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# Life history variation of invasive mosquitofish (*Gambusia holbrooki*) along a salinity gradient

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## ABSTRACT

The mosquitofish (*Gambusia holbrooki*) is among the most invasive fish worldwide, with well documented ecological impacts on ecosystem functioning and several native taxa. Although it has been suggested that salinity limits the invasive success of mosquitofish, there are few studies on how salinity affects its ecology. We examined differences in life history traits of mosquitofish at 19 sampling sites from fresh (438  $\mu\text{S}/\text{cm}$ ; ca. 0.2‰ of salinity) to polysaline waters (35.1  $\text{mS}/\text{cm}$ ; ca. 23‰). Salinity affected the density and life history traits of both male and female mosquitofish; density showed a unimodal response to salinity, stronger for females and thus affecting the sex ratio. On the other hand, the endemic *Aphanius iberus* was only present in polysaline and eusaline waters, where mosquitofish were generally absent. Mosquitofish increased reproductive investment (higher gonadal mass) in oligosaline waters, at the cost of somatic condition in females. The increase in reproductive investment also implied an increase in total mass in contrast to somatic mass, demonstrating that the latter is a better indicator of condition. Females from higher salinity waters presented more embryos in advanced developmental stages, indicating earlier reproduction than in fresh water. After correction for brood and fish sizes, females from saltier environments presented heavier embryos (independently of maturation state). In summary, salinity mainly affected mosquitofish females which, despite presenting earlier maturation and higher reproductive investment, reduced their condition and density, hence supporting the hypothesis that salinity limits mosquitofish invasive success.

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## 1. Introduction

Biotic homogenization through the introduction of invasive species and the extinction of native species is now recognized as one of the main threats to biodiversity and ecosystem function, particularly in freshwater ecosystems (Mack et al., 2000; Holway and Suarez, 2006; Marchetti et al., 2006). Invasions by non-native species are of widespread concern because of their detrimental effects on ecosystem processes

and their economic consequences (Mack et al., 2000; Lake and Leishman, 2004), and human activities have recently accelerated the intentional and accidental spread of species across their natural dispersal barriers (Alcaraz et al., 2005; Gido et al., 2004). The mosquitofish (in fact two very closely related species: *Gambusia holbrooki* and *Gambusia affinis*) is only native to the United States and Mexico but has been introduced into more than 50 countries (García-Berthou et al., 2005) in order to control mosquito populations and

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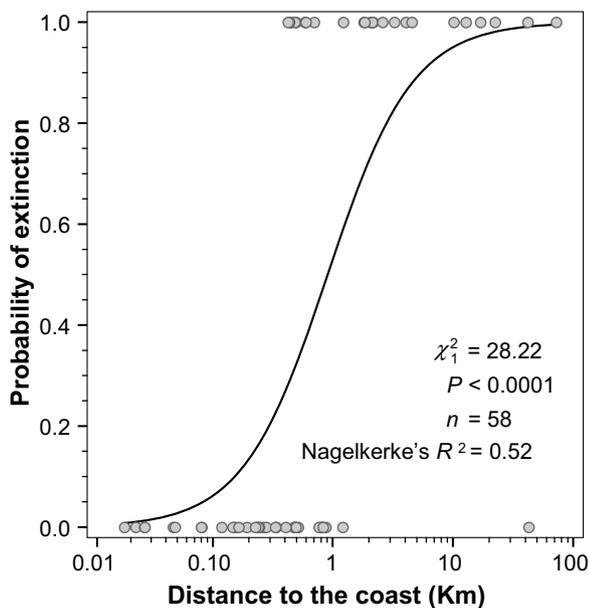
URL: <http://ciencias.udg.edu/w3/EGarcia/lab.html> (C. Alcaraz).

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hence malaria (Pyke, 2005). The mosquitofish is probably the most widely distributed freshwater fish in area occupied (Pyke, 2005) and considered to be one of the world's 100 worst invasive alien species by the GISP (Global Invasive Species Programme, <http://www.issg.org/database/>).

The strong ecological impact of introduced mosquitofish is quite well known. Mosquitofish profoundly alter ecosystem functioning through trophic cascades (Hurlbert et al., 1972) and several studies have demonstrated their effects on the decline of native amphibians (Kats and Ferrer, 2003) and small fish, such as the Mediterranean endemic cyprinodontid *Aphanius iberus* (Rincón et al., 2002). The Spanish toothcarp (*A. iberus*) originally inhabited a wide range of lowland waters but nowadays its distribution has been restricted to polysaline, eusaline and hypersaline waters (i.e. salinity > 18‰ according to Cowardin, 1979), mostly due to replacement by invasive *G. holbrooki* (García-Berthou and Moreno-Amich, 1992; Elvira, 1995) in fresh and oligosaline waters (i.e. salinity < 5‰). A review of the literature (Fig. 1) shows that most *A. iberus* extinctions have occurred far from the coast (> 1 km; i.e. mostly fresh waters) and that the probability of extinction significantly depends on distance to the coast (Wald's  $\chi^2 = 12.02$ ,  $P < 0.001$ ) and not latitude (Wald's  $\chi^2 = 0.03$ ,  $P = 0.96$ ). Some works have reported lower mosquitofish density with salinity and it is often suggested that salinity limits its invasive success (Elvira, 1995; Nordlie and Mirandi, 1996). However, there are few studies on the effects of salinity on mosquitofish biology, mostly on metabolism (Nordlie and Mirandi, 1996). Mosquitofish are able to cohabit in highly saline waters with other species that are displaced in fresh water (Al-Daham et al., 1977), but the ecological mechanism by which salinity limits the invasive success of mosquitofish is largely unknown. Although the life history plasticity of mosquitofish has been proposed as an important factor to understand its invasive



**Fig. 1** – Relationship of historical extinctions of *Aphanius iberus* with distance to the coast. The circles are known populations (0, extant; 1, extinct) and the line is the extinction probability fitted with logistic regression.

success (Haynes and Cashner, 1995), the only studies of the effects of salinity on mosquitofish life history traits are Stearns and Sage (1980) and Brown-Peterson and Peterson (1990), who studied short salinity gradients in North America (two populations from fresh (0‰) to mesosaline (10‰) and oligosaline (2.5‰) waters, respectively). The objective of our paper is to assess the variation of life history traits of invasive mosquitofish along a long salinity gradient (from 0‰ to 23‰) in northeastern Spain, in order to understand why salinity may limit its invasive success.

