Dilution stress facilitates colonization of invasive mosquitofish in a saline Mediterranean stream: population biology response

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ABSTRACT

1. Knowledge of the interplay of abiotic factors and the invasive success of exotic fish is fundamental both to proper management and the ability to predict consequences for native communities.

2. The highly invasive eastern mosquitofish (*Gambusia holbrooki*) has been introduced worldwide; however, although salinity could limit its invasive success, the influence of variations in water salinity on its population biology has been scarcely studied.

3. The present study aimed to analyse the effect of changes in water salinity on the life history and microhabitat use of a mosquitofish population inhabiting a hypersaline stream subjected to human-caused dilution stress.

4. Dilution process and associated habitat changes, such as increased aquatic vegetation, available food and the spread of invasive riparian vegetation (the common reed *Phragmites australis*), seem to have favoured mosquitofish proliferation and colonization.

5. Dilution caused an increased somatic condition for all individuals and a higher reproductive investment of males and larger females. This led to greater survival, recruitment rates, and abundance during the period of lower salinity.

6. The species showed changes in microhabitat use which could be advantageous in less saline conditions; for instance, the species preferentially used areas with the presence of *P. australis* as a refuge, and this pattern increased during the dilution period.

7. These results support the hypothesis that human activity is one of the main forces shaping the colonization and proliferation of exotic freshwater fishes. At least in a Mediterranean context, an increase in mosquitofish abundance and the improvement of its population status could be clear indicators of the degradation of natural saline systems. Copyright © 2012 John Wiley & Sons, Ltd.

INTRODUCTION

The spread of non-native, invasive species is a long-recognized problem and one of the main global threats to biodiversity, particularly in inland aquatic ecosystems (Gherardi, 2007). The ability to analyse the life-history and ecological responses of invasive species within new invaded habitats must surely be considered as a key to the success of future conservation and management programmes (Kopp *et al.*, 2009).

The eastern mosquitofish *Gambusia holbrooki* Girard is a poeciliid fish that has been introduced into fresh and saline inland aquatic systems worldwide as a mosquito control agent. Together with the closely related western mosquitofish *G. affinis* (Baird and Girard), they are considered

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among the world's 100 worst invasive alien species by the Global Invasive Species Programme (Lowe *et al.*, 2000) and their negative ecological impacts are well known (Pyke, 2005, 2008).

Inland aquatic ecosystems are among the most vulnerable environments in the world and have been demonstrated to be especially sensitive to non-native species invasions (Sala *et al.*, 2000). It has been suggested that human-caused habitat degradation has facilitated the establishment and success of non-native species, and their combined and interacting influences have resulted in population decline and range reduction of freshwater biodiversity worldwide (Mack *et al.*, 2000). The inland aquatic habitats in the Mediterranean region, and especially in the Iberian Peninsula, are subjected to severe human disturbances mainly related to the intensive use of water resources and changes in land use within catchments as a consequence of the expansion of irrigated agriculture and the associated hydraulic infrastructures (Clavero *et al.*, 2005). These disturbances have accelerated the intentional and accidental spread of invasive species across their natural dispersal barriers (García-Berthou *et al.*, 2005), adversely affecting the composition, structure and functioning of their biological communities (Clavero *et al.*, 2010).

Several Mediterranean streams located in arid and semiarid areas are natural saline systems that have a high ecological and biogeographical value because of their restricted biological communities (Millán *et al.*, 2011). Despite their ecological and conservation interest, the reduction of salinity in these naturally saline streams (dilution stress), mainly due to freshwater drainage of intensively irrigated agriculture and freshwater inputs from diversion channel losses, has been an increasingly common phenomenon in recent decades (Gómez *et al.*, 2005). Dilution processes are probably among the main adverse impacts on the composition and structure of their biological communities and, moreover, dilution stress favours the spread of invasive species. For instance, the expansion of the common reed *Phragmites australis* (Cav.) Trin. ex Steud. and the associated replacement of halophytic plant communities are a general phenomenon that negatively affects most of these saline systems (Gómez *et al.*, 2005).

Although records of mosquitofish in highly saline waters are scarce, the existence of viable populations of this species in aquatic habitats with salinities over 30 has been registered in the Iberian Peninsula (Alcaraz and García-Berthou, 2007; Ruiz-Navarro *et al.*, 2011). However, and despite its importance in their management, the influence of variations in water salinity on its population biology has been scarcely studied (Alcaraz and García-Berthou, 2007; Ruiz-Navarro *et al.*, 2011), and no field studies have used a temporal approach to analyse whether salinity mediates the invasive success of mosquitofish.

The present research focuses on the Rambla Salada, a hypersaline stream (salinity > 40) located in the south-eastern Iberian Peninsula that is regularly subjected to dilution stress due to agriculturally derived freshwater inputs. The aim of this 2-year study was to analyse the effect of temporal changes in water salinity on several life-history traits and microhabitat use in a population of mosquitofish that invaded this hypersaline system more than 10 years ago. On the assumption that temporal changes in salinity cause environmental stress that affects the population biology of the species, the specific objectives were (i) to test whether life history differed among the periods established according to the temporal variations in water salinity, regarding relative abundance, fish condition, reproductive investment, recruitment and sex ratio; and (ii) to characterize microhabitat use and analyse possible salinity-related variations. It was hypothesized that mosquitofish benefits from dilution phenomena in hypersaline streams, which favour its colonization in these systems. The understanding of its biology in saline habitats has important management implications for control programmes of this plague species.

