

Some Life-History Traits of *Gambusia holbrooki* (Pisces: Poeciliidae) from Bulgaria

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Abstract: The eastern mosquitofish *Gambusia holbrooki* (Girard, 1859) is worldwide spread invasive fish species and can be found in all Southern European countries. For almost 100 years since the species was introduced in Bulgaria there is no scientific study concerning the biology and ecology of this species and its influence on native taxocoenoses. The results of the current investigation confirm the successful adaptation of this fish species in Bulgarian ecosystems.

Key words: invasive species, life history, Poeciliidae, *Gambusia holbrooki*

Introduction

The eastern mosquitofish (*Gambusia holbrooki* Girard, 1859) is a small, sturdy fish, with deeply rounded body and flat dorsal surface. It belongs to family Poeciliidae Garman, 1895. LLOYD & TOMASOV (1985) stated that there are two close related but not synonymic species – eastern (*Gambusia affinis holbrooki* Krumholz 1948) and western mosquitofish (*Gambusia affinis affinis* Baird & Girard 1853). Their native range covers south-eastern states of USA and Mexico (LLOYD & TOMASOV 1985). Now eastern mosquitofish according to LLOYD & TOMASOV (1985) and ARTHINGTON *et al.* (1999) is not a subspecies but a different species *Gambusia holbrooki*.

Gambusia holbrooki inhabits slowly moving or stagnant waters with preference of areas densely covered with plants. The species can survive in heavily polluted waters, because has the ability to supply with oxygen from the surface layers of the water (CECH *et al.* 1985).

Eastern mosquitofish was introduced intentionally or accidentally in over 50 countries around the world (GARCÍA-BERTHOU *et al.* 2005). Intentional introductions were undertaken for using mosquitofish

as biological agent for prevention of malaria. In many countries introduction of this fish caused a negative impact on native ecosystems. There are records that confirm its negative impact and predatory behavior on many native fish and amphibians and their larvae (ARTHINGTON 1989, COURTENAY & MEFFE 1989, MINCKLEY *et al.* 1991). Eastern mosquitofish is included in Top 100 of the most dangerous invasive species of GISP (Global Invasive Species Programme, <http://www.issg.org/database/>).

In Europe *Gambusia holbrooki* was introduced first in Italy in 1921 with the presumption that it is a very good biological agent for prevention of malaria. In Bulgaria it was introduced in 1924. First successful attempts for breeding were made near river Tundzha by Slivensky (MIKOV, 2005). Then fish were massively breed and introduced in malaria rich areas, such as swamps, lakes, coastal lakes and wetlands near Black Sea shore and Danube River, as a biological agent in the fight with malaria mosquitoes from genus *Anopheles* Meigen, 1818.

The present distribution of mosquitofish in Bulgaria is well known (Fig. 1). Its range covers most

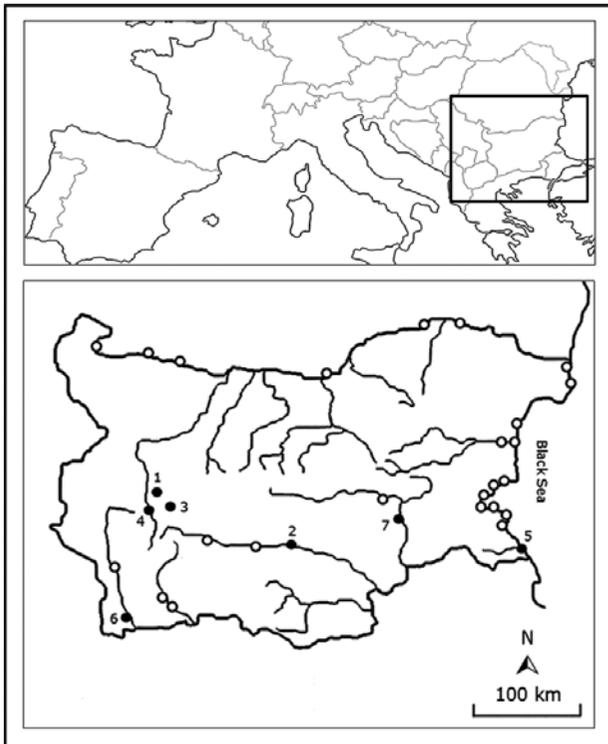


Fig. 1. Location of sampling sites (black dots) and known distribution (white dots) of *Gambusia holbrooki* in Bulgaria (South-Eastern Europe). Sampling sites are: Dolni Bogrov lake (1), Maritza river (2), Ognyanovo reservoir (3), Pancharevo reservoir (4), Potamyata river (5), Rupite hot springs (6), Tundzha river (7).

