Life history pattern and feeding habits of the invasive mosquitofish, *Gambusia holbrooki*, in Lake Pamvotis (NW Greece)

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The life history pattern and feeding ecology of introduced *Gambusia holbrooki* was studied in Lake Pamvotis, a shallow lentic ecosystem. The length frequency analysis identified two different age groups. The first recruits were recorded in July and were present thereafter until December. Females reached greater sizes, grew faster and lived longer than males. The largest sizes found were 43.06 mm for females and 34.34 mm for males. Sex-ratio deviated significantly from unity (1.65:1). Multiple spawning occurred throughout the reproductive cycle (from May to August). The average brood size per female reached its maximum in June (50.8 embryos per female). After reproduction, the percentage of parental individuals decreased drastically. Mosquitofish fed mainly on zooplankton (Copepoda, littoral Cladocerans), but surface insects (such as Nematoceran adults, basically Chironomid, collembolans, Hemiptera, spiders and worms) were additional food. Diet composition showed little seasonal variation. Trophic niche breadth was largest in summer (H = 0.68), and smallest in autumn (H = 0.46). The mean volume of stomach contents increased significantly for fish larger than 30 mm TL (Total Length). *Gambusia holbrooki* showed high intrapopulation dietary overlap and followed a specialist feeding strategy with adaptation to seasonal prey availability. Moreover, Culicidae larvae constituted a small quantitative fraction of the *G. holbrooki* diet which raised questions about the extensive use of mosquitofish for biological control of mosquitoes.

Key words: mosquitofish, growth, reproduction, diet, feeding strategy.

INTRODUCTION

The introduction of non-native species into new environments and their spread over natural areas pose important threats for the conservation of biodiversity and have caused declines or extinctions of native species worldwide (Clavero & García-Berthou, 2005). Most of the alien vertebrates that were introduced for beneficial outcomes and generalized vertebrate predators that were used as biological control agents, have revealed serious negative ecological and/or economic impacts and had unexpected consequences (Simberloff & Stiling, 1996). However, non-native species introductions are not always undesirable be-

cause not all introduced species become invasive; also, successful invaders do not constantly have negative ecological and economic impacts (Williamson, 1996; Leprieur *et al.*, 2009). In addition, success in predicting the undesired consequences of non native species is compromised by the fact that many ecosystems increasingly support multiple invasive species (Leprieur *et al.*, 2009).

The family Poeciliidae, belonging to the order of Cyprinodontiformes, is a large taxon of bony fish comprised many genera (*Gambusia* sp. included). Numerous species are included in this genus, which originated from the USA, Mexico, and Cuba (Gandolfi *et al.*, 1991), and are usually known as mosquitofish due to their ability to prey upon larval of the family of Culicidae. The mosquitofish is probably the most widely distributed freshwater fish of the globe (Pyke,

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2005) and is considered to be one of the world's 100 worst invasive alien species by the GISP (Global Invasive Species Program, http://www.issg.org/ database/).

Two species of mosquitofish, *G. affinis* (Baird & Girard, 1853) and *G. holbrooki* Girard, 1859, have been introduced into more than 50 countries to control mosquitoes, a vector of malaria (García-Berthou *et al.*, 2005). Eastern mosquitofish, *G. holbrooki*, was introduced in Lake Pamvotis, in the mid of 1950s. The population expanded rapidly in the lake and it is by now the most widespread fish species at the littoral zone together with the freshwater sand goby *Economidichthys pygmaeus* (Leonardos *et al*., 2008).

Gambusia holbrooki has a high level of plasticity and survival capability, and can easily colonize new habitats. It has a preference for stagnant waters with a muddy bottom and lush vegetation (Gandolfi *et al*., 1991) and withstands wide ranges of temperature and salinity (Alcaraz & García-Berthou, 2007). Moreover, the ovoviviparity of this species, with a particular maternal system (Balon, 1975), may have been a great advantage for its invasive success (McKay, 1978). In the non-native habitats, the mosquitofish is considered the main predator of mosquitoes and display different feeding habits, depending on season and feeding resources (Ferrante *et al*., 2002). Moreover, it is considered to have a negative impact on the indigenous aquatic community structure and dynamics because of its selective predation on zooplankton (Margaritora *et al*., 2001), eggs and fish larvae (Pen & Potter, 1991). Therefore, in many cases *G. holbrooki* seems to upset the native community, not only as a result of larva predation but also through direct trophic competition (Lloyd *et al*., 1986; Scalici *et al*., 2007). Yet, despite the fact that in the past the mosquitofish has been considered to be of great contribution against the proliferation of mosquito larvae (Tortonese, 1970), many studies have also shown that it had an impact on many native fish species (Mills *et al*., 2004).

Most studies of *Gambusia* have been documented in its natural range (Krumholz, 1948; Meffe, 1991; Haynes & Cashner, 1995) and in areas where it has been introduced (Brown & Fox, 1966; Sawara, 1974; Milton & Arthington, 1983); few studies have examined the life history of *G. holbrooki* in Europe (Fernandez-Delgado, 1987; Fernández-Delgado, 1989; Alcaraz & García-Berthou, 2007) while Crivelli & Boy (1987), García-Berthou (1999) and Specziar (2004) reported its feeding habits.

Here we present the results of an extensive field study for the investigation of some life-history parameters (i.e. length-mass relationship, well being indices, age, growth and fecundity) of Eastern mosquitofish population in Lake Pamvotis (NW Greece). We also provide information on the diet, its feeding behavior with special emphasis on seasonal and ontogenetic dietary variation, as well as its feeding strategy. The results of the current study can provide insights towards to understanding the structure of the introduced species population and its potential effects on the indigenous biota.

MATERIALS AND METHODS

Study site

The study was conducted in Lake Pamvotis, a shallow Mediterranean lake (39˚40′ N, 20˚ 53′ E). Its surface area is 22.8 km², the mean depth is 4.5 m and the maximum depth is 7.5 m (Fig. 1). It is an ancient lake ecosystem, situated in a topographically diverse landscape on the western flank of the Pindus mountain range (Tzedakis *et al*., 2002). Lake Pamvotis has recently been recognized as globally significant for its biodiversity (Krystufek & Reed, 2004) and, because of its great nature conservation value, is now listed in Natura Special Conservation areas according to the Habitats Directive EC 92/43 on the conservation of natural habitats and of wild fauna and flora. This inclusion is due to the presence of the following fish species: Trichonis roach *Rutilus panosi* (Bogutskaya & Iliadou, 2006), Epirus barbell or Maritsi *Luciobarbus albanicus* (Steindachner, 1870), Epirus minnow *Pelasgus epiroticus* (Steindachner, 1895) and habitat types of *Magnopotamion* or *Hydroharition* vegetation. According to Kagalou *et al*. (2001) the lake's mixing regime is polymictic with the appearance of an unstable, weak thermal stratification during the summer period. Generally, the lake is strongly wind-dependent with intensive sediment and algae stirring-up phenomena. During the last three decades, anthropogenic activity (for example irrigation and domestic sewage discharge) has altered the trophic status of Lake Pamvotis and caused serious eutrophication problems (Kagalou *et al*., 2001), while frequent cyanobacterial blooms occur during the warm months.

