**Responses of showy leopard frog larvae (*Lithobates spectabilis)* to the presence of exotic rainbow trout**

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**Abstract** (300 words or 200)

The introduction of exotic fish for aquaculture can potentially have a significant impact on native fauna, especially when these introductions occur in regions where native amphibians with a biphasic life cycle occur. We conducted laboratory experiments to evaluate possible pathways of impact of exotic rainbow trout (*Oncorhynchus mykiss*) aquaculture on a native frog species (*Lithobates spectabilis*) in Mexico. Our aim was to understand the effects of trout visual and chemical cues on tadpole behaviour, and long-term effects of prolonged exposure to chemical cues (in interaction with tadpole density) on tadpole growth, development and survival. In Experiment 1, we found that tadpoles did not alter use of refugia in response to trout visual cues but did select positions within tanks that were further from trout visual cues than positions in control tanks. In Experiment 2, we observed that tadpoles responded to water-borne trout chemical cues by increasing use of refugia. In Experiment 3, we found no effect of long-term exposure to trout chemical cues on tadpole growth, development or survival, but higher tadpole density produced lower survival rates. Our findings indicate that trout farming, either via escape of fish or discharge of farm water containing trout cues without treatment, could trigger behavioural responses by native amphibian larvae that increase local tadpole densities, and thereby compromise their survival and potentially, species persistence.

**Introduction**

Amphibian populations are declining due to both abiotic and biotic factors, including invasive species that are now the second leading cause of biodiversity loss (Kats and Ferrer 2003; Stuart et al. 2004; Whittaker et al. 2013). The most common direct negative effects of invasive alien species on native amphibian populations are via predation and competition for habitat and resources, especially when it comes to invasive species that, due to their biological characteristics, require conditions similar to those required by native amphibians (Bucciarelli et al. 2014; Kats and Ferrer 2003).

One of the vertebrate groups that have commonly been translocated and become successful invaders are fish (Courtenay, 1974; Mills et al., 1993; García, 2000). Although such fish translocations potentially have benefits for humans (e.g., aquaculture: Moyle, 1976; Garcia-Berthou and Moreno-Amich, 2000; Elvira and Almodovar, 2001), they can have negative effects on native organisms (Adams, 2000; Gillespie, 2000).

Despite proven negative ecological effects, during the last 20 years aquaculture has experienced an annual growth of 5.3% globally. In 2018, 114.5 million tons of live fish, including Nile tilapia (*Oreochromis niloticus*)*,* common carp(*Cyprinus carpio*) and rainbow trout (*Oncorhynchus mykiss*)*,* were produced in the five continents, of which 47 million tons were inland-farmed fish (FAO, 2020). In Mexico, rainbow trout aquaculture is of economic importance and has been developed since 1885 (García-Mondragón et al, 2013). For 2021, it was reported that 2480 tons of this fish were produced nationally (CONAPESCA, 2021).

Known negative effects of introduced trout on native amphibians include direct predation of eggs (Gall and Mathis, 2010) and larvae (Gillespie, 2001), changes in larval morphology and fungal infections of embryos (Martín-Torrijos et al., 2016), developmental changes (Kenison et al., 2016), displacement and competition for habitat (Zamora et al., 2018; Miró and Ventura, 2018; Bosch et al., 2019; Miloch et al., 2020) and food resources (Joseph et al., 2011), behavioral changes in larvae (Bosch et al., 2006), and changes in distribution, abundance (Matthews et al., 2001) and occupancy of habitat (Velasco et al., 2017).

Additionally, it has been reported that trout farms can negatively impact aquatic ecosystems by generating effluents that alter the physical and chemical characteristics of receiving waters, which in turn has the potential to alter amphibian communities (Sosa-Villalobos et al., 2016; Krynak et al., 2020; Preston and Lamb, 2021). However, responses of amphibian larvae to water discharged from trout farms have not previously been studied.

A common trout farm design used in many countries (Cinemre *et al.*, 2006; d’Orbcastel *et al.*, 2009) involves extracting water from streams, passing the water through several tanks or ponds where fish are held (where water is oxygenating and cooled), then releasing water back into the same stream from which it was extracted, generally without any treatment. As a result, discharged water transports trout chemicals into streams where many aquatic species, including amphibians, live. Additionally, despite anti-escape designs in aquaculture farms, exotic fish (including our study species: rainbow trout) can escape and establish populations in streams where they are not native (Charles et al. 2017; Garcia-Diaz et al. 2018; Føre and Thorvaldsen 2021; pers. obs.).

