

Deleterious effects by mosquitofish (*Gambusia affinis*) on the endangered fire salamander (*Salamandra atra*)

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Abstract

Invasive fishes, via competition and predation, may be an important factor in endangering populations of amphibians and other aquatic fauna. The mosquitofish, *Gambusia affinis*, commonly stocked into amphibian breeding sites for mosquito control worldwide, has recently been found in some breeding sites of the endangered fire salamander *Salamandra atra* in northern Israel. A comparison of *Salamandra* larvae in natural pools varying in *Gambusia* densities suggested that *Gambusia* negatively affects *Salamandra* by causing damage to its appendages, in particular, its tailfin (as manifested in a smaller tail:body ratio). Comparison of the same pool before and after mosquitofish introduction suggested strong negative effects on larval performance and number of emerging metamorphs. To explicitly test the hypothesis that *Gambusia* negatively affects *Salamandra* larval development, growth and survival, we conducted a replicated outdoor mesocosm experiment. In this experiment, we also tested how habitat structural complexity, in the form of radiating cords simulating artificial vegetation, might mediate damage caused by *Gambusia*. We used a two-by-two factorial design: the presence or absence of increased structural complexity crossed with the presence or absence of mosquitofish. After a short period, mosquitofish strongly reduced larval wet weight and survival, and increased body damage in the form of a reduced larval tail:body ratio and partially missing gills and limbs. No *Salamandra* larvae exposed to mosquitofish survived to metamorphosis in either habitat type. We conclude that *Gambusia* strongly and negatively affects *Salamandra* larvae and that the introduction of *Gambusia* into sites containing *Salamandra* is inconsistent with the goal of preserving this endangered urodele.

Introduction

Invasive predators may play an important role in population declines and perhaps extinctions of native species in general, including amphibian species (Skelly, 2001; Kats & Ferrer, 2003). Fish introductions are a global-scale phenomenon: Kiesecker (2003) reported the introduction of more than 160 species of predatory fishes in 120 different countries. Fishes are stocked in natural water bodies not only for fishing but also to biologically control mosquito populations, as is the case of stocking the mosquitofish *Gambusia affinis* (Courtenay & Meffe, 1989). Introduced biological control agents may have deleterious ecological impacts (Diamond, 1996; Simberloff & Stiling, 1996). In the case of *Gambusia* for mosquito control, accumulating evidence suggests that this small poeciliid fish is far from a panacea; due presumably to their generalist feeding habits (García-Berthou, 1999), they are not always effective at reducing

mosquitoes (Hoy, Kauffman & O'Berg, 1972; Arthington & Lloyd, 1989; Blaustein, 1992) and they may negatively impact non-target organisms including endangered amphibian species, fishes and invertebrates (Courtenay & Meffe, 1989; Alcaraz, Bisazza & García-Berthou, 2008). In the case of amphibians, negative effects of *Gambusia* species (Gamradt & Kats, 1996; Goodsell & Kats, 1999; Lawler *et al.*, 1999; Hamer, Lane & Mahony, 2002) and other fishes (Tyler *et al.*, 1998; Funk & Dunlap, 1999; Hoffman, Larson & Samora, 2004; Denoel, Dzukic & Kalezić, 2005) have been documented on populations of a number of amphibian species. Most of these studies have considered anurans, and not urodeles, and even fewer studies have considered how mosquitofish in particular affect urodeles. Moreover, the evidence accumulated thus far indicates that the effects of mosquitofish species on amphibians are not consistent across all amphibian species and systems, but may vary greatly among amphibian species from highly deleterious to

minimal (e.g. Baber & Babbitt, 2004; Ling, 2004; Gregoire & Gunzburger, 2008). The potential magnitude of the effect of *Gambusia* on endangered species may be influenced by habitat structure such as aquatic vegetation type and amount. Habitat structure may affect predator–prey interactions through the amount of prey refuge it provides. Submerged vegetation in aquatic systems has been suggested as the cause for reduced fish predation (Werner *et al.*, 1983; Baber & Babbitt, 2004; Lehtiniemi, 2005; Sass *et al.*, 2006). However, the overall effect of submerged vegetation in influencing the effect of *Gambusia* on amphibian larvae may be complex because not only may it provide a refuge for the amphibian larvae, it may also provide a refuge for *Gambusia* juveniles from cannibalistic adults, leading to a larger *Gambusia* population size (Blaustein, 1988; Benoit, Post & Barbet, 2000).

