

Assessing the potential for successful translocation and co-management of two endangered aquatic species

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Abstract

Translocation programmes for endangered species typically focus on a single species, but in areas where little native habitat remains, it may be necessary to translocate multiple species to the same sites. Interactions between translocated species, such as predation and competition, are among the factors that need to be considered when planning multispecies translocations. Translocation sites for aquatic species are particularly scarce in southern California, where a limited number of sites exist for historically co-occurring endangered mountain yellow-legged frogs *Rana muscosa* and unarmoured three-spine sticklebacks *Gasterosteus aculeatus williamsoni*. To determine how these species would interact if translocated to the same sites, we carried out experiments *ex situ* with *R. muscosa* tadpoles and a surrogate subspecies of stickleback (*G. a. microcephalus*). We found that (1) adult sticklebacks preyed on hatchling tadpoles but did not consume *R. muscosa* eggs or large tadpoles; (2) tadpoles did not consume stickleback eggs or disturb stickleback nests; and (3) both species' microhabitat use shifted slightly when the other was present. Our results suggest that these species can likely be co-managed successfully, if measures are taken to curb stickleback predation on tadpoles until the *R. muscosa* population is well established. Using *ex situ* studies to evaluate species interactions prior to translocation is an approach that could prove useful in other species recovery programmes. Multispecies translocations could make better use of available resources when habitat is limited and promote ecosystem recovery by re-establishing interactions among native species.

Introduction

Endangered species translocations typically focus on single species, but a scarcity of suitable habitat may necessitate translocating multiple endangered species to the same protected areas. In addition to making better use of available habitat and management resources, multispecies translocations could foster ecosystem recovery by re-establishing interactions among native taxa (Akçakaya *et al.*, 2018). However, species interactions that might stabilize populations at moderate densities could have the opposite effect in the early post-relocation phase, when population densities are typically quite low. For example, life-stage-restricted predation has the potential to stabilize coexistence between species that compete for common resources, but the predicted outcome depends on initial population densities (Polis & Holt, 1992). Species that directly interact might need to be translocated in a particular order or at particular life stages for multispecies translocations to be successful.

Translocation has become a necessary conservation strategy for many freshwater species impacted by the loss, degradation and fragmentation of native habitat (Olden *et al.*, 2011). Freshwater ecosystems are changing rapidly under pressure from anthropogenic activities (Vörösmarty *et al.*, 2010) and face alarmingly high rates of extinction (Ricciardi & Rasmussen, 1999; Kopf *et al.*, 2015). These changes are particularly striking in California, USA, where water diversion, introduced species and drought have had devastating effects on aquatic communities (Moyle, 2014). Nearly half of California's freshwater species are now at risk of extinction (Howard *et al.*, 2015).

At-risk species in southern California that might benefit from co-management include the mountain yellow-legged frog *Rana muscosa* and the unarmoured three-spine stickleback *Gasterosteus aculeatus williamsoni*. Historically, *R. muscosa* was abundant in the Palomar, San Bernardino, San Gabriel and San Jacinto Mountains, but today only nine small populations remain in southern California streams (Backlin *et al.*, 2015; USFWS, 2018). *G. a. williamsoni* was

once widespread in the Santa Clara River, Los Angeles River, San Gabriel River and Santa Maria River watersheds, but is now restricted to one creek in northern Santa Barbara County, the upper reaches of the Santa Clara River drainage in Ventura County and three ponds in San Bernardino County (USFWS, 2021). Both species are listed as endangered in the state of California (<https://wildlife.ca.gov/Conservation/CESA>) and federally (USFWS, 1970; USFWS, 2002). These species do not currently co-occur but likely did in the past (Moyle, 2002; USFWS, 2012). The remaining lotic populations of both species are especially vulnerable because streams that were perennial in the recent past have become intermittent, often drying out in large stretches during the summer months. Two protected lakes, one of which is managed for *G. a. williamsoni*, are within the historic range of *R. muscosa*. The recovery of both species could potentially be enhanced by managing all of the available protected habitat for both species, but they are not currently co-managed because it is not known whether the species would negatively impact each other and, if so, whether those impacts could be mitigated.

Introduced fish are partly responsible for population decline and range contraction in *R. muscosa* (Hayes & Jennings, 1986; USFWS, 2012; Backlin *et al.*, 2015; Shier *et al.*, 2021); with negative effects of fish on the distribution of mountain yellow-legged frogs evident at the landscape, watershed and individual water body levels, in large part due to ecological interactions such as predation (Bradford *et al.*, 2011) and competition for habitat (Knapp & Matthews, 2000). Typical prey for *G. a. williamsoni* includes benthic insects, snails and small crustaceans (USFWS, 2009), but amphibian eggs are vulnerable and often palatable to fish (Light, 1969; Gunzburger & Travis, 2005). Other subspecies of three-spine stickleback (*G. a. aculeatus*) prey on Ranid tadpoles (Laurila & Aho, 1997; Teplitsky *et al.*, 2005). Adult *R. muscosa* primarily feed on terrestrial and semi-aquatic insects, and tadpoles primarily graze on algae and detritus (USFWS, 2012). However, the tadpoles are also opportunistic cannibals and might eat stickleback eggs if they have the opportunity. These species differ in some key life history characteristics. Ranid frogs typically become reproductively ready at three years of age, produce a single clutch in a year (Zweifel, 1955) and rarely exhibit parental care (Furness & Capellini, 2019). By contrast, sticklebacks are capable of becoming reproductive in their first year, producing multiple clutches across multiple years (Baker *et al.*, 2015) and during fry development, male sticklebacks defend their nests against predators (Wootton, 1972). The time and energy males spend repelling tadpoles might interfere with other components of paternal care, such as egg fanning (Lissåker & Kvarnemo, 2006). Larvae of both species seek shelter from predation in shallow and vegetated areas (Babbitt & Tanner, 1998; USFWS, 2009; Brown *et al.*, 2019; Robak *et al.*, 2019; Davenport, Fishback, & Hossack, 2020). Thus, competition for space between tadpoles and fry could potentially reduce the recruitment rates of both species.