METHODS

Study area and sampling methods

The Rambla Salada is a hypersaline stream (i.e. mean salinity values from 47.5 to 76.4 during 2004) located in a semiarid ecological sector (Vidal-Abarca *et al.*, 1992) that belongs to the Segura River basin in the south-eastern Iberian Peninsula. Mean annual precipitation in the study area is about 200–300 mm, mainly concentrated in spring and autumn. Its 44.7 km^2 catchment is drained by a permanent 11.5 km-long main channel (third-order stream with intermittent flow at its head) and short ephemeral tributaries. More details about its environmental features are described in Velasco *et al.* (2006). The

established mosquitofish constitutes a monospecific fish assemblage in this system.

Since the 1980s, the Rambla Salada stream has experienced increased freshwater inputs owing to the expansion of intensively irrigated crops within the basin (Velasco et al., 2006; Millán et al., 2011). In addition, there have been several water losses over the years from a diversion–irrigation channel that crosses the stream. Breaks and maintenance operations on the channel have led to a fall in salinity values (dilution process) below 30 for relatively long periods (Velasco et al., 2006). During the present study, the diversion–irrigation channel added substantial freshwater inputs to the Rambla Salada continuously from autumn 2006 to spring 2007 and again in autumn 2007.

From February 2006 to May 2008, 61 samplings were carried out and, for each one, water temperature (°C) and salinity were registered by a multiparameter WTW-400® (Figure 1). Three salinity periods, with significant differences (analysis of variance, ANOVA: $F = 40.25$, $P < 0.001$), were

established according to the temporal variation in water salinity: a disturbance period (winter 2007 – autumn 2007), characterized by inputs of fresh water into the stream that reduced salinity to a minimum value of 9.7 in May 2007 and a mean water salinity of 19.1; a pre-disturbance period (winter 2006 – autumn 2006) and a recovery period (winter 2008 – spring 2008), both characterized by higher (mean values of 42.3 and 49.5, respectively) and more stable water salinity. Rainfall did not significantly contribute to temporal variations in water salinity (Pearson's correlation: $r = -0.32$, $P = 0.101$). Water temperature showed an annual variation pattern that made it possible to differentiate between the warm season (from late spring to autumn) and the cold season (from winter to early spring) (Figure 1). Water temperature did not show significant differences between salinity periods (ANOVA: $F = 0.62$, $P = 0.553$).

The sampling site was established in a representative stretch (150 m long) of the stream, where shallow pools (< 70 cm deep) and runs (< 20 cm deep, 1–2 m width) constitute the principal habitats. Pools are mainly composed of soft substrate covered by small patches of the filamentous algae Cladophora glomerata (Linnaeus) Kuetzing and the phanerogam Ruppia maritima Linnaeus. Runs present a mix of heterogeneous substrate with pebbles and boulders. As a consequence of the dilution process at the sampling site, riparian vegetation is mainly formed close to the banks by P. australis and several patches of Tamarix sp., although there are also small halophilic plants present (e.g. Sarcocornia fruticosa (Linnaeus) A.J. Scott, Arthrocnemum sp.).

Fish sampling consisted of setting 20 minnow-traps (30 mm diameter; 1 mm mesh size) twice per season, distributed only in pools, for roughly 24 h. Owing to the shallow depth in the majority of the sampling site, it was impossible to set minnow-traps at different depths. A sieving process of 20–30 min per sampling was performed with quadrangular hand nets (40 × 40 cm; 1 mm mesh size) to complete data on population structure. A hand net was also used in additional samplings in order to complete the samples necessary for the life-history analysis, carried out at least every 2 weeks during the reproductive period (April–October) and monthly for the rest of the year (a total of 41).

Fish were anaesthetized with benzocaine, fixed in neutralized formaldehyde (10%) and transported to the laboratory. All captured fish were sexed (male,
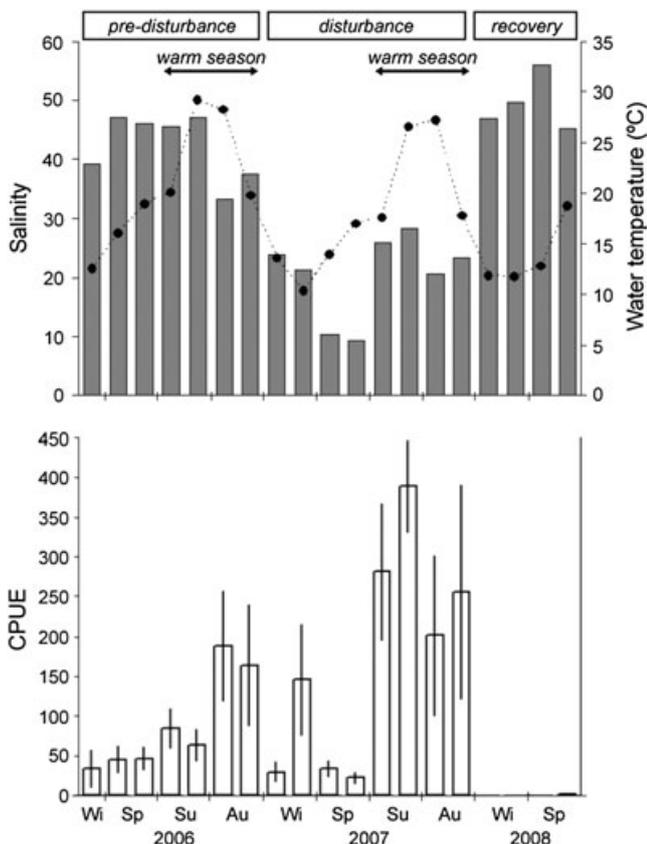

