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Call survey indicates rainbow trout farming alters glassfrog community composition in the Andes of Ecuador

[1,*]Katherine L. Krynak, [2]Dana G. Wessels, [3]Segundo M. Imba, [4]Timothy J. Krynak, [2]Eric B. Snyder, [3]Jane A. Lyons, and [5,6]Juan M. Guayasamin

[1]*Department of Biological and Allied Health Sciences, Ohio Northern University, Ada, Ohio, USA* [2]*Department of Biology, Grand Valley State University, Grand Rapids, Michigan, USA* [3]*Reserva Las Gralarias, Province of Pichincha, ECUADOR* [4]*Natural Resources, Cleveland Metroparks, Cleveland, Ohio, USA* [5]*Universidad San Francisco de Quito, Colegio de Ciencias Biológicas y Ambientales, Instituto Biósfera-USFQ, Laboratorio de Biología Evolutiva, Av. Diego de Robles y Vía Interocéanica, Campus Cumbayá, Quito, ECUADOR* [6]*Department of Biology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA*

***Abstract.*—Aquaculture, the farming of fish for human consumption and/or trade, is a growing industry throughout the world. The effects of farming on local ecosystems and wildlife are understudied, particularly in regions where farms are often limited to subsistence practices with little to no government regulation. The influence of Rainbow Trout (*Oncorhynchus mykiss*) farms on glassfrog community composition was assessed in the Mindo and Alambi regions of Ecuador. Call surveys were conducted during the dominant glassfrog reproductive season (March–May 2017) across 13 sites, six of which were in the immediate proximity of trout farms. Nonmetric multidimensional scaling ordination analyses and multiple response permutation procedures indicate that glassfrog communities differed between trout farm and non-trout farm sites (MRPP; A = 0.11, *P* = 0.04). Differences in glassfrog community composition were significantly or marginally correlated with percent canopy openness, dissolved oxygen (mg/L), conductivity (µS), and total dissolved solids (mg/L), environmental characteristics altered by the aquaculture practice. As the prevalence of trout farms increases across this region, it is likely that the glassfrog community composition will be altered, potentially resulting in a pattern of decreased species richness. It is also likely that habitat changes associated with trout farming practices including deforestation, water chemistry changes, and predation pressures by escaped trout will influence glassfrog species persistence. Mitigation strategies including improved barriers to decrease trout escape, the incorporation of settling ponds to decrease stream contamination, and the preservation of habitat in areas of high amphibian species richness are warranted.**

Keywords. Amphibians, Anura, aquaculture, cloud forest, conservation, habitat protection, introduced species, land management, water quality

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Introduction

Aquaculture, or the farming of fish for human consumption and/or trade, is a growing industry worldwide (Mantri et al. 2017). While the practice has economic benefit (Offemet al. 2010), the long-term costs to local wildlife and ecosystems are largely understudied and likely underestimated (Niklitschek et al. 2013). Aquaculture practices in regions with little to no government regulation may be particularly detrimental to the surrounding ecosystem because habitat protection practices are often not utilized, resulting in increased farmed-fish escapes and water contamination (Niklitschek et al. 2013).

Rainbow Trout, *Oncorhynchus mykiss* (Salmonidae), is a non-native predatory species currently being cultivated in Andean streams that also are the habitat for several of Ecuador's most endangered amphibians (Vimos et al. 2015), including glassfrogs (Centrolenidae). Whether introductions of *O. mykiss* have negatively affected glassfrog populations is currently not known; however, multiple studies indicate broad negative effects of this cultivated fish species on amphibians (Gall and Mathis 2010; Garcia et al. 2012; Ortubay et al. 2006; Pearson and Goater 2009; Vredenburg 2004). *Oncorhynchus mykiss* represents a direct threat to amphibian larvae via predation due to their biphasic life cycle (Garcia et

Correspondence. **k-krynak@onu.edu*

Fig. 1. Trout farming in the Mindo region of Ecuador utilizes a flow-through aquaculture technique. Stream water is diverted into tandem raceways/holding reservoirs and then flows through these reservoirs back into the natural stream system. This figure displays a panoramic view of Finca de Jaime's (FJ) set-up. *Photograph by Katherine L. Krynak.*

al. 2012; Pearson and Goater 2009). Many amphibian species have been shown to demonstrate a lack of predator avoidance in response to this introduced fish (Gall and Mathis 2010; Garcia et al. 2012), though this information is limited to temperate amphibian larvae and it is unknown whether predator avoidance is demonstrated in tropical amphibian larvae. However, in a recent laboratory study, Martín-Torrijos et al. (2016) found that the presence of *O. mykiss* altered larval morphology in *Nymphargus grandisonae*, a glassfrog species included in this survey. The extent to which *O. mykiss* presence may affect the glassfrog larvae *in situ* has yet to be determined. Additionally, *O. mykiss* can introduce pathogens to naïve amphibian communities, including aquatic fungal pathogens such as *Saprolegnia diclina* (Martín-Torrijos et al. 2016) and iridoviruses like ranavirus, a pathogen that has caused amphibian population declines and extirpations across the globe (Miller et al. 2011; Smith et al. 2017). Together, these studies suggest *O. mykiss* introductions may negatively affect glassfrog population persistence by decreasing larval survival through both direct (predation) and indirect means (aquatic pathogen introduction).

