



Habitat characterization, occupancy and detection probability of the Endangered and endemic Junín giant frog *Telmatobius macrostomus*

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ABSTRACT: The Junín giant frog *Telmatobius macrostomus* (Peters, 1873) is an Endangered and endemic frog in the central Andes of Peru. However, no quantitative ecological data exist to facilitate characterization of *T. macrostomus* habitat, nor do estimates of occupancy and detection probability, all of which are vital to inform appropriate management decisions. We present novel data on the current distribution and habitat of *T. macrostomus* in 3 natural protected areas in the regions of Junín and Pasco, Peru. To provide a general description of the areas where *T. macrostomus* occurred and did not occur, we measured a variety of physical, chemical, and biological conditions. We found *T. macrostomus* in fewer than half the streams surveyed for an estimated occupancy and detection probability of 0.40 ± 0.11 SE and 0.84 ± 0.06 SE, respectively. Akaike's information criterion (AIC) indicates that of the 20 single-variable models constructed during this study, 5 were substantially associated with *T. macrostomus* occurrence (Δ corrected AIC [Δ AIC_c] < 2). The % Chironomidae metric of benthic macroinvertebrate communities at sampled sites best predicted the occurrence of *T. macrostomus*, followed by pH. Specific conductance, % silt and rainbow trout *Oncorhynchus mykiss* were also associated with *T. macrostomus* occurrence. Although the most parsimonious multi-variable occupancy model only included % Chironomidae, pH and rainbow trout as predictive variables of *T. macrostomus* occurrence, the other single-variable models with apparent influence over *T. macrostomus* occurrence may also be used to identify adequate areas for Junín giant frog conservation.

KEY WORDS: Detection probability · Endangered amphibians · Habitat · Junín giant frog · Occupancy · *Telmatobius macrostomus* · Wildlife conservation

INTRODUCTION

Amphibians have a greater risk of extinction than any other major vertebrate taxon, with nearly one-third (32%) of described species being threatened (IUCN 2016). This global proportion of threatened species is unprecedented in known history (Wake & Vredenburg 2008), and human population growth

and anthropogenic activities are undoubtedly associated both directly and indirectly. Amphibian declines have been attributed to habitat loss, degradation and fragmentation, urbanization, invasive species, contamination and pollution, overharvesting, emerging infectious diseases, global climate change, and synergistic effects (Young et al. 2004, Zevallos et al. 2016). Of these factors, habitat loss is the principal

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threat to 9 out of every 10 Threatened amphibian species (Baillie et al. 2004), making it critical to identify, protect and conserve the habitats where Threatened amphibians occur (Silvano et al. 2007).

Peru is a megadiverse country, home to approximately 588 species of amphibians (Jarvis et al. 2015), and is especially important for the high-elevation genus *Telmatobius*, with 27 of the 63 known species occurring in the country (Ttito et al. 2016). Twenty of the 27 species of *Telmatobius* distributed throughout Peru are categorized as Threatened (IUCN 2016, Ttito et al. 2016). The regions of Junín and Pasco in the central Andes of Peru include the historic range of the Endangered and endemic Junín giant frog *Telmatobius macrostomus*, an exclusively aquatic frog with an elevational range of 3200–4600 m above sea level (masl; Fjeldsa 1983, Angulo et al. 2004, Angulo 2008). One of the direct threats affecting the Junín giant frog is habitat loss, mainly through degradation and fragmentation caused by overgrazing, industrial, agricultural and residential pollution, and fluctuations in water levels controlled by the Upamayo Dam (Shoobridge 2006). Additionally, information from climate change models suggests that increasing temperatures will be pronounced in the highest elevation regions of the tropical Andes (Bradley et al. 2006, Rodbell et al. 2014), thus directly affecting this high-elevation spe-

cies' habitat. Identifying suitable habitat that is already protected by local authorities is an important step in the conservation of *T. macrostomus*.

