

Life history and parasites of the invasive mosquitofish (*Gambusia holbrooki*) along a latitudinal gradient

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Abstract The eastern mosquitofish (*Gambusia holbrooki*) is among the most invasive fish worldwide and yet, while very abundant in most Mediterranean countries, it is unable to tolerate the colder winters of northern and central Europe. Understanding the effects of latitude on its life-history traits is essential to predict the potential for its invasion of central Europe in current scenarios of climate change. We studied the variation of life-history traits and parasite load in the eastern mosquitofish along a latitudinal gradient from southern France to southern Spain, sampling mosquitofish populations in eight Mediterranean river mouths ranging 5° in latitude. Southern mosquitofish populations displayed higher catch rates, allocated more energy to reproduction (gonadosomatic index and gonadal weight after accounting for fish size) and had a lower condition (total weight and eviscerated weight after accounting for fish size) than in northern populations. Despite variability among populations,

size-at-maturity (L_{50}) significantly varied with latitude and northern individuals matured at smaller size (lower L_{50}). Parasite prevalence ranged from 0.0 to 26.7% but parasite richness was very low; all the parasites identified were larvae of pleurocercoid cestodes belonging to the order Pseudophyllidea. The abundance of mosquitofish parasites decreased with latitude and the presence and number of parasites infecting the mosquitofish had a significant negative effect on fish condition. The significant effects of latitude on the catch rates, life history and parasites of mosquitofish highlight the importance of latitudinal studies of invasive species to understand the interactive mechanisms of climate change and biological invasions.

Keywords Temperature · Invasive species · Latitude · Reproductive investment · Poeciliidae · Iberian Peninsula

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Introduction

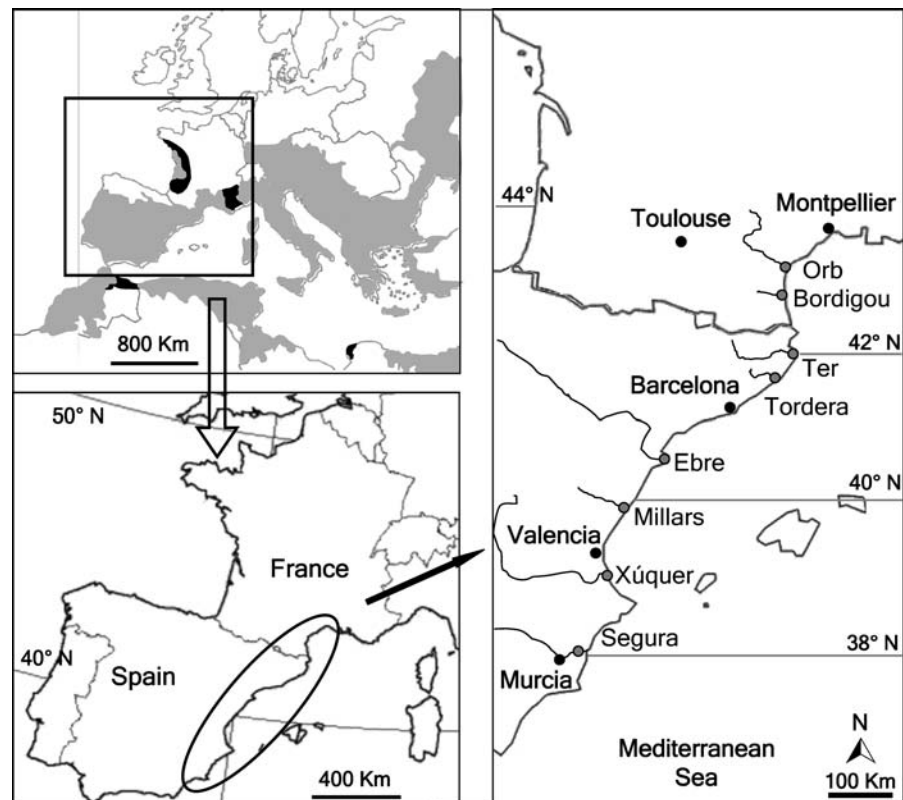
The latitudinal gradient of increasing biological diversity from polar to equatorial regions is ecology's longest recognized pattern but its explanation is still open to over thirty potential mechanisms (Hawkins et al. 2003; Willig et al. 2003). The effects of latitude on life history traits have been less studied but in a number of fish species are mainly mediated through

temperature and photoperiod (Garvey and Marschall 2003; Heibo et al. 2005). In the best studied case of several salmonids, temperature has also been shown to influence migration timing, smolting, growth rate, age-at-maturity, and fecundity (Power et al. 2005). For instance, the growth rate of brown trout (*Salmo trutta*) decreases and age-at-maturity increases with latitude but there is no overall effect on adult size (L'Abée-Lund et al. 1989). Latitudinal clines in egg number and size have also been widely reported in many animal species but those differences are even more enigmatic (Fleming and Gross 1990) since the relationship between egg number and size varies from positive to negative and is strongly dependent on multiple environmental factors and complex tradeoffs (Johnston and Leggett 2002; Power et al. 2005).

Latitudinal studies are important for understanding the potential effects of climate change at several levels of ecosystem structure because latitudinal variation in key environmental parameters (e.g. temperature) may mimic predicted climate-driven shifts in biological characteristics over time (Power et al. 2005).

