Potential effect of habitat disturbance on reproduction of the critically endangered harlequin frog *Atelopus varius* in Las Tablas, Costa Rica

D. A. Gómez–Hoyos, R. Seisdedos–de–Vergara, J. Schipper, R. Allard, J. F. González–Maya

Abstract

Potential effect of habitat disturbance on reproduction of the critically endangered harlequin frog Atelopus varius *in Las Tablas, Costa Rica*. We studied a population of *Atelopus varius* in Las Tablas Protected Zone in southwest Costa Rica, where we estimated occupancy rates of tadpoles along the Cotón River. In addition, we report the first tadpoles observed in the wild in 20 years. Tadpole rate of occupancy was greater in habitat containing native forest than in disturbed areas bordering cattle pasture. This same pattern was also reflected in adult hotspots, where encounter rates were higher for adults in habitat surrounded by forest versus pasture. We present evidence for the potential effect of habitat modification on the presence and reproduction of *A. varius* and suspect that over time this modification impacts the species' demography. However, further study is necessary before we can confirm that habitat change alone was the key factor involved in patterns of decline for the species.

Key words: Habitat disturbance, Hotspots, Occupancy, Tadpoles, Threats

Resumen

Posibles efectos de la perturbación del hábitat en la reproducción de la rana arlequín Atelopus varius *críticamente amenazada en Las Tablas, Costa Rica*. Estudiamos una población de *Atelopus varius* de la Zona Protegida Las Tablas, en el sureste de Costa Rica, donde estimamos la tasa de ocupación de renacuajos a lo largo del río Cotón. Además, reportamos los primeros renacuajos observados en el medio silvestre en 20 años. La tasa de ocupación de renacuajos fue mayor en los hábitats con bosque nativo que en las zonas perturbadas, aledañas a pastizales. Esta misma pauta se observa también en las zonas de alta concentración de adultos, donde la tasa de encuentro es más alta en los hábitats rodeados por bosque que en los pastizales. Presentamos algunos indicios de los efectos que la modificación del hábitat podría tener en la presencia y reproducción de *A. varius* y planteamos la posibilidad de que, con el tiempo, esta modificación pueda afectar también a su composición demográfica. Sin embargo, se necesitan más estudios antes de poder confirmar que el cambio de hábitat fue el único factor que determinó la tendencia decreciente de la especie.

Palabras clave: Perturbación del hábitat, Zonas de alta concentración, Ocupación, Renacuajos, Amenazas

Diego A. Gómez–Hoyos, Rocío Seisdedos–de–Vergara, Jan Schipper, José F. González–Maya, ProCAT Internacional / Sierra to Sea Institute Costa Rica, Las Alturas, Puntarenas, Costa Rica.– Diego A. Gómez–Hoyos, Grupo de Investigación y Asesoría en Estadística / Grupo de Herpetología, Universidad del Quindío, Armenia, Colombia.– Jan Schipper, Ruth Allard, Arizona Center for Nature Conservation / Phoenix Zoo, Phoenix, Arizona, U.S.A.

Corresponding author: Diego A. Gómez–Hoyos. E–mail: dgomez@procat–conservation.org

Introduction

The variable harlequin frog *Atelopus varius* is categorized as Critically Endangered on the IUCN Red List of Threatened Species following a drastic population decline since the 1980s (Pounds et al., 2010). Despite this decline, *Atelopus varius* is one of the few harlequin frog species remaining in Central America. Reproductive populations have recently been recorded in Costa Rica and Panama (González–Maya et al., 2013; Perez et al., 2014; Barrio–Amorós and Abarca, 2016), providing the opportunity to carry out research and conservation efforts to better understand the factors leading to the decline and how to address them.

Although *A. varius* is the best–known species within the genus (Lötters, 1996), information about current threats and their effect is scarce. Ongoing factors impacting the species include chytrid fungus, invasive species, and habitat loss and degradation (Richards–Zawacki, 2009; Pounds et al., 2010; Perez et al., 2014; González–Maya et al., 2018). Chytrid fungus is the most well–known and persistent threat affecting the species (Brem and Lips, 2008), and the *Atelopus* genus, while other described threats both directly and indirectly impact the species (Pounds et al., 2010).

