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Salinity mediates the competitive interactions between invasive mosquitofish and an endangered fish

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Abstract The interplay of abiotic factors and competition has a long history in ecology, although there are very few studies on the interaction of salinity and competition in fish. Mosquitofish (*Gambusia holbrooki*) are among the most invasive fish worldwide, with well documented ecological impacts on several taxa such as amphibians and small native fish. It has been previously hypothesized, based on field observations, that salinity limits the invasive success of mosquitofish and provides a competitive refuge for Mediterranean cyprinodonts. We experimentally tested this hypothesis by examining the agonistic behaviour and food competition between mosquitofish and an endangered native cyprinodont (*Aphanius fasciatus*) at three salinities (0, 15, 25‰). Intraspecific aggressive behaviour for both species was not significantly affected by salinity. As salinity increased, mosquitofish decreased their aggressive behaviour towards cyprinodonts and captured less prey. In contrast, the cyprinodonts did not change their behaviour with different salinity treatments, with the possible exception of increased defensive acts in higher salinities, but captured more prey with increasing salinity because of the reduced efficiency of mosquitofish. Our study confirms previous field observations that salinity limits the invasive success of mosquitofish and provides one of the few experimental demonstrations that it may mediate

behavioural and competitive interactions between fish species. Condition-specific competition of mosquitofish might be expected with other species and ecosystems worldwide and illustrates the importance of integrating biotic and abiotic factors in the study of interspecific interactions.

Introduction

The interplay of abiotic factors and competition or other biotic interactions has a long history in ecology. Some of the classical studies of competition are examples of condition-specific competition, i.e. how competitive superiority depends upon environmental conditions (Dunson and Travis 1991; Taniguchi and Nakano 2000; Thomas and Holway 2005). For instance, Connell (1961) experimentally demonstrated that the degree and outcome of interspecific competition between barnacles was mediated by water height in the intertidal zone and Tansley (1917) showed that competition between *Galium* plant species depended on soil characteristics. Our understanding of condition-specific competition is, however, still poor. There is an ongoing debate on whether competition is less important, or at least less intense, in more stressful abiotic conditions (Brooker et al. 2005; Lortie and Callaway 2006; Maestre et al. 2006). Very few studies have examined the interaction of competition and abiotic factors in freshwater organisms and particularly fish or salinity (Dunson and Travis 1991, 1994). Temperature and hence altitude seems to mediate competition among salmonids and cyprinids

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(de Staso and Rahel 1994; Taniguchi et al. 1998). Although the interplay of salinity and competition has been very well investigated in marsh plant assemblages (e.g. La Peyre et al. 2001; Crain et al. 2004), there is very little experimental work with fish (but see Dunson and Travis 1991 for a few examples).

Invasive species represent both a formidable opportunity to study ecology and evolution at unusual spatial and temporal scales (Rice and Sax 2005) and an urgent research agenda for the conservation of biodiversity (Dukes and Mooney 2004). Invasive species often displace native fauna by resource or interference competition, although behavioural traits have received little attention despite the mechanistic insight that they offer (Holway and Suarez 1999; Rehage et al. 2005). Two closely related mosquitofish species, the eastern mosquitofish *Gambusia holbrooki* and the western mosquitofish *Gambusia affinis*, are only native to the United States and Mexico but have been introduced into more than 50 countries (García-Berthou et al. 2005) in order to control mosquito populations and hence malaria (Krumholz 1948). In southern Europe the eastern mosquitofish was first introduced to the Iberian Peninsula in 1921, was transferred to Italy in 1922 and nowadays is very abundant in most Mediterranean countries (see e.g. Krumholz 1948). Several studies have demonstrated the role of mosquitofish in the decline and local extinction of native amphibians (Hamer et al. 2002; Kats and Ferrer 2003) and small fishes (Courtenay and Meffe 1989; Mills et al. 2004). Predation by mosquitofish on eggs and larvae, food competition and behavioural interference have been proposed as the causal mechanisms (Arthington and Lloyd 1989; Rincón et al. 2002; Mills et al. 2004).

