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ORIGINAL PAPERS

First Record of *Gambusia Holbrooki* (Eastern Mosquitofish), an Invasive Fish in the Tuzla Lake, Romania

Ana Bianca PAVEL[1],
Gabriel IORDACHE[1], Cătălina GAVRILA[1], George TIGANOV[2],

[1] PhD, Researcher, National Institute for Research and Development on Marine Geology and Geo-ecology- GeoEcoMar, 23-25 Dimitrie Onciul St, 024053 Bucharest, Romania
[2] Eng., PhD, Marine Resources Department, "National Institute for Marine Research and Development "Grigore Antipa", Constanta, Romania
Corresponding author: gtiganov@alpha.rmri.ro

Abstract. *The current study reveals the first record of Gambusia holbrooki (eastern mosquitofish), an invasive fish in the Tuzla Lake, Romania. The investigated area is located 150 m from the shore of the Black Sea and is adjacent to Lake Techirghiol. The formation of the lake occurred due to human factors and was favored by natural factors. During the field campaigns carried out by NIRD GeoEcoMar in Lake Tuzla, in October 2022, a series of measurements and samples collection were carried out. The individuals of Gambusia holbrooki were found in the samples collected with the limnological net. The main objective of this contribution is the presentation of the first occurrence of the invasive species of eastern mosquitofish in Tuzla Lake. It is recognized as "a superior invader" due to the "invasiveness" characteristics of the species. It is important to monitor this species in Tuzla Lake to observe the impact on the rest of the fish populations, considering that this lake is used as a fish farm.*

Keywords: invasive fish, littoral lake, freshwater, eastern mosquitofish

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1. Introduction

Gambusia holbrooki is a native species from North America [1]. Worldwide spreading of *Gambusia* has occurred since the first introduction into Hawaii in 1905 [2]. The mosquitofish have become the most widely distributed freshwater teleost in the world [2] mainly through deliberate human introductions [3] to control the mosquitoes in rice and natural waters [2, 4] and eradicate malaria (Figure 1).

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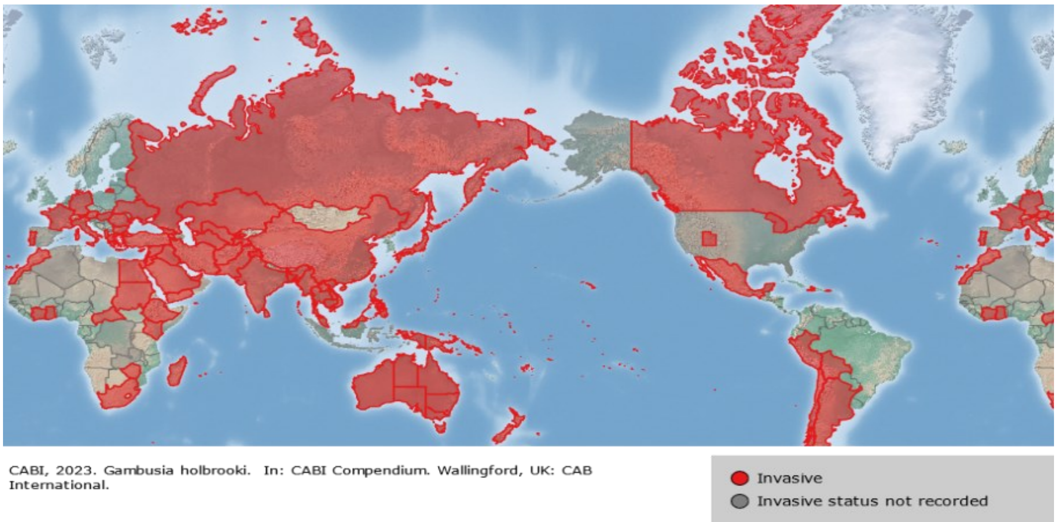

Fig. 1. Distribution of *G. holbrooki* after CABI, 2023

Mosquitofish have become the most widely distributed freshwater teleost in the world [2] mainly through deliberate human introductions. The native range of *G. affinis* is throughout the Mississippi Basin and the tributaries to the northern Gulf of Mexico. *G. holbrooki* ranges from New Jersey in the east to the Gulf of Mexico and northern Florida. In North America, in 1914 in Puerto Rico [5], in 1924 Canada [6], in 1927 in Utah [7], in 1931 in Mexico [5], 1941 in Michigan [2], in 1983 in Haiti [5]. In South America was first recorded in 1937 Chile and in 1940, 1943 in Peru, Argentina [5].

