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Scientific Editors

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Coimbra • Imprensa da Universidade

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ROLE ASSESSMENT OF AN EXOTIC FISH IN RICE FIELDS OF THE  
LOWER MONDEGO RIVER VALLEY: LIFE HISTORY, POPULATION  
DYNAMICS, PRODUCTION AND DIET OF EASTERN  
MOSQUITOFISH, *Gambusia holbrooki* (PISCES, POECILIIDAE)

**Abstract**

The introduced population of *Gambusia holbrooki* from the rice fields of the Lower Mondego River Valley, Portugal, was studied for 15 months, relating their life cycle and population dynamics with its production and diet, in order to assess the role of the species in the energy flow and secondary production in this type of agro-ecosystem. Two main annual cohorts (1995 and 1996 cohorts) were identified. The females outnumbered males and the average female/male-ratio was 4. The inspection of ovary developmental stages of this viviparous fish, revealed that the most important reproductive period was between April and August. The first recruits were recorded from June and were present thereafter until October. Females reached greater sizes, had a higher growth rate and lived longer than males. Annual production was estimated at  $3.101 \text{ g m}^{-2} \text{ year}^{-1}$  (ash-free dry weight, AFDW), the average biomass at  $2.896 \text{ g m}^{-2}$  (AFDW), and the P/B ratio was 1.071. *G. holbrooki* feeds mainly on copepods, cladocerans and rotifers. Surface insects, such as aphids, collembolans, adult (imago) chironomids and other dipterans, are additional food. Large *G. holbrooki* consumed greater amounts of cladocerans and adult chironomids than other smaller size groups, while small fish preferred rotifers. Gravid females ate copepods, cladocerans, adult chironomids and other dipterans in significantly greater amounts than immatures, males, and non-gravid females. A combination of life history, population dynamics, production and ecological traits (e.g. fast growth, reduced longevity, viviparity, high productivity, an intermediate position in food chain, plasticity and adaptability in its food use, and no special habitat requirements for reproduction) clearly show that the populations of *G. holbrooki*, introduced into rice fields all over the world, may play an

363

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important role in the structure and functioning of the biological communities of these important agro-ecosystems.

## Introduction

Eastern mosquitofish, *Gambusia holbrooki* (Girard) (Cyprinodontiformes: Poeciliidae), is native to the coastal region of the eastern United States, but was been widely introduced into warm temperate and tropical regions all over the world through mosquito control programs (Cech et al. 1992, Haynes and Cashner 1995, Homski et al. 1994, Hoy 1985, Lydeard and Belk 1993, Schaefer et al. 1994, Wurtsbaugh et al. 1980). The species was introduced in the Iberian peninsula in 1921 (Albuquerque 1956) and has invaded the lowest stream sections, wetlands and coastal lagoons (Vargas and Sostoa 1996). This viviparous fish is well known for its consumption of insect larvae, zooplankton and other invertebrates (Blaustein 1992, Cabral et al. 1998, Colwell and Schaefer 1983, Crivelli and Boy 1987, Daniels and Felley 1992, Hubs 1990, Hurlbert and Mulla 1981) but also as a threat to native fishes in habitats where mosquitofish have been introduced. Rupp (1996) reported that the harmful consequences of *Gambusia* introductions ranged from eating the eggs of economically desirable fishes to endangering rare indigenous species. Viviparity and a high reproductive effort may give such exotics an advantage over native oviparous species, because fry are larger, feed at birth, grow more quickly, and become predators faster (Rupp 1996). In Portugal, the most serious conservation problem for endemic fishes has been introductions of exotic species dating from the time of the Roman occupation of Iberia (Almaça 1995).

In the Lower Mondego River Valley (Western Portugal), the mosquitofish is very abundant, namely in rice fields (Cabral and Marques 1999). Rice fields are complex ecological systems with a variety of plant and animal species (Linden and Cech 1990). In the Lower Mondego River Valley, rice fields are linked by drainage and irrigation channels spread across the whole valley with other ecosystems, such as rivers, streams and wetlands (Anastácio and Marques 1995).

Mosquitofish is presently one of the most widely distributed species of freshwater fish in the world, and is believed to be the most widely disseminated natural predator in the history of biological control (Botsford et al. 1987). In the Iberian peninsula, there have been studies on the reproductive biology and population dynamics of *G. holbrooki* in rice fields (Fernández-Delgado 1989, Franca 1953, Franca and Franca 1954) and lagoons (Fernández-Delgado and Rossomano 1997, Vargas and Sostoa 1996), but its productivity has received little attention. This study addresses this information gap by relating life cycle and population dynamics of the mosquitofish with its production (Cabral and Marques 1999). The integration of this information with the existent knowledge of the relationships between mosquitofish and its main prey in rice fields of the Lower Mondego River Valley (Cabral et al. 1998), is an important requirement to assess the role of the species in the energy flow and secondary production in non-native rice fields.