The Mediterranean toothcarp (*Aphanius fasciatus*) is a small endemic Mediterranean fish, listed in Annex II (Strictly protected fauna species) of the Convention on the Conservation of European Wildlife and Natural Habitats (Bern Convention). As with other native Mediterranean cyprinodontiforms, its current distribution has mostly been reduced to polysaline, eusaline and hypersaline waters (i.e. waters with salinities ranging from 18 to 30‰, 30–40‰, and >40‰, respectively; Cowardin et al. 1979). Habitat alteration and the introduction of exotic species, mainly mosquitofish that have replaced native cyprinodontiforms in fresh and oligosaline waters (i.e. salinities <0.5 and 0.5–5‰, respectively), have been previously proposed as causes to explain this reduction (García-Berthou and Moreno-Amich 1992; Bianco 1995).

There are no previous studies on the competitive interactions between *A. fasciatus* and mosquitofish and the mechanisms that mediate these interactions. The competitive interactions between *G. holbrooki* and the endemic Spanish cyprinodontiforms *Aphanius iberus* and *Valencia hispanica* have been investigated by Rincón et al. (2002)

and Caiola and de Sostoa (2005), who experimentally demonstrated that the endemic cyprinodonts decrease feeding rates and receive more aggression in the presence of mosquitofish. The occurrence and intensity of these potentially harmful effects, however, seemed to be modulated by water temperature, reproductive condition, relative size of the interacting fishes and species-specific habitat preferences (Rincón et al. 2002). Whether or not salinity mediates these interactions and the invasive success of mosquitofish is largely unknown. The abundance of mosquitofish peaks at intermediate salinities, in contrast to *A. iberus* that has been mainly extirpated from fresh and oligosaline waters (Alcaraz and García-Berthou 2007). Although salinity is considered to limit the invasive impact of mosquitofish and to act as a refuge for native fauna (Bianco 1995; Nordlie and Mirandi 1996; Alcaraz and García-Berthou 2007), its importance in behavioural interactions with native species has not been previously investigated. The objectives of this paper were: (1) to experimentally study aggressive behaviour and food competition between mosquitofish (*G. holbrooki*) and toothcarp (*A. fasciatus*), and (2) to test the role of salinity, over the range of salinities encountered in the wild, in the mediation of the competitive interactions between mosquitofish and toothcarp. Condition-specific competition of mosquitofish might be expected with other species and ecosystems worldwide and illustrates the importance of integrating biotic and abiotic factors in the study of interspecific interactions.

Materials and methods

Fish capture and acclimation

Fish were collected in July 2005 with nets: adult eastern mosquitofish (*G. holbrooki*) from a channel near Padova, Italy (Idrovia Padova-Venezia, Camin) and toothcarp from the nearby Valle Millecampi (Venice lagoon system). All fishes were immediately brought to the laboratory, where they were acclimated before the experiments. Mosquitofish was captured in freshwater whereas Mediterranean toothcarp was captured at 24‰ salinity. For this reason, the latter species was transferred to freshwater (0‰) in a drop-by-drop process with dechlorinated tap water during 1 week, and then were allowed to acclimate to laboratory conditions for at least 2 weeks before the start of the salinity adaptation schedule. Until the experiments began, mosquitofish and toothcarp were separately maintained in 12 stock aquaria of 150 l capacity provided with natural gravel and an air filter. Each aquarium contained about 20–25 fish of a single species to be acclimated to one of the three experimental salinities (see below). The aquaria were

provided with artificial plants to avoid changing vegetation in the different salinity treatments, were illuminated with 15-W fluorescent lights and were maintained at a constant temperature ($25 \pm 1°C$) and photoperiod (13:11 h light:-dark cycle).

The salinity adaptation protocol was done in the same 12 aquaria (two per salinity and species) and consisted of the progressive renewal of the fresh water with hypersaline water (50‰, prepared by adding sea salt to dechlorinated tap water) through a drop-by-drop process until the experimental conditions (0, 15 and 25‰) were reached. For the two experiments, fish that were used in a specific salinity treatment had been previously acclimated to that same salinity only. Salinity was measured using an optical refractometer (with a precision of 1‰, compensated for temperatures of 10–30°C), 3 times per day to regulate the water input to a maximum salinity increase of 2–3‰ per day. After 10 days all fishes were at the necessary experimental salinity and were maintained in these conditions for at least 2 weeks before the experiment commenced. Mortality during acclimation was low (<5%). Fish were fed to satiation twice daily (in the morning and in the evening) with commercial food flakes, live *Artemia* nauplii and frozen dipteran larvae (*Chironomus salinarius*).