To assess the potential for co-managing translocated populations of these endangered species in lakes within their ancestral range, we studied interactions between *R. muscosa* and a closely related surrogate of *G. a. williamsoni* in aquaria. Specifically, we quantified interactions that were most likely to occur based on the species' natural history and the results of research on similar systems: (1) predation of frog eggs or tadpoles by stickleback; (2) predation of stickleback eggs or interference with male stickleback paternal behaviour by tadpoles; and (3) displacement of either species by the other along vegetation or depth gradients. The primary goal was to inform decision-making regarding co-management at translocation sites.

Materials and methods

Study animals

We obtained 250 4-week-old *R. muscosa* tadpoles from the conservation breeding facility at Omaha's Henry Doorly Zoo and Aquarium in June 2020, which we refer to as 'large tadpoles' or 'large tadpoles with hind limbs', depending on their developmental stage. The tadpoles were produced from a backcross of a male of mixed lineage (San Jacinto x City Creek population) and a San Jacinto female. We also acquired 336 unfertilized *R. muscosa* eggs from the San Diego Zoo Wildlife Alliance (SDZWA). In June 2021, we obtained 200 recently hatched San Jacinto tadpoles from SDZWA, which we refer to as 'hatchling tadpoles'.

Due to the endangered status of *G. a. williamsoni*, we used a closely related subspecies, the partially-armoured three-spine stickleback (*G. a. microcephalus*), which is similar to *G. a. williamsoni* in size, diet, habitat use, and breeding and nesting behaviour (Miller & Hubbs, 1969; Sánchez-González, Ruiz-Campos, & Contreras-Balderas, 2001), as a surrogate. Subspecies designations are defined by morphology (i.e. the presence and number of bony plates, or 'body armour', that line the lateral flanks), not geography or phylogenetics (Richmond *et al.*, 2015). One hundred and twenty *G. a. microcephalus* were collected from a tributary of the Santa Clara River (below the Piru gap) near Fillmore Fish Hatchery (Fillmore, CA) and immediately transported to UCLA (for husbandry details, see Supplementary Material). To obtain stickleback eggs, we isolated gravid females in separate tanks for up to 16 h, or until they laid eggs at the bottom of the tank. Unfertilized eggs were stored at -76°C until being used in Experiment 2. To induce nesting behaviour and obtain stickleback fry for Experiment 3, we used a modified version of a breeding protocol developed by the Santa Barbara Zoo for *G. a. microcephalus* (see Supplementary Material).

All experiments were carried out with permission from the United States Fish & Wildlife Service (Permit No. TE76006B-2) and the California Department of Fish and Wildlife (SCP – S-191150001-20066-001 and an MOU). Husbandry and experimental protocols were approved by the UCLA Institutional Animal Care and Use Committee.

Effects of stickleback on frog eggs and tadpoles

To examine the effects of adult stickleback on *R. muscosa* tadpoles and eggs, we carried out an experiment in summer 2020 with two treatments: ‘with stickleback’ (2 adults of each sex) ($n = 14$ trials) and ‘without stickleback’ ($n = 14$ trials). In each trial, we placed 12 large tadpoles (1.5–2.5 cm SVL, Gosner stages 25–37) and 12 unfertilized eggs (Gosner, 1960) in 151 L glass tanks (89 × 37 cm) with artificial plants. Unique groups of tadpoles and fish were used in each trial, but some individuals were used in multiple trials. To account for any pre-existing damage or damage sustained in earlier trials, a single observer estimated the percentage of the tail that remained intact by visually comparing it to a fully intact tail immediately before each trial. Trials began at approximately 5:00 PM and ended after 22 h, to allow time to set up the next trial. We used a video camera (GoPro HERO7) to record activity in the tank for 5 min between 09:00 and 10:00 on the second day (ca. 16 h after the start of the trial), and again for 5 min during the last hour of the trial. We later quantified the frequency of predatory and aggressive behaviour in each 30-s interval, which included fish biting, chasing and approaching tadpoles or other fish. At the end of the trial, we placed tadpoles and eggs in temporary containers and returned the fish to their holding tanks. We then counted the remaining tadpoles and eggs and again quantified tadpole tail damage.

Based on the results from 2020, we carried out a new experiment in summer 2021 using the same two treatments, except that we used 24 hatchling tadpoles (0.75–1.5 cm SVL, Gosner stage 25) per trial, and three sticklebacks (1 male, 1 female and 1 subadult) in the ‘with stickleback’ treatment.

To test for effects of stickleback presence on tadpole tail damage, we used a one-inflated beta generalized linear mixed model using the ‘GAMLSS’ package in the R programming environment (Rigby & Stasinopoulos, 2005, R version 4.0.0, Team, 2018). After verifying that the initial amount of tail damage did not differ between treatment groups, we modelled the effect of treatment on the final tail percentage estimate with the trial number as a random effect. To examine whether the number of surviving eggs and tadpoles differed between treatments, we used Mann–Whitney U tests. To determine whether the frequency of heterospecific and conspecific aggressive behaviours was positively correlated, as would be expected if heterospecific aggression is a byproduct of conspecific aggression (Peiman & Robinson, 2010), we computed the Spearman rank correlation.

Effects of tadpoles on stickleback eggs and paternal behaviour

To examine whether tadpoles would prey on stickleback eggs and whether the presence of tadpoles negatively affects stickleback paternal behaviour, we carried out nine trials each of two treatments: (1) 10 large tadpoles with hindlimbs (2.5–3.0 cm SVL; Gosner stages 38–41) were added to a

tank with a single male stickleback tending to his eggs in the nest at one end of the tank and a fake nest with 30 stickleback eggs at the other end; and (2) identical to (1) in all respects except that no tadpoles were added to the tank. The purpose of the fake nest was to assess whether the tadpoles would consume stickleback eggs in an undefended nest. We used a unique group of tadpoles in each trial, but individual tadpoles were used in multiple trials. Nine male sticklebacks were each used in one trial of each treatment, four with the tadpole treatment first, and five with the control treatment first.

The experiment was conducted in 151 L glass tanks (89 × 37 cm) containing gravel and sand substrate, one plastic and one live plant to serve as cover or nesting material, and one floating plastic ‘dock’ for tadpoles to rest against. Fake nests were made from unravelled cotton balls, weighted down with sand.

