Abstract
Little is known regarding the life history of *Gambusia holbrooki* in Europe, particularly in Italy, where it has been introduced in several wetlands since 1922. In order to increase the knowledge of its biology, we tried to clarify its life history in a Mediterranean wetland, comparing our results with those of other authors. The principal aim of this study was to describe the demographic potentialities of the studied population by modal progression analysis. Besides the curvature parameter (0.52 and 0.51 for females and males, respectively) and the asymptotic length values (39.01 and 30.45 for females and males, respectively), we observed a population divided into four age classes, for both sexes, and a longevity of 5 years, with only a limited number of mosquitofish reaching this age (due to a high mortality rate). In addition, reproduction observations were carried out for females. The most interesting aspect concerned the correlation between fecundity and body size, and the capacity of small females to absorb fertilized eggs.

Keywords: Cyprinodontiformes, Gambusia holbrooki, growth, life history, Pisces, population structure, reproduction

Introduction
The family Poeciliidae, belonging to the order Cyprinodontiformes, is a large taxon of bony fish including many genera, such as *Gambusia*. Many species are included in this genus, which originated in the USA, Mexico, and Cuba (Gandolfi et al. 1991), and are usually known as mosquitofish due to their ability to prey upon larval Culicidae.

Recent infestations of the tiger mosquito [*Aedes albopictus* (Skuse, 1894)] and other dipterans (e.g. *Culex pipiens* Linnaeus, 1758 and those belonging to the genus *Anopheles*) in many Italian urban areas and in small wetlands have stimulated a new interest in their biological control by using Poeciliidae species (Bellini et al. 1994; Cocchi et al. 1998). The principal aim of this activity is to solve problems underlying many human diseases, such as malaria. This illness is spread by the transmission of the parasite *Plasmodium* spp. via dipterans (Grassi 1900). In this way, the Poeciliidae species become a deterrent against the transmission of human illnesses, and for this reason they were believed to be of great help to
humans, producing, at the same time, the lowest possible environmental impact (Ewel et al. 1999).

This is the case of the mosquitofish *Gambusia holbrooki* Girard, 1859, once considered a subspecies of *Gambusia affinis* (Baird & Girard, 1859) and recently raised to the rank of species by Wooten et al. (1988). The latter suggested that all the European mosquitofish populations introduced from eastern USA should be included in the species *G. holbrooki*. Its native distribution covers a wide geographic area on the southern coast of the USA (Meffe 1991; Haynes & Cashner 1995). From there, it has been introduced to numerous inland waters in many countries. Its introduction to Italy was recorded for the first time in Latium (central Italy) in 1922 (Tortonese 1970), and subsequently in every kind of lentic and low-flow lotic waters of the national territory due to its ability to colonize all types of habitat (Weeks & Meffe 1996) and to tolerate wide ranges of temperature, salinity and oxygen (Otto 1973; Chervinski 1983; Meffe et al. 1995; Gandolfi et al. 2002a). It has a preference for stagnant waters with a mud bottom and lush vegetation (Gandolfi et al. 1991). Moreover, the ovoviviparity of this species, with a particular maternal system (Balon 1975), may have been a great advantage for the invasive process (McKay 1978).

In the non-native habitats, the mosquitofish displays different feeding habits, depending on season and feeding resources (Ferrante et al. 2002), and has a deleterious impact on aquatic community structure and dynamics because of its selective predation of zooplankton (Margaritora et al. 2001), eggs and fish larvae (Pen & Potter 1991). In fact, if in the past the mosquitofish has been considered to be of great help against the proliferation of mosquito larvae (Tortonese 1970), many studies have also shown that it had an impact on many native fish species. 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 (Myers 1965; Arthington et al. 1983; Meffe 1985; Lloyd et al. 1986).

In Italy, this species has only recently been taken into account in environmental monitoring for preservation purposes but, despite the fact that many ecological studies have been carried out in Europe, its life history is still poorly known (Paes da Franca & Da Franca 1953; Stella et al. 1984; Crivelli & Boy 1987; Fernández-Delgado 1989).