## 2. Material and methods

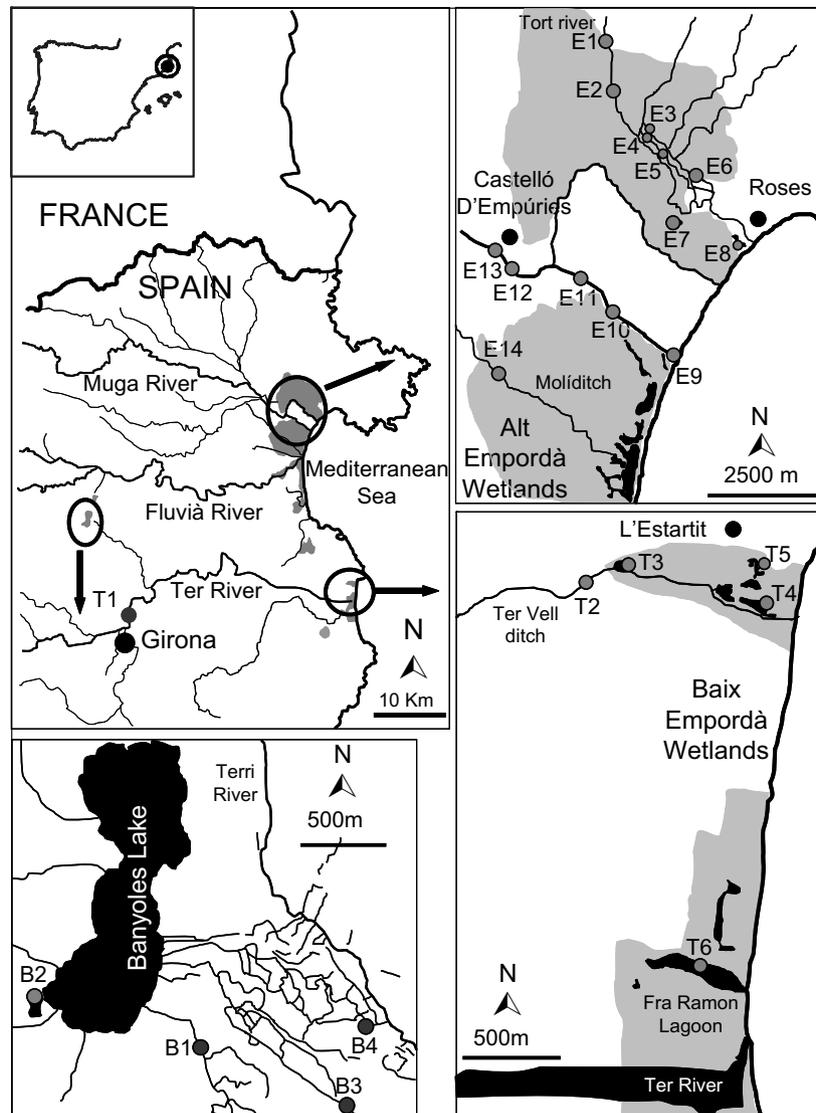
### 2.1. Study area and sampling methods

We studied the density and life history traits of mosquitofish at 24 sampling sites ranging from fresh water (438  $\mu\text{S}/\text{cm}$ ; ca. 0.2‰) to eusaline water (55,600  $\mu\text{S}/\text{cm}$ ; ca. 38.7‰) along three river basins in northeastern Spain (Fig. 2). The three river basins sampled were: the Ter River, with 208 km of length, 3010.5 km<sup>2</sup> of basin area, and 17.15 m<sup>3</sup>/s of average flow, from fresh water sites at 35 km from the coast (close to Banyoles Lake) to mixosaline and eusaline water sites at the Baix Empordà wetlands, close to the river mouth; the Muga River with a length of 58 km, 854 km<sup>2</sup> of area, and 3.34 m<sup>3</sup>/s of average flow; and the Tort River, a small stream in the Alt Empordà wetlands (Fig. 2).

Because the main objective was to assess the effect of salinity on life history traits, all samples were taken within a week, from 5 to 12 of May 2003, to minimize effects of seasonal variation and when the reproductive period of the mosquitofish that had survived the winter started but new born were still not present (Fernández-Delgado and Rossomanno, 1997). All the fish were captured during daylight hours using dip nets (60 cm diameter and a stretched mesh size of 1 mm) and preserved in situ in 10% formalin. The sampling time was measured in order to estimate fish abundance (catch per unit effort, CPUE, as the number of fish per minute). We measured in situ temperature and dissolved oxygen (with an Oxi 320 oximetre), electrical conductivity (EC<sub>25</sub>, with a Crison 524), and pH (Crison 507). An estimate of the water salinity concentration was obtained from conductivity with the conversion equations of APHA (2001). The distance to the coast was measured from maps as a perpendicular line from sampling sites to the sea.

### 2.2. Laboratory procedures

In the laboratory, all the fish were identified to species level, weighed (total mass to the nearest 0.1 mg) and measured (total and standard length (SL) to the nearest 0.01 mm with a digital caliper). All mosquitofish were sexed according to anal fin morphology (Turner, 1941); when this was not possible (usually fish with SL  $\leq$  11 mm), sex was determined by the direct observation of the gonads, and when it was not possible to discern the sex, the fish were classified as immature. For each sample, about 30 mosquitofish (15 mature males and 15 mature females, when available) were eviscerated, and their eviscerated and gonad masses were measured to the



**Fig. 2 – Location of the 24 sampling sites in the three study areas: the Alt Empordà wetlands (sites starting with E), Banyoles Lake drainage (B sites) and the Ter River basin that includes the Baix Empordà wetlands (T sites).**

nearest 0.1 mg. The gonadosomatic index (GSI) was calculated as the percentage of gonadal mass of total mass.