Farming of *O. mykiss* has been occurring for over 25 years in the Mindo and Alambi regions of Ecuador (western slope of the Andes Mountains, Province of Pichincha) and is increasing in prevalence; several farms in the region are fewer than 10 years old (Rolando Sanchez and JAL, pers. comm.). Trout farms in the Mindo and Alambi regions utilize a flow-through system of aquaculture. Natural stream water is diverted into tandem holding reservoirs (and/or raceways; Fig. 1); water then flows through these reservoirs back into the natural stream system. The system has no mechanism for preventing stream contamination other than the limited settling that occurs in reservoirs prior to outflow. Fish escapes are largely prevented by size sorting of trout between reservoirs (smaller fish being held in the first reservoirs, larger fish nearer the outflow) and wire screen barriers put in place to limit escape. Interviews of farm managers indicated that heavy rains (notably during the months of March–May) often result in large amounts of debris being swept into the diverted stream channels, which damages the wire barriers that contain the trout in the reservoirs. Managers estimated that 2–10% of farmed trout escape during these common rain events. Between 1 March 2017 and 22 May 2017, 588.70 mm of rain fell in this region (HOBO U30 Remote Monitoring System stationed at Reserva Las Gralarias: 0.0091S, 78.7375W, elevation 2,068 m).

Particular stream characteristics associated with stream diversion aquaculture may affect aquatic larval glassfrog survival and, over time, influence glassfrog community composition. Total dissolved solids, conductivity, and pH are often altered by trout farming (Boaventura et al. 1997; McNaughton and Lee 2010) and are known to influence amphibian fitness correlates including growth, development, and innate immune defenses (Krynak et al. 2015, 2016). Trout farming in this region may be particularly detrimental to water quality given that multiple trout farms often occupy the same stream, potentially causing a cumulative effect on water quality. Increased stream water nutrient loads can increase periphyton abundance (a larval glassfrog food source) and subsequently decrease dissolved oxygen levels (Selong and Helfrich 1998), thereby negatively affecting larval glassfrog survival (Gillespie 2002; Tattersall and Ultsch 2008). In temperate systems, canopy cover (or lack thereof due to deforestation) can also influence periphyton abundance by changing light availability, potentially altering available larval food sources (Skelly et al. 2002), though context dependency of this relationship may be greater in tropical ecosystems (Garcia et al. 2015). Furthermore, changes to stream canopy composition may negatively affect glassfrog persistence by decreasing suitable egg deposition sites (Arteaga et al. 2013).

The Mindo region of Ecuador is home to nine species of glassfrogs (Arteaga et al. 2013) that vary in their 2017 IUCN Redlist conservation status from Data Deficient (DD) to Critically Endangered (CR): Emerald Glassfrog (*Espadarana prosoblepon;* Least Concern [LC]), Red-spotted Glassfrog (*Nymphargus grandisonae*; LC), Pepper Glassfrog (*N. griffithsi*; Vulnerable [VU]), Las Gralarias Glassfrog (*N. lasgralarias*; DD), Lynch's Glassfrog (*Centrolene lynchi*; Endangered [EN]), Golden-flecked Glassfrog (*C. ballux;* CR), Dappled Glassfrog (*C. peristictum*; Near Threatened [NT]), Mindo Glassfrog (*Cochranella balionota;* VU), and Bumpy Glassfrog (*C. heloderma*; CR) [Table 1; Fig. 2]. Previous studies have suggested that glassfrog population declines might be partially associated with the introduction of predatory fish into streams, though this effect has not been quantified (Catenazzi et al. 2011; Merino-Viteri 2001). In comparison extensive work has been done in temperate systems indicating that introduced trout have devastated amphibian communities (Bosch et al. 2019; Knapp and Matthews 2000; Knapp et al. 2007; Pope 2008).

In this study, presence/absence acoustic surveys were conducted throughout the dominant glassfrog breeding months of March–May 2017 (Arteaga et al. 2013), to determine the influence of trout farms on glassfrog community composition in the Mindo region of Ecuador. The predictions were that trout farms would have decreased glassfrog species richness and that particular environmental characteristics (such as water chemistry, periphyton abundance, and canopy cover) would correlate to differences in glassfrog community composition between trout farms and non-trout farms.