## Material and methods

### Study site

The Lower Mondego River Valley (figure 1), in central Portugal (40°10'N, 08°41'W), consists of approximately 15,000 ha. The main agricultural crop is rice, occupying about 60% of the farmable area. Non-cultivated areas, such as swamps, appear in the periphery of the valley, and have characteristic wetland fauna and flora. Drainage channels are spread across the whole valley, constituting biological reservoirs for rice fields (rice paddies and irrigation channels) (Anastácio and Marques 1995).

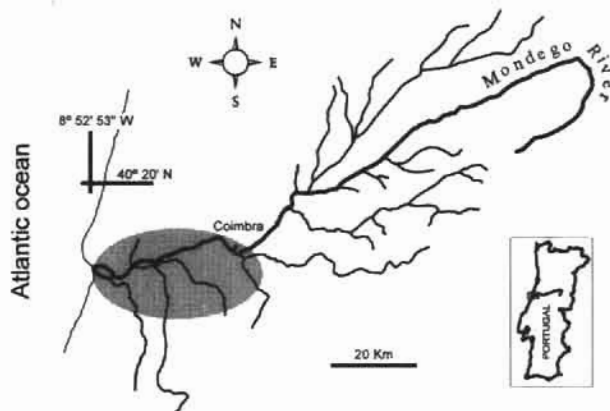


Figure 1. Location of the Lower Mondego River Valley (shaded area).

In the chosen study site, mosquitofish occur in the rice paddies and irrigation channels. However, since rice fields are exposed to dramatic manipulation of the water level, the paddies are dry or have little water during much of the year, whereas the main irrigation channels always have enough water to support a population of mosquitofish. The sampling program was therefore focused in the irrigation channels.

### Field program

The sampling program was carried out in a main irrigation channel from April 1996 to June 1997, fortnightly during the most important mosquitofish reproduction period (April - July), and monthly in the remaining period. Samples of mosquitofish, zooplankton, and macroinvertebrates, both benthic and associated with aquatic vegetation, were taken.

During each sampling event, mosquitofish were electrofished in three randomised areas confined by nets laid transverse across the irrigation channel. A semi-portable generator supplied a rectified DC current (350-600V). Sampling always took place between 10 a.m. and 1 p.m., corresponding to the most active mosquitofish feeding period (Crivelli and Boy 1987). Sampled areas ranged from 3 to 16 m<sup>2</sup> and were shocked during a period of 30 to 40 minutes, enough to catch virtually all the fish

present in each area. All mosquitofish caught were immediately preserved in 4% neutralised formaldehyde, while other fish were returned to the irrigation channel.

Prey samples were collected from the primary microhabitats in the irrigation channels, including the sediment, water phase, and aquatic vegetation. Three replicates were randomly sampled for each microhabitat as described by Cabral et al. (1998).

### Laboratory procedures

In the laboratory fish were washed, counted, and preserved in 70 % ethanol. A total of 5,003 fish were examined. All individuals were measured and sorted by standard length (SL) to the following size groups: 1 (till 10 mm), 2 (11-15 mm), 3 (16-20 mm), 4 (21-25 mm), 5 (26-30 mm), 6 (31-35 mm), 7 (36-40 mm) and 8 (41-45 mm). Moreover, fish were classified as (a) immature (normally with 15 mm or less, if sex could not be determined externally), (b) males (normally no longer than 30 mm and identified by the presence of a gonopodium), (c) non-gravid females and (d) gravid females. All females were dissected. Ova and embryos were removed from the ovaries, measured (maximum diameter) with an ocular calibrated micrometer, counted, and assigned to one of five developmental stages: (1) opaque, white non-fertilised eggs about 100  $\mu\text{m}$  in diameter (Thibault and Schultz 1978); (2) intermediated between immature ova (partially yolked) and mature ova with a clear amber color (complete complement of yolk but no embryonic structures visible) (Meffe 1987, Thibault and Schultz 1978); (3) a stage from the primitive streak to the early embryo (eyes not fully formed; little dorsal pigmentation); (4) middle embryo (eyes fully formed; heavier pigmentation; moderate amount of yolk remaining); and (5) late embryo (little or no yolk remaining; ready for parturition) (Meffe 1987). The females in the developmental stage 2 or in older stages were considered as gravid females. Sex-ratios were calculated as the number of females per male.

Five individuals per size group and from each sampled area were examined for gut contents, except for size groups 3, 4, and 5, where females and males had coincident sizes. In this case, five females and five males were examined per group. For each fish the gut tube was excised from the esophagus up to the point where it bends ventrally and dissected. Prey items were recorded and identified.

Length-weight relationships were determined to estimate production. One hundred fifty-six individuals were collected during the summer, the only period in which all size classes were present, to provide a single regression equation for SL-AFDW (ash-free dry weight in g) conversion. The following equation was obtained:  $\text{AFDW} = 1.56\text{E}^{-6} * \text{SL}^{3.24}$  ( $r^2 = 0.96, p < 0.001$ ). Specimens were dried in an oven for 48 hours at 60 °C and weighted, and ignited in a muffle furnace for 8 hours at 450 °C to obtain AFDW. Weight determinations were carried out with a 10<sup>-5</sup> g precision.

