Research Article

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Variation in Life History and Feeding Ecology of the Invasive Eastern Mosquitofish, *Gambusia holbrooki* Girard, 1859 (Poeciliidae), in a Groundwater-dependent Wetland in Turkey

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Abstract: We studied the variations in life history and feeding ecology of *G. holbrooki* in Acıgöl, a groundwater-dependent wetland and the only distribution area of the critically endangered *Aphanius transgrediens*. Fish were sampled from three sites with different ecological characteristics: Site 1 with stable conditions, small size and high fish density; Site 2 with stable conditions, large size and medium fish density; Site 3 with variable conditions, large size and low fish density. The scale readings showed two age groups in males and three in females. The reproduction period was from April to September. More embryos and heavier gonads were found in specimens at Site 3. The overall ratio of fertilised eggs and the absolute and relative fecundities were the lowest at Site 1. Two different trophic positions, plant-based omnivory and animal-based omnivory, were detected. The main food items at Site 2 and Site 3 were adults and larvae of Diptera, as well as zooplankton. At the same time, mosquitofish fed predominantly on plant sources and occasionally on the other ones at Site 1. Significant temporal and ontogenetic differences in feeding were also determined. The highest diet diversity and broadest trophic niche were found at Site 2.

Key words: *Gambusia holbrooki***,** biodiversity, feeding strategy, Acıgöl, *Aphanius transgrediens*

Introduction

The way to understand the impacts of invasive species on native fauna, in a broad sense on biodiversity, is to understand their biology. The invasive eastern mosquitofish, *Gambusia holbrooki* Girard, 1859, has been successfully introduced into freshwater and brackish ecosystems throughout the world as a malaria control agent (Fernández-Delgado 1989, Pyke 2005). Since the 1990s, its reputation as a 'successful mosquito control agent' has been disreputed thanks to recent studies (Arthington 1989, Rupp 1996, Cabral & Marques 1999, Garcia-Berthou 1999), which have disagreed on its efficiency in mosquito control.

Gambusia holbrooki was first introduced in Turkey in the 1930s (Erençin 1978) for the same purpose and now has spread throughout the country (Ekmekçi *et al.* 2013). While its biology and ecology has been well documented in its native range (Krumholz 1948, Brown-Peterson & Peterson 1990, Meffe 1991, Haynes & Cashner 1995, Matthews & Matthews 2011) and in Europe (Fernández-Delgado 1989, Vargas & de Sostoa 1996, Garcia-Berthou 1999, Specziár 2004, Scalici et al. 2007, Gkenas et al. 2012, Zarev 2012), much fewer studies have been conducted about the introduced populations in Turkey (Öztürk & İkiz 2002, Ergüden 2013).

In this study, we presented life history variations of *G. holbrooki* among habitats with different characteristics in Acıgöl, a shallow Mediterranean lake. In addition, we elaborate its feeding strategy with special emphasis on variations in spatiotemporal, ontogenetic and sexual traits. Given the fact that Acıgöl is the only distribution area of the critically endangered *Aphanius transgrediens* (Ermin, 1946) (Freyhof 2014, Yoğurtçuoğlu & Ekmekçi 2014), the results of the current study may also provide insights into key elements of conservation actions.

Materials and Methods

Study area

The study was carried out in Acıgöl, a shallow hypersaline (mostly Na_2SO_4 salinity) Mediterranean lake (N 37°48.53'; E 29°52.81', Fig. 1). It is located in an endorheic basin, on a tectonic depression between two fault lines. These faults give rise to several freshwater springs flowing into the lake with various flow rates. The area between discharge points of the springs and the lake margin constitutes the main wetland, where *G. holbrooki* and the critically endangered *A. transgrediens* coexist. The surface area of the lake together with the wetland is approximately 80 km^2 with a maximum depth of about 2 m. However, the area of the main lake body varies greatly throughout the year.

The emergent plant communities were dominated by *Phragmites australis*, *Typha sp.* and *Juncus* sp. with variable cover percentages depending on the season. The other most common species were the submerged *Nasturtium officinalis* and floating *Lemna minor* beside the epilithic and filamentous algal communities.

We examined three sites, which were spatially close but ecologically distinct. The distinctions between the sampling sites were due to their variability of the physicochemical properties, size and density of *G. holbrooki* (Table 1). Physicochemical characteristics of the water (e.g. salinity, pH, dissolved oxygen) were stable in all of the spring outlets, except the one at the northern side (hereafter, Site 3). This spring is connected with the outlet channel of one of the sodium sulphate plant ponds. Therefore, unpredictable salinity increases occurred throughout the sampling period depending on the pond discharge.

Data collection and statistical analysis

From September 2013 to September 2014, 934 fish specimens were caught by a beach seine net (3 m x 1.5 m x 1.5 m, 4 mm mesh size) from the three sites. The monthly sampling was possible only at Site 1, because of the insufficient number of fish caught in some months at Site 2 and Site 3. The fish were immediately anesthetised with clove oil, then fixed in 4% formaldehyde solution and transported to the laboratory, where they were measured by a digital calliper (total length, TL, to the nearest 0.01 mm) and weighed by a digital precision balance (to the nearest 0.001 g). Scales were used for age determination.

The length-weight relationship was calculated, using the equation $W = a \times L^b$, where W is the body weight, L the total length, *a* the intercept and *b* the slope. The parameter estimation was established using a linear regression analysis on log-transformed data (Froese 2006). Analysis of covariance (ANCOVA) was used for comparison of slopes between sites for each sex, with the total body weight as the dependent variable and total length as covariate.

The parameters of the von Bertalanffy growth equation (L_∞ – asymptotic length; *k* – growth coefficient; t_0 – hypothetical age at which length is 0) and the growth performance index ($\phi' = 2\log(L_\infty) + \log(k)$) were calculated using ELEFAN I method (Gayanilo et al. 1988).

The sex was determined by the anal fin morphology and by examining the gonad structure (usually in individuals < 20 mm TL). The gonads were dissected and weighed and the gonadosomatic index (GSI = gonadal weight/ total weight) was calculated for both sexes. Differences in GSI throughout the reproduction period were tested by one-way ANOVA for Site 1. Embryonic development and fecundity were studied over 102 females for Site 1, 20 females for Site 2 and 18 females for Site 3. The ovarian and embryonic developmental stages were modified from Haynes (1995) as follows: (1) small, granular, white in colour and opaque; (2) opaque, yellow or orange, yolked eggs; (3) recently fertilised ovum, embryo appears as a small white cap on the surface of the yolk; (4) embryo appears as a thin white streak, covering one-half of the yolk diameter; (5) optic vesicles begin to pigment; (6) eyes enlarged but not full sized, pigmentation process almost completed, yolk sac is partially absorbed; (7) eye pigmentation process fully completed, body begins to pigment; (8) caudal fin elongated enough to cover the head, paired fins appeared, yolk sac absorption almost completed; (9) yolk sac absorption fully completed, paired fins elongated, scales present, embryo resembles small adult. The absolute (total number of embryos) and relative (number of embryos per unit of body weight) fecundity was estimated from the gravid females, which possess fertilised eggs (from stage 3 and older stages). The difference in size of the gravid females during the reproductive period was tested by one-way ANOVA, followed by Tukey test. Linear regression analyses were used to describe the relationship between fecundity and total length.