of the territory of the country, as reported by several authors previously (APOSTOLOU 2005, APOSTOLOU *et al.* 2010, KARAPETKOVA & ZHIVKOV 2006, POLACIK *et al.* 2008, VASSILEV 1995, VASSILEV 1999, VASSILEV & PEHLIVANOV 2005). Until now there have not been conducted any studies on mosquitofish biology and ecology in Bulgaria and Eastern Europe, and its influence on native taxocoenoses. This paper presents the first data concerning the life-history of *Gambusia holbrooki* in Bulgaria.

Materials and Methods

The study was carried out in a total of seven model water bodies of different type in Bulgaria (Fig. 1, Table 1). The sampling sites were selected to have similar habitat features consisting of shallow areas ranging from 5 cm to 50 cm deep, low water velocity or standing water and abundant aquatic vegetation.

For the analyses were collected a total of 1484 mosquitofish, 208 of them were immature, 434 males and 842 females. The sampling was carried out from 2008 to 2010. The examined specimens

were captured along the shoreline using hand net and preserved in 4% buffered formalin solution. Geographical coordinates, altitude, water and air temperatures ($^{\circ}\text{C}$) were measured in each sampling event (Table 1). In the laboratory, sex was determined by using the morphology of the anal fin according to TURNER (1941) and by gonad examination. Males were classified as mature if a fully formed gonopodium was present; females were classified as mature if the ovaries contained developed eggs and as immature if the ovaries were absent or small and translucent (STRANGE, 1996). Total weight (TW), eviscerated weight (EW) and gonadal weight (GW) were measured to the nearest 0.1 mg; standard length (SL) (nearest 0.1 mm) was measured with a caliper.

Statistical analyses of covariance (ANCOVA) were applied to study variations of life-history traits among different investigated populations, for each sex separately. In the analyses SL was used as a covariate. Analyses include testing for interactions among covariates (e.g. SL, TW, EW) and categorical factors (e.g. latitude, temperature). If the covariate factors were not significant ($P > 0.10$), they were excluded from the analysis. The gonadosomatic index ($\text{GSI} = \text{gonadal weight}/\text{total weight}$) was calculated as an alternative to ANCOVA of GW according to GARCÍA-BERTHOU & MORENO-AMICH (1993). Standard length was not used as covariate for the analysis of GSI. Estimated adjusted means of the dependent variables were used to describe differences among populations. The relationship of the life-history variables with latitude and average year temperature was analyzed with multiple linear regression (MLR). All of the quantitative variables, except GSI and latitude, were \log_{10} -transformed for linear models, because homoscedasticity and linearity were clearly improved.

Results

Population structure and dynamics

Sex-ratio differed among the examined populations, as in Pancharevo Reservoir, Dolni Bogrov Lake, Maritza River it was two females per one male. In Ognyanovo Reservoir and Potamyata River it was three females per one male. In Tundzha River sex-ratio was 1.6 females per 1 male and in Rupite hot springs – one female per one male.

Population dynamics for Pancharevo Reservoir during the study period is illustrated in Fig. 5. In

Table 1. Characteristic of sampling sites and main features of studied mosquitofish populations.

