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First Record of a Feral Population of Green Swordtail (*Xiphophorus hellerii*) with an Additional Record of Guppy (*Poecilia reticulata*) in Turkish Freshwaters

Türkiye Tatlısularında Yeşil Kılıçkuyruk (*Xiphophorus hellerii*)'nin İlk ve Lepistes (*Poecilia reticulata*)'nın İlave Kaydı

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ABSTRACT

Livebearer poeciliids, with few exceptions, are among the most popular and highly traded ornamental fish species. Therefore, they are frequently introduced to natural waters outside their native range mostly by aquarium hobbyists. Here we report feral populations of two exotic poeciliid species, the green swordtail (*Xiphophorus hellerii*) and the guppy (*Poecilia reticulata*), established in a hot spring in the Eastern Turkey (38°34′ 32″N, 37°29′21″E). This is the first documented record of swordtail in Turkey and possibly the first records of the two species in the wider Euphrates Basin. More research is needed to address the potential impacts of these two poeciliids on the native biota, and also their further spread chance and management options.

Key Words

Aquarium fish, alien species, establishment, Euphrates Basin.

ÖZ

Canlı doğuran poeciliidler, birkaç istisna dışında en popüler ve en çok ticareti yapılan süs balığı türleri arasında yer almaktadır. Dolayısıyla, bu türler genellikle akvaryum meraklıları tarafından kendi doğal alanlarının dışındaki doğal sulara sıklıkla taşınmaktadır. Bu çalışmada, Türkiye'nin doğusunda (38°34′ 32″K, 37°29′21″D) bir sıcaksu kaynağında iki egzotik poeciliid türü, kılıçkuyruk (*Xiphophorus hellerii*) ve lepistes (*Poecilia reticulata*) türlerinin yabani popülasyonları kaydedilmiştir. Bu, Türkiye'deki belgelenmiş ilk kılıçkuyruk kaydı olup, muhtemelen belirtilen iki türün Fırat Havzası'ndaki ilk kayıtlarıdır. Bu iki poeciliid türünün doğal biyota üzerindeki potansiyel etkilerini belirlemek, ayrıca yayılma şanslarını ve yönetim seçeneklerini de ele alabilmek için daha fazla araştırmaya ihtiyaç vardır.

Anahtar Kelimeler

Akvaryum balığı, yabancı tür, yerleşme, Fırat Havzası .

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INTRODUCTION

Biological invasion has been recognized as one of the most serious environmental problems since the last few decades, and it is one of the most important issues in conservation biology today [1]. Invasive alien species (IAS) are also an important part of this issue in marine, brackish and freshwater ecosystems of the world; especially fishes are widely introduced and translocated globally [2-4]. One of the major pathways by which IAS are introduced and spread throughout the world is the ornamental fish trade and associated aquarium releases [5-8]. Today, more than 2 billion live ornamental fish are moved annually worldwide every year, with exports coming from 100 countries [9]. In recent years, much interest has been shown by Turkish hobbyists to keep ornamental fish. According to Turkmen and Alpbaz [10] the dealers have imported 227 freshwater and 51 marine species, and no recent records could be obtained. However, almost 30 freshwater fish species including the livebearers, neon, characids, angelfish, goldfish and zebra fish are major groups in the aquarium sector throughout the World [11]. With the growing pursuit of aquarium keeping, several ornamental fish species have recently been documented from Turkish freshwaters, all of which are directly associated with aquarium release. Some records are diagnosed as occasional, e.g. red piranha, *Pygocentrus nattereri* [12], jewel fish, *Hemicromis letourneuxi* [13] and giant pangasius, *Pangasius sanitwongsei* [14]; whereas, some others including *Pterygoplichthys* spp., and *Clarias batrachus* have established viable populations in natural water bodies [15, 16].

The latest record of established population of ornamental fish is the poeciliid guppy (*Poecilia reticulata*), reported from western Turkey [17]. The family Poeciliidae is a large group of fishes consisting of small-bodied and highly prolific species [18]. Many poeciliids, particularly guppies, mollies and swordtails, are also known as the most popular aquarium species; therefore, they are frequently released to and recorded from natural ecosystems in many countries [2, 19-21]. Moreover, some poeciliids are even known to be the worst invasive species in the world (e.g. *Gambusia* spp.). The occurrence of a feral population of *P. reticulata* in Turkey highlighted once again the problem of aquarium releases, yet too little attention has been paid to the possible consequences related to the IAS and the role of hot springs and anthropogenically modified habitats as recipient environments. Many successful introductions likely have gone unnoticed or remained localized in these habitats, especially of small species like poeciliids.

