmidae are well resolved based on mitochondrial rRNA sequences (Orti 2008).

In R. Macedonia the legislature forbids the trade with real piranhas, on the other hand, within the aquarium industry it is legal to trade with herbivore pacu (an Amazonian species which resemble as a real piranha). Evidently both species are found in the European waters but never previously have been reported for the open waters of Macedonia. This paper presents a first record of red belied piranha (*Pygocentrus naterreri*) in Dojran Lake, followed by a complete morphological analysis of the newly detected introduced species for R. Macedonia.

Material and Methods

The fish was collected in august 2016 by local fisherman from Dojran Lake, one of the three natural lakes in the country. It is a tectonic lake situated 148 m above sea level, with surface area of 28 km^2 and maximum depth of 5 m. As a transboundary lake (Fig. 1), is shared by Republic of Macedonia and Greece. The fish was collected at the profile site known as "Achikot" at the Macedonian side (Fig 1). The single sample was brought to the Institution for Animal Science in Skopje for determination. After initial identification of the specimen as a member of piranhas, the sample was killed and stored in 10% formaldehyde solution for further laboratory analyses to confirm species level at the Department for Comparative Anatomy (Faculty of Natural Science and Mathematics).

Figure 1. Location map showing the first record of *P. nattereri* in R. Macedonia.

Morphometric measurements from the specimen were taken from point to point using digital caliper with an accuracy of 0.1 mm. Meristic data were counted under binocular microscope. Vertebrae, supraneuralia, pterygophores and caudal rays were counted from radiographs. For age determination scales were stained with Alizarin Red "S" and analyzed under a stereomicroscope WILD M3C. The taxonomic identification to species level was performed using key characters provided by Fink (1993). Morphometric measurements follow the methodology by Hubbs & Lagler (1958) with modifications explained bellow: Total length (TL), from the anteriormost point of the snout to the longest caudal fin ray; Fork length (FL), from the anteriormost point of the snout to the shortest caudal fin ray; Body length (L), from the anteriormost point of the snout to the end of scale cover; Standard length (SL), from the anteriormost point of the snout to the end of the hypural complex; Praedorsal distance (aD), from the anteriormost point of the snout to the origin of the first dorsal fin ray, not including the spine; Praeventral distance (pV), from the anteriormost point of the snout to the origin of the first pelvic fin ray; Praeanal distance (pA), from the anteriormost point of the snout to the origin of the first anal fin ray, not including the spine; Distance between dorsal fin origin to adipose fin origin (DoM); Distance from dorsal fin insertion to adipose fin origin (DiM); Caudal peduncle length (plc), from the insertion of caudal fin to end of hypural comlplex; Caudal peduncle length (pls), from the insertion of caudal fin to end of cover scale; Maximal body depth (H), at level of first dorsal; Caudal peduncle depth (h) minimal body depth; Length of dorsal fin base (Dl); Length of anal fin base (Al); Length of pectoral fin (Pl); Length of pelvic fin (Vl); Length of adipose fin base (Ml); Depth of dorsal fin at 1st ray

(Dh); Depth of anal fin at 2nd ray (Ah); Length of anterior part of gas bladder (Ga); Length of posterior part of gas bladder (Gp); Head length (HL), from the tip of the snout to end of opercular bone including the membrane; Preorbital distance (s) or snout distance, from the anteriormost point of the mandible to anterior orbit of eye; Horizontal eye diameter (e); Postorbital distance (po) from posterior orbit of eye to end of opercular bone; Postorbital distance (pm), from posterior orbit of eye to operculum, including the membrane; Interorbital width (io), dorsal measurement of the skull between the left and right orbit of eyes; Head depth at the level of eye center (eH); Maxilla length (mxl), from middle of maxilla to the corner with mandibula (Fig. 2).