Figure 1. Water salinity (grey bars; mean values), temperature (closed circles; mean values) and relative abundance (CPUE; mean ± SE) of Gambusia holbrooki in the Rambla Salada stream from winter 2006 to spring 2008. Salinity periods (pre-disturbance, disturbance, and recovery) and warm seasons are indicated. Wi – winter; Sp – spring; Su – summer; Au – autumn.

female or juvenile) and measured for total length (TL; ±1 mm). A sample of 2648 individuals was weighed for total mass (TM; ±0.001 g), dissected, and their eviscerated mass (EM; ±0.001 g) and gonadal mass (GM; ±0.0001 g) were registered. The gonadosomatic index (GSI) was calculated as the gonadal mass as percentage of total mass. Age data from scales were obtained from Ruiz-Navarro *et al.* (2011).

For a sample of 1+ females (n = 171) captured between the end of April and May (first months of the reproductive period) and 0+ females (n =130) captured at the end of June and first days of July (first reproduction of the 0+ cohort), ovaries were processed and eggs were classified in six stages according to Reznick (1981). All of them were counted and, according to Fernández-Delgado and Rossomanno (1997), the number of eggs ready to be fertilized (stage I) determined potential fecundity and the number of fertilized eggs (stages II to VI) determined real fecundity. From 115 of these females, a sample of 20 eggs (all if n < 20) in the most abundant developmental stage of each female was dried and weighed to calculate mean dry oocyte or embryo mass (DM; ±0.0001 g).

Microhabitat measurements were recorded at trap level (20 samplings). Using the minnow-trap position as the centre of a 1-m-diameter circle (microhabitat unit), seven variables related to microhabitat structure and refuge presence were taken: depth (cm), distance to the nearest shore (cm), submerged vegetation cover (%), emergent macrophyte cover (%), substrate size, substrate heterogeneity and riparian vegetation shading. The assessment of submerged and emergent vegetation cover was made visually (two observers) and recorded as the percentage of the area covered by vegetation. Substrate types were categorized [1 = bedrock, 2 = mud (<0.06 mm), 3 = sand (0.06–1 mm), 4 = gravel (2–15 mm), 5 = pebble (16–63 mm) and 6 = boulder (>64 mm)] and estimated as substrate size (SS; average) and substrate heterogeneity (SH, standard deviation). Riparian vegetation shading was coded as an ordinate categorical variable from 1 (absence of canopy shelter) to 4 (complete canopy shelter).

Data analyses

The relative abundance of mosquitofish was expressed as catch per unit of effort (CPUE), defined as the number of fish captured in each minnow-trap per 24 h. Bivariate relationships between environmental variables (water salinity and temperature) and the species' relative abundance (Pearson's correlation coefficients) were analysed. A significance level of $P \leq 0.05$ was selected in this and all other analyses. Moreover, linear, quadratic, and cubic regressions were performed to describe the best fitted relationship between water salinity and abundance (ln-transformed data).

Variation in fish condition and reproductive investment between salinity periods (only pre-disturbance and disturbance periods because no significant captures were obtained in the recovery period) was studied with analyses of covariance (ANCOVA) using fish length as the covariate (ln-transformed data), except for the GSI, for which no covariate was used (i.e. an ANOVA was used). The adjusted or predicted means in ANCOVA, which are the means of values of the response variable adjusted for effects of the covariate (size-adjusted means), were used to describe variation between salinity periods, testing differences with ANOVA (Bonferroni *post hoc* tests). When the covariate was not significant ($P > 0.05$) in the ANCOVA it was removed from the model to increase statistical power and an ANOVA was used for the analysis. Condition and reproductive investment were studied separately in male and female mosquitofish (both with TL $\geq$ 20 mm) to account for significant sex-related effects. They were also studied independently for 0+ and 1+ individuals to avoid possible age-related differences in life-history traits (Tedesco *et al.*, 2008). Condition was analysed in cold and warm seasons (1300 males and 1246 females) and reproductive investment was only analysed during the first months of the reproductive period (end of April–May for 1+ individuals and end of June–beginning of July for 0+) (294 males and 301 females). Recruitment and sex ratio comparisons between salinity periods (pre-disturbance vs. disturbance) were achieved through Chi-square tests.

To analyse possible sex- and size-related variations in microhabitat use, mosquitofish were classified into three groups: juveniles (TL < 20 mm), males and females (both with TL $\geq$ 20 mm). The complex nature of microhabitat structure must be taken into account in selection studies, since many interacting variables can be simultaneously implied (Clavero *et al.*, 2009). This situation is best analysed through the use of multivariate statistics. In this study, a Principal Component Analysis (PCA) was applied to a matrix including both available and used

records (299 microhabitat units) of the microhabitat variables (ln- or arcsin-transformed data). The resulting microhabitat variation gradients (henceforth PCs) were selected for further analyses when the eigenvalues were greater than 1. The scores of these PCs were divided into three segments of equal amplitude, in each of which the number of available microhabitat units and the number of records of juvenile, male, and female mosquitofish was counted.