Materials and methods

Study area

Tuzla Lake, a 50.4 ha artificial body of water, has a reception basin of 5.4 km^2, was created to shield nearby villages from floods by diverting excess water from Lake Techirghiol. The latter, originally marshy, saw a reduction in salt concentration after Tuzla Lake's formation. In the 80s, a 400 m-long dike was built, forming Tuzla Lake and serving the purpose of accessing a television tower for Eforie (North and South), Techirghiol, and Tuzla. The lake, now bordered by tall reed bushes, is in the early stages of environmental improvement. It sits 150 m from the Black Sea shore, adjacent to Lake Techirghiol (Figure 2 & 3), and serves as a fish farm for sport fishing [8].

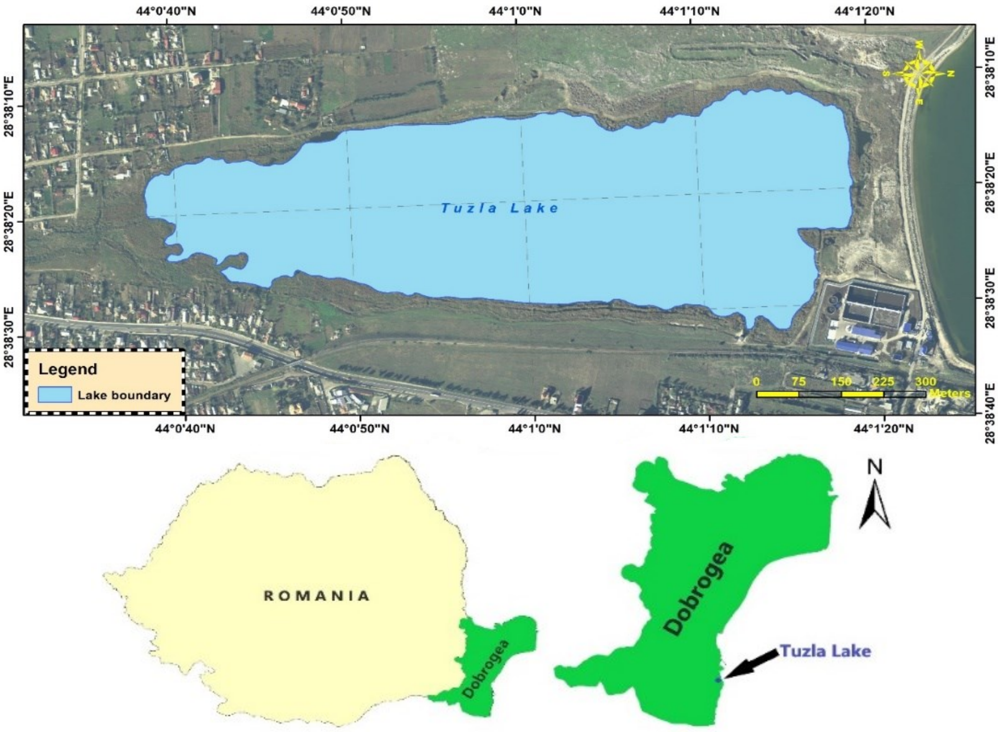

Fig. 2. Site Location

Fig. 3. The view of the Tuzla Lake, NE shore and NW shore (April and October 2022)

Specimen collection and processing

During the field campaigns carried out in October 2022 in the area by NIRD GeoEcoMar researchers, a series of measurements and samples were collected. Individuals of *G. holbrooki* were found in the samples collected with the limnological net. The specimens were fixed in 70% ethanol and morphology was examined.