For females, ovaries were removed and processed under a dissecting microscope. *G. holbrooki* is a live-bearer that develops one clutch of eggs at a time, i.e. there is no superfetation (Reznick, 1981). Consequently, the ovary of mature females typically shows two kinds of oocytes: a group in development and another group of immature oocytes that may develop when the first ones have been released. Oocytes were classified in six categories according to Reznick (1981): I, no development (yolked but non-fertilized, development is not apparent, pre-vitellogenic ones are not considered); II, uneyed (development is already apparent); III, early-eyed; IV, mid-eyed; V, late-eyed; VI, very late-eyed. Stages II–VI were considered fertilized eggs (Fernández-Delgado and Rossomanno, 1997). All the oocytes present in the ovaries were classified, counted and weighed (to the nearest 0.1 mg) by stage group, after elimination of intra-ovarian tissue. Malformed embryos were detected in a very low percentage of females

(<1%) and they represented a low percentage (<1%) of the total number of embryos. Following Fernández-Delgado and Rossomanno (1997), each group of oocytes (non-fertilized and fertilized) was used to calculate two fecundity measures: potential fecundity (including all the oocytes) and brood size (including only fertilized oocytes).

### 2.3. Statistical analyses

To further describe the relationship of mosquitofish abundance and one of the species most affected by it (the endemic cyprinodont *A. iberus*), we also fitted generalized additive models (GAMs) (Lepš and Šmilauer, 2003), as available in the CANOCO program, to fit the response of CPUE of both species to water conductivity. GAMs are an extension to generalized linear models that, unlike more conventional regression methods, do not require the assumption of a particular shape for the species distribution along the environmental gradient (Lepš and Šmilauer, 2003). The model complexity of GAMs

was selected by the stepwise selection procedure using the Akaike information criterion (AIC), as available in CANOCO 4.5. AIC considers not only the goodness of fit but also parsimony, penalizing very complex models (Burnham and Anderson, 1998).

Spearman's correlations coefficient ( $r_s$ ) was used to test differences in mean length with water conductivity. Variation of life history traits among populations were analyzed with analysis of covariance (ANCOVA) using fish length as the covariate, except for the gonadosomatic index (GSI), for which no covariate was used (i.e. an ANOVA was used). For mean number and mass of oocytes per developmental stage, an ANCOVA with fish length and brood size was used. When the covariate was not significant ( $P > 0.10$ ), it was deleted from the models (i.e. an ANOVA was used), to increase statistical power (García-Berthou and Moreno-Amich, 1993). The significant variation among populations was decomposed in a linear relationship with water conductivity and a residual component with polynomial orthogonal contrasts (Sokal and Rohlf, 1995) and the SPSS "metric" option (SPSS Inc., 2004). By default, polynomial contrasts assume equally-spaced levels, whereas with the metric option unequal spacing for the factor levels (real distances between log-transformed water conductivities) may be specified (SPSS Inc., 2004). Estimated marginal (or adjusted) means of a dependent variable are the means for each level of the factor, adjusted for covariates (see e.g., García-Berthou and Moreno-Amich, 1993) and were used to describe the differences among populations.

The sex group composition was compared among populations with a G-test of independence (Sokal and Rohlf, 1995),

followed with Spearman's correlation coefficient to test the relationship with water conductivity. Quantitative variables were log-transformed for parametric analyses because homoscedasticity and linearity were clearly improved. All statistical analyses were performed with SPSS 13.0.

### 3. Results

#### 3.1. Effects of water conductivity vs. other variables on life-history variation

Many environmental variables generally tend to covary in space and time. Sampling sites where mosquitofish were potentially present were selected to display a strong gradient of salinity, in part but not exclusively corresponding to river zonation or distance to the coast (e.g. sites T2 to T5 were within 1 km of distance and had similar depths and hence temperature regimes but differed more markedly in salinity than other sites, see Table 1). As expected, water conductivity of the sampling sites where mosquitofish were captured was significantly but not strongly related to distance to the coast ( $r_s = 0.55$ ,  $n = 19$ ,  $P = 0.015$ ), and not significantly related to temperature (Spearman's  $r_s = -0.42$ ,  $n = 19$ ,  $P = 0.08$ ) or pH ( $r_s = 0.14$ ,  $n = 19$ ,  $P = 0.57$ ). All the life history variables were more related to water conductivity than to the other measured physico-chemical variables. For instance, the adjusted means of gonadal mass were more related to water conductivity ( $r_s = 0.56$  for males and 0.47 for females) than to distance to the coast ( $r_s = -0.50$  and  $-0.09$ , respectively), pH ( $r_s = 0.40$  and 0.44), or temperature ( $r_s = 0.45$  and 0.36). Similar