Mosquitofish (in fact two close species, *G. holbrooki* and *Gambusia affinis*, that intergrade but since Wooten et al. (1988) are generally considered to have specific status) are live-bearing members of the family Poeciliidae that have been introduced worldwide since the 1920s in order to control mosquitoes as vectors of malaria. Although only native to the United States and Mexico, mosquitofish have been introduced to more than 50 countries (García-Berthou et al. 2005). Most introductions outside North America have been of eastern mosquitofish (*G. holbrooki*), which has been less studied than western mosquitofish (*G. affinis*) and exhibits different life-history patterns (Haynes and Cashner 1995). *G. holbrooki* was first introduced to Europe in 1921 through southwestern Spain, then transferred to other parts of the Iberian Peninsula, and nowadays is very abundant in most Mediterranean countries but is not able to tolerate the colder winters of northern and central Europe (Krumholz 1948, see also Fig. 1). The strong ecological impacts of invasive mosquitofish are well known and include changes in ecosystem

Fig. 1 Study area with the eight Mediterranean river mouths sampled. The top left map shows the current distribution of mosquitofish introduced to Europe and the Mediterranean region (in grey according to Krumholz 1948; in black a few areas that have been colonized thereafter). See Table 1 for precise location of the sampling sites (major cities are also shown with black circles)



functioning through trophic cascades (Hurlbert et al. 1972) and the decline and local extinction of a variety of native amphibians (Hamer et al. 2002; Kats and Ferrer 2003) and small fishes (Courtenay and Meffe 1989; Mills et al. 2004; Alcaraz et al. 2008). Although the life history of mosquitofish has been well studied both in native (Brown-Peterson and Peterson 1990; Haynes and Cashner 1995) and introduced populations (Vargas and Sostoa 1996; Fernández-Delgado and Rossomanno 1997), there are no specific studies on the effects of latitude beyond some brief data in a general review of Bergman's rule (Belk and Houston 2002). However, the direct effect of temperature on metabolism, feeding rates, and life history of *G. affinis* (Vondracek et al. 1988; Chipps and Wahl 2004) and on the reproductive and feeding behaviour of *G. holbrooki* (Rincón et al. 2002; Wilson 2005) have been experimentally demonstrated. Mosquitofish have several reproductive features that give them an enormous capacity to increase in population size and thus an exceptional invasive potential among fish: they produce litters of live born young approximately every 3 weeks, during a breeding season that ranges from 3 to 7 months depending on latitude, and the young can mature and reproduce just a few weeks after birth (Reznick et al. 2006).

The success of invasive species is often related to either the introduction of new pathogens or parasites into new areas and native species or by benefiting from enemy release in the introduced area (Prenter et al. 2004; Roessig et al. 2004). Although many introductions and translocations of pathogens in inland European aquatic ecosystems have been reported (Blanc 1997), there are no studies of pathogens of mosquitofish in Europe. The effects of latitude on mosquitofish parasite load have not been investigated in the native nor in other introduced ranges.

The objectives of this study are: (1) to evaluate the effects of latitude on the life-history traits and parasite load of *G. holbrooki* introduced in the Mediterranean region; and (2) to examine the parasite community of the mosquitofish introduced to Europe. Since mosquitofish invasive success seems clearly limited by temperature, the understanding of latitudinal variation is essential to predict its invasiveness and impact at different latitudes and the potential for its invasion of central Europe in current scenarios of climate change.

Materials and methods

Study area

A total of 929 *G. holbrooki* individuals were sampled in eight Mediterranean river mouths, including most of the largest rivers in eastern Spain (Fig. 1). The river mouths were situated in a latitudinal gradient ranging more than 5° from southern France to southern Spain (Table 1). Sampling sites were always situated in the last 1,500 m of river mouths, to control for the effect of river zonation, and consisted of reed (*Phragmites australis*) beds on the shore (where mosquitofish is generally found). Sites were sampled in early summer (July 2004); two sites were also sampled in spring and autumn (Table 1). July is the month with maximum fecundity for several Iberian populations of mosquitofish (Pérez-Bote and López 2005). Meteorological data from the closest stations to the sampling sites (generally <10 km apart) were provided by Météo France and the Instituto Nacional de Meteorología. As an estimate of the environmental temperature of the mosquitofish population, we used the mean from July 2003 to June 2004 because it presumably corresponds to the lifespan of most of the studied mosquitofish, which in Spain usually live less than 12 months and reproduce from mid-May to mid-September (Fernández-Delgado and Rossomanno 1997). The mean, minimum, maximum and range of air temperatures in this period (July 2003 to June 2004) were all highly correlated ($r > 0.96$, $n = 8$, $P < 0.0005$) with those of the ten previous years (1994–2003). We used air temperatures as a proxy to water temperatures because the latter were not available. As expected, the latitudinal gradient corresponded to a temperature gradient and latitude was highly correlated to mean temperature ($r = -0.936$, $n = 8$, $P = 0.001$) and minimum mean temperature ($r = -0.905$, $n = 8$, $P = 0.002$), and less so to maximum mean temperature ($r = -0.765$, $n = 8$, $P = 0.027$). However, there were some site-specific temperature anomalies (e.g. high temperatures in the Ebro River valley), which have been shown elsewhere (e.g. Font 1983) and allowed to assess the latitude-independent effects of temperature. Contrastingly, conductivity, which ranged in summer from 2.4 to 13.6 mS cm⁻¹ (ca. 1–9‰ of salinity), was not significantly related to the latitudinal gradient ($r = 0.37$, $n = 8$, $P = 0.36$) and was not considered

Table 1 Sampling dates and main features of the populations studied: mosquitofish CPUE (catch per unit effort), parasite prevalence (% of fish with parasites) and fish length

