



Published by the British Herpetological Society

## Morphological anomalies of tadpoles from a contaminated stream in the state of Tlaxcala, Mexico

Maribel Méndez-Tepepa<sup>1</sup>, Kevin I. Medina-Bello<sup>2</sup>, Edelmira García-Nieto<sup>1</sup> & Libertad Juárez-Santa Cruz<sup>1</sup>

<sup>1</sup>Centro de Investigación en Genética y Ambiente, Posgrado en Ciencias en Sistemas del Ambiente, Universidad Autónoma de Tlaxcala, Mexico

<sup>2</sup>Doctorado en Ciencias Biológicas, Centro Tlaxcala de Biología de la Conducta, Universidad Autónoma de Tlaxcala, Mexico

The contamination of aquatic systems by anthropogenic activities may impact amphibian populations by causing malformation and death in tadpoles. However, there often needs to be more information regarding environmental pollutants' effects on amphibians, such as contaminated systems from the state of Tlaxcala in Mexico. This study reports on morphological abnormalities observed in *Lithobates spectabilis* tadpoles found in a stream of three sites monitored in Tlaxcala. The tadpoles presented swollen heads, edema, intestinal hemorrhage, anomalies in the mouth, a deviated tail, and underdeveloped tail fins. We hypothesise that these abnormalities have been caused by exposure to contamination from nearby communities, including garbage and sewage pumped directly into the stream. This research provides the first report on the potential adverse effects of contaminant exposure on tadpole development and morphology in this region. Identifying malformations in tadpoles could help detect contaminants in aquatic systems for further analysis.

**Keywords:** *Lithobates spectabilis*, pollutants, anthropogenic, edema, abnormalities

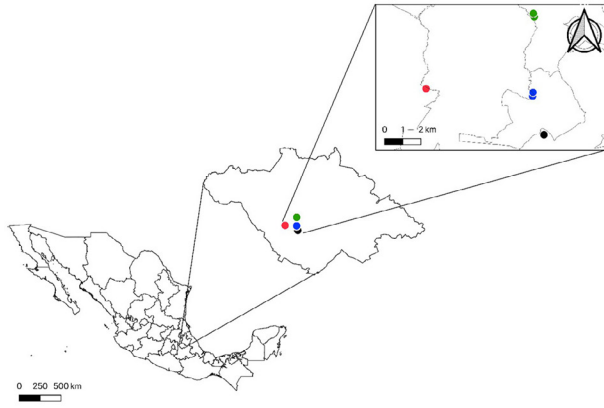
Amphibian populations are in decline and at risk worldwide (Wake & Koo, 2018), and one of the leading causes is the presence of environmental contaminants in aquatic systems (Stuart et al., 2004). Contaminant runoff affects the different stages of development in amphibian species that inhabit permanent systems, particularly in waterbodies where the accumulation of pollutants is much greater compared to smaller waterbodies (Barrett et al., 1995; Hu et al., 2020). Tadpoles are particularly vulnerable to contaminants during the early stages of development since they start their life cycle as aquatic organisms (Cooke, 1981; Rosenshield et al., 1999). Contaminants such as metals, microplastics, agrochemicals and radioactive have been shown to negatively impact the morphology, physiology, and behaviour of amphibians that begin their life cycles in aquatic systems (Mann et al., 2009; Ouellet et al., 1997). Tadpoles are very susceptible to contaminants due to their permeable skin, however, some tadpoles are

more vulnerable than others. This depends on the dose of the contaminant to which they are exposed and the organism's absorption, elimination, and biotransformation (Lotufo et al., 2015). In addition, it also depends on the octanol-water partition coefficient (Mackay et al., 2013). Therefore, some tadpoles that inhabit contaminated areas can experience harmful effects only if they are in constant exposure, preventing the concentrations of the contaminant in the tissue from persisting at levels that could cause a detrimental biological effect (Lotufo et al., 2015).