Within the time frame of survey, water temperature was highest during July (26.7˚C) and lowest during December (6.1˚C), pH values varied from 7.1 (April) to 8.6 (September), dissolved oxygen (DO) increased gradually from 2.8 mg l⁻¹ (August) to 10.4
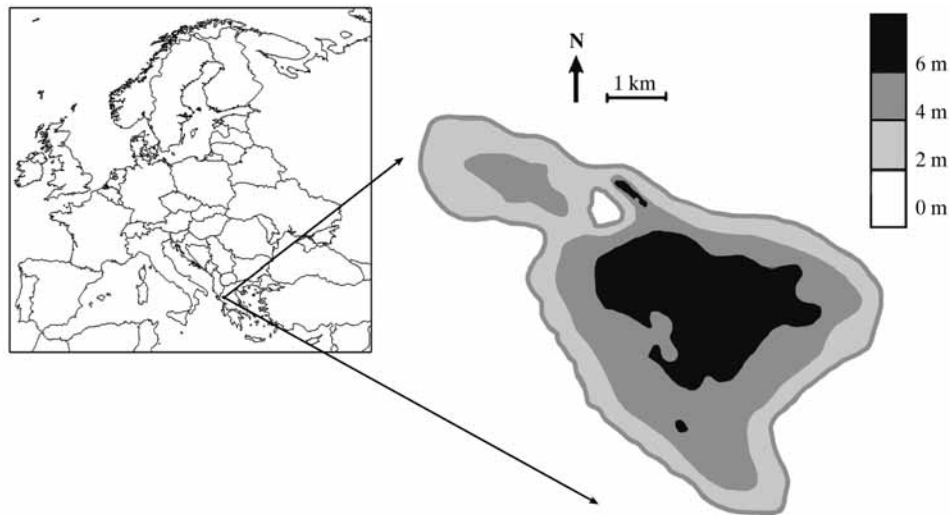
FIG. 1. Map of Lake Pamvotis indicating the sampling sites.

mg l^{-1} (January) and conductivity varied between 357 µS sec^{-1} (September) and 429 µS sec^{-1} (June). The bottom is covered mainly of pebbles mixed with coarse sand and characterized by dense aquatic vegetation (*Myriophyllum spicatum*) as well as the common reed (*Phragmites australis*). In addition to *G. holbrooki*, two other fish species co-exist in the littoral zone of Lake Pamvotis: *Economidichthys pygmaeus, Knipowitschia caucasica* as well as two shrimp species, *Atyaephyra desmarestii* and *Palaemonetes antennarius*.

Sampling procedure

Fish were sampled monthly with a hand net (2 mm mesh size) between May 2007 and April 2008 at the littoral zone of the lake. Fish were preserved in neutralized formaldehyde (4%) for later laboratory analysis. Sampling always took place between 10 am and 1 pm. According to Crivelli & Boy (1987), this period corresponds to the most active feeding period of mosquitofish.

Life history parameters and statistical analyses

In the laboratory, standard (SL) and total length (TL) of each individual were measured to the nearest 0.1 mm. Total (TM) and eviscerated (EM) masses were weighted to the nearest 0.01 mg. The gonads and the liver were removed and weighed separately to the nearest 0.01 mg. Mosquitofish were sexed as males if they possessed any evidence of a gonopodium, as juveniles if they were smaller than the smallest male in each sample, and as females in the case of the absence of gonopodia but were larger than the smallest male. Females were considered gravid if they possessed developing ova and as pregnant if they possessed eyed embryos. Sex ratios were calculated as the number of females per male, based on the above sex determination criteria. Length-frequency histograms were plotted as 1 mm size classes for females, juveniles and males (Haynes & Cashner, 1995).

Length-mass relationship (TM = aTL^b), was calculated, where TM is the total mass (g) and TL the total length (mm). In the linear (after logarithmic transformation) form of the equation, a is the intercept to the y-axis of the best-fit line and b is the slope of the line. The regressions were performed separately for males and females and then were tested for differences in slopes and intercepts both between sexes and seasons using Analysis of Covariance (ANCOVA; Zar, 1999). A t-test (Pauly, 1984) was used to test whether *G. holbrooki* grows isometrically ($b = 3$) or allometrically (positive: $b > 3$ or negative: $b < 3$).

For the calculation of condition factor (K and K$_{ev}$) the Fulton formula was used (Bagenal & Tesch, 1978): K = 10^5 (TM/TL^b) and K$_{ev}$ = 10^5 (EM/TL^b), where b is the slope of the regression line between mass and total length and EM is the eviscerated fish mass. The gonadosomatic index (GSI), commonly used as indicator of reproductive activity, was calculated as GSI = (GM×EM)×100, where GM is the gonad mass (g) and EM is the eviscerated mass (g). The hepatosomatic index (HSI), commonly used as a measure proportional to energy reserves stored in the liver was calculated as HSI = (LM×EM)×100, where

LM is the liver mass (g) and EM is the eviscerated mass (g). All indices were compared between sexes using the Mann-Whitney U-test and across months using the non-parametric Kruskal-Wallis test, since the assumptions of normality and homogeneity were not met after arc-sine or logarithmic transformation (Sokal & Rohlf, 1995). Homogeneity of variance and normality were tested with Levene's test and the Kolmogorov-Smirnov test, respectively.

Fecundity was determined for pregnant females as the number of embryos in the ovary. Analysis of Variance (ANOVA) was used to test for the significant differences in the mean size of pregnant females and in the mean brood size over the reproductive months. Normality and homogeneity of variance were tested with the Kolmogorov-Smirnov test and Levene's test, respectively. Regression analyses were used to describe the relationship of fecundity to the fish length and body mass. Descriptive statistics are reported as mean ± standard error.

Dietary habits and statistical analyses

The stomach contents of all captured mosquitofish were examined under a dissecting stereoscope with an ocular micrometer. Each stomach content was washed in a petri dish, where prey items were identified, separated, counted and measured (body length and body width). The volume of the food items was estimated by calculation of the volume of a geometric solid of similar size and shape (Bowen, 1983). The volume of uncountable categories (plant leaves) in the gut contents was estimated to the nearest 0.00025 mm^3 with a Neubauer counting chamber. Estimated wet volume later transformed to biomass using a conversion of 0.27 mg of dry mass mm^{-3} according to Lindegaard (1992) and Alcaraz & García-Berthou (2007). Fragments of organisms, such as heads were considered, as individuals. Animal prey remains were identified to the lowest readily recognizable taxon (usually family) and counted.

The seasonal feeding activity pattern was derived from the seasonal variation of percentage of empty guts. In order to determine prey importance in the diet of *G. holbrooki*, per cent number (%N), per cent biovolume (%V), per cent biomass (%M) and frequency of occurrence (%F) were used to analyze the dietary importance of each food category.

The contribution of each prey (dietary item) to the diet was estimated using three simple indices [number percentage (%N), volume percentage (%M) and frequency of occurrence percentage (%FO)] discussed and revised by Hyslop (1980) and Cortés (1997). The index of relative importance (IRI) (Pinkas *et al*., 1971) standardized to %IRI (Cortés, 1997) was also used because it is the best approach to gain an adequate description of the diet (Hyslop, 1980) and allows comparison between dietary items of the same species and between diets of different species (Cortés, 1997).

