

Population growth and behavioural interactions of a critically endangered fish with co-occurring native and exotic species

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Abstract

1. Invasive species represent a threat to biodiversity and ecosystem services and cost millions of dollars to the global economy. The viviparous Mexican fish known as the tequila splitfin (*Zoogoneticus tequila*) became extinct in the wild as a consequence of habitat loss and degradation, and interactions with invasive species. Tequila splitfins are native to the Teuchitlan River in Central Mexico; they were kept in captivity and reintroduced into their native distribution in 2016. Approximately 80% of the fish in the Teuchitlan River are exotic species, and over 50% are twospot livebearers (*Pseudoxiphophorus bimaculatus*).
2. We performed an ex-situ mesocosm experiment to explore whether tequila splitfin populations would establish and increase, and how fish would behave when introduced into sites already colonised by other species. We tested this idea by introducing tequila splitfin individuals into empty mesocosms, mesocosms where individuals of the native *Ameba splendens* had established, mesocosms with individuals of the native *Goodea atripinnis* and mesocosms with individuals of exotic invasive twospot livebearers. All heterospecific species have been recorded in tequila splitfin's native range, are viviparous fish and share ecological requirements with them.
3. We found that tequila splitfin abundance (number of individuals that survived and new individuals) was greater when sharing mesocosms with native species. Furthermore, they had reduced activity levels when inhabiting mesocosms with exotic invasive twospot livebearers, in comparison, interactions with natives proved to be beneficial.
4. Our results highlight the need to remove exotic invasive species and protect other native species to increase reintroduction success. Close monitoring is needed during the initial stages of the reintroduction, and several reintroduction events from captive breeding facilities may also be necessary. When planning a reintroduction, it is critical to remove exotic species and make an effort to restore the habitat as close as possible to the original conditions.

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KEYWORDS

endangered species, heterospecific interactions, restoration ecology, species reintroduction

1 | INTRODUCTION

The success of a reintroduction programme is multifactorial. Understanding the interactions between reintroduced individuals and the biotic and abiotic factors they encounter in their now modified native ranges, provides information that improves the chance of reintroduction success (Cochran-Biederman et al., 2015; Fischer & Lindenmayer, 2000; Pérez et al., 2012; Wedderburn et al., 2020). Behaviour is likely to be a critical factor impacting reintroduction, as it comprises the catalogue of immediate animal responses to the environment. Behaviour could therefore affect population dynamics and demography (Shumway, 1999). For example, Trinidadian guppies (*Poecilia reticulata*) have been documented as a highly successful species in almost every site where they have been introduced, in part because of their high behavioural plasticity, which allows them to adjust and modify their behaviour according to their environmental conditions (Dugatkin & Godin, 1992). Guppies and other successful species are bolder or take more risks, have low neophobia, evaluate predators, and exploit unknown resources (Deacon & Magurran, 2016). Additionally, guppies can use social learning to follow conspecifics to reach a new food source (Laland & Reader, 1999).

Some reintroduction programmes have failed due to a lack of previous knowledge of the interactions between the reintroduced species and conspecifics and heterospecifics living in a particular area (McPhee, 2004). For example, reintroduced Atlantic salmon in the Connecticut River basin experienced low recruitment and survival when coexisting with a native generalist predator, showing how interactions between species are important to reintroduction programmes (Ward et al., 2008). Exotic invasive species are a significant threat to ecosystem function, their impacts are generally irreversible, and humans often have no control over them (Lockwood et al., 2013). In successful invasions, the exotic species have behavioural, morphological, or even physiological characteristics that give them an advantage in terms of colonising and successfully surviving and reproducing (Lodge, 1993; Rehage & Sih, 2004). When a recently reintroduced species shares similar ecological niches with a native or invasive species that is already established in the habitat, the interactions with those species is a potentially critical factor to reintroduction success, and therefore needs further research prior to implementing reintroduction programmes.

Poeciliid fish are among the most common exotic and invasive species in Mexican freshwater ecosystems. They were introduced as mosquito biocontrol agents and by aquarists as pets and as bait for larger fish (De la Vega-Salazar, 2006; Ramirez-Garcia et al., 2018). Poeciliid fishes have been introduced to several aquatic systems throughout the country, usually with adverse effects on native species (Grapputo et al., 2006; Man & Hodgkiss, 1981; Vera et al., 2016). One such group of native species are members of the Goodeinae

subfamily, which comprises c. 40 species endemic to the Mexican Central Plateau. Exotic invasive species (including poeciliids) are the second main cause of their population declines, preceded only by habitat degradation (Gesundheit & Macias, 2018). As a result, most goodeids are endangered, according to the International Union for Conservation of Nature (IUCN, 2021).

Poeciliid and goodeid fish are ecologically similar. Both fish groups are of similar size and share food and habitat types, such that they compete directly for resources (Ramírez Carrillo & Macias Garcia, 2015). Behavioural responses from poeciliids such as aggressiveness and boldness reportedly contribute to their success as invasive species (Rehage et al., 2005; Rehage & Sih, 2004). For example, poeciliid aggression towards goodeid females may increase females' predation risk (Dill et al., 1999), reduce their foraging (Magurran & Seghers, 1994), injure them during forced copulation (Valero et al., 2008), and increase their metabolic demands (Valero et al., 2005). Therefore, heterospecific behavioural interactions of these groups should be evaluated during a reintroduction programme.

The tequila splitfin (*Zoogoneticus tequila*) is a fish species that is endemic to the Teuchitlan River in the state of Jalisco, Mexico (Miller et al., 2005; Webb & Miller, 1998). It is currently in the Endangered category (IUCN, 2021). This species has been at risk mainly because the Teuchitlan River suffered significant habitat degradation following the construction of a dam in the 1950s and the establishment of exotic fish (De La Vega-Salazar et al., 2003a; Mar-Silva et al., 2021). Tequila splitfins were reportedly extinct in the wild, even before being formally described from captive populations and museum collections in 1998 (Webb & Miller, 1998). However, in 2003 a population of fewer than 50 individuals was found in an isolated water body in the Teuchitlan springs (De La Vega-Salazar et al., 2003b). In 2006, tequila splitfins were documented as critically endangered, with 99.5% of their historically reported populations extinct, and the presence of invasive fish was recognised as a major threat (De la Vega-Salazar, 2006).