During field expeditions in the Euphrates River basin in November 2020 and June 2021, we noticed a breeding poeciliid populations in a hot spring near Tohma Stream (Malatya, in the Eastern Turkey). Here, we recognize and report these populations as the green swordtail (*Xiphophorus hellerii*) and the guppy (*P. reticulata*), with the former being the first record ever for Turkish freshwaters. Despite the prediction that *X. hellerii* may become established and spread easier than the guppy due to its higher ability to cope with the flow rate and water temperature [22], this remains untested and poorly understood. We also practically compare the establishment success of two co-occurring species in a narrow ditch with high flow rate by estimating some basic population parameters, including population size, sex ratio and gravidity of the females. We also had informed by the locals about the origin and timing of the introduction of these two ornamental poeciliids and asked about the eradication, control or monitoring attempts, if any. We suggest that early records on alien species are very important and useful to provide public awareness on illegal ornamental fish release and prevent range expansion of aquarium species.

MATERIALS and METHODS

Study area and data collection

The examined populations inhabit an artificial drainage canal (a narrow ditch with ca. 100 cm wide and 50 cm deep) (Figure 1a, b) and nearby karstic hollows (Figure 1c) fed by a hot spring near to Tohma Stream, in Euphrates River Basin in Darende-Malatya (38°34′ 32″N, 37°29′21″E).

The water temperature (T), specific electrical conductivity (SPC), dissolved oxygen and salinity were measured in situ using a multiparameter instrument (YSI Pro Plus, Yellow Springs Instruments, Yellow Springs, OH, USA). Fish were captured using a dipnet with 50 cm diameter and 1 mm mesh size on 04 June 2021. Catching effort was kept within comparable bounds as 15 minutes sampling that is corresponding to five net chasing events, with every chase the net was drifted ca. 1 m alongside the ditch. The collected fish were over-anaesthetized with clove oil and preserved in 4% formaldehyde solution and transported to the laboratory for further analysis. Fish collections were approved and