Materials and Methods

Call surveys were conducted across 13 sites in the Mindo region of Ecuador (six trout farms and seven without trout; Fig. 3), one of the most amphibian-diverse cloud forests in South America (Arteaga et al. 2013). Sites were chosen based upon habitat viability, elevation, and accessibility. Adult glassfrogs in the region inhabit forested habitats surrounding creeks, streams, and rivers. Sites included in the study ranged in elevation from 1,596–2,666 m, and habitat was considered to be viable for glassfrog presence if at least small remnants of forest surrounded the streams or their tributaries (for streams both with and without trout farms). Six sites were located at trout farms along the Río Alambi and Quebrada Santa Rosa waterways. The Río Alambi water system included trout farm sites referred to as El Paraíso del Pescador (EP), Finca de Jaime (FJ), Santa Teresita (ST), La Sierra (LS), and Verdecocha (VC). A single trout farm was located on Quebrada Santa Rosa system, the Lower Río Santa Rosa (LRSR) site. The trout farms ranged in age from 6–27 years. The non-trout farm sites included four sites along the Quebrada Santa Rosa stream system (upstream of LRSR), referred to as Río Santa Rosa (RSR), Michelle's (M), Five Frog Creek (5F), and Ballux Creek (Bcrk). Three additional non-trout farm sites were chosen that represent headwater streams not connected with Quebrada Santa Rosa or Río Alambi: Lucy's Creek (LC), Kathy's Creek (KC), and a small tributary of the Chalguayacu Grande River (C). Ballux Creek and Five Frog Creek also represent headwater stream systems forming Quebrada Santa Rosa (see Appendix 1 for details on site locations). The non-trout farm sites, with the exclusion of LRSR, are located on privately-owned protected land. Access to headwaters of trout farm streams was not possible due to transportation and permission constraints.

Fig. 2. Glassfrog species found during surveys. **(A)** *Centrolene heloderma*, **(B)** *Centrolene ballux*, **(C)** *Esparana prosoblepon*, **(D)** *Nymphargus lasgralarias*, **(E)** *Centrolene peristictum*, **(F)** *Nymphargus grandisonae*, **(G)** *Centrolene lynchi*, **(H)** Egg mass from *C. ballux*. *Nymphargus griffithsi* was not encountered during the 2017 survey but has been documented at Kathy's creek in 2012 and 2013 (by Jane A. Lyons). *Photographs by Dana G. Wessels (A–F) and Timothy J. Krynak (G–H).*

Overnight call surveys were conducted between 2000 h and 0200 h at each survey site (Mean: 3.38 ± 1.9 SD visits per site) on multiple dates during the rainy season, when glassfrogs are reproductively active (JMG, pers. comm.). Surveying included 2–8 visits per site with the exception of a single site (C) which was only visited on a single occasion due to safety concerns associated with heavy rains and steep, eroding terrain. The presence of each of the documented species was recorded at first visit at each site, therefore sampling effort did not bias the detection. It should be noted that the species recorded at site C on 30 March 2017 were the same as had been previously observed at the site in March–May in 2012 and 2013 (JAL, pers. comm.). Species presence was assessed

Table 1. Glassfrog species presence/absence data across sites in the Mindo region of Ecuador. Sites: RSR = Río Santa Rosa, LC = Lucy's Creek, Bcrk = Ballux Creek, KC = Kathy's Creek, M = Michelle's, C = Chalguayacu Grande River, 5F = Five Frog Creek, LRSR = Lower Río Santa Rosa, ST = Santa Terricita, FJ= Finca de Jaime, LS = La Sierra, VC = Verda Cocha, EP = El Paraíso del Pescador. Values of 0 indicate those "not detected," whereas values of 1 indicate those audibly detected. IUCN RedList status codes are listed below each species name.

	Species and IUCN RedList conservation status						
	Nymphargus grandsiosonae	*Nymphargus lasgralarias*	*Centrolene lynchi*	*Centrolene peristitum*	*Centrolene ballux*	*Centrolene heloderma*	*Espadarana prosoblepon*
Site	LC	DD	EN	NT	CR	CR	LC
RSR	1	0	1	1	0	0	0
LC	1	0	1	1	0	0	0
Bcrk	0	0	0	0	1	1	0
KC	1	1	0	1	1	0	0
M	1	0	1	0	0	0	0
C	1	0	1	1	1	0	0
5F	1	0	0	0	1	1	0
LRSR	1	0	0	0	0	0	1
ST	1	0	0	0	0	1	0
FJ	1	0	0	0	0	0	0
LS	0	0	0	0	0	0	0
VC	0	0	0	0	0	0	0
EP	0	0	0	0	0	0	0

based upon audible detection of calling males across an approximate 200 m distance (up to 100 m above and 100 m below the stream access point). For all sites, detectable species richness did not change as a function site visits. Audible recording of calls used for identification for each of the observed species can be referenced at http://lasgralariasfoundation.org/cantos-de-ranas.

The location and environmental characteristics recorded at each of the sites included water temperature (°C), conductivity (µS/cm), total dissolved solids (mg/L), dissolved oxygen (mg/L), pH, canopy cover (% openness), and average periphyton abundance (chlorophyll *a* mg/cm^2). Site elevation was determined by estimation via Google Maps™, which was then corroborated via topographical maps obtained from Ecuador's Ministry of the Environment. Minnow traps (Grayson and Row 2007) were deployed downstream of each trout farm (with the exception of LRSR) for 24-hr periods to assess the abundance of escaped trout (catch per unit effort).