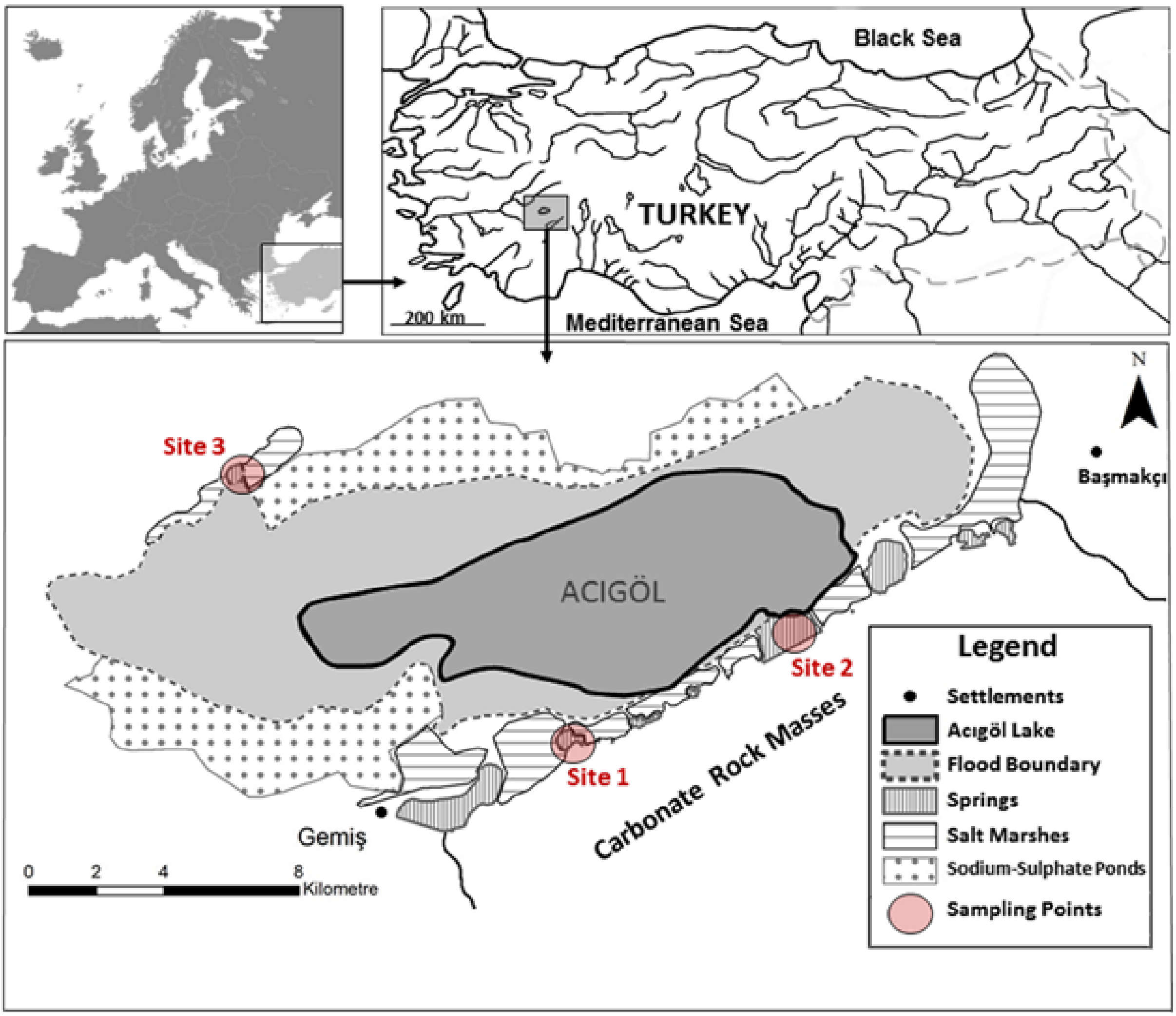

Fig. 1. Location of the sampling sites in Acıgöl, Turkey

Table 1. Some characteristics of the sampling sites, as measured and observed from September 2013 to September 2014. *x* – mean; min – minimum; max – maximum; Sd – standard deviation; D – depth; S – size; IsFD – intraspecific *Gambusia* density; AVD – aquatic vegetation density; *** – high; ** – medium; * – low

	Salinity (ppt)		**Temperature (°C)**		**Physical Features**		**Density**	
	***x* (min-max)**	**Sd**	***x* (min-max)**	**Sd**	**~D (cm)**	**~S (m^2)**	**IsGD**	**AVD**
Site 1	0.87 (0.72-0.95)	0.06	19.8 (19.2-20.7)	0.5	50-200	60	***	**
Site 2	0.71 (0.60-0.79)	0.05	19.9 (19.4-21.1)	0.4	50-100	2500	**	***
Site 3	10.17 (0.54-58.56)	17.35	18.6 (10.9-26.8)	5.4	100-200	1200	*	*

The difference between the slopes of the regression equations was tested by ANCOVA.

For analysis of the feeding ecology, 175 stomach samples were selected from coinciding sampling months in order to compare feeding habits among sampling sites. The entire gut was removed and dissected, and the gut contents were identified and counted under a dissecting microscope for macroscopic food items. A Sedgewick rafter chamber was used to count and identify smaller organisms. The volume of the gut contents and food items were estimated using two methods. Firstly, the individual gut contents and/or inseparable unique categories (e.g. detritus) were squashed on a plate to a uniform depth (here we used the Sedgewick rafter) and the area of the squash was measured (Hellawell & Abel 1971). Secondly, the volume of specific food items was estimated through calculation of a volume of geometric shape closest to it (Sun & Liu 2003). This procedure was carried out by a digital image analysis software – IMAGEJ (Schindelin et al. 2012). The identifiable parts of organisms, such as heads, were considered as individuals.

The dietary importance of each food category was expressed by percent number (%N), percent volume (%V), and frequency of occurrence (%FO), as follows: %N – number of prey *i*/total number of prey x 100; %V – volume of prey *i*/total volume of prey x 100; %FO – number of the guts containing prey *i*/total number of the guts containing prey x 100 (Hyslop 1980). The index of relative importance (IRI = (%N + %V) x %FO) was used to incorporate these indices (Pinkas et al. 1971).