**Table 1 – Features of the 24 sites sampled in May 2003**

Site	Latitude	Longitude	Gambusia CPUE	Sex proportion		Total fish CPUE	Aphanius CPUE	Water conductivity ( $\mu\text{S}/\text{cm}$ )	Salinity (‰)
				♂	♀				
E1	42°17'57"	3°6'15"	0.35	25.0	65.0	0.39	0	443	0.25
E2	42°17'23"	3°6'48"	0.16	11.1	88.9	0.22	0	438	0.24
E3	42°16'54"	3°7'6"	3.05	19.7	77.0	4.10	0	1127	0.62
E4	42°16'49"	3°7'4"	0.77	43.5	39.1	0.80	0	740	0.40
E5	42°16'34"	3°7'28"	0.92	47.8	43.5	1.20	0	698	0.36
E6	42°16'25"	3°7'58"	0			25.80	0	24200	17.45
E7	42°16'4"	3°7'37"	0			2.62	0.62	27400	17.54
E8	42°15'34"	3°8'50"	0			0.57	0.43	55600	38.68
E9	42°14'20"	3°7'30"	2.60	19.2	50.0	14.10	0	6350	3.57
E10	42°14'53"	3°6'23"	0.38	33.3	60.0	2.56	0	1546	0.78
E11	42°15'9"	3°5'57"	0.52	23.5	67.6	1.40	0	693	0.35
E12	42°15'22"	3°4'17"	0			0	0	718	0.38
E13	42°15'30"	3°4'9"	0.02	100.0	0.0	1.60	0	718	0.38
E14	42°14'3"	3°4'15"	0.53	24.1	17.2	0.75	0	888	0.61
B1	42°7'10"	2°44'55"	2.30	46.1	50.0	2.30	0	1332	0.74
B2	42°6'59"	2°45'38"	12.00	69.8	29.2	12.00	0	1330	0.72
B3	42°6'57"	2°46'38"	8.25	27.3	72.7	8.25	0	1326	0.74
B4	42°7'6"	2°46'40"	1.23	33.3	52.4	1.23	0	1406	0.78
T1	41°59'36"	2°49'24"	1.03	38.7	61.3	1.17	0	576	0.34
T2	42°2'49"	3°11'12"	23.00	60.0	35.7	23.00	0	1094	0.56
T3	42°2'55"	3°11'18"	2.84	56.8	42.0	2.84	0	1599	0.89
T4	42°2'46"	3°11'49"	15.20	72.4	27.6	15.20	0	35100	23.25
T5	42°2'55"	3°11'48"	8.67	59.6	40.4	8.67	0	10890	6.40
T6	42°1'50"	3°10'55"	0			2.21	2.21	32400	20.90

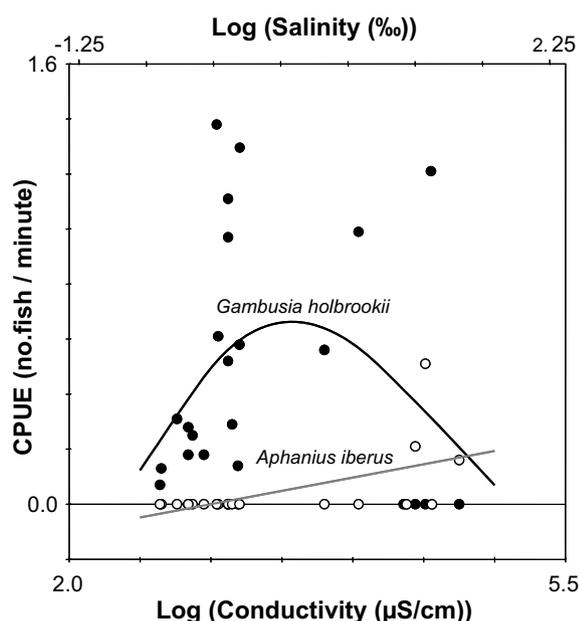
All values refer to the sampling date. See Fig. 1 for location of the sampling sites. CPUEs are number of fish captured per minute. ♂ is the percentage of male mosquitofish and ♀ the percentage of females (the rest is percentage of immature).

analyses (e.g. effect sizes of linear models) and other variables also showed that water conductivity had a stronger relationship with life-history traits than the rest of variables in the populations studied. Also, the elimination of the high salinity collection did not alter the results. Although life-history traits might depend on many environmental variables, we chose to analyse the relationship with water conductivity because its demonstrated stronger effect. Collinearity with water conductivity and the modest variation range of other environmental variables precludes the analysis of other environmental variables in this study.

### 3.2. Mosquitofish population structure

A total of 921 mosquitofish were captured; at only five of the 24 sites, mostly those with highest salinities, mosquitofish were not captured. The response curves (GAMs) of mosquitofish and toothcarp abundances (Fig. 3) showed a clear relationship with water conductivity. AIC selected a unimodal response for mosquitofish (non-linear  $F_{1,19} = 2.34$ ,  $P = 0.045$ ), its abundance peaking at intermediate water conductivity (ca. 3500  $\mu\text{S}/\text{cm}$ ), while toothcarp showed a linear abundance–conductivity relationship (linear  $F_{1,20} = 9.63$ ,  $P = 0.006$ ), being mostly present in polysaline and eusaline waters (i.e. salinity  $> 18\%$ ), where the mosquitofish was generally not present (Fig. 3).

The mosquitofish standard length was not correlated with water conductivity for females ( $r_s = 0.31$ ,  $n = 15$ ,  $P = 0.25$ ; mean  $SL = 21.9$  mm,  $SE = 0.1$ ), males ( $r_s = -0.30$ ,  $n = 15$ ,  $P = 0.27$ ; mean  $SL = 27.8$  mm,  $SE = 0.2$ ) or immatures ( $r_s = -0.10$ ,  $n = 15$ ,  $P = 0.78$ ). Mosquitofish abundance (CPUE) showed a parabolic relationship with water conductivity (see also Fig. 3), the quadratic component being significant ( $P \ll 0.05$ )



**Fig. 3** – Response of *Gambusia holbrooki* (●; deviance = 3.14, model  $df = 19$ ) and *Aphanis iberus* (○; deviance 0.20, model  $df = 20$ ) abundance (CPUE) with water conductivity. Lines are the generalized additive models selected by the Akaike information criterion.

for males (polynomial regression, adjusted  $r^2 = 0.16$ ), females (adjusted  $r^2 = 0.25$ ) and immatures (adjusted  $r^2 = 0.21$ ). Density reduction in higher salinity was more marked for females than for males and thus the sex ratio varied significantly among populations ( $G = 77.8$ ,  $df = 14$ ,  $P < 0.0001$ ) and the female proportion decreased with water conductivity (Spearman's  $r_s = -0.58$ ,  $n = 15$ ,  $P = 0.022$ ) (Table 1). Although the proportion of immature fish differed among populations ( $G = 76.7$ ,  $df = 14$ ,  $P < 0.0001$ ), these differences were not related to water conductivity ( $r_s = -0.14$ ,  $n = 15$ ,  $P = 0.62$ ).