Therefore, in order to increase our knowledge of the biology of *Gambusia holbrooki*, our aim in this study was to clarify its life history in a wetland with variable conditions, and then to compare the results with those carried out in different geographical areas. In particular, the main purpose of this study was to understand the demographic potentiality of this species in order to increase knowledge and to improve control activities of this species.

**Materials and methods**

**Study area**

The present study was carried out in a former clay mine near Rome (central Italy), located in a typical Mediterranean area, currently used as a water source for neighbouring irrigation systems. This zone is morphologically divided into different ponds (3 m maximum depth) and channels (1 m maximum depth) continuously fed by ground water sources, giving a low but constant supply. The water temperature reaches a maximum of 29°C in July and a minimum of 6°C in January, and its mean pH value was 7.5 (±0.5). The fish community is made up of alien species such as *Procambarus clarkii* (Girard, 1852), *Lepomis gibbosus* (Linnaeus, 1758), *Micropterus salmoides* Lacépède, 1802, and *Gambusia holbrooki*, and historically introduced species such as *Carassius carassius* (Linnaeus, 1758), *Carassius*
auratus (Linnaeus, 1758), and Cyprinus carpio Linnaeus, 1758. Recently, it has been suggested that this site should be included in the Pratica di Mare Archaeological Park, due to its national palaeontological importance, because of the presence of both gastropod and bivalve Pliocene fossils and for the presence of migratory birds, listed in the Conventions of Ramsar (1971), Berne (1979), and Bonn (1979).

Data collection and statistic elaboration

In 2005, four samples were carried out (14 February, 14 May, 31 August, and 13 November), in which temperature and pH were recorded. The mosquitofish were captured by hand-net and electrofishing, and preserved in a solution of 70% alcohol obtained by diluting absolute alcohol with an isotonic solution (NaCl 0.9%) in order to maintain the structure and weight of the animals (personal observation). Subsequently, each specimen was sexed and measured (standard length, SL, was recorded), and the relation of length and weight was calculated by the equation:

\[ W = a \times SL^b \]

where \( W \) is the weight and \( a \) and \( b \) are two specific constants. The coefficient of condition was then calculated by the following Fulton formula (Beckmann 1948; Bagenal & Tesch 1978):

\[ K_c = \frac{W}{(SL)^b} \times 10^5. \]

Scales were then removed in order to age mosquitofish individuals. This method was then compared to the length–frequency distribution analysis by the Bhattacharya method (1967), a routine of the FiSAT (FAO-ICLARM Stock Assessment Tools) computer program (Gayanilo et al. 1996). This method can decompose size–frequency distributions into diverse normal components (each component being identified as a cohort) and it is based on the assumption that the observed distribution in size classes results from the overlap of diverse normal distributions. The process converts normal distributions into lines that simplify the procedure, linearization being performed by computing the natural logarithms of frequencies. Intercepts and slopes of the regression lines were used to estimate the parameters of each normal distribution. Given a distribution in size classes, the Bhattacharya method allows for the iterative computation of regression lines until the total decomposition of the overall size–frequency distribution. The program provides values for each Gaussian component, i.e. means, standard deviations, numbers of individuals per size class, regression lines, and separation index values (S.I.) for each adjacent group. In particular, S.I. denotes when two adjacent Gaussians can be separated, i.e. S.I. \( \geq 2 \) (Sparre and Venema 1998). In a univoltine population, where S.I. values decrease below 2, the last class is included in the preceding component. At the end of the separation process, the program provides \( \chi^2 \) test values.