To test the intraspecific (among size groups and between sexes) and seasonal differences in the diet composition, permutational multivariate analysis of variance (PERMANOVA) was applied, using Bray-Curtis similarity resemblance matrices of square-root transformed volume data, with 9999 permutations. Three length classes (< 30 mm, 30-40 mm, and > 40 mm) were assigned to assess the ontogenetic variation in feeding habits. Canonical Analysis of Principal coordinates (CAP) was performed to display a visual ordination of the dietary pattern among sampling sites by using IRI data. This method was preferred against traditional ordination methods since it uncovers patterns that are masked in an unconstrained scaling ordination (Anderson & Willis 2003). PERMANOVA and CAP analysis were performed by a PERMANOVA+ v1.0.1. PRIMER v6 software package (Anderson et al. 2008).

The diet diversity was calculated using the Shannon index (H´):

$$(H')=-\sum_{i=1}^{S}(pi \times \ln pi),$$

where *p* is the proportion of the number of prey item '*i*' to the total number of prey organisms. The niche breadth, an indication of trophic generalisation level, was quantified by the Levins index (B) (Krebs 1989

$$B=\frac{1}{\sum_{i=1}^{n} P_i^2}$$

where P_i is the proportion of each food category '*i*' in the diet and *n* is the total number of food categories in the diet of *G. holbrooki*. The Levins index was also standardised by the equation (B-1) / (n-1) to represent the niche breadth on a scale from 0 (narrow niche) to 1 (broad niche). The fractional trophic level (TROPH) values were estimated using TrophLab (Stergiou & Karpouzi 2002).

Finally, to visualise the feeding strategy, the Costello graphical method (Costello 1990), modified by Amundsen et al. (1996) was used. Here, the prey-specific abundance (%P*i*) was plotted against the frequency of occurrence (%FO). The %P*i* is the percentage of prey category *i* to the total number of prey items in the stomachs, which contained the prey category *i*.

Results

Life history traits

Of the total fish captured, 622 were females and 371 were males (χ^2=62.9, P<0.001). This ratio differed between the sampling sites and the seasons with a decrease in female/male ratio in spring and summer (Table 2). Three age groups (0+, 1+ and 2+) in females and two (0+ and 1+) in males were determined. The dominant age group was 0+ in both sexes (42.4% in females and 58.0% in males). In fact, only two individuals of 1+ males were sampled from Site 3. The total length ranged between 14.0 mm and 61.4 mm in females and between 16.2 mm and 33.3 mm in males (Table 3). The analysis of covariance ANCOVA showed significant differences in length-weight relationships (Table 3) between the sampling sites for each sex (females F=11.79, P=<0.001; males F=5.64, P=0.004). The females were longer and heavier than the males of the same age (Student t-test for 0+ age group t=5.74, P<0.001 and for 1+ age group t=16.91, P<0.001). The seasonal growth varied between sexes as depicted by the mean monthly lengths of individuals (Fig. 2). The null growth in females initiated in February and followed by a notable secondary stage just before the beginning of summer (March-April). The initial growth stage in males occurred less obviously in October. The growth performance index was higher in females than in males, whereas the males reached to their asymptotic length at a faster rate (Table 4).

The smallest gravid female with mature embryos was with 28.6 mm TL, the smallest male with fully developed gonopodium was with 20.0 mm TL. The average GSI value started to increase from April and reached its maximum in June for both sexes at Site 3, where the monthly sampling was achieved completely (Fig. 3). The highest GSI value was recorded as 40.9% for a female captured from Site 3 in August. When assessing the GSI together with the frequency of gravid females (Fig. 4), the reproductive period of *G. holbrooki* in Acıgöl could be seen to commence in April and end in September.

The absolute and relative fecundity values and fertilisation success that was estimated for the females caught at the three sampling sites (Table 5) revealed the highest average absolute fecundity

Table 2. Seasonal sex ratio of *Gambusia holbrooki* caught in Acıgöl from September 2013 to September 2014. Significant differences from parity were tested by chi-square test

Season	**Location**	**Number of individuals**		**Ratio Females:Males**	χ^2	***P*-value**
		Females	**Males**			
Autumn	Site 1	109	69	1.58:1	8.55	**< 0.05**
	Site 2	89	44	2.02:1	14.56	< 0.05
	Site 3	100	44	2.27:1	21.01	**< 0.05**
Winter	Site 1	50	17	2.94:1	15.28	**< 0.05**
	Site 2	0	0	–	–	–
	Site 3	0	0	–	–	–
Spring	Site 1	70	46	1.52:1	4.56	**< 0.05**
	Site 2	34	35	0.97:1	0.01	0.95
	Site 3	20	12	1.67:1	1.53	0.216
Summer	Site 1	116	69	1.68:1	11.44	**< 0.05**
	Site 2	0	0	–	–	–
	Site 3	34	35	0.97:1	0.01	0.95

Table 3. Estimated length-weight relationship parameters for *Gambusia holbrooki* males and females in Acıgöl. F – females; M – males; C – sexes combined; n – sample size; TL – total length; W – total body weight; *a* and *b* – parameters of the equations (see Materials and Methods); Cl – confidence limits; *r*2 – coefficient of determination

			TL (cm)			**W (g)**		**Regression Parameters**			
Sites	**Sex**	**n**	**Min**	**Max**		**Min**	**Max**	*a*	*b*	***b* Cl %95**	r^2
Site 1	**F**	322	1.85	5.14		0.06	1.80	0.0064	3.48	3.44-3.53	0.988
	M	186	2.01	3.33		0.07	0.38	0.0071	3.27	3.14-3.39	0.936
	C	508	1.85	5.14		0.06	1.80	0.0055	3.57	3.53-3.61	0.985
Site 2	**F**	128	1.40	5.09		0.03	1.80	0.0077	3.25	3.17-3.32	0.983
	M	84	1.62	3.20		0.04	0.30	0.0094	2.95	2.85-3.10	0.974
	C	212	1.40	5.09		0.03	1.80	0.0074	3.26	3.20-3.33	0.981
Site 3	**F**	134	1.84	6.14		0.06	3.07	0.0071	3.33	3.24-3.42	0.976
	M	80	1.76	3.23		0.05	0.36	0.0083	3.09	2.88-3.29	0.918
	C	214	1.76	6.14		0.05	3.07	0.0067	3.36	3.28-3.43	0.974

Table 4. Estimated von Bertalanffy parameters and growth performance indices of *Gambusia holbrooki* in Acıgöl. L_∞ – asymptotic length; *k* – growth coefficient; t_0 – hypothetical age at which length is 0 (year); ϕ’ – growth performance index

