Poeciliid Research

Gambusia holbrooki Girard, 1859 - Biology of the species and its history in Romania as an introduced taxon

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Abstract. Eastern mosquitofish (*Gambusia holbrooki* Girard, 1859) is a small, viviparous, freshwater fish, member of the Poeciliidae family, living in shallow or slow-flowing waters. The paper aims to review several aspects related to the biology of the species and its history in Romania as an introduced species. *G. holbrooki* is an euribiont species, except for adapting to very low temperatures. The species was introduced in many locations in Romania at the beginning of the last century. Our attempts to identify the species in Transylvania failed, and the last water in Transylvania where it could still be found dried up.
Key words: mosquitofish, alien species, non-native, intentional release, Transylvania, Bihor.

Introduction. Eastern mosquitofish (*Gambusia holbrooki* Girard, 1859) is a small, viviparous, freshwater fish, member of the Poeciliidae family, living in shallow or slow-flowing waters (Pyke 2005; Petrescu-Mag 2007a; Mag et al 2009) (Figure 1). 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).

Lexical root for Gambusia: it comes from the word Gambusino (Cuban) = nothing; an allusion to someone who went fishing but did not catch anything (fishbase.ir).

The paper aims to review several aspects related to the biology and history of *Gambusia holbrooki* in Romania as an introduced species.

Distribution. The mosquitofish was introduced worldwide in tropical, subtropical and temperate climate countries (Raja & Ravikanth 2020; Innal & Giannetto 2020) (Figure 2). In North America, the distribution is Atlantic and Gulf Slope drainages from New Jersey south to Alabama in United States (Page & Burr 1991). It is established now throughout the southern part of Europe (Kottelat & Freyhof 2007). Being larvivorous, it was introduced for mosquito control, but had rare to non-existing effects on mosquitoes, and negative to possible neutral impact on native fishes (Kottelat & Whitten 1996; Petrescu-Mag 2007b) (see the original prey of the mosquitofish in Figure 3).

Figure 1. The mosquitofish, *Gambusia holbrooki* (source: wikipedia.org 2020).