Morphological examination

The preserved specimens (four individuals) were examined under a Carl Zeiss Discovery V.8 Stereomicroscope with Axiocam 208 color and also, photographed using a digital camera. Some morphometric measurements were taken.

Results and Discussion

Eastern mosquitofish (*Gambusia holbrooki* Girard, 1859) is a small, viviparous, freshwater fish, member of the Poeciliidae family, living in shallow or slow-flowing waters [9 - 11]. Mosquitofish is a member of Class Actinopterygii (ray-finned fishes), Order Cyprinodontiformes (Rivulines, killifishes and live bearers), Family Poeciliidae (a family of freshwater fishes of the order Cyprinodontiformes, the tooth-carps, and include well-known live-bearing aquarium fish, such as the guppy, molly, platy, and swordtail), and Subfamily Poeciliinae (group that includes species from both American continents which are collectively known as the livebearers due to the fact that many, but not all, of the species within the group are ovoviviparous).

Size and morphology.

Females measure 4-5 cm, while males, less numerous, are small, 2.5-3.5 cm [12]. The maximum reported length was 4.7 cm TL male/unsexed [13] 7.0-8.0 cm TL female [14, 15]. *Gambusia* has a thick, elongated body and is slightly compressed laterally. The dorsal fin is inserted in the posterior half of the body, following the ventral fins, which have an abdominal position [16, 17]. The male has a copulatory organ (called the gonopodium) [16, 17]. Through its peculiar body shape, in severe conditions of deprivation, *Gambusia* spp. procures necessary food and oxygen at or near the water surface [18, 19]. Specimens from Tuzla Lake match the description of *G. holbrooki* having a thick, elongated body and is slightly compressed laterally, with the head flattened dorso-ventral with slightly elongated snout, large eyes and wide mouth placed obliquely. The dimensions of the 4 specimens present in the samples show lengths that vary between 3 (3.5 - 3.7) and 4.6 (Figure 4), 3 individuals were males and the larger one was female, all of them have the characteristics of mature specimens, the proof that the lake condition are proper for their development. Mosquitofish collects small particles of fine food on the surface of the water [20], [21] and gulps air from the atmosphere when dissolved oxygen tends to zero [22], [23]. Their dorso-ventrally flattened head and dorsally oriented mouth help them use the surface water layer [19], [24]. *G. holbrooki* is a carnivorous fish, namely a larvivores one, consuming mainly mosquito larvae [25]. It also consumes small crustaceans, diatoms, and other algae, and, if necessary, becomes a cannibal. Adult mosquitofish feed on small terrestrial invertebrates usually in the drift and amongst aquatic plants [28], actively selecting very small prey [4]. It was introduced worldwide for mosquito control. Some researchers reported that the introductions to the European continent have severely threatened many freshwater endemic species [16]. *G. holbrooki* is a natural prey of some species of fishing spiders of the genus *Dolomedes* [30]. *Gambusia* spp. exhibits both social and aggressive behavior [9]. Some mosquitofish released into the wild by aquarium hobbyists may have exacerbated mosquito control issues, as their voracious appetite for natural invertebrate predators of mosquito larvae could outweigh any minimal positive effects [29]. *G. holbrooki* is a natural prey of some species of fishing spiders of the genus *Dolomedes* (Pisauridae, e.g., *D. okefinokensis* in United States, *D. plantarius* in Italy, and *D. triton* in United States) [30]. Mosquitofish exhibit both inter- and intra-specific aggressive behavior [31]. As a result of the aggressive behavior, the hierarchies of dominance are established with larger fish usually dominant over smaller fish [32- 37, 9]. Aggression is not used to defend a territory but is used by larger individuals to establish a hierarchy within a temporary group [38, 39]. When courtship and mating are less frequent, *Gambusia* spp. are often found in large shoals swimming together [40; 41; 42; 9].

