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Rainbow trout (*Oncorhynchus mykiss*) threaten Andean amphibians

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Amphibian populations are declining due to a myriad of abiotic and biotic factors, including invasive species and pathogens. In temperate freshwater ecosystems, for example, amphibian populations are threatened by the predation of introduced salmonids. Salmonids not only directly predate upon amphibian eggs and larvae but may also transport deadly pathogens into freshwater systems. Though most research has focused on temperate systems, much less is known about the effects of introduced species in Neotropical streams. We conducted two experiments to investigate the impacts of rainbow trout (*Oncorhynchus mykiss*) in two Neotropical anurans. First, we assessed the effect of the rainbow trout on tadpole survivorship and morphology in *Nymphargus grandisonae*, a glassfrog species endemic of the Andes. Tadpoles of *N. grandisonae* were subjected to three treatments involving the absence of rainbow trout (control) and its presence with different types of chemical cues – kairomones (from rainbow trout) and cue alarms (from tadpole prey); the results show that the presence of rainbow trout affects the larval morphology of this glassfrog. In the second experiment, to test whether rainbow trout is a vector of the pathogenic freshwater mold *Saprolegnia diclina* (Oomycetes), eggs of *Engystomops petersi* were placed with infected and uninfected rainbow trout. There was a high mortality rate in the embryos of *E. petersi* exposed to trout infected with *S. diclina*. This represents the first evidence that rainbow trout may have a direct negative effect on Neotropical amphibian populations, and thus should be considered a threat. Management programs should be implemented to eradicate trout from Andean rivers, especially in areas with high number of endangered amphibians.

Las poblaciones de anfibios están disminuyendo debido a una serie de factores, incluyendo las especies invasoras y patógenos. En los ecosistemas templados, por ejemplo, los anfibios están amenazados por salmónidos introducidos. Los salmónidos no solo depredan directamente los huevos y larvas, sino que también son vectores de patógenos en los ecosistemas acuáticos. A pesar de que existe una serie de investigaciones en zonas templadas, el efecto de las especies introducidas en sistemas neotropicales es prácticamente desconocido. En este trabajo estudiamos experimentalmente el efecto de la trucha arcoíris (*Onchorhynchus mykiss*) en dos especies de anuros neotropicales. Primero, determinamos el efecto de la trucha en la supervivencia y morfología de renacuajos de *Nymphargus grandisonae*, una rana de cristal endémica a los Andes. Los renacuajos fueron sometidos a tres tratamientos, los cuales combinan la ausencia de la trucha (control) y su presencia con diferentes señales químicas: cairomonas (de las truchas) y de alarma (producidas por los renacuajos depredados). Los resultados demuestran que la presencia de la trucha afecta la morfología de los renacuajos. En el segundo experimento, para determinar si la trucha actúa como vector del pátogeno *Saprolegnia diclina* (Oomycetes), los huevos del sapo *Engystomops petersi* fueron expuestos a la presencia truchas infectadas o no infectadas con *S. diclina*. El experimento muestra que la trucha actúa como vector de *S. diclina*, la cual produce una alta mortalidad en los huevos de *E. petersi*. Este es el primer estudio que claramente indica el efecto negativo de la trucha arcoíris en anfibios neotropicales. Recomendamos implementar programas de manejo dirigidos a erradicar a este pez introducido de los ríos andinos, especialmente en áreas con una alta presencia de especies amenazadas de anfibios.

Keywords: amphibian declines; invasive species; *Saprolegnia diclina*; phenotypic plasticity; emerging infectious diseases

Introduction

The decline of amphibian populations since the 1970s is a complex global phenomenon involving interactions among biotic and abiotic factors that affect amphibians at various stages of their life.[1–6] Habitat alteration and destruction, global environmental change, emergent infectious diseases, contaminants, and/or introduced species have been associated with amphibian population declines.[1,4,7–9]

The impact of alien species is a leading cause of biodiversity loss in freshwater systems,[10] particularly in amphibian populations, which can be driven to extinction.[7,11–13] The rainbow trout is one such alien species common in many freshwater ecosystems. Native

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to the Pacific Coast of North America, this fish has been introduced in at least 125 countries globally,[14] where it primarily is used for sport fishing and aquaculture. As a result, this species has become an exemplar of one of the world′s worst invasive alien species.[15–17]