Anuran larvae have innate predator recognition systems, therefore, it is necessary to understand if trout cues (visual, chemical) activate responses in species that have not co-evolved with trout. Among others, such anti-predatory responses include changes in motility and activity patterns, and selection of sites away from predators (Petranka and Sih, 1987; Hamer et al., 2002). These behavioural changes can have significant effects on larval growth and development (Skelly and Werner, 1990; Lima, 1998).

With the aim of increasing the understanding of effects of exotic trout farming and trout presence on native amphibians in Mexico, we explored: 1) whether rainbow trout visual cues induce behavioural (anti-predator) response in larvae of a native frog species (*Lithobates spectabilis*), 2) whether rainbow trout chemical cues induce behavioural (anti-predator) response in *L. spectabilis* larvae, and 3) whether long-term exposure to rainbow trout chemical cues, and their interaction with tadpole density, affect the growth, development and/or survival of *L. spectabilis* larvae.

**Materials and methods**

*Organisms for laboratory experiments*

To avoid the possibility that wild tadpoles have already learnt anti-trout behaviours, thereby affecting interpretation of our results, we used trout-naïve tadpoles reared in the laboratory for our experiments. Clutches were obtained from the field and from wild-caught frogs that reproduced in the laboratory. One clutch from the field was collected immediately after laid in a stream pond (collect permit SGPA/DGVS/09541/21) and transported to the amphibian ecology laboratory at CIIDIR-Oaxaca. Other clutches were obtained from adult frogs that were collected in the field and reproducedin the laboratory. Adult pairs of *L. spectabilis* were kept in 120 X 80 X 60 cm terrariums with water, rocks, sand and plants from the stream until they reproduced.Egg clutches were kept in filtered water with constant oxygenation. After hatching, tadpoles were reared in tanks with filtered water and were fed every three days with boiled lettuce, boiled spinach and fish food pellets (Kyorin food Ind. Ltd.) until they developed into fully mobile, early-stage tadpoles (Gosner 1960, stage 27).

Adult trout were captured from a stream near a local farm and kept in a 300 L container with aquaria oxygenators and filters, located inside a controlled temperature room (CTR) at 15.0 °C. Trout were fed twice a day with fish food.

All experiments were conducted in the laboratory at a temperature range from 15 to 20 degrees Celcius.

*Experiment 1: Response to trout visual cues*

To test whether *L. spectabilis* tadpoles are able to visually detect a trout and exhibit behavioural anti-predator responses, we ran an experiment that evaluated (1) tadpole refuge use, and (2) tadpole position relative to the (non-lethal) location of a trout.

For this, we built two 30 L plastic test arenas (one for the trout treatment and one for the control; hereafter, referred to as “tanks”, Figure 1A). Each tank was divided in half by a sealed antireflective clear glass partition to create two chambers: one for the trout (or no trout in control tanks) and one for tadpoles. In the centre of the tadpole section, we glued a plastic refuge (15 cm diameter) with two entrances (8 cm diameter, Figure 1A). In addition, the tadpole side was visually divided into four equal size sections by marking the bottom with lines parallel to the glass partition (Figure 1A). This grid marking allowed us to record tadpole location during the experiment. To ensure that tadpoles were only exposed to trout visual cues and not also chemical cues, the impermeability of the glass partition was verified by adding dye to the water on one side and ensuring that the water on the other side did not show any color change.

Before the trials, each side of the tank was filled with 10 L of water filtered by reverse osmosis. The water pH, TDS, conductivity, dissolved oxygen and temperature were measured, as well as CTR temperature and humidity.

For each replicate trial, a total of 10 tadpoles (5 for the trout treatment and 5 for the control) at stage 27 (Gosner, 1960) were haphazardly selected from a single clutch and placed in a 1 L plastic container inside the CTR for 30 minutes to allow them to acclimatize to the room temperature.

To start the experiment, the tadpoles were placed in the tadpole side of each tank (section 2, Figure 1A) and cameras were turned on. After 10 minutes, one trout was placed in section 1 of the trout treatment tank, while section 1 of the control tank remained empty. To avoid any effect of human presence on tadpole responses, trials were video recorded with a camera located directly above each tank. All trials lasted 15 minutes. We ran the trials using 2 tadpole clutches, with 3 or 7 replicates per treatment per clutch (as determined by tadpole availability).