The first reports of poeciliid introductions into the Teuchitlan River are from 1977 (Webb & Miller, 1998). *Poecilia mexicana* is recognised as one of the first exotic poeciliids to become established in the river around 1989. Given that declines in tequila splitfin populations started around the same time *P. mexicana* was introduced, it is believed that sharing habitat with *P. mexicana* contributed to its extinction (Escalera-Vazquez et al., 2016). Today, the Teuchitlan river hosts *P. mexicana* as well as several additional exotic poeciliid species: *Pseudoxiphophorus bimaculatus*, *Poecilia sphenops*, *Xiphophorus helleri*, and *Xiphophorus maculatus* (Hernandez-Morales et al., 2020; Mar-Silva et al., 2021; Ramirez-Garcia et al., 2018).

Tequila splitfins have been bred in captivity in museums, zoos, universities, and private aquariums in North America and Europe, mostly linked and organised by the Goodeid Working Group

(Arbuatti et al., 2013). In 2016, Domínguez-Domínguez led a project sponsored by several associations and societies to breed tequila splitfins and reintroduce them into the Teuchitlan River. The first steps of this project included a characterisation of the habitat where the population was to be reintroduced. The fish community in the Teuchitlan River was heavily dominated by twospot livebearers (*P. bimaculatus*, Heckel 1848), making up 59.8% of the individuals collected during their survey (Hernandez-Morales et al., 2020; Mar-Silva et al., 2021). The native goodeid species blackfin goodea (*Goodea atripinnis*), butterfly splitfin (*Ameba splendens*), and Tarascan splitfin (*Zoogoneticus purhepechus*) were also present at the site, but their abundances were significantly lower: 4.4%, 3.3%, and 1.9%, respectively (Hernandez-Morales et al., 2020; Mar-Silva et al., 2021).

Using an ex-situ mesocosm approach, we investigated how tequila splitfins behave and whether populations would tend to grow or decrease when being introduced into an environment colonised by other fish species. To further investigate whether population dynamics and behavioural responses were due to an exotic invasive species or simply to other ecologically similar species, we compared these dynamics when coexisting with native *A. splendens* or *G. atripinnis* versus when coexisting with the exotic invasive *P. bimaculatus*. Our results could help predict and explain the outcomes of the reintroduction project and design new reintroduction plans for tequila splitfins and other goodeid species that are extinct in the wild but have been bred in aquariums.

2 | METHODS

This experiment was carried out at the Instituto de Investigaciones en Ecosistemas y Sustentabilidad (IIES) at UNAM in Morelia, Mexico, from October 2015 to December 2016. Butterfly splitfins, blackfin goodeas, and twospot livebearers were obtained from the aquarium of the Aquatic Biology Lab of the Universidad Michoacana de San Nicolas de Hidalgo (UMSNH). Tequila splitfins were collected from a pond in the Botanical Garden of the UMSNH in Morelia, where they were reared. This species has been bred for research and reintroduction. The fish were carefully transported to the IIES in breathable bags. Twenty c. 100-L mesocosms (80×40×40 cm) were set up inside a shade house, which allowed them to experience outdoor environmental conditions while keeping birds and other animals from preying on experimental fish. The mesocosms were lined with a net (3-mm light mesh) to facilitate recording and identifying all fish accurately by lifting it. Each mesocosm contained two large plastic plants and a brick over the mesh, to include the same environmental complexity in each mesocosm. We drilled one 5-cm diameter hole at the top of each mesocosm for drainage in case of flooding due to rain. We filled all mesocosms with 90 L of tap water and left them open for 1 week to eliminate any traces of chlorine (i.e., aged tap water). The mesocosms were self-maintained; thus, no food, vitamins, or antibiotics were added at any time during the experiment.

We set up four mesocosm treatments with four replicates each: (1) control: only tequila splitfins; (2) five male–female butterfly

splitfin pairs; (3) five male–female blackfin goodea pairs; and (4) five male–female twospot livebearer pairs. Once in the shade house, the fish were introduced into their experimental mesocosms in floating plastic containers filled with water from their aquarium tanks to allow them to acclimate to the temperature. We based the fish density on a similar experiment with the same species in which 30 adult fish were introduced into a similar size mesocosm, which had fish survival probability near 100% (Escalera-Vazquez et al., 2016).

All fish except the tequila splitfins were introduced on the same date (11 October 2015) with 1 L of water from their original aquariums into the mesocosm. They were then allowed to establish for 3 weeks before introducing the tequila splitfins. After this period (11 October to 1 November 2015), we introduced three male–female tequila splitfin pairs into each of the 20 mesocosms. At the same time, we added 10 L of water from the captive breeding habitat of tequila splitfins (semi-natural pond at Botanical Garden of the Universidad Michoacana de San Nicolas de Hidalgo, UMSNH) to each mesocosm. Food was not actively added to the mesocosms, but this water could carry some invertebrate propagules, bacteria, and algae from the original habitat. Approximately once a week, aged tap water was added to the mesocosms to compensate for evaporation. We recorded the water temperature every hour during the entire duration of the experiment using data loggers (MicroLite Lite 5016, Fourier Systems).

2.1 | Population growth

The individuals in each mesocosm were counted and identified by lifting the mesocosm mesh during each visit. From 1 November to 6 December 2015 (37 days), we visited mesocosms to check on tequila splitfins' mortality during their establishment period. We replaced dead tequila splitfins weekly during this period in each mesocosm, since there was a high rate of mortality of the tequila splitfins during the first days after introduction. We calculated the mortality rate of tequila splitfins during its establishment period and tested whether mortality depended on temperature (max and min), date (categorical variable with five levels, corresponding to each of the five dates on which tequila splitfins were introduced), and/or treatment.

From 7 December 2015 (beginning of the experiment) to 1 September 2016 (end of the experiment), all individuals in each mesocosm were counted and identified every c. 45 days. Juveniles were recorded at approximately 1 cm standard length when they could be reliably identified to the species level. We calculated tequila splitfins' intrinsic population growth rate (r) over the entire experimental period (7 December 2015 to 1 September 2016) using the number of births and deaths in the mesocosms during each time interval (c. 45 days; Gotelli, 2008). We explored whether the r varied depending on the species in the mesocosm and the date. We also tested whether the mean abundance of tequila splitfins differed among treatments and time intervals. We implemented sets of linear mixed models using mesocosm identity as the random factor for these analyses.