The purpose of our paper is to present the results of Junín giant frog monitoring in 3 natural protected areas. Using statistical modeling of the species' occupancy, detection probability, and habitat use, we present an estimate of *T. macrostomus* occupancy and detection probability to identify associations between occupancy and habitat characteristics. Our results are based on 2 seasons of field surveys conducted by the United States Peace Corps and the Peruvian National Park Service (Servicio Nacional de Áreas Naturales Protegidas; SERNANP).

MATERIALS AND METHODS

Study sites

We conducted fieldwork in 3 protected areas – the Junín National Reserve (10° 59' 15.1" S, 76° 06' 31.5" W), the Historic Sanctuary of Chacamarca (11° 13' 22.0" S, 75° 59' 00.6" W), and the National Sanctuary of Huayllay (10° 59' 39.1" S, 76° 19' 18.3" W) – and their respective buffer zones in the regions of Junín and Pasco in the central Andes of Peru (Fig. 1). To assess

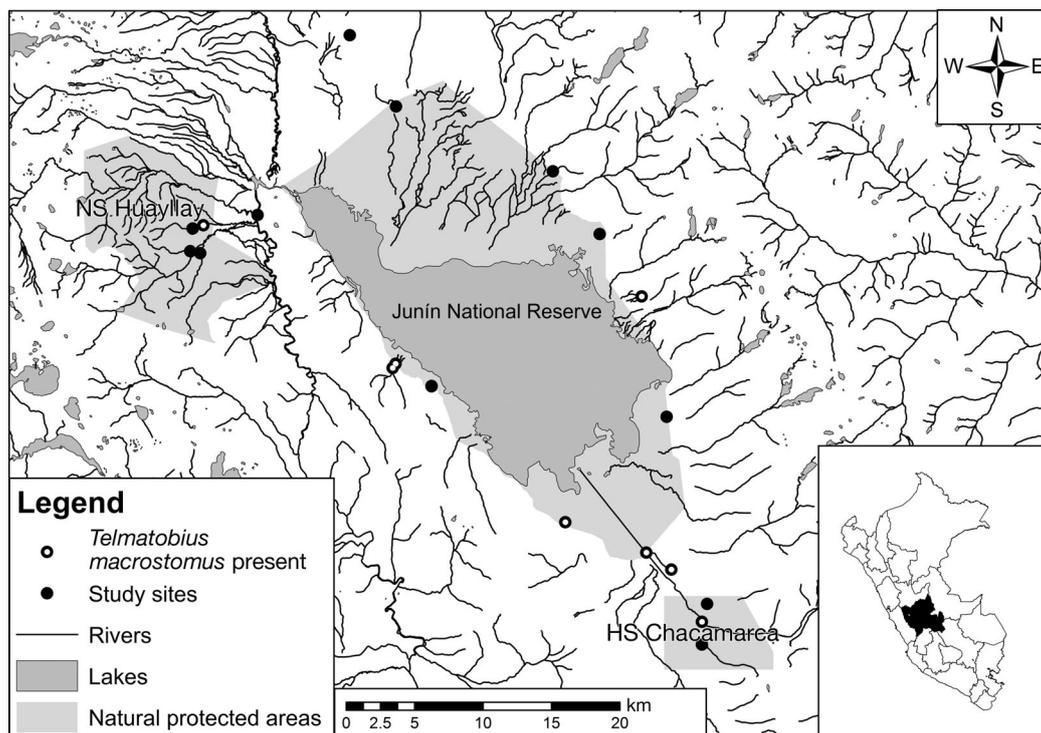


Fig. 1. Location of study sites within the Junín National Reserve, Historic Sanctuary (HS) of Chacamarca and National Sanctuary (NS) of Huayllay and their respective buffer zones. Insert: Peru with the regions Pasco and Junín shown in black

the proportion of sites occupied (occupancy) and the detection probability of *Telmatobius macrostomus*, we selected twenty 100 m transects throughout the study area based on accessibility and overall appearance of the site, which resulted in a variety of sampled habitats (Watson et al. 2017). Transects (sites) were independent of each other (i.e. 1 transect per stream) with the exception of 3 replicates (i.e. 2 transects per stream). Replicate transects were more than 500 m apart and were representative of different habitat types (e.g. natural rivers to canals). Sites ranged in elevation from 4080 to 4546 masl. Mean stream widths and depths ranged from 0.7 to 39.0 and 0.2 to 3.0 m, respectively. We conducted all surveys during the day due to the difficulty of accessing remote locations at night.