River basin	Country	Date	Latitude	UTM	Mean air temperature (°C)	CPUE (no. fish/minute)	Prevalence (%)	Mean total length (mm)		Total length range	Total catch
								Males	Females		
Bordigou	France	13 April 2004	42°45'12"	31T0502603/4733523	–	1.27	0.0	26.6	31.8	22–39	38
Tordera	Spain	7 April 2004	41°39'30"	31T0481284/4611946	–	0.17	0.0	18.0	27.0	18–31	6
Orb	France	28 July 2004	43°16'56"	31T052362/4792277	15.6	0.60	0.0	20.1	19.7	14–45	27
Bordigou	France	6 July 2004	42°45'12"	31T0502603/4733523	15.6	4.15	3.2	22.4	36.1	14–55	108
Ter	Spain	8 July 2004	42°1'35"	31T0512712/4652805	16.2	3.69	6.7	23.1	25.9	15–46	129
Tordera	Spain	8 July 2004	41°39'30"	31T0481284/4611946	16.1	3.04	10.0	23.2	27.5	19–59	76
Ebro	Spain	15 July 2004	40°41'11"	31T0318654/4506226	17.9	20.25	6.7	22.6	28.0	14–46	162
Millars	Spain	20 July 2004	39°54'58"	30S075877/4422801	18.2	10.87	6.7	18.3	19.3	16–24	87
Xúquer	Spain	20 July 2004	39°10'31"	30S0736288/4339877	17.8	9.80	10.0	23.7	26.2	13–40	98
Segura	Spain	20 July 2004	38°5'26"	30S0700096/4218388	18.4	10.50	26.7	21.9	28.6	15–59	105
Bordigou	France	5 October 2004	42°45'12"	31T0502603/4733523	–	4.90	0.0	22.8	20.8	17–28	49
Tordera	Spain	5 October 2004	41°39'30"	31T0481284/4611946	–	2.93	6.7	22.4	22.8	15–35	44

Mean air temperature refers to yearly average for the period July 2003 to June 2004

in the analyses (but see Alcaraz and García-Berthou 2007 for effects over long longitudinal gradients).

Field and laboratory methods

Mosquitofish were collected by two people during daylight hours with dip nets of 1 mm stretched mesh size, measuring the time used for sampling to estimate fish abundance (catch per unit effort, CPUE). Live specimens were transported to the laboratory for parasite examination and processing. All 929 caught mosquitofish were sexed and their total length and total weight were measured to the nearest millimetre and 0.1 mg, respectively. Sex was determined from the morphology of the anal fin (Turner 1941) and, when necessary (usually in individuals <15 mm), by dissection and examination of the gonads. Thirty specimens (if available) of each sampling site were dissected for parasite examination and measurement of eviscerated weight, gonadal weight, and (for females) ova number, embryo number and total weight of embryos. The ova corresponded to developmental stages I–III and the embryos to stages IV–XI of Haynes (1995), or to stages I and II–VI, respectively, of Reznick (1981). As in previous studies (Haynes 1995; Specziár 2004), only embryos were considered to be a measure of fecundity because in the ova development is not readily discernible with a dissecting microscope and it is not known whether they have been fertilized (Haynes 1995; Reznick 1981). As a descriptive alternative to the ANCOVA of gonadal weight (see García-Berthou and Moreno-Amich 1993), the gonadosomatic index ($GSI = 100 \times \text{gonadal weight} / \text{total weight}$) of the fish was also calculated. The mean embryo weight per female was calculated as the total weight of embryos divided by the number of embryos. Size-at-maturity (L_{50}) of a population is the length at which 50% of the individuals are mature and was estimated from the fit of logistic regressions (see below).

Parasite examination of dissected specimens included examination under a dissecting microscope of fins, tail, skin, eyes, gills, body cavity and major organs. The gastrointestinal tract was removed, split from mouth to rectum and examined for parasites in the lumen and attached to the walls. All parasites found were identified under a compound microscope, counted, and fixed in 70% ethanol.

Statistical analyses

The sex-ratio and percentage of mature individuals were first compared among populations with a *G*-test of independence (Sokal and Rohlf 1995). The association of these variables with latitude and temperature (controlling for total length for the size-at-maturity relationship) was then analyzed with generalized estimating equations (GEEs), with binomial errors and the logit link function. GEEs extend generalized linear models to correlated or clustered observations (such as individuals within populations) (Agresti 2002). The association of mosquitofish CPUE and parasite prevalence with latitude and temperature was analyzed with Spearman's correlation coefficient (r_s). Analysis of covariance (ANCOVA) was used to compare the condition (weight-length relationship) in the presence and absence of parasites and to compare reproductive variables among mosquitofish populations taking into account fish size (covariate). ANCOVA has several advantages over condition factors and similar indices (García-Berthou and Moreno-Amich 1993; García-Berthou 2001). The ANCOVA-adjusted population means were related to latitude with Pearson's correlation coefficients (r). The relationship of the life-history variables with total length and latitude was further analyzed with multiple linear regression analysis. Most of the quantitative variables (except embryo weight and latitude) were log-transformed for linear models, because homoscedasticity and linearity were clearly improved. All data analyses were performed with SPSS 15.

Results

Mosquitofish population structure

The overall percentage of mature individuals was significantly different among the eight populations ($G^2 = 182$, $df = 7$, $P < 0.0005$) but was not significantly related to latitude (GEE, Wald test, $\chi^2 = 0.34$, $df = 1$, $P = 0.56$) or temperature (GEE, $\chi^2 = 0.65$, $df = 1$, $P = 0.42$). The sex-ratio also varied among populations ($G^2 = 25.6$, $df = 7$, $P = 0.001$) but was not associated to latitude (GEE, Wald test, $\chi^2 = 0.10$, $df = 1$, $P = 0.75$) or temperature (GEE, Wald test, $\chi^2 = 0.34$, $df = 1$, $P = 0.56$) either.