Parameters	**Site 1**		**Site 2**		**Site 3**	
	Females	**Males**	**Females**	**Males**	**Females**	**Males**
L_∞ (mm)	55.57	34.10	58.57	35.63	64.29	38.38
k (yr^{-1})	0.61	0.89	0.36	0.69	0.36	0.76
t_0 (yr)	-0.29	-0.31	-0.63	-0.19	-0.37	-0.19
ϕ’	1.28	1.02	1.09	0.94	1.17	1.05

at Site 3. However, the average relative fecundity was the highest at Site 2. The fertilisation success was higher at Site 3 with the highest percentage of malformed eggs. The size of the gravid females was significantly different during the reproductive period (ANOVA, F=3.87, P=0.023; Site 1 = Site 2, Site 1 = Site 3 and Site 2 < Site 3, according to the Tukey test). The equations of the relationships between the fecundity (F) and total length (TL) of *G. holbrooki* at the three sites were:

F_{Site1} = -36.41 + 1.51 TL (r^2=22.9%; F=29.16, P<0.001),

F_{Site2} = -109.4 + 3.93 TL (r^2=64.3%; F=32.54, P<0.001) and

F_{Site3} = -92.70 + 3.39 TL (r^2=78.1%; F=57.19, P<0.001)

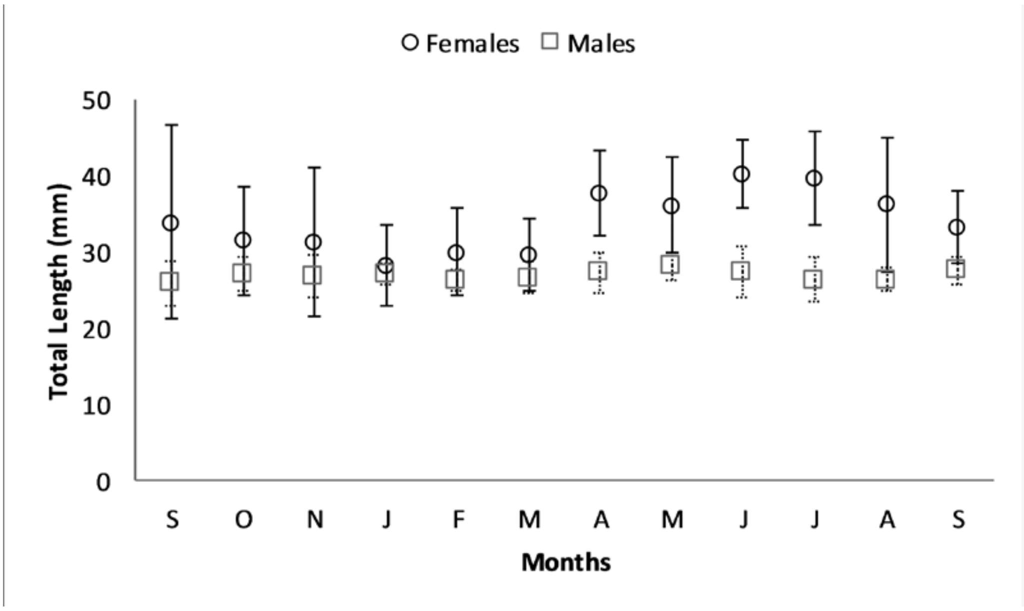

Fig. 2. Monthly growth pattern of *Gambusia holbrooki* at Site 1 in Acıgöl. Average total lengths with bars representing standard deviation

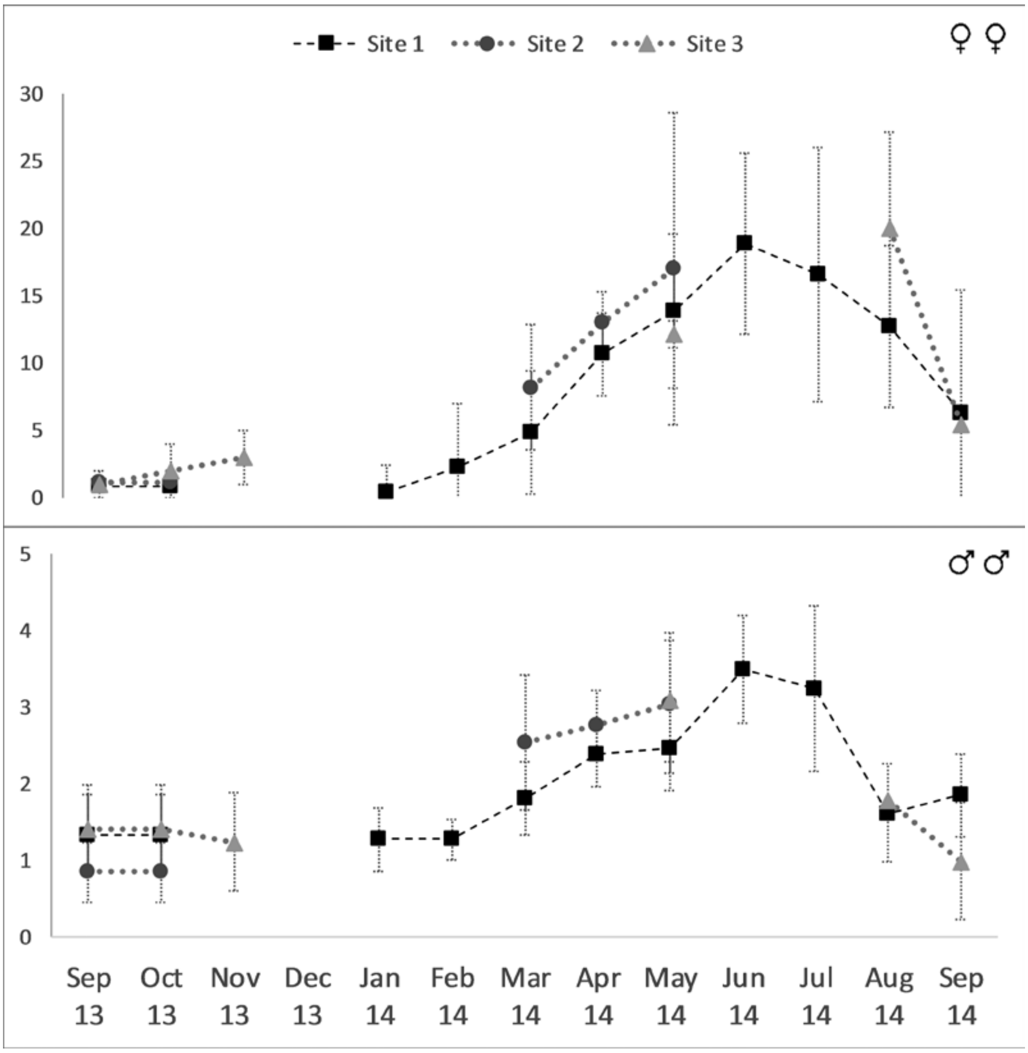

Fig. 3. Variation in mean (± standard deviation) gonadosomatic indices of male and female *Gambusia holbrooki*, sampled in Acıgöl, between September 2013 and September 2014