All statistical analyses were performed in R version 3.4.3 (R Development Core Team 2017). For all sites with detectable glassfrogs, the influence of trout farming on glassfrog species richness was assessed using a *t*-test (*t*-test function in the stats package by R Core Team 2014). Sites without detectable glassfrogs were not included in this analysis to avoid artificially inflating results of the test. Glassfrog community composition was assessed using nonmetric multidimensional scaling ordination using Jaccard distance (NMDS; metamds function in the vegan package, Oksanen et al. 2018) to visualize glassfrog community composition similarities across the 13 sites. Trout farms El Pariso del Pescador, La Sierra, and Verdecocha are not included in the analysis because glassfrogs were not observed at these sites. Multiple response permutation procedures (MRPP; 999 permutations; mrpp function in the vegan package, Oksanen et al. 2018) were used to quantify differences in glassfrog community composition between trout farms and non-trout farm sites. Pearson correlation tests (cor.test function in the stats package by R Core Team 2014) between axis scores and the environmental measures were performed to assess the potential influences of these environmental characteristics on glassfrog community similarity in the NMDS ordination. Moran's *I* was used to assess potential spatial autocorrelations between the environmental variables and the GPS locations of the sites (Moran.I function in the ape package, Paradis and Schliep 2018). Glassfrog taxonomy follows the proposal by Guayasamin et al. (2009). All surveys were conducted under permit MAE-DNB-CM-2015-0017 issued by Ecuador's Ministry of the Environment (Ministerio de Ambiente del Ecuador) and with permission of land owners.

Results

A total of seven glassfrog species was recorded across the 13 sites (Table 1). Species detected were *Centrolene heloderma, C. ballux, C. peristictum, C. lynchi, Nymphargus grandisonae, N. lasgralarias*, and *Espadarana prosoblepon*.

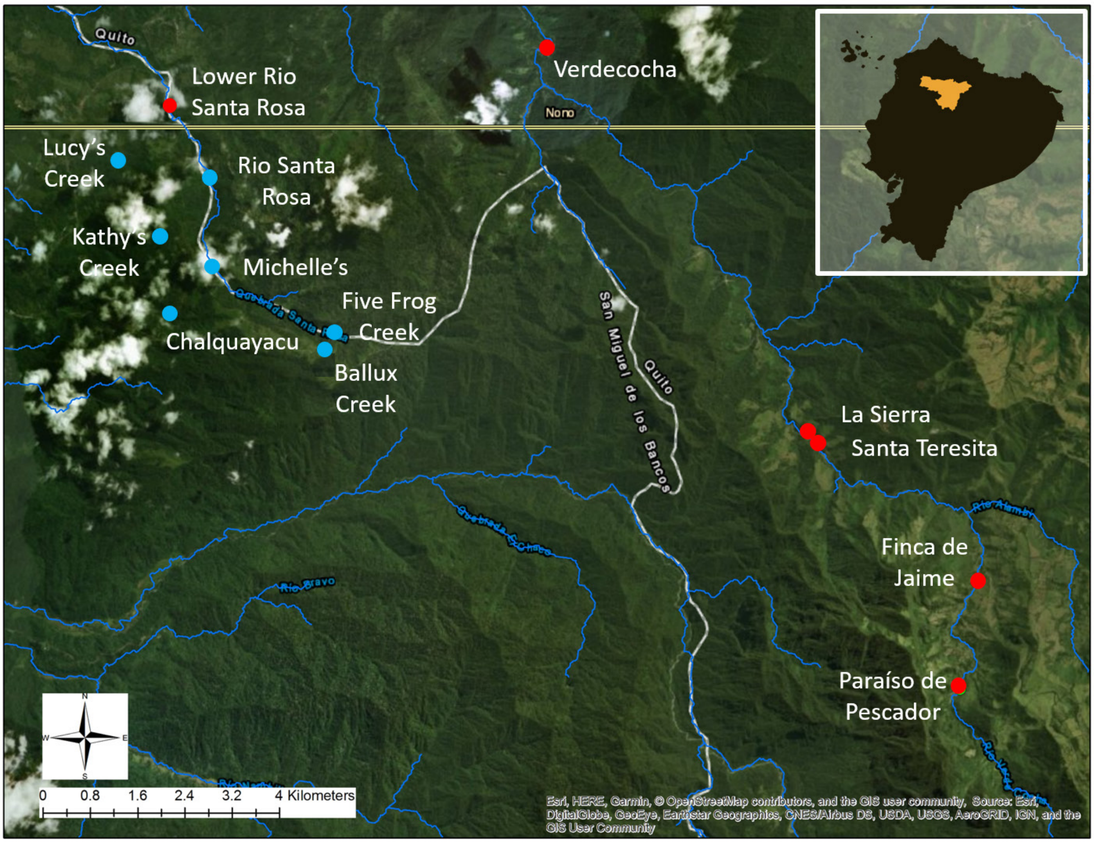

Fig. 3. Map of the study area. Inset: Pichincha Province, Ecuador. Main: Blue points indicate non-trout farm sites whereas red points indicate trout farm sites. Yellow line represents the equator (latitude 0).