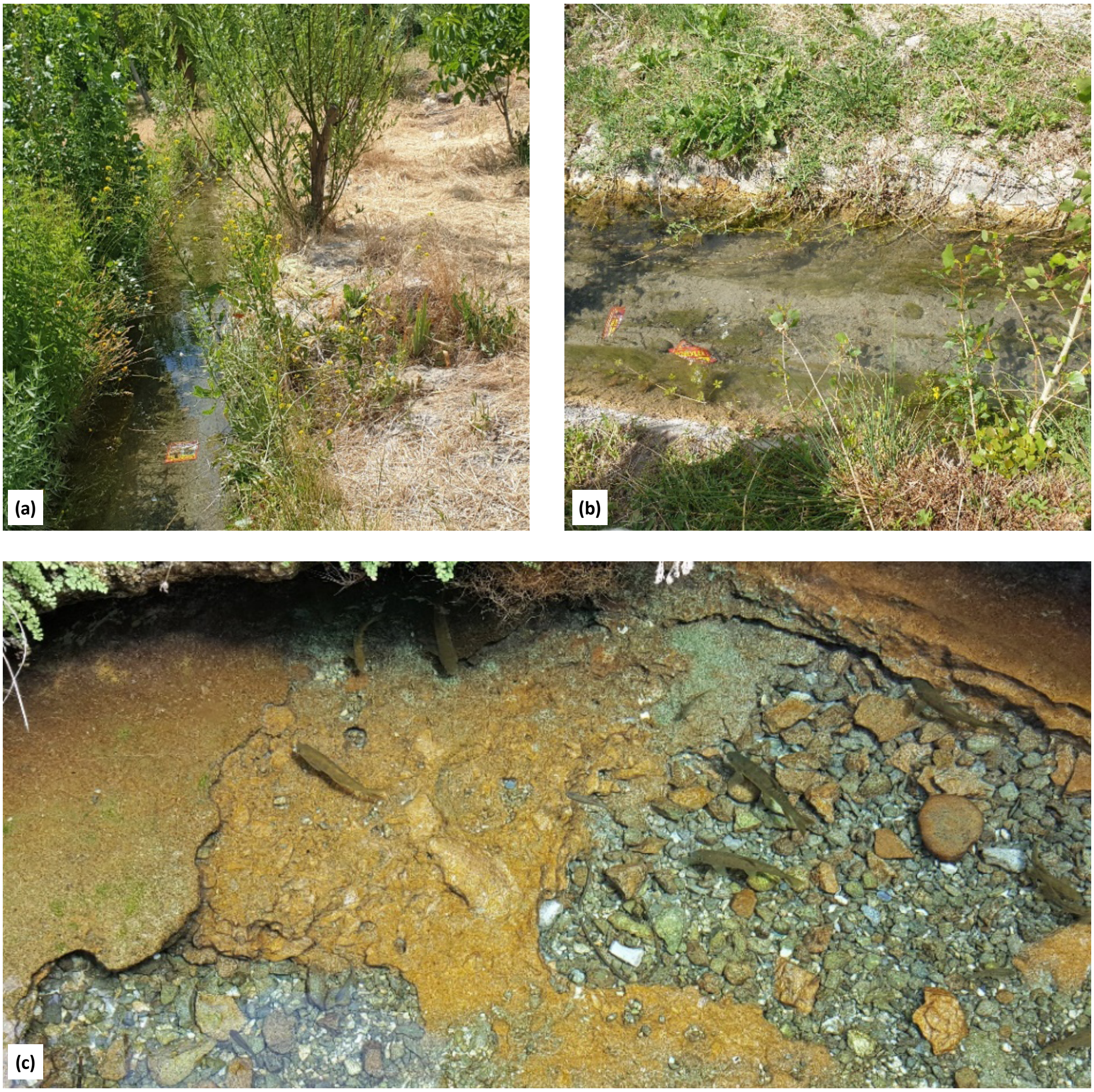

Figure 1. The artificial drainage channel (a, b) and the karstic hollows (c) near to Tohma Stream (Malatya, Turkey).

granted by the Ministry of Agriculture and Forestry, General Directorate of Fisheries and Aquaculture (codes for the protocols: B.12.0.KKG.0.17/106.01-11-01 and 67852565-140.03.03-E.4052273). All applicable international, national or institutional guidelines for the care and use of animals were followed.

Laboratory procedure

The standard length (SL) was measured using a digital caliper to the nearest 0.01 mm. The fish specimens were identified by examining morphological characteristics following Froese and Pauly [23] and diagnostic characters given by Kallman et al. [24] for *X. hellerii*, by McDowall [25], Poeser et al. [26] and Rosso et al. [27] for *P. reticulata*. Methods for counts and measurements follow Kottelat & Freyhof [18]. Standard length (SL) is measured from the tip of the snout to the posterior extremity of the hypural complex. The last two-branched rays articulating on a single pterygiophore in the dorsal and anal fins are counted as „1½". Sex was determined from external characteristics since both species exhibit sexual dimorphism (Figure 2), in which males possess a highly modified anal fin called a gonopodium. In order to examine reproductive status, females were dissected and total numbers of embryos at different developmental stages were determined and counted.

Figure 2. Green swordtail *(Xiphophorus hellerii*) (left) and guppy (*Poecilia reticulata*) (right) specimens from hot springs near Tohma Stream (arrows show gonopodium) SL: 40 mm (both sexes of swordtail), 20 mm (male guppy) and 30 mm (female guppy).

RESULTS

We observed co-occurring populations of *X. hellerii* and *P. reticulata* in the drainage channels of hot spring with high numbers of individuals. *Xiphophorus hellerii* was also found in the karstic hollows of this spring together with *Capoeta damascina*, *Alburnoides velioglui* and *Alburnus sellal*. Both of the species had wild-type ornamentation. In total, 58 green swordtail (45 females and 12 males) and 231 guppy (124 females and 107 males) individuals were collected by the five random net chasing events from the canals. Females were dominant in both populations. The SL ranged from 25.21 to 40.81 mm in males and from 12.03 to 45.60 mm in females for *X. hellerii*; and from 16.93 to 22.96 mm in males and from 12.99 to 35.02 mm in females for *P. reticulata* (Table 1). The largest specimen of *X. hellerii* was 45.60 mm SL, and the largest *P. reticulata* was 35.02 mm SL, both individuals were female.

Table 1. Standard length of *X. helleri* and *P. reticulata* specimens (SL: Standard length, n: number of specimens, min: minimum, max: maximum, SD: Standard deviation).

Species	SL_{MALE} (mm)				SL_{FEMALE} (mm)			
	n	min	max	Mean ±SD	n	min	max	Mean ±SD
Xiphophorus hellerii	6	25.21	40.81	37.56 ± 3.25	26	12.03	45.60	28.30 ± 9.30
Poecilia reticulata	25	16.93	22.96	19.78 ± 2.30	25	12.99	35.02	24.49 ± 7.99

Based on the species identification keys both species were easily distinguished by the species-specific characters; i.e. brightly colored males with a black spot on the flank in *P. reticulata*; and a long dorsal fin with 11½ – 12½ branched rays, a medium to large swordtail with a long straight caudal appendage; dusky, brownish or red midlateral stripe; 2 additional reddish stripes above midlateral line and one beneath in *X. hellerii*.