Subsequently, the Von Bertalanffy (1938) growth parameters (curving parameter \( k \), asymptotic length \( L_\infty \), and initial condition parameter \( t_0 \)) were then calculated. In particular, the asymptotic length was calculated by the Powell–Wetherall Plot method (Powell 1979; Wetherall 1986) while the ELEFAN I method (Electronic Length–Frequency Analysis) was used to evaluate the curvature parameter, both belonging to the FiSAT program. The ELEFAN I method provides growth parameters from sequential length–frequency data, by the scan of the \( k \) value. This method allowed us to estimate the \( k \)
value by a plot of $k$ versus fit index (i.e. $Rn=10^{ESP/ASP}/10$, where ESP is the explained sum of peaks and ASP the available sum of peaks—for more details see Gayanilo and Pauly 1997). Subsequently, the values of the amplitude constant ($C$) and the winter point ($W_p$) were calculated in order to establish the degree of seasonal influence on growth by utilizing the seasonalized Von Bertalanffy formula (Pauly et al. 1992):

$$L(t) = L_\infty[1 - \exp(- k(t-t_0) - (Ck/2\pi)(\sin 2\pi(t-t_s) - \sin 2\pi(t_0-t_s)))]$$

where two new parameters appear: $L(t)$, the length of the specimens at time $t$, and time $t_s$ which is applied to the variation of growth due to seasonal changes, and which fixes the winter point value ($W_p = t_s + 0.5$).

Once the Von Bertalanffy parameters were calculated, it was then possible to compare the growth potentialities by the growth performance index ($\Theta'$), calculated through the following formula (Gayanilo & Pauly 1997):

$$\Theta' = \log k + 2\log L_\infty.$$ 

Moreover, the total mortality index $Z$ (the sum of natural and fishing mortality) was calculated using the Powell–Wetherall Plot formula (Powell 1979; Wetherall 1986), a simple process to estimate the asymptotic length and the ratio between the mortality coefficient and the curvature parameter ($Z/k$), using only length–frequency data imported in the FiSAT program. In this study, total mortality equals natural mortality, since *G. holbrooki* in this area is not subject to fishing.

Finally, to analyse demographic aspects, the following formula was applied to assess longevity (Gayanilo & Pauly 1997):

$$t_{\text{max}} = (3/k) + t_0.$$ 

Moreover, observations regarding reproductive efficiency were carried out. The reproductive females (recognized by the presence of the latero-ventral spot), were dissected in order to extract gonads and record the number of embryos. The latter were divided into different stages: (1) preneurula; (2) neurula; (3) eyed larva; (4) fry (according to a modification of Reznick 1981). The diameter of a large number of eggs was measured. For females, $L_{50}$ was calculated (the length at which 50% of females had mature gonads), interpreted as the sexual maturity onset (Spedicato & Cannas 2000).

All the data were imported into the Statistica Statsoft program version 6.0 in order to carry out statistical analyses.

**Results**

In this study, 3177 individuals (1876 females and 1301 males) were caught and measured. The length–frequency diagrams and the numbers of specimens caught in any sample are shown in Figure 1. The sex-ratio (calculated as the female percentage) favoured females ($\chi^2=5.85; \text{df}=1; P<0.05$, with the Yates adjustment), due to the high number of females during February ($\chi^2=4.11; \text{df}=1; P<0.05$, with the Yates adjustment). Indeed, in the other samples, the sex-ratio did not show any difference in number between the sexes ($\chi^2=2.16, 1.84, 1.94$ with $P=0.089, 0.101, 0.074$, respectively, for May, August, and November, always with df=1).
After the body measurements, the length–weight relation and the Fulton coefficient condition values for both sexes were calculated in any sample (Table I). In Figure 2 the trend of the condition index during the study period is shown.

The age determination by scale observations was carried out in 107 females and 96 males (Figure 3).

A total of 69 (64.5%) females and 59 (61.5%) males showed one annulus (1+ age class), 21 (19.6%) females and 18 (18.8%) males no annuli (0+), and 17 (15.9%) females and 19 (19.8%) males two annuli (2+). Comparing the mean values of age classes by the two-way ANOVA test, the only significant difference has been observed between 2+ and 1+ females and the remaining classes of both sexes ($F_{5,197}=10.51, P=0.0314$; $F2+=F1+>F0+=M0+=M1+=M2+$, after the post hoc Tukey test).