In the Ecuadorian Andes, rainbow trout were introduced in 1920[14]; they have become the main fish predator of Andean tadpoles, because native fishes are mostly catfishes that feed on small insect larvae and annelids.[18,19] Several studies have shown that introduced salmonids have a negative effect on anuran populations.[20–25] This may reflect the aggressive feeding behavior of trout, along with the absence of a shared predator–prey evolutionary history. Although many anurans have evolved anti-predatory defenses against coexisting predators, these defenses seem to be ineffective with alien predators.[22,26–30] Alternately, it is possible that evolutionary history has endowed many organisms with the possibility of altering their phenotype in response to changes (i.e. new predators) in the environment that surround them.[31] These changes are known as phenotypic plasticity.[30,32] Anurans can exhibit anti-predator behavior (plastic defense traits) when exposed to chemical cues produced during predation events – kairomones (components from predators) and cue alarms (components from prey consumed).[30,32–35]

Among other threats to amphibian populations are some emerging infectious diseases that can be transported by migratory fishes.[1,36–38] For example, the oomycete *Saprolegnia* can parasitize freshwater animals and their eggs[3,39]; salmonids (specifically trout) are linked to this disease.[37,40] Also, *Saprolegnia* parasitizes anuran eggs and cause embryonic mortality, and thus, is associated with amphibian declines.[3,37,41–43]

While multiple causes are involved in amphibian declines,[43] it is necessary to determine the effect and extent of each contributing factor. Consequently, we first tested the hypothesis that the presence of exotic rainbow trout would affect the morphology and survivorship of tadpoles of *Nymphargus grandisonae*. We expected that tadpole survivorship would decrease and that morphological traits would be affected. Second, we tested whether rainbow trout can act as a vector of the pathogen *Saprolegnia diclina* and cause disease, by exposing *Engystomops petersi* eggs to the presence of rainbow trout infected and uninfected by this pathogenic oomycete. We expected that eggs exposed to the infected trout would have a higher mortality than eggs exposed to uninfected trout.

Methods
Biological collections
We obtained two clutches of *N. grandisonae* (Amphibia: Centrolenidae) from Reserva Las Gralarias (00°00′33″S,

78°44′15″W; 2150 m), Pichincha Province, Ecuador, on 8 July 2011. As other glassfrogs, *N. grandisonae* places the eggs on riparian vegetation; tadpoles hatch and fall into nearby streams. No parental care was evident during our fieldwork and collected clutches were unattended. Only native fish (*Astroblepus* sp.) were observed in the streams of Las Gralarias reserve. However, we did not conduct specific searches for trout. Since several trout fisheries are nearby the reserve, it is possible that trout have been introduced in the area.

On 15 August 2011, eggs of the Amazonian *E. petersi* (Amphibia: Leptodactylidae) were collected from a trout-free pond at Sacha Yaku Wildlife Rescue Center (01°24′07″S, 77°43′06″ W; 1078 m), Pastaza Province, Ecuador. At Sacha Yaku, this toad regularly reproduces in temporary ponds. Trout are absent from this lowland locality.

Twenty rainbow trout (*Oncorhynchus mykiss*: Family Salmonidae) of uniform size (body length of 16–18 cm) were obtained from the private fish hatchery La Quinta Pinta, located near Reserva Las Gralarias. Before experiments were conducted, all trout were disinfected with 4 ppm malachite green.[44]

Experimental design
All experiments took place at the Biodiversity Laboratory at Universidad Tecnológica Indoamérica, Quito, Ecuador. To determine the effect of trout on the survivorship and morphology of glassfrog tadpoles, we conducted an experiment consisting of a randomized design with three treatments replicated three times, for a total of nine experimental units (Figure 1), during a four-wk period. A replicate consisted of a central 45-l tank containing 1 trout connected by hoses to 10 independent containers (subunits, hereafter). Each tadpole tank had aerators and filters, as well as sand and rocks obtained from the stream from which the larva was collected. A fine mesh prevented tadpoles from swimming into the trout tank but permitted water and chemical cues to circulate among the tanks. All water was chlorine-free. Each treatment had a total of 30 tadpoles, 15 from each of the two clutches. Treatments were as follow: (1) tadpoles in the absence of trout (control); (2) tadpoles in the presence of one trout; and (3) tadpoles in the presence of one trout that fed on *N. grandisonae* tadpoles. In the case of the latter, we added two tadpoles directly into the tank at the beginning of every week.