Glassfrog species richness differed between trout farm and non-trout farm sites (t = 2.94, df = 5.54, P = 0.03; mean trout farm richness = 1.67 ± 0.58 SE species; mean non-trout farm richness = 3.0 ± 0.82 SE species) and the NMDS analyses indicated a difference in glassfrog community composition between trout farm sites and non-trout farm sites (MRPP; delta = 0.59, A = 0.11, P = 0.04; Fig. 4). Pearson correlation tests indicated correlations between multiple environmental variables and NMDS axis scores (NMDS Stress on 2D solution was 3% indicating good fit, Table 2, Fig. 4). Site elevation was found to be positively correlated with NMDS Axis 1 ($T_{(8,9)}$ = 2.73, P = 0.03). NMDS Axis 2 was correlated with canopy cover ($T_{(8,9)}$= 2.73, P = 0.03) and dissolved oxygen ($T_{(8,9)}$ = 3.16, P = 0.01; Table 2). NMDS axis 2 was marginally correlated with conductivity ($T_{(8,9)}$ = 2.0, P = 0.08) and total dissolved solids ($T_{(8,9)}$ = 2.12, P = 0.07). NMDS Axis 2 differentiates glassfrog communities observed at trout farms versus non-trout farms (Fig. 4). Moran's I tests revealed pH, chlorophyll *a*, canopy cover, and dissolved oxygen were not spatially autocorrelated (Moran's I test P > 0.05); while conductivity, total dissolved solids, elevation, and temperature did suffer from spatial autocorrelation (Moran's I test P < 0.05). Correlation analyses were conducted on all variables independently, including spatially autocorrelated variables, given that it is unknown whether the spatial autocorrelation was due to exogenous or endogenous factors and the small sample size.

Traps for quantifying Rainbow Trout abundance were not effective at the sites and therefore, this effort was discontinued after multiple attempts (see **Discussion**). These traps did, however, catch a single *Astroblepus* sp., a native (non-predatory) fish from the family Astroblepidae known for climbing waterfalls in these Andean streams at site FJ.

Table 2. Pearson correlation estimates between NMDS axis scores and environmental variables across sites. Statistically significant values are in bold (P < 0.05). Values of P < 0.1 are indicated with an asterisk (*), and are designated as such based upon their biological significance and the small sample size.

	pH	**Conductivity (µS/cm)**	**Total dissolved solids (mg/L)**	**Dissolved oxygen (mg/L)**	**Canopy openness (%)**	**Elevation (m)**	**Temperature (°C)**	**Chlorophyll a (mg/cm²)**
NMDS1	0.40	0.08	0.18	0.06	<0.01	**0.70**	-0.39	0.38
NMDS2	0.39	0.58*	0.60*	**0.75**	**0.69**	-0.07	-0.50	0.14

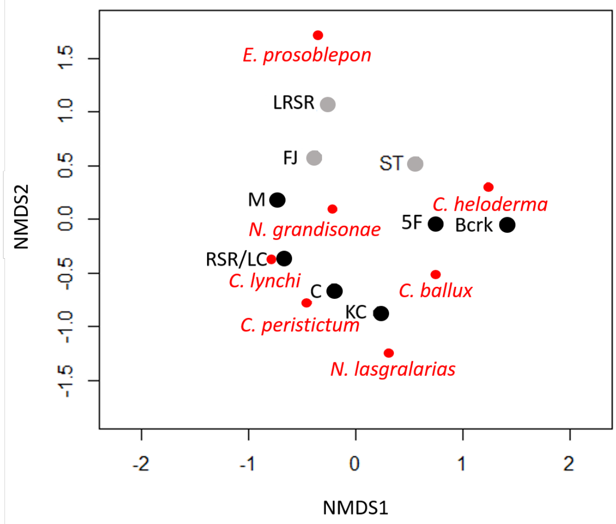

Fig. 4. Two-dimensional NMDS ordination of survey sites and glassfrog species based upon presence of frogs audibly documented in 2017 survey conducted in the Mindo region of Ecuador (Stress = 3%). Red points and labels represent glassfrog species; grey points represent trout farms; and black points represent non-trout farms. RSR = Río Santa Rosa, LC = Lucy's Creek, Bcrk = Ballux Creek, KC = Kathy's Creek, M = Michelle's, C = tributary of the Chalguayacu Grande River, 5F = Five Frog Creek, LRSR = Lower Río Santa Rosa, ST = Santa Teresita, FJ = Finca de Jaime, LS = La Sierra, VC = Verdecocha, EP = El Paraíso del Pescador. Trout farms EP, LS, and VC are not included in the analysis because glassfrogs were not observed at these sites. A significant difference in glassfrog community composition between trout farm and non-trout farm sites was indicated by MRPP (delta = 0.59, A = 0.11, *P* = 0.03). NMDS1 correlated with elevation; NMDS2 correlated with: percent canopy openness, dissolved oxygen (mg/L), total dissolved solids (mg/L), and conductivity (µS).