Among *X. hellerii* specimens, 10 females were gravid (Figure 3, Table 2). Embryo numbers in different developmental stages ranged between 13 and 42.

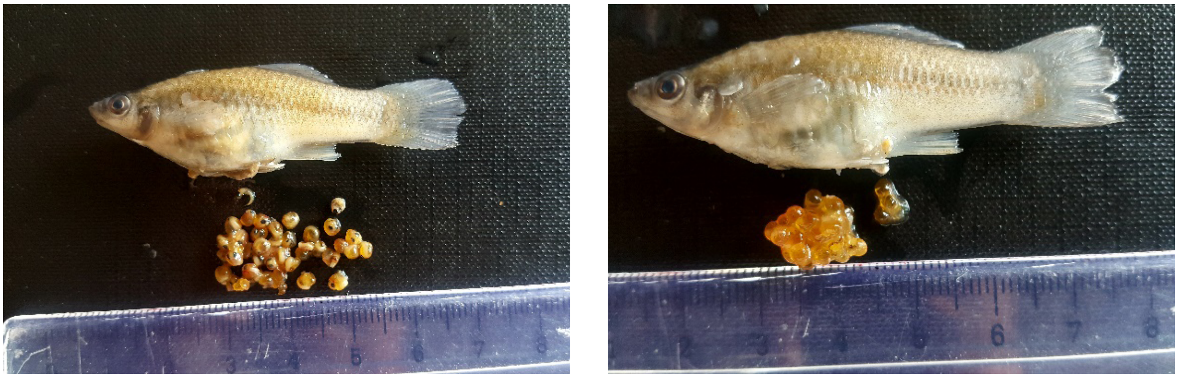

Figure 3. Eggs and embryos in different developmental stages in *X. helleri*. SL: 45,08 mm (left) and 45,60 mm (right).

Table 2. Embryo numbers in gravid females of *X. helleri* and *P. reticulata* specimens (n: number of specimens, min: minimum, max: maximum, SD: Standard deviation).

Species	n	Embryo Numbers		
		min	max	Mean ±SD
Xiphophorus hellerii	10	13	42	31.33 ± 6.55
Poecilia reticulata	16	3	27	14.83 ± 8.43

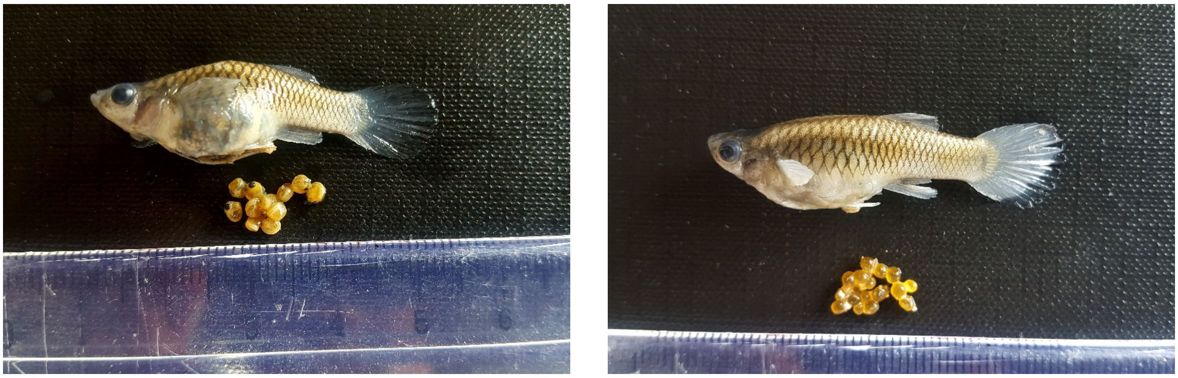

Figure 4. Eggs and embryos in different developmental stages in *P. reticulata*. SL: 30,51 mm (left) and 35,10 mm (right).

Poecilia reticulata females larger than 15 mm were gravid. It was determined that 16 of 124 guppy females were gravid, while rest of the females have eggs in different developmental stages (Figure 4).

Water temperature was measured as 21°C. Water was slightly alkaline, with a pH value of 7.84. Specific conductivity of the water was 1035 µS/cm. Dissolved oxygen content was 12.5 mg/L.

We were informed by the local administration that these species had been released to the hot spring system by the aquarium hobbyists nearly seven years ago.