Environmental contaminants can directly affect external morphological structures, leading to locomotion problems (Araújo & Malafaia, 2020), bent tails (Pérez-Iglesias et al., 2015; Sunderman et al., 1991; Montalvão et al., 2018), malformation of the mouthparts (keratodons) and changes in skin colouration (Schuytema & Nebeker, 1998; Egea-Serrano et al., 2012). In addition, contaminants affect internal morphological structures, leading to edemas (swelling of the body with liquid) (Wells et al., 2005; Liendro et al., 2015), intestinal malrotation and reddish colouration of the visceral (Severtsova & Aguillón-Gutiérrez, 2013; Lefcort et al., 1998; Snodgrass et al., 2005; Gutiérrez & Bautista, 2015; Peltzer et al., 2013; Christopher et al., 1996). These anomalies could affect ecological aspects such as patterns of relative abundance, dominance, and richness, among others (Bridges & Semlitsch, 2000; Ficken & Byrne, 2013) and physiological mechanisms such as metabolic responses and alterations during metamorphosis (Wells et al., 2005; Severtsova & Aguillón-Gutiérrez, 2013).

Mexico ranks fifth in amphibians worldwide, and approximately 69.5 % of the species are considered threatened. Contamination affects a relatively high number of Mexican amphibians threatened by toxic chemicals (Frías-Alvarez et al., 2010). The malformations in tadpoles have generated scientific interest, with increased detection of malformations within the last decades (Araújo & Malafaia, 2020; Wells et al., 2005; Peltzer et al., 2013). However, in the state of Tlaxcala, Mexico, there has yet been no report of contamination and abnormalities in tadpoles that inhabit aquatic systems. In this study,

Correspondence: Maribel Méndez-Tepepa (maribelmendezte@gmail.com)



**Figure 1.** Capture sites located in three locations in the state of Tlaxcala, México. The pink and blue dots correspond to the capture sites in STH, the green and yellow dots in SAT, and the purple in the LTT. In addition, downtown Tlaxcala is marked with a black dot.

we report on morphological abnormalities in tadpoles of *Rana manchada* (*Lithobates spectabilis*) located in a contaminated stream.

*Lithobates spectabilis* tadpoles were captured during amphibian monitoring from 1 May 2021 to 30 April 2022, at five sites located in three communities (i.e. human settlements): one site in La Trinidad Tenexyecac (hereinafter LTT; 19° 21'8.37" N, 98° 18'30.79" W), two sites in San Ambrosio Texantla (hereinafter SAT; 19° 21'16.343" N, 98° 15'5.348" W) and two sites in San Tadeo Huiloapan (hereinafter STH; 19° 23'46.131" N, 98° 15'42.101" W). The capture sites are found in the municipalities of Ixtacuixtla and Panotla, in the state of Tlaxcala, Mexico (Fig. 1). The capture sites are located at a distance from the downtown of Tlaxcala of ~7.6 km for LTT, ~4.6 km for SAT and ~8.01 km for STH. This distance between the capture sites and the downtown of Tlaxcala was measured linearly using the Qgis software (Quantum GIS Development Equipment). The vegetation is composed of *Quercus* spp.

The tadpoles were captured using a fishing net in the stream, and with the permission of the wildlife department (SEMARNAT SGPA/DGVS/03662). A group of biologists from the Universidad Autónoma de Tlaxcala determined the identity of the species. In addition, the species was determined by utilising the identification guide for *L. spectabilis*. The tadpoles of *L. spectabilis* are of the stream type; it has a long and muscular tail with low fins. In addition, tadpoles of this species grow to be quite large, and individuals with a total length of over 100 mm are common (Hillis & Frost, 1985).

Once captured, the tadpoles were immediately transported to the laboratory in two tanks (51 x 29.5 x 26 cm) with water from their respective sites. We obtained the tadpole's body mass (Mb) using a digital balance with a precision of 0.1 g (Ohaus, Newark, New Jersey, U.S.A.), and snout-vent length (SVL) using a caliper to the nearest 0.1 mm (Truper, CALDI-6MP; 14388). Morphological characteristics were determined with a binocular stereoscope (Mitoc®, 1272ZH), according to criteria reported by Gutiérrez & Bautista (2015). Photographs were taken with a Canon-DS126491 camera.