### Data analysis

Growth rates were deduced by tracking recognisable cohorts along size-frequency distributions (1 mm SL classes) from successive sample dates. All fish born during the



same reproductive period were assigned to the same cohort (Fernández-Delgado and Rossomano 1997). Mosquitofish length-frequency histograms, complemented with scale readings, allowed a visualisation of the population structure throughout the year. Since there is genetically-based size polymorphism in *Gambusia*, the growth process is different for each sex. Female mosquitofish have indeterminate growth and may attain a size greater than 50 mm SL, representing therefore a continuous range of growth from the first immature stages, whereas males tended to stop growing upon reaching maturity, approximately with 20 mm SL (Cabral and Marques 1999, Vondracek et al. 1988). Therefore, we considered separately immature and females combined (with continuous growth) and males because population structure analysis becomes difficult if males are considered together with the remainder of the population. The monthly average and standard deviations of SL to cohorts detected were calculated, which established the annual growth pattern for each sex. Since growth rates are normally not constant through the year, seasonal variations were taken into consideration using a model proposed by Gaschütz et al. (1980).

Size-frequency analysis also allowed an estimate of the density of each cohort at each sampling date. Daily mortality rate was estimated by the adjustment of a negative exponential curve to the densities of the cohort detected from the recruitment period, when it reached the maximum density value, to the period where all the individuals belonging to this cohort disappeared (Anastácio and Marques 1995, Cabral and Marques 1999). The instantaneous mortality rate ( $Z$ ) was also calculated for mean age and mean life span estimations.  $Z$  is equal to the natural logarithm (with sign changed) of the complement of the annual mortality rate (Ricker 1975). For exponential mortality at a constant rate, mean age and mean life span are equal and, except when growth is exponential, both are equal to the reciprocal of the instantaneous mortality rate (Allen 1971).

The length of the reproductive season was determined by calculating the time interval between 1 month before the presence of the first immature, since a typical gestation period is about 4 weeks (Meffe 1990), and the presence of the last newborn/small immature.

To identify the preferential prey items caught by mosquitofish we used the Ivlev's electivity index for fishes (Ivlev 1961), defined as  $E = (r - p) / (r + p)$ , where  $r$  = proportion of the number of a given prey in the mosquitofish gut content, and  $p$  = proportion of the number of the same organism in field samples. Positive values of  $E$  (0 to 1) indicate a preference, negative values (-1 to 0) indicate little or no representation in the gut content. We used the value -0.5 as lower limit to identify "preferential" prey.

The Kruskal-Wallis one-way analysis of variance by ranks followed by a non-parametric multiple comparisons test with unequal sample sizes (Zar 1984) was used to assess the significance of differences in the number of preferential prey items between the gut contents of different mosquitofish size and sex groups.

Production was estimated using Allen curves (Waters 1977). A year-to-year (or cohort-to-cohort) stability was assumed and the method was used for the mixed-age population. In these cases, a single cohort production will be equal to annual

production of all population (Waters 1977). The production estimate for the population was achieved by determining the average AFDW and density of mosquitofish, both against time. Then, density was plotted against average AFDW for each sampling date, where production was given by the integral of the curve adjusted to this plot.

The average biomass was determined by the ratio of the biomass integral within a given time interval and the time interval (Allen 1971). Assuming the same cohort-to-cohort stability presupposition used for production estimation, the total biomass in a given time was the product of individual average weight (AFDW) and the density of the mixed-age population.

## Results

### Population structure and reproduction

It was possible to recognise and track two annual cohorts for both sexes, the 1995 and 1996 cohorts. Recruitment occurred from June through October, at which time the last newborns were collected. Females outnumbered the males during the entire study period, with an average sex-ratio of 4:1 (Cabral and Marques 1999). Inspection of the developmental stage of the female intra-ovarian cycle revealed that the most important period for reproduction falls within the time interval between April and August. The proportion of non-gravid females (stage 1) decreased until the early June, whereas gravid females (specially stages 3, 4 and 5) increased (figure 2). After the beginning of recruitment the inverse trend occurs, and in August only a very small proportion of females were gravid. The average brood size (embryos) reached its maximum in July 1996 (32 embryos per female) for the 1995 cohort, at which time there were almost no unfertilised eggs in the ovaries, and in early June 1997 (34 embryos per female) for 1996 cohort (our last data).

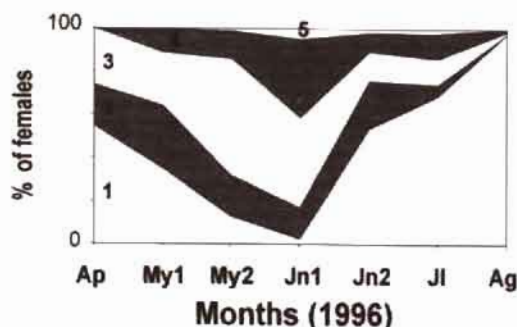


Figure 2. Changes in the percentage of female *Gambusia holbrooki* bearing eggs/embryos in each developmental stage (1, 2, 3, 4 and 5, as defined in Material and Methods) during the most important reproductive period (1402 females were examined). The initials of the months with numbers (1 and 2) correspond to samples made fortnightly.