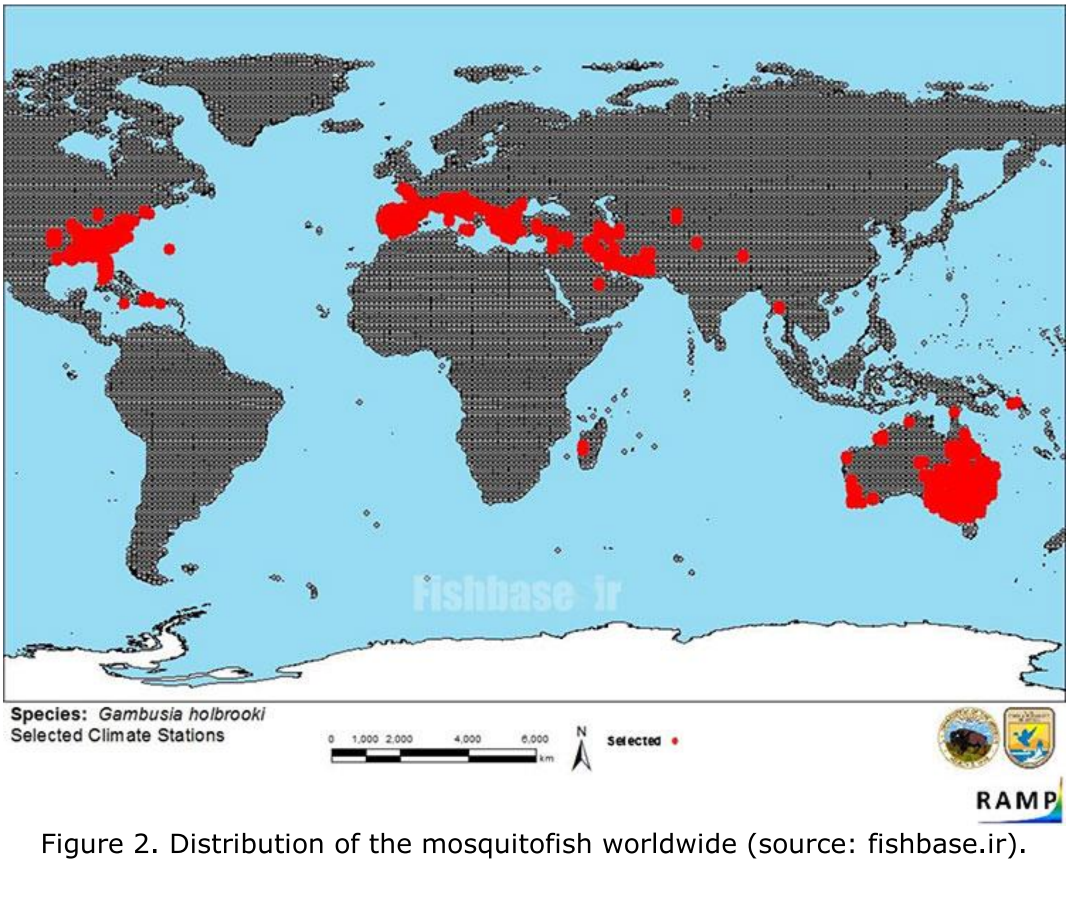

Figure 2. Distribution of the mosquitofish worldwide (source: fishbase.ir).

Figure 3. Mosquito larvae (Photo by Napat Polchoke, source: http://vtichthyology.blogspot.com/).

Size and morphology. Females measure 4-5 cm, while males, less numerous, are small, 2.5-3.5 cm (Iacob & Petrescu-Mag 2008). The maximum reported length was 4.7 cm TL male/unsexed (Tarkan et al 2006); 7.0-8.0 cm TL female (Gavriloaie & Berkesy 2013; Froese & Pauly 2020). Dorsal spines (total): 1; dorsal soft rays (total): 7; anal spines: 1; anal soft rays: 9 (Froese & Pauly 2020).

Gambusia has a thick, elongated body and is slightly compressed laterally. The head is flattened dorso-ventral with slightly elongated snout, large eyes and wide mouth placed obliquely and with small teeth on the jaws. The dorsal fin is inserted in the posterior half of the body, following the ventral fins, which have an abdominal position (Kottelat & Freyhof 2007; Wikipedia.org 2020). The caudal fin is rounded. The body is covered with olive-green scales, slightly darker on the back and whitish on the abdomen (Figure 1). The male has a copulatory organ (called the gonopodium), resulting from the transformation of the anal fin and by lengthening the 3-5 rays of this fin (Kottelat & Freyhof 2007; Wikipedia.org 2020).

It is very difficult to distinguish *G. holbrooki* from another member of the genus, *G. affinis* (Pyke 2005; Jouladeh-Roudbar 2015). For this reason, many previous studies cannot be considered to be carried out on the mentioned species.

Environmental parameters, ecology and behavior. Freshwater; brackish; benthopelagic; pH range: 6.0-8.8; dH range: up to 40; potamodromous (Riede 2004). Subtropical; 15-35°C (Riehl & Baensch 1996); 40-31°N, 89-74°W (Froese & Pauly 2020). In temperate regions, at a temperature below 10°C, the mosquitofish is buried in the sand, entering the winter sleep (Wikipedia.org 2020).

Mosquitofish stay on the surface of the water and swim in very large groups (Figure 4). Adults occur in standing to slow-flowing water, mostly in vegetated ponds and lakes, backwaters and quiet pools of steams (Page & Burr 1991), typically seen shoaling at the edges (Allen et al 2002). Although they are primarily freshwater fish, they are also present in brackish waters (Page & Burr 1991).

Mosquitofish are tolerant to salinity within fairly wide limits. The studies in Table 1 indicate that they can move from low to high salinity provided the transition is gradual, not abrupt (Pyke 2005).

Table 1
Experiments that highlight the adaptability to different levels of salinity of mosquitofish fish