Selection of microhabitat variables was analysed by applying Ivlev's electivity index (D) with Jacobs' modification (Jacobs, 1974) to each of the three segments defined in the different PCs. This index ranges from -1 (total avoidance) to 1 (absolute positive selection) and was calculated as: $D = (r\text{-}p)/(r + p\text{-}2rp)$, where D is the electivity measure, r is the percentage use of the resource (i.e. proportion of mosquitofish individuals included in a PC segment), and p is the percentage of the resource in the environment (i.e. proportion of available microhabitat units included in a PC segment). D values between 0.25 and 0.5 were considered to be a moderate positive selection, and those higher than 0.5 were a strong positive selection (negative selection in the case of negative D values). For the analysis of microhabitat use based on mosquitofish abundance (ln-transformed data), ANOVA was applied to test for differences in relative abundance between the three segments established in the selected PCs.

RESULTS

Population abundance

The temporal pattern of CPUE estimates for mosquitofish showed a high degree of seasonal variation (Kruskal–Wallis, season as a factor: $H = 147.52$, $P < 0.001$) (Figure 1). Maximum CPUE means appeared during the recruitment phase of the population included in the salinity disturbance period ($CPUE_{Jul2007} = 282.0$; $CPUE_{Aug2007} = 389.3$). Minimum values ($CPUEs < 1$) with a significant and drastic drop of CPUE values were detected in samples included in the recovery period (winter and spring 2008) (Figure 1). Mean abundance of mosquitofish was significantly higher during the disturbance period ($CPUE = 169.9$) than in the pre-disturbance ($CPUE = 88.9$) and recovery periods ($CPUE = 0.6$) (Kruskal–Wallis: $H = 99.28$, $P < 0.001$) (Figure 1).

During the study period, a bivariate relationship was detected between water salinity and species abundance (Pearson's correlation: $r = -0.53$, $P = 0.021$), but not with water temperature ($r = 0.28$, $P = 0.247$). Furthermore, regression analyses revealed a significant parabolic relationship between mosquitofish abundance and water salinity as the best significant model. Due to the great effect of seasonality on the abundance patterns of the species, independent regression analyses were carried out for each of the established water temperature seasons: warm periods, in which more than 82% of the total fish captures were obtained, and cold periods. A better fit of abundance data for the parabolic model was obtained for the warm period (warm period adjusted $R^2 = 0.93$, ANOVA: $F_{(2,5)} = 45.24$, $P = 0.001$; cold period adjusted $R^2 = 0.37$, ANOVA: $F_{(2,8)} = 3.97$, $P = 0.063$). Quadratic regression models were $\ln(CPUE + 1) = -19.76 + 17.03 \ln(\text{salinity} + 1) - 2.97 [\ln(\text{salinity} + 1)]^2$ for cold seasons and $\ln(CPUE + 1) = -33.22 + 24.15 \ln(\text{salinity} + 1) - 3.74 [\ln(\text{salinity} + 1)]^2$ for warm seasons. The lowest abundance values were reached at salinities higher than 45.

Life-history traits

Fish condition and reproductive traits, except GSI and oocyte dry mass (stage I), depended significantly on total length (Table 1). After accounting for fish length interaction, fish condition in terms of TM and EM for any age classes in both sexes showed a significant increase during the disturbance salinity period (Table 1; Figure 2). For males and older females (1+ age-class), GM and GSI were significantly higher during the disturbance period. However, these two parameters were similar between periods in the case of 0+ females (Table 1; Figure 3). Fecundity was also affected by salinity; potential fecundity for all of the females and real fecundity for the 0+ females in their first reproductive event were lower in the disturbance period. On the other hand, real fecundity and oocyte and embryo dry mass increased in 1+ females during the reproductive season included in the disturbance period, with a marginal significance in the case of real fecundity and embryo dry mass (Table 1; Figure 3). Therefore, during the disturbance period the total mosquitofish population significantly increased reproductive investment in terms of higher gonadal mass (except for 0+ females), together with real fecundity, oocyte and embryo dry mass in the case of older females (1+).

Table 1. Results of ANCOVA analyses of the life-history traits in *Gambusia holbrooki*. Total length (TL) is the covariate, and salinity period (pre-disturbance or disturbance) is the fixed factor. Cold and warm seasons were separated and reproductive traits were analysed only in warm seasons. No covariate was used for GSI analysis