Frog surveys

We searched each transect (N = 20) moving in the upstream direction, and carefully examined all available refugia for frogs (e.g. underneath rocks, inside vegetation, and underneath stream banks). Survey effort was 4 person-hours per transect, i.e. by 2 observers for 2 h or by 4 observers for 1 h.

To establish frog occupancy and detection probability, we conducted 2 field surveys at each study site during both the dry season (October 2015) and the wet season (April 2016) within a 10 d period. We conducted repeated searches at study sites as discrete visits (i.e. on different days) using multiple observers, and during the dry season of 2015 we used 3-pass depletion methods at study sites (transects) to estimate detection probability (Petty et al. 2014). We reduced survey bias by using different observers among different sites, and all observers were previously trained for frog surveys (MacKenzie et al. 2003).

Environmental characterization

To provide a general description of the areas where *T. macrostomus* occurred and did not occur, we measured a variety of physical, chemical, and biological conditions. We recorded descriptions of stream substrate, mean stream width and mean stream depth for each transect (site) using a modified version of the Wolman pebble count protocol (Wolman 1954). We established ten 10 m stations along each transect, and collected 10 measurements of stream substrate using a zigzag pattern, and 1 measurement of

stream width and depth at each station. We recorded measurements of water chemistry *in situ* at all sites on the day surveys were conducted. We measured water temperature (°C), pH, and specific conductance ($\mu\text{S cm}^{-1}$) using a water measurement pocket meter (ExStik EC500). In addition to presence/absence of *T. macrostomus*, we recorded presence/absence of dams and the exotic rainbow trout *Oncorhynchus mykiss* at each site.

Additionally, we sampled benthic macroinvertebrate communities at each site in October of 2015 and April of 2016 following the West Virginia Department of Environmental Protection (WVDEP) protocol for benthic macroinvertebrate collection (WVDEP 2014). We chose to use aquatic insects as environmental variables in our experimental design to assess the biological integrity of sampled sites. Aquatic insects are frequently used as indicators of biotic integrity because of their ubiquitous nature, and their susceptibility to environmental stressors in a variety of aquatic ecosystems (Rosenberg et al. 2008, Ríos-Touma et al. 2014). The great diversity of aquatic insects yields a range of responses to environmental stressors useful for habitat quality assessment (Rosenberg et al. 2008). Indicator communities such as the taxa belonging to the orders Ephemeroptera (E), Plecoptera (P), and Trichoptera (T) require high-quality water and consequently are pollution intolerant (Cushing & Allan 2001). In contrast, pollution-tolerant indicator organisms, such as chironomid midges, characterize impaired habitat conditions (Rosenberg et al. 2008). At each site, we obtained 11 samples with a D-frame dip/kick-net (net dimensions 0.3×0.3 m with 500 μm mesh) using a modified version of the multi-habitat approach for low gradient streams to sample a total of 1.0 m^2 (WVDEP 2014). We filtered all 11 samples through a 250 μm sieve, combined them into a single composite sample, and preserved the composite sample in 95% ethanol. To obtain a sub-sample that was both random and representative of the whole, we used a gridded sorting tray with a random number matrix on the bottom and picked 200 macroinvertebrates from randomly selected grid cells (WVDEP 2014). We identified all macroinvertebrates to family or the lowest possible taxonomic level using Domínguez & Fernández (2009). We calculated family richness, Ephemeroptera/Plecoptera/Trichoptera (EPT) richness, % EPT abundance, % E abundance, % Chironomidae, % 2 dominant families, Modified Hilsenhoff Index (MHI), and the Andean Biotic Index (ABI) (*sensu* Ríos-Touma et al. 2014) for each site.