Habitat disturbance is the main driver of amphibian population decline and extinction worldwide (Blaustein et al., 2011). However, the implications of habitat disturbance on population are unknown for many amphibians (Cushman, 2006), including the genus *Atelopus*. Understanding the effects of this threat factor is essential to develop and prioritize management actions to avoid the extinction of endangered species.

Because the species is restricted to a very limited area along riverbanks, riparian habitat disturbance may have a negative effect on many aspects of the ecology of *A. varius* (Richards–Zawacki, 2009). Unfortunately, the effect at the population level is unknown. Here, we show evidence of the impact of habitat disturbance on the reproduction of *A. varius* along the Cotón River, Las Tablas Protected Zone, Costa Rica. We also present the first records of harlequin frog tadpoles in the wild in at least 20 years.

Material and methods

Our study site is located at Las Tablas Protected Zone (LTPZ), Puntarenas, Costa Rica. LTPZ is in southeastern Costa Rica, specifically along the Pacific flanks of the Talamanca mountain range (8.93º N and 82.82º W). LTPZ spans for over 19,000 ha and is part of La Amistad Biosphere Reserve. Our specific study site is situated in and along the Cotón River (fig. 1), at 1,300 m a.s.l., with a mean annual precipitation of 3,500 mm and a mean annual temperature of 19 ºC (González–Maya and Mata–Lorenzen, 2008).

We conducted surveys along a 2.2 km section of the Cotón River, in LTPZ (fig. 1), along both river banks. Two experienced researchers (each with at least 1.5 years surveying the population) performed the surveys. One researcher focused on searching for adults and the other focused the search on tadpoles.

The same observers performed all the surveys during the study to minimize detection bias. Mean days surveyed per month were 7.4 (± 1.52) with a mean effort of 4.24 hours/day/person (± 1.61). The surveys were carried out monthly from October 2016 to April 2017, corresponding to the reproductive season of *A. varius*. The only break in surveys occurred following abnormally high water levels during November 2016 which prevented access to the study area due to hazardous conditions. All surveys consisted of visual encounter surveys (VES) along the river banks; for adults this entailed surveying at least 3 m from the water–line into the forest and for tadpoles we searched over and under rocks and in pools along the water's edge. Tadpoles of *A. varius* were identified by their exclusive ventral sucking disc and distinguishing chromatophores, which differ from larvae of other sympatric anuran species, as well as the depressed–flattened body and massive proximal caudal musculature (Lötters, 1996). Tadpole records include one or more individuals which were assigned to Gosner's stages (1960) per month through subaquatic photography. Each adult individual detected was captured and measured. We photographed each adult's ventral and dorsal surfaces to 1) identify individuals and to create a capture–recapture histories database, 2) define movement of individuals and 3) prevent pseudoreplication. Only adult detections between October and December 2016 were used in the analysis, because this is time period when aggregation for reproduction occurs. Uncertainty about the identification between males and females did not allow discrimination by sex categories in all cases; only females with snout to vent length (SVL) > 33 mm were identified.

Geographic location was recorded for each individual or tadpole and was georeferenced using QGIS software (QGIS Development Team, 2016). Each location was classified into one of two vegetation categories based on satellite imagery and verified using field observations, according to the composition and dominant vegetation elements: disturbed forest (perturbed) zone and late successional forest zone (fig. 1). The perturbed zone is dominated by pastures and scrublands with a few dispersed natural vegetation patches and crops. The late successional forest zone is dominated by natural vegetation that suffered selective wood extraction over 15 years ago and which is currently recovering.