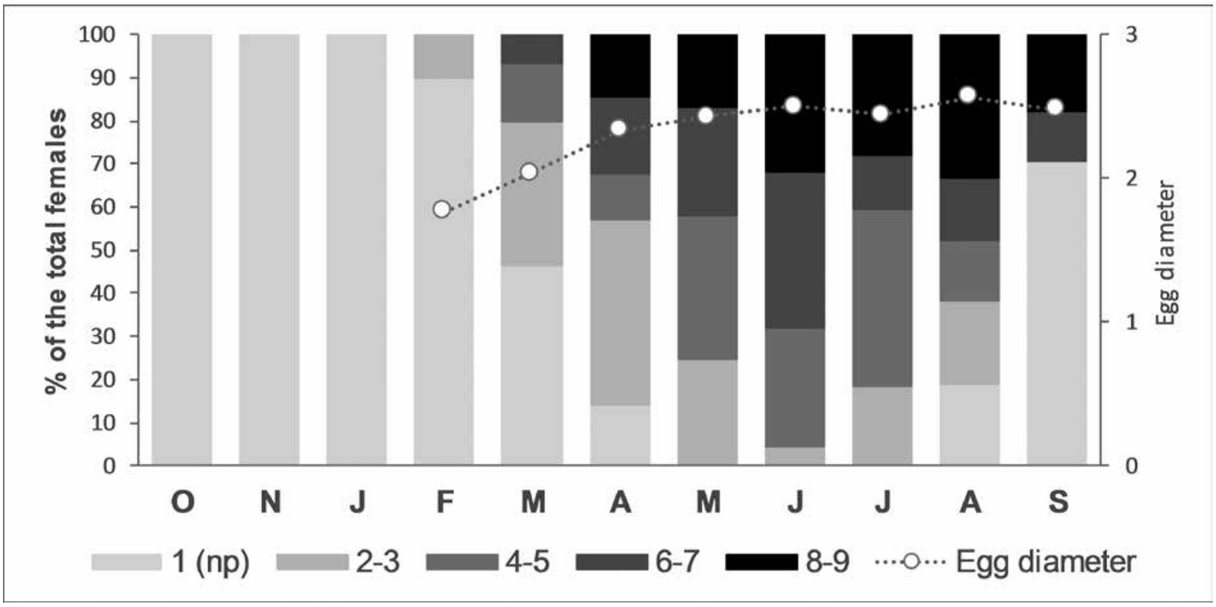

Fig. 4. Changes in the percentage of female *Gambusia holbrooki* bearing eggs/ embryos in each developmental stage as defined in Materials and Methods with the egg diameter change during the reproductive period. np – non-pregnant

Table 5. Absolute and relative fecundity and fertilisation success of *Gambusia holbrooki* in Acıgöl. AF – absolute fecundity; RF – relative fecundity; Nf – non-fertilised eggs; M – malformed eggs; n – number of females examined for fecundity; n' – number of eggs examined for fertilisation success

Fecundity						Fertilisation success		
Sites	**n**	**AF (Mean±sd)**	**Min-max**	**RF (Mean±sd)**	**Min-max**	**n'**	**Nf (%)**	**M (%)**
Site 1	102	26.3±15.2	1-76	27.1±14.5	1.4-85.5	2689	5.60	0.80
Site 2	20	43.1±32.2	10-125	54.2±21.7	32.1-127.7	862	3.55	0.78
Site 3	18	57.7±41.3	6-130	38.9±13.3	7.1-68.7	1039	1.93	2.48

The difference in the slopes of the regression equations was statistically significant (ANCOVA, F=29.01, P<0.001), due to the significance of difference between Site 1 and the other two sites (Tukey test).

Overall diet description and seasonal variation

None of the 175 stomach samples examined was completely empty. The dietary spectrum was summarised under 26 food categories (Table 6). Regarding the occurrence in the diet and the percent volume, the most common food items were plant materials (especially filamentous algae) and detritus at Site 1 and insect groups at Site 2 (mostly nematoceran Diptera) and Site 3 (mostly brachyceran Diptera). The most important contribution to the overall food components in terms of the percent number was made by invertebrate eggs at Site 1 and by zooplankton at Site 2.

The demonstration of the full seasonal variation in feeding was possible only for Site 1. IRI was significantly different between the seasons at this sampling site (PERMANOVA, pseudo-*F* = 4.17, P<0.001). The most important food component was the adult nematocerans in spring (Fig. 5). The diet shifted towards the plant sources and detritus in summer, followed by the dominance of nematoceran larvae in autumn.

Feeding ecology

Two different trophic positions were represented by *G. holbrooki* in Acıgöl: omnivory with a preference for plants at Site 1 and omnivory with a preference for animals at Sites 2 and 3. The main food items explaining the variation among dietary composition of the individuals are displayed in Fig. 6, after performing PERMANOVA to detect significant differences in the diet between the sites (pseudo-*F* = 11.56, P<0.001). Despite the spring feeding pattern seen at Site 1 (Fig. 5), most of the variation was contributed by filamentous algae, detritus and diatoms.

The diet composition was dominated by Hydracinidae, Cladocera and Copepoda in small individuals, whereas shifted towards different taxa, e.g., insects (at Site 2 and Site 3) and filamentous

Table 6. Overall diet composition of *Gambusia holbrooki* in Acıgöl: frequency of occurrence, % number and % volume of the main food categories. Estimated S-W diversity index and niche breadth with confidence intervals and trophic level ± standard error