DISCUSSION

The finding of a feral *X. helleri* population in a natural hot spring system in Tohma Stream drainage is the first record of the species in the Turkish freshwaters. The species is native to tropical Central America including Rio Nantla, Veracruz in Mexico to northwestern Honduras [28] and has been introduced more than 30 countries as an ornamental species [29]. It has established breeding invasive populations in many countries including Iran [19], Greece [30], Israel [23]. To the best of authors' knowledge, the current study also represents the first established record of *X. helleri* and *P. reticulata* in the wider Euphrates drainage. All of the established ornamental fish records, except the gold fish (*Carassius auratus*), in open water habitats in Turkey were documented from hot springs or their immediate outflows [13, 15-17]. The suitability of hot springs for tropical IAS has long been known by the frequent records worldwide. However, the impacts of these warm water species are supposed to remain local, and their spread from these springs is usually impossible [4, 31]. Nevertheless, native fauna with restricted distributions (i.e. to certain hot springs) have the potential to decline or even extirpate due to aquarium release of warm water IAS. In the present study, we found no such native fish restricted to the hot spring, or none recorded from Tohma Stream itself (Cüneyt Kaya, pers. comm., 2021).

We found color polymorphic male phenotypes in *P. reticulata* (Figure 5) similar to those found in natural guppy populations. Similarly, *X. hellerii* individuals had the wild-type morphology (greenish or grey vs. usually orange or red in captive-bred), with few individuals having minimal difference in color. This observation may support previous researchers whose findings suggest that fish possessing wild-form color are the most likely to survive introduction [32, 33]. Our data on maximum body size also indicated a feralization process for both species. Male and female individuals of *X. hellerii* can reach 140 and 160 mm total length (TL), though are usually much smaller (average approximately 50–70 mm) [23]. In the present study, the largest *X. hellerii* individuals were 40.81 mm SL (male) and 45.60 mm SL (female). Similarly, *P. reticulata* can reach 25–35 mm SL in males and 40–60 mm SL in females, whereas our largest individuals had 22.96 mm SL (male) and 35.02 mm SL (female) (Table 1). Previous studies have documented that domesticated strains of poeciliids are usually larger and more colorful than their wild populations likely due to the selecting forces (e.g. predation) acting upon the body size and shape (e.g. elongated fins) or ornamentation [34, 35]. Given this phenotypic change from domestic type to the wild type occurred throughout the observed individuals, we concluded that populations are self-sustaining at least over several generations.

Poeciliids can reproduction almost throughout the year [36, 37]. Additionally, fecundity is usually low in poeciliid species. Females of *X. hellerii* produces 20 to 240 young after a gestation period of 24 to 30 days [38], whereas *P. reticulata* can give birth to 20 to 40 live young after a gestation period of four to six weeks [23]. Coherently, we observed fewer fertilized eggs in *P. reticulata* than *X. hellerii* (Table 2), but higher population number of *P. reticulata* (Table 1), suggesting that the mortality would be higher in *X. hellerii.* This finding may be due to the larger body size and prolonged courtship behavior of *X. hellerii*, which may increase their susceptibility to predation [39].

The female-biased sex ratio in both of the species might be related to several factors including brighter colors, higher susceptibility to stressors or mating behaviors of males [23, 40].

Figure 5. Males with different phenotypes among *P. reticulata* samples.

Magalhães and Jacobi [37] stated that many species of Poeciliidae are considered among the top invasive freshwater fishes and have negative impact on zooplankton, damselfly/dragonfly populations, and may cause decline and extinction of native fishes; additionally they are known to prey upon amphibian eggs and larval stages. *X. hellerii* has fairly broader environmental tolerance, especially escaped populations are very tolerant to cold waters than aquaculture populations [22]. Plasticity in environmental preferences can facilitate spread of *X. helleri* in different habitats and geographical regions. On the other hand, *P. reticulata* has a wide salinity range, but needs strictly warm temperatures approximately 20–24°C, and it cannot tolerate water temperatures below 15°C [23]. Therefore, environmental conditions in the study area seems more favorable for *X. hellerii*. In fact, *X. hellerii* is a fish native to ponds, rivers, swift-flowing streams and other aquatic environments in its natural distribution area [22]. Negative impacts of *X. helleri* on aquatic ecosystems and indigenous fishes were observed in the eastern Australia [41, 42], Hawaii [43], Hong Kong [44], Israel [45] and the USA [46], where their impacts were prominent particularly when it co-occurs with other introduced poeciliids (*Gambusia*, *Poecilia* or *Xiphophorus* spp.) [29]. Both *X. helleri* and *P. reticulata* have established non-native populations in the wild and assumed to pose a high risk of invasiveness in Greece [30]. Non-native ornamental fish species could cause similar threats to biodiversity in neighbouring regions [47]. Therefore, Wei et al. [47] suggested that collaboration between neighbouring countries could be useful in order to develop management and regulation strategies for the conservation of native species.