## Growth

The average monthly lengths and their respective standard deviations depict a seasonal variation in growth for immature and females combined and males (figure 3). Therefore, we took seasonal variations into consideration, and growth data were used to calibrate a growth model proposed by Gaschütz et al. (1980). Two main cohorts were tracked for both sexes during a total period of 411 days. Since the study period was not sufficient to contain the entire life cycle of a single cohort, we adjusted a growth curve for each sex to the original data of 1996 cohort merged with the last data on 1995 cohort. The fit was compared based on  $r^2$  values. The growth-data-fitted Gaschütz's model predicted well for immature and female combined ( $r^2 = 0.96$  for 17 data points), but not for males ( $r^2 = 0.67$  for 13 data points). The growth pattern of males is different since some of them essentially stop growing after maturation (figure 3). Therefore, we used this method only for immature and females (figure 4), the fraction of the population with a continuous growth. The model parameters for this group were estimated as described by Cabral and Marques (1999).

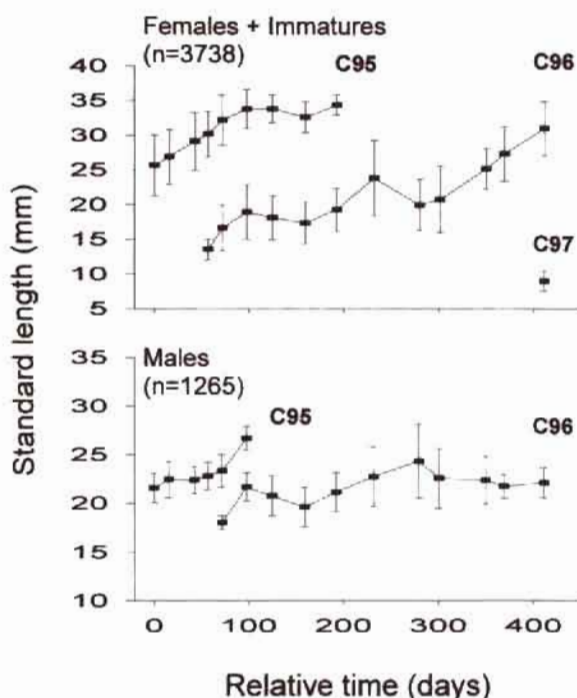


Figure 3. Annual growth pattern in females and immature combined and males from the 1995 (C95), 1996 (C96) and 1997 (C97) cohorts of *Gambusia holbrooki*, based on monthly averages of standard length (mm). The standard deviations from the mean are also indicated.

There was an initial stage with notable growth (0.076 mm day<sup>-1</sup>), corresponding to the months with mild temperatures (early first summer) and initial recruitment of a new cohort (figure 4). This period was followed by a second stage of extremely slow growth (0.014 mm day<sup>-1</sup>) between the end of summer and the "cold months" from autumn to mid-winter, and a final phase, with a recovery in growth (0.052 mm day<sup>-1</sup>) until the end of the second summer (figure 4). For males, we assumed a growth rate similar to the first stage until reaching maturity (approximately with 20 mm SL), and thereafter the second stage growth rate for the remain male life cycle.

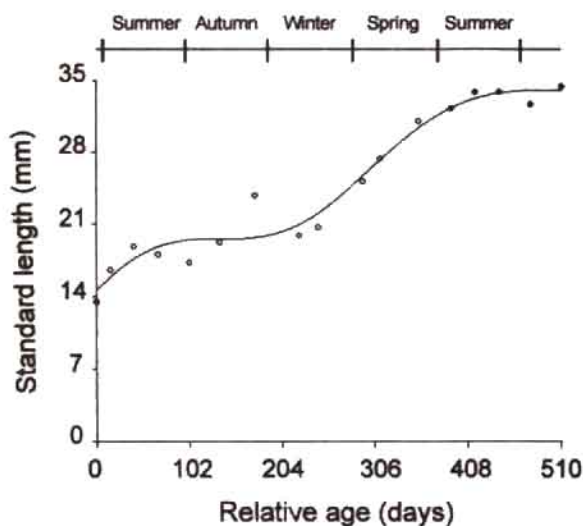


Figure 4. Gaschütz's growth model of standard length (mm) for the 1996 female+immature cohort, merged with the last data on 1995 cohort, of *Gambusia holbrooki*. Data points used to calibrate the model are also plotted (the last five black data points belong to 1995 cohort).

#### Density, mortality, mean age and mean life span

For all mixed-age population, the mosquitofish showed the same density patterns throughout the study period for the three main "sexual" categories considered: females (non-gravid and gravid), males and immature. The density of the 1996 cohort on each sampling date allowed an estimate of the daily mortality rate for both sexes. This cohort was merged with the last data on 1995 cohort, for the same reason explained for growth analysis. Since the sex-ratio at birth in mosquitofish is 1:1 (Krumholz 1948), we assumed that each sex contributed 50% of the immature density. The daily mortality rate was estimated by the adjustment of a negative exponential curve ( $y = a \cdot 10^{-bx}$ ) to the densities of each sex detected from the recruitment period, when they reached its maximum density value, to the period where all the respective individuals disappeared (figure 5). The mortalities, mean age and mean life span parameters estimated for both sexes from the density equations are given in table 1. Females were longer-lived, with smaller mortality rates than males.

