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Changes in diet, growth and survivorship of the native Tequila Splitfin *Zoogoneticus tequila* in co-occurrence with the non-native Shortfin Molly *Poecilia mexicana*

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With 3 figures and 3 tables

Abstract: In this study, captive-bred fishes were used in a mesocosm experiment in which the native Tequila Splitfin *Zoogoneticus tequila* and non-native Shortfin Molly *Poecilia mexicana* were held singly and jointly in different experimental tanks to assess changes in growth and diet composition between the two species. We also examined changes in growth and survivorship at various food abundances during co-occurrence. Experiments were performed at ≈ 25 °C in aquarium and ≈ 20 °C in ponds. In the single-species treatment, *Z. tequila* exhibited a more diverse diet composition, and *P. mexicana* exhibited higher survivorship at the lowest prey density. The non-native *Poecilia* had no effect on growth of the native species; this could be related to the possible elimination of smaller individuals of the native species in treatments with low chironomid densities. The Shortfin Molly also exhibited higher survivorship at low food availability in co-occurrence and single species treatment. These findings contribute to a better understanding of native species responses to non-native species. The experiments illustrate possible mechanisms by which non-native fish affect native species in freshwater environments where the effects of small non-native fish species have not been considered fully.

Keywords: goodeids; diet changes; survivorship; non-native species; México

Introduction

The introduction of non-native fish species has been recognized as a major threat to aquatic diversity (Wilcove et al. 1998; Zambrano & Macías-García 1999; Elvira & Almodóvar 2001; Kolar & Lodge 2002). Non-native fishes change and deplete host ecosystems through parasite introduction (Fernando 1991; Crowl et al. 1992; Bruton 1995; Hinojosa-Garro & Zambrano 2004), modify habitat and species-assemblage

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structure (Bain 1993; Propst et al. 2008; Mitchell & Knouft 2009), and change diet, survival, and food-web interactions in the local fauna through predation (Beisner et al. 2003; Townsend 2003) or competition pressure (Ogutu-Ohwayo 1990; Kaufman 1992; Townsend 1996; Strecker 2006). The effects and responses of non-native fishes in new environments have been studied primarily with species used in inland fisheries, aquaculture, and sport fishing activities (Courtenay 1993; Crossman & Cudmore 1999), relegating the study of the effects of small non-native fish species not consumed by humans to a lower priority. For example, poeciliids have been introduced intentionally and accidentally by fish hobbyists and governments worldwide (Miller 1966; Courtenay & Meffe 1989; Swift et al. 1993) and have been successful in many aquatic ecosystems in Mexico (Lyons et al. 1995; Aguilera-González et al. 1996). Nonetheless, the possible direct mechanisms by which small non-native fish affect native species have been poorly studied.

The "Mesa Central" of Mexico maintains a large number of endemic fish species, the family Goodeidae being one of the most diverse groups in this region (Doadrio & Domínguez-Domínguez 2004; Webb et al. 2004). Most of the species in this family are microendemic, such as the Tequila Splitfin, *Zoogoneticus tequila* (Webb & Miller, 1998). This species is presumed extinct in the wild where its distribution was restricted to the Teuchitlán River in the Ameca River drainage (Domínguez-Domínguez et al. 2008). The Mexican Ecological Norm (NOM-ECOL-059– 2010) and the IUCN Red List of Threatened Species consider *Z. tequila* to be endangered and critically endangered, respectively. Populations of *Z. tequila* have been dramatically reduced since 1990 (Webb & Miller 1998), and more recently a small population (less than 500 individuals) was found in the headwaters of the Teuchitlán River (De La Vega-Salazar et al. 2003a; De La Vega-Salazar et al. 2003b; Bailey et al. 2007).The causes of this population decrease in the wild is uncertain, but the most commonly suggested hypothesis involves a combination of the introduction of non-native species, pollution, deforestation, water extraction and habitat fragmentation in the Ameca River (Webb & Miller 1998; De La Vega-Salazar et al. 2003a; Domínguez-Domínguez et al. 2006; Domínguez-Domínguez et al. 2007). Biological information regarding its feeding habits and diet selection in the wild is scarce; however, its congeneric species, *Z. quitzeoensis* Bean, 1898, feeds on amphipods, insects, ostracods and detritus (Moncayo 2012). Presently, *Z. tequila* is maintained and bred in captivity by aquarists and scientific groups in North America and Europe (Miller 2005).

In the original habitat of *Z. tequila* in the Teuchitlán River and headwaters of the Ameca River, high abundances of small non-native fish species, such as the Shortfin Molly *Poecilia mexicana* Steindachner, 1863 have become established (Miller et al. 1989; Webb & Miller 1998). The extensive native range of the Shortfin Molly extends along the Gulf of Mexico and Atlantic slopes, from the lower Río Bravo basin southward into Costa Rica (Miller 2005). It has been distributed throughout the world (see Courtenay & Meffe 1989; Swift et al. 1993; Miller 2005) via a generally unregulated industry (i.e. the pet trade). This molly has been translocated in Mexico to the Rio Lerma (Lyons et al. 1995) and the Ameca River basin (Miller et al. 1989). It has a broad diet including filamentous and unicellular green algae and diatoms, aquatic and terrestrial arthropods, parts of vascular plants and seeds, and in some cases, sand and rock residuals (Menzel & Darnell 1973; Tobler et al. 2008; Scharnweber et al. 2011).