Fig. 4. Stereomicroscope photographs of lateral and dorsal view on a preserved *Gambusia holbrooki* individuals

Reproduction.

In temperate regions, it reproduces in summer, when the water temperature is 16-20°C [17]. In tropical regions, the reproduction is continuous. It matures at 4-6 weeks. About three generations can be produced in one year [15]. Like at other poeciliids, fertilization is internal, the male's gonopodium serving as a penis [43; 44]. Gestation lasts 3-4 weeks. Brood may reach up to 354 young, but it is generally around 40-60 [45; 16]. It multiplies very quickly, so that 1 m^3 of water can contain 1,000 specimens. After fertilization and hatching, the young are carried in the mother's abdomen until they can swim alone. Sexual maturity is reached in the very first year of life. At a temperature below 10°, the gambusia buries itself in the sand, entering hibernation [17].

Habitat.

The native habitat of mosquito fish is in the southern US, represented by lowland ponds, lakes, and streams [46]. Mosquito fish are particularly adapted to populate floodplains [47]. *G. holbrooki* is abundant in nearshore environments, preferring densely vegetated areas with slow waters [48]. It adapts to various habitats such as shallow water, dark substrates, and submerged vegetation [46; 49]. Mosquitofish are highly adaptable, thriving in diverse aquatic environments, from fresh to hyper-saline, cold temperate to tropical waters, inland, coastal, estuarine waters, and both still and slow-flowing waters. Fast-flowing water inhibits large population development [48]. Mosquitofish have invaded habitats worldwide, including hot springs, rivers, lakes, swamps, billabongs, cooling pondages, rice fields, ornamental ponds, estuaries, near-shore marine habitats, and salt lakes [48; 4]. They tolerate temperatures from 0.5°C to 39°C, with a preference for warm waters around 25°C [50]. Juveniles are more thermally tolerant than adults, allowing them to exploit warm patches with increased growth, survival, and maturation rates. They can even inhabit ice-covered waters in Japan and hot bores in central Australia [51; 52; 48]. Mosquitofish prefer Tropical/Mega thermal and Temperate/Mesothermal climates, tolerating Dry (arid and semi-arid) and Continental/Microthermal climates [9]. Their broad salinity tolerance extends to salt lakes, estuaries, and near-coastal marine environments [48]. They can tolerate oxygen concentrations as low as 1.3 mg/L and withstand virtual anoxia by utilizing the oxygen-rich surface water/air interface [19]. The salinity LD50 for mosquitofish is more than 58g/L and they can tolerate direct transfers to salinity differences of up to seawater (35 g/L) with few mortalities [53]. Mosquitofish swim in large groups at the water's surface, occurring in standing to slow-flowing water, mainly in vegetated ponds, lakes, backwaters, and quiet pools of streams [54]. Although primarily freshwater, they also exist in brackish waters [54]. Mosquitofish's tolerance to salinity and resistance to pollutants contribute to their successful invasion of various environments [9; 48; 55].

Importance.

G. holbrooki is used to fight against the larvae of mosquitoes that carry malaria and as an aquarium fish in the commercial aquarium industry (www.fishbase.org) but poor sales are likely given their noxious status in many countries, aggressive behavior, and poor appearance. *G. holbrooki* did not evolve spectacularly in the aquarium, if we compare it with other species of poeciliids. There are, however, a few varieties of color, which are rather melanic versions more or less distant from the wild variety.

Interspecific hybridization and intraspecific variability

G. holbrooki and *G. affinis* were reported to produce interspecific hybrids with other members of the genus, such as: *G. georgei* [56], *G. nobilis* [57] and *G. heterochir* [58; 59; 60]. Members of the *Gambusia* genus show patterns of geographic variation in terms of population genetics, external morphology, physiological processes, physiological tolerances to physical or chemical stress, biochemical traits and ethology, with the degree of dissimilarity between populations increasing with either the geographic distance between them or with the degree of habitat difference between the sites where they occur [9].