The response variables (refugia use, tadpole location) were measured by analyzing the videos with Avidemux 2.7.5 software (Mean, 2019). Every 30 seconds, the number of tadpoles inside and outside refugia were counted and the location of tadpoles throughout the tank was recorded. We subsequently categorized location data for each tadpole at each time interval as either “close to” or “far from” the visual fish cue based on whether the tadpole was in the half of the tadpole section closest to the fish, or in the half of the tadpole section furthest from the fish, respectively.

*Experiment 2: Response to trout chemical cues*

To evaluate whether *L. spectabilis* tadpoles detect and behaviourally respond to water-borne trout chemical cues, we built two gravitational flow systems (GFS) modified from Petranka and Sih (1987). Each GFS consisted of four plastic containers (phases) interconnected by a 1-inch hose with PVC valves to regulate flow (Figure 1B). Phase (a) was equipped with an aquarium oxygenator to hold a single live trout during the trials; phase (c) contained a plastic refuge for tadpoles (15 cm diameter) with two entrances (8 cm diameter) glued to the bottom. Phase (b) and (d) allowed the maintenance of constant water flow through the entire system, with phase (d) being equipped with an 18 W water pump. The GFS was placed inside a Controlled Temperature Room (CTR).

The experiment consisted of exposing groups of 10 tadpoles (stage 27, Gosner, 1960) to control water (control GFS) or "trout water" (treatment GFS) and recording tadpole behavioural response (number of tadpoles using the refugia). For each replicate, tadpoles from a single clutch were haphazardly selected. Tadpoles were placed in a 1 L plastic container and allowed to acclimatize to the room temperature for 30 minutes prior to each trial. After this period, we placed the tadpoles in phase (c) of each GFS and turned the pump on to allow water circulation. We then placed one trout in phase (a) for the trout GFS.

For all trials, we used water filtered by reverse osmosis (15 L in each phase). All water parameters were measured before each trial: pH, conductivity, total dissolved solids, dissolved oxygen and temperature and room conditions were the same among experiments. To avoid effects of human presence and to accurately count tadpoles using the refugia, all trials were recorded with a video camera located directly above phase (c).

Trials lasted for 20 minutes, which is the time that it took for water to circulate through the entire GFS. The response variable (refugia use) was measured by analyzing the videos with Avidemux 2.7.5 software (Mean, 2019) and counting the number of tadpoles inside and outside refugia every 5 minutes. We ran these trials using two tadpole clutches, with either 2 or 8 replicates per treatment per clutch (as determined by tadpole availability).

*Experiment 3: Effect of trout chemical cues and tadpole density on tadpole growth, development and survival*

Because tadpoles in nature might respond by avoiding stream sites where trout are present (i.e., have escaped) or are perceived to be present (trout cues discharged from trout farms), a possible consequence could be higher tadpole densities at certain sites. Such increased local densities could have negative effects on tadpoles due to increased stress.

We conducted a long-term experiment to evaluate the effects of exposure to trout chemical cues and tadpole density on growth, development and survival of *L. spectabilis* tadpoles. Tadpoles from single clutch (TRUE? BECAUSE MY DATA FILES DON’T IDENTIFY MULTIPLE CLUTCHES) were reared in containers filled with 4 L of water at three density levels: low (2 tadpoles), medium (4 tadpoles) and high (8 tadpoles). At each density level, tadpoles were exposed to one of two water treatments: control or trout cue (6 replicates per treatment, N = 36 containers total). The control containers contained no trout cue (YOU MEAN REVERSE OSMOSIS WATER LIKE IN EXPERIMENTS 1 AND 2?) while trout cue water was made by mixing 3750 ml of clean water with 250 ml of water obtained from a 300 L container where 3 live trout were kept.

Tadpoles at Gosner (1960) stage 27 were placed in each container and were left for 4.5 months in the CTR; every 5 days, tadpoles were fed 1 g of boiled lettuce and 0.1 g of algae powder per tadpole. By maintaining a constant food supply per tadpole, we aimed to investigate density effects *per se*, rather than competitive effects via reduced food supply at increased density. Every 15 days, the water in each container was replaced and tadpoles were weighed (Ohaus Scout digital scale), measured with digital calipers (CD-6”AX, Mitutoyo Corp: tail length, body length, total length) and assessed for developmental stage (Gosner, 1960). When a tadpole died, it was replaced with another tadpole marked by a scalpel incision at the tip of the tail. This replacement tadpole was not measured or considered in the statistical analyses; its role was to maintain treatment density.