In Israel, *G. affinis* has been stocked into water bodies for mosquito control since the turn of the 20th century (Kligler, 1930). As a consequence of these introductions and their invasion abilities (Moyle & Marchetti, 2006), they are widespread in Israel. Their recent occupancy includes several breeding sites of the fire salamander *Salamandra infraimmaculata* (L. Blaustein, pers. obs.), an endangered species in Israel (Dolev & Perevolotsky, 2002). Most permanent water bodies that serve as breeding sites for fire salamanders are isolated, thus limiting 'natural' invasions, but the threat of introductions into additional salamander breeding sites for mosquito control exists. Like mosquitofish (Blaustein, 1992; Courtenay & Meffe, 1989), *Salamandra* larvae are generalist intraguild predators (Blaustein, Friedman & Fahima, 1996; Eitam, Blaustein & Mangel, 2005; Segev & Blaustein, 2007). Consequently, mosquitofish may thus negatively affect *Salamandra* larvae via predation and competition.

To assess the potential effects of *G. affinis* on *S. infraimmaculata* larvae, we present some field observations that compare *Salamandra* abundance and size at the same site before and during the presence of *Gambusia*, and compare three *Salamandra* breeding sites simultaneously having different mosquitofish densities. Additionally, we present a mesocosm experiment designed to study the effects of *Gambusia* and habitat complexity on *Salamandra* larvae in a two-factorial design. The results of both field observations and experiment indicate that *Gambusia* has strong negative effects on larval *Salamandra* and that the introduction of *Gambusia* in permanent water bodies containing *Salamandra* is inconsistent with the goal of preserving this amphibian species.

Methods and materials

Natural pool observations

To collect preliminary field evidence that *Gambusia* influences *Salamandra*, we compared three breeding sites that differed in *Gambusia* densities ranging from none to high densities. We also compared some *Salamandra* data at one site before and after *Gambusia* was introduced. One pool in Alon Spring, located at Mount Carmel National Park

(32°43'30"N; 35°1'26"E; ~4 m² surface area and 0.4 m maximum depth), did not contain mosquitofish based on visual inspection and the sampling described below. The other two pools, ~40 m apart from each other, were located in the Lower Galilee Mountains at Kaukab Springs (32°49'23"N; 35°14'51"E; each ~15 m² surface area and ~0.8 m maximum depth). The two Kaukab pools, hereafter Kaukab 1 and Kaukab 2, are both independent end points of underground springs and they are not connected. Mosquitofish were stocked into the two Kaukab pools by an unknown source sometime in 2002, about a year before our post-introduction field observations.

On 28 May 2003, we sampled each pool for fish abundance by taking fifteen 1-m sweeps using a D-net (0.3 cm mesh; 1600 cm²) along the bottoms and sides of the pools. *Salamandra* larvae were sampled during late afternoon by entering the water, dislodging rocks (that exposed *Salamandra* larvae under rocks) and by sweeping for 15 min on 28 May, 10 July and 24 July 2003. This dislodging minimized any possibility of differential sampling efficiency of salamander larvae in the presence or absence of *Gambusia* due to antipredator behavior in response to *Gambusia* (Holomuzki, 1986; Blaustein & Spencer, 2005; Schmidt & Van Buskirk, 2005). On the first date, 15 larvae captured in each of the Kaukab pools and 10 larvae captured at Alon pool were also measured for wet weight, total length and snout–vent length. Larval lengths were measured using an electronic calliper and wet weights were taken using an electronic field scale. From the length data, we calculated the tail:body ratio [i.e. the tail fin length (from the tail fin distal end to the vent) divided by the snout–vent length]. Larvae were returned to the pools after measurements. From observations in previous years, we knew that *Salamandra* metamorphs would, during early fall, emerge and concentrate in a collection of ~15 rocks just behind a cave-like area of Kaukab 2 pool before dispersing during the rainy season. These collections of rocks, from just above the water surface to just about 10 cm above the highly consistent water level, were carefully lifted to count *Salamandra* metamorphs on 19 September and 9 October 2003. We then carefully returned the rocks.

We were able to compare the 2003 *Salamandra* data with some data collected from the Kaukab 2 pool in 1999, before the introduction of *Gambusia*. We sampled for larval abundance in the same way as described for 2003 on three dates: 20 May, 10 July and 24 July 1999. This allowed us to compare larval abundances on the three similar Julian dates for this pool before and after *Gambusia* introduction. We also compared the 28 May 2003 tail:body ratios and the wet weight from this Kaukab pool with measurements taken from nine *Salamandra* larvae collected from the same pool on 20 May 1999. A count of *Salamandra* metamorphs in the rock pile behind Kaukab 2 pool on 9, 16 and 30 October 1998, and 20 November 1998 (before mosquitofish introduction) allowed for comparisons of the 2003 counts made after mosquitofish introduction.