Effect of trout on the survivorship of tadpoles
We checked each subunit weekly to record the survivorship of the tadpoles, and used Fisher's exact test [45] to determine whether the treatments had a significant effect on tadpole survivorship.

Figure 1.   (a) Randomized design, with the nine experimental units. A replicate consisted of a 45-L tank (with or without a trout) connected by hoses to 10 independent containers (subunits), each of which housed a tadpole, with sand and rocks obtained from the stream of origin. (b) A fine mesh prevented tadpoles from swimming into the tank, but allowed the flow of water and chemical cues between the tank and surrounding containers. (c) Trout were kept with an aerator and filter, to limit water contamination.

Effect of trout on the morphology of tadpoles

To evaluate changes in tadpole morphology, we obtained dorsal and lateral photographs of each tadpole before and after the experiment. Photographs were processed with the morphometric software tps-Util and tps-Dig. We measured the following morphological variables: maximum body length (BL), maximum body width (BW), maximum eye width (EW), maximum tail muscle width (TW), maximum tail length (TL), maximum anterior tail depth (ATD), maximum body depth (BD), and maximum tail depth (TD) (Figure 2). To test phenotypic changes, we first calculated the differences between the initial and final measures of each variable. We removed body size differences among tadpoles by regressing all variables with the total length and saving the standard residual values.[46,47]

Because tadpoles from our subunits were not independent (i.e. flow of water and chemical cues were shared among the tank and subunits),[48] we conducted an ANOVA nested design with the standard residual values of the differences between initial and final measures, in which subunits were nested within tanks and treatments. Owing to the unequal number of tadpoles in both treatments, the degrees of freedom were adjusted for an unbalanced design using Satterthwaite's method.[49] To assess the effect of the treatments on morphology, we performed a principal components analysis (PCA).[50] The standard residual values for the eight variables were introduced into the PCA and recorded the scores from the first and second principal components (PC1 and PC2, respectively). With the resulting scores from the PCA, we conducted an ANOVA for each of the principal
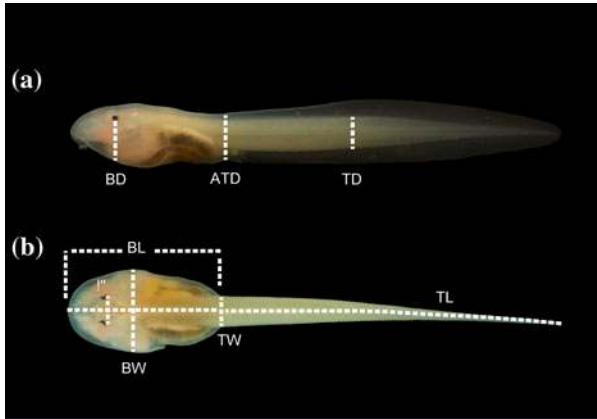
Figure 2. Morphological variables measured in tadpoles of the Red-spotted Glassfrog (*N. grandisonae*). (A) *Dorsal view*: maximum body length (BL), maximum body width (BW), maximum eye width (EW), maximum tail muscle width (TW), maximum tail length (TL). (B) *Lateral view*: maximum anterior tail depth (ATD), maximum body depth (BD), and maximum tail depth (TD).

components, including a *post-hoc* test to identify which treatments, if any, were significantly different.

With the standard residual values of the differences between final and initial measures, we conducted a MANOVA. *Post-hoc* tests were included to identify which treatments were significantly different. All statistical analyses were conducted in STATISTICA 7.

Transmissions experiments of Saprolegnia

To test whether the trout could act as vector of *S. diclina*, and to test the pathogenicity of *S. diclina*, we used a randomized design consisting of two treatments replicated two times, for a total of four experimental units. The eggs of *E. petersi* were exposed during 1 wk to the following treatments: (1) presence of one uninfected trout (control); and (2) presence of one trout infected with *S. diclina*.