It has been suggested that the persistence of two goodeid species in the Ameca River—the Butterfly Splitfin, *Ameca splendens* Miller & Fitzsimons, 1971, and the Blackfin Goodea, *Goodea atripinnis* Jordan, 1880 —is a result of weak trophic interactions with the non-native species in comparison to interactions between *Z. tequila* and *P. mexicana* (Escalera-Vázquez & Zambrano 2010). The introduction of poeciliids in the Teuchitlán River occurred as early as 1977 (Webb & Miller 1998), with the successful establishment of *P. mexicana* documented by 1989 (Miller 1989). Because the population of *Z. tequila* started to decline after 1990, the co-occurrence with *P. mexicana* in the Teuchitlán River may have driven the extinction of the native *Z. tequila*. However, lack of ecological and environmental data prior to the extinction of *Z. tequila* makes it difficult to determine the factors leading its demise. We wished to determine whether changes in the diet and survivorship of *Z. tequila* in the presence of *P. mexicana* may have been mechanisms that promoted its population decline. Therefore, we used mesocosms and experimental aquarium systems with captive native fish in this study to determine: (1) changes in the growth and diet of the native and non-native species, and (2) changes in the growth and survivorship of the native goodeid in co-occurrence with the non-native poeciliid at different food abundances.

Material and methods

Fish collection and breeding

Poecilia mexicana individuals were bred under laboratory conditions from mature adults obtained from the wild. A total of 32 females and 26 males of *P. mexicana* (standard length; SL > 40.0 mm) were caught in the Teuchitlán River at the locality "El Rincón", Jalisco (20° 41′ 22.27″ N, 103° 50′ 30.53″ W; 1311 m asl) in January 2005. *Zoogoneticus tequila* was not found in the Teuchitlán River and Ameca River tributaries; therefore, mature adults (40 females and 20 males; SL > 30.0 mm) were obtained from a stock maintained at the Laboratorio de Biología Acuática, Universidad Michoacana de San Nicolás de Hidalgo (LBAUMSNH). Adults of each species were kept indoors in 600 L round plastic tanks (diameter = 1.1 m, height = 0.63 m) with continuous air and water circulation. We used the female-biased sex ratio (2 females to 1 male) to reduce aggressive behaviour among males. Weekly, we replaced 20 % of the tank water, and temperature was maintained at 25.0 ± 1.0 °C by heaters (Aqua Top, 300 W, Model GH-300). Fishes were fed twice daily with a mixture of flake food and live crustaceans, such as *Artemia* sp., *Daphnia pulex* L., 1758, and *Bosmina* sp., obtained from stocks maintained in the laboratory. Mature adults were observed daily, and when a gravid female was found she was placed in an individual 5-L glass aquarium with internal filters and continuous air supply, and fed twice daily until the brood was produced. Fry were kept in the same aquarium where born and fed daily with a mixture of *Artemia* nauplii, *D. pulex* and *Bosmina* sp. until they reached the appropriate size for the experiments.

Mesocosm experiment

This experiment was designed to analyse changes in diet composition and growth for *Z. tequila* and *P. mexicana* in treatments in which they were held either together or with conspecifics. Therefore, a mesocosm design with fifteen ponds (i.e., concrete ponds of 1.0 × 0.4 × 0.4 m; 160.0 L each) was carried out outdoors at the LBAUMSNH on March 2005. Filtered water circulated through all ponds, creating a closed circulation system that recycled the entire water volume 15 times a day and maintained the same water level in all ponds. To generate biotic conditions for the fish without inoculating the ponds with zooplankton or bacteria, the ponds were filled 30 days prior to the start of experiment. To evaluate potential differences among water conditions in the ponds, nitrite (mg L^{-1}), ammonia (mg L^{-1}), pH, total hardness (°dH) and temperature (°C) were monitored weekly at 13:00 hours. Because of pond characteristics (e.g., water volume and placement outdoors), temperature could not be controlled as in the later experiments (see below). Zooplankton abundance and occurrence were monitored in each pond every five days by filtering 20 L of pond water through a net with 43 µm mesh.