### 3.3. Mosquitofish life history traits along the salinity gradient

All reproductive variables depended significantly on standard length (Table 2). After accounting for that, all reproductive variables for both sexes significantly differed among populations and for most of them the linear component with water conductivity was highly significant (Table 2). Only for total and eviscerated masses of males, potential fecundity and mean embryo mass the linear contrast was not significant; however, for these cases the deviation contrast was significant and in several cases a curvilinear relationship was observed.

For males, gonadal mass and the gonadosomatic index showed a significant increasing relationship with water conductivity (Table 2, Fig. 4); so, mosquitofish inhabiting fresh water showed lower gonadal mass (after accounting for fish length) and GSI values than mosquitofish populations of higher salinities (Fig. 4). For females, water conductivity had a positive linear effect on total mass, gonadal mass and GSI, but a negative one on eviscerated mass (Table 2, Fig. 5). Potential fecundity (total number of oocytes without pre-vitellogenic ones) was not related to water conductivity, but brood size (number of embryos at stages II to VI) significantly decreased with lower water conductivity (Table 2, Fig. 5, quadratic contrast  $P = 0.016$ ). On the other hand, mean oocyte mass was positively affected by water conductivity but mean embryo mass was not (Table 2, Fig. 5). Therefore, female mosquitofish from fresh waters significantly decreased reproductive investment, as indicated by lower gonadal mass (adjusted for length), GSI and total mass, but had better somatic condition (eviscerated mass adjusted for length) (Fig. 5). Potential fecundity did not vary with water conductivity but freshwater females significantly presented fewer embryos (brood size) and mean oocyte mass was lower than in higher salinity populations.

### 3.4. Oocyte developmental traits

The detailed analysis of oocyte developmental traits (ANCOVA with standard length) showed that the mean oocyte mass averaged for each developmental stage (from I–VI) was not related to water conductivity (linear and quadratic contrasts,  $P > 0.18$ ). Standard length was only significant for stage I oocytes ( $F_{1,172} = 49.81$ ,  $P < 0.0001$ ) because no relationship was observed for the embryos (i.e. stages II–VI) ( $P > 0.15$ ). However, oocyte mass averaged for each developmental stage was significantly correlated with brood size for all developmental stages (ANCOVA,  $P < 0.001$ ). After partialling out the effect of brood size, the same pattern (no variation

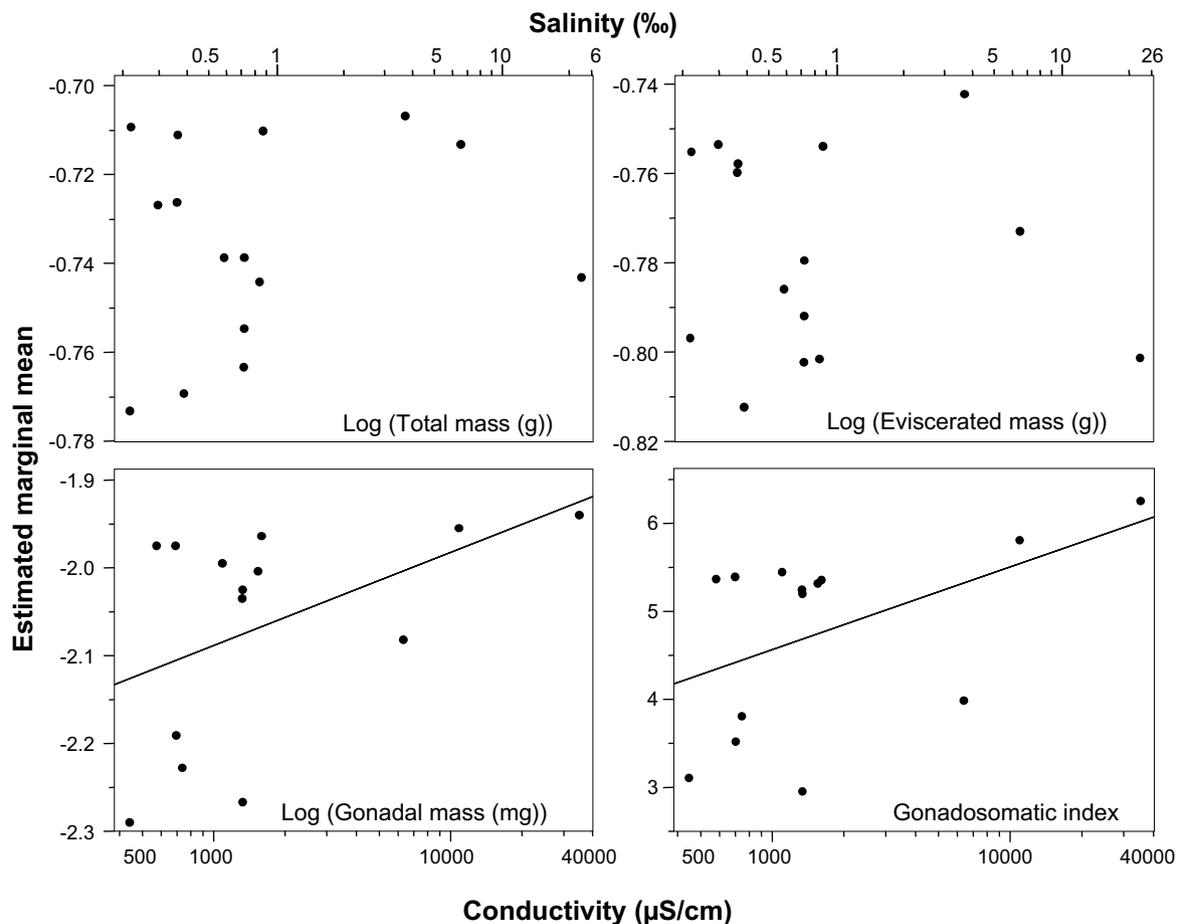
**Table 2 – ANCOVAs of the life history traits of mosquitofish with conductivity (factor) and standard length (covariate)**