We used the same experimental design tanks and subunits from the survivorship and morphological experiment, after having disinfected them with a 10% sodium hypochlorite solution. Isolates of *S. diclina* used to infect the trout came from the culture collection at the Real Jardín Botánico (Spain; available by request). Four rainbow trout were infected by exposure to *S. diclina* cultures (culture introduced in a 1mm-sieve size mesh enclosure) for 2 days. The control tanks contained uninfected trout (disinfected with malachite green 4 ppm). Each subunit contained between 4 and 13 clumped eggs of *E. petersi*. In the final phase, some of the eggs were removed from the subunits and washed with autoclaved river water with 100 mg l^{-1} Ampicillin to prevent bacterial growth; these eggs were placed in peptone glucose agar until they were

tested for *Saprolegnia* infection following the procedures described by Fernandez–Beneitez et al.[3] The remaining eggs were checked daily to record the number of live and dead embryos, to calculate survivorship prevalence of each treatment. We used an unpaired t-test to determine if our two treatments were significantly different.

Ethics statement

Animal research was performed under the approval and supervision of the Centro de Investigación de la Biodiversidad y Cambio Climático, Universidad Tecnológica Indoamérica. Research permits were issued by the Ministerio del Ambiente de Ecuador (N14-2011-IC-FAU-DPAP-MA).

Results

Effect of trout on the survivorship of tadpoles

Although both treatments containing trout had lower survivorship (80% survivorship) compared with the control group, in which no tadpoles died (100% survivorship), tadpole survivorship was not significantly affected according Fisher exact test ($p = 0.884$) (Figure 3).

Effect of trout on the morphology of tadpoles

The unbalanced nested ANOVA test showed no significant differences, between either the subunits or the tanks for the eight variables (Table 1). PCA produced two principal components for the morphological characteristics. The first principal component (PC1) explained 40% of the variation (eigenvalue = 4.421), whereas the second principal component (PC2) explained 29% of the variation (eigenvalue = 1.157). In PC1, seven morphological variables loaded strongly and positively (0.6–0.8), and tail length loaded strongly but negatively (−0.6). In PC2,
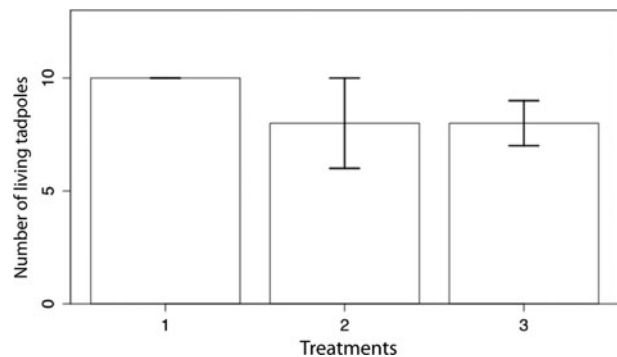

Figure 3. Tadpoles survivorship among treatments. Treatments are: 1 (absence of rainbow trout), 2 (presence of rainbow trout), and 3 (presence of rainbow trout preying on *N. grandisonae* tadpoles). Each treatment contained a total of 30 tadpoles.

Table 1. Results of the unbalanced nested ANOVA for eight morphological variables.

	df	BL F	BW F	EW F	TW F	ATD F	BD F	TD F	TL F
Subunits	9	1.02	0.88	0.65	1.05	0.89	1.56	1.13	1.24
Tanks	2	0.92	3.25	0.05	0.32	0.23	7.28	0.69	0.12
Treatments	2	6.8**	7.70**	1.20	4.38*	14.41***	5.36**	5.92**	1.92

Notes: Maximum body length (BL), maximum body width (BW), maximum eye width (EW), maximum tail muscle width (TW), maximum tail length (TL), maximum anterior tail depth (ATD), maximum body depth (BD), and maximum tail depth (TD). Levels of significance: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

tail length loaded strongly (0.7), five variables loaded weakly (0.2–0.3), and the two remaining variables loaded weakly and negatively (−0.1, −0.4) (Table 2). Thus, the PC1 should be interpreted as overall size and the PC2 as overall shape, in which tail length plays an important role on the size and shape of the tadpoles of this species. Subsequently, we tested residual factors scores extracted from the PCA analysis (PC1 and PC2) with an ANOVA analysis. For PC1, there were significant differences among treatments ($F_{2,75} = 6.370$; $p = 0.003$), with Treatment 1 (control) significantly differing from Treatment 2 (predator presence) and Treat-

Table 2. Eigenvalues, percentage of variance explained, and loadings of the tadpoles' phenotypic traits from the first principal component (PC1) and second principal component (PC2), as determined in the PCA.