*Statistical analyses*

All data was analyzed in R (R Core Team, 2021).

To test the effects of trout cues on tadpole behaviour (Experiments 1 and 2), we compared the proportion of tadpoles using refugia (response to visualand chemical cues) and tadpole location relative to the fish (response to visual cues only) between treatments. The response of each tadpole at each observation time was categorized as a binomial response (inside refugia *vs* outside refugia; close to fish *vs* far from fish). Data were analyzed using logistic regression (Warton and Hui, 2011; generalized linear mixed effects) with quasibinomial models to account for under- or over-dispersion (package MASS:glmmPQL, Venables and Ripley, 2021) followed by Anova (package car:Anova, Fox et al., 2021). All models included treatment (control *vs* trout cue) and observation time as fixed effects, with tank nested within tadpole clutch as a random effect to account for non-independence of observations from the same tank over time.

To test tadpole responses to prolonged exposure to trout chemical cues and density (Experiment 3), we analyzed data for body length, tail length, total length, weight and developmental stage using generalized linear mixed effects models (package lmerTest:lmer, Kuznetsova *et al.*, 2020) followed by Anova (package car:Anova, Fox *et al.*, 2021). These models included treatment (control *vs* trout cue), density (2, 4 or 8 tadpoles), the treatment x density interaction, and observation time as fixed effects. Tank was included as a random effect to account for non-independence of observations from the same tank over time. We analyzed survival data as a binomial response (tadpole alive *vs* dead, as described for above for binomial analyses in Experiments 1 and 2, followed by Tukey tests) using the same mixed effects model structure. To account for convergence issues in the survival analysis (due to 100% survival in the “2 tadpoles” density treatment), we allocated one tadpole to have died in this treatment to obtain a conservative estimate of effect (Warton and Hui 2011). The treatment x density interaction was not significant for any of the Experiment 3 analyses, and so was removed before we re-ran the models to test for main effects.

**Results**

*Response to trout visual cues*

Tadpoles did not significantly alter refuge use in response to trout visual cues, but refuge use did vary significantly over time (Table 1). However, tadpoles did significantly alter their location in response to trout visual cues: tadpoles positioned themselves further from the center of the tank in the trout visual cue treatment (i.e., further from the trout visual cue) than the control treatment (Table 1, Fig. 2A). Observation time was not a significant covariate in the tadpole location model (Table 1).

*Response to trout chemical cues*

There were significant effects of trout chemical cues and observation time on tadpole refuge use. A higher proportion of tadpoles used refugia in the presence of trout chemical cues than in the control treatment (Table 1, Fig. 2-B).

*Effect of trout chemical cues and tadpole density on tadpole growth, development and survival*

There were no significant effects of trout chemical cues or density on tadpole body length, tail length, total length, weight or developmental stage (Table 1). As would be expected, there was a significant effect of observation time on all tadpole growth and development variables (Table 1).

Tadpole survival was also not significantly affected by exposure to trout chemical cues, but survival did vary significantly with tadpole density and time (Table 1). Tadpole survival was negatively affected by tadpole density: Tukey tests showed that survival in the high density treatment (8 tadpoles) was significantly different (lower) from both the medium density (4 tadpoles: *t* = -3.592, *df* = 33, *p* = 0.0029) and low density (2 tadpoles: *t* = -3.600, *df* = 33, *p* = 0.0029) treatments (Fig. 2C). There was no significant difference between survival in the medium (4 tadpoles) and low (2 tadpoles) density treatments (*t* = -0.984, *df*= 33, *p* = 0.5915).

**Discussion**

Our results show that larvae of a native amphibian (*L. spectabilis*) are able to detect both visual and chemical cues of exotic rainbow trout and change their behaviour in response. However, long-term exposure to trout chemical cues did not directly affect larval growth, development or survival.

The experiment on visual cues indicated that in optimal visibility conditions (due to the clarity of the water used in the experiments: total dissolved solids <0.11 mg/L), tadpoles of *L. spectabilis* are capable of visually detecting rainbow trout and move away in response. This reaction is consistent with other tadpole-fish systems where predator detection through visual stimuli plays an important role in tadpole responses (Hettyey et al., 2012). However, another aspect that can influence the visual detectability of a predator is the distance between predator and prey (Takahara, 2012) and the size of the predator (Jara and Perotti, 2010). In our experiments, we used trout of relatively large size (total length ~25 cm) which could have contributed to the ability of tadpoles to visually detect trout. Other experiments with ranid species showed that visual signals alone produce a weak response in tadpoles, which has been interpreted as tadpoles having either poor or limited vision (Stauffer and Semlitsch, 1993). However, our results clearly demonstrate that *L. spectabilis* larvae are capable of visually detecting a potential fish predator.