Common experimental procedures

The two experiments were done in three adjacent aquaria, one for each salinity treatment, in a dark quiet room. The aquaria had a capacity of 96 l (60×40 and 40 cm high), were filled to a depth of 32 cm with water and 2 cm with natural gravel, and were illuminated by two 15-W fluorescent lights and maintained at the same laboratory temperature ($25 \pm 1°C$). A grey nylon net (2×2 mm) was attached to the inner surface of the glass wall of the tank as a one-way screen to prevent the fish from seeing the observer and from distractions produced by the mirror effect. All trials were videotaped for later observation and analysis. After testing, the standard length of the fish was measured with a digital calliper to the nearest 0.01 mm. To ensure that individual fish were used only once during the experiments, they were placed into a post-experimental tank after the trials. No mortality was observed during the tests.

Only females were used in the trials. Both mosquitofish and toothcarp are dimorphic species, females being larger than males, and the sex ratio is usually skewed in favour of females (Snelson 1989; Leonardos and Sinis 1999). Although both mosquitofish sexes are highly aggressive towards other species (including fish much larger than themselves), females are particularly aggressive, attacking other fishes, shredding their fins, and sometimes killing them (Courtenay and Meffe 1989). The effect on the

female toothcarp is more important in demographic terms as females are generally more abundant because of higher survival rates and greater longevity (Leonardos and Sinis 1998; 1999), like in many other cyprinodonts (Fernández-Delgado et al. 1988; Keivany and Soofiani 2004).

Experiment 1: aggressive behaviour

In this experiment, we studied the effects of salinity on agonistic interactions between mosquitofish and toothcarp. We used a two-way factorial design with three species treatments (one mosquitofish and one toothcarp; two mosquitofish and one toothcarp; and one mosquitofish and two toothcarp) and three salinity treatments (0, 15 and 25‰), with five replicates (sessions with different fish individuals) per treatment combination (i.e. a total of 45 replicates with 120 different fish). The fish used in a specific salinity treatment had been previously acclimated to that salinity (see above). The species treatment used is a kind of response surface experimental design, which in contrast to additive or substitutive designs varies the densities of the two competing species independently, hence allowing the effects of fish density to be distinguished from the effects of species composition within competitive interactions (Inouye 2001). No food was provided for 24 h before the experiment. The evening before the trial, fish were transferred to the experimental tanks, in which a transparent methacrylate wall separated the two species (but not individuals of the same species). Since the methacrylate wall allowed the two species to see each other, there could be some visual adaptation that might affect the overall rate of aggression although equally in the three salinity treatments. The following morning the wall was gently raised to the surface avoiding the fall of drops that might scare the fishes. We then waited for the fish to behave normally and for the first interaction between them. Thereafter, the following behavioural parameters were counted for each of the two species during a 30-min period: number of orientations (fish orienting itself and swimming towards another fish), nips, chases, and defensive acts (fish responding to an aggressive act by other fish). The standard length of mosquitofish and toothcarp (Table 1) used in the aggressive behaviour experiment did not show significant differences among treatment combinations (ANOVA, $P > 0.27$).

Experiment 2: food competition

In this experiment, we tested the effect of the same salinity treatments (0, 15 and 25‰) on the proportion of prey captured by mosquitofish and toothcarp. This experimental set-up was similar to that used in a previous study of *G. holbrooki*

Table 1 Standard lengths (mm) of fish used in the experiments (mean ± SD)

Experiment	Species	n	Salinity		
			0‰	15‰	25‰
Aggressive behaviour	*Gambusia holbrooki*	20	32.0 ± 2.3	32.6 ± 3.1	31.1 ± 2.2
	Aphanius fasciatus	20	37.6 ± 2.7	39.1 ± 3.0	36.6 ± 5.0
Food competition	*G. holbrooki*	24	35.0 ± 2.9	34.5 ± 2.9	33.5 ± 2.4
	A. fasciatus	24	37.7 ± 3.1	40.5 ± 4.1	38.5 ± 5.4