Table 1. Estimated parameters for mortalities, mean age and mean life span of both sexes from the functions describing the evolution of a *Gambusia holbrooki* cohort density. 50% of the immature density was considered as belonging to each sex.  $b$  is the parameter of the density equations of the type  $y = a \cdot 10^{bx}$ .  $D$  is the number of deaths during the year and  $N_0$  is the number of fish present at the start of a year.

Parameters	Equations	Females + 0.5 (immatures)	Males + 0.5 (immatures)
Daily mortality rate ( $m$ )	$m =  -b \cdot \ln 10 $	0.00746	0.00836
Annual mortality rate ( $A$ )	$A = D/N_0$	0.93432	0.95268
Instantaneous mortality rate ( $Z$ )	$Z = -\ln(1-A)$	2.72304	3.05081
Mean age and mean life span ( $L$ )	$L = (1/Z) \cdot 365$	134.04 days	119.64 days

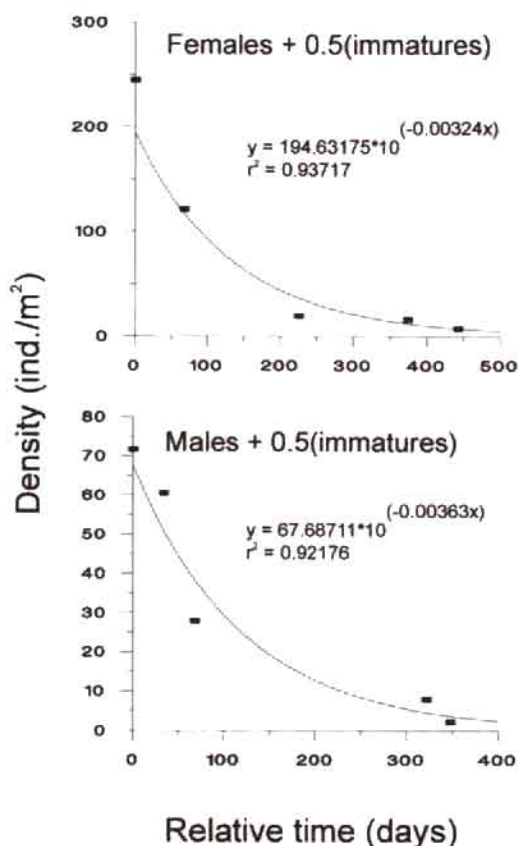


Figure 5. The survivorship curve for both sexes of a *Gambusia holbrooki* cohort (1996 cohort merged with the last data on 1995 cohort). A negative exponential curve ( $y = a \cdot 10^{bx}$ ) was adjusted to the densities throughout time.  $x$  and  $y$  are time in days and density (individuals  $m^{-2}$ ), respectively. 50% of the immature density was considered as belonging to each sex. The respective functions and  $r^2$  values are also indicated.

## Production estimates, average biomass and P/B ratio

Production was estimated from a plot of the relationship between the density and the average weight of the mixed-age population (Allen curve method). A curve of the type  $y = a \cdot 10^{-bx}$  was then adjusted to data points. The resulting figure is basically a growth-survivorship curve with the density of survivors plotted against mean individual ash-free dry weight (AFDW) (figure 6a), and the total production within the study period (411 days) being given by the integral of this function. Production (P) was then estimated as  $3.10 \text{ g m}^{-2} \text{ year}^{-1}$  (AFDW).

Total biomass values for each sampling date were estimated from the products of densities and average weights (AFDW) of the mixed-age population. A fourth order polynomial function was then adjusted to these data (figure 6b), and the average biomass (B) was calculated by dividing the integral of this function within the study interval by 411. The obtained value was  $2.90 \text{ g m}^{-2}$  (AFDW). The P/B ratio was then estimated as 1.07.

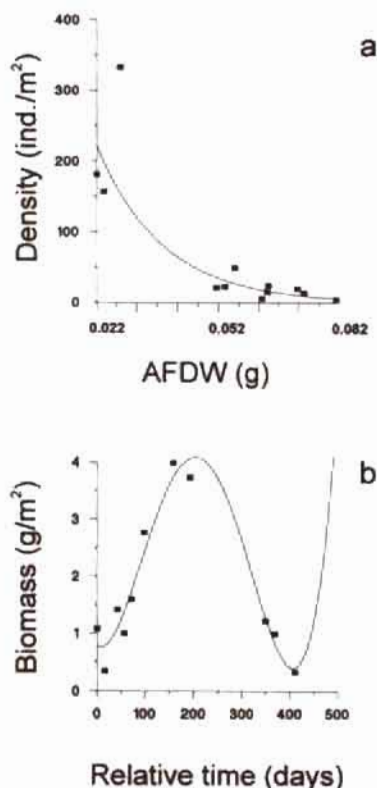


Figure 6. Models used to estimate *Gambusia holbrooki* total production and average biomass, respectively: a) adjustment of the Allen curve to the relationship between the density and the average ash-free dry weight (AFDW) of mixed-age population; and b) adjustment of a polynomial curve to the estimated values for total biomass throughout the study period. Data points used to calibrate the models are also plotted.