It is assumed that under natural conditions, multimodal signals obtained by prey about the presence of a predator cannot be isolated, and therefore a more effective response is expected when prey are simultaneously exposed to multiple predator cues (Ward and Mehner, 2010; Stynoski and Noble, 2012). Nonetheless, our experiments demonstrate that *L. spectabilis* tadpoles detect and respond to individual predator cues: tadpoles moved away from a potential predator when the only signal received was a visual stimulus, and increased refuge use when the only signal received was a chemical stimulus. The fact that *L. spectabilis* tadpoles did not alter refuge use in response to trout visual cues, but increased refuge use in response to trout chemical cues, suggests that chemical cues conveyed a signal of greater immediate threat to tadpoles than did visual cues. Increased use of refugia in response to predator chemical cues has been shown in other species (Laurila et al., 1997). Such hiding behavior is an effective defense mechanism in the short term since the reduction of movement is correlated with higher survival rates of prey (Lawler, 1989) and lower probability of detection by predators (Babbit and Jordan, 1996). However, costs for this behaviour are a reduction of available time to feed (Eklöv and Halvarsson, 2000) or changes in feeding schedules (Feminella and Hawkins, 1994) which can affect growth and development (Park and Do, 2023).

Even when anuran larvae have innate predator recognition systems (Rajchard, 2006), the effectiveness of this mechanism depends on the existence of constant predation regimes where prey and predators have co-existed for long periods of time, which in the context of introduced species, rarely occurs (Gall and Mathis, 2010). In this regard, studies on the detection of chemical signals emitted by native and introduced turtles showed that tadpoles recognize the former and reduce their mobility, but do not respond to the latter, which can explain the displacement of native anuran species by introduced predator species (Polo-Cavia et al., 2010). In contrast, we found that *L. spectabilis* tadpoles are able to detect and respond to chemical signals of exotic rainbow trout. However, there is a risk that this responsiveness can be reduced over time because other ranid tadpoles can become habituated to chemical cues emitted by salmonids (Polo-Cavia et al., 2020).

Our experiment on long-term exposure to trout chemical cues showed no significant effect on tadpole growth, development or survival. However, in this experiment, trout water was added every 15 days instead of constant exposure, which could have influenced our results. It is also possible that the concentration of trout cue we used was lower than what has been used in other studies or is found in nature where trout farms exist. It is important to obtain accurate measurements of the concentration of these chemicals in nature to better understand and predict effects of trout farms on native aquatic species.

Alternately, the results of our long-term experiment could be a consequence of tadpole habituation to trout chemical cues (Polo-Cavia et al., 2020). If that is the case, habituation could increase predation by trout if tadpoles reduce their responses to trout cues over time. It is common to find established escaped rainbow trout in the rivers of our study area, so long-term exposure to trout cues is a possible scenario in nature. Additionally, anuran larvae can respond to the presence of a predator via different mechanisms, such as accelerated time to metamorphosis (i.e., reduced larval period: Skelly and Werner, 1990) or changes in morphology (Thimann, 2000, (Buskirk and Yurewicz, 1998 (Teplitsky et al., 2003). Further investigation is required to see whether *L. spectabilis* tadpoles respond to trout cues via these mechanisms.

Our long-term experiment also demonstrated that density had a significant effect on tadpole survival, with tadpoles from the high density treatment having lower survival than tadpoles in medium or low density treatments. Other studies have also shown that increased density reduces the survival of anuran larvae (e.g., Newman, 1987). This result is important because our first two experiments showed that trout cues can increase local density of tadpole via behavioural changes (movement to areas further away from visual cues, increased refuge use in the presence of chemical cues). Furthermore, we have observed where trout are present in streams, *L. spectabilis* tadpoles congregate in high densities in shallow areas that are inaccessible to juvenile or adult trout (pers. obs). Thus, live trout in streams and trout cues in fish farm discharge have the potential to change the spatial distribution of tadpoles, increasing local density and thereby reducing tadpole survival.