(Pilastro et al. 2003). The fish used in a specific salinity treatment had been previously acclimated to that salinity and kept separately by species (see above). In each aquarium, we placed five equally spaced plastic barriers (11 × 40 cm) in order to simulate plants in the natural environment and thus increase spatial structure and the time needed to capture all the prey. Each barrier was composed of a series of elongated bars, 1 cm wide and 0.5 cm apart, partially dividing the aquarium into six identical sections. Three mosquitofish and three toothcarp were introduced into the aquarium 20 min before starting the test and allowed to freely move through the six sections of the aquarium and interact with each other. We used eight replicates, i.e. sessions with different fish individuals, for each of the three salinities (i.e. a total of 24 replicates with 144 different fish). After 20 min, ten prey items (frozen *Chironomus salinarius*) placed at equal distances on a methacrylate sheet (70 × 2.5 cm) were released at the water surface. The sheet was turned upside down and the food items were regularly scattered over the six sections: one item at the two end sections and two at each of the four central sections because previous studies using this experimental set-up (Pilastro et al. 2003) indicated that the two end sections were less visited by fish than the central ones. We recorded the time taken to eat each of the ten prey items and the species involved.

Statistical analyses

Differences among treatments in length of the fish used in the experiments, behavioural parameters and the proportion of prey items captured were analysed with ANOVA. Significant ANOVAs were followed by polynomial orthogonal contrasts (Sokal and Rohlf 1995) for the salinity factor and deviation contrasts (that compare each treatment with the overall mean) for the species treatment. Salinity and species treatments were considered as fixed effects. We used partial η^2 as a measure of effect size. Like r^2, partial η^2 is the proportion of variation explained for a certain effect {[effect sum-of-squares (SS)]/(effect SS + error SS)} and has the advantage over η^2 (effect SS/total SS) of not depending on the number of sources of variation used in the ANOVA design, because it does not use the total SS as the denominator (Tabachnick and Fidell 2001, p 191).

Experiment 2 is a kind of repeated measures design, in which we compared the time needed for different fish to capture the ten prey items in the three salinity treatments. Most prey were captured by mosquitofish, particularly in freshwater, thus creating many missing values mainly for toothcarp. Conventional repeated measures ANOVA or multivariate ANOVA is inefficient in these cases because it discards any individual with missing data, so we used mixed linear models, which include the following advantages over conventional ANOVA: (1) cases with missing values are included in the analyses, so the analysis is more powerful and unbiased; (2) many different variance–covariance structures can be used, including those of conventional repeated measures ANOVA as special cases, so the analyses is more efficient and with less assumptions (Bagiella et al. 2000; von Ende 2001). We used a heterogeneous first-order autoregressive relationship as a variance–covariance structure because its solution converged and had the best corrected Akaike information criterion.

For statistical analyses, lengths and times were log transformed, behavioural frequencies were square root transformed $\left(\sqrt{(X + 0.5)}\right)$, and the proportion of prey items captured was arcsine transformed $\left(\text{arcsine } \sqrt{X}\right)$ (Sokal and Rohlf 1995) because homoscedasticity and normality were clearly improved. All statistical analyses were performed with SPSS 14.

Results

Experiment 1: aggressive behaviour

The majority of aggressive acts were performed by mosquitofish on toothcarp (66.3%), whereas only about 21.1% of them were done by toothcarp on mosquitofish (Table 2). Mosquitofish performed significantly more orientations, chases and nips towards toothcarp in freshwater (0‰) and their aggressiveness decreased with increasing salinity (Table 2; Fig. 1). For both orientations and nips, the number of aggressive acts performed by mosquitofish on toothcarp significantly decreased (deviation contrasts $P < 0.05$) in the following species treatment sequence: 1 mosquitofish + 2 toothcarp > 2 mosquitofish + 1 toothcarp > 1 mosquitofish + 1 toothcarp (Fig. 2). Therefore,

there was a density effect as well as a species composition effect. Although the same pattern was observed for the number of chases among species treatments, no significant differences were observed (Table 2; Fig. 2). For the number of orientations and nips performed, salinity (partial $\eta^2 = 0.416$ and 0.187, respectively) was a more important factor than treatment (partial $\eta^2 = 0.238$ and 0.155) or their interaction (partial $\eta^2 = 0.026$ and $\eta^2 = 0.031$).