Capture–mark–recapture data were used to estimate the mean distance that *A. varius* individuals moved. We used the Points2One application (available at: https://plugins.qgis.org/plugins/points2one/) to calculate distance between capture and recapture locations for each individual. Mean distance covered by *A. varius* individuals was 76 m, so we established sub–transects of 100 m to divide the 2.2 km stretch we surveyed into 22 segments. The 100 m segments were used to identify aggregations of individuals of greater magnitude than that expected for random events. These aggregations were identified with the Getis–Ord Gi* statistic (Ord and Getis, 1995) return the z–score (GiZ) of each segment and 5 % significance, a GiZ greater than 1.96 indicates a hotspot. We used the
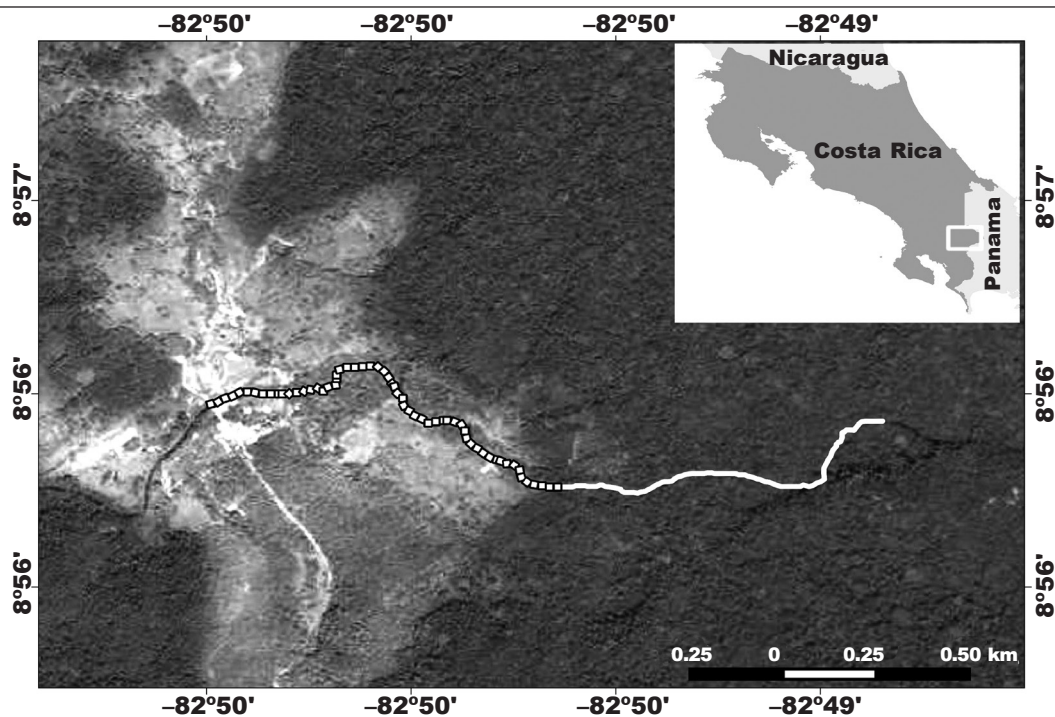
Fig. 1. Study area in the Cotón River, Las Tablas Protected Zone, Costa Rica: location of the protected zone (white rectangle), perturbed zone (dotted line) and late successional forest zone (continuous line) of the studied stretch of the Cotón River (Satellite image: Google Inc.).

Fig. 1. Zona de estudio en el río Cotón, en la Zona Protegida Las Tablas, Costa Rica: ubicación de la zona protegida (rectángulo blanco), de la zona perturbada (línea punteada) y de la zona de bosque en sucesión tardía (línea continua) del tramo estudiado del río Cotón (imagen satelital: Google Inc.).

fixed distance option of 200 m (two segments) to identify hotspots. This method works by evaluating each feature within the context of neighboring features, thus identifying the degree of spatial clustering. We used the Getis–Ord Gi* statistic because we assumed that the spatial distribution of harlequin frog (adult or tadpole) hotspots are spatially associated during the reproductive season since adults aggregate during these months (Lötters, 1996; Savage, 2002).

We also estimated the occupancy rate of tadpoles along the river. The occupancy was modelled through Unmarked package (Fiske and Chandler, 2011) for the R language (R Core Team, 2016). We tested the null model (constant detection and occupancy) against detection (p) and occupancy (Psi) depending on vegetation cover zone (perturbed or late successional forest areas). The best–fitting model was selected based on Akaike's Information Criterion (AICc), which was adjusted for small sample size and derived measures (Burnham and Anderson, 2002). Model selection was performed with *AICcmodavg* package (Mazerolle, 2016).

Our research permit was granted by the Sistema Nacional de Áreas de Conservación (SINAC) of Costa Rica (identification number: SINAC–ACLAP–GMRN–INV–105–2016).