2.2 | Behavioural observations

We conducted behavioural observations 13 times throughout the experiment to assess potential differences in tequila splitfins' behaviour when alone and in the company of natives or invaders. The observer (M.C.C.) sat in a chair in front of each mesocosm at 1 m distance. The observer was careful not to cast a shadow on the surface, but we could not account for the visibility of her body from the mesocosms, so she sat still for 3 min before recording fish behaviour to allow them to acclimatise to her presence. After this time, she recorded the number and species of fish visible at the surface, where they are more exposed to danger (risk-taking behaviour). During each visit, two fish were observed in each mesocosm. M.C.C. observed a first fish that she chose randomly, recording its behaviour (see below). Immediately after she visually lost this fish, she switched attention to another fish right away. This process ensured that no fish was observed twice. She recorded whether each focal fish followed other fish, had social encounters, and how much time it kept still or was swimming. We divided the behavioural observations into three types to improve clarity: risk-taking behaviour, sociability, and activity level.

2.2.1 | Risk-taking behaviour

Our proxy to assess fish risk-taking behaviour was tequila splitfins' tendency to remain visible on the water's surface, where they would be more vulnerable to predators (Ioannou et al., 2008). The number of tequila splitfins on the water's surface was used as a variable, where the number of visible fish was a proportion of the total fish in the mesocosm. We implemented a generalised linear mixed model with a binomial error distribution and logit link function to determine whether a similar proportion of tequila splitfins remained visible in all treatments. We used treatment as an independent factor and proportion of heterospecific fish at the surface of the mesocosm as a covariable. Given that we had mesocosms with no heterospecific fish, we were not able to test the interaction between the factor and the covariable. However, we implemented a second set of models where we excluded mesocosms with only tequila splitfins to test the interactions. Mesocosm identity was declared as the random factor in all models.

2.2.2 | Sociability

The tendency of tequila splitfins to socialise with other fish (i.e., follow or remain in close proximity to others) was compared among treatments and shoal composition (fish identity) as a categorical predictor (fish identity; conspecific, heterospecific, and mixed). We declared time of association and time following as binomial response variables (in independent analyses) composed of the time tequila splitfins associated/followed others and the total observation time. In this case, we first tested the interaction between treatments and shoal/fish identity, excluding mesocosms with only tequila splitfins

because there were no heterospecific fish. In a second set of models, we compared the time tequila splitfins associated with conspecifics among all treatments. In all models, mesocosm identity was declared as the random factor.

2.2.3 | Activity

Finally, to assess the level of activity tequila splitfins displayed during their time unaccompanied (when fish were not interacting with other individuals), we compared the time tequila splitfins remained still or were swimming when unaccompanied. Treatment and activity (swimming or still) were independent factors. This dependent variable was also declared as bimodal, formed by the time they were unaccompanied and the total observation time.

2.3 | Statistical analysis

To arrive at the most supported model we used a model selection approach based on the Akaike information criterion (adjusted for small sample sizes; AICc). Model selection using AICc allowed us to test a set of a priori hypotheses and quantify the evidence within our data to rank them (Burnham et al., 2011). We used the approach of competing hypothesis (information-theoretic inference) rather than frequentist distributions (null-hypothesis testing; please see Burnham et al., 2011).

AICc was used to select the models (i.e., hypotheses) that best explained our data (Burnham & Anderson, 2002). The lowest value of AICc indicates the model with the best fit to the data, and a difference larger than two units in the AICc values ($\Delta\text{AICc} > 2$) between each model and the best-fitting model indicates important differences in their fit (Johnson & Omland, 2004). When two or more models have lower values of ΔAICc , the most parsimonious model was chosen. Statistical analyses and plots were constructed in the program R (R Core Team, 2021); we provide the complete code implemented in the Supplementary Materials.

3 | RESULTS

3.1 | Population growth

Tequila splitfins' mortality during its establishment period was not related to treatment or temperature; however, it differed among the five dates tequila splitfins were introduced (model: mortality~date+mesocosm identity, AICc = -55.7, see the complete model selection in Table S1). The highest mortality occurred during the middle of the reintroduction establishment period (mean mortality rate \pm standard error; on 10 November mortality rate was 0.28 ± 0.04 , while on 2 November it was 0.10 ± 0.04 , 7 November 0.16 ± 0.05 , 20 November 0.05 ± 0.02 , and 1 December 0.08 ± 0.03 ; Figure S1).

The intrinsic population growth rate of tequila splitfins (7 December 2015 to 1 September 2016) did not differ among treatments or time intervals. Of the four models tested (see Table S2), the null model (intercept only) had the best fit (AICc for null model = 59.9), followed by the model that included the treatment ($\Delta\text{AICc} = 14.4$; Figure 1a).

Tequila splitfin abundance was best explained by the model including treatment (best model AICc = 404.5), followed by the model with treatment and date ($\Delta\text{AICc} = 5.18$; see Table S3). Tequila splitfins were most abundant when they were alone (mean abundance \pm standard error; 6.1 ± 0.27), followed by when they cohabited with blackfin goodea (5.58 ± 0.13), with butterfly splitfins (5.07 ± 0.32), while the mesocosms with the fewest tequila splitfins were those where they cohabited with twospot livebearers (3.8 ± 0.22 ; Figure 1b; see Table S3.1).

3.2 | Behavioural observations

3.2.1 | Risk-taking behaviour

The proportion of tequila splitfins visible at the surface was best explained by the model including mesocosm treatment and the proportion of heterospecific fish visible (AICc = 706.5), followed by the model with only the proportion of heterospecific fish ($\Delta\text{AICc} = 13.94$; see Table S4). The proportion of tequila splitfins at the surface was positively related to the proportion of heterospecific fish ($\beta = 1.92 \pm 0.32$; Figure 2a); more tequila splitfins were visible in mesocosms where they cohabited with blackfin goodea (mean proportion \pm standard error; 0.39 ± 0.03), followed by mesocosms with butterfly splitfins (0.34 ± 0.02), which were similar to mesocosms with only tequila splitfins ($\beta = 0.32 \pm 0.03$). Tequila splitfins were least likely to be visible in mesocosms with twospot livebearers (0.27 ± 0.03 ; Figure 2b).