Seasonal variation in diet was assessed by categorizing months into seasons (autumn = Sep-Nov; winter = Dec-Feb; spring = Mar-May; summer = Jun-Aug). To identify size-related feeding shifts, fish were grouped into six 5-mm length classes: small individuals (size I: < 20 mm; size II: 20-25 mm), intermediate individuals (size III: 25-30 mm; size IV: 30-35 mm) and large individuals (size V: 35-40; size VI: 40-45 mm) according to Mieiro *et al*. (2001). Effects of length-class and season on the mean biovolume per stomach (Vm/ST) were tested by Analysis of Variance (ANOVA). A logarithmic transformation was applied to meet the assumptions of normality of each variable and the homogeneity of variances (Sokal & Rohlf, 1995). Tukey HSD post-hoc test was employed to locate the source of significant differences (Zar, 1999).

Prey diversity in the diet, which corresponds to food niche breadth (Scrimgeour & Winterbourn, 1987), was calculated using the Shannon-Weaver index (H′): H′ = −∑p$_i$ lnp$_i$, where p$_i$ is the proportion of individuals of prey type *i* (Shannon & Weaver, 1963). This index has adequate sensitivity for detecting changes in species diversity and provides a general indication of the relative magnitude of trophic specialization (Berg, 1979). Kruskal-Wallis test was used for comparison of dietary breadth for each season and each size - class (Sokal & Rohlf, 1995), since the assumptions of normality and homogeneity were not met after arc-sine or logarithmic transformation (Sokal & Rohlf, 1995).

To calculate dietary overlap, %W was applied to the index proposed by Pianka (1980). Overlap index values range from 0 (no overlap) to 1.0 (complete overlap), and values higher than 0.6 are considered as "biologically significant" overlap (according to Pianka, 1980). To validate significance of these overlaps, the observed values were compared to a distribution of expected overlap values based on null-model simulations. The distribution of null-model data resulted from 1000 randomizations of the diet by using EcoSim v7.42 software (Gotelli & Entsminger, 2001). The observed value was considered statistically different from the null distribution if it was greater or

less than the simulated index in 95% of the permutations ($p < 0.05$; Winemiller & Pianka, 1990). An observed value significantly lower than the simulated index suggests differences in the diet or partitioning of food resources, while an observed value significantly higher than the simulated index suggests that the diets are similar or that there is strong resource competition (Gotelli & Graves, 1996).

The feeding strategy of *G. holbrooki*, in terms of specialization and generalization, and the importance of each prey category in the diet were identified by plotting the prey-specific abundance (P_i) of each prey category against frequency of occurrence (%FO) (Amundsen *et al*., 1996). P_i refers to the relative abundance among prey species found in the stomachs, and was calculated as the number of prey category *i* divided by the total number of prey in the stomachs that contained the prey category *i*, expressed as a percentage. Prey points located on the upper right of the diagram would be indicative of specialization of the predator population. In contrast, all prey points located along or bellow the diagonal from the upper left to the lower right would reflect a generalized feeding strategy of the predator population. Furthermore, the distribution of points along the diagonal from the lower left to the upper right corner provides a measure of prey importance, with dominant prey at the upper and rare prey at the lower end (Amundsen *et al*., 1996). Data analyses were carried out using SPSS 19 software package.

RESULTS

Life history traits

In this study, 909 individuals (549 females, 278 males and 82 juveniles) were caught and measured. The smallest male with gonopodium was 16.44 mm. The smallest gravid female was 20.35 mm and the smallest pregnant female was 24.52 mm. The largest female found was 43.06 mm while the largest male was 34.34 mm. Length frequency distributions for females, males and juveniles are shown in Figure 2. From January to June, only adult female and male specimens were recorded. After the start of reproduction, bimodal distributions were observed thereafter until December, which is the month where the last newborns were collected.

The overall sex ratio of females to males was 2.3:1, diverging significantly from 1:1 ($\chi^2 = 88.804$; $p < 0.001$). Monthly sex ratio (Table 1) differed significantly from 1:1, except for the period June to September.

ANCOVA analysis for total mass (TM) to total length (TL) regressions (Table 2) showed significant differences between sexes and seasons. The slopes and the intercepts were significantly different between sexes (ANCOVA: $F_{1,823} = 11.561$, $p = 0.001$; ANCOVA: $F_{1,824} = 130.410$, $p < 0.001$) and seasons (ANCOVA: $F_{3,819} = 17.786$, $p < 0.001$; ANCOVA: $F_{3,822} = 16.159$, $p < 0.001$). Comparison of the exponent (i.e., slope at the linear form of the equation) with the theoretical value 3 (Table 2) indicated nega-

TABLE 1. Monthly sex ratio of *G. holbrooki* from Lake Pamvotis (May 2007 to April 2008) tested by χ^2 analysis

|	Sex ratio	χ^2	No. of females	No. of males	*p*-value
May 2007	3.2:1	5.76	16	5	0.016*
June 2007	1.6:1	1.33	8	4	0.248
July 2007	0.76:1	0.29	14	17	0.59
August 2007	0.64:1	0	25	25	1
September 2007	0.86:1	0.03	16	15	0.857
October 2007	1.20:1	16.03	76	34	< 0.001*
November 2007	2.37:1	36.58	102	32	< 0.001*
December 2007	3.14:1	7.76	22	7	0.005*
January 2008	2.22:1	4.17	20	9	0.041*
February 2008	1.41:1	5.3	88	60	0.021*
March 2008	2.48:1	25.3	82	29	< 0.001*
April 2008	1.95:1	12.57	80	41	< 0.001*

* denotes *p*-values < 0.05
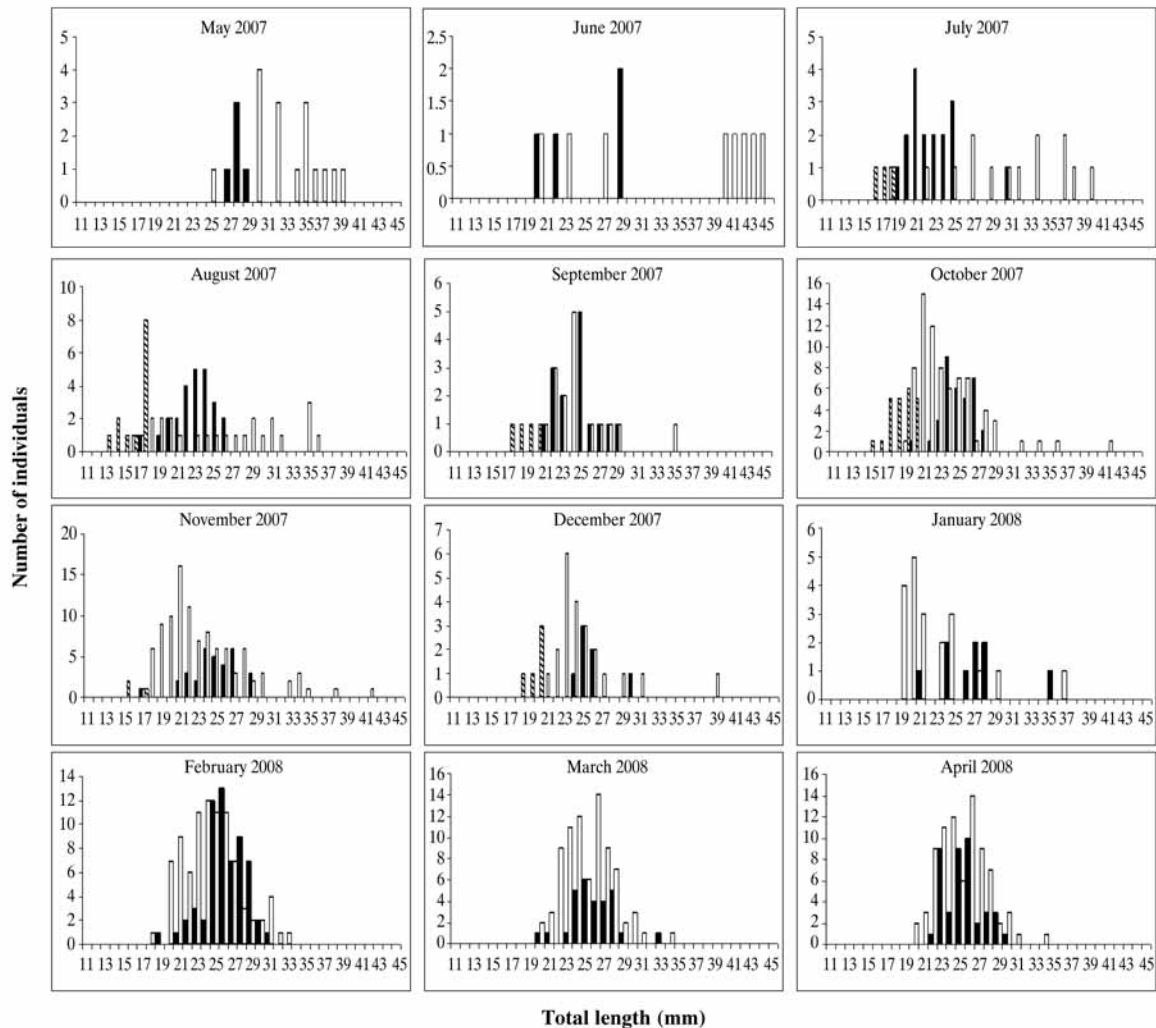
FIG. 2. Monthly pattern of length-frequency distribution of *Gambusia holbrooki*. Cross-hatched represent juveniles, black bars males and empty bars females.