Statistical analysis

We used the program PRESENCE to estimate the detection probability and occupancy of the Junín giant frog throughout our study sites (MacKenzie et al. 2002). To estimate occupancy, we applied the approach of Nájera-Hillman et al. (2009), where detection probability is assumed to be constant across surveys and also to be survey-specific. Detection probability was assumed constant across surveys because the reproductive cycle of *T. macrostomus* is not seasonally regulated due to stable temperatures in its habitat (De Macedo 1950, Vellard 1951). Additionally, we used the estimate of detection probability to determine the number of surveys necessary to establish whether a species is truly absent from a site (Kéry 2002). We used the approach of Pellet & Schmidt (2005) to calculate the minimum number of surveys necessary to be 95% certain that the Junín giant frog would be absent from a stream transect in the study area.

Our second objective was to identify associations between *T. macrostomus* occupancy and habitat characteristics. To meet this objective, we used an information-theoretic approach (Burnham & Anderson 2002). We used site-specific variables (e.g. benthic macroinvertebrate community metrics and stream substrate) to model *T. macrostomus* occupancy, and sampling occasion variables (e.g. water temperature and pH) to model *T. macrostomus* detection probability. Additionally, we used the average values of the sampling occasion variables from each site as site variables (Nájera-Hillman et al. 2009). We used Akaike's information criterion (AIC) for both occupancy and detection probability to compare models with different environmental variables. We adjusted AIC for small sample size (AIC_c) in the model selection process to account for the relatively low number of surveyed sites (Burnham & Anderson 2002). Next, we used the program PRESENCE to build single-variable models of the measured environmental variables, and compared their AIC_c values to determine which variables had the most substantial support. We then constructed multi-variable models of the most substantial variables to see whether their combinations produced a post-hoc model that better fit the data than the best single-variable model (Nájera-Hillman et al. 2009). To classify the level of empirical support for models that explained the occupancy and detection probability of *T. macrostomus*, we calculated AIC_c differences ($\Delta AIC_c = AIC_c - \text{minimum } AIC_c$) where values between 0 and 2 indicate substantial support, 4 and

7 some support, and >10 no support (Burnham & Anderson 2002).

Additionally, we calculated Akaike weights (w_i) to determine the weight of evidence supportive of each post-hoc model. Finally, to verify the strength of the best model we made sure that the error estimates (β) of the untransformed coefficients included in the models did not encompass zero (Nájera-Hillman et al. 2009).

RESULTS

We detected *Telmatobius macrostomus* at 8 of 20 sites. As a result, the average occupancy (naïve estimate) of *T. macrostomus* was 40%. When detection probability (0.84 ± 0.06 SE) was considered to be either constant or survey-specific, the estimated occupancy of *T. macrostomus* was 0.40 ± 0.11 SE. We found that the minimum number of surveys necessary to be 95% confident that *T. macrostomus* would be absent from a stream transect was 1.6, based on the average detection probability (0.84), indicating that 2 surveys would be sufficient to determine whether *T. macrostomus* is absent from a site. During this study, we found tadpoles (survey effort = 1.4 person-hours per tadpole) with more frequency than adults (survey effort = 22.9 person-hours per frog). Additionally, *T. macrostomus* was found present at the same sites during both the dry and wet seasons, and adults were not found at sites without the presence of tadpoles. Therefore, just the presence of tadpoles was sufficient to determine occupancy and detection probability.

Of the 20 environmental variables we measured (Table 1), only 5 were strongly associated with *T. macrostomus* occurrence ($\Delta AIC_c < 2$). Of these, % Chironomidae best predicted the occurrence of *T. macrostomus*, followed by pH. Specific conductance, % silt and rainbow trout *Oncorhynchus mykiss* were also substantially associated with *T. macrostomus* occurrence; however, these models did not present a better fit than the null model [$\Psi(\cdot)$, $p(\cdot)$; see Table S1 in the Supplement at www.int-res.com/articles/suppl/n032p429_supp.pdf]. The models that considered the effect of survey-specific variables (i.e. detection probability models) were not substantially associated with *T. macrostomus* occurrence ($\Delta AIC_c > 2$; see Table S1). Subsequently, the multi-variable models (post-hoc models) constructed only included combinations of the 5 most substantially supported site-specific variables (% Chironomidae, % silt, pH, specific conductance, and rain-