## Diet

Twenty-three invertebrate large groups were collected in the three microhabitats considered. For the present purposes it was considered enough to take into account high taxonomic levels. The most abundant invertebrates in the water phase were copepods and cladocerans. In the macrobenthos, oligochaets and chironomid larvae were the most abundant invertebrate groups. Adult hydrophilids, aphids, chironomids larvae, and crayfish (*Procambarus clarkii*) were very abundant on the aquatic vegetation, being found during the whole study period.

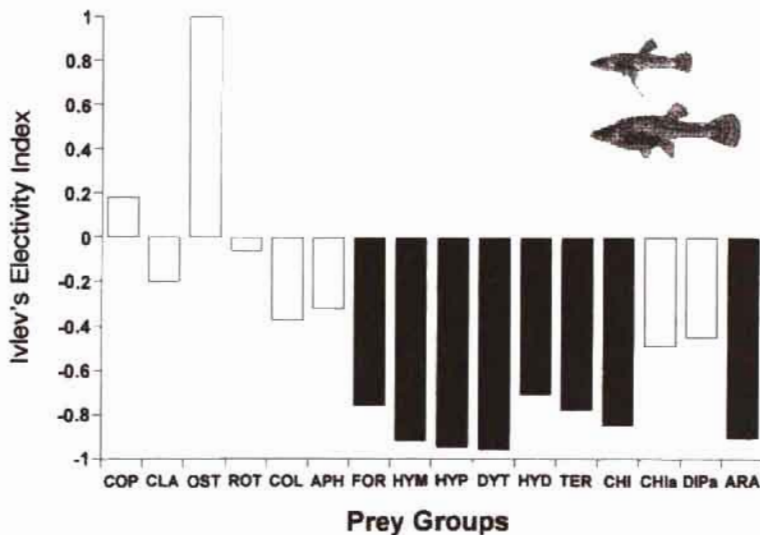


Figure 7. Prey groups Ilev's electivity index values calculated for the total population sampled of *Gambusia holbrooki*. The white polygons indicate a prey preference, taking into account the lower limit selected to identify "preferential" preys (-0.5). The codes of the sixteen prey groups are respectively: Copepods (COP), Cladocerans (CLA), Ostracods (OST), Rotifers (ROT), Collembolans (COL), Aphids (APH), Ants-Formicidae (FOR), other Hymenopterans (HYM), Hydrophilids (HYP), Dytiscids (DYT), Hydraenids (HYD), Terrestrial-Coleopterans (TER), Chironomids larvae (CHI), adult Chironomids (CHIa), other adult Dipterans (DIPa), and Arachnids (ARA).

The 16 groups found as prey items in the mosquitofish gut contents are given in figure 7. Taking the Ilev's electivity index values into account, for the population considered as a whole (figure 7), zooplankton (copepods, cladocerans, ostracods and rotifers) constituted the main feeding option for mosquitofish, followed by the insect groups aphids and collembolans, adult chironomids and other dipterans. Ostracods appeared as a preferential prey, although they were found in only 7.8 % of the fish analysed (Cabral et al. 1998). However, ostracods were excluded from the statistical analysis because of the lack of information regarding their occurrence in the environment. The absence of ostracods in the samples can be explained as a function of our sampling methodology for benthic organisms (Cabral et al. 1998). Copepods were the only other group with a positive Ilev's index (figure 7).

A Kruskal-Wallis one-way analysis of variance by ranks was used to assess the significance of differences in the numbers of preferential prey items between the gut contents of different mosquitofish size and sex groups. Only cladocerans, rotifers and adult chironomids were caught in significantly different quantities by distinct size groups (table 2). Since we analysed only a few fish from the peripheral size groups 1 and 8, they were pooled with groups 2 and 7 respectively. The multiple comparison test showed that large mosquitofish size groups (7+8) clearly consumed greater amounts of cladocerans and adult chironomids than the other size groups, whereas small and medium fish consumed greater amounts of rotifers (table 2). There were also differences between the sex groups regarding the type of prey caught (table 3). Copepods, cladocerans, adult chironomids and other dipterans were caught in significantly greater amounts by gravid females than by immature, males, and non-gravid females. Nevertheless, males and non-gravid females fed more on other adult dipterans than immature, showing also significant differences with respect to their diet (table 3). Non-gravid females ate collembolans in significantly greater quantities than any other sex group.

## Discussion

Like other populations of *Gambusia holbrooki* previously studied in the Iberian peninsula (Fernández-Delgado and Rossomano 1997, Vargas and Sostoa 1996), the population in the rice fields of the Lower Mondego River Valley consisted of two main annual cohorts with a cohort substitution during the reproductive season.