TABLE 2. Estimated relationship parameters between total length (TL, mm) and total mass (TM, g) for *G. holbrooki* males, females, juveniles and the whole sample

$TM = aTL^b$	log a	b	N	r^2	p-level	95% C.I. for b
Males	–4.735	2.852	278	0.95	< 0.001	2.738-2.965
Females	–5.031	3.096	549	0.97	< 0.001	3.036-3.156
Juveniles	–4.333	2.560	82	0.92	< 0.001	2.318-2.802
Total	–4.845	2.952	909	0.97	< 0.001	2.905-3.000

tive allometric growth for males (t = 2.55, $p < 0.05$, n = 278) and positive allometric growth for females (t = 3.09, $p < 0.05$, n = 549).

Condition factor followed a discrete seasonal pattern (for K: Z = –11.172, $p < 0.05$; for K_{ev}: Z = –6.767, $p > 0.05$) in both males and females. Significant differences were found between the months for females condition (for K: H = 124.43, $p < 0.001$; for K_{ev}: H = 108.46, $p < 0.001$). This occurred in both K and K_{ev}, so the variation in condition must have taken place at a somatic level. The condition of males with gonads (for K: H = 14.88, $p > 0.05$) was similar, from which it can be deduced that testicular mass has little influence on the condition factor. However it varied sig-
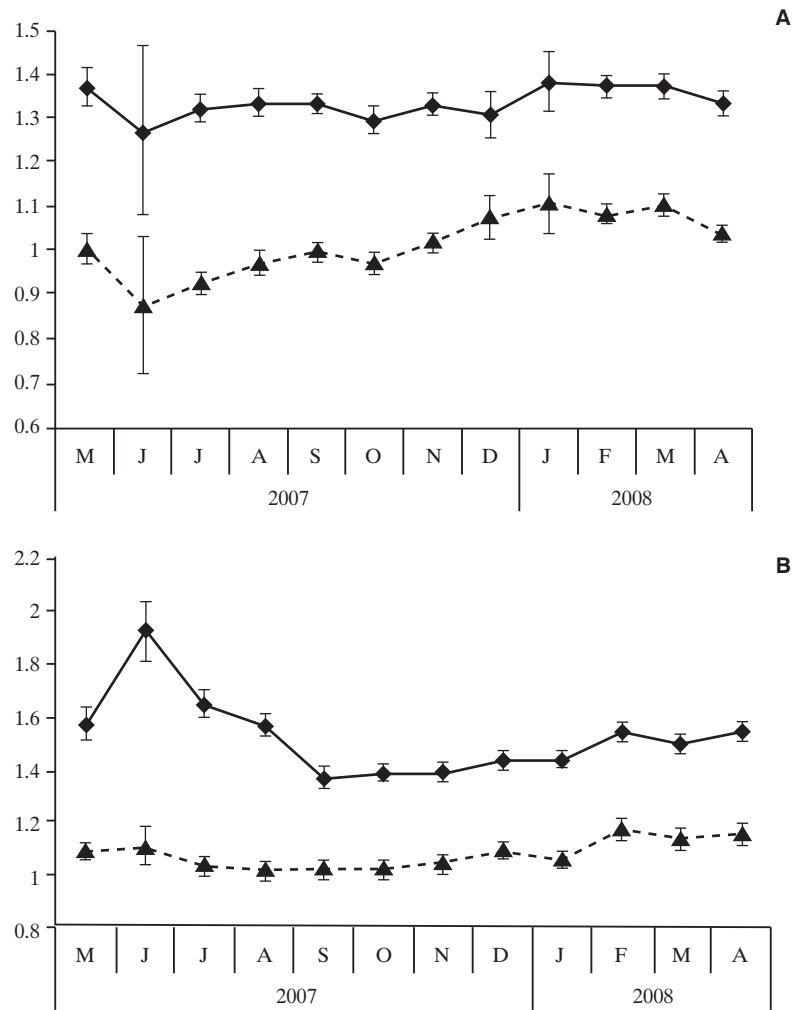
FIG. 3. Mean values (bars represent standard error) for monthly condition factor (CF), using total mass (K: solid line) and eviscerated mass (K_{ev}: broken line) for (A) males and (B) females *G. holbrooki* in Lake Pamvotis.
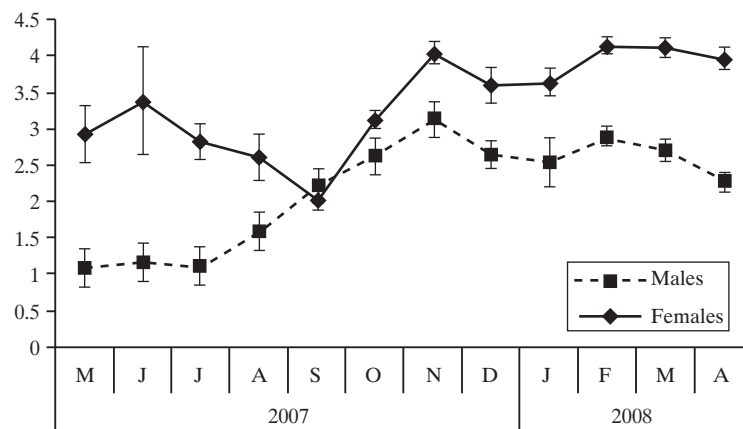

FIG. 4. Mean values (bars represent standard error) for monthly hepatosomatic index (HSI) of males and females *G. holbrooki* in Lake Pamvotis.

nificantly without gonads (for K_{ev}: $H = 58.340$, $p < 0.001$) across months. When the reproductive cycle began in May, condition factor was improved, which was more evident in the females for whom the energetic cost of embryo formation is high. The condition factor for males changed very little during the reproductive period, whereas for females the maximum condition factor value was achieved in June (1.93 ± 0.11) as a consequence of mass increase due to high water temperatures and embryo growth. Condition factor, then progressively declined to a minimum in September, when the quiescent period started (Fig. 3).