Mosquitofish CPUE in summer depended on mean temperature ($r_s = 0.833$, $P = 0.010$) rather than on latitude ($r_s = -0.690$, $n = 8$, $P = 0.058$) and increased in warmer sites (Fig. 2). There was no significant variation of mosquitofish CPUE among sampling dates (ANOVA: $F_{2,8} = 0.61$, $P = 0.57$). The mean total length of males or females in summer (Table 1) did not vary with temperature or latitude (all $r_s < 0.49$, $n = 8$, all $P > 0.26$).

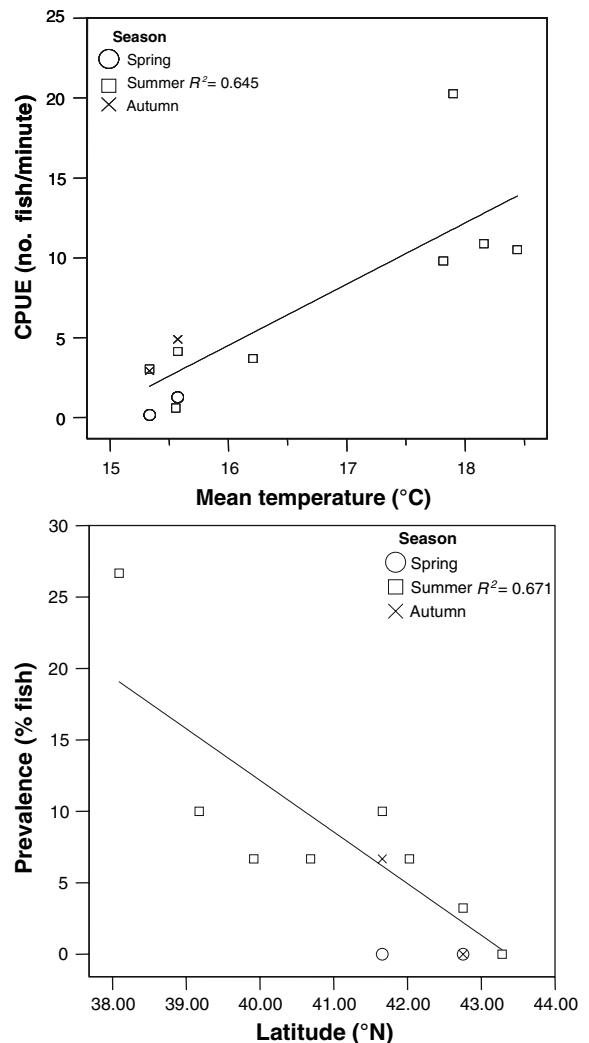


Fig. 2 *Top* Relationship of summer CPUE (catch per unit effort) of mosquitofish with annual mean air temperature. *Bottom* Relationship of parasite prevalence (% of infected fish) with latitude. Data for the two populations also sampled in spring and autumn are also given; the linear regression function corresponds to summer data only (see further statistics in the text)

Reproductive variables

Size-at-maturity significantly varied latitude in both males (GEE, Wald χ^2 : latitude, $P = 0.037$; total length, $P = 0.026$; latitude \times length, $P = 0.039$) and females (GEE: latitude, $P < 0.0005$; latitude \times length, $P < 0.0005$). Although some populations (namely no. 3 and 7) deviated from the general pattern, northern individuals matured at smaller size on average (lower L_{50}) (Figs. 3, 4). For instance, at a size of 20 mm TL, males and females of the two northernmost populations were all mature, whereas almost none was in the southernmost population (Figs. 3, 4). The L_{50} of both sexes (estimated from the

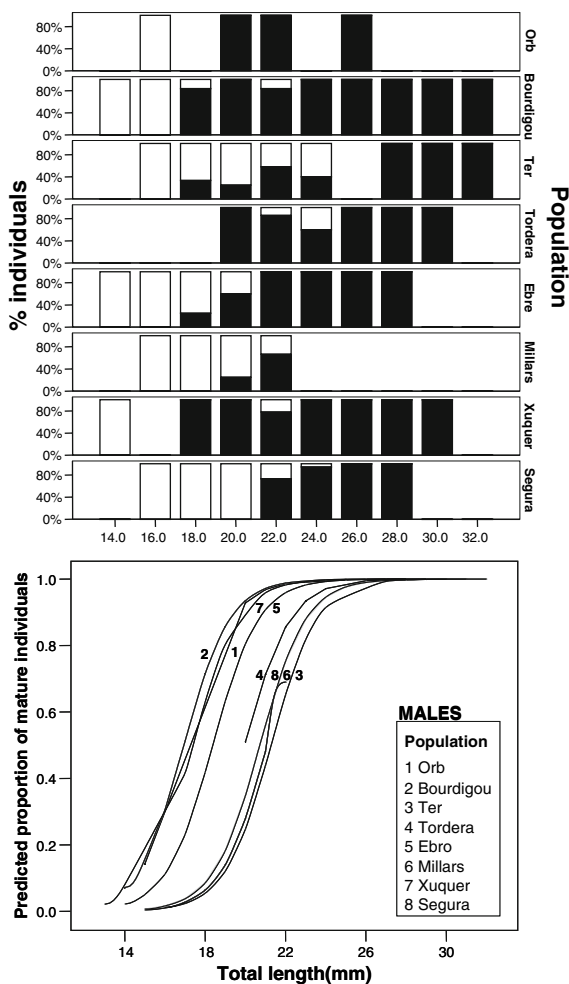


Fig. 3 Proportion of mature males observed (*top*; populations from north to south downwards) and predicted (by logistic regression; *graph on bottom*) by total length in the eight populations studied (\square immature, \blacksquare mature)

logistic models) were highly correlated among populations ($r_s = 0.881$, $n = 8$, $P = 0.004$) suggesting that the effect of latitude was similar on males and females.