Sampling site	Site code	Date	Average year air temperature (°C)	Altitude (m)	Longitude (°E)	Latitude (°N)	Males mean standard length (mm)	Males range (mm)	No of males	Females mean standard length (mm)	Females range (mm)	No of females
Dolni Bogrov Lake	1	July 2010	10.5	529	23.43	42.47	18.83	14.8 - 23.7	65	21.86	14.6 - 29.6	148
Maritza River	2	September 2009	11.2	135	25.14	42.14	17.74	12.0 - 21.4	8	21.00	12.6 - 28.5	15
Ognyanovo Reservoir	3	October 2009	10.5	624	23.52	42.50	18.61	16.2 - 21.7	16	19.32	16.0 - 29.0	61
Pancharevo Reservoir	4	October 2008 - June 2010	10.5	611	23.31	42.50	20.65	11.9 - 24.0	181	22.63	11.8 - 36.8	383
Potamyata River	5	July 2009	12.5	2	28.05	42.05	18.61	15.2 - 23.2	14	24.91	14.2 - 39.2	45
Rupite Hot Springs	6	April 2009; April 2010	14.4	88	23.28	41.35	22.21	18.4 - 26.5	78	27.99	21.3 - 36.1	70
Tundzha River	7	September 2009	11.2	114	26.32	42.27	20.61	17.0 - 25.0	72	22.57	15.4 - 34.7	111

December and March there was observed the lowest number of mosquitofish, probably because of their biological characteristic to hibernate in leaf litter on the bottom in winter months. In April no immature fishes were present, and females were relatively small in size (from about 15 mm to 23 mm SL). During May there were established larger females altogether with the smaller ones; their size can be divided in two classes ranging from 15 mm to 23 mm and from 24 mm to 36 mm in standard length. The first newborns appeared also in May. In June there was observed increased number of immature specimens and very few mature males and females. In October, most of males and females were **immature**. Most of the observed females were small-sized (from about 15 mm to 23 mm) with a few larger ones (from 24 mm to 30 mm).

Condition

The lowest condition values for both males and females were observed in March; then they increased gradually to June (Fig 2, 3). The periods with the highest values condition were from June to October (except August), then they decreased. The populations of Ognyanovo Reservoir and Tundzha River had the highest condition values, followed by Potamyata River, Dolni Bogrov Lake and Pancharevo Reservoir. The lowest condition values were observed in Rupite hot springs and Maritza River (Fig. 2, 3).

Significant correlations at $P \leq 0.05$ level were calculated (Table 3) between eviscerated weights of females and males ($r^2 = 0.829$, $P = 0.042$). Linear regression analyses showed high correlation rates at $P \leq 0.05$ level between total weight and latitude in males, with an established significant corresponding increase of these two parameters (Fig. 4).

Reproduction

The eggs' development generally started in mid-April when the water temperature was above 15 °C and continued until the end of September. In Pancharevo Reservoir population, two reproduction periods can be distinguished. The first one was marked to start in April and continued to June, when it was observed a peak in the immature mosquito fish number, with very few present mature fish. The second period, which should start in end of summer when the previous year cohort offspring will become mature as reported before (CABRAL & MARQUES 1999) was established by indirect data – presence of small-sized

juveniles in September sampling, on account of missing August sampling .

Gonadosomatic index started to increase from April to May, decreased in the period June-July and increased again in July. For females the highest average GSI values were observed in September (Fig. 3). For males the highest average GSI values were observed in June (Fig. 2). Highest GSI values showed females from Maritza River, followed by females from Potamyata River and Dolni Bogrov Lake.

Gonadal weight of females had lowest values in April, then increased and reached maximum values in the period from July to September (Fig. 3). In males well developed gonads can be found throughout the year and it seems that their gonadal weight is not closely related to temperature (Fig. 2, 3).

ANCOVA analyses of males showed more significant correlation between standard length and gonadal weight than in females (Table 2). Significant correlations at $P \leq 0.01$ level, were observed between altitude and GSI, also between latitude and GSI of the males (Table 3).

Discussion

The absence of data concerning the life-history traits of mosquitofish from neighboring countries led us to compare the characteristics of the examined Bulgarian populations with more distant ones living in different environmental conditions.

The observed overall ratio (two females per one male is similar to sex-ratio of mosquitofish populations from SW Iberian Peninsula (FERNÁNDEZ-DELGADO 1989) but showed significant difference compared to other populations of the species from NE Iberian Peninsula (VARGAS & SOSTOA 1996), where sex ratio is four females per one male. Other populations from SW Iberian Peninsula (PÉREZ-BOTE 2005) had sex-ratio 1:1. In mosquitofish populations from the Mediterranean region of Turkey (ÖZTÜRK 2002), sex-ratio was three females to one male.