In contrast, toothcarp did not modify their aggressive behaviour with salinity or species treatments, and only showed marginally significant effects of salinity on defensive acts (Table 2), in agreement with the less aggressive behaviour of mosquitofish at higher salinity. Moreover, we observed that the number of aggressive acts (orientations, nips and chases) performed by mosquitofish towards toothcarp was higher than the number performed by toothcarp on mosquitofish in freshwater (0‰) and 15‰ salinity (Student t tests, $P < 0.001$ and $P < 0.005$, respectively), but no significant differences were observed at 25‰ salinity ($P > 0.28$). Therefore, mosquitofish reduced their aggressive behaviour with salinity, whereas toothcarp hardly modified it, both species having the same aggressiveness at the highest salinity treatment.

Salinity × species interactions were not significant for all the behavioural variables (Table 2), so the effects of salinity were similar for the three species treatments.

Furthermore, salinity had no significant effect on intraspecific behaviour relationships for either species (Table 2).

Experiment 2: food competition

The proportion of prey items captured by the two species depended significantly on the salinity treatment (ANOVA, $F_{2,21} = 5.74$, $P = 0.010$), decreasing with increasing salinity in mosquitofish (linear contrast, $P = 0.006$) and thus increasing in toothcarp (Fig. 2). In freshwater (0‰), toothcarp did not capture any prey item because they were all captured by mosquitofish. The time needed by mosquitofish to find and capture prey (Fig. 2) significantly increased with salinity (linear mixed model, salinity, $F_{2,19.9} = 5.40$, $P = 0.013$; time × salinity, $F_{18,93.2} = 1.20$, $P = 0.28$), whereas toothcarp was not significantly affected (linear mixed model, salinity, $F_{1,405.3} = 0.30$, $P = 0.58$; time × salinity, $F_{7,31.4} = 1.89$, $P = 0.11$).

Discussion

We present the first experimental evidence that the competitive interactions between introduced mosquitofish

Table 2 Two-way ANOVAs of the behavioural variables with the experimental factors (salinity and species treatment)

Behavioural variables		Mean number	Salinity				Species treatment		Salinity × treatment	
			F	df	P	Linear contrast P	$F_{2,36}$	P	$F_{4,36}$	P
Gambusia on *Aphanius*	Or	72.4	12.8	2, 36	<0.0001	<0.0001	4.14	0.024	0.91	0.91
	Ni	32.7	5.61	2, 36	0.008	0.003	3.31	0.048	0.29	0.88
	Ch	32.3	3.31	2, 36	0.048	0.019	0.61	0.55	0.07	0.99
	DA	0.1	1.49	2, 36	0.24	0.093	1.05	0.36	1.31	0.29
Aphanius on *Gambusia*	Or	31.5	0.56	2, 36	0.58	0.42	1.41	0.26	0.64	0.64
	Ni	7.1	0.89	2, 36	0.42	0.60	0.31	0.74	0.31	0.87
	Ch	5.3	0.37	2, 36	0.69	0.87	1.57	0.22	0.54	0.70
	DA	9.3	2.92	2, 36	0.067	0.021	0.21	0.81	0.41	0.80
Gambusia on *Gambusia*	Or	24.1	0.10	2, 12	0.91	0.75				
	Ni	15.5	0.45	2, 12	0.65	0.83				
	Ch	14.1	0.65	2, 12	0.54	0.65				
	DA	0.2	1.20	2, 12	0.33	0.15				
Aphanius on *Aphanius*	Or	13.1	1.19	2, 12	0.83	0.55				
	Ni	7.5	0.49	2, 12	0.62	0.35				
	Ch	7.8	0.29	2, 12	0.75	0.71				
	DA	0.3	1.38	2, 12	0.29	0.13				

Only the linear component of the polynomial contrasts for salinity is shown because all quadratic contrasts were not significant ($P \gg 0.10$). Variables were square root transformed. The overall average (mean number of acts during the 30-min trial) for each behavioural variable is also given