	Explained variation (adjusted R <sup>2</sup> )	Among populations		Log conductivity ( $\mu\text{S}/\text{cm}$ )		Log standard length (mm)	
		F	df	Linear contrast	Deviation	F	df
				P	P		
<b>Males</b>							
Total mass	0.914	6.09 <sup>a</sup>	14, 380	0.237	a	3465.1 <sup>a</sup>	1, 380
Eviscerated mass	0.918	4.40 <sup>a</sup>	14, 158	0.548	a	1658.6 <sup>a</sup>	1, 158
Gonadal mass	0.611	9.26 <sup>a</sup>	13, 159	a	a	113.8 <sup>a</sup>	1, 159
GSI	0.450	11.75 <sup>a</sup>	13, 158	a	a		
<b>Females</b>							
Total mass	0.958	7.34 <sup>a</sup>	14, 341	0.002	a	5958.2 <sup>a</sup>	1, 341
Eviscerated mass	0.969	9.11 <sup>a</sup>	14, 201	0.002	a	5243.9 <sup>a</sup>	1, 201
Gonadal mass	0.799	3.33 <sup>a</sup>	14, 201	b	b	553.2 <sup>a</sup>	1, 201
GSI	0.268	6.65 <sup>a</sup>	14, 202	a	a		
Potential fecundity	0.546	6.37 <sup>a</sup>	14, 201	0.159	a	130.1 <sup>a</sup>	1, 201
Brood size	0.619	3.41 <sup>a</sup>	14, 201	b	b	189.9 <sup>a</sup>	1, 201
Mean oocyte mass	0.603	4.11 <sup>a</sup>	14, 200	0.010	a	189.5 <sup>a</sup>	1, 200
Mean embryo mass	0.596	11.41 <sup>a</sup>	13, 99	0.377	a	28.8 <sup>a</sup>	1, 99

The variation among populations was decomposed into a linear component and a residual term (deviation) with polynomial contrasts. All variables were  $\log_{10}$  transformed except GSI. No covariate was used for the analysis of GSI (i.e. ANOVA was used).

a  $P < 0.0001$ .

b  $P = 0.001$ .



**Fig. 4 – Relationship of conductivity with size-adjusted means (ANCOVAs of Table 2) of life history traits for male mosquitofish. The adjusted means are the population means after adjusting for fish length. For the gonadosomatic index the observed means without adjusting for length are given. Significant ( $P < 0.05$ ) linear or quadratic components are also shown.**

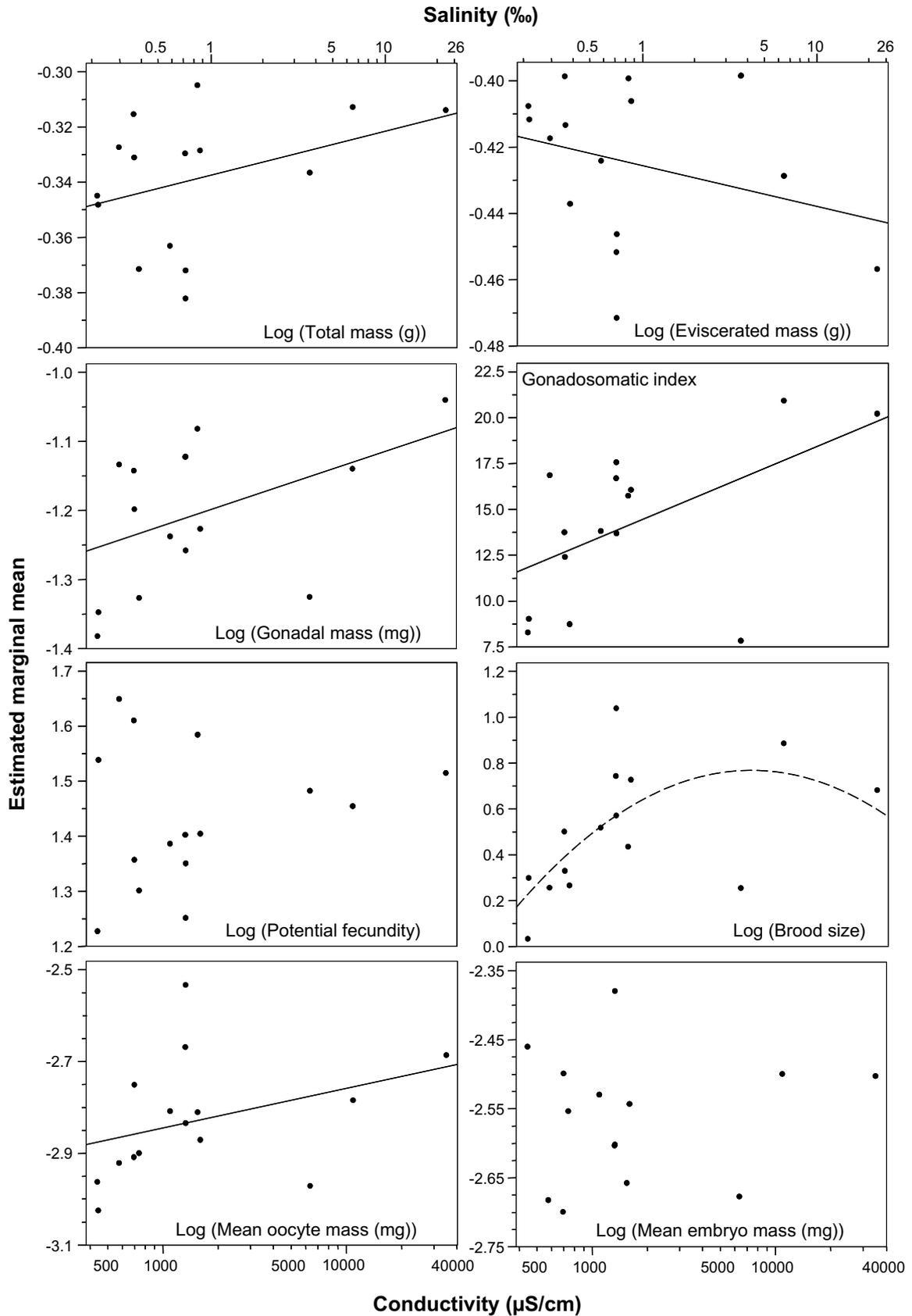
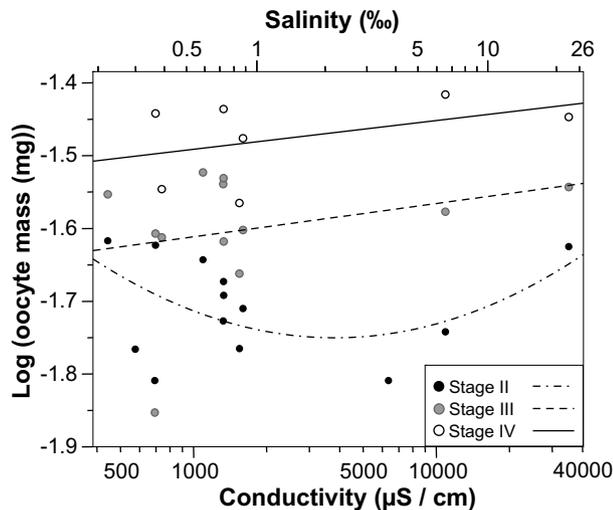