The population dynamics showed that in spring, females are presented only by small sized individuals (15 mm – 23 mm SL). The missing of immature mosquitofish in April is probably related to the fact that all fish are born during the previous year and had reached maturity at this point. The bigger females (24 mm – 36 mm SL) probably die during winter months. In the beginning of summer period in May, females were presented by two size classes

Table 2. ANCOVAs of \log_{10} transformed life-history variables (total weight (g), eviscerated weight (g), gonadal weight (g), gonadosomatic index (GSI)) with standard length (SL)(mm) as covariate. Standard length was \log_{10} transformed.

	Total mass			Eviscerated mass			Gonadal mass			GSI		
	SS	df	P	SS	df	P	SS	df	P	SS	df	P
Males												
SL	6.20	1	***	4.73	1	***	3.18	1	***	-	-	-
Site	0.22	3	***	0.22	3	***	0.56	1	***	5.32	1	**
Date	0.54	7	***	0.72	7	***	2.69	6	***	26.91	6	***
SE	0.56	419	-	0.44	319	-	6.02	152	-	82.24	153	-
R^2 adj.	0.951			0.950			0.503			0.249		
Females												
SL	53.02	1	***	35.48	1	***	0.96	1	**	-	-	-
SL ²	-	-	-	-	-	-	0.44	1	*	-	-	-
Site	0.65	3	***	0.36	3	***	9.67	2	***	1410.59	2	***
Date	0.88	7	***	0.89	7	***	4.60	4	***	574.75	4	***
SE	1.24	818	-	0.76	608	-	23.34	224	-	4264.37	233	-
R^2 adj.	0.987			0.988			0.885			0.481		

Significance (P) is presented with ** for $0.01 < P < 0.001$ level of significance and with *** for $P \leq 0.001$ level of significance.

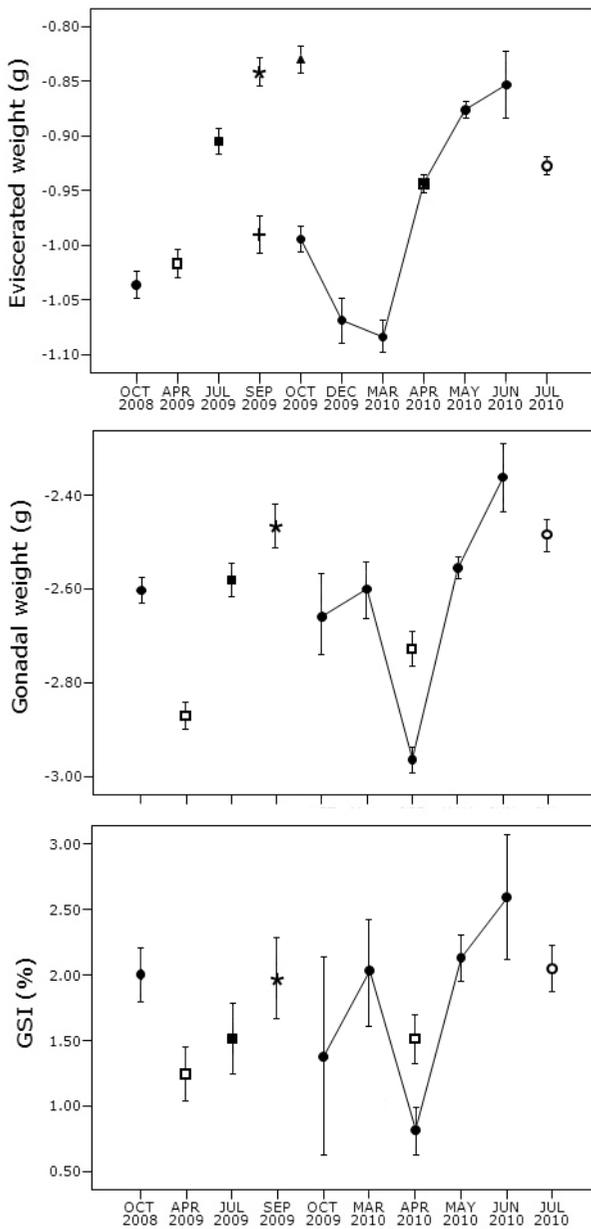


Fig. 2. Log₁₀ transformed adjusted means of eviscerated weight, gonadal weight and gonadosomatic index (GSI) for male mosquitofish. The symbols correspond to sampling sites as follows: ○ Dolni Bogrov lake, + Maritza river, ▲ Ognyanovo reservoir, ● Pancharevo reservoir, ■ Potamyata river, □ Rupite hot springs, ★ Tundzha river.