Variables	PC1	PC2
Eigenvalues	4.421	1.157
% Variance explained	40.071	29.651
Loadings		
Maximum body length	0.886	−0.165
Maximum body width	0.873	−0.41
Maximum eye width	0.746	0.214
Maximum tail muscle width	0.744	0.282
Maximum anterior tail depth	0.739	0.319
Maximum body depth	0.67	0.238
Maximum tail depth	0.603	0.353
Maximum tail length	−0.637	0.744

Table 3. Results of ANOVA and Bonferroni *post-hoc* test of residual values for the first principal component (PC1) and second principal component (PC2).

Factors	ANOVA			Bonferroni	
	df	F	p	Treatments	p
PC1	2, 75	6.37	**0.003**	1–2	**0.013**
				1–3	**0.008**
				2–3	1.000
PC2	2, 75	1.452	0.241	1–2	NA
				1–3	NA
				2–3	NA

Note: NA = not applicable. Significant values ($p < 0.05$) are shown in bold.

ment 3 (predator consuming conspecifics; $p < 0.05$, Bonferroni *post-hoc* test) (Table 3). For PC2, there were no significant differences among treatments ($F_{2,75} = 1.452$; $p = 0.241$) (Table 3). We found the same trend in the means and standard deviations for the PC1 and PC2 results: a decrease in the mean value in Treatments 2 and 3 compared to the control (Figure 4). The MANOVA test showed that treatments had a significant effect on five variables: BL, BW, TW, ATD, and TD ($p < 0.05$) (Table 4). In the *post-hoc* Bonferroni test, we found several significant differences ($p < 0.05$) among treatments in the five variables mentioned above (Table 4). The increase or decrease in all morphological traits is illustrated in Figure 5. The values for all variables (except TL) for the first treatment were higher than the values for the second and third treatments. On the other hand, the TL increased in Treatments 2 and 3, compared with the Treatment 1.

Transmission experiments of Saprolegnia

Eggs exposed to infected trout presented white cotton/wool-like patches, whereas eggs from the control container did not develop signs of *Saprolegnia* infections and no isolates were obtained. Trout exposed to *S. diclina* developed patches of white hyphal colonies. Alignment of ITS sequences of isolates from the treatment with infected rainbow trout and isolates from the infected trout revealed that they were identical (GenBank KF717795). A BLAST search of the sequence of isolates showed 100% similarity to Genbank sequence AM228818, corresponding to isolates Sa-017, Sa-025, Sat-009, Sat-015, Sax-005, Sax-009, and Sax-035 of *S. diclina* designated by molecular operational taxonomic units.[40] The survivorship of eggs from the control was significantly higher than in eggs in the exposure treatment. Unpaired *t*-test showed significant difference between the two treatments ($t = 12.3$; $p = 0.0001$) (Table 5).

Discussion

Introduced species have the potential to affect amphibians in a variety of ways.[1] Our study shows that
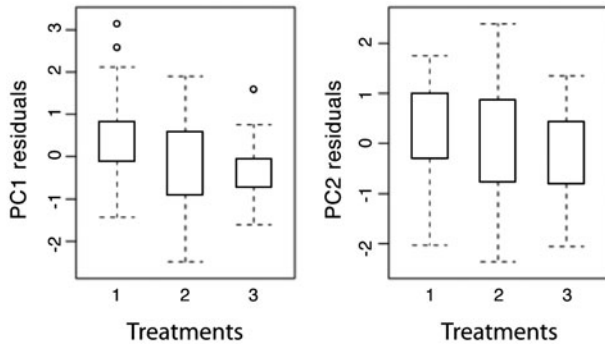
Figure 4. Trends of size (PC1) and shape (PC2) among treatments represented by means and standard deviations of residual values from the first principal component (PC1) and second principal component (PC2). Treatments are: 1 (absence of rainbow trout), 2 (presence of rainbow trout), and 3 (presence of rainbow trout preying on tadpoles of *Nymphargus grandisonae*).

rainbow trout have a clear effect on tadpole morphology and functions as a vector of the pathogenic freshwater mold *S. diclina*.