Fig. 5 – Relationship of conductivity with size-adjusted estimated means (ANCOVAs of Table 2) of life history traits for female mosquitofish. The adjusted means are the population means after adjusting for fish length. See Fig. 4 for statistics shown.



**Fig. 6** – Significant relationships of oocyte mass (size-adjusted means) per developmental stage with water conductivity.

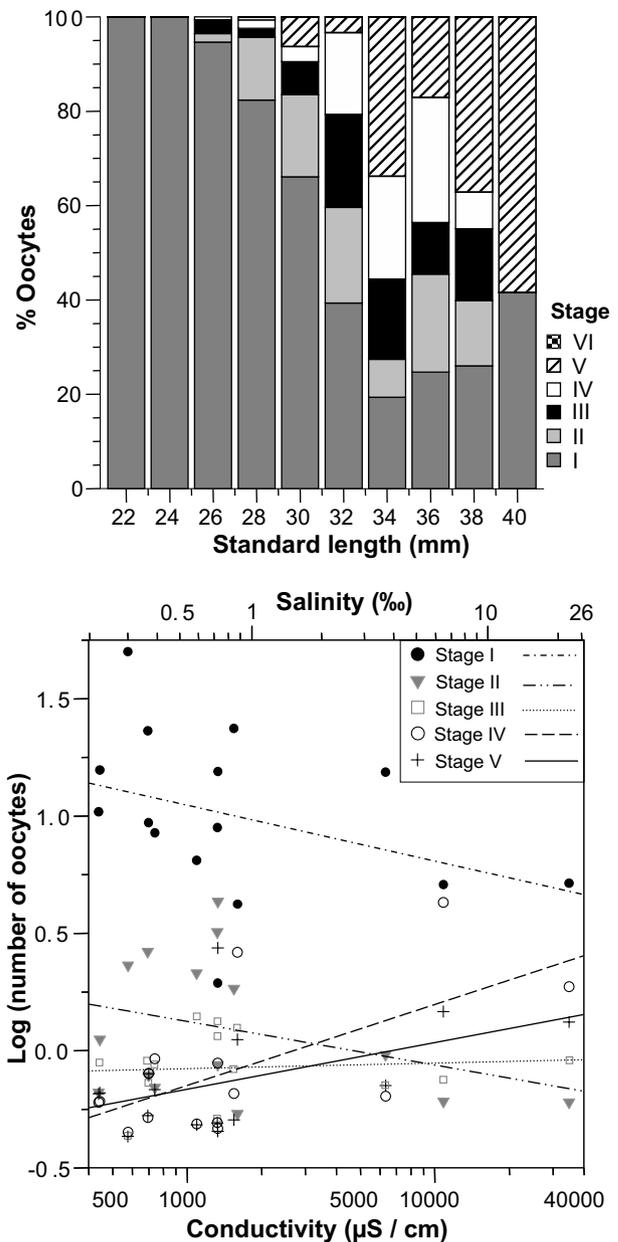
with water conductivity) was observed except that the mass of stage II oocytes (linear and quadratic contrasts:  $P = 0.93$  and  $P = 0.033$ , respectively) and stage III and IV oocytes (linear and quadratic contrasts:  $P < 0.05$  and  $P > 0.68$ , respectively) varied with water conductivity (Fig. 6), showing a mass gain, independent of female size, with water conductivity.

The number of oocytes per developmental stage (from I–V) significantly depended on female standard length (ANCOVA,  $P < 0.0001$  for all the stages). The number of stage I oocytes significantly decreased in larger females, whereas the number of the other stages significantly increased with female standard length (Fig. 7). After accounting for female size, all stages (except stage III) showed a significant linear relationship with water conductivity (linear and quadratic contrasts:  $P \ll 0.05$  and  $P \gg 0.05$ , respectively, for all the stages). The number of oocytes of stages I and II were negatively related to water conductivity (linear contrasts:  $P = 0.001$  and  $P = 0.016$ , respectively), while stages IV and V presented a significant positive relationship with water conductivity (linear contrasts:  $P < 0.0001$  and  $P = 0.001$ , respectively); stage III oocytes did not depend on water conductivity (linear contrast,  $P = 0.73$ ) (Fig. 7). Therefore, oocyte mass did not depend on standard length (except stage I), but females inhabiting saltier waters presented heavier embryos (stages II–IV, after correcting for brood size), less oocytes (after correcting for standard length) in initial developmental stages (I and II) and more embryos in the latest developmental stages (IV and V).