When we excluded the control mesocosms to consider the interaction between treatment (heterospecific species) and proportion of heterospecific fish visible, the model including the interaction was the best supported (AICc = 472), followed by the model containing the additive effect of the two variables ($\Delta\text{AICc} = 11.84$; Figure 2c; see Table S5). This interaction showed that the positive relationship between the proportion of visible tequila splitfins and proportion of visible heterospecific fish was stronger when the heterospecific fish were blackfin goodea than when the heterospecific fish were butterfly splitfins ($\beta = -2.39 \pm 0.80$) and was weakest when cohabiting with the invasive species than with either of the other two goodeids (compared with butterfly splitfins $\beta = 2.19 \pm 0.78$, and with blackfin goodeas $\beta = 2.39 \pm 0.80$).

3.2.2 | Sociability

Excluding the control mesocosms, we found that the time tequila splitfins associated with other individuals was explained by the interaction between treatment in the mesocosm (fish species cohabiting with tequila splitfins) and the fish species they were associating with (conspecific, heterospecific, or mixed individuals; AICc = 3,775.5) followed by the model with the variables as principal effects ($\Delta\text{AICc} = 477.73$; see Table S6). In treatments where they cohabited with goodeids, tequila splitfins spent more time in association with a mixed shoal (mean proportion of time \pm standard error; butterfly splitfins 0.38 ± 0.04 , blackfin goodeas 0.27 ± 0.03); meanwhile, in mesocosms where they cohabited with invaders, tequila splitfins associated only with conspecifics (0.01 ± 0.04 ; Table 1a and Figure 3a).

When comparing only the time of association with conspecifics in the analysis that included all treatments, we found that it varied among treatments (AICc = 2,201.6, followed by the intercept only model $\Delta\text{AICc} = 4.99$; see Table S7). Tequila splitfins spent more time associating with conspecifics in mesocosms with only

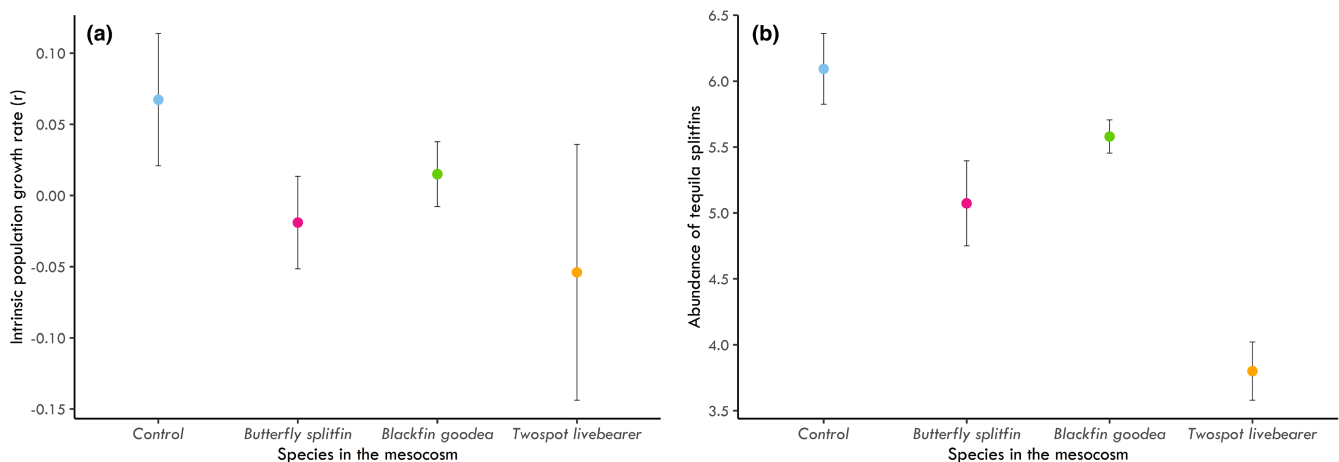


FIGURE 1 (a) Intrinsic population growth rate (r) of tequila splitfins among treatments in the mesocosms (mean \pm standard error); there were no statistically supported differences among treatments. (b) Abundance of tequila splitfins among treatments (butterfly splitfin 5.07 ± 0.32 , tequila splitfins 6.09 ± 0.26 , blackfin goodea 5.58 ± 0.13 , twospot livebearer 3.80 ± 0.22). Vertical lines represent standard errors.