Trials started at approximately 10:00 AM, one day after the onset of stickleback nesting behaviour and ran for 34 h to allow enough time for the animals to acclimatize to the tank and interact with each other. At the end of a trial, we removed the tadpoles (if present) and determined whether there were eggs remaining in the fake nest. We observed each tank for 5 min in the morning and afternoon on each day of the trial and recorded nesting behaviour and interactions between sticklebacks and tadpoles. Observation sessions were divided into 10 equal 30-s observation intervals. In each interval, the observer recorded: (1) the distance of the male stickleback from to his nest (≤ 15 , >15 cm, or both <15 and >15 cm in the same interval); (2) the behaviour and position of tadpoles relative to the real nest (resting ≤ 15 cm, resting >15 cm, swimming ≤ 15 cm and swimming >15 cm) and (3) aggressive responses of the male stickleback towards tadpoles (approaching, biting).

To test for the effect of tadpoles on the presence or absence of eggs in the fake nest, we used Fisher’s exact tests. To test if stickleback were more likely to approach or bite tadpoles when they were close to the nest, we constructed a mixed-effects binomial regression with fish ID as a random effect using the generalized linear mixed model (GLMM) function `glmer` in the R package ‘lme4’ (Bates *et al.*, 2015). To test for an effect of the presence of tadpoles on the proportion of intervals in which the male stickleback was >15 cm from his nest and the proportion of intervals in which the male briefly left the nest and returned to within 15 cm, we constructed mixed-effects logistic regressions with individual fish as a random effect using `glmer` with a ‘logit’ link (Bates *et al.*, 2015).

Effects of heterospecifics on habitat use

To determine whether microhabitat use changes in the presence of other species, we carried out an experiment with three treatments: stickleback only, tadpoles only and stickleback and tadpoles together in the same tank. We ran the experiment twice, with animals of different developmental stages and densities: (1) 30 stickleback fry and 30 large tadpoles (1.5–2.5 cm SVL; Gosner stage 25–37; 19 trials of

each treatment) and (2) 20 subadult nonbreeding stickleback and 20 large tadpoles with hind limbs (2.5–3.0 cm SVL; Gosner stages 38–41; 20 trials of each treatment). Each trial consisted of a unique group of animals, although individual animals were used in multiple trials.

These trials were conducted in 284 L tanks (119 × 29 cm) with a sloping gravel gradient ranging in water depth from 0 to 40 cm along the length of the tank (Fig. 1). The tank was divided into four equal quadrants, representing four different depths. Plastic vegetation was placed on one side of the tank such that one half of each quadrant was open and the other half included vegetation.

At the start of each trial, the animals were placed in the centre of the tank simultaneously. Trials began at

approximately 4:00 PM and lasted 22 h, which allowed the animals time to interact while leaving time to set up and start the subsequent trial. An observer conducted scan sampling approximately 17 h after the start of the trial (10:00 AM) and again at the end of the trial (2:00 PM) to record the number of individuals of each species in each quadrant and whether they were in the vegetated or open area. The scan sampling consisted of a series of three scan samples spaced 10 min apart, with the observer standing 1 m away from the tank.

While repeated scan samples facilitated an accurate assessment of the location of the animals in the tanks, assessing their behaviour required recording the animals in the tanks with a camera and reviewing the footage. We used a video