Further taxonomic analysis and gender determination required a subsequent dissection. Measurements of gas bladder were taken and gonad sample was obtained for histological analyses. The gonads were removed, cut into small fragments, less than 5mm thick, and fixed in Bouin's fluid for 48 hour. Thereafter the tissues were routinely processed for embedding in paraffin. Each block was cut into 5 μm thick sections. The obtained sections were stained with haematoxylin an eosin (H&E).

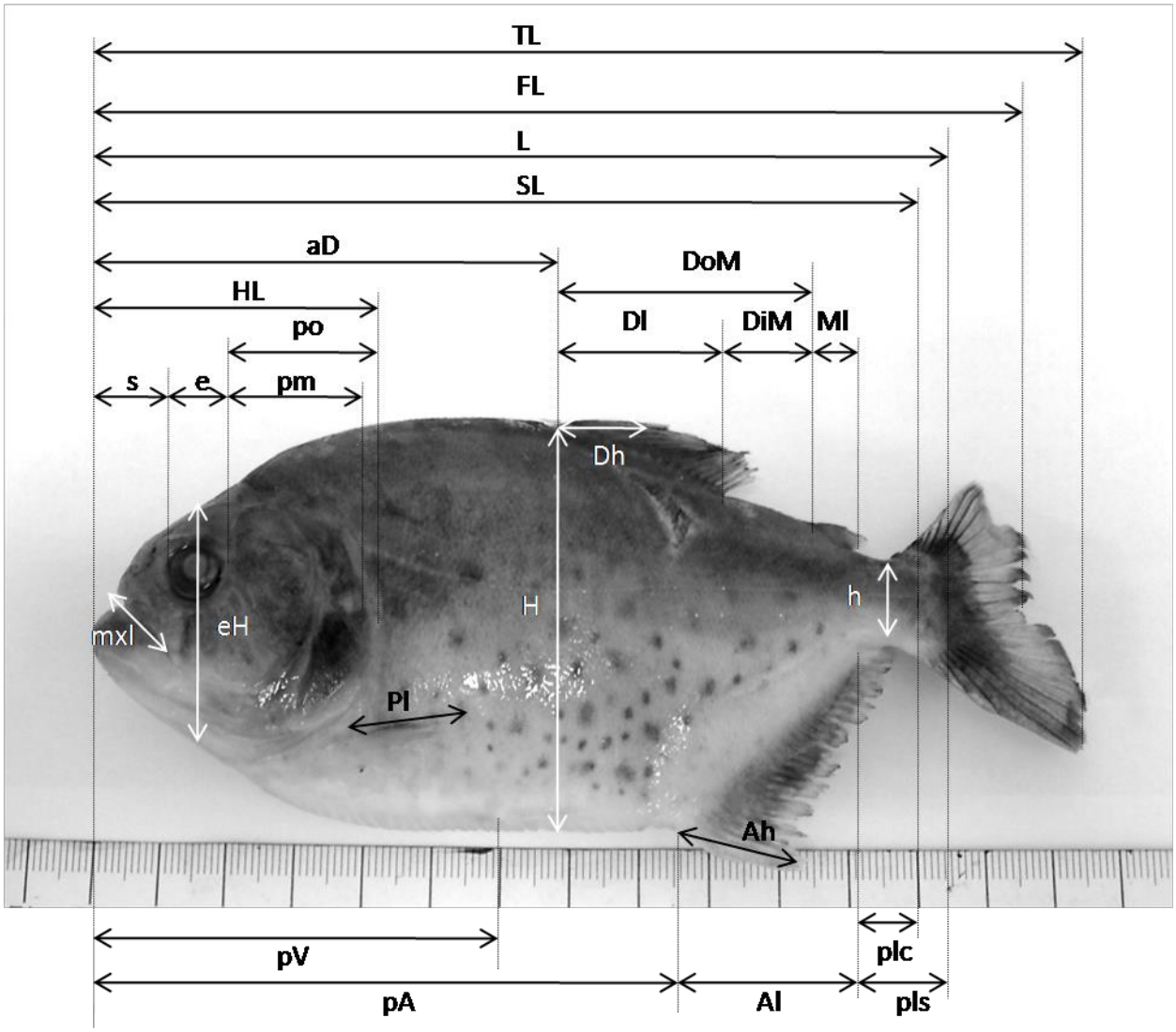

Figure 2. Morphometric measurements of *P. nattereri.* Abbreviations are given in material and methods. Length of pelvic fin was not possible to be marked in the photograph.