Or Orientations, *Ni* nips, *Ch* chases, *DA* defensive acts
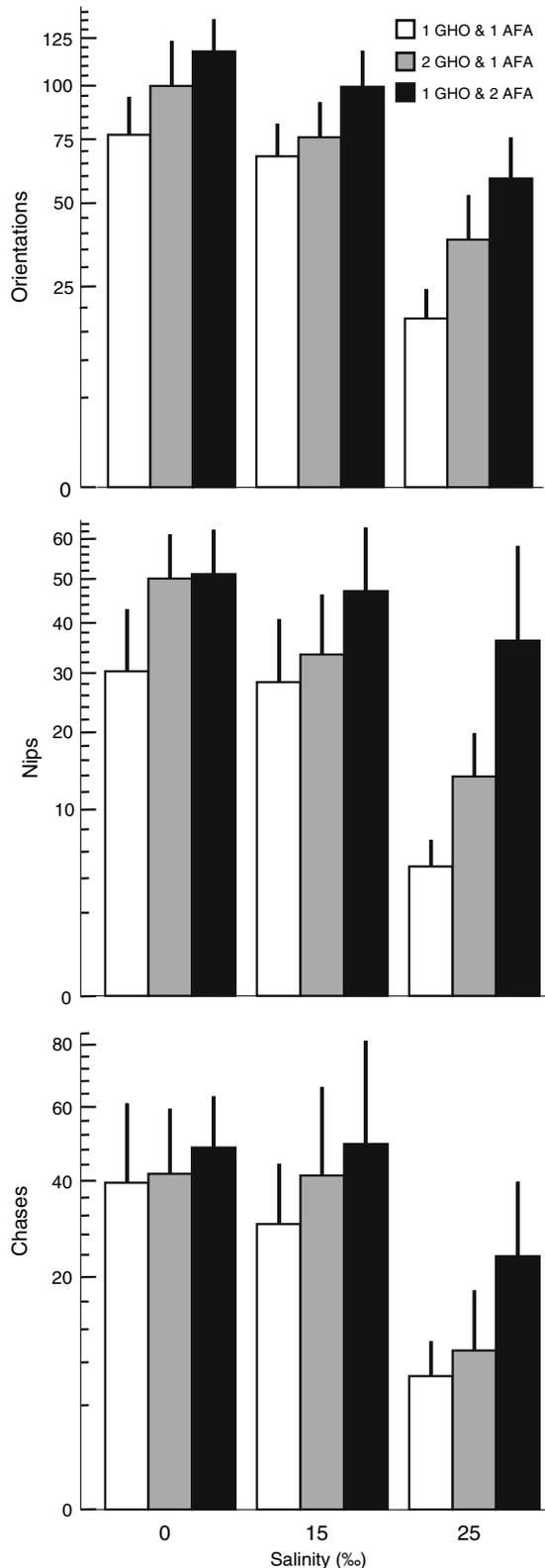
Fig. 1 Number of aggressive acts (orientations, nips and chases) performed by mosquitofish towards toothcarp under the different species combinations [*Gambusia holbrooki* (*GHO*), *Aphanius fasciatus* (*AFA*)] and salinity treatments. SE (*error bar*) is shown
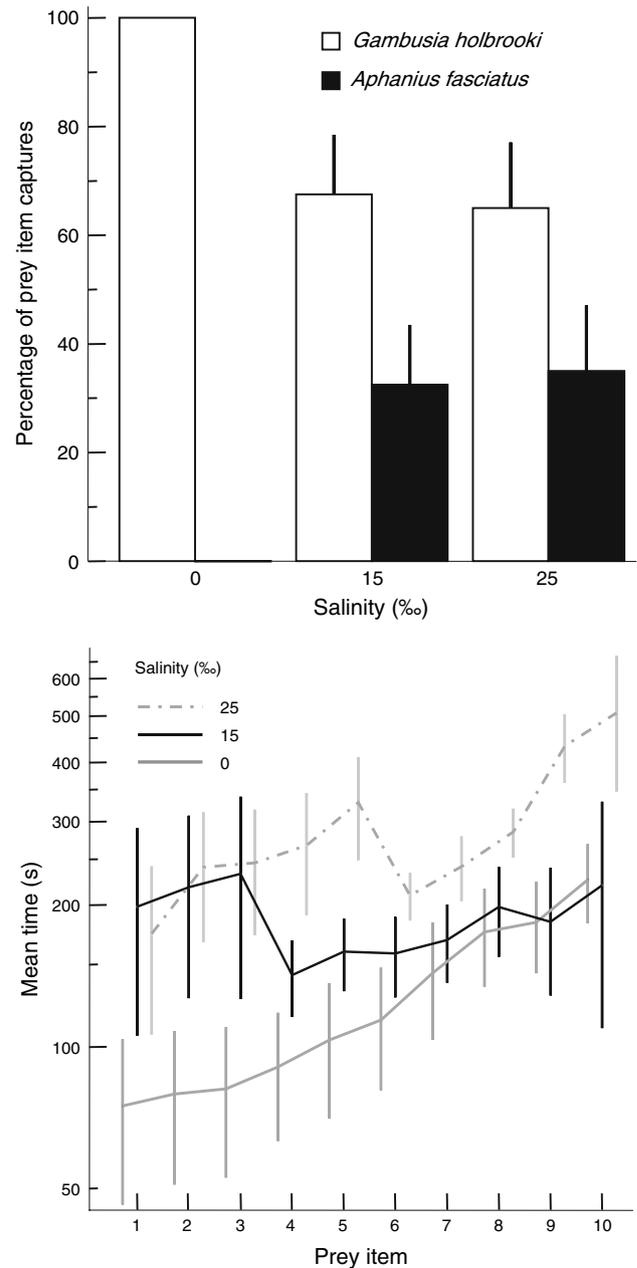
Fig. 2 Effects of salinity on the number of prey items captured by mosquitofish and toothcarp (*top*) and the mean time employed by mosquitofish to capture the ten prey items (*bottom*). SE (*error bar*) is shown