After accounting for fish size (covariate in ANCOVA), we found significant differences among populations in all reproductive variables. In males, total weight (ANCOVA: $F_{7,281} = 3.28$; $P < 0.0005$), eviscerated weight ($F_{7,118} = 7.93$; $P < 0.0005$) and gonadal weight ($F_{7,109} = 3.44$, $P = 0.002$) significantly varied among populations (Fig. 5). Similarly, for females, total weight (ANCOVA: $F_{7,287} = 20.91$, $P < 0.0005$), eviscerated weight ($F_{7,101} = 7.55$, $P < 0.0005$), gonadal weight ($F_{7,102} = 3.705$,

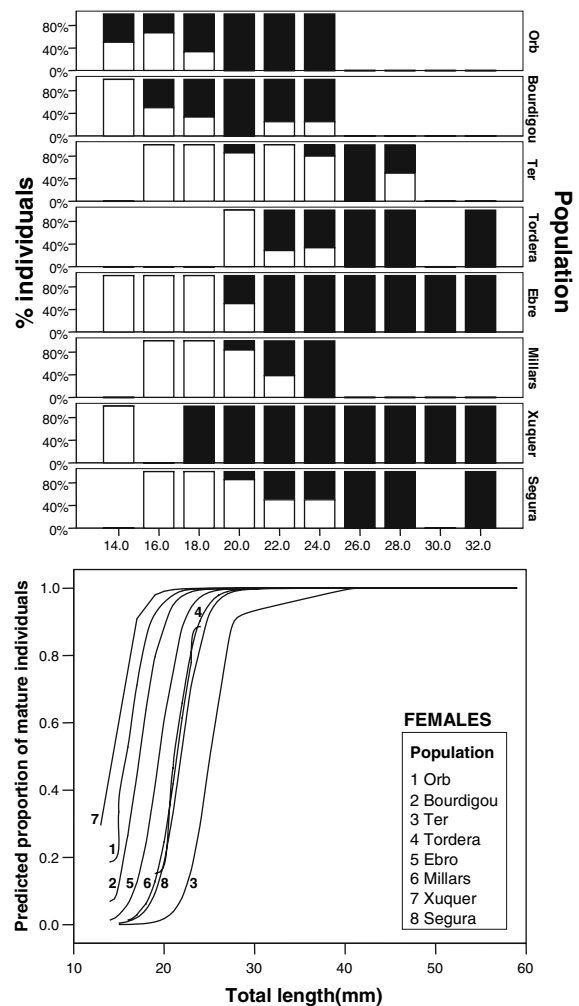
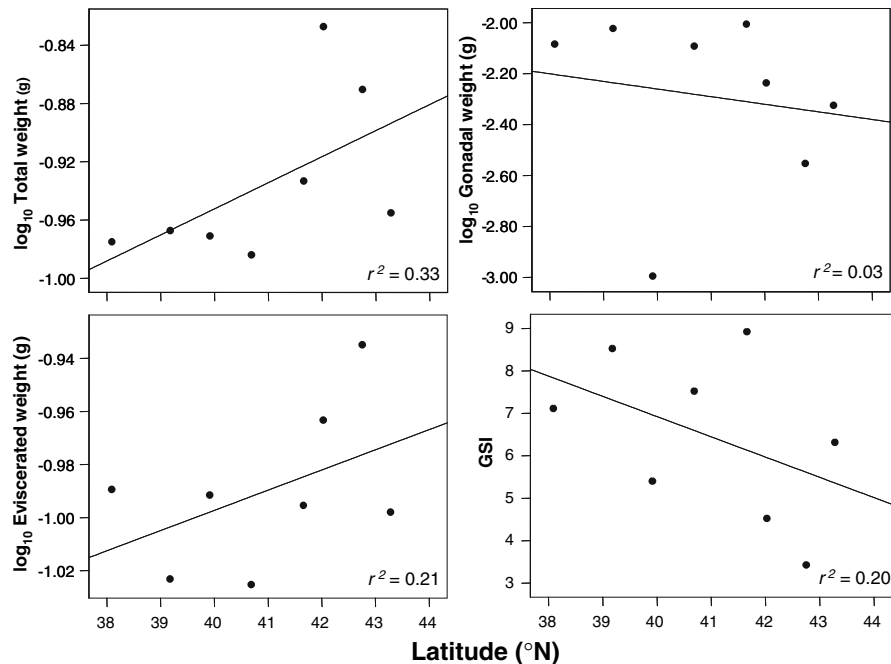


Fig. 4 Proportion of mature females observed (*top*; populations from north to south downwards) and predicted (by logistic regression; *graph on bottom*) by total length in the eight populations studied (\square immature, \blacksquare mature)

Fig. 5 Size-adjusted means (ANCOVA) of life-history variables (total weight, eviscerated weight and gonadal weight) with fish length (all \log_{10} -transformed) as the covariate and population as a factor for male mosquitofish. For the gonadosomatic index (GSI) only means (\pm SD) are given (no need to adjust with ANCOVA; ANOVA was used)