Results and Discussion

The alien fish specimen from Dojran Lake analyzed at Department for Comparative Anatomy was identified as *Pygocentrus nattereri* (Kner, 1858), in contrast to previous case (reported in the media) of identification

as red bellied pacu, *Piaractus brachypomus* (Cuvier, 1818). The specimen, identified as *P. nattereri* possess all features diagnostic of *Pygocentrus* such as: prepelvic serrae, 21 (within the range for the genus 13-21); lack of ectopterygoid teeth; gas bladder with the posterior chamber much truncated, as opposed to elongate in outgroups (Fig. 3); frontal, parietal, and pterotic bones with crests surrounding the sensory canals of the head; the head is substantially wider than in other piranhas, at comparable body sizes. In specimen measured for this study, the mean of head width as a percent of SL is 14.26 (within the range for the genus 10.7 - 19.5). According Fink (1993) in all non-*Pygocentrus* species, head width is less than in *Pygocentrus* specimens.

Figure 3. Red pigmentation of the anterior and posterior part of the orbit in *P. nattereri* from Dojran Lake.

Having in mind that *P. nattereri* and *Pygocentrus cariba* (Humboldt, 1821) are morphologically very similar and color patterns may vary considerably (Fink 1993), additional analysis were undertaken during the taxonomic determination of the specimen from Dojran Lake. Except standard morphomertic measurements, osteological analyses based on radiography were performed as well (Fig. 4). Morphometric measurements and meristic counts including osteological ones are presented in Tables 1 and 2.

The identification of *P. nattereri* to species level was performed based on the absence of humeral spot as well as the presence of 5 supraneural bones which lead to discrimination from *P. cariba.* The capture sample has oval-shaped body with convex profile, with SL of 121.72 mm. The dorsal part of the body possess predominantly dark grayish-silvery coloration, which becomes reddish in the region of the pectoral, pelvic, and anal fins. Redish colour is present anterior and posterior to the papilla (Fig. 3). First scale of the lateral line with a pore for the lateral line canal is the 8th scale, after which 77 scales follow to the end of hypural complex. Nine scales can be counted after the end of hypural complex, location easily perceptible by bending of the tail towards either side of the body. Although very small and on some places irregular, the flanks of the body below the dorsal fin did presented regular 36 scale rows above and below of the lateral line.

Dorsal fin ray are presented with 2 unbranched, but segmented and 15 branched and segmented rays, not counting the one modified anterior pointed spine (Fig. 4). Anal fin is presented with 3 unbranched rays, the first one is not segmented, while 2 are unbranched and segmented, 27 are branched and segmented. Preanal spine is paired, very small with flat endings and not included in the count. Last two rays of both anal and dorsal fin are attached to one pterygophore. First pterygophore of dorsal fin being between 6th and 7th neural spine (Fig. 4) clearly discriminate our specimen from *Pygocentrus piraya* a species, according to Fink (1993) with first pterygophore between 7th and 8th neural spine.