**Figure 2.** Morphological anomalies in *Lithobates spectabilis* tadpoles located in the contaminated stream SAT, Tlaxcala, Mexico. **(A)** Contamination mainly by garbage and **(B)** car washing in the stream. **(C)** Tadpole without abnormalities with normal head (arrowhead), long tail (black arrow) and caudal fin with melanophores (red arrow). **(D)** Tadpoles with morphological abnormalities folded fin and without melanophores (red arrow). **(E)** In addition, they had small tails that were thin, curved, and slightly crooked (arrow), **(F)** edema, deformation of labial papillae (arrowhead), and **(G)** intestinal bleeding (arrowhead).

We captured 100 tadpoles of species *L. spectabilis* (LTT n = 30; SAT n = 40; STH n = 30). We observed that three tadpoles presented morphological abnormalities at one site in particular (SAT), which is a stream contaminated by anthropogenic activities (Fig. 2A & B). During surveys, we observed abundant evidence of contamination of streams at SAT, primarily solid waste material (polystyrene, aluminium, plastics, glass, detergents, oils) resulting from various anthropogenic activities. In addition, people visit the stream to generate garbage (Fig. 2A), and wash their cars in the stream (Fig. 2B).

We found tadpoles without abnormalities (Fig. 2C), and the tadpoles with abnormalities had a caudal fin that was not well-formed and lacked melanophores (Fig. 2D). Additionally, the abnormal tadpoles had small tails that were thin, curved, and slightly bent (Fig. 2E). The tadpoles had a swollen head (edema in the body with a transparent liquid), deformation of labial papillae (Fig. 2F) and intestinal bleeding (Fig. 2G).

Although we did not obtain enough data from the tadpoles with abnormalities to carry out a statistical test, we observed that the Mb of these was substantially greater ( $0.29 \pm 0.1$  g) when compared to tadpoles without abnormalities ( $0.19 \pm 0.06$  g). Furthermore, snout-vent length (SVL) of tadpoles with abnormalities was lower (17.7

$\pm 6.1$  mm) concerning tadpoles without abnormalities ( $20.6 \pm 2.0$  mm) in SAT. While capturing the tadpoles with abnormalities, we observed a slow and unsynchronised swimming movement, which made capturing the tadpoles faster.

The present work is the first formal report of alterations in the morphology of tadpoles in a site that is part of the megalopolis of the Mexican highlands. Curiously, malformations were only observed in tadpoles found in the capture sites located in the SAT community, which is the closest to downtown Tlaxcala, unlike the other two study sites. The SAT site is constantly visited by the community people, so malformations in the tadpoles can be indicators of environmental health and habitat quality. Additionally, according to interviews with authorities, sewage is discharged directly from the community, without treatment, into this aquatic system. This finding may be important because surface-water discharge from industrial and municipal is one of the significant sources of contaminant mixtures in aquatic systems (Pal et al., 2010) and tadpoles exposed to these domestic residual releases result in developmental alterations, metabolic disruption, and behavioural effects (e.g. preference or avoidance of areas with high contaminants) (Melvin et al., 2016; Krishnamurthy et al., 2008; Pal et al., 2010). These results indicate the first study determining the effect of wastewater on tadpole development in the stream SAT and that the distance between SAT and downtown Tlaxcala could indicate more stream contamination and a possible increase in the frequency of abnormalities in the tadpoles.