Transfer from… to… salinity	*Survival*	*Reference*
From freshwater to salinity of 17 ppt	84% after 1 day	Congdon (1994)
From freshwater to salinity of 17 ppt	54% after 2 days	Congdon (1994)
From freshwater to salinity of 17 ppt	33% after 3 days	Congdon (1994)
From freshwater to salinity of 10 ppt	>90% after 6 days	Al-Daham & Bhatti (1977a)
From freshwater to salinity of 20 ppt	>90% after 6 days	Al-Daham & Bhatti (1977a)
From freshwater to salinity of 31 ppt	0% after 1 day	Al-Daham & Bhatti (1977a)
From freshwater to salinity of <6 ppt	100% after 14 days	Nordlie & Mirandi (1996)
From freshwater to salinity of 10 ppt	60% after 14 days	Nordlie & Mirandi (1996)
From freshwater to salinity of 12 ppt	37% after 14 days	Nordlie & Mirandi (1996)
From freshwater to salinity of 10 ppt	50% after 7 weeks	Ahuja (1964)
From freshwater to salinity of 16 ppt	5% after 7 weeks	Ahuja (1964)
From freshwater to salinity of 20 ppt	90% after 1-2 days	Chervinski (1983)
From freshwater to salinity of 24 ppt	0% after 1-2 days	Chervinski (1983)
From freshwater to salinity of 28 ppt	0% after 1-2 days	Chervinski (1983)
From freshwater to salinity of 17 ppt	67% after 3 days	Congdon (1994)
From freshwater to salinity of 30 ppt	0% after 3 days	Congdon (1994)
From freshwater to salinity of 20 ppt gradual transition	100% after 7 days	Chervinski (1983)
From freshwater to salinity of 20 ppt abrupt transition	90% after 7 days	Chervinski (1983)
From freshwater to salinity of 36 ppt gradual transition	80% after 7 days	Chervinski (1983)
From freshwater to salinity of 36 ppt abrupt transition	0% after 7 days	Chervinski (1983)
From freshwater to salinity of 40 ppt gradual transition	65% after 7 days	Chervinski (1983)
From freshwater to salinity of 36 ppt abrupt transition	0% after 7 days	Chervinski (1983)

Due to its peculiar body shape, in severe conditions of deprivation, *Gambusia* spp. obtains necessary food and oxygen at or near the water surface (Homski et al 1994; Lewis 1970). Mosquitofish collects small particles of fine food on the surface of the water (Hildebrand 1919; Vooren 1972), and gulps air from the atmosphere when dissolved oxygen tends to zero (Odum & Caldwell 1955; Sjogren 1972). Their dorso-ventrally flattened head and dorsally oriented mouth facilitate these uses of the surface water layer (Lewis 1970; Lloyd 1984).

G. holbrooki is a carnivorous fish, namely a larvivorous one, consuming mainly mosquito larvae (Gabrielyan 2001), the number of which it can reduce by 97% in a lake (Wikipedia.org 2020; Pyke 2005). However, this happens only when other types of prey are lacking (Kottelat & Whitten 1996). It also consumes small crustaceans (Cladocera), diatoms and other algae (green or blue algae), fish eggs, and, if necessary, becomes a cannibal. It can destroy the eggs and young of other fish in small ponds (Pyke & White 2000). Adult mosquitofish feed on small terrestrial invertebrates usually in the drift and amongst aquatic plants (Froese & Pauly 2020), actively selecting very small prey (Arthington 1989).

It was introduced worldwide for mosquito control, or even unintentionally (Figure 2). Some mosquitofish released into the wild are due to aquarium hobbyists. Introductions to the European continent have severely threatened many of the freshwater endemic species (Kottelat & Freihof 2007). It is now largely accepted that the mosquitofish introduction effect on mosquito control has been minimal and even may have exacerbated the problem due to their voracious appetite for natural invertebrate predators of mosquito larvae (Allen et al 2002).

G. holbrooki is a natural prey of some species of fishing spiders of the genus *Dolomedes* (Pisauridae, eg. *D. okefinokensis* in United States, *D. plantarius* in Italy, and *D. triton* in United States) (Nyffeler & Pusey 2014). IUCN Red List status: least concern (LC) (IUCN 2020).

Figure 4. The mosquitofish. Schooling in standing or slow flowing water (source: https://npr.brightspotcdn.com/).