Although the presence of trout had no significant effect on the mortality of tadpoles, our observation of lower absolute survivorship of individuals in the treatment trials may be a direct effect of the stress that a perceived predator causes on its prey. Many studies have reported that when predator cues (kairomones) are perceived, they act as stressors and can have a negative impact amphibian embryos and larvae.[28,30,51,52] The evolutionary context of communities is also relevant; Benard [53] demonstrated that tadpole survival is higher when the larvae are exposed to predators that usually are present in their habitat, rather than recently introduced predators. This is not the case in our experiment, because tadpoles were obtained from a historically trout-free area. The only native fish in the area where the study was performed belong to the genus *Astroblepus* (*pers. obs.*), a sucker-mouth catfish that feeds on arthropod larvae and annelids.[54] No member of *Astroblepus* is known to prey on anuran eggs or tadpoles. The only likely fish native to the Andes that might prey upon these glass-frogs may be *Grundulus quitoensis* (Characidae); however, the geographic range of this taxon is restricted to the El Angel paramo lacustrine system, which is well above the elevation where *N. grandisonae* is found.[18,19] The great adaptability of rainbow trout to different microhabitats and their abundance in Ecuadorian Andean rivers and streams within an altitudinal range of 2000–3900 m [55] makes this predator a likely threat to other anuran communities.

Glassfrog tadpoles expressed phenotypic plasticity when exposed to trout and to their depredated

Table 4. Results of MANOVA and Bonferroni *post-hoc* test of residual values for eight phenotypic variables.

Variables	MANOVA			Bonferroni	
	df	F	p	Treatments	p
Maximum body length	2, 75	4.370	**0.016**	1–2	0.124
				1–3	**0.019**
				2–3	1.000
Maximum body width	2, 75	5.488	**0.006**	1–2	**0.033**
				1–3	**0.011**
				2–3	1.000
Maximum eye width	2, 75	1.526	0.224	1–2	NA
				1–3	NA
				2–3	NA
Maximum tail muscle width	2, 75	5.736	**0.005**	1–2	**0.032**
				1–3	**0.008**
				2–3	1.000
Maximum anterior tail depth	2, 75	11.889	**0.000**	1–2	0.113
				1–3	**<0.0001**
				2–3	**0.032**
Maximum body depth	2, 75	2.612	0.080	1–2	NA
				1–3	NA
				2–3	NA
Maximum tail depth	2, 75	6.051	**0.004**	1–2	**0.012**
				1–3	**0.013**
				2–3	1.000
Maximum tail length	2, 75	1.568	0.215	1–2	NA
				1–3	NA
				2–3	NA

Note: NA = not applicable. Significant values ($p < 0.05$) are shown in bold.
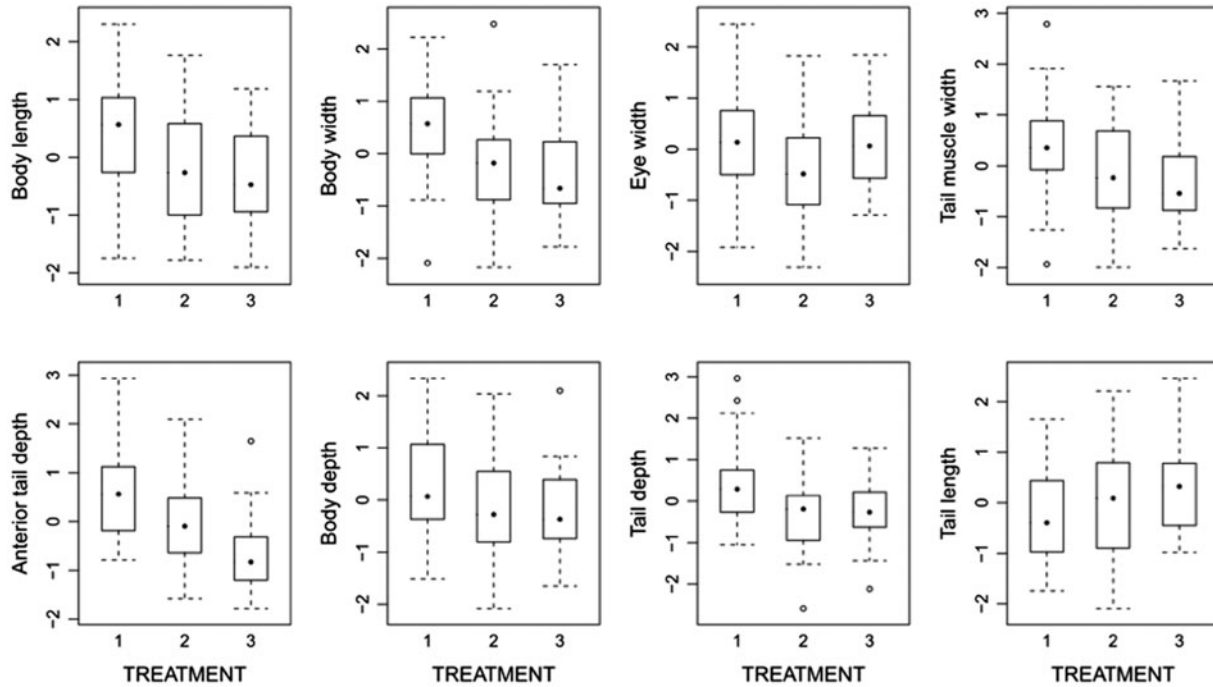
Figure 5. Trends of morphological changes in body measures as standardized residuals between final and initial values. Treatments are: 1 (absence of rainbow trout), 2 (presence of rainbow trout), and 3 (presence of rainbow trout preying on tadpoles of *Nymphargus grandisonae*).