The tadpoles showed edema, which can signify exposure to these particular chemicals. The plastics product of garbage generation (Rhodes, 2019), metals like aluminium (Ismail et al., 2019), and even different pesticides (Lenkowski et al., 2010) are widely present in the different aquatics system and causes different abnormalities such as edema. However, viruses, bacteria and fungi also can cause edema and other morphological abnormalities (Densmore & Green, 2007). On the other hand, intestinal hemorrhage affects their feeding and induces the death of individuals (Marco & Blaustein, 1999). The intestinal abnormalities are due to oxidative stress or necrosis in the cells (Wells et al., 2005; Liendro et al., 2015). The tadpoles with deformation of labial papillae have feeding problems (Pérez-Iglesias et al., 2015). Caudal fin malformation affects thrusting, bending, buckling, locomotion and escape from predators (Doherty et al., 1998). In addition, other researchers determined the curved and deflected tail may affect swimming activity and orientation (Cooke 1981; Rosenshield et al., 1999), just like foraging (Horat & Semlitsch, 1994).

Although we found damage to the morphology of the tadpoles, the usual concentrations of pollutants in the stream in SAT are unknown. Our results suggest that the anomalies found in the tadpoles may be a biological indicator of contamination in this small aquatic system. However, the specific contaminants responsible for these malformations were not identified. It is, therefore, essential to analyse the water and sediments of the aquatic

ecosystems of Tlaxcala, since there currently needs to be more information on water quality. For this reason, it is critical to carry out more ecotoxicological research and provide adequate solutions to avoid contamination and possible threat status of aquatic species at this site. Monitoring tadpoles with morphological anomalies can be a vital strategy for identifying potentially contaminated sites in Tlaxcala, México.

## ACKNOWLEDGEMENTS

We thank the authorities of San Tadeo, Huiloapan, San Ambrosio Texantla, and the Trinidad Tenexyecac community for their support in the logistics of this work. Likewise, to A. Popocatl and E. Chamorro for their support.

## REFERENCES

- Araújo, A. & Malafaia, G. (2020). Can short exposure to polyethylene microplastics change tadpoles' behavior? A study conducted with neotropical tadpole species belonging to order anura (*Physalaemus cuvieri*). *Journal of Hazardous Materials* 391, 122214.
- Barrett, M.E., Zuber, R.D., Collins, E.R., Malina, J.F., Charbeneau, R.J. & Ward, G.H. (1995). A review and evaluation of literature pertaining to the quantity and control of pollution from highway runoff and construction, 2nd Ed. Technical Report 239, Center for Research in Water Resource, The University of Texas at Austin.
- Bridges, C.M. & Semlitsch, R.D. (2000). Variation in pesticide tolerance of tadpoles among and within species of Ranidae and patterns of amphibian decline. *Conservation Biology* 14(5), 1490–1499.
- Densmore, C.L. & Green, D.E. (2007). *Diseases of Amphibians*. ILAR Journal, Volume 48, Issue 3. 235–254 pp.
- Christopher, R., Kinney, O.M., Fiori, A.M. & Congdon, J.D. (1996). Oral deformities in tadpoles (*Rana catesbeiana*) associated with coal ash deposition: effects on grazing ability and growth. *Freshwater Biology* 36, 723–730.
- Cooke, A.S. (1981). Tadpoles as indicators of harmful levels of pollution in the field. *Environmental Pollution Series A, Ecological and Biological* 25, 123–133.
- Doherty, P.A., Wassersug, R.J. & Lee, J.M. (1998). Mechanical properties of the tadpole tail fin. *The Journal of Experimental Biology* 201, 2691–2699.
- Egea-Serrano, A., Relyea, R.A., Tejedro, M. & Torralva, M. (2012). Understanding of the impact of chemicals on amphibians: a meta-analytic review. *Ecology and Evolution* 2, 1382–1397.
- Frías-Alvarez, P., Zúñiga-Vega, J.J. & Flores-Villela, O. (2010). A general assessment of the conservation status and decline trends of Mexican amphibians. *Biodiversity and Conservation* 19, 3699–3742.
- Ficken, K.L. & Byrne, P.G. (2013). Heavy metal pollution negatively correlates with anuran species richness and distribution in south-eastern Australia. *Austral Ecology* 38, 523–533.
- Gutiérrez, D.R.A. & Bautista, A.R. (2015). Anomalías frecuentes en una población de *Hyla plicata* (Anura: Hylidae) expuesta a plomo y hierro durante el desarrollo postembrionario. *Biocyt: Biología, Ciencia y Tecnología* 8, 515–529.
- Hillis, D.M. & Frost, J.S. (1985). Three new species of leopard