Discussion

Understanding the potential effects that trout farming has on glassfrog community structure is critical for improving species conservation efforts as this aquaculture practice is expected to increase in this region of Ecuador, and throughout the world (Diana 2009). Across the thirteen sites, the presence of seven glassfrog species is reported, two of which are listed as Critically Endangered (*C. ballux* and *C. heloderma*) and one as Endangered (*C. lynchi*; IUCN Redlist 2017). Notably, at Michelle's site, *Centrolene lynchi* was recorded at a much higher elevation (2,031 m) than previously documented for the species (published elevational range 1,520–1,858 m; Arteaga et al. 2013). Additionally, a previously undocumented population of *C. heloderma* (20+ calling males) was recorded between the trout farm sites La Sierra and Santa Teresita (and at Santa Teresita) along the Río Alambi system (Krynak et al. 2018). *Nymphargus griffithsi* was not observed at any of the sites, though the species has been recorded in Five Frog Creek at Reserva Las Gralarias in previous years (Hutter and Guayasamin 2012).

This survey found that (i) mean glassfrog species richness nearly doubled in non-trout farm sites compared to trout farm sites, (ii) glassfrog community composition differed between trout farm sites and non-trout farm sites (based on clear separation between these factors along NMDS2 in the ordination and quantitative confirmation via MRPP analyses), and (iii) multiple environmental measures (dissolved oxygen, canopy cover, total dissolved solids, and conductivity) were correlated with observed differences in glassfrog community composition (Table 2; Fig. 4). There are several possible explanations for these marked differences, as previous research has indicated amphibian community composition and larval performance are associated with water chemistry, riparian cover, and predator presence and the findings reported here provide additional support for these hypotheses (Gonzalez-Maya et al. 2018; Hecnar and M'Closkey 1996; Sebasti and Carpaneto 2004; Watling et al. 2011).

This study indicates that water chemistry (measures of dissolved oxygen, total dissolved solids, and conductivity) is associated with the difference in glassfrog community composition between trout farm and non-trout farm sites. It is probable that increased nutrient loads associated with uneaten food and fecal waste from the trout may be driving the increased total dissolved solids (TDS) content at trout farms sites (Selong and Helfrich 1998). Increased nutrients from flow-through aquaculture are known to negatively affect larval amphibian survival by increasing periphyton and thereby decreasing dissolved oxygen (DO) content (Gillespie 2002; Tattersall and Ultsch 2008). However, the measured DO levels were slightly higher at trout farm sites compared to non-trout farm sites (mean ± SE DO: trout farms = 7.9 ± 0.58 mg/L, non-trout farms = 7.4 ± 0.55 mg/L). This phenomenon may be common within tropical ecosystems, or context dependent (Garcia et al. 2015). An expanded sampling effort will be required to tease apart these possible relationships. Another possibility is that the cooler water temperatures associated with slighting higher elevations of one of the trout farm sites may be driving this difference in DO (Appendix 2, mean ± SE temperature: trout farms = 14.84 °C ± 0.65 °C, non-trout farms = 15.55 ± 0.93 °C; elevation range: trout farms = 1,593–2,666 m, non-trout farms 1,693–2,254 m; mean ± SE elevation: trout farms = 2,013 ± 16.7 m, non-trout farms = 2,020 ± 12.9 m). Surprisingly, periphyton abundances did not differ between trout farm and non-trout farm sites in this study. However, we suspect that the increased nutrient levels (as suggested by TDS) may be affecting water chemistry in terms of ammonia and nitrite levels in the system, which could in turn negatively affect larval glassfrog survival; although this hypothesis needs to be tested.

The correlation found between canopy cover and glassfrog community composition differences between non-trout farm and trout farm sites, as visualized by the separation along NMDS2 in the ordination and quantitatively confirmed by the MRPP analyses, may indicate the deforestation at trout farm sites influenced which glassfrog species inhabited the sites. Based upon previous literature, we initially hypothesized that the mechanism for this correlation is that decreased canopy cover causes increased periphyton abundance (increased food availability which may benefit only particular amphibian larvae); however, the results obtained here contradict this idea (i.e., periphyton measures not correlated with NMDS axis 2 scores; Skelly et al. 2002). The decreased canopy cover at trout farm sites may instead be detrimental to the glassfrog species because of the lack of egg deposition sites. Overhanging vegetation along streams is critical to glassfrog reproductive success. Glassfrogs of this region lay eggs on leaves overhanging streams (plant families include Araceae, Annonaceae, Euphorbiaceae, Capparaceae, Fabaceae, and Rubiaceae) and upon hatching, the rheophilic larvae drop into the stream below where they continue to grow and mature (Arteaga et al. 2013). Therefore, a decrease in the number or quality of egg deposition sites (canopy cover) may result in decreased glassfrog abundance. Furthermore, decreased canopy cover may also negatively affect glassfrogs by means of increased ultra-violet (UV) exposure, as UV radiation is known to negatively affect amphibians at all life stages (Blaustein et al. 2003). Finally, while generalized deforestation (and canopy cover loss) cannot be separated from the deforestation caused by the creation and maintenance of the trout farms, this lack of vegetation (or appropriate vegetation) does seem to negatively affect the glassfrog community richness.