Hepatosomatic index (HSI) (Fig. 4) for females (3.69 ± 0.06) and males (2.42 ± 0.07) was significantly different ($Z = -13.92$, $p < 0.05$). HSI of both sexes showed a clear seasonal pattern (females: $H = 110.32$, $p < 0.001$; males: $H = 101.85$, $p < 0.001$) (Fig. 4). Fe-

male HSI (4.04 ± 0.15) and male HSI (3.11 ± 0.24) peaked in November. The hepatosomatic index of both sexes decreased during the spawning season from April to July, when HSI of both sexes reached the lowest value, followed by a continuous recovery until the beginning of the next spawning season.

Gonadosomatic index for females (3.86 ± 0.34) and males (2.31 ± 0.09) was significantly different ($Z = -14.99$, $p < 0.05$). Female gonadosomatic index (GSI) (Fig. 5) varied significantly across months ($H = 124.68$, $p < 0.001$), as did male gonadosomatic index (GSI) ($H = 97.12$, $p < 0.001$). For males, the GSI varied slightly through the year, although it was possible to determine an upward trend from May to June, when the maximum was reached (Fig. 5). A second peak was reached in August and then the index remained low until November. Females in the resting period
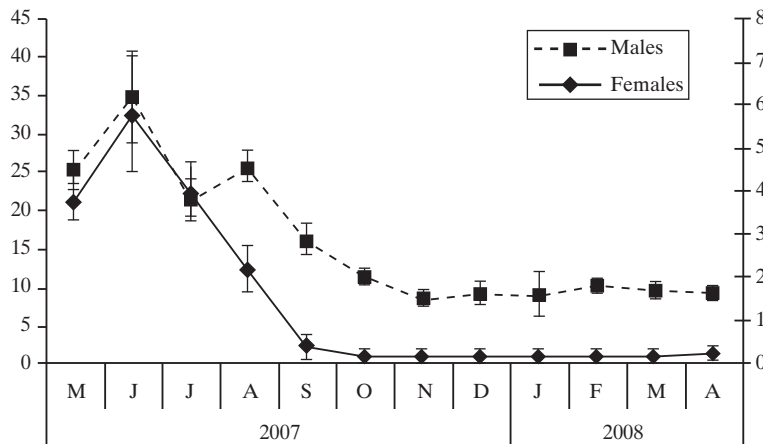

FIG. 5. Mean values (bars represent standard error) for monthly gonadosomatic index (GSI) of males and females *G. holbrooki* in Lake Pamvotis.
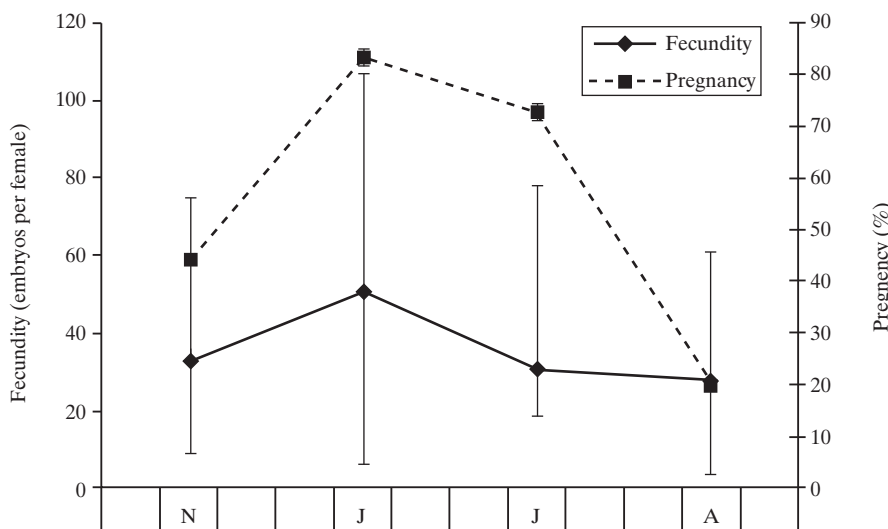

FIG. 6. Monthly pattern of fecundities (mean values and range) and the ratios of pregnancy in females of *G. holbrooki* in Lake Pamvotis.

(October-April) had lower GSI than males. The highest GSI occurred from May to August, decreasing rapidly during September. Female GSI reached its maximum in June.

Considering the monthly pattern of gonad indices in both sexes, as well as the presence of ripe females and males, the spawning season was spread over a rather long period, lasting from May to August. Mean size of pregnant females varied over the reproduction season (ANOVA: $F_{3,15} = 9.356$, $p < 0.001$), being highest in June and lowest in August (Fig. 6). The proportion of pregnant females increased rapidly during May; from June to July nearly all females were pregnant (Fig. 6). The proportion of pregnant females started to decrease in August while only 6% of the females bore developing embryos in September.

The monthly mean brood size varied over the reproduction period (ANOVA: $F_{3,15} = 6.581$, $p < 0.001$). It started to increase from the beginning of May and it reached a maximum in June (50.80 embryos per female) when the largest overwintered females were reproducing. From the end of June to September the mean brood size fluctuated within 30.7 to 28.5 embryos per female range. A positive relationship was found between TL/TW and fecundity, as summarized in the following equations:

$$E = -58.213 + 2.600 \times TL \ (r^2 = 0.78, p < 0.001)$$
$$E = 12.600 + 30.550 \times TW \ (r^2 = 0.77, p < 0.001)$$

However, according to r^2 values, size of the females explained 78% of the variation found in the embryo number.

Feeding habits

Of the 496 stomachs of mosquitofish examined, 52 were empty (~10.5%). The proportion of empty stomachs varied significantly through the year ($G = 9.687$, df = 3, $p < 0.05$), with a maximum of 20.35% during autumn and minimum of 8% in summer. The proportion of empty stomachs among size classes ranged from 7% (size-class < 20 mm) to 31% (size-class 35-40 mm) and differed significantly ($G = 9.687$, df = 5, $p < 0.001$).

The food spectrum of the eastern mosquitofish in Lake Pamvotis was broad. The fish consumed crustaceans (Copepoda, littoral Cladocerans), insect larvae and adults (Chironomidae, Chaoboridae, Culicidae, Corixidae, Hymenoptera, Ephemeroptera, Collembola), spiders, annelids (Oligochaeta), gastropods and plants (Table 3). Copepoda were the most preva-

lent food category, present in 48.48% of the fish stomach, comprising 24.95% of the diet by number, 7.52% by biomass and 38.25% by IRI. *Chydorus sphaericus* was the second most common prey item (29.39%) and accounted for 34.47% of the diet by number, 2.39% by biomass and 26.32% by IRI. Chironomidae larvae were also frequent (27.19%) but less important numerically (3.99%). When percentage by biomass (M) was considered, Diptera imagos (Chironomidae, 33.55%; Chaoboridae, 11.55%) were the most important prey categories, followed by Copepoda (7.52%), Chironomidae larvae (5.76%) and Hemiptera (Corixidae: 5.68%). Certain prey, such as *Bosmina longirostris* was less frequent (17.06 %FO) but more important in terms of numbers (21.69%). Culicidae (4.61%), Hymenoptera (5.16%) and also Ephemeroptera (2.08%) were more important by biomass than by occurrence (1.18%, 3.2%, 0.84%, respectively).