Table 5. Survivorship of embryos of the toad *Engystomops petersi* in two treatments: presence of uninfected rainbow trout (total control, where CT3 and CTR3 are replicates), and presence of rainbow trout infected with *S. diclina* (total *Saprolegnia*, where ST2 and STR2 are replicates).

Treatment	Nº Eggs	Eggs alive	Eggs death	(%) Embryos survivorship
CT3	*80*	*54*	*26*	67.5
CTR3	*64*	*46*	*18*	71.9
Total control	144	100	44	*69.0*
ST2	*51*	*8*	*43*	15.7
STR2	*149*	*22*	*127*	14.8
Total *Saprolegnia*	200	30	170	*15*

Note: The survivorship of eggs from the control was significantly higher than in eggs in the exposure treatment ($p < 0.001$).

conspecifics. It has been demonstrated that tadpoles react to kairomones by modifying behavior and, thus, their growth pattern.[34,56–58] Although we did not test directly for the presence or absence of kairomones in our experiment, tadpoles increased in size in all measured traits, except tail length in the absence of rainbow trout. In contrast, tadpoles that were exposed to trout had smaller bodies and longer tails, a trend that was even more pronounced in tadpoles that were exposed to trout that preyed on conspecific tadpoles (Figure 5). Similar morphological responses (shorter bodies and longer tails) to larval predators have been documented in numerous studies.[30,34,56–59] Although there were no significant differences in growth pattern between Treatments 2 and 3 (both containing trout), the observed trend (Figure 5) suggests that the chemical cues released by depredated

conspecifics may trigger a stronger phenotypic response in tadpoles. Longer tails and shorter bodies may be a non-adaptive response and an indicator of poor growth performance (consequence of a suboptimal feeding condition).[60] In our experiment, if the cause of the morphological changes is poor growth performance, it was not caused by the lack of food but rather because tadpole behavior changed in the presence of trout, resulting in less time spent foraging and feeding. Similarly, Relyea [30] showed that shorter bodies and longer tails is the adaptive response of Gray Treefrogs (Hylidae) to fish predators.

Another commonly observed morphological change is an increase in tail depth,[30,61] although this was not observed in the tadpoles of *N. grandisonae*. The increase in tail length and tail depth in tadpoles has been

associated with an increase in musculature to improve the thrust production and swimming speed during escape from a predator.[61] We did not study specifically the behavior of tadpoles. However, in natural conditions they often hide in leaf litter and sand at the bottom of streams (*pers. obs.*), a behavior that may require less allocation of energy than an increase in tail depth because individuals must balance the conflicting costs and benefits of anti-predator responses to maximize fitness.[30] It is clear that swimming speed is crucial when tadpoles are in the presence of predators.[62] When we placed tadpoles in the tank that contained trout, they were eaten in less than 24 h, even though each tank contained sand and rocks that could be used as a refuge. However, the morphological responses of glassfrog tadpoles (short bodies, longer tails) might well be an anti-predator response against rainbow trout that might be effective in natural conditions.