$P = 0.001$), number of embryos ($F_{7,86} = 3.28$, $P = 0.004$) and mean embryo weight ($F_{6,44} = 12.66$, $P < 0.0005$) were also significantly different among populations (Fig. 6). Multiple linear regressions provided predictive models of the latitudinal effects and showed that after accounting for total length, which as expected had a positive influence on all reproductive variables, latitude had highly significant effects on 6 out of 8 variables (Table 2). After accounting for fish size, total weight and eviscerated weight (i.e. somatic condition) significantly increased with latitude in both males and females (Figs. 5, 6). Latitude had a negative effect on size-adjusted gonadal weight in females and a marginally negative one ($P = 0.069$) in males (Table 2; Figs. 5, 6); the results for males were highly significant ($P = 0.002$) when removing an anomalous population (Millars, around 40° in Fig. 5). In contrast, the gonadosomatic index (GSI) was less related to latitude in females than in males, being more related in the latter to latitude ($r = -0.45$) than to temperature ($r = 0.24$), and increasing in southern populations (Figs. 5, 6). The GSI and the gonadal weight (ANCOVA-adjusted) provided different information because the former refers to total weight (and is not independent of fish size) whereas the latter adjusts for length (and removes allometric effects). Mean embryo weight

and embryo number varied significantly with female length but not with latitude (Table 2). A model of embryo weight controlling for embryo number and female size was marginally significant for latitude ($P = 0.097$), suggesting a possible trade-off between egg size and number. The percentage of variation explained by latitude (Figs. 5, 6) was higher for eviscerated and total weight (21–45%) and lower for gonadal variables and males in general. In summary, mosquitofish inhabiting northern latitudes matured at a smaller size, had less gonadal weight but better condition (total weight and eviscerated weight after accounting for fish size) than southern populations (Figs. 5, 6).

Preliminary data for the same eight populations also sampled in July 2005 also show that the adjusted means of gonadal weight decreased with latitude for females ($r = -0.66$, $n = 8$, $P = 0.074$) and less for males ($r = -0.39$) and that the variation among populations was considerably correlated both for males ($r = 0.69$, $n = 8$, $P = 0.058$) and females ($r = 0.36$), despite the low statistical power. The agreement of data between the 2 years suggests that despite the expected interannual and local variation in life-history traits, the latitudinal variation observed seems relatively stable and has an environmental and/or genetic basis.

Fig. 6 Size-adjusted means (ANCOVA) of life-history variables (total weight, eviscerated weight, gonadal weight, embryo weight, and embryo number) with fish length (\log_{10} -transformed) as the covariate and population as factor for female mosquitofish. For gonadosomatic index (GSI) only means (\pm SD) are given (no need to adjust with ANCOVA; ANOVA was used)

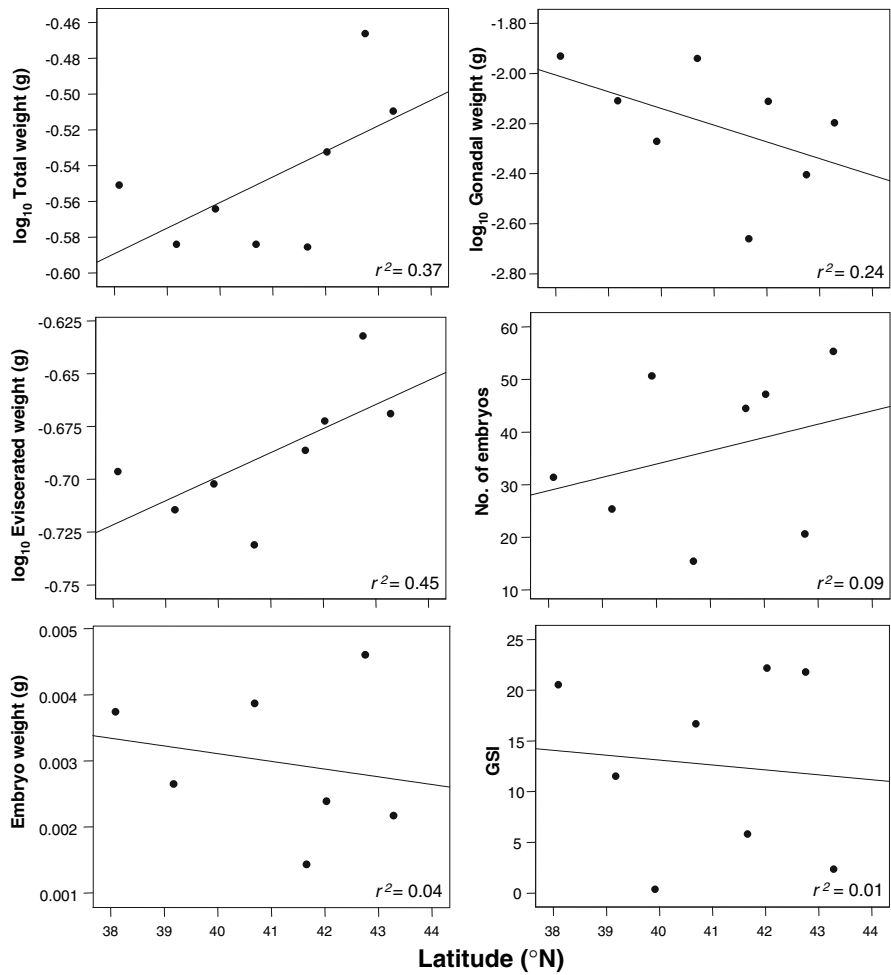


Table 2 Multiple linear regressions of reproductive variables with total length and latitude as independent variables for female and male mosquitofish