Results

We identified 87 adult individuals during the reproductive season (October–December 2016), where at least nine were females and three of them were in amplexus. Furthermore, 33 records of tadpoles with 195 individuals in total were taken (fig. 2). We detected between 1 and 55 individuals per record (mean: 5.9 ± 10.1). The tadpoles were between stages 26 in January 2016 and 39 in April 2016, Gosner's stages (1960).

Despite equal survey effort, tadpoles were recorded almost exclusively in the late successional forest zone. We identified two hotspots for tadpole records using the defined segments of the Cotón River. The lengths of these hotspot segments were 200 and 400 m with GiZ value > 2.19 (fig. 3A). On the other hand, only 22 % of the adults were recorded in the perturbed zone. One adult hotspot was identified, and it was exclusively within the late successional forest zone. This hotspot was 400 m long with GiZ value > 2.16 (fig. 3B). Adults and tadpoles hotspots hadan overlap of 293 m (50.56 % and 75.85 % of tadpole and adults hotspots, respectively).

The occupancy model supports the hypothesis that habitat disturbance has a negative effect on *A. varius*
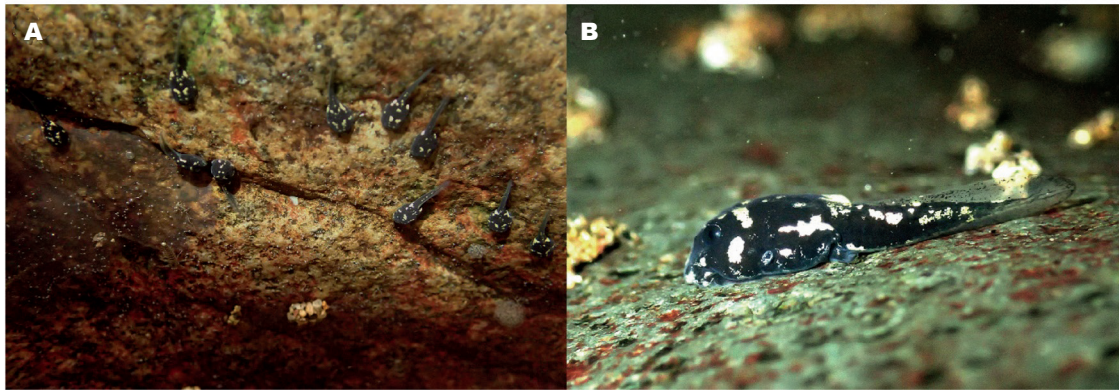
Fig. 2. Tadpoles of *Atelopus varius* from Las Tablas Protected Zone, Costa Rica: A, ten individuals recorded; B, detail of a tadpole.

Fig. 2. Renacuajos de Atelopus varius *de la Zona Protegida Las Tablas, Costa Rica: A, diez individuos registrados; B, detalle de un renacuajo.*

tadpole presence. The best–fitting model included both constant detection and occupancy rate depending on vegetation zone (table 1). Detection probability was 0.72 ± 0.07 (CI 95 %: 0.59–0.82). Occupancy in the perturbed zone was 0.095 ± 0.09 (CI 95 %: 0.02–0.36) and 0.98 ± 0.03 (CI 95 %: 0.84–0.99) in the late successional forest zone.

Discussion

Despite *A. varius* being the most studied species within the genus *Atelopus* (Lötters, 1996), ecological aspects are still almost entirely unknown. After the dramatic population decline and local extinctions in Costa Rica and Panama in the 1980s, remnant populations such as those studied here are an important opportunity for research, especially on demography and threat impacts (Muths et al., 2011).

Herein, we present the first records of *A. varius* tadpoles in the wild in at least 20 years (Lötters, 1996; La Marca et al., 2005; Lötters pers. comm.). It is important to consider that almost all tadpoles were restricted to the forest zone of the Cotón River, where sun exposure is less due to continuous forest canopy cover. Hotspots of adult individuals were also restricted to the forest zone, and tadpole occupancy was higher in the forest than in the perturbed zone. This spatial pattern is the first evidence indicating that habitat disturbance affects reproduction and could have a strong impact on demographic parameters in *A. varius*. However, to avoid premature generalizations we recommend a multi–year study in order to confirm that the spatial pattern is explained by habitat disturbance as a driver of reproduction site selection in *A. varius* and not by some other factor.