(*G. holbrooki*) and a native cyprinodont (*A. fasciatus*) are mediated by salinity: mosquitofish decreased their aggressiveness towards toothcarp and captured fewer prey, more slowly, at higher salinities. In contrast, toothcarp did not change their aggressive behaviour towards mosquitofish with salinity, except perhaps for increased defensive acts (marginally significant), and captured more prey at higher salinity because of the reduced mosquitofish efficiency. Several points indicate that these results are not artifactual:

(1) the speed and range of salinity changes in our experiments are well below those often experienced by both species in their natural environments; (2) the two intraspecific relationships and the agonistic behaviour and capture efficiency of toothcarp did not change with salinity, and the only significant results were mosquitofish capture efficiency and aggressive mosquitofish behaviour towards the toothcarp; and (3) salinity × species interactions were not significant. It is noteworthy that although the intraspecific orientations of mosquitofish on mosquitofish and the orientations of toothcarp on mosquitofish were also frequent, they did not vary with salinity, unlike the number of aggressive acts of mosquitofish towards toothcarp, suggesting that what was mainly affected was the relative performance of mosquitofish versus toothcarp. Note also that, in contrast to mosquitofish, toothcarp suffered two acclimation processes (from polysaline water to freshwater and then acclimation to experimental salinity) and yet did not change their aggressive behaviour with salinity treatments. It would be interesting to extend our experiments to populations from different origins to test if adaptation of mosquitofish to more salty environments modulates the competitive interactions. However, laboratory and field information does not indicate great variations in salinity tolerance among native populations of *G. holbrooki* (Nordlie and Mirandi 1996).