Table 1. Habitat characteristics of sites surveyed for *Telmatobius macrostomus* presence. EPT: Ephemeroptera, Plecoptera, Trichoptera; E: Ephemeroptera; MHI: Modified Hilsenhoff Index; ABI: Andean Biotic Index; ONMY: *Oncorhynchus mykiss* (rainbow trout)

Characteristic	Unoccupied (N = 12)		Occupied (N = 8)	
	Mean	SE	Mean	SE
Benthic macroinvertebrate metrics				
% EPT abundance	20.99	22.82	27.55	21.45
EPT richness	1.79	1.47	2.00	1.10
% E	9.13	15.41	20.05	18.98
% Chironomidae	22.67	18.19	13.64	14.73
% 2 dominant families	75.40	11.07	65.79	14.44
MHI	5.62	0.81	5.61	0.55
ABI	35.88	11.63	38.56	8.40
Family richness	8.08	2.02	9.25	1.84
Water chemistry				
Temperature (°C)	13.76	2.13	13.46	3.30
pH	8.04	0.52	8.38	0.52
Specific conductance (µS cm ⁻¹)	307.14	128.59	378.55	95.64
Physical habitat				
Mean stream width (m)	6.60	8.78	5.18	4.08
Mean stream depth (m)	0.89	0.72	0.60	0.18
% Cobble	6.68	0.12	11.31	0.11
% Gravel	10.27	0.22	23.05	0.22
% Sand	11.44	0.19	18.11	0.24
% Silt	53.84	0.42	26.87	0.26
% Clay	14.74	0.15	17.70	0.10
Dam present (no. sites; %)	33.33		12.50	
Predators				
ONMY present (%)	41.67		12.50	

Table 2. Summary of AIC_c (corrected Akaike’s information criterion) model selection for post-hoc models of stream occupancy by *Telmatobius macrostomus*. The global model includes all variables with substantial association with frog occurrence. The symbols Ψ and p indicate the occupancy and detection portions of the models, respectively. ΔAIC_c: AIC_c – minimum AIC_c; K: no. of parameters in the model; w: Akaike weights; (.): null model; ONMY: *Oncorhynchus mykiss* (rainbow trout); SpC: specific conductance

Model	AIC _c	ΔAIC _c	K	w
Ψ(% Chironomidae, pH, ONMY), p(.)	41.74	0.00	4	0.76
Ψ(% Chironomidae, pH, SpC, ONMY), p(.)	45.74	4.00	5	0.10
Ψ(% Chironomidae, pH, % silt, ONMY), p(.)	45.74	4.00	5	0.10
Ψ(% Chironomidae, pH, SpC), p(.)	49.78	8.04	4	0.01
Ψ(Global), p(.)	50.36	8.62	6	0.01
Ψ(% Chironomidae), p(.) ^a	58.27	16.53	2	0.00

^aBest model from Table S1 in the Supplement

bow trout; Table 2). Of the multi-variable models constructed, a majority offered a better fit to the data than the best single-variable model (Table 2). The multi-variable model that presented the best fit to the data included % Chironomidae, pH, and rainbow trout. This model was 7.6 times more likely to be the best explanation of *T. macrostomus* occur-

rence compared with the next-ranked multi-variable model, which included % Chironomidae, pH, specific conductance, and rainbow trout, as indicated by the Akaike weights (0.76/0.10; Table 2). Percent Chironomidae and rainbow trout were negatively associated with *T. macrostomus* occurrence, while pH was positively associated with *T. macrostomus* occurrence. Percent Chironomidae, pH and rainbow trout variables had a strong association with *T. macrostomus* occurrence given that their error estimates did not encompass zero (see Table S2 in the Supplement). In addition, % silt was negatively associated with *T. macrostomus* occurrence and specific conductance was positively associated with *T. macrostomus* occurrence.