The data were analyzed with *t*-tests for any pair-wise comparison and by ANOVA accompanied by Tukey's HSD test for multiple comparisons. The tail:body ratios were arcsine-square root transformed before analysis.

Mesocosm experiment

We used a two-by-two factorial design – the presence or absence of mosquitofish crossed with the presence or absence of increased structural complexity – to study the effects of these two factors on larval *Salamandra* survival and development. The experiment was conducted on the periphery of the University of Haifa Campus using 16 artificial pools (180 L rectangular-plastic containers; height, length, width: 35 × 91 × 57 cm) in a 2 × 8 array under a 90% shade net suspended at a height of 1.5 m. The distance between adjacent containers was 0.5 m and treatments were randomly assigned among containers. We filled the containers with tap water on 12 February 2005 and maintained maximum or near-maximum water levels throughout the experiment using some rainwater, and mostly aged tap water. Containers were open at the top to allow insect colonization and allochthonous input. For structural complexity, we used a black plastic cord (3.5 mm diameter). In half of the containers, we added thirty 1 m sections of cord, each tied to a brick. The strings, which were positively buoyant, radiated toward the surface, simulating submergent vegetation. In order to control for any possible chemical effects of the cord material, we also added the same amount of cord (one 30 m cord) to the eight low structural complexity pools by tightly rolling the cord around the brick. On 14 February, to provide food resources for the mosquitofish and *Salamandra* larvae, we inoculated equal aliquots of invertebrates (containing primarily cladocerans, ostracods and copepods) collected from a nearby winter pool to each experimental pool. Insect colonization/oviposition (mainly chironomids) also contributed to a prey base. Sweep samples with a small net (16 × 11 cm; 250 µm mesh) taken once per 4 weeks for 16 weeks did not reveal any treatment or time differences in invertebrate abundance (repeated measures ANOVA) and is not discussed further.

On 17 February 2005, we added eight male and eight female *Gambusia* collected from the Kaukab pools to each of four pools of each habitat structure type. This density was well below the densities reported elsewhere (Botsford *et al.*, 1987; Blaustein, 1992). Female mosquitofish averaged 48.1 mm in total length (SE: 1.18; $n = 64$) and 1.21 g in wet weight (SE: 0.11; $n = 64$). Males averaged 24.3 mm in total length (SE: 0.78; $n = 64$) and 0.12 g in wet weight (SE: 0.01; $n = 64$).

We used *Salamandra* newborn larvae all deposited in the lab by four mothers collected from a breeding site on Mt Carmel. On 21 February, we added 16 *Salamandra* larvae (mean total length ± SE: 31.44 ± 1.47 mm; mean wet weight ± SE: 0.18 ± 0.02 g; size estimates based on a subset of 40 newborns, 10 each from each mother) to each container. This is a moderate density (0.09 L⁻¹) compared with what we have found in natural pools where densities of newborns can be more than an order of magnitude higher (>> 1 L⁻¹; L. Blaustein, unpubl. data). Particularly because this species is listed as endangered, we used the minimum number of individuals and replicates that would likely lead to detection of moderate effects. Given that this particular population is > 500 adults with a 1:1 sex ratio as determined

by mark–recapture (Segev *et al.*, unpubl. data), and that females deposit on average > 100 larvae, the 96 individuals placed in *Gambusia* pools represented < 0.3% of the total deposited larvae for that one season. Each container included larvae from all four mothers (an experimental design that would also assess for maternal effects would have required many more pools and larvae).

Containers were sampled for mosquitofish monthly for 9 months. *Salamandra* were sampled every 3 weeks until metamorphosis began, and then additional observations were made more frequently. Fish and larval *Salamandra* abundances were assessed by first searching for and removing individuals with a net. We estimate that this resulted in the capture of most individuals, and for *Salamandra*, all of the larvae, because the water was clear and larvae were relatively large and visible. We systematically swept for five additional minutes after the last individual had been captured. Captured *Salamandra* larvae were counted and measured (wet weight, snout–vent length and tail fin length as described in the survey). Mosquitofish were classified as adults or juveniles and according to gender. After measuring, both mosquitofish and *Salamandra* larvae were returned to their respective pools. As in the field survey, these data allowed us to calculate a tail:body ratio. Additionally, we recorded the number of the *Salamandra* larvae containing missing or partially missing limbs and gills. Once metamorphosis began, in addition to the regular sampling, we made observations several times per week for new metamorphs. Completely metamorphosed individuals were measured and weighed and returned to their natural breeding site. These data provided us with survival to, time to and size at metamorphosis. Based on circumstantial evidence, some metamorphosing individuals apparently escaped before capturing and measuring. Therefore, survival to metamorphosis is probably not an exact estimate but a still a good comparative estimate among treatments.