Three treatments (i.e., two single-species and one two-species) with five replicates were randomly distributed across the fifteen-pond system. To mimic fish densities (ind L^{-1}) reported in the wild, we used a density of 0.2 ind L^{-1} in the tanks, according to the number of individuals and natural habitat characteristics provided in De La Vega-Salazar et al. (2003a). In the first treatment, we stocked 30 individuals/pond of *Z. tequila*, and in the second treatment 30 individuals/pond of *P. mexicana.* For the third treatment, we used 15 individuals/pond of *Z. tequila* and 15 individuals/pond of *P. mexicana* (total individuals = 450). Young fishes are more specialized in their diet than older individuals, making the strength of ecological interactions stronger in earlier stages (García-Berthou 1999; Persson & Brönmark 2002; Rezsu & Specziar 2006). Therefore, to reduce variability in these interactions, we used individuals with SL of 17.00 ± 4.40 S.D. mm for both species in all treatments. After 30 days, we removed all individuals from the ponds and measured their ultimate SLs to the nearest 0.01 mm using a digital calliper (Tresna® EC16; Trensnainstrument). Five individuals of each species per pond were randomly selected for gut-content analysis. Fishes were anesthetized with benzocaine (250.0 mg L^{-1}) then euthanized with 5 % formalin; gut contents were surgically extracted and preserved in absolute formalin for 15 minutes, and were subsequently moved to 70 % ethanol for preservation and examination. Each gut was dissected in a petri dish with a grid (1.0 mm grid size) to determine the volumetric percentages (%V) and occurrence of prey species. Prey taxa in the guts were identified to the lowest practical taxonomic level using Usinger (1956), Edmonson (1959), Pennak (1978), Korovchinsky (1992) and Fernando (2002).

Survivorship at different prey densities

This experiment was designed to explore differences in growth and survivorship of *Z. tequila* and *P. mexicana* at different prey densities when held either with conspecifics or with each other. In the first experiment, chironomids were one of the main prey taxa found in the gut contents of both fish species (see results in Table 3). Because of their relatively high protein content (73.6 %) and high digestibility, chironomids are a rich food source for many aquatic organisms (Yashouv 1956; Yashouv & Ben Shachar 1967) and are also easy to handle in the laboratory. Therefore, we selected chironomid larvae for use in this experiment. To promote stronger interactions related to prey abundance, a higher fish density was used (0.3 ind L^{-1}) in this experiment than in the mesocosm experiment. Each experimental unit consisted of a 40-L glass aquarium with internal filters and continuous air supply; gravel and pebbles were omitted to avoid the establishment of plankton. Once the experiment began, fish wastes were siphoned off weekly using a vacuum cleaner (Pen Plax, 16 inch gravel vac) from each aquarium, followed by replacement of 20 % of the water. In natural ponds, the mean temperature averages 25 °C ± 0.2 S.D. (López-López et al. 2004); therefore, water temperature in each experimental aquarium was maintained constantly at 25 (± 1.0) °C during the experiment using a heater (Aqua Top, 100 W, Model GH-100). Three treatments (i.e., two single-species and one two-species) with five replicates were established in June 2005; each treatment contained four different prey densities (PD): PD1 = 1 chironomid/fish; PD2 = 2 chironomids/fish; PD3 = 4 chironomids/fish; and PD4 = 8 chironomids/fish. This resulted in a total of 60 experimental units (i.e., 3 × 5 × 4). Fish density in each aquarium was calculated as 15.00 mm of fish length/1 L; therefore, we stocked 12 individuals/aquarium of *Z. tequila* in the first treatment and 12 individuals/aquarium of *P. mexicana* in the second treatment. In the third treatment, we stocked 6 individuals/aquarium of *Z. tequila* and 6 individuals/aquarium of *P. mexicana* with both species in the same aquarium (total individuals = 720) were used. Individuals of *Z. tequila* measured 19.66 mm ± 2.63 S.D. (SL) and *P. mexicana* 19.22 mm ± 1.76 S.D. (SL) were selected for this experiment. We fed the fish twice daily for 30 days with chironomid larvae (5.19 ± 1.12 S.D. mm length) at the established PDs. We calculated mortality rate (%) as a fish response and therefore, recorded the total number of individuals that died during each 30-day trial. The final SL of each survivor was measured to the nearest 0.01 mm with a digital calliper. We used no analgesic or anaesthetic for the fishes because that may have had an adverse effect on fish behaviour, producing bias in the results (Readman et al. 2013; Nordgreen et al. 2014). At the end of this experiment, surviving fishes were reintroduced into the stocks maintained at the LBAUMSNH.