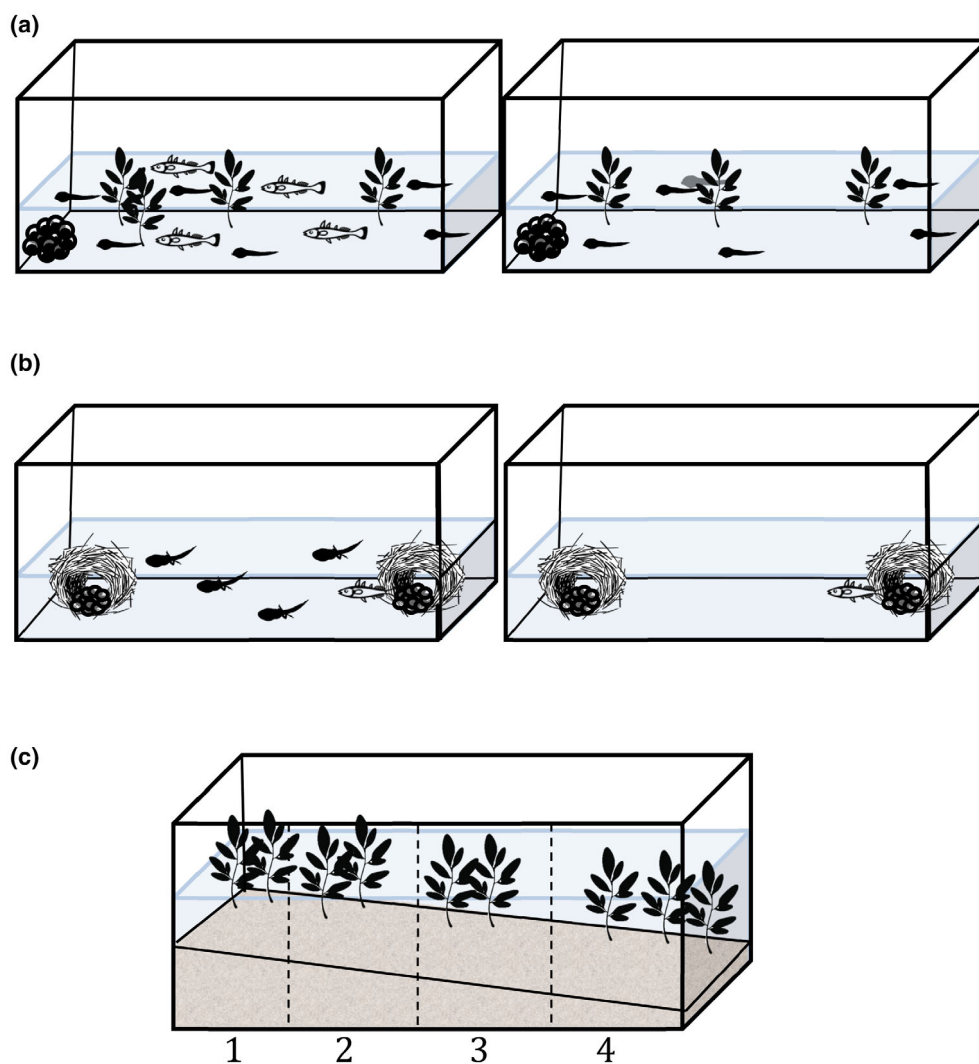


Figure 1 Experimental design or setup for each of the three experiments (a) effects of stickleback on frog eggs and tadpoles, (b) effects of tadpoles on stickleback eggs and paternal behaviour, and the tank setup for (c) heterospecific impact on habitat use in which a depth gradient was created using gravel along the length of the tank and the tank was divided into 4 quadrants: (1) 0–10 cm deep, (2) 10–20 cm deep, (3) 20–30 cm deep and (4) 30–40 cm deep. Along the width of the tank, half contained plastic plants and gravel, while the other half contained only gravel.

camera to film each quadrant for 60 s after the first scan sample, and again for 60 s after the second scan sample. Each 1-min video was divided into 15-s intervals. In each time interval, observers recorded the number of animals performing each behaviour, whether the animals were in the vegetation or the open, and the total number of animals. Stickleback behaviours included (1) shoaling (stationary in a group), (2) schooling (mobile in a group), (3) alone and stationary and (4) swimming alone. Tadpole behaviours included (1) aggregating (stationary in a group), (2) resting alone and (3) swimming (see Table S1 for Ethogram).

To compare the distribution of animals between treatments, we used negative binomial generalized linear mixed models with treatment, depth and vegetation as fixed effects, all two- and three-way interactions, and trial as a random effect in STATA 16.1 (StataCorp, 2019). To compare the frequency of behaviours between treatments and microhabitats, we used negative binomial generalized linear mixed models with treatment, depth and vegetation as fixed effects offset by the total number of animals of either species observed and with trial and observation sessions as random effects using the ‘glmmTMB’ package in R (Brooks *et al.*, 2017).

Results

Effects of stickleback on frog eggs and large tadpoles

All large tadpoles survived, but those in trials with adult sticklebacks lost tail tissue (mean ± SD: $-6.4 \pm 5.2\%$) while those in the control trials did not ($0.26 \pm 0.7\%$) (GLMM beta regression, coefficient estimate = -0.58 ± 0.08 , $t = -7.04$, $P < 0.001$; $n = 14$ trials; Fig. 2). The presence of adult stickleback did not affect the number of frog eggs remaining at the end of the trials (2.1 ± 2.0 [mean ± SD] of 12 eggs were lost in the absence of stickleback versus 2.7 ± 2.9 in the presence of stickleback; Mann–Whitney test, $W = 88.5$, $P = 0.67$, $n = 28$).

Sticklebacks were frequently aggressive towards conspecifics and occasionally to tadpoles (Table 1). Stickleback were observed biting tadpoles 17 times (57% of 14 trials) and the tadpoles usually responded by quickly swimming away (14 of 17 cases). Across trials, the rates of conspecific and heterospecific biting, chasing or approaching were not correlated [Spearman correlation, biting ($\rho = -0.05$, $S = 478.1$, $P = 0.9$, $n = 14$), chasing ($\rho = 0.20$, $S = 362.5$, $P = 0.5$), or approaching ($\rho = -0.2$, $S = 559.4$, $P = 0.4$)].

Effects of stickleback on hatchling tadpoles

More hatchling tadpoles died in trials with stickleback than in control trials without stickleback (Mann–Whitney test, $W = 42.5$, $P < 0.01$, $n = 28$). In total, seven hatchling tadpoles died in the control trials, while 44 died in the trials with stickleback (14 in the latter group were fully

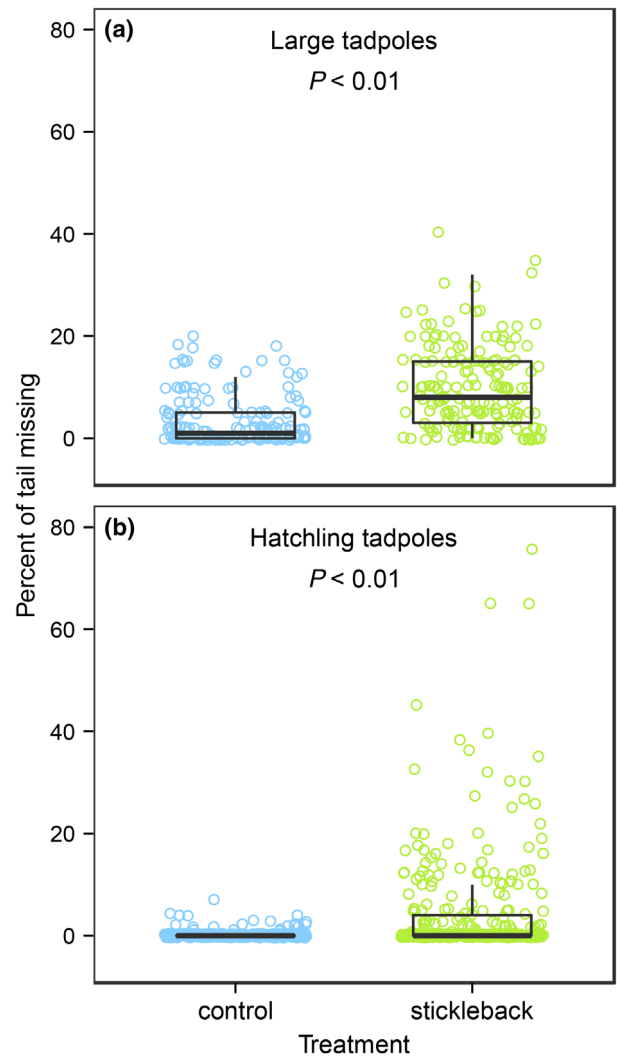


Figure 2 Boxplots illustrating that both large tadpoles (a) and hatchling tadpoles (b) suffered losses in tail tissue when housed in aquaria with adult sticklebacks. Boxplots depict the median (horizontal line within the box), interquartile range (box) and lower and upper adjacent values (whiskers).