DISCUSSION

Frog occupancy and detection probability

We found *Telmatobius macrostomus* in less than half of the streams surveyed. Using presence/absence data we were able to provide a reliable estimate of occupancy in the study area, and our detection probability estimate indicates that 2 frog surveys on a particular transect are enough to be 95% certain that *T. macrostomus* is absent from a site. This information, along with the information on documenting the various threats to *T. macrostomus*, will likely prove invaluable to conservation and management plans in and around the various protected areas where the Junín giant frog occurs. For example, if a mining lease is requested in the buffer zone of a protected area,

the developers should provide evidence of *T. macrostomus* absence, and we suggest that such evidence must be backed with statistical analyses before development activities are approved and initiated (Pellet & Schmidt 2005, Nájera-Hillman et al. 2009).

Our methodology for detecting *T. macrostomus* proved effective; however, it should be noted that

tadpoles were found in much higher quantities than adults. Using our sampling protocol, adults were rare and/or difficult to find. To this extent, we believe that the time of day should be taken into consideration when frog surveys are conducted, depending on the question asked and life history stage targeted for subsequent studies of this species. Nocturnal surveys could prove more effective for finding adult Junín giant frogs. Adults of the closely related species *T. culeus*, the Lake Titicaca water frog, have been observed to be more active during the night (Arturo Muñoz, Bolivian Amphibian Initiative, pers. comm.). Thus, additional studies comparing diurnal and nocturnal surveys of adult *Telmatobius* frogs are recommended for future research. Also, Junín giant frog adults were not found at sites without the presence of tadpoles. Therefore, if tadpoles are not present, the extra time needed to search for adults may not be worth the effort. Although *T. macrostomus* was present at the same sites in both the dry and wet seasons, most likely because their reproductive cycle is not regulated by seasonality, it should be noted that detection probabilities of amphibians have the potential to vary temporally year to year (MacKenzie et al. 2003, Nájera-Hillman et al. 2009), due to environmental conditions, behavior patterns (Bailey 2002), and seasonality. This information should be taken into consideration for research teams that wish to replicate our monitoring protocols for other species.

Relationship between frog distribution and environmental characteristics

The occurrence of *T. macrostomus* at surveyed sites is best described as negatively associated with % Chironomidae, % silt, and rainbow trout, and positively associated with pH and specific conductance. Our finding of *T. macrostomus* being negatively associated with % Chironomidae at surveyed sites suggests that *T. macrostomus* is relatively sensitive to water quality. Chironomids are known to be tolerant of poor water quality and have been found to dominate benthic communities where poor conditions exist. Our results show that in general, benthic macroinvertebrate metrics associated with better water quality (% EPT abundance, EPT richness, % E, and family richness) in frog-occupied sites were higher than unoccupied sites (Table 1). Additionally, we used the ABI as a monitoring tool to evaluate the biological quality of streams in our study. The ABI was developed to assess the effects of organic pollu-

tion and riparian alteration of Andean streams (Ríos-Touma et al. 2014). The ABI uses invertebrate family scores adapted for the Andean region to identify 5 quality classes: excellent, good, moderate, poor, and bad (Ríos-Touma et al. 2014). Frog-occupied sites on average possessed a higher ABI score than unoccupied sites (Table 1); however, on average, all sites scored within the quality class of moderate. These results suggest that *T. macrostomus* has greater affinity for streams with more sensitive benthic macroinvertebrate communities indicative of relatively better water quality. Interestingly, the mean % E metric at frog-occupied sites was more than double the mean % E at unoccupied sites (Table 1), and baetid mayflies (order: Ephemeroptera) are a known prey item for adult Junín giant frogs (Watson et al. 2017).