Data analyses

To compare physicochemical variables among ponds, homogeneity of variance (i.e., Bartlett's test) and normality (i.e., Shapiro-Wilk's test) tests were performed along with one-way analysis of variance (ANOVA). In cases where normality or homoscedasticity failed to meet assumptions, ANOVA by ranks (i.e., Kruskal-Wallis test) and Bonferroni correction for *post hoc* Mann–Whitney pair-wise comparisons were used. For prey-density comparisons in ponds during the experiment, a Repeated measures ANOVA was used, with the days of zooplankton sampling (day 0, 5, 10, 15, 20, 25 and 30) as factors. In the first experiment, non-metric multidimensional scaling (NMDS) based on ranked Bray-Curtis dissimilarity index was used to illustrate differences in fish-diet composition. This ordination procedure was not susceptible to the problems associated with zero truncation and does not require assumptions associated with general linear models and factor analyses (Jaworska & Chupetlovska-Anastasova 2009). Proportions of prey taxa found in the gut were square root-transformed, then arcsine-transformed to normalize distributions. Three-dimensional solutions were searched in order to reduce the stress level in the NMDS configuration (i.e., <0.15). To test for significant differences between fish species diets, an analysis of similarities (ANOSIM) based on the Bray-Curtis dissimilarity index with 1000 permutations was used. To identify the contribution percentage of the most important prey species in the diet composition, similarity percentages analyses (SIMPER) were calculated. Fish growth was calculated as the difference between the mean SL's before and after treatment for each replicate. Those data were aligned rank-transformed to achieve normality, and a two-way analysis of variance (two-way ANOVA, factors = treatment and species) used to test for differences in growth between the two species in the single-species and the two-species treatments.

In the second experiment, fish growth was calculated as in the first experiment, but in light of the experimental design, we used a three-way ANOVA (factors = treatment, species, and PD) to test for differences in growth between the two species at different PDs. For comparing species survivorship, the proportional data were square root–transformed, then arcsin-transformed, and a three-way ANOVA (factors = treatment, species, and PD) with a *post hoc* data analysis for paired comparisons (i.e. Tukey-Kramer HSD) was used. Statistical analyses were performed with the libraries stats, vegan (Oksanen et al. 2013) and MASS (Venables & Ripley 2002) using the statistical computer environment R 3.0.2 (R development core team; www.R-project.org/) and the software PRIMER 6 for Windows (PRIMER-Ltd, Plymouth, U.K.; http://www.primer-e.com/primer.htm).

Results

Diet and growth in mesocosm

Physicochemical variables related to water quality were not normally distributed (Shapiro-Wilk's test, $W > 0.40, p < 0.05$ for all variables). No differences for any variable were found among experimental ponds in the mesocosm experiment; therefore, the global mean values and S.D. are presented in Table 1. Temperature did not differ among ponds; however, daily changes of more than 10.0 °C were measured, with the lowest value occurring at 15.5 °C at 09:00 hours and the highest value of 24.9 °C at 16:00 hours, in response to ambient air temperature.

For zooplankton species densities, we detected no statistical differences during the experiment. The dominant taxa (in density) in the plankton community were ostracods, the amphipod *Hyalella azteca* Saussure 1858, the cladoceran *D. pulex,* and chironomid larvae (Table 2).

We identified 11 prey taxa in 83 fish gut contents (17 guts were empty). Insects were difficult to monitor in the experimental ponds; however, parts of insects were found in gut contents. Chironomids, parts of insects and *D. pulex* were the prey taxa that accounted for the highest %V of the diet in both species. Although *H. azteca* was the second most abundant prey,

Table 1. Global mean values (± S.D.) and Kruskal-Wallis test results for the physicochemical variables in ponds for the mesocosm experiment. No differences for all variables among ponds were obtained; NA = test was not performed.

Variable	Mean ± S.D.	H_{14}
pH	8.88 ± 0.21	1.7
NH_4 (mg L^{-1})	0.46 ± 0.61	1.86
NO_2 (mg L^{-1})	0.3 ± 0.0	NA
Total hardness (°dH)	8.62 ± 1.11	0.0
Temperature (°C)	20.06 ± 1.96	7.9

Table 2. Repeated measures ANOVA (factor: Days) and mean value ± S.D. of density of the species in the plankton community (ind/L) in the experimental ponds.

Zooplankton species	Mean ± S.D.	F-value	*p*-value
Ostracods	17.07 ± 11.3	0.88	0.51
H. azteca	6.36 ± 4.33	0.62	0.71
D. pulex	6.22 ± 6.65	1.37	0.23
Chironomids	3.31 ± 2.39	0.74	0.61
Bosmina sp.	1.16 ± 0.78	0.72	0.63
Alona sp.	1.13 ± 0.78	1.13	0.34
Copepods	0.08 ± 0.03	0.64	0.69

Table 3. Dissimilarity percentages of prey taxa in the gut contents of *P. mexicana* and *Z. tequila* in the mesocosm experiment. S.C. = similarity contribution, C.D. = contribution to dissimilarity, %V = percentage volume. The sum of the bold numbers represents >70 % of the dissimilarities in diet for each treatment.