Table 1. The main morphometric parameters and the body proportions of *P. nattereri* from Dojran Lake. Abbreviations are given in Material and Methods.

character	in mm	percentages
TL	147.05	
FL	135.57	
L	127.01	
SL	121.72	
		in % of SL
aD	74.36	61.09
pV	64.04	52.61
pA	86.66	71.20
DoM	38.63	31.74
DiM	12.72	10.45
plc	14.29	11.74
pls	18.91	15.54
H	58.8	48.31
h	12.59	10.34
Dl	25.34	20.82
Al	35.27	28.98
Pl	22.38	18.39
Vl	11.85	9.74
Ml	6.41	5.27
Hh	23.45	19.27
Ah	18.08	14.85
Ga	27.89	22.91
Gp	20.81	17.10
HL	41.48	34.08
io	17.36	14.26
		in % of HL
s	15.37	37.05
e	8.38	20.20
po	22.06	53.18
pm	23.31	56.20
io	17.36	41.85
eH	31.61	76.21
mxl	15.09	36.38

The teeth are resistant, sharp, strong, with tricuspidal shape, which reflects the voracious carnivorous feeding behavior of these fishes. The species lack ectopterygoid teeth. The gas bladder terminates posteriorly within the body cavity and is build by two differently shaped chambers - an anterior one with oval shape, and a truncated posterior one. Two extrinsic muscles are attached above anterior chamber (Fig. 5).

Age determination showed that the single specimen of Dojran Lake is 1+ year old while histological analyses of the gonad tissue show that fish is female from pre-vitellogenesis stage (Fig. 6).

Table 2. The main meristic parameters of *P. nattereri* from Dojran Lake.

character	results
Dorsal fin rays	2+15
Anal fin rays	3+27
Pectoral fin rays	15
Pelvic fin rays	7
Procurient rays of caudal fin in upper lobe	5
Main rays of caudal fin in upper lobe	12
Procurient rays of caudal fin in lower lobe	5
Main rays of caudal fin in lower lobe	11
Dorsal fin pterygophores	15 + stay
Anal fin pterygophores	27
Position of first dorsal pterygophore	between 6th and 7th
Lateral line scales	95 (7+79+9)
Scales above lateral line	36
Scales below lateral line	36
Scutes (serrae) prepelvic	21
Scutes (serrae) postpelvic	9
Vertebrae	36
Supraneuralia	5