For *Gambusia* abundance data, we conducted repeated measures analyses of variance on adults for 9-monthly samples and on juveniles for 5-monthly samples (because there were no juveniles for the first 4 months). We also conducted repeated measures analyses of variance on larval *Salamandra* survival (arcsin-square root-transformed proportions), wet weight (log-transformed) and tail:body ratios (arcsin-square root transformed) over three sampling dates – 3, 6 and 9 weeks after introducing the newborn *Salamandra*. After that, survival in all *Gambusia* pools reached zero, presenting homogeneity of variance problem for the survival variable, and there was an absence of any data in *Gambusia* pools for the other variables. We made Greenhouse–Geisser adjustments to degrees of freedom for within-subject sources of variation when data failed to meet sphericity assumptions.

In the cases of per cent limb damage, per cent gill damage and survival to metamorphosis, Fisher exact tests were used instead of analyses of variance for *Gambusia* effects, because of the absence of homogeneity of variance, and *t*-tests were used to assess structural complexity effects.

Results

Natural pools survey

Neither Alon pool sampled in 2003 nor Kaukab 2 pool sampled in 1999 had mosquitofish. In May 2003, Kaukab 1 pool had relatively low densities of *Gambusia* (hereafter 'low-fish-density pool'), while Kaukab 2 pool had relatively high densities (hereafter 'high-fish-density pool'), a statistically significant difference ($t = 3.16$, d.f. = 28, $P = 0.004$; Fig. 1a).

Several pieces of circumstantial evidence are in line with the hypothesis that mosquitofish negatively affect *Salamandra* larvae. First, damage consistent with biting was evident on the bodies of the salamander larvae when mosquitofish densities were high. Tail:body ratios of *Salamandra* larvae were highest (longest tails) when mosquitofish were absent (i.e. in Alon pool and the Kaukab 2 pool before mosquito-

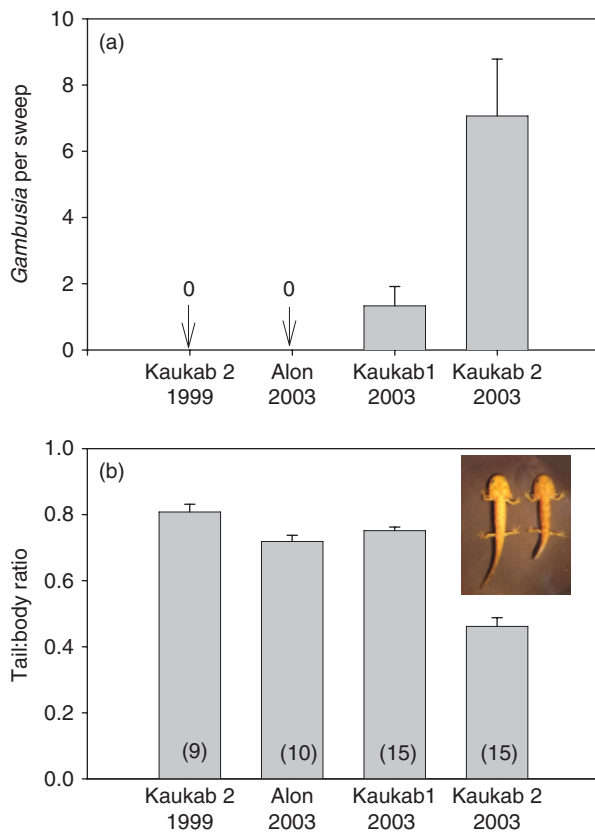


Figure 1 (a) Mosquitofish *Gambusia affinis* caught per sweep (mean of fifteen 1 m sweeps) at 'Kaukab 2' pool on 20 May 1999 (before mosquitofish introduction) and 'Alon', 'Kaukab 1' and 'Kaukab 2' pools on 28 May 2003. Error bars are one standard error; (b) tail:body (snout–vent) ratio of *Salamandra* larvae from the same pools and dates. Error bars are one standard error. Numbers in parentheses indicate the number of larvae measured. Photo inset: typical specimens of *Salamandra* larva with an intact tail from 'Kaukab 1', the low-fish-density pool (left), versus *Salamandra* larva with a truncated tail from 'Kaukab 2', the high-fish-density pool (right).