- frogs (*Rana pipiens* complex) from the Mexican plateau. *The Museum of Natural History*, University of Kansas Lawrence, Kansas.
- Horat, P. & Semlitsch, R.D. (1994). Effects of predation risk and hunger on the behaviour of two species of tadpoles. *Behavioral Ecology and Sociobiology* 34, 393–401.
- Hu, L., He, D. & Shi, H. (2020). Microplastics in inland small waterbodies. *Microplastics in Terrestrial Environments*, 93–110.
- Ismail, T., Lee, H.K., Kim, C. Kim, Y., Lee, H., Kim, J.H., Kwon, S., Huh, T.L., Khang, D., Kim, S.H. & Choi, S.C. (2019). Comparative Analysis of the Developmental Toxicity in *Xenopus laevis* and *Danio rerio* Induced by Al<sub>2</sub>O<sub>3</sub> Nanoparticle Exposure. *Environmental Toxicology and Chemistry* 38(12), 2672–2681. doi:10.1002/etc.4584.
- Krishnamurthy, S.V., Meenakumari, D., Gurushankara, H.P. & Vasudev, V. (2008). Nitrate-induced morphological anomalies in the tadpoles of *Nyctibatrachus major* and *Fejervarya limnocharis* (Anura: Ranidae). *Turkish Journal of Zoology* 32(3), 239–244.
- Lefcort, H.G., Meguire, R.A., Wilson, L.H. & Ettinger, W.F. (1998). Heavy metals alter the survival, growth, metamorphosis, and antipredatory behavior of Columbia spotted frog (*Rana luteiventris*) tadpoles. *Archives of Environmental Contamination and Toxicology* 35, 447–456.
- Lotufo, G.R., Biedenbach, J.M., Sims, J.G., Chappell, P., Stanley, J.K. & Gust, K.A. (2015). Bioaccumulation kinetics of the conventional energetics TNT and RDX relative to insensitive munitions constituents DNAN and NTO in *Rana pipiens* tadpoles. *Environmental Toxicology and Chemistry* 34(4), 880–886.
- Lenkowski, J.R., Sanchez-Bravo, G. & McLaughlin, K.A. (2010). Low concentrations of atrazine, glyphosate, 2, 4-dichlorophenoxyacetic acid, and triadimefon exposures have diverse effects on *Xenopus laevis* organ morphogenesis. *Journal of Environmental Sciences* 22(9), 1305–1308.
- Liendro, N., Ferrari, A., Mardirosian, M., Lascano, C.I. & Venturino, A. (2015). Toxicity of the insecticide chlorpyrifos to the South American toad *Rhinella arenarum* at larval developmental stage. *Environmental Toxicology and Pharmacology* 39, 525–535.
- Mann, R.M., Hyne, R.V., Choung, C.B. & Wilson, S.P. (2009). Amphibians and agricultural chemicals: Review of the risks in a complex environment. *Environmental Pollution* 157, 2903–2927.
- Mackay, D., Arnot, J.A., Gobas, F.A.P.C. & Powell, D.E. (2013). Mathematical relationships between metrics of chemical bioaccumulation in fish. *Environmental Toxicology and Chemistry* 32, 1459–1466.
- Marco, A. & Blaustein, A.R. (1999). The effects of nitrite on behavior and metamorphosis in cascades frogs (*Rana cascadae*). *Environmental Toxicology and Chemistry*, 18.
- Melvin, S.D., Lanctôt, C.M., van de Merwe, J.P. & Leusch, F.D. (2016). Altered bioenergetics and developmental effects in striped marsh frog (*Limnodonastes peronii*) tadpoles exposed to UV treated sewage. *Aquatic Toxicology* 175, 30–38.
- Montalvão, M.F., da Silva Castro, A., de Lima Rodrigues, A.S., de Oliveira Mendes, B. & Malafaia, G.C. (2018). Impacts of tannery effluent on development and morphological characters in a neotropical tadpole. *The Science of the Total Environment* 610, 1595–1606.