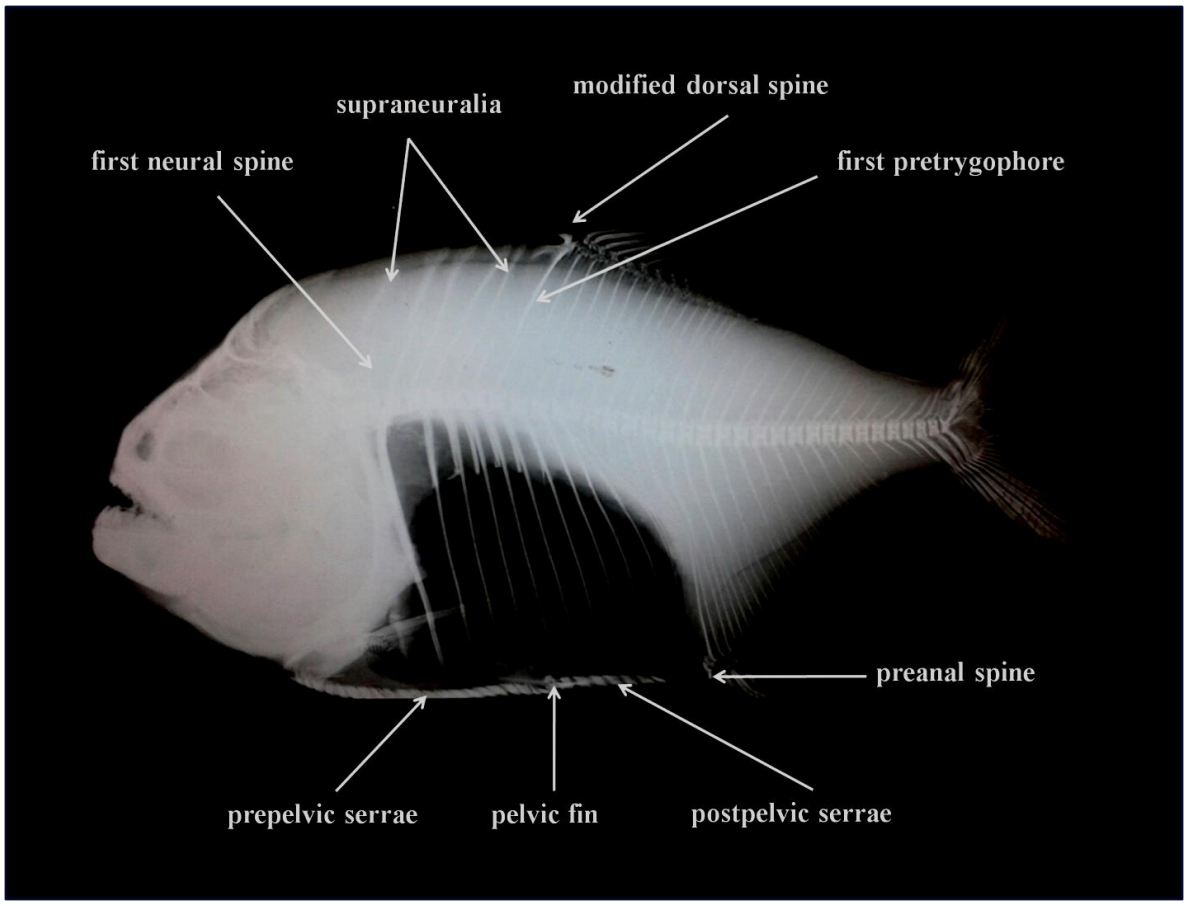

Figure 4. Some of the osteological characters of *P. nattereri* from Dojran Lake.

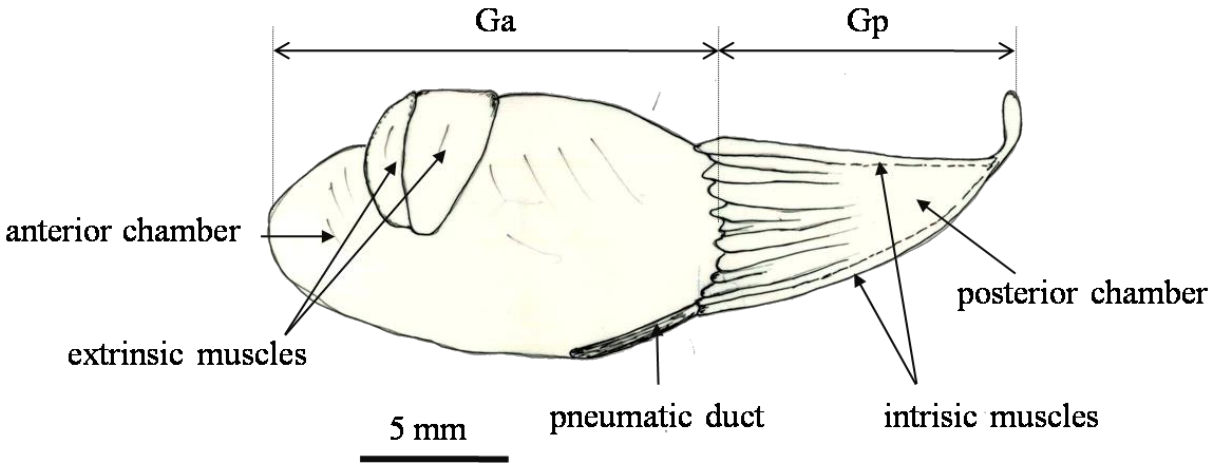

Figure 5. Gas bladder of *P. nattereri* from Dojran Lake. Abbreviations are given in material and methods.