Smell can be an important mechanism in determining the degree of risk of predation in fish. Many fish have developed such alarm signals (Nordell 1998), in which an individual can detect lesions of conspecifics by olfaction and respond behaviorally to the presence of the predator (Vanzwoll 2010). It was known that ostariophysians (which includes catfishes, minnows and characins) have a fright reaction to the appearance of conspecific wounds, which is achieved by specialized cells and alarm substances (Von Frisch 1942). Vanzwoll (2010) tested whether a non-ostariophysian like *G. holbrooki* had a similar behavioral reaction to injured conspecifics. He also examined the effects of exposure to this risk stimulus and other signs of predation during a single pregnancy cycle. He concluded and hypothesized that immediate exposure to predation indicators causes an obvious behavioral response, and long-term exposure would cause *G. holbrooki* to alter the nutrient supply of developing embryos, resulting in altered offspring morphology and performance (Vanzwoll 2010). *G. holbrooki* individuals exposed to the skin extract (an alarm substance from injured conspecifics) schooled significantly closer than individuals which were not exposed to the skin extract. *G. holbrooki* did not show any perceptible life-history plasticity in response to signs of predation during a single pregnancy (Vanzwoll 2010). However, they showed significant differences in the relationship between fertility and maternal size between two locations in coastal Georgia. These observations confirm the existence of an ethological alarm reaction in mosquitofish and different reproductive traits between geographical locations (Vanzwoll 2010). Many animals, including fish, experience morphological and life-history plasticity in response to changes to abiotic and biotic environmental factors. Further replication of the experiment is needed to determine whether this species alters the nutrient supply of embryos in response to the risk of predation (Vanzwoll 2010).

In sexually reproducing animals, larger individuals benefit from higher reproductive success (Bisazza & Marin 1995). In many species, female fertility correlates positively with body weight (Tilley 1968). Even the size of the brood is positively influenced by the size of the female, which produces more viable offspring (Congdon et al 1983). In males, the correlation between size and reproductive success may be caused by female preference (Valvo et al 2019), intrasexual competition (Devigli et al 2015), or both (Hughes 1985a), and this is also valid for *G. holbrooki*.

The courtship behavior (and/or mating) in *Gambusia* genus involves three distinct phases (Pyke 2005): the first one - association, the second – courtship, and the last one - mating stricto sensu. During the first phase, males and females tend to mix together, males often congregate around a female and males are generally aggressive towards one another, with the larger male in a group becoming dominant and chasing other males away from the vicinity of the female (Itzkowitz 1971; Hughes 1985b; Bisazza & Marin 1991). The association of individuals takes place in late spring or early summer (Pyke 2005). Courtship involves the female and dominant male of the group and may be followed by one or more matings (Bisazza & Marin 1988). Courtship and mating take place in late spring and early summer (Maglio & Rosen 1969; Martin 1975). Sexual courtship behavior in mosquitofish involves sexually aggressive approaches of the male against the female and acceptance or rejection by the female. The visible courtship behavior of the male may include gonopodial oscillation, when the gonopodium is raised but copulation is not attempted, the female is followed without lifting the gonopodium, and attempts to copulate with the erect gonopodium (Rosen & Tucker 1961; Haynes 1993). Before the copulation, the female usually either allows the male to approach her or she approaches and follows the male (Bisazza & Marin 1991; Pyke 2005). She may also exhibit an arching behavior which is translated as sexual receptivity (Bisazza & Marin 1991). She may also simply swim away (Rosen & Tucker 1961). Whether or not copulation occurs appears to be under the decision of the female (Pyke 2005).

Literature shows that *Gambusia* spp. exhibits both social and aggressive behavior. Both *G. holbrooki* and *G. affinis* are notorious for their aggressive behavior (Pyke 2005). Mosquitofish exhibits both inter- and intra-specific aggressive behavior (Courtenay & Meffe 1989). This aggressiveness includes biting and physical pushing or thrusting as reported by Caldwell & Caldwell (1962), Meffe et al (1983), Meffe (1985) and Lloyd et al (1986). As a result of the aggressive behavioral sequences, the hierarchies of dominance are established with larger fish usually dominant over smaller fish (Caldwell & Caldwell 1962; Bisazza & Marin 1991; Mills et al 2004; Pyke 2005). The aggression is not used to defend a territory as long as the species is not highly territorial (Itzkowitz 1971; Winkler 1979). The aggression is used by larger individuals to establish a hierarchy within a temporary group. This is on one side clearly stated in literature. On the other side, when courtship and mating are less frequent, *Gambusia* spp. is often found in aggregations or in schools that swim together (Figure 4). This tolerance to other individuals found in close proximity is very likely outside the mating season (Rees 1958; Maglio & Rosen 1969; Al-Daham et al 1977; Pyke 2005).