Single-species	**SC (Mean ± S.D.)**	***Z. tequila* Mean (%V)**	***P. mexicana* Mean (%V)**	**C.D. (%)**
Chironomids	0.09 ± 0.07	25.5	24.64	**16.43**
Parts of Insects	0.10 ± 0.08	21.66	0.0	**18.06**
D. pulex	0.10 ± 0.08	15.0	33.76	**17.92**
Bosmina sp.	0.05 ± 0.07	11.17	0.0	**9.70**
Ostracods	0.05 ± 0.04	19.83	19.63	**9.04**
Alona sp.	0.04 ± 0.05	0.0	7.89	7.96
Oscillatoria sp.	0.03 ± 0.05	1.0	5.94	6.50
Diatoms	0.02 ± 0.05	0.0	5.36	5.23
H. azteca	0.02 ± 0.06	5.0	0.0	3.57
Organic matter	0.02 ± 0.03	0.0	2.77	4.64
Plant debris	0.005 ± 0.02	0.84	0.0	0.89
Two-species	**SC (Mean ± S.D.)**	***Z. tequila* Mean (%V)**	***P. mexicana* Mean (%V)**	**C.D. (%)**
Chironomids	0.15 ± 0.13	36.36	20.32	**27.64**
Parts of Insects	0.009 ± 0.04	0.0	1.9	1.52
D. pulex	0.12 ± 0.1	21.97	22.22	**21.11**
Bosmina sp.	0.02 ± 0.05	3.79	0.0	3.82
Ostracods	0.11 ± 0.08	37.88	25.24	**18.98**
Alona sp.	0.03 ± 0.07	0.0	6.03	5.37
Oscillatoria sp.	0.02 ± 0.06	0.0	4.76	4.38
Diatoms	0.05 ± 0.09	0.0	10.95	**9.25**
H. azteca	0.00 ± 00	0.0	0.0	0.0
Organic matter	0.04 ± 0.08	0.0	7.62	6.45
Plant debris	0.008 ± 0.02	0.0	0.95	1.43

they were found only in *Z. tequila* in the single-species treatment. Chironomids, parts of insects and *D. pulex* contributed most to the differences in diet between treatments.

The number of prey taxa consumed by *P. mexicana* increased in the two-species treatment compared to the single-species treatment. Meanwhile, the number of prey taxa consumed by *Z. tequila* decreased (Table 3). The two treatments resulted in a good configuration (stress < 0.15) with the three-dimensional solution. Nested comparisons demonstrated differences in diet composition between treatments (ANOSIM, global $R = -0.50$, $p < 0.05$); paired comparisons revealed differences between the conspecific and the two-species treatments for both species (ANOSIM, *Z. tequila*: global $R = 0.24$, $p < 0.05$; *P. mexicana*: global $R = 0.76$, $p < 0.05$). The diets of the two fish species were also different in both treatments, and considering the R-value, were more similar in the two-species than in the single-species treatments (Fig. 1).

In this mesocosm experiment, *P. mexicana* showed a higher mean growth (single-species treatment: 6.51 mm ± 4.29 S.D.; two-species treatment: 9.04 mm ± 4.78 S.D.) compared to *Z. tequila* (single-species treatment: 5.59 mm ± 4.45 S.D.; two-species treatment: 6.33 mm ± 4.11 S.D.). The two-way ANOVA showed no differences in growth associated with species (ANOVA, $F_{1,16} = 0.04$, $p > 0.05$), treatment ($F_{1,16} = 0.04$, $p > 0.05$) or their interaction ($F_{1,16} = 0.04$, $p > 0.05$).

Survivorship and growth in aquariums

Survivorship of each species at each prey density is shown in Fig. 2. We detected significant differences in survivorship between the species using a three-way ANOVA ($F_{1,64} = 65.24$, $p < 0.001$) and among prey density levels ($F_{3,64} = 72.54$, $p < 0.001$) but not between treatments ($F_{1,64} = 2.21$, $p > 0.05$). There was also a significant interaction between species and prey density ($F_{3,64} = 21.76$, $p < 0.001$; $F_{1,64} = 1.63$, $p > 0.05$, respectively). In the single and two-species treatments, there was 100 % survivorship for both fish species at PD3 and PD4. However, the post hoc paired comparison showed that *P. mexicana* had higher survivorship at the two lowest chironomid availabilities (PD1 and PD2) in the single and two-species treatments com-