There was a little seasonal variation in food habits of *G. hobrooki* within the studied area. Copepoda were the dominant prey group during all seasons, except in autumn (Table 4). *Chydorus sphaericus* were also present in the stomachs throughout the year, with a peak value recorded in winter (35.92%). In autumn, fish fed chiefly on *Aloma rectangula* (37.29%) and Nematocera adults (17.52%), which were the highest percentages among the seasons. The mean volume (Vm/ST) of prey items varied significantly throughout the year (ANOVA: $F = 21.872$, $p < 0.001$) and was significantly greater (Tukey HSD post-hoc test) in the spring and summer seasons (Fig. 7A). The trophic niche breadth was largest in summer (H = 0.68), and smallest in autumn (H = 0.46) (Table 5). Kruskal-Wallis showed that there is a significant difference in the trophic niche breadth between seasons (H = 10.335, $p = 0.016$). The index of Pianka (Table 6) indicated a high degree of diet overlap between autumn and summer (0.95) and a low degree of dietary overlap between winter and summer (0.08). Simulation results of null model confirmed the high trophic overlap levels between the seasons given that overlap average observed was significantly different from expected average ($p < 0.05$).

Copepods (52.91% and 44.15%), followed by *C. sphaericus* (33.65% and 30%) were the most important prey groups in the smallest size-classes (size I and size II), while contributions of aquatic insects were comparatively low (Table 6). The IRI of Nematocerans (53.2%, size IV) and *B. longirostris* (40.48%, size V) increased with fish size, whereas the IRI of Copepods decreased. The mean volume of stomach

TABLE 3. Mean diet composition of *G. holbrooki* in Lake Pamvotis: % frequency of occurrence (FO), % number (N), % biomass (M) and % IRI of the main food items

Prey category	%FO	%N	%M	%IRI
Crustacea				
Copepoda	48.48	24.95	7.52	38.25
Chydorus sphaericus	29.39	34.47	2.39	26.32
Alona rectangula	13.85	6.57	0.21	2.28
Bosmina longirostris	17.06	21.69	1.69	9.69
Daphnia spp.	7.6	2.81	0.18	0.55
Diptera larvae				
Chironomidae	27.19	3.99	5.76	6.44
Chaoboridae	0.68	0.057	0.29	0.006
Dixidae	0.17	0.014	0.13	0.0006
Culicidae	0.34	0.028	0.33	0.003
Coleoptera larvae				
Coleoptera	2.7	0.24	1.09	0.088
Dytiscidae	0.34	0.028	0.93	0.008
Diptera pupae				
Culicidae	1.01	0.085	1.84	0.047
Diptera imagos				
Chironomidae	15.54	1.71	33.55	13.31
Chaoboridae	3.55	0.37	11.55	1.03
Dixidae	0.51	0.04	1.62	0.02
Culicidae	1.18	0.13	4.61	0.14
Other Diptera	5.41	0.5	4.94	0.71
Hymenoptera				
Formicidae	0.84	0.07	2.13	0.05
Other Hymenoptera	2.36	0.24	3.03	0.19
Hemiptera				
Corixidae	3.21	0.51	5.68	0.48
Coleoptera	0.84	0.07	2.29	0.05
Ephemeroptera	0.84	0.07	2.08	0.04
Thysanoptera				
Tripidae	0.34	0.03	0.01	0.0003
Trichoptera	0.68	0.07	1.56	0.03
Insects unidentified	2.36	0.2	0.001	0.01
Collembola	2.2	0.23	0.41	0.03
Arachnida	3.04	0.28	2.15	0.18
Oligochaeta	1.18	0.16	0.25	0.01
Nematoda	0.75	0.06	0.001	0.001
Plant material	2.6	0.3	0.1	0.023
Gastropods	0.2	0.02	1.05	0.007

contents (Vm/ST) varied significantly among size classes (ANOVA, F = 20.245, $p < 0.01$). Tukey HSD post-hoc test revealed that mean volume of stomach contents for specimens up to 30 mm TL (size I, II, III) differed significantly from the large ones (Fig. 7B). Although the trophic niche breadth varied in different size classes (Table 5), the Kruskal-Wallis test showed that there is no significant difference among them (H = 4.443, $p = 0.487$). The resource utilization of size I with the rest of size classes did not overlap (Table 6). The frequent preference of the intermediate and large mosquitofish for bigger prey items, hard-bodied insects instead of very small littoral crustaceans (*C. sphaericus*, *A. rectangula*) as well as, the simulation results of null model confirmed that the high trophic overlap levels between the size-class given were significantly different from expected average ($p < 0.05$).

TABLE 4. Seasonal and intraspecific diet composition of *G. holbrooki* based on %IRI values of major prey groups

| Prey category	Season				Size Classes					
	Autumn	Winter	Spring	Summer	I	II	III	IV	V	VI
Chydorus sphaericus	1.07	35.92	28.55	13.55	33.65	30	19.57	3.7	3.27	0.38
Alona rectangula	37.29	0.32	0.08	5.99	4.01	2.75	1.51	0.01	0.39	0
Bosmina longirostris	10.94	0.17	4.86	0.1	0.49	2.84	10.73	1.8	40.48	23.54
Daphnia spp.	0	0.14	1.43	0	0.01	0.35	0.7	1.47	0.11	0
Copepoda	18.04	62.22	48.6	39.87	52.91	44.15	36.85	27.17	3.23	0
Chironomidae larvae	12.17	0.99	0.55	23.31	8.45	4.88	6.34	6.58	1.65	4.32
Insects larvae	0.59	0.001	0.003	4.04	0.14	0.93	0.28	0.34	1.14	3.18
Nematocera adults	17.52	0	15.22	9.81	0	11.94	19.30	53.2	25.02	41.2
Diptera general	2.06	0.03	0.04	0.32	0.19	0.34	0.84	0.67	0.32	1.04
Collembola	0.005	0.004	0.02	0.08	0.03	0.01	0.03	0.07	0.17	0
Other Insects	0.28	0	0.59	2.75	0.003	1.77	3.6	4.71	19.75	26.35
Arachnida	0.01	0.06	0.03	0.08	0.11	0.002	0.15	0.13	4.49	0
Worms	0.03	0.14	0.02	0	0	0.02	0.05	0	0	0
Plants	0.01	0	0.001	0.11	0	0.01	0.02	0.16	0	0
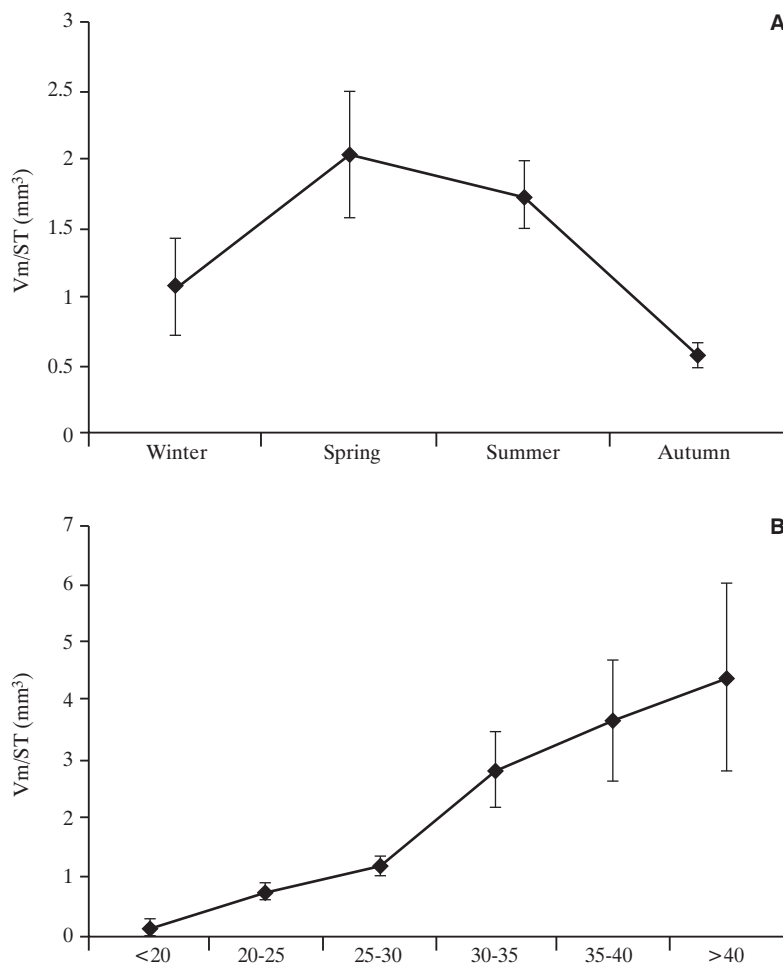
FIG. 7. Mean total biovolume of food in mosquitofish stomachs. (A) Seasonal variation in mean volume (± standard error) of prey per stomach (Vm/ST) of *G. holbrooki*, (B) Variation of mean volume (± standard error) of prey per stomach (Vm/ST) of *G. holbrooki* among size classes.