Figure 1. Size–frequency diagrams for both mosquitofish sexes (by month) (white, females; black, reproductive females; grey, males).
After the scalimetric analysis, length–frequency diagrams were plotted by the Bhattacharya method (Table II).

The mean values of the age classes were utilized to obtain the Von Bertalanffy parameters and the growth line by the ELEFAN I method (Figure 4). Using the three Von Bertalanffy parameters \(k\), \(L_\infty\), and \(t_0\), the \(\Theta'\) index, the mortality rate \(Z\), and longevity \(t_{\text{max}}\) were calculated for both sexes (Table III).

With regard to reproductive biology, the occurrence of 246 (64.91%) ovigerous females in May and 118 (32.07%) in August was shown in the length–frequency diagrams reported in Table I.
in Figure 1. The minimum size of the spotted females was 19.1 mm (in August), while the
$L_{50}$ value (Figure 5) was about 21 mm.

Among the ovigerous females in May, 254 individuals were dissected in order to observe
the total number of embryos and their development phase. A significant positive correlation
(described by the following formula: $y=2.4x+30.3$) between the mean values of the embryo
number and the fish standard length was observed ($R^2=0.96; P<0.05$). The correlation
between embryo number at each stage and body size is shown in Figure 6 (function is
reported in Table IV).

Moreover, the differences in egg diameter among females of different body size were
recorded. In particular, 30 eggs of females with $SL=21–25$ mm, 28 eggs of females with
$SL=26–30$ mm, and 37 eggs of females with $CL>31$ mm were measured. The Kruskal–
Wallis test did not show significant differences ($H(2, N=96)=11.27, P=0.18$).

**Discussion**

In this study, more than 3000 mosquitofish were collected, a sufficiently significant sample
to hit our target.

**Table II.** Results of the length–frequency analysis after the application of the Bhattacharya method (results always showed significance after $\chi^2$ test, $P<0.05$).

| Age class | No. ind. | % ind. | Mean | SD   | S.I. | $R^2$ |
|-----------|----------|-------|------|------|------|-------|
| **Females** |          |       |      |      |      |       |
| February  |          |       |      |      |      |       |
| 0+        | 598      | 79.52 | 20.52| 1.98 | –    | 0.88  |
| 1+        | 132      | 17.55 | 27.39| 1.87 | 3.57 | 0.71  |
| 2+        | 22       | 2.93  | 32.12| 0.96 | 3.33 | 0.65  |
| May       |          |       |      |      |      |       |
| 0+        | 60       | 16.26 | 9.86 | 1.18 | –    | 0.94  |
| 1+        | 105      | 28.46 | 21.54| 1.30 | 9.42 | 0.79  |
| 2+        | 204      | 55.28 | 28.30| 2.56 | 3.50 | 0.71  |
| August    |          |       |      |      |      |       |
| 0+        | 84       | 25.45 | 13.78| 20.8 | –    | 0.89  |
| 1+        | 108      | 32.73 | 23.43| 1.28 | 5.74 | 0.80  |
| 2+        | 138      | 41.82 | 30.67| 1.85 | 4.63 | 0.73  |
| November  |          |       |      |      |      |       |
| 0+        | 82       | 20.71 | 15.82| 1.24 | –    | 0.82  |
| 1+        | 178      | 44.95 | 25.56| 2.00 | 6.01 | 0.72  |
| 2+        | 131      | 33.08 | 30.34| 1.60 | 4.37 | 0.62  |
| 3+        | 5        | 1.26  | 35.50| 0.76 | 2.66 | 0.57  |
| **Males** |          |       |      |      |      |       |
| February  |          |       |      |      |      |       |
| 0+        | 40       | 12.01 | 16.62| 1.13 | –    | 0.90  |
| 1+        | 251      | 75.38 | 22.49| 2.07 | 3.67 | 0.81  |
| 2+        | 42       | 12.61 | 26.87| 1.56 | 2.41 | 0.75  |
| May       |          |       |      |      |      |       |
| 0+        | 60       | 16.81 | 9.86 | 1.18 | –    | 0.94  |
| 1+        | 63       | 17.65 | 18.25| 1.30 | 6.77 | 0.81  |
| 2+        | 234      | 65.55 | 22.81| 1.78 | 3.96 | 0.73  |
| August    |          |       |      |      |      |       |
| 0+        | 81       | 25.31 | 12.27| 1.33 | –    | 0.82  |
| 1+        | 169      | 52.81 | 19.80| 1.81 | 4.80 | 0.76  |
| 2+        | 70       | 21.88 | 24.09| 1.57 | 3.54 | 0.61  |
| November  |          |       |      |      |      |       |
| 0+        | 131      | 37.01 | 15.83| 1.85 | –    | 0.90  |
| 1+        | 113      | 31.92 | 21.69| 0.93 | 4.22 | 0.84  |
| 2+        | 96       | 27.12 | 25.02| 1.10 | 3.76 | 0.78  |
| 3+        | 14       | 3.95  | 28.50| 0.75 | 3.28 | 0.70  |