Table 1 Adult stickleback behaviour directed towards conspecifics and heterospecifics in Experiment 1, reported as number of events and percent of trials in which the interaction was observed

Behaviour	Conspecific		Heterospecific	
	No of events	% of trials	No of events	% of trials
Biting	45	64	17	57
Chasing	121	92	5	21
Approaching	237	100	95	85

consumed). Surviving tadpoles lost a larger percentage of tail tissue (mean ± SD: $5.2 \pm 5.2\%$) in the stickleback treatment than in the control treatment ($0.2 \pm 0.8\%$) (GLMM beta

regression, coefficient estimate = -0.71 ± 0.21 , $t = -3.34$, $P < 0.001$; Fig. 2)).

Effects of tadpoles on stickleback eggs and paternal behaviour

Nest-tending male sticklebacks were more likely to approach and bite tadpoles with hindlimbs that came close to the nest (≤ 15 cm) than those that stayed farther away (approaches, GLMM coefficient estimate = 5.34 ± 2.18 , $P = 0.01$; bites, coefficient estimate = 3.96 ± 1.58 , $P = 0.01$). Nest-tending sticklebacks were less likely to make brief (<30 s) forays away from the nest in trials with tadpoles ($12.00 \pm 0.13\%$; $n = 540$ observation intervals) than in control trials without tadpoles ($18.00 \pm 0.16\%$; $n = 540$) (GLMM coefficient estimate = -0.71 ± 0.17 , $P < 0.001$). However, the proportion of observation intervals in which the tending stickleback

remained near the nest (≤ 15 cm) did not differ significantly between trials in which tadpoles were present ($93.0 \pm 0.2\%$; mean \pm SE; $n = 540$) and control trials without tadpoles ($90.0 \pm 0.2\%$; $n = 540$) (GLMM coefficient estimate = -0.1 ± 0.23 , $P = 0.64$).

The tadpoles were not observed consuming eggs or otherwise disrupting the fake stickleback nests; the number of fake nests with eggs remaining at the end of the trials did not differ between treatments (10 of 14 trials with tadpoles; 7 of 14 control trials; Fisher's Exact test, $P = 0.44$, $n = 18$).

Effects of heterospecifics on habitat use

Stickleback fry shifted into the vegetation when large tadpoles were present (GLMM negative binomial regression; treatment by vegetation cover interaction: -0.33 ± 0.15 , $\chi^2 = 4.52$, d.f. = 1, $P = 0.033$; Table S2, Fig. 3a,c) and

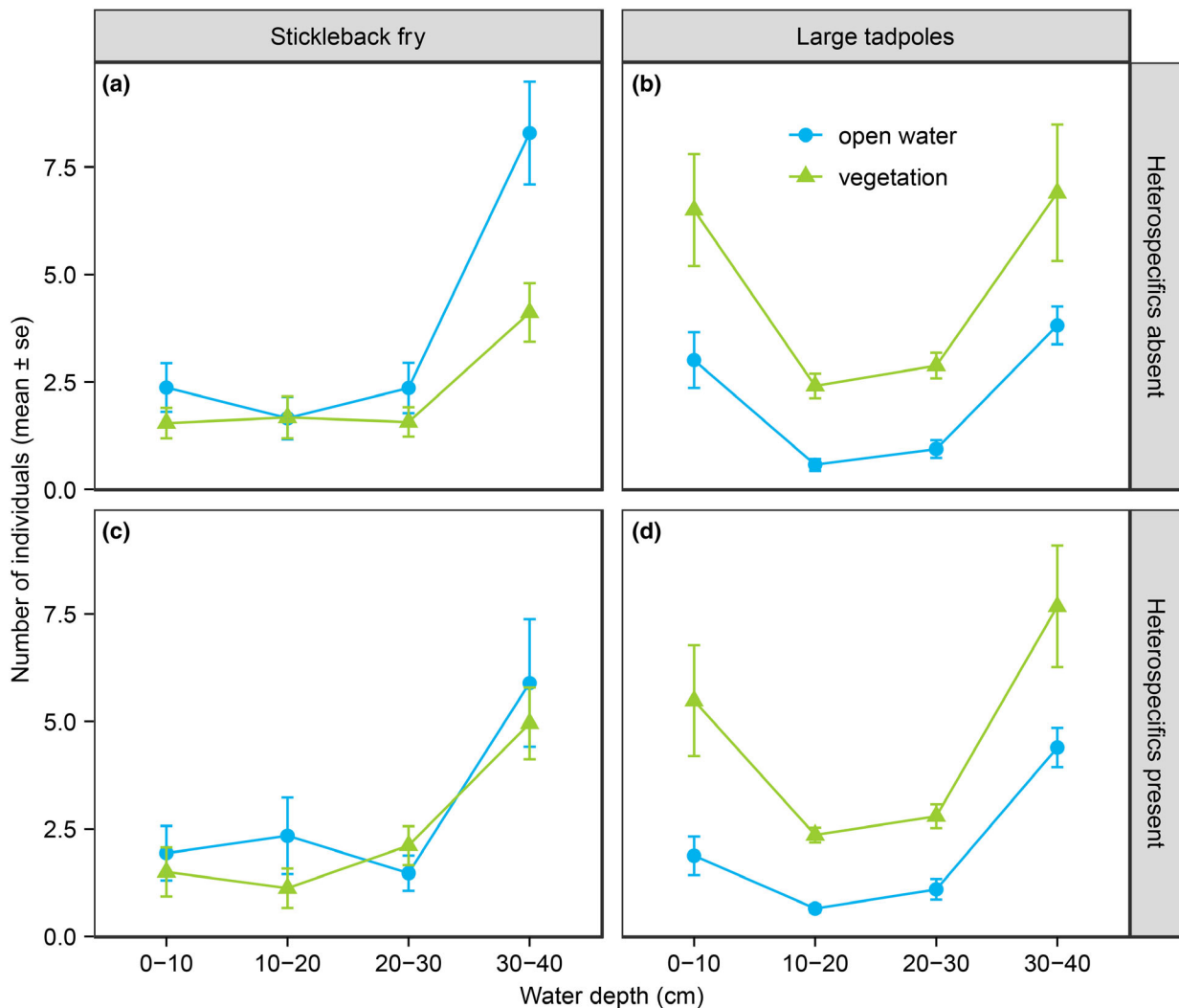


Figure 3 Effects of heterospecifics on the space use of stickleback fry and large tadpoles. Stickleback fry shifted into vegetation in the presence of large tadpoles (a, c). Large tadpoles shifted slightly to deeper water in the presence of stickleback fry (b, d). Plotted values are the means \pm SE of the trial means ($n = 19$) for each factor combination.