- Ouellet, M., Bonin, J., Rodrigue, J., DesGranges, J.L. & Lair, S. (1997). Hindlimb deformities (ectromelia, ectrodactyly) in free-living anurans from agricultural habitats. *Journal of Wildlife Diseases* 33, 95–104.
- Pal, A., Gin, K.Y.H., Lin, A.Y.C. & Reinhard, M. (2010). Impacts of emerging organic contaminants on freshwater resources: review of recent occurrences, sources, fate and effects. *Science of the Total Environment* 408, 6062–6069.
- Peltzer, P.M., Lajmanovich, R.C., Attademo, A.M., Junges, C.M., Cabagna-Zenkhusen, M.C., Repetti, M.R., Sigrist, M.E. & Beldoménico, H. (2013). Effect of exposure to contaminated pond sediments on survival, development, and enzyme and blood biomarkers in veined treefrog (*Trachycephalus typhonius*) tadpoles. *Ecotoxicology and Environmental Safety* 98, 142–151.
- Pérez-Iglesias, J.M., Soloneski, S., Nikoloff, N., Natale, G.S. & Larramendy, M.L. (2015). Toxic and genotoxic effects of the imazethapyr-based herbicide formulation Pivot H® on montevideo tree frog *Hypsiboas pulchellus* tadpoles (Anura, Hylidae). *Ecotoxicology and Environmental Safety* 119, 15–24.
- Rhodes, C.J. (2019). Solving the plastic problem: From cradle to grave, to reincarnation. *Science Progress* 102(3), 218–248.
- Rosenshield, M.L., Jofré, M.B. & Karasov, W.H. (1999). Effects of polychlorinated biphenyl 126 on green frog (*Rana clamitans*) and leopard frog (*Rana pipiens*) hatching success, development, and metamorphosis. *Environmental Toxicology and Chemistry* 18, 2478–2486.
- Schuytema, G.S. & Nebeker, A.V. (1998). Comparative toxicity of diuron on survival and growth of Pacific treefrog, bullfrog, red-legged frog, and African clawed frog embryos and tadpoles. *Archives of Environmental Contamination and Toxicology* 34, 370–376.
- Severtsova, E.A. & Aguillón-Gutiérrez, D.R. (2013). Postembryonic development of anurans in ponds littered with metal-containing refuse (simulation experiments). *Biology Bulletin* 40, 738–747.
- Snodgrass, J.W., Hopkins, W.A., Jackson, B.P., Baionno, J. A. & Broughton, J. (2005). Influence of larval period on responses of overwintering green frog (*Rana clamitans*) larvae exposed to contaminated sediments. *Environmental Toxicology and Chemistry* 24, 1508–1514.
- Stuart, S.N., Chanson, J.S., Cox, N.A., Young, B.E., Rodrigues, A.S., Fischman, D.L. & Waller, R.W. (2004). Status and trends of amphibian declines and extinctions worldwide. *Science* 306, 1783–1786.
- Sunderman Jr., F.W., Plowman, M.C. & Hopfer, S.M. (1991). Embryotoxicity and teratogenicity of cadmium chloride in *Xenopus laevis*, assayed by the FETAX procedure. *Annals of Clinical and Laboratory Science* 21, 381–391.
- Wake, D.B. & Koo, M.S. (2018). *Amphibians*. *Current biology* 28, R1237–R1241.
- Wells, P.G., Bhuller, Y., Chen, C.S., Jeng, W., Kasapinovic, S., Kennedy, J.C., Kim, P.M., Laposa, R.R., McCallum, G.P., Nicol, C.J. et al. (2005). Molecular and Biochemical Mechanisms in Teratogenesis Involving Reactive Oxygen Species. *Toxicology and Applied Pharmacology* 207, 354–366.

Accepted: 2 April 2023