fish introduction) and significantly lower in the high-fish-density pool ($F_{3,46} = 53.77$, $P < 0.0001$; Tukey's HSD; Fig. 1b). Second, larval densities were similar from late May through July 1999 in Kaukab 2 pool when *Gambusia* was absent, but in 2003, when *Gambusia* was present in high densities in this pool, no *Salamandra* were detected in the two July sampling dates (Fig. 2). Third, the larvae sampled on 20 May 1999 – that is, before the introduction of mosquitofish – were more than twice as heavy (mean = 3.30 g; SE = 0.30) as the larvae sampled in the same pool later during the same month (28 May 2003) after mosquitofish introduction (mean = 1.31; SE = 0.24) ($t = 5.17$; d.f. = 22; $P < 0.0001$). Fourth, we found many more metamorphosed *Salamandra* in the rock pile just above the water line in the high-fish-density Kaukab pool before the mosquitofish introduction (126, 106, 27 and 1 individuals on 9 October 1998, 16 October 1998, 30 October 1998 and 20 November 1998, respectively) than after introduction (2 and 1 individuals on 19 September 2003 and 9 October 2003, respectively). The drastic decline in metamorphs through time in 1998 almost certainly represents dispersal away from the breeding site during the rainy season.

Mesocosm experiment

A repeated measures analysis on all 9-monthly samples (with Greenhouse–Geisser adjustments of degrees of freedom) showed no statistically significant effects of structural complexity, time or the structural complexity \times time interaction on mosquitofish adult abundance (Table 1; Fig. 3). The first newborn mosquitofish appeared on 6 June. Juvenile mosquitofish abundance was higher in the increased structural complexity treatment over the final 5 months of the experiment (Table 1; Fig. 3).

A repeated measures analysis on three sample dates over the first 9 weeks showed that mosquitofish had a large negative effect on *Salamandra* survival, with the effect increasing with time as evidenced by the highly significant *Gambusia* \times time interaction (Table 2; Fig. 4a). By the next sample date, no salamander remained in the *Gambusia*

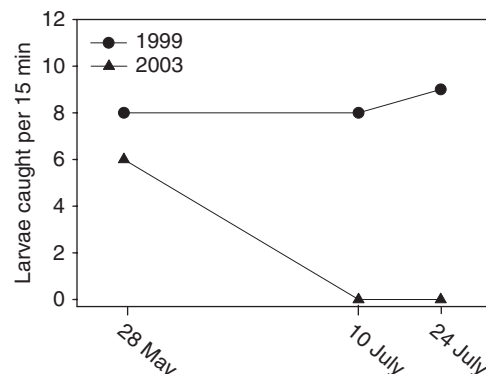


Figure 2 Number of *Salamandra* larvae caught per 15-min sweep in 'Kaukab 2' pool in 1999, before mosquitofish *Gambusia affinis* introduction and during 2003, after mosquitofish introduction.

Table 1 Repeated measures analysis of variance assessing structural complexity and time effects on adult (nine dates) and juvenile (five dates) mosquitofish *Gambusia affinis* abundance

	Adult mosquitofish			Juvenile mosquitofish		
	d.f.	F	P	d.f.	F	P
Structural complexity	1, 6	0.12	0.745	1, 6	29.28	0.0016
Time	1.27, 7.64	1.85	0.218	1.81, 10.85	1.68	0.232
Interaction	1.27, 7.64	0.42	0.583	1.81, 10.85	1.66	0.234

Greenhouse–Geisser adjustments of degrees of freedom (d.f.) were used because of violations of the sphericity assumptions.

Table 2 Repeated measures analysis of variance assessing effects of *Gambusia affinis* and structural complexity on larval *Salamandra* (a) survival (proportion, arcsin-square root transformed); (b) mass (natural log-transformed); (c) tail:body ratio (arcsin-square root transformed) during 3, 6 and 9 weeks after introducing *Salamandra* larvae

	Survival		Mass		Tail:body	
	F _{1,12}	P	F _{1,12}	P	F _{1,12}	P
<i>Gambusia</i>	134.12	<0.001	18.33	0.001	97.27	<0.001
Structural complexity	0.42	0.527	2.49	0.143	0.98	0.343
<i>Gambusia</i> × structural complexity	0.004	0.949	0.13	0.720	1.04	0.329
	F _{2,24}	P	F _{1,31,14,36}	P	F _{2,24}	P
Time	15.43	<0.001	30.62	<0.001	1.44	0.257
Time × <i>Gambusia</i>	7.73	0.003	2.08	0.169	0.28	0.758
Time × structural complexity	0.23	0.794	0.82	0.413	0.63	0.541
Time × <i>Gambusia</i> × structural complexity	0.60	0.560	0.51	0.532	0.50	0.614

Greenhouse–Geisser adjustments of degrees of freedom were used in analyzing mass due to violations of the sphericity assumptions.