exhibited a preference for deeper water (GLMM negative binomial regression; depth: $\chi^2 = 277.51$, d.f. = 3, $P < 0.0001$; Table S2) regardless of the presence or absence of tadpoles (GLMM negative binomial regression; treatment by depth interaction: $\chi^2 = 3.97$, d.f. = 3, $P = 0.27$; Table S2, Fig. 3a,c). The preference of the stickleback fry for the deepest section of the tank was stronger in the open water in the absence of heterospecifics, as shown by a significant 3-way interaction between treatment, vegetation and depth (Table S2). Large tadpoles were more often observed in the vegetated areas within the deepest and shallowest water (GLMM negative binomial regression; vegetation by depth interaction: $\chi^2 = 29.77$, d.f. = 3, $P < 0.0001$; Table S3, Fig. 3b,d) and shifted slightly to deeper water when stickleback fry were present (treatment by depth interaction: $\chi^2 = 10.20$, d.f. = 3, $P = 0.017$; Table S3, Fig. 3b,d).

Subadult sticklebacks preferred deeper water (GLMM negative binomial regression; depth: $\chi^2 = 519.71$, d.f. = 3, $P < 0.0001$; Table S4) but shifted to somewhat shallower depths when tadpoles with hindlimbs were present ($\chi^2 = 11.42$, d.f. = 3, $P < 0.01$; Table S4, Fig. 4a,c). Like large tadpoles, the large tadpoles with hindlimbs preferred the vegetated areas within the deepest and shallowest water (GLMM negative binomial regression; vegetation by depth interaction: $\chi^2 = 10.15$, d.f. = 3, $P = 0.017$; Table S5) and shifted slightly to deeper water when stickleback subadults were present (treatment by depth interaction: $\chi^2 = 9.73$, d.f. = 3, $P = 0.021$; Table S5, Fig. 4b,d).

The presence of heterospecifics did not affect the frequency of any behaviour observed in either size class of tadpoles or sticklebacks. Regardless of treatment, tadpoles with and without hindlimbs were more likely to aggregate when in the

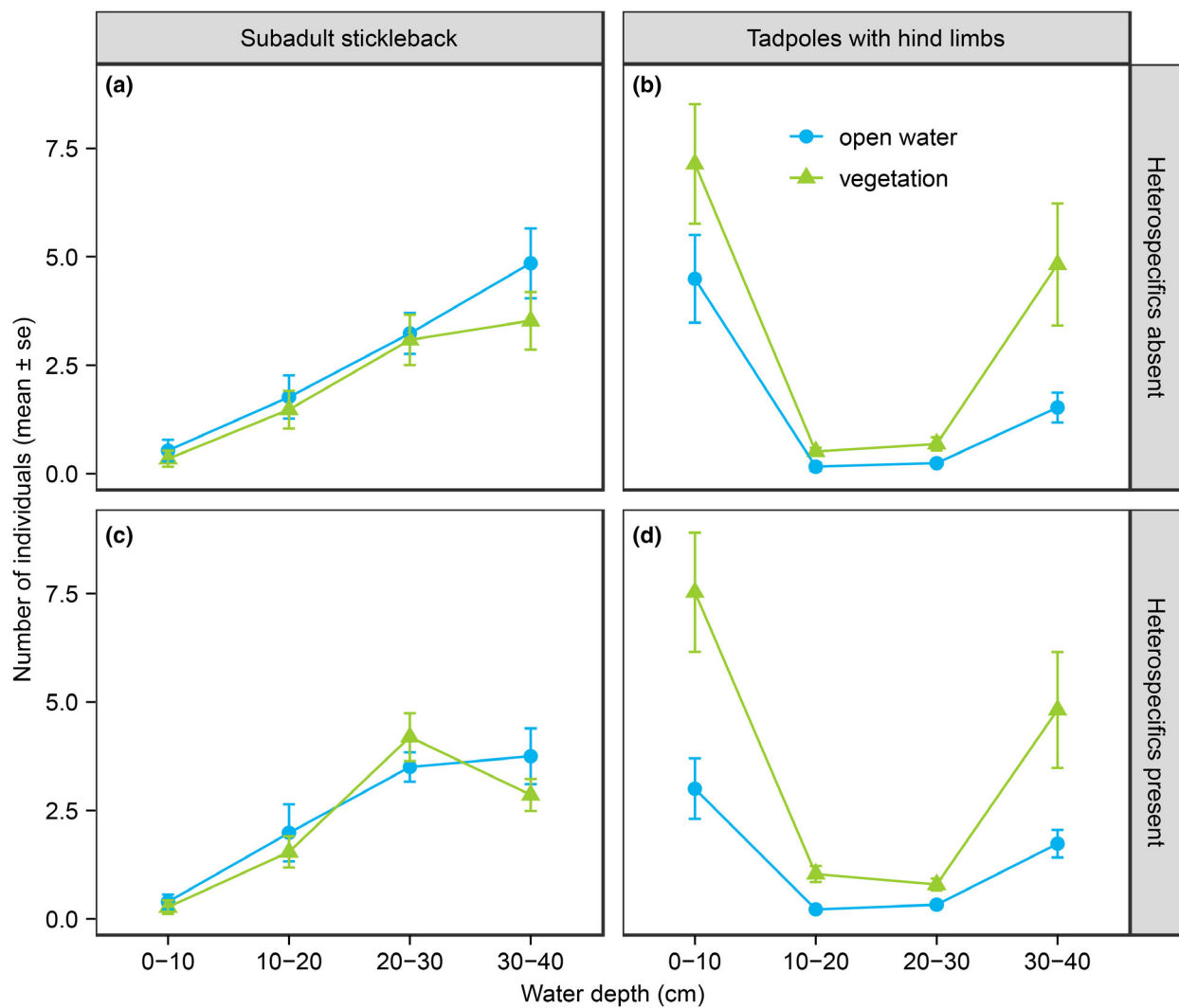


Figure 4 Effects of heterospecifics on space use of stickleback subadults and large tadpoles with hindlimbs. Subadult sticklebacks shifted slightly to shallower water in the presence of tadpoles with hindlimbs (a, c). Tadpoles with hindlimbs shifted into vegetation cover when in shallow water in the presence of subadult sticklebacks (b, d). Plotted values are the means \pm SE of the trial means ($n = 20$) for each factor combination.