Prey Categories	**Site 1**			**Site 2**			**Site 3**		
	% FO	**% N**	**% V**	**% FO**	**% N**	**% V**	**% FO**	**% N**	**% V**
Insecta	54.22	27.06	36.72	78.13	32.43	88.94	94.23	84.89	89.71
Coleoptera	2.41	0.66	3.55	12.50	3.75	46.05	19.23	6.58	8.80
Hemiptera	1.21	0.17	1.14	3.13	0.27	1.60	7.69	2.19	17.41
Homoptera	1.21	0.17	0.09	0.00	0.00	0.00	1.92	0.44	0.44
Brachycera	8.43	2.31	10.17	6.25	0.80	10.27	40.39	19.30	49.01
Nematocera (adult)	31.36	9.74	13.03	71.88	13.94	16.98	53.85	33.77	6.50
Nematocera (larvae)	18.07	3.47	7.02	46.88	10.99	6.68	5.77	2.87	0.67
Trichoptera (larvae)	3.61	0.83	0.96	0.00	0.00	0.00	0.00	0.00	0.00
Ephemeroptera (larvae)	0.00	0.00	0.00	9.38	1.88	6.79	1.92	1.32	5.47
Other (unidentified)	8.43	9.74	0.77	6.25	0.80	0.57	11.54	18.42	1.41
Zooplankton	18.07	8.09	0.03	53.13	41.56	2.23	9.62	7.02	0.10
Cladocera	14.46	6.77	0.03	37.50	25.74	0.75	5.77	3.51	0.01
Copepoda	0.00	0.00	0.00	31.25	15.55	1.42	7.70	2.63	0.08
Ostracoda	0.00	0.00	0.00	3.13	0.27	0.07	1.92	0.44	0.02
Rotifera	3.61	1.32	<0.00	0.00	0.00	0.00	1.92	0.44	<0.00
Other Invertebrates	13.25	59.08	0.17	31.25	21.72	2.58	23.08	6.14	7.90
Amphipoda	0.00	0.00	0.00	6.25	0.54	1.13	7.69	2.63	7.84
Gastropoda	1.21	0.17	0.07	6.25	0.54	1.10	0.00	0.00	0.00
Hydrachnidia	0.00	0.00	0.00	15.63	1.34	0.07	13.46	3.51	0.06
Invertebrate eggs	8.43	54.95	0.02	6.25	18.50	0.03	0.00	0.00	0.00
Other (unidentified)	1.21	3.96	0.08	3.13	0.80	0.25	0.00	0.00	0.00
Vertebrata	6.02	0.99	0.69	18.75	3.79	0.05	1.92	0.44	<0.00
Aphanius scales	3.61	0.66	0.01	12.50	3.22	0.04	1.92	0.44	<0.00
Aphanius adult	1.21	0.17	0.68	0.00	0.00	0.00	0.00	0.00	0.00
Gambusia scales	1.21	0.17	<0.00	3.13	0.57	0.10	0.00	0.00	0.00
Plant-Detritus	89.16	4.79	62.40	18.75	0.54	6.21	19.23	1.51	2.28
Detritus	39.76	–	11.96	9.38	–	1.07	5.77	–	0.07
Filamentous Algae	75.90	–	47.88	12.50	–	3.83	17.31	–	0.16
Diatoms	53.01	–	2.03	6.25	–	1.31	1.92	–	<0.00
Macrophytes	6.02	3.96	0.52	3.13	0.27	0.01	1.92	1.51	2.06
Pollen	2.41	0.83	<0.00	0.00	0.00	0.00	0.00	–	0.00
Shannon-Wiener *(H')*	2.14 (2.01-2.24)			2.59 (2.51-2.66)			2.18 (2.07-2.28)		
Levin's Niche Breadth *(B)*	6.39 (5.42-7.24)			10.65 (9.43-11.67)			6.53 (5.57-7.45)		
Std. Niche Breadth *(BA)*	0.23 (0.19-0.27)			0.42 (0.37-0.46)			0.24 (0.20-0.28)		
Trophic Level ± SE	2.42±0.25			3.04±0.39			3.09±0.41		

algae (at Site 1) in larger individuals. This ontogenetic pattern was evident between the >40mm and <30mm individuals, but not between these length classes and the 30-40 mm length class. Indeed, the factorial test with the length and sex as fixed factors indicated a significant effect of length rather than of sex or length x sex interaction on the overall diet of the species (Table 7).

The feeding strategy diagrams show similar feeding strategy patterns at all examined sites (Fig. 7). At all sites, a mixed-feeding strategy, with alternating levels of specialisation and generalisation on different prey types was presented. The percent of individual predators specialised on different types of prey, which indicates a broad niche, was relatively higher at Site 2. This pattern

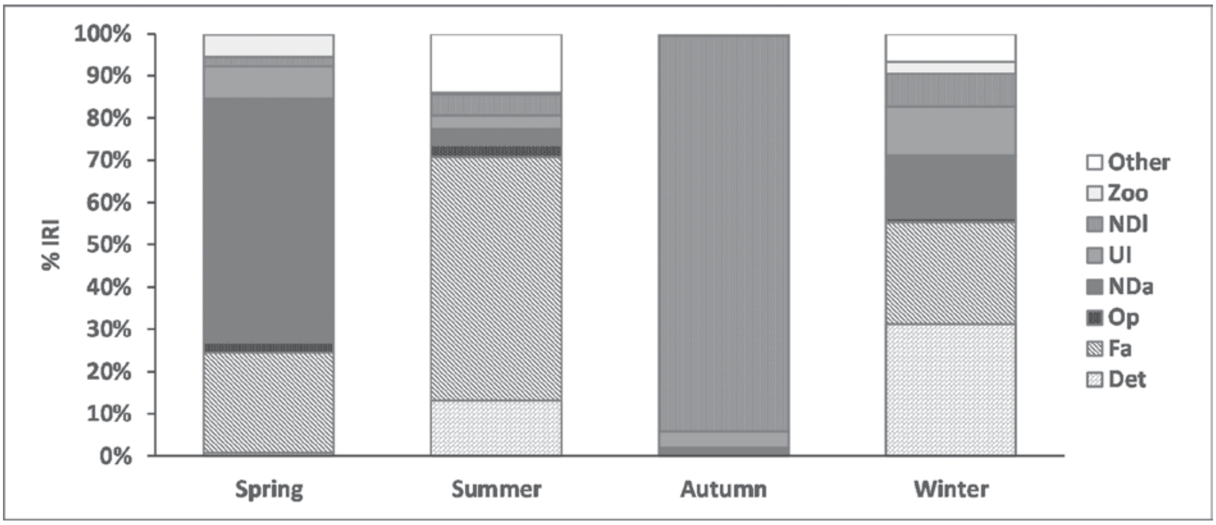

Fig. 5. Seasonal change in the percent relative importance index of *Gambusia holbrooki* at Site 1 in Acıgöl. IRI – index of relative importance; Zoo=zooplankton; NDl=Nematorecan Diptera (larvae); UI=unidentified Insecta; NDa=Nematorecan Diptera (adults); Op=other plant material; Fa=filamentous algae; Det=detritus