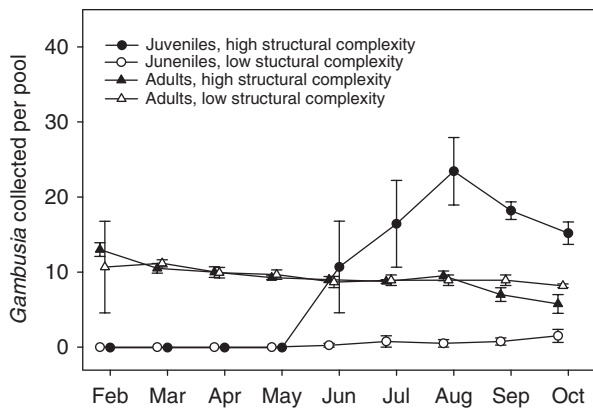


Figure 3 Mosquitofish *Gambusia affinis* adults and juveniles (mean ± se) caught per pool in pools with and without increased structural complexity.

pools. During these first three sample dates, structural complexity did not significantly influence the negative effect of mosquitofish on *Salamandra* survival (Table 1). *Salamandra* wet mass was strongly reduced by *Gambusia* but unaffected by structural complexity (Table 2; Fig. 4b). The magnitude of the mosquitofish effect on wet mass tended to increase with time, but the *Gambusia* × time interaction was not statistically significant ($P = 0.169$). The tail:body ratio (Fig. 4c) was strongly reduced by *Gambusia* but not by structural complexity (Table 2; Fig. 4c). The magnitude of the effect did not appear to increase with time (*Gambusia* × time interaction: $P \gg 0.05$).

The body damage caused by *Gambusia* was not limited to tail fins. To assess gill and limb damage, we used the Fisher exact tests on the last date where effects appeared greatest, instead of repeated measures ANOVA because of homogeneity of variance violations. The occurrence of larvae with partially missing limbs (Fisher exact test: $P = 0.0014$) and partially missing gills (Fisher exact test: $P = 0.0014$) was drastically higher in the mosquitofish treatments (Fig. 4d and e). In the pools containing mosquitofish, the proportion with damaged limbs and gills did not appear to be affected by structural complexity (Fisher exact tests: $P = 0.143$; Fig. 4d and e).

Observations and sampling indicated that it is likely that not a single *Salamandra* larva survived to metamorphosis in any of the *Gambusia* pools regardless of structural complexity while many metamorphosed in the absence of mosquitofish (Fisher exact test: $P = 0.0014$; Fig. 5). Because some metamorphosing individuals may have escaped the pools in between sampling events after 9 weeks, the number surviving to metamorphosis in *Gambusia*-free treatments shown in Fig. 5 was likely higher. Given that the sizes of the remaining larvae in *Gambusia* pools were considerably below the minimum size for metamorphosis (Eitam *et al.*, 2005), it is unlikely that any larvae survived to metamorphosis in mosquitofish pools. In *Gambusia*-free pools, habitat type did not affect survival to, time to or the tail:body ratio at metamorphosis, but *Salamandra* metamorphosed at a larger size in the lower structural complexity pools (Table 3).