In addition to being negatively associated with % Chironomidae at surveyed sites, we also found *T. macrostomus* to be negatively associated with % silt. De Macedo (1950) notes that the Junín giant frog can be found resting in habitats with thick layers of silt, and Vellard (1951) also describes *T. macrostomus* habitat as being associated with a silt substrate. Although in our study *T. macrostomus* occurrence was found to be negatively associated with % silt, frog-occupied sites on average were observed to have a 27% silt substrate, suggesting that *T. macrostomus* occurrence is associated with moderate levels of silt. During surveys it was common to locate tadpoles and adults in areas of heavy silt. Therefore, further studies assessing the microhabitat utilization of *T. macrostomus* could confirm their preference for silty substrates as refugia, and their utilization of more open areas for foraging, basking, or attracting mates. Our general habitat characterization describes the water bodies where they occur as moderately silty.

T. macrostomus occurrence was also found to be negatively associated with presence of rainbow trout, a predatory and invasive species. However, we did find Junín giant frog tadpoles at 1 of 6 sites where rainbow trout occurred. The other 5 transects where rainbow trout were present appeared to provide sub-optimal refugia for *T. macrostomus*. While the interactions between rainbow trout and these frogs require further study, the fact remains that the impact of invasive species is a primary driver of biodiversity loss in freshwater ecosystems (Kolar & Lodge 2000, Martín-Torrijos et al. 2016) and can push amphibian populations to extinction (Martín-Torrijos et al. 2016). Not only have invasive rainbow trout become the main fish predator of Andean tadpoles, but they can also serve as a vector of the

pathogenic freshwater mold *Saprolegnia diclina* (Oomycetes), which causes high mortality on amphibian eggs (Martín-Torrijos et al. 2016).

In terms of water quality, we found *T. macrostomus* to be positively associated with pH and specific conductance. Geology throughout our study area is primarily sedimentary limestone rock (Shoobridge 2006). Limestone (CaCO₃) is a source of alkalinity and water hardness. Consequently, the limestone geology is most likely keeping pH higher at study sites, and contributing to higher levels of specific conductance. One of the main sources of habitat loss in Lake Junín, historically an important habitat for the Junín giant frog (Arias Segura 2003), is decades of acid mine drainage entering the lake from the Cerro de Pasco region via the San Juan River (Rodbell et al. 2014). Acid mine drainage from the Cerro de Pasco region has made the sediments of Lake Junín among the most polluted in Peru (Rodbell et al. 2014). Concentrations of Cu, Zn, and Pb in water samples taken from Lake Junín all exceed maximum contaminant levels set by the United States Environmental Protection Agency (Rodbell et al. 2014). As such, the conservation and protection of Lake Junín's surrounding rivers, streams, wetlands, and lagoons are of utmost importance. We have identified these bodies of water as critical habitat for the conservation of *T. macrostomus* considering that the influx of metals to Lake Junín will continue until efforts are focused on preventing these sediments from entering the lake.

Implications for conservation

The resulting model of frog distribution and habitat use from this study provide a reliable characterization of the habitat requirements of the Junín giant frog throughout the protected areas where it occurs. This information can be used to assess future changes in habitat. Although the best parsimonious multi-variable occupancy model only included % Chironomidae, pH, and rainbow trout as predictive variables of *T. macrostomus* occurrence, the other single-variable models with substantial influence over *T. macrostomus* occurrence may also be used to identify adequate areas for Junín giant frog conservation. Thus, ideal stream habitat characteristics of the Junín giant frog are free of predatory, invasive rainbow trout with moderate levels of silt and relatively clean water. Unfortunately, this habitat is not very well represented throughout our study area, as reflected by the low occupancy of the Junín giant frog.

In conclusion, contamination and habitat alteration, which result in habitat loss and the introduction of exotic species, are identified as major threats for *T. macrostomus*. Protecting, conserving, and restoring Junín giant frog habitat is essential for effective management and maintenance of this species. Other important recommendations for the conservation of this species include: (1) identifying and engaging with stakeholders who have interest in raising public awareness on the conservation status of this Endangered amphibian; (2) better enforcement of the legislation prohibiting the harvesting, consumption, and trafficking of this species throughout its native range; and (3) promoting *in situ* research for the Junín giant frog. Continued long-term monitoring and an extinction risk assessment of the Junín giant frog is needed to inform appropriate conservation management of this Endangered, high-elevation, endemic species.

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