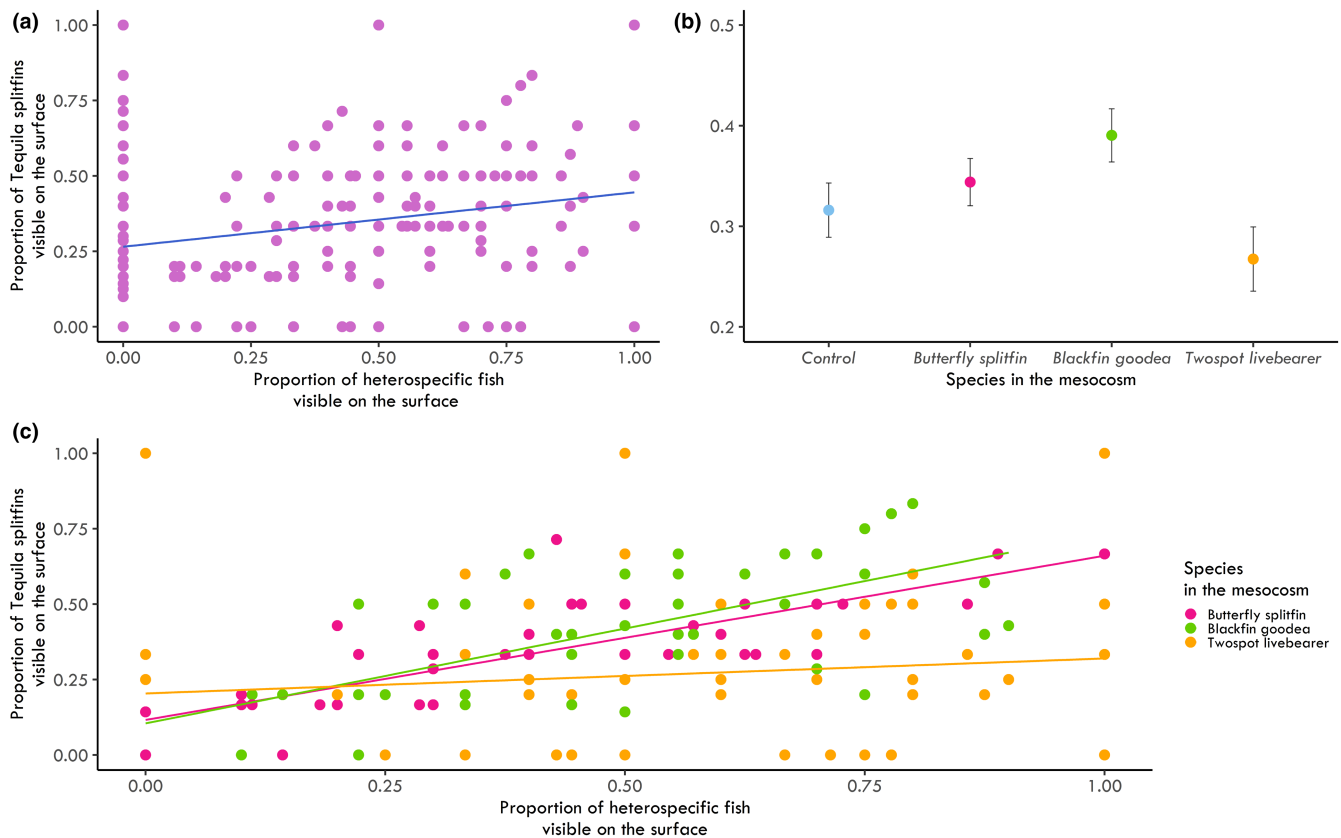


FIGURE 2 Risk-taking behaviour represented by the proportion of tequila splitfins at the surface of the mesocosms. When including all treatments, there was a positive relationship between the proportion of tequila splitfins at the surface and the proportion of heterospecific fish at the surface (a), and the proportion of tequila splitfins at the surface differed among the treatments (b; mean \pm standard error). (c) Among the four heterospecific treatments, the strength of the relationship between the proportion of tequila splitfins and the proportion of heterospecific fish at the surface of the mesocosms differed among the treatments (important interaction effect in analysis excluding control mesocosms).

conspecifics (mean proportion of time \pm standard error; 0.32 ± 0.05), followed by the treatment where they cohabited with blackfin goodeas (0.16 ± 0.03), then where they were with butterfly splitfins (0.09 ± 0.02) and associated less with each other when cohabiting with the invasive twospot livebearers (0.08 ± 0.04 ; Table 1b and Figure 3b).

The time tequila splitfins spent following other fish in each treatment depending on the fish species (model: treatment \times shoal/fish identity; $AICc = 1,327.3$, followed by the model with the same variables as principal effects $\Delta AICc = 11.74$; see Table S8). When tequila splitfins cohabited with butterfly splitfins, they spent the most time following conspecifics (mean proportion of time \pm standard error; 0.08 ± 0.02). In mesocosms where they cohabited with blackfin goodeas, they spent similar amounts of time following conspecifics (0.05 ± 0.01) and heterospecifics (0.05 ± 0.01). In mesocosms where they cohabited with invasive twospot livebearers, they spent more time following conspecifics (0.03 ± 0.05) than heterospecifics (0.01 ± 0.01 ; Table 2a and Figure 4a).

When comparing the time tequila splitfins followed conspecifics, we found differences among treatments ($AICc = 1,426.4$), followed by the model with the intercept only ($\Delta AICc = 8.58$; see Table S9). Tequila splitfins spent less time following conspecifics

when cohabiting with twospot livebearers (mean proportion of time \pm standard error; 0.03 ± 0.02), similar to mesocosms with blackfin goodeas (0.04 ± 0.01). They followed conspecifics for more time when they were alone (0.14 ± 0.04), followed by when they were with butterfly splitfins (0.08 ± 0.02 ; Table 2b and Figure 4b).

3.2.3 | Activity

The time tequila splitfins spent swimming or remaining still when they were unaccompanied depended on the mesocosm treatment ($AICc = 4,450.5$), followed by the model with the same variables as principal effects $\Delta AICc = 578.03$; see Table S10. In the treatment where tequila splitfins cohabited with twospot livebearers, they remained still longer than in any other treatment (mean proportion of time \pm standard error; 0.68 ± 0.06). Interestingly, the second treatment where they remained still was the control (0.28 ± 0.04). However, the time tequila splitfins spent swimming was similar in all treatments (blackfin goodeas 0.22 ± 0.02 , butterfly splitfins 0.20 ± 0.03 , twospot livebearers 0.20 ± 0.04 , control 0.25 ± 0.03 ; Figure 5 and Table 3).

TABLE 1 Results from best models exploring the sociability of tequila splitfins, quantified as the time they associated with others. (A) Analysis excluding the treatment with only tequila splitfins (model: time of association/total time observed ~ treatment × shoal composition + mesocosm identity). (B) Analysis including all treatments evaluating the time tequila splitfins spent with conspecifics (model: time of association/total time observed ~ treatment + mesocosm identity).