		Log TL (mm)			Among salinity periods		
		F	df	P	F	df	P
(a) Fish condition							
Cold season							
Males 0+	Total mass	632.53	1,363	<0.001	37.65	1,363	<0.001
	Eviscerated mass	1029.71	1,363	<0.001	5.60	1,363	0.018
Males 1+	Total mass	4049.09	1,332	<0.001	165.06	1,332	<0.001
	Eviscerated mass	4315.03	1,332	<0.001	82.19	1,332	<0.001
Females 0+	Total mass	3957.03	1,263	<0.001	33.61	1,263	<0.001
	Eviscerated mass	3872.97	1,263	<0.001	10.63	1,263	0.001
Females 1+	Total mass	12949.21	1,360	<0.001	55.91	1,360	<0.001
	Eviscerated mass	26621.74	1,360	<0.001	112.31	1,360	<0.001
Warm season							
Males 0+	Total mass	2835.36	1,542	<0.001	8.75	1,542	0.003
	Eviscerated mass	3343.50	1,543	<0.001	25.92	1,543	<0.001
Males 1+	Total mass	196.64	1,49	<0.001	55.86	1,49	<0.001
	Eviscerated mass	166.29	1,49	<0.001	41.45	1,49	<0.001
Females 0+	Total mass	13462.72	1,484	<0.001	18.86	1,484	<0.001
	Eviscerated mass	15907.00	1,484	<0.001	107.52	1,484	<0.001
Females 1+	Total mass	177.98	1,128	<0.001	5.54	1,128	0.020
	Eviscerated mass	482.20	1,127	<0.001	8.63	1,127	0.004
(b) Reproductive traits							
Males 0+	Gonadal mass	95.31	1,136	<0.001	31.05	1,136	<0.001
	GSI	-	-	-	5.47	1,137	0.021
Males 1+	Gonadal mass	43.73	1,151	<0.001	5.31	1,151	0.023
	GSI	-	-	-	8.52	1,153	0.004
Females 0+	Gonadal mass	465.04	1,126	<0.001	2.34	1,126	0.129
	GSI	-	-	-	1.71	1,128	0.193
	Potential fecundity	23.51	1,48	<0.001	15.40	1,48	<0.001
	Real fecundity	155.23	1,70	<0.001	5.06	1,70	0.028
Females 1+	Gonadal mass	102.69	1,167	<0.001	30.16	1,167	<0.001
	GSI	-	-	-	6.46	1,169	0.012
	Potential fecundity	137.90	1,103	<0.001	9.13	1,103	0.003
	Real fecundity	166.39	1,116	<0.001	3.07	1,116	0.082
	Oocyte dry mass	2.63	1,20	0.121	13.79	1,21	0.001
	Embryo dry mass	7.01	1,23	0.014	4.08	1,25	0.054

The relative frequency of juveniles (TL < 20 mm) between the end of May and June was lower for the pre-disturbance period (7.6%) than the disturbance period (15.9%) (Chi-square test: $\chi^2 = 68.88$, $P < 0.001$). Females were significantly dominant throughout the study period. The sex ratio varied significantly between the two salinity periods (Chi-square test: $\chi^2 = 25.57$, $P < 0.001$); the proportion of females was higher in the disturbance period (1.99:1) than the pre-disturbance period (1.67:1).

Microhabitat use

The PCA on the habitat characteristics of available microhabitat units produced two gradients (PC1 and PC2) that explained 49.0% of the original variation within the data set (Table 2). PC1 described a gradient running from microhabitat units with a rougher and more heterogeneous substrate to microhabitat units dominated by fine substrate (mainly mud). PC2 was positively associated with emergent macrophyte cover.

Mosquitofish showed size-specific microhabitat use along the gradient defined by PC1, although microhabitat use was also affected by season and salinity periods (Figures 4 and 5). During warm seasons, adults were equally present in all microhabitat segments regarding substrate size and heterogeneity in both pre-disturbance and disturbance salinity periods, although there was a marginal significance ($P = 0.096$ in males, $P = 0.086$ in females) indicating a higher abundance of adult fish in locations with a thinner substrate during the pre-disturbance period (Figure 5). During cold seasons, the negative selection of males and females of microhabitats with a rougher and more heterogeneous substrate was more significant during the disturbance period ($P < 0.001$ in Figure 5). In contrast, juveniles, generally captured during warm seasons (no significant abundance data), demonstrated a strong avoidance of areas with fine substrate and moderate or strong positive selection of areas with a more heterogeneous substrate (Figure 4). During the disturbance period, juveniles
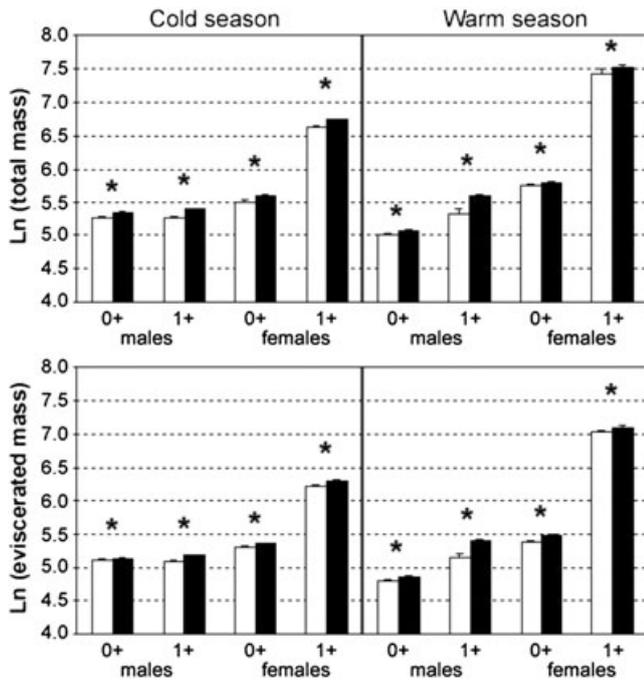
Figure 2. Comparison of condition with size-adjusted means (± 95% CI) between salinity periods (pre-disturbance in white bars; disturbance in black bars) for male and female *Gambusia holbrooki*. Cold and warm seasons were separated. Asterisks indicate significant differences ($P \leq 0.05$).