Risk of Introduction

Environmental Impact

G. holbrooki, highly invasive, colonizes new habitats swiftly, with characteristics such as high fecundity, juvenile survival, and rapid population growth [61; 62; 63]. Mosquitofish disperse through waters as shallow as 3 mm, using drains and natural channels [63]. Rehage and Sih [64] link dispersal behavior to "invasiveness," noting *G. affinis* spreads faster, but *G. holbrooki*, with superior fecundity and maximum population growth rates, is considered a superior invader [64]. Eastern mosquitofish rapidly increase in population size due to quick maturation and high young survival [65; 35; 66]. They are resilient to various pollutants and exhibit adaptability to different environments physiologically and genetically. Facing few predators, parasites, diseases, or competitors, *G. holbrooki* poses a threat by preying on invertebrates, potentially increasing malaria-carrying mosquito populations targeting insects, natural predators of mosquitoes.

Biodiversity Impact

G. holbrooki, found in invasive areas like Australia, faces minimal predators, parasites, diseases, or competitors [48]. Experiments reveal native Australian fish actively avoid consuming this mosquitofish [48]. Rehage et al. (2005a, b) propose *G. holbrooki* and *G. affinis* as more efficient predators than non-invasive relatives, with juveniles being more voracious than adults [4; 9; 35; 48; 49; 66-72]. Significant habitat overlaps and *Gambusia*'s competitive advantage result in the potential loss of native fish in Gambusia-dominated waters [4; 9; 35; 48; 49; 66-72]. *G. holbrooki's* voracious invertebrate predation may increase mosquito populations, as studies suggest *Gambusia* is less effective than native fish in controlling mosquito larvae [73]. In the River Murray, *G. holbrooki* proves a poor mosquito larvae predator compared to small native fish species [48]. *G. affinis* may pose a threat to native invertebrate grazers in New Zealand [74]. Gut contents assessment reveals *G. holbrooki* significantly preys on native fish in Australia

[75]. *G. holbrooki* populations impact native frog tadpoles [76; 77; 78; 27], as well as reproductive rituals, breeding success, and growth of native fish [79].

***G. holbrooki* - eastern mosquitofish in Romania.**

G. holbrooki was introduced to Romania in 1927 from Hamburg, then from Bulgaria and Italy [12; 80; 81]. It has populated various waters in Transylvania, Lake Pantelimon near Bucharest, and coastal lakes of the Black Sea [82; 83]. Recent inventories confirm *G. holbrooki* as an established species in Romania [84; 85]. Initial reports about Gambusia in Romania were made by Petru Bănărescu [80] and other colleagues [82; 86- 90]. The species is present in several locations, including Lake Mangalia, Mlaștina Hergheliei, coastal lakes, and waterbodies in Bucharest, Oradea, and Nicolae Romanescu Park in Craiova [14; 80; 85; 91-94]. Although it was present in Lake Tineretului Park, Bucharest, in the late 1990s–early 2000s, it was not found in 2020–2021 [85]. *G. holbrooki* is constrained by temperature and prefers Mediterranean and sub-Mediterranean areas or urban settings with a favorable microclimate [95; 96]. In Romania, it thrives in the littoral, thermal or thermal-influenced waters [91] and some city parks. Its impact on Lake Tuzla is challenging to assess due to the lack of pre-fish farm population data. The introduction hypothesis suggests accidental stocking with juvenile fish.

Fig. 5. Distribution of *G. holbrooki* in Romania

We can assume that the impact of the eastern mosquitofish in Tuzla Lake will be mostly on the population fish species that are the subject of aquaculture, mostly cyprinids species like carp, silver carp, bighead carp, through its predatory behavior consuming the larvae. It is important to monitor this species in the Tuzla Lake, to observe the impact on the rest of the fish populations, considering the history of this species.

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Conflict of interest. The authors declare that they have no conflict of interest.

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