Lastly, the direct effect of predation and indirect effects of perceived predation threat by trout on glassfrog larvae *in situ* remain in need of assessment. During the surveys, an attempt was made to quantify trout presence directly measured by catch per unit effort via direct trapping and indirectly measured via collection of *O. mykiss* DNA from the streams. However, both efforts were discontinued due to ineffectiveness. Although trout were not directly observed in the streams, and there was no success in capturing trout using the minnow traps (despite multiple attempts and equipment adjustments), local people were seen pole fishing in the streams for the trout at El Paraíso del Pescador and near Santa Teresita. While the use of environmental DNA (eDNA) has become a valuable tool for assessing species presence in stream habitats (Young et al. 2017), there are limitations which must be addressed to fully utilize this tool in these fast-flowing Andean streams. The 10µ nylon membranes used to filter the stream water to collect the DNA samples were found to clog rapidly, limiting the ability to standardize collection efforts and obtain enough samples for comparisons across streams. Such assessments may be better suited for times of the year when there is less rainfall, when larval trout are not being washed downstream and stream water is less turbid. Electrofishing was not used to sample the trout because this methodology may have negative effects on small vertebrates, including glassfrog larvae (Miranda and Kidwell 2010). Nevertheless, changes in tadpole survival, morphology, behavior, and fitness when fish predators are present has been documented extensively (Relyea 2001, 2004; Relyea and Hoverman 2003), and may be a widespread phenomenon in Andean amphibian communities (Martín-Torrijos et al. 2016). As such, the effects of *O. mykiss* presence on Andean stream inhabitants is deserving of further investigation, especially when an overall negative effect of trout farms on amphibian richness has been correlated to multiple environmental characteristics associated with trout farming, as demonstrated in this study.

Conclusions

As trout farming increases in the Andean cloud forests, environmental managers need to be concerned about direct and indirect effects the practice has on naïve communities. While the persistence of the few glassfrog populations found at the trout farm sites provides encouragement, the differences in glassfrog community composition indicate that areas of high glassfrog species richness should be protected from the farming of non-native predatory fish. While minimizing water contamination (e.g., implementation of settling pools) and preventing fish escapes may be enough to maintain the existing populations in the streams currently used for aquaculture, we suspect that naïve communities may undergo a decrease in diversity if new farms are constructed. The results of this study suggest that mitigation strategies need to be employed in streams currently used in aquaculture and that trout farming should be prohibited in areas of high glassfrog species richness in order to protect these species.

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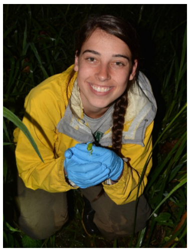
Katherine L. Krynak is an Assistant Professor of Biology at Ohio Northern University (ONU) and a founding board member of Las Gralarias Foundation (North Olmstead, Ohio, USA). Her research interests broadly span the fields of biodiversity conservation and eco-immunology. Katherine works with Las Gralarias Foundation to help protect the biodiversity of the Mindo region of Ecuador through research, environmental education, and land protection. Katherine was awarded a Fulbright Early Career Fellowship (Ecuador) in 2016 that supported the research presented in this article. Katherine teaches an undergraduate Tropical Biodiversity Conservation course in Ecuador for ONU.



Dana G. Wessels is a recent graduate of Grand Valley State University (Allendale, Michigan, USA) with a Master's in Biology and emphasis in aquatic science. Dana's research was supported by the President's Grant from Grand Valley State University and by the Michigan Space Grant.

Segundo M. Imba is a passionate conservationist, excellent field assistant, and long-time staff member at Reserva Las Gralarias (RLG), Ecuador. Segundo has assisted in a wide variety of research conducted at RLG including studies of birds, bats, terrestrial and aquatic insects, amphibians, and reptiles.

Timothy J. Krynak is a Natural Resources Manager for Cleveland Metroparks and a founding board member and President of Las Gralarias Foundation. Tim's research focuses on biodiversity preservation and bat ecology. Tim works to protect the environment through his land management, stewardship, and environmental education practices. As an adjunct professor at Grand Valley State University (Allendale, Michigan, USA), Tim co-instructs the Tropical Biology and Field Conservation Course in Ecuador with Eric Snyder.