Figure 3. Comparison of reproductive traits with size-adjusted means (± 95% CI) between salinity periods (pre-disturbance in white bars; disturbance in black bars) for male and female *Gambusia holbrooki*. For the GSI and dry mass of oocytes, the observed means are given without adjusting for fish length. Asterisks indicate significant differences ($P \leq 0.05$).

chose water of a maximum substrate heterogeneity, in contrast to the pre-disturbance period.

There was also size-specific microhabitat use along the gradient defined by PC2, but no clear differences between sexes were detected (Figures 4 and 5). Microhabitat units with an absence of emergent macrophytes were markedly avoided by juveniles that positively selected intermediate locations in the gradient defined by PC2, in both pre-disturbance and disturbance periods (Figure 4). Adults did not choose any of the three PC2 segments during warm seasons in both the pre-disturbance and disturbance periods, and there was no significant difference in fish abundance. However, during cold seasons, they negatively selected microhabitats without emergent macrophytes, and this microhabitat use was more pronounced during the disturbance period ($P = 0.042$ in males, $P = 0.019$ in females; Figure 5).

DISCUSSION

The great adaptability of life-history traits of some invasive species to extreme habitats makes knowledge of the species biology in such habitats imperative for their management. Identification of the most relevant habitat characteristics necessary for the success of potential invaders in recipient 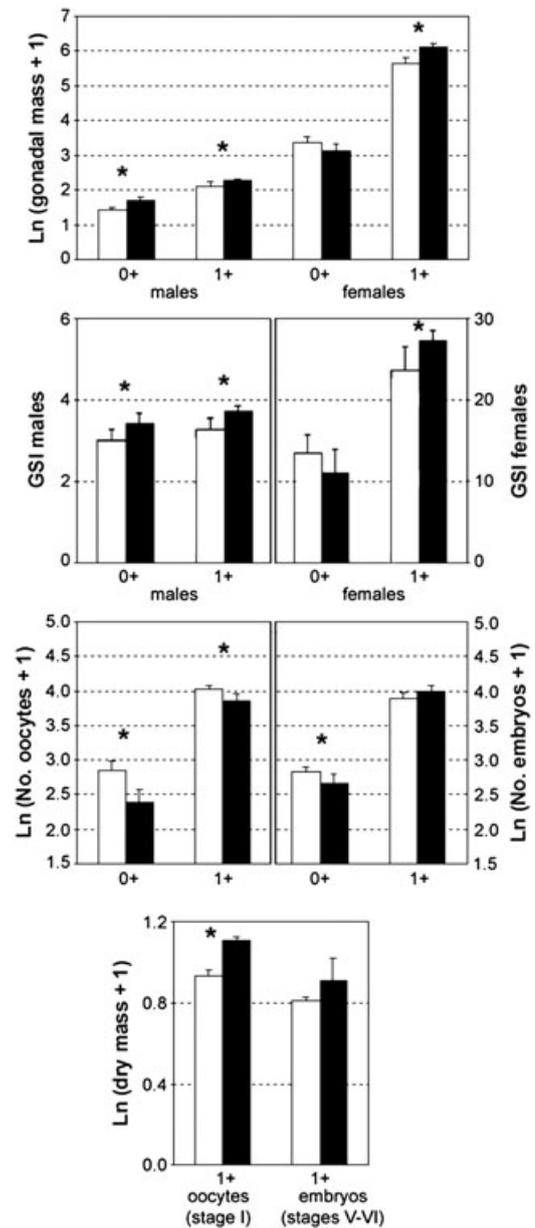systems is fundamental to understanding the invasive process (García-Berthou, 2007) and, thus, in designing effective control programmes (Britton *et al.*, 2010). Salinity is one of the most stressful factors for mosquitofish survival, since osmotic pressure strongly affects its metabolism (Nordlie and Mirandi, 1996; Alcaraz and García-Berthou, 2007). Previous studies have demonstrated that mosquitofish is better adapted to aquatic habitats with low salinity (2–10) than to fresh waters (0–0.5) or more saline waters (up to 38.7); survival rate and somatic and reproductive parameters indicate such

Table 2. Correlation coefficients between microhabitat variables and the two variation gradients (PC1 and PC2) with eigenvalues > 1 obtained from the PCA. Asterisks indicate significant correlations ($P \leq 0.05$)