Despite well documented negative effects of introduced trout on native amphibians, indirect effects of trout aquaculture are less understood. For trout farms with a design that requires the extraction of fresh water from a stream and its subsequent discharge into the same system, known impacts of discharge include increased concentration of nutrients (ammonium, nitrite, nitrate, phosphate), reduced concentration of dissolved oxygen, and altered composition of benthic macroinvertebrate species and bacterial communities (Boaventura et al., 1997; Camargo and Gonzalo, 2007). Our data show that that the establishment of such trout farms has the potential to negatively affect native amphibian larvae via behaviour-induced changes in behaviour due to direct introduction (escape) of trout into streams or release of trout chemicals into streams.

Although changes in native ecosystem structure due to aquaculture are known, and despite rainbow trout being listed among the 100 worst invasive species, the cultivation of rainbow trout continues to be promoted in several countries (Cruz-Castro et al., 2011; Hernández and Carrillo, 2018) but not always with effective regulation. In Mexico, for example, *O. mykiss* is included in the national list of invasive exotic species (DOF: 12/07/2016) but is not considered in the latest update of the National Aquaculture Act as it does not include a biosafety regulation that takes into account the risk of trout escaping into the environment (DOF: 09/09/2013), even when those recommendations are given for other exotic cultured species such as the Australian lobster *Cherax quadricarinatus* (DOF: 09/09/2013).

Our research offers evidence that a better evaluation of the impact of trout farming on native amphibian fauna in natural waterways is needed. Additionally, regulation of this practice needs to be improved to incorporate effective biosecurity protocols to prevent the release and establishment of rainbow trout populations outside their natural range, particularly in areas of high amphibian richness. It is also necessary to promote the use of water recycling systems that allow water to be treated with biofilters before being reintegrated into aquatic systems and, above all, reduce the escape of invasive alien species into ecosystems.

Finally, in Mexico there is little data on the distribution of *O. mykiss* in the natural environment, or on the responses of native amphibians which have been recently exposed to this exotic predator. The practice of trout farming in regions of Mexico with high amphibian diversity and a highly marginalized portion of society is increasing and has been promoted by government programs that do not consider the negative ecological effects of this farming. In addition, these aquaculture programs do not meet the objective of improving the welfare of people from poor rural communities (Tapia and Zambrano, 2003) and might represent a risk for amphibian permanence in those aquatic systems and thus for ecosystem functioning.

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**Authors’ contribution**

CAF, MA and EGB collected organisms, raised and maintained them in the laboratory and conducted the experimental trials. EGB, CAF and MRC designed the experiments. MRC and CAF conducted the statistical analyses. CAF, EGB and MRC wrote the first draft of the manuscript and all authors participated in the writing of the final draft of manuscript. All authors read and approved the final version of the manuscript.

**Ethics and permits**

All procedures were approved by the Escuela Nacional de Ciencias Biológicas ethics committee (ENCB/CEI/026/2022, CONBIOÉTICA-09-CEI-002-20190327). Permission for collection, transportation and maintenance in captivity of organisms was granted by Mexican Secretaría de Medio Ambiente y Recursos Nautales (SEMARNAT) permit number: SGPA/DGVS/09541/21.