Dependent variable	Intercept	Total length	Latitude	Adjusted R^2	n
<i>Females</i>					
Total weight	-6.380***	3.493***	0.017***	0.976	296
Eviscerated weight	-5.640***	3.045***	0.012***	0.988	110
Gonadal weight	-13.355***	9.666***	-0.075*	0.875	111
Embryo number	-7.323***	6.277***	-0.028	0.839	95
Mean embryo weight	-0.004	8.7×10^{-5} **	9.3×10^{-5}	0.212	52
<i>Males</i>					
Total weight	-5.557***	2.663***	0.024***	0.806	249
Eviscerated weight	-5.605***	3.058***	0.009**	0.923	93
Gonadal weight	0.017	-0.001***	-0.001*	0.158	93

All variables were \log_{10} -transformed except embryo weight, latitude and the gonadal weight–total length relationship of males. Weight variables in grams, length in millimetres, and latitude in degrees (* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$)

Parasites

All parasites identified were larvae of pleurocercoid cestodes belonging to the order Pseudophyllidea; further taxonomic precision was not possible due to the lack of adult cestodes. We did not find any other parasite taxa, despite thorough dissection of 929 fish. In our study, parasite prevalence ranged from 0 to 26.7% (median 10%) and varied significantly with the latitude of the population ($r_S = -0.847$, $n = 8$, $P = 0.008$) rather than with temperature ($r_S = -0.663$, $n = 8$, $P = 0.073$), and there was greater prevalence in southern latitudes (Fig. 2). In addition to latitude (GEE, Wald test, $\chi^2 = 14.5$, $df = 1$, $P < 0.0005$), there was also a positive effect of total length ($P = 0.036$) on parasite prevalence, and a marginal effect of sex ($P = 0.043$), with higher prevalence in males. Although prevalence was greater in summer, its seasonal variation was not statistically significant.

The presence of parasites negatively influenced the eviscerated weight–length relationship for males (ANCOVA: $F_{1,124} = 11.34$, $P = 0.001$) but not significantly for females (ANCOVA: $F_{1,173} = 2.72$, $P = 0.101$) (Fig. 7). The adjusted mean of eviscerated weight (controlling for length with ANCOVA) indicated that males without parasites (adjusted mean = -0.970) were 5.6% heavier (i.e. in better condition) than males with parasites (adjusted mean = -1.024). The number of parasites infecting a fish also had a significant influence on the eviscerated weight of mosquitofish after accounting for the significant effect of fish length (ANCOVA: $F_{1,200} = 4.24$, $P = 0.041$). Populations with higher prevalence also had a higher average number of parasites per fish ($r_S = 0.88$, $n = 8$, $P = 0.004$).

Discussion

Latitudinal variation of mosquitofish life history

Mosquitofish seemed more abundant (CPUE) in southern populations, where they allocated more energy to reproduction (GSI and gonadal weight after accounting for fish size) and less to condition (size-adjusted total weight and eviscerated weight), than in northern populations. In general, temperature and photoperiod are the main factors controlling the

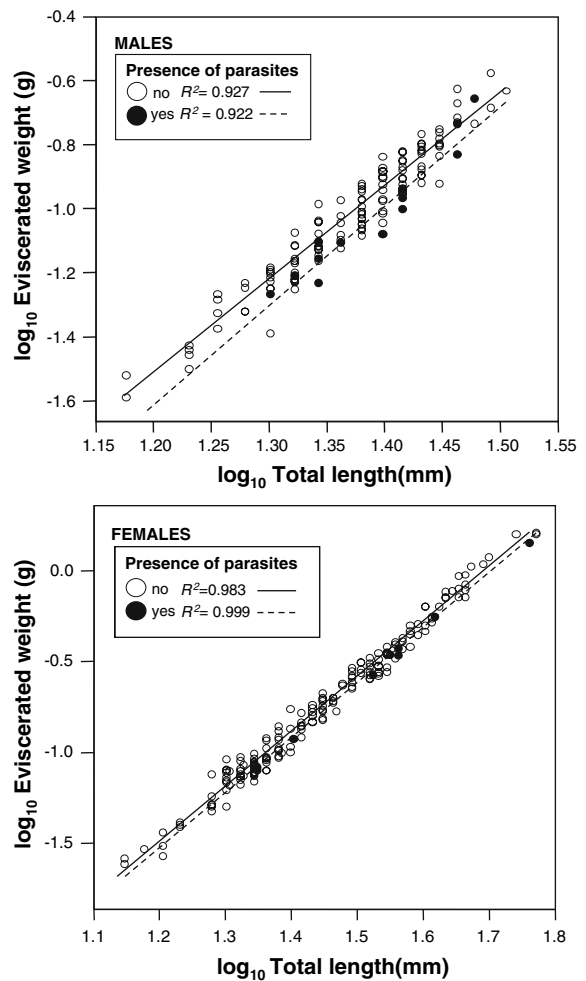


Fig. 7 Effect of parasite presence on the relationship of eviscerated weight with total length (both log-transformed) in male (*top*) and female (*bottom*) mosquitofish

reproduction of teleosts inhabiting the temperate zone (Haynes and Cashner 1995; Koya and Kamiya 2000). Koya and Kamiya (2000) experimentally demonstrated that the onset of reproduction in mosquitofish (*G. affinis*) is induced by a rise in temperature (vitellogenesis starts at ca. 14°C) and the end of reproduction is triggered by a shortening of day length (shorter than 12.5 h). In *G. affinis*, gonadal growth and GSI also increased with increasing temperatures (Vondracek et al. 1988; Koya and Kamiya 2000). The southern populations had better environmental conditions for reproduction and survival (high temperature and long photoperiod) and were thus more abundant. The negative effects of latitude on reproductive investment have also been

shown in several fish species. In perch (*Perca fluviatilis*), eelpout (*Zoarces viviparus*) and cod (*Gadus morhua*) reproductive investment decreased with increasing latitude (Pörtner et al. 2001; Heibo et al. 2005). The fecundity of Arctic char (*Salvelinus alpinus*) also declined with increasing latitude, possibly because of restricted growth opportunities (Power et al. 2005). Similarly to our findings, largemouth bass (*Micropterus salmoides*) at lower latitudes also invested more energy in growth in length and reproduction but less in fat reserves (Garvey and Marschall 2003).