Environmental variables	PC1	PC2
Depth	−0.57	−0.15
Distance to the nearest shore	−0.12	0.52
Submerged vegetation cover	−0.34	−0.14
Emergent macrophytes cover	0.13	0.77*
Substrate size	0.87*	−0.18
Substrate heterogeneity	0.83*	−0.23
Riparian vegetation shading	0.07	0.71
Eigenvalue	1.95	1.47
Explained variance	27.9%	21.1%
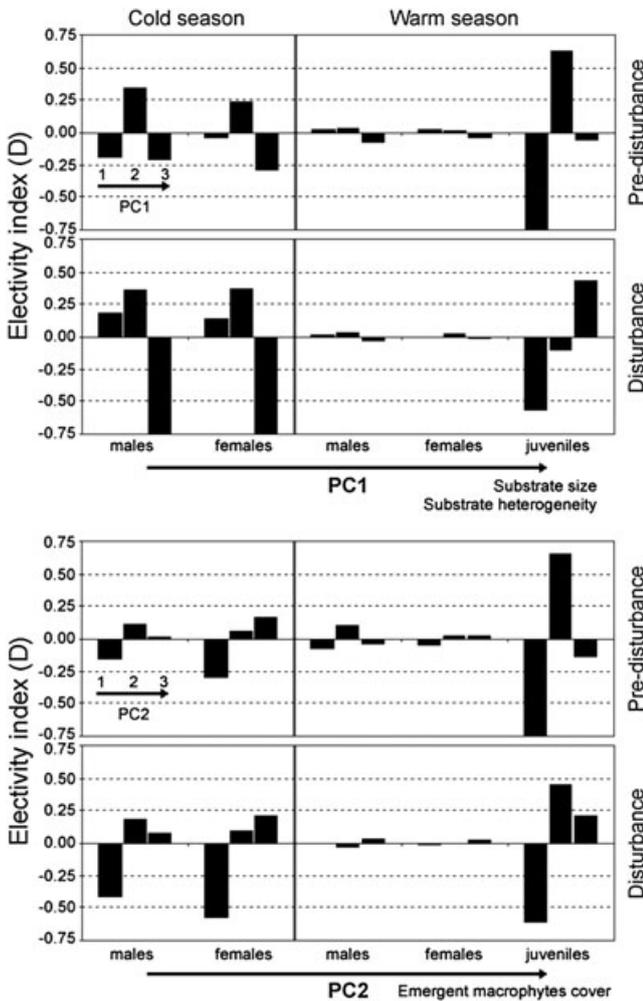
Figure 4. *Gambusia holbrooki* electivity (Ivlev's index, D) for each of the three segments (1, 2, 3) in which the gradients of variation of microhabitat features were divided (PC1 and PC2). Data are shown separately for males, females and juveniles captured in cold and warm seasons for each of the salinity periods (pre-disturbance and disturbance). The interpretation of PCs is based on the significantly correlated original variables.

Figure 5. Relative abundance of *Gambusia holbrooki* (CPUE ± SE) in each of the three segments (1, 2, 3) in which the gradients of variation of microhabitat features were divided (PC1 and PC2). Data are shown separately for males and females captured in cold and warm seasons for each of the salinity periods (pre-disturbance and disturbance). The interpretation of PCs is based on the significantly correlated original variables. Significance values (P) of the F-statistic of the one-way ANOVA tests used to compare abundance among segments are presented.

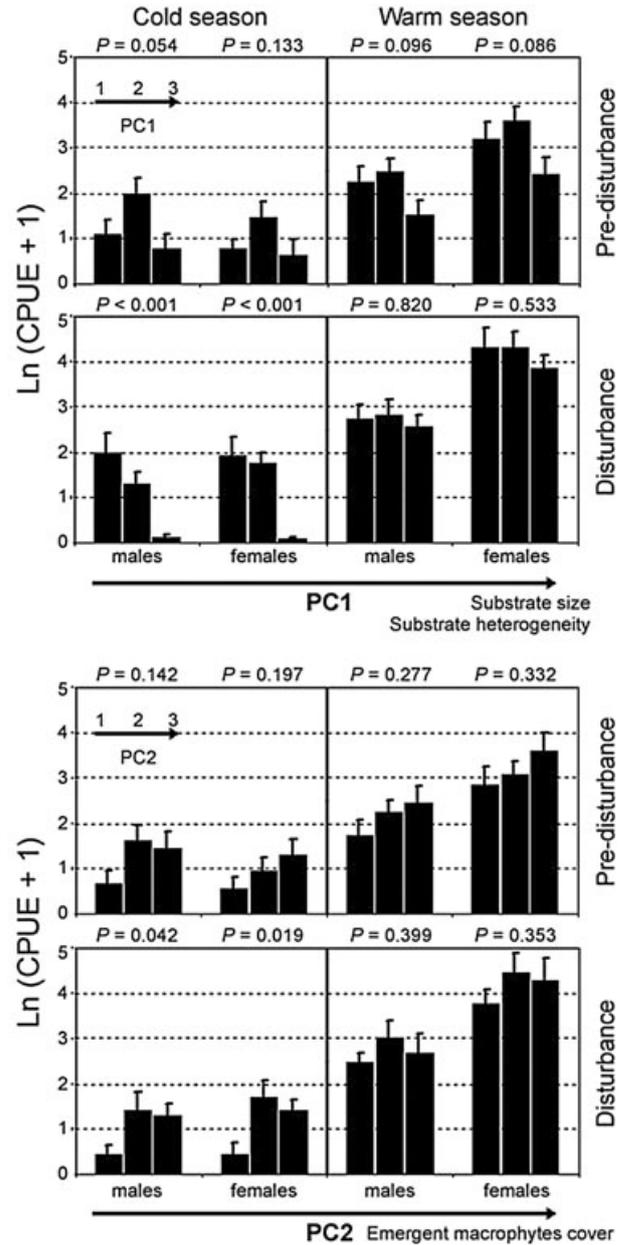adaptation (Brown-Peterson and Peterson, 1990; Alcaraz and García-Berthou, 2007), and salinity has been considered to limit the invasiveness and impact of mosquitofish (Nordlie and Mirandi, 1996; Alcaraz and García-Berthou, 2007). To delve into salinity limitation to mosquitofish invasion, this study provides new insights into the variation of life-history traits and microhabitat use of mosquitofish in a saline lotic system subjected to hydrological alteration and the subsequent dilution process.