FIGURES

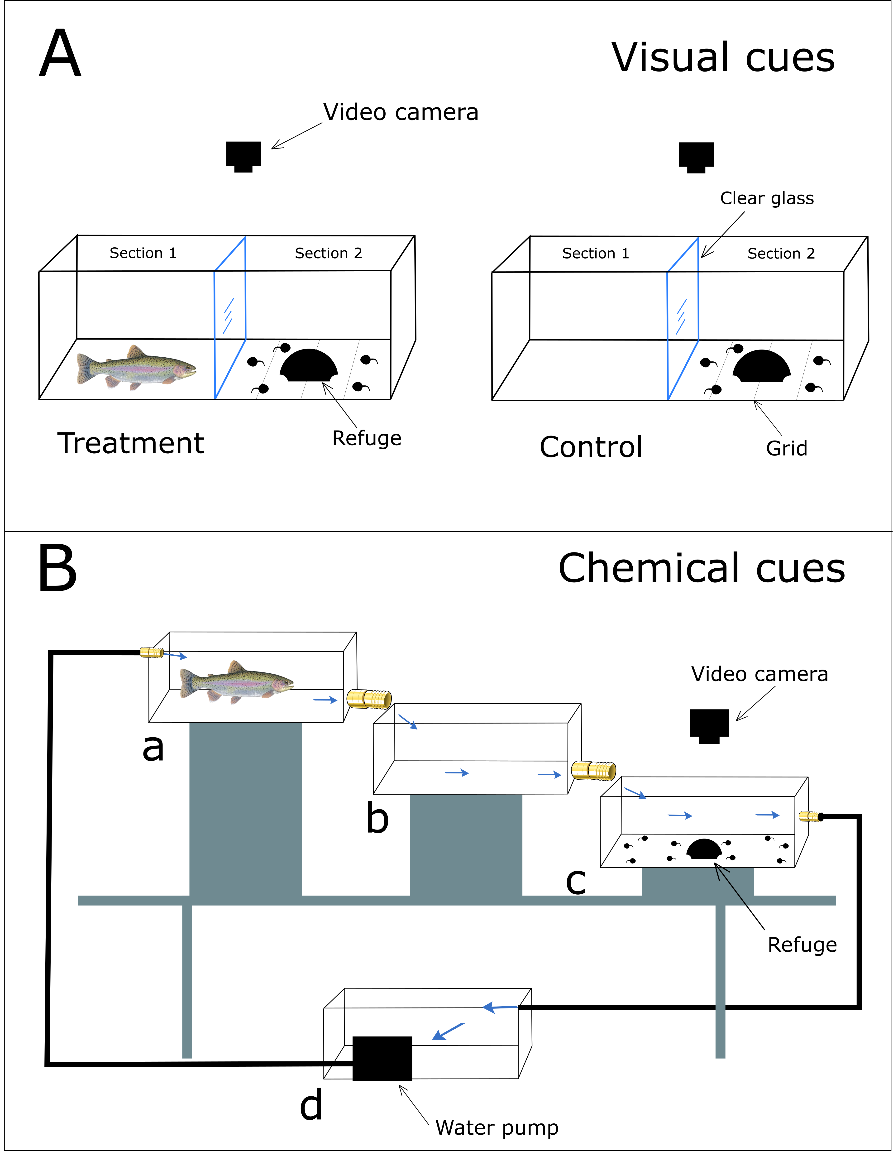
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Figure 1. A) Testing chamber for larvae response to visual cues. One tank for treatment (fish present) and one for control (fish absent). B) Gravitational flow system (GFS) for chemical cues testing: a) Test chamber where fish is placed in treatment and absent in control b) section for water flow balance c) chamber to evaluate tadpole response in presence or absence of fish d) section of GFS which contains a water pump to recirculate water.