– the smaller ones were young of the year and the bigger ones were born the previous year.

The mosquitofish condition from the examined sites varied in different populations, with highest values during late summer and autumn. This is because of physiological adaptation of the species to store fat reserves, as an energy source during cold periods (REZNICK & BRAUN 1987; MEFFE & SNELSON 1993). In both sexes the condition started to decline proportionally to the temperature, continued to de-

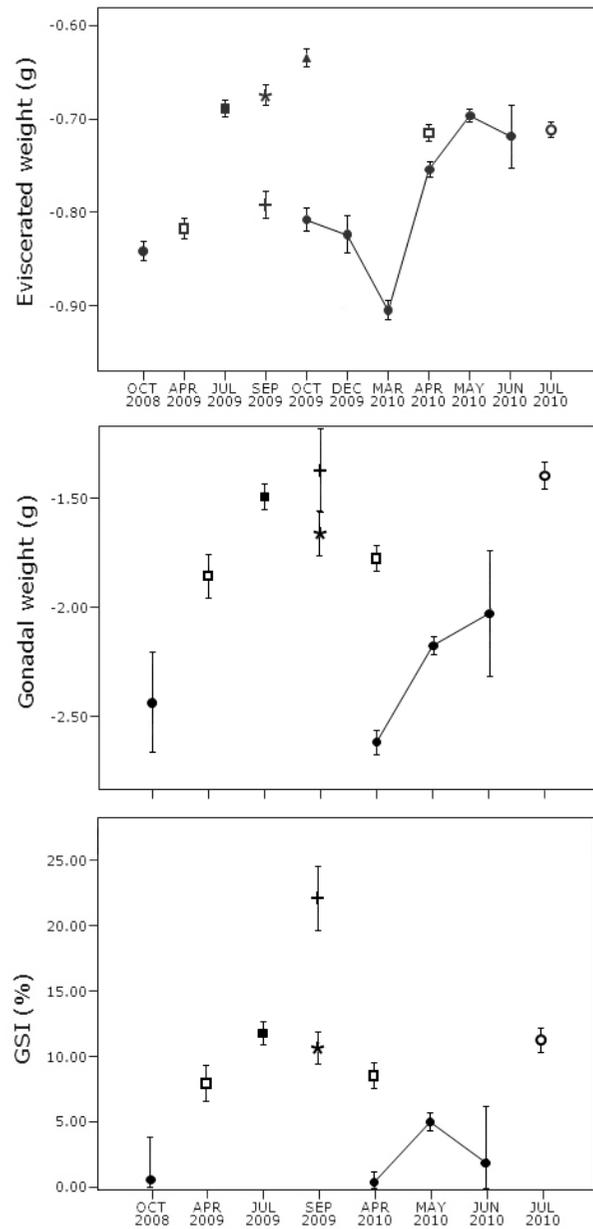


Fig. 3. Log₁₀ transformed adjusted means of eviscerated weight, gonadal weight and gonadosomatic index (GSI), for female mosquitofish. The symbols correspond to sampling sites as follows: ○ Dolni Bogrov lake, + Maritza river, ▲ Ognyanovo reservoir, ● Pancharevo reservoir, ■ Potamyata river, □ Rupite hot springs, ★ Tundzha river.

crease during winter months and started to increase in spring.

The relationship between total mass and latitude observed in male *Gambusia holbrooki* (Fig. 4) confirms the results of BENEJAM *et al.* (2008), who established a proportional increasing of body condition according to latitude. This can be explained with the thesis that in colder temperatures eastern mosquitofish invest more energy to get bigger, and spend less energy in reproduction.