The Rambla Salada system has suffered frequent dilution incidents (disturbance periods) as a consequence of human activities. Despite the fact that the population viability of mosquitofish was consistent during the pre-disturbance period (salinity range 33–47), reduced salinity during the disturbance (salinity range 10–28) resulted in a significant increase of its abundance and important changes in its life-history traits. During this period, the reduced salinity made it possible for all mosquitofish individuals to show higher condition and, with the exception of 0+ females, present higher gonadal mass and GSI. Although the number of mature oocytes was initially lower in 1+ females, their size and their subsequent viability were higher, so that these females contained more and larger viable embryos at the end of the development process. Owing to the fact that population fecundity from May to June in mosquitofish populations mostly relies on 1+ females (Fernández-Delgado and Rossomanno, 1997), population recruitment during these months in the Rambla Salada also increased during the disturbance period. However, recruitment rates also must have been affected by the negative effects of high water salinity on metabolism and survival of larvae and juveniles (Bohlen, 1999).

There was a significant relationship between water salinity and mosquitofish abundance, with maximum abundances during the disturbance period and a drastic fall during the recovery of salinity. This marked decline in population abundance was probably determined by rapidly increasing salinity, although it must have also been facilitated by the temporal dynamics of the species, with high winter mortality (Ruiz-Navarro et al., 2011). It has been stated that populations of mosquitofish subjected to high environmental stress produced by salinity are likely to adapt to gradual increases of that factor (Purcell et al., 2008). On the contrary, rapid salinity increases have also been related to higher mortalities (Chervinski, 1983). As the adverse effects of salinity are more conspicuous in mosquitofish females than males (Alcaraz and García-Berthou, 2007), the proportion of females in this study diminished in more saline conditions. Nevertheless, the greater proportion of females to males found during the entire study period could reflect a high reproductive investment of the population related to its establishment (Pyke, 2005), although this issue is currently slightly speculative and deserves further research.

In addition to the direct metabolic effects of water salinity, habitat changes produced by the dilution process and related mainly to food and refuge availability could have influenced the changes in life-history traits between pre-disturbance and disturbance periods. Velasco et al. (2006) and Gutiérrez-Cánovas et al. (2012) demonstrated that macroinvertebrate richness, diversity, and total biomass were higher during dilution periods than during natural hypersaline conditions in the Rambla Salada. This situation might enhance mosquitofish population viability through increased macroinvertebrate food availability.

Associated with the dilution process occurring in the Rambla Salada in recent decades, P. australis has colonized this stream and currently dominates the riparian plant community in some sectors (Millán et al., 2011). These results on microhabitat use suggest that mosquitofish benefited from the presence of P. australis, since it was used as a refuge by over-wintering individuals during cold seasons and by juveniles during warm seasons. Despite the fact that there were no significant differences, adult mosquitofish abundance during warm seasons was also greater in traps with higher refuge values. Since riparian vegetation in saline streams in the south-eastern Iberian Peninsula is usually scarce or absent (Gómez et al., 2005), the development and expansion of P. australis might offer suitable microhabitats for the establishment of mosquitofish, which may benefit from advantages provided by its stems, such as increased refuge and food availability (Cucherousset et al., 2008). In addition, the dilution process in the Rambla Salada has also been related to the proliferation of filamentous algae and R. maritima (Velasco et al., 2006), mainly in pools. Chapman and Warburton (2006) demonstrated that mosquitofish abundance was greater in pools with a high density of vegetation on the shore, and suggested that such habitat characteristics might facilitate the establishment of large populations. Submerged vegetation is also used by this species as a refuge (Pyke, 2008) and could be favouring its establishment in the Rambla Salada. Microhabitat use results also show that mosquitofish individuals, especially adults, are able to occupy all of the available microhabitat gradient in this system, which could partially reflect the high invasiveness of the species.

According to Kennard *et al.* (2005), the increases in the abundance of mosquitofish and the improvements in its population status could be evident indicators of the degradation of natural saline systems. Despite the time constraint of the study, these results provide evidence that habitat alteration due to anthropogenic factors aids the colonization of an exotic fish species. In this sense, the results are in accordance with the hypothesis that human activity is one of the main forces shaping the establishment and proliferation of exotic freshwater fishes in aquatic systems worldwide (Mack *et al.*, 2000) and especially in saline systems (Millán *et al.*, 2011). Similarly, these results suggest that the restoration of natural conditions restricts the proliferation of non-natives, in agreement with previous studies (Scoppettone *et al.*, 2005). Thus, the restoration of natural salinity levels should be used as a key tool in designing management programmes for mosquitofish in saline systems. This habitat management action, together with other actions such as the elimination of invasive vegetation (e.g. *P. australis*) or the physical extraction of mosquitofish individuals, can result in the success of the management programmes.

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