After customers have purchased the fishes, disposal of live fish to water bodies is considered the last step of aquarium trade [48]. Therefore, we should focus on the behavior of fish owners as a major cause of aquarium fish introductions to natural waters. As stated by Maceda-Veiga et al. [8] the aquarium trade has been identified as an important vector of invasive species that has been investigated mostly in North America and partly in southwestern Europe. In Turkey, the freshwater fish fauna is highly rich and unique that is nearly half of the species are endemic and one third of them placed in IUCN red list. Therefore, there is a need to increase public awareness for preventing the release of ornamental aquatic species to the natural waters to protect the biodiversity. Gertzen et al [48] suggested that modifying human behavior is the key to reducing the potential ecological and economic impacts of live aquarium release. Although many people tend to think that releasing the aquarium species to the nature is more humane than kill these animals, but the consequences of the introduction are poorly known. Public awereness through education programs and public service tools can be the first step for a proper management, particularly by targeting retailers and aquarium hobbyists [8]. Improvement of current legislation on the non-native species by integrating ornamental speies can be highly beneficial.

We continue further studies in the study area to get a better insight into the possible impacts of these populations in Tohma Stream drainage.

References

1. R.B. Primack, Essentials of Conservation Biology, 5th Ed. USA: Sinauer Associates, Inc., 2010.
2. R.L. Welcomme, International Introductions of Inland Aquatic Species, FAO Fisheries Technical Paper, No:294., 1988.
3. C.S. Kolar, D.M. Lodge, Progress in invasion biology: predicting invaders, Trends Ecol. Evol., 16 (2001) 199–204.
4. I.C. Duggan, The freshwater aquarium trade as a vector for incidental invertebrate fauna, Biological Invasions. 12 (2010) 3757–3770.
5. J.L. Lockwood, M.F. Hoopes, M.P. Marchetti, Invasion Ecology, Blackwell Publishing, UK, 2007.
6. R.E. Gozlan, Introduction of non-native freshwater fish: is it all bad? Fish and Fisheries. 9 (2008) 106–115.
7. G.H. Copp, L. Villizi, R.E. Gozlan, The demography of introduction pathways, propagule pressure and occurrences of nonnative freshwater fish in England. Aquatic Conservation- Marine and Freshwater Ecosystems. 20 (2010) 595-601.
8. A. Maceda-Veiga, J. Escribano-Alacid, A. De Sostoa, E. Garcia-Berthou, The aquarium trade as a potential source of fish introductions in Southwestern Europe, Biological Invasions. 15 (2013) 2707–2716.
9. P. Monticini, The Ornamental Fish Trade. Production and Commerce of Ornamental Fish: Technical-Managerial and Legislative Aspects. GLOBEFISH Research Programme, Vol. 102. (2010) Rome, FAO, p134, 2010.
10. G. Türkmen, A. Alpbaz, Studies on Aquarium Fish Imported to Turkey and the Results (Türkiye'ye İthal Edilen Akvaryum Balıkları ve Sonuçları Üzerine Araştırmalar), E.U. Journal of Fisheries & Aquatic Sciences. 18 (2001) 483-493. (in Turkish)
11. M.A. Hekimoğlu, F. Güleç, S. Engin, C. Süzer, Ş. Saka, K. Fırat, Development of Aquaculture and Aquarium sector in Turkey, Proceedings of Pakistan Congress of Zoology. 38 (2018) 17-29.
12. A.S. Tarkan, A story of piranha in Sapanca Lake, Av Doğa. 41 (2006) 75-77.
13. D. İnnal, F. Erk'akan, Effects of exotic and translocated fish species in the inland waters of Turkey, Reviews in Fish Biology and Fisheries. 16 (2006), 39-50.
14. B. Yoğurtçuoğlu, F.G. Ekmekçi, First record of the giant pangasius, *Pangasius sanitwongsei* (Actinopterygii: Siluriformes: Pangasiidae), from central Anatolia, Turkey, Acta Ichthyologica et Piscatoria. 48 (2018) 241–244.