Treatment compared to the intercept	Conspecifics Tequila splitfins	<i>Ameca splendens</i> Butterfly splitfins	<i>Goodea atripinnis</i> Blackfin goodea
(A)			
Conspecifics (intercept) versus Heterospecifics			
<i>A. splendens</i>			
Butterfly splitfins			
<i>G. atripinnis</i>			
Blackfin goodea		0.03 ± 0.16	
<i>Pseudoxiphophorus bimaculatus</i>			
Twospot livebearer		-20.39 ± 129.16	-17.2 ± 556.37
Conspecifics (intercept) versus Mixed			
<i>A. splendens</i>			
Butterfly splitfins			
<i>G. atripinnis</i>			
Blackfin goodea		-1.37 ± 0.12	
<i>P. bimaculatus</i>			
Twospot livebearer		-20.00 ± 69.04	-18.19 ± 596.94
Heterospecific (intercept) versus Mixed			
<i>A. splendens</i>			
Butterfly splitfins			
<i>G. atripinnis</i>			
Blackfin goodea		-1.40 ± 0.14	
<i>P. bimaculatus</i>			
Twospot livebearer		-1.74 ± 1,198.76	-0.67 ± 86.42
(B)			
<i>A. splendens</i>			
Butterfly splitfins	-1.81 ± 0.89		
<i>G. atripinnis</i>			
Blackfin goodea	-0.89 ± 0.83	0.93 ± 0.89	
<i>P. bimaculatus</i>			
Twospot livebearer	-3.14 ± 0.91	-1.33 ± 0.96	-2.25 ± 0.91

Note: In both A and B, the reference levels (intercept) are shown in columns. Numbers represent estimated differences (regression coefficient estimates ± standard errors).

4 | DISCUSSION

Exotic invasive species threaten native species, frequently by expressing behaviours that provide them with advantages in colonising heterogeneous and fluctuating habitats. The presence of invasive species should therefore be considered problematic for reintroduction programmes in which the genetic variability and plasticity of reintroduced species may be compromised. In our experiment, the presence of invasive twospot livebearers resulted in a reduced abundance of tequila splitfins and changed their behaviour. In the presence of invaders, tequila splitfins spent less time interacting between them. Tequila splitfins avoided interactions with twospot livebearers apparently preferring to interact with other species of goodeids, but in general when coexisting with invaders they were more time immobile. These changes in the

behaviour of tequila splitfins may restrict behaviours related to their reproduction and development, such as foraging, courtship, and mating, and this decrease in activity could contribute to their lower abundance in mesocosms where they cohabited with the invasive species. Escalera-Vazquez et al. (2016) found that tequila splitfins had a more diverse diet composition when alone in a mesocosm than when sharing it with the invasive twospot livebearers, and that twospot livebearers had higher survival rates than tequila splitfins when food abundance was low. Little research has been carried out on the mechanisms of twospot livebearers' invasion. However, guppies (*Poecilia reticulata*), a closely related species, benefit from interactions with heterospecific native goodeids, such as transmission of information (Camacho-Cervantes et al., 2015), foraging efficiency (Camacho-Cervantes et al., 2014), and showing more risk-taking behaviour (Santiago-Arellano

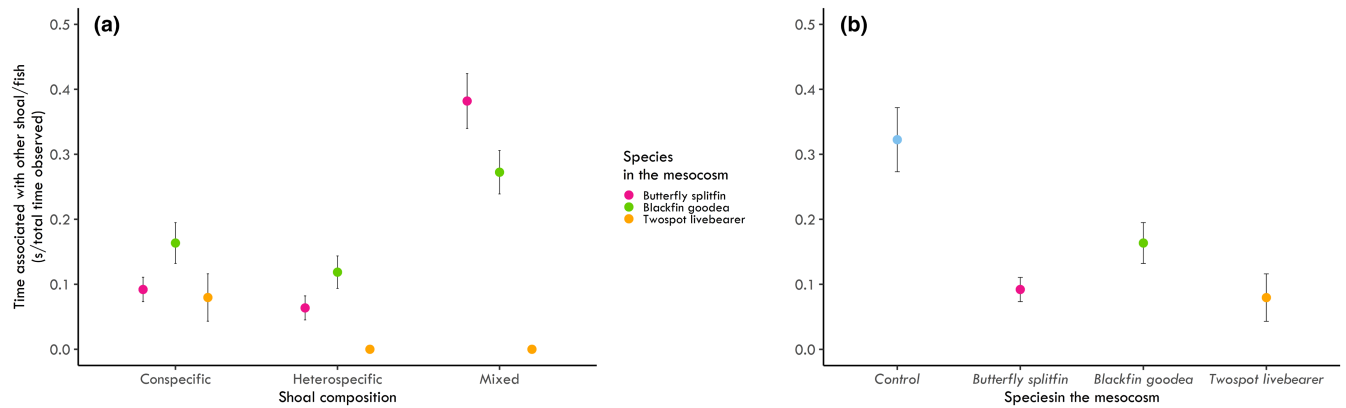


FIGURE 3 Sociability of tequila splitfins measured as the time they associated with other fish (a) as a function of the mesocosm treatment and the shoal composition (mean \pm standard error). In this analysis we excluded the mesocosms with only tequila splitfins to test the interaction between variables. (b) Time tequila splitfins associated with conspecifics among treatments. Vertical lines represent standard errors.

TABLE 2 Results from the best models exploring the sociability of tequila splitfins quantified as the time they spent following other fish. (A) Analysis excluding the treatment with only tequila splitfins (model: time following/total time observed \sim treatment \times shoal/fish identity + mesocosm identity). (B) Analysis including all treatments, but only conspecific interactions of tequila splitfins (model: time of association/total time observed \sim treatment + mesocosm identity).

	Conspecifics	<i>Ameba splendens</i>	<i>Goodea atripinnis</i>
Treatment compared to the intercept	Tequila splitfins	Butterfly splitfins	Blackfin goodea
(A)			
Conspecifics (intercept) versus Heterospecifics			
<i>A. splendens</i>			
Butterfly splitfins			
<i>G. atripinnis</i>		0.82 \pm 0.21	
Blackfin goodea			
<i>Pseudoxiphophorus bimaculatus</i>		0.03 \pm 0.36	-0.78 \pm 0.002
Twospot livebearer			
(B)			
<i>A. splendens</i>	-0.54 \pm 0.67		
Butterfly splitfins			
<i>G. atripinnis</i>	-1.35 \pm 0.64	-0.81 \pm 0.67	
Blackfin goodea			
<i>P. bimaculatus</i>	-2.89 \pm 0.74	-2.35 \pm 0.78	-1.54 \pm 0.75
Twospot livebearer			

Note: Numbers represent estimated differences (regression coefficients; estimates \pm standard errors).

et al., 2021). At the same time, goodeids often show adverse effects from interactions with poeciliids (e.g., decreased foraging efficiency; Camacho-Cervantes, 2019). Interestingly, the population growth rate of tequila splitfins did not vary among treatments, probably because there was high mortality rate as well as high birth rate and vice versa in all of the treatments. Nevertheless, we cannot ignore the large variability of the r within mesocosms where they cohabited with twospot livebearers, which together with the sample size, might not have allowed us to reach the statistical power required to detect differences.