As shown by Relyea [30], many trait correlations would favor selection as adaptive anti-predator traits. In glassfrog tadpoles, the traits significantly affected by treatments (BL, BW, TW, ATD, and TD) had medium to high positive correlations, which suggest that they may be selected as adaptive anti-predator traits. Special attention should be paid tail length because it is negatively correlated with all other traits, meaning that its increase relates to a decrease in all other variables, a tendency that is more pronounced in Treatments 2 and 3 (presence of trout; Figure 5). As shown in several studies [57,61,63], tail length is important in the development of anti-predator defenses, and it is possible that its increase in length is achieved by diverting resources from the body. As Van Buskirk and Relyea [62] demonstrated, phenotypic changes in response to presence of predators involve diversion of material and energy resources from optimal growth and/or reproduction. Therefore, it is expected that these deviations might affect fitness, resulting in delayed metamorphosis or incurring costs on post-metamorphic stages such as decreased size at maturity or reduction in egg production.[64–67]

Historically, wildlife diseases have been considered important mostly when agriculture or human health have been threatened, but because of the present-day translocation and introduction of species, such emerging infectious diseases are taken more seriously.[68,69] Our study demonstrates that rainbow trout can transmit *S. diclina*, and thereby cause a high mortality on amphibian eggs (Table 5). These experimental observations are likely to occur in other *Saprolegnia* because of their large distributions and similar life history traits.[70] The pathogenicity of *S. diclina* has been demonstrated in salmonids;[71,72] if the levels of mortality that *S. diclina* caused in eggs of *E. petersi* are indicative of its effect on other anurans, and given that the rainbow trout is an effective vector of *Saprolegnia*, the impact on amphibian

conservation efforts would be significant, as rainbow trout has been introduced in numerous Andean rivers and hatcheries are widespread in Ecuador.[14]

Until 1991, malachite green was used as against *Saprolegnia* infections in hatcheries, but because of its teratogenicity, its use is restricted to the treatment of non-food fish. Currently, hatcheries use other less effective compounds.[73] Therefore, *Saprolegnia* infections remain an unsolved problem.[1,74]

Aside from the pathogenicity of *Saprolegnia*, environmental changes may amplify the lethal effect of *Saprolegnia*.[2] Several studies [41,75] pose the idea that the differences in susceptibility to the infection depend on the ability of different species to cope with the synergistic effects of the pathogen and a stressful environment. For example, Kiesecker et al. [37] associated the mortality of amphibian embryos in Oregon caused by *Saprolegnia* with the combination of the 'El Niño Southern Oscillation' (ENSO) and global change. The increase in frequency, duration, and intensity during the past years of the ENSO phenomenon [76] and the prediction of an increase in its frequency [77] could result in catastrophic consequences for Neotropical amphibians.[76] As Blaustein and Bancroft postulated,[78] if a population faces many pressures and fails to adapt to just one of them, it may not persist, resulting in local declines. Thus, only individuals with rapid adaptation potential would cope with the continuous stressors, environmental changes, or emerging infectious diseases.

After showing the consequences in survivorship and phenotype that rainbow trout induces in *N. grandisonae* and given the morphological, ecological, and behavioral similarity of glassfrog tadpoles,[79,80] it seems likely that trout have a similar negative effect on other glassfrog species, many of which are endangered.[81] The effects of the rainbow trout in other amphibian groups with riparian tadpoles, such as harlequin toads (*Atelopus* spp.), which have suffered catastrophic declines,[82,83] and tree frogs (Hylidae) remain to be tested. Our results suggest that trout likely have negative effects on stream-breeding anuran species in general in the Andes.

Given that importance of the Andes in terms of amphibian diversity and endemicity,[84–90] we propose that steps should be taken to reduce the negative impacts of rainbow trout on Andean aquatic ecosystems. First, trout should be eliminated from public and private protected areas. The use of pesticides such as rotenone for this task is not recommended because of its high toxicity to non-target native species.[23] A feasible alternative to eradicate rainbow trout could be the combination of gill netting and electric fishing,[91,92] a mandated increase in security measures at fish hatcheries, and prevention of new introductions.[24] This is a complex task that concerns not only professional conservation biologists but also environmental authorities and administrators and

staff of protected areas, as well as stakeholders from aquaculture and sport fishing. Because trout is the source of food and recreation for people across the Andes, eradication programs need to be supplemented with environmental education programs.

Author contributions

LMT, JMG, JM, JDU, and JB conceived and designed the study. LMT, JM, JD analyzed the data. LMT wrote the first draft of the manuscript. JMG, JVSS, JM, JDU, JB made significant additions in the text.

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