15. Ö. Emiroğlu, F.G. Ekmekçi, S.Aksu, S. Başkurt, M.A. Atalay, A.S. Tarkan, Introduction and establishment of tropical ornamental fish, *Pterygoplichthys* spp. (Actinopterygii: Siluriformes: Loricariidae) in hot springs: aquarium trade as a potential risk for biodiversity in Turkey, Acta Ichthyologica et Piscatoria. 46 (2016) 351-356.
16. Ö. Emiroğlu, M.A. Atalay, F.G. Ekmekçi, S. Aksu, S. Başkurt, E. Keskin, E.M. Ünal, B. Yoğurtçuoğlu, A.S. Tarkan, One of the Worlds Worst Invasive Species, *Clarias batrachus* (Actinopterygii: Siluriformes: Clariidae), Has Arrived and Established a Population in Turkey, Acta Ichthyologica et Piscatoria. 50 (2020) 391-400.
17. G. Türkmen, First record of the Guppy (*Poecilia reticulata* Peters, 1859) in inlandwaters of Turkey, Ege Journal of Fisheries and Aquatic Sciences. 36 (2019) 397-400.
18. M. Kottelat, J. Freyhof, Handbook of European Freshwater Fishes. Kottelat, Cornol, Switzerland and Freyhof, Berlin, Germany, 646 p. (2007).
19. H.R. Esmaeli, A. Teimori, F. Owfi, K. Abbasi, B.W. Coad, Alien and invasive freshwater fish species in Iran: Diversity, environmental impacts and management, Iranian Journal of Ichthyology. 1 (2014) 61-72.
20. A. Maceda-Veiga, O. Domínguez-Domínguez, J. Escribano-Alacid, J. Lyons, The aquarium hobby: can sinners become saints in freshwater fish conservation?, Fish and Fisheries. 17 (2016) 860-874.
21. N. Koutsikos, A.N. Economou, L. Vardakas, D. Kommatas, S. Zogaris, First Confirmed Record of an Established Population of Sailfin Molly, *Poecilia latipinna* (Actiopterygii: Cyprinodontiformes: Poeciliidae), in Europe. Acta Ichthyologica et Piscatoria, 47 (2017) 311-315.
22. Q.M.Tuckett, J.L. Ritch, K.M. Lawson, L.L. Lawson, J.E. Hill, Variation in cold tolerance in escaped and farmed nonnative green swordtails (*Xiphophorus hellerii*) revealed by laboratory trials and field introductions, Biological Invasions. 18 (2016) 45-56.
23. R. Froese, D. Pauly. Editors. FishBase. World Wide Web electronic publication. www.fishbase.org, version (06/2021), 2021.
24. K.D. Kallman, R.B. Walter, D.C. Morizot, S. Kazianis, Two new species of *Xiphophorus* (Poeciliidae) from the Isthmus of Tehuantepec, Oaxaca, Mexico, with a discussion of the distribution of the *X. clemenciae* clade, Am. Mus. Novit. 3441 (2004) 1-34.
25. R.M. McDowall, Further feral poeciliid fish in New Zealand fresh waters, with a key to species, N. Z. J. Mar. Freshw. Res. 33 (1999) 673-682.
26. F.N. Poeser, M. Kempkes, I.J.H. Isbrücker, Description of *Poecilia* (Acanthophacelus) *wingei* n. sp. from the Paría Peninsula, Venezuela, including notes on *Acanthophacelus Eigenmann*, 1907 and other subgenera of *Poecilia* Bloch and Schneider, 1801 (Cyprinodontiformes, Poeciliidae), Contributions to Zoology. 74 (2005) 97-115.
27. J.J. Rosso, F. Rosso, E. Mabragana, N.F. Schenone, E. Avigliano, J.M.D. Astarloa, Molecular taxonomic characterisation of introduced specimens of *Poecilia reticulata* in the lower Paraguay River basin (Cyprinodontiformes: Poeciliidae), Neotropical Ichthyology.15 (2017) e170046.
28. R.R. Miller, Geographical Distribution of Central American Freshwater Fishes, Copeia. Vol. 1966, 4 (1966) 773-802.
29. M.G. Maddern, H.S. Gill, D.L. Morgan, Biology and invasive potential of the introduced swordtail *Xiphophorus hellerii* Heckel (Poeciliidae) in Western Australia, Aquatic Conservation: Marine and Freshwater Ecosystems. 21 (2011) 282–291.
30. C. Perdikaris, N. Koutsikos, L. Vardakas, D. Kommatas, P. Simonovic, I. Paschos, V. Detsis, L. Vilizzi, G.H. Copp, Risk screening of non-native, translocated and traded aquarium freshwater fishes in Greece using Fish Invasiveness Screening Kit, Fisheries Management and Ecology. 23 (2016) 32-43.
31. I.C. Duggan, C.A.M. Rixon, H.J. MacIsaac, Popularity and propagule pressure: determinants of introduction and establishment of aquarium fish, Biological Invasions. 8 (2006) 377-382.
32. D.M. D'Amore, V.D. Popescu, M.R. Morris, The influence of the invasive process on behaviours in an intentionally introduced hybrid, *Xiphophorus helleri–maculatus* Animal Behaviour. 156 (2019) 79-85.
33. Q.M. Tuckett, J.L. Ritch, K.M. Lawson, J.E. Hill, Landscape-scale survey of non-native fishes near ornamental aquaculture facilities in Florida, USA, Biological Invasions. 19 (2017) 223-237.
34. J. Jourdan, F.W. Miesen, C. Zimmer, K. Gasch, F. Herder, E. Schleucher, M. Plath, D. Bierbach, On the natural history of an introduced population of guppies (*Poecilia reticulata* Peters, 1859) in Germany, BioInvasions Records. 3 (2014) 175-184.
35. F.H. Rodd, D.N. Reznick, Variation in the demography of guppy populations: the importance of predation and life histories, Ecology. 78 (1997) 405–418.
36. T.D. Oliveira, A.C. Reis, C.O. Guedes, M.L. Sales, E.P.R. Braga, T.F Ratton, B.P. Maia, A.L.B. Magalhães. Establishment of non-native guppy *Poecilia reticulata* (Peters, 1859) (Cyprinodontiformes: Poeciliidae) in an Municipal Park located in Minas Gerais State, Brazil, PANAMJAS. 2014; 9 2014 21-30.
37. A.L.B. Magalhães, C.M Jakobi, Colorful invasion in permissive Neotropical ecosystems: establishment of ornamental non-native poeciliids of the genera *Poecilia*/*Xiphophorus* (Cyprinodontiformes: Poeciliidae) and management alternatives, Neotropical Ichthyology. 15 (2017) e160094.
38. C.M. Breder Jr, D.E. Rosen, Modes of Reproduction in Fishes, Jersey City, New Jersey, T.F.H. Publications, 941 pp., 1966.
39. R. Borowsky, K.D. Kallman, Patterns of mating in natural populations of *Xiphophorus* (Pisces: Poeciliidae). I: *X. maculatus* from Belize and Mexico, Evolution. 30 (1976) 693–706.
40. A.E. Deacon, I.W. Ramnarine, A.E. Magurran. How reproductive ecology contributes to the spread of a globally invasive fish, PLoS One. 6 (2011) e24416.
41. R.J. McKay, Introductions of exotic fishes in Australia. In Distribution, Biology and Management of Exotic Fishes, Courtenay WR, Stauffer JR (eds), John Hopkins University Press: Baltimore, pp: 177–199, 1984.
42. A.H. Arthington. Diet of *Gambusia affinis holbrooki*, *Xiphophorus hellerii*, *X. maculatus* and *Poecilia reticulata* (Pisces: Poeciliidae) in streams of southeastern Queensland, Australia, Asian Fisheries Science. 2 (1989) 193–212.