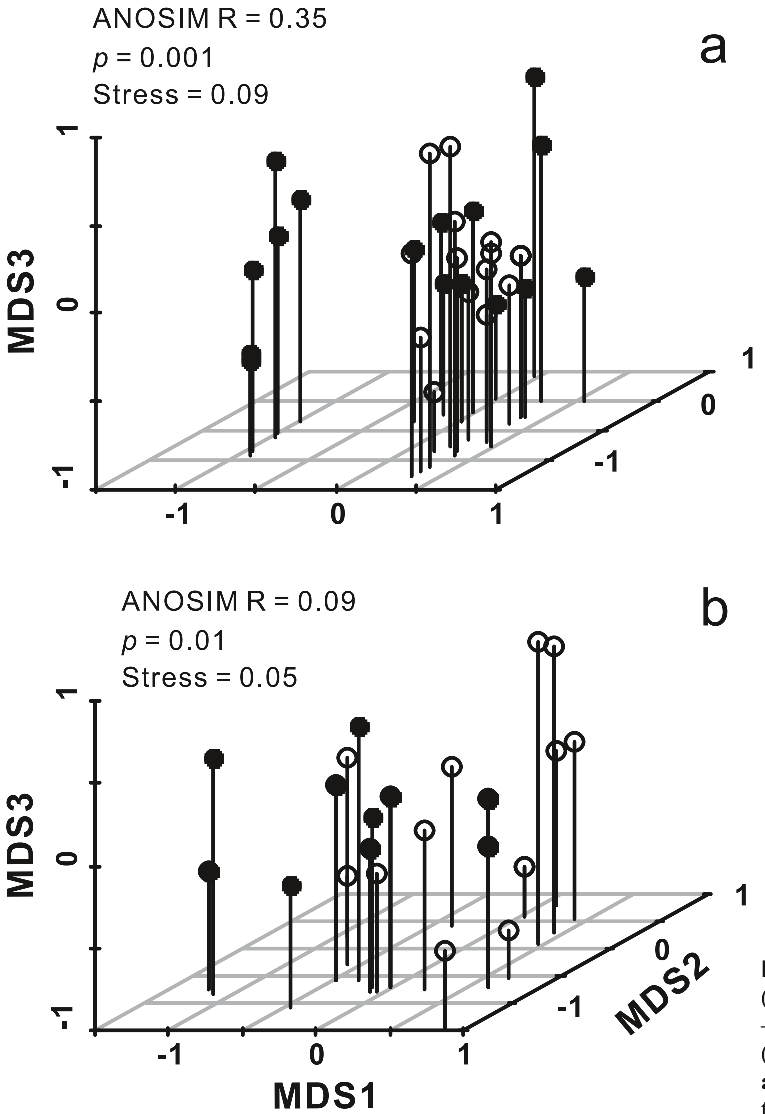

Fig. 1. Non-metric multidimensional scaling (NMDS) ordination of square root – and arcsine – transformed measures of the diets of *Z. tequila* (solid circles) and *P. mexicana* (open circles) in **a)** single-species treatments and, **b)** two-species treatments.

pared to *Z. tequila* at the same PDs. No differences between species were present at PD3 and PD4 in both treatments.

Growth of each species at each prey density is shown in Fig. 3. According to the three-way ANOVA (growth data were normally distributed), growth was affected by species (three-way ANOVA, $F_{1,64} = 22.38$, $p < 0.001$) and PD (three-way ANOVA, $F_{3,16} = 29.62$, $p < 0.001$) but not treatment (three-way ANOVA, $F_{1,16} = 0.28$, $p > 0.05$). The interaction of species-treatment had no effect (three-way ANOVA, $F_{1,16} = 0.786$, $p > 0.05$) on growth, but the other interactions were statistically significant. In the single-species treatment, *Z. tequila* had the lowest growth at PD1. *Poecilia mexicana* exhibited decreased growth at PD1 and PD2 compared with PD3 and PD4. However, *Z. tequila* grew less than *P. mexicana* at PD1 (p adj. = 0.001) in the single-species treatment. In the two-species treatment, *Z. tequila* showed a significantly higher growth than *P. mexicana* at PD1 (p adj = 0.017); at the other PDs, there was no difference in growth between the two species.

Discussion

The results in the present study suggest that *Z. tequila* alters its diet when it occurs with *P. mexicana*, supporting the hypothesis that non-native species influence the diet of native species, a phenomenon enhanced by diet overlap (Declerck et al. 2002; Vander Zanden et al. 2003). Also, the ordination analyses with permutations performed, allowed a robust interpretation of the dispersion data obtained from the diet of

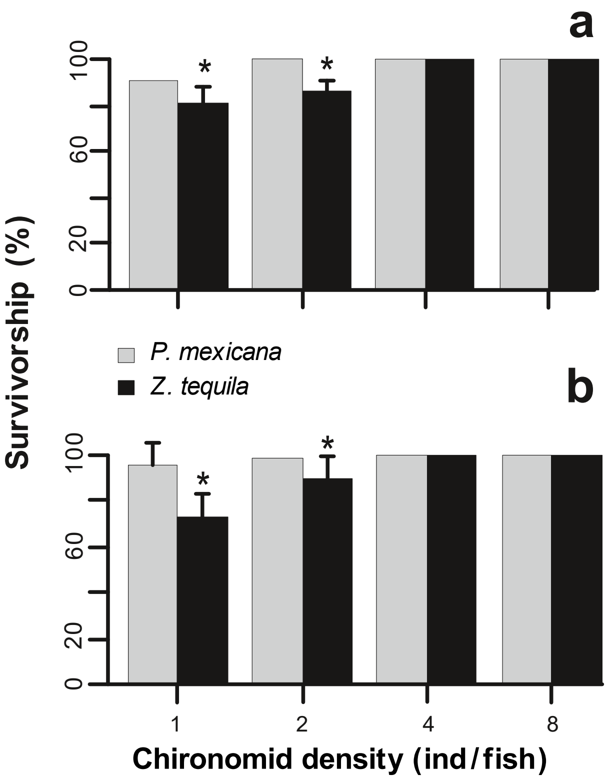

Fig. 2. Survivorship (%) of *Z. tequila* and *P. mexicana* in **a)** single-species and **b)** two-species treatment at different chironomid densities. *Z. tequila* = black bars and *P. mexicana* = grey bars. Error lines represent standard deviation, and asterisks (*) denotes significant differences in paired comparisons ($p < 0.05$).