As in *G. affinis* (Belk and Houston 2002), there was no significant relationship between mean size of *G. holbrooki* and latitude. Northern individuals, however, matured smaller on average (lower L_{50}). The size-at-maturity range (16.9–21.3 mm in males, 14.1–25.1 mm in females) was similar to previous studies (e.g. Fernández-Delgado and Rossomanno 1997; Stockwell and Weeks 1999). The smaller size-at-maturity at northern latitudes might be an adaptation to the shorter breeding and growing season. Vondracek et al. (1988) experimentally demonstrated that female *G. affinis* at higher temperatures reproduced at an earlier age but larger sizes, so that fecundity was higher. Stockwell and Weeks (1999) also observed increased size-at-maturity of introduced *G. affinis* with increasing temperature in thermal springs in Nevada. Guppies and many other fishes grow faster and mature at larger sizes but younger ages in more favourable environments (Reznick 1990). Age and size at maturity are pivotal in life history theory because fitness is generally more sensitive to changes in these traits than to changes in any other (Stearns 1992).

In contrast to size-at-maturity, offspring number and offspring size varied among populations but showed neither a clear pattern nor a trade-off with latitude or temperature. Trendall (1982) also reported the lack of a trade-off between fecundity and embryo weight in *G. holbrooki* (referred to as *G. affinis*) introduced to Australia. Similarly, Stockwell and Weeks (1999) demonstrated the rapid evolution in *G. affinis* of size-at-maturity and fat content but not offspring size.

Although temperature appears to be the most important factor causing latitudinal clines in life-history traits, particularly for freshwater organisms (L'Abée-Lund et al. 1989; Blanck and Lamouroux 2007), many factors covary with latitude and, in

addition to temperature, southern mosquitofish populations also have different photoperiods and possibly different resource availability (but probably similar predation pressure). Comprehensive field studies and experiments have demonstrated that Trinidadian guppies (*Poecilia reticulata*) from low predation sites have large age- and size-at-maturity, and produce smaller litters of larger offspring than guppies from high predation sites (Reznick and Endler 1982; Reznick et al. 1996a, b). However, other factors such as resource availability have been recently been shown to covary and interact with predation pressure (Reznick et al. 2001, 2002). The final mechanism of selection on guppy life-histories influenced by predation and resource availability is differential mortality rate (Reznick et al. 1996a). Given the negative relationship found between mosquitofish CPUE and latitude and how the latter strongly limits mosquitofish invasion of Europe, it is likely that mortality rates are also dependent on latitude and are driving the life history variation that we observed. Further studies, however, are needed to confirm these patterns and disentangle the underlying mechanisms.

Parasites

Parasites sometimes mediate the success of invasive species, either because introduced species bring alien parasite species with them, because they lose their native parasite species during introductions, i.e. the enemy release hypothesis, or through other less known mechanisms (Torchin et al. 2003; Prenter et al. 2004). Despite thorough dissection of 929 mosquitofish, we only found a single taxon (cestode larvae), although we were limited to identifying these parasites at the order level (Pseudophyllidea). Compared to previous studies, this is a poor parasite community with low parasite richness. For example, Dove (2000), confirming the enemy release hypothesis for *G. holbrooki*, found 11 parasite species of a variety of taxa in Australia and noted that 50 parasite species have been recorded from *G. holbrooki* in its native area (eastern USA). More data are needed to test the enemy release hypothesis in European mosquitofish but our preliminary results provide some credit to it. Concerning complex life-cycle parasites, the local absence of all the potential hosts for the cycle is usually the main reason given to explain the lack of parasites. Interestingly, we did not find generalist local parasite species

with complex life-cycles such as digenean metacercariae or native parasite species with direct life-cycles such as monogeneans. The absence of metacercariae of local digenean parasites may be due to several non-mutually exclusive reasons, whereas monogeneans may have not been introduced with the mosquitofish and, because of their usually strict specificity, new species have not been captured by *G. holbrooki*. Although prevalence was greater in summer, its seasonal variation was not statistically significant; however, we only studied two populations during three seasons. Studies on spatial and temporal variation of cestodes in fish have shown that growth and development in the fish hosts exhibit a seasonal pattern (Granath and Esch 1983; Nie and Kennedy 1992).

We found that the presence and number of parasites negatively influenced the eviscerated weight–length relationship for males but not for females (Fig. 7). Parasite prevalence in males of another well-studied poeciliid (*Poecilia reticulata*) has been shown to affect sexual selection by females (Kennedy et al. 1987). Although we did not find higher parasite prevalence in females as in Britton and Moser (1982), we found a significant increase of prevalence with fish size and female mosquitofish are much larger than males. In light of our results, we may hypothesize that parasite could have a selective pressure on males.

Overall, our study confirms the interest of latitudinal studies of invasive species (Huey et al. 2000; Gotelli and Arnett 2000), particularly given current scenarios of accelerated climate change. This knowledge is of enormous importance to understand the invasive potential of mosquitofish worldwide.

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