It has often been thought that salinity limits the invasive success of mosquitofish, displacing native cyprinodonts to saltier waters, which serve as a refuge from mosquitofish (Bianco 1995; Nordlie and Mirandi 1996; Doadrio 2002). There were no previous data on the mechanisms by which salinity mediates the spatial segregation of these species. Both toothcarp and mosquitofish and cyprinodontiform fishes in general are secondary freshwater fishes, i.e. fish species that generally inhabit freshwater but that can tolerate sea water for short periods (Myers 1949; Banarescu 1990). However, cyprinodontids are the most salt-tolerant family, whereas poeciliids are less salt tolerant (Banarescu 1990). The main experimental studies on the salinity tolerance of mosquito fishes are Chervinski (1983) and Nordlie and Mirandi (1996). Nordlie and Mirandi (1996) reported an upper tolerance limit under 30‰ in *G. holbrooki*, whereas Chervinsky (1983) showed that *G. affinis*, a very closely related species, was able to tolerate direct transfer to 19.5‰ salinity and that 65% of the fishes survived in sea water (39‰) while 50% survived in water at 58.5‰ salinity for 7 days. However, records of mosquitofish in eusaline or hypersaline waters are very scarce. In Spanish ecosystems, mosquitofish peak at intermediate salinities and are rare in coastal lagoons with salinities >15‰, where they are replaced by *Aphanius iberus*, which has been mainly extirpated from fresh and oligosaline waters (Alcaraz and García-Berthou 2007). There are no similar data available for *Aphanius fasciatus,* but experiments on similar species such as *Aphanius dispar* (Plaut 2000) and *Aphanius iberus* (Oltra and Todolí 2000), field observations of *A. fasciatus* in hypersaline lagoons with 4–5 times sea salinity (Bianco 1995; Leonardos and Sinis 1998), and our findings of no modification of *A. fasciatus* behaviour with salinity treatments support a higher upper salinity tolerance limit for *A. fasciatus* than for mosquitofish. Therefore, toothcarp is more euryhaline (i.e. tolerates a wider range of salinities) than mosquitofish and at our experimental salinities was still within its optimum.

Salinity adaptation by euryhaline teleosts is a complex process involving a suite of physiological and behavioural responses to environments with differing osmoregulatory requirements (Swanson 1998). Salinity is expected to affect metabolism through changes in energy expenditure for osmoregulation and energy costs are expected to increase in water that deviates from the isosmotic level (Swanson 1998; Plaut 2000). The metabolism response to an increase over the salinity tolerance limit is similar in all euryhaline cyprinodontiform fishes. A decrease in the routine metabolic rate and an increment of plasma osmotic concentration (a measure of stress) (Nordlie 1987; Nordlie et al. 1992; Haney et al. 1999) has been previously reported for several cyprinodontiform fishes. This decrease in metabolism has substantial ecological effects, since swimming capacity and activity rate are expected to decrease, reducing the ability of fish to capture prey, to avoid predation, and to perform other critical survival functions (Plaut 2000). A previous study of *G. holbrooki* (Nordlie and Mirandi 1996) reported that the mean plasma osmotic concentration for mosquitofish increased with salinity, showing the best osmotic regulation from freshwater to 10‰ and a loss of regulatory capability near the upper tolerance limit (25–30‰) and exhibiting limited tolerance and limited plasma osmotic regulatory abilities at elevated salinities near to those of the sea. There are no similar data available for *A. fasciatus* but a very close species (*A. dispar*) decreased its oxygen consumption, critical swimming speed and routine activity rate at extreme salinities over 105‰ (Plaut 2000). Therefore, since toothcarp is more euryhaline than mosquitofish, when both species were exposed to a salinity increase, the overall performance of mosquitofish was more affected than that of toothcarp and its overall performance significantly decreased, reducing the number of aggressive acts towards toothcarp and number of prey captured.

The reduced activity and behavioural changes experienced by mosquitofish with increasing salinity stress and the better relative performance of *A. fasciatus* under higher salinities determined in our study agrees with the current distribution of mosquitofish and Mediterranean native cyprinodontiforms. Mosquitofish are abundant in most

Mediterranean lowland waters. Native cyprinodontiforms are usually restricted to lagoons that present a wide range of salinities but are hypersaline in summer, thus not allowing mosquitofish survival. Our study gives the first experimental evidence that salinity mediates the competitive interactions between invasive mosquitofish (*G. holbrooki*) and native cyprinodontiforms and confirms the field observations that polysaline, eusaline and hypersaline waters constitute a competitive refuge for the latter. This condition-specific competition of mosquitofish might be expected with other species and ecosystems worldwide and points to the importance of salinity for an understanding of the impact and to control the spread of this worldwide invasive species.

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