To date, habitat disturbance (specifically habitat loss) has not been a factor considered in the decline and extinction of the *Atelopus* genus (La Marca et al., 2005). On the other hand, threat factors such as chytrid fungus have been well documented and have been associated with amphibian extinction and population decline processes (Ryan et al., 2008; Crawford et al., 2010), and more specifically for *Atelopus* species (Brem and Lips, 2008; La Marca et al., 2005). Although there is no doubt that habitat disturbance has been a factor involved in amphibian decline and extinction globally (Blaustein et al., 2011), including other *Atelopus* species (La Marca et al., 2005), we demonstrate a potential effect of habitat disturbance over *A. varius* reproduction and we suspect this disturbance also impacts population demography. However, we cannot confirm that habitat disturbance alone was the key factor involved in decline patterns for the species, although we suspect that it is a contributing factor.

Species' disturbance tolerance is widely used as an indicator of ecosystem conservation status (Segurado et al., 2011) and it is also used during routine assessment of species' conservation status and categorization on the IUCN Red List of Threatened Species (IUCN, 2016). However, disturbance tolerance of *A. varius* is based on expert inference, with little empirical evidence (Segurado et al., 2011). Expert opinion is generally based on anecdotic and limited records, although in the absence of information it is an important resource when inferring impacts of known threats. However, it can be easily misapplied from one species to the next in the absence of specific data on occupancy during different life stages, specifically on breeding populations.

This new information stands in contrast to inference sometimes made unknowingly by some experts. Therefore, we feel it is prudent and necessary to provide evidence that the presence of *Atelopus varius* individuals (especially adults) in a perturbed zone is not proof of a population' tolerance to disturbance,
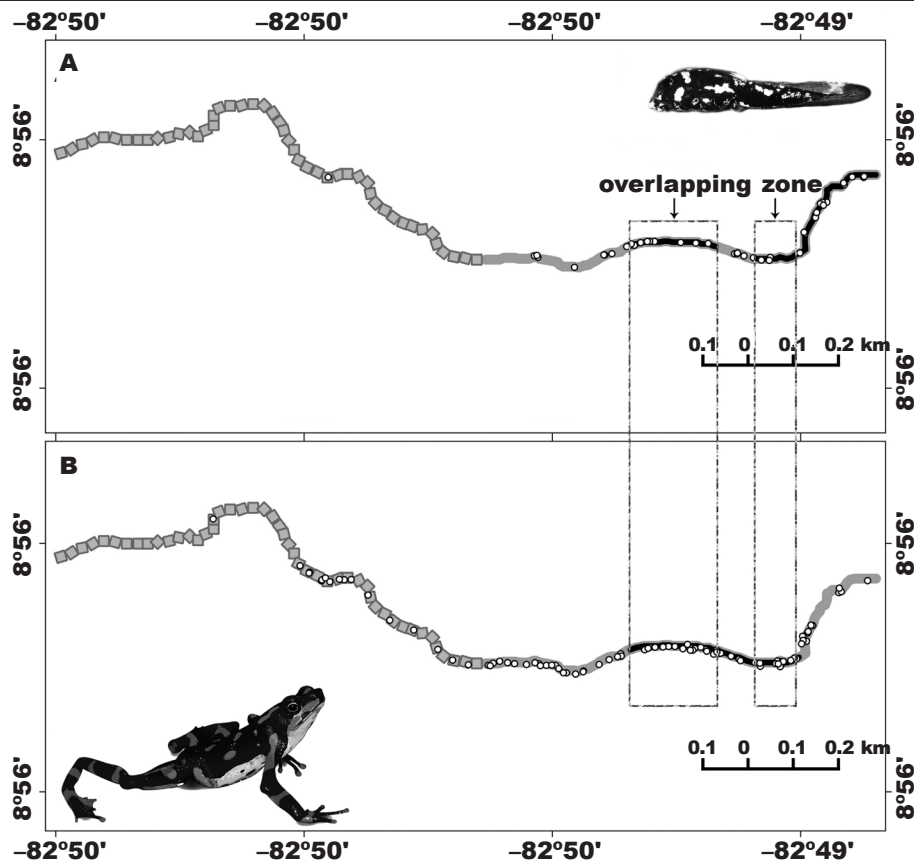
Fig. 3. Hotspots for tadpoles (A) and adults (B) of *Atelopus varius* in the Cotón River, Las Tablas Protected Zone, Costa Rica. Records (white dots); hotspots with GiZ value > 1.96 (black segments); perturbed zone (dotted line). Hotspots overlapping zones are indicated with arrows and rectangles.