43. R.A. Englund The impacts of introduced poeciliid fish and Odonata on the endemic megalagrion (Odonata) damselflies of Oahu Island, Hawaii. Journal of Insect Conservation. 3 (1999) 225–243.
44. D. Dudgeon, R. Corlett Hills and Streams: An Ecology of Hong Kong, Hong Kong University Press: Hong Kong, (1994).
45. M. Goren, B.S. Galil. A review of changes in the fish assemblages of Levantine inland and marine ecosystems following the introduction of non-native fishes. Journal of Applied Ichthyology. 21 (2005) 364–370.
46. W.R. Courtenay, C.R. Robins, R.M. Bailey, J.E. Deacon, Records of exotic fishes from Idaho and Wyoming, Great Basin Naturalist. 47 (1988) 523–526.
47. H. Wei, R. Chaichana, L. Vilizzi, P. Daengchana, F. Liu, M. Nimtim, Y. Zhu, S. Li, Y. Hu, G.H., Copp, Do non-native ornamental fishes pose a similar level of invasion risk in neighbouring regions of similar current and future climate? Implications for conservation and management, Aquatic Conservation Marine and Freshwater Ecosystems. (2021) 1-17, DOI: 10.1002/aqc.3609
48. E. Gertzen, O. Familiar, B. Leung, Quantifying invasion pathways: fish introductions from the aquarium trade, Canadian Journal of Fisheries and Aquatic Sciences. 65 (2008) 1265-1273.