Poeciliids in general, have been described as aggressive species, probably contributing to their success as invaders (Valero et al., 2008). Twospot livebearers share some ecological

requirements with *Girardinichthys multiradiatus*, a goodeid species that is closely related to tequila splitfins. Their similarities facilitated twospot livebearers' colonisation in the same habitats (Ramírez Carrillo & Macías García, 2015). Our results show that tequila splitfins avoid twospot livebearers. The risk-taking behaviour of tequila splitfins, which reflects the trade-off between, for example, foraging and danger of predation, was affected by the identity of the heterospecific fish present. Animals constantly assess costs and benefits of risk-taking behaviour, when benefits outweigh risks of encountering predators or competition animals behave bolder (Reale et al., 2007). Tequila splitfins approached the surface less often when twospot livebearers were present, and they were at the surface more when other heterospecific fish were visible. Exposing to the surface may

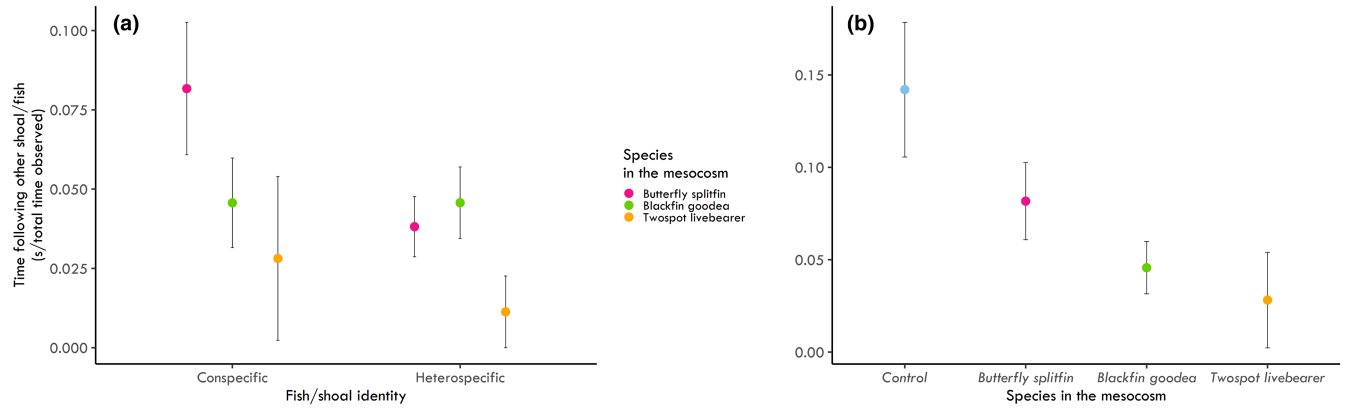


FIGURE 4 Sociability of tequila splitfins measured as the time they followed other fish differed among treatments depending on the identity of the fish (a). In this analysis, we excluded mesocosms with only conspecifics to test the interaction between variables. (b) Time tequila splitfins followed conspecifics differed among treatments. Vertical lines represent standard errors.

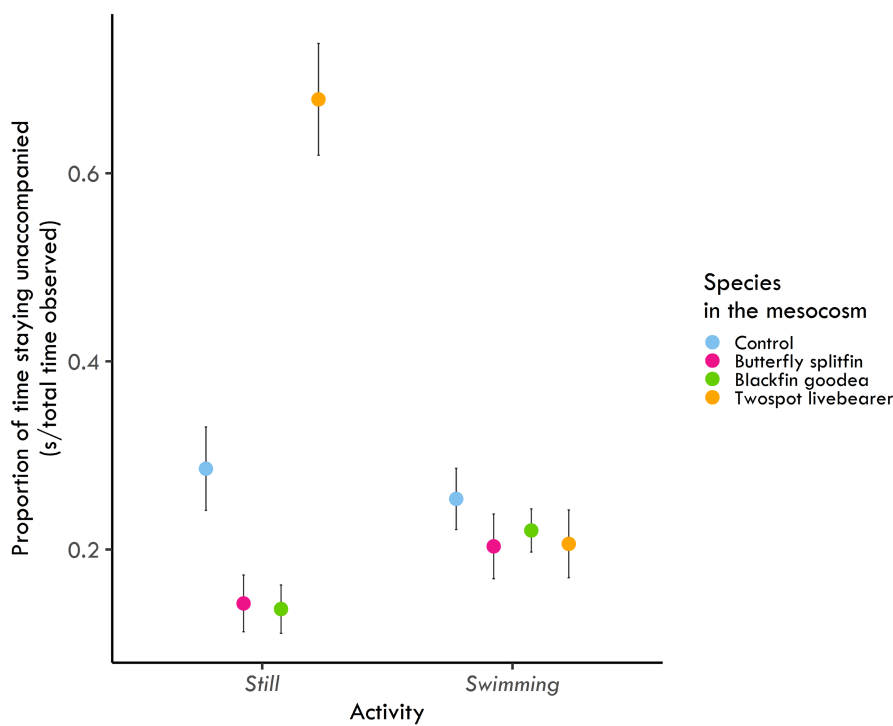


FIGURE 5 Activity behaviour of tequila splitfins measured as the time they remained unaccompanied as a function of the treatment in mesocosms and the activity they were doing (mean \pm standard error). Vertical lines represent standard errors.

Treatments compared to the intercept	Conspecifics	<i>Amea splendens</i>	<i>Goodea atripinnis</i>
	Tequila splitfins	Butterfly splitfins	Blackfin goodea
<i>A. splendens</i> Butterfly splitfins	0.64 \pm 0.11		
<i>G. atripinnis</i> Blackfin goodea	0.60 \pm 0.12	0.03 \pm 0.12	
<i>Pseudoxiphophorus bimaculatus</i> Twospot livebearer	-2.04 \pm 0.13	-2.65 \pm 0.14	-2.68 \pm 0.13

Note: Numbers represent estimated differences (regression coefficients; estimates \pm standard errors).