No. ind., theoretical number; % ind., theoretical percentage; S.I., separation index values; $R^2$, statistical output of the FiSAT computer program for each Gaussian component.
This population showed an unbalanced sex ratio, in favour of females, in accordance with studies carried out on the same species (Table V), due to the high number of females (2.5 times more abundant than males) in February. This disproportion could be due to several reasons, such as differences in habitat selection between the sexes, season, and

|       | k   | \( L_\infty \) | \( t_0 \) | C   | \( W_p \) | \( \theta' \) | Z     | \( t_{\max} \) |
|-------|-----|----------------|---------|-----|---------|---------|-------|--------------|
| Female| 0.52| 39.01          | -0.84   | 0.42| 0.2     | 6.7     | 4.71  | 4.93         |
| Male  | 0.51| 30.45          | -0.93   | 0.59| 0.2     | 6.2     | 5.16  | 4.95         |

See text for details of parameters.

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selective mortality (Rosen & Bailey 1963; Martin 1975; Casterlin & Reynolds 1977; Fernández-Delgado 1989).

Many aspects of our study show no differences to other observations. In particular, observing the number of ovigerous females and the Fulton index values, our results seem to be similar to those of other studies, also with regards to reproductive biology, brood size range, size at sexual maturity, and percentage of ovigerous females in May and August, which were in agreement with the observations of other authors (Vargas & de Sostoa 1996; Fernández-Delgado & Rossomanno 1997; see also Table V).

As far as reproductive biology is concerned, the breeding period did not seem to occur in two specific periods during the year, but only over a long time period (nearly 5 months). During this period we observed a particular phenomenon of parental care in spotted females. In *G. holbrooki*, the number of fertilized eggs and embryos increased positively with the fish standard length, according to Reznick (1981). However, small females seemed to absorb some fertilized eggs, probably because young females were unable to tolerate a great number of eggs. The young females lost fertilized eggs, almost at the first phase, after the neurula stage, while in old females, the number of fertilized eggs remained constant, especially after the ‘eyed-larvae’ phase.

Several interesting aspects were observed with regard to the dynamic potentialities. In this study no correlation was observed between size and annuli number, especially for male individuals. It was different from previous studies which described populations of *G. holbrooki* structured into almost two age classes (Paes da Franca & Da Franca 1953; Trendall 1982; Hughes 1985; Fernández-Delgado 1989) in that we observed four age classes, from 0+ to 3+, although few individuals were observed in the latter class (3.95% of males and 1.26% of females in November), due to the high mortality rate.