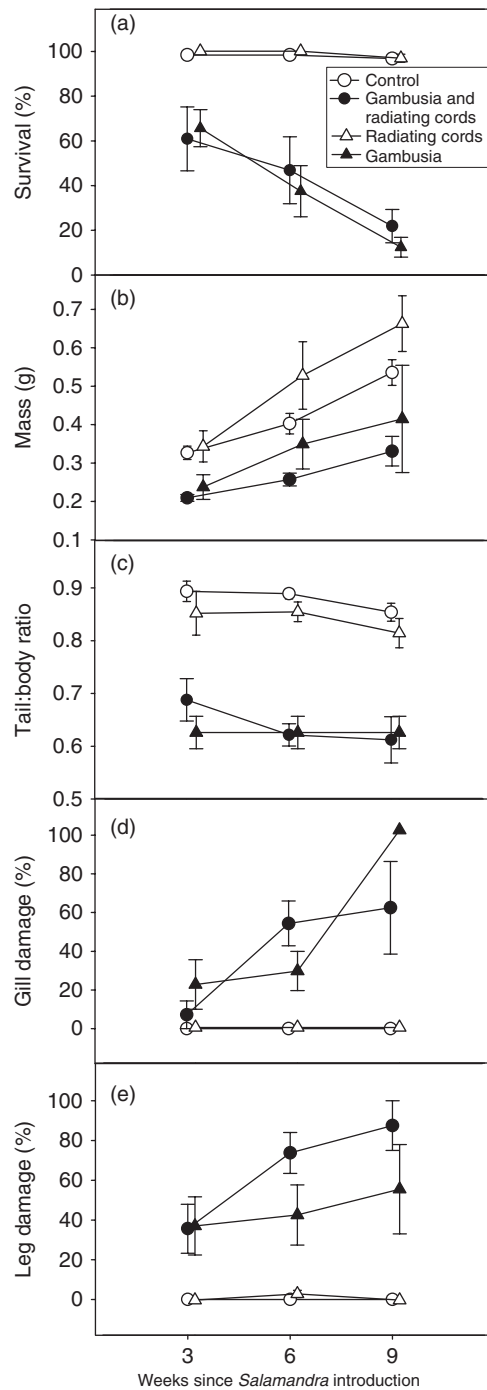


Figure 4 Mosquitofish *Gambusia affinis* and structural complexity effects on *Salamandra* larvae (mean \pm SE): (a) survival; (b) mass; (c) tail to body (snout-vent) ratio; (d) per cent of damaged gills; (e) per cent of damaged limbs, 3, 6 and 9 weeks following the introduction of *Salamandra* larvae.

Discussion

Invasive fishes in general, and mosquitofish in particular, by acting as predators and competitors, pose a concern regard-

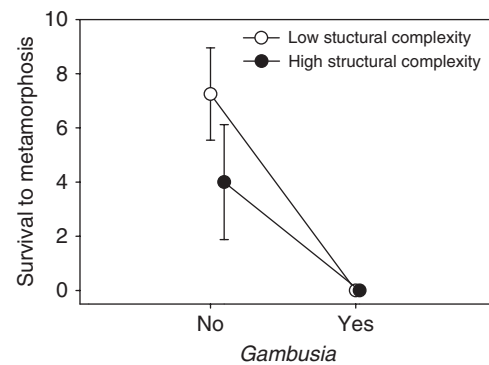


Figure 5 Mosquitofish *Gambusia affinis* and structural complexity effects on *Salamandra* survival to metamorphosis (mean \pm SE).

ing how they might negatively influence the native community including endangered fish and amphibian species (Kats & Ferrer, 2003; Rogowski & Stockwell, 2006). However, although there are clear demonstrations of *Gambusia* negatively affecting specific amphibian species (Courtenay & Meffe, 1989; Gamradt & Kats, 1996; Goodsell & Kats, 1999; Hamer *et al.*, 2002), the effect of mosquitofish, if any, varies greatly and depends on the specific amphibian of interest (Lawler *et al.*, 1999; Komak & Crossland, 2000; Walls, Taylor & Wilson, 2002; Ling, 2004). Here, we present convincing evidence for strong detrimental effects of the mosquitofish on fire salamander larvae, an endangered species in Israel. Our observations in natural *Salamandra* breeding pools provide circumstantial evidence of strong negative effects as follows: first, comparative temporal observations within the same pool during the same time of the year before and after mosquitofish introduction showed that larvae were in higher densities, were larger in size and had larger tail:body ratios before mosquitofish introduction. Moreover, high metamorph abundance in the rock pile before mosquitofish introduction, followed by a very low abundance after mosquitofish introduction suggested a drastic reduction of successful metamorphosis after mosquitofish introduction. Second, a simultaneous spatial comparison of three different pools containing no, low and high fish densities showed a negative correlation between fish density and tail:body ratio.

The mesocosm experiment results are congruent with our field survey findings and show a strong negative effect of *Gambusia* on *Salamandra* in the form of larval survival, size and body damage (larval tail:body ratio and the number of damaged appendages). A significant fraction of the reduced tail:body ratio cannot be attributed to some phenotypic plastic response to *Gambusia* – that is the tails were not shorter whole tails, but shorter because they were partial tails with raw wounds and absence of fin tissue at the distal end. *Gambusia*, being a gape-limited predator, cannot consume an entire larva and instead inflicts multiple injuries through the tail, limb and gill bites (Richards & Bull, 1990; Komak & Crossland, 2000). This damage likely eventually caused mortality in all or almost all larvae in all mosquitofish pools before any could complete metamorphosis.