TABLE 3 Results from the best model exploring the activity of tequila splitfins while remaining alone (model: time unaccompanied/total time observed \sim treatment \times still/swimming + mesocosm identity).

be beneficial behaviour for goodeids and poeciliids, for example for surface respiration (Chapman & McKenzie, 2009) and foraging (Horth, 2004). To balance the costs of risk-taking behaviours, fish shoal with others to decrease the probability of predation (Agrillo & Dadda, 2007). Since tequila splitfins decreased their activity and avoided twospot livebearers, it is possible that they gain more costs than benefits from being near to them. These results suggest tequila splitfins are more cautious to be around the invaders even when it implies losing benefits of being on the surface. Similarly, population decline of the European mudminnow (*Umbra krameri*), a threatened native fish endemic to the River Danube, is linked to the invasion of the Amur sleeper (*Perccottus glenii*); both species have similar ecological requirements and often compete for limiting food sources (Grabowska et al., 2019). The invasive Amur sleeper often prevented natives from accessing prey by behaving aggressively; interference competition for food might be the mechanism explaining the rapid decline of this species (Grabowska et al., 2019). It is possible that this is also the case for tequila splitfins when coexisting with twospot livebearers as they tend to be more aggressive than goodeids; by reducing tequila splitfins's activity twospot livebearers could be interfering with their foraging activities. In contrast, goodeids are more likely to socialise, at least under laboratory conditions (M.C.C., personal observation). Cohabiting with species with similar temperaments may benefit both parts, enabling the interactions we recorded among goodeids, which in the wild could be beneficial for locating resources, avoiding predators, etc.

The abundance of tequila splitfins was also lower when they cohabited with the other two goodeid species than when they were alone, but it was not so low as when they were with invaders. In our experiment, the goodeid species used were part of the same subfamily and share habitat in the Teuchitlan river; thus, they are expected to share at least some ecological requirements. As we did not supplement the experimental mesocosms with food, and they were the only vertebrates present in the mesocosms, competition among them may be acting as a natural population regulator. In addition, tequila splitfins associated more frequently with butterfly splitfins and blackfin goodeas than with twospot livebearers, and even more so than with groups of only conspecifics. Associating with heterospecific fish may transmit beneficial information when there are not many conspecifics (Averages-Weber et al., 2013; Damas-Moreira et al., 2018). In our study, this benefit may be reflected by the inclination of tequila splitfins to spend time at the surface of the water when cohabiting with other goodeids.

Human actions are increasingly needed to preserve species and counteract the biodiversity decline that results from anthropogenic stressors (Seddon et al., 2007). Reintroduction attempts require both good management and good research to increase their chances of success. Seddon et al. (2007) recognised reintroduction ecology as an emerging studying topic and suggested that plans must be tackled in a multidisciplinary way. Species can be reintroduced to places where they are extinct, but current conditions must be managed, including management of invaders that could act as predators or competitors (Rehm et al., 2018). Whether

the reintroduction of tequila splitfins is viable in the long term is uncertain. Some authors argue that populations kept in captivity can form viable populations (Arbuatti et al., 2013), while others have found that tequila splitfins already had a low effective population size before captive populations were established (Bailey et al., 2007). After the reintroduction of tequila splitfins in the Teuchitlan River, the species continues to be present at the reintroduction sites today (O.D.D., personal observation). In our mesocosms, once they became established, tequila splitfins reproduced and survived for the duration of the experiment. However, during the reintroduction establishment period at the beginning of the experiment, tequila splitfins struggled to colonise mesocosms, regardless of the species they cohabited with.

The presence of exotic invasive species is one of the most concerning aspects threatening the survival, growth, and reproductive success of reintroduced individuals due to predation and/or competition for resources (Cochran-Biederman et al., 2015). In our study, exotic twospot livebearers affected the abundance and behaviour of tequila splitfins, supporting previous findings that livebearers threaten Mexican native goodeids (Camacho-Cervantes, 2019; Valero et al., 2008), which may be critical for their reintroduction to their natural habitats. Therefore, when reintroducing tequila splitfins, and other extinct-in-the wild species, it may be necessary to remove invasive heterospecifics that represent competition. Furthermore, the presence of other native species may represent competition too; in our experiment, however, other goodeids seem to pose a null or a positive effect on the reintroduction effort.

5 | CONCLUSIONS

Sharing habitat with an invasive species negatively affected the abundance and behaviour of tequila splitfins, while sharing with other native species proved to be beneficial. Our results suggest that it is important to remove exotic invasive species to increase the probability of a successful reintroduction of tequila splitfins in the wild. We would argue that this includes not only invasive poeciliids but also other larger invaders that can alter habitat and act as predators. Also, it is important to integrate quantitative, interdisciplinary, and ecosystem perspectives (Malone et al., 2018) to establish a successful reintroduction programme, and it is necessary to keep track of the reintroduction events over the long term given the high mortality we detected at the beginning of the experiment and the susceptibility of tequila splitfins to being affected by other species. Similar to other studies suggesting that invasive poeciliids have negative effects on native goodeids (Camacho-Cervantes, 2019; Ramírez Carrillo & Macías García, 2015; Valero et al., 2008), in this study tequila splitfins avoided interactions with twospot livebearers, were less active and social, and their abundance was negatively affected. However, interactions with other native species are beneficial or at least not negative and, therefore, protecting the native community could

also increase chances of a successful reintroduction. We emphasise the need of studies of fish communities before reintroduction of species, given that when reintroducing a native species that has been absent from an environment, all the community has changed. Ecological and behavioural interactions may largely influence the success of a reintroduction event and the care of the integrity of the native fish community.

AUTHOR CONTRIBUTIONS

Conceptualisation: M.C.C. and A.F.O. Developing methods: M.C.C., A.F.O., and O.D.D. Conducting the research: M.C.C., E.D.V., and M.S.R. Data analysis: M.S.R. Data interpretation: M.C.C. and M.S.R. Preparation of figures and tables: M.S.R. Writing: M.S.R., E.D.V., A.F.O., O.D.D., and M.C.C.

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DATA AVAILABILITY STATEMENT

Data are available from the authors upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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