| Embryo stadium       | SL=aEN+b         | $R^2$ | $t_{(N-2)}$ | $P$  |
|----------------------|------------------|-------|-------------|------|
| Preneurola           | $y=0.47x+14.10$  | 0.88  | 9.82        | <0.05|
| Neurola              | $y=0.39x+15.59$  | 0.71  | 8.87        | <0.05|
| Larva occhiuta       | $y=0.72x+1.35$   | 0.92  | 10.12       | <0.05|
| Larva pronta         | $y=0.78x+0.80$   | 0.94  | 8.93        | <0.05|

The significance of the regression analysis by the Student’s $t$ test is shown in the last two columns.
The asymptotic length reached different values depending on sex, showing a larger size in females owing to the reproductive strategy of this species. The different asymptotic length between the two sexes determined a difference in growth performance (as shown by the $\bar{O}$ index in Table III), since the curvature parameter of females and males show similar values. The values of the $k$ parameter (ranging between 0.5 and 0.6) obtained in our study, together with longevity (estimated as nearly 5 years), confirm the difference in population structure among all the studies carried out on the mosquitofish (Table V). Observing the studies reported in Table V, $G. \text{holbrooki}$ seems to be a very short-lived species, and in this case it could have higher values of the curvature parameter and low values of longevity. In our study, $k$ and $t_{\text{max}}$ show different values from those expected when taking into account previous studies. This is probably due to the age determination by scale observation, a method we find to be unuseful in this species.

These results, besides representing a first attempt at describing the Von Bertalanffy parameters, provide the first evidence of seasonal influence on the growth of $G. \text{holbrooki}$, as amplitude ($C$) and winter point ($W_p$) values show. In fact, it is possible to note that Mediterranean seasonal changes cause a decrease in growth at the beginning of autumn and an increase in spring.

Growth parameters reflect the acclimation capability of the moquitofish in the new non-native habitat and show the ecological plasticity of this species, capable of colonizing many

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Table V. Comparison of some history parameters surveyed in different studies.

| Locality          | Age $\varphi$ | Age $\delta$ | Maximum size $\varphi$ | Maximum size $\delta$ | Spawning season | No. of broods | Brood size | Sex ratio ($\varphi:\delta$) | References$^a$ |
|-------------------|---------------|--------------|------------------------|------------------------|-----------------|--------------|------------|----------------------------|----------------|
| Natural           |               |              |                        |                        |                 |              |            |                            |                 |
| South Carolina (USA) | –             | –            | 60                      | 40                     | April–September | –            | ?–75       | –                          | 1, 2           |
| SW Louisiana (USA) | –             | –            | 35                      | 33                     | March–July      | –            | –          | 3:1                        | 3              |
| Introduced        |               |              |                        |                        |                 |              |            |                            |                 |
| Ebro (NE Spain)   | 2+            | 0+           | 63                      | 32                     | April–September | 3/2          | 3–181      | 4:1                        | 4              |
| Guadalquivir (SW Spain) | 2+        | 1+           | 65                      | 36                     | May–September   | 2/4          | –          | 2:1                        | 5              |
| Aquas de Moura (Portugal) | 3+       | 1+           | 70                      | 40                     | April–September | –            | –          | 5:1                        | 6              |
| Brisbane (Australia) | –             | –            | 39                      | 28                     | August–March    | –            | 3–108      | 1:1                        | 7              |
| Collie River (Australia) | –             | –            | 62                      | 35                     | August–March    | –            | –          | 4:1                        | 8              |
| Zoonar lagoon (Spain) | –             | –            | 53                      | 39                     | May–September   | –            | –          | 1:1                        | 9              |
| Vilademans (Spain) | 2+            | 2+           | –                       | 24.5                   | May–September   | –            | –          | –                          | 10             |
| Trasimeno lake (Italy) | –             | –            | –                       | –                      | May–September   | –            | –          | 6–73                       | 3:1            |
| Roma lake (Italy)  | 3+            | 3+           | $37^b$                  | $29^b$                 | May–August      | –            | 80–114     | 1.5:1                      | This study      |