TABLE 5. Dietary breadth of *G. holbrooki* expressed in terms of the Shannon-Weaver index (H′), which were classified according to sampling season, length-class and sex

Season	Autumn	Winter	Spring	Summer		
H′	0.46	0.50	0.54	0.68		
Size-class	Size I	Size II	Size III	Size IV	Size V	Size VI
H′	0.52	0.56	0.53	0.46	0.70	0.50

TABLE 6. Dietary overlap values between the seasons and size-classes of *G. holbrooki*. The overlap was calculated with Pianka's Index where 0 and 1 values correspond to the minimum and maximum of dietary overlap, respectively

Season	Autumn	Winter	Spring	Summer		
Autumn	–	0.10	0.89[*]	0.95[*]		
Winter		–	0.14	0.08		
Spring			–	0.90[*]		
Summer				–		
Size-class	Size I	Size II	Size III	Size IV	Size V	Size VI
Size I	–	0.33	0.29	0.10	0.06	0.03
Size II		–	0.99[*]	0.95[*]	0.84[*]	0.88[*]
Size III			–	0.95[*]	0.86[*]	0.90[*]
Size IV				–	0.79	0.84[*]
Size V					–	0.96[*]
Size VI						–

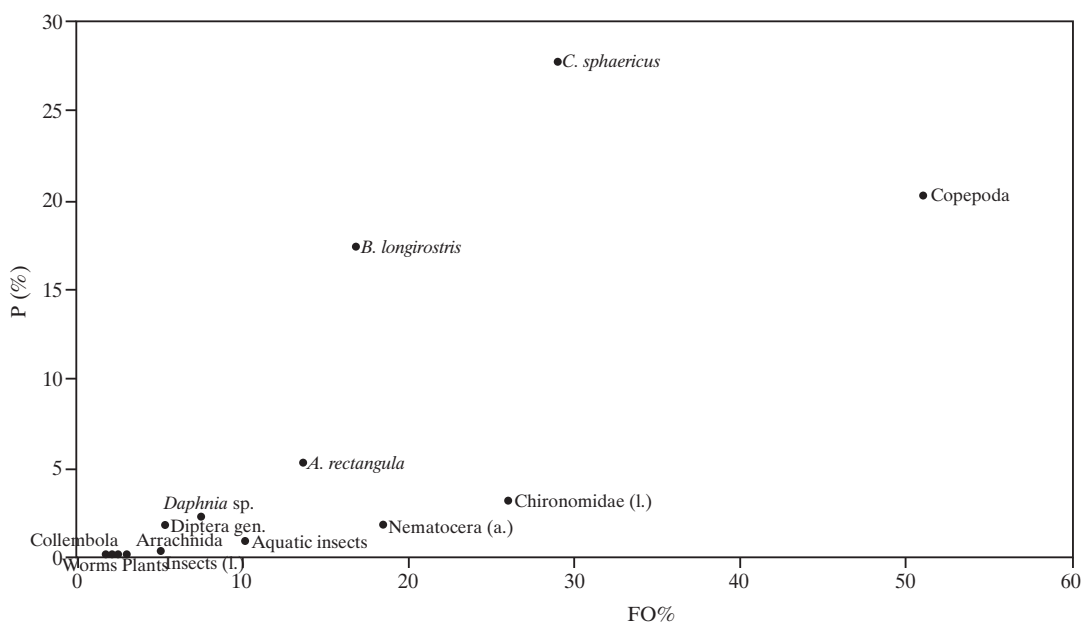[*] Values equal or greater than 0.6 are considered significant

FIG. 8. Relationship among prey specific abundance (% P$_i$) and frequency of occurrence (% FO) of food categories of *G. holbrooki* diet. Plots based on the modified Costello graphical method (Amundsen *et al.*, 1996).

The interpretation of the diagram of the modified Costello graphical method is shown in Figure 8. The plot of prey-specific abundance (%P_i) and frequency of occurrence (%FO) of the main components of the diet between May 2007 and April 2008, showed a strong specialization towards Copepods to have been eaten by more than half the individuals (FO = 51%) and to have moderate contribution in specific abundance (P_i = 20.2%). However, some littoral prey (e.g. *C. sphaericus*, *A. rectangula*, *B. longirostris*, Nematocera adults and Chironomidae larvae) presented a low FO and a low P_i displaying evidence of a generalist strategy.

DISCUSSION

The population of *G. holbrooki* in Lake Pamvotis develops a bimodal length frequency distribution with a generation substitution in the middle of the reproductive cycle, like other populations studied previously (Fernández-Delgado, 1989; Fernández-Delgado & Rossomanno, 1997).

In Lake Pamvotis *G. holbrooki* females live longer and reach larger sizes than males because, in general, females need longer time to mature and continue growing throughout their life (Fernández-Delgado, 1989; Specziar, 2004). Males stop growing, or exhibit a decelerating growth rate after the gonopodium has been completely formed and they do not live as long after reaching maturity (Krumholz, 1948). After the completion of the reproductive period the largest individuals (mostly females) become rare due to the high mortality which follows the reproduction (Fernández-Delgado, 1989; Vargas & de Sostoa, 1996; García-Berthou, 1999).