Reproduction. In temperate regions, it reproduces in summer, when the water temperature is 16-20°C (Wikipedia.org 2020). In tropical regions, the reproduction is continuous. It matures at 4-6 weeks. About three generations can be produced in one year (Froese & Pauly 2020). Like at other poeciliids, fertilization is internal, the male's gonopodium serving as a penis (Pen & Potter 1991; Mag-Mureșan & Bud 2004). Gestation lasts 3-4 weeks. Brood may reach up to 354 young, but it is generally around 40-60 (Riehl & Baensch 1991; Kottelat & Freyhof 2007). 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 (Wikipedia.org 2020).

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* (Hubbs & Peden 1969), *G. nobilis* (Baird & Girard 1853) and *G. heterochir* (Hubbs 1957a,b; Hubbs & Delco 1960; Yardley & Hubbs 1976).

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 (Pyke 2005).

Importance. *G. holbrooki* is used to fight the larvae of mosquitoes that carry malaria and as an aquarium fish. *G. holbrooki* did not evolve spectacularly in the aquarium, if we compare it with other species of poeciliids in the aquarium. There are, however, a few varieties of color, which are rather melanic versions more or less distant from the wild variety (Figure 5).

Figure 5. Ornamental, melanistic/spotted variety of *Gambusia holbrooki* (sources: https://livefins.com/ and https://www.tapatalk.com/).

Eastern mosquitofish in Romania. *G. holbrooki* is native to North America, from where it was brought to Europe (Bănărescu 1964). In Romania, *G. holbrooki* was first brought from Hamburg in 1927 by Professor D. Mezincescu, then from Bulgaria and in 1930 from Italy (Iacob & Petrescu-Mag 2008; Gavriloaie & Berkesy 2013). Lake Pantelimon near Bucharest, other lakes in Bucharest, various lakes and ponds in Transylvania and some coastal lakes of the Black Sea (Lake Mangalia, etc.) were populated (Nicolau 1946; Gavriloaie 2008). The most recent inventory of alien vertebrates in Romania lists *G. holbrooki* among the established species (Stănescu et al 2020).

The first reports about the presence of the genus *Gambusia* in Romania were made by the father of Romanian ichthyology, Petru Bănărescu (1964), but also by other colleagues and friends of his time (Nicolau 1946; Cărăușu 1952; Vasiliu 1959; Bușniță & Alexandrescu 1971; Lustun et al 1978; Bacalbașa-Dobrovici 1982). However, due to the instability of fish taxonomy in the past century, and the difficulty by which *G. affinis* and *G. holbrooki* can be discriminated, we will never be sure that the species they referred to have been determined by current standards, so we do not know which of the two species was reported by each of these authors. Several authors through recent meetings and studies have concluded that, most likely, the individuals reported at the beginning in Romania belonged to the species *G. holbrooki*, which is confirmed by most Romanian ichthyologists from the old school (Petrescu-Mag I. V., personal communication; opinion understood at the first Romanian Symposium of Ichthyology, held in Arad, 2004, where Professor Bănărescu was present).

Trying to understand the meaning of the term "Lakes in Transylvania" (Gavriloaie 2008), we concluded that the authors referred to certain thermal lakes, as it is more likely for poeciliids to establish self-sustaining populations in thermal lakes and thermal springs than under the ice bridge in winter. For this reason, we discussed with Ioan Valentin Petrescu-Mag, one of the researchers who investigated the fish fauna in the Transylvanian thermal waters in the last decades.

Petrescu-Mag stated that he always believed that the Băile Felix and Pețea Lakes (Bihor County) hosted mosquitofish, but the bans on fishing in the respective thermal waters did not allow the identification of this species in the Bihor lakes. For this reason, the author did not mention *G. holbrooki* in any of his works on the fish fauna of thermal springs (Mag et al 2009 – submitted in 2005, 2-3 April). However, his post-2005 attempts, along with his student Iacob Miruna, to identify *Gambusia holbrooki* at Băile Felix failed. The last location in Transylvania suspected of hosting mosquitofish was the Pețea Hot Spring (Pețea Lake). Lake Pețea ended its history due to the overexploitation of thermal water in 2013-2014 (Bunea et al 2014) (Figure 6). Therefore, we believe that we no longer have *Gambusia* in Transylvania.

Figure 6. The image of Lake Pețea, dried up (source: Cristian Horgoş, Bihor Online).

Conclusions. *G. holbrooki* is an euribiont species, except for adapting to very low temperatures. The species was introduced in many locations in Romania at the beginning of the last century. Our attempts to identify the species in Transylvania failed, and the last water in Transylvania where it could still be found dried up.

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