Table 3 Structural complexity (presence or absence of radiating cords) effects in the absence of *Gambusia* on *Salamandra* number surviving, time to (days), size (wet mass) and tail:body ratio at metamorphosis assessed by *t*-tests

	High structural complexity		Low structural complexity		Test	
	Mean	SE	Mean	SE	<i>t</i>	<i>P</i>
Survival	5.33	2.33	7.25	1.70	0.68	0.524
Time	137.71	50.75	240.10	64.47	1.17	0.294
Mass	0.73	0.05	0.98	0.04	3.78	0.013
Tail:body	0.77	0.06	0.80	0.03	0.49	0.645

Repeated non-lethal injuries can accumulate to cause mortality (Harris, 1989). Even under conditions where there is some survival to metamorphosis in the presence of *Gambusia*, the tissue damage inflicted by the fish likely causes reduced fitness for the surviving larvae. Predator-inflicted tail damage has been studied primarily in anuran tadpoles (Wilbur & Semlitsch, 1990; Blair & Wassersug, 2000) and has been shown to affect tadpole development and swimming performance (Figiel & Semlitsch, 1991; Parichy & Kaplan, 1992; Van Buskirk & McCollum, 2000). The first fish-inflicted damage in the mesocosm experiment was seen on the larval tail, while gill and limb damage followed only later. One possible explanation is that when a mobile salamander larva flees, its most vulnerable tissue to the pursuing predator is its tail fin. Then, significant tail damage caused a reduction in larval swimming performance, which increased larval vulnerability to bites in other, possibly more, vital body parts. The reduction in gill surface area due to gill biting may affect larval gas exchange performance and consequently fitness as urodele gills are responsible for 60–70% of O₂ uptake and CO₂ elimination (Burggren & Just, 1992).

Lower body weight in the presence of *Gambusia* in the mesocosm experiment and the field observations may be in part due to exploitative and interference competition. The two species are considered generalist predators showing a high overlap in their diets (Blaustein, 1992; Gophen, Malinkov & Degani, 1998; Mansfield & Mcardle, 1998).

In the natural pool survey, we initially surmised that the absence of any detectable effect of mosquitofish on *Salamandra* larvae in the low-fish-density pool was due in large part to the lower *Gambusia* densities (~4 × lower) but it might also have been due to increased refuge for *Salamandra* larvae; this pool had considerably denser algal filamentous mats (comprising >50% of the water volume) in contrast to the high-fish-density pool, which had low algal abundance (<5% of the water volume). Comprising part of the mosquitofish diet (Harrington & Harrington, 1982; Meffe & Snelson, 1989; Gophen *et al.*, 1998), the abundance of algae in the low-fish-density pool might even reduce exploitative competition between *Salamandra* and *Gambusia*. In the mesocosm experiment, increased structural complexity did not reduce the negative effect of *Gambusia* on *Salamandra* larvae. One possible reason for the lack of a refuge effect is that structural complexity in the form of artificial vegetation does not provide much of a refuge for amphibian larvae; *Gambusia* have been shown to be effective predators in vegetation on invertebrates (Linden & Cech, 1990) and on larger prey such as amphibian larvae (Baber & Babbitt, 2004). A second

possibility might be that other types or amounts of structural complexity not simulated by our radiating cords provide a better refuge for *Salamandra* larvae. Stones, beneath which *Salamandra* larvae are often found, may provide a better refuge than the plastic cords we used. These cords, bundled around the brick in the low-structural complexity treatment, might have also provided some additional refuge. Lastly, the artificial cords, unlike the filamentous algae, could not have provided an alternative food source for the *Gambusia*.

The radiating cords caused an increase in juvenile recruitment in *Gambusia*. Other studies have shown that vegetation in the water column causes increased survival and increased population growth rates of *Gambusia* (Blaustein, 1988; Winkelman & Aho, 1993; Benoit *et al.*, 2000). However, this increased indirect positive effect on mosquitofish abundance could not have had an effect in the mesocosm experiment; by the time there was mosquitofish reproduction, all *Salamandra* larvae had been killed.

The introduction of *Gambusia* into a specific permanent *Salamandra*-breeding site may not just threaten the salamander population at that breeding site; *S. infraimmaculata* is capable of long-term dispersal (Bar-David *et al.*, 2007). The absence of recruits from *Gambusia*-stocked pools to other breeding sites may endanger the long-term persistence of other small subpopulations.

With regard to mosquito control in permanent ponds, mosquitofish and *Salamandra* larvae may be ecologically redundant; *Salamandra* larvae themselves can be very effective predators of mosquitoes (L. Blaustein, unpubl. data). As a consequence of the results from this paper, the Israel Nature Reserve Authority has decided to remove *Gambusia* from *Salamandra*-breeding sites and not allow further introductions. *Gambusia* is widely distributed globally (e.g. Meffe & Snelson, 1989; Caiola & Sostoa, 2005; Alcaraz *et al.*, 2008) where various species and subspecies of salamandrids are found. Many urodele species that lay eggs instead of larvipositing may be even more vulnerable to mosquitofish because they undergo longer and probably more vulnerable stages. In summary, the evidence shown here clearly demonstrates that the goal of *Salamandra* preservation is inconsistent with the goal of using mosquitofish for mosquito control.

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