The recorded sex ratio was significantly female biased (2.31:1) is in accordance with what has been found in other poeciliids species such as *Poecilia sphenops* (5:1) (Martinez-Trujillo, 1983). This feature of preponderance of females is occurring in sexually dimorphic fishes, particularly those belonging to the Poeciliid family (Vargas & de Sostoa, 1996; Fernández-Delgado & Rossomanno, 1997; Scalici *et al.*, 2007). Snelson (1989) mentioned that the female preponderance might be attributed to the differential mortality of the sexes. Females have higher survivorship rates due to their larger sizes, longer lifespan, less conspicuous colors, and that they are more resistant to the rigors of reproductive effort and unfavorable environmental conditions. In this study, monthly variations in the sex ratios could be explained also by the effect of the mesh size and the shallowness of the lake, which make difficult the fish capture.

Respecting to the relationship between length and mass, the slope (*b*) values obtained for the species in this study is significantly different ($p < 0.05$) from 3, which shows that the growth of the fish species is allometric. Ricker (1975) mentioned that when the fishes have a coefficient *b* value significantly different from 3, this fact suggests that the body parts of the individuals do not grow at a similar rate. Yet, the mass increases allometrically with size and especially the females grow in mass more than in length. This difference is common in mosquitofish where females have higher energetic needs for their growth during the spring, since this leads to higher production of offspring and faster development of embryos or a quicker brood initiation (Vargas & de Sostoa, 1996).

The influence of both environmental and reproductive factors was reflected in the somatic condition of the Eastern mosquitofish in Lake Pamvotis. The maximum condition factor occurred in June coinciding with the high temperatures and the high reproductive investment. The condition factor then gradually declined to a minimum in September. For the Lake Pamvotis population, a drastic reduction of the larger individuals (sizes V & VI) due to the combination of the lower condition values and greater reproductive effort has not been observed, contrary to the observations reported from other *Gambusia* populations (Krumholz, 1948; Fernández-Delgado, 1989).

Lipidic storage is important for winter survival and reproduction (Reznick & Braun, 1987). HSI values for both males and females started to increase from October to April as a result of the accumulation of nutritive substances before the spawning season, when a great portion would be utilized and would allow the survival at the adverse winter period. In Lake Pamvotis the reproduction period of *G. holbrooki* was similar to the one observed in areas where the species is naturally distributed or introduced (Fernández-Delgado, 1989; Meffe, 1991; Vargas & de Sostoa, 1996). It is suggested that the main factor determining the timing of the reproductive cycle in mosquitofish is the photoperiod (Milton & Arthington, 1983). The pattern of the gonad cycle implied that the reproduction season begins at the beginning of May and ends by the end of August, corresponding to periods of 11 to 12 hrs of light.

In *G. holbrooki*, fecundity is a function of the female size (Milton & Arthington, 1983; Fernández-Delgado, 1989; Vargas & de Sostoa, 1996) and reproductive status (Haynes & Cashner, 1995), although the brood size is not constant in the largest and oldest females (Krumholz, 1948). The size at first maturity (20.35 mm) (Fernández-Delgado, 1989; Fernán-

dez-Delgado & Rossomanno, 1997) and the mean brood size (30 embryos per female) are similar to populations from other localities of the distribution area (Specziar, 2004) although brood sizes up to 100 embryos are also common (Krumholz, 1948; Milton & Arthington, 1983; Fernández-Delgado, 1989; Meffe, 1991; Fernández-Delgado & Rossomanno, 1997).

The minimum percentage of empty guts of *G. holbrooki*, in Lake Pamvotis, occurred in summer, when prey abundance was highest. The higher prey consumption in summer is thus a consequence of greater food availability and higher energy requirements for growth and reproduction (Caiola *et al*., 2001). Preferential prey categories were Copepods and Cladocerans, as also reported in studies carried out in many systems, such as rice fields (Blaustein & Karban, 1990), seminatural ponds (Miura *et al.*, 1979), and drainage canals (Crivelli & Boy, 1987). Nematocera adults were also present but constituted only a small fraction of the mosquitofish diet.

Values of Pianka's index (> 0.60) indicated high dietary overlap between seasons and this is in accordance with the little seasonal variation that was noticed in the diet of mosquitofish during this study. The small variations in the main prey items between seasons contributed to the high level of inter-season proportional overlap. Copepods dominated the diet composition throughout the year, except in autumn. The increased consumption of Nematocerans and Chironomids during the late spring and summer coincided with the period of new recruits of many insect species, which may be present in high densities (Kagalou *et al*., 2006). *Chydorus sphaericus* consumption also exhibited marked differentiation between seasons, as it was an important food item in winter and spring, reflecting seasonal availability. The observed greater dietary diversity in spring and summer could be attributed to the possible decrease in availability of particular prey during this season, which would force *G. holbrooki* to forage other preys that might be readily available. Furthermore, the prey diversity is at the highest levels in summer, probably in an effort to compensate the increased energy demands of the reproductive period.

Food composition and dietary overlap revealed significant changes in the *G. holbrooki* diet as it grows. Smaller specimens mainly preyed on Copepods and small littoral Cladocerans, contributing a small biomass. Wurstbaugh *et al*. (1980) explained that the immature mosquitofish could cause a higher impact on zooplankton populations than adult fish, because they are able to consume over 100% of their own mass dai-

ly. As the fish grew, the proportion of large Cladocerans (*B. longirostris*) and Nematocerans increased. In this respect, the mean volume of prey (Vm/ST) significantly increased towards the larger size-classes as a consequence of the presence of Nematocerans and other Insects in addition to large Cladocerans. This ontogenetic shift could be related to a change in prey categories and not a change in mean size of a certain prey (García-Berthou, 1999). The trophic niche breadth, as expressed by the Shannon-Weaver index, showed as weak tendency to increase with size, because of the ability of large specimens to utilize a wider range of habitat resources. Furthermore, the large intraspecific food overlap between intermediate and large size mosquitofish suggested potential competition for the same food items within food limited situations. On the other hand, the low diet overlap between juveniles with intermediate and adult *G. holbrooki* indicated different prey utilization. This could represent an intraspecific segregation in the differential consumption of the same prey items due to the different dimensions reached by small size mosquitofish.

On analyzing the diet composition, one can conclude that *G. holbrooki* has a homogeneous diet strategy and a broad niche width. The modified Costello method indicated a spectrum of feeding strategies for the mosquitofish population, but corresponded mainly to a specialist. Nevertheless, generalism also exists for a limited spectrum of accessible preys (Copepods, *C. sphaericus*). Contrariwise, *G. holbrooki* from Lake Heviz (Specziar, 2004) is a generalist opportunistic predator, whose diet composition reflects changes in prey availability and shows marked temporal and spatial variations.

In conclusion, the paper presents for the first time an extensive study of the life history and feeding pattern of *G. holbrooki*, in a small shallow eutrophic Mediterranean Lake (Lake Pamvotis). The species is characterized by fast growth, early maturation, high level of reproductive effort and short life-span. *Gambusia holbrooki* presented a high intrapopulation dietary overlap and adaptation to seasonal prey availability followed a specialist feeding strategy. In addition, Culicidae larvae had a negligible significance in mosquitofish diet. The results of the study are in accordance with the unfavorable reports from experiments using mosquitofish for mosquito control around the world (Rupp, 1996). This species potentially could be utilized as a biological control tool for mosquito control in particular ecosystems under certain conditions since it is still important to attempt to list the suspected ecological costs of the invasion and the

potential problems that a control agent might cause in addition to numerous introductions of species which are ecologically safe.

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