## 4. Discussion

### 4.1. Individual effects of salinity

Water conductivity had highly significant effects on the life history traits of both male and female mosquitofish at the start of the reproductive season, with both sexes increasing reproductive investment in higher salinity, at the cost of somatic condition (eviscerated mass adjusted for standard length) in females. The increase of female reproductive



**Fig. 7** – Top: variation of oocyte stage composition with standard length for female mosquitofish. Bottom: relationship of oocyte number (size-adjusted means) per developmental stage with water conductivity.

investment also implied a gain in total mass, in contrast to somatic mass, indicating that the latter is a better indicator of condition. Females from higher salinity waters produced more embryos (brood size) and had larger oocytes than those in freshwater, but did not differ in total brood size (potential fecundity) and total embryo mass. These differences could be explained by the detailed analysis of oocyte stages. After accounting for fish length, females from saltier waters had significantly less oocytes in initial developmental stages (I and II) and more in advanced stages (IV and V), showing that the increase in reproductive investment is partly due to an earlier start of reproduction. However, in addition to earlier reproduction, females from saltier environments also pre-

sented heavier embryos (after correction for brood size and mosquitofish length).

Although it has often been thought that salinity may limit the invasive success of the mosquitofish, there are few studies on how salinity affects its ecology. Our results are similar to those reported by Stearns and Sage (1980) and Brown-Peterson and Peterson (1990), the only similar previous studies. Although they studied a shorter salinity range, from fresh (0‰) to mesosaline (10‰; ca. 17 mS/cm) and oligosaline waters (2.5‰; ca. 4.7 mS/cm), respectively, they also found that at the start of reproductive season, females from brackish waters increased reproductive effort and had larger GSI and brood sizes (similar to our brood size due to different oocyte classification), but produced smaller embryos. The contrasting results on embryo size might be due to a variety of reasons, including a different classification of developmental stages or a longer salinity range. Brown-Peterson and Peterson (1990) also reported that females from fresh water were in better somatic condition and matured later, since ovarian development was significantly more advanced in oligosaline waters. Similar effects of salinity on life history traits have been observed in other fish species. For instance, both males and females of the poeciliid *Poecilia latipinna* grew faster and matured earlier in relatively salty water (Trexler and Travis, 1990), and environmental factors had a more pronounced influence on females than on males (Trexler et al., 1990), as we observed for mosquitofish. Frenkel and Goren (1997) showed that *Aphanius dispar* females from higher salinity had larger GSI and oocytes in more advanced developmental stages. Similarly, the fecundity and GSI values for both males and females of Caspian roach (*Rutilus rutilus caspicus*) (Naddafi et al., 2005) were greater in saline habitats than in fresh water; while in environments with high salinity the black-chinned tilapia (*Sarotherodon melanotheron*) and the bonga shad (*Ethmalosa fimbriata*) reduced size-at-maturity and increased fecundity (Panfili et al., 2004a,b). Therefore, the pattern of increased reproductive investment with salinity seems widespread among euryhaline fish.

Life history theory predicts that reduced adult survival will select for earlier maturation and increased reproductive effort, whereas fluctuating environments select for a configuration of traits marked by younger age- and smaller size-at-maturity, higher reproductive effort, larger brood sizes, and smaller offspring, relative to stable environments (Stearns, 1992). Salinity is one of the most stressful factors in mosquitofish survival, affecting it at two levels: because of osmotic pressure capabilities and because saline habitats usually undergo much greater salinity fluctuations than freshwater habitats (Nordlie and Mirandi, 1996). Both stress mechanisms may thus favor a similar response of life history traits. The above mentioned results, however, may also be due to indirect effects of salinity, because salinity is also related to distance to coast and, consequently, to temperature, pH and other water chemistry variables. For instance, Grether et al. (2001) and Reznick et al. (2001) have recently discussed that despite the well known effects of predation on the life history of guppies (*Poecilia reticulata*), others factors such as resource availability or forest cover co-vary with predation pressure. Thus, laboratory experiments are needed to further understand salinity effects on mosquitofish life history.

#### 4.2. Population effects of salinity

Because samples were taken at the start of the reproductive season, we only captured fish born at the end of the last reproductive season that had survived the winter, and the new born were still not present. Salinity effects are more important in female mosquitofish than in males (Trexler et al., 1990); in accordance with that, we found that the mosquitofish population size-structure did not vary with salinity but that sex ratio differed in saltier waters because of a stronger decrease of female density. Salinity affects mosquitofish metabolism (Nordlie and Mirandi, 1996), which is positively correlated with body mass (Hölker, 2003). Because female mosquitofish are much larger than males, higher energy expenditures and oxygen consumption to maintain osmotic regulation are expected. The somatic condition of females was also significantly reduced in saltier waters because of a stronger reproductive investment. Both factors suggest reduced female survival at higher salinities. Similarly, Timmerman and Chapman (2003) reported that *P. latipinna* increased routine metabolic oxygen consumption during brood development and showed a significant increase of routine metabolic rate during late gestation, whereas the metabolic rate of males was lower. Moreover, they suggested that the higher oxygen demand of females caused an increase in the time spent in aquatic surface respiration, hence affecting maternal predation risk.

Although the reproductive investment of mosquitofish increased with salinity, abundance showed a unimodal relationship, with a strong decline in highly polysaline waters, particularly of females. In contrast, the Spanish toothcarp were only present in high polysaline and eusaline waters where mosquitofish were not present. The toothcarp is endemic to the Mediterranean coast of Spain, considered in danger of extinction by the National Catalogue of Endangered Species and listed in the Annex II of the Bern Convention (Doadrio, 2002). It originally inhabited a wide range of lowland waters but nowadays its distribution is reduced to polysaline, eusaline and hypersaline waters. Several factors have been proposed to explain this decline (García-Berthou and Moreno-Amich, 1992; Elvira, 1995) but the impact of invasive mosquitofish plays a dominant role. The change from fresh to oligosaline waters implied the increased abundance and reproductive investment of invasive mosquitofish; these patterns do not continue, however, with further salinity. The contrasting response of mosquitofish and toothcarp abundance with salinity, in addition to the lower somatic condition of mosquitofish with increasing salinity, support the hypothesis that polysaline waters limit the invasiveness of mosquitofish and constitute a refuge for native fauna such as Mediterranean cyprinodontiform fishes.

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