Fig. 3. Zonas de alta concentración de renacuajos (A) y adultos (B) de Atelopus varius *en el río Cotón, en la Zona Protegida Las Tablas, Costa Rica: registros (círculos blancos), zonas de concentración con GiZ > 1,96 (segmentos negros), y zona perturbada (línea punteada). Los puntos en los que se solapan varias zonas de alta concentración se indican con flechas y rectángulos.*

Table 1. Model selection to explain detection and occupancy rate of *Atelopus varius* tadpoles in the Cotón River, Las Tablas Protected Zone, Costa Rica: K, number of parameter; AICc, Akaike's information criterion adjusted for small sample size.

Tabla 1. Selección de modelos para explicar la detección y la tasa de ocupación de renacuajos de Atelopus varius *en el río Cotón, en la Zona Protegida Las Tablas, Costa Rica: K, número de parámetros; AICc, criterio de información de Akaike ajustado para tamaños de muestra pequeños.*

Model	K	AICc	ΔAICc	AICc Weight	Cumulative Weight
p(.) Psi(zone)	3	71.4	0	0.81	0.81
p(zone) Psi(zone)	4	74.41	3	0.18	0.99
p(.) Psi(.)	2	81.88	10.48	0	1
p(zone) Psi(.)	3	84.58	13.18	0	1

nor does it indicate that a population is breeding or thriving in disturbed habitat. Evidence from this study suggests that successful reproduction occurs almost exclusively in the forest zone (i.e. native habitat). We urge biology researchers and conservation scientists to avoid speculation about the effects of habitat disturbance on species, especially those on the brink of extinction. Instead we need to increase ecological research efforts for remnant populations of threatened species, including *A. varius*, with the goal of conducting appropriate conservation and management actions to avoid their extinction.

Acknowledgements

References

Barrio–Amorós, C. L., Abarca, J., 2016. Another surviving population of the Critically Endangered *Atelopus varius* (Anura: Bufonidae) in Costa Rica. *Mesoamerican Herpetology*, 3: 128–134.

Blaustein, A. R., Han, B. A., Relyea, R. A., Johnson, P. T., Buck, J. C., Gervasi, S. S., Kats, L. B., 2011. The complexity of amphibian population declines: understanding the role of cofactors in driving amphibian losses. *Annals of the New York Academy of Sciences*, 1223: 108–119.

Brem, F. M. R., Lips, K. R., 2008. *Batrachochytrium dendrobatidis* infection patterns among Panamanian amphibian species, habitats and elevations during epizootic and enzootic stages. *Diseases of Aquatic Organisms*, 81: 189–202.

Burnham, K., Anderson, D., 2002. *Model selection and multimodel inference: a practical information and theoretic approach*. Springer, New York.

Crawford, A. J., Lips, K. R., Bermingham, E., 2010. Epidemic disease decimates amphibian abundance, species diversity, and evolutionary history in the highlands of central Panama. *Proceedings of the National Academy of Sciences*, 107(31): 13777–13782.

Cushman, S. A., 2006. Effects of habitat loss and fragmentation on amphibians: a review and prospectus. *Biological Conservation*, 128(2): 231–240.

Fiske, I., Chandler, R. B., 2011. Unmarked: An R package for fitting hierarchical models of wildlife occurrence and abundance. *Journal of Statistical Software*, 43: 1–23.

González–Maya, J. F., Belant, J. L., Wyatt, S. A., Schipper, J., Cardenal–Porras, J., Corrales, D., Cruz–Lizano, I., Hoepker, A., Escobedo–Galván, A. H., Castañeda, F., Fischer A., 2013. Renewing hope: the rediscovery of *Atelopus varius* in Costa Rica. *Amphibia–Reptilia*, 34(4): 573–578.