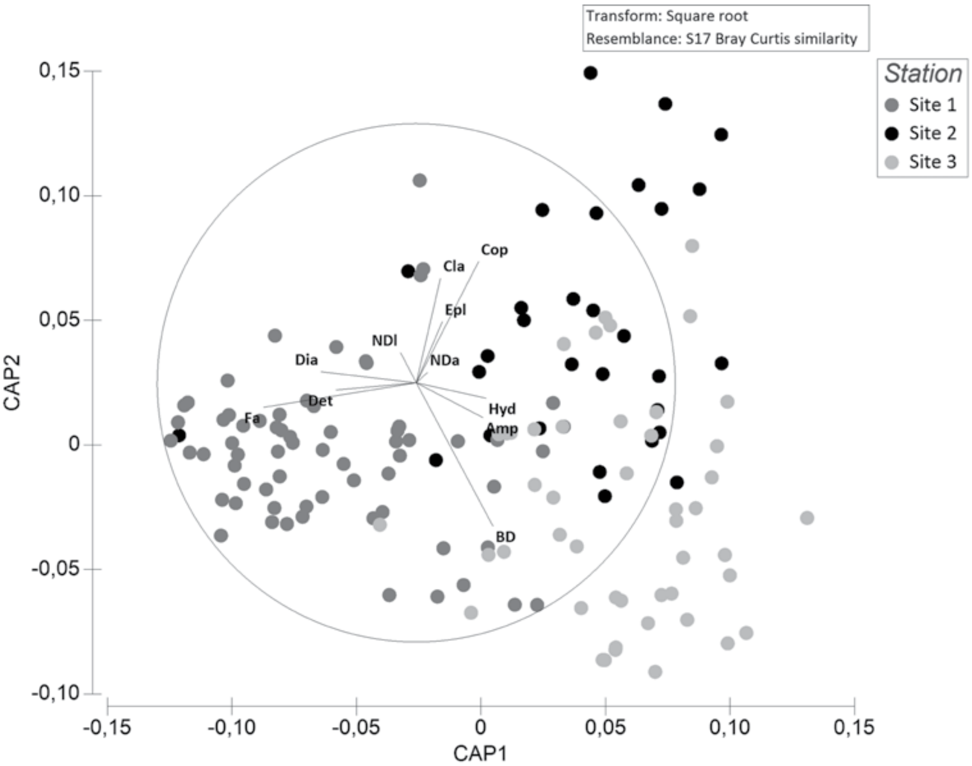

Fig. 6. Two-dimensional ordination plots resulting from the diet composition data of *Gambusia holbrooki* at all sites in Acıgöl. Amp=Amphipoda; BD=Brachyceran Diptera; Cla=Cladocera; Cop=Copepoda; Det=detritus; Dia=diatoms; Epl=Ephemeroptera (larvae); Fa=filamentous algae; Hyd=Hydracinidia; NDl=Nematorecan Diptera (larvae); NDa=Nematorecan Diptera (adults)

was also evident in the Shannon diversity function and Levins niche breadth estimations (Table 6). The trophic levels of the food items that dominated the population were different between the sites (filamentous algae and diatoms at Site 1 and Diptera groups at Sites 2 and 3).

Discussion

The most prominent contrasting factors between the studied sites were the water salinity and temperature, which greatly fluctuated at Site 3, and were highly stable throughout the year at Sites 1 and 2 (Table 1).

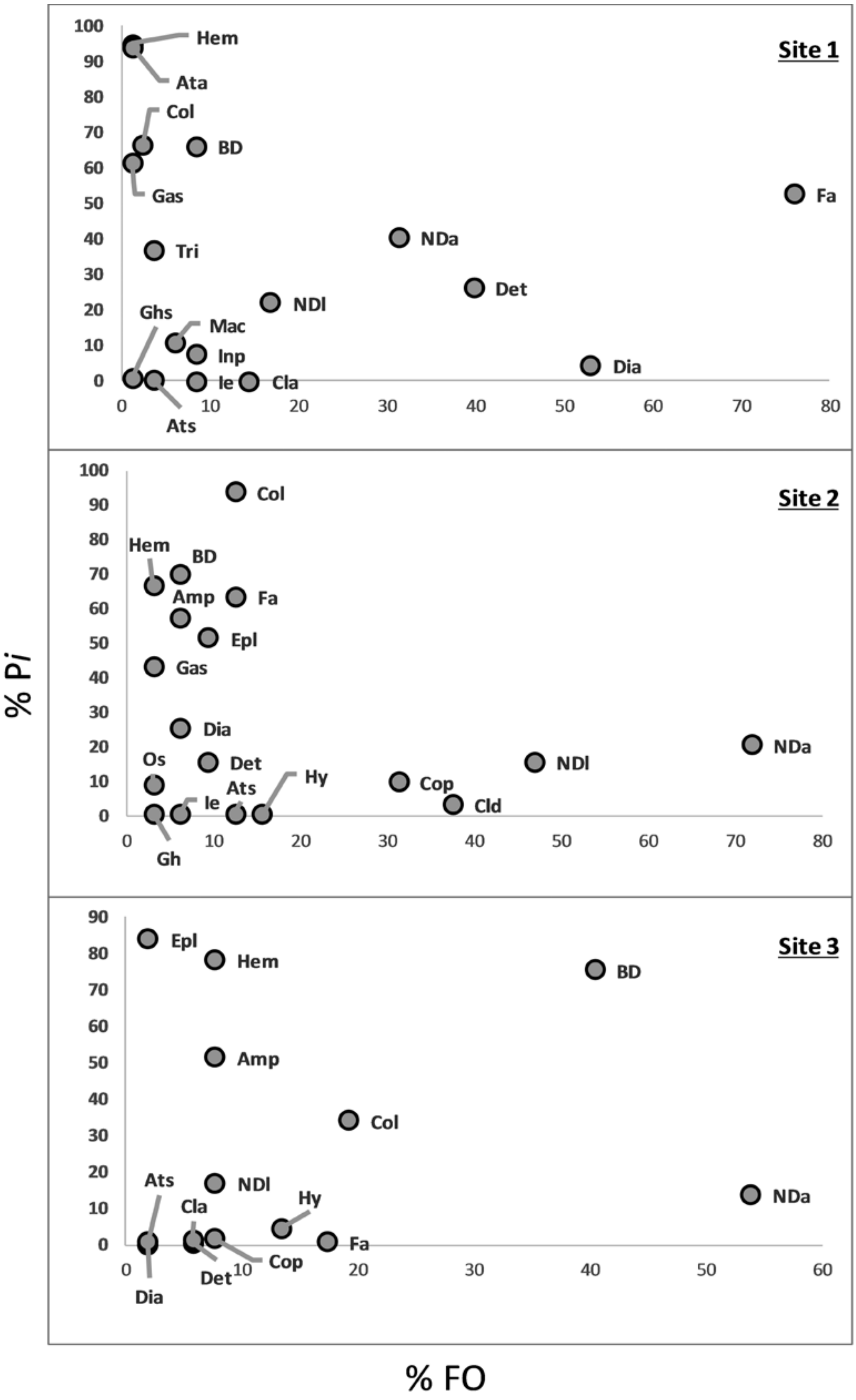

Fig. 7. Graphical analysis of *Gambusia holbrooki* feeding strategy in Acıgöl, using the modified Costello method. %P*i* – prey-specific abundance; %FO – frequency of occurrence; Amp=Amphipoda; Ata=*A. transgrediens* (adult); Ats=*A. transgrediens* (scale); BD=Brachyceran Diptera; Cla=Cladocera; Col=Coleoptera; Cop=Copepoda; Det=detritus; Dia=diatoms; Epl=Ephemeroptera (larvae); Fa=filamentous algae; Gas=Gastropoda; Ghs=*G. holbrooki* (scale); Hem=Hemiptera; Hy=Hydracinidia; Inp=insect part; Mac=macrophytes; NDl=Nematorecan Diptera (larvae); NDa=Nematorecan Diptera (adults); Os=Ostracoda; Tri=Trichoptera (larvae)