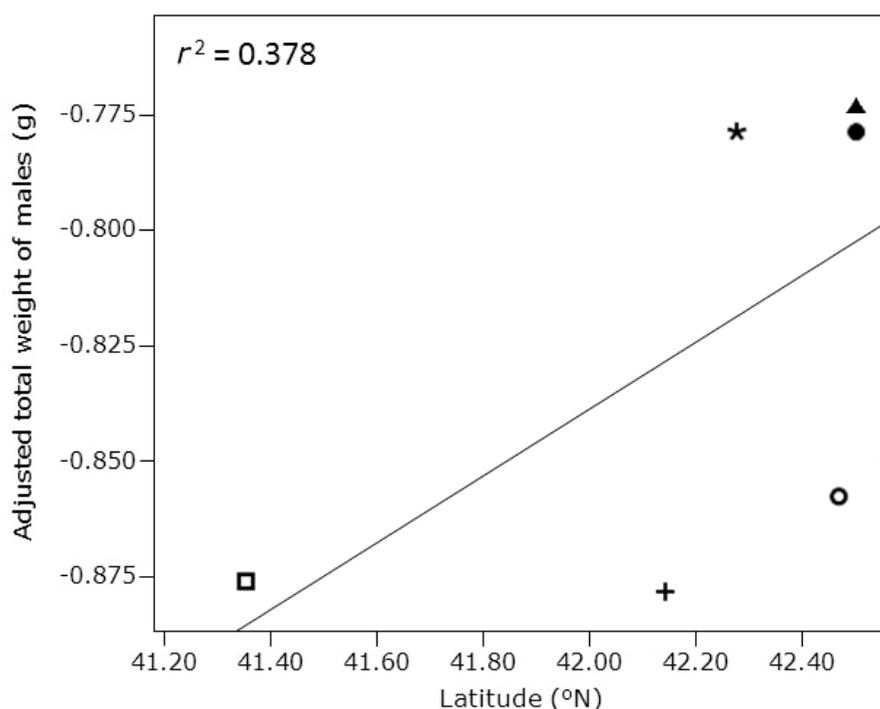


Fig. 4. Relationship between \log_{10} transformed adjusted means of total weight (g) of males and latitude ($^{\circ}$ N). The symbols correspond to sampling sites as follows: \circ Dolni Bogrov lake, $+$ Maritza river, \blacktriangle Ognyanovo reservoir, \bullet Pancharevo reservoir, \square Rupite hot springs, \star Tundzha river.

Table 3. Table of correlation coefficients of environmental factors compared to \log_{10} transformed life-history characteristics (total weight (TW) (g), eviscerated weight (EW) (g), gonadal weight (GW) (g), gonadosomatic index (GSI) for male (m) and female (f) mosquitofish.

	Water temperature ($^{\circ}$ C)	Average year air temperature ($^{\circ}$ C)	Altitude (m)	Latitude ($^{\circ}$ N)	EW (f)	GW (f)	TW (f)	GSI (f)	EW (m)	GW (m)	TW (m)	GSI (m)
Average year air temperature ($^{\circ}$ C)	0.15		**	**								
Altitude (m)	-0.26	-0.93		**								**
Latitude ($^{\circ}$ N)	-0.32	-0.94	0.93								*	**
EW (f)	-0.43	-0.28	0.26	0.38			*		*			
GW (f)	-0.30	0.05	-0.10	-0.30	-0.10			**				
TW (f)	-0.09	0.06	0.03	0.09	0.83	-0.40						**
GSI (f)	-0.30	0.05	-0.10	-0.30	-0.10	1	-0.40					
EW (m)	-0.60	-0.53	0.54	0.73	0.83	-0.50	0.60	-0.50			**	
GW (m)	-0.80	-0.63	0.80	0.80	-0.20	-0.40	-0.80	-0.40	0.80		**	
TW (m)	-0.43	-0.68	0.71	0.87	0.66	-0.70	0.49	-0.70	0.94	1		
GSI (m)	-0.40	-0.95	1	1	-0.40	-0.20	-1	-0.20	0.40	0.80	0.80	

Significance (P) is presented with * for $P \leq 0.05$ level of significance and with ** for $P \leq 0.01$ level of significance.