In the Lower Mondego River Valley, the reproductive season (April-October) was similar to those of populations in other areas of the Iberian peninsula (Fernández-Delgado and Rossomano 1997, Franca and Franca 1954, Vargas and Sostoa 1996).

In our study, the average sex-ratio of 4:1 (females per male) was similar to the value described for the Canal Vell lagoon (4:1) by Vargas and Sostoa (1996) in NE Spain, and smaller than the value described for Águas de Moura rice fields (5:1) by Franca and Franca (1954) in Portugal. Since equal numbers of male and female mosquitofish occur in the ovary and at birth (Krumholz 1948), the temporal variation in the adult sex-ratio must be attributed to the differential mortality of the sexes.

Maximum growth occurred during the early summer, when the temperatures become mild and the availability of food, specially zooplankton, increases (Cabral et al. 1998). These conditions are favourable to increase the mosquitofish metabolic rate (Cech et al. 1985) and food consumption (Linden and Cech 1990), promoting high growth rates. Females had a pattern of indeterminate growth with seasonal variation (Cabral and Marques 1999): growth slows during the "cold months" until the mid-winter and then recover with relatively high rates until the end of the second summer. The growth of adult males did not followed this pattern, because it is associated with the onset of maturation, growth cessation, or slow growth, after the gonopodium has been completely formed (Krumholz 1948, Vondracek et al. 1988). The estimated growth rates, taking into consideration seasonal variations by using the model



Table 2. Average number of prey items eaten (Mean  $\pm$  S.E.) by mosquitofish (*Gambusia holbrooki*) taking into account the size groups collected during the study period. The number of solid circles below the values indicates significant differences between size groups for the medians of a given prey group (Kruskal-Wallis and nonparametric multiple comparison tests). \*\* =  $p < 0.01$ ; \*\*\* =  $p < 0.001$  (n.s. = no significant). n is the number of individuals.

PREYS	MOSQUITOFISH SIZE GROUPS						$\chi^2$	p
	1 + 2 (n=39)	3 (n=120)	4 (n=137)	5 (n=101)	6 (n=53)	7 + 8 (n=34)		
Copepoda	16.4 $\pm$ 4.0	18.0 $\pm$ 3.9	31.6 $\pm$ 4.7	40.2 $\pm$ 8.5	37.0 $\pm$ 10.0	51.1 $\pm$ 13.1	9.27	n.s.
Cladocera	6.9 $\pm$ 1.9 •	7.9 $\pm$ 2.9 •	6.4 $\pm$ 1.8 •	7.4 $\pm$ 2.7 •	19.3 $\pm$ 7.6 ••	30.4 $\pm$ 10.1 •••	15.76	**
Rotifera	1.5 $\pm$ 1.2 •••	7.8 $\pm$ 3.4 •••	13.8 $\pm$ 4.8 ••••	0.6 $\pm$ 0.3 ••	0 •	0 •	15.36	**
Collembola	0.3 $\pm$ 0.2	1.1 $\pm$ 0.3	1.0 $\pm$ 0.3	1.1 $\pm$ 0.4	3.1 $\pm$ 1.2	0.8 $\pm$ 0.7	3.41	n.s.
Hemiptera Aphididae	1.8 $\pm$ 0.4	1.4 $\pm$ 0.2	2.6 $\pm$ 0.4	3.2 $\pm$ 0.6	3.7 $\pm$ 0.9	2.1 $\pm$ 1.1	4.52	n.s.
Diptera Chironomidae (AD) <sup>1</sup>	0.03 $\pm$ 0.03 •	0.02 $\pm$ 0.01 •	0.2 $\pm$ 0.1 •	0.4 $\pm$ 0.2 •	0.6 $\pm$ 0.3 •	1.7 $\pm$ 0.9 ••	22.22	***
Others (AD)	0.03 $\pm$ 0.03	0.07 $\pm$ 0.02	0.08 $\pm$ 0.04	0.3 $\pm$ 0.08	0.08 $\pm$ 0.05	0.09 $\pm$ 0.05	9.48	n.s.

<sup>1</sup> (AD) = Adult

Table 3. Average number of prey items eaten (Mean  $\pm$  S.E.) by mosquitofish (*Gambusia holbrooki*) sex groups considered during the study period. The number of solid circles below the values indicates significant differences between sex groups for the medians of a given prey group (Kruskal-Wallis and nonparametric multiple comparisons tests). \*\*\* =  $p < 0.001$  (n.s. = no significant). n is the number of individuals.