Table 7. The factorial PERMANOVA test with length and sex of *Gambusia holbrooki* as fixed factors. df – degrees of freedom; MS – mean square; Pseudo-*F* – permutational *F*-statistics; p(perm) – permutational *p* value

Source	**df**	**MS**	**Pseudo-*F***	**P(perm)**
Total length	2	9990	2.8083	0.0008
Sex	1	4853.2	1.3643	0.1868
Total length x Sex	1	6349.2	1.7848	0.0714
Residuals	139	3557.3		

Therefore, the individuals of *G. holbrooki* at Site 3 were exposed to a higher degree of environmental stress than those at the other sites. The lack of individuals at this site in most of the season was related to these conditions – mostly to the extreme salinity fluctuations.

Considering the overall sampling in the Acıgöl Lake, the females of *G. holbrooki* markedly dominated the sample with lower proportion in spring and summer. The sex ratio of *Gambusia* spp., as it is known, is 1:1 at birth (Krumholz 1948). The later stage imparity can often be linked to selective mortality, sampling bias and different habitat preferences of males and females (Fernández-Delgado 1989, Vargas & de Sostoa 1996, Fernández-Delgado & Rossomanno 1997). Approaching the 1:1 ratio in summer and spring in this study can be attributed to a combination of one or more factors: (i) mating aggregation; (ii) acting of selective mortality by predators at the expense of females due to pregnancy (Vargas & de Sostoa 1996, Cabral & Marques 1999); and (iii) the new generation represented in the sample (Haynes & Cashner 1995).

Many studies on *Gambusia* spp. have shown that females have higher survival rate and larger longevity than males (Pérez-Bote & López 2005, Patimar et al. 2011, Ruiz-Navarro et al. 2011). While females survived after the reproduction period in Acıgöl, a great proportion of males from the parental cohort did not survive the winter, especially those sampled at Site 3. The somatic costs of reproduction against unpredictable conditions, which can be related to high adult mortality, may explain this situation, considering that only two individuals of 1+ males have been caught.

Making a comprehensive comparison on the seasonal growth between the sampling sites was impossible, since we could not find fish in some months, except at Site 1. However, according to the estimated von Bertalanffy parameters, different asymptotic length values were estimated among the sampling sites, being the highest at Site 3, where the water temperature reached 10°C. The effect of temperature was more apparent on the asymptotic length than on the growth coefficient (k) and L∞ tended to be greater as temperature declined (Basoline et al. 2004). The seasonal growth pattern illustrated for Site 1 is similar to other Mediterranean populations (Cabral & Marques 1999, Pérez-Bote & López 2005).

It has been suggested that the timing of the reproductive cycling in *Gambusia* spp. is mainly related to photoperiod rather than temperature (Hubbs 1971, Milton & Arthington 1983, Cech et al. 1992). This was also proven for Acıgöl, as the gravidity pattern and GSI were similar between the sites even though the temperature was stable throughout the year at Site 1 and Site 2, but not at Site 3. The reproductive season started at the beginning of April and ended at the end of September as observed in many other populations (Vargas & de Sostoa 1996, Specziár 2004, Pérez-Bote & López 2005, Zarev 2012). Given that the pregnancy period suggested for mosquitofish is about four weeks (Krumholz 1948, Milton & Arthington 1983, Meffe 1990), it means that *G. holbrooki* females could release up to six broods in Acıgöl between April and September.

Like many other fish species, the fecundity has been known to be positively correlated with female length in *Gambusia* spp. (Krumholz 1948). The strongest correlation in the fecundity-length relationship was estimated at Site 3, with larger females and more embryos. Many studies have shown that the reproductive investment increases (e.g. more embryos, heavier gonads) with salinity (Brown-Peterson & Peterson 1990, Vargas & de Sostoa, 1996, Alcaraz et al. 2008, Swenton & Kodric-Brown 2012). Therefore, the highest absolute fecundity and the maximum number of embryos found in a female were obtained at Site 3, with the cost of the highest percent of malformed eggs.

Although *G. holbrooki* is known to prefer animal prey (Arthington 1989, Arthington & Marshall 1999, Garcia-Berthou 1999), a few studies suggest that the species feeds more on plant material (Gophen et al. 1998, Arthington & Marshall 1999, Specziár, 2004). Prey shifting in response to availability of sources is common in the mosquitofish (Pyke 2005). Most likely the plant-weighed feeding habit demonstrated at Site 1, but not at other sites, was associated with the high intraspecific density.

It is difficult to characterise the feeding of *G. holbrooki*. Even though some studies reveal that its feeding is based mainly on zooplankton (Bence & Murdoch 1986, Kramer *et al.* 1987, Singh & Gupta 2010), or on larvae of Diptera (Hoy *et al.* 1972, Peck & Walton 2008), it has a highly flexible diet depending on the available sources (Pyke 2005). Similarly, in Acıgöl, three distinct compositions for three spatially close sampling sites were identified (Table 6).

The ontogenetic shift in diet has been reported in previous studies (Miura et al. 1979, Farley 1980, Garcia-Berthou 1999). In Acıgöl, the diet composition of the small individuals was dominated by Hydracinidae, Cladocera and Copepoda, and shifted towards larger taxa specific to each sampling site. Although there was remarkable sexual dimorphism in terms of body size, the effect of the length on the overall diet variation was more significant than that of the sex. This may support the findings of Crivelli & Boy (1987) who hypothesised that the ontogenetic resource partitioning could involve different microhabitat use of different size classes.

The modified Costello method outlined a mixed-feeding strategy for *G. holbrooki* in Acıgöl, which could be unclear in the summary table of dietary composition. Relatively broader trophic niches with higher rate of specialised individuals were illustrated for Site 2, which was also evident from the diversity indices. This was not surprising, when considering the mild environmental conditions with a larger habitat size at Site 2.

Several studies have demonstrated the ability of *G. holbrooki* to outcompete native fishes (Pyke 2005, Alcaraz & Garcia-Berthou 2007, Alcaraz et al. 2008). Even though there is evidence that *G. holbrooki* and the critically endangered *A. transgrediens* (the only native fish in the lake) do not compete notably for food in Acıgöl (unpublished data), *Aphanius* scales were observed in the stomach of *G. holbrooki*, which indicates its aggressive pressure on *A. transgrediens*. This behaviour seems to be species-specific having in mind the lower occurrence of *Gambusia* scales in the examined stomachs.

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