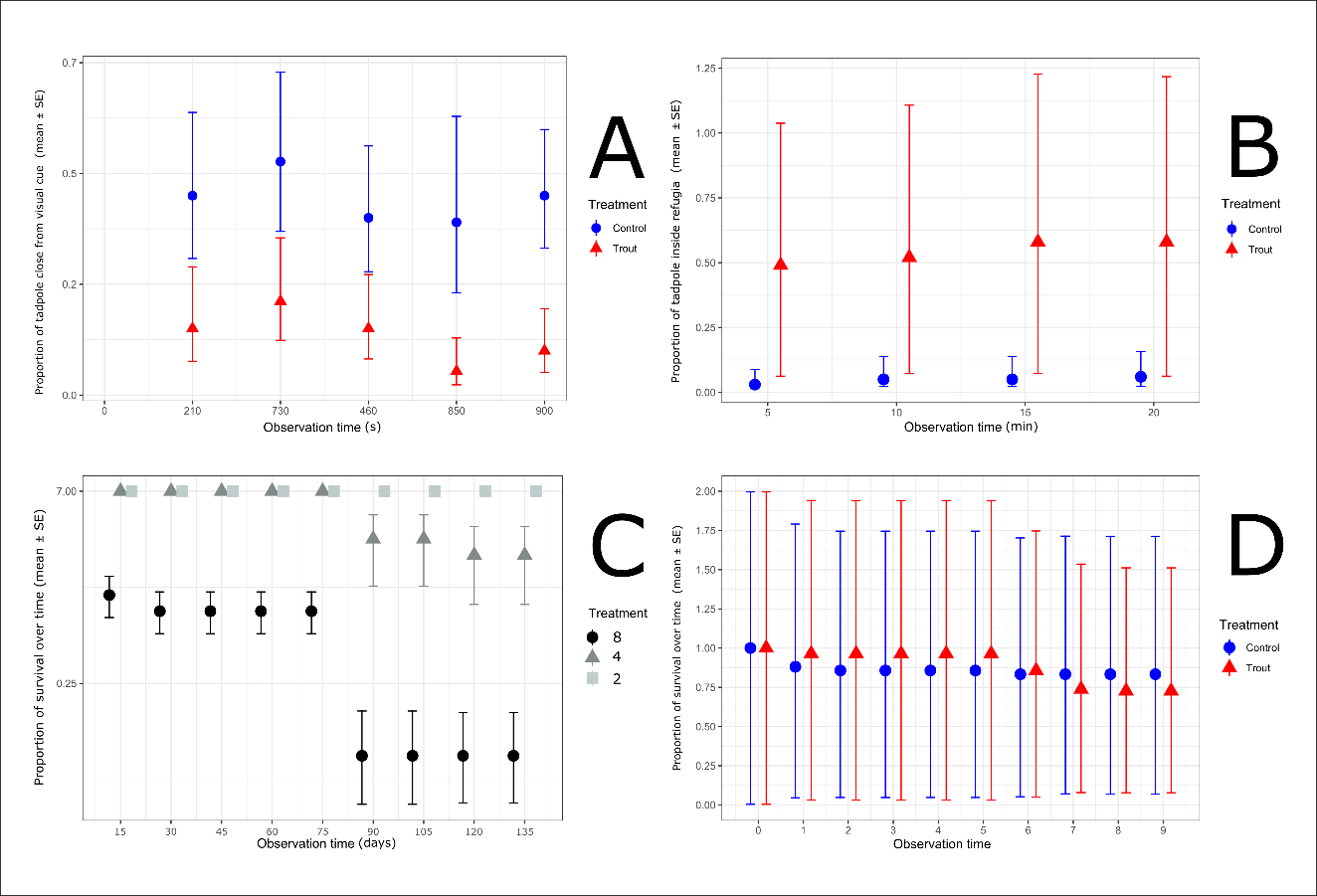
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Figure 2. A) Location of larvae in the presence or absence of trout visual cue. B) Refuge use by larvae in the presence of absence of trout chemical cues. C) Effect of density treatment on tadpole survival according to density treatments. D) Tadpole survival on prolonged exposure to a chemical cues emitted by a trout.

Table 1. Results of statistical models used in the three experiments.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| ***Experiment*** | ***Dependent variable*** | ***Fixed effect*** | ***Random effect*** | ***ANOVA*** | | | | | |
|  |  |  |  |  | ***X2*** | | ***df*** | ***p*** | | | |
| Visual cues | Refugia use | Treatment  Observation time | Clutch  /  Tank |  | 0.1648  40.400 | | 1  1 | 0.6848  <0.0001\* | | | |
| Location | Treatment  Observation time | Clutch  /  Tank |  | 21.4123    0.3702 | | 1  1 | <0.0001\*  0.5429 | | | |
| Chemical cues | Refugia use | Treatment | Clutch  / |  | 47.144    6.999 | | 1  1 | <0.0001\*  0.0081\* | | | |
| Observation time | Tank |
| Growth and development | Body length  Tail length  Total length  Weight  Stage | Treatment  Observation time  Density  Treatment *x* Density  Treatment  Observation time  Density  Treatment *x* Density  Treatment  Observation time  Density  Treatment *x* Density  Treatment  Observation time  Density  Treatment *x* Density  Treatment  Observation time  Density  Treatment *x* Density  Treatment  Observation time  Density  Treatment *x* Density | Tank  Tank  Tank  Tank  Tank  Tank |  | | 1.4243  1253.2  2.2867  1.1472  0.1203  1992.2  0.1620  1.7789  0.0900  2498.7  0.1288  1.3770  1.4143  25.991  1.0059  2.6754  1.3672  2132.5  0.5221  0.5002 | 1  1  2  2  1  1  2  2  1  1  2  2  1  1  2  2  1  1  2  2 | 0.2327  <0.0001\*  0.3188  0.5635  0.7287  <0.0001\*  0.9222  0.4109  0.7642  <0.0001\*  0.9376  0.5023  0.2343  < 0.0001\*  0.6048  0.2624  0.2423  <0.0001\*  0.7703  0.7787 | | |
| Survival |  | | 0.0406  247.65  12.392  0.5693 | 1  1  2  2 | 0.8403  <0.0001\*  0.0020\*  0.7522 |

*X*= interaction between variables, \* significant *p* values.

**CITED LITERATURE**

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