Eric B. Snyder is a Professor of Biology at Grand Valley State University (GVSU, Allendale, Michigan, USA). Eric's research interests include stream ecology, restoration ecology, aquatic entomology, lotic ecosystem metabolism, and trophic structure. Eric has been teaching a Tropical Biology and Field Conservation course in Ecuador for GVSU since 2014, and has mentored two graduate students, including Dana Wessels, in research conducted in Ecuador's cloud forest streams.

Jane A. Lyons is the founder and land manager of Reserva Las Gralarias, Ecuador. Jane is currently the majority owner of Mindo Bird Tours and serves as Vice-President of the Board of the Las Gralarias Foundation, Inc. (North Olmstead, Ohio, USA). Jane was the first President of Jocotours Cia Ltda., the tour agency of the Jocotoco Foundation (Quito, Ecuador), and also works as a consultant for birding tourism and supervisor for graduate students and other field research projects. Jane works daily to preserve and protect the great biodiversity of the Mindo region through her efforts in eco-tourism, land acquisition, land management, and environmental education.

Juan M. Guayasamin is an Ecuadorean herpetologist and Professor of Evolution, Biology, and Island Biogeography at Universidad San Francisco de Quito in Ecuador. Juan's research interests include taxonomy, ecology, evolution, and neotropical biodiversity conservation. Juan and his students also investigate the impact of introduced species to Andean cloud forest streams in an effort to protect these ecosystems.

Appendices

Appendix 1. Survey site locations in the Mindo region of Ecuador (Datum WGS 84). Site locations: RSR = Río Santa Rosa, LC = Lucy's Creek, ST = Santa Teresita, FJ= Finca de Jaime, KC = Kathy's Creek, M = Michelle's, C = Chalguayacu Grande River, 5F = Five Frog Creek, LRSR = Lower Río Santa Rosa, Bcrk = Ballux Creek, EP = El Paraíso del Pescador, LS = La Sierra, VC = Verdacocha.

Site	Site Name	Coordinates (decimal degrees)	Trout farm (y/n)
RSR	Río Santa Rosa	0.1302S, 78.8440W	N
LC	Lucy's Creek	0.0051S, 78.7383W	N
ST	Santa Teresita	0.0481S, 78.6317W	Y
FJ	Finca de Jaime	0.0468S, 78.6332W	Y
KC	Kathy's Creek	0.0167S, 78.7316W	N
M	Michelle's	0.0215S, 78.7240W	N
C	Chalguayacu Grande River	0.0287S, 78.7303W	N
5F	Five Frog Creek	0.0315S, 78.7052W	N
LRSR	Lower Río Santa Rosa	0.0032N, 78.7304W	Y
Bcrk	Ballux Creek	0.0360S, 78.7074W	N
EP	El Paraíso del Pescador	0.0121N, 78.6727W	Y
LS	La Sierra	0.0698S, 78.6073W	Y
VC	Verdecocha	0.0861S, 78.6100W	Y

Appendix 2. Environmental characteristics of sites included in the glassfrog call survey conducted March-May, 2017 Mindo region of Ecuador. Site abbreviations: VC = Verdacocha, LS = La Sierra, ST = Santa Teresita, FJ= Finca de Jaime, EP = El Paraíso del Pescador, C = Chalguayacu Grande River, LC = Lucy's Creek, Bcrk = Ballux Creek, 5F = Five Frog Creek, KC = Kathy's Creek, RSR = Río Santa Rosa, M = Michelle's, LRSR = Lower Río Santa Rosa. All measurements were collected during daylight hours.

Site type	Site code	Stream discharge (m^3/sec)	pH	Conductivity (µS/cm)	Total dissolved solids (g/L)	Dissolved oxygen (mg/L)	Canopy openness (%)	Elevation (m)	Temperature (°C)
Trout farm	VC	0.132	7.725	55	0.047	7.415	55.51	2,666	12.255
	LS	0.595	7.765	71.5	0.0595	7.245	28.86	2,483	13.585
	ST	1.352	8.125	128.5	0.1055	8.245	33.93	2,186	14.39
	FJ	1.421	8.08	131.5	0.107	7.58	60.21	2,160	14.31
	EP	281.25	7.74	78	0.0605	7.785	43.29	1,593	16.595
Non-trout farm	C	0.004	7.84	25	0.02	7.61	20.54	2,015	16
	LC	0.115	7.69	35	0.027	7.37	0.26	1,814	15.66
	Bcrk	—	8.61	43	0.036	7.41	22.88	2,254	15.16
	5F	0.22	7.7	42	0.034	7.48	32.5	2,167	14.86
	KC	0.23	7.115	28	0.02	6.9	1.04	2,053	15.8
	RSR	1.131	7.61	43	0.0034	7.37	15.08	1,811	15.97
	M	1.339	8.06	41	0.032	7.91	41.08	2,031	15.43
	LRSR	4.003	7.63	43	0.034	7.92	28.34	1,693	15.83