shallowest area (GLMM: negative binomial distribution; large tadpoles with hindlimbs: coefficient estimate (number of animals) = 1.54 ± 0.17 , $P < 0.001$; large tadpoles: coefficient estimate = 0.74 ± 0.16 , $P < 0.001$), and more likely to swim when in the intermediate depths (GLMM: negative binomial distribution; large tadpoles with hindlimbs: coefficient estimate = 0.29 ± 0.11 , $P = 0.01$; large tadpoles: coefficient estimate = 0.24 ± 0.05 , $P < 0.01$). Large tadpoles were less likely to aggregate in the vegetation versus open areas (coefficient estimate = -0.58 ± 0.1 , $P < 0.001$). Subadult sticklebacks and fry were more likely to shoal when in the vegetation versus open areas (GLMM: negative binomial distribution; subadult: coefficient estimate (number of animals) = 0.79 ± 0.22 , $P < 0.001$; fry: estimate = 0.49 ± 0.26 , $P = 0.07$). Subadults were more likely to be alone and stationary (likely hiding) in the vegetation versus open areas (GLMM: negative binomial distribution; coefficient estimate = 0.33 ± 0.07 , $P < 0.001$). Subadults were less likely to swim on their own in the shallowest area compared to shallower areas (GLMM: negative binomial distribution; coefficient estimate = -0.32 ± 0.18 , $P = 0.07$), and less likely to shoal in the deepest area compared to shallower areas (GLMM: negative binomial distribution; coefficient estimate = -0.53 ± 0.19 , $P < 0.01$). Fry were less likely to school when in the deepest area compared to shallower areas (GLMM: negative binomial distribution; coefficient estimate = -0.62 ± 0.22 , $P < 0.01$).

Discussion

Understanding how endangered species interact is critical for evaluating the viability of relocating them to the same sites with the goal of maximizing use of the limited available habitat while recovering the species' functional roles in ecological communities. We found that stickleback predation attempts on hatchling tadpoles are often lethal, predation attempts on larger tadpoles are sublethal and predation on frog eggs was not detected. This suggests that stickleback predation on tadpoles is limited by the tadpoles' size class, and lethal impacts become less likely as tadpoles grow. Tadpoles did not prey on stickleback eggs or affect the amount of time that male stickleback spent at their nest, although stickleback were less likely to make brief trips away from the nest when tadpoles were present. This slight shift in nesting behaviour could reflect a trade-off between nest tending and vigilance against territory intruders. Subadult sticklebacks were often observed in deep water, while tadpoles were more likely to use shallow areas, but the distribution of both species shifted somewhat when the other was present. These results indicate that the presence of either species could impact the other however, it may be possible to successfully co-manage relocated populations.

Lethal and sublethal effects of fish on tadpoles

Stickleback predation on tadpoles is restricted by tadpole size class, and lethal encounters become less likely as tadpoles grow.

This study was conducted in aquaria *ex situ*, so it is unclear how often predation would occur in the wild. However, these results are consistent with previous research indicating that hatchling tadpoles are more vulnerable to fish predation than larger size classes (Travis, Keen, & Julianna, 1985; Brodie & Formanowicz, 1987; Anholt, Skelly, & Werner, 1996). Sticklebacks are gape-limited predators; thus, the size of the refuge at which tadpoles avoid predation and the rate at which it can be reached are dependent on stickleback size and tadpole growth rate. In captivity, *R. muscosa* tadpoles typically reach maximum body size 4–6 months after hatching (L. Jacobs, personal communication, August 29, 2021). However, as a high-elevation species, wild mountain yellow-legged frog larvae can require two or more summers to develop through metamorphosis (Zweifel, 1955; Bradford, 1983; Knapp & Matthews, 2000), likely because low water temperatures or food availability result in slower growth and smaller size in overwintering tadpoles. Faster growth is favoured in amphibians that primarily face threats from gape-limited predators (Urban, 2007). Nevertheless, Ranid tadpoles exhibit an extended larval period and a smaller size at metamorphosis when raised in ponds with gape-limited predators, including stickleback (Lawler *et al.*, 1999; Davenport *et al.*, 2013). This probably reflects a trade-off among factors that affect larval amphibian growth rates, such as activity level and foraging efficiency. For example, tail injuries caused by predation attempts, which were commonly observed in our experiment, could result in reduced growth and developmental rates (Wilbur & Semlitsch, 1990). Although tail injury from attempted predation is common and often sub-lethal in tadpoles (Morin, 1985), an extended larval period and smaller size at metamorphosis could reduce adult survival and fecundity (Semlitsch, 1987; Smith, 1987; Chelgren *et al.*, 2006; Pechenik, 2006).

Effects of tadpoles on stickleback reproduction

Stickleback nest structure and parental behaviour appear to be effective in protecting eggs from potential predation by tadpoles. In addition to protective nest structure, stickleback males guard their nest and chase away potential predators and territory intruders (Wootton, 1976). Consistent with previous observations on nesting behaviour, paternal sticklebacks spent the vast majority of their time at the nest, either fanning water over the eggs or stationed above the nest, regardless of whether tadpoles were present (cf. Wootton, 1972). But the fish were less likely to make brief trips away from their nest when tadpoles were present, which is consistent with how the behaviour of nesting sticklebacks shifts in response to perceived predators (Stein & Bell, 2012; Gravalin *et al.*, 2021). However, three-spine sticklebacks demonstrate plasticity in time allocation to parenting behaviours, and can quickly return to baseline following the removal of potential predators (Stein & Bell, 2012).