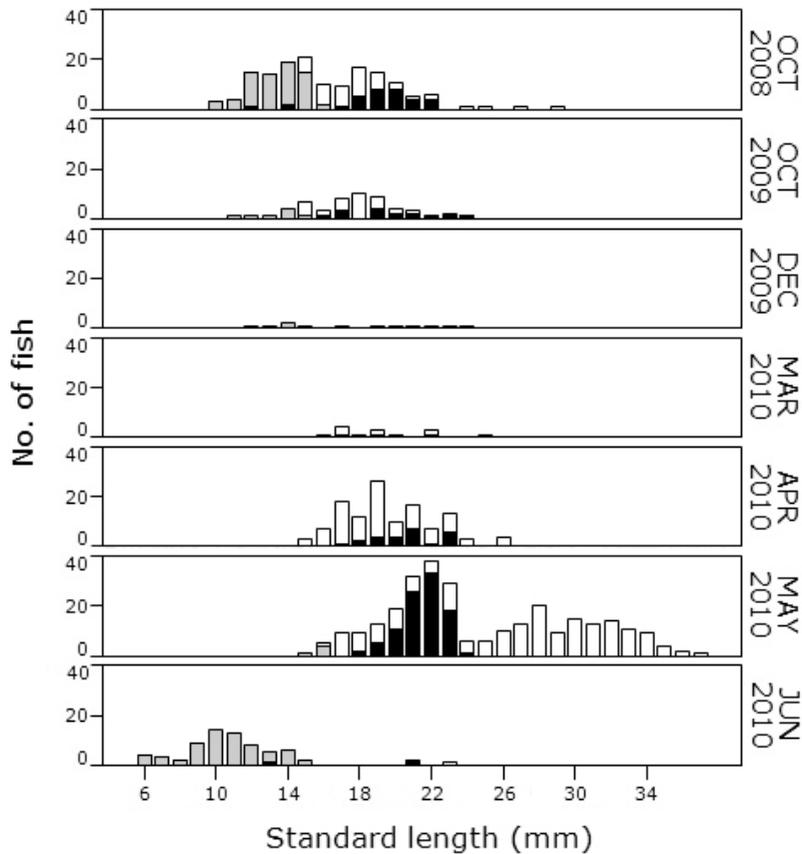


Fig. 5. Length-frequency distribution of immature (□), male (■) and female (□) *Gambusia holbrooki* at ponds near Panharevo Reservoir from October 2008 to June 2010.

The egg production started in April and the GSI values increase until the end of May. In June it was observed a decrease in GSI values similar to the results published by CABRAL & MARQUES (1999). According to KRUMHOLZ (1948) mosquitofish populations produce brood every 3 to 5 weeks, then for one breeding season of 5 months as it is in Bulgaria, it is possible that the local populations produce up to 6 broods.

In Panharevo Reservoir reproduction starts in April, when all the fishes are from 2009 cohort and are mature. In June it was observed a peak in the numbers of immature specimens on account of the present mature, which indicates that the 2009 cohort started to disappear. It is previously reported, that in other countries there is a second period of reproduction and it starts in the end of the summer (when the offspring of 2009 cohort had reached maturity and started breeding (CABRAL & MARQUES 1999, BOTSFORD *et al.* 1987).

In the end of September and until mid-October the average temperature is high enough for breeding to occur – 4-5 °C higher than in spring (when gonads

development normally starts) – but no gonads were detected then. This corresponds with the study of MEDLEN (1951) in which he proved that both lower temperature and short photoperiod inhibit the reproduction of mosquitofish. Compared to the breeding season of mosquitofish from Mediterranean regions of Turkey (ÖZTÜRK 2002), in Bulgaria it is shorter and starts a month later. In populations from Western Portugal (CABRAL & MARQUES 1999) the breeding season start in April and ends in October.

The population from Rupite hot springs is unique for Bulgaria. There was measured the highest temperature in which this species can be found in the country – 42.8 °C. It had lower condition compared to other studied populations, most probably because of the constant high water temperature, where they do not have to invest much energy to become bigger and to be able to withstand the typical for the region winter conditions.

In general the ability of *Gambusia holbrooki* to change its life history in order to adapt to different habitats (HAYNES & CASHNER 1995) explains the differences in the life-history traits between the stud-

ied Bulgarian populations. This study showed that it is very well adapted to the four-season climate in Bulgaria, as well to some specific conditions like brackish waters, hot springs or winter-frozen low-mountain reservoirs.

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