$^a$1, Meffe (1991); 2, Meffe et al. (1995); 3, Daniels and Felley (1992); 4, Vargas and de Sostoa (1996); 5, Fernández-Delgado (1989); 6, Paes da Franca and Da Franca (1953); 7, Milton and Arthington (1983); 8, Pen and Potter (1991); 9, Fernández-Delgado and Rossomanno (1997); 10, Puigcerver (1992); 11, Gandolfi et al. (2002b). $^b$Only in the present study has the standard length been used.
temperate and warm habitats worldwide (see the Introduction), the Mediterranean wetland included. Growth parameters represent the most useful parameter, providing an estimate of the fitness and adaptation of a population to its habitat, because animal size is always correlated to the main biological process, such as alimentation, predation and fecundity.

Unfortunately, as time goes on, the mosquitofishes become more invasive and are often capable of dominating indigenous populations and communities (Kolar & Lodge 2001; Crooks 2002). The spread of cosmopolitan invasive species, combined with the extinction or range contraction of indigenous species, also leads to a constant ‘homogenization’ of the native biota, i.e. the reduction of regional differences among faunas and floras (McKinney & Lockwood 1999). Recent analyses suggest that biodiversity in freshwaters is declining more and more quickly (e.g. Ricciardi & Rasmussen 1999), and that biotic homogenization is accelerating in several freshwater systems (Rahel 2000). Freshwater systems are also highly susceptible to the colonization of invasive species as a result of both the strong affinity of humans for water (for commerce, transportation, recreation, and sanitary and aesthetic reasons) and the dispersal ability of freshwater species (Lodge et al. 1998; Gherardi 2000; Beisel 2001). Moyle et al. (1986) maintained that deliberate introductions of non-indigenous species are often carried out to solve either local or regional problems, but if the broad-scale consequences of each introduction are not considered, they may ultimately cause more problems than they solve. The introduction of _G. holbrooki_ into Europe is an example of this phenomenon. This retrospective analysis of the _G. holbrooki_ history of invasion shows that several life-history traits (early maturity, several broods, rapid growth, large number of offspring, plastic life cycle) and biological features (tolerance to extreme environments, dispersal, polyphagy, predatory and competitive ability, behavioural flexibility) help this species become particularly invasive and enable the Eastern mosquitofish to inhabit different areas and exploit favourable environmental conditions. Practically the entire population is renewed during the reproductive period. Moreover, ovoviviparity provides _G. holbrooki_ with several advantages over oviparous species (Vargas 1993). Similar to other species of the genus _Gambusia_, each population of the Eastern mosquitofish may be able to alter its life history in order to adapt to a particular environment (Haynes & Cashner 1995). This could explain the differences in the life history between the population in the Pomezia cave delta and the others.

Indeed, invasion is a multifactorial process and the identification of species characteristics is only one component which might explain its potential success (Gherardi 2006a). The others include elements such as the locality of introduction (disturbance, anthropogenic impacts, available resources, or so-called empty niches), and interaction between species and environment which accounts for habitat and climate matching (Ruesink 2005). Furthermore, several confounding variables may hamper any predictive effort, such as the natural variability of the environment in space and time, synergistic effects of other established invaders, and the variable time lag between initial introduction and detectable impact (Gherardi 2006b). This means that generalizations cannot be made from single case studies, and predictions cannot be based on experiments and observations conducted on small temporal and spatial scales. An intensification of scientific research on mosquitofish today may soon lead to a broader understanding of invasions in this taxon, and provide the quantitative data needed to predict future mosquitofish invasions and, thus, to reduce their occurrence and impact.

In the light of the results obtained from this study and those of other researchers, a possible method for _in situ_ preservation of the fauna and the continued biological fight against the illness vector could involve the release of animals and, in particular, females of
over 30 mm standard length without spots, which have been selected during the end of the reproductive period. This activity would need constant monitoring of the introduced specimens in the area. In this way mosquitofish populations could contribute to the biological control of infective illnesses while completing their ecological role.

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