PREYS	MOSQUITOFISH SEX GROUPS				$\chi^2$	p
	Immature (n=37)	Males (n=138)	Non-Gravid Females (n=193)	Gravid Females (n=116)		
Copepoda	14.6 $\pm$ 4.0 •	14.2 $\pm$ 2.2 •	24.7 $\pm$ 3.8 •	65.8 $\pm$ 8.8 ••	39.56	***
Cladocera	6.9 $\pm$ 2.0 •	5.8 $\pm$ 1.8 •	5.2 $\pm$ 1.8 •	24.6 $\pm$ 5.0 ••	41.10	***
Rotifera	1.6 $\pm$ 1.3	7.3 $\pm$ 2.9	5.6 $\pm$ 2.1	6.9 $\pm$ 4.6	6.52	n.s.
Collembola	0.2 $\pm$ 0.1 •	0.8 $\pm$ 0.2 •	2.3 $\pm$ 0.4 ••	0.03 $\pm$ 0.02 •	45.28	***
Hemiptera Aphididae	2.2 $\pm$ 0.5	2.4 $\pm$ 0.4	2.6 $\pm$ 0.4	2.2 $\pm$ 0.5	2.15	n.s.
Diptera Chironomidae (AD) <sup>1</sup>	0.03 $\pm$ 0.03 •	0.1 $\pm$ 0.04 •	0.02 $\pm$ 0.01 •	1.2 $\pm$ 0.3 ••	38.30	***
Others (AD) <sup>1</sup>	0.03 $\pm$ 0.03 •	0.1 $\pm$ 0.04 •••	0.06 $\pm$ 0.03 ••	0.3 $\pm$ 0.06 ••••	16.71	***

<sup>1</sup> (AD) = Adult

proposed by Gaschütz et al. (1980), were about one order of magnitude smaller than the values described for the Canal Vell (Vargas and Sostoa 1996) and Zoñar lagoons (Fernández-Delgado and Rossomano 1997). The possible cause of this may be related with the fact that the adjustment of the seasonal growth curves to the original data for long periods (several months) may skew the growth rates to lower values than when they are directly estimated for short favourable periods (1-2 months), as performed in the studies mentioned above. Moreover, this type of adjustments to the monthly averages of length at successive cohorts allows correction of some of the bias in data (Ricker 1975).

With regard to mosquitofish annual production and P/B ratio, no estimates are available for other mosquitofish populations from the literature, so we have no idea of general levels to be expected. Nevertheless, the estimated mosquitofish annual production of  $3.10 \text{ g m}^{-2} \text{ year}^{-1}$  (AFDW), approximately  $12.63 \text{ g m}^{-2} \text{ year}^{-1}$  (wet weight) and  $126.26 \text{ kg ha}^{-1} \text{ year}^{-1}$  (wet weight), seem to be an extraordinary value for a very small fish species, much higher than, for instance, the values estimated for other planktivore populations studied in lakes from the ex-U.S.S.R. and reviewed by Waters (1977), ranging from  $9\text{-}24 \text{ kg ha}^{-1} \text{ year}^{-1}$ . The P/B ratio of 1.07, is also elevated when compared with values of those planktivore populations, ranging from 0.7-0.8, indicating in this case that mosquitofish have a faster growth and a shorter life span (Waters 1977).

Mosquitofish normally feed primarily near the surface on zooplankton, specially free-living Cyclopoid copepods and cladocerans (Cabral et al. 1998, Colwell and Schaefer 1983, Crivelli and Boy 1987, Daniels and Felley 1992). Hurlbert and Mulla (1981) and Crivelli and Boy (1987) found that copepods were much less affected than cladocerans by mosquitofish predation. However, we observed exactly the contrary, with copepods constituting the most important prey group during the study period. Cladocerans were clearly less important as prey items, and ostracods were found only in a few guts. The difference in these findings may be a function of the different availabilities of the prey groups to mosquitofish. In fact, among the water phase invertebrates, copepods composed 62.8 % of the samples, whereas the cladocerans accounted for only 16.1 % (Cabral et al. 1998). Although mosquitofish prey selectively on larger zooplankters, rotifers seemed to be a relative important prey group for immature fish as well as for young males and females, which constitute the small-medium size classes (table 2).

Throughout the year, surface insects, like aphids, collembolans, adult chironomids, and other dipterans, were an important additional food source. Nevertheless, mosquito larvae (chironomids and other dipterans), which were relatively abundant in the irrigation channel sediments (Cabral et al. 1998), constituted only a small quantitative fraction of the mosquitofish diet. This observation agrees with the disappointing reports from experiments using mosquitofish for mosquito control around the world (Rupp 1996).

During the non-reproductive season, our results showed a similar diet for immature, males and non-gravid females, which ate very close quantities of the preferential prey groups (table 3). During the reproductive season, from April to July,

most of the mature females were gravid. These females captured, in general, more prey items than immature, males and non-gravid females. Moreover, during this period of high reproductive investment, gravid females eat more surface insects, especially adult dipterans, than the other population groups (table 3). This may be explained by the larger size of gravid females that enable them to eat bigger prey items and the need for higher caloric intake (Harrington and Harrington 1961).

A combination of life history, population dynamics, production and eco-ethological traits (e.g., fast growth, reduced longevity, viviparity, high productivity, an intermediate position in food chain, plasticity and adaptability in its food use, and no special habitat requirements for reproduction) show that *Gambusia holbrooki*, introduced into rice fields all over the world, certainly induce an important impact in the structure and functioning of the native biological communities of these important agro-ecosystems, such as the Lower Mondego River Valley rice fields. This question is extremely important to reinforce the recommendation that *Gambusia*, the backbone of biocontrol for one-quarter of a century (Rupp 1996), not be introduced into new areas.

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