González–Maya, J. F., Gómez–Hoyos, D. A., Cruz–Lizano, I., Schipper, J., 2018. From hope to alert: demography of a remnant population of the Critically Endangered *Atelopus varius* from Costa Rica. *Studies on Neotropical Fauna and Environment*, 53(3): 194–200.

González–Maya, J. F., Mata–Lorenzen, J., 2008. Dung–beetles (Coleoptera: Scarabeidae) from the Zona Protectora Las Tablas, Costa Rica. *Checklist*, 4: 458–463.

Gosner, K. L., 1960. A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica*, 16(3): 183–190.

IUCN, 2016. *The IUCN Red List of Threatened Species*. Version 2016–3, http://www.iucnredlist.org. [Accessed on 07 December 2016].

La Marca, E., Lips, K. R., Lötters, S., Puschendorf, R., Ibáñez, R., Rueda–Almonacid, J. V., Schulte, R., Marty, C., Castro, F., Manzanilla–Puppo, J., García–Pérez, J. E., Bolaños, F., Chaves, G., Pounds, J. A., Toral, E., Young, B. E., 2005. Catastrophic population declines and extinctions in neotropical harlequin frogs (Bufonidae: *Atelopus*). *Biotropica*, 37(2): 190–201.

Lötters, S., 1996. *The Neotropical Toad Genus Atelopus. Checklist – Biology – Distribution* (M. Vences, F. Glaw, Eds.). Verlags GbR, Koln.

Mazerolle, M. J., 2016. AICcmodavg: Model selection and multimodel inference based on (Q)AIC(c). R package version 2.1–0. https://cran.r–project.org/package=AICcmodavg [Accessed on April 2018].

Muths, E., Scherer, R. D., Pilliod, D. S., 2011. Compensatory effects of recruitment and survival when amphibian populations are perturbed by disease. *Journal of Applied Ecology*, 48(4): 873–879.

Ord, J. K., Getis, A., 1995. Local spatial autocorrelation statistics: distribution issues and an application. *Geographical Analysis*, 27: 286–306.

Perez, R., Richards–Zawacki, C. L., Krohn, A. R., Robak, M., Griffith, E. J., Ross, H., Gratwicke, B., Ibáñez, R., Voyles, J., 2014. Field surveys in Western Panama indicate populations of *Atelopus varius* frogs are persisting in regions where *Batrachochytrium dendrobatidis* is now enzootic. *Amphibian & Reptile Conservation*, 8(2): 30–35.

Pounds, J., Puschendorf, R., Bolaños, F., Chaves, G., Crump, M., Solís, F., Ibáñez, R., Savage, J., Jaramillo, C., Fuenmayor, Q., Lips, K., 2010. *Atelopus varius*. In: IUCN Red List of Threatened Species. Version 2017.1, 2010, http://www.iucnredlist.org [Accessed on April 2018]

QGIS Development Team, 2016. QGIS Geographic Information System. Open Source Geospatial Foundation, http://qgis.osgeo.org [Accessed on April 2018]

R Core Team, 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, https://www.R–project.org/ [Accessed on April 2018]

Richards–Zawacki, C. L., 2009. Effects of slope and riparian habitat connectivity on gene flow in an endangered Panamanian frog, *Atelopus varius*. *Diversity and Distributions*, 15: 796–806.

Ryan, M. J., Lips, K. R., Eichholz, M. W., 2008. Decline and extirpation of an endangered Panamanian stream frog population (*Craugastor punctariolus*) due to an outbreak of chytridiomycosis. *Biological Conservation*, 141(6): 1636–1647.

Savage, J. M., 2002. *The amphibians and reptiles of Costa Rica: a herpetofauna between two continents, between two seas*. University of Chicago press, Chicago.

Segurado, P., Santos, J. M., Pont, D., Melcher, A. H., Jalon, D. G., Hughes, R. M., Ferreira, M. T., 2011. Estimating species tolerance to human perturbation: expert judgment versus empirical approaches. *Ecological Indicators*, 11(6): 1623–1635.