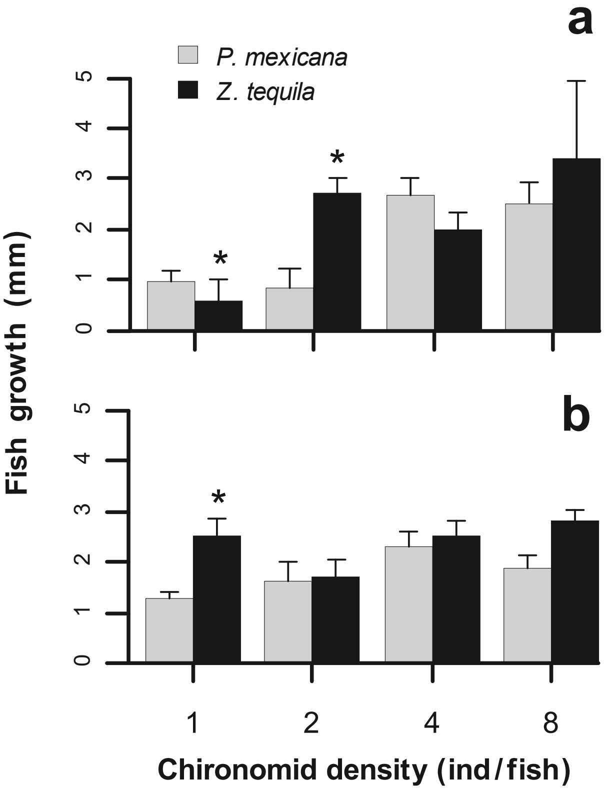

Fig. 3. Growth of *P. mexicana* and *Z. tequila* in **a)** single-species and **b)** two-species treatments varying chironomid densities. *Z. tequila* = black bars and *P. mexicana* = grey bars. Error lines represent standard deviation, and asterisks (*) denotes significant differences in paired comparisons ($p < 0.05$).

these two species. Using NMDS ordination to illustrate diet range, it appeared that there was higher overlap when species co-occurred. However, ANOSIM demonstrated that the diets of the two species did not significantly overlap in both treatments. This could be related to a higher variability in prey %V within than between the diets of the fishes and might explain the negative global *R*-value obtained (Chapman & Underwood 1999).

Food may be a commonly available resource for all individuals; however, it has been suggested that non-native species can force natives to shift their diet to less suitable resources (Werner & Hall 1977; Persson & Greenberg 1990). Chironomid larvae and *D. pulex* were the prey most often consumed by both species in both treatments. The protein content of chironomid larvae is 73.60 % (Yashouv 1956; Yashouv & Ben Shachar 1967), while it is between 62.50 % and 65.60 % for *D. pulex* (Watanabe et al. 1978; Webster et al. 1991). Thus both prey are good sources of protein for fish. If the consumption of chironomids and *D. pulex* increases in *Z. tequila* and decreases in *P. mexicana*, it is difficult to conclude that the native species is relegated to food sources with suboptimal properties when they occur with the non-native species. However, in the two-species treatment, insects were not found in *Z. tequila*, suggesting that *P. mexicana* may prevent *Z. tequila* access to prey from the surface and substrates of the waterbody to obtain insects, which represent 42–70 % of crude protein (Barroso et al 2014; Tran et al. 2015). This requires further testing to confirm whether that behaviour occurs.

It has been suggested that diet change in non-native species is a response to avoid interactions for food with the native fauna; in some cases this can result in a more diverse diet than that of the native species (Jackson & Britton 2013). In poecilids, gut plasticity and rapid changes in diet are related to changes in environmental conditions (Scharnweber et al. 2011; Hinojosa-Garro et al. 2013). *Poecilia mexicana* may be more efficient at modifying its diet to avoid competition and may be able to utilize different prey during periods of food scarcity, using a wider range of prey taxa than the native species. In turn, this practice could compensate for the decrease in consumption of other items. Non-native fish may consume different prey species and change food availability, affecting native fish growth (Carey & Wahl 2010). However, we did not find a direct effect on growth related to the co-occurrence of the non-native species. However, a lower growth in both species was observed at low PDs (i.e. PD1 and PD2). Therefore, differences in growth were related to prey abundance, not to interspecific interactions. The high protein content and digestibility of chironomid larvae promote growth in fish (Yashouv 1956; Yashouv and Ben Shachar 1967); therefore, in our experiments, growth was promoted even at low PDs. To find pronounced growth differences between native and non-native fish species, further experiments using prey species with essential or limited nutritional characteristics should be performed.