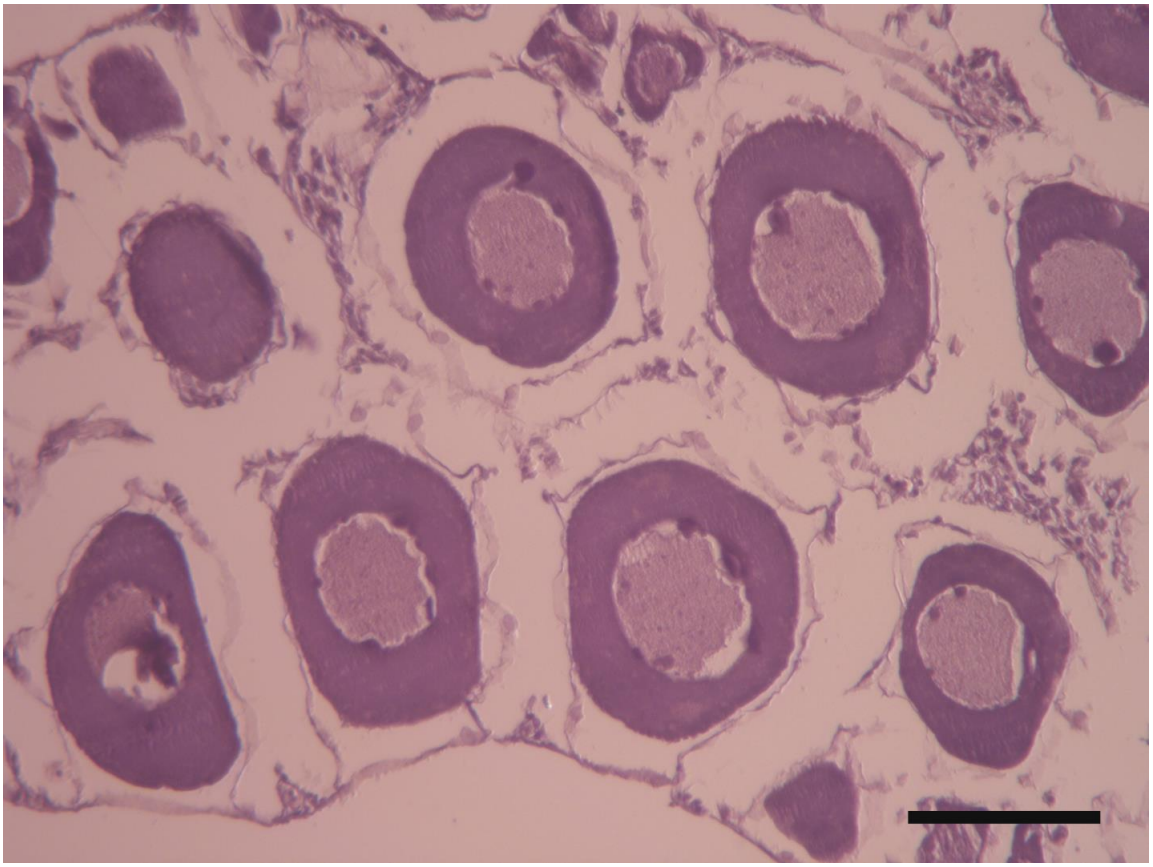

Figure 6. Light micrograph of the ovaries of a female *P. nattereri*. H&E. Bar line = 0.1mm.

The question is can this aquarium species breed in Dojran Lake and establish a viable population, knowing the fact that lake has a mean annual temperature of 14.2 ºC, with minimal temperature of 3.6 ºC in January and maximal 24.7 ºC for 120 days of the year. Being an eutrophic lake surely means a substantiate food source for this fish especially during the summer period. Despite the open speculations about the reasons of why someone introduced this species in Dojran Lake, and the questions of when and how many samples there might be, serious analyses need to be conducted regarding the biology and population in this environment. Finally, regulations to prevent the introduction of unwanted aquarium species, as well as education of all participants in the aquarium trade industry, should be implored with better authority (Padilla & Williams 2004; Knight 2010).

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