Heterospecific impact on habitat use

When sticklebacks were present, tadpoles shifted to deeper parts of the aquarium. In the wild, a shift to deeper, cooler water could potentially reduce growth rates and delay

metamorphosis (Smith-Gill & Berven, 1979; Bradford, 1984; Wheeler & Carde, 2014; Brown *et al.*, 2019). In the presence of tadpoles, stickleback fry were more often found in the vegetated area, while some subadults shifted away from the deepest area to intermediate depths. This pattern is consistent with observations that young *G. a. williamsoni* often inhabit shallow areas of streams and ponds with warmer water and dense vegetation, while adults are found across a wider range of microhabitat types, from shallow, vegetated areas to relatively deep, open water (USFWS, 2009).

Management recommendations

While the design of this study was limited by some logistical factors, the results are highly relevant to conservation efforts for the focal species. *G. a. williamsoni* differs slightly in morphology from the surrogate species used, *G. a. microcephalus*, but they share similar diets and habitat. *G. a. williamsoni* and *R. muscosa* no longer co-occur in the wild; thus, their interactions could only be studied ex-situ, which allowed for experimentation under controlled conditions. While some environmental factors could not be accounted for, such as space and the presence of other species, the laboratory environment replicated natural water temperature and light cycles.

Based on the results, *R. muscosa* reintroductions are unlikely to have deleterious effects on *G. a. williamsoni* reproductive behaviour, habitat use or survival and thus *R. muscosa* would not be expected to negatively affect *G. a. williamsoni* population establishment or persistence. However, *G. a. williamsoni* might impede the establishment of *R. muscosa*. Given the direction of impact, the much slower development of *R. muscosa* compared to *G. a. williamsoni* and the differences in reproductive output between the species, the frogs should be introduced first, and ideally, the newly created frog population should be allowed to establish and grow to medium to high density (Reintroduction success: Stage 3 recovery; Miller, Bell, & Germano, 2014) before introducing sticklebacks. If *G. a. williamsoni* must be reintroduced in advance of *R. muscosa* or before *R. muscosa* has an opportunity to reproduce, selecting a receiver site with habitat complexity and abundant invertebrate prey could reduce predation pressure on *R. muscosa*. In addition, installing a mesh enclosure along the shoreline to serve as a refuge area for small tadpoles could help reduce the impact of sticklebacks on tadpole survival. Our findings indicate that while *G. a. williamsoni* poses some risk to *R. muscosa*, the timing of relocations, size and growth of individuals, and quality of habitat could help facilitate their establishment and coexistence in the same lentic sites (Table 2).

Our results elucidate key considerations when multiple at-risk species require translocation to the same site. First, interspecific interactions between the target species are likely to influence the order and timing in which release into new sites should be conducted to maximize survival. Prey species may only successfully establish themselves at a receiver site if released in advance of predators (Hayward

Table 2 Potential impacts of three spined stickleback (*G. a. williamsoni*) on mountain yellow legged frog (*R. muscosa*) tadpoles, and suggested mitigations

Observed interaction	Potential impact	Suggested mitigation
Predation	Decreased survival and recruitment	Introduce the frogs before the stickleback; introduce the stickleback only after evidence of frog population growth; avoid introducing small tadpoles; choose a release site with abundant habitat refuge, structure and numerous shallow areas; ensure alternate prey is available; install an enclosure as an artificial refuge
Sublethal predation attempts	Decreased body size and fitness	Choose a release site with abundant habitat structure and refuge and numerous shallow areas; ensure alternate prey is available
Territorial or nest defence aggression	Spatial displacement	Choose a release site with abundant habitat structure and refuge and diverse microhabitats
Interference in habitat use	Decreased body size, survival and recruitment	Choose a release site with diverse microhabitats including abundant aquatic vegetation and numerous shallow areas

& Somers, 2009). Similarly, for species that compete for common resources and have negative direct interactions (e.g. interguild predation, Amarasekare, 2002; aggression, or reproductive interference, Grether *et al.*, 2017), introducing the species that is harmed more by the direct interactions in advance of the other species may be more likely to allow both species to become established within a single receiver site. The order of translocations and number of founder individuals could affect the outcome, particularly when introducing competing or consumer-resource species pairs, in which introducing the prey species first is beneficial (Plein *et al.*, 2016). Moreover, life history-directed management may improve co-management outcomes. Competitive species that reach sexual maturity quickly, reproduce across multiple seasons and/or produce large numbers of offspring may need to be released after species that develop more slowly or produce fewer offspring, simply because of the disparity between the species in terms of population growth. Harmful interactions might be mitigated by considering the animals' size class, timing of relocations and habitat characteristics at the receiver site. Further work is needed to monitor the long-term impacts of species co-occurrence on the stability of relocated populations.

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Author contributions

SKS, DMS and GFG conceived the ideas and designed the methodology; SKS and KMS performed the experiments and collected the data; SKS, GFG and DMS analysed the data; SKS wrote the first draft of the manuscript and DMS and GFG made major revisions. DMS coordinated the project, secured funding and permits. All authors contributed critically to the manuscript drafts and gave final approval for publication.

Conflict of interest

The authors have no conflicts of interest to declare.

Data availability statement

Original data of this study are available at Mendeley Data DOI: [10.17632/7f29n6d9yy.1](https://doi.org/10.17632/7f29n6d9yy.1)

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Supporting information

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

Table S1. Ethogram of behaviors scored.

Table S2. Habitat use of stickleback fry in the presence and absence of large tadpoles. For details on experimental design and statistical model, see Methods.

Table S3. Habitat use of large tadpoles in the presence and absence of stickleback fry. For details on experimental design and statistical model, see Methods.

Table S4. Habitat use of subadult stickleback in the presence and absence of large tadpoles with hind limbs. For details on experimental design and statistical model, see Methods.

Table S5. Habitat use of large tadpoles with hind limbs in the presence and absence of subadult stickleback. For details on experimental design and statistical model, see Methods.