The survivorship of *Z. tequila* was lower than *P. mexicana* at low prey densities regardless co-occurrence. As in the growth experiment, the survivorship might be related to prey abundance and not the presence of *P. mexicana*. However, the results of the survivorship experiments suggest that, during long periods of low resource availability, the probability that the non-native species might survive and displace populations of the native species in nature is high. A possible explanation could be the interspecific and intraspecific increase of aggressive behaviour at low food availability in the presence of high fish density. In goodeids kept in captivity, an increase in levels of aggression related to interspecific fish density has been reported (Jones & Magurran 2014), as has a similar increase in aggressiveness by poeciliids in the presence of native species (Warburton & Madden 2003). In our survivorship experiments, only one prey taxon was used; therefore, aggressiveness could be higher at lower prey densities. In fact, in the mesocosm experiment, some males of the native species showed damage to the caudal fin, and in the survivorship experiment, *P. mexicana* was observed to chase and nip *Z. tequila*; that behaviour may prevent the native species from feeding and the resultant wounds could result in mortality (pers. obs.). Among conspecifics chasing behaviour was observed, but is difficult to conclude if this was related to aggressiveness or mating behaviour.

In general, successful non-native species tolerate a wide range of environmental factors (Moyle & Light 1996; Kolar & Lodge 2002). For example, *P. mexicana* occurs in a variety of habitats from light-limited caves, sulfidic waters, coastal lagoons, rivers and shallow pools in freshwater and brackish environments (Miller 2005; Tobler et al. 2008; Scharnweber et al. 2011) and withstands a wide range of temperatures (11.3 to 41.6 °C; Bierbach et al. 2010). Unfortunately, there is no information regarding the tolerance of goodeids to environmental factors, but the high vulnerability of many goodeid species (e.g. Domínguez-Domínguez et al. 2005; Bailey et al. 2007) suggests a low tolerance to habitat changes. Despite the potential differences in altitude between the experimental ponds (1900 m a.s.l.) and the habitat where the two species may co-occur (Teuchitlán River; 1311 m a.s.l.), the temperature used in both experiments had ranges similar to the natural habitat (Kingston 1978; De La Vega-Salazar et al. 2003a; López-López et al. 2004; Tejeda-Vera et al. 2007); therefore, the high tolerance of non-native species to environmental factors, the wide differences in the thermal physiological traits and high plasticity in diet could be related to their ability to survive long periods of starvation when conditions become harsh. In addition, the diet and feeding habits of *Z. tequila* in the wild are unknown, but considering possible similarities with the diet of its congener, *Z. quitzeoensis*, it may have a narrow diet composed mainly of insects, amphipods and cladocerans, making this native species carnivorous and susceptible to low survivorship when high-protein invertebrates (e.g. insects such as chironomids) are scarce. Those traits may provide the non-native fish more advantages than native fish, enhancing the probability of establishment in new habitats even when the aquatic habitats are degraded.

This study demonstrated that a non-native poeciliid promoted changes in diet composition of a native species and exhibited higher survivorship at low food availability in comparison to the native species. It is important to note that we did not find major effects on the growth of the native species in the presence of the non-native species. The lack of effect could indicate that 30 days was not an adequate period during which strong inter- and intraspecific interactions might develop. In addition, potential competitive interactions in the mesocosm experiment could have been weak in relation to high prey density, or the fish density used (0.2 and 0.3 ind L^{-1}) in this study may have been low.

Future studies using a higher fish density and lower prey density are needed to evaluate if stronger effects result from the interaction between the native and non-native species. Furthermore, our results are based on observations and data obtained in laboratory conditions and from individuals bred after many generations of captivity. As mentioned above, there are no published data about tolerance of *Z. tequila* to environmental factors; therefore, our results may be applied regarding the temperature range used in the present study, since the temperature range may be higher in the experimental tanks and lower in the natural water bodies. Moreover, the behaviour of captive-reared goodeid populations may produce different results compared to wild populations (Kelley et al. 2005). Thus, to fully understand relationships between non-native poeciliids and goodeids, long-term experimental and environmental research in the wild and in the laboratory should be considered using goodeid species still common in the wild to compare with non-native species. Multiple interactions are most likely to occur between species with very similar life-history traits (Mills et al. 2004). Thus, goodeids may be more susceptible to extinction in freshwater environments where non-native poeciliids have been introduced. Poeciliids invaded the Teuchitlán River at least since 1977 (Webb & Miller 1998) with the establishment of *P. mexicana* around 1990 (Miller 1989), suggesting that biological interactions between *Z. tequila* and poeciliids could have occurred in freshwater localities. Because the ecological responses of non-native species to new environments, and their effects on native fauna (e.g., goodeids), are not fully understood, the findings in this study contribute to a better understanding of native species responses to non-native species, and provide evidence about the possible mechanisms by which non-native fish affect natives species in freshwater environments, where the impact of small non-native fish species has not been fully considered.

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